SPECIALIST PERIODICAL REPORTS

Carbohydrate Chemistry

VOLUME 14

Part II

MACROMOLECULES

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Volume 14

Part II

A Specialist Periodical Report

Carbohydrate Chemistry

Volume 14

Part II

Macromolecules

A Review of the Literature Published during 1980

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Preface

This Report, the fourteenth in the Series, covers the new literature available to us between January and December, 1980. Again we have continued to produce a comprehensive survey of publications, both primary and secondary, in a concise and readable form.

From conversations we have had with readers in various parts of the world, the almost unanimous opinion is one of satisfaction with the content of the Report. However, if the Series is to continue then it must receive greater support in terms of sales. There is a considerable threat to its continued life in view of the continually increasing costs of production not being paralleled by increase in sales. It is felt that greater price increases would be suppressive to the market.

As intimated in a preliminary way in the Preface to the previous Volume in the Series, an attempt is also being made to restore the balance of economy of production by introducing the camera-ready copy system. Thus this is the last Volume to be type-set. Although there are a large number of problems in setting up a successful word processing system which will allow editing and unification of the appearance of all chapters, not only have we surmounted this problem in Birmingham but we have developed a prototype microcomputer/word processor which will handle all the special symbols necessary for writing carbohydrate chemistry.

In an endeavour to restrain price increases, it has been decided to publish this Volume in two separately bound books – Parts I and II, and these parts correspond exactly in types of content with those of the existing Parts I and II of previous volumes. Thus continuity from previous volumes will not be broken, but personal purchases should be more economic since readers will have the option not to purchase one of the Parts if they feel they can, at least, make only limited use of it. Volume 15 will also be produced in two separately bound books.

We are grateful to those readers who responded to our enquiry in which the utility of the Author Index was questioned. Almost without exception, readers wished retention of the Index and we are pleased to announce that the Royal Society of Chemistry has accepted this view in spite of the increased cost of inclusion of an Author Index.

The team of reporters for Volume 14 was unchanged from that for Volume 13, and I gratefully recognize the considerable effort put in by every one of my colleagues, without whose sustained conscientious effort I would not be able to produce a Volume.

Finally, the services provided by Dr P. G. Gardam, Manager, Books, and his staff, particularly Mrs L. A. Turrell, Assistant Editor, Books, at the Royal Society of Chemistry in the production of this Report are much appreciated.

May 1983 JOHN F. KENNEDY

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Abbreviations

The following abbreviations have been used:

Chemicals and Chemical Groups

ADP	adenosine 5′-diphosphate
AMP	adenosine 5′-phosphate
ATP	adenosine 5′-triphosphate
CDP	cytidine 5′-diphosphate
CMP	cytidine 5′-phosphate
DEAE	*N*,*N*-diethylaminoethyl
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
GDP	guanosine 5′-phosphate
RNA	ribonucleic acid
THF	tetrahydrofuran
TMS	trimethylsilyl
UDP	uridine 5′-diphosphate

Physicochemical Methods

c.d.	circular dichroism
d.s.c.	differential scanning calorimetry
e.s.r.	electron spin resonance
g.c.-m.s.	gas–liquid chromatography–mass spectrometry
g.l.c.	gas–liquid chromatography
g.p.c.	gel permeation chromatography
h.p.l.c.	high performance liquid chromatography
i.r.	infrared
m.s.	mass spectrometry
n.m.r.	nuclear magnetic resonance
o.r.d.	optical rotatory dispersion
t.l.c.	thin-layer chromatography
u.v.	ultra-violet

Part II

MACROMOLECULES

By

J. F. Kennedy
I. M. Morrison
C. M. Sturgeon
R. J. Sturgeon

1

Introduction

BY J. F. KENNEDY

The Introduction to the Part II Report was revised extensively in Volume 12.[1] That Chapter, and the additions in Volume 13,[2] covered in detail: 'Scope and Coverage of the Report'; 'Organization, Nomenclature, and Use of the Report'; 'Significant Advances in Macromolecular Carbohydrate Chemistry'; and 'Conclusions and Readership', is classified as 'essential reading' for any new reader of 'Carbohydrate Chemistry' Part II Reports.

This present Chapter essentially builds upon the corresponding chapters of the two previous Volumes[1,2] the former of which may be regarded as the ground state.

1 Scope and Coverage of the Report

This is largely unchanged from that of the two preceding Volumes; again the journal coverage list has been modified to include new titles. However, the current trend of economies in various University and other institutional libraries will undoubtedly lead to cutbacks in their current holdings of less well read journals. If a considerable amount of material becomes unavailable directly to Reporters, it may be that again on grounds of cost it will not be possible to sustain back-up through the Inter Library Loans system.

The continuing inclusion of reports on principal carbohydrate (-containing) macromolecules in the more recently inaugurated Specialist Periodical Reports series entitled 'Macromolecular Chemistry',[3,4] is neither a duplication of, nor a competitor to, 'Carbohydrate Chemistry, Part II'. For the specialists, and the readers who want information on the broader field of both synthetic and neutral macromolecules, the similarities and differences between them, and an overview of the uses of all types of macromolecules including carbohydrates, proteins, and nucleic acids, a copy of each volume of 'Macromolecular Chemistry' is appropriate for their bookshelves. However, the relevance of that title is not

[1] J. F. Kennedy, in 'Carbohydrate Chemistry', Part II, Macromolecules, ed. J. F. Kennedy and N. R. Williams (Specialist Periodical Reports), The Royal Society of Chemistry, London, 1981, Vol. 12, p. 215.

[2] J. F. Kennedy, in 'Carbohydrate Chemistry', Part II, Macromolecules, ed. J. F. Kennedy and N. R. Williams (Specialist Periodical Reports), The Royal Society of Chemistry, London, 1982, Vol. 13, p. 233.

[3] A. D. Jenkins and J. F. Kennedy, ed., 'Macromolecular Chemistry', (Specialist Periodical Reports), The Royal Society of Chemistry, London, 1980, Vol. 1.

[4] A. D. Jenkins and J. F. Kennedy, ed., 'Macromolecular Chemistry', (Specialist Periodical Reports), The Royal Society of Chemistry, London, 1982, Vol. 2.

confined to the Chapter on carbohydrates – since numerous chapters include items on the chemistry and interactions of carbohydrates in general polymer chemistry and bio-medical applications.

2 Organization, Nomenclature, and Use of the Report

Draft documents from the Nomenclature Committee of the International Union of Pure and Applied Chemistry (IUPAC) for various aspects of carbohydrate nomenclature are under consideration. One final document, entitled 'Conformational Nomenclature for Five- and Six-Membered Ring forms of Monosaccharides and their Derivatives (Rec. 1980)', has been published[5] and definitive documents on abbreviated terminology of oligosaccharide chains, nomenclature of unsaturated monosaccharides, symbols for specifying the conformation of polysaccharide chains, nomenclature of branched-chain monosaccharides, and polysaccharide nomenclature can be expected to appear in the very near future.

In the area of nomenclature, there has been considerable discussion of late on the terminologies of the words phytohaemagglutinin, haemagglutinin, and lectin. Whereas the underlying etymology of some of these words is obvious, the relative utilities of the words has come into question. As new discoveries have been made, it has been necessary to adjust from the original belief that such types of molecules were only endogenous to plants. Various workers in the field[6] have discussed nomenclature under the heading 'What should be called a lectin?' as follows.

'During the past decade, interest in lectins (also referred to as agglutinins, phytohaemagglutinins, phytoagglutinins, and protectins) has increased enormously. The term lectin has frequently been used to describe substances that differ markedly from those classically considered lectins, that is plant seed carbohydrate-binding proteins. Nevertheless, the discovery of lectin-like substances in widely diverse sources (bacteria, fungi, fish-sera, snails, *etc.*) appears to warrant an expansion of the term. For the sake of clarity we would suggest the following definition of "lectin".

'A lectin (from the Latin *legere*: to choose) is a sugar-binding protein or glycoprotein of non-immune origin which agglutinates cells and/or precipitates glycoconjugates. Lectins bear at least two sugar-binding sites, agglutinate animal and plant cells (most commonly erythrocytes, unmodified or enzyme-treated) and/or precipitate polysaccharides, glycoproteins, and glycolipids. The specificity of a lectin is usually defined in terms of the monosaccharide(s) or simple oligosaccharides that inhibit lectin-induced agglutination (or precipitation or aggregation) reactions. Although first discovered in plants, lectins also have been found in many organisms from bacteria to mammals. A lectin may be soluble in biological fluids (*e.g.* concanavalin A) or membrane-bound (*e.g.* rabbit or rat liver lectins). There are several other types of sugar-binding proteins including sugar-specific enzymes (glycosidases, glycosyltransferases, glycosyl-

[5] 'Conformational Nomenclature for Five- and Six-Membered Ring Forms of Monosaccharides and Their Derivatives (Rec. 1980)', *Eur. J. Biochem.*, 1980, **111**, 295.

[6] I. J. Goldstein, R. C. Hughes, H. Monsigny, T. Osawa, and N. Sharon, *Nature (London)*, 1980, **285**, 66.

kinases, glycosylpermeases, glycosylepimerases, ...), transport proteins, hormones (thyroid-stimulating hormone, follicle-stimulating hormone,...), and toxins (ricin, abrin, modeccin,...), *etc.*

'Under some conditions, sugar-specific enzymes with multiple combining sites agglutinate cells (and/or precipitate glycoconjugates) and so act as a lectin. In spite of similarities to true lectins from the same source, toxins that bear only one sugar-binding site should not be called lectins since they do not agglutinate cells or precipitate glycoconjugates.'

In connection with use of Chapter 6 on Enzymes, readers are reminded of the existence of a special index suffixed to the Chapter. This index is invaluable since it enables readers to locate references to a particular enzyme speedily, and it also can be read as a listing of the enzymes which are principally related to carbohydrate hydrolysis, oxidation, and isomerization. As has been generally adopted in this Series, transferase enzymes are dealt with in conjunction with the macromolecular carbohydrate, the biosynthesis of which they contribute to, and therefore appear in the relevant Chapter rather than specifically and only in Chapter 6.

3 Significant Advances in Macromolecular Carbohydrate Chemistry

An increasing dislike by the legislative authorities for the use of polysaccharide (starch) derivatives in foodstuffs means that the development of derivatives for commercial applications must be confined to other uses. In particular, oxidized starches are coming to be questioned *re* their safety in foods such as bread, *etc.*

Mention should be made of the increasing use of ^{1}H and ^{13}C n.m.r. spectroscopy in structural studies on plant polysaccharides. Particular use has been made in studies on water-insoluble polymers where the polymer has been solubilized by derivatization. Anthraquinone and its 2-sulphonic acid derivative continue to be of use in preventing the β-elimination reaction of polysaccharides in alkaline solutions.

The inability of certain tomato fruits to ripen is caused by the absence of a specific poly-D-galacturonanase isoenzyme.

The number of citations in the field of glycoproteins, proteoglycans, and animal polysaccharides has increased dramatically, and for the first time there are more than one thousand references cited in Chapter 5. Techniques for the preparation of monoclonal antibodies are now being widely applied, and numerous examples of the isolation and application of monoclonal antibodies to well-defined glycoproteins and polysaccharides are reported.

The minimum sequence of monosaccharides in heparin necessary for binding to antithrombin III has now been identified as eight. The location of sulphate ester groups in the octasaccharide is also important for the retention of biological activity.

The amount of attention being afforded the types and levels of glycoside hydrolase enzymes involved in the manifestation of clinical conditions and related subjects continues to increase, and the various enzymes responsible for the breakdown of a number of carbohydrate(-containing) molecules have now been identified. The various amylolytic enzymes also attract attention and there

is considerable activity in the field of generation of new substrates particularly for fully automated systems. Undoubtedly, the number of identifiable polysaccharide-degrading enzymes is steadily increasing, but the fuller understanding of their properties, action patterns, and mechanisms of action follows at a slower pace. The field of cellulases still provides plenty of scope for elucidation of their functional relationships.

It has been established that Krabbes disease is caused by an accumulation of D-galactosylsphingosine (psychosine). The glycolipid reaches toxic levels and kills oligodendroglial cells.

The first report has been made of the presence of L-fucose residues in the more complex glycolipid fraction of human brain tissue.

Considerable interest continues to be taken in the synthesis of the repeating units of bacterial polysaccharides. The behaviour of cycloamylose inclusion complexes, particularly those of drugs, has been the subject of a number of studies. Polysaccharides have been used extensively as support matrices for the preparation of affinity chromatography media and of immobilized enzymes. Numerous immobilized derivatives of cells have been prepared and applied to problems in both research and industry.

Special Issues of *Carbohydrate Research* have continued in the pattern of being published in honour of persons with international reputations in carbohydrate chemistry and their achievements. Professor Stephen J. Angyal and his work on, *inter alia*, chromium trioxide oxidation of glycosides and the use of the reaction in structural studies on polysaccharides, and the complexation of sugar with alkaline-earth and rare-earth ions and the use of this complexation to facilitate the synthesis of rare methyl glycosides as well as in the separation of mixtures of methyl glycosides, have been so honoured.[7]

Published versions of the Tate and Lyle Carbohydrate Chemistry Award Lecture entitled 'Transition-metal oxide chelates of carbohydrate-directed macromolecules',[8] and 'From carbohydrates to enzyme analogues',[9] have appeared.

4 Conclusions and Readership

As already indicated, 'Carbohydrate Chemistry' enjoys a wide readership and utility. However, it is the experience of Senior Reporters and Reporters that well-established reputable groups overseas are not readily aware of the Series. Whereas the Royal Society of Chemistry has increased its publicity and sales, programme all readers are encouraged to support the Series by advertising it as widely as possible in the interests of economy and survival.

[7] L. Anderson, *Carbohydr. Res.*, 1980, **82**, 163.
[8] J. F. Kennedy, *Chem. Soc. Rev.*, 1979, **8**, 221.
[9] J. F. Stoddart, *Chem. Soc. Rev.*, 1979, **8**, 85.

2

General Methods

BY R. J. STURGEON

1 Gas-Liquid Chromatography

The use of g.l.c. for the measurement of free sugars and sugar alcohols in biological fluids has been reviewed.[1] A chiral stationary phase, *N*-propionyl-L-valyl-t-butylamide polysiloxane, has been used for the separation of monosaccharides as their alditol acetates by capillary column g.l.c.[2]

The alditol acetates of the common neutral sugars and three hexa-acetates, and the peracetates of 3-*O*-methyl- and 4-*O*-methyl-D-glucitol can be resolved in short analysis times. The determination by g.l.c. of neutral sugars as aldononitrile acetates, and of amino-sugars as *O*-methyl oxime acetates, in glycoproteins has been reported.[3] An improved method is also described for converting neutral sugars to oximes, which can be either converted to the trimethylsilyl derivatives or, upon acetylation, derivatized to aldononitrile acetates.

Levels of D-mannose in human serum have been measured using the aldononitrile acetate derivatives.[4] The method has been used for measuring D-mannose in patients as an indicator of the presence of invasive candidiasis. Dissolved carbohydrates in natural water have been estimated by g.l.c. of the derived alditol acetates.[5]

The use of peracetylated aldononitrile derivatives of aldoses and peracetylated ketose oxime derivatives of ketoses for g.l.c.–m.s. studies has been reported in an accurate discriminatory method for the identification and quantitation of aldoses, ketoses, and small oligosaccharides.[6] These derivatives may also be used in the separation and identification of products arising from methylation–fragmentation structural analysis.

D-Fructose has been determined as the *O*-methyl oxime trimethylsilyl derivative by g.l.c. using glass capillary columns.[7] The D-fructose derivative is readily separated from D-glucose, D-galactose, L-rhamnose, and L-arabinose.

Hexitols occurring in biological fluids have been analysed as their per-*O*-acetyl derivatives by g.l.c.–m.s. with selected ion-monitoring.[8] The method has been developed and applied to the analysis of D-galactitol and other polyols in

[1] M. F. Laker, *J. Chromatogr.*, 1980, **184**, 457.
[2] G. Holzer, J. Oro, S. J. Smith, and V. M. Doctor, *J. Chromatogr.*, 1980, **194**, 410.
[3] T. P. Mawhinney, M. S. Feather, G. L. Barbero, and J. Martinez, *Anal. Biochem.*, 1980, **101**, 112.
[4] T. P. Monson and K. P. Wilkinson, *Clin. Chem.*, 1979, **25**, 1384.
[5] M. Ochiai, *J. Chromatogr.*, 1980, **194**, 224.
[6] F. R. Seymour, E. C. M. Chen, and J. E. Stouffer, *Carbohydr. Res.*, 1980, **83**, 201.
[7] H. Zegota, *J. Chromatogr.*, 1980, **192**, 446.
[8] B. R. Pettit, G. S. King, and K. Blau, *Biomed. Mass Spectrom.*, 1980, **7**, 309.

amniotic fluid as an adjunct to enzyme studies on amniotic fluid cells in the antenatal diagnosis of galactosaemia and measurement of galactokinase activity.

The thermal degradation of mono- and oligo-saccharides occurring in culture fluids as a result of microbial growth has been studied by g.l.c.-m.s.[9] Aldopentoses degrade to furfural, whereas aldohexoses yield acetic acid, furfural, methyl furfural, levulinic acid, and hydroxymethylfurfural. Oligosaccharides give degradation products that reflect the structures of the component monosaccharides.

The carbohydrates and organic acids of uremic serum have been monitored using g.l.c.-chemical ionization m.s. of the *O*-TMS-ethers.[10]

2 Column and Ion-exchange Chromatography

Practical aspects of gel-permeation chromatography for separation of carbohydrates have been reviewed.[11] Molecular sieving and non-ionic adsorption in polysaccharide gels have been discussed in the XII Hopkins Memorial Lecture.[12]

The applications of a commercially available liquid chromatography analyser to provide a fully automated qualitative and quantitative analysis of neutral mono- and oligo-saccharides with on-line computer calculation of the data have been described.[13,14] Oligosaccharides (d.p. 2–15) may be routinely separated, with a degree of separation superior to that which is achieved using gel-filtration techniques under gravity or with peristaltic pump-aided flow.

Gels synthesized by cross-linking guar gum with epichlorhydrin in aqueous propan-2-ol have been described.[15] The flow properties of the gels are satisfactory only at moderate pressures. The degree of cross-linking was controlled and one of the products was used in the separation of proteins (mol. wt. 5×10^3–7×10^4). The problems associated with the column packings used in the characterization of dextran by aqueous gel-permeation chromatography have been investigated.[16] Fractionating range, efficiency, short analysis time, and long-term stability are the major factors taken into account, but none of the solumn packings investigated is considered to be ideal.

Liquid chromatographic separations of poly-, oligo-, and mono-saccharides have been achieved.[17] Polysaccharides and oligosaccharides are separated by gel-permeation chromatography, whereas monosaccharides are resolved on macroporous anion-exchange resins.

[9] K. Uobe, K. Nishida, H. Inoue, and M. Tsutsui, *J. Chromatogr.*, 1980, **193**, 83.
[10] A. C. Schoots and P. A. Leclercq, *Biomed. Mass Spectrom.*, 1979, **6**, 502.
[11] R. L. Whistler and A. K. M. Anisozzaman, *Methods Carbohydr. Chem.*, 1980, **8**, 45.
[12] J. Porath, *Biochem. Soc. Trans.*, 1979, **7**, 1197.
[13] J. F. Kennedy, J. E. Fox, and J. C. Skirrow, *Stärke*, 1980, **32**, 309.
[14] J. F. Kennedy and J. E. Fox, in ref. 11, p. 13.
[15] K. C. Gupta, M. K. Sahni, B. S. Rathaur, C. K. Narang, and N. K. Mathur, *J. Chromatogr.*, 1979, **169**, 183.
[16] P. E. Barker, B. W. Hatt, and S. R. Holding, *J. Chromatogr.*, 1979, **174**, 143.
[17] D. Noel, T. Hanai, and M. D'Amboise, *J. Liq. Chromatogr.*, 1979, **2**, 1325.

A new detection technique for the location of non-reducing oligosaccharides has been reported.[18] These sugars are hydrolysed by passing the eluant from liquid chromatographic columns through a small reaction column packed with a strongly acidic cation-exchanger. The reducing saccharides which are released may then be detected after reaction with 4-hydroxybenzoic acid hydrazide.

Oligosaccharides and their degradation products have been separated on Bio-Gel P-4.[19] Differences of one D-glucose equivalent could be distinguished for oligomers at least as large as 15 D-glucose equivalents. The method has been applied to the analysis of glycoprotein-derived oligosaccharides. Although various charged species such as phosphorylated or sialylated oligosaccharides show aberrant mobilities when eluted with water, their proper mobilities are obtained by removal of the charged function from the oligosaccharide or by using high salt concentrations.

The anomalous behaviour of some sugars on gel chromatography has been studied.[20] The separation of hexoses from 6-deoxyhexoses by gel filtration on Bio-Gel P-2 is not related solely to molecular size.

Six methyl ethers of methyl α-L-rhamnopyranoside have been isolated by liquid chromatographic separation on silica gel of the products of partial methylation of methyl α-L-rhamnopyranoside.[21]

Uronic acids and their oligomers derived from pectic substances have been separated and determined using a commercial liquid chromatograph.[22] Monosaccharides normally found in glycoprotein hydrolysates have been chromatographed as free sugars and as tritium-labelled alditols on anion-exchange resins.[23] The presence of amino-acids in the hydrolysates does not interfere with the analysis.

Details of a fully automated ion-exchange chromatographic analysis of neutral monosaccharides and oligosaccharides have been published.[24]

3 Thin-layer Chromatography

A separation of hexoses, polyols, and polyhydroxy-compounds has been achieved by t.l.c. on cellulose impregnated with tungstate.[25] The t.l.c. analysis of carbohydrates on sintered silica gel plates can be performed more simply and economically than that on other types of silica gel plates.[26] This is because of the simple impregnation of the sintered plates with salts, the low impregnation time required for a satisfactory separation, and the feasibility of re-using the plates

[18] P. Vrátný, J. Ouhrabková, and J. Čopiková, *J. Chromatogr.*, 1980, **191**, 313.
[19] M. Natowicz and J. U. Baenziger, *Anal. Biochem.*, 1980, **105**, 159.
[20] H. Yamada, Y. Ohsiima, K. Tamura, and T. Miyazaki, *Carbohydr. Res.*, 1980, **83**, 377.
[21] E. V. Evtushenko, N. M. Vakhrusheva, and Y. S. Ovodov, *J. Chromatogr.*, 1980, **196**, 331.
[22] H. Konno, Y. Yamasaki, K. Yoshida, and J. Ozawa, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 2387.
[23] J. Barr and P. Nordin, *Anal. Biochem.*, 1980, **108**, 313.
[24] J. F. Kennedy and J. E. Fox, in ref. 11, p. 3.
[25] J. Briggs, I. R. Chambers, P. Finch, I. R. Slaiding, and H. Weigel, *Carbohydr. Res.*, 1980, **78**, 365.
[26] N. Iizima, M. Fujihara, and T. Nagumo, *J. Chromatogr.*, 1980, **193**, 464.

repeatedly if they are re-conditioned with acid. 1,5-Anhydro-D-glucitol, present in cerebrospinal fluid, has been separated from other polyols and neutral monosaccharides by t.l.c. on silica gel impregnated with borate buffer.[27] The advantages of using unidimensional multiple-development chromatography for the separation of oligosaccharides by high-performance t.l.c. has been discussed.[28] Rapid and efficient separations of malto-oligosaccharides have been obtained by t.l.c. using multiple developments at an elevated temperature.

The microdetermination of reducing sugars on thin-layer chromatograms has been achieved by fluorodensitometry after treatment with 1,2-diaminoethane sulphate reagent.[29]

4 High-pressure Liquid Chromatography

Practical aspects of h.p.l.c. have been reviewed.[30] The main interaction between the stationary phase, a bonded-amine packing, and the solute in liquid chromatographic separations appears to be hydrogen bonding, with the hydrophobicity of the packing being negligible.[31] A separation of oligosaccharides up to d.p. 20 has been achieved using this packing with a colorimetric detection system using 3,3′-[(3,3′-dimethoxy-1,1′-biphenyl-4,4′-diyl)]bis(2,5-diphenyl-2*H*- tetrazolium) dichloride (Tetrazolium Blue).

The fluorescence produced in the reaction of ethanolamine and boric acid with reducing sugars has been applied to the post-column detection of carbohydrates after h.p.l.c. separation.[32] The method has been applied to the detection of mixtures of mono- and oligo-saccharides. D-Gluco-oligosaccharides (d.p. 2–20) may be resolved without derivatization using a simple isocratic system on silica columns dynamically modified with different di- or poly-amines by h.p.l.c.[33] The results agree with those obtained by automated gel-permeation chromatography. Oligosaccharides (up to pentasaccharides) present in corn syrups have been separated by h.p.l.c.[34]

High-performance gel chromatography on cation-exchange resins using acidic eluants has been used for the separation of neutral and acidic mono- and oligosaccharides.[35] The separation is based primarily on the differences in molecular size and its application to the analysis of the acidic hydrolysate of plant gum isolated from the sap of the laquer tree (*Rhus succedanea*) is illustrated.

Mono-, di-, and oligo-saccharides have been separated by reverse-phase h.p.l.c. using water as eluant.[36] The system is more useful for the separation of oligomers than for mono- and di-saccharides. Oligosaccharides and glyco-

[27] K. Lajunen, S. Purokoski, and E. Pitkanen, *J. Chromatogr.*, 1980, **187**, 455.
[28] D. Nurok and A. Zlatkis, *Carbohydr. Res.*, 1980, **81**, 167.
[29] J. Iwakawa, H. Kobatake, I. Suzuki, and H. Kushida, *J. Chromatogr.*, 1980, **193**, 333.
[30] G. D. McGinnis and P. Fang, in ref. 11, p. 33.
[31] M. D'Amboise, D. Noël, and T. Hanai, *Carbohydr. Res.*, 1980, **79**, 1.
[32] T. Kato and T. Kinoshita, *Anal. Biochem.*, 1980, **106**, 238.
[33] C. A. White, P. H. Corran, and J. F. Kennedy, *Carbohydr. Res.*, 1980, **87**, 165.
[34] L. E. Fitt, W. Hassler, and D. E. Just, *J. Chromatogr.*, 1980, **187**, 381.
[35] R. Oshima, Y. Kurosu, and J. Kumanotani, *J. Chromatogr.*, 1979, **179**, 376.
[36] A. Heyraud and M. Rinaudo, *J. Liq. Chromatogr.*, 1980, **3**, 721.

peptides, which accumulate in the liver and are excreted in the urine in several lysosomal storage disorders, have been separated by h.p.l.c.[37] Characteristic elution profiles were obtained for oligosaccharides, their borohydride-reduced products and glycopeptides.

The separation of mono- and oligo-saccharides and their anomers by h.p.l.c. on aminopropyl–silica gel has been optimized after determining the factors responsible for the changes in the separation process.[38] The separation of anomers if affected by binding of sulphate counterions on the 3-aminopropyl groups. A separation of anomers of some mono- and di-saccharides on a macroreticular anion-exchange resin with a relatively high degree of cross-linking has been achieved by h.p.l.c. using ethanol–water as eluant.[39]

L-Rhamnose, D-xylose, L-arabinose, D-mannose, D-glucose, and D-galactose have been separated by h.p.l.c.[40] Depending on the sugars in the sample it is possible to reduce the analysis time by increasing the proportion of water in the elution solvent. Compared with the normally used detection by refractive index measurement, u.v. detection (at 188 nm) has the advantage of a lower detection limit because of its higher sensitivity and because of the smaller disturbances to regular column flow caused by the pump.

An h.p.l.c. method of analysing hyaluronic acid oligosaccharides produced by bovine testicular hyaluronidase has been reported.[41] Using silica-gel-packed columns and an aqueous mobile phase, mono- to hexa-saccharide were separated from higher oligosaccharides and hyaluronic acid, thus allowing the enzymic conversion of polysaccharides to oligosaccharides to be monitored. Unsaturated sulphated disaccharides produced from chondroitin sulphates by treatment with chondroitin sulphate lyases ABC and AC have been separated by h.p.l.c.[42] The method has potential for the chemical diagnosis of patients with mucopolysaccharidoses and for the clarification of glycosaminoglycan structure.[43]

2-Amino-2-deoxy-D-galactose and 2-amino-2-deoxy-D-glucose have been converted to their toluenesulphonyl and dansyl derivatives.[44] Each type of derivative may be separated on reverse-phase h.p.l.c. Monosaccharides present in urine (D-xylose, L-arabinose, D-fructose, D-mannose, D-galactose, and D-glucose) have been separated by h.p.l.c. on a macroporous anion-exchange resin with acetonitrile–water as eluant.[45] The sugars were detected by means of a post-column reduction of Tetrazolium Blue. Absolute limits of detection were about 10 ng of monosaccharide. Particularly methylated sugars have been separated by

[37] N. M. K. Ng Ying Kin and L. S. Wolfe, *Anal. Biochem.*, 1980, **102**, 213.
[38] V. Kahle and K. Tesařik, *J. Chromatogr.*, 1980, **191**, 121.
[39] R. Oshima, N. Takai, and J. Kumanotani, *J. Chromatogr.*, 1980, **192**, 452.
[40] H. Binder, *J. Chromatogr.*, 1980, **189**, 414.
[41] P. J. Knudsen, P. B. Eriksen, M. Fenger, and K. Florentz, *J. Chromatogr.*, 1980, **187**, 373.
[42] G. J. L. Lee and H. Tieckelmann, *J. Chromatogr.*, 1980, **195**, 402.
[43] G. J. L. Lee, J. E. Evans, H. Tieckelmann, J. T. Dulaney, and E. W. Naylor, *Clin. Chim. Acta*, 1980, **104**, 65.
[44] A. Hjperpe, C. A. Antonopoulos, B. Classon, and B. Engfeldt, *J. Chromatogr.*, 1980, **202**, 453.
[45] M. D'Amboise, T. Hanai, and D. Noël, *Clin. Chem.*, 1980, **26**, 1348.

h.p.l.c. on reverse-phase C_{18} bonded columns.[46] The use of chemically bonded amine columns was avoided, as these tend to deteriorate with long use.

5 Electrophoresis

A discontinuous electrophoretic method has been described for fractionation of heparin into two or three different components and separation of these from other glycosaminoglycans.[47] The method consists of electrophoresis first in agarose containing barium acetate and then in 1,3-diaminopropane. Plasma membranes and seed proteins have been solubilized and analysed by two-dimensional polyacrylamide gel electrophoresis.[48] Classes of glycoproteins were detected by incubating with small volumes of ^{125}I-lectins. Plasma membranes from Chinese hamster ovary cells contain several high molecular weight acidic glycoproteins that bind to wheat-germ agglutinin but which do not stain with Coomassie Blue.

Paper electrophoresis in an electrolyte containing Ca^{2+} is suitable for the separation and characterization of many polyols.[49] Complexes form between the cation and at least two hydroxy-groups in the carbohydrate. The correlation of electrophoretic mobilities with the configuration and conformation of the compounds is discussed. Electrophoresis in sodium metavanadate solution has been advocated for the separation of hexitols, hex-2-uloses, and several di-saccharides.[50] The vanadium to polyol ratio in several complexes has been determined.

6 Analytical Methods

Several polysaccharides have been shown to interact in solution with the direct dyes Calcofluor White M2R and Congo Red.[51] Strongest interaction is shown by polysaccharides having contiguous (1→4)-β-linked D-glucopyranosyl residues, such as cereal β-D-glucans, xyloglucan, and substituted celluloses.

The hydrophilic *N,N,N′,N′*-tetramethyl-1,2-diaminoethane complex of osmium(VI) has been used to produce water-soluble osmate esters from cyclodextrins and amylase.[52] These osmium-labelled oligo- and poly-saccharides may be investigated by high-resolution scanning transmission electron microscopy.

Microheterogeneous populations of the glycoprotein fetuin bind to the D-galactosyl-binding lectin in proportion to the degree of desialylization and to the number of D-galactosyl residues exposed in the fetuin.[53] The binding of

[46] N. W. H. Cheetham and P. Sirimanne, *J. Chromatogr.*, 1980, **196**, 171.
[47] P. Bianchini, H. B. Nader, H. K. Takahashi, B. Osima, A. H. Straus, and C. P. Dietrich, *J. Chromatogr.*, 1980, **196**, 455.
[48] M. N. Horst, S. M. M. Basha, G. A. Baumbach, E. H. Mansfield, and R. M. Roberts, *Anal. Biochem.*, 1980, **102**, 399.
[49] S. J. Angyal and J. A. Mills, *Aust. J. Chem.*, 1979, **32**, 1993.
[50] F. Searle and H. Weigel, *Carbohydr. Res.*, 1980, **85**, 51.
[51] P. J. Wood, *Carbohydr. Res.*, 1980, **85**, 271.
[52] J. Resch, D. Tunkel, C. Stoeckert, and M. Beer, *J. Mol. Biol.*, 1980, **138**, 673.
[53] A. Surolia, P. S. Appukuttan, D. Pain, and B. K. Bachhawat, *Anal. Biochem.*, 1980, **105**, 436.

monovalent lectins to glycoproteins may provide a first approximation to the number of oligosaccharide chains in the glycoprotein.

Immobilized *Bandeiraea simplicifolia* I lectin can be used to separate structurally homologous sugar nucleotides (UDP-D-galactose from UDP-2-acetamido-2-deoxy-D-galactose) and oligosaccharide alditols (lactitol from melibiitol).[54] Differential precipitation with concanavalin A has been used as a method for the purification of glycoproteins.[55] In the case of alkaline phosphatase from human liver, this has been achieved both by fractional solution of the lectin–glycoprotein precipitate and by fractional precipitation of the enzyme with the lectin by varying the concentration of Ca^{2+} and Mn^{2+} required for binding.

The immobilized D-glucose oxidase method for the estimation of D-glucose has been shown to compare favourably with the free D-glucose oxidase method, especially in linearity over a greater range of D-glucose concentration.[56] The two-enzyme system using immobilized D-glucose oxidase–D-glucose isomerase has been found to improve the stability of a D-glucose sensor in the measurement of D-glucose.[57]

Whole cells of *Pseudomonas fluorescens* have been immobilized and used as a D-glucose sensor.[58] D-Glucose is measured amperometrically in the microbial electrode, which consists of the bacterial cells immobilized in a collagen membrane and an oxygen electrode. In a review on enzyme electrode probes, several examples of the use of D-glucose oxidase for the measurement of D-glucose, lactose, and maltose are cited.[59] A commercial analyser for the measurement of D-glucose has been evaluated for use with small volumes of body fluids.[60]

A kinetic determination of D-glucose from blood, urine, and spinal fluid in an autoanalyser with D-glucose dehydrogenase has been developed.[61] The NADH formed is measured by the increase in absorbance at 334 nm. A good correlation with the hexokinase–D-glucose 6-phosphate dehydrogenase end-point method is reported. Details of the simultaneous spectrophotometric determination of D-glucose, D-fructose, and D-mannose using the hexokinase–D-glucose 6-phosphate method have been published.[62] Hexokinase and D-glucose 6-phosphate dehydrogenase co-immobilized on the inner wall of plastic tubing have been used with continuous-flow analysers to measure the levels of D-glucose in serum.[63] Some problems encountered in optimizing the operating conditions for such bound-enzyme coils have been reported.

[54] D. A. Blake and I. J. Goldstein, *Anal. Biochem.*, 1980, **102**, 103.
[55] A. L. Latner and A. W. Hodson, *Anal. Biochem.*, 1980, **101**, 483.
[56] L. Sokol, C. Garber, M. Schults, and S. Updike, *Clin. Chem.*, 1980, **26**, 89.
[57] S. Gondo, M. Morishita, and T. Osaki, *Biotechnol. Bioeng.*, 1980, **22**, 1287.
[58] I. Karube, S. Mitsuda, and S. Suzuki, *Eur. J. Appl. Microbiol. Immunol.*, 1979, **7**, 343.
[59] G. G. Guilbault, *Enzyme Microb. Technol.*, 1980, **2**, 258.
[60] J. C. Cate, R. Hedrick, M. Taylor, and C. D. McGlothlin, *Clin. Chem.*, 1980, **26**, 266.
[61] A. Bruckner, *J. Clin. Chem. Clin. Biochem.*, 1980, **18**, 275.
[62] R. J. Sturgeon, in ref. 11, p. 135.
[63] L. P. Leόn, D. K. Chu, L. R. Snyder, and C. Horváth, *Clin. Chem.*, 1980, **26**, 123.

D-Galactose may be measured enzymically with D-galactose dehydrogenase,[64] or with D-galactose oxidase.[65] For the latter method, the enzyme is immobilized between cellulose acetate polycarbonate membranes and has subsequently been used for measuring D-galactose in serum. The method is reported to be superior to the use of homogeneous enzymic methods in terms of the simplicity of the analytical procedure and the volume of serum required.

Continuous-flow fluorimetry has been used for the measurement of low concentrations of D-galactose in blood or plasma.[66] The method involves the use of D-galactose oxidase and peroxidase, the latter being coupled to the fluorogenic substrate 4-hydroxyphenylacetic acid. A colorimetric procedure for measuring the D-galactose released enzymically from G_{M_1} ganglioside has been developed.[67] The NADH generated from the oxidation of D-galactose in the presence of NAD using D-galactose dehydrogenase is used to reduce 4-iodonitrotetrazolium to the formazan. D-Galactose has been measured in a coupled system by following the consumption of NADH using D-galactose oxidase and NAD^+ peroxidase (NADH : hydrogen-peroxide oxidoreductase, EC 1.11.1.1).[68] The use of similar coupled systems is suggested for the assay of other enzymes which produce hydrogen peroxide.

Microenzymic procedures based on the use of enzyme sprays of D-glucose oxidase and D-galactose together with paper chromatography has been used for identification of monosaccharide constituents of glycans, glycolipids, and glycoproteins.[69]

Free L-fucose in urine has been determined by an L-fucose dehydrogenase method.[70] In a tentative screening test, individuals with increased amounts of free L-fucose in urine were found. L-Fucose dehydrogenase has been used for the measurement of L-fucose after acid hydrolysis of glycoproteins.[71, 72] The assay method was linear up to 20 nmol α-L-fucose when measuring NAD fluorescence.

A new fluorogenic substrate, methylumbelliferyl 2-acetamido-2-deoxy-β-D-lactoside, has been prepared from methylumbelliferyl-2-acetamido-2-deoxy-D-glucoside and UDP-D-galactose in the presence of galactosyltransferase.[73] The merits of this new fluorogenic substrate for β-D-galactosidase assays have been assessed, together with the potential of this synthetic approach for a wider range of enzyme activities.

The continuous fluorimetric monitoring of the enzymic release of 4-methylumbelliferone from its corresponding glycoside has been found suitable for kinetic studies of various glycoside hydrolases.[74] The method has been found to

[64] R. J. Sturgeon, in ref. 11, p. 131.
[65] G. J. Buffone, J. M. Johnson, S. A. Lewis, and J. W. Sparks, *Clin. Chem.*, 1980, **26**, 339.
[66] J. M. Henderson and F. W. Fales, *Clin. Chem.*, 1980, **26**, 282.
[67] J. C. Urbanowski, T. M. Wunz, and J. A. Dain, *Anal. Biochem.*, 1980, **105**, 461.
[68] G. Avigad, *Anal. Biochem.*, 1978, **86**, 470.
[69] J. H. Pazur and K. L. Dreher, in ref. 11, p. 139.
[70] M. Endo, H. Matsue, S. Sato, M. Majima, and N. Hiyama, *Clin. Chim. Acta*, 1980, **103**, 69.
[71] T. Maler, *Anal. Biochem.*, 1980, **102**, 340.
[72] T. Maler, *Anal. Biochem.*, 1980, **105**, 476.
[73] S. Creme and D. H. Leaback, *Anal. Biochem.*, 1980, **103**, 258.
[74] M. L. Shulman, V. A. Kulshin, and A. Y. Khorlin, *Anal. Biochem.*, 1980, **101**, 342.

be sensitive and accurate for β-D-2-acetamido-2-deoxy-glucosidases A and B isolated from human placenta.

O-α-*N*-acetylneuraminyl-(2→3/6)-β-D-galactopyranosyl-(1→4)-D-glucose-(sialyl-lactose) has been reduced with sodium borotritide giving *O*-α-*N*-acetyl-neuraminyl-(2→3/6)-β-D-galactopyranosyl-(1→4)-D-glucitol(sialyl-lactitol) of high specific activity (>300 μCi/μmol).[75] The substrate may be used in a rapid and sensitive method for the estimation of neuraminidase activity. Hydrolysis of the substrate by the enzyme is monitored by measuring the radioactivity of the neutral reaction product which is separated from the charged substrate by passage over an anion-exchange column.

Interference with a procedure for measuring free neuramic acids using the thiobarbituric acid reagent has been shown to be due to the presence of certain carbohydrates capable of forming pigments with the reagents.[76] Glyceraldehyde, glycolaldehyde, and hydroxymalonaldehyde, which may be generated after periodate oxidation of glycoproteins, appear to be the substances responsible for the interfering colours. The chromogen produced in the Reissig method for 2-acetamido-2-deoxy-D-glycosyl sugars can be depressed by several divalent cations (Mg^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+}), cryoprotective agents such as DMSO, poly-(ethyleneglycol), and glycerol, and thiol compounds such as 2-mercaptoethanol, dithiothreitol, and cysteine.[77]

2-Amino-2-deoxy-D-glucose, 2-amino-3-*O*-[1-(*S*)-carboxyethyl]-2-deoxy-D-glucose (muramic acid), and the corresponding amino-deoxyalditols are converted into 2,4-dinitrophenyl derivatives using 1-fluoro-2,4-dinitrobenzene without undue formation of by-products if the conditions are carefully controlled.[78] The implications for quantitative analysis of amino-sugars are discussed.

1-Deoxy-D-fructose, 2-deoxyaldo-hexoses and -pentoses, 2,5-anhydro-D-mannose, and several 2-, 3-, 4-, and 5-mono- or bis-(hydroxymethyl)furans can be measured fluorimetrically with 3,5-diaminobenzoic acid.[79] The fluorescence properties of the products formed from all of the compounds are similar, suggesting structural similarities of the adducts formed and possible mechanistic similarities of the reactions involved.

Optimal conditions for the reaction of carbohydrates with indole in acidic medium have been established to make the reaction specific for uronic acids.[80] The interference from 2-deoxy-D-ribose and -D-glucose can be eliminated, and with the added stability of the chromogen, it would appear to be an attractive alternative to the carbazole reaction for the estimation of uronic acids. Reducing sugars have been analysed with a 2,2′-bicinchoninate reagent, which has a similar sensitivity to the Nelson reagent but is more convenient to use.[81] The effects of

[75] A. Frisch and E. F. Neufeld, *Anal. Biochem.*, 1979, **95**, 222.
[76] S. S. Kuwahara, *Anal. Biochem.*, 1980, **101**, 54.
[77] M. Rouleau, *Anal. Biochem.*, 1980, **103**, 144.
[78] M. J. Talieri and J. S. Thompson, *Carbohydr. Res.*, 1980, **86**, 1.
[79] W. L. Dills and G. M. McDonough, *Anal. Biochem.*, 1980, **108**, 325.
[80] B. E. F. de Arcuri, M. E. Fernandez de Recondo, and E. F. Recondo, *Carbohydr. Res.*, 1980, **85**, 165.
[81] R. F. McFeeters, *Anal. Biochem.*, 1980, **103**, 302.

1,2-dihydroxyethane concentration, reagent pH, heating time, and borate ion concentration on the colour development were evaluated.

An *N*-(1-naphthyl)ethylenediamine dihydrochloride reagent has been used for the determination of sugars on thin-layer plates.[82] Quantitation of the sugars was achieved by a mathematical calibration process using the equation (peak area)$^x = y$ (amount), where the parameters x and y are correlated to the number of hydroxy-groups of the molecules.

2-Cyanoacetamide reacts with reducing carbohydrates in borate buffer to give a fluorescence with excitation and emission maxima at 331 and 383 nm, respectively.[83] Under optimum conditions 1×10^{-10}–3×10^{-7} mol carbohydrate can be determined. The method has been applied to the automated analysis of mono- and oligo-saccharides which are separated by h.p.l.c. anion-exchange chromatography. In order to achieve high accuracy as well as high analytical capacity, a continuous-flow system based on the L-tryptophan–sulphuric acid method has been developed for the determination of total carbohydrates in sea water.[84]

The carboxymethyl ether groups in sodium *O*-carboxymethyl-cellulose have been determined using a uranyl acetate reagent.[85]

Various methods for the desulphation of polysaccharides have been described.[86] Glycosaminoglycuronans have been desulphated by solvolysis in DMSO.[87]

7 Structural Methods

The determination of polysaccharide structure with ^{13}C n.m.r. spectroscopy has been reviewed.[88] Aspects of ^{13}C n.m.r. spectroscopy, including the assignment of chemical shifts and the evaluation of the factors that influence the magnitude of coupling constants of isotopically enriched monosaccharides and some of their derivatives, have been reviewed.[89] The structural elucidation of bi-, tri-, and tetra-antennary oligosaccharides of the 2-acetamido-2-deoxy-4-*O*-β-D-galactosyl (*N*-acetyl-lactosamine) type using 500 MHz ^1H n.m.r. spectroscopy has been reported.[90] Higher resolution is obtained than with 360 MHz spectra.

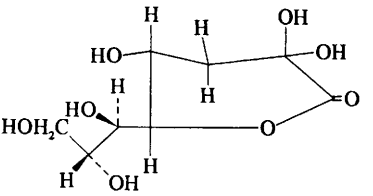
The ^{13}C n.m.r. signals of D-glucopyranosyl residues of acetylated oligosaccharides are more distinct than those of the parent hydroxylated compounds.[91] Changes in various signals with the degree of polymerization of the oligosaccharide were noted.

[82] M. Bounias, *Anal. Biochem.*, 1980, **106**, 291.
[83] S. Honda, Y. Matsuda, M. Takahashi, K. Kakehi, and S. Ganno, *Anal. Chem.*, 1980, **52**, 1079.
[84] K. Eberlein and K. D. Hammer, *Z. Anal. Chem.*, 1980, **301**, 17.
[85] D. F. Durso, in ref. 11, p. 127.
[86] E. Percival, in ref. 11, p. 281.
[87] L. Kenne and B. Lindberg, in ref. 11, p. 295.
[88] H. J. Jennings and I. C. P. Smith, in ref. 11, p. 97.
[89] R. Barker and T. E. Walker, in ref. 11, p. 151.
[90] H. Van Halbeck, L. Dorland, J. F. G. Vliegenthart, K. Schmid, J. Montreuil, B. Fournet, and W. E. Hull, *FEBS Lett.*, 1980, **114**, 11.
[91] D. Bassieux, D. Gagnaire, and M. Vignon, *Carbohydr. Res.*, 1977, **56**, 19.

^{13}C–^{1}H Coupling constants in oligosaccharides have been measured by two-dimensional ^{13}C n.m.r. *J* spectroscopy.[92] In order to illustrate the potential utility of this type of spectroscopy, two-dimensional spectra have been measured for solutions of raffinose and a tetrasaccharide repeating unit of *Klebsiella* K52 bacterial cell-wall polysaccharide. The ^{13}C n.m.r. signals of the pentitols and hexitols in aqueous solution, and of their acetates in chloroform solution, have been assigned by the use of specifically deuterium-substituted compounds.[93] Most of the data agree with assignments for these polyols in DMSO solution.

A 1:1 complex is formed between *N*-glycolylneuraminic acid with Ca^{2+} at pH 7, as shown by ^{1}H- and ^{13}C-n.m.r. spectroscopy.[94] The loss of the glycerol-1-yl side-chain (C-8 and C-9) decreased its Ca^{2+}-ion-binding capacity.

Ketodeoxyoctonoate, a component of bacterial lipopolysaccharides, has been submitted to spectroscopic examination (^{1}H n.m.r., c.d., and i.r.).[95] Evidence suggests that a 1,5-lactone (1) in which the keto-group exists as the hydrate is formed spontaneously in acid solution.



(1)

The ^{1}H n.m.r. spectra of various linear and branched D-manno-oligosaccharides have been assigned to the component sugars.[96] The chemical shifts, which range from δ 4.76–5.36, provide information regarding the linkages, sequences, and anomeric configurations of D-mannosyl residues. The method has been applied to the study of those oligosaccharides released from glycoproteins by *endo*-β-2-acetamido-2-deoxy-D-glucanase H.

Mass Spectrometry. – The chemical-ionization m.s. of *O*-trimethylsilyl ethers of aldose, alditols, aldonolactones, and aldonic acids using isobutane as the reagent gas have been reported.[97] Comparisons have been made with the corresponding electron-impact m.s.

A new glycosyl-sequencing method has been made possible by fractionation of complex mixtures of peralkylated oligosaccharides by reverse-phase h.p.l.c.[98] Subsequent unambiguous identification by g.l.c.–m.s. of disaccharides of almost

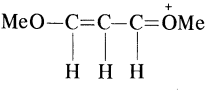
[92] L. D. Hall and G. A. Morris, *Carbohydr. Res.*, 1980, **82**, 175.
[93] S. J. Angyal and R. Le Fur, *Carbohydr. Res.*, 1980, **84**, 201.
[94] L. W. Jaques, B. F. Riesco, and W. Weltner, *Carbohydr. Res.*, 1980, **83**, 21.
[95] L. D. Melton, E. R. Morris, D. A. Rees, D. Thom, and S. M. Bociek, *Carbohydr. Res.*, 1980, **81**, 295.
[96] R. E. Cohen and C. E. Ballou, *Biochemistry*, 1980, **19**, 4345.
[97] A. C. Schoots and P. A. Lerclerq, *Biomed. Mass Spectrom.*, 1979, **6**. 502.
[98] B. S. Valent, A. G. Darvill, M. McNeil, B. K. Robertson, and P. Albersheim, *Carbohydr. Res.*, 1980, **79**, 165.

all trisaccharides and in some cases tetrasaccharides generated by successive partial acid hydrolysis, reduction, and ethylation of a permethylated polysaccharide has been achieved. Glycoproteins have been subjected to acetolysis, reduction with sodium borohydride, permethylation of the liberated oligosaccharides followed by g.l.c.–m.s. analysis.[99] The suitability of the spectral data to establish the position of the glycosidic bond and the monosaccharide sequence is discussed for each oligosaccharide.

Methane, isobutane, methanol, and ammonia have been used as reagent gases in the chemical-ionization m.s. of permethylated disaccharides.[100] With methane, more pronounced differences related to different glycosidic linkages especially between (1→2)- and (1→4)-linked disaccharides were obtained than for the corresponding electron-impact spectra. The chemical ionization isobutane spectra shows higher abundances for the $[M+H]^+$ ions than with methane and allows an easy differentiation of various glycosidic linkages. Reduced permethylated 2-acetamido-2-deoxy-D-glucosyl disaccharides have been separated by g.l.c. according to their anomeric configuration and the position of the glycosidic linkage within the disaccharide.[101] These disaccharides can be fully characterized and identified by their retention times and m.s. analysis.

The mass spectra of two peracetylated disaccharides and the penta-acetate of an aldobiouronic acid methyl ester all having a (1→2)-linkage are very similar to each other.[102] The fragmentation pathways can be classified into four classes common to all of the compounds examined.[102]

The structure of the fragment ion ($[C_5H_9O_2]^+$, *m/z* 101), the principal ion obtained on m.s. of permethylated saccharides, has been re-investigated.[103] The participation of the C-1 carbon atom in the formation of this ion has been determined quantitatively from the spectra of ^{13}C-labelled permethylated D-glucofuranosides. Investigation of the metastable ion and collisional activation spectra of *m/z* 101 has led to its characterization as having an acylic ion structure (2).



(2)

Collisional activation spectra of quasimolecular ions generated by chemical ionization can be used for the discrimination of stereoisomeric permethylated disaccharides.[104] The best choice of reagent gas has to be determined experi-

[99] B. Fournet, J. M. Dhalluin, G. Strecker, J. Montreuil, C. Bosso, and J. Defaye, *Anal. Biochem.*, 1980, **108**, 35.

[100] E. G. de Jong, W. Heerma, and C. A. X. G. F. Sicherer, *Biomed. Mass Spectrom.*, 1979, **6**, 242.

[101] M. Jensen, D. Borowiak, H. Paulsen, and E. T. Rietschel, *Biomed. Mass Spectrom.*, 1979, **6**, 559.

[102] T. Fujiwara and K. Arai, *Carbohydr. Res.*, 1980, **87**, 11.

[103] E. G. de Jong, W. Heerma, C. A. X. G. F. Sicherer, and G. Dijkstra, *Biomed. Mass Spectrom.*, 1980, **7**, 122.

[104] E. G. de Jong, W. Heerma, and G. Dijkstra, *Biomed. Mass Spectrom.*, 1980, **7**, 127.

mentally. A flow chart is given for the identification of an unknown permethylated disaccharide using this technique together with electron-impact and chemical-ionization m.s. The preparation and g.l.c.–m.s. analysis of partially methylated alditol acetates, *O*-trimethylsilyl ethers, and acetates of amino-sugars have been described.[105]

Using doubly-labelled 2-acetamido-2-deoxy-3-*O*-β-D-galactosyl-D-glucose (*N*-acetyl-lactosamine), procedures for the preparation of the partially methylated alditol acetates and their subsequent analysis by g.l.c. have been monitored quantitatively.[106] Although both D-galactosyl- and 2-acetamido-2-deoxy-D-glucosyl-residues are quantitatively converted to these derivatives, there is a preferential loss of the amino-sugar derivative on gas-chromatographic columns when the amount of derivative injected on to the column is less than a certain level. The molar response factor for the amino-sugar derivative is about 25% higher than that of the corresponding D-galactosyl derivative. Electron-impact m.s. of alditol acetates produces mainly odd-numbered fragments. The origin of the fragments having even numbers has been studied using 1-deoxy-hexitol acetates and some specifically deuteriated analogues.[107]

A general method for distinguishing between 4-linked aldopyranosyl residues and 5-linked aldofuranosyl residues, involving sequences of methylation, ethylation, reduction, and identification of fragments by g.l.c.–m.s., has been developed.[108] Per-*O*-methylated amino-oligosaccharide alditols have been prepared from lacto-*N*-tetraose, lacto-*N*-fucopentaose, and the mixed populations of oligosaccharide chains from α_1-acid glycoprotein and hog gastric mucin.[109] They have been used as model substrates to assess the scope of the reaction sequence of *N*-deacetylation–nitrous acid deamination, followed by derivatization, in the fragmentation of complex amino-oligosaccharides.

The products of nitrous acid deamination of per-*O*-methylated 2-amino-2-deoxy-D-glucitol and of 2-amino-2-deoxy-3-*O*-β-D-galactopyranosyl-D-glucitol and its per-*O*-methylated derivative have been characterized by g.l.c.–m.s. after treatment with sodium borodeuteride and further substitution by acetylation and methylation.[110] The most important reaction pathway involves a 1→2 hydride shift to give 2-deoxy-D-*arabino*-hexoses. Side reactions also occur, including solvolytic displacement at C-2, a 3→2 hydride shift to give 2-deoxy-D-*erythro*-3-hexuloses, and a C-4→C-2 migration to give 2-deoxy-2-*C*-(hydroxymethyl)-D-ribose. These latter two reactions result in elimination of the original 3-*O*-substituents, with the exposure of new reducing groups, from oligosaccharides terminated by 3-*O*-substituted 2-amino-2-deoxy-hexitols.

Methyl 2-amino-2-deoxy-β-D-glucopyranoside has been shown to undergo deamination with hydroxylamino-*O*-sulphonic acid to methyl 2-deoxy-β-D-

[105] C. G. Wong, S. S. J. Sung, and C. C. Sweeley, in ref. 11, p. 55.
[106] I. Funakoshi and I. Yamashina, *Anal. Biochem.*, 1980, **107**, 265.
[107] P. E. Jansson and B. Lindberg, *Carbohydr. Res.*, 1980, **86**, 287.
[108] A. G. Darvill, M. McNeil, and P. Albersheim, *Carbohydr. Res.*, 1980, **86**, 309.
[109] G. O. Aspinall, M. M. Gharia, R. G. S. Ritchie, and C. O. Wong, *Carbohydr. Res.*, 1980, **85**, 73.
[110] G. O. Aspinall, M. M. Gharia, and C. O. Wong, *Carbohydr. Res.*, 1980, **78**, 275.

arabino-hexopyranoside.[111] The method has been applied to the modification of *Streptococcus pneumoniae* type 14 capsular polysaccharide and *Vibrio cholera* *O*-antigen after *N*-deactylation of their respective amino-sugar residues.

Neither acetolysis nor strong acidic hydrolysis cause appreciable *N*-demethylation of methylated 2-deoxy-2(*N*-methylacetamido)hexitol derivatives.[112] Partial *O*-demethylation at C-1 occurs, therefore, on acetylation of 2-deoxy-1,3,4,5,6-penta-*O*-methyl 2-(*N*-methylacetamido)-D-glucitol and -D-galactitol, and the main product is 1-*O*-acetyl-2-deoxy-3,4,5,6-tetra-*O*-methyl-2(*N*-methylacetamido)hexitol. The previously postulated 2-(*N*,*N*-diacetyl)-2-deoxy-1,3,4,5,6-penta-*O*-methylhexitol derivatives have not been detected.

Partially methylated methyl α-D-xylopyranosides and partially methylated D-xyloses have been subjected to trifluoroacetolysis.[113] Methyl 2,3,4-tri-*O*-methyl-α-D-xyloside is completely destroyed, presumably *via* solvolysis and acid-catalysed elimination reactions occurring on the pertrifluoroacetate. For analogues having one free hydroxy-group, some unchanged glycoside could be recovered. The partially methylated D-xyloses were rapidly converted into their pertrifluoroacetate derivatives. The effect of hydroxy-groups in the aglycone in the solvation of D-glucopyranosides by trifluoroacetolysis has been reported.[114] The sequential degradation of methylated polysaccharides, by the oxidation of free hydroxy-groups followed by β-elimination, has been applied to the study of structures of polysaccharides.[115]

A method has been described for monitoring the rate and extent of the β-elimination reaction as applied to *O*-glycosyl-glycoproteins, utilizing alkaline solutions of sodium borotritide.[116] The released oligosaccharides are reduced to the corresponding tritiated alditols and can be separated from the protein moiety, which is also labelled. The stoicheiometry of the reaction has been established by the use of 2-acetamido-2-deoxy-D-galactosyl-*O*-glycoproteins.

A fingerprinting technique that may be of value in the differentiation of oligosaccharide chains of glycoproteins has been developed.[117] Alkaline borohydride is used to cleave those oligosaccharide chains linked to *O*-L-serine or *O*-L-threonine residues. Non-reducing terminal D-galactosyl residues on these oligosaccharides are oxidized by D-galactose oxidase and the ^{14}C-cyanohydrin derivative is then formed. Two-dimensional t.l.c. has been used with this method to demonstrate differences in oligosaccharide side-chains of human gastric mucins.

Potassium t-butoxide in DMSO has been used successfully as an alternative methylation reagent to the well known sodium hydride–DMSO reagent.[118] The methylsulphenyl carbanion is more rapidly and safely prepared from potassium t-butoxide than from sodium hydride. The presence of an excess of

[111] J. Arnarp, P. J. Garegg, B. Lengstad, and J. Lönngren, *Carbohydr. Res.*, 1980, **83**, 394.
[112] M. Caroff and L. Szabo, *Carbohydr. Res.*, 1980, **84**, 43.
[113] L. E. Franzén and S. Svensson, *Carbohydr. Res.*, 1980, **79**, 147.
[114] L. E. Franzén and S. Svensson, *Acta Chem. Scand., Ser. B*, 1980, **34**, 171.
[115] S. Svensson and L. Kenne, in ref. 11, p. 67.
[116] D. Aminoff, W. D. Gathmann, C. M. McLean, and T. Yadomae, *Anal. Biochem.*, 1980, **101**, 44.
[117] E. Moczar and D. Waldron-Edward, *J. Chromatogr.*, 1980, **181**, 108.
[118] J. Finne, T. Krusius, and H. Rauvala, *Carbohydr. Res.*, 1980, **80**, 336.

methylsulphenyl carbanion is necessary for the complete methylation of saccharides.[119] This can be demonstrated by the addition of triphenylmethane, which forms a red colour in the presence of the carbanion. Methylation of bacterial lipopolysaccharides has been accomplished using methyl trifluoromethane sulphonate in trimethylphosphate as a solvent.[120]

The determination of the sugar sequence and glycosidic bond arrangement of heteroglycans by an integrated analytical scheme, which includes methylation analysis, periodate oxidation, acetolysis, and β-elimination, has been proposed.[121] Structural information on polysaccharides may be obtained by the application of the oxidation of acetylated carbohydrates with chromium(III) oxide,[122] and of the specific cleavage of carbohydrates containing uronic acid residues by the Hoffmann–Veerman reaction.[123]

Recent advances in periodate-oxidation analysis as a tool for the study of carbohydrate structures have been reviewed.[124]

The chain lengths of hexosans may be obtained from enzymatic estimation of the glycerol produced after periodate oxidation, borohydride reduction, and acid hydrolysis of these polysaccharides.[125] Degrees of polymerization of D-glucans and D-xylans have been calculated after reduction of the polysaccharides with borohydride and enzymic estimation of the derived polyols.[126]

Glycoconjugates have been treated with sodium periodate to modify neuraminic acid residues or with D-galactose oxidase to modify D-galactosyl residues.[127] The aldehyde groups formed have then been reacted with rhodamine- and fluorescein-containing hydrazides, thus allowing the introduction of fluorescent markers into carbohydrate moieties on cell surfaces.

Neuraminic acid residues of glycoproteins and glycolipids in erythrocyte membranes have been selectively labelled with a nitroxide spin-label.[128] The possible conformations of neuraminic acid have been analysed using semi-empirical potential functions. In solution, neuraminic acid may exist preponderantly in two different conformations, which differ in orientation of the terminal hydroxymethyl group of the glycerol side-chain.[129] This model is consistent with ^{1}H and ^{13}C n.m.r. data.

Helical conformations of infinite polymer chains, using the helical parameters, n, the number of residues per turn of the helix and h, the translation of the corresponding residue along the helix, have been applied to the construction of general n, h maps.[130] The maps cover the whole range of predictable secondary

[119] H. Rauvala, *Carbohydr. Res.*, 1979, **72**, 257.
[120] P. Prehm, *Carbohydr. Res.*, 1980, **78**, 372.
[121] J. H. Pazur and L. S. Forsberg, in ref. 11, p. 107.
[122] J. Hoffman and B. Lindberg, in ref. 11, p. 117.
[123] N. K. Kochetkov, O. S. Chizhov, and A. F. Sviridov, in ref. 11, p. 123.
[124] S. Honda, *Yakugaku Zasshi*, 1980, **100**, 871.
[125] R. J. Sturgeon, in ref. 11, p. 73.
[126] R. J. Sturgeon, in ref. 11, p. 77.
[127] M. Wilchek, S. Spiegel, and Y. Spiegel, *Biochem. Biophys. Res. Commun.*, 1980, **92**, 1215.
[128] J. B. Feix and D. A. Butterfield, *FEBS Lett.*, 1980, **115**, 185.
[129] K. Veluraja and V. S. R. Rao, *Biochim. Biophys. Acta*, 1980, **630**, 442.
[130] D. Gagnaire, S. Pérez, and V. Tran, *Carbohydr. Res.*, 1980, **78**, 89.

structures of homopolysaccharides having the 1,2-, 1,3-, and 1,4-linkage types. A general strategy for investigating the secondary structures of 1,6-linked homopolysaccharides is proposed. A prediction of the possibility of a stable cyclic tetrasaccharide containing 1,6-linked β-D-glucosyl residues is proposed.

The determination of T_1 and nuclear Overhauser enhancement (n.O.e.) values for branched-chain polysaccharides (D-mannans and L-rhamno-D-mannans) have been reported.[131]

[131] P. A. J. Gorin and M. Mazurek, *Carbohydr. Res.*, 1979, **72**, C1.

3

Plant and Algal Polysaccharides

BY I. M. MORRISON

1 Introduction

The general structure and occurrence of plant and algal polysaccharides have been reviewed in Volume 5 of Comprehensive Organic Chemistry.[1] These polysaccharides have also been reviewed in an article on biopolymers in general.[2] The polysaccharides in food, with special mention of starch but discussing other polysaccharides, have been discussed in the proceedings of a conference on that subject.[3] The chemistry and chemical technology of plant polysaccharides in pulping and papermaking have been published in a book.[4] Carbohydrates in general including some of plant origin, have been reviewed.[5] This article has discussed their role in normal biological processes and disease.

2 Starch

The crystal structure of potassium hydroxide-complexed amylose, obtained by heterogeneous deacetylation of amylose acetate, has been determined through a combination of stereochemical structural refinement and *X*-ray diffraction analysis.[6] The complex crystallizes as an orthorhombic unit cell of dimensions $a = 8.84$, $b = 12.31$, and $c = 22.41$ Å. The crystals have $p2,2,2$ symmetry. The conformation of the amylose chain is a distorted left-handed helix with six D-glucose residues per turn. Each three-residue assymmetric unit is complexed with one molecule of potassium hydroxide and three molecules of water. The co-ordination of the K^+ ion probably causes the extension of the amylose chain. To elucidate the mechanism of the formation of crystalline polymorphs of starch granules, the effect of aliphatic alcohols and acids on the crystallization of Naegli-type amylodextrins has been studied.[7] Addition of the acids and alcohols to the crystallization media shifted the resultant structures towards the A-type from the B-type. The effect was more pronounced the greater the molecular size of the alcohol and its concentration. Decanoic and myristic acids shifted the

[1] J. F. Kennedy and C. A. White, 'Comprehensive Organic Chemistry', ed. E. Haslam, Pergamon, Oxford, 1979, Vol. 5, Chapter 26.3, pp. 755–815.
[2] J. F. Kennedy, *Chem. Br.*, 1978, **14**, 436.
[3] 'Polysaccharides in Food', ed. J. M. Blanshard and J. R. Mitchell, Butterworths, Kent, 1979.
[4] 'Pulp and Paper: Chemistry and Chemical Technology', 3rd Edn., ed. J. P. Casey, John Wiley and Sons Ltd, Chichester, England, 1980, Vol. 1.
[5] N. Sharon, *Sci. Am.*, 1980, **243**, 80.
[6] A. Sarko and A. Biloski, *Carbohydr. Res.*, 1980, **79**, 11.
[7] S. Hizukuri, Y. Takeda, S. Usami, and Y. Takase, *Carbohydr. Res.*, 1980, **83**, 193.

crystallization from the Cb-type to the Ca- and A-types, respectively. The effect of the cereal lipids suggested that they may have a role in the formation *in vivo* of the A-type of cereal starches.

Hydrated cyclomalto-octaose crystallizes as a monoclinic $P2_1$ space group with dimensions, $a = 20.25$, $b = 10.49$, and $c = 16.89$ Å with the angle β being 105.32°.[8] The macrocyte has an open conformation but displays significant deviations from ideal 8-fold molecular symmetry. Of the 19 water molecules so far isolated, 12 may be characterized as being in the torus of the cycloamylose.

The structure of the iodine complex of amylose, prepared either from amylose and iodine in iodide ion solution or iodine vapour has been studied by resonance Raman and iodine-129 Mössbauer spectroscopy.[9] The major chromophore in both studies was the I_5' anion. When prepared from iodine vapour, the iodine ion required for I_5' is produced by hydrolysis or alcoholysis of I_2. The other product of the reaction is hypoiodite and has been assigned by Mössbauer spectroscopy.

Aqueous solutions of the amylose–iodine complex, dialysed against either potassium iodide solutions or benzene, have been shown to have exceptional stability.[10] In the extreme case, only 20% of the total iodine is extracted with benzene. Dialysis against anionic surfactants and non-ionic surfactants affects the iodine-retention capacity of amylose as well as causing changes in the spectral behaviour of its complex. The addition of cosolvents makes the polymer chain more compact, the exceptions being high concentrations of urea and dimethylformamide.

The preferential solvation of glucans, including amylose, in binding solvent mixtures of dimethylsulphoxide (DMSO) and water have been studied by gel-permeation chromatography.[11] In solvent mixtures of DMSO:$H_2O < 1:2$ each glucan was solvated preferentially by water with a maximum of 2.5 mol $H_2O \cdot mol^{-1}$ D-glucose residues. With ratios $> 1:2$, DMSO solvates preferentially to 1 mol DMSO $\cdot$ mol^{-1} D-glucose residues.

The molecular weight distribution of legume starches has been studied by gel chromatography and the amylopectins all had molecular weights in excess of 2.0×10^7.[12]

An h.p.l.c. procedure has been developed that will provide information on the saccharide distribution of corn syrups up to the pentasaccharide.[13] The method employs a strong cation-exchange resin and detection is by a differential refractometer. The precision and accuracy of the results compare favourably with accepted and more time-consuming techniques. A similar report has been published on the analysis of cyclomalto-oligosaccharides, which also used a cation-exchange resin and a differential refractometer.[14]

[8] J. M. Maclennan and J. J. Stezowski, *Biochem. Biophys. Res. Commun.*, 1980, **92**, 926.
[9] R. C. Teitelbaum, S. L. Ruby, and T. J. Marks, *J. Am. Chem. Soc.*, 1980, **102**, 3322.
[10] S. P. Moulik and S. Gupta, *Carbohydr. Res.*, 1980, **81**, 131.
[11] P. R. Staub and D. A. Brant, *Biopolymers*, 1980, **19**, 639.
[12] C. G. Biliaderis, D. R. Grant, and J. R. Vose, *Cereal Chem.*, 1979, **56**, 475.
[13] L. E. Fitt, W. Hassler, and D. E. Just, *J. Chromatogr.*, 1980, **187**, 381.
[14] H. Hokse, *J. Chromatogr.*, 1980, **189**, 98.

The malto-series of oligosaccharides (from maltose to maltohexaose) have been subjected to pyrolysis–g.c.[15] The ratio of amounts of volatile products varied depending on the actual oligosaccharide examined.

An enzymatic procedure has been developed for the determination of starch in cereal products.[16] The procedure involves a short gelatinization step at 100 °C, α-amylase hydrolysis at 85 °C, and finally glucoamylase treatment. The result can be obtained in 4 h. A similar method has been reported in which a treatment with calcium chloride solution is added after the gelatinization step.[17] A rapid non-enzymatic procedure for the determination of damaged starch in flour has been published and is based on the iodometric determination of extractable amylose.[18] The comparison with the enzymatic method was better for soft wheats than hard wheats and it was suggested that the two methods differed in their sensitivity to the type of damage.

A proportion of the amylodextrin molecules in various samples with the degrees of polymerization *ca.* 25, have been found to contain two branch points by methylation and enzymatic analysis.[19] The finding was related to the resistance of these amylodextrins to debranching by pullulanase and to the overall structure of the original amylopectin.

The Naegli amylodextrins from waxy maize starch have been separated into three fractions by gel chromatography and the average structures of these fractions deduced by enzymatic degradation with α- and β-amylases and pullulanase.[20] The fraction with the highest molecular weight had a complex branching pattern, whereas that with the lowest molecular weight was mostly linear. The intermediate fraction had about one branch point per molecule. The formation of these fragments may be explained as arising from a cluster model for amylopectin in which the outer chains are in a double-helical crystalline arrangement.

The structural properties of maltodextrin hydrogels from potato starch have been investigated.[21] They possess weakly developed reticular structures, the structural elements of which are in globular form. These globular forms aggregate when the reticular structure breaks up on long-term storage.

The range of physical properties, such as water-binding capacity, gelatinization temperature, and swelling power, of starches from pearl millets have been determined.[22] Some starches tend to retrograde more easily than others. The small variations found in the amylose content indicated that some other physiocochemical factors, such as molecular dimensions, may be more important than the amylose content in determining these properties.

[15] B. J. Donnelly, J. E. Voigt, and B. L. Scallet, *Cereal Chem.*, 1980, **57**, 388.
[16] M. C. Baur and R. J. Alexander, *Cereal Chem.*, 1979, **56**, 364.
[17] S. E. Fleming and R. D. Reichert, *Cereal Chem.*, 1980, **57**, 152.
[18] E. E. MacDermott, *J. Sci. Food Agric.*, 1980, **31**, 405.
[19] R. S. Hall and D. J. Manners, *Carbohydr. Res.*, 1980, **83**, 93.
[20] T. Watanabe and D. French, *Carbohydr. Res.*, 1980, **74**, 115.
[21] E. E. Braudo, E. M. Belavtsera, E. F. Titova, I. G. Plahchina, V. L. Krylov, V. B. Tolstoguzov, F. R. Schierbaum, M. Richter, and C. Berth, *Starch*, 1979, **31**, 188.
[22] A. Beleia, E. Varriano-Marston, and R. C. Hosenay, *Cereal Chem.*, 1980, **57**, 300.

Starches have been isolated from three oat cultivars, fractionated into components and their physico-chemical and chemical properties have been compared with those of a hard red spring-wheat starch and its fractions.[23] During heating, the starch gels from oats showed a greater reduction in viscosity than did the wheat starch gels. The starch contents of the oat cultivars were similar to those of the wheat sample except for a high protein oat cultivar, which had a lower starch content.

The soluble starch content of wheat bread crumb decreases as the bread ages.[24] If baked with a surfactant and no shortening, the content was low. The soluble starch had a low iodine affinity value and had a higher β-amylolysis value and lower molecular weight than amylopectin. Debranching with pullulanase showed that all the soluble starch molecules were branched but had a shorter chain length and higher A:B ratio than normal wheat starch. It is suggested that the starch is degraded by amylases during baking.

The size distribution of starch granules during kernel development in wheat has been determined as has the actual starch content.[25] The results agree with the hypothesis that at least two populations of granules are present which have distinct initiation and growth characteristics. There also appears to be a seasonal effect. In a similar study on wheat endosperm development, the amylose content of the starch granules has been shown to increase during development with the content of the smaller granules increasing throughout development, whereas the content of the other granules only increased towards the end of grain development.[26]

Elutriation techniques have been used to prepare primary and secondary starch fractions from barley. Each fraction was homogeneous with sizes of 16.7 and 1.87 μm, respectively.[27] The secondary fraction was more efficient than the primary fraction in its ability to absorb α-amylase, due to its greater surface area. The greatest absorption occurred at 5 °C and pH 5–6.

A water-soluble polysaccharide, which resembles glycogen, has been found in fresh oyster mushrooms (*Pleurotus ostreatus*).[28] This polysaccharide was susceptible to post-harvest breakdown, as was some D-mannose-containing material that was also present.

Ciliates of the genus *Entodinium*, from the ovine rumen, when extracted with dimethylsulphoxide, have yielded oval granules that contain D-glucose residues and give a reddish-purple stain with iodine.[29] The average chain length was 22–25 residues and the chains consisted only of 1,4-linked α-D-glucopyranosyl residues joined *via* 1,4,6-linked α-D-glucopyranosyl residues as branch points as in amylopectin. The i.r. spectrum was similar to that from potato amylopectin and shellfish glycogen.

[23] L. A. MacArthur and B. L. D'Appolonia, *Cereal Chem.*, 1979, **56**, 458.
[24] K. Ghiasi, R. C. Hosenay, and D. R. Lineback, *Cereal Chem.*, 1979, **56**, 485.
[25] D. W. Baruch, P. Meredith, L. D. Jenkins, and L. D. Simmons, *Cereal Chem.*, 1979, **56**, 554.
[26] C. M. Duffus and S. M. Murdoch, *Cereal Chem.*, 1979, **56**, 427.
[27] A. W. MacGregor, *Cereal Chem.*, 1979, **56**, 430.
[28] J. B. W. Hammond, *Phytochemistry*, 1980, **19**, 2565.
[29] M. Wakita and S. Hoshino, *Comp. Biochem. Physiol.*, 1980, **65**, 571.

The gradual substitution of H^+ ions by Ca^{2+} or Mg^{2+} ions in plant materials decreased the swelling power, water-binding capacity, viscosity, and solubilization of starch.[30] Processing of potato tubers with added Ca^{2+} ions controlled the swelling power of the starch matrix as well as having effects on the integrity of the cell walls.

Wetting and air-drying or chlorine treatment of starches gives a product that has a higher β-amylolysis limit than the untreated starch.[31] Prior treatment with base reduced the effectiveness of the air drying. It was concluded that chlorine (or bromine) treatment or air-drying caused oxidative depolymerization of starch chains and oxidation of D-glucose residues within the chains.

An investigation has been made of the reaction products obtained from the hydrolysis of amylose in the presence of block copolymers of vinyl alcohol and styrene sulphonic acid.[32] Compared to hydrolysis with sulphuric acid, less random hydrolysis occurred. For products of the same total reducing power, the block copolymers produced more D-glucose, maltose, and amylose fragments of higher molecular weight than mineral acids.

The addition of anthraquinone drastically changed the anaerobic degradation of amylose in the presence of sodium hydroxide.[33] Chain-cleavage reactions took place and the new reducing groups were stabilized provided they needed anthrahydroquinone. Under aerobic conditions, the degree of polymerization of amylose was consistently half of that without anthraquinone indicating that anthraquinone and oxygen reduced the chain size.

Chromogenic and fluorogenic substrates have been prepared for the detection and determination of α-amylases.[34] Neither butan-1-ol nor cyclohexanol had any effect on the hydrolysis of amylopectin by α- or β-amylase in terms of either the optimum pH or the K_m values of the enzymes.[35] However, both alcohols interacted with amylose to give a product resistant to both of the amylases. Inhibition of these reactions was of the unco-operative type.

Culture filtrates of *Cladosporium resinae* ATCC 20495 contain a mixture of enzymes able to convert starch to D-glucose.[36] The mixture has been shown to contain at least four enzymes, namely α-D-glucosidase, α-amylase, and two glucoamylases, one of these being specific for the (1→6)-α-D-glucosidic linkages.

The α-amylase activity, sprouting damage, and α-amylase electrophoretic patterns of two varieties of hard white wheat have been compared with a standard red hard wheat.[37] One of the white wheats had a far higher α-amylase content and amount of sprouting damage. Of the two methods used for determining α-amylase, the falling-number method correlated better with

[30] M. Haydar, K. Moledina, B. Ooraikul, and D. Hadziyev, *J. Agric. Food Chem.*, 1980, **28**, 383.
[31] A. C. Johnson, R. C. Hosenay, and K. Ghiasi, *Cereal Chem.*, 1980, **57**, 94.
[32] K. Arai and Y. Ogiwara, *J. Polym. Sci., Polym. Chem. Ed.*, 1980, **18**, 1643.
[33] F. L. A. Arbin, L. R. Schroeder, N. S. Thompson, and E. W. Malcolm, *TAPPI*, 1980, **63**, 152.
[34] K. Wallenfels, B. Meltzer, G. Laule, and G. Janatsch, *Z. Anal. Chem.*, 1980, **361**, 169.
[35] S. V. Bhide, *Indian J. Biochem. Biophys.*, 1980, **17**, 73.
[36] B. V. McCleary and M. A. Anderson, *Carbohydr. Res.*, 1980, **86**, 77.
[37] G. Huang and E. Varriano-Marston, *J. Agric. Food Chem.*, 1980, **28**, 509.

sprouting damage than the reducing-sugar method. Gel-chromatographic evidence has indicated that the bimodal distribution of chains obtained from debranching wheat amylopectin probably resulted from a single normal population of amylopectin molecules.[38] Amylopectin β-limit dextrin had a normal distribution, but debranching both the leading and tailing edge of this distribution led to the bimodal distribution being preserved. The modes of action of pullulanase and isoamylase on amylopectin also differed, pullulanase producing a bimodal distribution throughout, whereas isoamylase only gave this distribution at the end of its action.

The minor component (α-amylase I) is a more efficient enzyme than α-amylase II from barley malt in hydrolysing large and small starch granules from normal and waxy barley cultivars.[39] The large starch granules from normal cultivars were the most difficult to hydrolyse. Small granules from both types were hydrolysed by surface erosion with no production of 'pinholes'.

The properties of two amylase activities from spinach leaves differed in their substrate specificity and subcellular localization.[40] Also reported was a chloroplast-associated debranching enzyme. The extrachloroplastic activities hydrolyse amylopectin and shellfish glycogen but are not very active against rabbit-liver glycogen, β-limit amylopectin, and amylose. In contrast, the major chloroplastic amylase attacks these D-glucans at comparable rates. The debranching activity acts on all the branched substrates except rabbit-liver glycogen.

Developing soybeans show a rapid increase in β-amylase activity that continues into early germination, but the distribution of this activity is not consistent with the supposed role of the enzyme in starch degradation.[41] The α-amylase and α-D-glucosidase activities are probably responsible for starch mobilization.

Digestion of amylomaize starch by rats has shown that starch is extracted from the faeces still containing digestible material.[42] The absorption spectra of the iodine complexes indicated that long-chain amylose components were missing from the faeces. An *X*-ray diffractogram showed that faecal starch granules were degraded in the amorphous part of the granule.

A rapid, sensitive assay for starch phosphorylase [(1→4)-α-D-glucan; orthophosphate α-D-glucosyltransferase, EC 2.4.1.1] has been developed and activities of less than 0.28 nM min^{-1} are readily measured.[43] Two isoenzymes of starch phosphorylase have been found in cotyledons of germinating seeds of *Voandezeia subterranea*.[44] The isoenzymes had pH optima of 5.5–5.6 and 6.1–6.3: the major component had a molecular weight of 2.04×10^5.

The starch synthetase activity from developing maize kernels, which is capable of synthesis in the absence of added primer, has been purified 10^3-fold

[38] W. A. Atwell, R. C. Hosenay, and D. R. Linebach, *Cereal Chem.*, 1980, **57**, 12.
[39] A. W. MacGregor and D. L. Ballance, *Cereal Chem.*, 1980, **57**, 397.
[40] T. W. Okita and J. Preiss, *Plant Physiol.*, 1980, **66**, 870.
[41] C. A. Adams, R. W. Rinne, and M. C. Fjerstad, *Ann. Bot. (London)*, 1980, **45**, 577.
[42] Y. Watanabe, F. Ohta, and Y. Ayano, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 2625.
[43] D. A. McCracken and W. M. Rutherford, *Anal. Biochem.*, 1980, **101**, 275.
[44] G. N. Umezurike and S. A. Ekhorutomwen, *Biochim. Biophys. Acta*, 1979, **567**, 331.

from the *amylose extender* (*ae*) mutant.[45] Since no branching enzyme is present, the citrate-stimulated reaction could be characterized. Malate could be used instead of citrate and added branching enzyme increased the velocity 4-fold but did not replace the requirement for citrate. Further studies have shown a 10-fold increase in velocity.[46] An analysis of the D-glucan–iodine complex and the enzymatic breakdown products patterns indicate a high degree of linearity and the results are discussed in relation to the biosynthesis of starch *in vivo*.

The ADP- or UDP-D-glucose-specific starch synthetase bound to sweet potato (*Ipomoea batatas*) starch granules has been shown to be localized within the granules and the UDP-D-glucose-specific enzyme was able to be solubilized by treatment of the starch granules with urea and pullulanase (EC 3.2.1.41).[47]

The levels of enzymes involved in starch metabolism, including starch synthetase, at different stages of grain development have been determined to ascertain whether they have any effect on the final grain size or starch content per grain in wheat.[48] Starch synthetase was observed after 14 days and continued to be present until 35 days after anthesis but no apparent influence on the grain size or starch content was noted.

A simple density-gradient technique has been developed for the isolation of starch granules from cotton (*Gossypium hirsutum*) leaves.[49] Starch granule-bound starch (bacterial glycogen) synthetase [ADP-glucose: 1,4-α-D-glucan 4-α-D-glucosyltransferase, EC 2.4.1.21] was isolated and is firmly bound to the granule and is free from soluble D-glucan synthetase. The activity of the enzyme is 10^2-fold better than that prepared by other procedures. Incubation of cotton leaves in the dark caused considerable starch degradation but no accumulation of sugars was detected except when the phloem transport was blocked.[50] Amylose was preferentially degraded, but the amylopectin component showed increasing resistance to degradation with leaf age.

The maltose series of oligosaccharides are accumulated by *Escherichia coli* up to maltoheptaose.[51] Larger oligosaccharides are bound to the outer envelope of intact cells. The high affinity binding has been shown to be dependent on the presence of a λ-receptor (an outer membrane protein) and a periplasmic maltose-binding protein. The sites are available to both utilizable and non-utilizable oligosaccharides. It has been shown further that amylose and amylopectin are bound to the λ-receptor.[52] This has been shown since both amylase and amylopectin inhibit the transport of maltose into *E. coli*, they prevent binding of non-utilizable maltodextrins and a fluorescent amylopectin derivative binds to *E. coli* in a reversible manner. Maltose and some other oligosaccharides are inhibitory to amylopectin binding but only at concentrations about 1 mM.

[45] C. D. Boyer and J. Preiss, *Plant Physiol.*, 1979, **64**, 1039.
[46] C. Pollock and J. Preiss, *Arch. Biochem. Biophys.*, 1980, **204**, 578.
[47] T. Sasaki and K. Kainuma, *Biochem. J.*, 1980, **189**, 381.
[48] R. Kumar and R. Singh, *Phytochemistry*, 1980, **19**, 2299.
[49] C. W. Chang, *Microchem. J.*, 1980, **25**, 179.
[50] C. W. Chang, *Plant. Physiol.*, 1980, **65**, 844.
[51] T. Ferenci, *Eur. J. Biochem.*, 1980, **108**, 631.
[52] T. Ferenci, M. Schwentorat, S. Ullrich, and J. Vilmart, *J. Bacteriol.*, 1980, **142**, 521.

Non-waxy milled brown rice (*Oryzae sativa*) samples, which have high amylose contents, contained proportionally more starch lipids than waxy rice.[53] The starch lipids were mainly lysophosphatidylcholine, lysophosphatidylenthanolamine, and free fatty acids. Native manioc starch, on twin-screw extrusion treatment, gave a product devoid of crystalline organization that was almost water-soluble, whereas the addition of fatty acids (C_2-$C_{18:2}$) gave a structurally ordered product that was water-insoluble.[54]

It has been suggested that the differences in water-binding capacity and other physical properties of starches from different plants may be more important than the granule size in determining the rheological characteristics of composite doughs.[55]

Some legume flours and starches have been investigated specifically for their breadmaking properties. The α-amylase content increased and the granule surface was altered after germination.[56] Germination had a detrimental effect on the quality of each flour especially that from yellow pea. Conditions are given for the production and functionality of starches and protein isolates from legume seeds, particularly field peas and horsebeans.[57]

3 Fructosans

The D-fructose content of fructosans has been estimated by g.l.c. using glass capillary columns in the form of *O*-methyloxime derivatives.[58] The anomeric form, ring structure, and linkage of oligosaccharides and polysaccharides containing D-fructose have been identified from their ^{13}C n.m.r. spectra.[59]

An homologous series of non-reducing oligosaccharides has been isolated from the roots of *Artemisia absinthium*. Their structures are based on chains of 2,1-linked β-D-fructofuranoside residues linked to the D-fructose residue of sucrose.[60]

Nine D-fructose-containing oligosaccharides have been synthesized *in vitro* from sucrose by an enzyme preparation from asparagus roots.[61] Their structures were identified as (1)–(9) by conventional techniques. All are found naturally in asparagus roots but it is the first report of (6), (7), and (9) being synthesized *in vitro*.

$$[\beta\text{-D-Fru}f\text{-}(2 \rightarrow 1)]_n\text{-}\beta\text{-D-Fru}f\text{-}(2 \leftrightarrow 1)\text{-}\alpha\text{-D-Glc}p$$

(1) $n = 1$; (2) $n = 2$; (3) $n = 3$

[53] N. H. Choudhury and B. O. Juliano, *Phytochemistry*, 1980, **19**, 1385.
[54] C. Mercier, R. Charbonniere, J. Grebaut, and J. F. De La Gueriviere, *Cereal Chem.*, 1980, **57**, 4.
[55] V. F. Rasper and J. M. De Man, *Cereal Chem.*, 1980, **57**, 331.
[56] M. M. Morad, H. K. Leung, D. L. Hsu, and P. L. Finney, *Cereal Chem.*, 1980, **57**, 390.
[57] J. R. Vose, *Cereal Chem.*, 1980, **57**, 406.
[58] H. Zegota, *J. Chromatogr.*, 1980, **192**, 446.
[59] H. C. Jarrell, T. F. Conway, P. Moyna, and I. C. P. Smith, *Carbohydr. Res.*, 1979, **76**, 45.
[60] M. Buffa, G. Gongiu, A. Lombard, and M. L. Tourn, *J. Chromatogr.*, 1980, **200**, 309.
[61] N. Shiomi, J. Yamada, and M. Izawa, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 2233.

$$[\beta\text{-D-Fru}f\text{-}(2 \rightarrow 1)]_n\text{-}\beta\text{-D-Fru}f\text{-}(2 \rightarrow 6)\text{-}\alpha\text{-D-Glc}p\text{-}(1 \rightarrow 2)\text{-}\beta\text{-D-Fru}f$$

(4) $n = 0$; (5) $n = 1$; (6) $n = 2$

$$[\beta\text{-D-Fru}f\text{-}(2 \rightarrow 1)]_m\text{-}\beta\text{-D-Fru}f\text{-}(2 \leftrightarrow 1)\text{-}\alpha\text{-D-Glc}p$$
$$6$$
$$\uparrow$$
$$2$$
$$[\beta\text{-D-Fru}f\text{-}(2 \rightarrow 1)]_n\text{-}\beta\text{-D-Fru}f$$

(7) $m = 1, n = 0$; (8) $m = 2, n = 0$, (9) $m = 1, n = 1$

The *in vivo* biosynthesis of D-gluco-D-fructosans by the D-fructosyltransferase of *Agave americana* from sucrose has been examined.[62] The products can contain up to 10 D-fructose residues but intermediate oligosaccharides could not serve as substrates.

The D-fructosyltransferase of *Fusarum oxysporum* has been purified by conventional techniques and shown to produce low molecular weight products.[63] The maximum enzymatic activity coincides with the maximum biosynthesis of polysaccharide.

The addition of a 'levan' type of fructosan as an acceptor has accelerated the polymerization of levan when catalysed by the levan-sucrase (EC 2.4.1.10) from *Bacillus subtilis*.[64] This effect was only seen under conditions of low ionic strength: at high ionic strength, a levan of d.p. *ca.* 120 was synthesized in reasonable yield. It was suggested that the d.p. of the synthesized levan is regulated by the ionic strength and that synthesis occurs in the absence of an acceptor. Further studies have shown that these levans contained a D-glucose residue at a terminal non-reducing position and that the molecular weight based on the D-glucose end units was similar to that based on gel-filtration studies.[65] The number of branch points in the polymer increased with an increase in molecular weight.

4 Cellulose

Pure crystals of cellulose IV_{II} have been prepared by deacetylation of cellulose acetate of a low degree of polymerization followed by precipitation.[66] For successful crystal formation, deacetylation was necessarily carried out at 150–160 °C. At temperatures between 90–150 °C hybrid crystals are obtained having cellulose II and cellulose IV_{II} domains cocrystallizing in syntaxy. When a solution leading to cellulose IV_{II} was seeded with native cellulose fibrils, a shish-kebab structure of microfibrils decorated with cellulose II lamellae was obtained.

The precipitation data for cellulose from diluted solutions of cadoxen are characterized by a positive temperature coefficient and the kinetics fitted a

[62] K. S. Nandra and I. S. Bhatia, *Phytochemistry*, 1980, **19**, 965.
[63] A. K. Gupta and I. S. Bhatia, *Phytochemistry*, 1980, **19**, 2557.
[64] T. Tanaka, S. Oi, and T. Yamamoto, *J. Biochem. (Tokyo)*, 1979, **85**, 287.
[65] T. Tanaka, S. Oi, and T. Yamamoto, *J. Biochem. (Tokyo)*, 1980, **87**, 297.
[66] A. Buleon and H. Chanzy, *J. Polym. Sci., Polym. Phys. Ed.*, 1980, **18**, 1209.

unidimensional nucleation rate law.[67] The low crystallinity of the product suggested that a dissociation of the cellulose–cadoxen complex and not a phase separation took place.

Cotton cellulose powders have been treated with various reagents to alter their degree of crystallinity and then subjected to enzymatic hydrolysis by the cellulase [1,4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase, EC 3.2.1.4] of *Aspergillus niger*.[68] In pure cellulose, a good correlation was obtained between the *X*-ray diffractograms and the susceptibility to microbial cellulase.

The structures of native and regenerated celluloses have been determined by ^{1}H n.m.r. spectroscopy.[69] Using deuteriated dimethyl sulphoxide, native cellulose, even in the swollen state, was devoid of a liquid-like mobile component, whereas regenerated cellulose contained much of a non-crystalline component which contained liquid-like segments.

Cross-polarization, magic angle-spinning ^{13}C n.m.r. spectroscopy of highly crystalline samples of cellulose I and II, together with vibrational spectra and unit cell dimensions have indicated that adjacent anhydro-D-glucose units are not equivalent and that anhydrocellobiose is required as a basic repeating unit in modelling cellulose structure.[70] Similar studies on cellulose I with the splitting of C-4 into two separate resonances, a shoulder associated with C-6, and the incipient splitting of the C-1 resonance into two peaks of nearly equal intensity has confirmed that the D-glucose residues are in two magnetically non-equivalent environments.[71]

N.m.r. spectroscopy has confirmed that mixtures of *N*-methyl-morpholine *N*-oxide and dimethyl sulphoxide, methylamine and dimethyl sulphoxide, and hydrazine and dimethyl sulphoxide are solvents for cellulose, but paraformaldehyde in dimethyl sulphoxide reacts with the cellulose and is not a true solvent.[72] Cellulose in the first-mentioned solvent is degraded by chain scission at 100 °C for >10 h. The ^{13}C n.m.r. spectra of cello-oligosaccharides and a low d.p. cellulose has shown that the higher oligosaccharides are very similar to cellobiose, and that variations in the spectral-line intensities has allowed the assignment of peaks to both terminal and internal residues.[73]

The solvation mechanism of the dimethyl sulphoxide–chloral–triethylamine system for cellulose has been studied.[74] U.v. spectroscopy showed that the coloration of the system was dependent on the amount of cellulose and cellulose did not degrade to a significant extent even when large quantities of triethylamine were present. From the intrinsic viscosity of cellulose in the system the

[67] M. H. Theil and E. T. Caviness, *J. Polym. Sci., Polym. Phys. Ed.*, 1979, **17**, 497.
[68] T. Sasaki, T. Tanaka, N. Nanbu, Y. Sato, and K. Kainuma, *Biotechnol. Bioeng.*, 1979, **21**, 1031.
[69] A. Hirai, F. Horii, and R. Kitamura, *J. Polym. Sci., Polym. Phys. Ed.*, 1980, **18**, 1801.
[70] R. H. Atalla, J. C. Gast, D. W. Sindorf, V. J. Bartuska, and G. E. Maciel, *J. Am. Chem. Soc.*, 1980, **102**, 3249.
[71] W. L. Earl and D. L. Van Der Hart, *J. Am. Chem. Soc.*, 1980, **102**, 3251.
[72] D. Gagnaire, D. Mancier, and M. Vincendon, *J. Polym. Sci., Polym. Chem. Ed.*, 1980, **18**, 13.
[73] J. C. Gast, R. H. Atalla, and R. D. McKelvey, *Carbohydr. Res.*, 1980, **84**, 137.
[74] K. Okajima, *Chem. Scr.*, 1979, **13**, 113.

molecular weight of the cellulose could be calculated.[75] The cellulose trichloroacetals in the system are as inflexible as hydroxyethyl-cellulose.

Wet milling of cellulosic materials enhanced the hydrolysis rate by a cellulase complex from *Trichoderma viride* over that obtained by ball-milling.[76] The advantage of wet milling and simultaneous hydrolysis depends on the continuous generations of accessible sites and a rapid rate of hydrolysis as the saccharification process proceeds.

Solutions of cellulose and its derivatives contain fully solvated macromolecules, as well as low and high molecular weight macrogel particles the latter can be distinguished by optical and rheological properties.[77] Considerable amounts of macrogel particles are present in cadoxen solutions depending on the source of cellulose.

Gel-permeation chromatography has been applied to polymers from wood, including cellulose which has been chromatographed as its methylol derivatives in dimethyl sulphoxide.[78] The column packing of choice was μ-Styragel® which satisfied the criteria of adequate pore size and stability in dimethyl sulphoxide. It was recommended that the cellulose methylol derivatives should be lyophilized before chromatography. The molecular-weight distribution of cellulose derivatives has been determined by gel-permeation chromatography using a hydrophilic-coated silica as packing material and buffers of high ionic strength as the mobile phase.[79] The high ionic strength eliminated viscosity effects.

Hydrolysis with trifluoroacetic acid followed by separation of the monomers by t.l.c. has been used to determine the degree of substitution in methyl cellulose.[80] The method agrees with the established method to within 4%. The degree of polymerization of cellulose has been determined as a function of degradation occurring during the isolation of cellulose as its nitrate derivative.[81]

A review of the use of inorganic acids and enzymes in the hydrolysis of wood has been published.[82]

Anthraquinone or its 2-sulphonic acid have been shown to stabilize the polysaccharides of wood in alkaline pulping reactions by suppressing the oxidative depolymerizations.[83] The yield of pulp that may be obtained with the 2-sulphonic acid derivatives as stabilizers is superior to that obtained using anthroquinone. In the quinone-stabilized system, the redox potential of the quinone is important especially in the breakdown of the lignin component.[84] The yield of pulp from alkaline treatment in the presence of anthraquinone was increased by

[75] K. Okajima, *Chem. Scr.*, 1979, **13**, 120.
[76] R. G. Kelsey and F. Shafizadeh, *Biotechnol. Bioeng.*, 1980, **22**, 1025.
[77] E. Gruber, *Cellul. Chem. Technol.*, 1979, **13**, 259.
[78] J. L. Minor, *J. Liq. Chromatogr.*, 1979, **2**, 309.
[79] H. G. Barth and F. E. Regnier, *J. Chromatogr.*, 1980, **192**, 275.
[80] B. Borrmeister, H. Dautzenberg, and B. Philipp, *Cellul. Chem. Technol.*, 1979, **13**, 683.
[81] G. Patscheke and S. Poller, *Cellul. Chem. Technol.*, 1980, **14**, 3.
[82] I. S. Golstein, *TAPPI*, 1980, **63**, 141.
[83] B. Bihani and O. Samuelson, *Sven. Papperstidn.*, 1980, **83**, 161.
[84] S. Lindenfors, *Sven. Papperstidn.*, 1980, **83**, 165.

a pretreatment in alkali at 60–100 °C in the presence of anthraquinone 2-sulphonic acid.[85]

The extent of chain end-initiated β-elimination ('peeling') reactions of cellulose can be measured from the yellow chromogen produced during the reaction but the results are difficult to evaluate when cleavage of non-terminal D-glucosidic linkages occur.[86] Ketocelluloses are largely resistant to β-elimination in dilute aqueous alkali and a mechanistic explanation of this stability is presented.

In the esterification of cotton fibres with acetic and sulphuric acids in dimethylformamide the effects of the acid, concentrations of dimethylformamide, treatment with water and alcoholysis have been studied with regard to the yield, degree of polymerization, degree of substitution, and solubility of the product.[87]

Pyrolysis of cellulosic materials proceeds through a complex series of chemical reactions to provide various products. 3-Hydroxy-6-methyl-2*H*-pyran-2-one, first produced from rhamnono-1,4-lactone has been isolated from the pyrolysis of phosphoric acid-treated cellulose materials.[88]

A low-pressure liquid chromatographic method has been developed for measuring cellulolytic activity.[89] The method takes only 20 min, gives good resolution, and is tolerant to extraneous salts and proteins.

No detectable differences were observed in the production of cellulase by the normal Rutgers and the isogenic non-ripening *nn* tomato.[90] The activity increased prior to the respiratory climax and continued to increase thereafter.

The normal inducer of extracellular cellulase from *Fusarium oxysporum* is not cellulose but its degradation products.[91] Carboxymethylcellulose is not a source of nutrients and there is no requirement for metal ions. Phenolics and related compounds are inhibitory to the enzyme but the effect is reduced by reagents like cysteine hydrochloride. The synergistic hydrolysis of cellulose by components of the extracellular cellulase system of *Talaromyces emersonii* have been studied.[92] The organism will grow equally well in an N_2 or an O_2 atmosphere and the complete enzyme complex is present in both atmospheres. A new type of celluolytic synergism has been postulated where an *exo-exo*-system has been reported to occur in the (1→4)-β-D-glucan cellobiohydrolases of *Trichoderma reesei* QM 9414.[93]

The effect of different precursors on the synthesis of D-glucan by intact cotton fibres at different stages of primary and secondary wall formation has been investigated.[94] UDP-D-glucose was superior for primary wall fibres but

[85] J. Basta and O. Samuelson, *Sven. Papperstidn.*, 1980, **83**, 281.
[86] T. Ziderman, *Carbohydr. Res.*, 1980, **81**, 196.
[87] K. Garves, *Cell. Chem. Technol.*, 1979, **13**, 299.
[88] M. Koos, M. Repas, M. Ciha, and P. Kocis, *Carbohydr. Res.*, 1980, **80**, 333.
[89] M. R. Ladisch, A. W. Anderson, and G. T. Tsao, *J. Liq. Chromatogr.*, 1979, **2**, 745.
[90] B. W. Poovaiah and A. Nukaya, *Plant Physiol.*, 1979, **64**, 534.
[91] G. L. Soni and I. S. Bhatia, *Indian J. Biochem. Biophys.*, 1979, **16**, 223.
[92] A. McHale and M. P. Coughlin, *FEBS Lett.*, 1980, **117**, 319.
[93] L. G. Fagerstam and L. G. Pettersson, *FEBS Lett.*, 1980, **119**, 97.
[94] C. Pillonel, A. J. Buchala, and H. Meier, *Planta*, 1980, **149**, 306.

sucrose was better for secondary fibres. However, much of the radiolabel was present in non-cellulosic glucans in the secondary walls.

A study has been made of the biosynthesis of cellulose by *Acetobacter xylinum* using D-glucose uniformly enriched in ^{13}C.[95] From a statistical analysis of the various ^{13}C–^{12}C coupling constants an estimate has been made of which various pathways for cellulose synthesis have been followed. The mechanism of the formation of cellulose-like microfibrils in a cell-free system from *A. xylinum* has been investigated by transmission electron microscopy and *X*-ray diffraction analysis.[96] After *ca.* 20 min, threads formed which then became entangled in clumps. After alkaline treatment, these threads had the characteristics of cellulose II. The possible similarity of cellulose synthesis in green plants was discussed.

Detachment of the cotton-fibre cell from the ovule has resulted in a loss of over 90% of the *in vivo* capacity for the synthesis of cellulose from D-glucose but over 50% of the capacity is protected when poly(ethyleneglycol) 4000 is present during detachment and incubation.[97] This latter value is obtained by full restoration of the activity in some cells and no recovery by others. The likely effect of the glycol is to assist the resealing of membranes.

Coumarin has been shown to affect the biosynthesis of cellulose in that protoplasts treated with it did not develop cell walls.[98] Studies with coumarin on *A. xylinum* has suggested that it is a general cell poison and not a specific inhibitor of cellulose synthesis.[99] The points of general inhibition must be before the formation of UDP-D-glucose, since coumarin has no effect on cellulose synthesis by a cell-free particulate suspension. Nonetheless, the pathway to cellulose in green plants must be more sensitive to coumarin than the pathways to hemicellulose or pectin. The fluorescent brightener Calcoflour white ST prevents the assembly of cellulose microfibrils by *A. xylinum.*[100] At concentrations of $>0.01\%$ synthesis of $(1\rightarrow4)$-β-D-glucan chains continues but there is no crystallinity in the wet state. Drying results in the formation of cellulose I. It is suggested that the dye hydrogen bonds with the D-glucan chains.

A survey has been carried out on the inhibitors of cellulose synthesis in cotton fibres.[101] The most potent inhibitor examined was 2,6-dichlorobenzonitrile, which was effective at 10^{-5} M, whereas coumarin required a concentration of 10^{-3} M to be effective. Bacitracin was not very effective.

Light and electron microscopic studies have been used to study the breakdown of cell walls of American Elm xylem tissues when infected by Dutch Elm disease.[102] No change was observed in the cellulose content of the hypocotyls of seedlings when grown in the dark.[103]

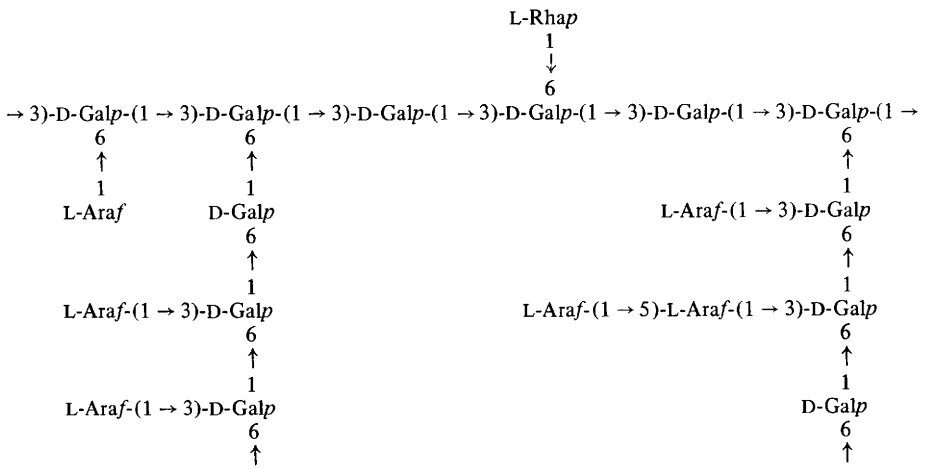
[95] D. Y. Gagnaire and F. R. Taravel, *Eur. J. Biochem.*, 1980, **103**, 133.
[96] J. R. Colvin, *Planta*, 1980, **149**, 97.
[97] N. C. Carpita and D. P. Delmer, *Plant Physiol.*, 1980, **66**, 911.
[98] H. J. Marchant, *Nature (London)*, 1979, **278**, 167.
[99] J. R. Colvin and D. W. Witter, *Plant Sci. Lett.*, 1980, **18**, 33.
[100] C. H. Haigler, R. M. Brown, jun., and M. Berziman, *Science*, 1980, **210**, 903.
[101] D. Montezinos and D. P. Delmer, *Planta*, 1980, **140**, 305.
[102] G. B. Oullette, *Can. J. Bot.*, 1978, **56**, 2666.
[103] G. J. Van Holst, F. M. Klis, F. Bouman, and D. Stegwee, *Planta*, 1980, **149**, 209.

Considerable interest has been shown in the analysis and composition of dietary fibre. One report leaves the final choice of method to the individual analyst.[104] Another shows the need to use α-amylase treatment when analysing breads.[105] Other methods have been presented with the emphasis on different aspects.[106–109] The effect on mice has shown that the higher the fibre content the lower the food consumption.[110] The various components did not have identical responses.

No clear relationship was observed between the ratio $^{13}C:^{12}C$ in cellulose from tree rings and the temperature to be useful as an indicator of past climates.[111]

5 Hemicelluloses

An L-arabinan has been isolated from the water-soluble pectic substances of cabbage (*Brassica oleracea*) cell wall.[112] Methylation analysis of the polysaccharide suggested that it possessed a highly branched structure of the same type as the L-arabinan associated with seed pectins. Although L-arabinose is the major sugar residue found in the water-soluble hemicellulose fraction of rice bran, it has not been established that an L-arabinan is present.[113]



(10)

[104] D. A. T. Southgate, G. J. Hudson, and H. Englyst, *J. Sci. Food Agric.*, 1978, **29**, 979.
[105] R. Mongeau and R. Brassard, *Cereal Chem.*, 1979, **56**, 437.
[106] O. Theander and P. Aman, *J. Sci. Food Agric.*, 1980, **31**, 31.
[107] M. A. Jeltema and M. E. Zabick, *J. Sci. Food Agric.*, 1980, **31**, 820.
[108] R. R. Selvendran and M. S. Du Pont, *J. Sci. Food Agric.*, 1980, **31**, 1173.
[109] R. R. Selvendran and M. S. Du Pont, *Cereal Chem.*, 1980, **57**, 278.
[110] K. Keim and C. Kies, *Cereal Chem.*, 1979, **56**, 73.
[111] T. Mazany, J. C. Lerman, and A. Long, *Nature (London)*, 1980, **287**, 432.
[112] B. J. H. Stevens and R. R. Selvendran, *Phytochemistry*, 1980, **19**, 559.
[113] R. R. Mod, E. J. Conkerton, R. L. Ory, and F. L. Normand, *Cereal Chem.*, 1979, **56**, 356.

The structure of an L-arabino-D-galactan, released from an extracellular, hydroxyproline-rich glycoprotein from suspension cultured tobacco cells has been determined (10).[114]

When the pistil is mature, the cells of the style canal of the *Gladiolus* family secrete a mucilage that contains, as a major component, an L-arabino-D-galactan-protein. The surface secretion of the stigma has now been shown to contain a closely related but distinct L-arabino-D-galactan as a major component.[115] The inner core of both polysaccharides appear to be very similar, but that from the stigma has a higher proportion of non-reducing terminal D-glucopyranosyl and L-arabinofuranosyl residues, whereas that from the style has more D-galacto-pyranosyl end-groups. A further comparison with the *Gladiolus* polymers and the L-arabino-D-galactan from *Lilium* has been made using the β-D-gluco-pyranosyl artificial antigen (Yariv antigen).[116] Those precipitated have a different D-galactose : L-arabinose ratio. Both *Gladiolus* preparations contain a single L-arabino-D-galactan, whereas that from *Lilium* contain at least two groups of L-arabino-D-galactans which could be separated by electrophoresis or lectin-affinity chromatography.

The turnover of cell-wall polysaccharides of a *Vinca rosea* suspension culture has been studied.[117] Active turnover was observed in the L-arabinose and D-galactose content of the hemicellulose fraction. No turnover of pectin components or D-xylose or D-glucose residues was observed. As the amount of radiolabel in the L-arabino-D-galactan of the cell wall declined, the amount in the extracellular L-arabino-D-galactan increased indicating that the L-arabino-D-galactan was synthesized, deposited in the cell wall, degraded rapidly, and made soluble in the medium as part of the extracellular secretion.[118]

Changes in the D-galactose content of the non-cellulose polysaccharides of tomato fruit cell walls have been analysed under various conditions.[119] In the normal plant, the D-galactan content decreases gradually during growth but the loss increased sharply as the fruits ripened. This was not observed with *nn* fruits. It was suggested that the decline in wall D-galactan is partly due to reduced synthesis in senescing normal fruits and in detached *nn* tomatoes.

A substance resembling callose is deposited during self-pollination of rye (*Secale cereale*).[120] Structural analysis has shown that both 1,4- and 1,3-linked β-D-glucopyranose residues, in ratio 77 : 9, are present in the same linear chain. Glutaraldehyde has been shown to induce the deposition of callose in *Abutilon* nectaries, probably in the plasmodesmata.[121] Since this is an artefact, care must be taken that the structure of the plasmodesmata is not wrongly interpreted.

[114] H. Hori, Y. Takeuchi, and T. Fujii, *Phytochemistry*, 1980, **19**, 2755.
[115] P. A. Gleeson and A. E. Clarke, *Carbohydr. Res.*, 1980, **83**, 187.
[116] P. A. Gleeson and A. E. Clarke, *Phytochemistry*, 1980, **19**, 1777.
[117] Y. Takeuchi, A. Komamine, T. Saito, K. Watanabe, and N. Morikawa, *Physiol. Plant.*, 1980, **48**, 536.
[118] Y. Takeuchi and A. Komamine, *Physiol. Plant*, 1980, **50**, 113.
[119] G. D. Lackey, K. C. Gross, and S. J. Wallner, *Plant Physiol.*, 1980, **66**, 532.
[120] H. I. M. V. Vithanage, P. A. Gleeson, and A. E. Clarke, *Planta*, 1980, **148**, 498.
[121] J. E. Hughes and B. E. S. Gunning, *Can. J. Bot.*, 1980, **58**, 250.

A (1→3)-linked β-D-glucan, curdlan, yields a thermo-irreversible gel on heating above 63 °C and a thermo-reversible one on heating to 63 °C and cooling.[122] Addition of NaCl or urea caused a shift in the critical temperature. The gelation was caused by swelling and the formation of a three-dimensional network due to micelle crosslinking by hydrophobic or hydrogen bonds.

The viscosity of an acid extract of barley flour is directly related to its content of soluble β-D-glucan ($r^2 = 0.99$).[123] Rain causes a reduction in the viscosity of the acid flour extract of barley flour, which is paralleled by a decrease in both soluble and total β-D-glucan content.[124] However, high-lysine varieties had a reduced viscosity that was not accompanied by a decrease in β-D-glucan content.

Cell-wall specimens from non-endospermic tissues of six grasses and nine other monocotyledons have been treated with a specific β-D-glucanase to liberate wall-bound non-cellulosic β-D-glucans.[125] From an analysis of the oligosaccharides released, all of the grasses contained a mixed-linkage (1→3)-(1→4)-β-D-glucan, which was not present in any of the monocotyledons. It was concluded that this D-glucan may be restricted to the Gramineae family.

An enzymatic method has been used to estimate the β-D-glucan content of cereal grains.[126] The crude β-D-glucanase from *Trichoderma viride* was separated from contaminating glucoamylase activity and the amount of β-D-glucan determined. The method has restrictions since the amount measured depended on the reaction temperature.

The presence of a mixed-linkage (1→3)-(1→4)-β-D-glucan has been confirmed in endosperm cell walls of wheat using the *endo*-β-D-glucanase of *Bacillus subtilis*.[127] From the oligosaccharides produced, at least 94% of non-cellulosic D-glucose residues were present in this polysaccharide.

The relationship between the β-D-glucan accumulation and that of other components in barley (*Hordeum vulgare*) endosperm has been studied.[128] The synthesis of this mixed-linkage β-D-glucan was the last to be initiated after fertilization and was also very dependent on the moisture content of the environment.

Two *endo*-(1→3)-β-D-glucanases have been identified in etiolated peas.[129] Enzyme I, found in the subapical regions, degrades substrates mainly to low molecular weight fragments and is best determined reductiometrically, whereas enzyme II, found in the basal regions, hydrolyses internal linkages of long chains so is best determined by changes in viscosity. Enzyme II, but not I, responds positively to the presence of auxin or ethylene.

[122] A. Konno, H. Kimura, T. Nakagawa, and T. Harada, *J. Agric. Chem. Soc. (Jpn.)*, 1978, **52**, 247.
[123] S. Aastrup, *Carlsberg Res. Commun.*, 1979, **44**, 289.
[124] S. Aastrup, *Carlsberg Res. Commun.*, 1979, **44**, 381.
[125] P. S. Stinard and D. J. Nevins, *Phytochemistry*, 1980, **19**, 1467.
[126] N. Prentice, S. Babler, and S. Faber, *Cereal Chem.*, 1980, **57**, 198.
[127] A. Bacic and B. Stone, *Carbohydr. Res.*, 1980, **82**, 372.
[128] G. Coles, *Carlsberg Res. Commun.*, 1979, **44**, 439.
[129] Y. S. Wong and G. A. MacLachlan, *Plant Physiol.*, 1980, **65**, 222.

Solutions of high ionic strength have the ability to extract β-D-glucan hydrolase activity from maize (*Zea mays*) seedlings cell walls.[130] Both *endo*- and *exo*-activities were present. The *exo*-enzyme was able to hydrolyse the β-D-glucan from *Avena* caryopsis to monosaccharides but was strongly inhibited by mercury(II) chloride such that only the *endo*-enzyme was active.

The profile of D-glucan synthase II, which is used as a marker to locate plasma membrane vesicles, has been shown to coincide with D-mannosyltransferase activity, suggesting that this latter enzyme is also located in the plasma membrane.[131]

The structure of the L-arabino-D-xylo-D-glucan from tobacco (*Nicotinia tabacum*) has been further studied by ^{1}H and ^{13}C n.m.r. spectroscopy and f.d. mass spectrometry after digestion with cellulase.[132] The results show that the polysaccharide is composed of two parts, namely a hexasaccharide component (L-Ara;D-Xyl: D-Glc, 1:2:3) and an unsubstituted (1→4)-β-D-glucan region, present in the ratio 1:2. There was some heterogeneity in the L-arabinofuranosyl substituents. Treatment of the D-xylo-D-glucan from jojoba (*Simmondsia chinensis*) seeds with cellulase and separation of the products has resulted in the preparation of six pure oligosaccharides whose structures have been identified.[133]

Golgi membranes of pea seedlings contain a UDP-D-xylose:polysaccharide D-xylosyltransferase, which is stimulated by UDP-D-glucose.[134] In the presence of both nucleotide sugars, a D-xylo-D-glucan is produced. This polysaccharide may be the final product of the (1→4)-β-D-glucan synthetase activity of Golgi membranes.

The seeds of *Anthocephalus indicus* contain a water-soluble polysaccharide, which consists of D-xylose, D-mannose, and D-glucose residues in the ratio 1:3:5.[135] Structural studies have indicated that the polysaccharide has a main chain of 1,4-linked β-D-mannopyranosyl and β-D-glucopyranosyl residues to which residues of D-xylose and D-glucose are linked at O-6 of some of the D-glucose residues in the main chain.

An unusual D-galacto-D-mannan has been isolated from the seeds of *Crotoharia juncea*.[136] The main chain was found to consist of 1,4-linked residues of D-galactose and D-mannose in the ratio 3:6 with other residues of these sugars, in the ratio 1:2, linked to O-6 of certain D-mannose residues in the main chain. The unit is repeated 19 times in the polysaccharide.

The ^{1}H and ^{13}C n.m.r. spectra of the D-galacto-D-mannans from guar, locust bean, lucerne, and clover seeds have been recorded after depolymerization to units of d.p. 20–40.[137] The resonances of the anomeric protons and the 12 carbon atoms were fully resolved and easily identified. The monomeric composition was in good agreement with that by chemical analysis. Other data

[130] D. J. Huber and D. J. Nevins, *Plant Physiol.*, 1980, **65**, 768.
[131] U. F. Klaus and H. Kindl, *Z. Anal. Chem.*, 1979, **360**, 1517.
[132] M. Mori, S. Eda, and K. Kato, *Carbohydr. Res.*, 1980, **84**, 125.
[133] T. Watanabe, K. Takahashi, and K. Matsuda, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 791.
[134] P. M. Ray, *Biochim. Biophys. Acta*, 1980, **629**, 431.
[135] O. C. D. Gupta, *Carbohydr. Res.*, 1980, **83**, 85.
[136] S. Bose and L. Singh, *Indian J. Chem.*, 1979, **18**, 59.
[137] H. Grasdalen and T. Painter, *Carbohydr. Res.*, 1980, **81**, 59.

fitted the recognized structures but did not exclude the possibility of more complex kinds of arrangements.

Neither locust bean or guar polysaccharide had any major effect on the viscosity of ice cream mixes.[138]

The major polysaccharide components of *Aloe saponaria* and *A. vanbalenii* are (1→4)-β-D-mannans in which about 90% of the D-mannose residues carry an *O*-acetyl substituent.[139]

The structure of the D-gluco-D-mannan from *Amorphophallus konjac* tubers has been investigated and the backbone linkage of a (1→4)-β-D-glycan confirmed.[140] Also confirmed was the presence of branching at O-3 of some of the D-mannose and D-glucose residues. Konjac D-gluco-D-mannans with high molecular weights and/or high D-mannose contents produce better flours.[141]

The ^{13}C n.m.r. spectra of the D-xylo-series of oligosaccharides has been recorded.[73] The variation in line intensities allowed the assignment of peaks to both terminal groups and internal residues. An important difference between the D-xylo- and the cello-series was the chemical shifts at internal C-4 atoms. This difference was interpreted as evidence of different linkage orientations or solvations, which is related to the absence of a C-6 in the D-xylo-oligosaccharides. A similar study has been made of other D-xylo-oligosaccharides.[142] As well as the (1→4)-linked β-series, the (1→3)-linked β-series was also examined as well as some mixed-linkage oligosaccharides. The data has been used to establish the structure of D-xylose-rich materials, including several D-xylans from red algae. Yet another report has considered the effects of substituents on the chemical displacement in the ^{13}C n.m.r. spectra of the D-xylose series.[143] This report considers both 1,3- and 1,4-substituted residues.

A ^{13}C n.m.r. spectral study has been made of the D-xylan from red lauan (*Shorea riboshorea*) wood.[144] The signals from the carboxy-group of the D-glucuronic acid residues in the polymer and the primary C-atom at C-5 of the D-xylose residues were good markers for the distinction of D-xylans from other polysaccharides. To assist structural studies on D-xylans, the series of D-xylobiose disaccharides containing the (1→2)-, (1→3)-, and (1→4)-linkages in both α- and β-anomeric forms have been synthesized.[145]

A series of water soluble L-arabino-D-xylans has been prepared from wheat flour L-arabino-D-xylan by partial removal of the L-arabinose side-chains.[146] The polymers assume an extended rod-like conformation in solution. The solubilizing effects of the L-arabinose side-chains was not the result of increased

[138] J. I. L. Cottrell, G. Pass, and G. O. Phillips, *J. Sci. Food Agric.*, 1980, **31**, 1066.
[139] D. C. Gowda, *Carbohydr. Res.*, 1980, **83**, 402.
[140] M. Meada, H. Shimahara, and N. Sugiyama, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 245.
[141] N. Kishida, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 2391.
[142] P. Kovac, J. Hirsch, A. S. Shashkov, A. I. Usov, and S. V. Yarotsky, *Carbohydr. Res.*, 1980, **85**, 177.
[143] J. P. Utille and P. J. A. Vottero, *Carbohydr. Res.*, 1980, **85**, 289.
[144] J. Y. Lee, A. Ishizu, S. Hosaya, and J. Nakano, *Cellul. Chem. Technol.*, 1979, **13**, 739.
[145] P. Kovac, *Collect. Czech. Chem. Commun.*, 1980, **45**, 892.
[146] K. A. Andrewatha, D. R. Phillips, and B. A. Stone, *Carbohydr. Res.*, 1979, **77**, 191.

hydration but due to the ability to prevent intermolecular aggregation of unsubstituted D-xylose residues. The extended conformation is consistent with its organization and function in the plant cell wall.

The principal hemicellulose of the floss of *Calotropis gigantea* is a 4-*O*-methyl-D-glucurono-D-xylan with the 12% of uronic acid residues being attached to O-2 of certain D-xylose residues in the main chain.[147]

The isolation and structural analysis of cell-wall material from beeswing wheat (*Triticum aestivum*) bran has been reported.[148] The major polysaccharides were confirmed as cellulose and an L-arabino-4-*O*-methyl-D-glucurono-D-xylan but it was also shown that phenolic ester linkages play a role in holding these polysaccharides together.

The relationship between the acetyl content and the sugar composition of expanding leaves of beech (*Fagus sylvatica*) has been investigated.[149] The accumulation of acetyl group closely paralleled that of D-xylose residues and the ratio was approximately equimolar except during the period of maximum leaf expansion. The results were discussed in relation to the known associations of acetyl groups and to the disappearance of constituents during cell-wall maturation.

The yield of holocellulose and hexosan from hornbeam wood decreased with increasing treatment with ammonia but the amount of pentosan increased.[150] The yield of cellulose also increased but its crystallinity and DP were both reduced.

Vapour-phase prehydrolysis of birch (*Betula papyrifera*) wood with sulphur dioxide solubilized 88% of the D-xylan and gave a solution of $>10\%$ D-xylose.[151] It is therefore a good method for preparing concentrated solutions of D-xylose.

Milled-wood lignin preparations from beech wood have been found to contain *ca.* 4% D-xylose residues, whereas cellulase lignin contained 11.2%.[152] The molecular weights were $3.9–5.1 \times 10^3$ and 3.2×10^4, respectively. There were also differences in the lignin structures.

The neutral sugar composition of the hemicellulose fractions from the piths of stalk of selected plants have been determined.[153] Maize and sugar cane had the highest D-xylan contents.

A g.l.c. method has been described for the determination of pentosan involving the measurement of the amount of furfural released by hydrolysis with 3.85 M-HCl.[154] The pentosan content of pearl millet has been determined.[155] Fractionation led to samples with wide variations in composition.

[147] D. C. Gowda and Y. V. Anjaneyalu, *Carbohydr. Res.*, 1980, **83**, 398.
[148] S. G. Ring and R. R. Selvendran, *Phytochemistry*, 1980, **19**, 1723.
[149] J. S. D. Bacon, *Biochem. J.*, 1980, **185**, 405.
[150] R. Solár and I. Melcer, *Cellul. Chem. Technol.*, 1980, **14**, 197.
[151] E. L. Springer and K. A. Libkie, *TAPPI*, 1980, **63**, 119.
[152] M. Fišerová and L. Šutý, *Cellul. Chem. Technol.*, 1980, **14**, 243.
[153] R. W. Jones, L. H. Krull, C. W. Blessin, and G. E. Inglett, *Cereal Chem.*, 1979, **56**, 441.
[154] D. J. Folkes, *J. Sci. Food Agric.*, 1980, **31**, 1011.
[155] A. V. Bailey, C. Sumrell, and C. W. Burton, *Cereal Chem.*, 1979, **56**, 295.

The changes in wheat flour pentosan as a result of dough mixing and oxidation have been investigated.[156] Flour had the highest pentosan content of the samples tested, mainly due to the loss of the high molecular weight L-arabino-D-xylan on over-mixing. The ferulic acid content was associated with this polymer only.

A comparison has been made of the non-starchy polysaccharides in oats and wheat.[157] More water-soluble material was obtained from oats than wheat but only wheat contained L-arabino-D-xylan. Oats did contain water-insoluble L-arabino-D-xylan.

The D-xylan-degrading activity of 95 strains of yeast has been investigated.[158] Of the 54 strains thriving on D-xylose, only 13 utilized D-xylan to give D-xylose and D-xylo-oligosaccharides as products. Two enzymes have been identified in *Cryptococcus albidus*, which convert (1→4)-β-D-xylan to D-xylose.[159] The enzyme almost completely secreted into the culture fluid is an *endo*-(1→4)-β-D-xylanase, which yields oligosaccharides that enter the cell and are hydrolysed by a cell-bound β-D-xylosidase. The enzymes are not produced constitutively and this yeast did not grow on cellulose. The *endo*-(1→4)-β-D-xylanase has been confirmed as inducible, with both D-xylan and its oligosaccharides being effective inducers.[160] Since (1→4)-β-D-xylobiose is not degraded, it is considered the natural inducer. Methyl-β-D-xylopyranoside also served as a non-utilizable inducer. Depending whether this yeast is grown on D-xylan or D-glucose, it differs markedly on its ability to take up inducers of *endo*-(1→4)-β-D-xylanase.[161] Increase in the rate of inducer uptake is due to the induction of an active transport system for the inducers, called β-D-xyloside permease. This example of the induction of a permease points to a new feature of induction of extracellular enzymes in eukaryotic micro-organisms. This *endo*-(1→4)-β-D-xylanase catalyses the degradation of aryl β-D-xylosides by other reactions than simple hydrolytic cleavage.[162] Some of the steps involve transglycosylation to oligosaccharides since D-xylose itself is not produced. It is proposed that the enzyme utilizes two pathways for the degradation of phenyl β-D-xylopyranoside which differ in the nature of the glycosyl donors.

The role of *myo*-inositol as a precursor of cell-wall polysaccharide in suspension cultures of wild carrot cells has been studied and the alcohol was found to be metabolized only into the pentosan and uronic acid groups.[163]

Among the thermophilic bacteria found in wood and bark compost, several genera of eubacteria (*Actinomycetes*) have been isolated that grow preferentially on hemicellulose.[164]

[156] Y. F. Yeh, R. C. Hoseney, and D. R. Linebach, *Cereal Chem.*, 1980, **57**, 144.
[157] L. A. MacArthur and B. L. D'Appolonia, *Cereal Chem.*, 1980, **57**, 39.
[158] P. Biely, Z. Krátký, A. Kocková-Kratochvilová, and S. Bauer, *Folia Microbiol.*, 1978, **23**, 366.
[159] P. Biely, M. Vršanská, and Z. Krátký, *Eur. J. Biochem.*, 1980, **108**, 313.
[160] P. Biely, Z. Krátký, M. Vršanská, and D. Urmaničová, *Eur. J. Biochem.*, 1980, **108**, 323.
[161] Z. Krátky and P. Biely, *Eur. J. Biochem.*, 1980, **112**, 367.
[162] P. Biely, M. Vršanská, and Z. Krátky, *Eur. J. Biochem.*, 1980, **112**, 375.
[163] D. C. Verma and D. K. Dougall, *Planta*, 1979, **146**, 55.
[164] A.-M. Deschamps and J.-M. Lebeault, *C.R. Hebd. Seances Acad. Sci.*, 1980, **291**, 485.

The hemicellulose fraction from wheat straw, after extraction with NaOH, has been used as a growth medium for the production of D-glucose isomerase by *Streptomyces flavogriseus*. Optimum conditions for production of the enzyme have been reported and, as a bonus, the hemicellulose-extracted residue can be used as an animal feed.[165]

High levels of dietary hemicellulose (*ca.* 20 g d^{-1}) has been shown to cause a zinc imbalance in man.[166]

6 Pectins

The separation of D-galacturonic acid oligosaccharides (up to d.p. 9) has been studied on Bio-Gel P-2 (a polyacrylamide gel).[167] Ionic exclusion of D-galacturonic acid was shown with water as eluant and the optimum conditions were obtained with acetate buffer (pH 3.6; $I = 0.1$) at 65 °C.

Pectin substances have been extracted from plum fruit tissue and separated into acidic and neutral fractions.[168] Each fraction contained residues of D-galacturonic acid, D-galactose, L-arabinose, D-xylose, and L-rhamnose in various proportions. High-voltage electrophoresis indicated the presence of neutral and negatively-charged material, and treatment with pectinesterase confirmed that the neutral component was fully esterified. The results were consistent with proposed models for pectin structure.

Fractionation of onion (*Allium cepa*) cell walls showed that they contained a range of pectin polysaccharides with varying proportions of neutral side-chains.[169] Methylation analysis of the alkali-extracted material indicated that (1→4)-linked D-galactans and a substituted D-xylo-D-glucan were major components. Onions therefore resemble dicotyledons more than the Gramineae in their cell-wall composition.

Methylation analysis of the cell-wall material from parenchymatous tissue of *Phaseolus vulgaris* and *P. coccineus* showed that the major component was based on residues of 1,4-linked D-galactopyranosyluronic acid with L-arabinosyl, D-xylosyl, and D-galactosyl residues being present mainly as non-reducing terminal residues.[170]

The solubilization of pectic substance from potato cell wall was suppressed during precooking by Ca^{2+} ion but not by Mg^{2+} ion and was best if the concentration of Ca^{2+} ion was greater than that of free carboxy-group.[30] It is believed that some Ca^{2+} ions bind to phosphate groups in the starch so both the starch and pectin content are important.

The kinetics of the degradation of pectic acid and its *O*-acetyl derivatives with the *exo*-D-galacturonanase from carrot have been studied.[171] With increasing

[165] W. P. Chen and A. W. Anderson, *Biotechnol. Bioeng.*, 1980, **22**, 519.
[166] C. Kies, H. M. Fox, and D. Beshgetoor, *Cereal Chem.*, 1979, **50**, 133.
[167] J.-F. Thibault, *J. Chromatogr.*, 1980, **194**, 315.
[168] D. Boothby, *Phytochemistry*, 1980, **19**, 1949.
[169] A. T. Mankarios, M. A. Hall, M. C. Jarvis, D. R. Threlfall, and J. Friend, *Phytochemistry*, 1980, **19**, 1731.
[170] M. A. O'Neill and R. R. Selvendran, *Carbohydr. Res.*, 1980, **79**, 115.
[171] K. Heinrichova and R. Kohn, *Collect. Czech. Chem. Commun.*, 1980, **45**, 427.

degree of acetylation (0.46–1.74), the initial and maximum degradation decreased, whereas the magnitude of the K_m value increased. Free hydroxy-groups on C-2 and C-3 of the terminal uronate residue are essential for the degradation.

In studies on the metabolism of cell walls from ripening fruit, the cell-wall changes in pears have been investigated.[172] As the cell walls ripened a substantial amount of D-galactopyranosyluronic acid and L-arabinose residues were lost from the wall but recovered in a form insoluble in 80% ethanol. Structural analysis indicated that it was a typical pectic backbone containing, as side-groups, variously linked L-arabinose residues. A study of the enzymes involved in ripening pears has shown the presence of poly-D-galacturonanase, pectin esterase, and seven glycosidases.[173] The poly-D-galacturonanase, α-D-galactosidase, and α-D-mannosidase activities increased as the fruits ripened, whereas cellulase and activity against wall D-xylose and L-arabinose residues was absent.

endo-D-Galacturonanase (EC 3.2.1.15) has been coupled to the cyanogen bromide-activated hydroxyalkyl methacrylate polymers Spheron® 300–1000, which differ in their porosity.[174] Although Spheron 300 bound most enzyme owing to its greater surface area, the Spheron 1000 preparation had the greatest relative activity owing to the better availability of the enzyme within the gel structure. The physical properties of the immobilized enzyme were determined.

The poly-D-galacturonanase activity of the normal Rutgers and a mutant (*nn*) tomato have been determined during ripening and it was concluded that the failure of the mutant fruits to ripen was related to their low D-galacturonanase activity during maturation as compared with normal fruit.[90] Similar studies have shown that two isoenzymes, D-galacturonanase 1 and 2, are produced on ripening and that 2 is absent from the non-ripening variety.[175] Although the two enzymes have different properties (*e.g.* mol. wts. 1.0×10^5 and 4.2×10^4, respectively), they gave rise to the same polypeptide on polyacrylamide-gel electrophoresis. Radioimmunoassay results suggest that the increase in enzyme activity during ripening is due to the net synthesis of protein.

The heat resistances of the *endo*-D-galacturonanases of the strawberry spoilage fungi (*Rhizopus sexualis*, *R. stolonifer*, and *Mucor piriformis*) have been compared with those of *R. arrhyus* and *R. oryzae*, which cause spoilage of apricots.[176] Each species produced the enzyme which showed marked heat tolerance and a bimodal heat stability. The maximum inactivation occurred at 50–60 °C but declined above that range to 100 °C. Some differences between fruits did occur. Only one peak of *endo*-D-galacturonanase activity was observed in macerated cotton hypocotyl.[177] The enzyme had a molecular weight of 4.2×10^4 and a pH optimum of 5.2. Infection of the seedlings with *Rhizoctonia solani* resulted in an extra peak of *endo*-D-galacturonanase activity.

[172] A. E. Ahmed and J. M. Labavitch, *Plant Physiol.*, 1980, **65**, 1009.
[173] A. E. R. Ahmed and J. M. Labavitch, *Plant Physiol.*, 1980, **65**, 1014.
[174] L. Rexová-Benkova, M. Mračková, and K. Babor, *Collect. Czech. Chem. Commun.*, 1980, **45**, 163.
[175] G. A. Tucker, N. G. Robertson, and D. Grierson, *Eur. J. Biochem.*, 1980, **112**, 119.
[176] J. E. Harris and C. Dennis, *J. Sci. Food Agric.*, 1980, **31**, 1164.
[177] L. W. Brookhouser, J. G. Hancock, and A. R. Weinhold, *Phytochemistry*, 1980, **70**, 1039.

The development of woolly breakdown of peaches has been shown to be accompanied by an increase in pectin esterase activity and an inhibition of D-galacturonanase activity.[178] Delaying the development of the woolly breakdown increased the D-galacturonanase activity to values found in normally-ripened fruit. It is suggested that an imbalance of pectolytic activity occurs at low temperatures, thereby inducing the pectin esterase activity and a build-up of de-esterified pectate.

The pectic enzyme complex from *Erwinia carotovora* has been investigated and discussed as a model for the degradation and assimilation of pectic fractions.[179] Four depolymerases (I–IV) were found in the intracellular complex. Depolymerase I has *endo*-lyase activity at pH 8.5 in the presence of Ca^{2+} ions and *endo*-hydrolase activity at pH 6.0 in the absence of Ca^{2+} ions. The activities have not been separated and the enzyme is also found extracellularly. Depolymerases II and III have *exo*-lyase activities, II requiring Mn^{2+} ions and III divalent cations. Depolymerase IV is a D-galacturonic acid oligosaccharide lyase.

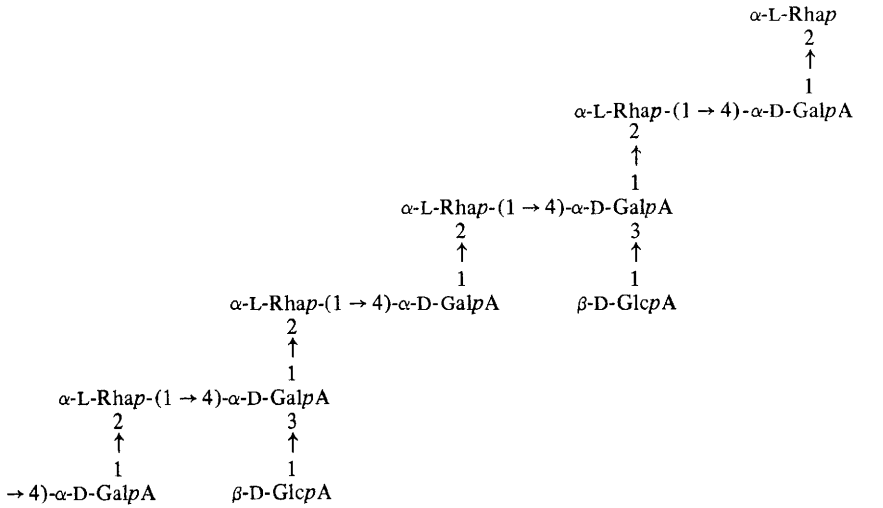
A comparison has been made of the biochemical and ultrastructural effects of *endo*-D-galacturonanase on cell walls of bean (*Phaseolus vulgaris*) and rice (*Oryzae sativa*).[180] Bean cell-walls were more susceptible and only D-galacturonic acid, L-rhamnose, D-galactose, and L-arabinose residues were solubilized from both preparations. Walls with secondary thickenings were not attacked but those with fibrillar appearances rapidly disappeared. The results suggest that the L-rhamno-D-galacturonan fraction contributes to the integrity of the cell wall.

7 Gums and Mucilages

The mucilage Abelmoschus-mucilage G from the roots of *Abelmoschus glutinotextilis* has been purified and its homogeneity confirmed by ultracentrifugation, electrophoresis, and gel chromatography.[181] It is a water-soluble acidic polysaccharide:protein complex in the ratio 3.9:1.0, with intrinsic viscosity of 52.8 and molecular weight of 6.8×10^4. The structure of the polysaccharide was confirmed as (11) by methylation and partial acid hydrolysis analyses.

The exudate gum from the bael (*Aegle marmelos*) tree contains residues of D-galactose, L-arabinose, L-rhamnose, and D-glucuronic acid in the ratio 9:1:3:3.[182] The structure of the gum was proposed from the results of methylation analysis of the whole gum, autohydrolysed gum, and the carboxy-reduced gum, together with periodate oxidation analysis, chromium(VI) trioxide analysis, and analysis of the oligosaccharides released by autohydrolysis.

[178] R. Ben-Arie and L. Sonego, *Phytochemistry*, 1980, **19**, 2553.
[179] J. P. Stack, M. S. Mount, P. M. Berman, and J. P. Hubbard, *Phytopathology*, 1980, **70**, 267.
[180] C. J. Baker, J. R. Aist, and D. F. Bateman, *Can. J. Bot.*, 1980, **58**, 867.
[181] M. Tomoda, M. Arai, Y. Suzuki, M. Ohmura, and H. Takayama, *Chem. Pharm. Bull. (Jpn.)*, 1980, **28**, 1546.
[182] P. K. Mandal and A. K. Mukherjee, *Carbohydr. Res.*, 1980, **84**, 147.



(11)

The structure of Junai mucilage has been proposed from successive Smith degradation and partial acid hydrolysis results.[183] The main chain consists of 1,3-linked β-D-mannopyranosyl residues to which are attached, at O-2, chains of 1,3-linked β-D-galactopyranosyl residues and single L-fucopyranosyl residues. Other peripheral side-chains were postulated. The oligosaccharides (12)–(14) were isolated after partial acid hydrolysis, (12) being a new trisaccharide. The results indicated that the Junai polysaccharide had an extremely complicated structure which reflects its unique physical properties.

α-L-Rha*p*-(1 → 3)-α-D-Gal*p*-(1 → 3)-L-Fuc*p*

(12)

α-D-Gal*p*-(1 → 3)-D-Gal*p*

(13)

β-D-Glc*p*A-(1 → 2)-D-Man*p*

(14)

The results of methylation and periodate oxidation analyses have shown that the degraded polysaccharide from the mucilage of the cactus *Opuntia ficus-indica* has a main chain based on alternating 1,4-linked α-D-galactopyranosyluronic acid and 1,2-linked β-L-rhamnopyranosyl residues.[184] Chains of 1,6-linked β-D-galactopyranosyl residues are attached to O-3 of most of the L-rhamnose residues.

[183] M. Kakuta and A. Misaki, *Agric. Biol. Chem. (Jpn.)*, 1979, 43, 1269.
[184] D. McGarvie and H. Parolis, *J. Chem. Soc.*, 1979, 1464.

The products of degradation of peach gum by a mixture of extracellular glycanohydrolases from *Aspergillus flavus* contained a high proportion of D-mannopyranosyl residues and a lower proportion of 1,3-linked D-galactopyranosyl residues.[185]

A polysaccharide has been purified, in 90% yield, from *Spondias dulcis* gum.[186] Autohydrolysis gave a degraded gum, which appeared to be homogeneous on electrophoretic and ultracentrifugation analyses. Methylation of the carboxy-reduced degraded gum followed by hydrolysis gave the following methylated sugars: 2,3,5-tri-*O*-methyl-L-arabinose (1.0), 2,3,4-tri-*O*-methyl-L-arabinose (0.9), 2,5-di-*O*-methyl-L-arabinose (0.9), 2,4,6-tri-*O*-methyl-D-galactose (0.5), 2,4-di-*O*-methyl-D-galactose (0.9), and 4,6-di-*O*-methyl-D-galactose (0.9). A structure for the degraded gum was proposed.

A glycopeptide has been isolated from Neem (*Azadirachta indica*) gum after digestion by pronase.[187] Ion-exchange chromatography showed that the product was a high molecular weight heteropolysaccharide associated with a small amount of protein. A glycopeptide, rich in 2-amino-2-deoxy-D-glucose, was also isolated in which the amino-sugar was linked to L-asparagine.

The major sugars known to be present in corn root-cap slime have been detected by the use of various lectins.[188] The absence of 2-acetamido-2-deoxy-D-glucose was confirmed but care has to be taken in interpreting the results. For example, the L-fucopyranosyl residues would appear to be fully accessible since they were determined in total both in sonicated and unsonicated slime, but the D-galactose residues must be protected in some way since they were only detected in sonicated slime.

The phenylalanine ammonia lyase from maize has been found to bind strongly to polysaccharides, residues of L-arabinose, D-ribose, L-fucose, D-galactose, D-mannose, and D-glucose all being found in hydrolysates of the complex.[189] Most (90%) of the associated polysaccharide could be removed by gel electrophoresis, diethylaminoethyl-Sephadex® chromatography, or trichloroacetic acid hydrolysis, but ammonium sulphate precipitation or chromatography on agarose or hydroxyapatite had no effect. The removal of polysaccharides containing L-arabinosyl and D-galactosyl residues caused large losses in activity. The enzyme was considered to bind specific polysaccharides.

The intracellular site of the synthesis of the L-fucose-rich polysaccharides from corn roots was found to be the particulate fraction.[190] Further fractionation showed that this activity was present in the smooth endoplasmic reticulum and a fraction rich in dyctyosome cisternae and vesicles. It was suggested that biosynthesis of the polysaccharide followed this route but the possibility of two sites of L-fucosyltransferase could not be ruled out. The method for the

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[186] S. Basu, *Carbohydr. Res.*, 1980, **81**, 200.
[187] B. Ramakrishna Nayak and T. N. Pattabiraman, *Indian J. Biochem. Biophys.*, 1978, **15**, 449.
[188] M. Rougier, C. Kieda, and M. Monsigny, *J. Histochem. Cytochem.*, 1979, **27**, 878.
[189] E. A. Havir, *Plant Sci. Lett.*, 1979, **16**, 297.
[190] D. W. James and R. L. Jones, *Plant Physiol.*, 1979, **64**, 914.

characterization of the above GDP-L-fucose : polysaccharide L-fucosyltransferase activity has also been published.[191]

In studies on the symbiosis of lichens, differences in the cell-wall properties of freshly isolated and cultured phycobionts were observed from a reduction in the number of concanavalin A binding sites.[192]

The 2-amino-2-deoxy-hexose content of various seeds and pollens has been determined.[193] Few differences were observed between mono- and di-cotyledonous plants.

The anthrone method has been applied to the determination of carbohydrate components present in humic acid fractions.[194]

8 Algal Polysaccharides

The ^{13}C n.m.r. spectroscopy of red seaweed galactans belonging to the agar and carrageenan groups has been studied.[195] Signal assignments could be made for most of the extreme structures of these galactans and is therefore a rapid method to determine the type of galactan structure, the absolute configuration of its constituents, and the position of sulphate and *O*-methyl groups in the polymer.

The agar composition of different generations of *Gracilaria verrucosa* has been determined.[196] There was little difference between yield, fractionation pattern, or properties of agars from fertile weeds but juvenile (sterile) plants gave agars of low yield and quality. The gel-forming properties could be improved by alkali treatment.

Chromium(VI) oxide has been used for the determination of the anomeric configuration of methyl 3,6-anhydro-D-galactopyranoside acetates and agarose.[197]

Artifacts have been described for agar–protein interactions that simulate a bacterial growth in haemocultures.[198]

The scope of circular dichroism (c.d.) for the characterization of alginate compositions and block structure has been assessed.[199] The three types of block structure have very different c.d. behaviours, that of poly-L-guluronate being entirely negative, whereas poly-D-mannuronate has a strong positive band. The peak at 200 nm and the trough at 215 nm can be used to determine the L-guluronate : D-mannuronate ratio. A measure of the block composition can also be obtained and the results are in good agreement with previous n.m.r. spectral

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[192] P. Burrick and M. Galun, *FEMS Microbiol. Lett.*, 1980, **7**, 311.
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[199] E. R. Morris, D. A. Rees, and D. Thom, *Carbohydr. Res.*, 1980, **81**, 305.

studies. N.m.r. spectroscopy has been used to study the interactions of metal ions with (1→4)-hexuronanates.[200]

The dynamics of the salt-induced disorder–order transition of κ-carrageenan have been studied by a polarimetric stopped-flow technique.[201] Formation of the ordered state is a second-order process; this supports the proposal that the aggregates of ordered polysaccharide chains contain double helical entities. The previous network model of polymer gel structure has been revised to take into account new evidence that different levels of chain association may be involved in the cation-specific aggregation of carrageenan helices.[202] The structure now has a microheterogeneous or domain structure. Limited numbers of chains are linked together through intermolecular double-helix formation but into small clusters that require further association by cation-mediated helix–helix aggregation to develop a cohesive network.

The thermally-induced order–disorder transitions of ι- and κ-carrageenans have been monitored by optical rotation and differential scanning calorimetry in various ionic environments.[203] Conformational ordering in κ-carrageenan is observed only in the presence of cations that promote helix–helix aggregation. ι-Carrageenan, in contrast, shows an order–disorder transition in the non-aggregating trimethylammonium salt form at a substantially lower temperature than κ-carrageenan and without hysteresis. In the presence of K^+ ions, i-carrageenan shows two distinct thermal transitions. These results suggest that the double helix of i-carrageenan can exist as a stable entity in isolation but can be further stabilized by aggregation, but κ-carrageenan helix is only stable when aggregated.

4,6-*O*-(1-Carboxyethylidene)-D-galactose has been identified as a component of the carrageenan of *Petrocellis middendorfii* by combined g.c.–m.s.[204] The red seaweed *Chondrus canaliculatus* contains a soluble polysaccharide that behaves like the carrageenan from *Chondrus crispus*.[205] From a methanolysate of the polysaccharide, methyl α-D-galactopyranoside, 3,6-anhydro-D-galactose dimethylacetal, and *O*-β-D-galactopyranosyl-(1→4)-3,6-anhydro-aldehydo-D-galactose dimethylacetal were obtained. The last sugar was identified by an ^1H n.m.r. spectral study of its per-*O*-acetyl derivative.

The polysaccharide material extracted from *Eucheuma spinosum* had a molecular weight of $1.5–2 \times 10^6$ by gel filtration, whereas an authentic sample of κ-carrageenan had a molecular weight of 2×10^7.[206] Viscosity measurements showed their relative molecular weights to be almost identical with those

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[202] E. R. Morris, D. A. Rees, and G. Robinson, *J. Mol. Biol.*, 1980, **138**, 343.
[203] E. R. Morris, D. A. Rees, I. T. Norton, and D. M. Goodall, *Carbohydr. Res.*, 1980, **80**, 317.
[204] V. L. Dininno, R. A. Bell, and E. L. McCandless, *Biomed. Mass Spectrom.*, 1978, **5**, 671.
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[206] H. K. Tong, K. H. Lee, and H. A. Wong, *Carbohydr. Res.*, 1980, **81**, 1.

obtained from gel filtration indicating that those polysaccharides could be related polymeric species.

The carrageenan from *Irideae undulosa*, devoid of a κ-fraction, is composed of so-called 'intermediate' fractions.[207] After fractionation, several structural features were predicted from their i.r. spectra. Alkaline treatment gave a κ-fraction in 53.6% yield. Two of the fractions have been subjected to methylation analysis and the identity of the methylated sugars produced indicated that the less soluble carrageenan had a simpler structure than the more soluble one.[208]

When the intrinsic viscosity of an anionic polysaccharide, *e.g.* carrageenan, is plotted against the reciprocal of the square root of the ionic strength, a linear plot of positive slope is obtained.[209] The slope reflects the ease with which the chains coil up as their effective charge density decreases and has been used to compare the stiffness of the chains in different ionic polysaccharides.

Antibodies prepared to λ- and κ-carrageenan have been shown to be essentially specific for the immogen used, whereas antibodies to ι-carrageenan displayed identical specificities to the anti-κ-carrageenan preparations.[210] The activity of the anti-λ preparation was apparently associated with the 6-sulphate of the 1,4-linked residue. Antibodies to κ-carrageenan reacted only with polymers containing the 1,4-linked 3,6-anhydro-L-galactose residue in association with 1,3-linked L-galactose 4-sulphate residues.

The carrageenan-induced aggregation of human blood platelets is inhibited by anti-thrombin III.[211]

Oligosaccharide (15), laminaritriose, has been isolated for the first time in crystalline form from a partial acid hydrolysate of *Schlerotina sclerotiorum* polysaccharide.[212]

β-D-Glc*p*-(1 → 3)-β-D-Glc*p*-(1 → 3)-D-Glc

(15)

A β-D-glucan of low molecular weight has been isolated from the siphonous green alga *Caulerpa simpliciuscule* and contains 30 D-glucose residues per molecule of which at least 27 at 1,3-linked and possess a β-configuration.[213] There are 1–2 branches at O-6 in the molecule with a maximum of four residues per side-chain. This D-glucan is associated in the ratio 3 : 1 with a (1 → 4)-linked α-D-glucan of the same molecular size.

The α-D-glucans of the prokaryotic green alga *Prochloron* have been extracted and separated by electrophoresis of their *sym*-triazine complexes.[214] A highly bra' ched D-glucan, similar to phycoglycogen, and an amylose type polymer were

[20' M. C. Matulewicz and A. S. Cerezo, *J. Sci. Food Agric.*, 1980, **31**, 203.
[21' M. C. Matulewicz and A. S. Cerezo, *Phytochemistry*, 1980, **19**, 2639.
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G. Kindness, F. B. Williamson, and W. F. Long, *Biochem. Soc. Trans.*, 1980, **8**, 84.
Y. Ueno and K. Kato, *Carbohydr. Res.*, 1980, **80**, 212.
D. B. Hawthorne, W. H. Sawyer, and B. R. Grant, *Carbohydr. Res.*, 1979, **77**, 157.
J. F. Fredrick, *Phytochemistry*, 1980, **19**, 2611.

detected. The existence of this unusual mixture suggested a possible mode of starch biosynthesis in algae in general.

Cellulose II (regenerated cellulose) has been isolated from mechanically prepared cell walls of the conchocelis phase of *Bangia fuscopurpurea* with Schweitzers reagent.[215] Its structure was confirmed by *X*-ray powder analysis and t.l.c. of the partial acid hydrolysis products. The presence of cellulose provided evidence that this bangiophycean life-cycle phase represents a transitional form or link between two classes of red algae, namely Bangiophyceae and Florideophyceae and also that the Bangiophyceae is a precursor of Florideophyceae.

Evidence has been presented to show that extracellular carbohydrate produced during the mating between gametes of the green algae *Chlamydomonas reinhardi* arises from lysis of the cell wall.[216] The major sugar residues found were D-mannose, L-arabinose, and D-galactose; an L-hydroxy-proline content was also identified.

Water-soluble and water-insoluble polysaccharides are released from the brown alga *Padina pavonia* by treatment with hydrochloric acid (pH 2.5).[217] Both fractions contain residues of D-glucopyranosyluronic acid, L-fucose, D-xylose, D-mannose, D-glucose, and D-galactose. The water-soluble fraction was separated into a neutral laminarin-type D-glucan, a sulphated heteropolysaccharide containing the above sugar residues and a protein moiety. The heteropolysaccharide had high anticoagulation activity. Structural studies showed that the main chain of the heteropolymer contained 1,4-linked β-D-glucopyranosyluronic acid, 1,4-linked β-D-mannopyranosyl, and 1,4-linked β-D-glucopyranosyl residues.[218] The exterior portion had residues of 1,4-linked β-D-galactose, 1,4-linked β-D-galactose 3-sulphate, 1,4-linked β-D-glucose, 1,2-linked α-L-fucose 4-sulphate, and 1,3-linked β-D-xylose.

The effect of temperature on the extraction of sulphated polysaccharides from three species of Ulvaceae and their physicochemical properties have been studied.[219] The physical properties of those from *Ulva pertusa* were independent of the extraction temperature, but the large molecular weight components from *U. conglobata* and *Entromorpha prolifera* were only extracted at temperatures above 40 °C. These larger molecules were thermostable at 80–90 °C. The sulphated polysaccharides from seven species of seaweed have been extracted at high temperatures (80–90 °C) and the molecular weight was found to be in the range 1×10^5–1×10^6.[220] Those from *U. pertusa* and *U. conglobata* were further characterized;[221] they contained 27% and 6% D-glucuronic acid, respec-

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[216] K. M. Solter, H.-C. Chow, D. A. Ray, and A. Gibor, *Phytochemistry*, 1980, **19**, 1943.
[217] M. Magdel-Din Hussein, A. Abdel-Aziz, and H. H. Salem, *Phytochemistry*, 1980, **19**, 2131.
[218] M. Magdel-Din Hussein, A. Abdel-Aziz, and H. M. Salem, *Phytochemistry*, 1980, **19**, 2133.
[219] M. Yamamoto and K. Mita, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 589.
[220] M. Yamamoto, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 673.
[221] M. Yamamoto, Y. Tadokoko, H. Iamai, and K. Mita, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 723.

tively, with molecular weights of 9.1×10^4 and 8.2×10^5 respectively. In spite of these differences, both polymers had similar physicochemical behaviour and similar random-coil conformations in acidic buffer solutions.

The extracellular polysaccharide of the diatom *Coscinodiscus nobilis* contains residues of L-fucose, L-ramnose, D-mannose, D-glucose, D-xylose, D-glucopyranosyluronic acid, D-galactose, and half-ester sulphate.[222] The positions of linkage between the monosaccharides have been obtained and evidence for the linkage of D-glucuronic acid has also been postulated. A chrysolaminarin and another polymer containing L-fucopyranosyl and D-mannopyranosyl residues were also detected.

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4

Microbial Polysaccharides

BY R. J. STURGEON

1 Teichoic Acids

A review has summarized and compared the known classes of amphiphiles (polymers that share specific physicochemical similarities, namely the presence of both hydrophilic and hydrophobic regions in their structures) on bacterial cell surfaces.[1] These include lipoteichoic acids and lipopolysaccharides. The distribution of lipoteichoic acids and other amphipathic antigens in oral streptococci has been reported.[2] Immunoperoxidase and electron microscopic studies have demonstrated that lipoteichoic acid of *Staphylococcus aureus* Cowan I is attached to the membrane and penetrates the whole cell wall.[3]

Octyl β-D-glucopyranoside has been found to give higher yields of lipoteichoic acid from *Lactobacillus casei* than other procedures.[4] Ester-linked D-alanyl residues in the *in vitro* synthesized lipoteichoic acid have been characterized. The increased cellular content of lipids and lipoteichoic acids in mutants of *Streptococcus faecium*, which have been shown previously to be potent inhibitors of the *N*-acetylmuramoyl hydrolase, are thought to contribute to the autolysis-defective phenotype of these mutants.[5] Esterification of the lipoteichoic acid of *Staphylococcus aureus* with L-alanine seems to be an ordered rather than a random process.[6] It is unlikely that a mixture of substituted and unsubstituted chains are synthesized but lipoteichoic acid molecules of both high and low L-alanine ester content are substituted within a narrow range.

Streptococcal lipoteichoic acid binds to albumin in a reversible and specific way involving the ester-linked fatty acids of the lipoteichoic acid molecule and the fatty acid binding sites of the albumin molecules.[7]

A set of glycerophosphoglycolipids isolated from *Streptococcus lactis* Kiel 42172 have been proposed to constitute a biosynthetic series of compounds involved in the synthesis of the lipoteichoic acid of that organism.[8] The membrane lipoteichoic acid of *S. faecium* (*S. faecalis* ATCC 9790) is a polymer of glycerol phosphate units that are linked at one end through a phosphodiester bond to phosphatidylkojibiosyl diacylglycerol.[9] Many of the glycerophosphate groups are substituted with kojibiose and L-alanine. Phosphatidylkojibiosyl

[1] A. J. Wicken and K. W. Knox, *Biochim. Biophys. Acta*, 1980, **604**, 1.
[2] S. Hamada, J. Mizuno, S. Kotani, and M. Torii, *FEMS Microbiol. Lett.*, 1980, **8**, 93.
[3] P. Aasjord and A. Grov, *Acta Pathol. Microbiol. Scand., Ser. B*, 1980, **88**, 47.
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[5] D. L. Shungu, J. B. Cornett, and G. D. Shockman, *J. Bacteriol.*, 1980, **142**, 741.
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[8] W. Fischer, D. Schuster, and R. A. Laine, *Biochim. Biophys. Acta*, 1979, **575**, 389.
[9] M. C. W. Ganfield and R. A. Pieringer, *J. Biol. Chem.*, 1980, **255**, 5164.

diacylglycerol functions as the acceptor of glycerol phosphate units from phosphatidylglycerol in the synthesis of this membrane lipoteichoic acid. The polymer product is devoid of any kojibiosyl- or L-alanyl-substituents on the glycerol phosphate chain, and therefore may be considered a nascent membrane teichoic acid.

Lipoteichoic acids oxidized with sodium periodate have been coupled to erythrocytes for use in the detection of antibody and antibody-producing cells specific for staphylococcal lipoteichoic acid.[10] The distribution of cross-reactive antigens A and B in *Streptococcus mutans* and other oral streptococci have been examined in relation to lipoteichoic acid and dextran.[11] Quantitative analyses of cell walls from *S. mutans* Ingbritt grown under carbohydrate limitation in the chemostat shows that growth conditions have no statistically significant effect on the composition of polysaccharide or peptidoglycan.[12] Differences have been noted in the immunogenicity of lipoteichoic acid under extremes of pH or dilution rate. Extracellular lipoteichoic acid together with small amounts of typing antigen are produced by *S. mutans* Ingbritt when grown in continuous culture with different carbohydrate sources.[13]

Fractionation of rabbit anti-lipoteichoic acid serum and precipitation reactions have shown that the immunoglobulin interacting with the lipoteichoic acid is IgM.[14] No anti-lipoteichoic acid activity was detected in the IgG fraction.

The existence of separate receptors for human immunoglobulin A (IgA) and immunoglobulin G (IgG) in alkaline-extracted group A streptococci cells has been shown to be related to group- and type-specific antigens, lipoteichoic acid, and peptidoglycan.[15]

Purified poly(ribitol phosphate) polymerase from *Staphylococcus aureus* H uses as acceptor a lipoteichoic acid carrier.[16] The specific structural requirement recognized by the enzyme is a segment of the hydrophilic chain with a minimum length of four glycerophosphate residues, which apparently must not be substituted. The terminal glycerol of this binding segment is not required for recognition but is indispensable for acceptor function. A non-specific hydrophobic portion of the carrier is also necessary for appropriate orientation at the lipid–water interface.

D-Alanine ester-containing lipoteichoic acids from *Staphylococcus aureus*, *Streptococcus lactis*, or *Lactobacillus helveticus* act as lipoteichoic acid carrier with poly(ribitol phosphate) polymerase, in contrast to the corresponding lipoteichoic acids from which the D-alanyl substituents had been removed.[17] It is

[10] P. R. Beining, G. M. Flannery, G. Caldes, B. Prescott, and P. J. Baker, *J. Immunol. Methods*, 1980, **32**, 167.

[11] R. R. B. Russell, *J. Gen. Microbiol.*, 1980, **118**, 383.

[12] K. W. Knox, N. A. Jacques, L. K. Campbell, A. J. Wicken, S. F. Hurst, and A. S. Beliweis, *Infect. Immun.*, 1979, **26**, 1071.

[13] N. A. Jacques, L. Hardy, L. K. Campbell, K. W. Knox, J. D. Evans, and A. W. Wicken, *Infect. Immun.*, 1979, **26**, 1079.

[14] P. Aasjord and A. Grov, *Acta Pathol. Microbiol. Scand., Ser. B*, 1980, **88**, 53.

[15] C. Schalen, P. Christensen, A. Grubb, G. Samuelsson, and M. L. Svensson, *Acta Path. Microbiol. Scand., Ser. C*, 1980, **88**, 77.

[16] W. Fischer, H. U. Koch, P. Rösel, F. Fiedler, and L. Schmuck, *J. Biol. Chem.*, 1980, **255**, 4550.

[17] W. Fischer, H. U. Koch, P. Rösel, and F. Fiedler, *J. Biol. Chem.*, 1980, **255**, 4557.

suggested that lipoteichoic acids are physiologically incapable of functioning as lipoteichoic acid carriers in the biosynthesis of wall teichoic acids.

Charged functional groups arising from both teichoic acids and peptidoglycans are involved in the interactions between cell walls and metal ions.[18] Hill plots of equilibrium dialysis data suggest that metal binding to cell walls involves negative co-operativity.

A lipid intermediate (1), additional to those already described, has been characterized as essential for the biosynthesis of teichoic acid in *Micrococcus varians*.[19] The synthesis of this intermediate is dependent upon UDP-2-acetamido-2-deoxy-D-glucose and is inhibited by tunicamycin. All three glycerol phosphate residues in the linkage unit are derived from CDP-glycerol.[20]

G-P-G-P-G-P-Glc*p*NAc-P-P-prenyl

G = glycerol

(1)

The biochemical and immunological properties of cell-surface teichoic acid preparations of different strains of *Staphylococcus aureus* have been reported.[21] Three strains prove to be of the ribitol phosphate-type containing, in addition, residues of D-glucose, D-galactose, and 2-acetamido-2-deoxy-D-galactose, whereas in another strain the amino-sugar is 2-acetamido-2-deoxy-D-galactose on a glycerol-phosphate type of teichoic acid.

The cell-wall teichoic acid of *Staphylococcus simulans* has been characterized as containing a glycerol phosphate backbone to which β-2-acetamido-2-deoxy-D-glucosyl residues are glycosidically linked.[22] Serological similarities of this teichoic acid to that of *S. saprophyticus* were noted.

Two types of D-glucosylated teichoic acids have been isolated after incubation of *Streptococcus sanguis* particulate preparations with UDP-D-glucose and phosphatidylglycerol.[23] The first type, readily soluble in aqueous salt solutions, contains no fatty acids, whereas the second type is bound to particulate matter and is only solubilized by sodium dodecyl sulphate. The second differs only from the saline extractable teichoic acid in possessing fatty acids. Teichoic acids appear to be widely spread in the genus *Streptomyces*.

The D-glycosyltransferase from *Streptococcus mutans* interacts with both lipoteichoic acid and deacylated lipoteichoic acid.[24] Most of the strains examined contain either glycerol or ribitol teichoic acids in the mycelia.[25]

[18] R. J. Doyle, T. H. Matthews, and U. N. Streips, *J. Bacteriol.*, 1980, **143**, 471.
[19] H. A. I. MacArthur, I. C. Hancock, F. M. Roberts, and J. Baddiley, *FEBS Lett.*, 1980, **111**, 317.
[20] F. M. Roberts, H. A. I. MacArthur, I. C. Hancock, and J. Baddiley, *FEBS Lett.*, 1979, **97**, 211.
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[23] D. J. Mancuso, D. D. Junker, S. C. Hsu, and T. H. Chiu, *J. Bacteriol.*, 1979, **140**, 547.
[24] H. K. Kuramitsu, L. Wondrack, and M. McGuinness, *Infect. Immun.*, 1980, **29**, 376.
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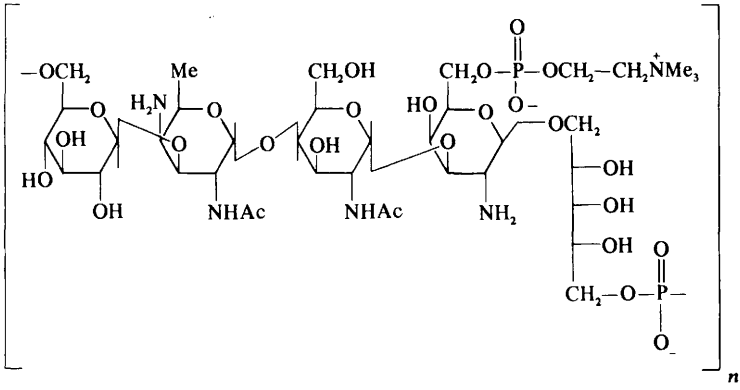
The structure of the cell-wall glycerol teichoic acid of *Streptomyces antibioticus* 39 has been reported.[26] Interpretation of the ^{13}C n.m.r. spectrum of the teichoic acid confirms the presence of phosphodiester groups between C-2 and C-3 of the glycerol molecules. The spectral data are also in accord with the results of methylation analysis and enzymic degradation studies, which indicate that the repeating monomeric unit of the teichoic acid is *O*-α-D-galactopyranosyl-(1 → 3)-*O*-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1 → 1)-glycerol monophosphate.

The complete structural analysis of the complex polysaccharide C-substance from *Streptococcus pneumoniae* type 1 includes the identification of a D-glucopyranosyl residue and a 2-acetamido-2-deoxy-D-galactosyl residue in its repeating unit.[27] By application of methylation analysis and ^1H and ^{13}C n.m.r. spectroscopy to a series of oligosaccharide fragments obtained by selective degradation of the *N*-acetylated antigen, which contains in addition residues of 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose, ribitol, and phosphate, the structure (2) is assigned to the native C-substance. Phosphocholine substituents are situated at O-6 of the unacetylated 2-amino-2-deoxy-D-galactosyl residues and the repeating units are linked through the diphosphate ester from ribitol to O-6 of the β-D-glucopyranosyl residue.



(2)

A phosphorus-rich polymer, which is not a conventional teichoic acid and which consists of a poly(mannitol phosphate) chain to which D-glucosyl substituents and acetal-linked pyruvic acid residues are attached, has been isolated from the cell wall of bacterium NCTC 9742.[28] Minor components of glycerophosphate and 2-amino-2-deoxy-D-galactose may form a linkage unit for the covalent attachment of the poly(mannitol phosphate) chain to peptidoglycan.

[26] A. S. Shashkov, M. S. Zaretskaya, S. V. Yarotsky, I. B. Naumova, O. S. Chizhov, and Z. A. Shabarova, *Eur. J. Biochem.*, 1979, **102**, 477.
[27] H. J. Jennings, C. Lugoswki, and N. M. Young, *Biochemistry*, 1980, **19**, 4712.
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2 Peptidoglycans

Conditions for the separation of muramic acid, DL- and *meso*-lanthionine, 2-amino-2-deoxy-D-glucose, and diaminopimelic acid from other naturally occurring amino-acids in bacterial cell-wall peptidoglycans have been reported.[29]

Lanthionine, a sulphur-containing diamino-acid not previously reported as one of the main amino-acids of bacterial cell-wall peptidoglycans, has been identified in *Fusobacterium nucleatum* peptidoglycan.[30] L-Lysine, diaminopimelic acid, and L-ornithine were absent. Lanthionine appears to be an essential dibasic amino-acid involved in cross-linkages between peptide subunits in the peptidoglycan. The distribution of products in mouse urine following intravenous administration of peptidoglycan monomer, D-Glc*p*NAc-MurNAc-L-Ala-D-isoglutamine-*meso*-diaminopimelic acid-D-Ala-D-Ala, include the recovery of about 50% of the product unchanged and 20% pentapeptide.[31]

Evidence for the existence in *Escherichia coli* of a soluble polymeric peptidoglycan containing covalently attached lipoprotein has been reported.[32] This macromolecular product appears to be a late intermediate in peptidoglycan synthesis, which subsequently becomes covalently bound to the existing sacculus. Proteolysis, followed by treatments with trichloroacetic acid and then mild alkali, have been used in the isolation of the intact globular cell-wall peptidoglycan from *Micrococcus lysodeikticus*.[33] The purified preparation retained the chemical and morphological integrity as judged by chemical analyses and electron microscopic evidence.

A water-soluble, tuberculin-active peptidoglycan (mol. wt. 2.8×10^4) has been isolated from the culture filtrate of *Mycobacterium tuberculosis*.[34] Analysis of the peptidoglycan showed that it contains L-proline, L-glutamic acid, L-alanine, diaminopimelic acid, L-tyrosine, L-threonine, 2-amino-2-deoxy-D-glucose, L-arabinose, and D-galactose.

Muramic acid has been detected in the tissues of rats with streptococcal cell-wall-induced polyarthritis by g.l.c.-m.s. of its alditol acetate.[35] Because the measurement of muramic acid reflects the bacterial biomass in tissue, it can be used in the study of bacterial infections. The cell walls of *Bacillus subtilis*, extracted to remove teichoic acids, retain a high affinity for some cations, although the stoicheiometry is generally reduced.[36] Functional groups of the peptidoglycan may contribute to the surface binding of metals by bacteria. The cell walls of *Methanobacterium thermoautotrophicum* contain 'pseudomurein' in place of peptidoglycan in which the polymer contains no D-amino-acids and in which muramic acid is replaced by 2-acetamido-2-deoxy-taluronic acid.[37] The L-configuration of this amino-sugar has now been demonstrated.

[29] E. Vasstrand, H. B. Jensen, and T. Miron, *Anal. Biochem.*, 1980, **105**, 154.
[30] K. Kato, T. Umemoto, H. Sagawa, and S. Kotani, *Curr. Microbiol.*, 1979, **3**, 147.
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[32] H. Mett, R. Bracha, and D. Mirelman, *J. Biol. Chem.*, 1980, **255**, 9884.
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[34] K. C. Gupta and S. Landi, *Infect. Immun.*, 1980, **27**, 344.
[35] A. Fox, J. H. Schwab, and T. Cochran, *Infect. Immun.*, 1980, **29**, 526.
[36] T. H. Matthews, R. J. Doyle, and U. N. Streips, *Curr. Microbiol.*, 1979, **3**, 51.
[37] H. König and O. Kandler, *Z. Phys. Chem.*, 1980, **361**, 981.

The extent of peptide cross-linking in the peptidoglycan of *Neisseria gonorrhoeae* has been assessed.[38] Gonococcal peptidoglycan is more extensively cross-linked than most Gram negative bacteria with about 40% of the ε-amino groups on diaminopimelic acid residues involved in cross-linking bonds. Confirmation of previous observations that multiple antibiotic resistance in gonococci is correlated with an increase in the extent of peptide cross-linking has been made.

An autolytic glycosidase from a lysozyme-resistant strain of *Bacillus cereus* can cleave the glycosidic linkages of *N*-unsubstituted 2-amino-2-deoxy-D-glucose in the cell-wall peptidoglycan.[39] The major oligosaccharide fragments resulting from digestion of untreated non-*N*-acetylated cell-wall peptidoglycan of *B. cereus* with this enzyme were identified as 2-amino-2-deoxy-3-*O*-(*N*-acetylmuramyl)-D-glucose and its dimer.

The action of lysozyme towards peptidoglycans and related oligosaccharides with several different *N*-acyl substituents at the 2-amino-2-deoxy-D-glucosyl residues has been reported.[40] The results obtained with *N*-acylated cell-wall peptidoglycan preparations are explained by the importance of the interaction of the alkyl moieties of the *N*-substituents with the enzyme at subsite C.

When the purified peptidoglycan from *Escherichia coli* K12 is degraded into disaccharide–peptide fragments by *endo-N*-acetylmuramidase from a *Chalaropsis* species, a small proportion of the peptidoglycan fragments are recovered as peptide cross-linked tridisaccharide peptide trimers.[41]

The action of lysozyme on *Bacillus cereus* 569, which is known to be resistant to lysis by lysozyme due to the presence of deacetylated 2-amino-2-deoxy-D-glucosyl residues in its peptidoglycan, releases some free reducing groups, indicating limited breakage of the peptidoglycan chains.[42] The modification of the peptidoglycan makes it more susceptible to autolysin(s). Lysozyme also enhances the rate of septum separation and the probable significance of the results in relation to the control of cell separation is discussed.

The absence of peptidoglycan turnover has been demonstrated for several strains of *Streptococcus mutans* and *S. sanguis*.[43] This is in contrast with the loss of streptococcal surface components, such as membrane-associated lipoteichoic acid.

The membrane-bound enzymes of peptidoglycan synthesis in *Escherichia coli* can be dissociated with solutions of cholate and lithium chloride.[44] The active enzymes can be separated further from the lipids necessary for synthesis. A protein, which is essential for peptidoglycan polymerization, was separated from the other synthetic enzymes by chromatography on hydroxyapatite. The formation of cross-linkages in the biosynthesis of peptidoglycan of *E. coli* is sensitive to penicillin G and to cephalexin.[45]

[38] R. S. Rosenthal, R. M. Wright, and R. K. Sinha, *Infect. Immun.*, 1980, **28**, 867.
[39] S. Kawagishi, Y. Araki, and E. Ito, *J. Bacteriol.*, 1980, **141**, 137.
[40] K. Amano, Y. Araki, and E. Ito, *Eur. J. Biochem.*, 1980, **107**, 547.
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[44] A. Taku, T. M. Hirsch, and D. P. Fan, *J. Biol. Chem.*, 1980, **255**, 2848.
[45] F. Ishino and M. Matsuhashi, *Agric. Biol. Chem., (Jpn.)*, 1979, **43**, 2641.

Phospho-*N*-acetylmuramyl-pentapeptide translocase, the initial membrane enzyme in the biosynthesis of peptidoglycan, requires a lipid micro-environment for function.[46] Lipid-translocase interactions can be perturbed by butan-1-ol, resulting in an increase in fluidity in the microenvironment of the enzyme. This change in fluidity correlates with a stimulation of enzymatic activity. The regulation of the terminal steps in peptidoglycan synthesis during amino-acid deprivation in a mutant of *Escherichia coli* results in a marked decrease in D-alanine carboxypeptidase activity.[47] An associated increase in the cross-linkage of peptidoglycan and a decrease in the peptidoglycan biosynthetic activity also occurs.

The peptidoglycan precursor UDP-muramylpentapeptide has been purified by affinity chromatography on immobilized vancomycin, utilizing the affinity of the antibiotic for the D-alanyl-D-alanine residues in the precursor.[48] The biosynthesis of cell-wall-bound material in *Micrococcus luteus* has been examined by studying the incorporation of UDP-2-acetamido-2-deoxy-D-[^{14}C]-glucose, both into peptidoglycan and into the link region that ultimately joins the teichuronic acid to the peptidoglycan.[49] Tunicamycin at low concentrations can inhibit the linkage-unit synthesis, while having little effect on peptidoglycan synthesis.

In the cell-wall polymer assembly in *Bacillus licheniformis*, *B. subtilis*, and *Staphylococcus aureus*, tunicamycin has been shown to inhibit the biosynthesis of both peptidoglycan and the linkage units involved in the attachment of peptidoglycan to teichoic acids.[50] In the biosynthesis of peptidoglycan of *Escherichia coli* K-12, the properties of the *in vitro* polymerization reaction by transglycosylation, which involves the lipid intermediate 2-acetamido-2-deoxy-D-glucosyl-*N*-acetylmuramyl-pentapeptide pyrophosphoryl undecaprenol, have been reported.[51] Penicillin binding protein 1b of *E. coli* K-12 can catalyse polymerization from this lipid intermediate.[52]

The exocellular DD-carboxypeptidase of *Streptomyces albus* G has been studied by *X*-ray crystallography, which has permitted visualization of the enzyme active centre and localization of the Zn^{2+} binding site.[53] The native enzyme (mol. wt. 1.8×10^4) contains one equivalent of bound Zn^{2+} per molecule.[54] The Zn^{2+} cofactor is required for both DD-carboxypeptidase activity and for binding of benzylpenicillin. LD-Carboxypeptidase acts in conjunction with DD-carboxypeptidase and peptidoglycan transpeptidase in the incorporation of nascent peptidoglycan into pre-existing peptidoglycan of the bacterial

[46] P. P. Lee, W. A. Weppner, and F. C. Neuhaus, *Biochim. Biophys. Acta*, 1980, **597**, 603.
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[49] H. R. Perkins, S. J. Thorpe, and C. A. Brown, *Biochem. Soc. Trans.*, 1980, **8**, 163.
[50] J. B. Ward, A. W. Wyke, and C. A. M. Curtis, *Biochem. Soc. Trans.*, 1980, **8**, 164.
[51] Y. Van Heijenoort and J. Van Heijenoort, *FEBS Lett.*, 1980, **110**, 241.
[52] H. Suzuki, Y. Van Heijenoort, T. Tamura, J. Mizoguchi, Y. Hirota, and J. Van Heijenoort, *FEBS Lett.*, 1980, **110**, 245.
[53] O. Dideberg, P. Charlier, L. Dupont, M. Vermeire, J. M. Frere, and J. M. Ghuysen, *FEBS Lett.*, 1980, **117**, 212.
[54] O. Dideberg, B. Joris, J. M. Frere, J. M. Ghuysen, G. Weber, R. Robaye, J. M. Delbrouk, and I. Roelandts, *FEBS Lett.*, 1980, **117**, 215.

cell wall.[55] The enzyme is sensitive to sulphydryl reagents and heat labile, and may play a role in the relationship between membrane and wall.

The major outer membrane proteins of 23 enterobacterial strains have been shown to contain one or more peptidoglycan-associated proteins, cross-reactive with the peptidoglycan-bound protein I of *Escherichia coli*, and one non-peptidoglycan-bound heat-modifiable protein cross-reactive with protein II of *E. coli*.[56] The antigenic cross-reactivity of these proteins is a general phenomenon in the family of Enterobacteriaceae, independent of any molecular weight variation of the corresponding proteins in different bacterial strains.

In *Escherichia coli* K-12 the product of gene *lam* B is an outer membrane protein.[57] Much of this protein has been solubilized and found to be associated with peptidoglycan. The construction of multiple penicillin-binding protein mutants of *E. coli* has facilitated the purification of these proteins, which are essential for cell elongation and division.[58] Penicillin-binding proteins have been isolated by affinity chromatography on immobilized β-lactam antibiotics.

Three penicillin-binding proteins, 1a, 1b, and 3, have been isolated from multiple mutants of *Escherichia coli*.[59] None of the three proteins shows D-alanine carboxypeptidase activity with UDP-*N*-acetylmuramoyl pentapeptide as substrate or endopeptidase activity. A review has summarized the role of penicillin and other β-lactam antibiotics in the acylation of penicillin-binding proteins in the bacterial membrane.[60] These penicillin-binding proteins are thought to be penicillin-sensitive enzymes involved in the cross-linking step of cell-wall biosynthesis, including transpeptidase and D-alanine carboxypeptidase. Some speculations on the biological role of the penicillin-binding proteins 4 and 5 in *E. coli* have been published.[61]

3 Lipopolysaccharides

A preliminary structural survey of the lipopolysaccharides obtained from eight different meningococcal sero-groups has been reported.[62] Although the cores of the different lipopolysaccharides contain identical components (D-glucose, D-galactose, 2-amino-2-deoxy-D-glucose, heptose, and 2-keto-3-deoxyoctonate) they can be separated into three distinct categories according to their ratios of D-galactose : D-glucose contents, and also according to the results of methylation analysis. Electrophoresis of lipopolysaccharides in the presence of sodium dodecylsulphate has been used for studies of lipopolysaccharide heterogeneity.[63]

[55] F. C. Neuaus, C. E. Tobin, and J. A. Ahlgren, *J. Bacteriol.*, 1980, **143**, 112.
[56] H. Hofstra and J. Dankert, *J. Gen. Microbiol.*, 1980, **119**, 123.
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[62] H. J. Jennings, A. K. Bhattacharjee, L. Kenne, C. P. Kenny, and G. Calver, *Can. J. Biochem.*, 1980, **58**, 128.
[63] E. T. Palva and P. H. Mäkelä, *Eur. J. Biochem.*, 1980, **107**, 137.

The dissociation of lipopolysaccharide resulting from the removal of divalent cations and the addition of the surfactant sodium deoxycholate has been re-examined.[64] Although other workers have suggested that the basic lipopolysaccharide unit is a trimer, the present work suggests that it exists as a dimer.

The mode of interaction of the ordered hexagonal lattice structure with a lattice constant of about 7 nm, which can be reconstituted from the outer membrane protein O-8 and the lipopolysaccharide of *Escherichia coli* K-12, has been studied by electron microscopy.[65] Both the fatty acid region and the polysaccharide region of the lipopolysaccharide are involved in an interaction of lipopolysaccharide with the membrane protein.

The feasibility of a bioluminescence assay for the determination of lipopolysaccharides of Gram negative bacteria has been demonstrated.[66] The assay depends on the myristic acid content of the lipopolysaccharide and thus gives a direct measurement of lipid A.

Gel immunoradioassay (GIRA) has provided a means of detecting human antibody activities against individual meningococcal cell-surface antigens.[67] Serum from a patient with meningitis caused by group C meningococci showed antibody activity against capsular polysaccharides, lipopolysaccharides, and outer membrane proteins of homologous and heterologous strains. Immunoelectron microscopic studies on lipopolysaccharides of *Salmonella typhimurium* have confirmed the localization of these antigens to two zones within the outer layer of the cell wall.[68]

Lipopolysaccharides have been extracted from estuarine sediments, and their distribution and biodegradation have been studied.[69]

The extent of heterogeneity in lipopolysaccharide preparations containing antigenic side-chains is far more than previously believed.[70] *Escherichia coli* 0111, thought to have only two size classes of lipopolysaccharide, contains more than 40 discrete size classes as shown by gel electrophoresis. Similar results are shown for *Salmonella typhimurium* LT2 and *E. coli* 086. Group A strains of *Neisseria meningitidis* have been distinguished on the basis of their lipopolysaccharides.[71] By use of a solid-phase radioimmunoassay inhibition method, three serologically distinct lipopolysaccharide antigens and several protein antigens of *N. meningitidis* have been characterized by a combination of immunodiffusion and an electrophoretic immunoperoxidase system.[72] It is confirmed that the serotype antigens of the outer membrane of some serotypes are proteins, whereas on other serotypes they are lipopolysaccharides.

The membranes of the stable protoplast-L-form of *Proteus mirabilis* strain VI are highly immunogenic carriers of lipopolysaccharide when compared with the

[64] J. W. Shands and P. W. Chun, *J. Biol. Chem.*, 1980, **255**, 1221.
[65] H. Yamada and S. Mizushima, *Eur. J. Biochem.*, 1980, **103**, 209.
[66] S. Ulitzur, B. Yagen, and S. Rottem, *Appl. Environ. Microbiol.*, 1979, **37**, 782.
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[70] R. C. Goldman and L. Lieve, *Eur. J. Biochem.*, 1980, **107**, 145.
[71] W. D. Zollinger and R. E. Mandrell, *Infect. Immun.*, 1980, **28**, 451.
[72] J. T. Poolman, C. T. P. Hopman, and H. C. Zanen, *J. Gen. Microbiol.*, 1980, **116**, 465.

immune responses to lipopolysaccharides in the cell walls of the bacterial form of this organism.[73]

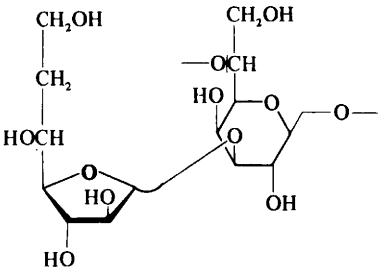
In the induction of the hypersensitive response in tobacco plants by *Pseudomonas solanacearum* the recognition between host and pathogen is thought to involve an interaction between plant lectins and bacterial lipopolysaccharide.[74] From a study of different strains of *P. solanacearum*, which are either capable or incapable of inducing the response, it appears that differences in lipopolysaccharide structure correlate strongly with host recognition of the bacterium. The mapping of genes determining penicillin-resistance and serum sensitivity in *Salmonella enteritidis* has been reported.[75] The chemical composition of two presumptive single-step mutants, their phage-sensitivity patterns, and their serological properties show one to be 'part-rough' and the other smooth.

Endotoxin prepared from several *Bordetella pertussis* strains contain two lipopolysaccharides and protein.[76] By use of ^{125}I-labelling of the protein moiety followed by column chromatographic procedures, it has been shown that these proteins are not linked by covalent bonds to the lipopolysaccharide.

Antibody and transferrin, acting together, exert a bacteriostatic effect on certain pathogenic *Escherichia coli*.[77] The antigen against which the antibody is directed is the *O*-specific side-chain of the lipopolysaccharide. The bacteriostatic effect can be abolished by 3,6-dideoxy-L-galactose (colitose), which is the terminal monosaccharide of the *O*-specific side-chain of the lipopolysaccharide of *E. coli* 0111.

Structural studies on the *Eubacterium saburreum* strain O2 antigen have confirmed that the polysaccharide has a comb-like structure and is composed of a disaccharide repeating unit (3).[78]

A lipopolysaccharide isolated from phase II *Coxiella burnetii* shows some differences in sugar composition when compared with that found in the



(3)

[73] H. Karch and K. Nixdorff, *Infect. Immun.*, 1980, **30**, 349.
[74] M. H. Whatley, N. Hunter, M. A. Cantrell, C. Hendrick, K. Keegstra, and L. Sequeira, *Plant Physiol.*, 1980, **65**, 557.
[75] D. E. Myers, B. A. D. Stocker, and R. J. Roantree, *J. Gen. Microbiol.*, 1980, **118**, 367.
[76] A. Le Dur, R. Chaby, and L. Szabo, *J. Biotecnol.*, 1980, **143**, 78.
[77] S. P. Fitzgerald and H. J. Rogers, *Infect. Immun.*, 1980, **27**, 302.
[78] J. Hoffman, B. Lindberg, N. Skaug, and T. Hofstad, *Carbohydr. Res.*, 1980, **84**, 181.

corresponding lipopolysaccharide of phase I cells.[79] Immunodiffusion studies demonstrated immunological identity of the two lipopolysaccharides.

The lipopolysaccharide of *Escherichia coli* K-12 CR34 has been shown to contain the β-(1→6)-linked disaccharide of 2-amino-6-*O*-[2-amino-2-deoxy-β-D-glucopyranosyl]-2-deoxy-D-glucose in the lipid A backbone.[80] The ^{13}C n.m.r. spectrum of this disaccharide has been used to characterize the disaccharide. From a study of the neutral sugars and fatty acids present in lipopolysaccharides isolated from several *Fusobacterium* species, the structures of these lipopolysaccharides may be less complex than those of most other bacteria.[81] L-*Glycero*-D-*manno*-heptose was present in abundance in all lipopolysaccharides examined.

Phenol–water extraction of *Haemophilus influenzae* type *a* solubilizes a lipopolysaccharide-type material devoid of heptose and 2-keto-3-deoxy-octonic acid, but containing neutral sugars, 2-amino-2-deoxy-D-glucose, amino-acids, and fatty acids.[82] The biological activity indicated that this preparation is not a classical endotoxin but rather an endotoxin-like substance. The toxicity of the polysaccharide is decreased without significant loss of antigenicity by mild acid hydrolysis.[83] The resulting preparation contained L-rhamnose, D-glucose, D-galactose, D-mannose, and 2-amino-2-deoxy-D-glucose (molar ratio 1 : 4 : 1 : 2 : 2).

Studies on the antigenic structure of the polysaccharide component of the lipopolysaccharide of *Neisseria gonorrhoeae* have indicated that the non-serogroup antigen structure is antigenically heterogeneous.[84]

The chemical composition of lipopolysaccharides isolated from 12 strains of fast-growing rhizobia have been reported.[85] Comparison of the total composition of the *Rhizobium* lipopolysaccharides shows many differences among different species and among strains of a single species. In view of a possible role of surface carbohydrates of *Rhizobium* in root nodule symbiosis, the specificity of the binding of legume lectins with *exo*- and lipo-polysaccharides of *Rhizobium* is discussed.

Information on the internal (D-gluco-heptose) region of the *Proteus mirabilis* R core has been obtained by methylation analysis of the core oligosaccharide of the *P. mirabilis* mutant R4 derived from serotype *O*-28.[86] A combination of data from the methylation analysis and ^1H n.m.r. spectroscopy was used to obtain the structure (4).

The polysaccharide chain of the *O*-serogroup 7 (Lanyi) lipopolysaccharide of *Pseudomonas aeruginosa* contains equimolar proportions of 2-acetamido-2,6-dideoxy-D-galactose (*N*-acetyl-D-fucosamine), 2-acetamido-2,6-dideoxy-L-

[79] O. G. Baca, I. L. Martinez, A. S. Aragon, and D. Klassen, *Can. J. Microbiol.*, 1980, **26**, 819.
[80] D. Blanche, M. Bruneteau, and G. Michel, *Biochimie*, 1980, **62**, 191.
[81] T. Hofstad and N. Skaug, *Acta Pathol. Microbiol. Scand., Ser. B*, 1980, **88**, 115.
[82] D. Raichvarg, C. Brossard, and J. Agneray, *Infect. Immun.*, 1979, **26**, 415.
[83] D. Raichvarg, C. Brossard, and J. Agneray, *Infect. Immun.*, 1980, **29**, 171.
[84] M. A. Apicella and N. C. Gagliardi, *Infect. Immun.*, 1979, **26**, 870.
[85] L. P. T. M. Zevenhuizen, I. Scholten-Koerselman, and M. A. Posthumus, *Arch. Mikrobiol.*, 1980, **125**, 1.
[86] J. Radziejewska-Lebrecht, U. Feige, M. Jensen, K. Kotelko, H. Friezolin, and H. Mayer, *Eur. J. Biochem.*, 1980, **107**, 31.

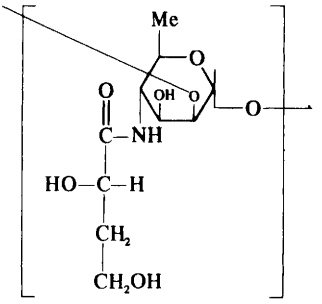
$$\beta\text{-D-Glc}p\text{-}(1 \rightarrow 3/4)\text{-}\alpha\text{-LD-Hep}p\text{-}(1 \rightarrow ?)\text{=}\alpha\text{OclA}$$
$$\begin{matrix} & & &

glucose I. The effect of the *rfa*H$^-$ character on the sensitivity of these mutants to various antibiotics is also reported.[91]

The *O*-polysaccharide of *Salmonella johannesburg* 5.58(R) is a receptor for phage ϕ1(40).[92] In an assay system, the receptor is enzymically degraded by the phage particles due to an *endo*-(1→3)-2-acetamido-2-deoxy-β-D-galactanase activity.

The *O*-specific side-chains of *Shigella sonnei* phase 1 lipopolysaccharide are composed of disaccharide repeating units containing 2-acetamido-2-deoxy-α-L-altruonic acid linked through O-4 and 2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactopyranosyl residues linked through O-3.[93] The pyranosyluronic acid residue assumes the $^4C_1$ conformation in the polymer with the carboxy-group in the axial position.

The compositions of *O*-specific side-chains and core polysaccharides of several strains of *Vibrio cholerae* lipopolysaccharide have been reported.[94] 2-Amino-2,6-dideoxy-D-glucose (D-quinovosamine) is present exclusively in *O*-specific side-chain polysaccharides, whereas 4-amino-4,6-dideoxy-D-mannose (D-perosamine) is concentrated in the *O*-specific side-chain. The *O*-antigen is a polysaccharide (mol. wt. 9.0×10^3), which on acid hydrolysis releases 3-deoxy-L-*glycero*-tetronic acid and 4-amino-4,6-dideoxy-D-mannose.[95] By use of methylation analysis, g.l.c.-m.s., and ^{1}H and ^{13}C n.m.r. spectroscopy, the polysaccharide has been identified as a homopolymer (8). This appears to be the first report of the existence of 3-deoxy-L-*glycero*-tetronic acid, and of a homopolymer of D-perosamine.



(8)

The mutation of the S-form of *Vibrio cholerae* to the R-form involves the total elimination of 2-amino-2,6-dideoxy-D-glucose (quinovosamine) and 4-amino-4,6-dideoxy-D-mannose (perosamine) from the lipopolysaccharide.[96]

[91] B. A. D. Stocker, B. M. Males, and W. Takano, *J. Gen. Microbiol.*, 1980, **116**, 17.
[92] R. Chaby and R. Girard, *Virology*, 1980, **105**, 136.
[93] L. Kenne, B. Lindberg, K. Petersson, E. Katzenellenbogen, and E. Romanowska, *Carbohydr. Res.*, 1980, **78**, 119.
[94] S. Raziuddin, *Infect. Immun.*, 1980, **27**, 211.
[95] L. Kenne, B. Lindberg, P. Unger, T. Holme, and J. Holmgren, *Carbohydr. Res.*, 1979, **68**, C14.
[96] K. Hisatsune and S. Kondo, *Biochem. J.*, 1980, **185**, 77.

This elimination results in the loss of *O*-specificity of S-form lipopolysaccharides and concomitant appearance of strong cross-reactivity among R-form lipopolysaccharides regardless of the sero-types (Inaba and Ogawa) of their *S* parent strains. The structures of five oligosaccharides (9)–(13) isolated from the *O*-antigen of *V. cholerae* Inaba 569B have been elucidated.[97]

β-D-Glc*p*A-(1 →)-D-Man

(9)

β-D-Glc*p*A-(1 → 6)-D-*glycero*-L-*manno*-Hep

(10)

D-*Glycero*-β-L-*manno*-Hep*p*-(1 → 2)-D-Man

(11)

α-D-Glc*p*A-(1 → ?)-D-*glycero*-L-*manno*-Hep

(12)

α-D-Glc*p*-(1 → 4)-α-D-Glc*p*NAc-(1 → 2)-D-*glycero*-L-*manno*-Hep

(13)

→ 3)-α-L-Fuc*p*-(1 → 3)-α-D-Gal*p*NAc-(1 → 2)-α-D-Man*p*-(1 →
4
↑
1
α-D-Par*p*

(14) D-Par*p* = 3,6-dideoxy-D-*ribo*-hexopyranose (paratose)

Lipopolysaccharides from various strains of *Yersinia pseudotuberculosis* type V have been isolated and characterized.[98] Differences in sugar composition and serological activity of lipopolysaccharides from various strains within the same subtype of the organism have been revealed. The repeating unit (14) of the *O*-specific side-chain polysaccharide of the lipopolysaccharide from the pathogenic *Y. pseudotuberculosis* type III has been determined.[99] The structure shows both similarities to and some differences from the trisaccharide repeating unit proposed for non-pathogenic *Y. pseudotuberculosis* type III.

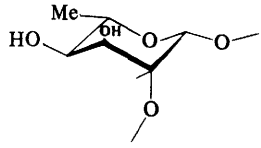
The combined evidence from methylation analysis, n.m.r. spectroscopy, and optical rotational measurements demonstrate that the *O*-antigen of *Yersinia enterolytica* Ye 128 is a homopolymer composed of 1,2-linked 6-deoxy-β-L-altropyranosyl residues and that these residues are in the $^1C_4$ conformation (15).[100] A purified envelope antigen of *Y. pestis* has been characterized as a

[97] A. K. Sen, A. K. Mukherjee, B. Guhathakurta, A. Dutta, and D. Sasmal, *Carbohydr. Res.*, 1980, **86**, 113.

[98] R. P. Gorshkova, N. I. Korchagina, T. A. Medonova, E. N. Kalmykova, N. N. Besednova, and Y. S. Ovodov, *Carbohydr. Res.*, 1980, **84**, 237.

[99] R. P. Gorshkova, N. A. Komandrova, A. I. Kalinovsky, and Y. S. Ovodov, *Eur. J. Biochem.*, 1980, **107**, 131.

[100] J. Hoffman, B. Lindberg, and R. R. Brubaker, *Carbohydr. Res.*, 1980, **78**, 212.



(15)

lipid-polysaccharide consisting of residues of D-galactose and L-fucose, together with phosphatidylethanolamine and phosphatidylserine.[101] This product differs from true lipopolysaccharide in containing no amino-sugars or D-glucose.

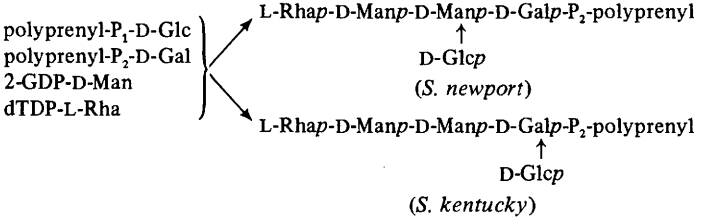
3-Deoxy-D-*manno*-octulosonate 8-phosphate synthetase has been purified and characterized from *Escherichia coli*.[102] No other sugar or sugar phosphate can substitute for D-arabinose 5-phosphate as a substrate.

The incomplete lipid A precursor produced by a mutant of *Salmonella typhimurium* conditionally defective in synthesis of 3-deoxy-D-*manno*-octulosonate (KDO) is rapidly converted to lipopolysaccharide when the mutant culture is shifted from nonpermissive to permissive conditions.[103] It is likely that some structural features of the 3-deoxy-D-*manno*-octulosonate-lipid region, possibly the KDO trisaccharide unit, are required for effective translocation of lipopolysaccharide to the outer membrane. Direct evidence has been obtained that incorporation of 3-deoxy-D-*manno*-octulosonate precedes addition of the saturated *O*-acyl chains to lipid A *in vivo* and that a previously described product of the CMP-3-deoxy-D-*manno*-octulosonate : lipid A transferase system represents a normal intermediate in lipopolysaccharide biosynthesis.[104]

Cell-surface expression of the *ompA* gene of *Escherichia coli* appears to depend upon the ability of the gene product to bind lipopolysaccharide, suggesting that an interaction between protein and lipopolysaccharide plays an essential role in biosynthesis of the outer membrane protein.[105]

Cell envelope and soluble glycosyltransferase preparations from *Salmonella newport* (sero-group C_2) and *S. kentucky* (sero-group C_3) catalyse the formation of polyprenyl pyrophosphate tetrasaccharides corresponding to the structure of the repeating unit of the main chain of *O*-specific polysaccharides.[106] The D-galactosyl residue is an initiator of a chain growth, with the transfer of D-galactosyl phosphate from UDP-D-galactose being followed by two consecutive transfers of D-mannosyl residues from GDP-D-mannose and the transfer of an L-rhamnosyl residue from dTDP-L-rhamnose. UDP-D-Glucose and polyprenyl phosphate are converted by the enzyme preparations into polyprenyl monophosphate D-glucose, which may transfer a D-glucosyl residue on to the polyprenyl pyrophosphate oligosaccharides (Scheme 1). The resulting penta-

[101] R. Glosnicka and E. Gruszkiewicz, *Infect. Immun.*, 1980, **30**, 506.
[102] P. H. Ray, *J. Bacteriol.*, 1980, **141**, 635.
[103] M. J. Osborn, P. D. Rick, and N. S. Rasmussen, *J. Biol. Chem.*, **225**, 4246.
[104] R. W. Walenga and M. J. Osborn, *J. Biol. Chem.*, 1980, **255**, 4252.
[105] M. Beher, A. Pugsley, and C. Schnaitman, *J. Bacteriol.*, 1980, **143**, 403.
[106] V. N. Shibaev, T. N. Druzhinina, A. N. Popova, S. S. Rozhnova, and V. A. Kilesso, *Eur. J. Biochem.*, 1979, **101**, 309.



Scheme 1

saccharide derivatives are polymerized by enzymes present in the cell-envelope preparations.

The incorporation of L-rhamnose and D-glucose into the core part of the lipopolysaccharide of *Pseudomonas aeruginosa* has been studied using enzyme preparations from one strain and from a lipopolysaccharide-defective mutant derived from it.[107] The core of the lipopolysaccharide is synthesized by the sequential addition of D-glucose followed by L-rhamnose, both from their nucleotide derivatives. An inability to synthesize dTPD-L-rhamnose produces a defect in the lipopolysaccharide core of the mutant.

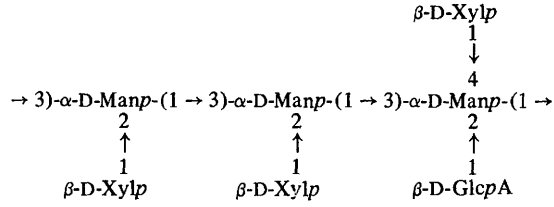
4 Capsular Polysaccharides

The capsular polysaccharide of *Cryptococcus neoformans* serotype A is a D-glucurono-D-xylo-D-mannan containing D-xylose, D-mannose, D-glucuronic acid, and *O*-acetyl (molar ratio 2 : 5 : 1 : 2).[108] The structure is composed of a linear (1 → 3)-linked D-mannan substituted at *O*-2 of every second D-mannopyranosyl residue by D-xylopyranosyl- or D-glucopyranosyl-uronic acid residues. Antigenic activity of this polysaccharide is directed toward the D-xylopyranosyl- and D-glucopyranosyl-uronic acid residues.

Of the two capsular polysaccharides isolated from *Cryptococcus neoformans* serotype A, which have been shown to be chemically equivalent, one has been shown[109] to have a structure almost identical to that identified in Ref. 108.

The capsular material from *Cryptococcus bacillisporus* serotype B contains a polysaccharide whose structure (16) has nonreducing D-xylopyranosyl-, as well as, D-glucuronopyranosyl-uronic acid residues attached to O-2 of D-mannopyranosyl residues of the linear backbone of (1 → 3)-α-D-mannopyranosyl residues.[110] Every third D-mannopyranosyl residue is doubly substituted with a D-xylopyranosyl residue at O-2 and a D-glucopyranosyl-uronic acid residue at O-4. The polysaccharide also contains three *O*-acetyl groups per repeating unit of seven sugars, but their location has not been identified.

[107] T. M. Asonganyi and P. M. Meadow, *J. Gen. Microbiol.*, 1980, **117**, 1.
[108] R. Cherniak, E. Reiss, M. E. Slodki, R. D. Plattner, and S. O. Blumer, *Mol. Immunol.*, 1980, **17**, 1025.
[109] E. H. Merrifield and A. M. Stephen, *Carbohydr. Res.*, 1980, **86**, 69.
[110] A. K. Bhattacharjee, K. J. Kwon-Chung and C. P. J. Glaudemans, *Carbohydr. Res.*, 1980, **82**, 103.



(16)

Various aspects of the structure of the capsular K2 antigen of *Escherichia coli* have been elucidated.[111] It is a linear polymer consisting of equimolar amounts of D-galactose, glycerol, and phosphate. The K2 antigen cross-reacts serologically with the *E. coli* K62 antigen, which has the same composition, but in addition contains equimolar amounts of *O*-acetyl groups. Both antigens contain phosphogalactopyranosyl-glycerol units and phosphogalactofuranosyl-glycerol units in the ratio of 2:1. Crossed immunoelectrophoresis has shown that the *E. coli* polysaccharide K2 exists in two forms, one being an *O*-acetylated version of the other.[112] D-Galactosyl residues, glycerol, and phosphate groups are common to the two forms.

^{13}C Two-dimensional *J* spectroscopy has been used to measure the ^{1}H–^{13}C couplings of the tetrasaccharide repeating unit of *Klebsiella* K32 polysaccharide.[113]

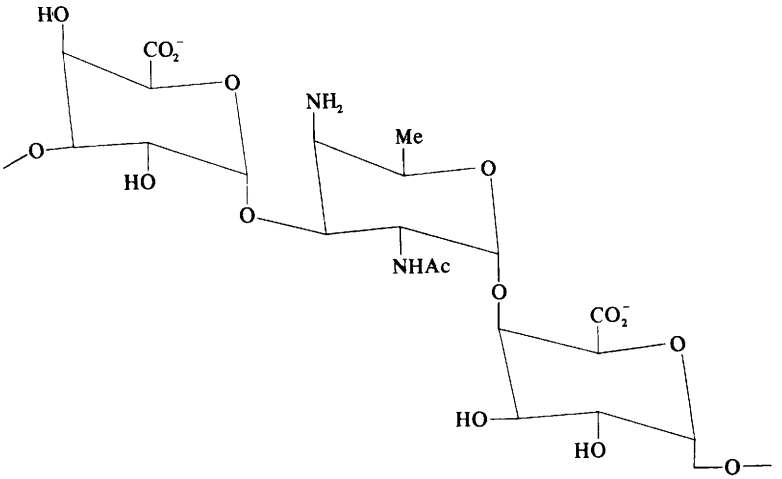
Capsule bacteriophage of *Escherichia coli*, which depolymerizes its host capsular polysaccharide, also depolymerizes the structurally similar *Klebsiella* K31 capsular polysaccharide by splitting the 3-*O*-β-D-glucosyl-D-glucopyranosyluronic acid linkage in the main chain.[114] Depolymerization of *Klebsiella* K21 and *Klebsiella* K32 by phage enzymes is a convenient method of preparing oligosaccharides that correspond to one, or several, repeating unit(s).[115] The oligosaccharides isolated from both sero-types contain acetal substituents, but whereas the 4,6-*O*-(1-carboxyethylidene)-D-galactopyranosyl residue in the K21 structure is relatively acid-stable, the corresponding 3,4-*O*-(1-carboxyethylidene)-L-rhamnopyranosyl residue in K32 is extremely acid-labile. Thus phage degradation may be the only way by which an oligosaccharide corresponding to an intact repeating unit may be obtained.

X-Ray diffraction studies on the capsular polysaccharide of *Klebsiella* K5 have been reported.[116] The occurrence of pyruvic acid acetal in *Klebsiella* capsular polysaccharides has been assessed.[117]

[111] K. Jann and M. A. Schmidt, *FEMS Microbiol. Lett.*, 1980, **7**, 79.
[112] J. C. Larsen, F. Ørskov, I. Ørskov, M. A. Schmidt, B. Jann, and K. Jann, *Med. Microbiol. Immunol.*, 1980, **168**, 191.
[113] L. D. Hall and G. A. Morris, *Carbohydr. Res.*, 1980, **82**, 175.
[114] Y. M. Choy, S. L. Wong, and C. Y. Lee, *Mol. Immunol.*, 1980, **17**, 391.
[115] G. G. S. Dutton, K. L. Mackie, A. V. Savage, D. Rieger-Hug, and S. Stirm, *Carbohydr. Res.*, 1980, **84**, 161.
[116] D. H. Isaacs, K. H. Gardner, C. Wolf-Ullish, E. D. T. Atkins, and G. G. S. Dutton, *Int. J. Biol. Macromol.*, 1979, **1**, 107.
[117] W. Nimmich, *Z. Allg. Mikrobiol.*, 1979, **19**, 343.

The capsular polysaccharide of *Streptococcus pneumoniae* type 27 (S-27), which contains residues of the pyruvic acid acetal of 2-acetamido-2-deoxy-D-glucose, D-galactose, D-glucose, and 2-*O*-phosphorylcholine-L-rhamnose, has been fragmented into oligosaccharides by controlled degradations.[118] Binding of these ligands with heterogeneous rabbit anti-(S-37) antibodies of selected phosphoryl choline affinity indicates that a major immunodominant group of S-37 is the phosphorylcholine moiety. However, the antibodies are also capable of binding to the polysaccharide from which the phosphorylcholine has been removed.

The capsular polysaccharide of *Streptococcus pneumoniae* type 1 is composed of D-galactopyranosyluronic acid and 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranosyl residues.[119] The amino-sugar, which was not isolated but was identified from products obtained on deamination of the polymer, has not been reported previously in nature. Using n.m.r. spectroscopy, methylation analysis, and Smith degradation studies, the structure (17) is assigned to the polysaccharide.

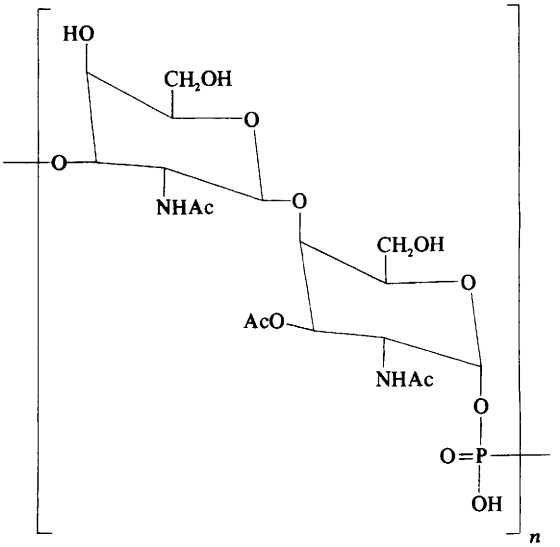


(17)

The comparative immunogenicity of group 6 pneumococcal type 6A and type 6B capsular polysaccharides has been reported.[120] The structural similarity of the pneumococcal group 6 polysaccharides confers extensive cross-reactivity with hyperimmune typing antisera.

[118] L. G. Bennett and C. P. J. Glaudemans, *J. Immunol.*, 1979, **122**, 2356.
[119] B. Lindberg, B. Lindqvist, J. Lönngren, and D. A. Powell, *Carbohydr. Res.*, 1980, **78**, 111.
[120] J. B. Robbins, C. J. Lee, S. C. Rastogi, G. Schiffman, and J. Henrichsen, *Infect. Immun.*, 1979, **26**, 1116.

Two groups of workers have independently established the structure (18) of the *Haemophilus influenzae* type f capsular polysaccharide.[121,122] This is a revised structure, since in earlier studies no *O*-acetyl group was detected and it was thought that the polysaccharide contained two non-equivalent (1→1)-linked 2-amino-2-deoxy-D-galactosyl residues, and that it possessed a (3→4)-phosphodiester linkage.



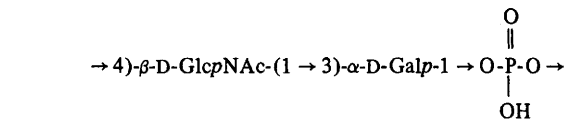
(18)

The repeating unit (19) of *Haemophilus influenzae* type c capsular polysaccharide contains about 80% of the 2-acetamido-2-deoxy-D-glucopyranosyl residues acetylated at *O*-3.[123] Competitive inhibition of antigen–antibody precipitation shows that the *O*-acetyl group confers an antigenic specificity on the type c capsular polysaccharide.

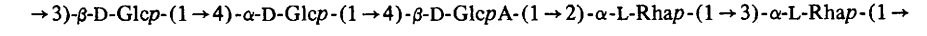
Klebsiella K44 capsular polysaccharide has a structure (20) based on a pentasaccharide repeating unit, to which must be added on average one *O*-acetyl group for every three repeating units.[124]

Serological cross-reactions of *Klebsiella pneumoniae* K47 antiserum with antigens of *Sporothrix chenckii*, *S. curviconia*, and *S. inflata* have been demonstrated.[125] The three cross-reactive *Sporothrix* species may have L-rhamnopyranosyl determinants that are related to the 1,2-linked L-rhamnopyranosyl

[121] W. Egan, F. P. Tsui, and R. Schneerson, *Carbohydr. Res.*, 1980, **79**, 271.
[122] P. Branefors-Helander, L. Kenne, and B. Lindqvist, *Carbohydr. Res.*, 1980, **79**, 308.
[123] W. Egan, F. P. Tsui, P. A. Climenson, and R. Scheerson, *Carbohydr. Res.*, 1980, **80**, 305.
[124] G. G. S. Dutton and T. E. Folkman, *Carbohydr. Res.*, 1980, **78**, 305.
[125] H. Ishizaki, Y. Kurata, Y. Nakamura, and R. W. Wheat, *Curr. Microbiol.*, 1979, **2**, 355.

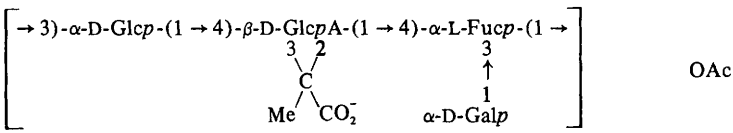


(19)



(20)

side chains of the capsular polysaccharide. *Klebsiella* K58 capsular polysaccharide consists of a tetrasaccharide repeating unit (21) with one *O*-acetyl group per repeating unit.[126] A uronic acid residue bearing a 1-carboxyethylidene group has previously been found, in this series, only in the polysaccharide from *Klebsiella* K1.



(21)

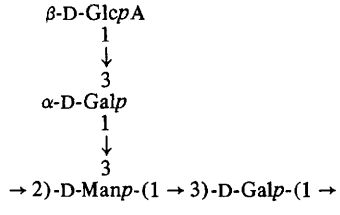
The capsular polysaccharides of group B, C, W-135, and Y meningococci have been distinguished on the basis of their pyrolysis mass spectra.[127] The presence of the free hydroxy-group at position 9 of the *N*-acetylneuraminic acid moieties in the group B polysaccharides as opposed to the glycosidically bound hydroxy-group at the same position in the group C polysaccharides appears to result in the increased formation of methanol in the pyrolyzate of the former polysaccharides. The method appears to be suitable for batchwise quality control of meningococcal polysaccharide preparations for use as vaccines as well as for screening preparations from new strains for the presence of structural differences.

The conformation of crystalline type III pneumococcal polysaccharide has been studied by *X*-ray diffraction and stereochemical analysis.[128] One of two left-hand helical models is favoured.

3-*O*-Methyl L-rhamnose has been identified as a constituent of the capsular polysaccharide of a *Rhizobium* isolated from the root nodules of *Acacia decurrens*.[129] The structures (22)–(32) of the repeating units of the following capsular polysaccharides have also been established: *Escherichia coli* K30 (22),[130]

[126] G. G. S. Dutton and A. V. Savage, *Carbohydr. Res.*, 1980, **84**, 297.
[127] J. Haverkamp, H. L. C. Meuzelaar, E. C. Beuvery, P. M. Boonekamp, and R. H. Tiesjema, *Anal. Biochem.*, 1980, **104**, 407.
[128] R. H. Marchessault, K. Imada, T. L. Bluhm, and P. R. Sundarajan, *Carbohydr. Res.*, 1980, **83**, 287.
[129] L. K. Jackson, M. E. Slodki, M. C. Cadmus, K. A. Burton, and R. D. Plattner, *Carbohydr. Res.*, 1980, **82**, 154.
[130] A. K. Chakraborty, H. Friebolin, and S. Stirm, *J. Bacteriol.*, 1980, **141**, 971.

Klebsiella K12 (23),[131] K17 (24),[132] K27 (25),[133] K46 (26),[134] K53 (27),[135] K63 (28),[136] K74 (29),[137] K60 (30),[138] *Streptococcus pneumoniae* type 19 (31) and type 56 (32),[139] and *S. pneumoniae* (S XIX) (33).[140]



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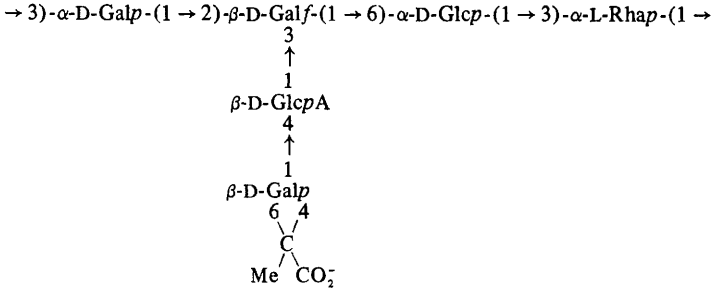
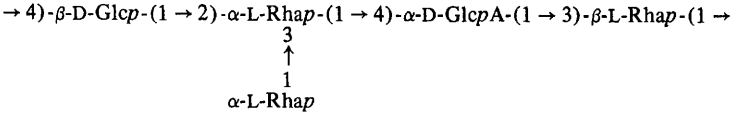


(23)



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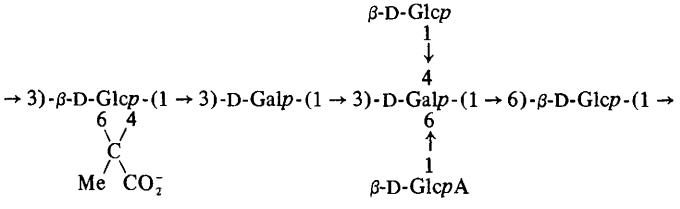
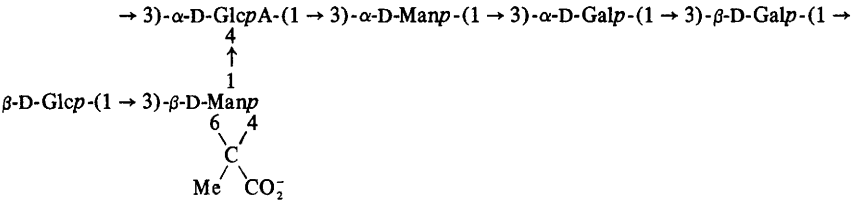


(25)

[131] G. G. S. Dutton and A. V. Savage, *Carbohydr. Res.*, 1980, **83**, 351.
[132] G. G. S. Dutton and T. E. Folkman, *Carbohydr. Res.*, 1980, **80**, 147.
[133] S. C. Churms, E. H. Merrifield, and A. M. Stephen, *Carbohydr. Res.*, 1980, **81**, 49.
[134] K. Okutani and G. G. S. Dutton, *Carbohydr. Res.*, 1980, **86**, 259.
[135] G. G. S. Dutton and M. Paulin, *Carbohydr. Res.*, 1980, **87**, 107.
[136] G. G. S. Dutton and E. H. Merrifield, *Carbohydr. Res.*, 1980, **85**, C13.
[137] G. G. S. Dutton and M. Paulin, *Carbohydr. Res.*, 1980, **87**, 119.
[138] G. G. S. Dutton and J. Di Fabio, *Carbohydr. Res.*, 1980, **87**, 129.
[139] C. J. Lee and B. A. Fraser, *J. Biol. Chem.*, 1980, **255**, 6847.
[140] N. Ohno, T. Yadomae, and T. Miyazaki, *Carbohydr. Res.*, 1980, **80**, 297.



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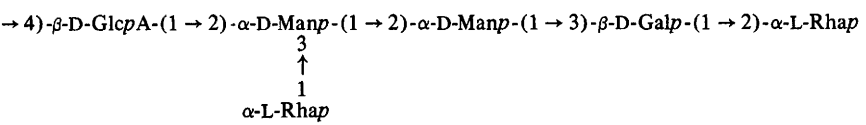

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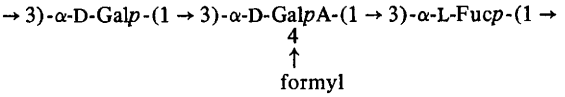

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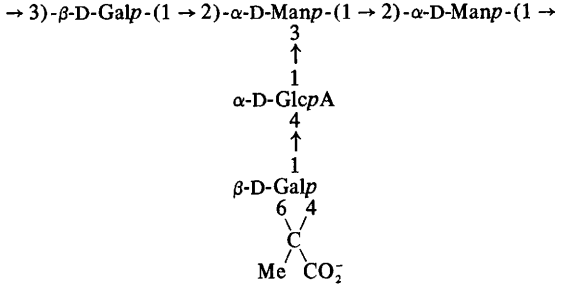
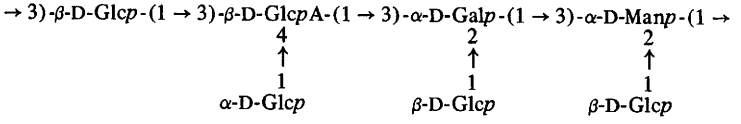
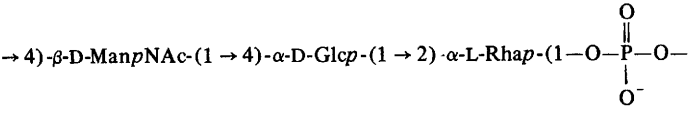


(29)



(30)



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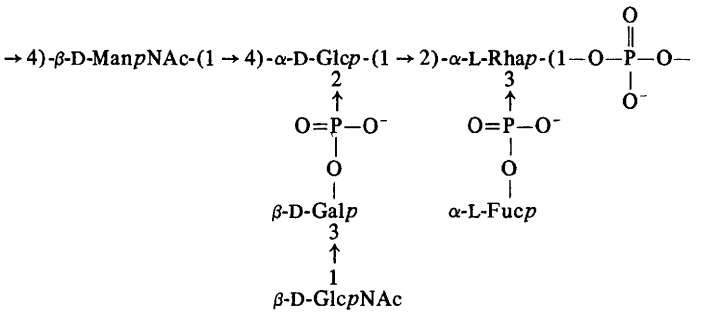

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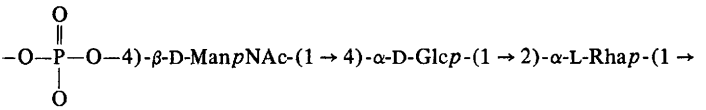


(33)

5 Intracellular and Extracellular Polysaccharides

Fractional solution, fractional precipitation, and fractionation by incompatibility with poly(ethylene glycol) are described for the preparation of dextran fractions (mol. wt. 1.0×10^4–5.0×10^5).[141] Dextrans with molecular weights less than 2.0×10^3 are almost perfect statistical coils in solution and no association occurs. A transition to rod-like conformation occurs at higher molecular weights. The results of intensity fluctuation spectroscopic studies of dextran-induced aggregation of *Streptococcus mutans* show that the aggregation is inhibited by the negatively charged surfaces of the cells, whereas dextran polymers effectively bind organisms together.[142]

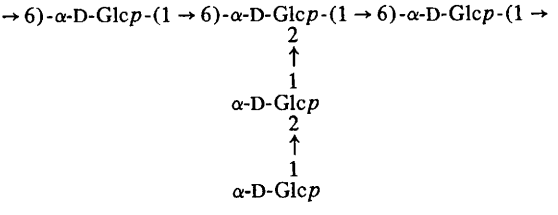
Single crystals of dextran, suitable for electron diffraction analysis, can be prepared from a native bacterial dextran as well as a synthetic dextran.[143] The crystals give well resolved electron diffraction diagrams from which the unit-cell parameters have been determined. Fourier-transform infrared difference spectra have been used in the measurement of the mol. percent of contiguously 3-mono-*O*-substituted α-D-glucopyranosyl residues in dextrans from *Leuconostoc mesenteroides* and of D-glucans from strains of *Streptococcus mutans*.[144]

High-temperature ^{13}C n.m.r. spectrometry of aqueous solutions has been used for structural determinations of dextrans from strains of *Streptococcus mutans*,[145] and the α-D-glucans from *Leuconostoc mesenteroides* and *Strepto-*

[141] A. M. Basedow and K. H. Ebert, *J. Polym. Sci., Polym. Symp. Ed.*, 1979, **66**, 101.
[142] V. Ryan, T. R. Hart, and R. Schiller, *Biophys. J.*, 1980, **31**, 113.
[143] H. Chanzy, C. Guizard, and A. Sarko, *Int. J. Biol. Macromol.*, 1980, **2**, 149.
[144] F. R. Seymour, R. L. Julian, A. Jeanes, and B. L. Lamberts, *Carbohydr. Res.*, 1980, **86**, 227.
[145] F. R. Seymour, R. D. Knapp, and B. L. Lamberts, *Carbohydr. Res.*, 1980, **84**, 187.

bacterium dextranicum have been studied using ^{13}C n.m.r. spin–lattice relaxation methods.[146] Relaxation measurements were employed to establish the relative position of *O*-substituted residues in the average repeating unit of the polysaccharides. The method has been applied to the analysis of α-D-glucans that contain 3-*O*-α-D-glucosylated α-D-glucopyranosyl residues at the branch points or in consecutive linear positions.[147]

Detailed structural features of dextran B-1355-S have been revealed by chemical investigations and enzymic degradation studies.[148] The findings confirm the overall arrangement of alternating 1,6- and 1,3-linked α-D-glucopyranosyl residues, and the absence of consecutive 1,3-linked α-D-sugar residues. The water-soluble extracellular α-D-glucan elaborated by *Streptococcus mutans* GS-5 contains 1,6- and (1,3,6)-linked α-D-glucopyranosyl residues.[149] Structural similarities were observed between this α-D-glucan and those of *Leuconostoc mesenteroides* NRRL B-152 and NRRL B-1375. The borate-soluble fraction of the dextran elaborated by *L. mesenteroides* NRRL B-1299 has been submitted to acetolysis and several oligosaccharides derived therefrom have been identified.[150] In addition to the previously identified branches of 1,2 single α-D-glucopyranosyl groups, it is now evident that the polysaccharide also contains more complex segments, *i.e.* (34) and (35).



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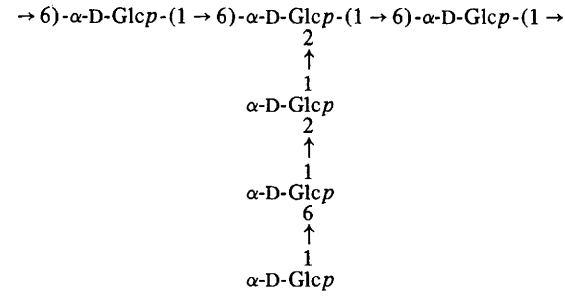


(35)

[146] F. R. Seymour and R. D. Knapp, *Carbohydr. Res.*, 1980, **81**, 67.
[147] F. R. Seymour and R. D. Knapp, *Carbohydr. Res.*, 1980, **81**, 105.
[148] A. Misaki, M. Torii, T. Sawai, and I. J. Goldstein, *Carbohydr. Res.*, 1980, **84**, 273.
[149] F. R. Higton and H. Weigel, *Carbohydr. Res.*, 1980, **86**, 105.
[150] T. Watanabe, M. Chiba, Y. Matsuba, F. Sakurai, M. Kobayashi, and K. Matsuda, *Carbohydr. Res.*, 1980, **83**, 119.

Biological, immunological, and cariogenic aspects of *Streptococcus mutans* have been reviewed.[151]

The adherence of *Streptococcus sanguis* (an organism frequently associated with infective endocarditis in humans) to damaged canine heart valves is dependent on dextrans of high molecular weight, including a commercial dextran and D-glucans from *S. sanguis* and *S. mutans*.[152] A two-reaction model of *S. mutans* adherence to smooth surfaces has been developed.[153] In one reaction, attachment of the cells to the tooth pellicle is mediated by cell-surface proteins rather than D-glucans or teichoic acids. The other reaction is cellular accumulation mediated by sucrose-derived D-glucans and cell-surface lectins.

The presence of sucrose in chemically defined synthetic medium used for growing *Streptococcus mutans* B13 determines whether the D-glucosyltransferase is produced extracellularly or is cell-associated.[154] The binding of enzyme depends on cell-surface D-glucan subsequent to D-glucan synthesis and *in vitro* adherence. Clinical dextran, partially oxidized with sodium periodate, but not similarly treated glycogen or amylose, acts as a potent inhibitor of the extracellular glucosyltransferase of several cariogenic strains of oral *S. mutans*.[155] The inhibitory effect probably involves the formation of a Schiff's base or a carbinolamine derivative between dialdehyde groups and reactive functional groups close to the dextran-binding site of the enzyme.

The interactions between cell-free D-glucosyltransferase of *Streptococcus mutans* (sero-type *d*) and water-insoluble and water-soluble α-D-glucans of different origin have been reported.[156] The effects of these interactions on *de novo* α-D-glucan synthesis and adherence to glass surfaces has been considered. D-Glucosyltransferase from *S. mutans* interacts with both lipoteichoic acid and deacylated lipoteichoic acid.[24] The interaction with the lipoteichoic acid results in inhibition of enzymic activity, whereas interaction with the deacylated lipoteichoic acid does not produce comparative inhibitory effects. Thus the fatty acid moieties of the lipoteichoic acid are not required for binding to the enzyme but are necessary for enzyme inhibition. Incubation of sucrose and isomaltose oligosaccharides containing more than six D-glucosyl residues in the presence of an α-D-glucosyltransferase of *S. mutans* produces branched saccharides by the introduction of a D-glucosyl side-chain on to the D-glucosyl residue penultimate to the reducing terminal.[157]

Biochemical aspects of the dextransucrase of *Streptococcus mutans* have been reviewed.[158] Dextran biosynthesis and dextransucrase production have been studied by continuous culture of *Leuconostoc mesenteroides*.[159] Purified

[151] S. Hamada and H. D. Slade, *Microbiol. Rev.*, 1980, **44**, 331.
[152] C. H. Ramirez-Ronda, *Infect. Immun.*, 1980, **29**, 1.
[153] R. H. Staat, S. D. Langley, and R. J. Doyle, *Infect. Immun.*, 1980, **27**, 675.
[154] S. Hamada and H. D. Slade, *Arch. Oral Biol.*, 1979, **24**, 399.
[155] M. Inoue and E. E. Smith, *Carbohydr. Res.*, 1980, **80**, 163.
[156] S. Hamada and M. Torii, *J. Gen. Microbiol.*, 1980, **116**, 51.
[157] G. W. Walker, *Carbohydr. Res.*, 1980, **82**, 404.
[158] T. J. Montville, C. L. Cooney, and A. J. Sinskey, *Adv. Appl. Microbiol.*, 1978, **24**, 55.
[159] G. R. Lawford, A. Kligerman, T. Williams, and H. G. Lawford, *Biotechnol. Bioeng.*, 1979, **21**, 1121.

dextransucrase exhibited both dextran biosynthetic activity and a β-D-fructofuranosidase(invertase)-like activity.

Further evidence has been reported for the suggestion that soluble and insoluble α-D-glucans of *Streptococcus mutans* are synthesized by interconvertible forms of the same D-glucosyltransferase.[160] The aggregated form, mutansucrase, preferentially catalyses (1 → 3)-α-bond formation but dissociates during gel filtration to the dextransucrase form which catalyses (1 → 6)-α-bond formation. The production of dextransucrase by *S. mutans* 6715 is related to the availability of lipid during growth of the organism.[161] Cerulenin, an inhibitor of fatty acid synthesis, suppresses the production of this extracellular enzyme.

Dextransucrase of *Lenconostoc mesenteroides* has been immobilized by covalent coupling to a porous amino-silica support.[162] The dextran produced by the immobilized enzyme in the presence of sucrose had a greater molecular weight than that dextran produced by the free enzyme. This is explained by diffusional limitations that prevent the acceptor molecules approaching the enzyme–dextranosyl complex, favouring the growth of dextran chains. A dextran-debranching enzyme has been isolated from *Flavobacterium* species.[163] The debranching enzyme has a strict specificity for the (1→ 2)-α-D-glucosidic linkage at branch points of dextrans and related branched oligosaccharides, and produces D-glucose as the only reducing sugar.

D-Glucans, isolated from the fish pathogen *Aeromonas hydrophila*, have been shown to be dependent on whether or not D-glucose was included in the growth medium.[164] Under conditions of low-carbohydrate growth, *A. hydrophila* produces two similar but distinguishable (1 → 4)-α-D-glucans, one substituted by single 1,6-linked α-D-glucosyl residues and the other by short chains of 1,6-linked D-glucosyl residues to give an amylopectin-type of polysaccharide.

The ADP-D-glucose pyrophosphorylase (EC 2.7.7.27) from *Salmonella typhimurium* LT-2 is very similar to the corresponding enzyme from *Escherichia coli* and correlates with the close supposed evolutionary relatedness of these two organisms.[165]

Pyridoxal phosphate has been used as a site-specific probe into the allosteric activator site of the ADP-D-glucose synthetase of *Escherichia coli*.[166]

The action patterns and other properties of 'amylomaltase' and a dextrin glycosyltransferase of *Pseudomonas stutzeri* have been shown to differ.[167] A scheme for the metabolism of starch in this micro-organism is proposed.

Several strains of *Bacillus laevolacticus* elaborate water-insoluble D-glucan and D-fructan.[168] The D-glucan can be solubilized by mild acid hydrolysis to give a product (mol. wt. 6.4×10^5) that is partially hydrolysed by dextranase. Both

[160] B. M. Newman, P. White, S. B. Mohan, and J. A. Cole, *J. Gen. Microbiol.*, 1980, **118**, 353.
[161] W. L. S. Leung, S. K. Harlander, and C. F. Schachtele, *Infect. Immun.*, 1980, **28**, 846.
[162] A. Lopez and P. Monsan, *Biochimie*, 1980, **62**, 323.
[163] Y. Mitsuishi, M. Kobayashi, and K. Matsuda, *Carbohydr. Res.*, 1980, **83**, 303.
[164] D. H. Shaw and M. J. Squires, *Arch. Mikrobiol.*, 1980, **125**, 83.
[165] M. Lehmann and J. Preiss, *J. Bacteriol.*, 1980, **143**, 120.
[166] T. F. Parsons and J. Preiss, *J. Biol. Chem.*, 1975, **253**, 6197.
[167] J. Schmidt and M. John, *Biochim. Biophys. Acta.*, 1979, **566**, 100.
[168] Y. Amemiya and O. Nakayama, *J. Gen. Appl. Microbiol.*, 1980, **26**, 159.

the soluble α-D-glucan and the D-fructan exhibit anti-tumour activity against mouse sarcoma 180.

Levans elaborated by two oral strains of *Streptococcus salivarius* have high relative molecular masses and are composed of β-D-fructofuranose residues linked through C-2, C-2, and C-6, as well as C-1, C-2, and C-6.[169] The molecular weight of levans synthesized by the levan sucrase of *Bacillus subtilis* is regulated by the ionic strength of the growth medium.[170] Conditions required for the selective synthesis of high or low molecular weight levans are described.

A β-D-fructofuranosidase from *Streptococcus salivarius* is capable of hydrolysing levan, and inulo-biose, -tetraose, -pentaose, and -hexaose, but has negligible effects on inulin, inulotriose, and inuloheptaose.[171] Enzymic degradation of the levan elaborated by *S. salivarius* followed by linkage analysis reveals that the levan possesses multiply-branched chains linked through C-1 and C-2.[172]

The extracellular polysaccharide of *Rhizobium trifolii* Bart A has been partially hydrolysed with acid and the neutral oligosaccharides therefrom have been characterized as (36)–(41), cellobiose, cellotriose, and gentiobiose.[173]

β-D-Gal*p*-(1 → 3)-D-Glc
(36)

β-D-Gal*p*-(1 → 3)-β-D-Glc*p*-(1 → 4)-D-Glc
(37)

β-D-Glc*p*-(1 → 4)-β-D-Glc*p*-(1 → 6)-D-Glc
(38)

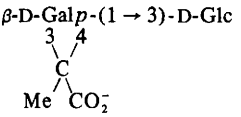
β-D-Gal*p*-(1 → 3)-β-D-Glc*p*-(1 → 4)-β-D-Glc*p*-(1 → 4)-D-Glc
(39)

β-D-Glc*p*-(1 → 4)-β-D-Glc*p*-(1 → 4)-β-D-Glc*p*-(1 → 6)-D-Glc
(40)

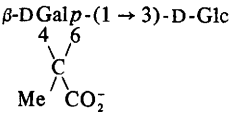
β-D-Gal*p*-(1 → 3)-β-D-Glc*p*-(1 → 4)-β-D-Glc*p*-(1 → 4)-β-D-Glc*p*-(1 → 6)-D-Glc
(41)

It is proposed that the sugar residues of the tetrasaccharide (39) form the side-chains of the polysaccharide. After partial acid hydrolysis of the extracellular acidic polysaccharide from *R. trifolii* Bart A, 3,5-*O*-(1-carboxyethylidene)-D-galactose, 4,6-*O*-(1-carboxyethylidene)-D-galactose, and oligosaccharides (42)–(45) were identified.[174]

[169] K. Marshall and H. Weigel, *Carbohydr. Res.*, 1980, **80**, 375.
[170] T. Tanaka, S. Oi, and T. Yamamoto, *J. Biochem., (Jpn.)*, 1980, **87**, 297.
[171] K. Marshall and H. Weigel, *Carbohydr. Res.*, 1980, **83**, 315.
[172] K. Marshall and H. Weigel, *Carbohydr. Res.*, 1980, **83**, 321.
[173] R. Sømme, *Carbohydr. Res.*, 1980, **85**, 156.
[174] R. Sømme, *Carbohydr. Res.*, 1980, **80**, 325.



(42)



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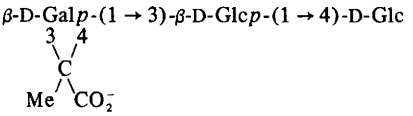

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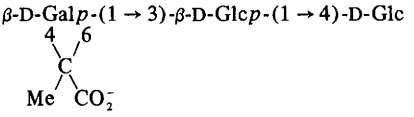


(45)

Three fast-growing species of *Rhizobium* have been found to secrete low molecular weight (1→2)-linked β-D-glucans when cultured in synthetic medium.[175] These β-D-glucans are indistinguishable from the (1→2)-β-D-glucan produced by *Agrobacterium tumefaciens*. No reducing terminus has been detected in these β-D-glucans. Two extracellular β-D-glucans (mol. wt. 4.5×10^3 and 1.2×10^4) with different proportions of 1,3- and 1,6-linked β-D-glucosyl residues have been isolated from *R. japonicum*.[176]

The results of differential thermal analysis of an extracellular linear (1→4)-linked β-D-glucan from *Acetobacter xylinum* having single D-glucosyl residues at C-2 of every third D-glucosyl residue of the main chain are consistent with this structure.[177] ^{1}H N.m.r. spectroscopy results confirm that all the glycosidic linkages have the β-D-configuration. No evidence has been obtained to support earlier suggestions that this branched β-D-glucan is a precursor of bacterial cellulose.

A sample of cellulose has been synthesized by *Acetobacter xylinum* from equal amounts of D-glucose and D-[^{13}-C]-glucose.[178] During the biosynthesis of

[175] W. S. York, M. McNeil, A. G. Darvill, and P. Albersheim, *J. Bacteriol.*, 1980, **142**, 243.
[176] W. F. Dudman and A. J. Jones, *Carbohydr. Res.*, 1980, **84**, 358.
[177] J. R. Colvin, L. C. Sowden, V. Daoust, and M. Perry, *Can. J. Biochem.*, 1979, **57**, 1284.
[178] D. V. Gagnaire and F. R. Taravel, *Eur. J. Biochem.*, 1980, **103**, 133.

cellulose, 23% of the D-glucosyl residues come from an unmodified molecule of glucose, 26% have been involved at least once in the pentose cycle, and 10% at least twice, and 30% of the D-glucosyl residues come from triose recombination.

A re-examination of the extracellular β-D-glucan from *Agrobacterium tumefaciens* has revealed the presence of a linear chain of 1,2-linked β-D-glucosyl residues with limited degree of branching at O-6 and O-3 of β-D-glucosyl residues.[179] The (1→2)-β-D-glucan of *A. radiobacter* contains a small proportion of 1,4-linked and 4,6-di-*O*-substituted β-D-glucosyl residues. In addition the non-reducing terminal residues are substituted with pyruvic acid acetal on the 4- and 6-positions.

The effects of applied hydrostatic pressure on the gel structures of the bacterial (1→3)-β-D-glucan, curdlan, have been reported.[180] The elasticity decreases initially with increasing pressure attributed to a decrease of free water as solvent, but then increases as the pressure is increased due to the promotion of hydrogen bonding by compression.

Six strains of slow-growing rhizobia contain *O*-methyl sugars in their extracellular polysaccharides.[181] Their identification as 3-*O*-methyl-L-fucose, 3-*O*-methyl-L-rhamnose, and 2-*O*-methyl-L-rhamnose is described.

The binding of fluorescent-labelled lectins to various *Rhizobium* isolates has been reported.[182] Observed variations in the binding of different lectins to *Rhizobium* species is determined not only by the sugar specificity of the lectin but also by its physical characteristics. A method based on haemagglutination inhibition for the assay of polysaccharides, which bind soya bean lectin during the growth of *Rhizobium japonicum*, has been developed.[183] Lectin-binding polysaccharide is detected in preparations during both exponential and stationary growth phases. Although culture conditions affect the yield of total polysaccharide produced, the formation of the lectin-binding polysaccharide is not affected.

The role of lectins and their saccharide receptors as determinants of the specificity in the *Rhizobium*–legume symbiosis has been reviewed.[184] Mutants of *R. leguminosarum*, which are deficient in normal polysaccharide production, have been isolated and characterized.[185] A correlation between diminished production of extracellular polysaccharide and reduced infection and nodulation efficiency on pea plants was observed.

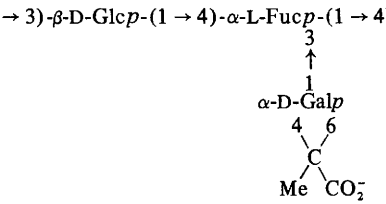
Cellulase causes a random breakdown of the main chain of the xanthan from *Xanthomonas campestris* when the polysaccharide is in the unordered conforma-

[179] E. Barreto-Bergter, C. R. Camargo, L. R. Hogge, and P. A. J. Gorin, *Carbohydr. Res.*, 1980, **82**, 366.
[180] S. Fujii, K. Miyagawa, and T. Watanabe, *Carbohydr. Res.*, 1980, **84**, 265.
[181] L. D. Kennedy, *Carbohydr. Res.*, 1980, **87**, 156.
[182] P. P. Wong, *Plant Physiol.*, 1980, **65**, 1049.
[183] H. C. Tsien and E. L. Schmidt, *Appl. Environ. Microbiol.*, 1980, **39**, 1100.
[184] F. B. Dazzo, in 'Cell surface, mediator of developmental processes', ed. S. Subtelny and N. K. Wessells, Acad. Press, NY, 1980, p. 227.
[185] C. Napoli and P. Albersheim, *J. Bacteriol.*, 1980, **141**, 1454.

tion.[186] It is shown that there is no hydrolysis on the ordered helical conformation of the polysaccharide. Quasi-elastic light-scattering studies of aqueous solutions of xanthan reveal the existence of two different structural forms characterized by different values of the translational diffusion coefficient D_t^0.[187] Xanthan (mol. wt. 2.16×10^6) undergoes a self-association in aqueous solution, an effect which is inhibited in 4 M urea solution. Convenient procedures have been developed for using rate equations to describe the time variation of biomass, product, and substrate concentrations in xanthan fermentation.[188] A novel characterization of the time evolution of the shear viscosity behaviour for xanthan and pullulan fermentation has been reported.[189]

An assignment of absolute configuration of the acetal carbon atom of pyruvic acid acetals in bacterial polysaccharides has been made by comparing signals in the ^{13}C and ^1H n.m.r. spectra of the polysaccharides with those of model compounds.[190] The *S*-configuration was demonstrated in polysaccharides in which the pyruvic acid is linked to O-4 and O-6 of D-glucopyranosyl or D-mannopyranosyl residues. The *R*-configuration was demonstrated in those polysaccharides in which it is linked to O-4 and O-6 of D-galactopyranosyl residues. The *S*-form was also demonstrated in polysaccharides in which the pyruvic acid is linked to O-3 and O-4 of D-galactopyranosyl residues. A general kinetic model for microbial fermentations that produce exopolysaccharides has been established.[191] Using published data for exopolysaccharide production from *Azotobacter vinelandii*, *Pseudomonas* sp., and *Aureobasidium pullulans*, the model provides a reasonable description for biomass and polysaccharide production.

The extracellular polysaccharide from *Corynebacterium insidiosum* is composed of tetrasaccharide repeating units having the structure (46).[192]



(46)

[186] M. Rinaudo and M. Milas, *Int. J. Biol. Macromol.*, 1980, **2**, 45.
[187] J. G. Southwick, H. Lee, A. M. Jamieson, and J. Blackwell, *Carbohydr. Res.*, 1980, **84**, 287.
[188] R. M. Weiss and D. F. Ollis, *Biotechnol. Bioeng.*, 1980, **22**, 859.
[189] N. Thomson and D. F. Ollis, *Biotechnol. Bioeng.*, 1980, **22**, 875.
[190] P. J. Garegg, P. E. Jansson, B. Lindberg, F. Lindh, J. Lönngren, I. Kvarnström, and W. Nimmich, *Carbohydr. Res.*, 1980, **78**, 127.
[191] J. Klimek and D. F. Ollis, *Biotechnol. Bioeng.*, 1980, **22**, 2321.
[192] P. A. J. Gorin, J. F. T. Spencer, B. Lindberg, and F. Lindh, *Carbohydr. Res.*, 1980, **79**, 313.

A bacterium, tentatively identified as *Enterobacter cloaceae*, when induced by alkyldimethylbenzyl ammonium chloride, produced an extracellular acid polysaccharide containing residues of D-galactose, D-glucose, D-glucuronic acid, L-fucose, pyruvate, and acetate.[193] The physical and chemical properties of this polysaccharide resemble those of colanic acid produced by *Escherichia coli* K235.

The fine structure of the extracellular polysaccharide of virulent and avirulent strains of *Erwinia amylovora* have been shown to be respectively encapsulated and unencapsulated.[194] In certain defined culture media, *E. amylovora* produces the same polysaccharide as that found in the ooze produced during fireblight infection of plant tissue, and it is highly likely that the ooze polysaccharide is of bacterial origin.[195] Two distinct polysaccharides are produced by *E. amylovora in vitro*, one a D-fructan and the other a heteropolysaccharide, containing residues of D-galactose, D-glucose, D-mannose, and uronic acid.[196]

Mucoid variants of *Pseudomonas aeruginosa* have been classified into two phenotypic groups based on whether or not alginic acid is produced on a chemically defined medium.[197] The control of alginate production in the bacterium appears to involve at least one chromosomal locus. An assay has been developed to measure the rate of exopolysaccharide formation by non-proliferating suspensions of *Pseudomonas* NCIB 11264 cells.[198] The specific activities of certain enzymes involved in the formation of the sugar nucleotide precursors of polysaccharide biosynthesis have also been measured in steady-state populations.

6 Miscellaneous Polysaccharides

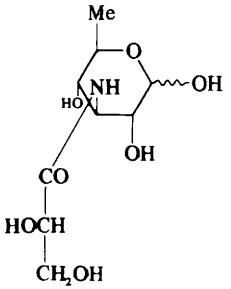
Membranous sialyltransferase complexes from *Escherichia coli* K-235 catalyse the synthesis of surface polymers of 2,8-α-ketosidically linked poly(*N*-acetyl-neuraminic acid), having an average degree of polymerization of 200 residues.[199] *In vitro* pulse-chase experiments confirm that the polymer elongation occurs by the addition of *N*-acetyl-neuraminosyl residues to the non-reducing termini of growing nascent chains. Solvent effects on the c.d. of colominic acid have been reported.[200]

Chemical and biological aspects of the enterobacterial common antigen have been reviewed.[201] In rabbits immunized with intact *Escherichia coli* cells, the immune response to the enterobacterial common antigen predominantly consists of the production of IgM antibodies.[202] The purified IgM, after conjugation to ferritin, has been used to locate the antigen on the surface of the bacteria. The

[193] K. Nishikawa, S. Oi, and T. Yamamoto, *Agric. Biol. Chem., (Jpn.)*, 1979, **43**, 2305.
[194] D. J. Politis and R. N. Goodman, *Appl. Environ. Microbiol.*, 1980, **40**, 596.
[195] R. A. Bennett and E. Billing, *J. Gen. Microbiol.*, 1980, **116**, 341.
[196] R. A. Bennett, *J. Gen. Microbiol.*, 1980, **116**, 351.
[197] J. A. M. Fyfe and J. R. W. Govan, *J. Gen. Microbiol.*, 1980, **119**, 443.
[198] A. G. Williams and J. W. T. Wimpenny, *J. Gen. Microbiol.*, 1980, **116**, 133.
[199] T. E. Rohr and F. A. Troy, *J. Biol. Chem.*, 1980, **255**, 2332.
[200] K. Gekko, *Agric. Biol. Chem., (Jpn.)*, 1980, **44**, 1183.
[201] H. Mayer and G. Schmidt, *Curr. Top. Microbiol. Immunol.*, 1979, **85**, 99.
[202] J. Rinno, J. R. Golecki, and H. Mayer, *J. Bacteriol.*, 1980, **141**, 814.

antigen is localized in the outer membrane of the cell envelope of *Proteus mirabilis* wild-type strains and their various derived L-forms.[203] In strains where the outer membrane is lacking (stable protoplast L-forms) or where its amount is reduced, no antigen or only limited levels of antigen could be detected.

3,6-Dideoxy-3-(L-glycerylamido)-D-glucose (47) has been identified as a sugar component of the antigenic polysaccharide of *Eubacterium saburreum* strain V5.[204]



(47)

The receptor for the adsorption of PL-1 phage to *Lactobacillus casei* ATCC 27092 cells is thought to be a polysaccharide containing residues of L-rhamnose, D-glucose, and 2-acetamido-2-deoxy-D-galactose.[205] The most effective inhibitor among lectins tested for phage adsorption is *Streptomyces* haemagglutinin, which is specific for L-rhamnosyl residues.

The (1→4)-linked polymer of 3-*O*-methyl-α-D-mannosyl residues regulates the fatty acid synthetase from *Mycobacterium smegmatis* by forming stoicheiometric complexes with the long-chain acyl-CoA synthetase products.[206] ^{13}C N.m.r. studies have supported earlier claims that the polysaccharide, a random coil in its free form, undergoes a major conformational transition upon enclosing long-chain acyl-CoA. The polysaccharide, probably in helical conformation in the complexed form, interacts with both the hydrocarbon chain and the fatty acid moieties of the included fatty acyl thioester.

3-*O*-Methyl-D-mannan, elaborated by *Mycobacterium smegmatis*, is postulated to be involved in the regulation of fatty acid synthesis in mycobacteria because of a stimulation of acetate incorporation and altered fatty acid product distribution.[207] However, in a mutant of *M. smegmatis*, which is capable of producing only 7% of the normal level of the methylated polysaccharide, fatty acid synthesis is not diminished. The mycobacterial polymethyl-polysaccharides, 3-*O*-methyl D-mannose polysaccharide and methyl D-glucose polysaccharide, which form stable complexes with palmityl-coenzyme A, are potent inhibitors

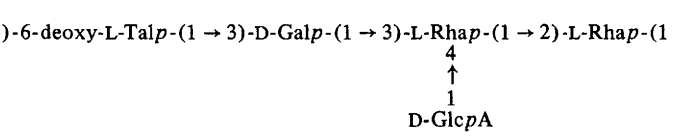
[203] J. Rinno, J. Gmeiner, J. R. Golecki, and H. Mayer, *J. Bacteriol.*, 1980, **141**, 823.
[204] W. Kondo, F. Nakazawa, and T. Ito, *Carbohydr. Res.*, 1980, **83**, 129.
[205] K. Ishibashi, S. Takesue, and K. Watanabe, *Agric. Biol. Chem., (Jpn.)*, 1980, **44**, 1689.
[206] J. E. Maggio, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 2582.
[207] D. H. Maloney and C. E. Ballou, *J. Bacteriol.*, 1980, **141**, 1217.

of the thioesterase-catalysed hydrolysis of palmityl-coenzyme A.[208] The polysaccharides have no effect on the initial rate of hydrolysis of lauryl-coenzyme A, a compound forming very weak complexes with the polysaccharides.

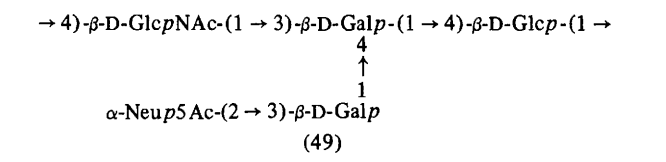
The group G antigen of lactobacilli has been identified as a cell-wall polysaccharide that does not have the properties of the teichoic acids or lipoteichoic acid group antigens of lactobacilli.[209] The components of the antigen prepared from *Lactobacilli salivarius* are D-glucose, D-galactose, L-rhamnose, 2-acetamido-2-deoxy-D-glucose, and phosphate, with L-rhamnose being the immunodominant sugar in serological studies.

The monosaccharide sequence (48) of an immunogenic tetraheteroglycan from the cell walls of *Streptococcus bovis* have been established by a combination of alkaline β-elimination, oxidation with ruthenium tetraoxide, and methylation analysis.[210] This is a revision of an earlier proposal involving a reversal of the D-galactopyranosyl residue and the 3-substituted L-rhamnopyranosyl residue.

The native polysaccharide antigen isolated from type 1a group B *Streptococcus* contains residues of D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, and neuraminic acid (molar ratio 2 : 1 : 1 : 1).[211] The structure of the antigen can be represented by the repeating unit (49) in which all the side-chain β-D-galactopyranosyl residues are masked by neuraminic acid residues. One basic



(48)



(49)

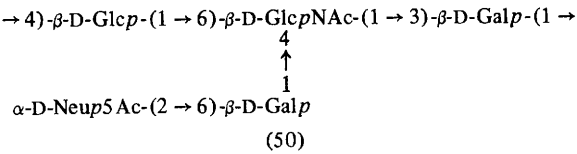
difference between the type 1a and type III native antigens is that the neuraminic acid residues of the former are linked to O-3 and the latter to O-6 of their respective side-chain β-D-galactopyranosyl residues. A sero-type-specific polysaccharide of *Streptococcus mutans* AHT (serotype *a*) composed of residues of D-galactose and D-glucose is loosely associated with the cell surface of the organism.[212] The antigen, which was formerly considered to be primarily a cell-wall constituent, also exists as part of a dense microcapsule. On examination for the presence of antigens cross-reacting with antigens A and B from *S. mutans*

[208] K. K. Yabusaki and C. E. Ballou, *J. Biol. Chem.*, 1979, **254**, 12 314.
[209] K. W. Knox, L. K. Campbell, J. D. Evans, and A. J. Wicken, *J. Gen. Microbiol.*, 1980, **119**, 203.
[210] J. H. Pazur and L. S. Forsberg, *Carbohydr. Res.*, 1980, **83**, 406.
[211] H. J. Jennings, K. G. Rosell, and D. L. Kasper, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 2931.
[212] R. A. Craig, D. H. Riege, and A. S. Bleiweis, *Infect. Immun.*, 1979, **26**, 1177.

sero-type *c*, antigens cross-reacting with A have been found only in *S. mutans* sero-type *b*, whereas antigens cross-reacting with B have been detected in sero-types *a*, *c*, *d*, *e*, and *f*.[213] An L-rhamno-D-glucan cell wall antigen has been isolated from *S. mutans* B13 (sero-type *d*).[214]

Using the direct immunoferritin technique, the type-specific polysaccharides of group B streptococci (*Streptococcus agalactial*) have been shown to be located exclusively on the outer side of the cell wall on which they form a capsule.[215] The group-specific polysaccharide has been found to be located on the inner surface in all strains tested.

Two soluble polysaccharide antigens of type III group B *Streptococcus* have been isolated from the culture medium after growth of the strain M732.[216] One of the antigens (the type III polysaccharide) contains residues of D-glucose, D-galactose, 2-amino-2-deoxy-D-glucose, and neuraminic acid as the immunodominant sugars. The group B polysaccharide contains residues of D-galactose, 2-amino-2-deoxy-D-glucose, and L-rhamnose as the immunodominant sugar. This polysaccharide appears to be both chemically and immunologically distinct from the sero-type *d* antigen. The native polysaccharide antigen having the structure (50) has been isolated from type III-group B *Streptococcus*.[217] By cleavage of all the labile neuraminic acid end-groups the incomplete type III-polysaccharide, which is structurally identical to the *S. pneumoniae* type 14 polysaccharide, is obtained.



(50)

A polysaccharide antigen containing residues of L-rhamnose and D-glucose together with protein and phosphorus has been isolated from *S. mutans* 6715-T_2 (sero-type *g*).[218] Immunological characterization with respect to its relationship to sero-type *g* and *d* polysaccharide antigens is reported.

7 Fungal Polysaccharides

In a review on the dimorphism of pathogenic fungi, changes in cell-wall polysaccharide composition during morphogenesis have been discussed.[219] The regeneration of cell walls of *Candida* species protoplasts have been studied by electron microscopy.[220] Changes in the levels of D-glucan and D-mannan were

[213] R. R. B. Russell, *J. Gen. Microbiol.*, 1980, **118**, 383.
[214] A. Prakobphol, R. Linzer, and R. J. Genco, *Infect. Immun.*, 1980, **27**, 150.
[215] M. Wagner, B. Wagner, and V. Kubin, *J. Gen. Microbiol.*, 1980, **120**, 369.
[216] R. B. Carey, T. K. Eisenstein, G. D. Shockman, T. F. Greber, and R. M. Swenson, *Infect. Immun.*, 1980, **28**, 195.
[217] H. J. Jennings, K. G. Rosell, and D. L. Kasper, *Can. J. Biochem.*, 1980, **58**, 112.
[218] A. Prakobhol and R. Linzer, *Infect. Immun.*, 1980, **30**, 140.
[219] J. W. Rippon, *C.R.C. Crit. Rev. Microbiol.*, 1980, **8**, 49.
[220] B. Popov, G. Reuter, and H. W. Meyer, *Z. Allg. Mikrobiol.*, 1980, **20**, 47.

monitored with time. Protoplasts have been obtained from the yeast and mycelial forms of various strains of *Candida albicans*, and are capable of regenerating the cell wall.[221] The resistance of *C. albicans* to amphotericin B methyl ester increases as the cultures enter the stationary phase of growth.[222] This resistance is decreased by treatment with cell-wall degrading enzymes such as chitinase, α-D-mannosidase, and (1→3)- and (1→6)-β-D-glucanases, singly or in mixtures.

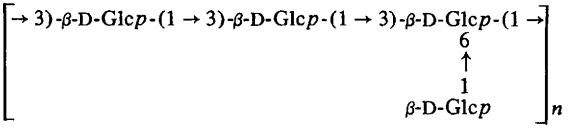
The cell wall of *Cryptostroma corticale* contains chitin, present as microfibrils embedded within a matrix of D-glucan.[223] The effects of several antibiotics on the cell-wall morphology have been recorded.

A distinction between the chemical composition of ultrastructurally modified regions and the rest of the cell wall ('canals') of *Schwanniomyces occidentalis* is indicated by cytochemical staining of the cell-wall polysaccharides.[224] The formation of 'canals' is introduced by cultivation of the yeasts on hydrocarbons and is parallelled by the enhancement of β-D-glucosidase, β-D-glucanase, and α-D-mannosidase activities, which are assumed to modify definite cell-wall regions, transforming them into 'canals'.

D-Glucans. – The ^{13}C n.m.r. spectrum of nigeran, a regular copolysaccharide having alternating 1,3- and 1,4-linked α-D-glucopyranosyl residues, shows a difference in intensities of two carbon atoms linked to the same number of protons and having the same environment.[225] This difference may come from either the T_1 relaxation time difference and, because of a main dipole–dipole relaxation phenomenon, from a correlation time of segmental-motion difference, or from differences in nuclear Overhauser enhancement. An alkali-insoluble fraction from the cell walls of *Coprinus macrorhizus* var. *microsporus* is a highly branched D-glucan containing α-1,3-, β-1,3-, and β-1,6-linkages, as shown by methylation analysis, partial acid hydrolysis, and enzymic hydrolysis.[226] Most of the α-1,4-linked segments are attached though an occasional β-(1→3)-glycosidic bond.

The sclerotia of *Grifora umbellata* contain an alkali-soluble homogeneous β-D-glucan (mol. wt. 1.2×10^6).[227] Methylation analysis, Smith degradation, and enzymic hydrolysis indicate that the polysaccharide is composed of a backbone of 1,3-linked β-D-glucopyranosyl residues joined through O-6 of every third D-glucopyranosyl residue of the backbone (51). This structure is very similar to that observed for sclerotan and schizophyllan, although both the latter polysaccharides have a much smaller molecular weight. A crystalline D-glucotetraose,

[221] L. J. Torres-Bauza and W. S. Riggsby, *J. Gen. Microbiol.*, 1980, **119**, 341.
[222] E. F. Gale, J. Ingram, D. Kerridge, V. Notario, and F. Wayman, *J. Gen. Microbiol.*, 1980, **117**, 383.
[223] S. D. Abbey and R. J. Stratton, *Microbios*, 1980, **27**, 41.
[224] V. Dmitriev, A. Tsiomenko, I. Kulaev, and B. Fikhte, *Eur. J. Appl. Microbiol.*, 1980, **9**, 211.
[225] T. F. Bobbitt, J. H. Nordin, D. Gagnaire, and M. Vincendon, *Carbohydr. Res.*, 1980, **81**, 177.
[226] C. B. Bottom and D. J. Siehr, *Can. J. Biochem.*, 1980, **58**, 147.
[227] Y. Ueno, M. Abe, R. Yamauchi, and K. Kato, *Carbohydr. Res.*, 1980, **87**, 257.



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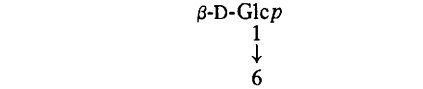


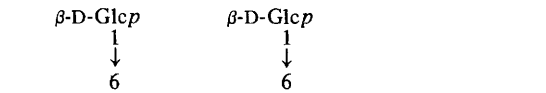
(52)

6^2-β-D-glucopyranosyl-laminaritriose (52) has been obtained by acid hydrolysis of the sclerotia of *Sclerotinia sclerotiorum*.[228]

The (1 → 3)-β-D-glucan synthetase from *Saccharomyces cerevisiae* is located on the plasma membrane.[229] No evidence is recorded for the involvement of lipid-linked intermediates. The sugar donor is UDP-D-glucose and the reaction requires the addition of glycerol, bovine serum albumin, and ATP or GTP for maximal activity. The activity of the D-glucan synthetase can be reversibly modified by a number of conditions.[230] In a working hypothesis, it is envisaged that GTP stimulation occurs simply by binding to a site in the membrane.

ATP in an enzymically catalysed reaction can convert a loosely bound phosphorylated factor into a product tightly attached to the enzyme and endowed with stimulatory properties similar to those of GTP. In the presence of Mg^{2+}, an endogenous hydrolytic enzyme converts the ATP product back into the ATP substrate, thus allowing for reversible activation–inactivation of D-glucan synthetase. This mechanism may be involved in regulation of wall synthesis during the yeast cycle. The kinetics of β-D-glucan and chitin formation by cells and protoplasts of *Saccharomyces cerevisiae* have been studied.[231] The composition of the cell walls newly formed on the surface of the protoplasts differs from that of the normal cell walls.

A water-insoluble β-D-glucan isolated from *Omphalia lepidescence* possesses a highly branched structure (53) consisting of 1,3- and 1,6-linked β-D-glucopyranosyl residues.[232]



(53)

[228] Y. Ueno and M. Abe, *Carbohydr. Res.*, 1980, **85**, 151.
[229] E. M. Shematek, J. A. Braatz, and E. Cabib, *J. Biol. Chem.*, 1980, **255**, 888.
[230] E. M. Shematek and E. Cabib, *J. Biol. Chem.*, 1980, **255**, 895.
[231] V. Farkas and A. Svoboda, *Curr. Microbiol.*, 1980, **4**, 99.
[232] T. Miyayaki and M. Nishijima, *Chem. Pharm. Bull. (Jpn.)*, 1980, **28**, 3118.

The major (1→3)-linked β-D-glucan produced by the laboratory cultivation of *Poria cocos* is almost identical to the pachyman isolated from natural sources.[233]

In the polymorphic fungus *Aureobasidium pullulans*, the commencement of pullulan excretion, which has been associated with blastospore production in the early phases of the growth cycle, probably arises through some stimulation concurrent with, though not necessarily identical to, the initiation of spore formation.[234]

Amylolysis of the glycogen from *Neurospora crassa* cells cultured in the presence of [^{32}P]-phosphate results in the release of D-glucose 6-[^{32}P]phosphate.[235]

Pullulan has been degraded with pullulanase and β-amylase to produce 6^3-α-D-glucosyl maltotriose.[236]

The higher the degree of crosslinking of glycogen, the lower is its affinity for glycogen synthetase.[237] The stability of the complex formed is maximal at neutral pH, and an enhanced stability of the complex occurs in the presence of D-glucose 6-phosphate, UDP-D-glucose, and UDP.

Mycolaminarans, soluble (1→3)-β-D-glucans, are the storage polysaccharides in mycelium, sporangia, zoospores, cysts, and chlamydospores of species of *Phytophthora*.[238] In the mycelium of six species, neutral mycolaminaran is the sole reserve polysaccharide, whereas in the sporangia, zoospores, and cysts the major reserve polysaccharide is mycolaminaran phosphate.

Aqueous solutions of schizophyllan, an extracellular (1→3)-β-D-glucan elaborated by *Schizophyllum commune*, exist in a triple helical structure similar to that proposed for a crystalline (1→3)-β-D-xylan.[239] In aqueous dimethyl sulphoxide solution, the helix rapidly and irreversibly rearranges to single linear chains at a critical dimethyl sulphoxide concentration.

A (1→3)-linked β-D-glucan and four glycoproteins have been identified from the extracellular material elaborated by *Sclerotium rolfsii*.[240] An extracellular β-D-glucan has been isolated from the viscous culture filtrate of *Sclerotinia libertiana*.[241] The polysaccharide is composed of a backbone of 1,3-linked β-D-glucopyranosyl residues with single β-D-glucopyranosyl residues attached through O-6 of every third D-glucopyranosyl residue of the backbone. The molecular weight of this polysaccharide is larger than the structurally identical β-D-glucan sclerotan, which is isolated from the sclerotia of the fungus.

D-Mannans. – D-Mannan of *Saccharomyces cerevisiae* and *Candida albicans* has been chemically and enzymically removed from the cell walls.[242] The resulting changes in the cell wall have been followed by scanning electron microscopy.

[233] T. Narui, K. Takahashi, M. Kobayashi, and S. Shibata, *Carbohydr. Res.*, 1980, **87**, 161.
[234] B. J. Catley, *J. Gen. Microbiol.*, 1980, **120**, 265.
[235] J. D. Fontana, *FEBS Lett.*, 1980, **109**, 85.
[236] B. V. McCleary, *Carbohydr. Res.*, 1980, **85**, 160.
[237] J. Zemek, S. Bauer, and L. Kuniak, *Biopolymers*, 1979, **18**, 2135.
[238] M. C. Wang and S. Bartnicki-Garcia, *Exper. Mycol.*, 1980, **4**, 269.
[239] T. Norisuye, T. Yanaki, and H. Fujita, *J. Polym. Sci., Polym. Phys. Ed.*, 1980, **18**, 547.
[240] G. Kritzman, I. Chet, and Y. Henis, *Can. J. Bot.*, 1979, **57**, 1855.
[241] Y. Ueno, Y. Hachisuka, H. Esaki, R. Yamauchi, and K. Kato, *Agric. Biol. Chem., (Jpn.)*, 1980, **44**, 353.
[242] Y. Koch and K. H. Rademacher, *Can. J. Microbiol.*, 1980, **26**, 965.

The results have been used to construct a model for the yeast cell-wall structure. Purified cell-wall D-mannan of type A *C. albicans* has been conjugated with tyramine and labelled with ^{125}I for use in a radioimmunoassay procedure for the measurement of serum anti-D-mannan levels.[243]

D-Mannan from *C. albicans* has been measured by immunodiffusion, counter-immunoelectrophoresis, and enzyme-linked immunoassay.[244] Anti-D-mannan immunoglobulin G levels of a normal population and of diseased patients have been measured by these methods.[245]

The fruit bodies of *Dictyophora indusiata* contain an α-D-mannan.[246] Structural studies indicate the presence of an essentially linear chain of 1,3-linked α-D-mannopyranosyl residues. The existence of a homoglycan of this structure has not been previously reported, although more complex heteropolysaccharides containing a backbone of 1,3-α-D-mannopyranosyl residues have been recognized in fungi.

Wild-type *Kluyveromyces lactis* cells bind three times more fluorescent-labelled wheat-germ agglutinin than do mutants whose D-mannoprotein lacks terminal 2-acetamido-2-deoxy-D-glucopyranosyl groups.[247] This observation has been used to develop a new procedure in which D-mannoprotein mutants have been isolated by separating mutagenized cells labelled with the fluorescent-labelled lectin using a fluorescent-activated cell sorter.

The structure of a highly branched D-mannan from rose wine is essentially identical to that of the D-mannan from *Saccharomyces uvarum*, which is used in the preparation of the wine, and is probably released from autolysed yeast cells during fermentation.[248] The antigenicity of yeast D-mannan preparations is significantly less than that of intact yeast cells against antiserum to intact yeast cells.[249] Although D-mannan has so far been regarded as the only antigen of the yeast cell-surface, other cell-surface antigens may exist.

The immediate precursor in the synthesis of the heterogeneous core region (D-Man*p*)$_{12-17}$(D-Glc*p*NAc)$_2$ of yeast mannoproteins is a D-glucopyranosyl-oligosaccharyl lipid with the composition (D-Glc*p*)$_3$(D-Man*p*)$_9$(D-Glc*p*NAc)$_2$.[250] This accounts for only part of what has been defined as inner core, which is built on the lipid carrier. After transfer to protein, the oligosaccharide is modified by removal of the D-glucosyl residues, followed by further elongation from GDP-D-mannose to give the size of the oligosaccharide chains found in native glycoproteins.

The *mmn*2 mutant of *Saccharomyces cerevisiae* has been employed as a parent strain in order to select double mutants that are altered more deeply in the

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[244] P. F. Lehmann and E. Reiss, *Mycopathologia*, 1980, **70**, 83.
[245] B. F. Lehmann and E. Reiss, *Mycopathologia*, 1980, **70**, 89.
[246] S. Ukai, C. Hara, T. Kiho, and K. Hirose, *Chem. Pharm. Bull. (Jpn.)*, 1980, **28**, 2647.
[247] R. H. Douglas and C. E. Ballou, *J. Biol. Chem.*, 1980, **255**, 5979.
[248] J. C. Villettaz, R. Amado, H. Neukom, M. Horisberger, and I. Horman, *Carbohydr. Res.*, 1980, **81**, 341.
[249] Y. Tamai, T. Nakashima, M. Takakuwa, and A. Misaki, *Agric. Biol. Chem., (Jpn.)*, 1980, **44**, 49.
[250] L. Lehle, *Eur. J. Biochem.*, 1980, **109**, 589.

polysaccharide component of the D-mannoprotein.[251] A new class of D-mannoprotein has been isolated that appears to lack most of the outer chain while retaining an apparently unaltered core structure. The mutants are defective in cell-wall synthesis and have altered morphologies, and most homozygous diploids form defective spores.

A new D-mannoprotein mutant, *mnn*5 has been isolated from a mutagenized culture of *Saccharomyces cerevisiae* X2180.[252] The properties of the mutant are consistent with the loss of the (1 → 2)-α-D-mannosyltransferase II activity that is responsible for the addition of the second D-mannopyranosyl residues to the polysaccharide side-chains.

The α-D-mannan of a mutant of *Saccharomyces cerevisiae* X2180, which possesses a main chain of 1,6-linked D-mannopyranosyl residues and a small proportion of branch chains composed of 1,2- and 1,3-linked D-mannopyranosyl residues, exhibits strong growth inhibitory activity against mouse implanted Sarcoma 180 and Ehrlich-carcinoma solid tumour.[253] Although the molecular size of the α-D-mannan is an important factor for anti-tumour activity, the presence of branches is not important, since a highly branched α-D-mannan from the wild-type strain has nearly identical biological activity.

Lysed spheroplasts of *Saccharomyces cerevisiae* contain a heat-labile trypsin-sensitive inhibitor of D-mannan synthesis.[254] This inhibitory protein may bind to one or more D-mannosyltransferases present and form an inactive complex, and its activity may be modulated by varying the available Mn^{2+} concentration.

Chitin. – The properties of L-glutamine-D-fructose 6-phosphate aminotransferase (EC 2.6.1.16) and chitin synthetase (EC 2.4.1.16) in blastospores and germ tubes of *Candida albicans* have been described.[255] These enzymes catalyse the first and last steps in the pathway of chitin synthesis, and their involvement in the regulation of chitin synthesis are discussed. Chitosomes, the smallest structures with chitin synthase activity detected in cell-free extracts of *Mucor rouxii*, can be dissociated into smaller particles containing chitin-synthetase activity by treatment with digitonin.[256] The product synthesized by the chitosome subunits was characterized by *X*-ray diffractometry as α-chitin.

Two different pools of the zymogen of chitin synthetase in *Mucor rouxii* have been reported.[257] One pool is inactivated by treatment of the whole germlings at low pH, suggesting an external location. Although the precise location of both pools has not been rigorously characterized, it is postulated that one is located in the plasma membrane and the other associated with internal structures.

[251] L. Ballou, R. E. Cohen, and C. E. Ballou, *J. Biol. Chem.*, 1980, **255**, 5986.
[252] R. E. Cohen, L. Ballou, and C. E. Ballou, *J. Biol. Chem.*, 1980, **255**, 7700.
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[254] C. R. Harrington and L. J. Douglas, *J. Bacteriol.*, 1980, **142**, 852.
[255] Y. Y. Chiew, M. G. Shepherd, and P. A. Sullivan, *Arch. Mikrobiol.*, 1980, **125**, 97.
[256] J. Ruiz-Herrera, S. Bartnicki-Garcia, and C. E. Bracker, *Biochim. Biophys. Acta*, 1980, **629**, 201.
[257] A. Flores-Carréon, G. Larriba, and R. Sentandreu, *FEMS Microbiol. Lett.*, 1980, **8**, 151.

The hypothesis that the initiation of the chitinous primary septum of *Saccharomyces cerevisiae* occurs by localized activation of the uniformly distributed zymogen has been confirmed.[258] When intact yeast plasma membranes synthesize chitin, the newly formed polysaccharide may be detected either by the fluorescence produced by a brightener or by autoradiography at many sites on each membrane. The activation and degradation of chitin synthase in *S. cerevisiae* by trypsin and pronase has been described.[259] The low catalytic levels and stability of the synthetase found in actively growing cells of the yeast may be due to the restrictions induced in the system by its membrane location.

The measurement of levels of chitin in barley has been used to determine the degree of infection of the cereal by *Puccinia hordei*.[260]

8 Miscellaneous Fungal Polysaccharides

The D-galacto-D-mannan-rich hyphal polysaccharide of *Aspergillus niger* contains a core with a main chain of 1,6-linked α-D-mannopyranosyl residues substituted at O-2 with side-chains of α-D-mannopyranosyl, *O*-α-D-mannopyranosyl-(1 → 2)-*O*-α-D-mannopyranosyl, and *O*-α-D-mannopyranosyl-(1 → 2)-*O*-α-D-mannopyranosyl-(1 → 2)-*O*-α-D-mannopyranosyl units (54).[261] Evidence from methylation analysis and ^{13}C n.m.r. data indicate that side-chains of 1,5-linked β-D-galactofuranosyl residues having an average chain-length of four residues are linked to the D-mannan core at O-6 (55). Polysaccharides having similar structural features probably exist in the hyphae of *A. fumigatus*, *A. terreus*, *A. flavus*, and *A. nidulans*.

α-D-Man*p*
1
↑
2
(α-D-Man*p*)$_n$
1
↑
2
→ 6)-α-D-Man*p*-(1 →

n = 0–2

(54)

β-D-Gal*f*
1
↑
5
(β-D-Gal*f*)$_m$
1
↑
6
→ 6)-α-D-Man*p*-(1 →

m = average of 3

(55)

Oligosaccharides have been isolated from the L-fuco-D-mannan D-galactan obtained from the fruit bodies of *Flammulina velutipes*.[262] The identification of a homologous series of 1,6-linked α-D-galactosyl oligosaccharides together with 3-*O*-α-D-mannopyranosyl-L-fucose confirmed earlier structural proposals.

[258] A. Duran, E. Cabib, and B. Bowers, *Science*, 1979, **203**, 363.
[259] A. Dominguez, M. V. Elorza, J. R. Villanueva, and R. Sentandreu, *Curr. Microbiol.*, 1980, **3**, 263.
[260] J. M. Whipps and D. H. Lewis, *Trans. Br. Mycol. Soc.*, 1980, **74**, 416.
[261] E. M. Barreto-Bergter, L. R. Travassos, and P. A. J. Gorin, *Carbohydr. Res.*, 1980, **86**, 273.
[262] T. Mukumoto and H. Yamaguchi, *Carbohydr. Res.*, 1980, **79**, 142.

The water-soluble glycuronan, 'protuberic acid', isolated from *Kobayasia nipponica* has a (1 → 4)-linked linear structure consisting of D-glucopyranosyluronic acid and L-idopyranosyluronic acid residues.[263]

A peptido-polysaccharide, isolated from the culture medium of *Paracoccidioides brasiliensis* contains residues of D-mannose (88%) and D-galactose (12%).[264] Only four amino-acids, L-alanine, L-aspartic acid, L-glutamic acid, and L-threonine, are found in the peptide portion of the molecule.

Structural studies of the extracellular polysaccharide elaborated by *Rhinocladiella eliator* indicate that the polysaccharide is a (1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucuronan.[265] The results of methylation analysis suggest that the corresponding polysaccharide from *R. mansonii* is composed of trisaccharide repeating units (56).

→ 3)-β-D-Glc*p*NAcA-(1 → 3)-β-D-Glc*p*NAc-(1 → 3)-β-D-Glc*p*NAc-(1 →

D-Glc*p*NAcA = 2-acetamido-2-deoxy-D-glucuronic acid

(56)

The synthesis of L-asparagine-linked glycoproteins in *Saccharomyces cerevisiae* proceeds in a way that closely resembles that described in animal tissues.[266] The precursors of the sugar residues in the vicinity of the polypeptide chain are dolichyl pyrophosphate derivatives of *NN'*-diacetylchitobiose containing about nine to 12 D-glucosyl- and D-mannosyl-residues. The transfer of the oligosaccharide from the lipid intermediate to protein is followed by the excision of the D-glucosyl residues and either the addition or removal of D-mannosyl residues, depending probably on the glycoprotein being synthesized.

An alkali-soluble polysaccharide has been isolated from the oak lichen *Cetraria islandica*.[267] On the basis of methylation, periodate oxidation, and partial acid hydrolysis studies, the polysaccharide has been shown to have a branched structure composed of a main chain of 1,3-linked D-glucopyranosyl and D-glucopyranosyluronic acid residues, with 1,4- and/or 1,6-linked D-glucopyranosyl residues at branch points.

[263] H. Tsuchihashi, T. Yadomae, and T. Miyazaki, *Carbohydr. Res.*, 1980, **84**, 365.
[264] V. J. Rodriguez, V. C. De Bièvre, and F. Mariat, *C.R. Hebd. Seances. Acad. Sci., Ser. D*, 1980, **290**, 1177.
[265] L. Kenne, B. Lindberg, K. Peterson, and P. Unger, *Carbohydr. Res.*, 1980, **84**, 184.
[266] A. J. Parodi, *J. Biol. Chem.*, 1979, **254**, 10 051.
[267] M. Hranisavljević-Jakovljević, J. Miljković-Stojanović, R. Dimitrijević, and V. M. Mićović, *Carbohydr. Res.*, 1980, **80**, 291.

5

Glycoproteins, Glycopeptides, and Animal Polysaccharides

BY R. J. STURGEON

1 Introduction

A treatise on the biochemistry of glycoproteins and proteoglycans has been published.[1] Individual chapters deal with the structure of glycoproteins, the function of lipid intermediates in the synthesis of oligosaccharides, the role of mammalian glycosyltransferases in synthesis, surface carbohydrates, carbohydrate recognition systems, and the structure and metabolism of connective tissue proteoglycans.

The primary structure of glycoprotein glycans (oligosaccharide chains) as a basis for the molecular biology of glycoproteins has been reviewed.[2] In a review on glycoproteins, structural aspects of the L-asparagine-linked oligosaccharide chains have been discussed.[3] A survey of glycoproteins has attempted to identify the role of neuraminic acids in influencing the viscosity of these macromolecules.[4]

2 Microbial Polysaccharides

Mycelial antigens (glycoproteins) have been prepared from *Aspergillus fumigatus*, *Trichophyton rubrum*, *Petriellidium boydii*, and *Mucor pusillus* after detergent extraction and affinity chromatography on immobilized concanavalin A.[5] Antigenic activity resides in both the protein and the carbohydrate moieties of these molecules. Tunicamycin has been used to inhibit the glycosylation of several extracellular enzymes produced by *Aspergillus niger*.[6] Both the intracellular and extracellular activities of β-D-2-acetamido-2-deoxyglucosidase are inhibited in the presence of the antibiotic. Various possible explanations for the decreased enzyme levels are discussed.

Changes in the glycoprotein content of *Dictyostelium discoideum* during development have been studied by direct binding to ^{125}I-labelled lectins after sodium dodecyl sulphate–gel electrophoresis.[7] At least halfway through the developmental cycle a minimum of 14 new glycoproteins reacting with wheat

[1] 'Biochemistry of glycoproteins and proteoglycans', ed. W.J. Lennarz, Plenum Press, New York, 1980.
[2] J. Montreuil, *Adv. Carbohydr. Chem. Biochem.*, 1980, **37**, 157.
[3] T. Mizuochi and A. Kobata, *Seikagaku*, 1980, **52**, 162.
[4] F. Ahmad and P. McPhie, *Int. J. Biochem.*, 1980, **11**, 91.
[5] V. M. Hearn and D. W. R. MacKenzie, *Mol. Immunol.*, 1980, **17**, 1097.
[6] B. K. Speake, F. W. Hemming, and D. A. White, *Biochem. Soc. Trans.*, 1980, **8**, 166.
[7] K. Burridge and L. Jordan, *Exp. Cell Res.*, 1979, **124**, 31.

germ agglutinin accumulate. In contrast, glycoproteins capable of binding to concanavalin A are unaffected during development. A membrane glycoprotein of aggregating *D. discoideum* cells with properties of contact sites has been purified and shown to contain residues of D-mannose, 2-acetamido-2-deoxy-D-glucose, and L-fucose.[8] The protein moiety contains 8% L-proline and is rich in hydroxyamino-acids. The loss of the lectin activity of a carbohydrate-binding protein (mol. wt. 2.6×10^4) results in the loss of aggregation competence and cell–cell adhesiveness in developing cells of *D. discoideum*.[9] The binding activity appears to be involved in the acquisition of cohesiveness and may be directly involved in the intercellular cohesion process. 2-Acetamido-2-deoxy-D-glucose is transferred to glycoprotein acceptors without the involvement of lipid intermediates in preparations from both growing and developing cells of *D. discoideum*.[10] Both growth-phase and aggregation-competent cells appeared to contain the same glycoproteins. Striking changes in the glycoproteins of plasma membrane during the course of development of *D. discoideum* have been observed from analysis of gel electrophoresis patterns of labelled cells.[11]

The phytopathogenic fungus *Fusarium solani pisi* produces two extracellular glycoproteinaceous enzymes, cutinases I and II.[12] Identification of the labelled carbohydrates released from the enzymes by alkaline sodium borotritide show that each mole of cutinase I has one mole of D-mannose, L-arabinose, 2-acetamido-2-deoxy-D-glucose, and D-glucuronic acid, *O*-glycosidically linked to L-serine, L-threonine, β-hydroxy-L-phenylalanine, and β-hydroxy-L-tyrosine. In addition the *N*-terminal glycine residue of the protein forms an amide linkage with D-glucuronic acid.

The main component of the *Halobacterium halobium* cell wall is a sulphated glycoprotein.[13] Sulphate is linked to *O*-glycosidically bound di- or tri-saccharides and also to a much higher molecular weight *N*-glycosidically linked hetero-saccharide.

Natural abundance ^{13}C n.m.r. spectroscopy has been used to substantiate and further characterize structural aspects of the phosphogalactomannan region of the glycopeptide from *Penicillium charlesii*.[14] All of the D-galactosyl residues appear to be in the furanosyl form as a (1 → 5)-β-D-galactan.

Neutral and acidic glycopeptides are synthesized at different periods during the growth of *Phialophora cirrerescens*.[15] These glycopeptides do not appear to be autolytic products of the fungal cells. A glycoprotein (mol. wt. 7.1×10^4) is involved in the specific cell adhesion required for cell aggregation during development of the cellular slime mould *Polysphondylium pallidum* cells.[16] This glycoprotein resembles the contact site A glycoprotein of *Dictyostelium*

[8] K. Müller, G. Gerisch, I. Fromme, H. Mayer, and A. Tsugita, *Eur. J. Biochem.*, 1979, **99**, 419.
[9] T. M. Shinnick and R. A. Lerner, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 4788.
[10] E. V. Crean, J. P. Lagerstedt, and E. F. Rossomando, *J. Bacteriol.*, 1979, **140**, 188.
[11] K. Toda, K. I. Ono, and J. Ochia, *Eur. J. Biochem.*, 1980, **111**, 377.
[12] T. S. Lin and P. E. Kolattukudy, *Eur. J. Biochem.*, 1980, **106**, 341.
[13] F. Wieland, W. Dompert, G. Bernhardt, and M. Sumper, *FEBS Lett.*, 1980, **120**, 110.
[14] C. J. Unkefer and J. E. Gander, *J. Biol. Chem.*, 1979, **254**, 12131.
[15] A. Pugin, *Mycopathologia*, 1980, **72**, 55.
[16] C. Steinemann, R. Hintermann, and R. W. Parish, *FEBS Lett.*, 1979, **108**, 379.

discoideum in being lost from the plasma membrane at the completion of aggregation when its synthesis ceases.

A purified plasma membrane fraction from *Saccharomyces cerevisiae* can transfer 2-acetamido-2-deoxy-D-glucose from UDP-2-acetamido-2-deoxy-D-glucose to polyprenoid lipids and from glycolipid to endogenous protein.[17]

Two glycoprotein fractions from *Ustilago maydis* are capable of solubilizing nucleic acids in dilute acid.[18] The two glycoproteins are distinguished by their ability to bind to immobilized concanavalin A and their differential binding to double- and single-stranded DNA and to RNA.

The biosynthesis and structure of myxovirus glycoproteins have been reviewed.[19] Two other reviews have discussed the co-translational and post-translational processing of viral glycoproteins[20] and the immunochemistry of herpes simplex viral glycoproteins.[21] Cell-free translation of adenovirus type 2-specific early messenger RNA in the presence of rough microsomes from dog pancreas has located the gene(s) for the adenovirus type 2 early glycoproteins within the early region 3 of the Ad2 genome.[22] The non-glycosylated form of the early glycoproteins (mol. wt. 1.7×10^4 and 1.9×10^4, respectively) of the non-defective Ad2-SV40 virus is a protein (mol. wt. 1.55×10^4).[23] The gene for the higher molecular weight glycoprotein is in early region 3 of the virus.

The glycosylation patterns of glycoproteins (mol. wt. 7.0×10^4) of xenotropic and dual-tropic murine leukaemia virus have been compared with those of ecotropic viruses.[24] Neither xenotropic nor dual-tropic viruses possess glycopeptides (mol. wt. 5.1×10^3) on proteolytic digestion of the glycoprotein. Like ecotropic viral glycoproteins, glycosylation of these two viral types appears to be controlled by features of the viral polypeptide sequence.

The major envelope glycoprotein of avian myeloblastosis virus has been isolated and partially characterized.[25] Determination of the hydrodynamic volumes in the presence of sodium dodecyl sulphate or guanidinium chloride yielded apparent molecular weights of 5×10^4 and 4.3×10^4, respectively. The lipophilic nature of the glycoprotein was shown by its binding to non-ionic detergent.

Two envelope glycoproteins and the viral nucleocapsid of a coronavirus have been isolated by solubilization of the viral membrane with detergent.[26] A schematic model for the location of the two glycoproteins in relation to the viral envelope and RNA is proposed.

[17] G. W. Welten-Verstegen, P. Boer, and E. P. Steyn-Parvé, *J. Bacteriol.*, 1980, **141**, 342.
[18] P. Unrau, D. R. Champ, J. L. Young, and C. E. Grant, *J. Biol. Chem.*, 1980, **255**, 614.
[19] H. D. Klenk, W. Garten, T. Kohama, R. T. C. Huang, and R. Rott, *Biochem. Soc. Trans.*, 1980, **8**, 419.
[20] H. D. Klenk and R. Rott, *Curr. Top. Microbiol. Immunol.*, 1980, **90**, 19.
[21] B. Norrild, *Curr. Top. Microbiol. Immunol.*, 1980, **90**, 68.
[22] H. Persson, M. Jansson, and L. Philipson, *J. Mol. Biol.*, 1980, **136**, 375.
[23] T. G. Storch and J. V. Maizel, *Virology*, 1980, **103**, 54.
[24] M. C. Kemp, N. G. Famulari, P. V. O'Donnell, and R. W. Compans, *J. Virol.*, 1980, **34**, 154.
[25] D. J. Marciai and J. D. Papmatheakis, *J. Biol. Chem.*, 1980, **255**, 1677.
[26] L. S. Sturman, K. V. Holmes, and J. Behnke, *J. Virol.*, 1980, **33**, 449.

No differences have been observed in the glycopeptide and glycoprotein patterns between two nuclear polyhedrosis viruses grown in two lepidopteran cell lines.[27] However, both viruses synthesize a glycoprotein that is not observed in either insect line and may be virus-directed. Glycoproteins of ortho- and para-myxoviruses have been solubilized with octyl β-D-glucopyranoside and separated by affinity chromatography on immobilized sulphanilic acid.[28] In this way neuraminidase activity is separated from the haemagglutinin activity. Glass wool column chromatography has been used for the separation of two glycoproteins of western equine encephalitis virus.[29] Cross-contamination of each protein was found to be negligible by polyacrylamide gel electrophoresis. A single glycoprotein (mol. wt. 2.5×10^5) has been identified as a membrane antigen of Epstein–Barr virus by immunoprecipitation with monoclonal antibody.[30]

Two glycoproteins (mol. wt. 2.7×10^4 and 6.8×10^4) having almost identical amino-acid and carbohydrate composition have been isolated from hepatitis B surface-antigen.[31] An antigen-specific method has been developed for the direct detection and quantitation of hepatitis B surface-antigen–immune complexes.[32]

The gA and gB glycoproteins of the major virus-specific glycoprotein region from herpes simplex virus type 1 infected cells have been separated and purified.[33] They are antigenically similar and may represent two different forms of the same polypeptide which differ in their degree of glycosylation. Membrane glycoproteins isolated from this virus bind tightly to immobilized concanavalin A and can only be dissociated from the lectin by treatment with sodium dodecyl sulphate in urea.[34] Herpes simplex virus tumour-associated antigen has been isolated from human kidney carcinoma. The antigen (mol. wt. 7.0×10^4) behaves as a glycoprotein, in binding to concanavalin A.[35]

A comparative structural analysis of glycoprotein SD of herpes simplex virus types 1 and 2 has been reported.[36] Detailed tryptic peptide analysis of the two glycoproteins indicate that the molecules are very similar, though not identical. Individual viral glycoprotein antigens of the virus type 1 are demonstrable early in the infectious cycle as determined by antibody-dependent cell-mediated cytotoxicity studies.[37] Fractionation of the major glycoprotein complex of type 1 has been reported and antisera to two glycoprotein antigen components

[27] N. I. Goldstein and A. H. McIntosh, *Arch. Virol.*, 1980, **64**, 119.
[28] F. X. Bosch, A. Mayer, and R. T. C. Huang, *Med. Microbiol. Immunol.*, 1980, **168**, 249.
[29] K. Yamamoto and B. Simizu, *Appl. Environ. Microbiol.*, 1980, **40**, 240.
[30] G. J. Hoffman, S. G. Lazarowitz, and S. D. Hayward, *Proc. Natl. Acad. Sci., USA*, 1980, **77**, 2979.
[31] H. Shiraishi, R. Shirachi, K. Akiyama, and N. Ishida, *J. Gen. Virol.*, 1980, **48**, 31.
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[33] P. Eberle and R. J. Courtney, *J. Virol.*, 1980, **36**, 665.
[34] L. Poliquin and G. C. Shore, *Anal. Biochem.*, 1980, **109**, 460.
[35] R. Cocchiara, G. Tarro, G. Flaminio, M. Di Gioia, R. Smeraglia, and D. Geraci, *Cancer*, 1980, **46**, 1594.
[36] R. J. Eisenberg, M. Ponce De Leon, and G. H. Cohen *J. Virol.*, 1980, **35**, 428.
[37] D. Norrild, S. L. Shore, T. L. Cromeans, and A. J. Nahmias, *Infect. Immun.*, 1980, **28**, 38.

have been prepared.[38] These glycoproteins have been shown to be expressed on the surface of virions as well as on the surface of infected cells. Glycoproteins with type-common and type-specific antigenic sites are excreted from cells infected with herpes simplex virus types 1 and 2.[39]

Antibodies have been prepared against the major envelope glycoprotein (mol. wt. 1.26×10^5) of herpes virus.[40] The glycoprotein was also purified from a host of virus-induced proteins with a specific immunoadsorbent prepared from the antibodies. Immune cytolysis mediated by antibody and complement is directed against components of the major herpes simplex virus glycoproteins.[41] A procedure for the enrichment of viral mutants defective in the expression of specific glycoproteins at the cell surface has been described.

Herpes simplex virus may influence a glycosylation of proteins by modifying the glycosyl-transferases of the infected baby hamster kidney and green monkey kidney cells.[42] The apparent K_m values calculated for *N*-acetylneuraminate-transferases and D-galactosyltransferases differ significantly from the corresponding values of uninfected cells. Two glycoproteins of herpes simplex virus are synthesized at different times in the infectious cycle.[43] Processing of both molecules appears to involve changes in both molecular weight and charge due to the addition of neuraminic acid. Tunicamycin and 2-deoxy-D-*arabino*-hexose inhibit the formation of cell-surface glycoproteins of the virus containing 2-amino-2-deoxy-D-glycosyl residues.[44] Evidence for differing pathways of metabolism of the amino-sugar in the presence of the inhibitors is given.

The haemagglutinin from the Hong Kong influenza virus A/Memphis/102/72 contains seven *N*-glycosidically linked oligosaccharide units, six of which are attached to the heavy chain (HA 1) and one to the light chain (HA 2).[45] As found for other glycoproteins, the glycosylated L-asparagine residues in the haemagglutinin occur in tripeptide sequences of the L-Asn-X-(L-Ser/L-Thr) type. However, unlike most other glycoproteins, all such potential glycosylation sites in this protein carry carbohydrate. The heavy chain contains 328 amino-acid residues and, compared with the polypeptides of similar chains from other influenza strains, has an additional 10 residues at its *N*-terminus, including a glycosylated L-asparagine residue.[46] The structure of the light chain and the arrangement of the disulphide bonds have been established.[47] No carbohydrate groups appear to be attached to either L-serine or L-threonine residues. Predictions of the secondary structures of the two chains of the haemagglutinin, based on the known amino-acid sequences, reveal a striking contrast between

[38] R. Eberle and R. J. Courtney, *J. Virol.*, 1980, **35**, 902.
[39] R. E. Randall, R. A. Killington, and D. H. Watson, *J. Gen. Virol.*, 1980, **48**, 297.
[40] C. Y. Ching and C. Lopez, *J. Immunol. Methods.*,1980, **32**, 383.
[41] N. A. Machtiger, B. A. Pancake, R. Eberle, R. J. Courtney, S. S. Tevethia, and P. A. Schaffer, *J. Virol.*, 1980, **34**, 336.
[42] S. Olofsson, B. Khanna, and E. Lycke, *J. Gen. Virol.*, 1980, **47**, 1.
[43] G. H. Cohen, D. Long, and R. J. Eisenberg, *J. Virol.*, 1980, **36**, 429.
[44] S. Olofsson and E. Lycke, *Arch. Virol.*, 1980, **65**, 201.
[45] C. W. Ward, P. A. Gleeson, and T. A. Dopheide, *Biochem. J.*, 1980, **189**, 649.
[46] C. W. Ward and T. A. Dopheide, *Virology*, 1980, **103**, 37.
[47] T. A. Dopheide and C. W. Ward, in 'Structure and variation in influenza virus', ed. G. Laver and G. Air, Elsevier, NY, 1980, p. 21.

the potential configurations of the two chains.[48] The heavy chain is high in β-structure whereas the light chain is highly helical. It is suggested that the coil–coil-type interactions may hold the haemagglutinin monomers together in the virus. Three disulphide bonds link the two chains and the heavy chain contains three intrachain disulphide bonds.[49]

The surface-membrane-bound glycoproteins, haemagglutinin and neuraminidase, of influenza virus strain 3QB have been isolated in biologically active forms.[50] A 16 residue antigenically active peptide was isolated from the purified haemagglutinin.

Trypsin releases five major classes of glycopeptides from the HA_1 subunit and a single major glycopeptide from the HA_2 subunit of influenza haemagglutinin glycoprotein.[51] Complex glycopeptides are present in three of the glycopeptides from HA_1 as well as from the HA_2 glycopeptide. The various glycopeptides also exhibit differences in the degree of sulphation.

In contrast to 2-deoxy-D-*arabino*-hexose, D-fluoroglucose inhibits the formation of D-mannosylphosphoryl dolichol and D-glucosylphosphoryl dolichol and therefore the synthesis of the normal lipid-linked or protein-linked oligosaccharides in the formation of influenza virus glycoproteins.[52] The inhibition is not complete and the oligosaccharides that are transferred to protein in the presence of D-fluoroglucose come directly from dolichol-linked oligosaccharides, which are smaller than the normal high D-mannosyl oligosaccharides and are resistant to digestion by *endo*-2-acetamido-2-deoxy-D-glucanase H.[53]

Nonspecific protection against infectious aerosols of influenza A virus is obtained in Swiss mice after vaccination with aerosols of bacterial ribosomes together with membranal glycoproteins from *Klebsiella pneumoniae* as the adjuvant.[54]

Measles virus has been labelled in its carbohydrate portion with tritium *in vitro* and *in vivo*.[55] The virus contains only one major glycoprotein (mol. wt. 7.9×10^4), which is exposed on the surface. Very little neuraminic acid could be detected in the molecule, which contained D-galactosyl residues at the non-reducing terminus of an oligosaccharide chain linked *N*-glycosidically to L-asparagine in the polypeptide.

Human peripheral blood lymphocytes from healthy donors express enhanced natural cytotoxicity to target cells after exposure to mumps virus.[56] The virus-dependent toxicity is significantly reduced when one of the viral glycoproteins

[48] C. W. Ward and T. A. Dopheide, *Aust. J. Biol. Sci.*, 1980, **33**, 441.
[49] T. A. Dopheide and C. W. Ward, *FEBS Lett.*, 1980, **110**, 181.
[50] A. Aitken and C. Hannoun, *Eur. J. Biochem.*, 1980, **107**, 51.
[51] K. Nakamura, A. S. Bhown, and R. W. Compans, *Virology*, 1980, **107**, 208.
[52] R. Datema, R. T. Schwarz, and A. W. Jankowski, *Eur. J. Biochem.*, 1980, **109**, 331.
[53] R. Datema, R. T. Schwarz, and J. Winkler, *Eur. J. Biochem.*, 1980, **110**, 355.
[54] D. Robert, J. P. Quillon, B. Ivanoff, Y. Beaudry, R. Fontanges, G. Normier, A. M. Pinel, and L. Dussourd D'Hinterland, *Infect. Immun.*, 1979, **26**, 515.
[55] O. Anttonen, M. Jokinen, A. Salmi, R. Vainionpä, and C. G. Gahmberg, *Biochem. J.*, 1980, **185**, 189.
[56] B. Harfast, C. Orvell, A. Alsheikhly, T. Andersson, P. Perlmann, and E. Norrby, *Scand. J. Immunol.*, 1980, **11**, 391.

(mol. wt. 7.5×10^4), which contains both neuraminidase and haemagglutinin activities, is selectively removed.

The Friend spleen focus-forming virus contains a glycoprotein (mol. wt. 5.5×10^4), which is expressed on the surface of cells infected with the virus.[57] A method for immunoselection of the glycoprotein synthesis mutants has been described. A similar glycoprotein is encoded by the Rauscher strain of acute erythroleukaemia virus.

Dolichos biflorus agglutinin, which is specific for terminal 2-acetamido-2-deoxy-α-D-galactosyl residues, binds to a spontaneous leukaemia cell of GRS/A mice but not to lymphoid cells of the host.[58] The major component of the receptors is a glycoprotein (mol. wt. 1.0×10^5), which releases oligosaccharides (mol. wt. 1.0×10^3) on treatment with alkali. Complexes of Friend leukaemia virus envelope glycoprotein bind specifically to mouse spleen leukocytes.[59] The putative receptor is identified as a protein (mol. wt. 1.4×10^4).

The major murine mammary-tumour virus envelope glycoprotein is synthesized by cells from a lymphoma cell line and released into the medium, but little of this is incorporated into the virions.[60] In spite of this deficiency, the virions produced by the cells are capable of inducing mammary tumours in an appropriate host. Monoclonal antibodies that identify individual determinants of a mouse mammary-tumour virus glycoprotein (mol. wt. 5.2×10^4) have been isolated.[61]

A model for the synthesis of viral glycoproteins has been suggested based on studies using mouse mammary-tumour virus glycoprotein precursors.[62] The precursor protein (mol. wt. 7.3×10^4) is synthesized on the rough endoplasmic reticulum as a transmembrane protein with a carboxy-tail of at least 2×10^3 daltons remaining on the cytoplasmic side. During synthesis at least five oligosaccharide core units are added to the growing chain at the appropriate L-asparagine residues. Soon after synthesis, cleavage of the glycoprotein occurs yielding the intracellular forms (mol. wt. 5.2×10^4 and 3.6×10^4). Further glycosylation of these two glycoproteins occurs followed by transport to the plasma membrane where they aggregate and associate with intracytoplasmic A type particles in the initiation of the budding process. Exogenous mouse interferons do not affect the synthesis of mouse mammary tumour virus, although alterations in the profiles of glycoproteins and polypeptides were observed.[63]

[57] D. Kabat, M. Ruta, M. J. Murray, and E. Polonoff, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 57.

[58] T. Muramatsu, H. Muramatsu, M. Kasai, S. Habu, and K. Okumura, *Biochem. Biophys. Res. Commun.*, 1980, **96**, 1547.

[59] P. J. Robinson, G. Hunsmann, J. Schneider, and V. Schirrmacher, *J. Virol.*, 1980, **36**, 291.

[60] A. B. Vaidya, C. A. Long, J. B. Sheffield, A. Tamura, and H. Tanaka, *Virology*, 1980, **104**, 279.

[61] R. J. Massey, L. O. Arthur, R. C. Nowinski, and G. Schochetman, *J. Virol.*, 1980, **34**, 635.

[62] C. Dickson and M. Atterwill, *J. Virol.*, 1980, **35**, 349.

[63] M. J. Yagi, N. W. King, and J. G. Bekesi, *J. Virol.*, 1980, **34**, 225.

The sequence relationship of glycosylated and unglycosylated *gag* proteins of Moloney murine leukaemia virus has been identified.[64] The glycoprotein (mol. wt. 8.0×10^4) contains additional polypeptide sequences that are located at or very near the *N*-terminus of the unglycosylated protein (mol. wt. 6.5×10^4), with the carbohydrate attached to polypeptide sequences held in common between the two.

An analysis of spleen focus-forming virus-specific RNA sequences coding for a spleen focus-forming virus-specific glycoprotein has been reported.[65] A study of plasma-membrane glycoproteins encoded by cloned Rauscher and Friend spleen focus-forming viruses suggest that very similar *env* gene recombination events are independently involved in the origins of different acute erythroleukaemia viruses.[66] Monospecific hybridomas directed against Rauscher murine leukaemia virus glycoprotein (mol. wt. 7.0×10^4) have been prepared.[67] The antibodies have been coupled with specific cleavage fragments and partial sequence data have been utilized to order the peptides within the molecule. A protein (mol. wt. 1.0×10^4) that binds this envelope glycoprotein has been purified from the culture medium used for growth of BALB/c 3T3 mouse cells.[68]

The H/N and F_1 glycoproteins responsible for haemagglutinin and neuraminidase (H/N) activity and fusion (F_1) activity have been purified from several Newcastle disease virus strains and used to produce antisera.[69] These antisera could be used to distinguish the virus strains by a number of techniques including radioimmunoassay and so provide a means of classifying new virus isolates. The location of post-translational cleavage events within the fusion glycoprotein and two minor glycoproteins of Newcastle disease virus have been reported.[70] Polypeptides synthesized in Newcastle disease virus-infected cells in the presence of tunicamycin have been shown to be relatively stable.[71]

Proteolytic digestion of Rabies virus glycoprotein releases glycopeptides (mol. wt. 3.8×10^3).[72] A partial structural study of the oligosaccharide portion of the molecule using *exo-* and *endo*-polysaccharide hydrolases indicates the presence of structures very similar to those described for vesicular stomatitis virus and numerous mammalian glycoproteins: di- or tri-antennary structures attached to poly(D-mannosyl-*N*,*N*′-diacetyl-chitobiose).

The synthesis and processing of viral glycoproteins has been studied in two nonconditional mutants of Rous sarcoma virus.[73] Among several purified proteins from this virus, only two external glycoproteins (mol. wt. 3.5×10^4 and

[64] S. A. Edwards and H. Fan, *J. Virol.*, 1980, **35**, 41.
[65] M. Yoshida and H. Yoshikura, *J. Virol.*, 1980, **33**, 587.
[66] M. Ruta and D. Kabat, *J. Virol.*, 1980, **35**, 844.
[67] H. L. Niman and J. H. Elder, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 4524.
[68] B. Landen and C. F. Fox, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 4988.
[69] R. J. Avery and J. Niven, *Infect. Immun.*, 1979, **26**, 795.
[70] A. C. R. Samson, P. Chambers, and J. H. Dickinson, *J. Gen. Virol.*, 1980, **47**, 19.
[71] T. G. Morrison and D. Simpson, *J. Virol.*, 1980, **36**, 171.
[72] P. Perrin and P. Atanasiu, *Microbiologia*, 1980, **3**, 57.
[73] M. Linial, J. Fenno, W. N. Burnette, and L. Rohrschneider, *J. Virol.*, 1980, **36**, 280.

8.0×10^4) can be methylated *in vitro* by protein methylase II (*S*-adenosyl-L-methionine:protein carboxy-*O*-methyl transferase, EC 2.1.1.24).[74]

Tunicamycin and 2-deoxy-D-*arabino*-hexose severely inhibit the synthesis of infectious Rous sarcoma virus without significantly affecting the synthesis of the virions.[75] In the presence of either inhibitor a partially glycosylated precursor (mol. wt. 9.5×10^4) to two glycoproteins is no longer synthesized, and a new polypeptide (mol. wt. 5.7×10^4), which is specifically recognized by antisera to the two glycoproteins, has been identified. Somewhat similar results have been obtained using tunicamycin to inhibit the glycosylation of herpes simplex virus.[76]

Glycoproteins of Sendai virus are trans-membrane proteins, a conclusion which is consistent with the observations that the glycoprotein of vesicular stomatitis virus and PE_2-glycoprotein of Sindbis virus appear to exist as trans-membrane proteins in rough microsomes involved in glycoprotein synthesis.[77]

Sendai viral glycoproteins have been separated by ion-exchange chromatography.[78] Retention of biological activity of both the haemagglutinin–neuraminidase (H/N) and fusion (F) glycoproteins was achieved. The haemagglutinin and fusion glycoproteins may be separated from each other by adsorption of the former on glutaraldehyde-treated chicken erythrocytes.[79] Monospecific antisera have been prepared by using these fractions as immunogens.

The E1 glycoproteins of Semliki Forest virus and Sindbis virus have much more homologous *N*-terminal amino-acid sequences than the two E2 glycoproteins.[80] This observation is in accord with the finding that the E1 glycoproteins are immunologically closely related whereas the E2 glycoproteins are type-specific.

Three individual membrane glycoproteins of Semliki Forest virus contain glycans with a monosaccharide sequence characteristic of lactosamine-type oligosaccharides.[81] Using stepwise degradation with *exo*-polysaccharide hydrolases, L-fucose is identified as being linked to the 2-acetamido-2-deoxy-D-glucosyl residue, which is in turn linked to L-asparagine. The newly-formed oligo-D-mannosyl-type glycans of the P62 protein of Semliki Forest virus, which are destined to become the oligo-D-mannosyl glycans of the E2 protein in the mature virus, decrease in size, with a net loss of two monosaccharide units.[82] This maturation process is limited to part of the glycosylation sites of the E2 portion of the P62 polypeptide.

The release of intact infectious snowshoe hare bunyavirus requires glycosylation of the viral G1 and G2 polypeptides.[83] The stage at which tunicamycin or

[74] A. Pierré and M. Robert-Gérd, *FEBS Lett.*, 1980, **113**, 115.
[75] R. Stohrer and E. Hunter, *J. Virol.*, 1979, **32**, 412.
[76] L. I. Pizer, G. H. Cohen, and R. J. Eisenberg, *J. Virol.*, 1980, **34**, 142.
[77] D. S. Lyles, *Proc. Natl. Acad. Sci. USA.*, 1979, **76**, 5621.
[78] Y. Fukami, Y. Hosaka, and Y. Yamamoto, *FEBS Lett.*, 1980, **114**, 342.
[79] Y. Hosaka, *Infect. Immun.*, 1980, **30**, 212.
[80] N. Kalkkinen, H. Jörnvall, H. Söderlund, and L. Kääriäinen, *Eur. J. Biochem.*, 1980, **108**, 31.
[81] M. Pesonen, *J. Gen. Virol.*, 1979, **45**, 479.
[82] M. L. Rasilo and O. Renkonen, *J. Gen. Virol.*, 1980, **47**, 525.
[83] P. Cash, L. Hendershot, and D. H. L. Bishop, *Virology*, 1980, **103**, 235.

2-amino-2-deoxy-D-glucose inhibits the virus particle formation is unknown. One possibility is that the non-glycosylated forms fail to insert (or remain inserted) in the cell membrane. However, with other viruses this step can proceed correctly. Alternatively the two polypeptides may have to be fully glycosylated in order that they function correctly as recognition sites for virus particle formation.

A discrete portion of Sindbis virus glycoprotein PE_2 (comprising the bulk of the molecule including its *N*-terminus) is selectively translocated across the microsomal membrane of dog pancreas.[84] The *C*-terminal portion of the glycoprotein remains untranslocated and exposed to attack by proteolytic enzymes outside the vesicle. A mutant of the virus has been characterized as producing a glycoprotein that contains a polypeptide chain 70 amino-acids shorter than that produced by the parent virus.[85]

The effect of cationic ionophores on the replication of Sindbis and vesicular stomatitis virus in cultures of baby hamster kidney and chicken embryo fibroblast cells is in blocking the movement of viral glycoproteins from the Golgi apparatus to the cell-surface membrane where budding and release of these two viruses occur.[86] The majority of the carbohydrate of vesicular stomatitis virus is attached to two different locations on the peptide backbone.[87] In addition, six different peptides containing small amounts of neuraminic acid, L-fucose, and D-mannose have been detected after treatment of the glycoprotein with cyanogen bromide, thus confirming earlier reports of the existence of more than two oligosaccharide chains in the molecule. An integral membrane glycoprotein of the plasma membrane of vesicular stomatitis virus is transported from the site of synthesis in the endoplasmic reticulum within small vesicles in two successive stages: first to the Golgi apparatus where oligosaccharide chains on the glycoprotein are processed, and secondly from the Golgi apparatus to the plasma membranes.[88]

A cell-free system has been isolated in which a membrane glycoprotein of vesicular stomatitis virus is transported to the membranes of the Golgi complex by a process resembling intracellular protein transport.[89] Transport *in vivo* is energy-dependent and is accompanied by terminal glycosylation of the membrane glycoprotein. The glycoprotein appears to be anchored in the viral membrane by a hydrophobic domain near the *C*-terminus.[90] Altered synthesis and processing of oligosaccharides of vesicular stomatitis virus glycoprotein in Chinese hamster ovary cell lines resistant to different lectins has been observed.[91] The intracellular glycosylation of the viral glycoprotein in a mutant cell line resistant to phytohaemagglutinin is identical to that in the normal cells except for a complete block in processing at a specific step in the final trimming

[84] S. Bonatti and G. Blobel, *J. Biol. Chem.*, 1979, **254**, 12261.
[85] A. Leone, V. Colantuoni, G. Pontarelli, and R. Cancedda, *J. Virol.*, 1980, **34**, 598.
[86] D. C. Johnson and M. J. Schlesinger, *Virology*, 1980, **103**, 407.
[87] L. Kingsford, S. U. Emerson, and J. M. Kelley, *J. Virol.*, 1980, **36**, 309.
[88] J. E. Rothman and R. E. Fine, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 780.
[89] E. Freis and J. E. Rothman, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 3870.
[90] J. K. Rose, W. J. Welch, B. M. Sefton, F. S. Esch, and N. C. Ling, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 3884.
[91] L. A. Hunt, *J. Virol.*, 1980, **35**, 362.

of the oligomannosyl core from five to three D-mannosyl residues. For a double mutant cell line selected from the phytohaemagglutinin-resistant cells for resistance to concanavalin A, an additional defect is apparent in one of the earliest stages of glycosylation, resulting in smaller precursor oligosaccharides linked to protein. Selective inhibition of glycoprotein and membrane protein of vesicular stomatitis virus from interferon-treated cells is reported.[92] The findings resemble those reported in interferon-treated cells infected with murine leukaemia viruses. The synthesis of the functional M protein but not G protein (a glycoprotein) is essential for maturation of the virions.[93] The M protein is not required for the assembly of G protein into the retrovirus virions.

3 Plant and Algal Glycoproteins

The biosynthesis and role of 4-hydroxy-L-proline in plant glycoproteins and animal systems have been compared.[94]

Glycoproteins from sarrasin (*Fagopyrum esculentum*) have been isolated using columns of immobilized concanavalin A.[95] A protocol has been produced to obtain optimum recovery of the glycoproteins. A glycoprotein from opium poppy capsules contains residues of L-arabinose, L-rhamnose, D-xylose, D-mannose, D-glucose, D-galactose, D-galactopyranosyluronic acid, D-glucopyranosyluronic acid, and 4-*O*-methyl-D-glucopyranosyluronic acid.[96] Highly-branched polysaccharide chains are linked to protein *via* D-galactopyranosyl-*O*-L-serine linkages. Streptovirudin and antibiotic 24010 act on glycoprotein biosynthesis of mung beans, *Vigna radiata*, in a similar manner to tunicamycin.[97] The use of tunicamycin is discussed with respect to its importance in studying glycoprotein biosynthesis and function in animal and plant tissues.

The endoplasmic reticulum of castor bean endosperm cells is the major particulate location of L-fucose-containing glycoproteins.[98] It is probable that the L-fucose is transferred directly from GDP-L-fucose to a core glycosylated acceptor by a glycoprotein L-fucosyltransferase located in the endoplasmic reticulum.

Glycoproteins having hydroxy-L-proline and L-serine as the most abundant amino-acids, and L-arabinose, D-galactose, and D-galactopyranosyluronic acid as the predominant monosaccharides, have been isolated from the cell wall of *Phaseolus coccineus*.[99] One of the glycoprotein fractions shows many structural similarities with the glycopeptide portion of the potato lectin. Glycoprotein II, isolated from the seeds of *P. vulgaris*, exists as a microheterogeneous population of molecules in which the protomers are made up of three subunits formed in various combinations from the existing subunits.[100]

[92] R. K. Maheshwari, F. T. Jay, and R. M. Friedman, *Science*, 1980, **207**, 540.
[93] R. A. Weiss and P. L. P. Bennett, *Virology*, 1980, **100**, 252.
[94] D. Ashford and A. Neuberger, *Trends Biochem. Sci.*, 1980, **5**, 245.
[95] C. Akiki, R. Bourbouze, C. Luporsi, and F. Percheron, *J. Chromatogr.*, 1980, **188**, 435.
[96] A. Hillestad, *Phytochemistry*, 1980, **19**, 1711.
[97] D. W. James and A. D. Elbein, *Plant Physiol.*, 1980, **65**, 460.
[98] L. M. Roberts, R. B. Mellor, and J. M. Lord, *FEBS Lett.*, 1980, **113**, 90.
[99] M. A. O'Neill and R. R. Selevendran, *Biochem. J.*, 1980, **187**, 53.
[100] A. Pusztai and J. C. Stewart, *Biochim. Biophys. Acta*, 1980, **623**, 418.

Contrary to other reports, legumin, from *Pisum sativum*, contains no covalently bound carbohydrate.[101] Extensive incorporation of D-[^{14}C]mannose and 2-acetamido-2-deoxy-D-[^{14}C]glucose into proteins associated with various membrane fractions isolated from the pea cotyledon has been observed.[102] Although labelled glycoproteins associated with the rough endoplasmic reticulum were not characterized, they could represent integral membrane proteins or glycoproteins destined for secretion. A partial characterization of lipids and glycoproteins labelled *in vivo* from exogenously supplied D-[^{14}C]-mannose and 2-acetamido-2-deoxy-D-[^{14}C]glucose in the cotyledons of *P. sativum* has been reported.[103]

From purified cell walls and various polysaccharide fractions of *Chara aspera* and *Nitella translucens* glycoproteins have been isolated after affinity chromatography on immobilized concanavalin A.[104] The protein moieties appear similar in both algae although differences in the component sugars were detected. Gelonin, a glycoprotein (mol. wt. 2.8×10^4) from the seeds of *Gelonium multiflorum*, and composed of a single polypeptide chain, acts in a cell-free system like the A chains of abrin, ricin, and modeccin as an inhibitor of protein synthesis.[105] However, gelonin lacks the ability to bind to the cell surface and to enter the intact cells, except when it is coupled to concanavalin A. Under these conditions the glycoprotein is rendered toxic to intact cells.

A salt-extractable glycoprotein isolated from aerated carrot discs consists of equal amounts of carbohydrate and protein.[106] The predominant amino-acid is hydroxy-L-proline.

The term 'lectin' has frequently been used to describe additionally substances that differ markedly from the plant seed carbohydrate-binding proteins classically considered to be lectins. For clarity, and in order to include lectin-like substances from diverse sources (micro-organisms, fish sera, snails, *etc.*) a new definition of 'lectin' has been proposed.[107] Lectins should bear at least two sugar-binding sites, agglutinate animal and plant cells and/or precipitate polysaccharides, glycoproteins, and glycolipids. In spite of similarities to true lectins from the same source, toxins, which bear only one sugar-binding site, should not be considered as lectins since they do not agglutinate cells or precipitate glycoconjugates.

Abstracts of papers delivered at a symposium on lectins have been published.[108] They include reports of new methods and areas of application for lectins, the diagnostic value of lectins for the estimation of serum glycoproteins and microsomal enzymes, and the histochemical investigation using labelled lectins of the location of antigens in disease states.

[101] J. A. Gatehouse, R. R. D. Croy, and D. Boutler, *Biochem. J.*, 1980, **185**, 497.
[102] J. Nagahashi, S. K. Browder, and L. Beevers, *Plant Physiol.*, 1980, **65**, 648.
[103] S.K. Browder and L. Beevers, *Plant Physiol.*, 1980, **65**, 924.
[104] M. F. Alary-Bernard, M. Briens, M. M. G. Quillet, and M. Goas, *Phytochemistry*, 1980, **19**, 111.
[105] F. Stirpe, S. Olsnes, and A. Pihl, *J. Biol. Chem.*, 1980, **255**, 6947.
[106] D. A. Stuart and J. E. Varner, *Plant Physiol.*, 1980, **66**, 787.
[107] I. J. Goldstein, R. C. Hughes, M. Monsigny, T. Osawa, and N. Sharon, *Nature (London)*, 1980, **285**, 66.
[108] G. Uhlenbruck, *J. Clin. Chem. Clin. Biochem.*, 1980, **18**, 375.

A new method to quantitate lectin activity based on haemagglutination assay in microtitre plates has used an electronic particle counter for counting non-agglutinated cells in the microtitre wells, in addition to the normal method of visual titre evaluation.[109] This allows a rapid quantitative determination of the amount of lectin required to agglutinate 50% of the countable single cells.

The addition of specific sugars to solutions of several lectins has induced u.v.-difference spectra.[110] Difference spectra for *Lens culinaris*, *Sophora japonica*, *Solanum tuberosum* lectins, and wheat germ agglutinin have peaks that are characteristic of the L-tryptophan residue, whereas that of *Arachis hypogaea* agglutinin has peaks characteristic of L-tyrosine residues. The binding constants for the lectins with specific sugars may be determined from the intensities of the difference spectra. Lectins may be used for the differentiation of serum enzymes.[111] Lectins from *Canavalia ensiformis*, *Phaseolus vulgaris*, and *Triticum vulgare* react with arylamidase, alkaline phosphatase (EC 3.1.3.1), γ-glutamyl-transferase (EC 2.3.2.2), and cholinesterase (EC 3.1.1.8) of human sera by formation of enzymically active complexes. Arylamidase, alkaline phosphatase, and cholinesterase react more intensely in sera of patients with liver and neoplastic diseases.

Antibodies against various plant lectins have been isolated from mice ascitic fluid.[112] Serological evidence shows that all of the Solanaceous lectins studied so far are related and that the degree of similarity is consistent with the phylogenetic relationships of these genera.[113] Lectins isolated from all of the seeds have antigenic properties in common, which are distinct from those of lectins occurring in other tissues of the same plant.

The role of lectins and their saccharide receptors as determinants of the specificity in the *Rhizobium*-legume symbiosis has been reviewed.[114] The effects of alkali on the conformation of eight lectins has been reported.[115] A surprising individuality of the various lectins in the resistance to alkali and in the conformational transitions is observed.

Ellipsometry has been used to assess the degree of interaction of lectins with carcinoembryonic antigen adsorbed on nickel-coated glass slides.[116] The method has also been applied to study different parameters of the reaction using, as a model system, the binding of the tetrameric concanavalin A and dimeric succinyl-concanavalin A to a film of yeast D-mannan.[117] The technique shows promise for the study of the interaction of lectins with films of glycopeptide and glycoprotein.

[109] H. Köhle and H. Kauss, *Anal. Biochem.*, 1980, **103**, 227.
[110] I. Matsumoto, A. Jinbo, H. Kitagaki, A. M. Golovtchenko-Matsumoto, and N. Seno, *J. Biochem. (Jpn.)*, 1980, **88**, 1093.
[111] K. Lorentz, B. Flatter, and F. W. Kolle, *J. Clin. Chem. Clin. Biochem.*, 1979, **17**, 757.
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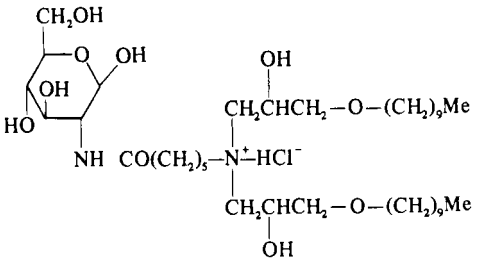
[113] D. C. Kilpatrick, C. E. Jeffree, C. M. Lockhart, and M. M. Yeoman, *FEBS Lett.*, 1980, **113**, 129.
[114] F. B. Dazzo, in 'Cell surface, mediator of developmental processes', ed. S. Subtelny and N. K. Wessells, Academic Press, NY, 1980, p. 277.
[115] B. Jirgensons, *Biochim. Biophys. Acta*, 1980, **625**, 193.
[116] M. Horisberger, *Physiol. Chem. Phys.*, 1979, **11**, 527.
[117] M. Horisberger, *Biochim. Biophys. Acta*, 1980, **632**, 298.

The effect of ionic strength on the interaction of lectins with carbohydrates has been determined by affinity electrophoresis.[118] Ionic strength appears to have no effect on the interaction of concanavalin A or of lentil lectin with immobilized α-D-mannosyl residues. In contrast, a weak dependence on the action of soya bean lectin with immobilized α-D-galactosyl residues was observed.

Peanut lectin, conjugated with horseradish peroxidase, has been used for the specific localization of D-galactose-rich sites in Golgi zones, secretory bodies, and cell surfaces in rodent tissues.[119]

A non-bonded method for the immobilization of concanavalin A has been reported.[120] The derivative (1) of 2-amino-2-deoxy-D-glucose dissolves in the hydrophobic grease Apiezon L, which in turn is adsorbed on Chromosorb W forming a support that will bind concanavalin A. The lectin may be recovered in the normal way by elution with methyl α-D-mannopyranoside.



(1)

A physical study for concanavalin A-induced agglutination using dextran gel spheres is in agreement with data using red blood cells.[121] The rates of formation and dissociation of concanavalin A with some 4-methylumbelliferyl and 4-nitrophenyl derivatives of α- and β-D-mannopyranosides and α- and β-D-glucopyranosides have been measured by fluorescence and spectral stopped-flow methods.[122] The effects of temperature, pH, and salt concentration of sugar-binding interactions were also examined. The association constants for binding of methyl α-D-mannopyranoside, and the (1 → 2)-α-D-manno-oligosaccharides, mannobiose, and mannotriose to concanavalin A have been determined over a range of temperatures by substitution titration using 4-methylumbelliferyl α-D-mannopyranoside as a carbohydrate-specific and fluorescent indicator.[123]

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The data obtained is consistent with an earlier suggestion that concanavalin A specifically binds a single D-mannopyranosyl residue in (1→2)-α-D-manno-oligosaccharides. Kinetic studies with the 4-methylumbelliferyl α-D-mannobioside and 4-methylumbelliferyl α-D-mannotrioside to concanavalin A also agree with the binding of the lectin to a single D-mannopyranosyl residue.[124]

Differences in the chromatographic behaviour of rat α-fetoprotein on several commercially available samples of immobilized concanavalin A have been demonstrated.[125] These differences should be taken into account in structural studies on microheterogeneous glycoproteins. Association constants for lectin–carbohydrate interactions have been determined by a competitive enzyme inhibition assay.[126] D-Glucose oxidase is adsorbed on immobilized concanavalin A and is in close proximity to an oxygen sensor, which is used as a detection device. Association constants obtained for maltose and maltotriose are comparable to values quoted in the literature.

Co^{3+} Ions have been stoicheiometrically incorporated into concanavalin A but the binding is not at the S1 site of the lectin.[127] The Co^{3+} protein still possesses a binding site for an additional divalent transition-metal ion, which together with Ca^{2+} can induce the sugar-binding activity. Metal-binding sites of concanavalin A have been examined using ^{113}Cd n.m.r. spectroscopy.[128] Rapid exchange of metal ions between the solution and the binding sites occurs. The binding of Cd^{2+} to concanavalin A at pH 6.5 involves both metal sites S1 and S2.[129] Full saccharide-binding activity is obtained only when the total bound Cd^{2+} stoicheiometry reaches 2 ions/concanavalin A subunit. The binding of Cd^{2+} to the S1 site induces a form of the lectin similar to that induced by Zn^{2+}, Ni^{2+}, or Co^{2+} but different from that induced by Mn^{2+}. A temperature-dependent conformational change occurs following the binding of only one Mn^{2+} to a concanavalin A monomer at pH 7.0.[130] This induces saccharide-binding activity and is followed by a slow conformational change, possibly involving an L-proline amide *cis–trans*-isomerization. No change in saccharide-binding activity accompanies this but a Ca^{2+} can bind to the new site formed.

Differential scanning calorimetry has been used to measure conformational changes in concanavalin A caused by different ligands.[131] The role of the bivalent cations Ca^{2+} and Mn^{2+} in lectin–carbohydrate interaction was also studied. In the presence of Mn^{2+} ions alone, concanavalin A cannot form the necessary conformation for binding the carbohydrate. However, with Ca^{2+} ions alone,

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concanavalin A can bind carbohydrate ligands, as judged by the shift in its transition temperature. A competitive-binding liquid-phase radioimmunoassay has been used in studies on the localization of wheat germ agglutinin in wheat plants.[132] The pattern of distribution is markedly different from that of legume lectins. Actively growing regions of the plant such as the bases of leaves, and rapidly growing adventitious roots, contain the highest levels of the lectin. Wheat germ agglutinin can be completely reduced in the absence of denaturing agents.[133] Specific binding of sugars to the lectin protects against reduction. Partially reduced and carboxymethylated lectin is a mixture of the unmodified protein and the fully reduced and carboxymethylated protein, suggesting that the reduction is an 'all-or-none' process, in which once the first disulphide bridge is opened the remaining ones are reduced very easily.

Significant changes are observed in the fluorescence polarization when *N,N'*-diacetylchitobiose or *N,N',N''*-triacetylchitotriose bind to wheat germ agglutinin.[134] These changes have been compared to similar changes upon adding the oligosaccharides to lysozyme, an enzyme in which tryptophan–sugar interactions at the active site have been well established. The binding of 1-*O*-methyl-di-*N*-trifluoroacetyl β-chitobioside to wheat germ agglutinin has been followed by fluorine n.m.r. spectroscopy.[135] *X*-Ray crystallographic studies on several complexes between wheat germ agglutinin and specific sugars have been reported.[136] The possible relevance of their observed binding mode to available solution data and to current theories relating to the complex phenomenon of lectin–cell interactions is discussed.

The crystal structure of the non-covalent complex between wheat germ agglutinin and *N*-acetylneuraminic acid has been determined from an electron density difference map at 2.8 Å resolution.[137] The map exhibits two strong binding sites on the lectin dimer molecule, which are located in corresponding faces of the protomer–protomer interface. The *N*-acetylneuraminic acid molecule is oriented such that its acetyl group becomes essentially buried upon binding, whereas the charged carboxylate and hydrophilic chain (C_7-C_9) point away from the protein surface. Model binding studies show that substituents of the pyranose ring, which have been predicted as essential for binding from solution studies, are situated favourably to allow interactions to be made with main- and side-chain atoms of the protein molecule.

Peanut lectin purified from the seed meal of the Jumbo Virginia and Spanish varieties of *Arachis hypogaea* L has been resolved into various isolectins.[138] Rabbit antibodies against the Jumbo Virginia seed isolectin preparation reacted with seed, cotyledon, and hypocotyl lectin preparations from both varieties of *A. hypogaea*. The carbohydrate-binding properties of peanut agglutinin have been measured by u.v.-difference spectroscopy.[139] Methyl α- and methyl β-D-

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galactopyranosides and lactose perturb the u.v. spectra of the lectin in the aromatic region and their association constants have been determined. An improved method for the isolation of *Bandeiraea simplicifolia* II lectin has been reported using affinity chromatography on columns of [(4-aminobenzyl) 1-thio-2-acetamido-2-deoxy-β-D-glucopyranoside]-succinylaminohexyl-agarose.[140]

Further characterization of the combining site of the D-galactopyranosyl- and 2-acetamido-2-deoxy-D-galactopyranosyl-specific lectin from *Bauhinia purpurea alba* seeds has been reported.[141] Although the available data from precipitin and precipitin-inhibition assays suggest that the combining site may be most complementary to the structure (2), several other possibilities remain to be tested when suitable oligosaccharides become available.

β-D-Gal*p*-(1 → 3)-β-D-Gal*p*NAc-(1 → 3)-D-Gal

(2)

Large-scale preparations of the lectin from *Crotalaria juncea* have involved fractionation by ammonium sulphate precipitation followed by affinity chromatography on columns of immobilized D-galactose.[142]

The isolectins from *Dolichos biflorus* have been studied by affinity electrophoresis.[143] Association constants have been measured and states of aggregation reported. A glycoprotein from the stems and leaves of *D. biflorus* that cross-reacts with antibodies to the seed lectin binds to blood group A + H substance under conditions of low ionic strength.[144] The specificity of this lectin-like glycoprotein differs from that of the seed lectin.

An immunological investigation has confirmed earlier reports of the absence of soya bean lectin in the seeds of several soya bean (*Glycine max*) lines.[145] No immunologically related materials were detected, indicating that the seeds of these lines lack non-haemagglutinating precursors of the lectin. A bacterial agglutinin, having properties differing from those of the major soya bean lectin, has been isolated from the seeds of the soya bean cultivar Clark.[146] In the undissociated state, the lectin agglutinates *Xanthomonas phaseoli* var. *sojensis*, the causal agent of bacterial pustule disease of the soya bean. No evidence has been obtained for the lectin having a role in determining pathogen specificity.[147]

The subunit structure and *N*-terminal sequences of a mitogenic lectin from sweet pea (*Lathyrus odoratus*) seeds have been reported.[148] A lectin isolated from the fruit juice of the tomato (*Lycopersicon esculentum*) is a glycoprotein with agglutinating activity towards human erythrocytes.[149] Inhibition of the

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agglutination activity can be achieved with oligomers of 2-acetamido-2-deoxy-D-glucose. The lectin cross-reacts immunologically with the lectin from *Datura stramonium* (thorn-apple).

Two lectins from lima bean (*Phaseolus lunatus*) have been characterized as a tetramer (mol. wt. 1.2×10^5) and an octomer (mol. wt. 2.4×10^5).[150] Using equilibrium dialysis methods the demetallized tetramer was shown to bind either two Mn^{2+} or four Ca^{2+} ions. The Ca^{2+} binding is strongly co-operative whereas the Mn^{2+} binding is not. After presaturation of the demetallized tetramer with Mn^{2+} it will bind only two Ca^{2+} in a non-co-operative manner. The metal-ion stoicheiometry of the octamer is double that for the tetramer. Leucoagglutinin, the isolectin of phytohaemagglutinin from *P. vulgaris*, is practically devoid of haemagglutinating activity with human erythrocytes.[151] The haemagglutinating activity appears after treatment of the erythrocyte with neuraminidase. The specificity of the desialylized receptor for the leucoagglutinin appears to be due to the presence of 2-acetamido-2-deoxy-D-galactosyl residues in the erythrocyte glycoprotein. The agglutinating activity of the lectin is temperature dependent.[152] Differences in the haemagglutinating activity of the lectin may be related to a temperature-induced change in the conformation of the lectin when bound to the glycosidic receptors.

Covalent modification of the phytohaemagglutinin of *Phaseolus vulgaris* impairs its ability to stimulate cultured human lymphocytes.[153] In the human lymphocyte transformation assay, the mitogenic property of the phytohaemagglutinin appears to depend on intact L-aspartic acid, L-glutamic acid, and L-tyrosine residues, whereas L-lysine residues do not appear to be essential. The structure (3) of the carbohydrate unit of Tora bean (*P. vulgaris*) lectin has been elucidated.[154] Microheterogeneity may exist in the oligosaccharide chains of this lectin, particularly with regard to the D-xylopyranosyl and L-fucopyranosyl residues. The lectin still exhibits potent haemagglutinin activity after enzymic removal of five of the nine D-mannopyranosyl residues.

Pea (*Pisum sativum*) root slime contains at least two D-glucose-binding proteins, one of which is a *Rhizobium leguminosarum*-agglutinating lectin, with the same electrophoretic pattern as a glycosylated lectin from the pea seed.[155] The role of pea lectins in the recognition of *R. leguminosarum* is discussed.

Potato lectin, which binds to oligomers of 2-acetomido-2-deoxy-D-glucose, has been purified from potato tubers by affinity chromatography on an immobilized fetuin matrix.[156]

Sophora japonica lectin, purified by affinity chromatography, is composed of the non-covalent association of two dissimilar subunits.[157] One subunit consists of two identical polypeptide chains attached by two disulphide bonds, and the

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α-D-Manp
1
↓
3
α-D-Manp-(1 → 2)-α-D-Manp-(1 → 6)-α-D-Manp-(1 → 6)
β-D-Manp-(1 → 4)-β-D-GlcpNAc-(1 → 4)-β-D-GlcpNAc-L-Asn
2
↑
3
α-D-Manp-(1 → 2)-α-D-Manp-(1 → 6)-α-D-Manp-(1 → 3)
3
↑
1
β-D-Xylp
1
α-D-Manp
3
↑
1
α-L-Fucp

(3)

other subunit of two identical polypeptide chains is stabilized by a single cysteine bridge.

An L-fucose-binding lectin from *Ulex europaeus* has been purified by affinity chromatography using an L-fucose–starch complex.[158] The purified lectin (mol. wt. 6.0×10^4) contains two glycoprotein subunits.

A blood-group A-specific lectin (mol. wt. 1.0×10^5) has been isolated from *Vicia cracca* seeds and has been shown to contain one oligosaccharide chain (with residues of D-mannose and 2-amino-2-deoxy-D-glucose) per subunit.[159] The lectin of *V. graminea* with blood-group N specificity has mol. wt. 1.06×10^5 and exists as a tetramer of four identical chains non-covalently linked.[160]

The haemagglutinating and toxic lectin from *Viscum album* inhibits protein synthesis in lysed rabbit reticulocytes in a manner similar to ricin.[161]

The specificity of *Wisteria floribunda* haemagglutinin purified by adsorption on insoluble polyleucyl hog gastric mucin blood-group A + H substance has been studied immunochemically by quantitative precipitin and precipitin-inhibition assays.[162] The combining site of the haemagglutinin is at least as large as the disaccharide 6-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-D-galactose, and hydrophobic interaction and shape appear to be important for binding.

The c.d. spectra of lectins from *Dolichos biflorus*, *Helix pomatia*, *Lotus tetragonolobus*, *Phaseolus vulgaris* (*erythro*-agglutinin), *Pisum sativum*, *Sophora japonica*, and *Ulex europaeus* have been recorded before and after the conformational transitions, which are effected by sodium dodecyl sulphate (SDS) and change of pH.[163] The conformation of the lectins is perturbed by SDS, resulting in the reorganization of the main chains into new forms having approximately 15–40% of α-helix. The binding-site specificity of lectins from *Bauhinia purpurea alba*, *Sophora japonica*, and *Wistaria floribunda* has been determined by haemaglutination-inhibition assay using various saccharides as inhibitors.[164] Many of the conclusions confirm earlier reports on the binding sites.

The properties of lectins from plant, animal, and microbial sources are listed in Table 1.[165–181]

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Table 1 *Composition and properties of some purified lectins*

Source	*Mol. wt.*	*Subunits*	*Carbohydrate (%)*		*Human blood type*	*Sugar(s)*	*Ref.*
			Neutral	*Amino-sugar*			
Plants							
Butea frondosa	1.4×10^5	3	4.3	0.5	ABO	α-D-Gal	165
Erythrina indica	6.6×10^4	2	5.6	0.46	ABO	α-D-Gal	165
Hura crepitans	1.2×10^5	4	9.0	1.0	ABO	D-GalNAc, D-Gal	166
Lathyrus sativus	4.3×10^4		2.0		ABO	α-D-Glc	168
L. silvestris	5.3×10^4		3.1		ABO	α-D-Glc, α-D-Man	167
L. odoratus	5.3×10^4		2.5		ABO	α-D-Glc, α-D-Man	167
Lycopersicon esculentum	7.1×10^4		50.0			Chito-oligosaccharides	169
Momordica charantia	1.29×10^5	4	4.1	0.5		α-D-Gal	165
Phaseolus vulgaris (Tora bean)	1.3×10^5	4	7.8		A, B, O, AB	β-D-Gal, β-D-Glc*p*NAc-D-Man	170
P. vulgaris (Tora-mame)	1.2×10^5	4	5.6	2.3	A, AB, O, B	D-GalNAc	171
Psophocarpus tetragonolobus	4.6×10^4	2	Nil	Nil	ABO	α-D-Gal, lactose	172
Vicia cracca	1.0×10^5	4	3.5	1.2	A	?	173
V. sativa	4.0×10^4	4			B	α-D-Glc, α-D-Man	174
Viscum album	6.0×10^4	2	11	?		β-D-Gal	175
Animals							
Human hepatic lectin	4.1×10^4		6.0	9.5		D-Gal, D-GalNAc	176
Chicken liver	3.1×10^4	2				Lactose	177
Chicken intestine	1.4×10^4					Lactose	177
Carcinoscorpius rotunda cauda	4.2×10^5	16				Neuraminic acid	178
Bothrops atrox (thrombolectin)	3.0×10^4	2				β-D-Gal	179
Sarcophaga peregrina larvae	1.9×10^5	6				D-Gal, lactose	180
Micro-organisms							
Aleuria aurantia	7.2×10^4	2	Nil	Nil	ABO	α-L-Fuc	181

Physical properties, including circular dichroism and fluorescence, have been examined for three lectins from *Abrus precatorius*.[182] There is a D-galactose-induced conformational change which buries several partially exposed tryptophan residues. A comparable analysis of the closely related *Ricinus* agglutinin revealed that its tryptophan residues are more deeply buried than those of the *Abrus* agglutinin and, unlike the *Abrus* agglutinin, there is no saccharide-induced change in tryptophan accessibility. The lectin has also been subjected to crystallographic analysis by *X*-ray diffraction and electron microscopy.[183] The molecule appears to consist of four similar domains grouped in a roughly planar diamond-shaped arrangement having a local intramolecular dyad axis.

Modeccin, the toxin purified from the roots of *Adenia digitata*, like other toxins consists of an A and a B subunit of which the former inhibits protein synthesis and the latter binds to cells. Two forms of modeccin have been isolated.[184] One is designated modeccin 4B, due to its affinity for agarose 4B and the other modeccin 6B binds to agarose 6B only if the gel is first subjected to mild acid hydrolysis.

Two agglutinins and two toxic ricins have been purified from the seeds of *Ricinus communis* by improved procedures based on ion-exchange chromatography and gel filtration.[185] Only one of the two ricins binds to agarose. The physicochemical properties of the four lectins are presented and their possible relationships to those previously described are discussed. The agglutinin from *R. communis* dissociates to lower molecular weight forms when heated in sodium dodecyl sulphate in the absence of reducing agents, but ricin is little affected by such treatment.[186] Protease inhibitors such as di-isopropylfluorophosphate, phenylmethanesulphonyl fluoride, and H_4 edta do not prevent this dissociation, although thiol-specific reagents are effective in the dissociation.

Treatment of ribosomes with either ricin or pokeweed anti-viral protein produce identical inhibitory effects of the elongation factor 2-catalysed translocation of acceptor site aminoacyl tRNA to the donor site.[187] Highly toxic cell-type specific reagents have been prepared by coupling of the proper receptor-binding moiety, a monophosphoryl-D-mannopentaose (4) to ricin.[188]

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P
↓
6
α-D-Man*p*-(1 → 3)-α-Man*p*-(1 → 3)-α-D-Man*p*-(1 → 3)-α-D-Man*p*-(1 → 2)-D-Man

(4)

The native ricin exhibits equal toxicity towards fibroblasts and HeLa cells. The added *monophospho-D-mannopentaosyl residues* provide ricin with the recognition factor common to lysosomal hydrolases and enable the modified ricin to bind to fibroblast D-mannosyl 6-phosphate receptor and inhibit protein synthesis in the cells *via* this receptor.

The A chain of ricin toxin has a higher affinity to lipids than the A fragment of diphtheria toxin.[189] When the A chain of ricin and the A fragment of diphtheria toxin are each conjugated with a subunit of *Wistaria floribunda* lectin through their intrinsic thiol groups, the toxicity of the ricin conjugate is much greater than that of the diphtheria conjugate. From association studies of the ricin A chain with lipid vesicles it appears that a hydrophobic region of this toxin subunit is essential for entry of the toxin into cells.

Human skeletal muscle contains two soluble lectins, which can be detected by their haemagglutination activities.[190] One of the lectins is specific for β-D-galactopyranosyl residues, as described for lectins from other mammalian species. The other lectin is strongly inhibited by heparin and heparan sulphate. A lectin from human liver, purified to apparent homogeneity, shows specificity for both terminal D-galactopyranosyl and 2-acetamido-2-deoxy-D-galactopyranosyl residues.[176] Although similar to the rabbit hepatic lectin in amino-acid composition and specificity for terminal D-galactopyranosyl residues, the human lectin differs in subunit composition. Human lung lectin is composed of two identical subunits whereas the corresponding rat lectin exists both as a dimer and a tetramer.[191] Both lectins bind to disaccharides or oligosaccharides containing terminal β-D-galactosyl residues. The carbohydrate moiety may be free (lactose), protein-bound (asialofetuin), or lipid-bound.

No differences in the binding specificity between rat hepatic and Kupffer cell receptors have been detected.[192]

A binding protein that recognizes D-mannosyl- and 2-acetamido-2-deoxy-D-glucosyl residues has been isolated from rabbit serum.[193] The serum binding protein is very similar to the α-D-mannan binding protein from rabbit liver.

The cell surface of peritoneal macrophages contains a receptor with specificity for D-galactosyl- and D-glucosyl-residues.[194] The receptor is able to mediate cell contacts between macrophages and erythrocytes.

[189] T. Uchida, E. Makada, and Y. Okada, *J. Biol. Chem.*, 1980, **255**, 6687.
[190] G. I. Franklin, F. S. Walsh, and E. J. Thompson, *FEBS Lett.*, 1980, **118**, 200.
[191] J. T. Powell, *Biochem. J.*, 1980, **185**, 123.
[192] H. Kolb, D. Vogt, L. Herbertz, A. Corfield, R. Schauer, and J. Schlepper-Schäfer, *Z. Physiol. Chem.*, 1980, **361**, 1747.
[193] Y. Kozutsumi, T. Kawasaki, and I. Yamashina, *Biochem. Biophys. Res. Commun.*, 1980, **95**, 658.
[194] Y. Nagamura and H. Kolb, *FEBS Lett.*, 1980, **115**, 59.

Antibodies to electrolectin, a lectin endogenous to embryonic skeletal muscle, have been used to study the distribution of electrolectin during myogenesis in the L_6 line of myogenic cells, and rat primary skeletal muscle cells as they undergo differentiation.[195]

Proteins (mol. wt. $1.0–6.0 \times 10^4$) with binding specificity for the fetal glycoprotein fetuin have been isolated from murine thymocytes and splenocytes by affinity chromatography on immobilized fetuin.[196] The binding specificity is probably directed to the oligosaccharide chains of the fetuin molecule. A lectin that may have a role in cell-matrix interactions is released into the medium of cultured chick embryo muscle cells after the occurrence of cell infusion.[197] The activity of this lectin is inhibited by glycosaminoglycan.

The spawn of the water snail, *Biomphalaria straminea*, contains agglutinins which are active against A and B normal erythrocytes.[198] Subgroups A_1 and A_2 may be differentiated with cells treated with proteolytic enzymes. A lectin has been detected in extracts of early cleavage stage amphibian *Rana pipiens* embryos that are capable of agglutinating formalinized sheep erythrocytes.[199] The apparent restriction of the amphibian embryo lectin to only the adhesive surfaces of the superficial cell suggests that it could play a role in the adhesive interactions of these cells.

The species-specific reactivity of a lectin from the sponge *Geodia cydonium* has been reported.[200] *Geodia* lectin agglutinates cells from the siliceous sponge *Tethya lyncurium*. This agglutination process leads to a reduction in viability as well as an inhibition of syntheses in these cells. A lectin has been isolated from extracts of several axenically-grown trophozoites of *Entamoeba histolytica* strains.[201] The lectin, which may mediate part of the adherence process of the amoeba, has a specificity similar to that found for wheat germ agglutinin, potato lectin, and *Datura stramonium* lectins.

The sugar specificity of haemagglutinins of soil bacteria for various erythrocytes has been reported.[202] All the haemagglutinins examined are inhibited by D-galactose and 2-acetamido-2-deoxy-D-galactose. The haemagglutination of human AB and horse erythrocytes by *Actinomyces viscosus* and *A. naeslundii* proceeds *via* a two-step mechanism.[203] Initially terminal neuraminic acid residues on the erythrocytes are removed by the action of a neuraminidase elaborated by the bacteria followed by lectin-like binding to exposed β-D-galactosyl residues on the erythrocytes. Strains differ in the extent to which they can perform the two functions, and this specificity may relate to their taxonomic classification.

[195] T. R. Podleski and I. Greenberg, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 1054.
[196] J. M. Decker, *Mol. Immunol.*, 1980, **17**, 803.
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[202] A. Takenaka, K. Oishi, and K. Aida, *J. Gen. Appl. Microbiol.*, 1979, **25**, 397.
[203] R. P. Ellen, E. D. Fillery, K. H. Chan, and D. A. Grove, *Infect. Immun.*, 1980, **27**, 335.

Commercially available samples of D-mannose derived from plant seeds may contain a low molecular weight protein-associated agglutinin for *Actinomyces viscosus* and *A. naeslundii* cells.[204] The agglutinin is thought to function *via* a 'lectin-like' selective affinity for the unique cell-wall sugar 6-deoxy-L-talose.

During fruiting-body formation of *Myxococcus xanthus* a major new development-specific protein is synthesized that has lectin-like activity.[205] This myxobacterial haemagglutinin binds to complex receptors that contain β-D-galactosyl residues. The observation that the reduced *O*-glycosidically linked trisaccharide from fetuin did not inhibit the lectin activity but the corresponding *O*-linked trisaccharide glycopeptide did inhibit is taken to suggest that the receptor may contain a peptide sequence in addition to the carbohydrate residues.

4 Collagen

Collagen–proteoglycan interaction in tissue sections has been measured by the reaction of the dye Sirius Red with the basic groups of collagen, after proteolytic digestion of proteoglycans.[206] The interaction of the two macromolecules is variable depending on the tissue and the species studied. Problems associated with the localization of collagens and proteoglycans in tendon using electron microscopy have been assessed.[207]

Collagen has been isolated from segments of thoracic aorta of normal and diabetic rats.[208] The extent of glycosylation of the collagen was estimated by a procedure which appears to be specific for hexoses bound to proteins by a ketoamine linkage. In this procedure, 5-hydroxymethylfurfural, generated from the carbohydrate moieties on heating under acidic conditions, is estimated with a 2-thiobarbituric acid reagent. Diabetic rats exhibited a significant increase in glycosylated collagen as compared to normal animals. The non-enzymic glucosylation of insoluble human tendon collagen increases with age of the individuals and increases markedly in juvenile onset diabetes.[209] The level of glycosylation of glomerular basement membrane collagen purified from diabetic rats is significantly greater than that of non-diabetic animals.[210] The increased levels of glycosylation, which occur *via* non-enzymic procedures, may alter structure-function relationships of the capillary filtration barrier.

The B chain of collagen from human placental tissue has been cleaved with cyanogen bromide and the resulting peptides have been characterized with regard to molecular weight and amino-acid composition.[211] The distribution of

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[211] R. K. Rhodes and E. J. Miller, *J. Biol. Chem.*, 1979, **254**, 12 084.

type I and type III collagens, glycosaminoglycans, and non-collagenous glycoproteins in calf skin has been reported.[212] The ratios of total glycosaminoglycans to collagen and of glycoproteins to collagen is higher in the upper dermis, but the ratio of dermatan sulphate to collagen remains constant. Antisera, which recognize specifically native type I and type II collagens and proteoglycan, have been used in immunofluorescence studies to investigate the distribution of collagens and proteoglycan in invertebral discs from adult and newborn pigs.[213] A component (mol. wt. 7.5×10^4) has been isolated from the collagenous D chain of porcine kidney cortices.[214] Seven peptides were released on treatment with cyanogen bromide and were characterized for amino-acid contents and molecular weights.

Investigation of the structure of the intestinal basement membrane of *Ascaris suum* demonstrates the existence of a collagenous domain.[215] The molecular features of this invertebrate basement membrane are much less complex than those of the vertebrate.

A polymeric form of the α_1-chain *C*-terminal peptide α_1CB6 (poly-α_1CB6) has been purified after digestion of insoluble bovine tendon type 1 collagen with cyanogen bromide.[216] Two unknown amino-acids in alkali hydrolysates of this material are believed to be components of the stable cross-link.[217] Concentration-dependent aggregation effects observed on gel filtration of this material, which contains residues of D-glucose and D-galactose, result from interaction with the agarose of the gel-filtration medium.

Very little, if any, carbohydrate is associated with the amino-terminal propeptides of either pro-α1 or pro-α2 chains of chick embryo tendon collagen.[218] Each carboxy-terminal propeptide in extracellular type 1 procollagen contains a single 'high mannose'-type oligosaccharide unit consisting of approximately eight to nine monosaccharides. The triple-helix formation in type III collagen and type III pN-collagen starts exclusively at the set of three disulphide bridges which link the three chains at the *C*-terminal end.[219] The growth of the helix proceeds from this single nucleus at a rather uniform rate in a zipper-like fashion. Collagen immunotyping in human liver has been achieved using specific antibodies to types I, III, IV, and AB collagens.[220]

The preparation of two structurally different collagenous components resembling type IV collagen from basement membrane collagen of bovine kidney cortices has been reported.[221] A continuous human tumor cell line, previously assumed to represent a fibrosarcoma, produces large amounts of base-

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[213] H. K. Beard, R. Ryvar, R. Brown, and H. Muir, *Immunology*, 1980, **41**, 491.
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[217] N. D. Light and A. J. Bailey, *Biochem. J.*, 1980 **189**, 111.
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[221] S. N. Dixit, *Eur. J. Biochem.*, 1980, **106**, 563.

ment membrane proteins.[222] This cell line was used to study several aspects of the chemistry and biochemistry of human type IV collagen. Smooth muscle cells from rat heart secrete extracellular matrix components, including glycoproteins and collagen.[223]

The main source of urinary hydroxy-L-lysyl glycosides is collagen of bone and skin.[224] The normal ratio of D-glucosyl-D-galactosyl-hydroxy-L-lysine to D-galactosyl-hydroxy-L-lysine demonstrates that both bone and skin collagens have a similar decreased turnover in rats exhibiting protein malnutrition.

Cockroach collagen contains a high carbohydrate content (8.8%).[225] A different pattern of peptides is released on treatment with cyanogen bromide from that of known collagens.

The cuticle collagen of clam worm (*Nereis virens*) contains the disaccharide 6-*O*-α-D-glucopyranosyluronic acid-D-mannose linked *O*-glycosidically to L-threonine.[226,227] Comparative studies on *Nereis* (a polychaete) and *Lumbricus* (an oligochaete) indicate the differences in the nature as well as the number of *O*-glycosidically linked carbohydrate units in the cuticle collagens of the two annelids. *Lumbricus* does not contain the acidic disaccharide but has more D-galactosyl-containing mono-, di-, and tri-saccharides.

Both procollagen and fibronectin respond to inhibition by the monovalent ionophore, monensin, in a similar fashion, accumulating in the same subcellular fractions in human fibroblasts.[228] Both glycoproteins share a common intracellular route before entering the Golgi region of the cell where inhibition takes place.

The chemical composition of bovine vitreous humour collagen fibres has been reported.[229] The α-chain fraction contains increased levels of D-glucose and D-galactose compared to the corresponding fraction from articular cartilage.

A new type of collagenous structure, 7S collagen, has been isolated from a mouse tumour basement membrane, mouse and human placenta, bovine lens capsule, and human kidney.[230] It has an amino-acid composition typical of basement membrane collagen and contains 22% carbohydrate mainly as D-glucopyranosyl-D-galactopyranosyl groups attached to hydroxy-L-lysine, although some residues of D-mannose and 2-amino-2-deoxy-D-glucose were also detected.

An O^5[2-*O*-α-D-glucopyranosyl-*O*-β-D-galactopyranosyl]-hydroxy-L-lysine glucohydrolase has been isolated from rat spleens.[231] The enzyme is responsible for catabolism of the hydroxy-L-lysine-linked disaccharide unit derived from

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[231] H. Hamazaki and K. Hotta, *Eur. J. Biochem.*, 1980, **111**, 587.

mammalian collagens. The regulation of the glycosylations of collagen hydroxy-L-lysine in chick embryo tendon and cartilage cells has been reported.[232] Rat kidney cortex contains an α-D-glucosidase with a high specificity for D-gluco-pyranosyl-D-galactopyranosyl-hydroxy-L-lysine.[233] However, a β-D-galactosidase, appropriate for the scission of D-galactopyranose from D-galactopyranosyl-hydroxy-L-lysine, does not appear to be present in this tissue.

Conflicting reports on the effects of tunicamycin upon collagen biosynthesis still appear.[234] When human fibroblasts in cell culture are used, tunicamycin impairs glycosylation of procollagen and a decreased content of carbohydrate occurs concomitantly with severe retardation of the intracellular movement of the protein and marked retardation of its secretion.

Changes in the articular cartilage collagen of dogs have been measured after the onset of osteoarthrisis.[235] The newly synthesized collagen is less glycosylated than in the controls. D-Galactopyranosyl-hydroxy-L-lysyl-D-gluco-pyranosyltransferase binds at least two Mn^{2+}.[236] The binding of the second Mn^{2+} decreases the K_m of UDP-D-glucose whereas the velocity of the enzyme at saturating concentrations of UDP-D-glucose is unaffected. It is likely that the enzyme operates at low and high Mn^{2+} concentrations through the ordered binding of Mn^{2+}, UDP-D-glucose and collagen, and Mn^{2+}, Mn^{2+}, UDP-D-glucose and collagen, respectively. In both cases the binding of Mn^{2+}, which occurs before that of UDP-D-glucose, is at thermodynamic equilibrium.

5 Glycogen

Rat liver glycogen, containing residues of 2-amino-2-deoxy-D-glucose, synthesized *in vivo* after injection of 2-amino-2-deoxy-D-galactose, has been examined for its potential resistance to phosphorylysis and the consequent ability of incorporated 2-amino-2-deoxy-D-glucose to act as a metabolic block causing a glycogen-storage disease.[237] The amino-sugar is released from this glycogen by phosphorylase as 2-amino-2-deoxy-α-D-glucose 1-phosphate and can be reincorporated into glycogen by the same enzyme, thus no metabolic block in glycogenolysis occurs.

The phosphohydrolase activities in the microsomes of fresh liver from normal individuals and patients with type III and type Ib glycogen-storage disease have been examined.[238] Type Ib glycogen-storage disease is caused by a defect in the D-glucose 6-phosphate translocase of the microsomal D-glucose 6-phosphatase system.

A sensitive method for the activity staining of (1 → 4)-linked α-D-glucan branching enzyme (EC 2.4.1.18, 1,4-α-D-glucan: 1,4-α-D-glucan 6-D-gluco-

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pyranosyltransferase) on polyacrylamide gels has been developed.[239] Rabbit skeletal muscle glycogen phosphorylase *b* is entrapped within running gels before electrophoresis in order to catalyse the co-operative synthesis of glycogen with branching enzyme in the absence of primer glycogen. The glycogen branching enzyme purified from rabbit skeletal muscle is a monomeric protein (mol. wt. 6.0×10^4).[240]

Phosphorylase kinase associated with liver glycogen has regulatory features similar to muscle phosphorylase kinase.[241] Porcine polymorphonuclears and lymphocytes show a lower glycogen content than human leucocytes, although glycogen synthase activity in leucocytes from both sources is similar.[242] Human polymorphonuclears contain an inhibitor for the conversion of porcine polymorphonuclear glycogen synthase D to I. The inter-relationship between polysaccharide and pyridoxal phosphate binding sites in plant and animal phosphorylases (EC 2.4.1.1) from potato tubers and rabbit skeletal muscle has been studied by affinity electrophoresis.[243] In each case the active site is adjacent to the co-factor site. Structural changes in glycogen phosphorylase have been revealed by cross-linking with bifunctional di-imidates.[244] The interconversion of the two forms of glycogen phosphorylase and glycogen synthase has been investigated in the adipose tissue of re-fed animals with a methodology that makes it possible to measure phosphorylase *a* with little or no interference from phosphorylase *b*.[245]

A modified form of glycogen synthetase D (EC 2.4.1.11) exists in the liver of the adrenalectomized and fasted rat.[246] The enzyme is altered in such a way that its phosphate moiety is no longer accessible to synthetase phosphatase and thus it cannot be converted to synthetase I. Lithium ions stimulate net glycogen production from D-glucose and glycogen synthetase activity in fasted rats, probably by a mechanism in which D-glucose 6-phosphate is diverted from glycolysis and towards glycogen synthesis.[247]

The catalytic properties of liver glycogen synthases of adult and newborn rats have been compared.[248] Glycogen synthetase *a*, the dephosphorylated enzyme, is that which is physiologically active *in vivo* when liver glycogen is synthesized. Glycogen synthetase from the newborn, which does not show an endogenous *b* to *a* transformation, has catalytic characteristics similar to the adult *a*-type synthetase. The divalent cation dependence of rat liver glycogen synthetase phosphatase activity has been investigated.[249]

[239] K. Satoh and K. Sato, *Anal. Biochem.*, 1980, **108**, 16.
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6 Glycosaminoglycuronans, Glycosaminoglycans, and their Protein and Peptide Derivatives

Reviews on glycosaminoglycans have dealt with their structure and function,[250] and the role of vessel proteoglycans and thrombogenesis.[251] The structure and biological activity of heparin and other glycosaminoglycans have been reviewed.[252] An overview of glycosaminoglycans and lipoprotein metabolism has been published.[253]

Analysis. – Proteoglycans trapped in polyacrylamide gel particles have been used as substrates for both proteases and polysaccharidases.[254] Enzymes can diffuse into these particles, and the digestion products that diffuse out are quantitated by reaction with a metachromatic dye or by assay of their uronic acid content with carbazole reagent. An immunoassay for purified link protein of proteoglycans from bovine nasal cartilage has been developed using Laurell rocket electrophoresis.[255]

A procedure has been described enabling measurement of microgram quantities of cartilage glycosaminoglycans by using microscopic sections, 10 μm thick, from anatomical, defined zones of heterogeneous connective tissues.[256] Glycosaminoglycans separated by electrophoresis on cellulose acetate have been stained using Alcian blue in the presence of different concentrations of magnesium chloride.[257] The procedure results in elution of those glycosaminoglycans whose critical electrolyte concentration is below that of the magnesium chloride solution, leaving glycosaminoglycans with higher critical electrolyte concentrations stained on the strip.

Glycosaminoglycans, solubilized from articular cartilage and synovial fluid by proteolysis, have been separated by electrophoresis, allowing microgram quantities of individual polysaccharides to be detected.[258] The electrophoretic separation of glycosaminoglycans in cationic media may not represent a normal electrophoresis but has more in common with phenomena that are associated with electrofocusing techniques.[259] Caution in interpreting data from this and similar electrophoretic techniques has been advocated. Heparin and other glycosaminoglycans have been identified following separation by discontinuous electrophoresis.[260]

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[259] E. M. Noordegraaf, *Int. J. Biol. Macromol.*, 1979, **1**, 93.
[260] P. Bianchini, H. B. Nader, H. K. Takahashi, B. Osima, A. H. Strauss, and C. P. Dietrich, *J. Chromatogr.*, 1980, **196**, 455.

An automated gel-filtration procedure has been used for the estimation of molecular weights of human aorta glycosaminoglycans.[261] On gel filtration hyaluronic acid resolved into two components (mol. wt. 3.8×10^4 and 8.0×10^3). Heparan sulphate was shown to have a molecular weight of 2.2×10^4, and chondroitin 6-sulphate and dermatan sulphate gave a similar value (1.8×10^4).

A method for the detection of urinary glycosaminoglycans by radial diffusion in gels followed by precipitation and staining has been described.[262] *N*-Sulphated glycosaminoglycans have been characterized by a rapid and multisample nitrous acid treatment during an electrophoretic run.[263] When applied to biological samples it was found unnecessary to use dialysis or gel filtration prior to electrophoresis.

An automated periodate–thiobarbituric acid assay of Δ-4,5-unsaturated uronic acids that avoids extraction of chromogen has been developed and applied to the analysis of hyaluronic acid and chondroitin sulphates.[264] By measuring the adsorption of ^{125}I-labelled hyaluronate-binding cartilage proteins to a hyaluronate-substituted gel in the presence of competing free hyaluronate, nanogram amounts of free hyaluronic acid have been determined.[265]

Hybrid tetra-, hexa-, octa-, and deca-saccharides prepared from chondroitin 4- and 6-sulphates have been separated by anion-exchange h.p.l.c.[266] The enzymic conversion of hyaluronic acid to oligosaccharides has been followed by h.p.l.c. of the released oligosaccharides.[267] Mono-, di-, tetra-, and hexa-saccharides were separated from higher oligosaccharides. The separation is achieved by size exclusion on three silica-gel-packed columns. A mobile phase with the same composition as the medium used for the enzymic reaction is advantageous.

H.p.l.c. has been used for the separation of four sulphated disaccharides, 4-*O*-(α-L-idopyranosyluronic acid 2-sulphate)-2,5-anhydro-D-mannitol 6-sulphate, 4-*O*-(α-L-idopyranosyluronic acid 2-sulphate)-2,5-anhydro-D-mannitol, 4-*O*-(α-L-idopyranosyluronic acid)-2,5-anhydro-D-mannitol 6-sulphate and 4-*O*-(β-D-glucopyranosyluronic acid)-2,5-anhydro-D-mannitol 6-sulphate, arising from treatment of heparin with nitrous acid followed by reduction of the products with sodium borohydride.[268]

The trisaccharide (5), which has been prepared from heparin by treatment with nitrous acid, sodium borohydride reduction, and partial acid hydrolysis, acts as a substrate for acetyl coenzyme A : 2-amino-2-deoxy-α-D-glucoside *N*-acetyltransferase, an enzyme that is deficient in the Sanfilippo syndrome.[269] This enzyme participates in the degradation of *N*-sulphated 2-amino-2-deoxy-D-glucopyranosyl residues in heparan sulphate and heparin, after the removal of the *N*-sulphate groups by sulphamidase.

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[265] A. Tengblad, *Biochem. J.*, 1980, **185**, 101.
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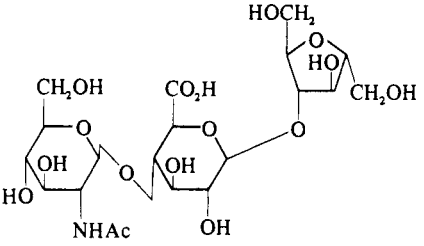

(5)

Heparin has been chromatographed on columns of immobilized L-lysine.[270] The relevance of the fractionated products to thrombin and antithrombin III binding are discussed.

An assay for 2-acetamido-2-deoxy-D-glucosyl *N*-deacetylase, based on the liberation of [^{3}H]acetate from *N*-[^{3}H]acetylated heparin, has been reported.[271] The enzyme, isolated from a microsomal fraction of mouse mastocytoma tissue, was active only on polysaccharides structurally related to heparin.

Procedures for the enzymic analysis of acidic glycosaminoglycans,[272] the constituent analysis of glycosaminoglycans,[273] the desulphation of glycosaminoglycan sulphates,[274] and the de-*N*-sulphation of glycosaminoglycan sulphates,[275] have been published.

Proteoglycans. – By using a vertical tube rotor for isopycnic centrifugation, the time taken for the isolation of proteoglycans from various tissues can be shortened and the resolution of proteoglycans improved.[276] Antibodies against the unsaturated oligosaccharide products of chondroitin sulphate lyase ABC digestion of bovine nasal cartilage recognize the β-D-gluco-4-enepyranosyluronic acid residue linked to 2-acetamido-2-deoxy-D-galactopyranosyl 4-sulphate residues.[277] The antibodies will be of value in the identification of chondroitin 4-sulphate-containing proteoglycans in tissues. Antibody binding to both the monomeric and aggregated forms of sulphated proteoglycans from chick embryonic sternal cartilage has been demonstrated.[278]

Some important structural features of cartilage proteoglycans have been measured by radioimmunoassay using antibodies specifically reacting with the link proteins, the hyaluronic acid in binding region and chondroitin sulphate-peptides.[279] Electric birefringence studies on cartilage proteoglycan confirm the

[270] M. W. C. Hatton, L. R. Berry, H. Kaur, A. Koj, and E. Regoeczi, *Can. J. Biochem.*, 1979, **57**, 1183.
[271] J. Reisenfeld, M. Höök, and U. Lindahl, *J. Biol. Chem.*, 1980, **255**, 922.
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[274] K. Nagasawa and Y. Inoue, in ref. 272, p. 287.
[275] K. Nagasawa and Y. Inoue, in ref. 272, p. 291.
[276] B. Radhakrishnamurthy, F. Smart, and G. S. Berenson, *Prep. Biochem.*, 1980, **10**, 151.
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[278] K. J. Sparks, P. L. Lever, and P. F. Goetinck, *Arch. Biochem. Biophys.*, 1980, **199**, 579.
[279] J. Wieslander and D. Heinegard, *Biochem. J.*, 1980, **187**, 687.

existence of dimers under conditions of low concentration and ionic strength.[280] A dependence of the relative proportions of dimer and homopolymer on the solution concentration was noted.

The products released by digestion of proteoglycans with clostripapain, a protease from *Clostridium histolyticum*, have revealed some new properties of these molecules.[281] Several distinct molecular weight intermediates (1.25×10^5, 1.0×10^5, and 8.5×10^4) containing hyaluronic acid-binding sites have been identified.

Proteoglycans have been isolated from the human meniscus and compared with those of human articular cartilage.[282] Detectable differences in the composition of the proteoglycans from the two tissues include the presence of dermatan sulphate in the meniscus preparation. Hyaluronic acid, heparan sulphate, chondroitin 4- and 6-sulphates, and dermatan sulphate have been identified from proteolytic digests of the human greater omentum.[283] The relative proportion of the hyaluronic acid fraction was found to increase with age, whereas that of the chondroitin sulphates decreased.

The degradation of proteoglycan aggregate in cartilage matrix by chondrocytes occurs in a pericellular region mediated by the action of proteinases.[284] The extracellular catabolism of matrix macromolecules by soluble tissue proteinases seems unlikely. Myogenic cells release into their culture medium a glycoprotein complex that mediates cellular adhesion.[285] Glycosaminoglycans, and several proteins, including fibronectin and collagen, have been detected in the complex.

A comparative study of proteoglycans isolated from bovine lung, tracheal mucosa, and aorta in 4 M guanidinium chloride indicate that only the chondroitin sulphate proteoglycans have high molecular weights.[286] The L-iduronic acid-containing species have molecular weights of at least an order of magnitude lower.

Proteoglycans from bovine lung have been isolated.[287] Their effects on platelet aggregation, and on certain biological properties such as anti-coagulant activity, are described. The core protein from bovine nasal proteoglycan has a flexible extended chain containing β-turns, with the *C*-terminal region lying in the region of the hyaluronic acid backbone.[288] Enzymatic cleavage of the core protein, which occurs in proteoglycan turnover, ageing, and degenerative diseases, probably does not occur by a stepwise cleavage from the *N*-terminus but by a more drastic degradation process.

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[287] B. Radhakrishnamurthy, F. Smart, E. R. Dalferes, and G. S. Berenson, *J. Biol. Chem.*, 1980, **255**, 7575.
[288] A. G. Walton, H. G. Volger, and E. N. Jaynes, *Int. J. Biol. Macromol.*, 1979, 1, 89.

The solubility and functional properties of the link protein from bovine nasal cartilage has been studied in several associative solvents.[289] A soluble form of the link protein, with a sedimentation coefficient of 8S, has been isolated and shown to possess the functional capacity to associate noncovalently with proteoglycan monomers and hyaluronate to form proteoglycan aggregates and to increase the stability of these aggregates.

The use of caesium sulphate as a gradient-forming salt, instead of caesium chloride, to give improved separations of proteoglycans from bovine nasal cartilage has been advocated.[290] The distribution of the link proteins in different proteoglycan extracts may be followed. Using monospecific antisera and immunofluorescence microscopy, proteoglycan monomer and link proteins have been identified throughout the extracellular matrix of bovine articular cartilage.[291]

Amino-acid sequencing of the two link proteins from bovine nasal cartilage proteoglycan complex suggest a possible structural identity (or at least high homology) of the two link proteins.[292] Conditions have been established for separation of proteoglycan from a bovine nasal septum extract by associative rate zonal sedimentation on a sodium chloride gradient.[293]

Chondroitin sulphate proteoglycan subunits, but not keratan sulphate proteoglycan subunits, from bovine cornea interact with collagen immobilized on macroporous agarose.[294] Digestion of bovine corneal proteoglycans with chondroitin sulphate chains are mainly responsible for the interaction with collagen. A high molecular weight heparin proteoglycan has been isolated from bovine duodenal mucosa.[295] The heparin released after proteolytic digestion possesses up to three-fold less lipoprotein lipase-releasing activity and a three-fold more anti-coagulant activity than the intact proteoglycan. Proteoglycans from bovine nucleus pulposus contain longer keratan sulphate chains than those from bovine nasal cartilage, as shown by gel chromatography of the products obtained by digestion of the original proteoglycans with chondroitin sulphate lyase ABC, trypsin and chymotrypsin.[296]

The equilibrium-binding behaviour of hyaluronate towards pig laryngeal cartilage proteoglycans has been examined.[297] Each proteoglycan monomer binds only one hyaluronate oligosaccharide molecule of about 18 monosaccharide residues. A hyaluronate oligosaccharide containing 56 monosaccharide residues was found to be capable of binding to more than one proteoglycan molecule.

[289] L. H. Tang, L. Rosenberg, A. Reiner, and A. R. Poole, *J. Biol. Chem.*, 1979, **254**, 10523.
[290] F. Bonnet, J. P. Périn, and P. Jollès, *Biochim. Biophys. Acta*, 1980, **623**, 57.
[291] A. R. Poole, I. Pidoux, A. Reiner, L. H. Tang, H. Choi, and L. Rosenberg, *J. Histochem. Cytochem.*, 1980, **28**, 621.
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[293] P. Hoffman, *J. Biol. Chem.*, 1979, **254**, 11854.
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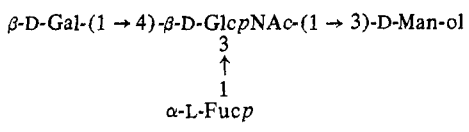
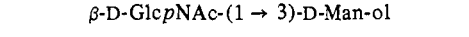
[296] D. Heinegard, I. Axelsson, and S. Ineroi, *Biochim. Biophys. Acta*, 1980, **581**, 122.
[297] I. A. Nieduszynski, J. K. Sheehan, C. F. Phelps, T. E. Hardingham, and H. Muir, *Biochem. J.*, 1980, **185**, 107.

Proteoglycan populations of baboon (*Papio papio*) cartilages from different anatomical sites have been purified by ion-exchange chromatography and assessed by gel electrophoresis.[298] Proteoglycans derived from different cartilages and different anatomical sites exhibit a similar basic pattern. The proteoglycans of the Rhesus monkey corneal stroma synthesized in organ culture are similar to their counterparts in the stroma, although they are synthesized in different proportions *in vitro* than they are accumulated *in vivo*.[299] The corneal stroma contains two proteoglycans: a chondroitin sulphate–dermatan sulphate proteoglycan (mol. wt. 1.0×10^5–1.5×10^5) containing 70% protein and a keratan sulphate proteoglycan (mol. wt. 4.0×10^4–7.0×10^4) containing 74% protein. Amino-acid analyses of these proteoglycans indicate that their protein cores are also different. Proteoglycans from rachitic rat growth cartilage have been studied by ultracentrifugal analysis.[300] Only one link protein (mol. wt. 4.5×10^4) was detected, a finding similar to that for swarm rat chondrosarcoma but in contrast to that for bovine nasal proteoglycan. The presence of chondroitin 4-sulphate associated with proteoglycan has been established in rat incisor hard tissue.[301] Differences between proteoglycans isolated from control and fluoride-fed animals is reported. Proteoglycans in rat tail tendon and rabbit achilles tendon have been localized by electron microscopy with a cationic phthalocyanin-like dye based on chinchomeronic acid.[302] The patterns observed have led to the proposal of a model based on protein–protein interactions between the proteoglycan core and collagen. Mild alkaline borohydride treatment of rat brain proteoglycan has released D-mannitol and several oligosaccharides terminated in D-mannitol, (6) and (7).[303] Evidence was also obtained for the occurrence of a



(6)



(7)

neuraminic acid-containing oligosaccharide and another (possibly sulphated) oligosaccharide, both having the structure of oligosaccharide (7) at their proximal ends. These novel alkali-labile oligosaccharides appear to be integral components of the proteoglycan, rather than being associated with some other co-purified material. Endogenous heparan sulphate proteoglycans have been shown to be associated with the cell-surface receptors of rat hepatocytes.[304]

[298] V. Staneseu, P. Maroteaux, and E. Sobczak, *Biochim. Biophys. Acta*, 1980, **629**, 371.
[299] J. R. Hassell, D. A. Newsome, and V. C. Hascall, *J. Biol. Chem.*, 1979, **254**, 12 346.
[300] J. C. Pita, F. J. Muller, S. M. Morales, and E. J. Alarcon, *J. Biol. Chem.*, 1979, **254**, 10313.
[301] J. W. Smalley and G. Embery, *Biochem. J.*, 1980, **190**, 263.
[302] J. E. Scott, *Biochem. J.*, 1980, **187**, 887.
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[304] L. Kjellen, A. Oldberg, and M. Höök, *J. Biol. Chem.*, 1980, **255**, 10407.

Heparin-like polysaccharides of high sulphate content are preferred as ligands. The ability of heparin to displace endogenous heparin sulphate increases with increasing molecular weight. A model for measuring the rate of release of sulphated compounds from extracellular cartilage matrix in organ culture has been developed.[305] These cultures contain sufficient endogenous enzyme to degrade and release their cartilage proteoglycans, but when treated with exogenously added hyaluronidase or protease, they show a much more rapid loss of sulphated macromolecules. Factors that alter the release of proteoglycans in culture, the kinetics of their release, and the nature of the released sulphated macromolecules were studied.

The recoveries of glycosaminoglycans following extraction of adult human articular cartilage with 10 separate solvents have been recorded.[306] Chondroitin 6-sulphate, dermatan sulphate, and hyaluronate affect the kinetics of fibril formation and the thermal stability of the *in vitro* assembly of collagen fibrils.[307] Under physiological conditions of ionic strength and pH the glycosaminoglycans influence the kinetics of collagen precipitation but not the thermal stability of the *in vitro*-assembled fibrils. Glycosaminoglycans interact with and stabilize fibronectin–collagen complexes.[308] Heparin as well as highly sulphated heparan sulphate and hyaluronic acid bring about the agglutination of plastic beads coated with gelatin when fibronectin is present. Experimental enthalpies of mixing of glycosaminoglycans with sodium chloride agree reasonably well with estimates from the finite line charge theory and are consistent, within the framework of that theory, with previous data for coefficients of sodium chloride in glycosaminoglycan solutions.[309]

Methyl glycosides of di- and higher oligo-saccharides have been prepared from glycosaminoglycuronans by solvolysis with dimethyl sulphoxide containing methanol.[310] Cleavage of the ester sulphate groups and of the 2-amino-2-deoxy-glycoside bonds of chondroitin sulphates and dermatan sulphate occur. For hyaluronic acid, a similar solvolytic depolymerization proceeds in the presence of pyridine–sulphur trioxide. The uronic components are neither degraded nor esterified, and the *N*-acetyl groups are markedly more stable under these solvolytic conditions.

Changes in the proportions of different types of glycosaminoglycans during the development of skeletal myogenic cells have been reported.[311] Total concentration of glycosaminoglycans in rabbit lung alters with age, in addition to changes in the levels of individual glycosaminoglycans having specific physiological functions.[312]

[305] C. B. Caputo and L. G. Raisz, *Connect. Tissue Res.*, 1980, **7**, 81.
[306] R. J. Elliott and D. L. Gardner, *Biochem. Soc. Trans.*, 1980, **8**, 446.
[307] J. M. Snowden and D. A. Swann, *Biopolymers*, 1980, **19**, 767.
[308] E. Ruoslahti and E. Engvall, *Biochim. Biophys. Acta*, 1980, **631**, 350.
[309] R. L. Cleland, *Biopolymers*, 1979, **18**, 2673.
[310] Y. Inoue and K. Nagasawa, *Carbohydr. Res.*, 1980, **85**, 107.
[311] M. Pacifici and M. Molinaro, *Exp. Cell Res.*, 1980, **126**, 143.
[312] B. Radhakrishnamurthy, J. Williams, and G. S. Berenson, *Proc. Soc. Exp. Biol. Med.*, 1980, **164**, 287.

The glycosaminoglycans that are bound to the plasma membranes of liver and renal tubule cells of the guinea pig have been identified.[313] Plasmalemma of renal tubule cells contain more total glycosaminoglycans, hyaluronic acid, chondroitin 4- and 6-sulphates, and less dermatan sulphates and heparin sulphates than liver plasma membranes. Glycosaminoglycans have been recognized as being integral constituents of renal glomerular basement membrane.[314] The ability of isolated rat glomeruli to incorporate radioactive sulphate *in vitro* was also demonstrated. A predominant feature of the changing glycosaminoglycan pattern as exponentially growing and non-proliferating BHK-21/C13 cells become confluent is a decrease in the proportion of chondroitin sulphates present.[315] The results also demonstrate the presence of dermatan sulphate.

Glycosaminoglycan–protein complexes of the comb, wattle, skin, and heart of the laying hen contain between 10–50% of carbohydrate, most of which is amino-sugar and uronic acid.[316] The corresponding complexes of the liver, kidney, spleen, lung, heart, ovaries, egg yolk, and different parts of the oviduct contain low levels of carbohydrate. The uptake and incorporation of 2-amino-2-deoxy-D-[1-^{14}C]glucose and 2-amino-2-deoxy-D-[1-^{14}C]galactose into these complexes is reported.[317]

The ^{13}C n.m.r. spectra of hyaluronate and chondroitin sulphates have been interpreted to give further evidence of an alkali-induced conformation change.[318] Interactions between the carbohydrate chains of hyaluronate and chondroitin sulphate are indicated from studies of the agglutination of hyaluronic acid-derivatized beads with chondroitin sulphate-derivatized beads.[319] Lipoprotein lipase (EC 3.1.1.34), which has previously been shown to bind to immobilized heparin, also binds to heparan sulphate and dermatan sulphate and to a lesser extent to chondroitin sulphate.[320] The structural features required for binding of various glycosaminoglycans to the enzyme have been studied.

Chondroitin sulphates have been extracted from bovine and whole nasal septum by treatment with alkaline sodium borohydride.[321] The reducing-end xylitol of the glycosaminoglycans was then determined by g.l.c., thus enabling the molecular weight of the glycosaminoglycans to be obtained. Chondroitin 4-sulphate has been degraded by an isolated perfused rat liver preparation.[322] The amino-sugars are secreted by the liver in a low molecular weight form and as components of glycoproteins.

[313] D. Lis and B. Munis, *J. Supramol. Struct.*, 1979, **12**, 15.
[314] M. Cohen, *Biochem. Biophys. Res. Commun.*, 1980, **92**, 343.
[315] H. H. K. Watson, M. Edward, F. B. Williamson, and W. F. Long, *Biochem. Soc. Trans.*, 1980, **8**, 134.
[316] K. F. Ng Kwai Hang and P. A. Anastassiadis, *Can. J. Biochem.*, 1980, **58**, 319.
[317] K. F. Ng Kwai Hang and P. A. Anastassiadis, *Can. J. Biochem.*, 1980, **58**, 325.
[318] S. M. Bociek, A. H. Darke, D. Welti, and D. A. Rees, *Eur. J. Biochem.*, 1980, **109**, 447.
[319] E. A. Turley and S. Roth, *Nature (London)*, 1980, **283**, 268.
[320] G. Bengtsson, T. Olivecrona, M. Höök, J. Riesenfeld, and U. Lindahl, *Biochem. J.*, 1980, **189**, 625.
[321] H. Yamaguchi, *J. Biochem. (Jpn.)*, 1980, **87**, 969.
[322] A. D. Macnicholl, F. S. Wusteman, P. J. Winterburn, G. M. Powell, and C. G. Curtis, *Biochem. J.*, 1980, **186**, 279.

Commercial samples of heparin have been tritiated by reduction with sodium borotritide.[323] It is believed that incorporation takes place by reduction of the small proportion (4–6%) of heparin molecules possessing a terminal monosaccharide. The behaviour of the labelled heparin compares closely with that of the native heparin on chromatography on columns of immobilized antithrombin III and immobilized thrombin. Heparin (mol. wt. 2.0×10^4) isolated from human lung fragments shows heterogeneity of binding to human antithrombin in a manner analogous to that observed with porcine heparin.[324] In addition, heparin (mol. wt. 6.0×10^4) has been isolated from dispersed enriched populations of human lung mast cells and shown to contain glycosaminoglycan side-chains (mol. wt. 2.0×10^4). The distribution of heparin in 12 tissues of eight mammalian species has been reported.[325] Heparin is present in high amounts in the lymph node and thymus of most species and is absent in rabbit tissues.

Heparan sulphates from beef lung have been fractionated and characterized following periodate oxidation, alkaline elimination, re-oxidation, and deaminative cleavage.[326] The studies have confirmed and provided more detail of the complex structure of this polysaccharide.

Heparin, heparan sulphate, and various derivatives thereof have been oxidized with periodate at pH 3.0 and 4 °C and at pH 7.0 and 37 °C.[327] Whereas oxidation under the latter conditions destroys all the nonsulphated uronic acids, treatment with periodate at low pH and temperature causes selective oxidation of uronic acid residues. The reactivity of the uronic acid residues depends on the nature of neighbouring 2-amino-2-deoxy-D-glucopyranosyl residues. D-Glucopyranosyluronic acid residues are susceptible to oxidation when flanked by *N*-acetylated amino-sugars but resistant when adjacent residues are either unsubstituted or *N*-sulphated. L-Iduronic acid residues in their natural environment (2-deoxy-2-sulphoamido-D-glucose) are resistant to oxidation, whereas removal of the *N*-sulphate groups renders a portion of these residues sensitive to periodate. Oxidized uronic acid residues in heparin-related glycans have been cleaved by alkali, producing a series of oligosaccharide fragments.

Further structural characterization of L-iduronate- and D-glucuronate-containing sequences in aggregating and nonaggregating chains of heparan sulphate has been reported.[328] In heparan sulphate III an alternating or mixed arrangement of L-idopyranosyluronic acid and D-glucopyranosyluronic acid residues is achieved mainly *via* the presence of alternating 2-acetamido-2-deoxy-D-glucopyranosyl and 2-deoxy-2-sulphamido-D-glucopyranosyl residues. Evidence

[323] M. W. C. Hatton, L. R. Berry, R. Machovich, and E. Regoeczi, *Anal. Biochem.*, 1980, **106**, 417.

[324] D. D. Metcalfe, R. A. Lewis, J. E. Silbert, R. D. Rosenberg, S. I. Wasserman, and K. F. Austen, *J. Clin. Invest.*, 1979, **64**, 1537.

[325] H. B. Nader, M. K. Takahashi, A. H. Straus, and C. P. Dietrich, *Biochim. Biophys. Acta*, 1980, **627**, 40.

[326] L. A. Fransson, I. Sjöberg, and B. Havsmark, *Eur. J. Biochem.*, 1980, **106**, 59.

[327] L. A. Fransson, A. Malmström, I. Sjöberg, and T. N. Huckerby, *Carbohydr. Res.*, 1980, **80**, 131.

[328] L. A. Fransson, B. Havsmark, I. A. Nieduszynski, and T. N. Huckerby, *Biochim. Biophys. Acta*, 1980, **633**, 95.

for a calcium-induced co-operative conformational transition of heparin has been reported.[329] The characteristics of the complex allow suggestions to be made regarding the backbone structure of the glycosaminoglycan. It appears to be an example of a polysaccharide undergoing a cation-induced co-operative intramolecular disorder–order process.

The chiroptical properties of heparin in salt-free water solutions at various degree of neutralization and for different counter-ions have been reported.[330] O.r.d. and c.d. data are discussed on the basis of a randomly coiled structure for macromolecules composed of rigid heterocyclic repeating units that are independent of each other in so far as electronic transitions of chromophore groups contributing to optical activity are concerned.

The polyelectrolytic properties of heparin and of some heparinates in salt-containing systems (alkali and alkaline-earth chlorides) have been monitored by c.d. spectrometry.[331] This technique can distinguish carboxy from sulphate groups, in so far as their ionization and the nature of their counter-ions are concerned. Using the line-charge model of Manning and, where appropriate, a form of empirical corrections, the ion-condensation phenomenon for four purified calcium heparin preparations and similar calcium heparin–calcium chloride mixtures in aqueous solutions have been reported.[332] This has been expressed in terms of the single-ion activity coefficient of the total calcium counter-ion present.

Controlled sedimentation of its barium salt from water–ethanol mixtures in a preparative ultracentrifuge has been used for the purification of hog mucosal heparin.[333] Heparin samples from different sources, which have been subjected to cellulose acetate electrophoresis, appear to consist of families of well differentiated species.[334]

Self-associating heparan sulphate from bovine lung contains both D-glucopyranosyluronic acid and L-idopyranosyluronic acid-containing repeating units, which are arranged in an alternating or mixed fashion.[335] These characteristics are analogous to those observed with self-associating dermatan sulphate species.[336]

Heparin has been fluorescently labelled in a manner that does not alter the functional properties of the polysaccharide.[337] The labelled polysaccharide has been used in conjunction with fluorescence-polarization spectroscopy to monitor the binding of heparin to the L-serine proteases thrombin, factor XIa, factor Xa, and plasmin. The stoicheiometry and dissociation constants of the

[329] J. Boyd, F. B. Williamson, and P. Gettins, *J. Mol. Biol.*, 1980, **137**, 175.
[330] C. Villiers, C. Braud, and M. Vert, *Carbohydr. Res.*, 1980, **83**, 335.
[331] C. Braud, C. Villiers, and M. Vert, *Carbohydr. Res.*, 1980, **86**, 165.
[332] D. J. Wedlock, G. P. Diakun, H. E. Edwards, G. O. Phillips, and J. C. Allen, *Biochim. Biophys. Acta*, 1980, **629**, 530.
[333] L. Ayotte, E. Mushayakarara, and A. S. Perlin, *Carbohydr. Res.*, 1980, **87**, 297.
[334] P. Oreste and G. Torri, *J. Chromatogr.*, 1980, **195**, 398.
[335] L. A. Fransson, I. A. Nieduszynski, and J. K. Sheehan, *Biochim. Biophys. Acta*, 1980, **630**, 287.
[336] L. A. Fransson and L. Coster, *Biochim. Biophys. Acta*, 1979, **582**, 132.
[337] R. E. Jordan, G. M. Oosta, W. T. Gardner, and R. D. Rosenberg, *J. Biol. Chem.*, 1980, **255**, 10073.

interactions have been measured. The kinetics of inactivation of the four proteases by antithrombin as a function of heparin concentration have also been measured.[338] Evidence shows that the direct binding of heparin to antithrombin is probably responsible for the polysaccharide-dependent acceleration of hemostatic enzyme-inhibitor reactions.

Heparin has been fractionated by ion-exchange chromatography with gradient elution in a fully automated system.[339] The system is suitable for the characterization on an analytical scale and fractionation on a preparative scale. Heparin (mol. wt. 2.0×10^4) is more reactive in inducing platelet aggregation than is the corresponding polysaccharide fraction of lower molecular weight (7.0×10^3).[340] Anti-coagulant activity does not parallel the platelet reactivity of the heparin fractions. An octasaccharide (8) representing the antithrombin binding site has been isolated from pig mucosal heparin following treatment with nitrous acid.[341] The 2,5-anhydromannitol residue corresponds to an *N*-sulphated

$$\beta\text{-L-Id}p\text{A-}(1\rightarrow4)\text{-}\alpha\text{-D-Glc}p\text{NAc-}(1\rightarrow4)\text{-}\beta\text{-D-Glc}p\text{A-}(1\rightarrow4)\text{-}\alpha\text{-D-Glc}p\text{NSO}_4$$

$$\text{-}(1\rightarrow4)\text{-}\beta\text{-L-Id}p\text{-}(1\rightarrow4)\text{-}\alpha\text{-D-Glc}p\text{NSO}_4\text{-}(1\rightarrow4)\text{-}\beta\text{-L-Id}p\text{A-}(1\rightarrow4)\text{-2,5-anhydromannitol}$$

$$\text{O-6} = \text{H or SO}_4 \text{ in residues 2, 4, 6, and 8}$$

(8)

2-amino-2-deoxy-D-glucopyranosyl residue in the intact polysaccharide. The interaction of heparin with plasma proteins has been studied by gel chromatography using elution-profile analysis to determine the binding constants.[342] Heparin purified by chromatography on immobilized antithrombin has a greater affinity for antithrombin and a greater anti-coagulant activity than unfractionated heparin, but the same K_i for thrombin (using a peptide nitroanilide substrate). Heparin purified on immobilized thrombin also has the same K_i for thrombin as unfractionated heparin, but a lower affinity for antithrombin and a lower anti-coagulant activity. These findings suggest that heparin binds to antithrombin with a greater specificity than it does to thrombin. The property of immobilized L-lysine to bind heparin has been compared with the affinities of the glycosaminoglycan for both thrombin and antithrombin III. Evidence has been presented suggesting the involvement of L-lysine residues of antithrombin III in this process.[343] L-Lysine residues, in addition to L-arginine residues of

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[339] R. Noreika, E. Stepaniukiene, and L. Lashas, *J. Polym. Sci., Polym. Symp. Ed.*, 1979, No. 66, 357.

[340] A. W. Salzman, R. D. Rosenberg, M. H. Smith, J. N. Lindon, and L. Favreau, *J. Clin. Invest.*, 1980, **65**, 64.

[341] L. Thunberg, G. Bäckström, H. Grundberg, J. Riesenfeld, and U. Lindahl, *FEBS Lett.*, 1980, **117**, 203.

[342] M. O. Langas, W. S. Ferguson, and T. H. Finlay, *Arch. Biochem. Biophys.*, 1980, **200**, 595.

[343] M. W. C. Hatton, L. R. Berry, H. Kaur, A. Koj, and E. Regoeczi, *Can. J. Biochem.*, 1980, **57**, 1183.

thrombin, are also involved in heparin binding. When two of four L-lysine residues of thrombin are modified on treatment with nitrous acid, the heparin-binding capacity of the enzyme and its plasma-clotting activity are largely destroyed, although the esterase activity is retained.

Heparin, due to its binding to thrombin, has been shown to interfere with the inhibition of thrombin by α_1-proteinase inhibitor.[344] Other glycosaminoglycans and carboxy-modified heparin do not have the same effect as heparin. There are similarities in the structural requirements in heparin, for anti-coagulant activity, and for the inhibition of α_1-proteinase-inhibitor interaction with thrombin. Platelets contain and release a heparin-neutralizing activity termed platelet factor 4.[345] This protein has been purified from rabbit platelets and its homology with human platelet factor 4 recorded.

Fibronectin binds to both native and denatured collagen immobilized on dextrans in reactions that exhibit different kinetics.[346] The rates of both reactions are enhanced by the presence of heparin or highly sulphated dextran sulphate but not by other glycosaminoglycans.

Dielectric changes in aqueous solutions of hyaluronic acid as a function of concentration at frequencies near 9 GHz have been reported.[347] An observed temperature-dependent behaviour is related to the orientational polarizability term in the Debye theory of polar molecules in liquids. A model for hyaluronic acid incorporating four intramolecular hydrogen bonds has been proposed.[348]

Evidence for specific intermolecular interactions in hyaluronate solutions has come from competitive inhibition studies.[349] It is suggested that hyaluronate chains interact by formation of specific co-operative junctions analogous to those characterized for plant structural polysaccharides. Conformational and rheological properties of hyaluronate solutions over a range of conditions, of concentration, pH, ionic strength, and molecular weight, together with an appraisal of viscoelastic and flow behaviour, have been examined in order to distinguish between general polymer solution properties and novel effects that might come from conformational ordering.[350]

A model for the binding of hyaluronic acid to the surface of Simian virus 40-transformed 3T3 cells, in which a single molecule of hyaluronic acid interacts with more than one receptor, has been proposed.[351] Temperature and ionic strength markedly influence the degree of binding. Hyaluronic acid and oligomers derived therefrom have been partially *N*-deacetylated in the presence of hydrazine in order to study the role of the acetamido-groups in interactions of hyaluronic acid with proteoglycans.[352]

[344] I. Danishefsky and R. Pixley, *Biochem. Biophys. Res. Commun.*, 1980, **91**, 862.
[345] M. H. Ginsberg, R. Hoskins, P. Sigrist, and R. G. Painter, *J. Biol. Chem.*, 1979, **254**, 12 365.
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[350] E. R. Morris, D. A. Rees, and E. J. Welsh, *J. Mol. Biol.*, 1980, **138**, 383.
[351] C. B. Underhill and B. P. Toole, *J. Biol. Chem.*, 1980, **255**, 4544.
[352] J. E. Christner, M. L. Brown, and D. D. Dziewiatkowski, *J. Biol. Chem.*, 1979, **254**, 12303.

For the maximal interaction between hyaluronic acid and proteoglycan at least two of the 2-amino-2-deoxy-D-glucopyranosyl residues within a decasaccharide unit of the hyaluronate must be *N*-acetylated.

Hyaluronic acid may be determined in tissues and body fluids using a radioassay method, which is based on the partition of a ^{125}I-labelled cartilage protein with affinity for hyaluronate between hyaluronate in free solution and immobilized hyaluronate.[353] The radioactivity bound to the immobilized hyaluronate is related to the amount of free hyaluronate in the system. The method is approximately 100 times more sensitive than previously described methods. Keratan sulphate from bovine cornea has been degraded by a defined chemical method to enable the oligosaccharide linking the protein to the disaccharide units of the keratan sulphate to be isolated.[354] The oligosaccharides forming the linkage region do not appear to be homogeneous and evidence for at least four components is given.

Biosynthesis. – Newly synthesized human nucleus pulposus proteoglycan is similar in size and function to proteoglycan from hyaline cartilage.[355] No evidence was found for the synthesis of large aggregating proteoglycans and smaller non-aggregating proteoglycans. Chondrocytes of adult mammalian cartilages have the capacity to respond quickly to rapid loss of extracellular proteoglycan with an increase in biosynthetic rate.[356] This increase apparently involves a de-repression of core protein synthesis and the accelerated synthesis of chondroitin sulphate-rich proteoglycans with abnormally long glycosaminoglycan chains. Under conditions of reduced proteoglycan synthesis, fewer proteoglycan subunits are synthesized by chondrocytes. However, the molecular size distribution of these macromolecules is similar to that of those synthesized by untreated cells.[357]

Undersulphated proteoglycans are secreted by cultured chondrocytes in the presence of low concentrations (3×10^{-8} M) of the ionophore monensin.[358] At higher concentrations (10^{-7} and 10^{-6} M) monensin inhibits secretion of type II collagen and proteoglycan synthesis is inhibited.

Proteoglycans, synthesized in calluses at various stages in fracture healing in rats, have been identified.[359] The sequential proteoglycan transitions observed in fracture healing differ from those in the endochondral ossification of embryonic skeletal tissues but resemble those in bone-matrix-induced bone formation. Rat granulosa cells, when grown in culture, secrete two proteoglycan species into the medium.[360] The larger molecular weight species contained dermatan sulphate chains (mol. wt. 3.8×10^4) and oligosaccharides bound to the

[353] U. B. G. Laurent and A. Tengblad, *Anal. Biochem.*, 1980, **109**, 386.
[354] A. Brekle and G. Mersmann, *Z. Physiol. Chem.*, 1980, **361**, 31.
[355] T. R. Oegama, D. S. Bradford, and K. M. Cooper, *J. Biol. Chem.*, 1979, **254**, 10579.
[356] J. D. Sandy, H. L. G. Brown, and D. A. Lowther, *Biochem. J.*, 1980, **188**, 119.
[357] C. J. Handley, G. Speight, H. C. Robinson, and D. A. Lowther, *Biochim. Biophys. Acta*, 1980, **631**, 124.
[358] K. Tajiri, N. Ughida, and M. L. Tanzer, *J. Biol. Chem.*, 1980, **255**, 6036.
[359] T. Yokoboro, A. Oohira, and H. Nogami, *Biochim. Biophys. Acta*, 1980, **628**, 174.
[360] M. Yanagishita and V. C. Hascall, *J. Biol. Chem.*, 1979, **254**, 12355.

core protein near sites of attachment of these chains. The second proteoglycan contained very few dermatan sulphate chains (mol. wt. 3.2×10^4).

Direct evidence has been provided for the synthesis of link protein by cultured chondrocytes derived from the Swarm rat chondrosarcoma.[361] The aggregate structure, formed by proteoglycan interaction with hyaluronic acid and the link protein, contains one link protein for each bound monomer. The active binding sites for monomer and link protein to hyaluronic acid in aggregates are located within a length of less than 30 monosaccharide residues. The two classes of oligosaccharides were also identified in proteoglycan fractions from Swarm rat chondrosarcoma.[362] Treatment of monomer proteoglycans with alkaline borohydride releases three oligosaccharides, each of which is linked to the proteoglycan core protein by an *O*-glycosidic bond between 2-acetamido-2-deoxy-D-galactose and L-serine and L-threonine. Their structures (9)–(11) and distribution on the core protein of cartilage proteoglycans suggest that they are related to the linkage region between keratan sulphate chains and the core protein of cartilage proteoglycans. The second class consists of oligosaccharides linked *N*-glycosidically to L-asparagine. Approximately 15 of these oligosaccharides are present per proteoglycan molecule, primarily located on the core protein near to the hyaluronic acid binding site.

Neu*p*5Ac-(2 → 3)-D-Gal*p*-(1 → 3)-D-GalNAc-ol

(9)

Neu*p*5Ac-(2 → 3)-D-Gal*p*-(1 → 3)-D-GalNAc-ol
6
↑
2
Neu*p*5Ac

(10)

Neu*p*5Ac-(2 → 3)-D-Gal*p*-(1 → 4)-D-Glc*p*NAc
1
↓
6
Neu*p*5Ac-(2 → 3)-D-Gal*p*-(1 → 3)-D-GalNAc-ol

(11)

Proteoglycans synthesized during chondrogenesis in chick embryo have been characterized physically and immunochemically.[363] Proteoglycans synthesized by chondrocytes in cultures derived from chick limb bud mesenchyme cells contain two distinct classes of oligosaccharides, a D-mannosyl-rich class and a mucin-type class, in addition to chondroitin sulphate and keratan sulphate.[364]

From a study of the metabolism of cell-surface-associated sulphated glycosaminoglycans in cultured human smooth muscle cells, it appears that these

[361] J. H. Kimura, T. E. Hardingham, and V. C. Hascall, *J. Biol. Chem.*, 1980, **255**, 7134.
[362] L. S. Lohmander, S. De Luca, B. Nilsson, V. C. Hascall, C. B. Caputo, J. H. Kimura, and D. Heinegard, *J. Biol. Chem.*, 1980, **255**, 6084.
[363] P. D. Royal, K. J. Sparks, and P. O. Goetinck, *J. Biol. Chem.*, 1980, **255**, 9870.
[364] S. De Luca, L. S. Lohmander, B. Nilsson, V. C. Hascall, and A. I. Caplan, *J. Biol. Chem.*, 1980, **255**, 6077.

compounds are neither precursors of the extracellular glycosaminoglycans nor do they represent mainly extracellular glycosaminoglycans which are in the process of endocytosis.[365] The net synthesis and distribution of sulphated glycosaminoglycans and hyaluronic acid are markedly altered by culturing skin fibroblasts on a collagen gel substratum instead of a plastic substratum of the type used in conventional cell culture.[366]

High-density lipoproteins decrease the synthesis of cellular glycosaminoglycans in cultured bovine smooth muscle cells, but the addition of low-density lipoproteins increases their secretion into the medium.[367] The phenomena are discussed in relation to the opposite roles that the two classes of lipoproteins are considered to play in the pathogenesis of early atherosclerotic lesions. The relationship between the rates of incorporation of precursors into newly synthesized glycosaminoglycans and the amounts of different glycosaminoglycans synthesized during various stages of an incubation period by confluent rat muscle fibroblasts has been defined.[368]

The synthesis of extracellular glycosaminoglycans in cultured pig epidermis requires the dermis to be present during culture although the polysaccharides are probably synthesized in the epidermis rather than the dermis.[369] The distribution of the glycosaminoglycans secreted by rabbit aortic smooth muscle cell into the medium is different from that retained by the cell layer.[370] A high percentage of the total hyaluronic acid and dermatan sulphate synthesized is secreted into the medium. Most of the heparan sulphate and chondroitin 4- and 6-sulphate seems to be associated with the cell layer.

The analysis of glycosaminoglycans synthesized by rat liver parenchymal cell clones has been reported.[371] Liver parenchymal cells synthesize a major part of the glycosaminoglycans of the liver and chondroitin sulphate production is closely related to cellular proliferation.

Consistent differences in the levels of sulphated glycosaminoglycans together with an absence of both keratan sulphate and heparin in prostatic tissue and secretions on incorporation of ^{35}S sulphate into glycosaminoglycans of mouse prostates in organ culture.[372] The sulphated glycosaminoglycan composition of fibroblast-like cells from embryonic guinea pig kidney, grown in culture, and variations that occur during sub-culturing, have been reported.[373] Developmental changes in the synthesis of glycosaminoglycans and collagen have been followed in embryonic chick skin.[374] A major change occurring during development is

[365] F. Middendorf, K. Von Figura, D. Zach, and H. Kresse, *Z. Physiol. Chem.*, 1980, **361**, 135.
[366] J. T. Gallagher, N. Gasiunas, and S. L. Schor, *Biochem. J.*, 1980, **190**, 243.
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[368] L. Hronowski and T. P. Anastassiades, *J. Biol. Chem.*, 1980, **255**, 9210.
[369] I. A. King and A. Tabiowo, *Biochim. Biophys. Acta*, 1980, **632**, 234.
[370] O. Namiki, B. Faris, F. Tschopp, P. Füglistaller, W. Hollander, C. Franzblau, and K. Schmid, *Biochemistry*, 1980, **19**, 1900.
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[372] C. S. Sato and F. Gyorkey, *Proc. Soc. Exp. Biol. Med.*, 1980, **163**, 1.
[373] P. A. S. Mourao, G. M. Machado-Santelli, and D. M. S. Toledo, *Biochim. Biophys. Acta*, 1980, **629**, 259.
[374] T. Nakamura and Y. Nagai, *J. Biochem. (Jpn.)*, 1980, **87**, 629.

the shift of glycosaminoglycan development from the non-sulphated type (hyaluronic acid) to the sulphated type (dermatan sulphate) together with an accelerated deposition of collagen.

The effect of cell density on net rates of hyaluronic acid and chondroitin sulphate synthesis and secretion by cultured rat fibroblasts has been studied.[375] For rat muscle, skin, and heart fibroblasts, the net rate of hyaluronic acid synthesis decreases with increasing cell densities. The biosynthesis of chondroitin sulphate occurs mainly in the Golgi apparatus of cultures of mouse mastocytoma cells.[376] Since sulphation accompanies or follows chondroitin synthesis it is probable that sulphation also occurs in the Golgi apparatus. A four-fold enhancement of the biosynthesis of chondroitin 6-sulphate occurs after stimulation of human T-lymphocytes by phytohaemagglutinin.[377]

Basic proteins and polyamines interact with acceptor polysaccharide (chondroitin), thereby causing an increase in the affinity of the glycosaminoglycan sulphotransferase (3′-phosphoadenosylsulphate : chondroitin 4′-sulphotransferase, EC 2.8.2.5) toward adenosine 3′-phosphate 5′-*P*-(dihydrogen phosphatosulphate).[378] The rates of synthesis and secretion of chondroitin sulphate and type II collagen by monolayer cultures of chick embryo chondrocytes have been compared.[379] One intracellular and two extracellular pools were identified as containing a major high molecular weight chondroitin sulphate proteoglycan.

Heparosan *N*-sulphate D-glucuronosyl 5-epimerase, which catalyses the conversion of β-D-glucuronosyl to α-L-isopyranuronosyl residues in the course of heparin biosynthesis, has been purified from mouse mastocytoma.[380] A protein factor is necessary for full activity of the purified enzyme. Incubation of *O*-desulphated heparin in ^3H_2O with the heparosan *N*-sulphate D-glucuronosyl 5-epimerase introduces tritium at C-5 of the glycuronosyl moieties of the substrate.[381] No exchange of hydrogen atoms at C-2, C-3, or C-4 with protons of the medium or with the C-5 hydrogen occurs during the reaction.

The substrate specificity of *O*-sulphotransferases involved in the biosynthesis of heparin has been reported.[382] 2-*O*-Sulphation of L-iduronic acid residues is coupled to the formation of these units by epimerization of the D-glucopyranosyl uronic acid residues. Both processes are completed before 6-*O*-sulphation of the polysaccharide is initiated. At an earlier stage of polymer modification, the production of non-sulphated L-iduronic acid residues occurs, and these residues appear to remain non-sulphated throughout the subsequent modification reactions.

[375] L. Hronowski and T. P. Anastassiadis, *J. Biol. Chem.*, 1980, **255**, 10091.
[376] J. E. Silbert and L. S. Freilich, *Biochem. J.*, 1980, **190**, 307.
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[379] J. J. Kim and H. E. Conrad, *J. Biol. Chem.*, 1980, **255**, 1586.
[380] A. Malmström, L. Rodén, D. S. Feingold, I. Jacobsson, G. Bäckström, and U. Lindahl, *J. Biol. Chem.*, 1980, **255**, 3878.
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[382] I. Jacobsson and U. Lindahl, *J. Biol. Chem.*, 1980, **255**, 5094.

An almost exclusive synthesis of heparan sulphate has been demonstrated by cultures of rat hepatocytes.[383] More than half of the synthesized glycosaminoglycan remains within the hepatocytes with the remainder associated with the cell surface and a minor proportion excreted into the medium. In all three pools, heparan sulphate chains (mol. wt. 2.4×10^4–3.0×10^4) are part of an alkali-labile proteoglycan.

The synthesis of hyaluronic acid in teratocarcinoma stem cells is stimulated by retinoic acid.[384] Since the target of retinoic acid seems to be the cell nucleus, the induction of hyaluronic acid synthesizing genes could be closely associated with differentiation.

Pathology. – In human osteoarthritic cartilage the proteoglycan aggregates are smaller and more heterogeneous and the chondroitin sulphate side-chains are shorter than in normal articular cartilage.[385] The osteoarthritic cartilage also contains two populations of proteoglycan, one with its hyaluronate-binding protein region of core protein intact and the other either possessing an inactive binding region or totally lacking it.

Human cartilage proteoglycans have been isolated from normally ossifying and congenitally malformed leg bones.[386] Alterations in proteoglycan metabolism might be involved in the pathogenic mechanisms producing the congenital limb defect. No differences have been detected in the amount of glycosaminoglycan excreted in the urine by normal or osteogenesis imperfecta affected individuals as measured by uronic acid or by Alcian Blue staining.[387]

The changes that take place in the structure of the proteoglycan subunit in human articular cartilage from foetal development to the mature adult have been described, and the possible relationship of these changes to the functional properties of the cartilage has been discussed.[388]

A comparison of bovine articular cartilage from immature and adult animals has revealed changes in proteoglycan structure.[389] The chondroitin sulphate-rich region becomes smaller on maturation because both the chondroitin sulphate chains and the core protein are smaller. These changes are accompanied by an enrichment of keratan sulphate chains in both the chondroitin sulphate-rich and keratan sulphate-rich regions. Variations in the composition of glycosaminoglycans from normal and arthritic bovine articular cartilage of various ages have been detected.[390] With increasing age the relative proportion of 4-sulphated disaccharide units in the total glycosaminoglycan decreased, whereas that of

[383] R. Prinz, U. Klein, P. R. Sudhakaran, W. Sinn, K. Ullrich, and K. Von Figura, *Biochim. Biophys. Acta*, 1980, **630**, 402.
[384] P. Prehm, *FEBS Lett.*, 1980, **111**, 295.
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[387] R. E. Hurst, J. M. Settine, W. M. Floyd, and A. E. Lorincz, *Clin. Chim. Acta*, 1980, **100**, 307.
[388] P. J. Roughley and R. J. White, *J. Biol. Chem.*, 1980, **255**, 217.
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[390] K. Murata and A. O. Bjelle, *Connect. Tissue Res.*, 1980, **7**, 143.

6-sulphated disaccharide units increased. The relative proportions of hyaluronic acid and keratan sulphate increased with age. Structural differences of proteoglycans synthesized by rat cartilages of different ages have been studied. An unusual finding is that on density-gradient centrifugation, a light component that contains dermatan sulphate–chondroitin sulphate copolymer chains, is isolated from all the cartilages examined.[391] The major proteoglycan in costal cartilage decreases in size with advance of age whereas the corresponding proteoglycan in newly induced ectopic cartilages is not subject to such age-related changes.

Proteoglycan isolated from rachitic chick growth cartilage is of decreased size relative to that isolated from tissue of normal chicks.[392] The two proteoglycan populations are of similar average chemical composition and similar in size with respect to their chondroitin sulphate chains. The difference in size in the rachitic state appears to result from an alteration in synthesis rather than extracellular proteolytic degradation of the normal proteoglycan. A unique basement membrane proteoglycan (mol. wt. 7.5×10^5) from the Engebreth-Holm-Swarm sarcoma has been characterized as containing equal amounts of protein and covalently-linked heparan sulphate.[393] Antibody prepared against this proteoglycan reacts with the basement membrane matrix in the tumour and with the basement membranes in skin, kidney, and cornea. The heparan sulphate proteoglycan appears to be a normal constituent of basement membranes with a role in the organization of basement membrane components and may determine the permeability of basement membranes to acidic molecules.

A proteoglycan (mol. wt. 7.1×10^4) isolated from plasma membranes of an ascites hepatoma, AH66, is composed of three heparan sulphate chains linked to the core protein.[394] The molecular model proposed for this proteoglycan resembles that proposed for a proteoheparan sulphate isolated from rat liver membranes.[395]

Increased levels of glycosaminoglycans have been detected in the urine of children with diabetes mellitus compared with levels from normal subjects.[396] A four- to six-fold increase in the concentration of total glycosaminoglycans in the neuronal perikarya of patients with mucopolysaccharidosis types IH, II, and IIIA has been detected.[397] The increased glycosaminoglycans are heparan sulphate in the case of type IIIA and dermatan sulphate plus heparan sulphate in the cases of types IH and II.

The isomeric chondroitin sulphates in urines from patients with mucopolysaccharidoses have been studied by digestion with chondroitin sulphate lyases

[391] A. Oohira and H. Nogami, *J. Biol. Chem.*, 1980, **255**, 1346.
[392] P. Roughley and I. Dickson, *Biochem. J.*, 1980, **185**, 33.
[393] J. R. Hassell, P. G. Robey, H. J. Barrach, J. Wilczek, S. I. Rennard, and G. R. Martin, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 4494.
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[397] G. Constantopoulos, K. Iqbal, and A. S. Dekaban, *J. Neurochem.*, 1980, **34**, 1399.

followed by h.p.l.c. determination of the sulphated unsaturated disaccharides produced.[398] Large amounts of dermatan sulphate were found in Hurler, Hunter, and Maroteaux-Lamy urines, whereas chondroitin 4-sulphate predominated in Sanfilippo urines, and chondroitin 4- and 6-sulphates were present in equal amounts in the urines of Morquio patients.

Intravenous administration of the anti-trypanosomal drug suramin to rats causes marked accumulation of glycosaminoglycans in the liver and excessive secretion in the urine.[399] The stored glycosaminoglycans were identified as heparan sulphate and dermatan sulphate. The suramin-treated rat may be a useful experimental animal model for mucopolysaccharidosis.

The incorporation of [^{35}S]sulphate into total- and specific-types of serum glycosaminoglycans has been studied in rats with acute, sub-acute or chronic liver injury (liver cirrhosis) and compared with that of normal rats.[400] There is a large increase in the rate and extent of [^{35}S]sulphate incorporation into total glycosaminoglycans for acutely liver-damaged rats but a decrease for sub-acutely and chronically liver-damaged rats. In comparison with serum glycosaminoglycans, the labelling profile of glycosaminoglycans in cirrhotic liver is quite different.

A diversity of sulphated glycosaminoglycans have been identified in rat leukaemic basophils, in contrast to a predominance of heparin in the rat mast cell.[401]

Dermatan sulphate and heparatin sulphate are the major sulphated glycosaminoglycans of a normal Swiss 3T3 cell line, whereas chondroitin 4-sulphate replaces dermatan sulphate in the STI variant.[402] Changes in the cellular glycosaminoglycans of cultured mastocytoma cells induced by sodium butyrate have been observed.[403] Approximately 30% of the total glycosaminoglycan fraction isolated from butyrate-treated cells is heparin or heparin-like material.

Dermatan sulphate–chondroitin sulphate copolymers with a high content of dermatan sulphate are the characteristic proteoglycans stored in cultured human skin fibroblasts from patients with mucopolysaccharidosis VI (Maroteaux–Lamy disease).[404] Comparative analysis of the molecular weight distribution and the reducing end-groups of the stored dermatan sulphate and compared with that isolated from the culture medium provided evidence that hyaluronidase-like enzymes are not present in these fibroblasts. Dematan sulphate-like material has been observed to represent the predominant component of the glycosaminoglycan fraction of a cultured murine mastocytoma, in contrast to three other

[398] G. J. L. Lee, J. E. Evans, H. Tieckelmann, J. T. Dulaney, and E. W. Naylor, *Clin. Chim. Acta*, 1980, **104**, 65.

[399] B. Constantopoulos, S. Rees, B. G. Cragg, J. A. Barranger, and R. O. Brady, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 3700.

[400] A. M. Gressner, W. Köster-Eiserfunke, E. Van de Leur, and H. Greiling, *J. Clin. Chem. Clin. Biochem.*, 1980, **18**, 279.

[401] D. D. Metcalfe, S. I. Wasserman, and K. F. Austen, *Biochem. J.*, 1980, **185**, 367.

[402] C. P. Dietrich and H. A. Armelin, *Biochem. Biophys. Res. Commun.*, 1978, **84**, 794.

[403] N. Nakamura, Y. Mori, Y. Tanigaki, and J. Kojima, *Biochim. Biophys. Acta*, 1980, **627**, 60.

[404] U. Klein and K. Von Figura, *Biochim. Biophys. Acta*, 1980, **630**, 10.

murine mastocytomas where the major constituent is heparin-like in nature.[405] Only small amounts of chondroitin sulphate are detectable.

A patient with multiple myeloma has developed an anti-coagulant with properties similar to heparin. A proteoglycan (mol. wt. 1.16×10^5) containing two polysaccharide units (mol. wt. 3.8×10^4) attached to protein (mol. wt. 3.9×10^4) was isolated.[406] The polysaccharide component appears to be of the heparan sulphate-type.

The difference in the extent of sulphation between the heparan sulphate isolated from Swiss 3T3 mouse cells and that from Swiss 3T3 cells transformed by DNA virus SV40 has been shown to reside in a higher *O*-sulphate content in the untransformed-cell heparan.[407] The cell-free synthesis of hyaluronic acid by extracts of human skin fibroblasts has been reported.[408] Hyaluronic acid produced from normal fibroblasts and those derived from patients with Marfan syndrome showed no significant differences in properties other than the rate of synthesis. Thus the nature of the genetic defect that results in the increase in hyaluronic acid synthesis remains unknown. A relative increase, when compared with controls, in the level of hyaluronic acid synthesis in skin fibroblast cultures from patients with osteogenesis imperfecta has been reported.[409]

7 Cell, Tissue, and Fluid Glycoproteins

The development of methods for cell separation by selective agglutination with lectins or on columns of immobilized lectins has been reviewed.[410] Special mention is made of the fractionation of murine and human lymphocytes into biologically distinct subpopulations.

Human brain glycoprotein NSA3 binds to immobilized hyaluronic acid.[411] The presence of glycosaminoglycans other than hyaluronic acid does not inhibit the binding. A glycoprotein (mol. wt. 3.6×10^4), isolated from the lung lavage of patients with aleovar proteinosis, contains hydroxy-L-proline, glycine, neuraminic acid, D-mannose, D-galactose, L-fucose, and 2-acetamido-2-deoxy-D-glucose.[412] This glycoprotein is composed of alternating short collagen- and non-collagen-like peptide sequences.

An α_1-antitrypsin-like glycoprotein, having similar molecular weight, chemical and immunological properties to human plasma α_1-antitrypsin, has been isolated from human liver.[413]

By use of a 'sandwich' radioimmunoassay in which anti-blood group A, B, and H antibodies are coupled to a solid-phase, it has been demonstrated that

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carcinoembryonic antigen contains blood group A antigenic determinants and that these determinants do not arise from contamination.[414] Carcinoembryonic-reactive antigen from breast neoplasms differs both physicochemically and antigenically from colon antigen.[415] The antigen was purified on columns of immobilized wheat germ agglutinin and monospecific antibodies. Glycoproteins of whole platelets have been solubilized, separated by electrophoresis, and identified by their interaction with ^{125}I-labelled lectins.[416] The method may be useful as a means of screening lectins for their binding to platelet glycoproteins so that they can be used in affinity chromatographic isolation of the glycoproteins.

The production and properties of interferons, glycoproteins with demonstrated anti-tumour and anti-viral properties,[417] and the intraspecies molecular heterogeneity and trans-species activity of mammalian interferons,[418] have been reviewed. Human lymphocyte cultures produce large amounts of interferon after treatment with D-galactose oxidase.[419] Interferon production was inhibited by pre-treatment of the cells with β-D-galactosidase or with neuraminidase followed by β-D-galactosidase, suggesting that the critical event for activation of interferon production is the oxidation of exposed D-galactopyranosyl residues on the lymphocyte membrane.

A large-scale purification of human fibroblast interferon has been achieved after affinity chromatography on immobilized concanavalin A followed by hydrophobic chromatography.[420] Improvements in the production and purification of human and murine type II interferons have been reported.[421] Human leucocyte interferon has been purified by a series of techniques involving precipitation, gel filtration, affinity chromatography with copper-chelate, blue dextran, and antibody.[422] The majority of biological activity is associated with two proteins (mol. wt. 1.8×10^4 and 2.0×10^4). Interferons have been produced from mixed leucocytes infected with Newcastle disease virus and resolved into three fractions by affinity chromatography on immobilized concanavalin A. Although the major portion of the interferon (70–75%, mol. wt. 1.6×10^4) does not bind to the column, two glycosylated fractions (25–30%, mol. wt. 2.1×10^4, and 15–20%, mol. wt. $2.1–2.3 \times 10^4$) are bound. Differences in the biological and antigenic properties of the fractions were also noted.[423]

Chromatography of human fibroblast interferon by thiol-exchange chromatography yields two classes of material exhibiting different stabilities to inactivation

[414] R. Magous, C. Lecou, and J. P. Bali, *Mol. Immunol.*, 1980, **17**, 1039.
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by mechanical stress.[424] Neither leucocyte interferon nor fibroblast interferon treated with thiol-blocking agents bind to the support. Chromatography on controlled pore glass and immobilized concanavalin A has been used in the purification of human fibroblastoid interferon.[425] The L-leucine-rich glycoprotein contains a high percentage of residues of L-glutamic acid and L-glutamine in addition to disulphide bridges, which are important for the biological activity of the molecule. 2-Amino-2-deoxy-D-galactopyranosyl and/or 2-amino-2-deoxy-D-mannopyranosyl residues are also present.

Tunicamycin, when used to suppress the glycosylation of human leucocyte interferon, results in the production of one molecular weight component of 1.6×10^4.[426] In the absence of antibiotic, non-glycosylated interferon (mol. wt. 1.6×10^4) and a glycosylated form (mol. wt. 2.1×10^4) are produced.

A non-glycosylated mouse L cell interferon has also been produced by the action of this antibiotic.[427] Inhibition of glycosylation has also been achieved with 2-amino-2-deoxy-D-glucose and 2-deoxy-D-*arabino*-hexose, both of which prevent the formation of large molecular weight interferon without affecting the production of lower molecular weight interferon.[428] The molecular weight heterogeneity of the glycoprotein may thus be attributable to differences in carbohydrate contents.

Interferon can be obtained from cells of different species and some of these interferons can be effective across species lines.[429] A prediction has been made that the ability to cross or not to cross species lines lies in the carbohydrate moiety, and the cross-species biological activity is a property of the polypeptide.

Interferon with relatively high specific activity has been produced in embryonic rat cells treated with Newcastle disease virus.[430] The interferon has no cross-activity in human or mouse cells.

Murine neuroblastoma cells in culture produce glycoproteins characteristically found in basement membranes.[431] Fibronectin, laminin, and type IV collagen have been identified. Laminin is involved in the increased cell adhesiveness during early aggregation of the nephrogenic mesenchyme of mouse embryos.[432] Cultured mouse endodermal cell laminin interacts with heparin and heparan sulphate, as evidenced by binding to immobilized heparin, and inhibition of this binding.[433] The interaction of laminin with glycosaminoglycans may be important in the formation of basement membranes.

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Cultured fibroblasts from a patient with mannosidosis accumulate oligosaccharides (12)–(14) with structures which are probably the same as those isolated from urine.[434] Addition of human liver acidic α-D-mannosidases to the culture medium partially correct the metabolic defect.

α-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-D-GlcNAc

(12)

α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-D-GlcNAc

(13)

α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-D-GlcNAc

(14)

A marked difference in the incorporation of 2-amino-2-deoxy-D-[^{14}C]glucose between five lines of Huntington's chorea fibroblasts and controls has been reported.[435] The differences have been characterized by a degree of protein and lipid glycosylation greater than that for normals and patients with other neurological diseases.

Human K-562 chronic myelogenous leukaemia-derived cells synthesize a unique long-chain oligosaccharide, which appears to be mainly attached to a polypeptide (mol. wt. 1.05×10^5).[436] This glycoprotein resembles erythroglycan which has been found on erythrocytes. The presence of high levels of lymphocyte receptors for peanut agglutinin has been identified on leukaemic cells from T-cell acute lymphoblastic leukaemia.[437] The analysis of these receptors may be a useful adjunct to the existing clinical and immunological prognostic indicators.

Glycopeptides derived from proteolysis of human teratocarcinoma cells have been fractionated by gel filtration and by affinity chromatography on immobilized concanavalin A.[438] Large molecular weight oligosaccharides (mol. wt. $> 7.4 \times 10^3$) have been isolated from the glycoproteins of whole cells derived from human ovarian teratocarcinoma.[439] The oligosaccharides contain residues of D-mannose, L-fucose, D-galactose, and 2-acetamido-2-deoxy-D-glucose.

Four major oligosaccharides from high D-mannose glycopeptides prepared from embryonal carcinoma cells are indistinguishable from (D-Man*p*)$_9$-D-GlcNAc, (D-Man*p*)$_8$-D-GlcNAc, (D-Man*p*)$_7$-D-GlcNAc, and (D-Man*p*)$_6$-D-GlcNAc isolated from fibroblasts.[440] The biosynthetic pathway of L-asparagine-linked oligosaccharides in early embryonic cells may be controlled as in adult cells, at least to the initial stage of processing of the nascent oligosaccharide transferred from

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[439] M. L. Rasilo, J. Wartiovaara, and O. Renkonen, *Can. J. Biochem.*, 1980, **58**, 384.
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lipid-linked intermediate. Partial acid hydrolysis of blood-group-specific glycoproteins from human ovarian cyst fluids removes about 80% of the carbohydrate moieties and releases non-diffusible polydisperse glycopeptides containing 2-acetamido-2-deoxy-D-galactopyranosyl residues as the predominant sugar.[441]

Doubt has been cast on the existence of structural glycoprotein as a major component of bovine aorta.[442] Exhaustive extraction of the tissue with agents previously claimed to extract structural glycoprotein results in the solubilization of mixtures of proteins consisting largely of actin, collagen, myosin, and other known proteins.

The effects of tunicamycin on the glycosylation of bovine rhodopsin have been studied.[443] In the presence of the antibiotic, the attachment of 2-amino-2-deoxy-D-glucose to the core-sites of the glycoprotein is eliminated although glycosylation of rhodopsin is not required for its insertion into the disc membrane.

Oocytes isolated from pig ovaries have been used in the preparation of zona pellucida 'ghosts'.[444] The zona pellucida is composed of several types of glycoprotein whose interaction forms supramolecular complexes.

The synthesis of glycoproteins containing L-fucosyl residues has been shown to be a feature of nerve terminals in sheep brain.[445] The turnover of L-fucose in synaptosomal glycoprotein appears to occur every 47 hours.

A homogeneous fraction of structural glycoproteins purified from the connective matrix of rabbit skin exerts a strong inhibitory influence in the protein syntheses that occur in cultured fibroblasts both from rabbit and human skin.[446]

Sulphated glycoproteins have been isolated from the brush border fraction and soluble fraction of rabbit small intestine.[447] The major constituent sugars in purified fractions were D-galactose, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2-deoxy-D-galactose together with small quantities of D-mannose, L-fucose, and neuraminic acid.

Rabbit testis contains a single molecular form of proacrosin, a glycoprotein (mol. wt. 6.7×10^4).[448] Amino-acid and carbohydrate analysis revealed the presence of 514 amino-acid and 25 carbohydrate residues per molecule. Rabbit bone marrow accretes and degrades asialotransferrin *in vivo* through a mechanism that recognizes the exposed β-D-galactopyranosyl residues in this glycoprotein.[449] After the liver, rabbit bone marrow is the second mammalian tissue now being identified as possessing a D-galactopyranosyl-specific pathway for the elimination of plasma glycoproteins. Comparative studies with other

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asialoglycoproteins containing bi-, tri-, and tetra-antennary glycans indicate that the bone marrow recognizes fewer glycan structures than does the liver.

Peripheral nervous system myelin of rabbit, like central nervous system myelin, consists of a heterogeneous population of particles containing lipid and glycoprotein, although their density and composition differ from central nervous system myelin.[450] The PO glycoprotein is exclusively localized in rabbit sciatic nerve myelin.[451] Digestion of the glycoprotein with trypsin releases a glycopeptide that contains a single monosaccharide entity, linked *N*-glycosidically to the protein chain.

The high affinity calcium-binding glycoprotein isolated from rabbit skeletal muscle is localized in the interior of the sarcotubular system.[452] It is suggested that this glycoprotein might be common to both sarcoplasmic reticulum and transverse tubular membranes.

Glycoproteins of the parallel fibres of the developing rat cerebellum are synthesized at an early post-natal age and have a short lifetime.[453] First exposure of 20 day-old dark-reared rats to light leads to an increase in the incorporation of L-[^{3}H]fucose into soluble glycoproteins of both the visual and motor cortex and into particulate glycoproteins of the visual cortex only.[454] These results are in contrast with those found with 50-day old animals and suggest that the effects of light-exposure on L-fucose incorporation may be age-dependent.

The relative proportion of the major high molecular weight glycoprotein of rat optic nerve and tract decreases during myelination.[455] The proportion of several lower molecular weight glycoproteins varies during development of the cells. Non-enzymatic glycosylation does not seem to be responsible for the modification of rat lens proteins in experimental cataract formation.[456]

The incorporation of L-[^{3}H]fucose and [^{32}P]orthophosphate into myelin proteins of isolated rat sciatic nerve during development has been reported.[457] An association of incorporation of sugar and phosphorylation with some step in the formation of myelin is suggested.

The nephritogenic glycopeptide isolated from rat glomerular basement membrane contains a trisaccharide (15) *N*-glycosidically linked to the peptide backbone.[458] Evidence for this structure was obtained by ^{13}C n.m.r. spectroscopy and interaction of the glycopeptide with concanavalin A.

$$\alpha\text{-D-Glc}p\text{-}(1 \rightarrow 6)\text{-}\beta\text{-D-Glc}p\text{-}(1 \rightarrow 6)\text{-D-Glc}$$

(15)

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The nature and synthesis of rat intestinal glycoproteins using vitamin A-deficient rats has been re-investigated.[459] The chemical composition and physical characteristics of a high molecular weight glycoprotein fraction were not changed by the deficiency.

In vivo glycoprotein synthesis and secretion has been studied in rat colonic epithelial cells.[460] Several low molecular weight membrane glycoproteins, possibly derived from the glycocalyx, are shed into the colonic lumen and can be separated from high molecular weight mucin by gel filtration and affinity chromatography. The consequences of injected 2-deoxy-D-*lyxo*-hexose (2-deoxy-D-galactose) on the biosynthesis of glycoproteins have been studied in rat liver and Morris hepatoma 9121.[461] Incorporation of the deoxy-sugar specifically modifies the oligosaccharide moiety of the glycoprotein *in vivo*. By blocking the O-2 for the attachment of L-fucose, this analogue of D-galactose may serve as an experimental tool for studying the significance of terminal L-fucosyl residues in secreted and membrane-bound glycoproteins. The rat mitochondrial Ca^{2+}-binding glycoprotein is a necessary component both in active uptake and passive diffusion of Ca^{2+} across the mitochondrial membrane.[462] The effector of this phenomenon is both the oxidized and reduced forms of nicotinamide adenine, with the former binding in a co-operative manner, resulting in a decreased affinity of the glycoprotein for Ca^{2+}.

The uptake of serum glycoproteins and of membrane-derived glycoproteins mediated by the hepatic lectin has been studied using asialoglycophorin and perfused rat liver and isolated hepatocytes.[463] The cellular uptake of the glycoprotein could be inhibited by other glycoproteins.

Asialoglycoproteins derived from fetuin and α_1-acid glycoprotein have been taken up in isolated rat hepatocytes.[464] Low-affinity binding of the asialoglycoproteins to hepatocytes was demonstrated at concentrations of asialoglycoproteins above 50 mM. Some speculations about the biological role of the hepatic receptor for these glycoproteins is reported. Pre-incubation of rat hepatocytes with dolichol leads to the phosphorylation and D-mannosylation of the dolichol in the endoplasmic reticulum.[465]

An α-D-mannosidase that is capable of processing L-asparagine-linked oligosaccharides has been isolated from rat liver Golgi apparatus,[466] and a glycoprotein from the microsomal fraction of the liver has been characterized as a nucleoside diphosphatase.[467]

A method has been developed for the preparation of rat liver lysosomes free from mitochondria, peroxisomes, and microsomes.[468] The major glycoproteins

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found in the lysosomal membranes were of higher molecular weight than those found in plasma membranes. A preneoplastic antigen, isolated from the microsomal fraction of the hyperplastic nodules of rat liver, is a glycoprotein (mol. wt. 1.45×10^5) that occurs as a dimer and contains 25–28% α-helix.[469] A similar protein is present in the endoplasmic reticulum of normal rat liver, but in a much lower concentration.

The involvement of carbohydrates in cell adhesion has been studied by degradation of rat neoplastic cells with specific glycosidases and by oxidation with sodium periodate.[470] Neuraminic acid residues appear to play a regulatory role, and D-galactopyranosyl residues may have a ligand function.

A protein and a glycoprotein (mol. wt. 2.0×10^5) with highly specific binding capacity for serotonin in the presence of Fe^{2+} have been isolated from rat platelets.[471] Their properties differ considerably from those of serotonin-binding protein of brain. The brush border antigen of the autologous immune complex glomerulonephritis of rats is a glycoprotein.[472] Binding studies with lectins indicate the presence of α-D-glucopyranosyl- or α-D-mannopyranosyl-residues.

Acid phosphatase has been purified from Dunning R3327H rat prostatic adenocarcinoma.[473] Although the enzyme possesses the same molecular weight as the corresponding enzyme from human malignant prostate, it differs in isoelectric point, and does not cross-react with antiserum raised against human prostatic acid phosphatase. No differences have been detected in the levels of L-fucose *O*-glycosidically bound to protein in normal and virus-transformed rat cells grown in culture.[474]

The axonal transport in guinea-pig spiral ganglion cells has been analysed.[475] A glycoprotein with a lifespan much shorter than any other major fast-component was detected.

Oligosaccharides have been released from L-fucose-labelled glycopeptides obtained from normal and polyoma-transformed baby hamster kidney cells.[476] Transformed cells are richer in oligosaccharides with highly branched outer chains than normal cells. An L-asparagine-linked oligosaccharide with an unusual structure has been isolated from proteolytic digests of Chinese hamster ovary cell glycoproteins.[477] The oligosaccharide has predominantly a tri-antennary structure consisting of repeating 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucopyranosyl units in the outer branches (16). It resembles oligosaccharides recently identified on human erythrocytes and corneal keratan sulphate.

Glycoproteins from mitogen-activated mouse lymphoid cells have been metabolically labelled with residues of D-mannose, D-galactose, or L-fucose.[478]

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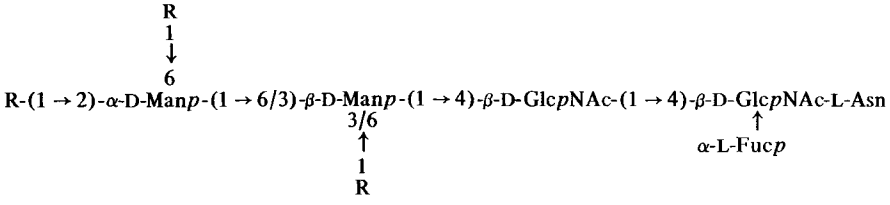
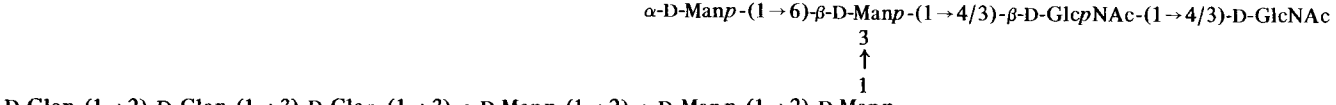


(16) R = [β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 →]$_3$



(17)

By using a combination of these labels it is possible not only to differentiate between activated T and B cells but also between certain subpopulations.

Proteins and glycoproteins have been isolated from phagosome membranes derived from mouse L cells.[479] A comparison was made of the major glycoprotein components associated with the L cell surface and the phagosome membranes. Mouse lymphoma cells of the class E complementation group have a block in the synthesis of the normal (D-Glc*p*)$_3$-(D-Man*p*)$_9$-(D-Glc*p*NAc)$_2$ lipid-linked oligosaccharide and synthesize instead a truncated molecule (17).[480,481] This oligosaccharide is then transferred *en bloc* to newly synthesized protein and is then processed to complex-type oligosaccharides with bi-antennary and tri-antennary structures.[482,483] The three D-glucopyranosyl- and two D-mannopyranosyl-residues are first removed, and the resulting oligosaccharide serves as an acceptor for UDP-2-acetamido-2-deoxy-D-glucose:glycoprotein 2-acetamido-2-deoxy-D-glucopyranosyltransferase. The biosynthesis of the complex oligosaccharide is then completed by the addition of one or two more outer residues of 2-acetamido-2-deoxy-D-glucose, and the D-galactopyranosyl, L-fucopyranosyl, and neuraminic acid residues are added to give rise to the final bi-antennary or tri-antennary structures.

The sialoglycoprotein Thy-1 antigen is a differentiation antigen on mouse thymocytes and brain cells.[484] Neuraminic acid residues may be necessary but not sufficient for the expression of Thy-1 antigenicity.

The glycoproteins of Balb/c 3T3 and AD^6, a glycosylation-defective, low adherent mutant mouse cell, have been compared.[485] Treatment of the two cell types with trypsin show that the mutant cells have a substantially different distribution of glycopeptide from transformed cells. A murine B16 melanoma-associated antigen has been isolated and purified.[486] The antigen is a glycosaccharides, which consist mainly of those terminating with the sequence Neu*p*5Ac-D-Gal*p*-D-Glc*p*NAc, although some are terminated with D-mannopyranosyl residues. These oligosaccharides are presumed to be linked *via* *N*,*N*'-diacetylchitobiose to L-asparagine residues. A few *O*-glycosidically linked oligosaccharides are terminated with 2-acetamido-2-deoxy-D-galactopyranosyl residues.

A glycoprotein, isolated from the egg coat jelly of the toad (*Bufo melanostictus*), contains residues of D-galactose, L-fucose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, and *N*-acetylneuraminic acid.[487] Some structural aspects of the carbohydrate moiety of the glycoprotein have been recorded.[488]

[479] M. L. Nagpal and J. C. Brown. *Int. J. Biochem.*, 1980, **11**, 127.
[480] I. Trowbridge and R. Hyman, *Cell*, 1979, **17**, 503.
[481] A. Chapman, I. Trowbridge, R. Hyman, and S. Kornfeld, *Cell*, 1979, **17**, 509.
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[486] V. P. Bhavanandam, J. G. Kemper, and J. C. Bystryn, *J. Biol. Chem.*, 1980, **255**, 5145.
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[488] M. S. Seshadri and M. S. Reddy, *Indian J. Biochem. Biophys.*, 1980, **17**, 24.

Embryonic chick lung and liver incorporates [$^{35}SO_4$] into oligosaccharide chains of glycoproteins.[489] In contrast to the results with these embryonic organs, adult lung and liver synthesize much lower levels of these sulphated glycoproteins.

The ganglion cell layer of goldfish retina contain at least 10 glycoproteins that have been identified by selective binding to ^{125}I-concanavalin A.[490] Axons of ganglion cells outside the retina are surrounded by glia and make up the optic fibres of the visual system and contain three major myelin proteins, one of which (mol. wt. 2.3×10^4) is characterized as a glycoprotein. The occurrence of sequences of polyneuraminosyl residues has been shown in the carbohydrate units of a glycoprotein isolated from the eggs of rainbow trout.[491] Reduced oligosaccharides obtained by alkaline borohydride treatment of the glycoprotein contain significant amounts of (2 → 8)-linked homopolymers of *N*-glycolylneuraminic acid with more than 15 neuraminosyl residues.

The binding of *Lens culinaris* lectin to sea urchin embryo chromatin has been reported.[492] Scatchard plot analysis of the binding reaction suggests the existence of a single binding site.

Sialoglycoproteins have been isolated from the eggs of the Pacific herring.[493] Their carbohydrate composition is similar to that of the glycoproteins known to have complex types of heterosaccharide units, whereas the amino-acid composition is unique.

8 Cell-surface Glycoproteins

Methods for the purification of cell-membrane glycoproteins by lectin affinity chromatography have been reviewed.[494] Reviews have highlighted studies on membrane variants of mammalian cells resistant to cytotoxic lectins,[495] the structures and functions of the oligosaccharide chains of cell-surface glycoproteins,[496] cell-surface components, and cell recognition.[497]

The optimum conditions for lectin affinity chromatography in the presence of different detergents commonly used to solubilize membrane constituents have been determined.[498] At constant detergent concentration, the buffer pH is of primary importance, whereas the maximum recovery of bound product may require a change of pH between fixation and elution.

Rhodamine and fluorescein hydrazides have been synthesized and used as fluorescent markers of glycoconjugates on cell surfaces or in solution.[499] The

[489] A. Heifetz, W. H. Kinsey, and W. J. Lennarz, *J. Biol. Chem.*, 1980, **255**, 4528.
[490] D. Guilian, H. Des Ruisseaux, and D. Cowburn, *J. Biol. Chem.*, 1980, **255**, 6486.
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[493] S. Inoue and M. Iwasaki, *Eur. J. Biochem.*, 1980, **111**, 131.
[494] R. Lotan and G. L. Nicholson, *Biochim, Biophys. Acta*, 1979, **559**, 329.
[495] J. A. Wright, *Int. J. Biochem.*, 1979, **10**, 951.
[496] A. Kobata, *Cell Struct. Funct.*, 1979, **4**, 169.
[497] W. Frazier and L. Glaser, *Annu. Rev. Biochem.* 1979, **48**, 491.
[498] M. Dodeur and M. A. Jacquet, *J. Chromatogr.*, 1980, **195**, 197.
[499] M. Wilchek, S. Spiegel, and Y. Spiegel, *Biochem. Biophys. Res. Commun.*, 1980, **92**, 1215.

procedure involves the oxidation of the neuraminic acid residues of glycoconjugates with sodium metaperiodate or the D-galactopyranosyl residues with D-galactose oxidase before interaction with the fluorescent derivatives. The method may also be used for the staining of membrane glycoproteins on polyacrylamide gels.

3,5-Di-iodo-L-tyrosine hydrazide has been used in the specific labelling of human platelet membrane glycoproteins which have been reacted with sodium periodate.[500] Two different labelling patterns were obtained when platelets were labelled with sodium borotritide of di-[^{125}I]iodo-tyrosine hydrazide. A novel covalent electron dense reagent for the ultrastructural localization of cell-surface sialoglycoconjugates has been developed.[501] Ferritin hydrazide, prepared by coupling horse spleen ferritin with an excess of bishydrazides, reacts with periodate-oxidized neuraminyl residues or other aldehyde groups.

The relationship between cell-surface fibronectin and oncogenic transformation has been reviewed,[502] as has the cellular and metabolic specificity of the interaction of adhesion proteins with collagen and with cells.[503] Aspects of the chemistry and biochemistry of fibronectin,[504] and the involvement of fibronectin and proteoglycans as determinants of cell-substratum adhesion,[505] have been reviewed.

Studies on the possible role of fibronectin in malignancy have been summarized.[506] Many tumour cell lines do not express fibronectin in tumours *in situ*, even though some of them express fibronectin in culture. Cell shape and hormones appear to influence the expression of this glycoprotein in culture, although it is unclear how its expression is regulated *in vivo*. Developing glial cells from embryonic chick and mouse cerebral cells produce cell-surface-associated fibronectin.[507] In contrast, fibronectin is not detectable in pure neuronal cultures. The implications of glial fibronectin for neuronal adhesion and migration during normal development and also in neoplasia are considered. Human platelets contain fibronectin. Immunofluorescent staining is minimal in intact cells, with a portion of the glycoprotein in a storage granule.[508] Using affinity-purified antibodies, fibronectin of chick embryo fibroblasts has been shown to be localized intracellularly at the rough endoplasmic reticulum and the Golgi apparatus.[509]

[500] A. Rotman, S. Linder, and V. Pribluda, *FEBS Lett.*, 1980, **120**, 85.
[501] E. Roffman, Y. Speigel, and M. Wilchek, *Biochem. Biophys. Res. Commun.*, 1980, **97**, 1192.
[502] R. O. Hynes, A. T. Destree, M. E. Perkins, and D. D. Wagner, *J. Supramol. Struct.*, 1979, **11**, 95.
[503] H. K. Kleinmann, A. T. Hewitt, J. C. Murray, L. A. Liotta, S. I. Rennard, J. P. Pennypacker, E. B. Goodwin, G. R. Martin, and P. H. Fishman, *J. Supramol. Struct.*, 1979, **11**, 69.
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[505] L. A. Culp, B. A. Murray, and B. J. Rollins, *J. Supramol. Struct.*, 1979, **11**, 401.
[506] L. B. Chen, I. Summerhayes, P. Hsieh, and P. H. Gallimore, *J. Supramol. Struct.*, 1979, **12**, 139.
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[508] M. H. Ginsberg, R. G. Painter, C. Birdwell, and E. F. Plow, *J. Supramol. Struct.*, 1980, **11**, 167.
[509] S. S. Yamada, K. M. Yamada, and M. C. Willingham, *J. Histochem. Cytochem.*, 1980, **28**, 953.

The collagen-binding fragment of chicken cellular fibronectin consists of a single polypeptide chain (mol. wt. 4.0×10^4).[510] Collagen-binding fragments of a similar size have also been isolated from chicken and human plasma. The collagen-binding domain is now considered to be located near the *N*-terminus of fibronectin and not in the *C*-terminal region as originally reported.[511] Fibronectin appears to contain two protease-sensitive regions. One region, some 30 000 daltons from the *N*-terminus is susceptible to cleavage by plasmin, thrombin, trypsin, chymotrypsin, and subtilisin, and a second region 72 000 daltons from the *N*-terminus is cleaved by trypsin, chymotrypsin, and cathepsin D. The location within fibronectin at which the glycoprotein binds to collagen and fibrin and the region at which these proteins are cross-linked to fibronectin by factor XIII transglutaminase have been established.[512] The cross-linking region is in the *N*-terminal fragment (mol. wt. 2.5×10^4) and the binding site is immediately adjacent in the cystine-rich region of a fragment (mol. wt. 2.0×10^5).

Physiological concentrations of polyamines specifically inhibit the factor XIII-catalysed cross-linking between fibronectin and collagen by interaction with the glycoprotein-factor XIIIa acylenzyme intermediate, and compete with L-lysyl residues of collagen as acyl acceptors in the cross-linking reaction.[513]

When ϵ-(γ-L-glutamyl)-L-lysine cross-links are formed between fibronectin and collagen, fibronectin contributes L-glutaminyl residues and collagen contributes L-lysyl residues.[514] Only two L-glutaminyl residues are susceptible to Factor XIIIa (transglutaminase), and they are localized in a 2.7×10^4 molecular weight segment of the fibronectin molecule. The part of the fibronectin molecule that contains the site of cross-linking to collagen is different from the part that is responsible for tight binding to collagen.

Proteolytic digestion of fibronectin by chymotrypsin or pronase releases two fragments (mol. wt. 1.6×10^5 and 5.0×10^4, respectively) both of which have affinity for heparin.[515] The possible involvement of such high affinity binding sites of fibronectin in the binding of glycosaminoglycans to the cell surface or in the organization of extracellular matrices are discussed. One of the pronase-resistant polypeptide domains (mol. wt. 5.0×10^4) has been purified by affinity chromatography on immobilized heparin.[516] This domain of fibronectin appears to be a unique region, free of carbohydrate, that is a globular, protease-resistant functional site.

From an examination of the maturation or processing of the oligosaccharides of cellular fibronectin in cultured chick embryo fibroblasts, it has been concluded that the carbohydrate portion of the glycoprotein is synthesized as a

[510] L. H. E. Hahn and K. M. Yamada, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1160.
[511] G. Balian, E. M. Click, and P. Bornstein, *J. Biol. Chem.*, 1980, **255**, 3234.
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[513] D. F. Mosher, P. E. Schad, and H. K. Kleinman, *J. Supramol. Struct.*, 1979, **11**, 227.
[514] D. F. Mosher, P. E. Schad, and J. M. Vann, *J. Biol. Chem.*, 1980, **255**, 1181.
[515] K. M. Yamada, D. W. Kennedy, K. Kimata, and R. M. Pratt, *J. Biol. Chem.*, 1980, **255**, 6055.
[516] M. Hayashi, D. H. Schlesinger, D. W. Kennedy, and K. M. Yamada, *J. Biol. Chem.*, 1980, **255**, 10 017.

'high-D-mannose' intermediate and is subsequently processed to give the characteristic complex oligosaccharide chains of fibronectin.[517]

Human fibrosarcoma cells produce a polypeptide (mol. wt. 1.0×10^4), which acts as a protease in releasing fibronectin from cultured human fibroblasts.[518] The polypeptide(s) may represent a family of gene products responsible for maintenance of low levels of surface-associated fibronectin in fibrosarcoma cells and thus be related to their infiltrating properties by preventing the formation of the extracellular matrix.

The quantity of collagen and fibronectin matrix around Rous sarcoma virus-transformed fibroblasts is not regulated by co-ordinate control of the synthesis of the two protein components.[519] The levels of collagen are at least partly regulated by control of procollagen synthesis, while levels of fibronectin are regulated by control of degradation or shedding.

Newly synthesized fibronectin of NIL8 hamster cells appears as a dimer both at the cell surface and secreted into the culture medium at an early stage in the metabolic labelling of these cells.[520] Processing of the high mannose form to a complex form of the oligosaccharide chains occurs. The dimeric fibronectin slowly forms high molecular weight aggregates requiring reduction of disulphide bonds for dissociation.

The expression of cell-surface glycoprotein antigens in cultured human cell lines has been examined.[521] The set of glycoproteins studied was defined by their immunoreactivity with antiserum to placental brush border membranes. The existence of a receptor specific for transferrin on the human brush border membrane has been reported.[522] The receptor exhibited properties similar to sialoglycoprotein 15b, which has been identified previously in a study characterizing the surface glycoproteins of the term human placenta.

An evaluation of membrane glycoproteins in platelet adhesion and aggregation has been published.[523] Lentil lectin binds tightly to human platelets, mainly to surface glycoprotein II.[524] The lectin neither causes aggregation nor does it inhibit platelet aggregation by other agents. It has no influence on the binding of thrombin to platelets or on thrombin-induced clot retraction. The hypothesis that glycoprotein II plays a crucial role in platelet aggregation by all physiological agents may have to be re-evaluated. Receptors on the plasma membrane of human platelets for wheat germ agglutinin, concanavalin A, and *Ricinus communis* lectins bound to gold granules have been located by scanning and transmission electron microscopy.[525]

Platelets from a patient with Bernard–Soulier syndrome and normal donors have been compared after surface labelling of neuraminic acid and D-galacto-

[517] K. Olden, V. A. Hunter, and K. M. Yamada, *Biochim. Biophys. Acta*, 1980, **632**, 408.
[518] J. Keski-Oja, H. Marquardt, J. E. De Larco, and G. J. Todaro, *J. Supramol. Struct.*, 1979, **11**, 217.
[519] G. Parry, W. J. Soo, and M. J. Bissell, *J. Biol. Chem.*, 1979, **254**, 11763.
[520] M. G. Choi and R. O. Hynes, *J. Biol. Chem.*, 1979, **254**, 12 050.
[521] T. A. Hamilton, H. G. Wada, and H. H. Sessman, *J. Supramol. Struct.*, 1979, **11**, 503.
[522] H. G. Wada, P. E. Hass, and H. H. Sussman, *J. Biol. Chem.*, 1979, **254**, 12 629.
[523] D. R. Phillips, *Progr. Hermostasis Thromb.*, 1980, **5**, 81.
[524] P. Ganguly and N. G. Fossett, *Biochem, Biophys, Res. Commun.*, 1980, **92**, 499.
[525] A. T. Nurden, M. Horisberger, E. Savariau, and J. P. Caen, *Experientia*, 1980, **36**, 1215.

pyranosyl or 2-acetamido-2-deoxy-D-galactopyranosyl residues.[526] Reduction in the levels of a glycoprotein were noted, with an additional possibility of an altered glycosylation pattern in one glycoprotein in the syndrome platelets. The antigenic complexity of human platelet membranes has been determined by crossed immunoelectrophoresis.[527] Platelet membranes from patients with Glanzmann's thrombasthenia and Bernard–Soulier syndrome have been shown to contain diminished amounts of major antigen content.

Glycocalicin, a glycoprotein located on the surface of intact human platelets, has been purified by affinity chromatography on immobilized wheat germ agglutinin.[528] Its membrane association and solubilization in aqueous media have been studied. Binding studies of native lectins and their ferritin conjugates to dispersed pancreatic cells have shown that the conjugation procedure does not significantly alter either the affinity constant of the lectin for its receptor on the cell surface or the number of sites detected.[529]

The sodium periodate–sodium borotritide procedure for surface labelling of sialoglycoproteins has been modified to eliminate interference from non-specifically labelled tritium.[530] Glycoproteins from synaptic membranes, labelled in such a procedure, are separated by polyacrylamide gel electrophoresis, and individual slices of the gels are treated with neuraminidase to release selectively [^{3}H]neuraminic acid.

The plasma membrane components of five human B-cell lines and three human T-cell lines, after electrophoretic separation, have been interacted with various radioactively-labelled lectins.[531] The lentil, castor bean, and wheat germ lectins bound to multiple components (mol. wt. 2.0×10^4–2.0×10^5) within the plasma membranes, whereas peanut lectin bound preferentially to glycoproteins of molecular weight 1.5×10^5 and 8.3×10^4 in B-cells and 1.5×10^5 and 1.3×10^5 in T-cells. Gorse lectin bound to a 2.2×10^5 component in B-cells that was not labelled in T-cells. Most of the lectin-binding sites identified within the plasma membrane preparations are likely to be constituents of the cell surface.[532] However, contamination by membranes from the endoplasmic reticulum, Golgi cisternae, and vesicles cannot be excluded.

Human peripheral blood T-lymphocytes and human T-lymphoblastoid cells of a MOLT 4B cell line, after surface labelling with either the lactoperoxidase or the D-galactose oxidase–sodium borotritide procedures have been analysed by polyacrylamide gel electrophoresis.[533] The major sialoglycoproteins from both cell types are very similar if not identical.

The reactivity of human lymphoid cells fractionated by sedimentation and affinity methods has been reported, and the nature of peanut agglutinin binding

[526] J. L. McGregor, K. J. Clemetson, E. James, E. F. Luscher, and H. Dechavanne, *Thromb. Res.*, 1980, **17**, 715.
[527] S. Shulman and S. Karpatkin, *J. Biol. Chem.*, 1980, **255**, 4320.
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[529] M. F. Maylie-Pfenninger and J. D. Jamieson, *J. Cell Biol.*, 1979, **80**, 69.
[530] T. F. Cruz and J. W. Gurd, *Anal. Biochem.*, 1980, **108**, 139.
[531] L. G. Gürtler, B. Sramota, and H. Cleve, *Z. Physiol. Chem.*, 1979, **360**, 1819.
[532] L. G. Gürtler, S. M. Lefranc, and H. Cleve, *Z. Physiol. Chem.*, 1979, **360**, 1829.
[533] M. Saito and T. Osawa, *Carbohydr. Res.* 1980, **78**, 341.

to blood cells that lack HLA membrane determinants discussed.[534] Glycoproteins on the surface of lymphoblastoid cells have been labelled in their neuraminic acid and D-galactopyranosyl residues.[535] Those cell lines that harbour the Epstein–Barr virus genomes do not appear to express a cell-surface sialoglycoprotein related to the virus.

The presence of the carbohydrate precursor T-antigen of human blood-group system MN can be demonstrated by an immunohistochemical technique.[536] Malignant breast lesions show a pattern of cell-membrane-associated T antigen expression different from that of benign breast tissues.

In the biosynthesis of the histocompatibility antigens HLA-A and -B by the lymphoblastoid cell line BRI8, the heavy chains are synthesized as transmembrane polypeptides and inserted asymmetrically into rough endoplasmic reticulum.[537] The majority of newly synthesized heavy chains become associated with β_2-microglobulin at an early stage at which time they carry the high D-mannose form of the oligosaccharide unit. This oligosaccharide moiety is then converted into the complex form, resulting in a shift in the mobility of the heavy chains in which the apparent molecular weight increase is primarily a result of the addition of terminal neuraminic acid residues to the oligosaccharide unit.

Comparison of the levels of carcinoembryonic antigen (CEA) and major histocompatibility antigens (HLA) on the surface of five cultured human adenocarcinoma cell lines shows an inverse type of relationship between amounts of CEA and HLA, suggesting that CEA may inhibit the effective expression of the activities of major histocompatibility antigens.[538]

Studies on the biosynthesis and regulation of expression of transplantation antigens as detected by a monoclonal antibody to human leukocytic antigens (HLA-A, B, and C) of human lymphoid cells have been reported.[539] Tunicamycin markedly decreases the levels of HLA heavy chains, both on the cell surface and intracellularly, and glycosylation of these chains appears to be an essential requirement for the normal expression of HLA-ABC antigens.

Granulocytes from normal, leukocytotic, and chronic myelocytic leukaemic patients have been examined for lectin-binding sites related to D-galactopyranosyl and D-mannopyranosyl residues.[540] In chronic myelogenous leukaemia, neuraminic acid or hypersialylated glycoproteins may mask cell surface receptors that may mediate granulocyte adhesion.

The existence of reversible clustering of lentil lectin membrane receptors on HeLa cells has been suggested.[541] The kinetics of this process are fast and temperature-dependent. The use of lithium di-iodosalicylate for extraction followed by affinity chromatography on immobilized wheat germ agglutinin has

[534] J. J. Ballet, M. Fellous, N. Sharon, Y. Reisner, and M. Agrapart, *Scand. J. Immunol.*, 1980, **11**, 555.
[535] S. W. L. Lui and M. H. Ng, *Arch. Virol.*, 1980, **63**, 31.
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[541] A. B. Schreiber, J. Hoebeke, B. Vray, and A. D. Strosberg, *FEBS Lett.*, 1980, **111**, 303.

given good yields of gamma protein, the major transmembrane sialoglycoprotein of the HeLa cell.[542]

A specific elevation of cell-associated α-D-mannosidase activity is observed in human skin fibroblasts cultured with concanavalin A.[543] The elevation of this enzyme activity is a result of a specific binding of the enzyme to the cell surface and is mediated by concanavalin A.

Tunicamycin interferes with the expression of phosphomannose receptors on the cell surface of cultured skin fibroblasts and the synthesis of phosphomannosyl residues on lysosomal enzymes after an initial lag phase.[544] These alterations are associated with an increased accumulation of β-D-2-acetamido-2-deoxyglucosidase extracellularly and a loss of that enzyme from intracellular sites and from the cell surface. Phosphomannosyl residues within *N*-glycosidically linked oligosaccharide side-chains on lysosomal enzymes function intracellularly or on the cell surface as a signal for the transfer of lysosomal enzymes into lysosomes.

A human cell-surface glycoprotein with unusual properties has been detected on cultured human haematopoietic cell lines by means of a monoclonal antibody.[545] Preliminary chemical characterization suggests that the glycoprotein may be identical to the abnormal glycoprotein previously reported to be associated with malignancy.

Cell-surface changes related to glycoproteins in Epstein–Barr virus-positive and -negative human haematopoietic cell lines have been observed.[546] Virus infection of B lymphocytes leads to the alteration in molecular size of glycoproteins containing L-fucosyl residues in a manner similar to those demonstrated in other neoplastic cell lines.

A surface glycoprotein from the spermatozoa of the Bonnet monkey, *Macaca radiata*, is unlike that of either the *N*-glycosyl or the *O*-glycosyl type as it contains residues of 2-acetamido-2-deoxy-D-galactose, L-fucose, and D-glucose, in addition to D-mannose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and neuraminic acid.[547]

Microsomal membranes from bovine mammary tissue contain an alkaline phosphatase (mol. wt. 7.0×10^4) with 35% carbohydrate.[548] Deoxycholate has been used in the extraction and gel-filtration chromatography of bovine brain synaptosomal plasma membrane glycoproteins.[549] Membrane glycoproteins solubilized by deoxycholate appear to exist in equilibrium between a dissociated form and one of random association.

Three major glycoproteins from calf thyroid plasma membranes have been isolated and characterized.[550] They contain an unusually high carbohydrate

[542] R. H. Kramer and E. S. Canellakis, *J. Supramol. Struct.*, 1979, **12**, 435.
[543] Y. Suzuki, H. Sakuraba, and K. Ohga, *Biochem. Biophys. Res. Commun.*, 1980, **92**, 1066.
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[547] Nasir-Ud-Din, E. W. Nasir, R. W. Jeanloz, and M. Shalev, *Carbohydr. Res.*, 1980, **85**, C7.
[548] R. B. O'Keefe and J. E. Kinsella, *Int. J. Biochem.*, 1979, **10**, 125.
[549] K. C. Leskawa, M. Saito, and A. Rosenberg, *J. Chromatogr.*, 1980, **193**, 316.
[550] Y. Okada and R. G. Spiro, *J. Biol. Chem.*, 1980, **255**, 8865.

content (56–79%). Some of the D-galactosyl residues are terminally located in α-glycosidic linkage. Polyacrylamide gel electrophoresis has been used in the identification of the glycoproteins.[551] The presence of high levels of D-galactose and 2-amino-2-deoxy-D-glucose in some of the glycoproteins was noted.

The glycoproteins in calf thymocyte plasma membrane contain L-asparagine-linked sugar chains.[552] The oligosaccharides were liberated by hydrazinolysis and were shown to contain a complex mixture of acidic and neutral components. Twelve oligosaccharides have been released from the glycoprotein.[553]

The occurrence of a number of sulphated glycoproteins associated with ovine synaptic plasma membranes has been reported.[554] Glycoproteins, characterized by their affinity for concanavalin A, exist on the ram spermatozoal plasma membrane.[555] Molecular differentiation occurs in these membrane glycoproteins on the maturing male gamete.

Cell-surface glycoconjugates in the epidermis of cultured pig skin slices have been metabolically labelled with L-[^{3}H]fucose and 2-amino-2-deoxy-D-[^{3}H]-glucose, with a view to detecting possible changes to cell-surface components in small biopsy samples of diseased human epidermis.[556] Both sugars are incorporated into the same major integral glycoproteins present in the plasma membranes of living epidermal cells. However, the amino-sugar is also incorporated into high molecular weight extracellular material, possibly proteoglycan in nature.

The major glycoprotein of rabbit adipocyte plasma membrane can be obtained in an electrophoretically homogeneous form by a combination of techniques, including detergent treatment, preparative electrophoresis, and lectin affinity chromatography.[557] The fat cell glycoprotein appears similar to the hepatic binding protein in containing more D-mannosyl than D-galactosyl residues, little or no 2-amino-2-deoxy-D-galactosyl residues, and primarily L-asparagine-linked carbohydrate chains.

Neoglycoproteins containing D-galactosyl residues have been shown to be good inhibitors of asialoorosomucoid binding to the rabbit liver plasma membrane.[558] The ability of a neoglycoprotein to inhibit the asialo-orosomucoid binding was directly related to the density of D-galactosyl residues covalently attached to the protein. Asialotransferrin, which is a poor inhibitor, becomes a good inhibitor after coupling D-thiogalactosyl residues to it. The soluble, rabbit hepatic asialoglycoprotein binding protein associates spontaneously with lipid vesicles either below or above the lipid phase-transition temperature.[559] Concomitant conformational changes accompanying the association of protein and

[551] G. Kidroni, M. J. Spiro, and R. G. Spiro, *Arch. Biochem. Biophys.*, 1980, **203**, 151.
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vesicles are shown in c.d. studies, which reveal a change in the overall secondary structure, predominantly an increase in β-pleated sheet.

A secretory component, a glycoprotein (mol. wt. 8.0×10^4), is secreted by epithelial cells of the rabbit mammary gland and is associated with the cell surface of mammary cells and plasma membrane-enriched fractions.[560] The binding capacity of membranes for immunoglobulin A dimer is directly related to the amount of free secretory component bound to membranes. Vitamin A deficiency affects the composition of glycoproteins, in particular the neuraminic acid residues, on either side of rat liver lysosomal membranes.[561] Neuraminic acid appears to be a recognition marker for binding aryl sulphatase B on the membrane. The specific binding activity in *N*-2-acetylaminofluorene-induced neoplastic nodules and primary hepatocellular carcinoma has been related to the content of hepatic binding protein in rat liver.[562] The loss of binding capacity for serum asialoglycoproteins is proportional to the decreased concentration of immunologically detectable lectin in the cultured tissues.

Some properties and characteristics of asialoglycoprotein receptors on freshly isolated rat hepatocytes have been reported.[563] Difficulties in the quantitation of asialoglycoprotein receptors on the rat hepatocyte have been highlighted.[564] The method of hepatocyte preparation, and conditions for binding studies, alter the apparent number of receptors identifiable on the hepatocyte cell surface.

The average degradation rates (half-lives) of glycoproteins and proteins in whole homogenates and plasma membranes from normal and regenerating rat livers have been measured.[565] Contrary to that of proteins, the half-life of glycoproteins remains unchanged during liver regeneration and the degradation of plasma membrane glycoproteins is identical to that found in whole homogenate under both normal and regenerating conditions.

Different half-lives of the carbohydrate and protein moieties of a glycoprotein (mol. wt. 1.1×10^5) isolated from rat liver plasma membranes have been reported.[566] L-Fucose and neuraminic acid, the terminal sugars of the oligosaccharide chains, turn over several times in the life-span of this protein. This process may be operative during membrane recycling mechanisms. Differences in the half-lives and rate constants of degradation of L-fucosyl residues in glycoproteins from plasma membranes and total cell homogenates of rat liver and Morris hepatoma 7777 demonstrate the existence of at least two dynamically different classes of glycoproteins in both liver and hepatoma plasma membranes.[567]

Phosphomannosyl receptors for lysosomal hydrolases may exist in the lysosomal membrane of rat liver as well as in the plasma membrane.[568] Most of

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the soluble hydrolase activity of broken lysosomes is bound to the lysosomal membrane and can be displaced by D-mannose 6-phosphate. Partially purified β-D-2-acetamido-2-deoxyglucosidase from human fibroblast secretions binds to membranes of rat tissues and subcellular fractions from rat liver.[569] The studies confirm the involvement of D-mannosyl 6-phosphate residues on lysosomal enzymes in their binding to receptors in rat liver.

The interaction with a specific cell-surface lectin is essential for removal of asialoglycoproteins from the circulation in rat liver.[570] Antibody specific to this lectin inhibits its binding of desialylized glycoproteins. A mammalian hepatic-binding protein specific for desialylized glycoproteins bearing terminal non-reducing D-galactosyl residues is located on the external or cytosolic surface of membranes in the lysosomal fraction.[571] Evidence has been obtained for the stability of the binding receptor under conditions where asialoglycoprotein is being continually destroyed in lysosomal catabolism. Evidence from *in vivo* and *in vitro* studies show that soluble immunoglobulin G-immune complexes, formed in antibody excess, are recognized and removed from the circulation by the receptor in rat hepatic parenchymal cells specific for D-galactosyl residues on the immunoglobulin G.[572]

Two rat liver plasma membrane glycoproteins have been identified as a nucleotide pyrophosphatase and a dipeptidyl peptidase, respectively.[573] Monospecific rabbit antibodies against the two glycoproteins participated the antigens without affecting their enzymic activities. The biogenesis of the two glycoproteins has been studied.[574]

Rat hepatocyte surface membranes have been separated into three major sub-fractions, and the distribution of their L-fucosyl-containing glycoproteins reported.[575]

Five glycoproteins (mol. wt. 6.9×10^4, 9.0×10^4, 1.0×10^5, 1.14×10^5, and 1.32×10^5) of the microvillus membrane of rat small intestinal epithelial cells can bind cholera toxin.[576] The presence of these glycoproteins was shown by anti-cholera toxin antibody experiments and by localization of glycoproteins on SDS-gels using iodinated cholera toxin. Differences in the binding of the soluble and membrane-bound forms of maltase(α-D-glucosidase)-glucoamylase in the suckling rat intestine to concanavalin A have been observed.[577] This may be due to differences in the carbohydrate structure of these two enzyme glyco-

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proteins, which in the case of the soluble form of the enzyme may prevent or at least weaken the attachment of the enzyme to the brush border membrane.

The principal surface glycoprotein, which specifically binds immunoglobulin E, has been isolated from rat basophilic leukaemia cells.[578] One molecule of receptor (mol. wt. 3.0×10^4) binds one molecule of immunoglobulin E.

The rat glomerular basement membrane glycopeptide has an *N*-α-D-glucosyl linkage to an unnamed amino-acid.[579] This is a new type of carbohydrate-protein linkage, and quite different from the more common N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl) hydrogen L-asparaginate system.

Several glycoproteins have been isolated from rat adipocyte plasma membranes and separated from the D-glucose transport system, which is apparently devoid of oligosaccharide units that can bind to concanavalin A, a lectin known to activate hexose transport in intact fat cells.[580] A glycoprotein (mol. wt. 9.4×10^4) and several minor components of the membrane represent the major antigens which react with anti-intrinsic membrane protein antibodies.[581] The insulin-like activity of these antibodies is not elicited by direct interaction of the antibodies with either the insulin receptor or the D-glucose transport system.

Ectoglycosyltransferases involved in the dolichol cycle are present at the outer surface of rat spleen lymphocytes.[582] The ability of rat lymph node cells to undergo oxidative mitogenesis is correlated with the presence of four high molecular weight cell surface sialoglycoproteins.[583] This group of glycoproteins is sensitive to a wide range of proteases and is present on the subpopulation of responder lymphocytes.

The early steps in the biosynthesis of glycoproteins associated with the plasma membranes of rat hepatoma tissue culture cells has been identified.[584] Not all the membrane glycoproteins behave identically during biogenesis. There are differences in the relative rates of synthesis, in the extent of modification of these proteins into charge-heterogeneous forms, and in the release into the environment of some membrane-associated glycoproteins.

Changes in expression of a major sialoglycoprotein associated with ascites forms of a mammary adenocarcinoma have been studied by analyses for peanut agglutinin receptors, and by 2-amino-2-deoxy-D-[^{3}H]glucose-labelling and lactoperoxidase-labelling.[585] The various ascites sublines of the 13762 rat mammary adenocarcinoma differ in their ability to survive rejection upon transplantation into a foreign host.[586] No evidence has been found to indicate

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that transplantation differences between sublines is due to the presence or absence of a dominant sialoglycoprotein. To explore cell-surface differences between MAT-B1 and MAT-C1 sublines of this mammary adenocarcinoma, sulphate incorporation into the sialoglycoproteins has been studied. Although both lines synthesize small amounts of sulphated glycosaminoglycans, only the MAT-B1 cells synthesize a sulphated sialoglycoprotein.[587] When oligosaccharides derived from this glycoprotein are isolated, about 20% of them are found to be sulphated and both sulphate and neuraminic acid are detected on some oligosaccharides. The major glycoproteins have been purified from membranes of the sublines MAT-B1 and MAT-C1 by density-gradient centrifugation.[588] Although the glycoproteins from the two lines are similar in composition and physical properties, there are distinct differences in the oligosaccharides attached to the polypeptide.

Glycoside hydrolases and lectins have been used to study the nature of the carbohydrate residues involved in insulin binding to normal and transformed rat ascitic hepatoma cells.[589] The importance of sialoglycopeptides for insulin binding is discussed, and a model for the role of glycoconjugates in the insulin-binding process is described.

Plasma membrane sialoglycoconjugates of Novikoff tumour cells are accessible to exogenous neuraminidase and proteases.[590] The glycoproteins have been radiolabelled using the lactoperoxidase-[^{125}I] method and have been resolved by affinity chromatography on carrier-bound lectins and by polyacrylamide gel electrophoresis.[591]

The amount of *Ricinus* lectin required to cause toxicity to hepatoma cells is able to enter neuraminidase-treated and neuraminidase- and D-galactose oxidase-treated cells more rapidly than untreated cells.[592] The difference agrees with the notion of possible clustering of the binding sites on the cells for the lectin that would occur during the positive co-operative process of lectin binding to enzyme-treated cells, since it has been demonstrated that lectins such as concanavalin A and ricin enter the cells by endocytotic vesicles only after clustering of the binding sites.

Four clonal variants of fibrosarcoma cells with differing degrees of tumorigenicity have been isolated from rats.[593] Protein and glycoprotein profiles have been studied by surface-labelling techniques. The cell lines show a difference in degree of sialylation in one glycoprotein species (mol. wt. 1.5×10^5). The possible role of glycosylation in the ectoprotein in defining the tumourigenic property of the variant cell lines is discussed.

Changes in the cell-surface glycopeptides during myogenesis in wild-type and non-fusing mutant L6 rat myoblasts have been observed.[594] The surface glyco-

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peptides of multinucleated myolubes are more heavily glycosylated than those from mononucleated cells. Several new size classes of glycopeptides appear on the surface of cells during myogenesis.

Cell-surface glycopeptides from mouse cerebrum inhibit protein synthesis and mitosis in several cell types.[595] Their mechanism of action is by inhibition of translation by acting against elongation of nascent polypeptide chains. Membrane interaction and/or cellular activation is required in order for the inhibitory activity to be expressed at the intracellular level.[596] Glycopeptides (mol. wt. 2.5×10^4–3.0×10^4) have been isolated from the cell surfaces of mouse brain cortical tissue.[597] They inhibit both cell division and protein synthesis by cells in culture.

Murine lung macrophages have a receptor system that mediates the binding of glycoproteins.[598] This receptor may be responsible for opsonin-independent phagocytosis-dependent clearance of certain lysosomal enzymes.

Over 30 lectins have been screened for their ability to distinguish mouse thymocyte subsets.[599] Fluorescein-labelled lectins and flow microfluorimetry reveal that there are subpopulations in the thymus that quantitatively differ in various cell-surface carbohydrate components. Cell subpopulations of different sizes can exhibit marked differences in the level of binding such that the amount of lectin bound per cell is often independent of cell size.

Murine cytotoxic T lymphocytes contain a cell-surface glycoprotein (mol. wt. 1.45×10^5), which binds to the lectin from *Vicia villosa*.[600] A lectin affinity absorbent has been prepared for the isolation of T lymphocytes containing this glycoprotein.

Some studies on the structures of the Thy-1 glycoprotein from mouse thymic (immature) and peripheral (mature) T lymphocytes have been reported.[601] The lymphocyte differentiation involves the modification of carbohydrate structures of individual glycoproteins since Thy-1 molecules on peripheral T cells contain more residues of neuraminic acid than do the immature Thy-1 molecules.

Alterations in expression of murine thymocyte cell-surface proteins and glycoproteins during foetal thymus development have been detected.[602] Proteins with lectin-like properties have been identified on murine thymocytes and splenocytes.[603] They have been isolated following affinity chromatography of solubilized cell material on immobilized fetuin.

[595] R. J. Kinders, J. V. Hughes, and T. C. Johnson, *J. Biol. Chem.*, 1980, **255**, 6368.
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Some structural properties of the H-2D^b and H-2K^d murine histocompatibility complex antigens have been reported.[604] Cyanogen bromide cleavage of both antigens yielded two glycopeptides that are homologous to glycopeptides from the H-2K^b molecule.

The structure and metabolism of the oligosaccharide moieties of the membrane glycoproteins in three clonal strains of contact-deficient mouse L cells have been compared with several contact-competent rodent fibroblasts.[605] The absence of functional contacts in the L cells is associated with the synthesis of incomplete glycopeptides expressed at the cell surface.

A cell-surface glycoprotein (mol. wt. 1.8×10^5) related to cell-substratum adhesion during differentiation of mouse myeloid leukaemia cells has been isolated.[606] The glycoprotein, which binds to concanavalin A, may be detected by lactoperoxidase-catalysed iodination and metabolic labelling with L-fucose.

Mouse anti-micrococcus antibodies recognize a membrane marker of murine lymphoid cell lines.[607] This marker, referred to as the confluence antigen, is only exhibited after transition from non-confluent to confluent culture conditions and its expression is correlated with a slowing down or cessation of DNA synthesis.

Similarities in the carbohydrate structures of the murine H-2 antigens, H-2K, H-2D, and H-2I glycoproteins have been demonstrated from lectin-binding studies.[608] A murine cell-surface glycoprotein (mol. wt. 4.8×10^4) having an affinity for exogenously added β_2-microglubulin has been isolated.[609] The receptor reacted with heteroantiserum raised against H-2K and D antigens but did not exhibit alloantigenic determinants shared with H-2K, D, or Ia antigens.

Histocompatibility antigens (H-2^b) have been purified from highly purified plasma membranes from murine tumour cells grown in ascites, using a combination of lectin-affinity chromatography and immunoadsorbent-affinity chromatography,[610] and the interactions between a cell-surface glycoprotein and the murine H-2K and H-2D antigens on myeloma tumour cells have been observed.[611]

The oligosaccharide chains (18)–(21) of epiglycanin, the major cell-surface glycoprotein of the TA3-HA ascites tumour cell of the strain A mouse have been isolated by β-elimination from the protein under reductive conditions, and the structures for four of the *O*-glycosidically linked products have been elucidated.[612]

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α-D-Gal*p*NAc-L-Ser(L-Thr)

(18)

β-D-Gal*p*-(1 → 3)-α-D-Gal*p*NAc-L-Ser(L-Thr)

(19)

α-Neu*p*5Ac-(2 → 3)-β-D-Gal*p*-(1 → 3)-α-D-Gal*p*NAc-L-Ser(L-Thr)-L-Ser(L-Thr)

(20)

α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-
6
↑
2
α-Neu*p*5Ac

(21)

The relative proportions of specific receptors at the cell surfaces of two murine mammary carcinoma ascites sublines have been identified by the adsorption of ^{125}I-labelled lectins and by measuring the relative agglutinability of the two cell lines with various lectins.[613] Determination of the inhibitory activity of epiglycanin for these agglutination reactions suggests the presence of new carbohydrate structures in epiglycanin.

T-200 Glycoprotein, a major cell-surface component of murine haematopoietic cells, is a phosphorylated transmembrane glycoprotein.[614] Two distinct regions of the molecule have been defined by radiolabelling with various metabolic precursors. A relatively protease-resistant domain is exposed on the cell surface and contains the antigenic site. The remaining portion of the molecule contains a region, which is exposed on the cytoplasmic side of the plasma membrane, and contains phosphoserine residues.

6-Thioguanine causes a decrease in the incorporation of D-[2-^{3}H]mannose into glycoproteins of sarcoma 180 treated mice.[615] The decrease appears to be related to a corresponding decrease in the number of receptor sites for concanavalin A available on the plasma membrane of these neoplastic cells.

Differences are observable in the cell-surface glycoproteins of a metastasizing line of B16 mouse melanoma and a poorly metastasizing wheat germ agglutinin-resistant clone.[616] Structural studies reveal that in the altered glycopeptides of the resistant cells decreased levels of neuraminic acid with concomitantly increased levels of L-fucose occur. The lost neuraminic acid originates from attachment to C-3 of D-galactosyl residues, whereas the additional L-fucosyl residues are bound to C-3 of 4-substituted 2-acetamido-2-deoxy-D-glucosyl residues. A possible basis for the glycosylation change and its relation to the biological behaviour are discussed.

Variants of mouse melanoma cells selected for resistance to wheat germ agglutinin toxicity undergo a stable surface change correlated both to lectin

[613] I. Matsumoto, J. F. Codington, M. R. Jahnke, R. W. Jeanloz, and T. Osawa, *Carbohydr. Res.*, 1980, **80**, 179.
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resistance and reduced metastasizing potential.[617] The surface alteration is restricted to glycoproteins able to interact with wheat germ agglutinin.

Cell-surface glycoproteins from Chinese hamster ovary plasma membranes have been isolated by affinity chromatography on immobilized concanavalin A.[618] Monoclonal antibodies, prepared from such receptors are capable of precipitating a cell-surface component (mol. wt. 2.65×10^5).

Adenosine 3′,5′-cyclic monophosphate markedly induces the cell-surface expression of a membrane glycoprotein (mol. wt. 1.35×10^5) in Chinese hamster ovary cells.[619] Colchicine-resistant mutants of Chinese hamster ovary cells with reduced permeability to the drug express an extra glycoprotein (P-glycoprotein) on their surfaces.[620] The glycoprotein (mol. wt. 1.7×10^5) has been purified from isolated plasma membrane vesicles.

The bleaching of fluorescein-conjugated concanavalin A-labelled plasma membranes from Chinese hamster lung fibroblasts with laser light leads to a loss of protein bands and the appearance of high molecular weight material as shown by polyacrylamide gel electrophoresis, indicating membrane glycoprotein cross-linking.[621]

A method has been devised to label specifically neuraminic acid-containing glycoproteins on the surface of baby hamster kidney fibroblasts in culture.[622] Gel electrophoresis enabled comparisons to be made and several differences to be detected in the labelled glycoproteins in the surface membranes from viral-transformed cells and their normal counterparts. When incubated with neuraminic acid or CMP–neuraminic acid, intact hamster fibroblasts incorporate the sugar into acceptors on the cell surface by direct transfer of the sugar moiety.[623] Loosely attached cell-surface neuraminyltransferase may play a role in catalysing the transfer reaction.

A comparison of glycopeptides from control and virus-transformed baby hamster kidney fibroblasts reveal that most of the differences observed between the two sources are quantitative in nature and involve several glycopeptides.[624]

Membrane glycoproteins have been isolated from control and Rous sarcoma virus-transformed baby hamster kidney cells.[625] Three classes of carbohydrate groups have been identified on these molecules: *N*-glycosidically linked, *O*-glycosidically linked, and covalently linked glycosaminoglycans of relatively small molecular weight. Differences between the carbohydrate groups derived from glycoproteins from control and transformed cells are mainly qualitative.

[617] J. E. Jumblatt, T. W. Tao, V. Shulp, J. Finne, and M. M. Buger, *Biochem. Biophys, Res. Commun.*, 1980, **95**, 111.
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The biosynthesis and processing of L-asparagine-linked oligosaccharides of cellular membrane glycoproteins has been examined in monolayer cultures of BHK21 cells and human diploid fibroblasts.[626] The heterogeneity of the oligo-D-mannosyl core components in both neutral and acidic oligosacharides linked to L-asparagine in the cellular membrane glycoproteins is analogous to the heterogeneity reported for the oligosaccharides of avian RNA tumour virus glycoproteins.[627]

Differential lectin-binding patterns observed in acinar, centroacinar, and endocrine cells from the pancreas of adult guinea pig and rat reflect surface carbohydrate developmental changes expressed during morphogenesis and cytodifferentiation of the gland.[628]

In fibroblasts from chick embryos, at different stages of development and subjected to treatment with trypsin, restoration of cell adhesion, cell morphology, and cell-surface glycoproteins occur very nearly in parallel.[629] A possible mechanism for cell-surface recovery is proposed.

Using an indirect ferritin-labelled antibody technique, the localization and distribution of chicken histocompatibility antigens of the H-B system have been studied in bone marrow cells and peripheral blood membranes.[630]

In the ascidian, *Ciona intestinalis*, residues of L-fucose present on the plasma membrane of the spermatozoon and/or on the outer surface of the chorion are functionally involved in the recognition between spematozoa and eggs.[631]

Mucous secretion in the urn cell complex of the murine invertebrate *Sipunculus nudus* may be initiated by the interaction of mucous-releasing stimuli, such as the lectins from *Lotus tetragonolobus* and *Ricinus communis*, with L-fucosyl and D-galactosyl residues of specific membrane receptors.[632]

A cell-surface glycoprotein receptor from *Tethya lyncurium* that functions as a receptor for the D-galactose-specific *Geodia* lectin has been isolated.[633] The glycoprotein (mol. wt. 1.7×10^5) contains neutral carbohydrate (53%), protein (35%), and lipid (6.5%).

Acetylcholine receptor, purified from the electric organs of *Torpedo marmorata*, binds to concanavalin A.[634] A steric inter-relationship between concanavalin A-binding sites and antigenic binding sites of the receptor has been demonstrated. The former do not contain its major antigenic determinants.

The potential role of surface glycoproteins in mediating intercellular adhesion has been investigated through study of cell reaggregation of *Strongylocentrotus*

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[629] M. Vernay, M. Cornic, M. Aubery, and R. Bourrillon, *Biochim. Biophys. Acta*, 1981, **640**, 31.
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[631] F. Rosati and R. De Santis, *Nature (London)*, 1980, **283**, 762.
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[634] S. Wonnacott, R. Harrison, G. G. Lunt, and T. Barkas, *Eur. J. Biochem.*, 1980, **108**, 621.

purpuratus (sea urchin) embryo cells in the presence of specific sugars.[635] D-Galactose and 2-acetamido-2-deoxy-D-galactose were found to inhibit the aggregation to the greatest extent.

Membranes of the parasite *Toxoplasma sondii* contain neither D-galactosyl nor neuraminic acid residues.[636] Low levels of surface glycoconjugates appear to be present.

Carbohydrate-containing macromolecules on the plasma membrane of the epimastigote forms of *Trypanosoma cruzi* have been identified as a result of D-galactose oxidase–sodium borotritide labelling.[637]

8 Hormonal Glycoproteins

A simple effective solvent system for the recovery of gonadotrophins from columns of immobilized concanvalin A has been developed.[638] Rapid chromatographic procedures have been developed that permit the isolation of pregnant mare serum gonadotrophin and subunits derived therefrom.[639]

An *in vitro* biological characterization of the lutrophin–follitrophin dual activity in pregnant mare serum gonadotrophin as expressed in rat tissue assays has been reported.[640]

All five disulphide bonds in the α-subunit of human chorionic gonadotrophin have been assigned.[641] A technique involving partial reduction and *S*-[^{14}C]-carboxymethylation has been used to determine their positions. The hormone undergoes a conformational transition in acid, which, at low temperatures, is characterized by a reversible increase in the polarization of L-tyrosyl fluorescence, together with an altered elution profile on gel chromatography, and changes in the u.v. and c.d. spectra.[642]

Placental human chorionic gonadotrophin, like the corresponding urinary hormone contains sialylized forms of three neutral oligosaccharides linked to L-asparagine residues of the polypeptide subunits.[643] The distribution of the three oligosaccharide chains on the α- and β-subunits has been studied.

Previous observations of selective retention by the kidney and liver of the β-subunit of human chorionic gonadotrophin have been confirmed.[644] The granulosa cells in preovulatory follicles process the two subunits in dissimilar ways and the processing is different from that observed for the hormone bound to other target cells. The synthesis and secretion of human chorionic gonado-

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[638] S. Matsuura and H. C. Chen, *Anal. Biochem.*, 1980, **106**, 402.
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[640] W. T. Moore and D. N. Ward, *J. Biol. Chem.*, 1980, **255**, 6930.
[641] T. Mise and O. P. Bahl, *J. Biol. Chem.*, 1980, **255**, 8517.
[642] H. Forastieri and K. C. Ingham, *Arch. Biochem. Biophys.*, 1980, **205**, 104.
[643] T. Mizuchi and A. Kobata, *Biochem. Biophys. Res. Commun.*, 1980, **97**, 772.
[644] K. L. Campbell, T. D. Landefeld, and A. R. Midgley, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 4793.

trophin subunits in eutopic and ectopic human chorionic gonadotrophin subunit-producing human malignant cell lines have been compared.[645] A hypothesis has been proposed for the synthesis of both the α- and β-subunits in cultured malignant cells.

Glycosylation of the α-subunit of human chorionic gonadotrophin and the β-subunit of bovine luteinizing hormone is inhibited by DL-β-hydroxynoravaline, an analogue of L-threonine.[646] The analogue is incorporated into nascent protein and replaces L-threonine in the L-Asn-X-L-Thr recognition site for glycosylation. The complete integrity of the oligosaccharide moieties of the α- and β-subunits of ovine pituitary lutrophin is not essential for intersubunit recognition.[647] The carbohydrate units appear to play an important role in the activation process that leads to the expression of the full hormone response.

2-Acetamido-2-deoxy-D-galactose has been identified previously as an integral component of the α-subunits of lutrophin and thyrotrophin.[648] Structural studies on bovine and ovine luteinizing hormones have resulted in the identification of the location of this sugar (22). The D-galactopyranosyl residue is present only in the ovine luteinizing hormone. An acid-labile constituent (R^1), whose precise identification is not established, is present in both hormones.

By use of subunit immunoassays that also measure high concentrations of complete glycoprotein hormones, the common α-subunits, and β-subunits of chronic gonadotrophin, luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone have been determined in sera from patients with various cancers with a view to identifying potential tumour markers.[649] Both the α-subunit of these glycoproteins and the β-subunit of chorionic gonadotrophin when used in combination may be potentially useful markers for certain malignant neoplasms.

The immunoperoxidase technique has been used to identify luteinizing hormone-releasing factor and the α-subunit of glycoprotein hormones in ductal carcinoma of the mammary gland.[650] On the basis of interaction with antibodies to α- and β-subunits, the free α-subunit, rather than any of the native glycoprotein hormones that share the common α-subunit, may be identified.

The location of all four disulphide bonds in the α-subunit and three of the six disulphide bonds in the β-subunit of human follicle-stimulating hormone have been reported.[651] The α- and β-subunits of the hormone have been individually conjugated by photoactivation to an azidobenzoyl derivative of a glycopeptide isolated from fetuin.[652] Both the conjugated hormone and the conju-

[645] R. W. Ruddon, C. A. Hanson, A. H. Bryan, G. J. Putterman, E. L. White, F. Perini, K. S. Meade, and P. A. Aldenderfer, *J. Biol. Chem.*, 1980, **255**, 1000.
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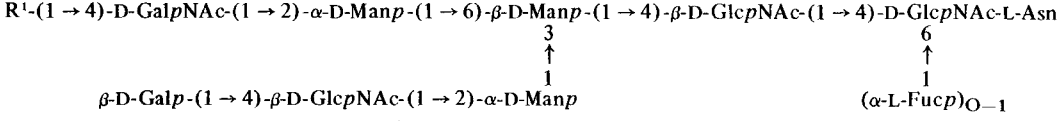
[648] O. P. Bahl, M. S. Reddy, and G. S. Bedi, *Biochem. Biophys. Res. Commun.*, 1980, **96**, 1192.
[649] M. R. Blackman, B. D. Weintraub, S. W. Rosen, I. A. Kourides, K. Steinwascher, and M. H. Gail, *J. Natl. Cancer. Inst.*, 1980, **65**, 81.
[650] M. Seppala and T. Wahlstrom, *Int. J. Cancer*, 1980, **26**, 267.
[651] Y. Fujiki, P. Rathnam, and B. B. Saxena, *Biochim. Biophys. Acta*, 1980, **624**, 428.
[652] P. Rathnam and B. B. Saxena, *Biochim, Biophys. Acta*, 1980, **624**, 436.



(22) R^1 = acid-labile substituent

gated α-subunit after recombination with untreated β-subunit showed increased biological activity. Although the photoactivation procedure is random and site-nonspecific in nature, it provides a method for the improvement of biological activity in hormonal glycoproteins.

Immunological relationships among ovine glycoprotein hormones have been demonstrated. Antiserum to ovine follicle-stimulating hormone binds to ovine-leuteinizing hormone or bovine thryoid-stimulating hormone.[653] This appears to be due to the presence of an antibody specific to the common α-subunit of these hormones. The binding sites of antisera to the β-subunits of these three hormones in the rat have been localized in rat anterior pituitaries by immuno-cytochemistry using light and electron microscopy.[654]

Asialo-human chorionic gonadotrophin is a competitive antagonist of thyroid-stimulating hormone action on its hormone receptor-adenylate cyclase system.[655]

The cDNAs to both mouse α- and β-thyrotrophin mRNAs have been successfully synthesized, cloned, and identified.[656] The cloning will allow for the determination of the nucleotide sequence of these cDNAs and thus, most of the amino-acid sequence of mouse thyroid-stimulating hormone, which is unknown.

Using a mouse thyrotropic pituitary tumour, which produces large amounts of thyroid-stimulating hormone without the structurally related gonadotrophins, the messenger ribonucleic acid-directed biosynthesis of both α- and β-subunits of the hormone has been studied.[657] Separate biosynthetic pathways for the two subunits were identified, and the processing of the pre-subunits to mature glycosylated forms was characterized. The *de novo* biosynthesis of the hormone in mouse pituitary tumour cells has been studied.[658] Inhibition of subunit glycosylation by tunicamycin results in the secretion of an α-subunit (mol. wt. 1.1×10^4) instead of the normal subunit (mol. wt. 1.8×10^4). The combination of the modified α-subunit with the β-subunit is inhibited, thus implying that specific glycosylation may be necessary for the biological activity of the hormone. Studies on the biosynthesis of thyroid-stimulating hormone in cell-free systems indicate that the α- and β-subunits are synthesized from separate messenger ribonucleic acid.[659]

9 Milk Glycoproteins

Publications on the composition and characteristics of goat milk have been reviewed.[660]

[653] M. R. Sairam, *J. Endocrinol.*, 1979, **83**, 149.
[654] C. Tougard, R. Picart, and A. T. Vidal, *J. Histochem. Cytochem.*, 1980, **28**, 101.
[655] P. Carayon, S. Amr, and B. Nisula, *Biochem. Biophys. Res. Commun.*, 1980, **97**, 69.
[656] N. C. Vamvakopoulos, J. J. Monahan, and I. A. Kourides, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 3149.
[657] I. A. Kourides, N. C. Vamvakopoulos, and G. M. Maniatas, *J. Biol. Chem.*, 1979, **254**, 11107.
[658] B. D. Weintraub, B. S. Stannard, D. Linnekin, and M. Marshall, *J. Biol. Chem.*, 1980, **255**, 5715.
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[660] R. Jenness, *J. Dairy Sci.*, 1980, **63**, 1605.

A study of glycopeptides obtained after enzymic hydrolysis of human caseinoglycopeptide from pooled milk has resulted in the complete sequence of its *C*-terminal moiety and the presence of several prosthetic carbohydrate groups being identified.[661] An important difference between cow or sheep and human caseinoglycopeptides is the possession of a single *O*-glycosidically linked L-threonyl residue in the former and up to ten *O*-glycosidically linked L-threonyl residues in the latter. The predicted secondary structure of the glycoprotein is in accordance with the high number of sugar groups situated all but one in β-turns.

A simple model to describe protein glycosylation in mammary tissue has been proposed on the basis of biosynthetic studies in the presence and absence of tunicamycin.[662] Casein is glycosylated, probably in the Golgi, by sequential transfer of monosaccharide units from nucleotide sugars to the protein. Several other polypeptides (mol. wt. 4.0×10^4–8.0×10^4) are glycosylated either during or soon after their synthesis, by transfer of an oligosaccharide from dolichol pyrophosphate.

The structures of three oligosaccharide alditols isolated from bovine κ-casein glycopeptide have been investigated by 360 MHz ^{1}H n.m.r. spectroscopy.[663] The oligosaccharide (23) represents a novel carbohydrate chain of κ-casein. The sites of attachment of the three oligosaccharide chains to the peptide backbone of bovine κ-casein have been identified as LThr-131, L-Thr-133, and L-Thr-135 or 136.[664] The structure (24) of the trisaccharide obtained as the main sugar moiety after treatment of bovine casein with alkaline borohydride has been identified.[665]

β-D-Gal*p*-(1 → 3)-D-GalNAc-ol
6
↑
2
α-Neu*p*5Ac

(23)

α-Neu*p*5Ac-(2 → 3)-β-D-Gal*p*-(1 → 3)-D-GalNAc-ol

(24)

A neuraminyltransferase (CMP-*N*-acetylneuraminate : D-galactosyl-glycoprotein *N*-acetylneuraminyltransferase, EC 2.4.99.1), which transfers neuraminic acid to the terminal end of the carbohydrate chain of κ-casein has been located in Golgi apparatus-enriched fractions of bovine mammary gland.[666] Some properties of

[661] A. M. Fiat, J. Jollès, J. P. Aubert, M. H. Loucheux-Lefebre, and P. Jollès, *Eur. J. Biochem.*, 1980, **111**, 333.
[662] B. K. Speake, F. W. Hemming, and D. A. White, *Biochem. Soc. Trans.*, 1980, **8**, 166.
[663] H. van Halbeek, L. Dorland, J. F. G. Vliegenthart, A. M. Fiat, and P. Jollès, *Biochim. Biophys. Acta.*, 1980, **623**, 295.
[664] M. Kanamori, N. Kawaguchi, F. Ibuki, and H. Doi, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 1855.
[665] T. Saito, T. Itoh, and S. Adachi, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 1023.
[666] S. J. Keller, T. W. Keenan, and W. N. Eigel, *Biochim. Biophys. Acta*, 1979, **566**, 266.

the enzyme are reported. A neuraminyl oligosaccharide chain of χ-casein, isolated from ovine colostrum, has been cleaved from the protein backbone by alkaline reduction.[667] The structures of three derived oligosaccharide alditols (25)–(27) were established.

β-D-Gal*p*-(1 → 3)-D-GalNAc-ol

(25)

β-D-Gal*p*-(1 → 3)-D-GalNAc-ol
6
↑
1
β-D-Glc*p*NAc
4
↑
1
β-D-Gal*p*
3
↑
1
β-D-Gal*p*

(27)

β-D-Gal*p*-(1 → 3)-D-GalNAc-ol
6
↑
1
β-D-Glc*p*NAc
4
↑
1
β-D-Gal*p*

(26)

Three glycoproteins (mol. wt. 6.7×10^4, 4.9×10^4, and 4.6×10^4) have been isolated from bovine milk fat globule membrane.[668] The membrane components were solubilized by detergent and separated by preparative electrofocusing. The selective solubilization of glycoproteins of bovine milk fat globule membranes with potassium chloride and magnesium chloride has been reported.[669]

Concanavalin A has been used to study the binding of prolactin to its receptors in liver membranes from female rats.[670] The binding sites for concanavalin A and prolactin appear to be independent but closely related.

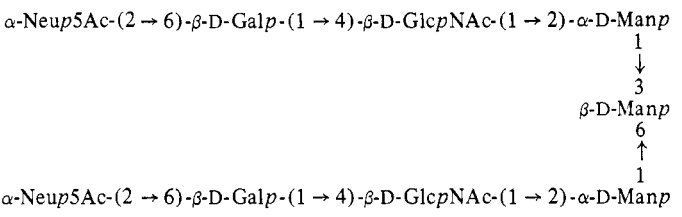
Amino-acid sequence studies have shown that the location of the two carbohydrate prosthetic groups is different in serum- and lacto-transferrins.[671] No sequence homology can be demonstrated, whereas a close relationship between a glycopeptide of human lactotransferrin and a glycopeptide of hen ovotransferrin is observed.

The region of bovine α-lactalbumin that interacts with bovine colostrum D-galactopyranosyltransferase in the lactose-synthetase complex has been examined by differential labelling of the amino-groups with acetic anhydride.[672] A region in α-lactalbumin corresponding to part of the active cleft region in the homologous lysozymes may function in interacting with D-galactopyranosyltransferase and monosaccharides.

[667] S. Soulier, R. S. Sarfat, and L. Szabo, *Eur. J. Biochem.*, 1980, **108**, 465.
[668] I. H. Mather, C. B. Tamplin, and M. G. Irving, *Eur. J. Biochem.*, 1980, **110**, 327.
[669] C. Kanno, S. Nakamura, and K. Yamauchi, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 1193.
[670] M. E. Costlow and P. E. Gallagher, *Biochim. Biophys. Acta*, 1979, **587**, 192.
[671] M. H. Metz-Boutigue, J. Jollès, P. Jollès, J. Mazurier, G. Spik, and J. Montreuil, *Biochim. Biophys. Acta*, 1980, **622**, 308.
[672] R. H. Richardson and K. Brew, *J. Biol. Chem.*, 1980, **255**, 3377.

The effect of acylation of goat α-lactalbumin on lactose synthetase activity and the ability of α-lactalbumin to inhibit the transfer of D-galactose to 2-acetamido-2-deoxy-D-glucose is biphasic.[673] Approximately 15% of the lactose synthetase activity of goat α-lactalbumin and 10% of its inhibitory power is lost in the initial phase, with corresponding losses of 65 and 30% in the second phase. The differential effect of acylation of α-lactalbumin on lactose synthase and inhibitory properties appears to be a result of differential changes in the affinity of the UDP-D-galactose:galactopyranosyltransferase-α-lactalbumin ternary complex for monosaccharides.

Form II of rat α-lactalbumin, which comprises about 80% of the total α-lactalbumin, contains a single oligosaccharide unit that is attached to the polypeptide chain at L-Asn-45.[674] The structure (28) of the unit was determined



(28)

using specific exoglycosidases, endoglycosidases, and methylation analysis.

Purified α-lactalbumin isolated from a rat mammary adenocarcinoma has the same amino-acid and carbohydrate composition, and specific activity in the lactose synthetase reaction, as does α-lactalbumin isolated from normal rat milk.[675]

No evidence has been found to verify an earlier report that one form of the Wistar rat α-lactalbumin has a higher molecular weight than the other form.[676] The molecular weights, amino-acid compositions, and partial amino-acid sequences at the *N*- and *C*-terminal ends of the two forms of α-lactalbumin are identical.

Three charged forms of rat α-lactalbumin have been separated by ion-exchange chromatography.[677] Although the forms have a similar amino-acid composition, they differ in carbohydrate composition. The existence of the three forms was found to reflect, in part, a variation in the number of neuraminic acid residues per polypeptide chain. The site of attachment of the carbohydrate is at L-Asn-45. Secondary structure calculations predict that this amino-acid is in a β-bend conformation, whereas this residue in bovine α-lactalbumin, a poorly glycosylated protein, is not in a bend conformation.

[673] P. Holohan, W. B. Hoffman, and M. J. Kronman, *Biochim. Biophys. Acta*, 1980, **621**, 333.

[674] R. Prasad, B. G. Hudson, D. K. Strickland, and K. E. Ebner, *J. Biol. Chem.*, 1980, **255**, 1248.

[675] R. Prasad and K. E. Ebner, *Cancer Res.*, 1979, **39**, 3598.

[676] R. Prasad and K. E. Ebner, *J. Biol. Chem.*, 1980, **255**, 5834.

[677] R. Prasad, B. G. Hudson, P. Butkowski, J. W. Hamilton, and K. E. Ebner, *J. Biol. Chem.*, 1979, **254**, 10 607.

A radioimmunoassay for mouse α-lactalbumin has been developed.[678]

α-Lactalbumin purified from mouse milk exists in several species with different charges and in two molecular size forms.[679] More than 90% of the α-lactalbumin exists in a form (mol. wt. 1.4×10^4) containing no significant amount of carbohydrate, whereas the remainder exists in a form (mol. wt. 1.85×10^4) that interacts with concanavalin A. All species are active in the lactose synthetase reaction and show identical immunological properties.

10 Serum Glycoproteins

The classification of human serum glycoproteins into those containing 2-acetamido-2-deoxy-D-galactosyl residues and those lacking this amino-sugar has been proposed.[680] Those glycoproteins containing 2-acetamido-2-deoxy-D-galactose have alkali-labile carbohydrate chains with the amino-sugar or 2-acetamido-2-deoxy-3-*O*-D-galactosyl-D-galactosyl residues linked *O*-glycosidically to hydroxy-amino-acids. Serologically, these two types of sugar chains, which usually occur together on the same molecule, can be characterized by their reaction with the lectins from *Helix pomatia*, *Agaricus bisporus*, and *Arachis hypogaea*.

An analysis of the carbohydrate structures from whole unfractionated rat plasma has been undertaken to obtain a quantitative measure of the occurrence of different carbohydrate structures.[681] The most abundant fraction is that of the biantennary *N*-glycosidic-type (71%), followed by the triantennary *N*-glycosidic-type (24%) and *O*-glycosidic chains (3%).

A comparative investigation of serum glycoproteins has identified levels in schizophrenic patients significantly elevated over those for normal individuals.[682] The contents of D-glucose and L-arabinose were higher in serum glycoproteins from schizophrenic patients.

The serum levels of the acute-phase proteins haptoglobin and α-acid glycoprotein in patients with operable solid malignancies correlate inversely with both *in vitro* and *in vivo* parameters of cellular immunity.[683] Serum levels of α_2-HS glycoprotein correlate directly with these parameters.

Eighty per cent of the sera from patients with progressive adenocarcinomas exhibit a unique electrophoretic pattern for glycoproteins as compared to patients in remission or those with some other types of cancer.[684] The pattern is characterized by the presence of two α_2-glycoprotein bands. Further characterization of the glycoproteins in one of the bands indicates that α_1-acid

[678] M. M. Zamierowski and K. E. Ebner, *J. Immunol. Methods*, 1980, **36**, 211.
[679] Y. Nagamatsu and T. Oka, *Biochem. J.*, 1980, **185**, 227.
[680] G. Uhlenbruck, I. Reese, P. Vaith, and H. Haupt, *J. Clin. Chem. Clin. Biochem.*, 1979, **17**, 29.
[681] J. Finne and T. Krusius, *Eur. J. Biochem.*, 1979, **102**, 583.
[682] R. Varma, A. Y. Hoshino, and J. R. Vercellotti, *Carbohydr. Res.*, 1980, **82**, 343.
[683] A. M. Baskies, P. B. Chretien, J. F. Weiss, R. W. Makuch, R. A. Beveridge, W. J. Catalona, and H. G. Spiegel, *Cancer*, 1980, **45**, 3050.
[684] G. B. Dermer, L. M. Silverman, S. J. Gendler, and Z. A. Tokes, *Clin. Chem.*, 1980, **26**, 392.

glycoprotein, and haptoglobin β-chains, are the principal components. These components are markedly less apparent in, or are absent from, this band of normal sera.

Human plasma angiotensinogens at various physiological states have been purified.[685] Microheterogeneity of the molecules is revealed by isoelectric focusing even after removal of the neuraminic acid with neuraminidase, implying that the heterogeneity is due to structural characteristics other than neuraminic acid.

The fate of some defined serum asialoglycoproteins in the circulation of the mouse has been recorded.[686] Different mechanisms to explain the uptake of glycoproteins bearing D-galactosyl residues on different types of oligosaccharide chains are discussed.

One of the significant effects in isolated rat hepatocytes of exposure to adenosine 3′,5′-phosphate is the continuous inhibition of glycoprotein secretion.[687] The normally excreted glycoproteins are synthesized but accumulate within the hepatocytes in the membrane-associated fractions.

A kinetic evaluation of the capacity of human serum to form complexes with trypsin has generated partition profiles that may be approximated by a series of four intersecting straight lines.[688] Such profiles are suggested to reflect the binding of trypsin to α_2-macroglobulin in a kinetically preferred mode (α-sites) followed by a subsidiary mode (β-sites) and finally to α_1-antitrypsin. The form of the profile indicates that saturation of the α-sites corresponds to a molar binding ratio of α_2-macroglobulin to trypsin of 1 : 2.

Relationships between structural variation of α_1-antitrypsin and disease have been reviewed.[689]

The plasma protease inhibitors, α_1-antitrypsin, inter-α-trypsin inhibitor, and C1-esterase activity are associated with unwashed human platelet surfaces.[690] No evidence for the location of α_2-macroglobulin, α_1-antichymotrypsin, or antithrombin III at these surfaces has been obtained.

The microheterogeneity of human plasma α_1-acid glycoprotein has been investigated by isoelectric focusing.[691] Variations in the ratio of (2→3)- and (2→6)-linked neuraminic acid residues, together with amino-acid replacements in the polypeptide, are considered to be the likely cause of the heterogeneity. Human α_1-acid glycoprotein can be separated on immobilized concanavalin A into two fractions, one that binds and one that does not bind to the lectin.[692] The concanavalin A-reactive variant contains only identical doubly-branched oligosaccharide chains.

[685] A. A. Faiers, A. Y. Loh, and D. H. Osmond, *Can. J. Biochem.*, 1978, **56**, 892.
[686] R. A. Newman, U. Fricke, P. J. Klein, G. Uhlenbruck, and A. L. Devries, *J. Clin. Chem. Clin. Biochem.*, 1980, **18**, 31.
[687] G. L. Firestone and E. C. Heath, *Arch. Biochem. Biophys.*, 1980, **201**, 453.
[688] R. M. Topping and S. Seilman, *Biochem. J.*, 1979, **177**, 493.
[689] R. W. Carrell and M. C. Owen, *Essays Med. Biochem.*, 1979, **4**, 83.
[690] G. K. M. Endresen, *Thromb. Res.*, 1980, **19**, 157.
[691] E. G. Berger, S. R. Wyss, R. B. Nimberg, and K. Schmid, *Z. Physiol. Chem.*, 1980, **361**, 1567.
[692] B. Baynard and J. P. Kerckaert, *Biochem. Biophys. Res. Commun.*, 1980, **95**, 777.

The labelling of α_1-acid glycoprotein by sodium borotritide or ^{125}I for use in radioimmunoassay procedures has been compared.[693]

Evidence for uniformity of the carbohydrate chains in individual glycoprotein molecular variants of α_1-acid glycoprotein has been obtained from affino-rocket immunoelectrophoresis studies.[694] The structures of the carbohydrate units from the glycosulation site 1 of human α_1-acid glycoprotein have been elucidated.[695] These units can be grouped into those with bi- (class A) and tri-antennary (class B) structures and the triantennary structure with an L-fucose residue (class FB). The structural variability of the carbohydrate chains at glycosylation site I and also at four other sites already reported account for the microheterogeneity of the glycoprotein.

The serum form of rat α_1-acid glycoprotein as well as its putative precursor form from liver have been isolated and purified.[696] During secretion of α_1-acid glycoprotein from the liver cell, several of the neutral sugars initially attached to the glycoprotein are removed, and subsequently other sugars such as 2-amino-2-deoxy-D-glucose and neuraminic acid are added.

The removal of residues of neuraminic acid is associated with an increased expression of activity inhibitory to platelet aggregation.[697] Asialo-α_1-acid glycoprotein is much more inhibitory than the α_1-acid glycoprotein in the inhibition of platelet response to collagen or thrombin. Evidence for reduced clearance of asialo-α_1-acid glycoprotein during the acute-phase response to inflammation in rats is reported.[698]

α_1-Acid glycoprotein has been identified as a cofactor in the lipoprotein lipase reaction.[699] The elevated levels of plasma glyceride in human nephrotic patients may be the direct result of an excessive loss of α_1-acid glycoprotein from the plasma to urine.

Glycopeptides have been isolated from proteolytic digests of human plasma α_1-antitrypsin.[700] Oligosaccharides were prepared from the glycopeptides by alkaline borohydride cleavage of the L-asparagine-oligosaccharide bond. The asialoglycopeptides and the asialo-oligosaccharides derived therefrom, but not the materials containing a full complement of neuraminic acid, are rapidly metabolized by the liver after injection into rats. The integrity of terminal D-galactosyl residues in both the asialoglycopeptides and asialo-oligosaccharides is required for recognition and uptake by the liver.

Structural characterization of the oligosaccharides of liver Z variant α_1-antitrypsin has been reported.[701] The findings suggest that the precursors of the

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[694] B. Baynard and J. P. Kerckaert, *Biochem. Biophys. Res. Commun.*, 1980, **95**, 777.
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complex oligosaccharides of the secretory glycoprotein plasma α_1-antitrypsin are D-mannose-rich oligosaccharides. Glycoside hydrolases, which are necessary for processing the precursor, are active in the rough endoplasmic reticulum of Pi (protease inhibitor) ZZ individuals. Most of the Z variant α_1-antitrypsin appears unable to migrate at a normal rate to the smooth endoplasmic reticulum and Golgi apparatus for eventual excretion from the liver.

The inhibition of thrombin by α_1-proteinase inhibitor can be prevented by heparin.[702] There are similarities in the structural requirements of heparin for anticoagulant activity and for the inhibition of the α_1-proteinase inhibitor interaction with thrombin.

Three large molecular weight peptides have been isolated from normal human α_1-protease inhibitor by treatment with cyanogen bromide.[703] The carbohydrate composition of these peptides accounts for the total carbohydrate content of the native protein. Human α_1-protease inhibitor has three oligosaccharide side-chains attached to three separate L-asparaginyl residues of the protein by *N*-glycosyl linkages.[704] Two of the oligosaccharide side chains have structures of the biantennary type and the third is of the triantennary type commonly found in other human plasma glycoproteins.

Human inter-α-trypsin inhibitor has been purified by zinc chelate and hydrophobic interaction chromatographies.[705]

The stability of complexes between some proteinases and the proteinase inhibitor α_2-macroglobulin has been examined.[706] The cleavage of a proteinase-susceptible site may lead to covalent-bond formation between the enzyme and α_2-macroglobulin at a region not involving the active site of the enzyme. Although the ability to form covalent links is not essential for the inhibitory capacity of the α_2-macroglobulin, it may help to stabilize the complexes against dissociation or proteolysis. A stoicheiometric enzyme–inhibitor complex is formed with α_2-macroglobulin and *Crotalus adamanteus* venom protease.[707] This is in contrast to the action of the protease on other plasma proteinase inhibitors, in which enzymatic digestion of the inhibitor results in a gradual loss of all inhibitory activity.

Purified α_2-macroglobulin as well as α_2-macroglobulin-trypsin complexes are quantitatively bound to rabbit anti-human α_2-macroglobulin antibody that is immobilized on a gel matrix.[708] Heparin protects thrombin from inhibition by α_2-macroglobulin, whereas it increases the enzyme inhibition by antithrombin III.[709] This observation may be of value in the treatment of thrombosis in patients with high levels of α_2-macroglobulin and low levels of antithrombin III,

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such as occurring in the nephrotic syndrome. α_2-Macroglobulin and α_1-proteinase inhibitor are both isolated from the same batch of pooled human plasma following chromatography on zinc chelate columns.[710, 711] The α_2-macroglobulin is homogeneous as determined by polyacrylamide gel electrophoresis and the α_1-proteinase inhibitor requires two further chromatographic steps to achieve homogeneity. Human α_2-macroglobulin undergoes autolytic peptide cleavage to give two large molecular weight peptides.[712] Fragmentation occurs at an alkylamine-reactive site, and it is proposed that this unique chemically reactive site in the native protein may be an internal pyroglutamyl residue. The complete amino-acid sequence of the 56 residue glycopeptide containing the alkylamine-reactive centre has been elucidated.[713]

Cultured fibroblasts bind, take up by endocytosis, and degrade α_2-macroglobulin.[714] Binding and endocytosis of the glycoprotein by a cell may be a means of modulating proteases in the macroenvironment of the cell and during endocytosis.

α_2-Macroglobulin from serum of multiple sclerosis patients has been characterized immunochemically.[715] Gross heterogeneity exists since isoelectric focusing reveals the presence of at least seven α_2-macroglobulin bands. Electrophoretic examination of α_2-macroglobulin preparations from normal and cystic fibrosis patients has shown no consistent differences in the number or position of electrophoretic species, or in the ability of these species to bind trypsin, to be cleaved by bound proteinase, or to change conformation.[716] Similarly, no differences were detected on comparing the proteolytic subunit cleavage patterns on reaction with trypsin. As no differences are observed between the two groups, a primary genetic defect affecting α_2-macroglobulin subunit cleavage in cystic fibrosis is unlikely.[717, 718] The binding of concanavalin A to α_2-macroglobulin, immunoglobulin G, or immunoglobulin M from cystic fibrosis patients is significantly higher than in the control groups.[719] It is suggested that these alterations in glycoprotein binding might be a general phenomenon in cystic fibrosis glycoproteins.

The Zn-α_2-glycoprotein of human serum consists of four major antigenically identical components.[720] Variations in the concentrations of the individual components were detected during ontogenesis.

Patients suffering from rheumatoid arthritis show a marked increase in pregnancy-associated α_2-glycoprotein.[721] The inhibitory effect of the glyco-

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protein on the lymphocyte response to phytohaemagglutinin is also shown. Two-dimensional immunoelectrophoresis has been used to study a morphological abnormality of α_2-glycoprotein in patients afflicted by hereditary angioneurotic oedema.[722] In some cases it has been demonstrated that there exists a double abnormality in the glycoprotein, associated with a deficiency in post-translational glycosylation accompanied by a loss of the inhibitory function of the esterase.

The synthesis, glycosylation, and secretion of α_{2u}-globulin in primary monolayer cultures of adult hepatocytes derived from normal and vitamin A-deficient rats has been examined.[723] The carbohydrate moiety of the glycoprotein aids in maintaining a critical level of the globulin in serum and the non-glycosylated form is cleared rapidly from circulation. The secreted glycoprotein did not bind to steroids under the assay conditions used, although the results do not preclude the possibility that α_{2u}-globulin may bind steroids in other situations.[724] Using primary cultures of rat hepatocytes as a model for the synthesis, secretion, and modification of α_{2u}-globulin, two major glycosylated species, which are resistant to dissociation by reducing agents and which contain a carbohydrate moiety that is similar to those reported previously for other serum glycoproteins, can be identified.[725] The high molecular weight glycoproteins do not appear to serve as precursors for the non-glycosylated protein.

A report has described a radioimmunoassay for the β-component of pregnancy-specific β_1-glycoprotein.[726, 727] The effects of variation of the ratios of two maternal plasma proteins (α_2 and β_1) with similar antigenic determinants has been assessed. Electro-immunoassay, radial immunodiffusion, and nephelometry have also been applied to the measurement of the glycoprotein.[728] Kinetic immunoturbidimetry has been applied to the measurement of pregnancy-specific β_1-glycoprotein.[729] The technique is compared with other published methods. An immunoassay has been described for human pregnancy-specific β_1 glycoprotein utilizing fluorescein-labelled antibodies to the glycoprotein.[730] However, although this technique has the advantage of avoiding radiation hazards associated with radioimmunoassay, it requires relatively large quantities of antibody. Analytical crossed immunoelectrophoresis has been performed to examine the relationship of two molecular variants (SP1α and SP1β) of

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pregnancy-specific β_1-glycoprotein.[731] The relative rates of disappearance of the two components from the maternal circulation after delivery is reported.

β_2-Glycoprotein-1, a constituent of human plasma chylomicrons and very low-density lipoprotein, activates the enzymic activity of lipoprotein lipase.[732] The glycoprotein is present in all major lipoprotein density fractions isolated from human serum, and preferentially binds to triglyceride-rich lipid emulsions.[733] In contrast to the binding of human serum β_2-glycoprotein I to mitochondria, its binding to platelets is an equilibrium binding and can be correlated with inhibition of adenylate cyclase activity.[734] β_2-Glycoprotein-1 has been isolated from rat serum also.[735] The strong binding of this glycoprotein to heparin has allowed its isolation and purification to be achieved by affinity chromatography on immobilized heparin. An artificial triglyceride emulsion has a high association with β_2-glycoprotein-1, thus providing further evidence for the importance of the glycoprotein in triglyceride metabolism.[736] Isoelectric focusing of β_2-glycoprotein-1 reveals four major bands all giving an immunochemical identity with a monospecific antiserum.[737] From its unique amino-acid composition, the glycoprotein is shown to be distinct from all apolipoproteins described previously.

The formation of serum β_2-microglobulin is not directly tumour-associated in gastrointestinal cancer as has been previously reported.[738]

Subcomponents $C1_q$, $C1\bar{r}$, and $C1\bar{s}$ of the first component of complement have been purified from Cohn fraction 1 by affinity chromatography on immobilized immunoglobulin G.[739] Mouse $C1_q$ has been isolated from serum and ascites fluid by affinity chromatography on human immunoglobulin G-coated latex beads.[740] Structural and functional similarities to human and rabbit $C1_q$ were demonstrated. The fourth component of complement (Ss protein), synthesized by murine macrophages, is a glycoprotein containing two different types of oligosaccharide units.[741] The α-chain oligosaccharide appears to be of a complex-type, whereas the β-chain is a high D-mannose unit containing eight or nine D-mannosyl residues, and the γ-chain is devoid of carbohydrate. Glycosylation of the precursor P-C4(185) is important for the secretion of the fourth component of complement.[742] Tunicamycin inhibits the glycosylation of this precursor by cultured peritoneal macrophages.

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The kinetics of secretion and glycosylation of the subunits of mouse complement component C4 by peritoneal macrophages have been reported.[743] The amino-acid sequence of the *N*-terminal regions of the intracellular precursor of the fourth component of guinea-pig complement (pro-C4) and isolated α-, β-, and γ-chains of native C4, synthesized by peritoneal macrophages in culture, have been determined.[744]

The third (C3) and fifth (C5) components of human complement have been purified by hydrophobic chromatography;[745] C3 is probably a protein and C5 is a glycoprotein, the carbohydrate moiety of which is responsible for variations in its apparent molecular weight.

Several aspects of the current understanding of the nature of the fibroblast cell–substratum interactions and the role of plasma fibronectin have been reviewed.[746] An electron microscopic study of plasma fibronectin has shown the glycoprotein to have a compact elongated structure with an axial ratio of 2 : 1.1, a length of 155 ± 13 Å, and a width of 88 ± 17 Å.[747] High concentrations of fibronectin-like antigens have been detected in human seminal plasma.[748] These antigens constitute a major fraction of the few protein antigens in common with blood and seminal plasma. Purified plasma fibronectin has a tendency to form filamentous protein polymers, which are produced in high yields by aggregation of fibronectin with polyamines.[749] The observed polymers of soluble fibronectin resemble the filamentous fibronectin-containing pericellular structures in fibroblast cultures and may provide a model for studies on the deposition of fibronectin in matrix form. Dextran sulphate (mol. wt. 5.0×10^5), as well as heparin, interacts with fibronectin and fibronectin–gelatin complexes and competes with heparin for binding to fibronectin bound to immobilized gelatin.[750]

The carbohydrate moiety of fibronectin is not required for the mediation of several biological activities characteristic of this glycoprotein.[751] When tunicamycin is used to inhibit glycosylation, fibroblast attachment to collagen and agglutination of formalin-fixed sheep erythrocytes are not impaired. Bovine brain gangliosides block fibronectin-mediated cell adhesion to collagen.[752] Specific gangliosides or related neuraminic acid-containing glycoconjugates at the cell surface may act as receptors for fibronectin, since destruction of the neuraminic acid residues on these glycoconjugates abolishes the inhibition of the

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cell adhesion. Human plasma fibronectin has been shown to retain its biological function as a mediator of adhesion and spreading of trypsinized fibroblasts on native or denatured collagen, after chemical treatment to alter specific constituent amino-acids or to oxidize much of the carbohydrate part of the glycoprotein.[753] Either the secondary or tertiary structure appears not to be essential for its *in vitro* activity or the glycoprotein possesses a biologically active domain relatively resistant to chemical modification.

^{125}I-Labelled fibronectin binds to both native and denatured collagen immobilized on macroporous agarose in reactions that exhibit different kinetics.[754] The rates of both reactions were enhanced by heparin and by highly sulphated dextran sulphate.

Several proteins (mol. wt. 3.2×10^4–2.2×10^5) of human plasma are capable of binding putrescine by the action of a Ca^{2+}-activated L-glutaminyl-peptide γ-glutamyltransferase (EC 2.3.2.13).[755] One of these proteins has been identified as fibronectin on the basis of its interaction with anti-fibronectin antiserum. The binding of plasma fibronectin to denatured collagen by macrophages has been reported.[756] Evidence is given for a limited number of receptors on the macrophages with higher affinity and a large number of sites with lower affinity for the fibronectin.

A tryptic fragment (mol. wt. 2.7×10^4) derived from the *N*-terminus of the fibronectin subunit inhibits the binding of intact fibronectin to *Staphylococcus aureus*.[757] The fragment can be cross-linked to *S. aureus* by blood coagulation factor XIIIa. Interactions of fibronectin with *S. aureus via* this fragment may be important for bacterial opsonization and attachment.

Hamster plasma fibronectin has been fragmented with trypsin and thermolysin into three distinct structural domains.[758] One of these domains, which contains essentially all the carbohydrates, exhibits gelatin-binding activity, whereas the other two domains bind to heparin. The ability to promote cell-attachment and spreading is localized in one of the glycosaminoglycan-binding domains.

Proteolytic digestion of human plasma fibronectin and *N*-terminal sequence analysis of some of the fragments produced have been used to locate the gelatin-binding region of the glycoprotein.[759] Cathepsin D cleaves fibronectin to release a fragment (mol. wt. 7.2×10^4) with gelatin-binding activity. This fragment is further split by thrombin to produce a polypeptide (mol. wt. 2.9×10^4) located at the *N*-terminus of fibronectin and an adjacent fragment (mol. wt. 4.3×10^4) containing the gelatin-binding site. The affinity centres of plasma fibronectin interacting with fibrin and denatured collagen are located in two different adjacent domains, although a weak affinity for fibrin is also exhibited by the

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collagen-binding site.[760] Glycopeptides isolated from human plasma fibronectin have similar compositions and structures to glycopeptides from transferrin.[761]

Structural studies on the L-asparagine-linked oligosaccharide chains released after hydrazinolysis and borohydride reduction of human plasma fibronectin have identified two acidic oligosaccharides (*29*a) and (29b).[762] The sugar chains have the typical complex structure found in human transferrin, human antithrombin III, and some other serum glycoproteins, although minor differences exist.

Human plasma fibronectin contains two polypeptide chains (mol. wt. 2.15×10^5 and 2.20×10^5), which are separable by electrophoresis after reduction of the disulphide bonds in the parent glycoprotein.[763] The two polypeptide chains are similar in structure but not identical to each other. Fibronectin from human amniotic fluid and from plasma are indistinguishable both immunologically and by amino-acid composition. However, carbohydrate analysis demonstrates the presence of additional saccharides in the amniotic fluid fibronectin.[764] The peptide pattern produced by limited cleavage with two proteases also highlighted differences in the two glycoproteins. Preliminary evidence for the interaction of cellular fibronectin with proteoglycans is also discussed.

Limited proteolysis of plasma fibronectin by human leucocyte elastase results in the release of three distinct biologically active peptides, which contain L-glutaminyl-peptide γ-glutamyltransferase (EC 2.3.2.13)-reactive L-glutaminyl residues, gelatin binding, and cell-adhesive activity.[765] The release of univalent, biologically active fibronectin fragments by the enzyme, which is released from leucocytes by physiological stimulation and is an important mediator of inflammatory tissue injury, has resulted in the proposal of a mechanism for alteration of cell–connective tissue interactions at sites of inflammation *in vivo*.

The interaction of proteases, antithrombin III, and heparin has been the subject of a review.[766] Heparin accelerates the rate of reaction of antithrombin with thrombin, an effect which is abolished by mild reduction of the antithrombin with dithiothreitol.[767] The cleavage of one of three disulphide bonds in antithrombin results in reduced affinity for heparin and the loss of heparin-accelerated antithrombin activity, and imply that the heparin and thrombin bind at different sites on the antithrombin molecule. The binding of heparin to the high-affinity binding site on antithrombin III does not directly enhance or inhibit the antithrombin III/thrombin reaction rate.[768]

Human antithrombin III–thrombin complex has been cleaved with [^{14}C]-methoxyamine into inactive thrombin and [^{14}C]-labelled antithrombin III.[769]

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During formation of the antithrombin III–thrombin complex, the inhibitor is cleaved at an L-arginine-X bond. This L-arginine residue forms a carboxylic ester with the enzyme, whereas the excised polypeptide remains bound through a disulphide bridge.

Antithrombin from mouse plasma has been purified by affinity chromatography on a highly stable immobilized heparin column.[770]

Human antithrombin III contains four L-asparagine-linked oligosaccharide chains per molecule.[771] Quantitative release of the oligosaccharides from the polypeptide portion has been achieved by hydrazinolysis followed by *N*-acetylation and reduction with sodium borohydride. Structural analysis of the oligosaccharides have shown the presence of typical complex-type structures as found in numerous other animal glycoproteins.

Human antithrombin III contains four identical *N*-glycosidically linked carbohydrate chains per molecule.[772] No evidence of heterogeneity in the carbohydrate chains was found and the structures (29) of these oligosaccharide chains are similar to those found in human plasma fibronectin.

L-Tryptophanyl residues of antithrombin have been modified with *N*-bromosuccinimide.[773] Chemical modification of antithrombin III with dimethyl(2-hydroxy-5-nitrobenzyl)sulphonium bromide results in the incorporation of one hydroxynitrobenzyl moiety per molecule of glycoprotein at an L-tryptophan residue.[774] When the modification is performed in the presence of heparin, the extent of modification is decreased and the heparin-promoted enhancement of thrombin inactivation is preserved.

Chemical modifications of antithrombin III have demonstrated that the u.v.-difference spectrum produced when heparin interacts with this proteinase inhibitor is due primarily to changes in the tryptophan environment.[775] The L-tryptophan-modified antithrombin III is still capable of binding to thrombin even when it has lost 85% of heparin co-factor activity.

Sodium pentosan polysulphate (SP-54), a semi-synthetic polysaccharide ester (mol. wt. 2.0×10^3) consisting of a chain of (1→4)-linked D-xylopyranose residues, has biological properties similar to those of heparin.[776] SP-54 markedly potentiates the inhibition of factor IIa, factor Xa, and plasmin by antithrombin III.

Removal of the carbohydrate portion of prothrombin fragment 1 does not alter the calcium-binding or membrane-binding properties of the protein.[777] The deglycosylated protein, however, undergoes rapid self-association.

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$$R^1\text{-}(1\rightarrow 6)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\rightarrow 2)\text{-}\alpha\text{-D-Man}p\text{-}(1\rightarrow 6)\text{-}\beta\text{-D-Man}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\rightarrow 4)\text{-D-GlcNAc-ol}$$

$$\begin{matrix} & & &

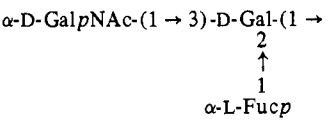
By use of lectins (*Bandeiraea simplicifolia* I-B_4 isolectin and castor bean agglutinin) and α- and β-D-galactosidases, it has been shown that the terminal non-reducing D-galactopyranosyl residues of asialoprothrombin possess the β-anomeric configuration rather than the α-anomeric form as previously reported.[778]

The receptors for factor VIII/von Willebrand factor that are present on human platelets are directly proportional to the extent of ristocetin-induced platelet-aggregating activity.[779, 780] Removal of the neuraminic acid residues from and modification of the resulting D-galactopyranosyl residues of the factor VIII/von Willebrand glycoprotein result in a reduced binding affinity for the receptors.[781] A specific receptor for factor VIII/von Willebrand factor protein exists on the surface of human platelets.[782] Two classes of binding sites, low and high affinity receptor sites, are present and the binding of the factor VIII/von Willebrand factor protein is highly dependent on ristocetin concentration and on the carbohydrate content of the factor VIII/von Willebrand factor protein.

Antisera against Ca^{2+}-dissociated factor VIII procoagulant activity (VIII:C) has been immobilized and used to study the removal of VIII:C, factor VIII-related antigen, and von Willebrand factor from normal human plasma.[783] The removal of VIII:C and the related antigen from the sera follow similar patterns but less von Willebrand factor can be removed.

Further studies support the hypothesis that the underlying defect in von Willebrand type II disease may be incomplete assembly of factor VIII/von Willebrand factor monomer molecules into oligomers at the quaternary level of molecular organization.[784] In von Willebrand type I disease, reduced synthesis of the factor VIII/von Willebrand factor subunit or of primary polymers (mol. wt. 1.0×10^6) may be the defect.

The carbohydrate composition and identification of blood group A, B, and H oligosaccharide structures on human factor VIII/von Willebrand factor have been reported.[785] Terminal oligosaccharide sequences of (30) and 2-*O*-α-L-fuco-



(30)

pyranosyl-D-galactose, respectively, are present on normal factor VIII/von Willebrand protein in a (1→4)-β-D-glycosyl linkage to the penultimate sugar residue. Oligosaccharides containing these structures exist in both alkali-stable

[778] A. E. Eckhardt, I. J. Goldstein, and G. L. Nelsestuen, *Arch. Biochem. Biophys.*, 1980, **199**, 635.
[779] K. J. Kao, S. V. Pizzo, and P. A. McKee, *J. Clin. Invest.*, 1979, **63**, 656.
[780] K. J. Kao, S. V. Pizzo, and P. A. McKee, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5317.
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[783] H. A. Cooper, D. Lee, M. A. Lamb, and R. H. Wagner, *Br. J. Haematol.*, 1980, **44**, 149.
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[785] J. M. Sodetz, J. C. Paulson, and P. A. McKee, *J. Biol. Chem.*, 1979, **254**, 10754.

and labile linkages to the protein. No evidence for any significant decrease in the carbohydrate content in factor VIII/von Willebrand factor in von Willebrand's disease has been recorded.[786]

Causes for the discrepancies in the measurements of factor VIII antigen have been investigated.[787] The antibody used in solid-phase radioimmunometric assay systems shows selection towards large molecular weight forms of factor VIII. This selection together with the 'two antibody sandwich' technique used in the assay may explain the loss of reactivity towards the smaller molecular weight forms of factor VIII. Purified and reduced human factor VIII exhibits an eight-band subunit structure (mol. wt. 3.0×10^4–2.3×10^5) as shown by polyacrylamide gel electrophoresis.[788] Rabbit antibody to factor VIII reacts with all of the subunits, whereas haemophilic antibody reacts with low molecular weight subunits only. Subunits derived from the largest factor VIII multimers have a higher binding affinity for D-galactose-specific lectins than those obtained after reduction of smaller oligomers.[789] Ristocetin co-factor activity of purified factor VIII is competitively inhibited by two *Ricinus communis* lectins and by concanavalin A.

Blood-coagulation factor VII has been purified from human plasma.[790] Factor VII (mol. wt. 4.8×10^4) is composed of a single polypeptide chain (mol. wt. 4.0×10^4). The carbohydrate composition of this glycoprotein was not reported.

Bovine blood-coagulation factor X contains both L-asparagine-linked and L-threonine-linked oligosaccharides.[791] The structures of the L-asparagine-linked chain (31) and (32), and the L-threonine-linked chains (33)–(36) have been elucidated.

The plasma partition system of dextran–poly(ethylene glycol) (2 : 1 w/w) has been used in the separation of rat α-fetoprotein from rat serum albumin.[792] The purity of the product was demonstrated by its binding parameters and by analytical gel electrophoresis. Mouse α-fetoprotein has been effectively separated from contaminating proteins by gel-entrapped antibody filtration.[793]

Affinity experiments with lentil (*Lens culinaris*) lectin have revealed the existence of two distinct molecular populations of α-fetoprotein.[794] By use of a combination of crossed lectin immunoelectrophoresis and radio-immunoelectrophoresis, one of the populations was shown to decrease linearly with gestational age.

[786] T. S. Zimmerman, R. Voss, and T. S. Edgington, *J. Clin. Invest.*, 1979, **64**, 1298.
[787] M. A. Howard, L. Hau, J. Perkin, K. B. Thomas, B. G. Firkin, and J. Koutts, *Thromb. Res.*, 1980, **19**, 63.
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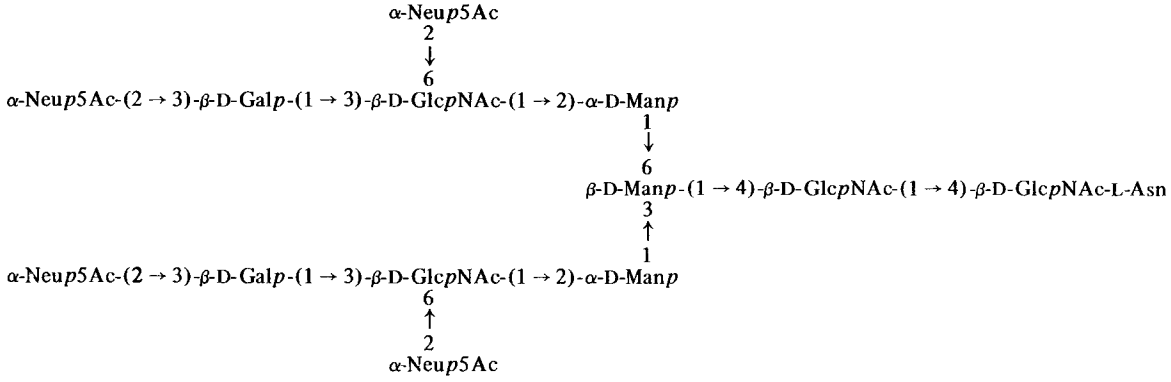
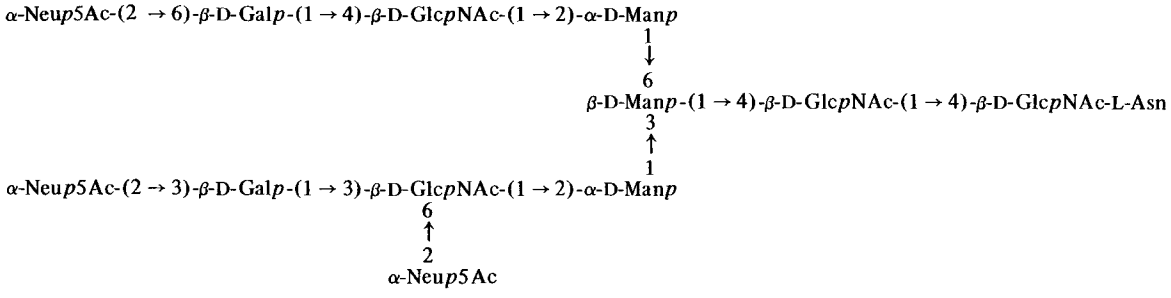
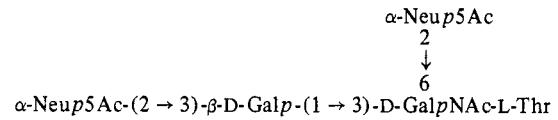
[792] P. Urios and N. Cittanova, *Biochim. Biophys. Acta*, 1980, **621**, 63.
[793] G. J. Mizejewski, R. Simon, and M. Vonnegut, *J. Immunol. Methods*, 1980, **31**, 333.
[794] J. P. Kekaert, B. Bayard, F. Puech, X. Codaccioni, and G. Biserte, *Clin. Chim. Acta*, 1980, **104**, 245.


α-Neup5Ac
2
↓
6
α-Neup5Ac-(2 → 3)-β-D-Galp-(1 → 3)-β-D-GlcpNAc-(1 → 2)-α-D-Manp
1
↓
6
β-D-Manp-(1 → 4)-β-D-GlcpNAc-(1 → 4)-β-D-GlcpNAc-L-Asn
3
↑
1
α-Neup5Ac-(2 → 3)-β-D-Galp-(1 → 3)-β-D-GlcpNAc-(1 → 2)-α-D-Manp
6
↑
2
α-Neup5Ac

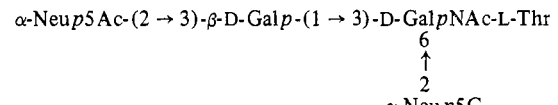
(31)


α-Neup5Ac-(2 → 6)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 2)-α-D-Manp
1
↓
6
β-D-Manp-(1 → 4)-β-D-GlcpNAc-(1 → 4)-β-D-GlcpNAc-L-Asn
3
↑
1
α-Neup5Ac-(2 → 3)-β-D-Galp-(1 → 3)-β-D-GlcpNAc-(1 → 2)-α-D-Manp
6
↑
2
α-Neup5Ac

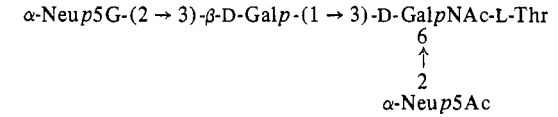
(32)



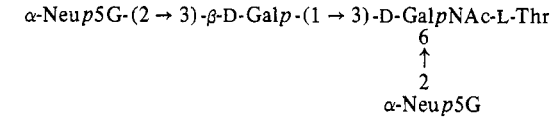
(33)



(34) G = glycolyl



(35)



(36)

The nucleotide sequence corresponding to the 3′-terminal region of rat α-fetoprotein mRNA has been determined, and the amino-acid sequence of the *C*-terminal portion of the glycoprotein has been inferred.[795] A striking homology in amino-acid sequence between this segment and the corresponding portions of rat, bovine, and human albumin was observed.

The importance of a careful selection of experimental conditions for the immunolocalization studies on exportable liver glycoproteins such as α-fetoprotein and albumin has been stressed.[796]

A single anionic binding site on bovine α-fetoprotein is responsible for the binding of both polyunsaturated fatty acids and bilirubin.[797] It is proposed that the function of this anionic binding site on the glycoprotein is for the transport of these two metabolites in foetal serum, as well as for their cross-placental transfer.

The interaction of rat α-fetoprotein with bilirubin has been measured from changes in the absorption spectrum, and by the appearance of two intense bands in c.d. spectra.[798]

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[797] J. C. Hsia, S. S. Er, C. T. Tan, T. Estes, and E. Ruoslahti, *J. Biol. Chem.*, 1980, **255**, 4224.
[798] V. Versée and A. O. Barel, *Biochem. J.*, 1979, **179**, 705.

A human plasma glycoprotein (mol. wt. 6.0×10^4) with affinity for L-lysine binding sites in plasminogen has been isolated and is probably identical to a previously described plasma protein with an unknown biological function called L-histidine-rich glycoprotein.[799] The role of the glycoprotein in the regulation of fibrinolysis is discussed.

Ceruloplasmin, the major antioxidant in serum, is able to dismutate superoxide radicals.[800] Ceruloplasmin is a multifunctional blue copper-containing enzyme, which shows oxidase activity directed towards aromatic amines and ferrous ions. An L-histidine-rich polypeptide fragment (mol. wt. 1.8×10^4) has been isolated from ceruloplasmin.[801] It consists of 159 amino-acids and contains a possible copper-binding site. Amino-acid sequence studies of tryptic peptides isolated from this L-histidine-rich fragment have been described.[802]

A single iron-binding fragment (mol. wt. 3.56×10^4) isolated from human transferrin by proteolysis, is derived from the *N*-terminal domain of the molecule and is devoid of carbohydrate.[803] Some physical characteristics of human transferring have been measured from small-angle neutron-scattering studies.[804]

Removal of the majority of the sugars from human thyroxine-binding globulin by glycoside hydrolases has no effect on the stoicheiometry of the binding of L-thyroxine, as shown by the quenching of L-tryptophan fluorescence and equilibrium dialysis.[805]

Thyroglobulin has been used as a model in the study of the synthesis of glycoproteins in follicular cells of the thyroid gland.[806]

Immunological and biochemical evidence has been reported for the identity of tartrate-resistant isoenzymes of acid phosphatases (EC 3.1.3.2) from human serum and tissue.[807]

Two molecular forms (mol. wt. 9.5×10^4 and 1.75×10^5) of alkaline phosphatase have been isolated from bull seminal plasma.[808] The enzyme activity is lowered after treatment with neuraminidase.

Diffusion control of the binding of carcinoembryonic antigen with immobilized anti-carcinoembryonic antigen antibody has been reported.[809] It is suggested that diffusion control of ligand binding to insoluble receptors may be a very general biological phenomenon.

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Fetuin and other proteins have been identified in cerebrospinal fluid and plasma of foetal pigs during development.[810] A radioimmunoassay for fetuin has been described, and used in the comparison of the immunological properties of ovine and bovine fetuin.[811]

Bovine plasma prekallikrein (Fletcher factor) is a glycoprotein (mol. wt. 8.2×10^4) consisting of a single polypeptide chain with the first 15 amino-acids at the *N*-terminus identical to those of human factor XI.[812]

Human α-2HS glycoprotein binds Zn^{2+} and thus has been purified by metal chelate affinity chromatography.[813] A study of the variations in the level of the glycoprotein in plasma under pathological conditions has been reported.

Bovine high molecular weight kininogen is a glycoprotein co-factor in the kaolin-mediated activation of factor XII (Hageman factor) and is composed of a single polypeptide chain containing four domains, including a heavy-chain, a kinin moiety, fragment 1.2, and a light-chain region.[814] Both fragment 1.2 and the light-chain region in the glycoprotein are essential for the activation of factor XII and prekallikrein.

Purified urinary Tamm–Horsfall glycoprotein has been used to develop a radioimmunoassay for its measurement in human serum or plasma.[815] An L-serine-L-threonine-rich D-galactopyranosylglycoprotein (mol. wt. 8.1×10^4) has been isolated from pooled normal human plasma.[816] The chemical composition of this glycoprotein is unusual, with the carbohydrate moiety accounting for 76% of its weight and consisting of neuraminic acid, D-galactosyl, D-mannosyl, L-fucosyl, 2-acetamido-2-deoxy-D-galactosyl, and 2-acetamido-2-deoxy-D-glucosyl (64 : 105 : 12 : 6 : 50 : 65) residues per molecule.

The rate of synthesis of a rat serum glycoprotein increases greatly during inflammation.[817] The molecular properties of this glycoprotein differ from those of any other rat protein hitherto described.

Non-enzymatic glycosylation of haemoglobin has been reviewed.[818]

Radioactively labelled D-glucose from several manufacturers contains radioactive impurities of unknown structure that bind covalently to proteins, thus simulating non-enzymic glycosylation.[819] Therefore, to prove non-enzymatic glycosylation of proteins, evidence in addition to incorporation of radioactivity from labelled D-glucose should be presented.

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[815] A. Dawnay, C. McLean, and W. R. Cattell, *Biochem. J.*, 1980, **185**, 679.
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Improved conditions have been reported for converting ketoamine-linked hexoses of glycosylated haemoglobin to hydroxymethyl-furfuraldehyde followed by determination with 2-thiobarbituric acid.[820] Glycosylated haemoglobins have been measured in an autoanalyser using a thiobarbituric acid reagent.[821] The values obtained on total haemolysate haemoglobin using this method correlate with the values obtained by an ion-exchange chromatographic method.

The specificity of nonenzymatic glycosylation of human haemoglobin A has been examined both *in vivo* and *in vitro* and the particular sites that are modified have been determined.[822] Significant differences between the *in vivo* and *in vitro* sites of glycosylation were observed.

Functional properties of three glycosylated minor haemoglobins have been studied by electron paramagnetic resonance (e.p.r.) spectroscopy.[823] The e.p.r. spectra of the components in the nitrosyl form account for their unique oxygen-binding characteristics in the framework of a two-state allosteric model.

The non-enzymatic glycosylation of haemoglobin in normal and diabetic subjects has been compared.[824]

Glycosylated haemoglobin A has been determined by scanning densitometry after agar gel electrophoresis.[825] The method is suited to laboratory monitoring of diabetic control.

Non-enzymatic glucosylation of rat albumin *in vitro* is first-order with respect to D-glucose and albumin and occurs primarily at interchain L-lysine residues.[826] Enhanced levels of glucosylated albumin have been detected in diabetic rats, indicating that measurements of albumin glycosylation in humans may also be useful in the detection and management of diabetes.

The effects of different freezing conditions on the activities of the anti-freeze glycoproteins and glycopeptides from polar fish have been reported.[827] Supercooling of solutions has been found to have a large effect on the apparent activities of two of the glycoproteins. The carbohydrate portions of these molecules are indispensable for activity although the size and structure of the peptide chain are subject to variation under conditions of freezing.[828]

[820] C. V. Subramaniam, B. Radhakrishnamurthy, and G. S. Berenson, *Clin. Chem.*, 1980, **26**, 1683.
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[822] R. Shapiro, M. J. McMannus, C. Zalut, and H. F. Bunn, *J. Biol. Chem.*, 1980, **255**, 3120.
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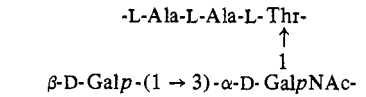
Natural abundance ^{13}C n.m.r. spectroscopy has been used to study aqueous solutions of anti-freeze glycoproteins.[829] Assignments of resonances specific to carbons of the repeating unit (37) are presented. The results are consistent with a flexible random-coil structure.

The anti-freeze activities of two anti-freeze glycoproteins from polar fish blood are lower in D_2O than in water.[830]



(37)

The synthesis and secretion of anti-freeze glycoproteins has been examined by L-[^{14}C]alanine pulse-labelling in the Antarctic cod (*Dissostichus mawsonii*).[831] Differences in the synthesis of this glycoprotein from that of other plasma proteins synthesized in the liver were observed.

11 Immunoglobulins

Reviews on immunoglobulins have dealt with antigenic features of immunoglobulins,[832] the three-dimensional structure of immunoglobulins,[833] and monoclonal antibodies to Ia antigens from rat thymus.[834]

Crude IgG, after precipitation with poly(ethylene glycol), has been purified on columns of carboxymethyl-Sephadex on which the poly(ethylene glycol) and contaminating proteins do not bind.[835]

The purification of rabbit immunoglobulin molecules expressing kappa (κ) light chains, utilizing the allotypic specificity b4, has been achieved in stages involving isolation of specific antibody, preparation of a solid-phase immunoadsorbent of coupled antibody and subsequent isolation of b4 (κ) IgG.[836]

Subclasses 1 and 2 of human IgG have been isolated following affinity chromatography on immobilized subclass-specific anti-IgG1 and anti-IgG2.[837] The procedure has been used for the removal of contaminating IgG1 and IgG2 from isolated myeloma proteins. The physicochemical characterization of an IgG1, monoclonal cryoimmunoglobulin exhibiting marginal low temperature-dependent insolubility, has been described.[838] In comparison with noncryo-

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[830] A. I. Ahmed, D. T. Osuga, and R. E. Feeney, *Biochem. Int.*, 1980, **1**, 41.
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globulin IgGs, amino-sugars, L-fucose, and neuraminic acid are present in reduced amounts.

A study of the three-dimensional structures of the pFc′ fragment of guinea-pig IgG1, a non-covalent dimer of the C_H3 homology subunit, is reported.[839] From quantitative ^1H n.m.r. studies on the Fc and pFc′ fragments of rabbit immunoglobulin G, it is concluded that there is a two-fold axis of symmetry in these fragments in solution, and that there is internal mobility in these fragments which leads to reduced line widths.[840]

A study of the interaction of human red cells and IgG alloantibodies directed against the membrane glycoproteins glycophorin A and glycophorin B, which vary in their site number, lateral membrane mobility, and possible intrinsic multivalency has been reported.[841] In each instance the Fc region of the antibody in the presence of ligand is found to impose significant restrictions on the segmental flexibility of the Fab regions.

Aggregation appears to be a necessary condition for staphylococcal protein A binding to the Fc region of IgG antibodies.[842] Two molecules of IgG must be bound in proximity to one molecule of protein A.

The primary structure of the variable region of human λ-type Bence-Jones protein NIG-48 has a segment of 112 amino-acid residues having a unique sequence and a low degree of homology with λ-chains of five other subgroups.[843] The addition of two amino-acid residues around position 65 may represent a new subgroup, V_λ VI. The kinetics of refolding reactions of type λ Bence-Jones protein, V_L and C_L fragments have been studied by c.d., u.v. absorbance, and fluorescence spectroscopy.[844] Refolding cannot be expressed as a simple sum of the refolding reactions of the V_L and C_L fragments.

The dimer of Bence-Jones protein has been reduced to generate one thiol group per monomer.[845] After alkylation, the modified protein can be conjugated to preformed liposomes and erythrocyte ghosts.

Mouse myeloma MOPC-46 produces a κ-type immunoglobulin light chain.[846] Glycosylation of the protein may be regulated in these cells when grown in culture by addition or deletion of D-glucose in the incubation medium. These cells are naturally deficient in their ability to glycosylate proteins when incubated in a defined medium containing only pyruvate as an energy source.

A copper–immunoglobulin complex, containing one atom of copper per Fab fragment, has been isolated from a myeloma patient.[847] The abnormal copper-binding affinity is a property of the unique primary structure of this fragment.

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An X-ray crystallographic study of the Fab fragment of the D-galactan-binding mouse immunoglobulin J539 has revealed a general similarity to mouse myeloma protein MCPC603.[848]

Further evidence for the *de novo* biosynthesis of a precursor form of K-46 immunoglobulin light chain has been presented.[849] In the presence of microsomal membranes, the precursor protein is proteolytically cleaved and glycosylated. Partial disruption of the integrity of microsomal membrane vesicles appears to be an essential prerequisite for the efficient utilization of the membrane-associated processing steps involved in co-translational modification of the newly synthesized protein.

Bovine serum albumin and keyhole limpet haemocyanin have been coupled to the 2-(4′-aminophenyl)ethylamine derivatives of sialo-oligosaccharides *via* phenylisothiocyanate intermediates under conditions that preserve labile sugar linkages.[850] Rabbits immunized with these synthetic glycoproteins produce antibodies, which are directed against the oligosaccharides and which distinguish positional isomers of α-neuraminosyl(2 → 3/6)-β-D-galactosyl groups.

The activation of the classical complement pathway by homogeneous anti-pneumococcal SIII polysaccharide antibody complexed with oligosaccharide antigens of different size has been reported.[851] The size of soluble antigen–antibody complexes is the major factor determining its capacity to trigger the complement pathway activation. Serum anti-D-mannan antibodies against *Candida albicans* D-mannan have been measured in a radioimmunoassay procedure using radiolabelled D-mannan-tyramine conjugate.[852] In pooled normal human serum, most anti-D-mannan antibody is of the IgG class.

Monoclonal antibodies have been prepared against cell-surface glycoproteins from Chinese hamster ovary plasma membranes.[618] Monoclonal IgG antibody against Epstein–Barr virus identifies a membrane antigen (mol. wt. 2.5×10^5).[30] The antibody can significantly reduce the infectivity of some independent strains of the virus.

Soluble IgG-immune complexes formed in antibody excess are recognized and removed from the circulation by the receptor in hepatic parenchymal cells specific for D-galactosyl residues in the immunoglobulin.[572]

Type-specific antiserum has been prepared against the major envelope glycoprotein of herpes simplex virus type 1.[40] The glycoprotein was also purified from a host of virus-induced proteins with a specific immunoadsorbent prepared from the antibodies. Antibodies to glycoproteins from herpes simplex virus type 1 have been used to demonstrate the location of the glycoproteins on the surface of the virions as well as on the surface of infected cells.[38]

Hepatitis B surface-antigen immune complexes have been determined by an antigen-specific method.[32]

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[851] G. A Medgyesi, G. Füst, J. Gergely, and J. C. Jaton, *Mol. Immunol.*, 1979, **11**, 949.
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Monospecific hybridomas directed against Rauscher murine leukaemia virus glycoprotein have been prepared.[67] The antibodies have been coupled with specific cleavage fragments and partial sequence data have been used to order the peptides within the molecule.

IgG, purified from antisera raised against human thrombin–anti-thrombin complex, has been used in a radioimmunoassay for the measurement of nanogram quantities of thrombin–anti-thrombin complex.[853] The assay system has been employed to assess the importance of anti-thrombin as a plasma thrombin inhibitor under conditions approaching the *in vivo* situation.

Antibodies to a purified glycopeptide derived from an avian riboflavin-binding protein have been raised in rabbits.[854] Radioimmunoassay indicated that the glycopeptide, although a structural component of the riboflavin-binding protein, did not contribute to the major antigenic determinants of the native protein.

Both murine complex formation and the binding of haptens, natural antigens, or bivalent affinity labels were unaltered by complete removal of oligosaccharides from rabbit IgG.[855] However, removal of oligosaccharides with L-aspartyl-β-D-2-acetamido-2-deoxyglucosidase irreversibly alters the abilities of the antibodies to interact with C_{1q}. Several possibilities are discussed in relation to the stability of C_{1q} binding regions on IgG antibodies.

The precipitation of a monoclonal IgG2 crystalline cryoglobulin has been shown to occur *via* a nucleation mechanism.[856] Cleavage of only one or two disulphide bonds between the two heavy chains appears to abolish cryoprecipitation. No structural changes between reduced (noncryoprecipitable) and native (cryoprecipitable) IgG2 have been detected by laser Raman spectroscopy, suggesting that perturbation at a local site is responsible for loss of cryoprecipitation.

A thermodynamic investigation of the solubility of monoclonal cryoimmunoglobulins as well as a large number of normal (cold-soluble) immunoglobulins has been reported.[857] The atypical behaviour of monoclonal cryoimmunoglobulins has been explained by the formation of one or a few additional electrostatic contacts, or, less frequently, a larger number of van der Waals interactions in the protein-rich solid phase relative to normal immunoglobulin.

An enzyme-linked immunosorbent (ELISA) assay has been developed to detect serum IgG and IgM antibodies against individual serotypes of pneumococcal polysaccharide antigens.[858] In contrast to other assays of antibody to pneumococcal polysaccharides, ELISA is capable of detecting IgG and IgM classes of antibody.

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A polysaccharide fraction from the parasite *Toxoplasma gondii* has been used for the ELISA assay of anti-IgG and anti-IgM.[859]

Goat anti-lacto-*N*-difucohexaose I IgG does not agglutinate human erythrocytes.[860] Upon polymerization of the antibody with dimethyl suberimidate, the higher oligomers agglutinate erythrocytes from donors belonging to the Le($a-b+$) blood group, thus acting as a specific Le^b agglutinin. Oligomers smaller than pentamers are not agglutinins.

Gangliosides covalently attached to bovine serum albumin have been used to elicit antibodies.[861] Antibodies to ganglioside G_{D_3} are highly specific and do not cross-react with G_{M_3}, lactosylceramide, or other glycolipids. Purified antibodies to G_{M_1}, in contrast, exhibit a marked heterogeneity.

A system suitable for the study of the core glycosylation activity of lymphoid cell membranes has been described.[862] The MOPC-46B light chain precursor is used as a cotranslational acceptor for the transfer of endogenous lipid-linked core oligosaccharide present in microsomal membranes from plasmacytoma tissue as well as those from canine pancreas and Krebs II ascites tumour cells.

Antibodies capable of precipitating a wide variety of oncoviral glycoproteins have been detected in human sera.[863] These antibodies are directed against cellular, rather than the virus-encoded antigenic determinants. The findings exclude the possibility that this immune response is elicited as a consequence of oncovirus exposure. Similar conclusions have been proposed from studies on the precipitation of human antibodies with purified envelope glycoprotein from the primate oncovirus, simian sarcoma virus–simian sarcoma-associated virus.[864]

The binding constants of levans and D-fructo-oligosaccharides to BALB/c and NZB D-fructan-specific myeloma anti-D-fructan antibodies have been determined by affinity electrophoresis, and the results shown to confirm earlier findings on the specificity of binding.[865]

Antibodies against plant lectins have been isolated from mice ascitic fluid.[866]

Monoclonal antibodies which identify individual determinants on a mouse mammary tumour virus glycoprotein (mol. wt. 5.2×10^4) have been isolated.[61] Type-specific, class-specific, and group-specific determinants are recognized on the glycoprotein.

Two D-mannose-rich glycopeptides have been isolated from a monoclonal human IgM, and their positions of origin on the polypeptide chain have been determined.[867] Structural studies, mainly using ^1H n.m.r. spectroscopy, show that each glycopeptide possesses a unique set of oligosaccharides. In addition, isomeric fragments, not previously observed, were detected, and the authors suggest a modification of the scheme previously proposed for the processing of

[859] J. R. Mineo, M. E. Camargo, and A. W. Ferriera, *Infect. Immun.*, 1980, **27**, 283.
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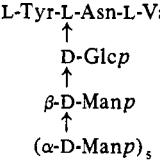
D-mannose-rich IgM oligosaccharides. Hybrid cells formed between human lymphocytes and mouse myeloma cells produce human immunoglobulin in culture. A hybrid producing human IgM reactive with the terminal disaccharide of the Forsman glycolipid has been isolated.[868] The findings illustrate the potential of human–mouse hybrid cells as a source of human monoclonal antibodies for therapeutic and diagnostic purposes.

A glycopeptide (38) from human IgM derived from a patient with Waldenstrom macroglobulinemia has been identified as containing an unusual oligosaccharide core of only one 2-acetamido-2-deoxy-D-glucopyranosyl residue and six D-mannopyranosyl residues.[869] Amino-acid sequence studies and other evidence show that the oligosaccharide chain is located on the C-terminal portion of the IgM heavy chain at L-asparagine-563.

The structures of the oligosaccharides present in the mouse IgM secreted by the plasma cell tumour MOPC 104E have been determined.[870] This mouse IgM contains four complex oligosaccharides and one high D-mannose-type oligosaccharide per heavy chain. The complex oligosaccharides have structures of two types, those with two outer branches which are similar to the complex oligosaccharides of human immunoglobulins (39) and others with three outer branches which have not been previously reported in immunoglobulins (40). The high D-mannose oligosaccharides liberated by *endo*-β-2-acetamido-2-deoxy-D-glucanase have structures also seen in human IgM, varying in size from D-Man*p*$_8$-D-Glc*p*NAc to D-Man*p*$_5$-D-Glc*p*NAc. An oligosaccharide with high D-mannosyl content is resistant to attack by this enzyme and apparently has a 'hybrid' or atypical structure.



(38)

The complete amino-acid sequence of the 432 residue heavy (α) chain of mouse myeloma MOPC 511 immunoglobulin has been determined.[871] The carbohydrate composition indicates the presence of two *N*-glycosidically linked units in the chain. These are positioned at L-asparagine 162 and L-asparagine 419. No *O*-glycosidically linked units in the hinge region (residues 225–247) are postulated.

Although tunicamycin blocks the glycosylation of immunoglobulins, it does not prevent the synthesis of heavy chains either directly or by feedback.[872]

[868] R. Nowinski, C. Berglund, J. Lane, M. Lostrom, I. Bernstein, W. Young, S. I. Hakomori, L. Hill, and M. Cooney, *Science*, 1980, **210**, 537.
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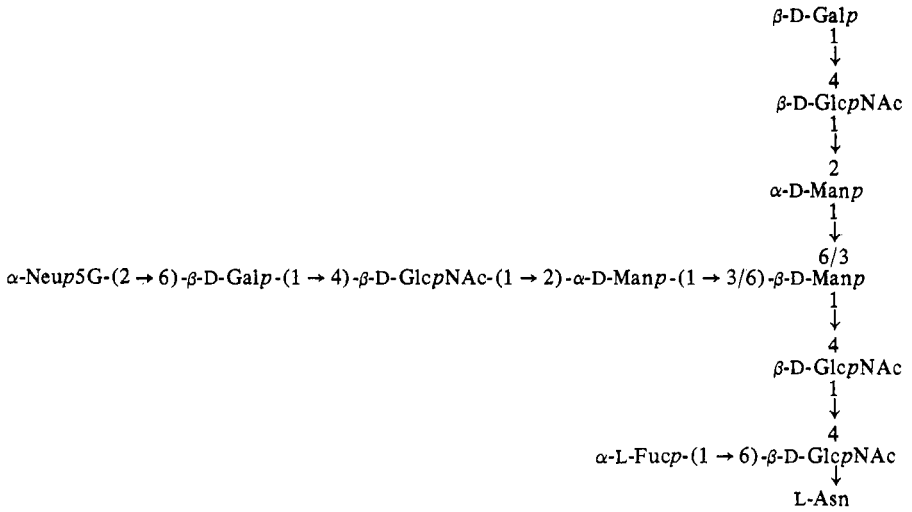
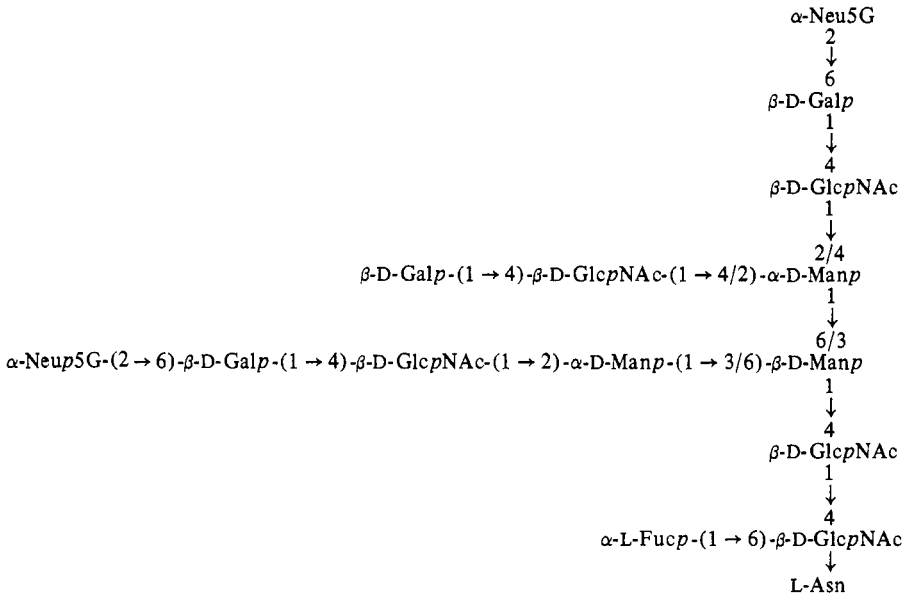


(39)



(40)

Since glycosylation affects overall protein three-dimensional structure, differences in the structure of non-glycosylated immunoglobulins might be responsible for the failure of non-glycosylated IgM to be transferred to the cell surface, and for alterations in the details of assembly and secretion of non-glycosylated immunoglobulin.

Soluble IgM–bovine serum albumin complexes formed in antibody excess are cleared from the circulation *via* the D-mannosyl recognition system in non-parenchymal cells in liver and other organs of the reticuloendothelial system.[873] Conformational changes on the IgM molecules are induced by antigen binding, resulting in the exposure of D-mannosyl-containing oligosaccharides on the immunoglobulins.

Significant IgM antibody responses to pneumococcal capsular polysaccharides have been demonstrated in nurse sharks immunized with pneumococcal vaccines.[874] The antibodies, isolated from immune sera by affinity chromatography, are exclusively of the 19S, IgM variety. Heterogeneity of ligand binding exhibited by the antibodies to capsular polysaccharide of type III pneumococcus is attributed to intermolecular heterogeneity.

Some conformational changes in the structure of human IgA have been induced by treatment with glycoside hydrolases.[875] The susceptibility of the immunoglobulin to hydrolysis by cathepsin B is increased after removal of the carbohydrate.

The kinetics and thermodynamics of binding of the hapten *O*-β-D-galactopyranosyl-(1 → 6)-*O*-β-D-galactopyranosyl-(1 → 6)-D-galactose to the homogeneous immunoglobulin IgA T-601 and to heterologous recombinants of heavy and light chains prepared from mouse myeloma IgA's, which all have the same D-galactan specificity, have been studied by a chemical relaxation method.[876] The heterologous recombinants exhibit the same kinetic pattern of binding as their parent proteins. Thus, the conformational transitions linked to hapten binding appear to be a general property of the antibody molecule, probably implemented as changes in the interactions between heavy and light chain domains.

12 Erythrocyte Glycoproteins

A method for selectively labelling neuraminic acid residues of glycoproteins in erythrocyte membranes with a nitroxide spin-label has been developed.[877] The heterobifunctional reagent 2-[^{14}C]acetamido-4-mercaptobutyric acid hydrazide has been applied to the labelling of periodate-generated aldehydes on the human erythrocyte membrane.[878] The levels of incorporation are comparable to those of the periodate–borotritide technique. Hydrazone formation appears to be the dominant mode of covalent attachment.

Nylon fibres coated with various lectins have been used for the specific selection from mixed populations of erythrocytes or tissue culture cells with

[873] J. F. Day, R. W. Thornburg, S. R. Thorpe, and J. W. Baynes, *J. Biol. Chem.*, 1980, **255**, 2360.
[874] T. V. Shankey and L. W. Clem, *Mol. Immunol.*, 1980, **17**, 365.
[875] E. Ganea, *Rev. Roum. Biochim.*, 1980, **17**, 31.
[876] R. Zidovetzki, Y. Blatt, C. P. J. Glaudemans, B. N. Manjula, and I. Pecht, *Biochemistry*, 1980, **19**, 2790.
[877] J. B. Feix and D. A. Butterfield, *FEBS Lett.*, 1980, **115**, 185.
[878] K. E. Taylor and Y. C. Wu, *Biochem. Int.*, 1980, **1**, 353.

lectin receptors.[879] The evaluation of the utility of such a system for studies of proliferating cells is reported.

A new type of cleavable heterobifunctional and photosensitive reagent, *N*-[4-(4′-azidophenylazo)benzoyl]-3-aminopropyl-*N*′-oxysuccinimide ester has been synthesized and attached *via* its activated ester group to peanut agglutinin.[880] The lectin derivative binds specifically and reversibly to neuraminidase-treated human erythrocyte ghosts, and, upon photo-irradiation, covalent attachment of over 35% of the bound lectin occurs. The lectin–receptor complexes were isolated by immunoprecipitation with antibodies against peanut agglutinin, and partially characterized as asialoglycophorin A (mol. wt. 4.1×10^4 and 8.5×10^4) and a second glycoprotein (mol. wt. 5.8×10^4).

Human and rabbit erythrocytes have been partitioned in an aqueous two-phase system.[881] The cell-partition behaviour has been examined with respect to neuraminic acid content of the cell surface. The existence of two main groups of neuraminic acids differing in their topochemical arrangement on the human erythrocyte surface is proposed. Human erythrocyte ghosts have been disrupted under pressure and the resulting fragments have been fractionated.[882] The sialoglycoprotein contents of individual fractions have been reported.

Changes in the carbohydrate structure of membrane glycoproteins during the development and ageing of human erythrocytes have been reviewed.[883, 884] The ageing of human erythrocytes is accompanied by an overall reduction in the surface carbohydrate content, which includes a decrease in neuraminic acid, with an increase in D-galactosyl and 2-acetamido-2-deoxy-D-galactosyl residues in the non-reducing terminal positions on the oligosaccharide chains of the glycoproteins.[885] These newly exposed residues in the desialylised protein may serve as recognition signals triggering the elimination of senescent erythrocytes from circulation. The complete carbohydrate composition of young and old human erythrocyte membranes demonstrates that, during ageing, a significant decrease of membrane carbohydrate occurs.[886]

During the development of Ehrlich ascites tumour in mice, a progressive decrease in red cell count is observed, which is accompanied by a transformation of erythrocytes to acanthocytes.[887] Desialylization of the glycoproteins in the plasma membranes is suppressed while a decrease in D-galactosyl and 2-acetamido-2-deoxy-D-galactosyl residues would be in accord with the observed reduced rate of erythrocyte clearance during tumour development.

[879] S. G. Phillips, E. A. Kabat, and O. J. Miller, *Exp. Cell Res.*, 1980, **127**, 361.
[880] C. L. Jaffe, H. Lis, and N. Sharon, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 402.
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[882] S. L. Schrier and I. Junga, *J. Supramol. Struct.*, 1980, **13**, 1.
[883] M. Fukuda, *Seikagaku*, 1980, **52**, 244.
[884] L. Gattegno, F. Fabia, D. Bladier, and P. Cornillot, *Biomedicine*, 1979, **30**, 194.
[885] Y. M. Choy, S. L. Wong, and C. Y. Lee, *Biochem, Biophys. Res. Commun.*, 1979, **91**, 410.
[886] D. Bladier, L. Gattegno, F. Fabia, G. Perret, and P. Cornillot, *Carbohydr. Res.*, 1980, **83**, 371.
[887] Y. M. Choy, S. Wong, and C. Y. Lee, *Biochem. Biophys. Res. Commun.*, 1980, **92**, 1238.

The survival of rabbit erythrocytes in the circulation is protected not only by neuraminosyl but also by α-D-galactosyl residues of the cell membrane.[888] A mechanism of erythrocyte clearance based on these findings is discussed. The metabolic fate of asialoglycophorin-containing liposomes has been examined in the perfused rat liver.[889] The uptake of the liposomes with subsequent digestion of their protein components required Ca^{2+} as well as the desialylized glycoprotein.

Intact erythrocytes have been labelled with D-galactose oxidase–sodium borotritide and the radiolabelled monosaccharide transporter glycoprotein has been isolated.[890] The transporter appears to be a heterogeneously glycosylated transmembrane protein and is probably a source of erythroglycan.

An *endo*-β-D-galactanase, capable of hydrolysing the (1→4)-linkages between β-D-galactosyl and 2-acetamido-2-deoxy-D-glucose in glycoconjugates, has been used to probe the plasma membrane of human erythrocytes.[891] By means of D-galactose oxidase–sodium borotritide labelling of erythrocytes, followed by treatment with *endo*-β-D-galactanase, it is apparent that at least three components of the erythrocytes contain *N*-acetyl-lactosamine as a structural entity.

A minor sialoglycoprotein of the human erythrocyte membrane has been shown to have a transmembrane location.[892] Examination of peptides released on proteolytic digestion indicates that the primary sequence of this polypeptide can be distinguished from dimeric or monomeric forms of the major erythrocyte sialoglycoprotein.

9-*O*-Acetyl-*N*-acetylneuraminic acid has been identified as almost the sole neuraminic acid released by mild acid hydrolysis of BALB/c mouse erythrocytes.[893] The mouse erythrocytes contain almost equal quantities of this sugar and *N*-acetylneuraminic acid, but in human erythrocytes only *N*-acetylneuraminic acid is found.

Senescent human erythrocytes have 10 to 15% less neuraminic acid per cell than young cells.[894] This is attributed to a loss of intact glycophorin from the cells during senescence. The removal of desialylized and senescent red blood cells from the circulation proceeds by different routes. Three major forms of glycophorin A have been determined by sedimentation equilibrium.[895] One of these forms (mol. wt. 6.5×10^4) is a dimer and the other two (mol. wt. 3.11×10^4 and 2.79×10^4) are monomers. The association of glycophorin A with band 3 in erythrocyte membranes has been demonstrated by combining protein diffusion measurements with antibody-induced cross-linking.[896] Human glycophorin, but not the asialoglycoprotein, binds to freshly grown and harvested cells of *Myco-*

[888] G. Pfannschmidt and R. Schauer, *Z. Physiol. Chem.*, 1980, **361**, 1683.
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[892] J. W. Owens, T. J. Mueller, and M. Morrison, *Arch. Biochem. Biophys.*, 1980, **204**, 247.
[893] G. Reuter, J. F. G. Vliegenthart, M. Wember, R. Schauer, and R. J. Howard, *Biochem. Biophys. Res. Commun.*, 1980, **94**, 567.
[894] H. U. Lutz and J. Fehr, *J. Biol. Chem.*, 1979, **254**, 11177.
[895] J. C. Dohnal, L. A. Potempa, and J. E. Garvin, *Biochim. Biophys. Acta*, 1980, **621**, 255.
[896] E. A. Nigg, C. Bron, M. Girardet, and R. J. Cherry, *Biochemistry*, 1980, **19**, 1887.

plasma gallisepticum, as measured by direct-binding assays with ^{125}I-labelled glycoproteins, suggesting that binding is mediated through neuraminic acid groups.[897] Although other sialoglycoproteins do not appear to bind *M. gallisepticum*, some inhibit the binding of glycophorin.

The structures (41) and (42) of the L-asparagine-linked oligosaccharide chains of human erythrocyte glycophorin A have one non-reducing terminal 2-acetamido-2-deoxy-β-D-glucopyranosyl residue at O-4 of the β-D-mannopyranosyl residue of the core.[898] This structure is very similar to that found in immunoglobulin A produced by human myeloma cells.

O-Glycosidically linked carbohydrate units of glycophorin, one tetrasaccharide (43), two trisaccharides (44) and (45), and two disaccharides (46) and (47), have been isolated from horse erythrocyte membranes as reduced oligosaccharides.[899] Oligosaccharide components with similar structures are found on human erythrocyte membranes, except that *N*-glycolylneuraminic acid is replaced by *N*-acetylneuraminic acid.

The effects of glycophorin on lipid molecular motion in reconstituted membranes have been studied by ^{13}C n.m.r. spectroscopy.[900] About 30 lipid molecules are immobilized by one glycophorin monomer. Glycophorin has been assembled into liposomes that may be handled like intact cells.[901] Under appropriate conditions the binding of wheat germ agglutinin to these simple model cells accurately mimics the positive co-operative behaviour commonly reported for binding of various lectins to naturally occurring cells.

Haemagglutination by polyoma virus is known to require the presence of neuraminic acid residues on cell-surface oligosaccharides.[902] The adsorption is not due simply to a non-specific electrostatic interaction but requires the presence of specific sialyloligosaccharide structures. Thus replacement of the neuraminic acid on neuraminidase-treated erythrocytes, using a β-D-galactoside α-2,3-sialyltransferase, completely restores their susceptibility to haemagglutination by polyoma virus. In contrast, replacement of neuraminic acid using β-D-galactoside 2,6-sialyltransferase or 2-acetamido-2-deoxy-D-galactosyl-α-2,6-sialyltransferase does not restore haemagglutination by the virus.

Oligosaccharides have been isolated from band 3 glycoprotein of human blood-group A erythrocyte membranes.[903] Structural studies, which include methylation analyses and enzymic degradation studies, indicate the existence of a core protein consisting of three D-mannosyl and two 2-acetamido-2-deoxy-D-glucosyl residues to which a variable number of repeating units at the peripheral portion of the oligosaccharides are attached.

[897] L. R. Glasgow and R. L. Hill, *Infect. Immun.*, 1980, **30**, 353.
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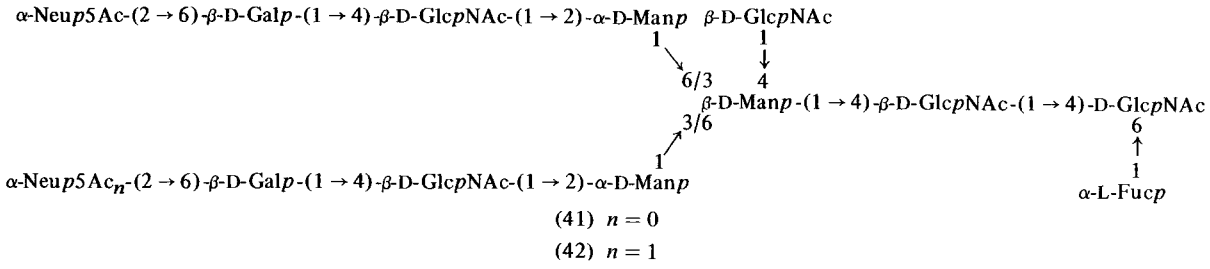
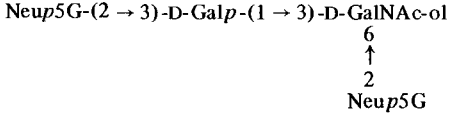


(41) $n = 0$

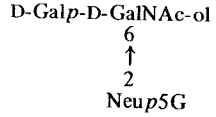
(42) $n = 1$



(43)

Neup5G-(2 → 3)-D-Galp-(1 → 3)-D-GalNAc-ol

(44)



(45)

Neup5G-(2 → 3)-D-Gal-ol

(46)

D-Galp-(1 → 3)-D-GalNAc-ol

(47)

Leukaemic blast cells from some patients having acute leukaemia express on their surface the major erythrocyte sialoglycoprotein glycophorin A.[904] Since this glycoprotein is exclusively present on erythrocytes and their precursors, the findings indicate that a higher proportion of the blast leukaemias than previously recognized show features of early erythroid differentiation. The glycoprotein profile and carbohydrate structure of human erythroblasts from three different ontogenic stages have been reported.[905] In contrast to mature erythrocytes, erythroblasts have barely detectable amounts of band 3 and band 4.5 glycoproteins but contain glycophorin and two other glycoproteins (mol. wt. 9.5×10^4 and 1.45×10^5).

The human continuous leukaemia cell line K562, previously thought to be myeloid, is in fact erythroid and synthesizes and expresses on its surface the major red cell sialoglycoprotein glycophorin A, composed of one *N*-glycosidic and 15 *O*-glycosidic oligosaccharides.[906] The synthesis of the *N*-glycosidic oligosaccharide is inhibited by tunicamycin, whereas the *O*-glycosidic oligosaccharides are not affected. Incomplete glycophorin A, lacking the *N*-glycosidic oligosaccharide is apparently incorporated normally into the surface membrane.

Reviews dealing with the biochemistry and genetics of ABO, Lewis, and P blood-group systems,[907] and with the role of lectins in blood-group serology,[908] have been published.

Blood-group A-activity in human erythrocyte membranes is confined to a few glycoproteins, including band 3.[909] Glycophorin does not seem to contain A-activity. Blood-group ABH-active protein components of the human erythrocyte membrane have been identified after polyacrylamide gel electrophoresis using blood-group-specific lectins.[910] The presence of two groups of blood-group-active glycoproteins with different lectin-binding properties could explain the controversy regarding the carbohydrate structure of these glycoproteins.

Euronymus europaeus lectin, which is specific for L-fucosyl-containing B and H blood-group-active oligosaccharides, has been used for immunochemical characterization of the alkali-stable oligosaccharides from the major sialoglycoprotein from human O erythrocytes.[911] The chemical composition of the alkali-stable polysaccharides and their pattern of inhibition of precipitation suggest that non-reducing terminal α-L-fucosyl residues are immunodominant groups. Blood-group H sites of human O cells have been converted *in vitro* into group A sites by the incorporation of 2-acetamido-2-deoxy-α-D-galactopyranosyl residues

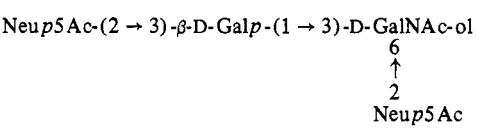
[904] L. C. Andersson, C. G. Gahmberg, L. Teerenhovi, and P. Vuopio, *Int. J. Cancer*, 1979, **24**, 717.
[905] M. Fukuda, M. N. Fukuda, T. Papayannopoulou, and S. I. Hakomori, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 3474.
[906] C. G. Gahmberg, M. Jokinen, K. K. Karhi, and L. C. Andersson, *J. Biol. Chem.*, 1980, **255**, 2169.
[907] W. M. Watkins, *Adv. Human Genetics*, 1980, **10**, 1.
[908] W. J. Judd, *CRC Crit. Rev. Biochem.*, 1980, **12**, 171.
[909] K. K. Karhi and C. G. Gahmberg, *Biochim. Biophys. Acta*, 1980, **622**, 344.
[910] J. Finne, *Eur. J. Biochem*, 1980, **104**, 181.
[911] J. Petryniak, B. Petryniak, K. Wasniowska, and H. Krotkiewski, *Eur. J. Biochem.*, 1980, **105**, 335.

from UDP-2-acetamido-2-deoxy-D-galactose in the presence of a human plasma 2-acetamido-2-deoxy-D-galactopyranosyltransferase.[912] The bulk of the incorporated amino-sugar can only be released from the membranes using proteolytic enzymes, suggesting that most of the blood-group H determinants are bound to glycoprotein and not to glycolipid.

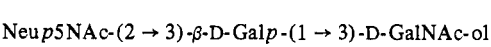
Both polyglycosylceramides and polyglycosylpeptides are linear molecules in erythrocytes with i phenotype.[913] This contrasts with the corresponding glycopeptides from control I erythrocytes in which there are no branch points.

The incorporation of *N*-acetylneuraminic acid from CMP-*N*-acetylneuraminic acid by human serum sialyltransferases has been determined by the uptake of *N*-acetyl-[^{14}C]neuraminic acid into human erythrocyte T, NN, and MM antigens.[914] The transferases in sera from donors of either N- or M-type incorporate *N*-acetylneuraminic acid into all T antigens, but only sera from donors possessing the M gene transfer the sugar to the NN antigen with the concomitant biosynthesis of M-specific immunodeterminants. The latter donors have a sialyltransferase modifier in their serum which is absent in persons without the M gene.

The structures (48) and (49) have been proposed for the alkali-labile oligosaccharide chains of M- and N-blood-group glycopeptides from human erythrocyte membrane.[915]



(48)



(49)

The c.d. spectra of M and N human blood-group glycoproteins indicate that these groups contain a low content of α-helix.[916]

The Ss blood-group antigen determinants are associated with the *N*-terminal tryptic and chymotryptic glycopeptides (residues 1–35 or 1–32) of the Ss sialoglycoprotein of human erythrocyte membranes.[917] Amino-acid sequencing studies on the *N*-terminal portion of the glycopeptides from SS and ss erythrocytes show only one difference, an L-methionine/L-threonine polymorphism

[912] H. Schenkel-Brunner, *Eur. J. Biochem.*, 1980, **104**, 529.
[913] E. Zdebska, A. Magnuska, G. Kuśnierz, H. Seyfried, and J. Koscielak, *FEBS Lett.*, 1980, **120**, 33.
[914] P. R. Desai and G. F. Springer, *J. Immunogenetics*, 1980, **7**, 149.
[915] E. Lisowska, M. Duk, and W. Dahr, *Carbohydr. Res.*, 1980, **79**, 103.
[916] J. Lisowski, K. Wasniowska, and E. Liskowska, *Biochim. Biophys. Acta*, 1980, **622**, 365.
[917] W. Dahr. W. Gielen, K. Beyreuther, and J. Kruger, *Z. Physiol. Chem.*, 1980, **361**, 145.

at position 29.[918] As judged from previous work, this amino-acid heterogeneity represents the structural difference between the Ss antigens.

A new human erythrocyte variant (PL) contains an abnormal neuraminic acid-rich glycoprotein (mol. wt. 3.2×10^4) that is stable to digestion with trypsin.[919] The presence of this component in the erythrocytes does not correspond to any of the known blood-group MNSs-related antigens examined.

Human erythrocyte membranes of different blood-group P phenotypes have been degraded by trifluoroacetolysis at conditions that will effect transamidation, releasing oligosaccharide from both glycoproteins and glycolipids.[920] Certain highly branched carbohydrate structures are specifically degraded into smaller units due to lack of protection. The blood-group ABH activity is determined preferentially by group-specific glycoproteins rather than glycolipids.[921]

13 Salivary and Mucous Glycoproteins

A sensitive technique for the measurement of respiratory mucous glycoproteins involves their precipitation with Alcian Blue.[922] The mucous–dye complexes are then dissociated in sodium dibutylsulphosuccinate solution, allowing the absorbance of the dye to be measured.

Review articles dealing with aspects of the biochemistry of mucins in the human gastrointestinal epithelium,[923] the histochemistry of colonic mucins,[924] and viscometric and chemical characterization of sputum from patients with chronic obstructive lung disease,[925] have been published.

β-D-Gal*p*-(1 → 3)-D-GalNAc-ol

(50)

β-D-Glc*p*NAc-(1 → 3)-D-GalNAc-ol

(51)

D-Gal*p*-(1 → 4)-D-Glc*p*NAc-(1 → 3)-D-GalNAc-ol

(52)

D-Glc*p*NAc-(1 → 3)-D-Gal*p*-(1 → 3)-D-GalNAc-ol

(53)

α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 3)-D-GalNAc-ol

(54)

[918] W. Dahr, K. Beyreuter, H. Steinbach, W. Gielen, and J. Kruger, *Z. Physiol. Chem.*, 1980, **361**, 895.
[919] M. J. A. Tanner, D. J. Anstee, and W. J. Mawby, *Biochem. J.*, 1980, **187**, 493.
[920] A. Lundblad, S. Svensson, B. Löw, L. Messeter, and B. Cedergren, *Eur. J. Biochem.*, 1980, **104**, 323.
[921] H. Schenkel-Brunner, *Eur. J. Biochem.*, 1980, **104**, 529.
[922] R. L. Hall, R. J. Miller, A. C. Peatfield, P. S. Richardson, I. Williams, and I. Lampert, *Biochem. Soc. Trans.*, 1980, **8**, 72.
[923] M. I. Filipe, *Invest. Cell Pathol.*, 1979, **2**, 195.
[924] C. F. A. Culling and P. E. Reid, *J. Histochem. Cytochem.*, 1979, **27**, 1177.
[925] A. O. Jenssen, O. Harbitz, and O. Smidsrød, *Scand. J. Resp. Diseases*, 1978, **59**, 141.

Human bronchial glycoproteins from the sputum of a patient suffering from chronic bronchitis have been subjected to alkaline borohydride degradation.[926] The structures of two disaccharides (50) and (51) and three trisaccharides (52)–(54) have been established.

Human cervical mucous, collected from different ovulatory donors during the follicular, ovulatory, and luteal phases of the menstrual cycle has been examined for viscoelasticity and composition.[927] The viscoelasticity is related to the carbohydrate composition and/or structure and is unique for each donor.

A correlation of chemical and histochemical studies of the glycoproteins from both normal and diseased human colonic epithelial tissues has been reported.[928]

Oligosaccharides have been obtained from human gastric mucin of A, B, and O blood-group donors, both by treatment of the mucins with alkaline borohydride and by oxidation of terminal D-galactosyl residues with D-galactose oxidase followed by formation of the [^{14}C]cyanohydrin derivatives.[929] The reaction mixtures obtained from the A, B, and O secretor groups contain different oligosaccharides, implying that the major oligosaccharide chain of these three types of mucin is different.

A glycoprotein present in the articular lubricating fraction of bovine synovial fluid contains about 110 trisaccharide side-chains linked primarily to L-threonine.[930] Structural studies have identified the trisaccharide units as being *O*-*N*-acetylneuraminosyl-(2 → 3)-*O*-β-D-galactopyranosyl-(1 → 3)-*O*-2-acetamido-2-deoxy-D-galactosyl-(1 → *O*-L-Ser/L-Thr. Gastric mucins of sheep express blood-group-I and -i activities.[931] Additional blood-group-I and -i activities are revealed by one cycle of Smith degradations of blood-group A-active sheep gastric glycoproteins.

A protein (mol. wt. 7.0×10^4), joined by disulphide bridges to pig gastric mucous glycoprotein, is released from the glycoprotein by reduction or proteolysis.[932] Immunodiffusion studies show that the protein is not serum albumin. It has similarities in molecular size, resistance to peptic digestion, and dependence on disulphide bridges to the secretory component, which is present in mucous secretions.

Hog gastric mucosa has been treated firstly by partial acid hydrolysis, then by action of β-D-galactosidase, to produce a precursor protein that is capable of acting as a substrate for a β-D-galactopyranosyltransferase and UDP-D-galactose.[933]

[926] G. Lamblin, M. Hermitte, A. Boersma, P. Roussel, and V. Reinhold, *J. Biol. Chem.*, 1980, **255**, 4595.
[927] D. P. Wolf, J. E. Sokolski, and M. Litt, *Biochim. Biophys. Acta*, 1980, **630**, 545.
[928] P. E. Reid, C. F. A. Culling, W. L. Dunn, M. G. Clay, and C. W. Ramey, *J. Histochem. Cytochem.*, 1978, **26**, 1033.
[929] E. Moczar and D. Waldron-Edward, *J. Chromatogr.*, 1980, **181**, 108.
[930] H. G. Garg, D. A. Swann, and L. R. Glasgow, *Carbohydr. Res.*, 1980, **78**, 79.
[931] E. Wood, E. F. Hounsell, J. Langhorne, and T. Feizi, *Biochem. J.*, 1980, **187**, 711.
[932] J. P. Pearson and A. Allen, *Biochem. Soc. Trans.*, 1980, **8**, 388.
[933] H. Schenkel-Brunner, E. A. Kabat, and J. Liao, *Eur. J. Biochem.*, 1979, **98**, 573.

High molecular weight glycoproteins have been isolated and purified from canine antral and fundic mucosal tissue using non-degrading techniques.[934] The glycoproteins were then degraded by proteolytic enzymes yielding low molecular weight glycopeptides rich in residues of L-serine, L-threonine, and L-proline. Glycoproteins of similar size and composition were also isolated from human gastric gel mucin.

Sulphated glycoproteins from the brush border fraction and the soluble fraction of rabbit small intestine have been isolated and characterized with respect to their monosaccharide and amino-acid composition.[935]

An acidic glycoprotein (mol. wt. 3.4×10^4) has been isolated from the armadillo submandibular gland.[936] Amino-sugars, neuraminic acid, and neutral sugars represent 7% of the glycoprotein, which also contains an unusual amino-acid composition.

Changes in the mucous glycoprotein concentration in the gastric juice of cats have been compared with the specific viscosity of the juice.[937] Glycoprotein concentrations are similar during infusion of pentagastrin alone, and infusion with pentagastrin plus secretin. The specific viscosity is only high when secretin is present, indicating that there must be a change in the structure of the glycoprotein. The structures of the carbohydrate units (55)–(61) of rat colonic mucous glycoproteins, which result from the reductive cleavage by alkaline borohydride, have been reported.[938] Rat colonic mucous glycoprotein, like that of rat sublingual glycoprotein, contains 3-*O*-β-(2-acetamido-2-deoxy-D-glucosyl)-2-acetamido-2-deoxy-D-galactosyl residues in the region adjacent to the protein core.

Non-steroid anti-inflammatory and analgesic drugs which in addition are ulcerogenic cause a marked inhibition of the incorporation of [^{35}S]sulphate into mucous glycoproteins.[939]

A sulphated glycoprotein, isolated from a transplantable colorectal adenocarcinoma of rats, contains *O*-glycoside linkages between 2-acetamido-2-deoxy-D-galactose and L-serine and L-threonine.[940] Similar glycoproteins have been described in mucous carcinoma of human stomach and tumour tissues of alimentary tract. Mucous glycoproteins from rat stomach have been characterized after their isolation from homogenates of the superficial gastric mucosa by equilibrium centrifugation.[941] One of the glycoproteins expressed blood-group A activity and contained residues of D-galactose, L-fucose, 2-amino-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-galactose (4 : 2 : 4 : 1), whereas another glycoprotein contained the same sugars (3 : 2 : 3 : 1) and showed mainly blood-group Le^b activity.

[934] F. M. Fouad and D. Waldron-Edward, *Z. Physiol. Chem.*, 1980, **361**, 703.
[935] H. Munakata and Z. Yosizawa, *J. Biochem. (Jpn.)*, 1980, **87**, 1559.
[936] A. M. Wu, C. E. Malinowski, and A. Herp, *Biochim. Biophys. Acta*, 1979, **581**, 189.
[937] R. Kaura, A. Allen, and B. H. Hirst, *Biochem. Soc. Trans.*, 1980, **8**, 52.
[938] B. L. Slomiany, V. L. N. Murty, and A. Slomiany, *J. Biol. Chem.*, 1980, **255**, 9719.
[939] K. D. Rainsford, *Biochem. Pharmacol.*, 1978, **27**, 877.
[940] H. Munakata and Z. Yosizawa, *Biochim. Biophys. Acta*, 1980, **623**, 412.
[941] R. Spee-Brand, G. J. A. M. Strous, and M. F. Kramer, *Biochim. Biophys. Acta*, 1980, **621**, 104.

α-Neu*p*5Ac-(2 → 6)-D-GalNAc-ol

(55)

β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 3)-D GalNAc-ol

(56)

β-D-Glc*p*NAc-(1 → 3)-D-GalNAc-ol
6
↑
2
α-Neu*p*5Ac

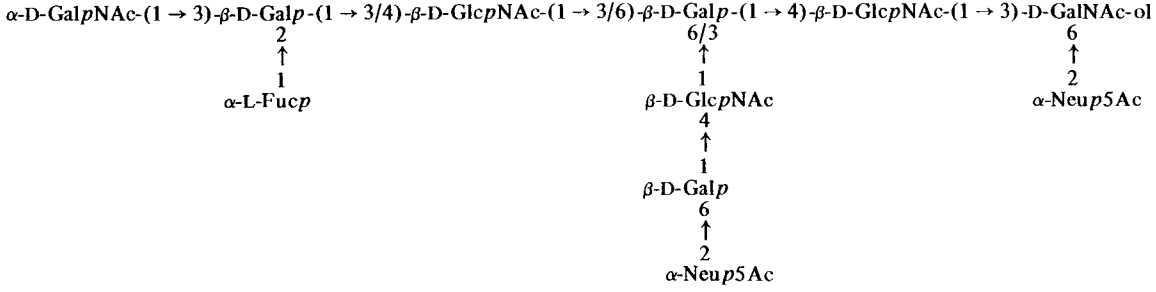
(57)

β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 3)-D-GalNAc-ol
6
↑
2
α-Neu*p*5Ac

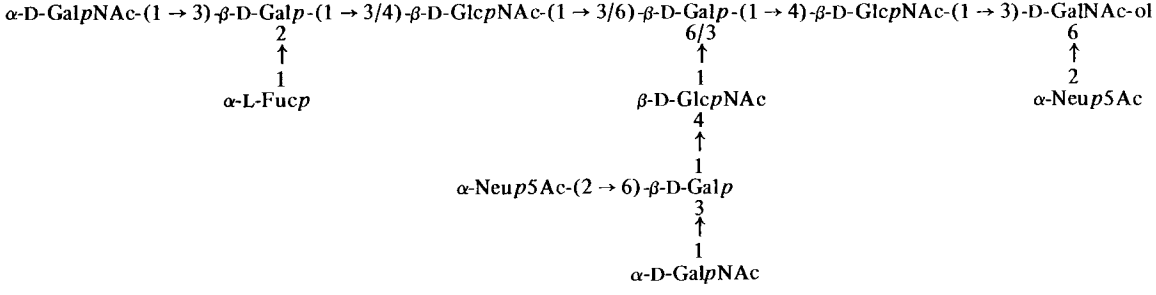
(58)

β-D-Gal*p*NAc-(1 → 3)-β-D-Gal*p*-(1 → 3/4)-D-Glc*p*NAc-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 3)-β-D-GalNAc-ol
2 6 6
↑ ↑ ↑
1 2 2
α-L-Fuc*p* α-Neu*p*5Ac α-Neu*p*5Ac

(59)


α-D-GalpNAc-(1 → 3)-β-D-Galp-(1 → 3/4)-β-D-GlcpNAc-(1 → 3/6)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 3)-D-GalNAc-ol
2
1
α-L-Fucp
6/3
1
β-D-GlcpNAc
6
2
α-Neup5Ac
4
1
β-D-Galp
6
2
α-Neup5Ac

(60)


α-D-GalpNAc-(1 → 3)-β-D-Galp-(1 → 3/4)-β-D-GlcpNAc-(1 → 3/6)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 3)-D-GalNAc-ol
2
1
α-L-Fucp
6/3
1
β-D-GlcpNAc
6
2
α-Neup5Ac
4
1
α-Neup5Ac-(2 → 6)-β-D-Galp
3
1
α-D-GalpNAc

(61)

An electrophoretically pure mucous glycoprotein has been isolated from the submandibular gland of the mouse.[942] Its chemical composition (high carbohydrate, lacking L-fucose and sulphate) differs from those of most salivary-mucous glycoproteins.

A sialoglycoprotein extracted from avian mucous material (edible bird's nest) has been shown to possess alkali-labile 2-acetamido-2-deoxy-3-*O*-β-D-galactosyl-D-galactose chains as well as alkali-stable chains.[943] Unexpected binding properties to peanut lectin were demonstrated in the alkali-stable glycoprotein.

Human parotid saliva mucin glycoproteins bind to some strains of oral streptococci.[944] Treatment of the cell surfaces of these streptococci with proteolytic enzymes abolishes the binding activity.

A comparative study of the nature of the glycoproteins secreted by various cultured mammalian tracheal explants and by bronchial explants from humans has been presented.[945]

Skin mucous from the char (*Salmo alpinus*) collected at time intervals after death of the fish, has been investigated for mucin glycoprotein composition.[946] At least some of the secreted glycoprotein contains incompletely glycosylated polypeptide chains.

14 Urinary Glycoproteins

Oligosaccharides and glycopeptides, which accumulate in the liver and are excreted in the urine of patients with one of several lysosomal storage disorders, have been separated by h.p.l.c.[947] Characteristic elution profiles were obtained for oligosaccharides and their borohydride-reduced products, and glycopeptides in mannosidosis, fucosidosis, G_{M_1}- and G_{M_2}-gangliosidoses, sialidosis, and aspartylglucosaminuria.

Gel-filtration data suggest that the α_1-acid glycoprotein fraction from urine of nephrotic patients consists of two components: free α_1-acid glycoprotein and a glycosaminoglycan–α_1-acid glycoprotein complex.[948] Since the glycosaminoglycan associated with the α_1-acid glycoprotein in urine appears to have a co-factor function in the lipoprotein lipase enzyme reaction, it is postulated that it is the urinary loss of plasma glycosaminoglycan that may be responsible for the hyperlipaemia associated with nephrotic syndrome.

Thirteen patients with Salla disease, a new type of lysosomal-storage disorder, all excrete approximately ten times more free neuraminic acid than normal individuals.[949] The isolated neuraminic acid has been characterized by t.l.c.,

[942] P. A. Denny, P. C. Denny, and K. Jenkins, *Carbohydr. Res.*, 1980, **87**, 265.
[943] G. H. Farrar, G. Uhlenbruck, and D. Karduck, *Z. Physiol. Chem.*, 1980, **361**, 473.
[944] S. Shibata, R. Nakamura, A. Tsunemitsu, and A. Misaki, *J. Dental Res.*, 1979, **58**, 2006.
[945] T. Kaizu, S. A. Lyons, C. E. Cross, M. D. Jennings, and J. A. Last, *Comp. Biochem. Physiol. B*, 1979, **62**, 195.
[946] J. K. Wold, and R. Selset, *Comp. Biochem. Physiol. B*, 1978, **61**, 271.
[947] N. M. K. Ng Ying Kin and L. S. Wolfe, *Anal. Biochem.*, 1980, **102**, 213.
[948] I. Staprans, C. D. Anderson, F. W. Lurz, and J. M. Felts, *Biochim. Biophys. Acta*, 1980, **617**, 514.
[949] M. Renlund, M. A. Chester, A. Lundblad, P. Aula, K. O. Raivio, S. Autio, and S. L. Koskela, *Eur. J. Biochem.*, 1979, **101**, 245.

optical rotation, ^{13}C and ^{1}H n.m.r. spectroscopy, and mass spectrometry of its permethylated derivative as *N*-acetylneuraminic acid.

A sialylated glycopeptide isolated after proteolytic digestion of human Tamm–Horsfall glycoprotein behaves as a powerful monovalent hapten in the precipitin reaction between human Tamm–Horsfall glycoprotein and leucoagglutinin, but fails to inhibit the interaction of the glycoprotein with rabbit anti-(human Tamm–Horsfall glycoprotein) antibodies.[950] The glycopeptide is much less active than the intact glycoprotein as an inhibitor of lymphocyte transformation induced by leucoagglutinin. Tamm–Horsfall glycoprotein has been purified from urine and used in the development of a radioimmunoassay procedure for the glycoprotein in serum.[951] The freezing salt-free solutions of the glycoprotein lead to changes that alter its ability to interact with antibody raised against it.[952] The implications of this finding for the radioimmunoassay of the Tamm–Horsfall glycoprotein have been studied. Sd^a blood-group activity is a hitherto unrecognized marker on the Tamm–Horsfall glycoprotein.[953] The Sd^a determinant is carried on the glycoprotein and a difference in the 2-acetamido-2-deoxy-D-galactosyl content of two variant forms of the glycoprotein correlates with the Sd^a activity of the preparations.

A tetrasaccharide has been isolated and serologically characterized from the urine of some but not all blood-group A secretors.[954] Although the data from sugar and methylation analyses of the tetrasaccharide are compatible with two different structures, that having structure (62) is probable on the basis of the known structure of the blood-group A hapten.

$$\begin{array}{c} \alpha\text{-D-Gal}p\text{NAc-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 4)\text{-D-Glc} \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad 2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad \uparrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad 1 \\ \qquad\qquad\qquad\qquad\qquad\qquad \alpha\text{-L-Fuc}p \end{array}$$

(62)

The urine of two mannosidosis patients has been purified and the structures of 16 oligosaccharides derived therefrom have been identified.[955] All of the oligosaccharides were found to have a 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-glucosyl residue at their reducing termini.

The level of β_2-microglobulin in urine has been determined by a radioimmunoassay in patients with Waldenstrom's macroglobulinaemia.[956] In 50% of the patients the level of the glycoprotein was significantly increased.

[950] A. Abbondanza, C. Franceschi, F. Licastro, and F. Serafini-Cessi, *Biochem. J.*, 1980, **187**, 525.
[951] A. Dawnay, C. McLean, and W. R. Cattell, *Biochem. J.*, 1980, **185**, 679.
[952] A. A. Goodall and R. D. Marshall, *Biochem. J.*, 1980, **189**, 533.
[953] P. C. Soh, W. T. J. Morgan, W. M. Watkins, and A. S. R. Donald, *Biochem. Biophys. Res. Commun.*, 1980, **93**, 1132.
[954] C. Derappe, A. Lundblad, L. Messeter, and S. Svensson, *FEBS Lett.*, 1980, **119**, 177.
[955] K. Yamashita, Y. Tachibana, K. Mihara, S. Okada, H. Yabouchi, and A. Kobata, *J. Biol. Chem.*, 1980, **255**, 5126.
[956] W. Engstrom and P. Wahrgren, *Clin. Chim. Acta*, 1980, **105**, 123.

The regulation of glycosylated α_{2u}-globulin, the major glycoprotein in adult male urine, has been shown to be regulated differently from the synthesis of the non-glycosylated form of the α_{2u}-globulin.[957] Characteristics of the carbohydrate moiety of rat α_{2u}-globulin have been investigated.[725] The oligosaccharide moiety of the glycoprotein is similar to those oligosaccharides found in other serum proteins. The formation of the two species of the α_{2u}-globulin (mol. wt. 7.5×10^4 and 5.0×10^4) requires either the attachment of many oligosaccharide chains to a single polypeptide (mol. wt. 2.0×10^4) or the covalent linkage of polypeptides containing one or more oligosaccharide chains to form dimers or trimers.

The immunoglobulin secreted by mouse myeloma MOPC 47A is a defective urinary IgA protein, having the light chain of normal size but a heavy chain with a deletion of approximately 100 amino-acids.[958] The protein has a single *N*-glycosidically linked oligosaccharide unit lying in the middle of the C_{H1} domain at L-asparagine-155.

α_1-Microglobulin has been isolated from urine of guinea pigs with renal damage.[959] The glycoprotein is homologous to human α_1-microglobulin, both in physicochemical properties and immunological activity.

15 Avian Glycoproteins and Chitin

The mechanism of the formation of the heat-stable form of ovalbumin (S-ovalbumin) from normal ovalbumin has been studied.[960] Although gross structural differences were not observed, some minor differences such as the elution patterns on ion-exchange chromatography, the isoelectric focusing properties, and titration curves of both proteins were noted.

The spatial relationship between protein synthesis and nascent chain glycosylation of ovalbumin has been defined.[961] At least 30 amino-acid residues of nascent ovalbumin must exist from the ribosomal cleft subsequent to synthesis of the glycosylation site before the site is glycosylated. This is a segment of sufficient length to allow the acceptor site to be accessible to the luminal face of rough endoplasmic reticulum. The presence of a second unglycosylated acceptor site is also demonstrated. This site is not glycosylated *in vivo* but can be glycosylated *in vitro*. This finding is discussed in terms of the potential role of nascent chain folding in determining whether or not glycosylation of a particular acceptor site will occur *in vivo*.

Ovomucoid from Japanese quail, *Coturnix coturnix japonica*, contains significantly lower levels of hexoses and amino-sugar than chicken ovomucoid.[962] Differences in isoelectric points, migration on gel electrophoresis, and antitryptic activities were also noted. Japanese quail ovomucoid exists in two polymorphic forms.[963] One has L-serine, the other glycine at position 162. The

[957] L. J. Harrs and H. C. Pitot, *Arch. Biochem. Biophys.*, 1980, **201**, 556.
[958] E. A. Robinson and E. Appella, *J. Biol. Chem.*, 1979, **254**, 11418.
[959] B. Åkerstrom and I. Berggard, *Eur. J. Biochem.*, 1979, **101**, 215.
[960] R. Nakamura, M. Hirai, and Y. Takemori, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 149.
[961] C. G. Glabe, J. A. Hanover, and W. J. Lennarz, *J. Biol. Chem.*, 1980, **255**, 9236.
[962] T. Itoh, H. Sugawara, and S. Adachi, *Comp. Biochem. Physiol. B.*, 1980, **65**, 563.
[963] W. C. Bogard, I. Kato, and M. Laskowski, *J. Biol. Chem.*, 1980, **255**, 6569.

quantitative distribution of these two amino-acids at position 162 in ovomucoid derived from numerous different hens is consistent with the interpretation that the ovomucoid gene exists in two co-dominant allelic forms at one locus. Immunization of rabbits with a purified glycopeptide from proteolytic digests of avian riboflavin-binding protein has resulted in the production of carbohydrate-specific antibodies.[964] β-D-Galactosyl residues in the glycopeptide proved to be the immunodominant determinants. Radioimmunoassay indicated that the glycopeptide, although a structural component of the riboflavin-binding protein, did not contribute to the major antigenic determinants of the native protein.

In a re-examination of *X*-ray diffraction data for chitin–protein systems, the complex from the ovipositor of the ichneumon fly *Megaryssa* has been used.[965] It is proposed that the crystalline α-chitin fibril is surrounded by a protein sheath of higher electron density to give a total fibril radius of 725 nm. Fibrils of this type are then packed on a hexagonal network with one fibril per unit cell.

The internal skeletal bones of the squid (*Sepia esculenta*) contain chitin.[966] Viscometric measurements of the polysaccharide dissolved in formic acid estimate the molecular weight to be 4×10^5.

The degree of acetylation of chitin, partially deacetylated chitins, and chitosan have been determined by g.l.c.[967] The retention time for methanol is proportional to the degree of acetylation of the chitin used as a column packing.

16 Miscellaneous Glycoproteins

A distinct relationship between chicken ovalbumin and two human plasma protease inhibitors has been revealed by computer analysis of their protein sequences.[968] A new protein super-family containing at least three families – ovalbumin (and probably gene X and gene Y proteins), antithrombin III, and α_1-protease inhibitor – has been proposed. Although these families may have diverged from a common ancestor 500 million years ago, they may still share similarity in gene structure as well as in protein sequence.

Sericin, a glycoprotein from the silkworm *Bombyx mori*, interacts with peanut agglutinin and the 2-acetamido-2-deoxy-D-galactosyl-specific lectins from the snails *Helix pomatia* and *Cepea nemoralis*.[969]

The jelly coat substance of sea urchin eggs of *Pseudocentrotus depressus* (Okayama) contain sulphated sialoglycoproteins.[970] *N*-Glycolylneuraminic acid

[964] L. Ramanathan, R. B. Guyer, W. W. Karakawa, E. G. Buss, and C. O. Clagett, *Mol. Immunol.*, 1980, **16**, 935.
[965] J. Blackwell and M. A. Weih, *J. Mol. Biol.*, 1980, **137**, 49.
[966] I. Cho and Y. C. Lee, *Int. J. Biol. Macromol.*, 1980, **2**, 52.
[967] R. A. A. Muzzarelli, F. Tanfani, G. Scarpini, and G. Laterza, *J. Biochem. Biophys. Methods*, 1980, **2**, 299.
[968] L. T. Hunt and M. O. Dayhoff, *Biochem. Biophys. Res. Commun.*, 1980, **95**, 864.
[969] G. Uhlenbruck, D. Karduck, and H. Sinohara, *Naturwissenschaften*, 1980, **67**, 146.
[970] J. P. Kamerling, R. Schauer, J. F. G. Vliegenthart, and K. Hotta, *Z. Physiol. Chem.*, 1980, **361**, 1511.

and 9-*O*-acetyl-*N*-glycolylneuraminic acid (in the molar ratio of 1:1) were identified by g.l.c.–m.s., t.l.c., and colorimetric analysis. The snail D-galactans of *Helix pomatia* and *Biomphalaria glabrata* have been isolated using immobilized D-galactosyl-specific lectins from the sponge *Axinella polypoides*.[971] The binding of *H. pomatia* galactans to the affinity matrix and precipitin formation with the sponge lectin is affected strongly by Ca^{2+}.

The propagation of mycoplasma cells is usually achieved in growth medium containing whole animal serum, and glycoproteins derived from the growth medium have been identified as contaminants in membrane glycoprotein preparations from several *Mycoplasma* species.[972]

The establishment of highly synchronized cultures of *Plasmodium falciparum* has enabled identification of stage-specific proteins, glycoproteins, and antigens.[973] Antigens unique to schizonts and merozoites were identified.

Glycoprotein antigens have been isolated from the outer envelope of *Leptospira interrogans* serovar. *canicola*.[974] Extraction of the outer envelope by ethanol, or by alkali released both high and low molecular weight glycoproteins, containing residues of L-arabinose, L-rhamnose, L-fucose, D-xylose, D-mannose, D-glucose, D-glucuropyranosylonic acid, and 2-amino-2-deoxy-D-glucose.

At least six distinct glycoproteins have been recognized in preparations of soluble egg antigen of *Schistosoma mansoni* after chromatography on immobilized concanavalin A followed by electrophoresis in sodium dodecylsulphate.[975] Several antigenic components of the soluble egg antigens have also been purified using immunoaffinity chromatography and are similar to those isolated by affinity chromatography on immobilized concanavalin A.[976]

Treponema pallidum synthesizes viscous acidic polysaccharides that are degraded by hyaluronidase.[977] In infected tissues, the organisms localize in areas containing relatively large amounts of acidic polysaccharides.[978]

Costae, intracellular organelles common to all trichomonads, of *Tritrichomonas foetus* are composed of carbohydrate (95%) and protein (5%).[979]

The existence of several glycoproteins in the plasma membrane of *Trypanosoma brucei* has been reported.[980] Elongation of the oligosaccharide chains occurs while the glycosyl acceptor is attached to its respective polypeptide exclusively while those polypeptides are resident in the plasma membrane. The elongation process is shown to use nucleotide sugar donors from the cytoplasmic side of the plasma membrane without participation of dolichyl phosphate as an intermediate sugar carrier. The attachment of the initial sugar

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[972] J. Nicolet, P. Paroz, and B. Kristensen, *J. Gen. Microbiol.*, 1980, **119**, 17.
[973] A. Kilejian, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 3695.
[974] A. Palit and P. M. Harrison, *J. Gen. Microbiol.*, 1980, **117**, 141.
[975] C. E. Carter and D. G. Colley, *J. Immunol.*, 1979, **122**, 2204.
[976] D. J. Harrison, C. E. Carter, and D. G. Colley, *J. Immunol.*, 1979, **122**, 2210.
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[978] T. J. Fitzgerald, R. C. Johnson, and D. M. Ritzi, *Infect. Immun.*, 1979, **24**, 252.
[979] W. E. Sledge, A. D. Larson, and L. T. Hart, *Science*, 1978, **199**, 186.
[980] C. T. Brett and H. P. Voorheis, *Eur. J. Biochem.*, 1980, **109**, 139.

residue directly to the polypeptide was not observed and may occur by means of a different glycosylation system, possibly located in the Golgi apparatus or endoplasmic reticulum rather than the plasma membrane. A major cell-surface glycoprotein (mol. wt. 9.0×10^4) has been isolated following affinity chromatography on columns of immobilized lentil lectin.[981]

Evidence for the presence of D-galactofuranosyl residues in the lipopeptidophosphoglycan from *Trypanosoma cruzi* has been based on partial acid hydrolysis and periodate oxidation studies.[982]

17 Analysis of Glycoproteins

The 500 MHz ^{1}H n.m.r. spectra of complex carbohydrate structures of glycoproteins show a much higher resolution than the corresponding 360 MHz spectra.[983] The improvement is due to the stronger magnetic field, with a contribution from a more sophisticated resolution enhancement computer program. Resolution in combination with line widths allowed the assignment of all individual D-galactosyl and 2-acetamido-2-deoxy-D-glucosyl H-1′ values in bi-, tri-, and tetra-antennary glycopeptides.

A new approach to the study of molecular protein interactions in biological membranes is based on measuring the rotation of a membrane protein in the presence and absence of specific antibodies directed toward a purported complex partner.[896] This is illustrated in the association of band 3 glycoprotein with glycophorin A in the human erythrocyte membrane.

The conformation of the glycopeptide linkage region of the serum-type glycoproteins has been studied by c.d. and n.m.r. spectroscopy of the linkage compound N^4-(2-acetamido-2-deoxy-β-D-glycopyranosyl)hydrogen L-asparaginate and on the model compound 1-*N*-acetyl-2-deoxy-2-acetamido-β-D-glycopyranosylamine.[984] On the basis of the published amino-acid sequence analysis of several glycoproteins, which indicate that the peptide chains is in a β-turn conformation in the region of glycosylation, and the spectroscopic evidence, it is proposed that the 2-acetamido-2-deoxy-D-glucosyl residue is in a $^4C_1$ chair and the amide exocyclic dihedral angles τ_1 and τ_2 are both 120°. Both L-asparagine side-chain dihedral angles x^1 and x^2 are placed at 60° and the glycosylated L-asparagine residue is in the second corner position of a type 1 β-turn.

Glycoconjugates, which have been treated with sodium periodate to modify neuraminic acid residues or with D-galactose oxidase to modify D-galactosyl residues, react with rhodamin- and fluorescein-containing hydrazides.[985]

Glycoproteins have been radiolabelled with high specific radioactivity after oxidation of the neuraminic acid residues with periodate followed by sodium

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[982] R. M. de Lederkremer, O. L. Casal, M. J. M. Alves, and W. Colli, *FEBS Lett.*, 1980, **116**, 25.
[983] H. Van Halbeek, L. Dorland, J. F. G. Vliengenthart, K. Schmid, J. Montreuil, B. Fournet, and W. E. Hull, *FEBS Lett.*, 1980, **114**, 11.
[984] C. A. Bush, A. Duben, and S. Ralapati, *Biochemistry*, 1980, **19**, 501.
[985] M. Wilchek, S. Spiegel, and Y. Spiegel, *Biochem. Biophys. Res. Commun.*, 1980, **92**, 1215.

borotritide reduction.[693] The method is recommended for glycoprotein antigens which cannot be labelled with ^{125}I.

Glycoproteins, after oxidation of sugar residues with sodium periodate, have been reacted with 3,5-[^{125}I]iodo-L-tyrosine hydrazide with the formation of a Schiff's base.[500] The method has been used to identify various membrane glycoproteins in intact cells.

A specific covalent labelling of neuraminic acid residues of erythrocyte membrane glycoproteins has been accomplished by sequential periodate oxidation and reductive amination with the introduction of a nitroxide spin-label.[877]

The heterobifunctional reagent 2-[^{14}C]acetamido-4-mercaptobutyric acid hydrazide forms hydrazones with periodate-generated aldehydes of glycoproteins.[878] An adaptation of the sodium periodate–sodium borotritide procedure for the identification of sialoglycoproteins that eliminates interference from non-specifically incorporated tritium has been described.[986] Following incorporation of the label and electrophoresis of the glycoprotein on polyacrylamide gels, slices of the gels are treated with neuraminidase to release selectively [^{3}H]neuraminosyl derivatives.

The products of hydrazinolysis of 1-*N*-acetyl- and 1-*N*-(4-L-aspartyl)-derivatives of 2-amino-2-deoxy-D-glucose cannot be converted quantitatively into 2-amino-2-deoxy-D-glucose under mild conditions.[987] Hydrazinolysis of 2-acetamido-1-*N*-acetyl-2-deoxy-β-D-glucopyranosylamine yields the hydrazone of 2-amino-2-deoxy-D-glucose. The implications of these results in relation to the hydrazinolysis of glycoproteins and glycopeptides with subsequent deamination of amino-sugars with nitrous acid are discussed.

Glycopeptides containing high levels of D-mannose, and complex oligosaccharides linked to L-asparagine, have been purified by high voltage paper electrophoresis.[988] The technique has been used for the preparation of glycopeptide substrates for glycosyltransferases and glycosidases.

General procedures for the quantification of the products of solvolysis of permethylated glycopeptides and glycoproteins have been described.[989]

The neutral and amino-sugar composition of glycoproteins and glycopeptides has been measured after h.p.l.c. separation on anion-exchange columns of the component monosaccharides or alditols.[990] The presence of amino-acids in the hydrolysates does not interfere with the analysis.

Urinary glycopeptides and oligosaccharides have been separated by h.p.l.c.[991] Characteristic elution profiles are obtained for oligosaccharides, their borohydride reduction products, and glycopeptides.

[986] T. F. Cruz and J. W. Gurd, *Anal. Biochem.*, 1980, **108**, 139.
[987] M. S. Saeed and J. M. Williams, *Carbohydr. Res.*, 1980, **84**, 83.
[988] S. Narashimhan, N. Harpaz, G. Longmore, J. P. Carver, A. A. Grey, and H. Schachter, *J. Biol. Chem.*, 1980, **255**, 4876.
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[991] N. M. K. Ng Ying Kin and L. S. Wolfe, *Anal. Biochem.*, 1980, **102**, 213.

The determination by g.l.c. of neutral sugars, as aldononitrile acetates, and amino-sugars as *O*-methyl oxime acetates, in glycoproteins has been reported.[992]

Interference with a procedure for measuring free neuraminic acids using the thiobarbituric acid reagent is due to the presence of certain carbohydrates capable of forming pigments with the reagents.[993] Glyceraldehyde, glycolaldehyde, and hydroxymalonaldehyde, which may be generated after periodate oxidation of glycoproteins, appear to be substances responsible for the interfering colours.

A method has been described for monitoring the rate and extent of the β-elimination as applied to *O*-glycosyl-glycoproteins, using alkaline solutions of sodium borotritide.[994] The stoicheiometry of the reaction has been established by the use of 2-acetamido-2-deoxy-D-galactosyl-*O*-glycoproteins.

A fingerprinting technique used in the differentiation of oligosaccharide chains of glycoproteins has been developed.[995] After alkaline cleavage of *O*-glycosyl residues, non-reducing terminal D-galactosyl residues on these oligosaccharides are first oxidized with D-galactose oxidase and then converted to the [^{14}C]cyanohydrin derivatives. Two-dimensional t.l.c. has been used to demonstrate differences in the oligosaccharide side-chains in glycoproteins.

18 Biosynthesis of Glycoproteins

In a review of glycoprotein biosynthesis, the role of oligosaccharide chains in influencing the ability of a protein to fold properly has been discussed.[996] The large size of the precursor L-asparagine-linked oligosaccharides may be essential if certain proteins are to achieve the correct tertiary structure. The role of lipid-linked saccharides in the biosynthesis of complex carbohydrates has been reviewed.[997]

After preparation, rat hepatocytes require a short recovery period of one hour for the stabilization of levels of nucleotide and nucleoside diphosphate sugar concentrations.[998] Once stability has been established, the isolated hepatocytes preserve this state for several hours during which time they are synthesizing and secreting glycoproteins. Although tunicamycin inhibits protein glycosylation, it also has a marked effect on protein synthesis in the isolated hepatocytes.[999] Furthermore, the release of proteins from the cells is affected.

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[995] E. Moczar and D. Waldron-Edward, *J. Chromatogr.*, 1980, **181**, 108.
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[998] T. Howe, J. R. Voisey, and P. J. Winterburn, *Biochem. Soc. Trans.*, 1980, **8**, 189.
[999] T. Howe, J. R. Voisey, and P. J. Winterburn, *Biochem. Soc. Trans.*, 1980, **8**, 190.

The kinetics of glycosylation and intracellular transport of sialoglycoproteins in mouse liver have been reported.[1000] Most glycoproteins are sialylated in the Golgi apparatus and are subsequently transported from there to the plasma membrane.

3-Hydroxy-3-methylglutaryl coenzyme A reductase (the rate-controlling enzyme of cholesterol biosynthesis) does not regulate the rate of dolichyl phosphate biosynthesis in rat liver.[1001] The regulation of this lipid intermediate is probably at the level of dolichyl phosphate synthetase.

Membrane preparations of three independently selected Chinese hamster ovary cell lines that are resistant to concanavalin A incorporate lower levels of GDP-D-mannose into lipid, oligosaccharyl lipid, and protein than do preparations from parental wild populations.[1002] This defect is probably responsible, at least in part, for the property of resistance to concanavalin A. The accompanying complex phenotype variants can also convert D-mannose to L-fucose to greater extents than the parental wild-type cells.

The polyisoprenoid carrier of 2-acetamido-2-deoxy-D-glucose in *Glycine max* contains a chain of 18 isoprene units and is similar in size to the carrier of D-glucose.[1003] Both lipids may be replaced by dolichol phosphate from liver.

Cultured human lymphocytes contain phosphatases for dolichol phosphate and dolichol pyrophosphate.[1004] The reactions of these enzymes are similar to those previously found in the cycle of bacterial cell-wall peptidoglycan biosynthesis.

Dolichol phosphate synthesized by means of a kinase in calf brain membranes is formed at a membrane locus where it can be utilized by the glucosyltransferase synthesizing D-glucosyl-dolichyl phosphate.[1005]

P^1-Diacetylchitobiosyl-P^2-dolichyl pyrophosphate acts as a substrate for the soluble enzyme D-galactosyltransferase. Using this enzyme as a probe, the topological arrangement of chitobiosyl lipid in both artificial and natural membranes has been examined.[1006] When incorporated into liposomes, the disaccharide residue of the glycolipid adopts a random trans-bilayer orientation. The glycolipid is a relatively static component of the endoplasmic reticulum of sealed microsomes from hen oviduct and is oriented with its disaccharide residue facing the lumen of this organelle.[1007]

The capacities of membrane preparations from actively growing regions of pea stems to synthesize lipid-linked saccharides from UDP-2-acetamido-2-deoxy-D-glucose, UDP-D-glucose, and GDP-D-mannose have been investigated.[1008] The pea stem preparations are compared to those from mouse L cells, which they resemble in their ability to generate complex membrane-bound oligosaccharides from exogenously added nucleotide sugars.

[1000] D. J. Carey and C. B. Hirschberg, *J. Biol. Chem.*, 1980, **255**, 4348.
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[1004] J. F. Wedgwood and J. L. Strominger, *J. Biol. Chem.*, 1980, **255**, 1120.
[1005] M. G. Scher, W. A. Burton, and C. J. Waechter, *J. Neurochem.*, 1980, **35**, 844.
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[1008] D. S. Bailey, M. Dürr, J. Burke, and G. Maclachlan, *J. Supramol. Struct.*, 1979, **11**, 123.

The enzymatic synthesis of P^1-3-*O*-β-D-mannopyranosyl-D-cellobiosyl-P^2-dolichyl pyrophosphate in *Acetobacter xylinum* has been reported.[1009] Cellobiosylpyrophosphoryl prenol and GDP-D-mannose are the precursors of this trisaccharide lipid intermediate. β-D-Mannopyranosyl-dolichyl phosphate is also synthesized by the enzyme preparation but does not seem to participate in the synthesis of the trisaccharide. A possible role for these lipid intermediates in the biosynthesis of extracellular polysaccharides is discussed.

The conditions for the synthesis of oligosaccharide-lipids of high molecular weight in microsomal preparations of *Saccharomyces cerevisiae* have been described.[1010] The primary species transferred to protein contains three D-glucosyl, nine D-mannosyl, and two 2-acetamido-2-deoxy-D-glucosyl residues. The trans-bilayer orientation of the oligosaccharide chain transferred from oligosaccharide–lipid to endogenous protein acceptors in sealed hen oviduct microsomes has been examined.[1011] Although sugar nucleotides are presented to the cytoplasmic face of the microsomes the oligosaccharides formed and transferred to endogenous proteins faces the luminal face. The endogenous acceptors have been demonstrated to be membrane-associated proteins, although not a part of the ribosome-tRNA–nascent-chain complex.

P^1-2-Acetamido-2-deoxy-D-glucosyl-P^2-dolichyl pyrophosphate is the product resulting from the transfer of 2-acetamido-2-deoxy-D-glucose 1-phosphate from UDP-2-acetamido-2-deoxy-D-glucose to dolichyl phosphate by a transferase from *Acanthamoeba castellanii*.[1012] The antibiotic diumycin was shown to inhibit selectively polyprenyl phosphate : sugar nucleotide transglycosidases.

Incubation of hen oviduct membranes with UDP-2-acetamido-2-deoxy-[^{14}C]D-glucose leads to the formation of four endogenously labelled lipids, three of which have been characterized previously.[1013] The fourth compound is unusual in that it has characteristics of a 2-acetamido-2-deoxy-β-D-glucopyranosyl-prenyl phosphate in which the α-isoprene unit is unsaturated. This is the first example from a eukaryotic source of this amino-sugar linked to the lipid *via* a monophosphate diester.

GDP-mannose, in addition to acting as a substrate for D-mannosyltransferase reactions in embryonic chick retina, acts in an indirect manner to stimulate the biosynthesis of the non-D-mannosyl 2-acetamido-2-deoxy-D-glucosyl lipids, a process which may be an aspect of the regulation of glycoprotein biosynthesis.[1014]

The biosynthesis of the major lipid-linked oligosaccharide of Chinese hamster ovary cells occurs by the ordered addition of D-mannosyl residues, and a scheme for the sequence of addition of the sugar residues to the lipid-linked oligosaccharide has been proposed.[1015]

[1009] R. O. Couso, L. Ielpi, R. C. Garcia, and M. A. Dankert, *Arch. Biochem. Biophys.*, 1980, **204**, 434.
[1010] R. B. Trimble, F. Haley, and A. L. Tarentino, *J. Biol. Chem.*, 1980, **255**, 10232.
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[1014] E. L. Kean, *J. Biol. Chem.*, 1980, **255**, 1921.
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Whole rat spleen lymphocytes are capable of synthesizing D-mannosyl dolichyl phosphate and P^1-oligosaccharyl-D-mannosyl-P^2-dolichyl pyrophosphate.[1016] These P^1-oligosaccharyl-P^2-dolichyl pyrophosphates are degraded into oligosaccharide phosphates and oligosaccharides which are released into the medium. The oligosaccharide phosphates may be cleaved either by alkaline phosphatase to give neutral oligosaccharides or by *endo*-β-D-2-acetamido-2-deoxy-glucanase H, to give 2-acetamido-2-deoxy-D-glucose 1-phosphate and neutral oligosaccharides. The splitting of the phosphodiester bond of the P^1-oligosaccharyl-P^2-dolichyl pyrophosphates by dephosphorylation and/or hydrolysis by the *endo*-β-D-2-acetamido-2-deoxy-D-glucanase of the phosphorylated oligosaccharides may represent the beginning of the catabolic pathway of these lipid-linked intermediates.

An enzyme present in rabbit liver microsomes has been found to catalyse the transfer of D-mannose from GDP-D-mannose to an exogenously added oligosaccharyl-lipid acceptor, resulting in the formation of an α-D-mannosyl-(1→2)-D-mannose linkage.[1017] The product is a heptasaccharide containing a single (1→6)-linked branched glycosyl unit with the majority of the newly added D-mannosyl residues in a terminal non-reducing position.

Membrane preparations of rat adipose tissue can synthesize D-mannosyl dolichyl phosphate.[1018] The endogenous concentrations of dolichyl phosphate and 3-mannosyltransferase activity can be altered in response to dietary manipulation. A D-mannosyltransferase from pig aorta transfers D-mannose from GDP-D-mannose but not from D-mannosyl dolichylphosphate to a lipid-linked oligosaccharide acceptor to form a heptasaccharide-lipid.[1019]

Partial purification and characterization of the D-glucosidases involved in the processing of L-asparagine-linked oligosaccharides has been achieved.[1020] A minimum of two D-glucosidases appear to be involved in removing D-glucosyl residues from nascent glycopeptide molecules.

The characterization from hen oviduct membranes of a D-glucosidase involved in an initial step in the processing of oligosaccharide chains of glycoproteins has been reported.[1021] The purified enzyme removes only one of the three D-glucosyl residues on the oligosaccharide chain, indicating that there are at least two D-glucosidases with different substrate specificities involved in the processing of the D-glucosyl-oligosaccharide after transfer to its protein acceptor. Two such D-glucosidases have been isolated from rat liver microsomes, and appear to interact with extensive portions of oligosaccharide structure in addition to the D-glucosyl residues which they hydrolyse.[1022] They have a specific interaction with the 6′-penta-D-mannosyl branch (63) of their oligosaccharide substrates, the activity being reduced when D-mannosyl residues were removed from this branch. Both D-glucosidases recognized features at the reducing end of the

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[1017] J. S. Schutzbach, J. D. Springfield, and J. W. Jensen, *J. Biol. Chem.*, 1980, **255**, 4170.
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[1022] L. S. Grinna and P. W. Robbins, *J. Biol. Chem.*, 1980, **255**, 2255.

D-Glc*p*-(1 → 2)-D-Glc*p*-(1 → 3)-D-Glc*p*-(1 → 3)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*

The dotted line indicates the 6′-penta-D-mannosyl branch.
R corresponds to D-Glc*p*NAc, D-Glc*p*NAc-lipid, and D-Glc*p*NAc-peptide.

(63)

oligosaccharide substrates, with the activity being lower towards lipid-linked and peptide-linked substrates than towards oligosaccharide substrates.

The high D-mannose-type oligosaccharides of the biosynthetic intermediates of β-D-glucuronidase contain phosphate groups in diester linkage between D-mannosyl residues and outer 2-acetamido-2-deoxy-α-D-glucosyl residues.[1023] Rat liver as well as human diploid fibroblasts contain a 2-acetamido-2-deoxy-α-D-glucosyl phosphodiesterase that is capable of removing the 2-acetamido-2-deoxy-D-glucosyl residues, leaving phosphomonoester groups on the high D-mannose oligosaccharide units.[1024] This enzyme may be involved in the 'unmasking' of the phosphomannosyl recognition marker on newly synthesized acid hydrolases, which can then direct the targeting of these enzymes to lysosomes. The purification and characterization of a rat liver α-D-mannosidase capable of processing L-asparagine-linked oligosaccharide chains of glycoproteins has been reported.[1025, 1026]

The biosynthetic addition of three D-glucosyl residues to P^1-oligosaccharyl-P^2-dolichyl pyrophosphate has been demonstrated by incubation of D-glucosyl-dolichyl phosphate and UDP-D-glucose with rat liver microsomes.[1027] The presence of the three D-glucosyl residues on the lipid-linked oligosaccharide appears to be necessary for transfer of the oligosaccharide to protein. Enzymic *N*-glycosylation of the tripeptide L-Asn-L-Ala-L-Thr by oligosaccharide transferases of pig thyroid microsomes has been demonstrated.[1028] Introduction of an L-proline residue between the L-asparagine and L-threonine residues renders the tripeptide sequence inadequate for attachment of the saccharide moiety.

N-Glycosylation of peptides bearing the recognition sequence L-Asn-X-L-Thr/L-Ser is affected by the chain length of the acceptor peptides as well as by the type of the amino-acid, X, in the 'marker' sequence.[1029]

The importance of L-threonine in the recognition sequence for glycosylation has been examined by studying the effect of DL-β-hydroxynorvaline, an analogue of L-threonine, on co-translational glycosylation in Krebs II ascites tumour lysates.[1030] DL-β-Hydroxynorvaline is incorporated into and inhibits the glycosylation of the α-subunit of human chorionic gonadotrophin and the β-subunit of bovine luteinizing hormone. The tripeptide recognition site appears to be sensitive to steric hindrance.

Using synthetic peptides, the structural requirements for enzymic *N*- and *O*-glycosylation *via* dolichol-linked sugar derivatives in membranes from *Saccharomyces cerevisiae* have been established.[1031] The structural requirement of the carbohydrate acceptor for the transfer of *N,N'*-diacetylchitobiose from P^1-(*N,N'*-diacetylchitobiosyl)-P^2-dolichyl pyrophosphate is the tripeptide

[1023] I. Tabas and S. Kornfeld, *J. Biol. Chem.*, 1980, **255**, 6633.
[1024] A. Varki and S. Kornfeld, *J. Biol. Chem.*, 1980, **255**, 8398.
[1025] I. Tabas and S. Kornfeld, *J. Biol. Chem.*, 1979, **254**, 11655.
[1026] I. Tabas and S. Kornfeld, *J. Biol. Chem.*, 1980, **255**, 4386.
[1027] R. J. Staneloni, R. A. Ugalde, and L. F. Leloir, *Eur. J. Biochem.* 1980, **105**, 275.
[1028] C. Ronin, S. Bouchilloux, C. Granier, and J. Van Rietschoten, *FEBS Lett.*, 1978, **96**, 179.
[1029] E. Bause and H. Hettkamp, *FEBS Lett.*, 1979, **108**, 341.
[1030] G. Hortin and I. Boime, *J. Biol. Chem.*, 1980, **255**, 8007.
[1031] E. Bause and L. Lehle, *Eur. J. Biochem.*, 1979, **101**, 531.

sequence L-Asn-X-L-Ser/L-Thr. The rate of glycosylation is affected by the chain length of peptides. In contrast to the L-asparagine sequon, a marker sequence for the formation of the *O*-glycosidic linkage *via* D-mannosyl dolichyl phosphate dolichol has not been deduced, although glycosyl transfer requires the minimum chain length of a tripeptide.

Under conditions in which dolichol-linked saccharides are involved in the *N*-glycosylation of proteins using oviduct membrane preparations, no lipid-linked intermediates for *O*-glycosylation can be detected.[1032] The enzymes responsible for *N*-glycosylation are located on the rough endoplasmic reticulum, whereas the 2-acetamido-2-deoxy-D-galactosyl : polypeptidetransferase, which is responsible for *O*-glycosylation, is localized primarily in membranes derived from the smooth endoplasmic reticulum and Golgi apparatus.

In the biosynthesis of O^3-(2-acetamido-2-deoxy-D-galactosyl)-L-serine/L-threonine linkages in submaxillary mucin, no evidence has been obtained for the involvement of lipid-linked 2-acetamido-2-deoxy-D-galactose.[1033] It is likely that the addition of monosaccharides in the mucin is achieved by step-by-step addition of monosaccharides.

The role of sugar analogues and a few antibiotics, which interfere in the formation of lipid intermediates and therefore in protein glycosylation, has been reviewed.[1034]

The incorporation of D-mannose and D-glucose into lipid-linked intermediate in chick embryo cells is inhibited by 2-deoxy-2-fluoro-D-glucose.[1035] This leads to the formation of smaller lipid-linked oligosaccharides which are biosynthetic intermediates. 2-Amino-2-deoxy-D-glucose, 2-deoxy-D-*arabino*-hexose, and 2-amino-2-fluoro-D-mannose also inhibit the formation of lipid-linked oligosaccharides in this system.[1036] Cycloheximide or puromycin inhibit protein synthesis in canine kidney cells and also inhibit the synthesis in canine linked oligosaccharides.[1037] The formation of the lipid-linked oligosaccharides may be regulated by end-product inhibition.

Mosquito cells synthesize *N*-glycosidically linked carbohydrate chains that are assembled through tunicamycin-sensitive steps, probably involving 2-acetamido-2-deoxy-D-glucosyl pyrophosphoryl dolichol and oligosaccharide intermediates, and *O*-glycosidically linked chains rich in 2-acetamido-2-deoxy-D-galactose, the assembly of which is unaffected by tunicamycin.[1038]

Bacitracin inhibits the synthesis of D-mannosyl-phosphoryl dolichol in rat liver microsomes by blocking the formation of dolichol phosphate from endogenous precursors.[1039] A bacitracin-sensitive phosphatase activity toward dolichol pyrophosphate can be detected in the microsomes, consistent with the

[1032] J. A. Hanover, W. J. Lennarz, and J. D. Young, *J. Biol. Chem.*, 1980, **255**, 6713.
[1033] P. Babczinski, *FEBS Lett.*, 1980, **117**, 207.
[1034] R. T. Schwarz and R. Datema, *Trends Biochem. Sci.*, 1980, **5**, 65.
[1035] R. Datema, R. T. Schwarz, and A. W. Jankowski, *Eur. J. Biochem.*, 1980, **109**, 331.
[1036] R. Datema and R. T. Schwarz, *Biochem. J.*, 1979, **184**, 113.
[1037] J. W. Schmitt and A. D. Elbein, *J. Biol. Chem.*, 1979, **254**, 12291.
[1038] T. D. Butters and R. C. Hughes, *Biochem. Soc. Trans.*, 1980, **8**, 170.
[1039] S. Kato, M. Tsuji, Y. Nakanishi, and S. Suzuki, *Biochem. Biophys. Res. Commun.*, 1980, **95**, 770.

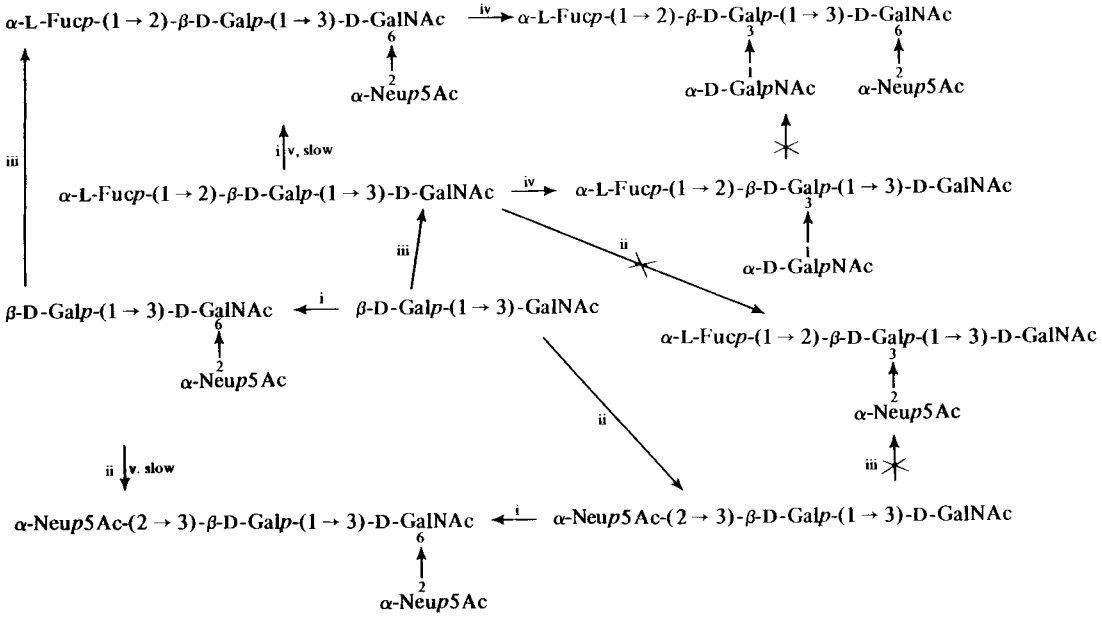
view that the control of dolichol phosphate concentrations is linked with changes in the rate of dolichol pyrophosphate dephosphorylation. Diumycin inhibits D-mannosyl transfer reactions in yeast membrane preparations at different steps.[1040] The formation of D-mannosyl-dolichyl phosphate and to a lesser extent the transfer of D-mannose from the lipid intermediate to L-serine or L-threonine residues of acceptor protein are inhibited, whereas neither the D-mannosylation of protein by GDP-D-mannose nor the formation of lipid-bound trisaccharide is impaired. Glycosyltransfer reactions from GDP-D-mannose either to D-mannosyl protein with the formation of D-manno-oligosaccharides or to P^1-(*N,N'*-diacetylchitobiosyl)-P^2-dolichyl pyrophosphate with the formation of lipid-linked trisaccharide are not inhibited by the antibiotic.

In a study of the subcellular localization of sugar nucleotide synthetases of mouse L cells, the pyrophosphorylases of GDP-L-fucose, GDP-D-mannose, and UDP-D-glucose have been detected only in the cytoplasm but the majority of CMP-*N*-acetylneuraminic acid synthetase is found in the nucleus.[1041]

Six purified glycosyltransrerases [a β-D-galactosyl α-(2→6)-sialyltransferase, an α-2-acetamido-2-deoxy-D-galactosyl-α-(2→6)-sialyltransferase, a β-D-galactosyl-α-(2→3)-sialyltransferase, a β-D-galactosyl-α-(1→2)-L-fucosyltransferase, a β-2-acetamido-2-deoxy-D-glucosyl-α-(1→3)-L-fucosyltransferase, and an L-fucosyl-α-(1→2)-D-galactosyl-α-(1→3)-2-acetamido-2-deoxy-D-galactosyltransferase] have been used in a study of the biosynthetic pathways for formation of the non-reducing terminal oligosaccharide sequences in mammalian glycoproteins.[1042] The results imply that oligosaccharide biosynthesis *in vivo* is regulated not only by the glycosyltransferases available but also by the order in which they act. Based on these findings, pathways are proposed (Scheme 1) for the biosynthesis of many of the structures found at non-reducing termini of the oligosaccharide chains in mammalian glycoproteins.

Affinity chromatography on immobilized UDP-hexanolamine has been used in the purification from bovine colostrum of 2-acetamido-2-deoxy-D-glucosyltransferases I and II.[1043] By using glycopeptides of known structure, it can be shown that relatively minor changes in oligosaccharide structure can prevent glycosyltransferase reaction, thus creating control points that channel glycoprotein assembly along certain well defined pathways. The physiological substrate for the 2-acetamido-2-deoxy-D-glucosyltransferase I has the structure (64).[1044] Numerous reports have shown that this oligosaccharyl peptide is an intermediate in the processing of high D-mannosyl-oligosaccharides to 'complex' oligosaccharides. Rat liver Golgi membranes contain one or two α-D-mannosidases dependent on the prior action of the 2-acetamido-2-deoxy-D-glucosidase I. Scheme 2 summarizes the combined evidence for the sequence of events in the synthesis of complex oligosaccharides.

[1040] P. Babczinski, *Eur. J. Biochem.*, 1980, **112**, 53.
[1041] S. W. Coates, T. Gurney, L. W. Sommers, M. Yeh, and C. B. Hirschberg, *J. Biol. Chem.*, 1980, **255**, 9225.
[1042] T. A. Beyer, J. I. Rearick, J. C. Paulson, J. P. Prieels, J. E. Sadler, and R. L. Hill, *J. Biol. Chem.*, 1979, **254**, 12531.
[1043] N. Harpaz and H. Schachter, *J. Biol. Chem.*, 1980, **255**, 4885.
[1044] N. Harpaz and H. Schachter, *J. Biol. Chem.*, 1980, **255**, 4894.



Reagents: i, 2-acetamido-2-deoxy-D-galactoside α-2,6-sialyltransferase; ii, β-D-galactoside α-2,3-sialyltransferase; iii, β-D-galactoside α-1,2-fucosyltransferase; iv, α-1,2-fucosylgalactoside α-1,3-(2-acetamido-2-deoxy)-D-galactosyltransferase

Scheme 1

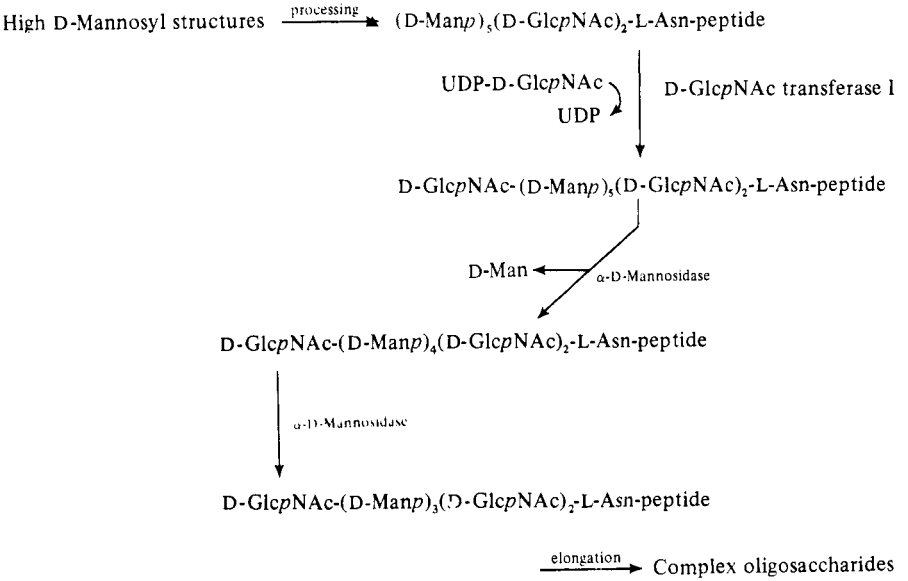
α-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 4)-D-Glc*p*NAc-1 → L-Asn
6
↑
1
α-D-Man*p*-(1 → 3)-D-Man*p*
6
↑
1
α-D-Man*p*

(64)

α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-R^1
6
↑
1
α-D-Man*p*
3
↑
1
R^2

R^1 = β-D-Glc*p*NAc-(1 → 4)-β-D-Glc*p*NAc-*P*,*P*-dolichol
R^2 = H

(65)



Scheme 2

Thy-1 negative mutant mouse lymphoma cells of the class E complementation group are unable to synthesize the normal lipid-linked oligosaccharide (D-Glc*p* : D-Man*p* : D-Glc*p*NAc, 3 : 9 : 2) but instead accumulated a smaller lipid-linked species (65).[1045,1046] The 1,3-α-D-mannosyltransferase necessary for the formation of higher members of this series $R^2 = \alpha$-D-Man*p*-(1→3)- is present in the cells but the Thy 1⁻E mutant cells are unable to synthesize D-mannosyl-dolichyl phosphate.[1047] It is postulated that GDP-D-mannose is the probable donor for the formation of oligosaccharide (65) and its precursors, while D-mannosyl-phosphoryl dolichol donates the sixth D-mannosyl residue and probably also the seventh, eighth, and ninth D-mannosyl residues.

Only one product, β-D-glucosyl-dolichyl phosphate, has been detected in the reaction between dolichyl phosphate and UDP-D-glucose and a D-glucosyltransferase from the cysts of *Acanthamoeba castellani*.[1048]

The existence of a β-2-acetamido-2-deoxy-D-galactosyltransferase active at the surface of embryonic chick neural retina cells has been reported.[1049] The enzyme utilizes a macromolecular endogenous acceptor and the glycosylated product is released into the medium. The activity of sialytransferase in human serum has been determined by radiochemical assay using desialylized fetuin as acceptor.[1050] The precision of this method was found to be low.

Some physical parameters for purified porcine submaxillary sialyltransferases have been obtained from sucrose density-gradient centrifugation data.[1051] Colchicine inhibits secretion of proteins from liver, intestine, and other tissues, and sialyltransferase activity increases as much as ten-fold.[1052] Rat intestine has a latent sialyltransferase different from that of serum. It is suggested that in colchicine-treated rats this enzyme and/or acceptor becomes exposed after preincubation. As a consequence, an increase of this enzyme activity is observed.

Conditions for solubilizing human liver asialofetuin : sialyltransferase activity has been described.[1053] The kinetic properties of the enzyme have been compared with those of the membrane-bound form.

Synthetic 4-*O*-methyl-*N*-acetylneuraminic acid has been incorporated into desialylated fetuin by sialyltransferase activities in microsomal fractions of equine liver.[1054] The isolated reaction products show similar neuraminic acid release by treatment with acid or fowl-plague virus neuraminidase, but display a marked resistance to desialylation by *Vibrio cholerae* neuraminidase.

[1045] I. Trowbridge and R. Hyman, *Cell*, 1979, **17**, 503.
[1046] A. Chapman, I. Trowbridge, R. Hyman, and S. Kornfeld, *Cell*, 1979, **17**, 509.
[1047] A. Chapman, K. Fujimoto, and S. Kornfeld, *J. Biol. Chem.*, 1980, **255**, 4441.
[1048] C. L. Villimez and P. L. Carlo, *J. Biol. Chem.*, 1979, **254**, 4814.
[1049] J. Balsamo and J. Lilien, *Biochemistry*, 1980, **19**, 2479.
[1050] A. Wilhelm, P. G. Berge, and H. Schreiwer, *J. Clin. Chem. Clin. Biochem.*, 1980, **18**, 271.
[1051] J. E. Sadler and J. I. Rearick, *J. Biol. Chem.*, 1979, **254**, 4443.
[1052] I. H. Fraser, S. Ratnam, J. M. Collins, and S. Mookerjea, *J. Biol. Chem.*, 1980, **255**, 6617.
[1053] J. A. Alhadeff and R. T. Holzinger, *Biochim. Biophys. Acta*, 1979, **570**, 56.
[1054] J. M. Beau and R. Schauer, *Eur. J. Biochem.*, 1980, **106**, 531.

6
Enzymes

J. F. KENNEDY

1 Introduction

General Aspects and Nomenclature. – As reported in Volume 13, page 417 one of the features of *Enzyme and Microbial Technology*, a multipurpose journal that features reviews, original papers, international comment, industrial comment, patent reports, and conference reports, is the provision of a literature survey that provides rapid access to the literature on immobilization methods and phenomena as a whole rather than to isolated parts, such as enzyme immobilization.[1-4] A further four surveys, covering much of the work appearing in the literature during recent months, have been presented. For simplicity and clarity the information is arranged in three tables. The first concerns the immobilization of enzymes, the second the immobilization of proteins, glycoproteins, and polysaccharides, and the third the immobilization of other molecules. The substance immobilized, the support material used, and the method of coupling adopted are outlined. Also included are brief comments about the applications of the immobile product as discussed in the original paper. Whereas the tables are indexed under the name of the molecule immobilized, searches for references on particular support matrices or applications may be conducted conveniently by running down the second and third columns respectively of the tables.

'Supplement 1: Corrections and Additions to "Enzyme Nomenclature, Recommendations 1978" ' includes corrections to two entries for carbohydrate-orientated hydrolases, *viz* to 'β-D-galactosidase' EC 3.2.1.23 and 'hyaluronoglucuronidase' EC 3.2.1.26.[5]

An edited volume on soil enzymes includes chapters on the origin and range of enzymes in soil, kinetics and consecutive reactions of soil enzymes, soil polysaccharidases: activity and agricultural importance, and methodology of soil enzyme measurement and extraction.[6]

An edited book, being the proceedings of a conference on mechanisms of saccharide polymerization and depolymerization, covers glycosyl transferase reactions and saccharide biosynthesis, enzymic degradation of starch, starch

[1] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Technol.*, 1980, **2**, 66.
[2] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Technol.*, 1980, **2**, 244.
[3] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Technol.*, 1980, **1**, 155.
[4] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Technol.*, 1980, **2**, 318.
[5] Supplement 1 to Enzyme Nomenclature, *Eur. J. Biochem.*, 1980, **104**, 1.
[6] 'Soil Enzymes', ed. R. G. Burns, Academic Press, New York, 1978.

biosynthesis, enzymic process in glycogen metabolism, and mechanisms of action of glycoside hydrolases.[7]

A book on the biochemistry of ageing includes a chapter that discusses changes in enzymes during ageing under such headings as: changes in enzyme levels, changes in isoenzyme patterns, induction of enzymes, and molecular properties of enzymes.[8]

The most recent volume in the 'Methods of Carbohydrate Chemistry' series includes sections on the analysis of polysaccharides by enzyme methods and on the synthesis of glycosides potentially usable as enzyme substrates.[9]

In a recent Tate and Lyle Carbohydrate Chemistry Award Lecture on the subject 'From Carbohydrates to Enzyme Analogues',[10] the following conclusions were drawn: (i) Constitution, configuration, and conformation define the structures of non-covalently bonded species in much the same way as they define the structures of covalently bonded species. Non-covalent bonds are highly directional! (ii) By utilizing secondary stabilizing interactions, keys can locate the heteroptopic faces of certain chiral asymmetric locks with high selectivity (>1.4 kcal mol^{-1}). (iii) Reorganization of the non-covalent bonding pattern within complexes is generally fast. The key spins in the lock! (iv) Some chiral symmetrical locks show modest enantiomeric differentiation (0.3–0.5 kcal mol^{-1}) towards racemic keys. (v) Complexes seek all opportunities (the counterion or additional binding sites) to become destructured. (vi) Carbohydrates are a convenient source of chiral locks. Principal objectives in the area at the moment are to (a) construct highly structured complexes in which the locks will show high chiral recognition towards racemic keys, and (b) construct highly structured complexes in which the locks will catalyse reactions that show high regioselectivity and stereoselectivity towards heterotopic and enantiotopic ligands and faces in appropriate keys. In short, our task is to build enzyme analogues. In principle, many chemical reactions are amenable to this kind of investigation; the challenges are both scientific and artistic in so far as our understanding of reaction mechanisms and stereochemistry is fast approaching the point where rapidly advancing synthetic skills can be put to the test in the design and preparation of man-made catalysts exhibiting the characteristics of enzymes.

A recent volume of the Advances in Carbohydrate Chemistry and Biochemistry Series includes an article on the biochemistry of α-D-galactosidic linkages in the plant kingdom.[11] A book on polysaccharide metabolism includes chapters on the biosynthesis and enzymic degradation of polysaccharides.[12]

[7] 'Mechanisms of Saccharide Polymerization and Depolymerization', ed. J. John Marshall, Academic Press, New York, 1980.
[8] 'Biochemistry of Ageing', ed. M. S. Kanungo, Academic Press, New York, 1980.
[9] 'Methods in Carbohydrate Chemistry', ed. R. L. Whistler, Academic Press, New York, 1980, Vol. 8.
[10] J. F. Stoddart, *Chem. Soc. Rev.*, 1979, **8**, 85.
[11] 'Advances in Carbohydrate Chemistry and Biochemistry', ed. R. S. Tipson and D. Horton, Academic Press, New York, 1980, Vol. 37.
[12] 'Polysaccharide Metabolism' by R. W. Stoddart, Department of Pathology, University of Manchester, Croon-Helm Ltd., 1981.

Many substances in the plant kingdom and in man's diet occur as glycosides.[13] Recent studies have indicated that many glycosides that are not mutagenic in tests such as the *Salmonella* test become mutagenic upon hydrolysis of the glycosidic linkages. The *Salmonella* test utilizes a liver homogenate by approximate mammalian metabolism but does not provide a source of the enzymes present in intestinal bacterial flora that hydrolyse the wide variety of glycosides present in nature. A description has been presented of a stable cell-free extract of human faeces, faecalase, which is shown to contain various glycoside hydrolases that allow the *in vitro* activation of many natural glycosides to mutagens in the *Salmonella*/liver homogenate test. Many beverages, such as red wine (but apparently not white wine) and tea, contain glycosides of the mutagen quercetin. Red wine, red grape juice, and tea were mutagenic in the test when faecalase was added, and red wine contained considerable direct mutagenic activity in the absence of faecalase. The implications of quercetin mutagenicity and carcinogenicity are discussed.

Recently appeared new titles in the established series 'Methods in Enzymology' are those which go into areas beyond the normal of methodology and enzymology, *viz* 'Biomembranes: Bioenergetic Biogenesis of Mitochondria Organization, and Transport' (biogenesis, genetic approaches, membrane organization, transport, specialized techniques);[14] 'Bioluminescence and Chemiluminescence' (firefly luciferase, bacterial luciferase, *Renilla semiformis* luciferase, aequorin, earthworm bioluminescence, chemiluminescence techniques, *etc.*);[15] 'Cell Culture' (basic methods, laboratories and media, cell-culture techniques, genetics, hybridization and transformation, virus preparation, specific cell lines);[16] 'Nucleic Acids and Protein Synthesis, Part G' (transfer RNA and aminoacyl-tRNA synthetases, ribosomes);[17] 'Nucleic Acids and Protein Synthesis, Part H' (initiation of protein synthesis peptide chain elongation);[18] 'Enzyme Structure, Part H' (molecular weight determinations and related procedures, interactions, conformation and transitions);[19] 'Vitamins and Coenzymes, Part D' (L-ascorbic acid, thiamine: phosphates and analogues, lipoic acid and derivatives, pantothenic acid, coenzyme A and derivatives, biotin and derivatives, pyridoscine, pyridoxamine and pyridoxal: analogues and derivatives);[20] 'Nucleic Acid and Protein Synthesis, Part I' (assays for [class II restriction]

[13] G. Tamura, C. Gold, A. Ferroluzzi, and B. N. Ames, *Proc. Natl. Acad. Sci. USA*, 1980, 77, 4961.

[14] 'Biomembranes, Part G', in 'Methods of Enzymology', ed. S. Fleischer, Academic Press, New York, 1979, Vol. 56.

[15] 'Bioluminescence and Chemiluminescence', in 'Methods in Enzymology', ed. Marlene A. DeLuca, Academic Press, New York, 1978, Vol. 57.

[16] 'Cell Culture', in 'Methods in Enzymology', ed. W. B. Jakoby and I. H. Pastan, Academic Press, New York, 1979, Vol. 58.

[17] 'Nucleic Acids and Protein Synthesis, Part G', in 'Methods in Enzymology', ed. K. Moldave and L. Grossman, Academic Press, New York, 1979, Vol. 59.

[18] 'Nucleic Acids and Protein Synthesis, Part H', in 'Methods in Enzymology', ed. K. Moldave, Academic Press, New York, 1979, Vol. 60.

[19] 'Enzyme Structure, Part H', in 'Methods in Enzymology', ed. C. H. W. Hirs, Academic Press, New York, 1979, Vol. 61.

[20] 'Vitamins and Coenzymes, Part D', in 'Methods in Enzymology', ed. D. B. McCormick, and L. D. Wright, Academic Press, New York, 1979, Vol. 62.

endonucleases, techniques for labelling termini, purification of restriction enzymes, purification and properties of enzymes acting at sites with altered bases, endonucleases cleavage mapping techniques, determination of DNA fragment sizes, determination of fragment ordering, nucleotide sequencing techniques, localization of functional sites on chromosomes);[21] 'Vitamins and Coenzymes, Part E' [nicotinic acid: analogues and coenzymes, flavins and derivatives, pteridines, analogues and pterin coenzymes (folate)];[22] 'Vitamins and Coenzymes, Part F' [cobalamins and cobamides (B_{12}), ubiquinore group, tocopherols, vitamin K group, vitamin A group, vitamin D group, miscellaneous vitamins and coenzymes];[23] 'Recombinant DNA' (enzymes used in recombinant DNA research, synthesis, isolation and purification of DNA, vehicles and hosts for cloning, screening and selection of cloned DNA, detection and analysis of expression of cloned genes);[24] 'Photosynthesis and Nitrogen Fixation, Part C' (mutants, cellular and subcellular preparations, components, methodology, inhibitors, nitrogen fixation).[25]

The enzymes referred to in this Chapter are listed and indexed at the conclusion of the Chapter, pages 342–343, together with their systematic names, EC number, and initial page of citation.

Methods of Assay. – A further volume in the series 'Methods in Enzymology', on 'Enzyme Kinetics and Mechanism', covers initial rate and inhibitor methods, and contains sections on: techniques in coupled-assays; summary of kinetic-reaction mechanisms; derivation of initial velocity and isotope-exchange rate equations; computer-assisted derivation of steady-state equations; statistical analysis of enzyme-kinetic data; plotting methods for analysing enzyme-rate data; kinetic analysis of progress curve; effects of pH on enzymes; temperature effects in enzyme kinetics; approaches to kinetic studies on metal-activated enzymes; stability constants for biologically important metal ligand complexes; cryoenzymology study of enzyme catalysis at subzero temperatures; anomeric specificity of carbohydrate-utilizing enzymes; reversible enzyme-inhibition; product inhibition and abortive complex formation, and practical considerations in the design of initial velocity enzyme-rate assays.[26]

Isolation and Purification. – An edited symposium volume on affinity chromatography and molecular interactions contains sections on new supports and new techniques in affinity chromatography, affinity chromatography in protein

[21] 'Nucleic Acids, Part 1', in Methods in Enzymology', ed. L. Grossman, Academic Press, New York, 1980, Vol. 65.

[22] 'Vitamins and Coenzymes, Part E', in 'Methods in Enzymology', ed. D. B. McCormick and L. D. Wright, Academic Press, New York, 1980, Vol. 66.

[23] 'Vitamins and Coenzymes, Part F', in 'Methods in Enzymology', ed. D. B. McCormick and L. D. Wright, Academic Press, New York, 1980, Vol. 67.

[24] 'Recombinant DNA', in 'Methods in Enzymology', ed. R. Wu, Academic Press, New York, 1980, Vol. 68.

[25] 'Photosynthesis and Nitrogen Fixation, Part C', in 'Methods in Enzymology', ed. A. San Pietro, Academic Press, New York, 1980, Vol. 69.

[26] 'Enzyme Kinetics and Mechanism, Part A, Initial Rate and Inhibitor Methods', in 'Methods in Enzymology', ed. D. L. Purich, Academic Press, New York, 1979, Vol. 63.

purification, hydrophobic interaction chromatography, and affinity electrophoresis: all of which is relevant to the isolation and purification of enzymes.[27]

Properties. – A book on the theory and practice of kinetics of fast enzyme reactions describes how to use rapid reaction techniques to perform measurements of fast enzyme reactions, analyse data, and interpret results in order to clarify the mechanisms of enzyme reactions.[28]

Kinetics. – Comments on and corrections to two previously reported[29a,b] theoretical models of enzymic hydrolysis of polysaccharides have been reported.[29c]

Mechanisms of Action. – New titles in the established series 'Methods in Enzymology' deal with 'Enzyme Kinetics and Mechanism, Part A' (initial rate methods, inhibitor and substrate effects)[30] and 'Enzyme Kinetics and Mechanism, Part B' (isotope probes of mechanism, complex enzyme systems).[31]

Applications. – A review on the use of *endo*- and *exo*-polysaccharide hydrolases for structural studies on glycoconjugates includes sections on the principle of sequencing of sugar chains by using '*exo*-glycosidases', aglycon specificities of *exo*-glycosidases, '*endo*-glycosidases', *etc.*[32]

The first volume of a new series entitled 'Progress in Biomass Conversion to Fuels and Chemicals' includes discussion on enzymic aspects of the title subject.[33]

Volume 2 of the Applied Biochemistry and Bioengineering series is entitled 'Enzyme Technology' and covers production of intracellular microbial enzymes; production, isolation and economics of extracellular enzymes; extraction and purification of enzymes using aqueous two-phase systems; D-glucose isomerase production of high-D-fructose syrups; development of an immobilized D-glucose isomerase for industrial application; starch hydrolysis with soluble and immobilized glucoamylase; industrial applications of immobilized enzymes: a commercial overview; industrial applications of fibre-entrapped enzymes; energy-related

[27] 'Affinity Chromatography and Molecular Interactions', in 'Inserm Symposia Series, No. 86', ed. J. M. Egly, Institut National de la Sante de la Recherche Medicale, Paris, 1979.

[28] 'Kinetics of Fast Enzyme Reactions: Theory and Practice', ed. K. Hiromi, J. Wiley and Sons Ltd., Chichester, 1980.

[29] (*a*) K. Suga, G. Van Dedem, and M. Moo-Young, *Biotechnol. Bioeng.*, 1975, **15**, 433; (*b*) S. E. Lee, W. B. Armiger, C. M. Watteeuw, and A. E. Humphrey, *ibid.*, 1978, **20**, 141; (*c*) T. A. Hsu and G. T. Tsao, *ibid.*, 1979, **21**, 2369.

[30] 'Enzyme Kinetics and Mechanism, Part A', in 'Methods in Enzymology', ed. D. L. Purich, Academic Press, New York, 1979, Vol. 63.

[31] 'Enzyme Kinetics and Mechanism, Part B', in 'Methods in Enzymology', ed. D. L. Purich, Academic Press, New York, 1980, Vol. 64.

[32] A. Kobata, *Anal. Biochem.*, 1979, **100**, 1.

[33] 'Progress in Biomass Conversion to Fuels and Chemicals, Vol. 1', ed. K. V. Sarkanen and D. A. Tillman, Academic Press, New York, 1979.

applications of immobilized enzymes; transformation of steroids by immobilized living micro-organisms.[34]

An article on enzymatic approaches to production of sucrose from starch reports that the cost of sucrose has fluctuated widely in recent years owing to political as well as climatic and agricultural factors.[35] The successful commercial conversion of starch to high-D-fructose corn syrups, with a composition approximately equivalent to hydrolysed sucrose, led to the examination of processes by which sucrose might be obtained from starch. The reversal of enzyme-catalysed hydrolytic reactions after replacing water with nonaqueous solvents has already been demonstrated. Attempts were made to reverse the invertase (β-D-fructofuranosidase) reaction and produce sucrose from high-D-fructose corn syrup. This investigation did not result in sucrose synthesis due to unfavourable enzymes specificity as well as thermodynamic considerations. Thus, a more attractive approach, the 'phosphate process' depicted in Figure 1, which integrates sucrose synthesis with the process of starch digestion and preserves some of the energy of the glycosidic bonds of starch was developed.

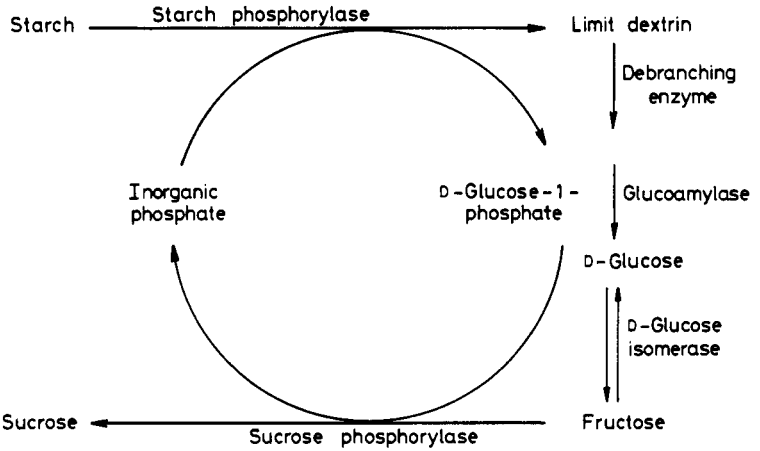


Figure 1 *Phosphate process for sucrose production from starch*

[34] 'Enzyme Technology, production of intracellular microbial enzymes; production, isolation, and economics of extracellular enzymes; extraction and purification of enzymes using aqueous two-phase systems; glucose isomerase production of high-fructose syrups; development of an immobilized glucose isomerase for industrial application; starch hydrolysis with soluble and immobilized glucoamylase, industrial applications of immobilized enzymes: a commercial overview; industrial applications of fiber-entrapped enzymes; energy-related applications of immobilized enzymes; transformation of steroids by immobilized living microorganisms', in 'Applied Biochemistry and Bioengineering', ed. L. B. Wingard, jun., Academic Press, New York, 1979, Vol. 2.

[35] S. J. Kelly and L. G. Butler, *Biotechnol. Bioeng.*, 1980, **22**, 1501.

A volume in the Biological Techniques series is entitled 'Immunochemical Methods in the Biological Sciences: Enzymes and Proteins.'[36] A further volume in the Topics in Enzyme and Fermentation Biotechnology series contains review chapters on enzymes in therapy – theory and practice, medical uses of proteolytic enzymes, and immobilization of microbial cells and their applications.[37]

An article on the subject of clinical enzymology summarizes its view of the subject as follows.[38] The most familiar aspect of clinical enzymology is its diagnostic function in which the clinical enzymologist attempts to infer the nature and extent of intracellular changes by measuring the activities of enzymes in extracellular fluids, usually serum. The steady development of this aspect, particularly in the last 25 years, has placed enzyme tests firmly amongst the most useful aids that the clinical chemist can offer to his clinical colleagues. However, clinical enzymology in its widest sense encompasses more than the diagnostic use of enzyme activity measurements. The aim of diagnostic medicine is to identify and classify diseases, in the hope that further study will reveal the nature of pathogenic changes and suggest ways of preventing or reversing them. Since life itself consists of a series of integrated chemical reactions, each catalysed by a specific enzyme, it is obvious that clinical enzymology has a significant role to play in these wider investigations. The classical view of the progress of science is one of a steady accumulation of new facts which, as they are confirmed, are added to the edifice of knowledge and form the foundations for further advances. Trends of thought and practice can be discerned in this gradual evolution, by examining the history of science, which can be extrapolated to provide indications of the likely future course of development. However, this steady progress may from time to time be interrupted by unforeseen observations that bring fresh insights and divert the evolution of science into new channels. Examples of both these aspects of the progress of science can be found in the history of clinical enzymology: although the unforeseen developments – the breakthroughs – of the past provide little guidance as to the likely breakthroughs of the future, because of the essentially unpredictable nature of these events, it is possible by the examination of past trends to form some picture of the clinical enzymology of the future.

In conclusion to a summary of a Tate and Lyle Carbohydrate Chemistry Award Lecture entitled 'Transition metal oxide chelates of carbohydrate directed macromolecules' it was stated that hydrous oxides of titanium and zirconium in various forms are effective matrices for immobilization.[39] Their advantages include their low cost, their convenient preparation (which may be conducted in any location without specialized facilities), the absence of any need for pre-preparation, their ability to couple enzyme at neutral pH, the high retentions of specific activity of the enzyme on immobilization, and the ability of modification

[36] 'Immunochemical Methods in the Biological Sciences: Enzymes and Proteins', in 'Biological Techniques Series', ed. R. J. Mayer and J. H. Walker, Academic Press, New York, 1980.

[37] 'Topics in Enzyme and Fermentation Biotechnology', ed. A. Wiseman, J. Wiley and Sons Ltd., Chichester, 1980, Vol. 4.

[38] D. W. Moss, *Enzyme*, 1980, **25**, 2.

[39] J. F. Kennedy, *Chem. Soc. Rev.*, 1979, **8**, 221.

to exert microenvironmental effects on, and thereby alter the characteristics of, the immobilized enzyme. The success of the preparation and activity of the matrix is guaranteed and, unlike the majority of immobilization matrices, does not require use of spectroscopy, titration, *etc.* to confirm the reactivity of the preparation in hand. The form of the hydrous oxide may be modified with ease to give high- or low-density, magnetic or non-magnetic, products of various sizes, shapes, and uses. Although much of the work conducted hitherto has employed (carbohydrate-directed) enzymes as models, on account of the additional biological-testing parameter afforded thereby, it is clear that the hydrous metal oxide matrices are equally suitable for the immobilization, removal, and recovery of antibodies, antigens, affinants, amino-acids, antibiotics, carbohydrates, cells, enzymes, glycoproteins, lectins, peptides, *etc.* This necessarily gives rise to several possible investigations of the properties of such compounds in the fields of pure, biological, and clinical chemistries.

A book on the fundamentals and applications of immobilized enzymes in analytical clinical chemistry systematically assesses the strengths and weaknesses of various immobilized-enzyme techniques and establishes a theoretical framework for development of analytical immobilized-enzyme systems.[40] Topics include basic enzymology and enzyme kinetics, the complexities of reactions in open systems, the process of dispersion in flowing streams and packed columns, immobilization chemistry, enzyme electrodes, and immobilized-enzyme reactors.

A topical article entitled 'Current frontiers and quests in immobilized cell technology' in addition to asking the question 'Why immobilize cells?' discusses general principles in methods of immobilization, effects of immobilization on the cell, and applications.[41] The article concludes that the immobilization of cells is new and the details unknown, so the subject provides a wealth of research possibilities with plenty of independent scope for large numbers of investigators. Researchers have first picked on the microbial cells first since these are most easily produced and immobilized. In cell-immobilization technology we are handling some of the chemical and biological treasures of life which will undoubtedly give rise to some of the most significant biological discoveries this century.

2 β-D-2-Acetamido-2-deoxygalactosidases, β-D-2-Acetamido-2-deoxyglucosidases, and β-D-2-Acetamido-2-deoxyhexosidases

A simple and rapid microfractionation procedure that enables the separate analysis of β-D-2-acetamido-2-deoxyhexosidase A and B activities in as little as a few hundred to a thousand cultured human cells has been described.[42] Cell homogenate (25 μl) is added to a pellet of DEAE-cellulose and 50 μl 0.1 M-NaCl in buffer. After centrifugation the B and I forms are measured in the supernatant, whereas form A is determined by direct incubation of the DEAE pellet

[40] 'Immobilized Enzymes in Analytical Clinical Chemistry: Fundamentals and Applications', ed. P. Carr and L. D. Bowers, J. Wiley and Sons Ltd., Chichester, 1980.
[41] J. F. Kennedy, *Enzyme Microb. Technol.*, 1980, **2**, 164.
[42] A. D'Azzo, A. Hoogeveen, and H. A. DeWit-Verbeek, *Clin. Chim. Acta*, 1978, **88**, 1.

with methylumbelliferyl glycoside substrate. The reliability and reproducibility of the method is compared with that of heat inactivation and column chromatography. The application of the procedure is illustrated by analyses of fibroblasts and cultured amniotic fluid cells from pregnancies at risk for Tay–Sachs disease and by serum assays for the diagnosis and heterozygote testing of this disease.

Hydrophobic chromatography was investigated as a purification procedure for human liver β-D-2-acetamido-2-deoxyhexosidases.[43] Both phenyl-Sepharose® and valine-Sepharose® have a high binding capacity for β-D-2-acetamido-2-deoxyhexosidases. A degree of resolution between the A and B isoenzymes is achieved with phenyl-Sepharose. Both hydrophobic supports must be used close to their capacity in order to recover the applied enzyme. Two purification procedures for human liver β-D-2-acetamido-2-deoxyhexosidases B were employed, which resulted in recoveries of approximately 48 and 24% with final specific activities of 33 400 and 4840 nmol min^{-1} mg^{-1}, respectively.

Human placental β-D-2-acetamido-2-deoxyhexosidases A was labelled with ^{125}I to high specific activity with the retention of conformational integrity as judged by the retention of enzymatic activity.[44] The oligosaccharide structure also appeared to be intact since the labelled enzyme was cleared from the circulation of the rat with a half-life identical to that of the unlabelled enzyme and an excess of unlabelled enzyme effectively blocked the clearance of the labelled form. Furthermore, the pattern of inhibition of clearance of the native and labelled enzymes by asialofetuin and D-mannans was identical. This useful and mild procedure for labelling enzymes may be of general importance in the preparation of enzymes for metabolic studies in normal animals and animal models of genetic lysosomal storage disorders.

Lysosomal β-D-2-acetamido-2-deoxyglucosidase in fibroblasts and leucocytes exhibited the same isoelectric focusing pattern as found in liver.[45] Treatment with neuraminidase had a great effect on the pattern of several glycoside hydrolases, whereas treatment with alkaline phosphatase did not significantly change the pattern. These findings are discussed with special reference to the different 'uptake' systems that exist for 'acid' hydrolases.

Human fibroblasts contain intracellular β-D-2-acetamido-2-deoxyhexosidase, which bears the phosphohexosyl recognition marker (β-hex_{pm}).[46] The evidence for this includes the high efficiency of uptake of the enzyme by Sandhoff cells, the saturation of uptake rates by high concentrations of enzyme, and the specific inhibition by D-mannose 6-phosphate. The mixture of intracellular β-D-2-acetamido-2-deoxyhexosidase forms appear to contain low levels of β-hex_{pm}. The pool size of β-hex_{pm}, although small, is not depleted by extensive incubation of donor cells in the presence of D-mannose-6-phosphate. This small pool is selectively secreted. Its fractional secretion rate is greater, depending on cell-line and monolayer age, by a factor of 3–10 than that for other forms of β-D-2-acetamido-2-deoxyhexosidase. This favoured secretion and the invariant

[43] J. Hardwick and P. Hechtman, *J. Chromatogr.*, 1980, **190**, 385.
[44] J. W. Kusiak and J. A. Barranger, *Clin. Chim. Acta*, 1979, **97**, 155.
[45] H. Kondo, H. Nakarani, and K. Hiromi, *Agric. Biol. Chem.*, 1977, **41**, 631.
[46] A. Kaplan, *Arch. Biochem. Biophys.*, 1980, **202**, 76.

fractional rate of secretion of total β-D-2-acetamido-2-deoxyhexosidase superimposed on a variable fractional rate for $\beta\text{-hex}_{prm}$ suggest routes for the secretion of β-D-2-acetamido-2-deoxyhexosidase by human fibroblasts.

The sex and age dependence of activity of eight glycoside hydrolases including β-D-2-acetamido-2-deoxygluco- and galacto-sidase and acid phosphatase was assayed in serum samples using the 4-methylumbelliferyl substrates.[47] The activity of these enzymes does not change in relation to sex and to age except for acid phosphatase and β-D-galactosidase, which show significantly higher values in children as compared to adults. The usefulness of the 4-methylumbelliferyl substrates for the detection of homozygotes for those lysosomal diseases involving one of the glycoside hydrolases studied is discussed.

The isoenzyme profiles of β-D-2-acetamido-2-deoxyglucosidase were determined using semi-automated DEAE-cellulose chromatography.[48] The major proportion of β-D-2-acetamido-2-deoxyglucosidase activity in synovial, seminal, and cerebrospinal fluid was present as the serum A^S-form.[48] Neonate and infant, but not adult kidney, contained significant amounts of intermediate activity, in addition to the A and B forms. Intermediate activity was also present in serum, synovial, and seminal fluids, but only low or trace amounts were recovered in urine and cerebrospinal fluid. No differences were found between the isoenzyme profiles of kidney cortex and medulla. β-D-2-Acetamido-2-deoxyglucosidase isoenzymes were affected by neuraminidase treatment in different ways. Renal β-D-2-acetamido-2-deoxyglucosidase A activity was unaffected but the intermediate forms present in neonate kidney were lost as the proportion of B activity increased. The β-D-2-acetamido-2-deoxyglucosidase A activity in urine was converted to B and I_2, whereas the A^S-form of serum was recovered mainly as the B form. Five patients with abnormal aspartate aminotransferase activity in their serum also had elevated β-D-2-acetamido-2-deoxyglucosidase activity. The sera of these patients could be further distinguished from normal on the basis of β-D-2-acetamido-2-deoxyglucosidase isoenzymes. The pathological sera contained elevated B and intermediate activities. The elevation of activity in body fluids in disease is accompanied by a change in the type and relative activities of the isoenzyme present. The use of semi-automated DEAE-cellulose chromatography has potential in the further characterization and diagnosis of disease.

Assay conditions have been developed for the determination of urinary β-D-glucuronidase, β-D-galactosidase, α-D-galactosidase, and β-D-2-acetamido-2-deoxyhexosidase using fluorimetric substrates.[49] The assay conditions for β-D-glucuronidase overcome interference by both low and high molecular weight inhibitors, a problem that has caused confusion in earlier studies of enzyme excretion. The four lysosomal enzymes are excreted co-ordinately, although their absolute levels (in units per milligram of creatinine) vary during the day and, from one day to the next, the ratio of one enzyme to another remains relatively constant. The lack of correlation between plasma and urine enzyme

[47] P. Annunziata and G. Dimatteo, *Clin. Chim. Acta*, 1978, **90**, 101.
[48] S. M. Tucker, R. J. Pierce, and R. G. Price, *Clin. Chim. Acta*, 1980, **102**, 29.
[49] K. Paigen and J. Peterson, *J. Clin. Invest.*, 1978, **61**, 751.

levels, together with the high molecular weights of these enzymes, suggests that the urinary enzymes are not derived by glomerular filtration. The lack of co-ordinancy with lactate dehydrogenase suggests they are not derived from exfoliated cells. By analogy with experimental animals, they may be derived from lysosomes extruded into the lumen of the proximal tubule by epithelial cells. There is considerable variation among a population of 125 healthy adult subjects for total enzyme excretion. Both total enzyme excretion and co-ordinancy ratios are log-normally distributed, suggesting that they are the resultants of many factors, each of which has a relative, or proportional, effect on enzyme excretion. About one-half the population variation resides in a process common to the excretion of all four enzymes (possibly the lysosome extrusion pathway), and about one-half resides in factors affecting each enzyme independently.

The effects of surfactants on the human liver β-D-2-acetamido-2-deoxyhexosidase A-catalysed hydrolysis of G_{M_2} ganglioside were assessed.[50] Some non-ionic surfactants, including Triton X-100 and Cutscum, and some anionic surfactants, including sodium taurocholate, sodium dodecyl sulphate, phosphatidylinositol, and *N*-dodecylsarcosinate, were able to replace β-D-2-acetamido-2-deoxyhexosidase A-activator protein and also stimulated the enzymic hydrolysis of substrate in the presence of saturating concentrations of activator. Other non-ionic surfactants, such as Tween 80, Brij 35, and Nonidet P40, and anionic surfactants, such as phosphatidylethanolamine, did not enhance enzymic hydrolysis of G_{M_2} ganglioside and inhibited hydrolysis in the presence of activator. The concentration of surfactants at which micelles form was determined by measurements of the minimum surface-tension values of reaction mixtures containing a series of concentrations of surfactant. In the case of Triton X-100, Cutscum, sodium taurocholate, *N*-dodecylsarcosinate, and other surfactants, the concentration range at which stimulation of enzymic activity occurs correlates well with the critical micellar concentration. None of the surfactants tested affected the rate of β-D-2-acetamido-2-deoxyhexosidase A-catalysed hydrolysis of 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside. Both activator and surfactants that stimulate hydrolysis of G_{M_2} ganglioside decrease the K_m for G_{M_2} ganglioside. Inhibitory surfactants are competitive with the activator protein. Evidence for a direct interaction between surfactants and G_{M_2} ganglioside was obtained by comparing gel-filtration profiles of ^{3}H-labelled G_{M_2} ganglioside in the presence and absence of surfactants. The results are discussed in terms of a model wherein a mixed micelle of surfactant or activator and G_{M_2} ganglioside is the preferred substrate for enzymic hydrolysis.

The biosynthesis of three lysosomal enzymes, β-D-2-acetamido-2-deoxyhexosidase, α-D-glucosidase, and cathepsin D, was studied in intact human diploid skin fibroblasts.[51] In a 3-hour pulse, β-D-2-acetamido-2-deoxyhexosidase was labelled in chains of 67 000 and 63 000 apparent molecular weight; during a subsequent chase, these chains disappeared, to be replaced by chains of the size seen in placental enzyme: 54 000, 29 000, and some smaller fragments. Antisera

[50] P. Hechtman and Z. Kachra, *Biochem. J.*, 1980, **185**, 583.
[51] A. Hasilik and E. F. Neufeld, *J. Biol. Chem.*, 1980, **255**, 4937.

raised separately to the α and β subunits of placental β-D-2-acetamido-2-deoxyhexosidase A allowed the identification of the 67 000 chains as a precursor of the 54 000 (α), and the 63 000 as a precursor of a transient 52 000 as well as of the 29 000 (β) chains. Labelling patterns of fibroblasts derived from patients with inherited disorders of G_{M_2}, metabolism (Tay–Sachs disease, Sandhoff's disease, and AB variant) confirmed this identification. Precursor chains accumulated in small amounts in the medium, but processed chains were not seen extracellularly.

β-D-2-Acetamido-2-deoxyglucosidase activity has been measured fluorimetrically in lymphocytes from 20 human donors, enriched B and T lymphocyte populations prepared by E rosette sedimentation from eight normal subjects, and lymphocytes from 15 untreated B-cell chronic lymphocytic leukaemic patients.[52] The pH profiles and optima (4.7) were similar in all preparations. Normal B lymphocytes had higher activity than normal T lymphocytes and both these preparations and normal unfractionated lymphocytes had significantly higher activity than chronic lymphocytic leukaemic lymphocytes. The apparent Michaelis constants were similar in normal unfractionated, B-enriched, and T-enriched lymphocytes, whereas a reduced affinity for the enzyme was observed in the leukaemic lymphocytes. The difference in enzyme content between normal and chronic lymphocytic leukaemic lymphocytes cannot therefore be explained on the basis of a high B-cell percentage in patients with chronic lymphocytic leukaemia.

The β-D-2-acetamido-2-deoxyhexosidase isoenzymes in tissues, cultured cells, and media from human foetal intestine and colonic adenocarcinoma have been studied.[53]

In a comparative study of eight glycoside hydrolases in serum samples from 72 cystic fibrosis patients, 85 cystic fibrosis parents, and 34 healthy and disease controls, the levels of β-D-2-acetamido-2-deoxygluco- and galacto-sidase activities were found to be unchanged.[54]

The specific activities of several lysosomal hydrolases in normal and cystic fibrosis livers have been investigated.[55] Neuraminidase activity with respect to two natural substrates (fetuin and bovine submaxillary mucin) is normal in cystic fibrosis livers. No significant differences were found between cystic fibrosis and normal livers in 4-methylumbelliferyl α-D-mannosidase specific activities determined at three different pH values (4.3, 5.65, and 6.5) corresponding to lysosomal, golgi, and cytosolic components of the enzyme, respectively. The specific activities of '4-nitrophenyl β-D-mannosidase' and '4-methylumbelliferyl 2-acetamido-2-deoxyglucosidase' were also both similar in cystic fibrosis and normal livers.

Secretion of the lysosomal β-D-2-acetamido-2-deoxyglucosidase by normal and cystic fibrosis fibroblasts has been compared.[56] In contrast to an earlier

[52] A. D. Crockard, M. H. R. Lewis, and J. M. Bridges, *Clin. Chim. Acta*, 1979, **93**, 151.
[53] D. Tsao, H. J. Freeman, and Y. S. Kim, *Cancer Res.*, 1970, **39**, 3405.
[54] L. Casola, G. Di Matteo, M. Romano, B. Rutigliano, and G. Mastella, *Clin. Chim. Acta*, 1979, **94**, 83.
[55] J. A. Alhadeff, *Clin. Chim. Acta*, 1978, **89**, 469.
[56] P. Willcox, *Clin. Chim. Acta*, 1979, **91**, 81.

report, no differences were found in either the rate of secretion, or in the molecular forms of the enzyme secreted by normal fibroblasts cultures and cultures initiated from patients with cystic fibrosis. The B isoenzyme of β-D-2-acetamido-2-deoxyglucosidase from cystic fibrosis fibroblasts was converted to a more negatively charged form during preincubation for 3 h at pH 5.0 and 37 °C. The enzyme from normal fibroblasts was unaffected by this treatment.

In a study of 10 normal controls, seven pregnant women, and 16 diabetic patients, β-D-2-acetamido-2-deoxyhexosidase in tears and 2,3-diphosphoglycerate in blood were investigated for possible use as indexes for diabetic retinopathy during pregnancy.[57] Tears were used as source for β-D-2-acetamido-2-deoxyhexosidase because the concentration in plasma and the isoenzyme pattern are affected by the occurrence of a placental isoenzyme in pregnancy. It has been found that in diabetes the concentration of β-D-2-acetamido-2-deoxyhexosidase in plasma may be elevated, but that it remains unchanged in tears and that there is no correlation between the plasma and tear values in all individuals. Neither β-D-2-acetamido-2-deoxyhexosidase in tears nor 2,3-diphosphoglycerate in blood appears as indicator for the onset or development of diabetic retinopathy.

β-D-2-Acetamido-2-deoxyglucosidase activity has been measured in the serum and urine of diabetics.[58] Results have shown significantly higher levels of serum β-D-acetamido-2-deoxyglucosidase in newly diagnosed diabetics (945 ± 372 units ml^{-1}) compared to non-diabetic controls (668 ± 225, $p < 0.005$) and the levels were reduced by treatment (778 ± 218, $p < 0.05$). Changes occurred in the same direction when urinary β-D-2-acetamido-2-deoxyglucosidase was measured falling from a mean of 572 ± 298 units mg^{-1} urinary creatinine, on diagnosis to 291 ± 176 (after treatment ($p < 0.005$), as compared with 177 ± 86 in non-diabetic controls. Established insulin-treated diabetics had a urinary β-D-2-acetamido-2-deoxyglucosidase activity of 461 ± 440 and a serum β-D-2-acetamido-2-deoxyglucosidase activity of 790 ± 245. No correlation was found between urine β-D-2-acetamido-2-deoxyglucosidase activity and urine D-glucose ($r = 0.315$) or serum β-D-2-acetamido-2-deoxyglucosidase and serum D-glucose ($r = 0.273$). An assessment of this enzyme was made in relation to early microangiopathy.

Human β-D-2-acetamido-2-deoxyglucosidase activity has been measured in the serum and urine of primary and secondary diabetics and in primary diabetics with microangiopathy.[59] β-D-2-Acetamido-2-deoxyglucosidase activity has also been measured in the tears of diabetics with ocular complications and diabetics with no ocular changes. Results have shown significantly higher levels of urinary β-D-2-acetamido-2-deoxyglucosidase in diabetics with proteinuria ($p < 0.001$) and proteinuria and retinopathy ($p < 0.001$). There was no correlation between urinary β-D-2-acetamido-2-deoxyglucosidase activity and serum creatinine ($r = 0.28$) or urinary β-D-2-acetamido-2-deoxyglucosidase and the degree of proteinuria ($r = 0.24$). Increased urinary β-D-2-acetamido-2-deoxyglucosidase levels were also observed in secondary diabetes associated with haemochromatosis and acromegaly. Significantly higher serum β-D-2-acetamido-2-deoxyglucosidase levels were found in newly diagnosed diabetics ($p < 0.01$) and signifi-

[57] N. J. van Haeringen, J. L. Vrooland, and E. Glasius, *Clin. Chim. Acta*, 1978, **86**, 333.
[58] P. H. Whiting, I. S. Ross, and L. J. Borthwick, *Clin. Chim. Acta*, 1979, **92**, 459.
[59] P. H. Whiting, I. S. Ross, and L. J. Borthwick, *Clin. Chim. Acta*, 1979, **97**, 191.

cantly lower levels in chemical diabetics ($p < 0.01$). Compared to non-diabetic controls tear β-D-2-acetamido-2-deoxyglucosidase levels were significantly higher in the diabetic controls ($p < 0.01$), in diabetics with retinopathy ($p < 0.01$), and in diabetics with cataract formation ($p < 0.05$). An assessment of this enzyme is made in relation to the development of diabetic microangiopathy.

The activities of several glycoside hydrolases (α-L-fucosidase, α-D-mannosidase, α-D-galactosidase, β-D-galactosidase, β-D-2-acetamido-2-deoxyglucosidase, and β-D-glucuronidase) were determined in human sera from 10 normal subjects and in three groups each of 10 patients with diabetes mellitus, hepatic cirrhosis, and gastric carcinoma.[60] The results show significantly higher activities in the patients for α-L-fucosidase ($p < 0.001$) and for β-D-2-acetamido-2-deoxyglucosidase ($p < 0.01$, $p < 0.001$, and $p < 0.05$, respectively), and smaller or not significantly greater values for the other glycosidases.

Optimal assay conditions are described for plasma α-D-galactosidase, β-D-galactosidase, β-D-glucuronidase, α-D-mannosidase, α-D-glucosidase, β-D-2-acetamido-2-deoxyglucosidase, α-L-fucosidase, α-D-2-acetamido-2-deoxyglucosidase, acid phosphatase, and arylsulphatase A.[61] The levels of these activities in normal adults and children, and the stabilities of the activities on storage at −20 °C or 4 °C, are reported. The levels of these enzymic activities in plasma from patients with Fabry, Pompé, Sanfillipo A, Sanfillipo B, Tay-Sachs, and Hunter diseases, G_{M_1}-gangliosidosis, and metachromatic leucodystrophy are described, and the possibility of using plasma-hydrolase activities in the diagnosis of these conditions is discussed.

In normal fibroblasts, radioactive phosphate was incorporated into β-D-2-acetamido-2-deoxyhexosidase, cathepsin D, and α-D-glucosidase.[62a] Nearly all [^{3}H]leucine-labelled chains previously identified with these enzymes,[62b] were also labelled with phosphate: precursor chains in cells and medium, as well as partially and fully processed intracellular chains. The enzymes were also phosphorylated in the presence of chloroquine or ammonium chloride, even though intra- and extra-cellular distribution of the enzymes was altered by the drugs and processing was impaired. However, in cultures from two patients with I-cell disease (a genetic disorder with numerous lysosomal abnormalities including abnormal distribution and processing of the three enzymes), phosphorylation was essentially undetectable. To determine the position of the phosphate residues, the precursor chains of cathepsin D and of the α- and β-chains of β-D-2-acetamido-2-deoxyhexosidase, labelled with D-[2-^{3}H]mannose and $^{32}P_i$, were obtained from the medium of normal fibroblasts exposed to NH_4Cl and isolated by polyacrylamide-gel electrophoresis.

Molecular forms of β-D-2-acetamido-2-deoxyhexosidase and α-D-mannosidase were studied using isoelectric focusing in fibroblasts and culture medium from patients with mucolipidosis types II and III.[63] Compared to controls, no significant aberrations could be found in the isoelectric focusing isoenzyme

[60] A. Reglero, M. I. Carretero, and J. A. Cabezas, *Clin. Chim. Acta*, 1980, **103**, 155.
[61] P. A. Griffiths, J. P. Milsom, and J. B. Lloyd, *Clin. Chim. Acta*, 1978, **90**, 129.
[62] (*a*) A. Hasilik and E. F. Neufeld, *J. Biol. Chem.*, 1980, **255**, 4937; (*b*) *ibid.*, p. 4946.
[63] B. Hultberg, *Clin. Chim. Acta*, 1980, **103**, 159.

pattern. D-Mannose 6-phosphate, which inhibits the uptake of lysosomal enzymes into the cells, was added to the cell cultures from controls without any significant effect on the isofocusing isoenzyme pattern.

Using concanavalin A–Sepharose®, the elution pattern of plasma α-D-mannosidase, β-D-2-acetamido-2-deoxyglucosidase, acid phosphatase, and α-L-fucosidase from patients with mucolipidosis II and III was comparable to that of plasma of a normal individual.[64] The elution of urinary β-D-2-acetamido-2-deoxyglucosidase and α-D-galactosidase was the same in mucolipidosis II as in normal controls. Mucolipidosis II patients' fibroblasts have a somewhat lower percentage elution of β-D-2-acetamido-2-deoxyglucosidase and 'acid' phosphatase than do normal controls.

Using a column of L-fucose-binding lectin, no activity could be found for eight lysosomal enzymes, which were tested in the elution fractions of plasma from patients with mucolipidosis II and normal controls. Elution of seven plasma lysosomal enzymes was obtained using a D-galactose-binding lectin. No significant differences were noted between plasma of normal persons and patients with mucolipidosis II. Results indicate that the lysosomal enzymes tested do not adsorb to L-fucose-binding lectin and that there are no profound differences in the elution of lysosomal enzymes from concanavalin A–Sepharose® and D-galactose-binding lectin between normal persons and patients with mucolipidosis II and III.

A comparative study has been made of the diagnostic value of disc electrophoresis of urinary proteins and measurement of the excretion of β-D-2-acetamido-2-deoxyglucosidase for the detection of renal tubule damage in chronic polyarthritis.[65]

A family is described in which alleles for two different β-D-2-acetamido-2-deoxyhexosidase β-chain variants are segregating.[66] When they co-exist in the same individual, Sandhoff's disease results. In the heterozygous state one of the variant alleles results in the production of an unstable β-D-2-acetamido-2-deoxyhexosidase B and a β-D-2-acetamido-2-deoxyhexosidase A with an altered K_m for the substrate 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-galactopyranoside. The other allele, when heterozygous with a normal allele, does not produce unstable isoenzymes with altered kinetics. Like many rare recessive diseases the affected children in this family would appear to have been compound heterozygotes and not true homozygotes.

β-D-2-Acetamido-2-deoxyhexosidase A is present in relatively low concentrations in cell-free amniotic fluids from pregnancies with Tay–Sachs foetuses.[67] This isoenzyme was determined by an immunological procedure, radial immunodiffusion by which β-D-2-acetamido-2-deoxyhexosidase A can be directly and specifically detected, even in the presence of excess amounts of β-D-2-acetamido-2-deoxyhexosidase B. No β-D-2-acetamido-2-deoxyhexosidase A could be detected by the same procedure in Tay–Sachs foetal tissues, implying that this

[64] W. R. Den Tandt, *Clin. Chim. Acta*, 1980, **102**, 199.
[65] E. Knoll, H. Wisser, and H. Rautenstrauch, *J. Clin. Chem. Clin. Biochem.*, 1980, **18**, 53.
[66] A. B. Lane and T. Jenkins, *Clin. Chim. Acta*, 1978, **87**, 219.
[67] B. Geiger, R. Navon, and R. Arnon, *Clin. Chem.*, 1978, **24**, 1131.

isoenzyme in the amniotic fluid originates from the mother. Identification of carriers of the Tay–Sachs gene during pregnancy is difficult because of the increase in serum of a heat-stable β-D-2-acetamido-2-deoxyhexosidase isoenzyme I (or P) as well as changes in the relative and absolute activities of the various molecular forms of the enzyme with advancing pregnancy.[68] In contrast, isolation of blood platelets followed by ion-exchange chromatographic separation and assay of the β-D-2-acetamido-2-deoxyhexosidase isoenzymes in platelet extracts by an automated method provides a sensitive and reliable method for heterozygote identification during pregnancy. This method appears superior to procedures involving thermal inactivation of extracts of peripheral blood leukocytes because of significant difference in the content of the β-D-2-acetamido-2-deoxyhexosidase isoenzymes in granulocytes, lymphocytes, and other cell types, as well as variations in the proportion of these cell types in samples of peripheral blood. It also alleviates the problem, inherent in any method involving thermal inactivation of β-D-2-acetamido-2-deoxyhexosidase A, of possible intercoversion of the various molecular forms of the enzyme on heating.

The molecular weights and subunit compositions of bovine liver β-D-2-acetamido-2-deoxyhexosidases A and B were studied using enzyme preparations purified by octyl-Sepharose® chromatography.[69] Analysis by gel filtration showed that the A enzyme had a molecular weight of *ca.* 310 000 (hexamer) at pH 5, 6, and 6.5, and was dissociated into dimers at pH 7 and 8, whereas the molecular weight of the B enzyme, *ca.* 310 000, remained constant in the pH range of 5–8. The molecular weights of forms A and B subunits as determined by electrophoresis in sodium dodecyl sulphate were both *ca.* 52 000, and this value remained unaltered when the enzyme was treated with a reducing agent or denaturant.

Some unusual molecular weight forms of β-D-2-acetamido-2-deoxyhexosidase have been encountered during the assessment of the enzyme from intestinal mucosa of newborn pigs.[70]

α-D-2-Acetamido-2-deoxyglucosidase was purified from porcine liver by acid and ammonium sulphate precipitation, followed by chromatography on various matrices including concanavalin A–Sepharose®.[71] 4-Nitrophenyl 2-acetamido-2-deoxy-α-D-glucopyranoside and Forssmann hapten hydrolysing activities were purified 3300-fold and 19 600-fold, respectively. Native gel electrophoresis with 7% polyacrylamide at pH 8.3 gave two protein bands with 4-nitrophenyl 2-acetamido-2-deoxy-α-D-glucopyranoside hydrolysing activity. The native enzyme and its subunits were estimated to have molecular weights of 102 000 and 52 000, respectively. The kinetic behaviour of this enzyme preparation was tested towards different substrates with 2-acetamido-2-deoxy α-D-galactopyranose and α-D-galactose residues at the non-reducing end, including 4-nitrophenyl

[68] S. Nakagawa, S. Kumin, P. Chandra, and H. M. Nitowsky, *Clin. Chim. Acta*, 1978, **88**, 249.
[69] M. Tanaka, S. Kyosaka, and S. Yamashiro, *Chem. Pharm. Bull. (Jpn.)*, 1980, **28**, 301.
[70] E. A. Munn, C. A. Greenwood, A. Orlacchio, and G. Porcellati, *Int. J. Biochem.*, 1979, **10**, 1045.
[71] S. S. J. Sung and C. C. Sweeley, *J. Biol. Chem.*, 1980, **255**, 6589.

2-acetamido-2-deoxy-α-D-glucopyranoside, Forssman hapten, *N*-acetyl-sphingosyl-Forssman pentasaccharide, porcine submaxillary mucin, 2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-D-galactose, and 4-methylumbelliferyl α-D-galactopyranoside. Apparent K_m values for these substrates were 2.5×10^{-3}, 2.6×10^{-4}, 2.5×10^{-4}, 2.3×10^{-6}, 4.8×10^{-3}, and 1×10^{-2} M, and the V_{max} values were 14.8, 4.2, 0.81, 0.42, 1.1, and 3.0 μmol min^{-1} mg^{-1}, respectively.

The immunological characteristics of feline β-D-2-acetamido-2-deoxyhexosidase isoenzymes A and B were studied.[72] Immunization of rabbits and goats with either cat form A or B produced antibodies that reacted with a common antigenic marker shared by both the A and B enzyme. With properly absorbed antisera, a unique antigenic marker was demonstrated on cat form A, but not on B. This antigenic profile is comparable to that of the human isoenzymes, in which both β-D-acetamido-2-deoxyhexosidase A and B share the antigenic determinant, β, whereas only the A form possesses the antigenic determinant, α. No cross-reactivity between the two species could be demonstrated using goat or rabbit antisera to either feline or human β-D-2-acetamido-2-deoxyhexosidase. These immunological data validate feline G_{M_2} gangliosidosis as a model for human G_{M_2} gangliosidosis type II, and facilitate the investigation of enzyme-replacement therapy.

Five glycoside hydrolases (α- and β-D-mannosidase, β-D-galactosidase, β-D-2-acetamido-2-deoxyglucosidase, and α-L-fucosidase) were studied during the post-natal development of the rat cerebellum.[73] Each glycosidase has a particular developmental pattern. Transient decreases in the specific activities of β-D-mannosidase, β-D-galactosidase, and β-D-2-acetamido-2-deoxyglucosidase could be correlated with the phase of massive cell multiplication. A possible more specific role for α-D-mannosidase is discussed.

Suckling rat liver β-D-2-acetamido-2-deoxyhexosidase activity undergoes considerable fluctuation during the first two weeks of life.[74] As two major forms of the enzyme (A, heat-labile, and B, heat-stable) are known to exist in both human and adult rat liver, the effect of the maturative hormones, thyroxine and cortisone, upon these isoenzymes during the suckling period was examined. Between days 7 and 15, the observed developmental change is attributable solely to an increase in the 'A-like' (heat-labile) form of the enzyme; an enhanced response is seen in thyroxine-injected 11–15 day old animals. The response may be considered 'age-independent', as adult animals react in the same manner. In contrast, cortisone-injected sucklings show a decrease in both A and B isoenzymes, whereas in adults no change in total activity or isoenzyme distribution are evoked. The ratio of isoenzyme A to isoenzyme B in suckling rat liver appears to shift in favour of the labile (A) isoenzyme early in development.

The kinetics of rat liver G_{M_2} ganglioside β-D-2-acetamido-2-deoxyhexosidase have been reported upon.[75]

[72] D. C. O'Neil, W. R. Bartholemew, and M. C. Rattazi, *Biochim. Biophys. Acta*, 1979, **580**, 1.
[73] J. P. Zanetta, A. Federico, and G. Vincendon, *J. Neurochem.*, 1980, **34**, 831.
[74] L. V. Uberkotter, R. Kern, and D. Koldovsky, *Biochim. Biophys. Acta*, 1980, **630**, 279.
[75] A. Novak and J. A. Lowden, *Can. J. Biochem.*, 1980, **58**, 82.

An *endo*-β-D-2-acetamido-2-deoxyglucosidase activity towards an asialo-*N*-acetyl-lactosaminic-type glyco-L-asparagine substrate was demonstrated in rat liver.[76] This activity was optimal at pH 7.0 and was predominantly present in the soluble (cytosolic) fraction.

Two different enzymes which cause the breakdown of nutritional chitin in the hunting spider *Cupiennius salei*, were purified to homogeneity and characterized.[77] One chitinase (EC 3.2.1.14) was enriched about 80-fold (molecular weight 48 000) (see p. 311). β-D-2-Acetamido-2-deoxy-D-glucosidase was purified about 160-fold (molecular weight 108 000) and is characterized by kinetic and thermodynamic parameters and inhibitors.

The influences of sex, developmental time, and food intake on the β-D-2-acetamido-2-deoxyglucosidase activity in the rice weevil *Sitophilus oryzae* have been investigated.[78]

A study has been made of the cytochemical localization of β-D-2-acetamido-2-deoxyglucosidase in the hyphae of *Mucor racemosus*.[79]

3 α-D- and -L-Arabino(furano)sidases

The subject of arabinosidases has been reviewed.[80]

An affinity chromatography technique that allows preparation of highly purified α-L-arabinofuranosidase from *Aspergillus niger* by using either arabinan cross-linked by epichlorohydrin or arabinan linked to epoxy-activated macroporous agarose *via* ether linkages as affinity carriers has been described.[81] These specific insoluble adsorbents allow a single-step separation of this enzyme from a mixture of extracellular enzymes.

4 β-D-Fructofuranosidases

A continuous optical method for the assay of D-glucose-releasing hydrolases, α-D-glucosidase, αα-trehalase, and β-D-fructofuranosidase, is reported.[82] Particular emphasis is given to the assay of purified sucrase from rabbit small intestine. The procedure requires D-glucose dehydrogenase and mutarotase. In the presence of the latter enzyme, the initial lag is substantially shortened when D-glucose is released as the α-anomer. Under the test conditions used, the method shows a good proportionality up to an activity of 0.2 units/3 ml and may also be applied for measuring the activity in crude homogenates.

When maize scutellum slices were incubated in solutions of sucrose or maltose, there was a release of D-glucose into the bathing solution.[83] The pH optima for D-glucose release were 2.5 for sucrose and 3.5 for maltose. From measurement of rates of D-glucose uptake into slices in the presence or absence

[76] R. J. Pierce, G. Spik, and J. Montevil, *Biochem. J.*, 1980, **185**, 261.
[77] T. P. Mommsen, *Biochem. Biophys. Acta*, 1980, **612**, 361.
[78] C. Wicker, *Experientia*, 1980, **36**, 1059.
[79] H. C. Hoch, G. Hanssler, and H. J. Reisener, *Exp. Mycol.*, 1979, **3**, 164.
[80] A. Kaji, *J. Agric. Chem. Soc. (Jpn.)*, 1980, **54**, 561.
[81] R. Waibel, R. Amado, and H. Neukom, *J. Chromatogr.*, 1980, **197**, 86.
[82] P. Vanni and G. M. Handzet, *Experientia*, 1980, **36**, 1035.
[83] T. Humphreys and E. Echeverria, *Phytochemistry*, 1980, **19**, 189.

of sucrose, it is calculated that D-glucose uptake will introduce errors of 3–9%, depending on the sucrose concentration, in estimates of free-space sucrose-hydrolase activity at pH 2.5. At their respective pH optima, maltose was hydrolysed at a rate 2.5 times that of sucrose. When frozen–thawed slices were used, the same pH optima were obtained, but rates of hydrolysis were increased. Raffinose and melezitose also were hydrolysed with pH optima of 2.5 and 3.5, respectively: methyl α-D-glucopyranose was not hydrolysed. A 60-min HCl treatment (pH 2) of scutellum slices destroyed 69% of the sucrose-hydrolase activity and 100% of the maltose-hydrolase activity. In contrast, sucrose uptake and sucrose synthesis from exogenous fructose were not affected by HCl treatment. It is concluded that there are two hydrolases, acid β-D-fructofuranosidase and α-D-glucosidase; that they are either on or outside the plasmalemma (in the free space); and that they are not necessary to the disaccharide-uptake processes either by supplying exogenous hexose or by acting as transporters.

It was found that certain substances that made the bound β-D-fructofuranosidase insoluble were released together with the enzyme by treating cell wall of sugar beet seedlings with salt.[84] Insolubilization of the enzyme was extremely reduced by pretreatment of the substance with either deoxyribonuclease or hot perchloric acid, but not affected by the substance pretreated with ribonuclease, alkali, or pronase. U.v. spectra of the purified substance showed a maximal absorption at 260 nm and a minimum at 238 nm. The diphenylamine test for deoxyribose and the Fiske–Subbarow test for phosphorus were both positive. These results indicate that the substance causing insolubilization of the enzyme is DNA.

The present paper deals with the formation of altered molecules of β-D-fructofuranosidase in the cell-wall fraction of tomato fruit in relation to ageing.[85] The monospecific antibody prepared from rabbits was used to characterize enzymes at ripened and senescent stages of tomato fruits. Although the activity on a fresh weight basis and the specific activity of the crude extract declined as the fruit aged, no difference was observed in the amount of the enzyme protein on a fruit weight basis between the two stages. With purified enzyme, there was little difference in such properties as K_m, heat stability, and optimum pH. However, the purified β-D-fructofuranosidase from the senescent fruits had a lower specific activity. It is concluded from the results that the decline in the enzyme activity in the senescent fruits is due to the occurrence of immunologically active but catalytically inactive molecules of β-D-fructofuranosidase.

Raffinose was converted enzymatically in a hollow-fibre reactor to melibiose, sucrose, D-galactose, D-glucose, and D-fructose.[86] The enzymes were a crude extract of α-D-galactosidase and β-D-fructofuranosidase produced by *Aspergillus awamori* NRRL 4869 on a solid substrate, wheat bran. With a concentration of raffinose, C_0, entering the reactor at a flow rate Q, and with C being the concentration of raffinose leaving the reactor, the conversion (C/C_0), was

[84] H. Masuda and S. Sugawara, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 69.
[85] H. Nakagawa, K. Iki, M. Hirata, S. Ishigami, and N. Ogura, *Phytochemistry*, 1980, **19**, 195.
[86] R. W. Silman, L. T. Black, J. E. McGhee, and E. B. Bagley, *Biotechnol. Bioeng.*, 1980, **22**, 533.

studied as a function of Q at two levels of C_0. The data could be fairly well fitted using the analysis of Waterland *et al.* even though a mixed crude enzyme system was being investigated. It was found empirically that $\ln(C/C_0)$ was linear in Q^{-1}, with the absolute value of the slope decreasing with increasing C_0. The linearity of such plots were predicted by Lewis and Middleman from Waterland *et al.* for a single enzyme system obeying first-order kinetics, the slope being independent of C_0. Although the assumptions involved in this approximate analytical solution are not valid, the observed linearity of the $\ln(C/C_0)$ *vs.* Q^{-1} plots is excellent and should prove useful in reactor design considerations.

The production of some properties of the β-D-fructofuranosidase isoenzymes of *Fusarium oxysporum* have been investigated.[87]

Fusarium β-D-fructofuranosidase appears to exist in two forms, mycelial (P-1) and conidial (P-2) types.[88] They were purified and partially characterized, but their specific activities were too low when compared with yeast and *Neurospora* β-D-fructofuranosidases. Present studies describe a method for isolation of highly purified enzymes and their properties. The enzymes are homogeneous by several criteria. Estimation of molecular weights revealed the subunit of each invertase, and the association–dissociation of subunits seem to occur as temperature varies. Amino-acid compositions and other properties have been studied. The two β-D-fructofuranosidases are glycoproteins that contain 36% (P-1) and 23% (P-2) carbohydrates (predominantly mannose with small percentages of D-glucose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2-deoxy-D-galactose). Comparison with the properties of the previous preparations is also described.

β-D-Fructofuranosidase activity was measured in cell-free extracts from early log phase *Saccharomyces cerevisiae* cultured in media containing either 1% D-glucose or 1% raffinose.[89] The mechanism for the apparent repression of β-D-fructofuranosidase by D-glucose was investigated by measuring β-D-fructofuranosidase synthesis at the level of transcription and translation with β-D-fructofuranosidase-specific antiserum and sodium dodecyl sulphate–polyacrylamide gel electrophoresis. No significant difference in the level of β-D-frucofuranosidase-specific mRNA was observed when cells were grown in D-glucose or D-raffinose, a sugar which does not repress the synthesis of β-D-fructofuranosidase. The immunoprecipitated β-D-fructofuranosidase polypeptide resulting from the completion of nascent peptide chains associated with endogenous polysomes was 60 000 daltons in D-glucose-grown cells, which is equivalent in size to the carbohydrate-depleted β-D-fructofuranosidase subunit (R. B. Trimble and F. Maley, *J. Biol. Chem.*, 1977, **252**, 4409). In contrast, the corresponding species obtained from the endogenous polysomes of D-raffinose-grown cells had a molecular weight of about 85 000, but this could be trimmed to 60 000 by treatment with *endo*-β-*N*-acetylglucosaminidase H.

Poly(A)-containing RNA from *Saccharomyces cerevisiae* was translated in the wheat germ protein-synthesizing system and yielded as one of its products a

[87] Y. Maruyama and K. Onodera, *J. Appl. Gen. Microbiol.*, 1979, **25**, 361
[88] M. Nishikawa, Y. Maruyama, and M. Nakamura, *Agric. Biol. Chem.*, 1980, **44**, 489.
[89] F. K. Chu and F. Maley, *J. Biol. Chem.*, 1980, **255**, 6392.

polypeptide that was identified as β-D-fructofuranosidase.[90] This characterization was based on the presence of a protein band at 60 000 daltons following immunoprecipitation with a specific β-D-fructofuranosidase antibody, and the formation from this protein of staphylococcal V8 protease and cyanogen bromide peptides, which were similar in size to those from carbohydrate-depleted β-D-fructofuranosidase. The observed increase in β-D-fructofuranosidase activity from the early to the mid logarithmic stage of cell growth was synchronous with the appearance of translatable β-D-fructofuranosidase mRNA in these cells. No translatable β-D-fructofuranosidase mRNA, however, was detected in late logarithmic cells, although a constant level of β-D-fructofuranosidase activity was present in the culture medium.

β-D-Fructofuranosidase, extracted from broken cells of *Saccharomyces cerevisiae* X-2180 *mnn*2 mannan mutant, was separated into a fraction insoluble in 75% ammonium sulphate (P75 β-D-fructofuranosidase, 36% carbohydrate) and a soluble fraction (S75 β-D-fructofuranosidase, 53% carbohydrate).[91] The latter reacted with antibodies specific for the (1→6)-linked α-D-mannose of the D-mannoprotein outer chain, whereas the P75 β-D-fructofuranosidase failed to react with this antiserum although it did react with serum against terminal (1→3)-linked α-mannose units that are characteristic of the D-mannoprotein core. A bacterial *endo*-(1→6)-α-D-mannanase removed the outer chains from the S75 β-D-fructofuranosidase and converted it to a form that was similar in electrophoretic and immunochemical properties to the P75 β-D-fructofuranosidase, whereas the *endo*-(1→6)-α-D-mannanase had little effect on the latter β-D-fructofuranosidase. The results suggest that the P75 β-D-fructofuranosidase is a form of the enzyme to which only the core oligosaccharide units had been added, and the S75 β-D-fructofuranosidase represents an enzyme fraction to which the polysaccharide out chains were also attached. The overall results suggest that the external β-D-fructofuranosidase molecules contain some core units without attached outer chains, and that the cells contain a precursor form of the enzyme to which only the core units have been added. In support of this conclusion, p.m.r. spectra and chromatographic patterns show that the core fragments from the P75, S75, and wild type β-D-fructofuranosidases are essentially identical.

Routes to the recovery of β-D-fructofuranosidase from industrial broths of baker's yeast have been described.[92]

Three β-D-fructofuranosidase isoenzymes extracted from derepressed yeast cell membranes with deoxycholate were separated on sodium dodecyl sulphate polyacrylamide gels.[93] A dye-producing method for the location of isoenzymes after gel electrophoresis has been developed by using a coupled enzymatic reaction including the formazan reaction. By the incorporation of β-D-glucose dehydrogenase as the D-glucose-utilizing auxiliary enzyme into the test system the problem of contaminating sucrase activities in commercial D-glucose oxidase

[90] F. K. Chu and F. Maley, *J. Biol. Chem.*, 1980, **255**, 6387.
[91] L. Lehle, R. E. Cohen, and C. E. Ballou, *J. Biol. Chem.*, 1979, **254**, 12 209.
[92] T. G. Villa and H. J. Phaff, *Eur. J. Appl. Microbiol. Biotechnol.*, 1980, **9**, 9.
[93] P. Babelinski, *Anal. Biochem.*, 1980, **105**, 328.

preparations has been overcome (the latter enzyme had often been used earlier in activity-staining procedures of glycoside hydrolases). Production of formazan dye is dependent on the presence of sucrose in the test system. By quantitative densitometry proportionality of dye intensity with incubation time, amount of deoxycholate extract, and amount of protein applied on to the gel were determined.

5 α-D-, β-D-, and α-L-Fucosidases

α-L-Fucosidase was one of eight glycoside hydrolases studied, and it was found to have activity levels independent of sex and age.[47] α-L-Fucosidase levels also remained unchanged in cystic fibrosis patients compared with healthy controls.[54]

Comparable α-L-fucosidase activity was found for normal and cystic fibrosis sera, 7.8 ± 3.3 and 9.8 ± 4.7 nmol min^{-1} ml^{-1}, respectively.[94] Isoelectric focusing of normal and cystic fibrosis sera α-L-fucosidase revealed similar isoelectric profiles with most activity between isoelectric points of 4.7–5.4. Kinetic analysis of normal and cystic fibrosis sera α-L-fucosidase revealed identical apparent Michaelis constants for the 4-methylumbelliferyl substrate (51 ± 6 μmol l^{-1} and 50 ± 8 μmol l^{-1}, respectively), similar pH optima curves (both have broad optima between pH 4.8 and 6.5), and identical thermostability curves at three different reincubation temperatures (37, 45, and 55 °C).

Cultured skin fibroblasts and primary amniotic fluid cell α-L-fucosidase had a double optimum of pH 5.0 and 6.0.[95] α-L-Fucosidase was largely bound as a single peak to DEAE-cellulose at pH 6.6. Sucrose density isoelectric focusing revealed up to seven components with p*I* values of 4.9, 5.2, 5.4, 5.8, 6.1, 6.5, and 7.1 with their apparent K_m values (77–500 μmol l^{-1}) being higher than that (57 μmol l^{-1}) of the unfocused enzyme. Liver, skin, fibroblasts, and amniotic fluid cell α-L-fucosidase was separated into two peaks by gel filtration. Peak one was more active and stable at low pH and more thermostable at 50 °C than peak two, and both peaks had an apparent K_m of 52 μmol l^{-1}. Apart from the different proportions of the peaks separated by gel filtration, the results for the three tissues were similar. The properties of α-L-fucosidase studied were similar for control and cystic fibrosis liver or skin fibroblasts. α-L-Fucosidase activity was found to be higher in human sera ($p < 0.001$) for patients with diabetes mellitus, hepatic cirrhosis, and gastric carcinoma than in healthy controls.[60]

Optimal assay conditions for α-L-fucosidase, the levels of activities in normal adults and children, and the stabilities of the activities on storage at −20 °C or 4 °C, are reported.[61] The levels of enzymic activity in plasma from patients with a wide range of conditions, and the possibility of using plasma hydrolase activities in the diagnosis of these conditions is discussed.

An antiserum has been raised to purified human α-L-fucosidase. Levels of cross-reaction with serum of two unrelated fucosidosis patients and normal individuals with low activity are consistent with the presence of very low

[94] J. A. Alhadeff and P. Watkins, *Clin. Chim. Acta*, 1980, **105**, 131.
[95] J. Butterworth and C. J. Guy, *Clin. Chim. Acta*, 1979, **92**, 109.

amounts of normal enzyme.[57] Similarly no cross-reacting material could be found in cultured fibroblasts from fucosidosis patients. It is deduced that in these cases there is no production of mutant enzyme in quantities comparable to normal levels. Some observations on the inter-relations of fucosidases I and II are reported.

α-L-Fucosidase was partially purified from the liver of a fucosidosis patient by column chromatography either on Agarose–ε-amino-hexanoylfucosamine or on concanavalin A–Sepharose®, despite no apparent enzymic activity in the crude liver supernatant.[96] Mixing studies indicated that the liver of the fucosidosis patient did not lack activators or contain inhibitors of α-L-fucosidase activity. The partially purified α-L-fucosidase from the liver of fucosidosis patient exhibits a 4–5-fold-increased Michaelis constant for the 4-methylumbelliferyl substrate (700–750 μm) and a greatly decreased thermostability at 55 °C compared with the normal liver enzyme. The pH-activity curve is similar to that for the normal enzyme between pH 5 and 8, but quite dissimilar in the acid region (pH 3.0–4.5): below pH 4.5 the α-L-fucosidase shows no activity, whereas the normal enzyme retains considerable activity ($\geqslant$ 50% of maximal activity). Isoelectric focusing of the α-L-fucosidase revealed one major form with p*I* 5.8 and other possible minor forms. No cross-reacting material was detected when the α-L-fucosidase was run in double-immunodiffusion experiments against the immunoglobulin-G fraction of anti-(α-L-fucosidase) antibodies, but the enzyme was immunoprecipitated by this immunoglobulin-G fraction. For at least the fucosidosis patient being studied here, all the data suggest retention of a thermolabile portion of normal α-L-fucosidase (with characteristics Michaelis constant and pH-activity curve) or production of a kinetically altered α-L-fucosidase with decreased catalytic activity but antigenic similarity to the normal enzyme.

The molecular defect responsible for I-cell disease and pseudo-Hurler polydystrophy is unknown but may result in an altered carbohydrate composition on the lysosomal hydrolases.[97] This concept was investigated by purifying α-L-fucosidase to apparent homogeneity from normal and I-cell liver samples and pseudo-Hurler polydystrophy urine samples. Gas–liquid chromatographic analysis of the sugar residues on the purified α-L-fucosidase preparations revealed an increase in the quantity of D-mannose, D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, and neuraminic acid found on the I-cell and pseudo-Hurler polydystrophy enzymes when compared to the normal liver α-L-fucosidase. The α-L-fucosidase from normal liver was composed of a major subunit with a molecular weight of 5.1×10^4 and a minor subunit of molecular weight 5.7×10^4, whereas the enzyme from pseudo-Hurler polydystrophy urine exhibited a minor subunit at $M_r = 51\,000$ and a major subunit at $M_r = 57\,000$. The heterogeneity of the isoelectric focusing profiles of the normal I-cell and pseudo-Hurler polydystrophy α-L-fucosidase was caused in part by alterations in the neuraminic acid content of the enzyme. The purified I-cell liver α-L-fucosidase displayed normal binding properties to concanavalin A, whereas the pseudo-Hurler polydystrophy urine enzyme revealed an altered interaction with this lectin. Com-

[96] J. A. Alhadeff and G. L. Andrews-Smith, *Biochem. J.*, 1980, **187**, 45.

[97] B. C. Kress, H. H. Freeze, J. K. Herd, J. A. Alhadeff, and A. L. Miller, *J. Biol. Chem.*, 1980, **255**, 955.

parisons of apparent K_m and pH optimum of the α-L-fucosidases revealed no significant differences between the normal and I-cell samples. The results indicated that the biochemical features of I-cell disease and pseudo-Hurler polydystrophy may result from an altered carbohydrate composition of these enzymes.

A comparison of the α-L-fucosidase activities in normal and chronic lymphocytic leukaemic lymphocytes has been reported.[98]

α-L-Fucosidase was found to have a particular developmental pattern from studies of post-natal development of the rat cerebellum.[73]

6 α- and β-D-Galactosidases and D-Galactolipid-oriented Galactosidases

Assay conditions have been developed for the determination of urinary β-D-glucuronidase, β-D-galactosidase, α-D-galactosidase, and β-D-2-acetamido-2-deoxyhexosidase using fluorimetric substrates (see p. 239).[49]

Under experimental conditions optimal for the assay of D-galactosyl-*N*-acylsphingosine galactohydrolase (EC 3.2.1.46) activity, homogenates of neurologically normal human brain tissue could transfer galactose from D-galactosyl ceramide, lactosyl ceramide, 4-methylumbelliferyl β-D-galactoside, or 4-nitrophenyl β-D-galactoside to [1-^{14}C]oleoyl sphingosine, but homogenates of brain tissue from patients with Krabbe's disease lacked this ability.[99] The rate of hydrolysis of ganglioside G_{M_1} and, to a lesser extent, of 4-nitrophenyl β-D-galactopyranoside by homogenates of Krabbe's brain tissue was also decreased. Activity of β-D-galactosidase (active against 4-nitrophenyl β-D-galactopyranoside) in normal brain tissue, like that of cerebroside β-D-galactosidase from the same source, was considerably more heat-stable than the activity of either β-D-galactosidase (active against the 4-methylumbelliferyl glycoside) or the predominant G_{M_1} β-D-galactosidase (EC 3.2.1.23). All four substrates were competitive inhibitors of human-brain cerebroside β-D-galactosidase. These findings confirm the ability of mammalian cerebroside β-D-galactosidase to catalyse a trans-galactosylation reaction and provide additional information on the substrate specificity of human brain cerebroside β-D-galactosidase.

α-D-Galactosidase A was purified approximately 67 000-fold from normal human liver by a procedure that included hydroxylapatite and ampholyte displacement chromatography.[100] The enzyme catalysed the hydrolysis of a fluorogenic substrate, 4-methylumbelliferyl α-D-galactopyranoside, with a K_m of 2.9 mM and a V_{max} of 28.7 μmol min^{-1} mg^{-1} at pH 4.6. Hydrolysis of this substrate was inhibited by sodium taurocholate (9.3 mM); the degree of inhibition was dependent on the pH of the reaction mixture, diminishing as the pH increased from 3.7 to 4.8. The enzyme also catalysed the hydrolysis of the glycolipids, galabiosylceramide, and globotriglycosylceramide, in the presence of sodium taurocholate. The apparent K_m values with these glycosphingolipids

[98] A. D. Crockard, J. M. Bridges, and M. H. R. Lewis, *Biochem. Soc. Trans.*, 1980, **8**, 439.
[99] T. P. Carter, D. W. Beblowski, M. H. Savage, and J. N. Kawfer, *J. Neurochem.*, 1980, **34**, 189.
[100] K. J. Dean and C. C. Sweeley, *J. Biol. Chem.*, 1979, **254**, 9994.

were 0.28 and 0.18 mM and the apparent V_{max} values were 1.09 and 1.16 μmol min^{-1} mg^{-1}, respectively. Hydrolysis of the glycolipids was optimal at pH 4.1. Hydrolysis of the related water-soluble oligosaccharides, galabiaose and globotriaose, was optimal at pH 4.5. The K_m values with these substrates were 5.5 and 4.1 mM and the V_{max} values were 15.8 and 13.8 μmol min^{-1} mg^{-1}, respectively. It was concluded that differences between the pH optimum, apparent K_m, and V_{max} values observed with glycolipid substrates and those with the water-soluble fluorogenic substrate and the oligosaccharides were caused by the electrostatic interaction of the enzyme with anionic detergent–glycolipid mixed micelles.

Lysosomal β-D-galactosidases in fibroblasts and leukocytes exhibited the same isoelectric focusing pattern as found in liver (see p. 238).[45]

Trihexosylceramide, isolated from human kidney and labelled in the terminal D-galactose position by oxidation with D-galactose oxidase and reduction with sodium borotritide, has been used to study some of the properties of human leukocyte α-D-galactosidase.[101] The enzyme was inactive in the absence of detergent. Of all the detergents tested, a crude sodium taurocholate preparation displayed the greatest activity. The optimal detergent concentration varied from 2 to 4 mg ml^{-1} depending on the protein concentration and indicating that the enzyme activity was dependent on the protein : detergent ratio. Because of its influence in regulating enzyme activity, it is essential that care must be taken to ensure that the protein : detergent ratio of all incubation mixtures is kept relatively constant whenever the diagnosis of Fabry's disease is attempted.

The properties and bile salt specificities of galactosylceramide- and lactosylceramide-β-D-galactosidase activities ('GC- and LC'-β-D-galactosidases) of human leukocytes and fibroblasts were compared and several differences observed.[102] Under the standard assay conditions the former activity was more sensitive to Zn^{2+} and Triton-X100. Glycholate and cholate were more active stimulators of the GC-β-D-galactosidase than the more frequently used taurocholate, which was the most effective stimulator of LC-β-D-galactosidase activity. It is postulated that some of the apparent differences in the properties of GC- and LC-β-D-galactosidase activities may be attributed to the different micellar properties of the lipid substrates. Experiments with fibroblasts from patients with Krabbe's disease confirmed an almost total absence of GC-β-D-galactosidase whichever bile acid was employed. Residual LC-β-D-galactosidase activity detected in these cells was much higher ranging from 13% of the lowest measured value when measured with taurocholate to approximately normal values with glycocholate. Fibroblasts obtained from patients with G_{M_1}-gangliosidosis displayed close to normal GC- and LC-β-D-galactosidase activity under the experimental conditions. The data suggest that diagnoses of Krabbe's disease should be performed with D-galactosylceramide rather than lactosylceramide as substrate.

β-D-Galactosidase showed significantly higher values in children as compared to adults when tested in serum samples using 4-methylumbelliferyl substrates.[47]

In a comparative study of eight glycoside hydrolases in serum samples from 72 cystic fibrosis parents and 34 healthy and diseased controls the levels of β-D-galactosidase were found unchanged.[54]

[101] A. Poulos and K. Beckman, *Clin. Chim. Acta*, 1978, **89**, 35.
[102] A. Poulos and K. Beckman, *Clin. Chim. Acta*, 1980, **101**, 277.

The activities of several glycosidases (including α-D-galactosidase and β-D-galactosidase) were determined in human sera from 10 normal subjects and in three groups each of 10 patients with diabetes mellitus, hepatic cirrhosis, and gastric carcinoma.[60] The results did not show significantly higher activities in the patients for α-D- and β-D-galactosidases.

α-D-Galactosidase B (α-D-2-acetamido-2-deoxygalactosidase, 2-acetamido-2-deoxy-α-D-galactoside acetamidodeoxygalactohydrolase), was purified 70 000-fold to apparent homogeneity from normal human liver.[103] The enzyme catalysed the hydrolysis of both 4-methylumbelliferyl α-D-galactopyranoside (K_m = 6.8 ± 1.3 mM; V_{max} = 18.7 ± 1.2 μmol min^{-1} mg^{-1}) and 2-nitrophenyl 2-acetamido-2-deoxy-α-D-galactopyranoside (K_m = 1.3 ± 0.1 mM; V_{max} = 60.0 ± 1.9 μmol min^{-1} mg^{-1}) Optimal hydrolysis of 4-methylumbelliferyl α-D-galactopyranoside occurred at pH 4.8, and the hydrolysis of 2-nitrophenyl 2-acetamido-2-deoxy-α-D-galactopyranoside was optimal at pH 4.3. α-D-Galactosidase B also catalysed the hydrolysis of the water-soluble oligosaccharides derived from globotriglycosylceramide and globopentaglycosylceramide (Forssman antigen). The pH optimum for the hydrolysis of globotriaose corresponded to that of 4-methylumbelliferyl α-D-galactopyranoside (pH 4.8), the K_m was 9.1 ± 1.1 mM, and the V_{max} was 4.1 ± 1.1 μmol min^{-1} mg^{-1} The pH optimum for the hydrolysis of globopentaose was approximately the same as that of the corresponding artificial substrate, 2-nitrophenyl 2-acetamido-2-deoxy-α-D-galactopyranoside (pH 4.4), the K_m was 3.7 ± 0.5 mM, and the V_{max} was 9.2 ± 0.5 μmol min^{-1} mg^{-1}. Hydrolysis of the glycolipid substrates, globo-tri- and -penta-glycosylceramide, required sodium taurocholate, at 9.3 and 7.4 mM, respectively, to solubilize the glycolipids in the aqueous reaction mixtures. The kinetics for the hydrolysis of these glycolipids were quite different from those of the water-soluble substrates. The failure of this enzyme to prevent the accumulation of globotriglycosylceramide in Fabry's disease and its activity with substrates containing 2-acetamido-2-deoxy-α-D-galactopyranosyl residues suggest that its normal role *in vivo* is the hydrolysis of glycoconjugates containing terminal 2-acetamido-2-deoxy-α-D-galactopyranosyl groups, such as Forssman antigen and blood-group A-active glycolipids and glycoproteins.

Optimal assay conditions are described for plasma α- and β-D-galactosidase, and the levels of enzymic activity in plasma in patients recorded.[61]

G_{M_1}-Ganglioside hydrolysis of leukocytes and fibroblasts, tissues easily obtainable from patients, was investigated using ^{3}H-labelled G_{M_1} was found to be at least as active as that reported for any other tissue.[104] Sodium taurocholate was required for the reaction, the crude bile salt at an optimum concentration of 0.4% producing twice as much activity as pure taurocholate at its optimum concentration of 0.8%. Leukocyte G_{M_1}-ganglioside β-D-galactosidase and 4-methylumbelliferyl β-D-galactopyranoside cleaving activities were similar, 134.5 ± 23.3 and 179.8 ± 25.4 nmol h^{-1} mg^{-1} protein, respectively. In cultured skin fibroblasts and amniotic fluid cells these enzyme activities were four to five times higher. Homozygotes for G_{M_1}-gangliosidosis showed negligible activity, whereas in

[103] K. J. Dean and C. C. Sweeley, *J. Biol. Chem.*, 1979, **254**, 10 001.
[104] S. Raghavan, A. Gajewski, and E. H. Kolodny, *Clin. Chim. Acta*, 1977, **81**, 47.

heterozygotes the leukocyte G_{M_1}-cleaving activity was reduced to one-third of control values. In leukocytes from patients with four other sphingolipid storage diseases the activity was either normal (Krabbe's, Tay–Sachs, metachromatic leukodystrophy) or increased (adult Gaucher's).

'Acid' β-D-galactosidase activity from autopsied I-cell disease brain and spleen tissues was 28% and 35%, respectively, or normal activity.[105] Acid β-D-galactosidase from two I-cell disease brains demonstrated a five-fold increase over normal for the proportion of enzyme activity which did not adsorb to concanavalin A–Sepharose®, whereas acid β-D-galactosidase from two I-cell disease spleens demonstrated a 21–35-fold increase in the proportion of unadsorbed enzyme activity. Normal and I-cell disease acid β-D-galactosidase present in crude brain and spleen supernatant fluids and in preparations partially purified on concanavalin A–Sepharose® had similar apparent K_m values with respect to 4-methylumbelliferyl β-D-galactopyranoside and G_{M_1}-ganglioside. Isoelectric focusing profiles of normal and I-cell disease acid β-D-galactosidase from crude brain and spleen's supernatant fluids and partially purified preparations were similar. Neuraminidase treatment and subsequent isoelectric focusing of the partially purified normal and I-cell disease enzyme preparations from brain and spleen revealed increases in the proportion of I-cell β-D-galactosidases found at neutral pH values, suggesting that the electrophoretic variations observed for the I-cell enzymes may not be attributed solely to changes in neuraminic acid composition.

Except for β-D-galactosidase activity, the activity of other brain lysosomal enzymes of a patient with I-cell disease were within the normal range. This finding was similar to that for Hurler's syndrome.[106] Only β-D-galactosidase activity was reduced to less than 10% of normal in the patient's brain. To examine the possible metabolic significance of β-D-galactosidase deficiency in I-cell disease, the physical characteristics of this enzyme, isolated from tissues from I-cell, Hurler and control patients, were compared using isoelectric focusing, concanavalin A–Sepharose and Sephadex® G-150 chromatography. The isoelectric point and the binding-affinity of I-cell β-D-galactosidase with concanavalin A–Sepharose was comparable to normal. However, the isoenzyme patterns of brain and liver I-cell β-D-galactosidase with Sephadex® G-150 gel filtration revealed decreased acid β-D-galactosidase. Effects of the addition of sodium chloride on each fraction of β-D-galactosidase isoenzymes isolated from I-cell tissues were markedly different from controls, whereas the pH optimum of these enzymes were similar to normal. These enzyme characteristics in I-cell tissues were different from normal and Hurler's syndrome. These findings suggest that β-D-galactosidase deficiency in I-cell disease is a more specific phenomenon rather than secondary inhibition as found in genetic hyperglycosaminoglycanuria (the mucopolysaccharidoses) and thus may have an important role for the pathogenesis of brain damage and disease occurrence.

[105] A. L. Miller, P. Levitt, H. Ingraham, J. Converse, and L. Lewis, *J. Neurochem.*, 1979, **32**, 1479.

[106] Y. Eto, M. Owada, T. Kitagawa, Y. Kokubin, and O. M. Rennert, *J. Neurochem.*, 1979, **32**, 397.

β-D-Galactocerebrosidase activity of brain and liver preparations from normal individuals and patients with Krabbe's disease (globoid-cell leukodystrophy) have been separated by gel filtration into four different molecular-weight forms.[107] The apparent molecular weights were $7.60 \pm 0.34 \times 10^5$ and $1.21 \pm 0.1 \times 10^5$ for the high- and low-molecular-weight forms (peaks I and IV, respectively) and $4.99 \pm 0.22 \times 10^5$ (mean ± S.D.) and $2.56 \pm 0.12 \times 10^5$ for the intermediate forms (peaks II and III, respectively). On examination by sodium dodecyl sulphate–polyacrylamide gel electrophoresis, the high- and low-molecular-weight forms revealed a single protein band with a similar mobility corresponding to a molecular weight of about 1.25×10^5. Antigenic identity was demonstrated between the various molecular-weight forms of the normal and the mutant β-D-galactocerebrosidases by using antisera against either the high- or the low-molecular-weight enzymes. The high-molecular-weight form of β-D-galactocerebrosidase was found to possess higher specific activity toward natural substrates when compared with the low-molecular-weight form. It is suggested that the high-molecular-weight enzyme is the active form *in vivo* and an aggregation process that proceeds from a monomer (mol. wt. approx. 2.50×10^5) and from the dimer to either a tetramer (mol. wt. approx. 5.00×10^5) or a hexamer (mol. wt. approx. 7.50×10^5) takes place in normal as well as in Krabbe's-disease tissues.

A chromogenic substrate, 2-hexadecanoylamino-4-nitrophenyl β-D-galactopyranoside, has recently been described for the diagnosis of Krabbe's disease.[108] Hydrolysis of this substrate by extracts of cultured cells and tissues was compared with the activities of lactocerebrosidase I and non-specific β-D-galactosidase. Under appropriate conditions, hydrolysis of the chromogenic analogue was markedly reduced in extracts of cultured amniotic fluid cells and skin fibroblasts derived from cases of Krabbe's disease. Activity was also markedly deficient in extracts of Krabbe's brain, although only a partial reduction was measured in liver extracts. Generally activities were higher in tissues of foetal origin. Unfortunately, the new analogue proved less specific and less sensitive than the natural substrates used to diagnose Krabbe's disease. Consequently, the analogue does not provide a satisfactory alternative substrate for the prenatal diagnosis of Krabbe's disease.

The elution of urinary β-D-2-acetamido-2-deoxyglucosidase and α-D-galactosidase was the same in mucolipidosis II patients as in normal controls (see p. 244).[64]

The enzyme properties of human leukocyte β-D-galactosidase and the effects of macromolecular compounds on its activity were studied in detail.[109] It was demonstrated that various macromolecular compounds markedly influence the activity of β-D-galactosidase. It was stated that these phenomena should be taken into account when considering the biochemical abnormalities of tissues of patients with genetic hyperglycosaminoglycanuria (mucopolysaccharidoses) and mucoplipidoses.

[107] Y. Ben-Yoseph, M. Hungerford, and H. L. Nadler, *Biochem. J.*, 1980, **189**, 9.
[108] G. T. N. Besley and A. D. Bain, *Clin. Chim. Acta*, 1978, **88**, 229.
[109] K. Kiguchi, Y. Eto, and K. Aoki, *Clin. Chim. Acta*, 1978, **85**, 151.

The properties of two forms of purified β-D-galactosidases from monkey brains have been studied.[110]

Bovine liver β-D-glucuronidase and testicular β-D-galactosidase were assimilated by generalized gangliosidosis fibroblasts at respective rates of 90 and 464 times the rate of assimilation of horseradish peroxidase.[111] Assimilation of either of the two enzymes by the fibroblasts was saturable, suggesting the participation of receptor-mediated adsorptive endocytosis for internalization. The rate of assimilation of either enzyme was not affected by high levels of the other enzyme, suggesting that distinct receptors for each enzyme occur on the fibroblasts cell surface. Furthermore, although assimilation of β-D-galactosidase was inhibited by D-mannose, methyl D-mannopyranosides, 2-α-D-mannopyranosyl D-mannose, and D-mannose 6-phosphate, these compounds did not detectably inhibit the assimilation of β-D-glucuronidase. These results suggest that testicular β-D-galactosidase was assimilated by the well established phosphomannosyl recognition system. However, liver β-D-glucuronidase was assimilated by a distinct, non-competing, and as yet undefined, recognition system.

β-D-Galactosidase was one of five glycoside hydrolases studied during the postnatal development of the rat cerebellum and found to have a particular development pattern.[73] Transient decreases in the specific activity of β-D-galactosidase could be correlated with the phase of massive cell multiplication.

Effects of the pesticides phosalone and lindane on the activity of some dipeptidases, and on disaccharidases, namely β-D-galactosidase, α-D-glucosidase (maltase), β-D-glucosidase (cellobiose, palatinase), sucrose α-D-glucohydrolase (sucrase) and αα-trehalase in rat intestinal mucosa have been studied.[112]

β-D-Galactosidase has been purified from macrophages of thioglycollate-treated mice using concanavalin A chromatography and immunoprecipitation.[113] The apparent molecular weight of the β-D-galactosidase subunit, as determined by sodium dodecylsulphate–polyacrylamide gel electrophoresis, changed during a long-term pulse-chase experiment. Following a 1-h pulse with L-[^{3}H]leucine, radiolabel was present exclusively in an $M_r = 82\,000$ form. However, after a 3-h chase in medium containing unlabelled leucine, most label migrated at $M_r = 63\,000$, and at 24 h, all label was in the $M_r = 63\,000$ form. Electrophoresis of peptides produced by cyanogen bromide cleavage of immunoprecipitates demonstrated structural similarities between precursor and mature forms. A mutation in the mouse, which is known to depress the rate of synthesis of β-D-galactosidase in many cell types, proportionately decreased incorporation of [^{3}H]leucine into both the $M_r = 82\,000$ and 63 000 forms. Therefore, by kinetic, structural, and genetic evidence, the large molecular weight β-D-galactosidase is a precursor of mature macrophage enzyme. No precursor of the $M_r = 75\,000$ subunit of β-D-glucuronidase was detected.

[110] T. Alam and A. S. Balasubramanian, *Indian J. Biochem. Biophys.*, 1980, **17**, 176.
[111] V. Hieber, J. Distler, R. Myerowitz, R. D. Schmickel, and G. W. Jourdian, *J. Clin. Invest.*, 1980, **65**, 879.
[112] N. Nedkova-Bratanova, E. Ivanov, G. Savov, Z. Michalova, and S. Petrova, *Enzyme*, 1979, **24**, 281.
[113] M. D. Skudlarek and R. T. Swank, *J. Biol. Chem.*, 1979, **254**, 9939.

In a rapid and relatively simple procedure described for kinetic measurement of low activities of enzymes hydrolysing phenol conjugates, phenol generated by the enzyme reactions in the pH-optimum range 5.0–9.5 is continuously monitored by an oxygen-membrane electrode of the Clark type.[114] The surface of the electrode exposed to the solution is coated by a layer of chemically insolubilized polyphenol oxidase. The current response of the electrode, indicating the uptake of oxygen (diffusing from the solution together with phenol into the enzyme membrane) is proportional to the enzyme concentration, according to the kind of hydrolase in the range from 0.2 to at least 2–9 ncat. The dependences of the initial rate of enzyme reaction on enzyme and substrate concentration and the K_m and V_{max} values at optimal pH were measured with emulsin (β-D-glucosidase) and β-D-glucuronidase. The kinetic measurements showed that sweet almond emulsin contains two enzymes differing in their affinity toward phenyl β-D-galactopyranoside (β-D-galactosidase activity).

The intracellular distribution of α-D-galactosidase in leaves of *Cucurbita pepo* has been reported upon.[115]

Four legume species (four genera) were examined and found to contain haemagglutinins with properties similar to those which have previously been described for the enzymic haemagglutinin in *Vigna radiata*.[116] Examination of extracts by gel filtration and ion-exchange chromatography showed that an α-D-galactosidase activity exactly co-purified with a haemagglutinin activity in each of the four species. The α-D-galactosidase activities in the four species were virtually identical to each other with respect to substrate and inhibitor specificity as well as kinetic behaviour. Additionally the haemagglutinin activities in all four species displayed very similar carbohydrate specificities. The inhibitor specificities displayed by the enzymes and the haemagglutinins were qualitatively and quantitatively very nearly identical to each other. The remarkable similarities of these proteins, both to each other and to the previously described *Vigna* enzymic haemagglutinin, suggest that each of these plants may contain a homologue from a specific class of enzymic haemagglutinin.

Twenty different legume species (20 genera) were examined for α-D-galactosidase and haemagglutinin activities.[117] Although all of the species contained enzyme activity, only 13 of 20 contained haemagglutinin activities and none displayed a haemagglutinin activity comparable to the previously described α-D-galactosidase-haemagglutinins. The α-D-galactosidase activities in the 20 species possessed remarkably similar kinetic behaviour and carbohydrate specificities. All were inhibited by D-galactose, D-xylose, and inositol (very similar K_i values from plant to plant) and had very similar K_m values for the substrate, 4-nitrophenyl α-D-galactopyranoside. Gel-filtration analysis of extracts from nine species suggests that legume α-D-galactosidase activities may frequently reside in two molecular weight forms. However, all these species contained a large molecular weight enzyme activity with a size comparable to the α-D-galacto-

[114] L. Macholan, *Collect. Czech. Chem. Commun.*, 1979, **44**, 3033.
[115] B. Thomas and J. A. Webb, *Can. J. Bot.*, 1979, **57**, 1904.
[116] C. N. Hankins, J. I. Kindinger, and L. M. Shannon, *Plant Physiol.*, 1980, **65**, 618.
[117] C. N. Hankins, J. I. Kindinger, and L. M. Shannon, *Plant Physiol.*, 1980, **66**, 375.

sidase-haemagglutinins. Immunochemical studies reveal that the α-D-galactosidases in these plants are immunologically related to an α-D-galactosidase-haemagglutinin and, therefore, are related to one another. These studies suggest that each of the legume species studied (and perhaps all members of this plant family) contain a homologue from a specific class of α-D-galactosidase. Although the previously described α-D-galactosidase-haemagglutinins appear to be members from this enzyme class, these proteins most frequently occur as forms devoid of haemagglutinin activity.

The purification of enzymes was investigated by high-speed gel filtration on TSK-GEL G3000SWG columns packed with porous silica gel deactivated by chemically bonded hydrophilic compounds.[118] Crude β-D-galactosidase from bacterial cells was purified *ca.* 15-fold in a single gel filtration. The enzyme was eluted within an hour from the column and the recoveries of enzymatic activity was almost 100% although the operation was carried out at room temperature (22 °C). Samples up to 100 mg could be applied to the column without loss of separation efficiency.

Raffinose was converted enzymatically in a hollow-fibre reactor to melibiose, sucrose, D-galactose, D-glucose, and D-fructose.[86] The enzymes were a crude extract of α-D-galactosidase and β-D-fructofuranosidase produced by *Aspergillus awamori* on a solid substrate, wheat bran.

The isoenzymes of α-D-galactosidase from *Bacillus stearothermophilus* have been investigated.[119]

Several lysosomal glycosidase activities were examined *in vitro* during heat-induced germination of *Dictostelium discoideum* spores and were found not to be co-ordinately controlled.[120] The level of β-D-glucosidase activity increased significantly during the emergence stage of germination. Both α-D-glucosidase and β-D-2-acetamido-2-deoxyglucosidase activities remained relatively constant until post-emergence, when they increased slightly; α-D-mannosidase activity decreased during all stages of germination. The activity of β-D-galactosidase increased slightly during spore swelling, fell below the level initially found in spores at zero time, and increased slightly during post-emergence. The expression of all these enzyme activities, except the increase in β-D-galactosidase, appeared to require protein synthesis. Spores in the lag phase of germination that were exposed to severe environmental stress were deactivated and exhibited reduced levels of α-D-glucosidase, β-D-glucosidase, and β-D-2-acetamido-2-deoxyglucosidase activities. Prolonged heat-activation treatment reduced the levels of lysosomal glycosidase activities in post-activated spores but did not change the subsequent enzyme patterns during the spore-swelling and emergence stages of germination.

Effect of carbon sources on the rates of adenosine 3′,5′-phosphate synthesis, excretion, and degradation, and the ability to produce β-D-galactosidase in *Escherichia coli* have been studied.[121] Using a method developed for determining

[118] Y. Kato, K. Komiya, Y. Sawada, M. Sasaki, and T. Hasimoto, *J. Chromatogr.*, 1980, **190**, 305.
[119] D. M. Pederson and R. E. Goodman, *Can. J. Microbiol.*, 1980, **26**, 978.
[120] L. S. Tisa and D. A. Cotter, *J. Bacteriol.*, 1980, **141**, 436.
[121] A. D. E. Fraser and H. Yamazaki, *Can. J. Biochem.*, 1979, **57**, 1073.

the cellular efficiency of enzyme production (termed 'cellular' rate), the relationship between adenosine 3′,5′-phosphate pool size and the rate of β-D-galactosidase production has been re-examined. Although there exists an overall trend of increasing rate of β-D-galactosidase production with increasing adenosine 3′,5′-phosphate pool size, large variations in the rates of β-D-galactosidase production are seen even under culture conditions, which yield similar adenosine 3′,5′-phosphate pool sizes. This suggests that the intracellular level of adenosine 3′,5′-phosphate cannot be the unique regulator of β-D-galactosidase production.

Continuous culture has been used to study the expression of β-D-galactosidase in *Escherichia coli*.[122] In these experiments the cultures were grown on carbon-limited media in which half of the available carbon was supplied as glycerol, D-glucose, or D-glucose 6-phosphate, and the other half as lactose. Lactose itself provided the sole source of inducer for the *lac* operon. The steady-state specific activity of the enzyme passed through a maximal value as a function dilution rate. The differential rate of β-D-galactosidase synthesis was an apparently linear function of the rate of lactose utilization per milligram protein regardless of the composition of the input medium. That is, it is independent of the rate of metabolism of substrates other than lactose that are concurrently being utilized and the enzyme level appears to be matched to the metabolic requirement for it. If this relationship is taken to indicate the existence of a fundamental control mechanism, it may represent a form of attenuation of the rate of β-D-galactosidase synthesis that is independent of adenosine 3′,5′-phosphate levels.

Plasmid-encoded α-D-galactosidase served as a marker enzyme for the recognition and comparison of raffinose (Raf) plasmids present in strains *Escherichia coli*.[123] Immunochemical relationships were established among raffinose plasmids of 39 independent isolates from man and domestic animals (from three continents) by using α-D-galactosidase antiserum. Immunodiffusion revealed three serological subclasses of α-D-galactosidase, which are correlated with the biological and geographical origin of the host strains. It is concluded that the *raf* determinants of all raffinose plasmids tested have evolved from a common ancestor.

Escherichia coli strains have been constructed in which *lacZ*, the gene for the cytoplasmic enzyme β-D-galactosidase, is fused to *lamB*, the gene for an outer membrane protein.[124] One such strain produced a β-D-galactosidase that remained cytoplasmic, even though it possessed the complete signal sequence of the *lamB* protein precursor at the amino-terminal end. Thus it was concluded that a signal sequence is not sufficient to lead β-D-galactosidase out of the cytoplasm.

The DNA-dependent *in vitro* synthesis of *Escherichia coli* β-D-galactosidase rquires the presence of a soluble protein referred to as L factor.[125] Comparison of physical, immunological, and biological properties shows that L factor is the

[122] S. S. Smith and D. E. Atkinson, *Arch. Biochem. Biophys.*, 1980, **202**, 573.
[123] K. Schmid, S. Ritchewald, and R. Schmitt, *J. Gen. Microbiol.*, 1979, **114**, 477.
[124] F. Moreno, A. V. Fowler, M. Hall, T. J. Silhavy, I. Zabin, and M. Schwartz, *Nature (London)*, 1980, **286**, 356.
[125] J. Greenblatt, J. Li, S. Adhya, D. I. Friedman, L. S. Baron, B. Redfield, H. F. Kung, and H. Weissbach, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 1991.

product of the *E. coli nusA* gene. The *nusA* gene product is known to interact with bacteriophage N gene protein and to prevent premature termination of transcription from the early λ promoters. Results suggest that premature transcription termination in the *lac* operon of *E. coli* may also be overcome by *nusA* protein.

A previous study described the purification of a factor, called L factor, that was required for the DNA-directed *in vitro* synthesis of β-D-galactosidase (H. F. Kung, C. Spears, and H. Weissbach, *J. Biol. Chem.*, 1975, **250**, 1556).[126] A further report characterizes the effect of L factor on β-D-galactosidase synthesis. Although L factor stimulated β-D-galactosidase synthesis using DNA from λp*lac*5, λh80dtrp-*lac*, λh80dtrp-*lac*, λh80d-*lac*p^5, or λh80d-*lac*UV5 phage, the synthesis of most other proteins directed by the DNA templates was not affected. The requirement of L factor for β-D-galactosidase synthesis was related to the amount of RNA polymerase present in the system. It was estimated that approximately 2 mol of L factor was required for each mole of RNA polymerase present. In addition, an interaction between L factor and RNA polymerase has been obtained by gel filtration. Although L factor resulted in a 5- to 15-fold stimulation of β-D-galactosidase synthesis, the synthesis of the α-peptide directed by pmc3 plasmid DNA was stimulated less than two-fold by the addition of L factor. These results suggest that the mode of action of L factor is mediated by an interaction with RNA polymerase, and that L factor may regulate the synthesis of β-D-galactosidase by affecting transcription termination.

Three hypotheses to explain the amino-acid composition of proteins are inconsistent ($P \simeq 10^{-9}$) with the experimental data for β-D-galactosidase from *Escherichia coli*.[127] The exceptional length of this protein, 1021 residues, permits rigorous tests of these hypotheses without complication from statistical artifacts. Either this protein is not at compositional equilibrium, which is unlikely from knowledge about other proteins, or the evolution of this protein and its coding gene have not been selectively neutral. However, the composition of approximately 60% of the molecule is consistent with either a selectively neutral or non-neutral evolutionary process.

Some 4-deoxy-analogues of methyl β-D-galactopyranoside, particularly the 4-amino- and 4-fluoro-derivatives, have been used to assess the influence of changes in the substituent at C-4 of D-galactose on the substrate specificity of *Escherichia coli* β-D-galactosidase.[128] As a complement to the study, analogous glycosides, methyl, methyl 4-amino-4-deoxy-, and methyl 4-deoxy-4-fluoro-α-D-galactopyranosides, in the α-D-series have been synthesized and tested with *Aspergillus fumigatus* α-D-galactosidase. The effect of replacing the ring-oxygen atom by a sulphur atom was also examined, using methyl 5-thio-β- and -α-D-galactopyranosides.

The interaction of *Escherichia coli* β-D-galactosidase with D-galactal, a 'transition-state analogue', is strongly dependent upon the presence of Mg^{2+} ions.[129]

[126] H. F. Kung and H. Weissbach, *Arch. Biochem. Biophys.*, 1980, **201**, 544.
[127] R. Holmquist, *Science*, 1979, **203**, 1012.
[128] J. E. Nam Shin, A. Maradufu, J. Marion, and A. S. Perlin, *Carbohydr. Res.*, 1980, **84**, 328.
[129] O. M. Viratelle and J. M. Yon, *Biochemistry*, 1980, **19**, 4143.

In the presence of 1 mM Mg^{2+}, D-galactal interacts with β-D-galactosidase with a K_i value of 5 μM. The binding of D-galactal can be analysed as a single exponential process occurring with a rate constant of 4.5×10^2 M^{-1} s^{-1}. This low value, along with spectral difference evidence, indicates the existence of an isomerization step, probably occurring after the formation of the Michaelis complex. The reappearance of free enzyme was tested by recording the appearance of 2-nitrophenol during 2-nitrophenyl β-D-galactopyranoside hydrolysis. It occurs as a single-exponential phenomenon ($k = 2.5 \times 10^{-3}$ s^{-1}) and can be accounted for mainly by the hydration of the enzyme–D-galactal complex. In the absence of magnesium, D-galactal exhibits an increase of the K_i value by three orders of magnitude. The effect arises from a large decrease in the binding rate, which precludes the study of binding kinetics. The hydration of the D-galactal–enzyme complex occurs in a time range similar to the one observed in the presence of Mg^{2+} ions. But the reappearance of enzyme activity, as recorded by the appearance of 2-nitrophenol, is no longer a simple process: following an activation process, a decrease of the enzymatic activity is observed. By quantitative analysis, it is shown that the enzyme exhibits temporarily a higher activity than it usually does in the absence of magnesium. The rate of the inactivation step is very similar to the one observed when Mg-activated enzyme isomerizes into Mg-free enzyme, suggesting that D-galactal induces an active conformation state similar to the one induced in the presence of Mg^{2+} ions. These data are discussed in the light of the known positive influence of Mg^{2+} ions upon an isomerization step during enzyme catalysis.

Klebsiella sp. strain CT-200 lacks both its plasmid-borne *lac* operon, which specifies β-D-galactosidase I, and its chromosomal *lac* operon, which specifies β-D-galactosidase II, but it expresses a gene for a third β-D-galactosidase, β-D-galactosidase III, constitutively.[130] CT-200 was examined to determine whether there was a β-D-galactoside permease associated with the β-D-galactosidase III gene. The failure of CT-200 to transport thiomethyl β-D-galactopyranoside, 2-nitrophenyl β-D-galactopyranoside, phenyl β-D-galactoside, lactulose, or D-galactosyl-L-arabinose was taken as evidence that β-D-galactoside permease is not part of a β-D-galactosidase III operon. Optimal assay conditions for β-D-galactosidase III, whose activity was used as a measure of β-D-galactoside transport, are reported here, as are an improved purification method and some physical and catalytic properties of the enzyme not previously reported.

The effect of temperature and pH on the kinetic behaviour of α-D-galactosidase of *Mortierella vinacea* was investigated on the hydrolysis of 4-nitrophenyl α-D-galactopyranoside.[131] A very unusual kinetic behaviour was observed for soluble α-D-galactosidase, *i.e.* substrate inhibition diminished gradually with increasing temperature or near the neutral pH range, and the kinetics approached the ordinary Michaelis–Menten type. On the other hand, with decreasing temperature or in acidic pH range, substrate inhibition was accelerated. Therefore, Arrhenius plots based on the initial reaction rate did not give straight lines. Furthermore, the slope in the Arrhenius plot changed with substrate concentration, which would make the determination of a characteristic value using con-

[130] B. G. Hall, *J. Bacteriol.*, 1980, **142**, 433.
[131] H. Kobayashi and H. Suzuki, *Biotechnol. Bioeng.*, 1980, **22**, 505.

ventional methods meaningless. However, the Arrhenius plots of individual kinetic parameters in the rate equation resulted in straight lines in the temperature range 15 to 50 °C. From this, the drastic change in kinetic behaviour could be explained in connection with the temperature and pH dependence of kinetic parameters in the model. For mould pellets (whole-cell enzyme), however, the influence of temperature and pH was less apparent than that of soluble enzyme because of the limitation in intraparticle diffusion. By using the rate equation that was determined for soluble enzyme and the theoretically derived effectiveness factor, the overall reaction rate for mould pellets at various temperatures and pHs could be predicted to some extent.

Synthesis of β-D-galactosidase by *Streptomyces violaceus* was induced by D-galactose and L-arabinose, and to a lesser extent by lactose, D-arabinose, and methyl β-D-galactopyranoside.[132] The synthesis of the enzyme was linear and started to increase 2–3 h after induction by D-galactose, reaching a maximum after 5–7 h. The highest level of specific activity was observed in 2% D-galactose, with an increase of 45 times over the basal level in glycerol. Isopropyl β-D-thiogalactopyranoside and methyl β-D-thiogalactopyranoside inhibited induction by D-galactose, but did not influence enzymatic activity. Cellular extracts hydrolysed 2-nitrophenyl β-D-galactopyranoside, but did not significantly hydrolyse lactose, melibiose, 4-nitrophenyl α-D-galactopyranoside, 4-nitrophenyl β-D-fucopyranoside, or 4-nitrophenyl β-D-glucopyranoside. Rifampicin and chloramphenicol inhibited β-D-galactosidase synthesis in non-preinduced and preinduced cells. The inhibition of chloramphenicol was reversible.

An active immobilized form of β-D-galactosidase has been produced by interaction of the enzyme with DEAE-cellulose.[133]

7 α- and β-D-Glucosidases and D-Glucolipid-oriented Glucosidases

A continuous optical method for the assay of D-glucose-releasing hydrolases including α-D-glucosidase, αα-trehalase, and β-D-fructofuranosidase has been reported (see p. 247).[82]

The effects of organic solvents, particularly dimethylsulphoxide, on the β-D-glucosidase-enhanced hydrolysis of genistin has been examined.[134]

Isoenzymes of α-D-glucosidases from various human organs and body fluids from foetuses and adults were separated by ion-exchange chromatography and gel filtration.[135] A minicolumn was used for the DEAE-cellulose column chromatography of extracts from tissues as well as cultivated cells of skin fibroblasts and amniotic fluid. The enzyme activity in the eluates was measured by the use of a methylumbelliferyl derivative as substrate and a very sensitive microscope fluorimeter. In most tissue samples α-D-glucosidase was eluted mainly as a single peak when monitored at acid pH and as two peaks when the activity was measured at neutral pH in both columns. Another small peak

[132] J. Sanchez and C. Hardisson, *Can. J. Microbiol.*, 1979, **25**, 833.
[133] M. W. Griffiths and D. D. Muir, *J. Sci. Food Agric.*, 1980, **31**, 397.
[134] M. Iio, Y. Yamamoto, and N. Ohta, *J. Agric. Chem. Soc. (Jpn.)*, 1978, **52**, 493.
[135] Y. S. Shin-Buehring, M. Drefers, H. Kroenner, M. Osang, and J. Schaub, *Clin. Chim. Acta*, 1978, **89**, 393.

representing α-D-glucosidase was found in fresh extracts of cultured cells on DEAE-cellulose columns. Neutral α-D-glucosidase especially in fibroblasts was extremely sensitive to storage at −20 °C. DEAE-cellulose column chromatography of plasma and amniotic fluid showed similar elution patterns of α-D-glucosidase. Differences were noticed in the elution pattern of urine from infants and adults. The tissue distribution and the different characteristics of the enzyme in samples of various origins and ages were discussed.

Some molcular properties of the purified 'neutral' α-D-glucosidase from human kidney were studied.[136] The enzyme is a glycoprotein with high molecular weight (315 000–352 000 according to the method used). Its sedimentation coefficient is 12.9 S and it exhibits at least three peaks of activity in isoelectric focusing experiments. This heterogeneity appears to be related to neuraminic acid residues from the carbohydrate moiety. The effect of an anti-(human renal α-D-glucosidase) antiserum on human intestinal maltases was studied in immunodiffusion experiments. An identity pattern was observed between renal neutral α-D-glucosidase and intestinal glucoamylase. No precipitation occurred with intestinal sucrase. Renal neutral α-D-glucosidase and intestinal glucoamylase were both completely precipitated by the antiserum, their 'maltase' activity being only slightly inhibited in the antigen–antibody complex. From their molecular and immunological properties a large homology appears between human renal α-D-glucosidase and intestinal glucoamylase.

A paper reports studies on the β-D-glucosidase activity in human placenta.[137]

The activity of α-D-glucosidase was assayed in serum samples using the 4-methylumbelliferyl substrates and it was found that it does not change in relation to sex or age.[47]

The biosynthesis of three lysosomal enzymes, β-D-2-acetamido-2-deoxyhexosidase, α-D-glucosidase, and cathepsin D, was studied in intact human diploid skin fibroblasts.[51] α-D-Glucosidase was synthesized as a precursor of 95 000 molecular weight, and processed through several intermediates to a mixture of chains (major components 79 000 and 76 000) that were similar, although not identical, with the major components of the placental enzyme. None of these chains were seen in a cell line from a patient with genetic α-D-glucosidase deficiency disease.

In normal fibroblasts, radioactive phosphate was incorporated into β-D-2-acetamido-2-deoxyhexosidase, cathepsin D, and α-D-glucosidase (see p. 243).[62]

In a study of eight glycosidases in serum samples from 72 cystic fibrosis patients, 85 cystic fibrosis parents, and 34 healthy and diseased controls, significant elevations of mean α-D-glucosidase levels were found in cystic fibrosis patients.[54] All other D-glucosidases did not show any significant change. Mean α-D-glucosidase levels in obligate heterozygotes were the same as in control individuals. Mean α-D-glucosidase levels in obligate heterozygotes were the same as in control individuals. Moreover, α-D-glucosidase levels in cystic fibrosis patients correlated with the degree of clinical impairment as measured by the Schwachman score.

[136] G. De Burlet, C. Vannier, J. Giudicelli, and P. Sudaka, *Biochimie*, 1979, **61**, 1177.
[137] R. S. Labow, D. G. Williamson, and D. S. Layne, *Can. J. Biochem.*, 1980, **58**, 23.

Optimal assay conditions are described for plasma α-D-glucosidase, β-D-2-acetamido-2-deoxyglucosidase, α-D-2-acetamido-2-deoxyglucosidase among others (see p. 243).[61]

Gaucher's disease is an inborn error of metabolism in which D-glucocerebroside accumulates in cells of the reticuloendothelial system owing to a marked deficiency in the activity of lysosomal D-glucocerebrosidase.[138] Five different published fluorometric assay procedures that utilize 4-methylumbelliferyl β-D-glucopyranoside as the substrate to determine β-D-glucosidase activity in fibroblasts, leukocytes, and liver from patients with heterozygote carriers of Gaucher's disease were compared with the results obtained using a D-glucocerebrosidase assay that employed authentic radiolabelled D-glucocerebroside as the substrate. When cultured skin fibroblasts were used as a source of enzyme, fluorometric β-D-glucosidase assays performed either at pH 4–4.05 in the absence of sodium taurocholate or at pH 5.4–5.5 in the presence of the bile salt, all proved effective in confirming the diagnosis of Gaucher's disease and in identifying heterozygous carriers of the disease. The fluorimetric β-D-glucosidase assay procedures that were most effective in evaluating relative D-glucocerebrosidase activity in leukocytes from patients and carriers of Gaucher's disease were those that included sodium taurocholate in the incubation medium. When human liver biopsy served as the source of enzyme, only the D-glucocerebrosidase assay employing authentic D-glucocerebroside substrate proved effective in confirming the diagnosis of Gaucher's disease: fluorometric assays performed at pH 4 or pH 5.4–5.5 in the presence or absence of the bile salt sodium taurocholate do not always identify patients with Gaucher's disease.

Gaucher's disease is a group of lipid storage diseases in which the glycosphingolipid D-glucocerebroside accumulates in tissues because of deficiency of the enzyme D-glucocerebrosidase.[139] Radioactively labelled glucocerebroside and the artificial fluorogenic substrate 4-methylumbelliferyl β-D-glucopyranoside are commonly used for its diagnosis. The use of a new chromogenic substrate, 2-hexadecanoylamino-4-nitrophenyl β-D-glucopyranoside for enzyme assay in cultured skin fibroblasts was studied. The amount of reaction product, 2-hexadecanoylamino-4-nitrophenol, increase linearly with incubation time for at least 4 hours and was proportional to the amount of fibroblast protein added up to 150 μg per incubation. The pH optimum was 4.8. The K_m was 0.19 mmol l^{-1}. The mean activity of control cultured skin fibroblasts was 22.9 ± 5.4 nmol of product formed per mg fibroblast protein per hour under standard conditions. Cultured skin fibroblasts from patients with adult non-neuropathic Gaucher's disease had reduced activity, 6.4 ± 2.4 nmol mg^{-1} h^{-1} or 28% of control activity. This compared well with mean enzyme activity in the same patients determined using the natural substrate, β-D-[^{14}C]glucocerebroside: 28% of control activity. Heterozygotes had reduced activity with the new substrate.

Normal human spleen contains two forms of membrane-bound β-D-glucosidase, distinguishable by their thermostability and kinetic properties.[140] The

[138] Y. B. Chiao, R. H. Clew, W. F. Diven, and R. E. Lee, *Clin. Chim. Acta*, 1980, **105**, 41.
[139] W. G. Johnson, A. E. Gal, A. F. Miranda, and P. G. Pentchev, *Clin. Chim. Acta*, 1980, **102**, 91.
[140] M. Yaqoob and M. Carroll, *Biochem. J.*, 1980, **185**, 541.

spleen from a patient with adult Gaucher's disease was deficient in the major, thermolabile, form of the enzyme.

Enzymic diagnosis in 27 cases of Gaucher's disease is the subject of a paper on this form of β-D-glucosidase deficiency.[141]

A revised fluorimetric assay for Gaucher's disease using conduritol β-epoxide with liver as the source of β-D-glucosidase has been described.[142]

The assay of β-D-glucosidase activity with natural, labelled, and artificial substrates in leukocytes from homozygotes and heterozygotes with the Norrbothniam type (Type 3) of Gaucher's disease has been described.[143]

A simple method is described for the isolation of the lysosomal enzyme 'acid' α-D-glucosidase from normal human liver is based on antibodies raised against the purified enzyme immobilized by covalent coupling to macrophorous agarose.[144] Acid α-D-glucosidase can be quantitatively removed from normal urine by incubating with an excess of immobilized antibody. With 4-nitrophenyl α-D-glucopyranoside as substrate, acid α-D-glucosidase accounts for 91 ± 3% of the total α-D-glucosidase activity at pH 4.0 in normal urine. In urine from a patient with the infantile form of Pompé's disease ('acid maltase deficiency'), no α-D-glucosidase activity could be removed by the immobilized antibody, in agreement with the fact that acid α-D-glucosidase is absent in these patients.

α-D-Glucosidase activity has been assayed in polymorphonuclear cells and lymphocytes from human peripheral blood with 4-methylumbelliferyl α-D-glucopyranoside as substrate in the presence of sodium taurocholate.[145] The pH activity curve of the α-D-glucosidase indicated that differential estimation between 'acid' and 'neutral' α-D-glucosidases was difficult to perform with polymorphonuclear cells, but easily accessible with lymphocytes. The use of peripheral blood lymphocytes for the enzymatic diagnosis of Pompé's disease seemed to be more reliable than those of whole leukocytes; this was also the case with a classical Pompé's patient. The lymphocytes from the parents had normal or low normal activity of acid α-D-glucosidase in the freshly isolated state, but when cultured with phytohaemagglutinin for 72 h, the stimulated lymphocytes of both parents showed about half the enzyme activity of the cultured controls. It was deemed possible in all probability to identify the carrier state by assay of the enzyme activity in phytohaemagglutinin-stimulated lymphocytes.

Activities of two major forms of α-D-glucosidase in cultured amniotic fluid cells have been measured by the 4-methylumbelliferyl α-D-glucopyranoside assay after 3, 6, and 9 weeks of culturing.[146] Activity of the pH 4 form of α-D-glucosidase, which is deficient in Pompé's disease, was low in early culture but increased rapidly as the culture time was increased. The cells harvested at 3 weeks had a low absolute activity of the pH 4, as well as a low ratio of the pH 4 to

[141] K. Harzer, *Clin. Chim. Acta*, 1980, **106**, 9.
[142] L. B. Daniels, R. H. Glew, N. S. Radin, and R. R. Vunnam, *Clin. Chim. Acta*, 1980, **106**, 155.
[143] L. Svennerholm, G. Hakansson, and S. Dreborg, *Clin. Chim. Acta*, 1980, **106**, 183.
[144] A. W. Schram, B. Brouwer-Kelder, W. E. Donker-Koopman, C. Loonen, M. N. Hamers, and J. M. Tager, *Biochim. Biophys. Acta*, 1979, **567**, 370.
[145] N. Taniguchi, E. Kato, H. Yoshida, S. Iwaki, T. Ohki, and S. Koizumi, *Clin. Chim. Acta* 1978, **89**, 293.
[146] A. Fujimoto and A. L. Fluharty, *Clin. Chim. Acta*, 1978, **90**, 157.

pH 6 enzyme. The pH 6 form is not affected in Pompé's disease. The results suggest cautions when attempting early diagnosis by use of microtechniques and re-emphasizes the need for differentiation of these two forms of α-D-glucosidases in prenatal diagnosis of Pompé's disease.

A horse kidney neutral α-D-glucosidase was purified about 580-fold with a yield of 33% by an affinity chromatography technique using the 4-aminophenyl β-D-maltoside, a substrate derivative, as ligand.[147] The purified enzyme, homogeneous in polyacrylamide-gel electrophoresis, was a glycoprotein with a molecular weight of 2.8×10^5 as calculated by gel filtration and its isoelectric focusing point was found to be p*I* 4.1. The purified enzyme was able to hydrolyse various substrates having (1→2)-, (1→3)-, (1→4)-, and (1→6)-α-D-glucosidic linkages. The V/K_m ratio shows that the (1→4) linkages are the best substrates. The pK_m of the purified enzyme determined at different pH values indicated that two ionizable groups with p*K* values 5.2 and 6.9 could be essential in the active site. Enzyme modification with carbodi-imide abolished the maltase activity. The turanose, a substrate analogue, protected the enzyme against this inactivation.

An article on the solubilization of oligosaccharide transferase and α- and β-D-glucosidase activities from porcine thyroid rough microsomes points out that the oligosaccharide transferase is extracted together with two D-glucosidases of distinct specificities.[148]

A D-glucosidase preparation with activity toward certain D-glucose-containing oligosaccharides was partially purified from calf liver membranes by Triton X-100 solubilization and DEAE-cellulose and hydroxyapatite chromatography.[149] The enzyme preparation hydrolysed the glucose residues from (D-glucose)$_1$(D-mannose)$_9$(2-acetamido-2-deoxy-D-glucose)$_1$ and (D-glucose)$_2$(D-mannose)$_9$(2-acetamido-2-deoxy-D-glucose)$_1$ but was totally inactive towards (D-glucose)$_3$(D-mannose)$_9$(2-acetamido-2-deoxy-D-glucose)$_1$. In contrast, crude membrane preparations of the calf liver were active toward all three substrates. The partially purified enzyme had a pH optimum of 6.7 and was very unstable in the absence of added 20% glycerol. The rate of D-glucose release from the one- and two-D-glucose-containing oligosaccharides was significantly decreased when four or five of the D-mannose residues were first removed from the substrate. The release of D-glucose from (D-glucose)$_1$(D-mannose)$_9$(2-acetamido-2-deoxy-D-glucose)$_1$ was inhibited by 4-nitrophenyl α-D-glucopyranoside much more effectively than by 4-nitrophenyl β-D-glucopyranoside, suggesting that this D-glucose residue may be linked α to the D-mannose residue.

A β-D-glucosidase that catalyses the hydrolytic cleavage of the terminal D-glucose residue of amygdalin (mandelonitrile-β-gentiobioside) has been demonstrated in cat, rat, and rabbit kidney tissue.[150] The enzyme was partially purified from feline kidney and its properties were determined. Although the natural substrates for the enzyme are unknown at this time, this β-D-glucosidase requires

[147] J. Giudicelli, E. Emiliozzi, C. Vannier, G. de Burlet, and P. Sudaka, *Biochem. Biophys. Acta*, 1980, **612**, E84 85.
[148] C. Ronin, *FEBS Lett.*, 1980, **113**, 340.
[149] J. M. Michael and S. Kornfeld, *Arch. Biochem. Biophys.*, 1980, **199**, 249.
[150] A. Freese, R. O. Brady, and A. E. Gal, *Arch. Biochem. Biophys.*, 1980, **201**, 363.

an aryl or unsaturated alkyl aglycone moiety for catalytic function. The unusual species and tissue distribution of the enzyme have considerable implication for cancer chemotherapeutic trials with amygdalin.

The effect of the pesticides phosalone and lindane on the activity of some dipeptidases and disaccharidases, including α-D-glucosidase (maltase), β-D-glucosidase (cellobiase, palatinase), and sucrose α-D-glucohydrolase (sucrase) and αα-trehalase in rat intestinal mucosa have been studied.[112]

α-D-Glucosidase (maltase-glucoamylase) from the rat intestinal brush-border membrane was solubilized by homogenization of the intestinal mucosa in buffer containing 0.5% Triton X-100.[151] The enzymes from papain and detergent solubilization were compared. Both migrated as single bands with the same mobility on sodium dodecyl sulphate–polyacrylamide gel electrophoresis, were eluted at the same volume Sepharose® 4B, and had the same sedimentation pattern in mannitol gradients. The amino-acid composition was similar. The only difference noted was the tendency of the detergent-solubilized enzyme to aggregate on storage, whereas the papain-solubilized enzyme remained unchanged. Both enzymes had two *N*-termini, glycine and L-arginine. When the two enzymes were dissociated by boiling in sodium dodecyl sulphate, each exhibited the same five species on sodium dodecyl sulphate–polyacrylamide gel electrophoresis. Aggregation in aqueous solutions does not depend on a proteolytically susceptible peptide fragment at the *N*-terminus of either subunit.

The distal intestine of suckling rats contains an anomalously high proportion of its brush-border membrane-typical enzymes in soluble, or easily released form.[152] As a sensitive probe of carbohydrate structure, a comparison was made of the binding of four forms of maltase-glucoamylase (I, membrane-bound, proteolytically solubilized, adult rat; II, membrane-bound, detergent solubilized, adult rat; III, membrane-bound, proteolytically solubilized, suckling rat; IV, soluble, suckling rat) to concanavalin A–Sepharose®, eluted by gradient methyl α-D-mannopyranoside. The three membrane-bound enzymes were readily eluted at low concentrations of methyl α-D-mannopyranoside in an identical manner, whereas the soluble enzyme contained a component that was more tightly bound. These results suggest that a portion of the soluble maltase-glucoamylase in the suckling rat intestine has a carbohydrate structure, which differs from that of the membrane-bound enzyme. This difference may prevent or at least weaken attachment of the enzyme to the brush-border membrane.

A novel α-D-glucosidase has been found in rat renal cortex which on the basis of its specificity for D-glucosyl-D-galactosyl-hydroxy-L-lysine is believed to be involved in the catabolism of the hydroxy-L-lysine-linked disaccharide units of basement membranes and collagens.[153] This enzyme, which has been designated as a 2-*O*-α-D-glucopyranosyl-5-*O*-β-D-galactopyranosylhydroxy-L-lysine glycohydrolase has been purified 176-fold from kidney cortex by ammonium sulphate fractionation, gel filtration, and ion-exchange chromatography. The α-D-gluco-

[151] L. M. Y. Lee, A. K. Salvatore, P. Flanagan, and G. G. Forstner, *Biochem. J.*, 1980, **187**, 437.

[152] A. Salvatore, L. Lee, J. Forstner, and G. Forstner, *Biochem. Biophys. Res. Commun.*, 1980, **93**, 315.

[153] M. Sternberg and R. G. Spiro, *J. Biol. Chem.*, 1979, **254**, 10 329.

sidase had maximal activity between pH 4.4 and 4.7 and acted optimally with D-glucosyl-D-galactosyl-hydroxy-L-lysine as substrate ($K_m = 5.7$ mM), yielding D-galactosyl-hydroxy-L-lysine and D-glucose. *N*-Acetylations of the hydroxy-L-lysyl disaccharide protected it from enzymatic hydrolysis and indicated that the specificity of the α-D-glucosidase was partly dependent on the presence of an unsubstituted ε-amino-group. The enzyme was inactive toward all β-D-glucosides and other α-D-glucosides tested, with the exception of kojibiose, maltose, and isomaltose, which were hydrolysed to a small extent.

Developmental changes and hormonal control of β-D-glucosidase activity in the rat kidney have been investigated.[154]

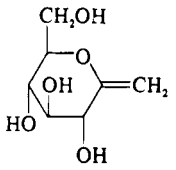
The ability of a series of phlorizin-like glycosides to interact with the active centre of the β-D-glucosidase (phlorizin hydrolase) present in hamster intestinal brush-border membranes has been estimated from K_m and K_i measurements.[155] All of the glycosides except paraphlorizin, were cleaved at the same V although large differences in their apparent K_m values were found. The inhibitors of D-[^{3}H]glucose-labelled phlorizin hydrolysis all acted competitively and their K_i values equalled their respective K_m values with two exceptions: (1) paraphlorizin, an isomer with its sugar residue in a different orientation than any of the other derivatives, is a better inhibitor than substrate and (2) phloretin 2′-galactoside appeared to be a better substrate than inhibitor: it was extensively hydrolysed, more than would have been predicted from its apparent K_i value. This 'extra' hydrolysis was probably due to lactase action. The affinities of these compounds for the D-glucose transport system and phlorizin hydrolase were compared. The relative inhibitory potency of all but two of the analogues was the same in either system suggesting that there are similarities in the architecture of the two receptors. However, there are differences that indicate that these two membrane components did not share a common site (1) the K_i values of the glycosides as sugar carrier blockers are 100-fold smaller than their K_i values as hydrolase inhibitors, (2) 4-methoxyphlorizin has somewhat greater affinity for the β-D-glucosidase than the transporter, and (3) *para*-phlorizin has essentially no affinity for the D-glucose carrier but is a good substrate and potent inhibitor of phlorizin hydrolase. Studies designed to isolate and affinity label the phlorizin-receptor component of the transport system must take into account that the intestinal brush-border membrane possesses a β-D-glycosidase, whose active site will probably also be labelled and at which phlorizin-like ligands could be inactivated.

A study of the mechanism of inhibition of honey-bee (*Apis mellifica*) haemolymph α-D-glucosidase by D-glucose *in vitro* has been reported.[156]

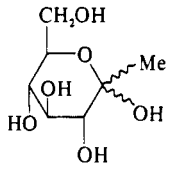
Sweet almond emulsin (β-D-glucosidase) and β-D-glucuronidase have been studied by a rapid and relatively simple procedure for kinetic measurement of low activities of enzymes hydrolysing phenol conjugates: phenol generated by the enzyme reactions in the pH-optimum range 5.0–9.5 is continuously monitored by an oxygen membrane electrode of the Clark type.[114]

[154] K. Hatakeyama, M. Hiramatsu, and N. Minami, *Enzyme*, 1980, **25**, 37.
[155] J. O. Evans and D. F. Diedrich, *Arch. Biochem. Biophys.*, 1980, **199**, 342.
[156] M. Bounias, *C. R. Seances Soc. Biol.*, 1979, **173**, 580.

D-Glucosylases of several types have been found to catalyse specific hydration and glycosyl transfer reactions (the former predominating) when incubated with 2,6-anhydro-1-deoxy-D-*gluco*-hept-1-enitol (1).[157] α-D-Glucosidases from *Candida tropicalis* and from rice convert this substrate into 1-deoxy-α-D-*gluco*-heptulose (2). The β-D-glucosidase from sweet almonds and an *exo*-(1→6)-D-glucanase (glucodextranase) from *Arthrobacter globiformis* convert the substrate to 1-deoxy-β-D-*gluco*-heptulose. The anomeric form of the sugar was identified



(1)



(2)

by following ^{1}H n.m.r. spectra of enzyme–enitol digests. The C-1 methyl group protons of the α- and β-anomers resonated at 1.45 and 1.40 p.p.m., respectively. Non-enzymatic anomerization of the β- to the α-form of the sugar was noted and, at equilibrium, ^{13}C and hydroxyl ^{1}H n.m.r. spectra showed the presence of only the α-anomer. The work provides the first demonstration of the ability of an 'inverting' D-glucanase to create specific anomeric configuration *de novo* from a substrate lacking α- or β-configuration. Indeed, the action of glucodextranase on this enolic glycosyl donor lead not only to 1-deoxy-β-D-*gluco*-heptulose but also to a 1-deoxy-α-D-*gluco*-heptulosyl transfer product when enitol is the acceptor. These results reveal a catalytic mechanism that is able to convert a substrate to products of either configuration, depending on the type of acceptor. A mechanism is proposed for the stereospecific hydration of 2,6-anhydro-1-deoxy-D-*gluco*-hept-1-enitol by each enzyme, involving an incipient glycosyl carbonium ion and assuming the presence at the active site of two carboxy-groups arranged to account for catalysis of reactions with appropriate glycosidic substrates. These observations provide strong evidence for the functional flexibility of the catalytic groups of glycosylases.

Two forms of α-D-glucosidase have been isolated from sugar-beet (*Beta vulgaris*) seeds by a procedure including fractionation with ammonium sulphate and ethanol, carboxymethylcellulose column chromatography, and preparative disc gel electrophoresis.[158] The two enzymes were homogeneous by polyacrylamide disc gel electrophoresis. Their molecular weights were 98 000 (I) and 60 000 (II). α-D-Glucosidase I readily hydrolysed, maltose, isomaltose, kojibiose, maltotriose, panose, amylose, soluble starch, amylopectin, and glycogen. α-D-Glucosidase II also hydrolysed maltose, kojibiose, and maltotriose but hydrolysed the other substrates only very weakly or not at all. α-D-Glucosidase I hydrolysed soluble starch at a faster rate than maltose. It produced isomaltose and panose as

[157] E. J. Hehre, C. F. Brewer, T. Uchiyama, P. Schlesselmann, and J. Lehmann, *Biochemistry*, 1980, **19**, 3557.

[158] Y. Yamasaki and Y. Suzuki, *Planta*, 1980, **148**, 354.

the main α-D-glucosyl-transfer products from maltose, whereas maltotriose was the main product of α-D-glucosidase II. α-D-Glucosidase I hydrolysed amylose liberating α-D-glucopyranose. The neutral-sugar content was calculated to be 2.7% for α-D-glucosidase I and 8.8% for α-D-glucosidase II. The main neutral sugar was D-mannose in α-D-glucosidase I, and D-glucose in α-D-glucosidase II.

The involvement of D-glucosidases in indole alkaloid biosynthesis has been studied using a specific assay system with structosidine as substrate.[159] The discovery and characterization in *Catharanthus roseus* plant cells of two highly glucoalkaloid-specific β-D-glucosidases are reported.

A β-D-glucosidase, which rapidly hydrolyses the cinnamyl alcohol D-glucopyranosides coniferin and syringin, has been purified from cell cultures, hypocotyls, and roots of *Glycine max*.[160] Isoelectric focusing in a column separated the enzyme from several other β-D-glucosidases that were inactive against either substrate. Syringin and coniferin were the best substrates tested. Both exhibited identical V_{max} values, whereas the K_m of coniferin (0.6 mM) was twice that of syringin (0.3 mM). The widely used synthetic substrates 4-nitrophenyl β-D-glucopyranoside and 4-methylumbelliferyl β-D-glucopyranoside were poorly utilized. D-Glucono-1,5-lactone was an effective competitive inhibitor with a K_i of 0.01 mM. From the observed substrate specificity, a role in the lignification process of higher plants may be predicted for this β-D-glucosidase.

Incubation tests on maize scutellum slices in maltose, *etc.* solutions have led to the conclusion that α-D-glucosidase is present (see p. 247).[83]

An intracellular α-D-glucosidase (α-glu_I) of *Aspergillus niger* was purified and its properties were compared to those of a secreted α-D-glucosidase (α-glu_E).[161] The estimated molecular weight of α-glu_I was 95 000 by gel filtration (α-glu_E = 63 000); it is a glycoprotein possessing 29 mol of D-mannose, 6 mol of 2-amino-2-deoxy-D-glucose, and 14 mol of D-glucose (α-glu_E has 5–6 and 2 mol of D-mannose and 2-amido-2-deoxy-D-glucose, respectively). The K_m values of α-glu_I for 4-nitrophenyl α-D-glucopyranoside and maltose were 1.49 and 1.04, respectively, slightly lower than those of α-glu_E. In addition, at 65 °C α-glu_I enzymatic activity decayed five-fold faster than that of α-glu_E, and anti-(α-glu_E) antibody did not recognize α-glu_I. Although some of these distinctions between the enzymes could be ascribed to conformational differences, the great dissimilarity in molecular weight (approximately 32 000) and lack of reactivity with anti-(α-glu_E) argue against α-glu_I being related to α-glu_E. The antibody covalently coupled to horseradish peroxidase (Ab-Px) was used as a probe to determine the cellular location of α-glu_E by electron microscopic immunocytology. It was found on both sides of the plasma membrane and in the outer two layers of the cell wall. This may mean that α-glu_E is synthesized at the inner surface of the plasma membrane, is extruded through the plasma membrane, becomes associated with the outer layer of the cell wall (perhaps as enzyme–substrate complex), and is eventually released into the growth medium.

[159] T. Hemscheidt and M. H. Zenk, *FEBS Lett.*, 1980, **110**, 187.
[160] W. Mosel and R. Todenmagen, *Phytochemistry*, 1980, **19**, 1349.
[161] M. J. Rudick, Z. E. Fitzgerald, and V. L. Rudick, *Arch. Biochem. Biophys.*, 1979, **193**, 509.

α-Deuterium kinetic isotope effects on V_{max} for hydrolysis of both β-D-glucopyranosylpyridinium ions and aryl β-D-glucopyranosides by β-D-glucosidase A_3 of *Aspergillus wentii* are in the range k_H/k_D 1.08–1.14, indicating that bond-breaking limits the rate of hydrolysis of both sets of substrates.[162] The variation of k_{cat} with aglycone acidity, expressed by $\log k_{cat} = A + \beta_{lg} pK_a$, is governed by a β_{lg} value of -0.96 ± 0.19 for the *N*-D-glycosides and -0.05 ± 0.05 for the *O*-D-glycosides. The latter, low value of $|\beta_{lg}|$ is evidence for extensive proton donation to the oxygen at the transition state, even for the departure of acidic aglycones. The dependence of rate on protonation of a group of $pK_a < 6$ required by this idea is indeed observed. Comparison of $\log k_{cat}$ values with spontaneous hydrolysis rates indicates that nucleophilic and non-covalent interactions accelerate C–N bond cleavage in the enzyme–substrate complex by a factor of $10^{(8+0.3[pK_a]N)}$ for D-glucopyranosyl pyridinium salts, where $[pK_a]$ is the pK_a of the pyridine. On the assumption that these interactions are similar for *O*-D-glucosides, proton donation to the aglycone oxygen atom can be estimated to contribute a rate-enhancement of $10^{(0.1+0.7[pK_a]_o)}$, where $[pK_a]_o$ is that of the free phenol. D-Glucono-1,4-lactone and 5-amino-5-deoxy-D-gluconolactam are bound $10^{2.8}$ and $10^{2.2}$ times, respectively, more tightly than β-D-glucopyranose, because of their similarity to a transition state in which α-deuterium kinetic isotope effects have shown the anomeric carbon atom to have substantial sp^2 character. Cationic inhibitors are bound $10^{2.5}$–$10^{3.5}$ times more tightly than directly comparable neutral ones, if the positive charge resides on the equatorial substituent at C-1, but protonated 2-amino-2-deoxy-β-D-glucopyranoside if bound to be more tightly than D-glucose. This indicates the presence of a negative charge in the enzyme–inhibitor complex near C-1 on the a face of the pyranose ring (an aspartate residue previously identified by covalent labelling), which is partly compensated by a positive charge near C-2.

D-Glucosidase inhibitors have been obtained from bacilli.[163]

A 4-nitrophenyl α-D-glucopyranoside-hydrolysing α-D-glucosidase from an obligate thermophile, *Bacillus thermoglucosidius*, gave a triphasic relationship at pH 6.8 in the van't Hoff plot of K_m, in the Arrhenius plot of the first-order rate constant of inactivation with 0.1% sodium dodecyl sulphate, and in the logarithmic plot of the maximal fluorescence intensity at 346 nm *versus* reciprocal of temperature.[164] The respective plots exhibited two breaks at 40 and 61 °C, 43 and 62 °C, and 40 and 61 °C. However, the Arrhenius plot of the molecular activity at pH 6.8 had a single discontinuity at 64 °C. These findings, together with thermodynamic quantities for the enzyme, suggest that the thermal conformational changes in the enzyme protein occur around 40–43 °C and 61–64 °C. The Arrhenius plot of the rate constant of heat inactivation at pH 6.8 was bent at 73 °C. Thermodynamic data indicate that the enzyme is transformed from a heat-stable form into a heat-unstable form at 73 °C with

[162] G. Legler, M. L. Sinnott, and S. G. Withers, *J. Chem. Soc., Perkin Trans. 2*, 1980, 1376.
[163] D. D. Schmidt, W. Frommer, L. Muller, and E. Truscheit, *Naturwissenschaften*, 1979, **66**, 584.
[164] Y. Suzuki, N. Nakarmura, T. Kishigami, and S. Abe, *J. Biochem. (Tokyo)*, 1980, **87**, 745.

temperature elevation. The critical points localized near the minimum, optimum, and maximum temperatures (40, 60, and 72 °C) of the cell growth, respectively.

Culture filtrates of *Cladosporium resinae* contain a mixture of enzymes able to convert starch and pullulan efficiently into D-glucose.[165] Culture conditions for optimal production of the pullulan-degrading activity have been established. The amylolytic enzyme preparation was fractionated by ion-exchange and molecular-sieve chromatography, and shown to contain α-D-glucosidase, α-amylase, and two glucoamylases. The glucoamylases have been purified to homogeneity and their substrate specificities investigated. One of the glucoamylases (termed P) readily hydrolyses the (1→6)-α-D-linkages in pullalan, amylopectin, isomaltose, panose, and 6^3-α-D-glucosylmaltotriose. Each of the glucoamylases cleaves the (1→6)-α-D linkage in panose much more readily than that in isomaltose.

Several lysosomal glycosidase activities were examined *in vitro* during heat-induced germination of *Dictyostelium discoideum* spores and were found not to be co-ordinately controlled (see p. 260).[120]

The presence of two forms of β-D-glucosidase in the culture filtrate of *Macrophomina phaseoline* has been investigated.[166]

The localization of cellulase components in a *Pseudomanas* sp. isolated from activated sludge was studied.[167] *endo*-D-Glucanases were found in the culture media during growth on cellulose but not on cellobiose. Gel filtration and electrophoresis indicated that the extracellular enzyme contained two components, EI and EII. During growth on both substrates intracellular *endo*-D-glucanases and aryl-β-D-glucosidases were formed. Late-exponential-phase organisms from media containing cellulose or cellobiose were subjected to osmotic-shock treatment or to spheroplast formation. Osmotic-shock fluid contained *endo*-D-glucanases and aryl-β-D-glucosidases. Spheroplast formation also released these enzymes. The β-D-glucosidases could be separated into aryl-β-glucosidase I and aryl-β-glucosidase II by ion-exchange chromatography. *endo*-D-Glucanase was located differently on the cell surface depending on whether the growth substrate was cellulose or cellobiose. The cytoplasm contained another *endo*-D-glucanase and aryl-β-glucosidase I. None of the enzymes hydrolysed Avicel, but all except aryl-β-glucosidase III hydrolysed carboxymethylcellulose. All aryl-β-glucosidases, but none of the *endo*-D-glucanases, hydrolysed cellobiose.

Kinetic studies of the two β-D-glucosidase isoenzymes, GB-1 and GB-2, which were purified from the culture filtrate of a phytopathogenic fungus *Pyricularia oryzae*, revealed that the latter isoenzyme was an allosteric protein with two substrate-binding sites.[168] The homotropic effects of 2- and 3-nitrophenyl β-D-glucosides on GB-2 showed positive co-operativity, whereas that of cello-oligosaccharide showed negative co-operativity. The affinity of GB-2 for cello-oligosaccharide tended to increase with decreasing chain length, in contrast to that of GB-1. D-Glucono-1,4-lactone and D-glucose acted as competitive

[165] B. V. McCleary and M. A. Anderson, *Carbohydr. Res.*, 1980, **86**, 77.
[166] S. C. Saha, A. Sanyal, S. Dube, and D. K. Dube, *Curr. Sci.*, 1980, **49**, 102.
[167] K. Ramasamy and H. Verachtert, *J. Gen. Microbiol.*, 1980, **117**, 181.
[168] T. Hirayama, H. Nagayama, and K. Matsuda, *J. Biochem. (Tokyo)*, 1980, **87**, 1203.

inhibitors of GB-1 and GB-2. As regards the control of the level of D-glucose formed by the cellulase system, it appears that the rate of formation of D-glucose by β-D-glucosidase is reduced by the presence of the substrate, cello-oligosaccharide, as well as by the product, D-glucose.

A new α-D-glucosyl-2-deoxy-D-glucose was enzymatically synthesized by the transglucosylation of *Schizosaccharomyces pombe* α-D-glucosidase under the co-existence of maltose and 2-deoxy-D-glucose.[169] The disaccharide was chromatographically isolated in a semicrystalline powder (hemihydrate). It was concluded that the sugar was 6-*O*-α-D-glucopyranosyl-2-deoxy-D-glucose, which was the main product formed in this transglucosylation.

The induced β-D-glucosidase from *Stachbotrys atra* hydrolyses aryl β-D-glucopyranosides and aryl β-D-xylopyranosides by the same basic two-step mechanism.[170] In the first step the aglycone group is split off with simultaneous formation of an enzyme–glycopyranosyl complex. In the second step this intermediate complex reacts with water yielding β-D-glucose or β-D-xylose. For β-D-xylopyranoside hydrolysis each of the two steps is partially rate-controlling, whereas for β-D-glucopyranoside hydrolysis the second step is rate-limiting. The enzyme is inhibited by high concentrations of substrate and the exact rate-concentration equation is a second-order equation. 1-Thio-β-D-glycopyranosides with an aromatic aglycone inhibit the reaction in both a competitive and non-competitive way. A tentative mechanism is proposed to explain all types of inhibition. In this mechanism substrates and inhibitors with an aromatic aglycone group bind through hydrophobic forces to the 'aglycone subsite' of the intermediate enzyme–glycopyranosyl complex. Binding of the second substrate molecule or of the inhibitor to this complex does not prevent the reaction of the glycopyranosyl moiety with water, it only decreases the rate of the second step.

The location of the β-D-glucosidase activity in a whole culture broth of the thermophilic organism *Thermoactinomyces* has been studied.[171] Little β-D-glucosidase activity was found in the culture filtrate, whereas the culture solids contained the major part of the activity of the whole culture broth. The activity does not appear to be adsorbed to the culture solids; rather there is evidence that it is an intracellular soluble enzyme(s). The pH and temperature optima for a crude β-D-glucosidase preparation were determined to be pH 6.5 and 50–55 °C. Enzyme activity studies indicate that the same enzyme(s) accounts for the β-D-glucosidase and the cellobiose activities. The validity of using the filter paper activity of culture filtrates from *Thermoactinomyces* to predict the total saccharification of cellulosic materials to D-glucose is discussed.

The carboxymethylcellulase and Avicelase (cellulase) activities of *Thermoactinomyces* sp., strain YX, were produced simultaneously with cell growth.[172] Throughout the growth phase these activities were primarily, extracellular, with up to 50% adsorbed to residual cellulosic substrate at any one time. On the other hand, the β-D-glucosidase activity was associated with the culture solids through-

[169] S. Chiba and O. Yamana, *Agric. Biol. Chem.*, 1980, **44**, 549.
[170] C. K. De Bruyne, G. M. Aerts, and R. L. De Gussem, *Eur. J. Biochem.*, 1979, **102**, 257.
[171] B. Hagerdal, H. Harris, and E. K. Pye, *Biotechnol. Bioeng.*, 1979, **21**, 345.
[172] B. G. R. Hagerdal, J. D. Ferchak, and E. K. Pye, *Appl. Environ. Microbiol.*, 1978, **36**, 606.

out the entire fermentation and appears to be intracellular. Preparative isoelectric focusing of the culture filtrate, in the pH range 3 to 5, separated three major fractions with cellulolytic activities towards both carboxymethylcellulose and Avicel. Based on the carboxymethylcellulase and Avicelase activities of these separated fractions, it was not possible to discriminate between *endo*- and *exo*-D-glucanases produced by *Thermoactinomyces* sp. Analytical isoelectric focusing of culture filtrates obtained throughout the growth phase of *Thermoactinomyces* indicated that all the extracellular cellulolytic enzymes are produced and released into the culture filtrate simultaneously, with no evidence of a sequential appearance of the various enzymes or isoenzymes.

The stabilities and optima with respect to temperature and pH of the β-D-glucosidase, Avicelase, and carboxymethylcellulase (CMCase) activities of *Thermonospora* sp., in the culture filtrate, culture whole broth, and filtrate after sonication of culture solids, are reported.[173] The β-D-glucosidase is cell-associated and has an optimal activity at about pH 6.5 and 55 °C. In the whole culture broth, it has a half-life of about 8 h at 55 °C and less than 1 h at 60 °C, whereas the half-life of the activity in the sonicated, cell-free filtrate is less than 1 h at 55 °C.

A new intracellular β-D-glucosidase was isolated from *Trichoderma reesei* and purified.[174] The enzyme has a molecular weight of 98 000, optimal activity at pH 6.5, p*I* 4.4 and K_m values of 6.7 mM and 3.3 mM for sophorose and cellobiose, respectively. Possible functions of the enzyme may be regulation of cellulase induction and/or to serve as a proenzyme.

A new procedure using high-performance liquid chromatography for the rapid separation of cellulase proteins is described.[175] The cellulase components of *Trichoderma reesei* are fractionated on a DEAE-cellulose anion-exchange column using a phosphate buffer at pH 6.2. Activities of the individual components obtained from *T. reesei* QM6a, a wild strain, and several mutant strains have been determined. Each system examined contained β-D-glucosidase, at least two *exo*-(1→4)-β-D-glucanases and five *endo*-(1→4)-β-D-glucanases with the *endo*-(1→4)-β-D-glucanases accounting for 20–36% and the *exo*-(1→4)-β-D-glucanases for 64–80% of the soluble protein.

Glucanohydrolase from *Trichoderma reesei*, having a molecular weight of 52 000, was evaluated from kinetic properties with respect to cellobiose.[176] Results from this work include (1) initial rate studies that show that glucanohydrolase hydrolyses cellobiose by a competitive mechanism and that the product, D-glucose, inhibits the enzyme; (2) low-pressure aqueous liquid chromatography that shows that formation of a reversion product, cellotriose, is minor and occurs in detectable amounts only at very high (90 mM) cellobiose concentrations; (3) development of an equation based on the mechanism of glucanohydrolase action as determined by initial rate kinetics, which accurately predicts the time course of cellobiose hydrolysis; and (4) derivation of an initial rate

[173] B. Hagerdal, V. D. Ferchak, and E. K. Pye, *Biotechnol. Bioeng.*, 1980, **22**, 1515.
[174] M. Inglin, B. A. Feinberg, and J. R. Loewenberg, *Biochem. J.*, 1980, **185**, 515.
[175] F. H. Bissett, *J. Chromatogr.*, 1979, **178**, 515.
[176] M. R. Ladisch, C. S. Gong, and G. T. Tsao, *Biotechnol. Bioeng.*, 1980, **22**, 1107.

expression for the combined activity of cellobiose and glucanohydrolase on cellobiose. Based on data in this paper it is shown that the difference in inhibition patterns of the two enzymes could be used for determining the contamination of one enzyme by small quantities of the other.

Trichoderma viride secretes a cellulase complex that is rich in β-D-glucosidase and therefore well suited for the saccharification of cellulosic materials.[177] The cellulase was investigated with respect to optimum conditions of reaction and enzyme stability (see p. 310). Avicelase, carboxymethylcellulase, and β-D-glucosidase differed considerably in their physiochemical properties. At temperatures above 50 °C, β-D-glucosidase is not very stable.

An α-D-glucosidase was purified from baker's yeast.[178] The molecular weight was approximately 44 000. Sodium dodecyl sulphate–disc gel electrophoresis suggested that the enzyme consisted of four subunits. The isoelectric point was at pH 5.4. The K_m values for 4-nitrophenyl α-D-glucopyranoside and maltose were 2.9×10^{4} and 2.5×10^{-2} M, respectively. Binding of 2-(4-toluidino)naphthalene-6-sulphonate (TNS) to the α-D-glucosidase was associated with a strong increase in fluorescence. The dissociation constant of the enzyme–TNS complex was 8×10^{-5} M. The fluorescent probe did not interfere with the binding of D-glucose to the enzyme although the α-D-glucosidase was inhibited by high concentrations of TNS. The formation of an enzyme–D-glucose complex was indicated by an increase of fluorescence and by a shift in the wavelength for maximal emission, which suggests that the binding process is associated with a change in conformation. The dissociation constant of the D-glucose α-D-glucosidase complex $K_D = 0.57 \times 10^{-3}$ M, was calculated from the increase in fluorescence as a function of D-glucose concentration.

Enzymically active but immobilized α-D-glucosidase has been prepared in bead shape by radiopolymerization of synthetic monomers such as acrylates, particularly of a mixture of acrylamide, methylenebisacrylamide, and potassium and magnesium acrylates.[179]

8 β-D-Glucuronidases

The inhibition of β-D-glucuronidase activity by albumin of human synovial fluid has been investigated.[180]

In a study to evaluate the properties of β-D-glucuronidase in human synovial fluid, the enzyme was shown to have a pH requirement of 5.0 and a K_m value of about 8.0×10^{-3} M using phenolphthalein β-D-glucopyranosyluronic acid as the substrate.[181] At low substrate concentration an endogenous inhibitor was demonstrable. The inhibition is of the competitive type and is removed by

[177] D. Herr, *Biotechnol. Bioeng.*, 1980, **22**, 1601.
[178] M. -R. Siro and T. Lovgren, *Acta Chem. Scand.*, 1978, **32**, 447.
[179] K. Kanaya, M. Sasaki, K. Kawashima, S. Chiba, and T. Shimomura, *J. Agric. Chem. Soc. (Jpn.)*, 1979, **53**, 385.
[180] L. Moro, B. De Bernard, P. Inaudi, and F. Gonano, *Proc. Soc. Exp. Biol. Med.*, 1978, **159**, 403.
[181] 'Clinical Enzymology Symposia, Vol. 2, 8th International Symp. on Clinical Enzymology, Venice, Italy, 1978', ed. A. Burlina and L. Galzigna, Piccin Medical Books, Padua, 1979.

proteolytic digestion of synovial fluid, whereas hyaluronidase digestion and addition either of Triton X-100 or of various salts to the assay mixture are ineffective. The inhibitor has been extracted and purified by gel filtration and chromatography: upon immunological evidence it appears to be albumin. The authors discuss the role of some proteins in the modulation of the activity of enzymes degrading cartilage.

The sex and age dependence of activity of β-D-glucuronidase was assayed in serum samples using the 4-methylumbelliferyl substrates and found not to change.[47]

Optimal assay conditions are described for plasma β-D-glucuronidase among others (see p. 243).[61] Assay conditions have also been developed using fluorometric substrates for the determination of urinary β-D-glucuronidase, which overcome interference by both low and high molecular weight inhibitors: a problem that has confused earlier studies of enzyme excretion (see p. 239).[49]

Adsorptive pinocytosis of lysosomal enzymes by fibroblasts depends on phospho-D-mannosyl recognition markers on the enzymes and on high affinity pinocytosis receptors on the cell surface.[182] β-D-Glucuronidase from human spleen was shown to bind specifically to receptors on membranes prepared from diploid human fibroblasts. The binding was saturable, and was competitively inhibited by D-mannose 6-phosphate and a phospho-D-mannan fragment from *Hansenula holstii* phospho-D-mannan, two compounds that have previously been shown to inhibit enzyme binding to and pinocytosis by fibroblasts. Comparable concentrations of D-mannose 1-phosphate, D-glucose 6-phosphate, and D-mannose were non-inhibitory. Pretreatment of the enzyme with alkaline phosphatase or *endo*-glycosidase H, which led to loss of susceptibility to pinocytosis by fibroblasts, also led to loss of specific binding to fibroblast membranes. Specific enzyme-binding was linearly dependent on the amount of added membrane protein, and could be destroyed by pretreatment of the membranes with trypsin. Dissociation of bound enzyme was slow, but was greatly accelerated by D-mannose 6-phosphate or by lowering the pH to levels comparable to intralysosomal pH. Studies of enzyme binding to trypsin-treated and untreated cells and to membranes prepared from trypsinized and untrypsinized cells indicated an internal pool of enzyme-binding sites at least four-fold larger than the number of binding sites on the cell surface.

Lysosomal β-D-glucuronidase in fibroblasts and leukocytes exhibited the same isoelectric focusing pattern as found in liver.[183] Treatment with neuraminidase had a great effect on the pattern of several glycosidases, whereas treatment with alkaline phosphatase did not significantly change the pattern. These findings are discussed with special reference to the different 'uptake' systems that exist for acid hydrolases.

In a comparative study of eight glycoside hydrolases in serum samples from 72 cystic fibrosis patients, 85 cystic fibrosis parents, and 34 healthy and diseased controls, the levels of β-D-glucuronidase were found to be unchanged.[54]

The activity of β-D-glucuronidase and other glycoside hydrolases was determined in human sera from 10 normal subjects and in three groups each of 10

[182] H. D. Fischer, A. Gonzalez-Noriega, and W. S. Sly, *J. Biol. Chem.*, 1980, **255**, 5069.
[183] B. Hultberg, *Clin. Chim. Acta*, 1979, **93**, 221.

patients with diabetes mellitus, hepatic cirrhosis, and gastric carcinoma. The results did not show significantly higher activities in the patients for β-D-acetamido-2-deoxyglucosidase or β-D-glucuronidase.[60]

Bovine liver β-D-glucuronidase and testicular β-D-galactosidase were assimilated by generalized gangliosidosis fibroblasts at respective rates of 90 and 464 times the rate of assimilation of horseradish peroxidase.[111] Assimilation was saturable, suggesting the participation of receptor-mediated adsorptive endocytosis.

The circadian rhythm of rat liver β-D-glucuronidase has been studied in animals kept under high standardized laboratory conditions. A clear 24 h rhythm has been observed for this enzyme with a peak activity of 01.00 h and a trough at 13.00 h.[184]

β-D-Glucuronidase has been purified from macrophages of thioglycollate-treated mice using concanavalin A chromatography and immunoprecipitation.[113] The apparent molecular weight of the β-D-glucuronidase subunit was determined by sodium dedecyl sulphate–polyacrylamide gel electrophoresis during a long-term pulse-chase experiment as for β-D-galactosidase (see p. 258).

It has been proposed that the recognition marker for the endocytotic uptake, and intracellular targeting of lysosomal enzymes, resides in phospho-D-mannosyl moieties on these glycoproteins.[185] To investigate the biosynthesis of the oligosaccharides of a lysosomal enzyme, mouse lymphoma cells were labelled for 3 h with D-[2-^{3}H]mannose, and the lysosomal enzyme β-D-glucuronidase was purified by immunoprecipitation. Oligosaccharides were prepared by sequential pronase and *endo*-β-D-2-acetamido-2-deoxyglucanase H digestions of the immunoprecipitate. Approximately 50% of the D-[^{3}H]mannose-labelled oligosaccharides contained a net negative charge as determined by binding to QAE–Sephadex® and were either entirely or partially resistant to jack bean α-D-mannosidase digestion. The data suggest that the 3 h D-[^{3}H]mannose-labelled oligosaccharides of β-D-glucuronidase consist of high D-mannose-type units in which D-mannose residues located at or near the non-reducing termini are linked by phosphodiester bonds to another moiety, which, at least in some of the oligosaccharides, is 2-acetamido-2-deoxy-α-D-glucopyranose. Upon maturation of the enzyme, both the blocking moieties and the phosphate residues are lost.

The occurrence of two molecular forms, I and II, in the β-D-glucuronidase of the liver (hepatopancreas) from the marine mollusc *Littorina littorea* has been demonstrated for the first time.[186] The two forms have been purified 355-fold and 1262-fold, respectively. Form I and II of β-D-glucuronidase behave differently on DEAE-cellulose chromatography, polyacrylamide gel disc electrophoresis, isoelectric focusing (pH 5.5 and 4.2, respectively), optimum pH (4.4 and 3.4–4.1, respectively), thermal stability K_m (1.2 mM and 0.5 mM with 4-nitrophenyl β-D-glucopyranosyluronic acid 0.3 mM and 0.15 mM with phenolphthalein β-D-glucopyranosyluronic acid as substrates for form I and II, respectively) and V. Their molecular weight, estimated by gel filtration through

[184] R. Dalwadibhattacharya, *Experientia*, 1980, **36**, 74.
[185] I. Tabas and S. Kornfeld, *J. Biol. Chem.*, 1980, **255**, 6633.
[186] T. Diez and J. A. Cabezas, *Eur. J. Biochem.*, 1979, **93**, 301.

Sephadex® G-200, was about 250 000 for both forms. Several subunits were separated by polyacrylamide gel electrophoresis in presence of sodium dodecyl sulphate. This β-D-glucuronidase is a glycoprotein but neuraminic acid(s) were not detected. The enzyme was very active on synthetic substrates and also on hexasaccharides and tetrasaccharides containing β-D-glucopyranuronic acid residues with (1→3)-linkages; it had practically no activity on certain glycosaminoglycans. Hg^{2+} and D-glucaro-1,4-lactone were very effective inhibitors of this enzyme; the latter by a competitive mechanism.

In a rapid and relatively simple procedure described for kinetic measurement of low activities of enzymes hydrolysing phenol conjugates, such as β-D-glucuronidase, phenol generated by the enzyme reactions in the pH-optimum range 5.0–9.5 is continuously monitored by an oxygen membrane electrode of the Clark type.[114]

9 α-L-Iduronidases

Procedures have been described for the preparation of two disaccharides, 4-*O*-α-L-iduronosyl-2,5-anhydro[^{3}H]mannitol and 3-*O*-α-L-iduronosyl-2,5-anhydro-[^{3}H]talitol, from heparin and dermatan sulphate, respectively.[187] These disaccharides lend themselves to an easy assay of α-L-iduronidase, which is based on the fractionation of the liberated neutral anhydro[^{3}H]mannitol or anhydro[^{3}H]-talitol from the unreacted substrate by adsorption of the latter to Dowex 1. Investigation of the reaction conditions showed that the α-L-iduronidase activity (enzyme from human fibroblasts and *Helix pomatia*) was optimal at pH 3.6 in acetate buffer containing 0.01 M-NaCl with 4-*O*-α-L-iduronosyl-2,5-anhydro-[^{3}H]mannitol as substrate. For 3-*O*-α-L-iduronosyl-2,5-anhydro[^{3}H]talitol the pH optimum was 4.0 with the *H. pomatia* enzyme. The K_m for 4-*O*-α-L-iduronosyl-2,5-anhydro[^{3}H]mannitol was 0.23 mM with human fibroblasts and 0.04 mM with *H. pomatia* enzyme; a K_m value of 0.02 mM was determined for 3-*O*-α-L-iduronosyl-2,5-anhydro[^{3}H]talitol with the *H. pomatia* α-L-iduronidase.

10 α-D-Mannosidases

Three-dimensional structure analyses of two 'polysaccharide-degrading' enzymes, α-amylase and α-D-mannosidase, though not yet complete, reveal that both enzymes are structural tandem duplicates and that the two similar domains within each are related by a dyad axis of symmetry.[188]

Human α-D-mannosidases A, B, and C have been partially purified from liver by conventional and affinity chromatographic procedures.[189] The kinetic, physical, and immunologic properties of the A, B, and C isoenzymes were determined including pH optima, K_m, effects of various inhibitors and activators, thermal stabilities, electrophoretic mobilities, native molecular weights, and antigenic relationships. By gel filtration, the apparent native molecular weights

[187] J. N. Thompson, *Clin. Chem. Acta*, 1978, **89**, 435.
[188] P. M. D. Fitzgerald, P. J. Stankiewicz, S. C. Smith, and A. McPherson, *J. Mol. Biol.*, 1979, **135**, 753.
[189] G. A. Grabowski, J. U. Ikonne, and R. J. Desnick, *Enzyme*, 1980, **25**, 13.

were 2.4×10^5, 3.0×10^5, and 4.2×10^5 for the A, B, and C isoenzymes, respectively. By polyacrylamide gel electrophoresis, the A and B isoenzymes had native molecular weights of approximately 150 000 and appeared to be charged isomers. Antibodies to the purified A and B isoenzymes were produced; cross-reactivity between the A and B isoenzymes against anti-A and anti-B immune sera were observed by double immunodiffusion and immunoprecipitation. In contrast, no reactivity of the C isoenzyme was detected with either immune serum. Based on these studies, models are presented for the molecular interrelationship of α-mannosidases A and B.

Lysosomal α-D-mannosidase in fibroblasts and leukocytes exhibited the same isoelectric focusing pattern as found in liver.[183] Treatment with neuraminidase had a great effect on the pattern of several glycosidases, whereas treatment with alkaline phosphatase did not significantly change the pattern. These findings are discussed with special reference to the different 'uptake' systems that exist for acid hydrolases.

The activity of α-D-mannosidase was assayed in serum samples using the 4-methylumbelliferyl substrates and there was found to be no change in relation to sex or age.[47]

No significant differences were found between cystic fibrosis and normal livers in 4-methylumbelliferyl α-D-mannosidase specific activities determined at three different pH values (4.3, 5.65, and 6.5) corresponding to lysosomal, golgi, and cytosolic components of the enzyme, respectively.[55] The specific activities of 4-nitrophenyl β-D-mannosidase and 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucosidase were also both similar cystic fibrosis and normal livers.

In a study of eight glycosidases in serum samples from 72 cystic fibrosis patients, 85 cystic fibrosis parents, and 34 healthy and diseased controls, the α-D-mannosidase levels were unchanged.[54]

α-D-Mannosidase was determined in human sera from 10 normal subjects and in three groups each of 10 patients with diabetes mellitus, hepatic cirrhosis, and gastric carcinoma. The results did not show significantly higher activities in the patients for β-D-2-acetamido-2-deoxyglucosidase or α-D-mannosidase.[60]

Optimal assay conditions have been described for plasma α-D-mannosidase (see p. 243).[61]

α-D-Mannosidase studied using isoelectric focusing in fibroblasts and culture medium from patients with immunolipidosis Types II and III contained no significant aberrations compared to controls even when D-mannose 6-phosphate was added to the latter.[63]

On concanavalin A–Sepharose®, the elution pattern of α-D-mannosidase from patients with mucolipidosis II and III was comparable to that of plasma of a normal individual.[64]

The intra- and extra-cellular 'acid' α-D-mannosidase in cultures of fibroblasts from mucolipidosis II patients has normal kinetics.[190] The extracellular activity in cultures of cells from mannosidosis patients is normal, but a mutant enzyme

[190] D. J. J. Halley, B. G. Winchester, L. J. Burditt, A. d'Azzo, D. Robinson, and H. Galjaard, *Biochem. J.*, 1980, **187**, 541.

is associated with the cell surface and intracellular fraction. The results support the involvement of membrane cycling and recognition markers in lysosomal enzyme localization.

There is a marked increase in the 'acid' α-D-mannosidase in the plasma of a patient with mucolipidosis II and of a patient with mucolipidosis III.[191] A small proportion (3–4%) of the 'acid' α-D-mannosidase does not bind to concanavalin A–Sepharose®, suggesting an alteration in the glycosylation of some of the enzyme in these disorders. A slight elevation in intermediate α-D-mannosidase was also demonstrated in these samples by using a differential assay for the acidic and intermediate α-D-mannosidase activities. A combination of chromatography on concanavalin A–Sepharose and Sephadex® G-200 showed that intermediate α-D-mannosidase components I_2 and I_4, which account for approximately 80% of the intermediate activity in normal plasma, were also present in mucolipidoses II and III plasmas. The minor intermediate α-D-mannosidase components in normal plasma, I_1 and I_3 were either present in small amounts or not detected. These results suggest that a defect in intermediate α-D-mannosidase is unlikely to be the primary defect in these disorders.

A comparison has been made of residual 'acid' α-D-mannosidase in three cases of mannosidosis.[192]

Five glycoside hydrolases including α- and β-D-mannosidase were studied during the post-natal development of the rat cerebellum (see p. 246).[73]

Studies in intact rat liver golgi cells have shown that α-D-mannosidase is capable of processing L-asparagine-linked oligosaccharides biosynthetically.[193] The enzyme shows specificity for (1→3)-linked α-D-mannopyranose residues. Structural analysis of the *in vitro* reaction products reveal that specific intermediates are formed in the conversion of the (D-Man)$_9$D-GlcNAc oligosaccharide to the (D-Man)$_5$D-GlcNAc oligosaccharide. Heat-inactivation studies are consistent with the possibility that one enzyme activity is responsible for this conversion. The (1→2)-specific-α-D-mannosidase described here appears to be distinct from two other rat liver Golgi α-D-mannosidase activities based on differential substrate specificity, inhibitor susceptibility, and detergent extractability.

The structure to 3.0 Å resolution of the protein canavalin from Jack beans has been determined by conventional *X*-ray crystallographic techniques.[194] We present evidence that canavalin is a proteolytically modified form of the enzyme α-D-mannosidase. This is based on the facts that the purified precursor protein possesses substantial α-D-mannosidase activity, it co-migrates on sodium dodecyl sulphate–polyacrylamide gel electrophoresis with Jack bean α-D-mannosidase prepared by other means, and its oligomer molecular weight and Zn^{2+} content are the same as those of Jack bean α-D-mannosidase.

α-D-Mannosidase of *Medicago sativa* (alfalfa) was purified to homogeneity by polyacrylamide gel disc electrophoresis.[195] The purified enzyme has a molecular

[191] S. Hirani and B. Winchester, *Clin. Chim. Acta*, 1980, **101**, 251.
[192] L. Burditt, K. Chotai, D. Halley, and B. Winchester, *Clin. Chim. Acta*, 1980, **104**, 201.
[193] I. Tabas and S. Kornfeld, *J. Biol. Chem.*, 1979, **254**, 11655.
[194] A. McPherson and S. Campbell-Smith, *Phytochemistry*, 1980, **19**, 957.
[195] A. Curdel and F. Petek, *Biochem. J.*, 1980, **185**, 455.

weight of 2.30×10^5 as estimated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. The enzyme comprises four subunits of different molecular weight. Optimum pH and K_m values were determined with 4-nitrophenyl α-D-mannopyranoside as substrate. When incubated at a temperature between 20 and 62 °C before assay, α-D-mannosidase initially shows an increase in activity. α-D-mannosidase is stable when the pH is about neutral; it can be activated by several metal ions, including Zn^{2+}. At a pH below 5 the enzyme undergoes irreversible inactivation. The presence of H_4-e.d.t.a. at acid pH considerably enhances the inactivation of the enzyme. This inactivation due to H_4-e.d.t.a. can be specifically reversed by incubation with Zn^{2+}.

The formation of α-D-mannosidase of *Athrobacter* species has been studied.[196]

α-D-Mannosidase activity decreased during all stages of heat-induced germination of *Dictyostelium discoideum* spores.[120] Prolonged heat activation treatment reduced the levels of lysosomal glycosidase activities in postactivated spores but did not change the subsequent enzyme patterns during the spore-swelling and emergence stages of germination.

11 Neuraminidase (Sialidases)

Two neuraminidase components A and B were distinguished in cultured human skin fibroblasts on the basis of thermolability at 37 °C.[197] The more labile component (A) $t_{1/2} = 4.7$–5.3 min at 37 °C, comprises 66–90% of total neuraminidase activity when determined using sodium (4-methylumbelliferyl α-D-*N*-acetylneuraminate) as substrate. Activity was assayed at 0 °C for 18 h instead of 37 °C to determine fully both thermolabile and thermostable components. Diminished activity was noted in cultured fibroblasts from mucolipidoses I, II, and III (ML I, ML II, ML III) and the cherry-red spot myoclonus syndrome patients when assayed at both 0 and 37 °C with either 4-methylumbelliferyl α-D-*N*-acetylneuraminate or each of a series of (2→3)- and (2→6)-linked *N*-acetylneuraminyl-oligosaccharides. Increased sensitivity and rapidity of analyses were achieved using 4-methylumbelliferyl α-D-*N*-acetylneuraminate as substrate in determining neuraminidase activity. Results from two obligate heterozygote MLI cell lines (14.5 and 8.0% of control activity) indicate that the 4-methylumbelliferyl α-D-*N*-acetylneuraminate substrate could be useful for heterozygote detection.

The specific activity of several lysosomal hydrolases in normal and cystic fibrosis livers has been investigated.[55] Neuraminidase activity with respect to two natural substrates (fetuin and bovine submaxillary mucin) is normal in cystic fibrosis livers.

The activity of neuraminidase in liver and brain from I-cell disease (mucolipidosis II) was investigated.[198] Neuraminidase activities using two substrates [α-L-*N*-acetylneuraminosyl-(2→3)-lactose and α-L-*N*-acetylneuraminosyl-(2→6)-lactose] were reduced in the supernatant and sedimentable fraction obtained in isotonic KCl. The activity of β-D-galactosidase was also reduced in the liver; on

[196] W. Hampel, *Monatsch. Chem.*, 1980, **111**, 443.

[197] M. Potier, G. Beauregard, M. Belisle, L. Mameli, V. N. Hong, S. B. Melancon, and L. Dallaire, *Clin. Chim. Acta*, 1979, **99**, 97.

[198] R. Minami, T. Kudoh, K. Oyanagi, and T. Nakao, *Clin. Chem. Acta*, 1979, **96**, 107.

the other hand, both neuraminidase fractions were normal, although β-D-galactosidase activities were markedly reduced. In view of these results, it is suggested that the defect of neuraminidase is not directly responsible for the primary etiology of I-cell disease.

Neuraminidase was assayed in the frozen autopsy tissues from three patients with I-cell disease and an adult patient with cherry-red spots, myoclonus, cerebellar ataxia, and β-D-galactosidase deficiency.[199] Both diseases showed normal neuraminidase activity toward neuramine lactose and fetuin in cerebral grey matter, liver, and kidney. These results suggest that the neuraminidase deficiency is limited only to some tissues and that this biochemical abnormality is not caused by a primary genetic mutation in these diseases.

Methods were developed for the purification of the surface membrane-bound glycoproteins, haemagglutinin and neuraminidase, of influenza virus strain 3QB in antigenically active forms.[200] The methods employed in the purification included selective removal of the neuraminidase with the proteinase, bromelain, and subsequent disruption of the residual virus particle with the detergent Sarkosyl to release the haemagglutinin.

In connection with a study of genetic expression in mice, a profound neuraminidase deficiency in the liver of SM/J mouse strain has been demonstrated.[201]

A radioimmunoassay capable of measuring nanogram quantities of *Arthrobacter sialophilus* neuraminidase was developed. Neuraminidases from several influenza viruses, *Clostridium perfringens*, *Vibrio cholerae*, *Diplococcus pneumoniae*, several group B streptococcal isolates, and human fibroblasts each failed to react with antisera raised in guinea pigs against the homogeneous *A. sialophilus* enzyme.[202] Cross-reactivity was limited to neuraminidases from other *Arthrobacter* isolates and, weakly, also with a *Corynebacterium diphtheria* enzyme preparation. The substrate-dependent differential inhibition apparent supports a steric model for inhibition of enzyme catalysis. Each of the cross-reacting *Arthrobacter* enzymes showed differential inhibition with neuraminyl-lactose, indicating variation in the positioning of one or more determinants with respect to their active sites. Further applications for this radioimmunoassay in relation to the use of neuraminidase in cell and molecular biology are discussed.

The purification and properties of two neuraminidases from *Streptococcus viridans* have been investigated with a view to their application in improved structural assessment of glycoprotein structures.[203]

Trichomonas fetus, a protozoon belonging to the class of flagellates, causes vaginal infections in cows, leading to sterility or abortion in early stage of pregnancy.[204] Two neuraminidases were isolated from the culture medium and purified by various procedures of gel chromatography, ion-exchange chromatography, and by affinity chromatography on *N*-(4-nitrophenyl)oxamic acid–Sepharose® 4B. The molecular weights of the two neuraminidases were deter-

[199] Y. Suzuki and K. Fukuoka, *Clin. Chim. Acta*, 1979, **99**, 107.
[200] A. Aitken and C. Hannoun, *Eur. J. Biochem.*, 1980, **107**, 51.
[201] M. Potier, D. Lu Shun Yan, and J. E. Womack, *FEBS Lett.*, 1979, **108**, 345.
[202] R. Huchzermeier, S. W. Tanenbaum, and M. Flashner, *Anal. Biochem.*, 1980, **105**, 454.
[203] H. von Nicolai, H. E. Muller, and F. Zilliken, *FEBS Lett.*, 1980, **117**, 107.
[204] M. Crampen, H. V. Nicolai, and F. Zilliken, *Z. Phys. Chem.* (*Leipzig*), 1979, **360**, 1703.

mined as 320 000 (enzyme I) and 38 000 (enzyme II), respectively. However, enzyme I seems to consist of two isoenzymes containing four subunits of almost equal molecular weight. The pH optima of both enzymes depend on the substrates and range from pH 4.7 to 5.5. The Michaelis constants (K_m) vary between 5.0×10^{-2} M and 6.6×10^{-3} M for enzyme I and between 1.4×10^{-2} M and 4.9×10^{-3} M for enzyme II, owing to the type of substrate. Among the different groups of neuraminic acid-containing substrates, *i.e.* glycoproteins, glycolipids, oligosaccharides, and synthetic ketosides, enzyme I preferably cleaves high-molecular weight glycoprotein-type substrates, whereas enzyme II shows higher affinities to low-molecular weight oligosaccharides. Both enzymes show differences in the specificity towards (2→3)-, (2→6)-, and (2→8)-glycosidic linkages of neuraminic acid.

An enzymically active but immobilized form of neuraminidase has been obtained by reaction of the enzyme with agarose cyclic imidocarbonate.[205]

12 β-D-Xylosidases

β-D-Xylosidase from a commercial *Aspergillus niger* preparation was purified by differential ammonium sulphate precipitation and either gel permeation or cation-exchange chromatography, giving 16-fold purification in 32% yield for the first technique or 27-fold purification in 19% yield for the second.[206] The second method in addition almost completely removed interfering β-D-glucosidase activity.

Aspergillus niger β-D-xylosidase has been characterized (activation energy 13.4 kcal mol, pH optimum 3.0, K_m for 2-nitrophenyl β-D-xylopyranoside 0.200 mM).[207] The enzyme was highly stable at pH 4, where lowest rates of decay occurred, and at temperatures of 65 °C and below.

Five different D-xylanases and a β-D-xylosidase in the culture medium of *Aspergillus niger* have been purified to homogeneity from 13- to 52-fold by a procedure of gel and hydroxyapatite chromatography.[208] The strain was isolated from soil of the African equatorial forest. Gel chromatography of the purified enzymes indicates that three of the D-xylanases have molecular weights of 31 000 and the other two D-xylanases have molecular weights of 50 000. β-D-Xylosidase has a molecular weight of 78 000. The pH curves of the D-xylanases were quite diverse and showed pH optima ranging from 4.0 to 6.5. Characteristic action patterns were obtained for each of the purified D-xylanases by gel chromatography of the D-xylan digests on Bio-Gel P-2. The enzymes degraded L-arabino-D-xylan by an endo-mechanism, producing L-arabinose, D-xylose, xylobiose, and a mixture of branched L-arabinose-D-xylose and D-xylose oligosaccharides. All D-xylanases seemed to be capable of liberating L-arabinose from either L-arabino-D-xylan or the L-arabinose-D-xylose oligosaccharides. Branched L-arabinose-containing D-xylose oligosaccharides were slowly hydrolysed, so that these sugars accumulate in the digest. Two D-xylanases showed relatively broad

[205] A. P. Corfield, J.-M. Beau, and R. Schauer, *Z. Phys. Chem.* (*Leipzig*), 1978, **359**, 1335.
[206] G. B. Oguntimein and P. J. Reilly, *Biotechnol. Bioeng.*, 1980, **22**, 1127.
[207] G. B. Oguntimein and P. J. Reilly, *Biotechnol. Bioeng.*, 1980, **22**, 1143.
[208] M. John, B. Schmidt, and J. Schmidt, *Can. J. Biochem.*, 1979, **57**, 125.

substrate specificity and were able to degrade also crystalline cellulose. β-D-Xylosidase showed optimal activity at pH 6.7 to 7.0 and at 42 °C. The K_m for 2-nitrophenyl β-D-xylopyranoside was 0.22 mM and xylotriose was hydrolysed more rapidly than xylobiose.

For several alkyl β-D-xylopyranosides having branched-chain cyclic, and substituted aglycon groups, and binding to β-D-xylosidase from *Bacillus pumilus* the binding constant K_i and (for some of them) the thermodynamic equilibrium ΔH^0, ΔS^0, and ΔG^0 have been determined.[209] Although the aglycon is bound through hydrophobic forces, no simple relationships between the binding parameters and the relative hydrophobicity of the alkyl β-D-xylopyranosides could be demonstrated. All of the available evidence suggests that the aglycon subsite has a highly specific structure, which forces the atoms of the aglycon group to occupy well defined positions. The supplementary energy-requirements resulting from the imposed restrictions seem to be the main reason for the irregular way in which the binding parameters depend on the aglycon structure.

The binding of D-xylose and of a series of n-alkyl β-D-xylopyranosides and their 1-thio-analogues to β-D-xylosidase from *Bacillus pumilus* has been investigated.[210] The binding constants and thermodynamic equilibrium parameters ΔH^0 and ΔS^0 have been determined. The enzyme does not distinguish between α- and β-D-xylopyranose. Although the enthalpy of binding of D-xylose is very favourable, the overall free-energy is small, owing to a large decrease in entropy. Furthermore, all of the evidence available suggests that the aglycon group is bound by unspecific hydrophobic forces. However, simple correlations between the binding parameters and the relative hydrophobicity of the compounds could not be found. Unexpectedly, no parallelism between binding of n-alkyl β-D-xylopyranosides and the corresponding 1-thio-derivatives was found.

The binding and hydrolysis of alkyl and aryl β-D-xylopyranosides and 1-thio-β-D-xylopyranosides of the β-D-xylosidase of *Penicillium wortmanni* have been studied.[211]

Enzymatically active immobilized forms of β-D-xylosidase have been obtained by glutaraldehyde cross-linking to alkylamine porous silica[206] and to alumina with titanium(IV) chloride.[207]

13 *endo*-α-D-2-Amino-2-deoxygalactanases

Culture fluid of *Clostridium perfringens* hydrolysed the synthetic chromogenic substrates, 1-*O*-(2- or 4-nitrophenyl)- and 1-*O*-phenyl-2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl α-D-galactopyranoside to 3-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl α-D-galactopyranoside and the aglycon. Such assays facilitated the characterization and purification of this *endo*-α-D-2-acetamido-2-deoxygalactanase activity.[212] This activity was purified 1200-fold by fractionation with

[209] E. Van Doorslaer, H. Kersters-Hilderson, and C. K. De Bruyne, *Carbohydr. Res.*, 1980, **78**, 317.

[210] H. Kersters-Hilderson, E. van Doorslaer, and C. K. De Bruyne, *Carbohydr. Res.*, 1980, **78**, 163.

[211] F. Deleya, M. Claeyssens, and C. K. De Bruyne, *Can. J. Biochem.*, 1980, **58**, 5.

[212] R. A. Di Cioccio, P. J. Klock, J. J. Barlow, and K. L. Matta, *Carbohydr. Res.*, 1980, **81**, 315.

ammonium sulphate and chromatography on columns of Sephadex®-G200, DEAE-Sephadex, and hydroxyapatite. The final preparation showed activity over a broad range of pH, with an optimum at 9.0, but less-pure material had two pH optima, 4.0 and 9.0.

14 *endo*-β-D-2-Acetamido-2-deoxyglucanases

An autolytic glycoside hydrolase (probably an *endo*-2-amino-2-deoxyglucanase) from a lysozyme-resistant strain of *Bacillus cereis* capable of cleaving the glycosidic linkages of *N*-unsubstituted 2-acetamido-2-deoxy-D-glucose in the cell-wall peptidoglycan was studied.[213] This glycosidase activity, together with *N*-acetylmuramyl-L-alanine amidase activity was found in an autolytic enzyme preparation obtained from the 20 000 × *g* precipitate fraction by means of autolysis followed by ammonium sulphate fractionation. The major saccharide fragments resulting from digestion of the untreated, non-*N*-acetylated, cell-wall peptidoglycan of *B. cereus* with the autolytic enzyme preparation were identified as *N*-acetylmuramyl-2-amino-2-deoxy-D-glucose and its dimer. The peptidoglycan *N*-acetylated with acetic anhydride could also be digested with the same enzyme preparation, giving *N*-acetylmuramyl-2-acetamido-2-deoxy-D-glucose and its dimer as the major saccharide fragments.

A novel *endo*-β-D-2-acetamido-2-deoxyglucanase has been characterized in a culture filtrate from a Basidiomycete.[214] This enzyme hydrolyses biantennary monosialo- and asialo-glycoasparagines of the *N*-acetyl-lactosaminic type. *endo*-β-D-2-Acetamido-2-deoxyglucanase from the Basidiomycete released from different glycoasparagines, β-D-Glc*p*NAc-(1→4)-*N*-[^{14}C]acetyl-L-Asn, α-L-Fuc*p*-(1→6)-β-D-Glc*p*NAc-(1→4)-*N*-[^{14}C]acetyl-L-Asn and oligosaccharides, which have been separated by paper and thin-layer chromatography.

Clostridium perfringens and isolated walls of this organism autolysed rapidly when incubated in buffer at pH 7.0 with the release of free-reducing groups but no *N*-terminal amino-acids.[215] The predominant autolytic enzyme was an *endo*-β-D-2-acetamido-2-deoxyglucanase, and an *endo*-β-*N*-acetylmuramidase was also present. The autolytic enzymes could be solubilized by extraction of the organisms with 5 M-LiCl and would then subsequently bind to and rapidly lyse walls of *Micrococcus luteus* and, more slowly, formamide-extracted walls of *C. perfringens* and walls of *Bacillus subtilis*. Lysis of *C. perfringens* walls by these extracted enzymes could not be demonstrated.

15 α-Amylases

Kinetic aspects of the enzymic hydrolysis of starch have been given an extended mathematical treatment.[216]

[213] S. Kawagishi, Y. Araki, and E. Ito, *J. Bacteriol.*, 1980, **141**, 137.
[214] S. Bouquelet, G. Strecker, J. Montreuil, and G. Spik, *Biochimie*, 1980, **62**, 43.
[215] R. Williamson and J. B. Ward, *J. Gen. Microbiol.*, 1979, **114**, 349.
[216] T. V. Subramanian, *Biotechnol. Bioeng.*, 1980, **22**, 661.

The expression of the activity of α-amylase samples in international units has been discussed.[217]

The summary of a conference on α-amylase assay: current state and future development concluded that α-amylase can be measured continuously with the aid of 4-nitrophenyl D-glucosides especially 4-nitrophenyl maltotrioside; the large-scale enzymatic synthesis of this compound seems to be possible.[218] Another method, which does not suffer from interference by endogenous glucose, consists of the hydrolysis of maltotetraose by α-amylase, followed by the determination of maltose. The substrate and auxiliary enzymes are, however, relatively expensive. Continuous methods, based on the measurement of the glucose released by α-amylase, are more sensitive. However, they suffer from interference by blood sugar, with exception of mechanized procedures, which remove D-glucose by gel filtration of the sample. Moreover these methods need α-D-glucosidase, which degrades malto-oligosaccharides consisting of less than seven glucose units, whereas higher polymerized substrates show slower degradation rates by amylase, and the kinetics are not easy to understand.

The Beckman Enzymatic Amylase-DS Method with maltotetraose as substrate for α-amylase measurement has been evaluated. Findings indicate that the method is approximately twice as sensitive as the old method involving soluble starch (*Clin. Chem.*, 1978, **24**, 762).[219] The new method also shows a linear rate of reaction, in contrast to the curvilinear rate previously observed with the old method. The K_m with maltotetraose is $0.77\ g\ l^{-1}$, or about twice that with soluble starch ($0.42\ g\ l^{-1}$). The method correlates well with the Phadebas α-amylase method ($r = 0.974$ and 0.991 on 84 serum and 52 urine specimens, respectively). Of 43 specimens with high concentrations of pyruvate examined for interference with α-amylase activity, only six showed interference when maltotetraose was the substrate. (With both Beckman methods the reaction of pyruvate with NADH-produced lactate and NAD^+ in the presence of lactate dehydrogenase as contaminant.) Pyruvate interference decreased with increases in (*a*) the α-amylase activity of the specimen, (*b*) the amount of NADH initially present in the maltotetraose reagent, and (*c*) the length of time the reconstituted maltotetraose reagent was allowed to stand before being used.

The Harleco α-D-glucosidase–hexokinase–D-glucose 6-phosphate dehydrogenase-coupled α-amylase method has been assessed by use of the GEMSAEC centrifugal analyser.[220] Performance evaluation included kinetic studies of substrate and maltose hydrolysis as well as effects of endogenous D-glucose and D-fructose. The reagent was found to give a linear response with α-amylase activity to greater than $1200\ U\ l^{-1}$. Within-run precision resulted in coefficients of variation (CV) of 0.9 to 3.2% over the range studied. Day-to-day precision corresponded to CV values of 2.4 to 4.4% over the same range of α-amylase activity. Method correlation with the DuPont aca α-amylase procedure was found to be good ($r = 0.997$) for patients' sera examined.

[217] J. J. Marshall, *Clin. Biochem.*, 1980, **13**, 4.
[218] K. Lorentz, *J. Clin. Chem. Clin. Biochem.*, 1979, **17**, 499.
[219] N. Q. Hanson and W. G. Yasmineh, *Clin. Chem.*, 1979, **25**, 1216.
[220] W. H. Porter and R. E. Roberts, *Clin. Chem.*, 1978, **24**, 1620.

Human serum and urine specimens have been assayed for amylase activity by the nephelometric (I), dyed-starch (Amylochrome®) (II), and amyloclastic (III) techniques.[221] For serum, the correlation coefficients of the regression lines were: I *vs.* II, 0.978 ($n = 106$); I *vs.* III, 0.736 ($n = 110$); and II *vs.* III, 0.736 ($n = 108$). For urine, they were I *vs.* II, 0.938 ($n = 49$); and I *vs.* III, 0.752 ($n = 46$). Because calculation of the Kolmogorov–Smirnov statistic showed the distributions to be non-Gaussian, Spearman rank correlation coefficients were determined and showed that I and II correlated well but neither method correlated with III. The clinical data show that I and II gave above-normal activities in every case of pancreatitis, but III gave normal values in two of eight cases. In all cases, I and II were more sensitive, giving higher amylase activities (as compared with the upper limit of normal) than did III. The nephelometric procedure is most suitable for routine and emergency testing; the dyed-starch assay is equally sensitive and reliable, but less convenient. The amyloclastic procedure appears to be less reliable.

The results of an enzymic assay for α-amylase adapted to a centrifugal analyser (GEMSAEC), have been compared with results by the manual dyed-starch (Phadebas) amylase assay.[222] Linearity, precision, and the stability of the reagent were assessed. The kinetic procedure is rapid, precise, requires only 25 μl of serum and thus is evidently suited for both paediatric and emergency use.

The dilution–activity curve for the DuPont aca kinetic amylase method is linear to approximately six times the upper limit of the expected range.[223] Total reaction time on the aca is 4.3 min: however, there is a non-linear rate of change in absorbance for at least the first 8 min, which is consistent with a lag phase. The within-run and day-to-day coefficients of variation were 2.4% at 100 U l^{-1} and 3.6% at 114 U l^{-1}, respectively. There was no interference by marked haemolysis (haemoglobin 4 g l^{-1}) or by glucose concentrations of 10–50 g l^{-1}. Comparisons by the split-sample method between the Amylochrome procedure and the aca procedure gave correlation coefficients of 0.992 for serum and 0.989 for urine. A second comparison, between an Autoanalyser I method and the aca, gave correlation coefficients of 0.998 for serum and 0.909 for urine. The expected range for serum was 18–106 U l^{-1} and for urine 2–33 U/2 h.

New chromogenic substrates have been developed for the quantitative assay of α-amylase and (1→4)-β-D-glucanase.[224] These were prepared by chemically modifying amylose or cellulose before dyeing, to increase solubility. After dyeing, the substrates were either soluble or could be readily dispersed to form fine, gelatinous suspensions. Assays based on the use of these substrates are sensitive and highly specific for either α-amylase or (1→4)-β-D-glucanase. The method of preparation can also be applied to obtain substrates for other *endo*-hydrolases.

A new starch derivative for the determination of amylase activity has been synthesized by coupling Procion Yellow dye with starch.[225] The product of this

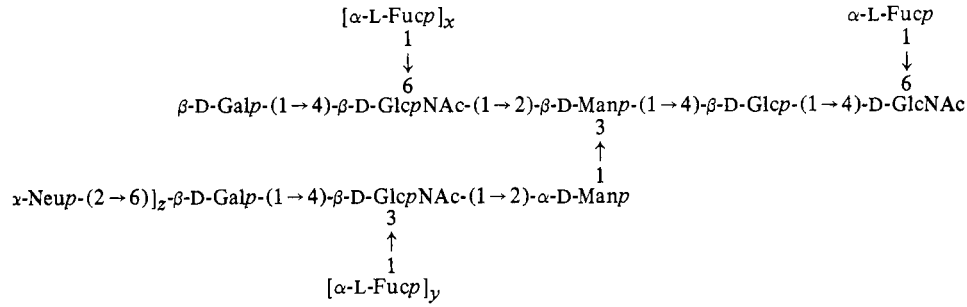
[221] D. P. Lehane, P. J. Wissert, G. Lum, and A. L. Levy, *Clin. Chem.*, 1977, **23**, 1061.
[222] M. Iosefsohn and J. M. Hicks, *Clin. Chem.*, 1978, **24**, 1644.
[223] R. M. Balkcom, C. M. O'Donnell, and E. Amano, *Clin. Chem.*, 1979, **25**, 1831.
[224] B. V. McCleary, *Carbohydr. Res.*, 1980, **86**, 97.
[225] D. H. Jung, *Clin. Chim. Acta*, 1980, **100**, 7.

reaction is intensely yellow and is easily suspended in water, in neutral buffer solution, and also in acidic solution. Amylase from pancreatin, saliva, urine, and serum readily hydrolyses this chromogenic substrate. A method for determining activity based on the use of the Procion Yellow Starch substrate is described. The procedure requires 0.1 ml of sample and an incubation time of 30 min. The soluble chromogen, which is liberated by enzymatic hydrolysis, is measured at 420 nm and the amylase activity–absorbance relationship at this wavelength is linear. The normal range for this method is 50–150 Somogyi units dl^{-1}.

A method has been reported for the simultaneous and continuous determination of lipase and amylase *in vivo*.[226]

Three-dimensional structure analyses of two 'polysaccharide-degrading' enzymes, α-amylase and α-D-mannosidase, though not yet complete, reveal that both enzymes are structural tandem duplicates and that the two similar domains within each are related by a dyad axis of symmetry.[188]

Human parotid amylase can be separated into three families of isoenzymes (A′, A, and B) by Sephadex G-75 column chromatography.[227] Isoenzymes in family B were free from carbohydrate, whereas those in family A were all glycoproteins. The carbohydrate moieties of family A isoenzymes were released from their polypeptide portions by hydrazinoylsis and labelled by reduction with sodium [$^3H_4$]borohydride. The yield of total radioactive oligosaccharides indicated that family A isoenzymes all contain single L-asparagine-linked sugar chains in one molecule. The radioactive oligosaccharides were fractionated into one acidic and two neutral oligosaccharide fractions by paper electrophoresis and paper chromatography. Their structures (3a–d) were determined by sequential glycosidase digestion in combination with a methylation study.



(3) a; $x = 1$, $y = 1$, $z = 0$
b; $x = 1$, $y = 0$, $z = 0$
c; $x = 0$, $y = 1$, $z = 0$
d; $x = 1$, $y = 0$, $z = 1$

[226] C. Trendelenburg, P. Greber, H. Munchherrmann, and J. D. Krusejarres, *Z. Anal. Chem.*, 1980, **301**, 167.
[227] K. Yamashita, Y. Tachibana, T. Nakayama, M. Kitamura, Y. Endo, and A. Kobata, *J. Biol. Chem.*, 1980, **255**, 5635.

Pure pancreatic juice (PPJ) from healthy human volunteers and from patients with pancreatic of liver disease was subjected to isoelectric focusing and assayed for α-amylase activity.[228] In pancreatic juice from most normals, a single predominant form of amylase was found, comprising 83% of the total activity recovered, and having $pI_{app} \simeq$ pH 6.8. In pancreatic juice from six normals, variant principal forms of amylase were at found at pH ≃ 6.4 or pH ≃ 7.3, in addition to the peak at pH 6.8. Isoelectric focusing patterns of pancreatic juice from individuals with pancreatic or liver disease were indistinguishable from patterns obtained with pancreatic juice from the control group of healthy volunteers.

Human salivary amylase and pancreatic amylase were purified and characterized.[229] These amylases gave two bands and one band, respectively, each staining for both protein and sugar, after electrophoresis on sodium dodecyl sulphate-polyacrylamide gel. The relative molecular mass (M_r) of pancreatic amylase was calculated to be 60 000; for the two components (A and B) of salivary amylase the M_r were 61 000 and 64 000. The two salivary amylases were separated by chromatography on concanavalin A-Sepharose®; only component B bound to concanavalin A. The carbohydrate content of pancreatic amylase was 1.61 ± 1.02% (SD), and of salivary amylases A and B 2.18 ± 0.71% and 8.77 ± 2.28%, respectively. The salivary and pancreatic amylases had completely identical antigenicities against antibody to either. On isoelectric focusing, pancreatic amylase showed one peak at pH 7.0, salivary amylase A showed a major peak at pH 6.4 with a trace material of pH 5.9, and salivary amylase B a major peak at pH 5.9 and one minor peak at pH 6.4. Serum amylase was separated into two major peaks with isoelectric points (p*I*) of 6.4 and 7.0, respectively, and one minor peak, with a p*I* of 5.9. Only a small part of the serum amylase with a p*I* of 5.9 combined with concanavalin A; the two other serum amylases did not.

The hydrolytic properties of the isoenzymes of human pancreatic and salivary α-amylase were studied.[230] The eight pancreatic isoenzymes split glycogen and starch into D-glucose, maltose, maltotriose, maltotetraose, and oligosaccharides of 5–10 D-glucose units. Maltotetraose is further digested to lower homologues. The percentage of conversion to these products is dependent on the substrate and varies from one isoenzyme to another. The six salivary isoenzymes split glycogen and starch into maltose, maltotriose, maltotetraose, panose, and oligosaccharides of 5–10 glucose units. Maltotetraose and panose are further digested to lower homologues. The percentage of conversion to these products is dependent on the substrate and is specific for each isoenzyme.

The kinetic studies on the reactions of human pancreatic and salivary α-amylases with several malto-oligosaccharides (maltotetraose, maltopentaose, maltohexaose, and maltoheptaose) were carried out.[231] The susceptibility to hydrolysis with human pancreatic α-amylase decreased in the order of maltopentaose, maltohexaose, maltotetraose, and maltoheptaose, while with human salivary α-amylase maltopentaose was hydrolysed slightly lower than malto-

[228] I. G. Renner, S. B. Abramson, and A. P. Douglas, *Clin. Chim. Acta*, 1979, **99**, 259.
[229] T. Takeuchi, *Clin. Chem.*, 1979, **25**, 1406.
[230] M. J. Kaczmarek and H. Rosenuund, *Clin. Chim. Acta*, 1977, **79**, 69.
[231] N. Saito, T. Horiuchi, M. Yoshida, and T. Imai, *Clin. Chim. Acta*, 1979, **97**, 253.

hexaose but fairly faster than maltotetraose or maltoheptaose from a viewpoint of the rates of reactions based on the amount of substrate changed. The relative rates of production of substrates, utilized in the coupled yeast α-D-glucosidase reaction, increased in the order of maltoheptaose, maltohexaose, maltotetraose, and maltopentaose with human pancreatic α-amylase, whereas with human salivary α-amylase in the order of maltoheptaose, maltotetraose, maltohexaose, and maltopentaose. Thus maltopentaose was considered to be the best substrate over maltotetraose, maltohexaose, or maltoheptaose for the α-D-glucosidase-coupled method of α-amylase determination.

A comparative study of salivary α-amylase from infants less than 15 days old and from adults has been performed. Amylase-specific activity of adult saliva was almost nine times higher than in the newborn.[232] Molecular forms of amylase from infant saliva showed higher electrophoretic mobility than those from adults, shown by the glycosidated, odd fractions. Amylase from infants presented a greater proportion of the faster, deamidated forms and showed greater stability to heating. There were no differences in action patterns or in the effect of chloride concentration upon enzymic activity. The findings could indicate differences in the carbohydrate moiety of the enzyme that impose conformational changes upon its molecule.

Ectopic production of salivary-type amylase was demonstrated in a human gastric carcinoma cell line (KMK-2) maintained *in vitro* for more than 3 years.[233] Electron-dense granules, which appeared as zymogens, were observed in the tumour tissue, in cancer cells in the peritoneal fluid (from which the present cell line had been derived), and in cultured cells in early passages. These granules, however, decreased and gradually disappeared during cultivation. Although such a morphologic alteration was recognized, the property of amylase synthesis has been maintained. The presence of α-amylase in the cultured cells was demonstrated cytochemically by the immuno-peroxidase method. The enzyme secreted and accumulated in the culture medium was partially purified and characterized. α-Amylase of KMK-2 cells closely resembled salivary-type amylase in gel filtration profile and disc gel electrophoresis. Immunological cross-reaction was observed between these enzymes. Secretion into the medium was constant, and the enzyme concentration in the cytoplasm was relatively high when the cells had reached confluence. Prednisolone increased the α-amylase production two-fold in the cells.

A simplified and relatively rapidly performed chromatographic method for measuring the principal isoenzymes of α-amylase in human serum and urine has been developed.[234] It is hoped that this method may help make amylase isoenzyme analysis more readily applicable in the ordinary clinical setting.

A DEAE-cellulose mini-column method has been developed, which allows for the separation and quantitation in human sera and urine of pancreatic- and salivary-type α-amylase isoenzymes.[235] Determination of the α-amylase iso-

[232] S. L. Bellavia, J. Moreno, E. Sanz, E. I. Picas, and A. Blanco, *Arch. Oral. Biol.*, 1979, **24**, 117.
[233] H. Nomura, S. Tokumitsu, and T. Takeuchi, *J. Natl. Cancer Inst.*, 1980, **64**, 1015.
[234] L. Fridhandler and J. E. Berk, *Clin. Chim. Acta*, 1980, **101**, 135.
[235] J. Stepan and J. Skrha, *Clin. Chim. Acta*, 1979, **91**, 263.

enzymes was found to be of value in differentiation of hyperamylasemias owing to disorders of the pancreas and parotid gland.

In a study of continuously measuring procedures for the determination of α-amylase in serum comparison was made with amyloklastic and chromolytic methods.[236] α-Amylase has been measured in 57 patients' sera with normal to highly elevated amylase activities. The results obtained with four enzymatic-kinetic test-kits have been compared with those from five conventional marketed procedures. The results are evaluated by regression analysis. The specificity of the methods was analysed with amylase samples from human pancreas, human saliva, and pig pancreas. The susceptibility of the kinetic procedures to interferences is smaller. The problems of conversion of activity units and reference ranges are discussed. The UltraZyme and the Monamyl new methods have been found superior to the other procedures because of their good practicability, precision, and broad linear range; their specificity for diagnostic purposes in pancreatic diseases is less favourable compared with that of conventional procedures.

In a study of the kinetic determination of α-amylase in serum and urine with an oligosaccharide as substrate, modification for a fully mechanized enzyme-measuring device, the author reports a modification of the u.v. method Ultra-Zyme Plus α-Amyl Harleco and the adaptation to the Eppendorf Enzymautomat 5010.[237] α-Amylase acts on an oligosaccharide mixture yielding maltose, which is hydrolysed by α-D-glucosidase. The liberated D-glucose is determined specifically by the hexokinase–glucose-6-phosphate dehydrogenase (NAD^+-dependent) method. NADH, resulting from the reaction of high concentrations of endogenous D-glucose, *e.g.* in urine, is oxidized to NAD^+ by addition of pyruvate, lactate dehydrogenase, and ATP. Thereafter the lactate dehydrogenase reaction is stopped by addition of oxamate and the α-amylase activity is measured.

A direct, sensitive, quantitative method for determining serum amylase isoenzyme activity with commercially available reagents has been reported.[238] Day-to-day reproducibility (CV) was 3–4% for the isoenzymes in normal serum; within-run precision was 8, 3, and 2% for low, normal, and high isoenzyme activities. Amylase isoenzymes, separated into the pancreatic and salivary types by electrophoresis in polyacrylamide gel, are then quantified by directly incubating the gels in soluble-starch solution, staining with iodine, and densitometry. The proportion of pancreatic isoenzyme (47 normal sera) was 43 ± 8% (mean ± SD). Isoenzyme activities as low as 2% of normal can be measured accurately in 10 μl of serum. The reproducibility, precision, and sensitivity indicate that the method is applicable to differential diagnosis of hyperamylasemia or hypoamylasemia, and is suited for monitoring the subtle changes in serum amylase isoenzyme distribution that may accompany disease progression or therapy.

[236] H. Meier, E. Henkel, and H. Dankert, *J. Clin. Chem. Clin. Biochem.*, 1979, **17**, 709.
[237] E. Henkel, *J. Clin. Chem. Clin. Biochem.*, 1979, **17**, 705.
[238] B. K. Gillard, *Clin. Chem.*, 1979, **25**, 1919.

A radioimmunoassay for human pancreatic amylase has been developed for the determination of human serum α-amylase content.[239] The assay was shown to be sensitive (7 ng ml^{-1}), reproducible and specific, but human pancreatic amylase and salivary amylase could not be distinguished by the antiserum used. In normal subjects, the mean concentration of amylase determined was found to be 122.1 ng ml^{-1} (range: 55–250 ng ml^{-1}). A good correlation was observed between the concentration of amylase and its enzymatic activity in normal subjects. In some instances with high amylase activity, however, the rise in enzymatic activity was not accompanied by increasing amount of amylase content.

A new method, which uses a differential inhibitor to measure pancreatic and salivary type α-amylase, was applied to serum samples from 46 cystic fibrosis patients (age range 4–14 years) and 50 controls of the same age group.[240] The levels of pancreatic type amylase were lower in the cystic fibrosis patients (median 26.5 i.u. l^{-1}) than the controls (median 81.5) ($P < 0.001$). The results for salivary-type enzyme, however, did not support the previously reported finding of higher than usual levels in cystic fibrosis patients. This discrepancy is probably due to differences in analytical methods.

In a patient with lung metastasis amylase isoenzyme analysis has been made of the amylase-producing tumour (plasma and pulmonary tumour).[241] An abnormal and neuraminidase-sensitive isoenzyme was noted both in plasma and tumour. Homogenates obtained from the tumour by two different mechanic disruption procedures denote an easier liberation for the salivary-type isoenzymes than for pancreatic types. It is suggested that the salivary enzymes are synthesized in the cytoplasm during many inflammatory processes and secreted without cellular necrosis.

A method for the purification of α-amylase from bovine pancreas homogenate has been described.[242] This enzyme has the same molecular weight as the porcine isoenzymes but its isoelectric point is more basic. Its amino-acid sequence is similar to that of porcine, ovine, rat, and mouse α-amylases.

The peptides obtained from a digest of isoenzyme A_{II} of porcine pancreatic α-amylase have been investigated.[243]

The role of L-histidine side-chains in reactions catalysed by porcine pancreatic α-amylase, sweet-potato β-amylase, and *Aspergillus niger* glucoamylase has been studied by using diethyl pyrocarbonate, a specific protein reagent.[244] Changes in the activity, binding affinity, and apparent kinetic parameters owing to ethoxycarbonylation have been determined. For pancreas α-amylase, four of the eight L-histidine groups, for sweet-potato β-amylase, six of the seven L-histidine groups, and for *A. niger* glucoamylase, four of the six L-histidine groups

[239] Y. Takatsuka, T. Kitahara, K. Matsuura, M. Ogawa, M. Azukizawa, K. Miyai, and G. Kasaki, *Clin. Chim. Acta*, 1979, **97**, 261.
[240] D. Kenny, A. Cooke, E. Tempany, and K. F. McGeeney, *Clin. Chim. Acta*, 1978, **89**, 429.
[241] M. Le Cam-Sagniez and J. Y. Le Reste, *Clin. Chim. Acta*, 1978, **90**, 225.
[242] C. Seigner and G. Marchis-Mouren, *C. R. Hebd. Seances Acad. Sci.*, 1980, **290**, 1541.
[243] B. Meloun, I. Klum, and L. Moravek, *Collect. Czech. Chem. Commun.*, 1980, **45**, 2572.
[244] A. Hoschke, E. Laszlo, and J. Hollo, *Carbohydr. Res.*, 1980, **81**, 145.

were shown to be ethoxycarbonylated. Ethoxycarbonylation occurred as an apparent first-order reaction, with rate constants in the range $3.6–4.9 \times 10^{-2}$ min^{-1}. Ethoxycarbonylation of the L-histidine group at the active centre rapidly inactivated α-amylase, whereas the other three groups are not located in the active centre, although activity and substrate binding are only slightly affected by their modification. For β-amylase and glucoamylase, only slight or no change in activity could be detected on ethoxycarbonylation, whereas significant changes were observed in the binding affinity.

The role of exposed L-tyrosine side-chains in enzyme-catalysed reactions has been studied for porcine-pancreatic α-amylase, sweet-potato β-amylase, and *Aspergillus niger* glucoamylase using *N*-acetylimidazole as the specific protein reagent.[245] The changes in activity, binding affinity ($\Delta K_{-1}/k_{+1}$), and kinetic parameters (K_m, k_2) owing to acetylation of the phenolic hydroxy-groups have been determined. Acetylation of each enzyme occurred by an 'apparent' first-order reaction with a rate constant of $0.72–1.4 \times 10^{-1}$ min^{-1}. Acetylation increased the apparent K_m (soluble starch as the substrate) for each enzyme (appreciably for α-amylase and glucoamylase), whereas k_2 remained unchanged. Similarly, for each enzyme, the binding affinity for immobilized cyclomaltohexaose decreased appreciably, whereas the catalytic activity was reduced only to a small degree (and remained unchanged for β-amylase). It is concluded that the L-tyrosine groups located in the active centre of each enzyme have a substrate-binding function.

In dispersed acini from guinea-pig pancreas several pancreatic secretagogues increased calcium outflux, cyclic guanosine monophosphate, and amylase secretion, whereas nitroprusside and hydroxylamide increased cyclic guanosine monophosphate but did not increase calcium outflux or amylase secretion and did not alter the action of secretagogues on calcium outflux or amylase secretion.[246]

A study of the distribution of some adsorbed and intrinsic enzymes between the mucosal cells of the rat small intestine and the apical glycocalyx separated from them has included work on α-amylase.[247]

Biochemical and immunochemical studies of α-amylase from the salivary glands of the mouse have been reported.[248]

A study has been made of changes in amylase activity in submandibular salivary glands of puberal male mice following castration;[249] although there was an initial decrease in the mean amylase activity in the experimental gland compared with controls up to 6 weeks postcastration, by 8–12 weeks postcastration it returned to control level. Postcastration levels of amylase activity varied considerably, indicating that its synthesis in the submandibular salivary gland was disturbed, but not permanently suppressed by castration.

[245] A. Hoschke, E. Laszlo, and J. Hollo, *Carbohydr. Res.*, 1980, **81**, 157.
[246] J. D. Gardner and A. J. Rottman, *Biochim. Biophys. Acta*, 1980, **627**, 230.
[247] A. M. Ugolev, L. F. Smirnova, N. N. Iezuitova, N. M. Timofeeva, N. M. Mityushova, V. V. Egorova, and E. M. Parshkov, *FEBS Lett.*, 1979, **104**, 3.
[248] A. V. Nieuw Amerongen, C. H. Oderkerk, A. P. Vreugaenhil, and P. A. Rookema, *Arch. Oral. Biol.*, 1979, **24**, 945.
[249] M. Shear, L. V. Christensen, M. Haralambous, and F. Barbakow, *Arch. Oral. Biol.*, 1979, **24**, 185.

The levels of α-amylase in submandibular salivary glands of castrated and control male mice have been compared.[250] The amylase activities in the glands of the intact and castrated animals did not differ significantly until the animals were 28–30 weeks old, and then activity was greater in the castrated animals. In the control animals, there appeared to be an age-dependent rate of amylase synthesis, which was possibly disturbed by castration. It is suggested that castration disclosed the contributing, or compensating, influence of one or more regulatory factors, other than testosterone, pertaining to the synthesis of amylase.

As part of a programme of studies on natural plant enzyme inhibitors, α-amylase inhibitors and amylases in plant tumours have been investigated.[251] Among 13 tubers and bulbs investigated, colocasia (*Colocasia antiquorum*) and local yam (*Dioscorea alata*) tubers exhibited the presence of high molecular weight proteinaceous material inhibiting human salivary, human pancreatic, and porcine pancreatic amylases. The ratios of magnitude of inhibition of these three enzymes were 6.0 : 3.5 : 1.0 by colocasia and 1.7 : 1.4 : 1.0 by yam inhibitors. The inhibitor of colocasia was more heat-stable and acid-stable than the yam inhibitor. Pepsin, trypsin, and chymotrypsin had no action on colocasia inhibitor, whereas the yam inhibitor was inactivated by trypsin and chymotrypsin. Tubers of colocasia, *D. esculenta* and *Alocasia macrorhiza* possessed amylase activity. *Dioscorea esculenta* amylase was highly heat-stable, whereas the alocasia enzyme was labile. [H_4]-e.d.t.a. treatment had no effect on these two analyses but caused 30% loss of enzyme activity in colocasia.

Conquest barley kernels were germinated for five days on moist filter paper or steeped for two days and then germinated in moist air for three days.[252] Samples of kernels were collected each day and freeze-dried. Isoelectric focusing analysis showed that three main groups of α-amylase were synthesized in both series. Rocket-line immunoelectrophoresis and combined isoelectric focusing and crossed immunoelectrophoresis showed that (*a*) α-amylase activity and the amount of α-amylase protein increased in parallel during germination, (*b*) steeping preferentially inhibited the synthesis of α-amylase I, and (*c*) steeping decreased the α-amylase II : α-amylase III ratio during germination.

The ability of pullulanase to stimulate the enzymic digestion of raw starch by barley-malt α-amylase and sweet potato β-amylase has been investigated.[253] The pullulanase had little effect on the α-amylolysis of waxy-maize starch granules but stimulated β-amylase greatly.

The effects of barley malt, fungal α-amylase, and bacterial α-amylase on starch crystallization and organization in staleing breads were studied by *X*-ray diffraction.[254] Comparison of *X*-ray patterns of fresh and stored breads showed an order of decreasing degree of starch crystallinity as follows: bread with bacterial α-amylase, bread with cereal α-amylase, bread with fungal α-amylase,

[250] M. Shear, L. V. Christensen, M. Haralambous, and F. Barbakow, *Arch. Oral. Biol.*, 1979, **24**, 113.
[251] B. Shivaraj, K. K. Sharma, and T. N. Pattabiraman, *Indian J. Biochem. Biophys.*, 1979, **16**, 52.
[252] A. W. MacGregor and J. Daussant, *Cereal Chem.*, 1979, **56**, 541.
[253] S. Ueda and J. J. Marshall, *Carbohydr. Res.*, 1980, **84**, 196.
[254] R. D. Dragsdorf and E. Varriano-Marston, *Cereal Chem.*, 1980, **57**, 310.

and unsupplemented bread. Those results were in direct contradiction to bread-firming data, indicating that starch crystallinity and bread firming are not synonymous. Starch crystalline organization also was affected by enzyme supplementation. Starches from breads containing bacterial enzyme supplements exhibited 'V_h' and 'A' structures, whereas the control had 'V_h' and 'B' structures. A possible role of starch crystal structure in bread firming was postulated.

Using a crude membrane fraction prepared from isolated aluerone layers, the secretory tissue of barley grains,[255] it was found that tunicamycin inhibited the secretion of glycoprotein-α-amylase by 60 to 80%. Intracellularly, however, no α-amylase was found to accumulate. On the other hand, tunicamycin did not inhibit the rate of total protein synthesis by more than 10%. The possibility is discussed that the synthesis of the protein portion of glycoproteins is specifically inhibited, when glycosylation is prevented.

During germination the aleurone layer of barley (*Hordeum distichon*) grains becomes progressively less able to form more α-amylase in response to a dose of gibberellic acid (GA_3).[256] This decline appears to be linked to the presence of a growing embryo. In whole grains the embryo 'modulates' the response (α-amylase formation) to controlled external applications of GA_3 in a dose-dependent manner. Sugars, and some other metabolites, repress α-amylase formation in transected grains, apparently by reducing levels of endogenously produced gibberellins. This effect is partly, but not completely, reversed by additions of GA_3. External applications of GA_3 augment the levels of several gibberellin fractions within the grain. The nature of the gibberellin material remaining on the surface of the grains alters with time. Grains treated with GA_3 contain a conjugate of low biological activity, possibly a glycoside, that is hydrolysed by a mixed glycosidase preparation to release a biologically-active gibberellin resembling GA_3.

Antibody directed against the purified α-amylase inhibitor from *Phaseolus vulgaris* was obtained from rabbits and characterized by immunoelectrophoresis and inhibitor neutralization.[257] Polyvalent serum diffusing against homogeneous inhibitor and monovalent serum diffusing against a crude bean extract each afforded a single precipitin band. After electrophoresis, α-amylase-inhibitor activity was detectable at the same position as the immunoprecipitate. Crude bean meal extracts obtained with different extraction media cross-reacted with anti-inhibitor serum. When compared in a double diffusion assay, fused single precipitin bands were seen. Within the limits of the technique it has been shown that the inhibitor is apparently not grossly modified by either of the extraction methods and, further, that multiple forms of the inhibitor do not occur. Analysis by this procedure of seven different cultivars of *P. vulgaris* suggest that, although the relative amount may vary, these inhibitor proteins are immunologically identical. The extract from one variety of *Vicia faba* contained neither inhibitor activity nor immunologically cross-reactive material. Leaves and immature fruits of bean plants raised from these seeds were also examined for α-amylase-inhibitor activity. Leaves of all plants are devoid of antigenic material and of inhibitor

[255] H. Schwaiger and W. Tanner, *Eur. J. Biochem.*, 1979, **102**, 375.
[256] M. T. Smith and D. E. Briggs, *Phytochemistry*, 1980, **19**, 1025.
[257] K.-H. Pick and G. Wober, *Z. Phys. Chem. (Leipzig)*, 1978, **359**, 1379.

activity, whereas a weak cross-reaction was observed with fruit extracts from two varieties. It is concluded that α-amylase inhibitor is synthesized in the final stage of seed maturation.

α-Amylase inhibitors were extracted from kernels of five hard winter wheat varieties that were grown at different locations in two crop years and that had been evaluated for susceptibility or resistance to the rice weevil, *Sitophilus oryzae*.[258] Inhibitor activity was assayed with larval α-amylase from two species of stored grain pests, the rice weevil and the yellow mealworm, *Tenebrio molitor*. Correlation in some wheat varieties was observed *in vivo* resistance to the insect and the extent of *in vitro* inhibition of the insect larval α-amylase by the extracted inhibitors. These results indicate that α-amylase inhibitors in wheat could be involved in the resistance of wheat to postharvest infestation.

α-Amylase from germinating tubers of a yam *Dioscorea dumetorum* was extracted and purified by four steps of purification. A total yield of 23.1% was obtained with over 1600-fold increase in specific activity.[259] Three distinct amylolytically active protein forms were resolved upon treatment of the preparation on DEAE-cellulose ion-exchange chromatography at pH 8.3. All the partially purified α-amylase fractions have similar physical properties with respect to pH optimum, K_m values, molecular weights, and energies of activation. Qualitative analysis of the α-amylase–amylose digest revealed variable product specificity for the three α-amylase fractions. One form exhibited a dual product specificity for the formation of maltose and maltohexaose, whereas another form produced exclusively maltopentaose from polysaccharide substrates. The third α-amylase fraction showed usual action pattern characteristic of most α-amylases.

Enzymes of starch synthesis and degradation were identified in crude extracts of the unicellular green alga *Dunaliella marina*.[260] By polyacrylamide gel electrophoresis and specific staining for enzyme activities, four multiple forms of starch synthetase, two amylases, and at least two forms of α-D-glucanphosphorylase were visible. Using specific α-D-glucans incorporated into the gel before electrophoresis a tentative correlation of α-amylase and β-amylase with both hydrolytic activities was made. The activities of α-D-glucan phosphorylase and amylase(s) were measured quantitatively in crude extracts, and the concomitant action of α-D-glucan phosphorylase and amylase(s) was found to account for the fastest rate of starch mobilization observed *in vivo*. Isolated chloroplasts retained both typical plasmid marker enzymes and ADP-D-glucose pyrophosphorylase, starch synthetase, amylase(s), and α-D-glucan phosphorylase to a similar percentage. Gel electrophoresis analysis followed by staining for enzyme activity of a stromal fraction resulted in a pattern of multiple forms of starch-metabolizing enzymes analogous to that found in a crude extract. The combined data was interpreted as indicating the exclusive location *in vivo* of starch-metabolizing enzymes in chloroplasts of *D. marina*.

[258] M. A. Yetter, R. M. Saunder, and H. D. Boles, *Cereal Chem.*, 1979, **56**, 243.
[259] L. O. Emiola, *J. Biochem. (Tokyo)*, 1980, **87**, 289.
[260] E. Kombrink and G. Wober, *Planta*, 1980, **149**, 130.

The α-amylase activities of thermophilic fungi causing deterioration of tobacco products in Nigeria have been assessed.[261]

The nature of thermophilic amylolytic enzymes produced by a strain of *Aspergillus niger* was influenced by media composition.[262] Although D-glucose, maltose, dextrin, and soluble starch induced the production of glucoamylase, pectin, and sodium polypectate almost exclusively induced for formation of α-amylase. Alkali ions suppressed the formation of glucoamylase. NH_4NO_3, $(NH_4)_2HPO_4$, and $(NH_4)_2C_4H_4O_6$ served as good inorganic nitrogen sources, whereas casein, groundnut protein, and corn steep liquor served to a similar capacity as nitrogen sources from organic material. Deletion of the growth factors pyridoxine hydrochloride and inositol suppressed enzyme production, but the absence of riboflavin had the opposite effect.

The molecular structure of Taka-amylase A, an α-amylase from *Aspergillus oryzae*, has been studied at 6 Å resolution by *X*-ray diffraction analysis.[263] The electron density map showed a non-crystallographic three-fold screw arrangement of the molecules in the crystal. The molecule is an ellipsoid with approximate dimensions of $80 \times 45 \times 35$ Å and contains a hollow which may correspond to the active centre. The inhibitor molecules bind to Taka-amylase A at four different sites, one of which is located in the hollow of the enzyme. The probable position of a thiol group is discussed in connection with heavy-atom binding.

Hydrolyses of amylose with a number-average degree of polymerization (DP_n) of 17 by a single- and a mixed-amylase system containing *endo*- and *exo*-type amylases were quantitatively followed by the measurement of total reducing power, determination of D-glucose using the D-glucose oxidase–peroxidase method and of oligosaccharides by high-performance liquid chromatography, and the fluorescence method using a fluorescent dye, 2-(4-toluidinyl)-naphthalene-6-sulphonate.[264] The amylases used in this study were Taka-amylase A, bacterial liquefying α-amylase, and glucoamylase from *Rhizopus niveus*. It has been indicated that change in product distribution with time can be predicted theoretically for the amylase-catalysed reaction by using a small number of parameters (subsite parameters), based on the subsite theory (R. Matsuno *et al.*, *J. Biochem.*, 1978, **83**, 385; H. Kondo *et al.*, *J. Biochem.*, 1978, **84**, 403). It was found that this theoretical treatment could be successfully applied to the mixed amylase system by taking into account the 'simple hydrolysis', disregarding transglycosylation, condensation, and multiple attack, see Figure 2.

The structure of a major oligosaccharide of Taka-amylase A (4) is proposed based on the results of chemical (methylation and acetolysis) and enzymatic

[261] V. W. Ogundero, *Mycopathologia*, 1979, **69**, 131.
[262] N. Ramachandran, K. R. Sreekantiam, and V. S. Murthy, *Starch*, 1979, **31**, 134.
[263] Y. Matsuura, M. Kusunoki, W. Date, S. Harada, S. Bando, N. Tanaka, and M. Kakudo, *J. Biochem.* (*Tokyo*), 1979, **86**, 1773.
[264] H. Kondo, H. Nakatani, R. Matsumo, and K. Hiromi, *J. Biochem.* (*Tokyo*), 1980, **87**, 1053.

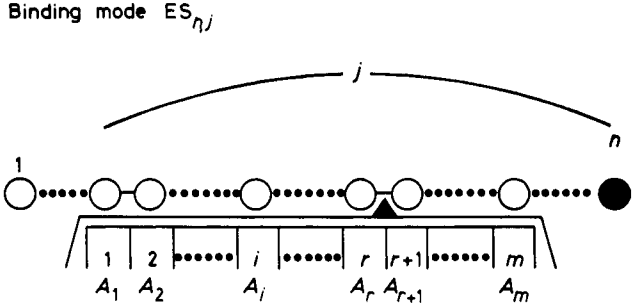
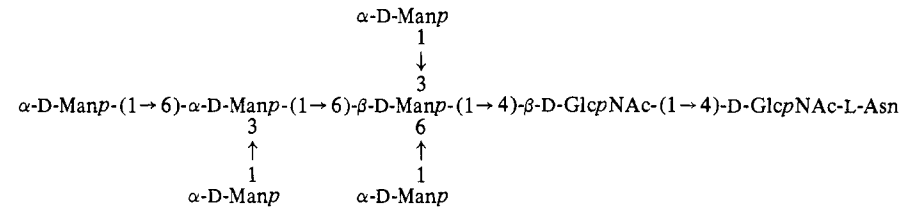


Figure 2 *Schematic illustration of the active site of an amylase and binding mode of a linear substrate. The box represents a subsite, which is numbered as indicated. The wedge (▲) shows the catalytic site at which the substrate linkage is cleaved. ○ represents a D-glucose residue of a linear substrate, for which the reducing end (●) is on the right-hand side of the figure. Subscript j represents the binding mode and is taken to be equal to the number of residues counting from the reducing end of substrate to subsite 1 of the enzyme*



(4)

(digestions with '*exo*- and *endo*-glycosidases') analyses.[265] This structure is an amendment of that proposed by Yamagushi *et al.*, *J. Biochem.*, 1971, **70**, 587, in which one more D-mannose residue is attached through a (1→2)-linkage to the α-D-mannopyranose residue, which is (1→3)-linked to the innermost α-D-mannopyranose residue.

The thermoacidophilic amylases produced by *Bacillus acidocaldarius* strains have very acid pH optima as expected but the temperature optima are not much higher than that of *Bacillus amyloliquefaciens*, which is a mesophile, and they are not as high as that of *Bacillus licheniformis*, which is a facultative thermophile.[266] Alkaline amylases produced by *Bacillus alcalophilus* subsp. *halodurans* strains, which are insensitive to sequestering agents and stable at high temperatures, expand the range of potential amylase uses to conditions of high alkalinity. Comparison of the pH and temperature profiles of different *Bacillus* species amylases demonstrates that amylases have evolved in a single genus in such a way that they can function optimally at almost any pH value and temperature. The properties of these amylases reflect extensive microbial adaptation

[265] S. Minobe, H. Nakajima, N. Itoh, I. Funakoshi, and I. Yamashina, *J. Biochem. (Tokyo)*, 1979, **86**, 1851.

[266] E. W. Boyer, M. B. Ingle, and G. D. Mercer, *Starch*, 1979, **31**, 166.

and demonstrate the wide variety of enzymes potentially available for biochemical processing.

Bacillus amyloliquefaciens α-amylase, which is commercially supplied as '*Bacillus subtilis* α-amylase', does not cross-react immunologically with *B. subtilis* α-amylase.[267] This enzyme (from *B. amyloliquefaciens*) was cleaved by treatment with cyanogen bromide into seven fragments. Peptide A was selected for sequence determination. It is the longest one, containing 185 amino-acids (*i.e.* approx. 50% of the total molecule) and connects to the hexapeptide of the *N*-terminus. Its primary structure was aligned by use of various proteolytic enzymes. The sequence of amino-acids 181–184 is identical with that of amino-acids 14–17 of the α-amylase isolated from *B. subtilis* (except that amino-acid 183 is L-asparagine rather than L-aspartic acid).

A paper on the stability of bacterial α-amylase in the starch liquefaction process states that nowadays, starch is normally liquefied by an all enzymatic process at a high temperature.[268] In 1973 a new α-amylase was introduced, which is more heat-stable than the products so far used. It is produced by a strain of *Bacillus licheniformis*. Since then, this enzyme has been recommended for use in a single dosage jet cooking process, operating at 105 °C for a few minutes followed by a 90–95 °C dextrinization period. This process is easy and more economic in terms of energy than earlier processes. This lecture deals with the stability of this enzyme, which depends on temperature, pH, starch dry substance, and salt content. A formula for the calculation of stability is given, as a function of these parameters. The degree of hydrolysis, very often measured as dextrose equivalent, can for a given process be estimated also by a formula. Results from pilot-plant trials confirming the laboratory investigations are presented. Based on the stability data it is possible to estimate optimum design parameters for the use of this enzyme in individual processes, thus minimizing enzyme cost.

The heat stability of a bacterial α-amylase is important for industrial starch utilization.[269] Although extensive studies have been done on heat stable α-amylase from various bacterial species little is known about the α-amylases of *Bacillus licheniformis*. In order to get better understanding of thermostable amylases produced by different strains of *B. licheniformis* and provide information how to utilize the enzyme in starch processing, studies on purification and characterization of a commercial heat-stable bacterial α-amylase from *B. licheniformis* BLM 1777 are reported.

New strains of *Bacillus licheniformis* and *Bacillus coagulans* producing thermostable α-amylase active at alkaline pH have been discovered and reported upon.[270]

Flow microfluorimetry, which provides detailed information on the state of a microbial population, has been employed to characterize the *Bacillus subtilis* population during time intervals in which significant changes in the culture α-amylase activity occur.[271] Four different batch experiments have been con-

[267] H. Chung and F. Friedberg, *Biochem. J* , 1980, **185**, 387.
[268] P. Rosendal, B. H. Nielson, and N. K. Lange, *Starch*, 1979, **31**, 368.
[269] J. P. Chiang, J. E. Alter, and M. Sternberg, *Starch*, 1979, **31**, 86.
[270] S. Medda and A. K. Chandra, *J. Appl. Bacteriol.*, 1980, **48**, 47.
[271] J. Fazel-Madjless and J. E. Bailey, *Biotechnol. Bioeng.*, 1980, **22**, 1657.

ducted, and the influences of inoculum age, fermentation temperature, and aeration rate on microbial population dynamics and amylase activity have been examined. Relatively high rates of amylase activity increase are observed twice during the batch, first as double cells initiate sporulation and later during germination. Rapid decreases in amylase activity are observed in highly (25–50%) sporulated populations, and, in at least one experiment, during a transition from large, rounded protoplast forms to normal rod morphology.

The amylolytic preparation of culture filtrates of *Cladosporium resinae* were fractionated by ion-exchange and molecular-sieve chromatography, and shown to contain α-D-glucosidase, α-amylase, and two glucoamylases (see p. 274).[165]

The effects of medium composition on excretion and biosynthesis of the amylases of *Schwarmiomyces castella* have been reported.[272]

The action of purified α-amylase of *Streptomyces precox* on starch granules of normal maize and potato has been studied.[273] The hydrolysis was followed by measuring total soluble carbohydrates and D-glucose released. Patterns of degradation were observed by scanning electron microscopy. The main soluble product was maltose, small amounts of D-glucose and higher oligosaccharides were always observed. By scanning electron microscopy, attack started small pits on the surfaces of maize starch granules, pits increased in number and in size, and pores penetrated into the inner portions of the granules toward the centre. The enzyme seemed to degrade the inner portion of starch granules better than the well known general α-amylases.

Selective isolation of derepressed mutants of an α-amylase yeast by use of 2-deoxy-D-glucose has been reported.[274] A relatively large number of derepressed and hyperproductive mutant strains with a relatively small investment of time, work, and material were isolated. Results with an α-amylase yeast, in addition to the results obtained earlier with β-D-fructofuranosidase yeast raise hopes that non-metabolizable repressors may become generally useful in the selective isolation of microbial mutants derepressed and hyperproductive with respect to repressible enzymes of industrial interest.

α-Amylase has been used in enzymically active immobilized forms obtained by the following routes: glutaraldehyde cross-linked on(to) silica[275] and covalent binding to hydroxyalkyl methacrylate gels.[276]

16 β-Amylases

The effects of metal ions on the determination of β-amylase activity with 3,5-dinitrosalicylic acid have been investigated.[277] These results suggest that although

[272] K. Oteng-Gyang, G. Moulin, and P. Gaizy, *Eur. J. Appl. Microbiol. Biotechnol.*, 1980, **9**, 129.
[273] T. T. Akaya, Y. Sugimoto, K. Wako, and H. Fuwa, *Starch*, 1979, **31**, 205.
[274] N. Van Uden, C. Cabeca-Silva, A. Madeira-Lopes, and I. Spencer-Martins, *Biotechnol. Bioeng.*, 1980, **22**, 651.
[275] C. G. Bohnenkamp and P. J. Reilly, *Biotechnol. Bioeng.*, 1980, **22**, 1753.
[276] K. Babor and L. Rexova-Benkova, *Collect. Czech. Chem. Commun.*, 1980, **45**, 617.
[277] R. Shinke, S. Mizuno, K. Aoki, H. Nishira, S. Giri, and Y. Singh, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 2393.

metal ions do affect the 3,5-dinitrosalicylic acid–maltose reaction, β-amylase activity can be correctly measured by using calibration curves obtained in the presence of the same metal ions and in the same concentrations as far as metal ions have no effect on the enzyme activity itself. However, it is difficult to know beforehand accurate concentrations of metal ions in an enzyme preparation. Conveniently, lower concentrations of metal ions may have little effect on the 3,5-dinitrosalicylic acid–maltose reaction. In practice, concentrations of metal ions in conventional culture media are often less than 2.5×10^{-4} M and usually lowered by dilution when the enzyme is assayed. However, when β-amylase preparations contain higher concentrations of such metal ions as Mn^{2+}, Ca^{2+}, *etc.*, much care should be taken to avoid their effects on the 3,5-dinitrosalicylic acid–maltose reaction, which bring about deviations from an accurate enzyme activity.

The β-amylase, protease, cellulase, and hemicellulase activities of eight cultivars of proso (*Panicum miliaceum*) millets were determined.[278] Two of the cultivars were grown on different plots to permit study of the effects of different locations on enzyme levels. All of the cultivars showed β-amylase, protease, cellulase, and hemicellulase activities, with the exception of one sample that showed no hemicellulase activity. Optimum pH for β-amylase activity was approximately 5.0.

The ability of pullulanase to stimulate the enzymic digestion of raw starch by sweet potato β-amylase and barley-malt α-amylase has been investigated.[253] The pullulanase greatly stimulated the action of the β-amylase on waxy-maize starch granules but had little effect on α-amylolysis.

The role of L-histidine side-chains in reactions catalysed by sweet potato β-amylase has been studied by using diethyl pyrocarbonate and also *N*-acetylimidazole, as specific protein reagents.[244,245] The changes in activity, binding affinity ($\Delta k_{-1}/k_{+1}$), and kinetic parameters (K_m, k_2) have been determined in both cases (see p. 295).

Two β-amylase components, I and II, were separated from wheat flour by DEAE-cellulose and CM-cellulose ion-exchange chromatography.[279] Component I contained three main forms and component II contained one form when analysed by polyacrylamide slab isoelectric focusing. A papain or a malted wheat extract converted the three β-amylases in component I to five forms with more basic p*I* values and the one β-amylase in component II to three forms with p*I* values identical to those arising from degradation of component I. The new β-amylases resulting from proteolytic attack were identical in p*I* values to β-amylases in germinating wheat, thus indicating that the mechanism of formation of such enzymes is *via* limited proteolytic degradation.

Specific α-D-glucans were incorporated into the gel before electrophoresis of the unicellular green alga *Dunaliella marina* and a tentative correlation of α-amylase and β-amylase with both hydrolytic activities was made.[260] Two amylases were visible on polyacrylamide gel electrophoresis and specific staining for enzyme activity (see p. 298).

[278] J. Skovron and K. Lorenz, *Cereal Chem.*, 1979, **56**, 559.
[279] J. E. Kruger, *Cereal Chem.*, 1979, **56**, 298.

Enzymically active, immobilized forms of β-amylase have been prepared by a covalent binding to hydroxyalkyl methacrylate gels,[276] by glutaraldehyde cross-linking on(to) silica,[275] and by glutaraldehyde coupling to alkylamine porous silica and to porous silica.[280]

17 Amylo-1,6-D-glucosidases

The development of amylo-1,6-D-glucosidase activity in foetal rat liver has been studied. The activity of control foetuses is high on day 17.5, decreases from day 17.5 to day 19.5, and then rises during the next days.[281] In hypophysectomized foetuses, the increase of the activity is suppressed but not the decrease. Moreover, if the mother is adrenalectomized, the decrease and the increase are abolished in hypophysectomized foetuses. Growth-hormone administration is quite effective in preventing the decrease in enzyme activity but cortisol treatment does not prevent it. In contrast, cortisol produces a precocious decrease of the activity in intact foetuses. These findings suggest that during foetal life, two hormonal regulation mechanisms are involved in the regulation of amylo-1,6-D-glucosidase activity: cortisol has a repressive effect on the enzymic activity, whereas growth hormone acts as an inducer.

18 Cellulases

The scope of improving cellulase enzyme productivities from the cellulose fermentation process is examined in laboratory-scale fermentors.[282] The maximum productivity (30 i.u. l^{-1} h^{-1}) is attained in a continuous-culture process with a cell recycler using modified medium containing 0.5% cellulose. Optimum dilution rate and recycle ratio are determined as 0.025 and 1.2 h^{-1}, respectively, for the process. The system is analysed and steady-state equations for predicting enzyme protein concentrations in the fermenter are developed. In fed-batch cultures, slow addition of cellulose at high concentrations can improve enzyme productivity by as much as 33% over a batch process. The scope and results of using modified medium for cellulase production are also presented.

A new chromogenic substrate has been developed for the quantitative assay of (1→4)-β-D-glucanase (see p. 289).[224]

A cellulase assay was developed for the continuous measurement of coloured cellulose oligosaccharides (total carbohydrates) released during enzymatic hydrolysis of dyed crystalline cellulose.[283] Several cellulosic substrates were uniformly dyed by Remalzol brilliant blue R salt without altering their physical properties. Dyed Avicel (6.5%, wt./wt.) was selected as the most representative substrate for the assay procedure. The assay was performed continuously in a simple, thermally controlled apparatus designed for filtration of the reaction mixture *via* a 5 μm-pore-size nylon filter to retain the crystalline dyed cellulose,

[280] C. C. Hon and P. J. Reilly, *Biotechnol. Bioeng.*, 1979, **21**, 505.
[281] R. Vaillant, P. Bourgeois-Victor, and A. Fairand, *Can. J. Biochem.*, 1979, **57**, 1245.
[282] T. K. Ghose and V. Sahai, *Biotechnol. Bioeng.*, 1979, **21**, 283.
[283] T. K. Ng and J. G. Zeikus, *Anal. Biochem.*, 1980, **103**, 42.

while spectrophotometrically monitoring the absorbance at 595 nm of the reaction filtrate. Crude supernatant cellulase of *Trichoderma viride* was used to test the assay procedure. The continuous assay has utility for the study of cellulase kinetics and for the comparison of activities from different micro-organisms.

The susceptibility of cellulose to enzymatic hydrolysis is affected by the structural features of cellulosic materials.[284] It has been suggested that the crystallinity and surface area of cellulose fibres are the most important structural features in this regard. This study investigated in depth the relative effects of these two structural features upon the enzymatic hydrolysis of cellulose and the change of the structural parameters of cellulose during the course of hydrolysis. It was found that the hydrolysis rate is mainly dependent upon the fine structural order of cellulose, which can best be represented by the crystallinity rather than the simple surface area. Monitoring the changes in the structural parameters during the course of reaction showed that surface area is not a major limiting factor that slows hydrolysis in its late stages as has been suggested. This information concerning structural features is used to elucidate the mode of action of cellulase.

Most cellulosic substances contain appreciable amounts of cellulose and hemicellulose, which on enzymatic hydrolysis mainly yield a mixture of D-glucose, cellobiose, and D-xylose.[285] In this paper, studies on the mechanisms of hydrolysis of bagasse (a complex native cellulosic waste left after extraction of juice from cane sugar) by the cellulase enzyme, components are described in light of their adsorption characteristics. Simultaneous adsorption of *exo*- and *endo*-glucanases on hydrolysable celluloses is the causative factor of the hydrolysis that follows immediately after. It supports the postulate of synergistic enzyme action proposed by Eriksson. D-Xylanase pretreatment enhanced the hydrolysis of bagasse owing to the creation of more accessible cellulosic regions that are readily acted upon by *exo*- and *endo*-D-glucanases. The synergistic action of the purified *exo*-D-glucanase, *endo*-D-glucanase, and D-xylanase has been found to be most effective for hydrolysis of bagasse but not for pure cellulose. Significant quantities of D-glucose are produced in β-D-glucosidase-free cellulase action on bagasse. Individual and combined action of the purified cellulase components on hydrolysis of native and delignified bagasse are discussed in respect to the release of sugars in the hydrolysate.

Cellulose-decomposing fungi have been harvested from barley grains in Egypt.[286] Their cellulolytic properties were examined.

Eight cultivars of proso (*Panicum miliaceum*) millets showed cellulase and hemicellulase activities, with the exception of one sample that showed no hemicellulase activity (see p. 303).[278]

An article, which discusses the current biological research in conversion of cellulose-orientated polysaccharide and glycoside hydrolases into liquid fuels, seeks to answer the question 'How far have we come?'[287]

[284] L. T. Fan, Y.-H. Lee, and D. H. Beardmore, *Biotechnol. Bioeng.*, 1980, **22**, 177.
[285] T. K. Ghose and V. S. Bisaria, *Biotechnol. Bioeng.*, 1979, **21**, 131.
[286] S. I. I. Abdelhafez and M. I. A. Abdelkader, *Mycopathologia*, 1980, **70**, 77.
[287] M. C. Flickinger, *Biotechnol. Bioeng.*, 1980, **22**, 27.

A satisfactory screening method for cellulolytic bacteria has been achieved with a cellulose-azure test.[288]

The cellulase activities of thermophilic fungi causing deterioration of tobacco products in Nigeria have been assessed.[261]

The cellulase production exhibited by *Acetivibrio cellulolyticus* has been reported upon.[289]

The degradation of cellulose by a newly isolated mesophilic anaerobe of the *Bacteriodaceae* has been investigated.[290]

The cellulose activity in haustoria of *Cassytha fibiformis* has been investigated.[291]

The degradation of crystalline cellulose by a strain of *Cellulomonas* and its mutant derivatives has been investigated.[292]

The decomposition of lignocellulose by *Cyathus steroreus*, a 'white rot' fungus from cattle dung has been investigated.[293]

In a study of the dynamics of cellulase synthesis in *Fusarium* species cultures particular account was taken of the influence of the cellulose substrate structure,[294] and of the influence of cellulose concentration in the culture media.[295]

The normal inducer of extracellular cellulase (C_x) from *Fusarium oxysporum* is the degradation product(s) of cellulose rather than the cellulose itself.[296] Though this enzyme is continuously produced by the fungus when grown on carboxymethylcellulose, the fungus fails to utilize carboxymethylcellulose as a source of nutrient. This enzyme does not require metal ions for its activity. Phenolics and related compounds are inhibitory to this enzyme but the inhibition is reversible by cysteine hydrochloride, sodium thioglycollate, and hydroxylamine to a significant extent. Catechol is the best inhibitor of the tested compounds. The enzyme preparation appears to consist of several components.

Two types of cellulases, Avicelase and carboxymethylcellulase from a thermophilic fungus *Humicola insolens* YH-8 were glycoenzymes.[297] Avicelase and carboxymethylcellulase contained 26.1% and 39.0%, respectively, of carbohydrate residues which consisted of D-mannose and 2-amino-2-deoxy-D-glucose. These enzymes, especially carboxymethylcellulase, showed remarkable thermal and pH-stability. The partial removal of the carbohydrate residues, 60% with mixed-glycosidase and 90% with periodate oxidation and Smith degradation, resulted in a significant decrease in thermal and pH-stability. The carbohydrate moiety was suggested to be a stabilizer of cellulases.

[288] A. M. Deschamps and J. M. Lebault, *Ann. Microbiol.*, 1980, **131A**, 77.
[289] J. N. Saddler and A. W. Khan, *Can. J. Microbiol.*, 1980, **26**, 760.
[290] A. W. Khan, J. N. Saddler, G. B. Patel, J. R. Colvin, and S. M. Martin, *FEMS Microbiol. Lett.*, 1980, **7**, 47.
[291] A. S. Reddy, M. Komariah, and S. M. Reddy, *Curr. Sci.*, 1980, **49**, 670.
[292] K. D. Haggett, P. P. Gray, and N. W. Dunn, *Eur. J. Appl. Microbiol. Biotechnol.*, 1979, **8**, 183.
[293] D. T. Wicklow, R. W. Detroy, and B. A. Jessee, *Appl. Environ. Microbiol.*, 1980, **40**, 169.
[294] Z. Targonski and C. Szajer, *Biotechnol. Lett.*, 1979, **1**, 75.
[295] Z. Targonski and C. Szajer, *Biotechnol. Lett.*, 1979, **1**, 439.
[296] G. L. Soni and I. S. Bhatia, *Indian J. Biochem. Biophys.*, 1979, **16**, 223.
[297] S. Hayashida and H. Yoshioka, *Agric. Biol. Chem.*, 1980, **44**, 481.

A cellobiohydrolase component was isolated from a *Pennicillium funiculosum* cellulase preparation by ion-exchange chromatography and purified by isoelectric focusing.[298] Purified in this way, the enzyme was homogeneous as judged by electrophoresis on sodium dodecyl sulphate–polyacrylamide gels and isoelectric focusing in polyacrylamide gels. Acting in isolation, the enzyme had little hydrolytic activity to highly ordered celluloses such as cotton fibre, but, when recombined in the original proportions with the other components [*endo*-(1→4)-β-D-glucanase and β-D-glucosidase] of the complex, 98% of the original activity was recovered. Synergistic effects were also observed when the enzyme was acting in concert with *endo*-(1→4)-β-D-glucanase from other fungal sources. Less-well-ordered celluloses, such as that swollen in phosphoric acid, were extensively hydrolysed, the principal product being cellobiose. Attack on carboxymethylcellulose (CM–cellulose), which is the substrate normally used to assay for *endo*-(1→4)-β-D-glucanase activity, was minimal. The enzyme was associated with 9% of neutral sugar, 88% of which was D-mannose. It was isoelectric at pH 4.36 (4 °C) and had a mol. wt. of 4.6×10^4.

The localization of cellulase components in a *Pseudomonas* sp. isolated from activated sludge was studied (see p. 274).[167]

In the control of the level of D-glucose formed by the cellulase system in the fungus *Pyricularia oryzae*, it appears that the rate of formation of D-glucose by β-D-glucosidase is reduced by the presence of the substrate, cello-oligosaccharide, as well as by the product, D-glucose (see p. 274).[168]

The enhanced cellulase production of a mutant of *Sclerotium rolfsii* has been noted.[299]

Sporocytophaga myxococcoides utilized several cellulosic substrates and produced extracellular carboxymethylcellulase and activity towards Avicel (a milled microcrystalline cellulose powder).[300] Both types of enzyme were synthesized in media containing D-glucose but the activity was influenced by the nature and concentration of the carbon source. The ratio of carboxymethylcellulase activities, measured by reducing-sugar production and by viscosity decreased, was also influenced by the nature of the cellulosic substrate supplied to cultures. The greatest extracellular enzyme production occurred in cultures grown on microcrystalline cellulose powders such as Avicel and Whatman CC31 at concentrations up to 4% (wt./vol.). The greatest degradation rate observed was in cultures supplied with Avicel where 60% of the substrate was degraded in 4 days.

The simultaneous production of a cellulase complex and D-glucose isomerase by *Streptomyces flavogriseus* has been reported.[301]

A saccharification of cellulosic material using culture filtrate from the stationary phase of a culture of *Thermomonospora* sp., produced primarily cellobiose up to levels inhibitory to further saccharification, whereas the use of

[298] T. M. Wood, S. I. McCrae, and C. C. Macfarlane, *Biochem. J.*, 1980, **189**, 51.
[299] J. C. Sadana, J. G. Shewale, and M. V. Deshpande, *Appl. Environ. Microbiol.*, 1979, **38**, 730.
[300] I. Vance, C. M. Topham, S. L. Blayden, and J. Tampon, *J. Gen. Microbiol.*, 1980, **117**, 235.
[301] D. Kluepfel, L. Biron, and M. Ishaque, *Biotechnol. Lett.*, 1980, **2**, 309.

whole broth resulted in the production of D-glucose as well.[302] D-Glucose production was enhanced and continued throughout the saccharification (24–36 h) by several additions of cellobiase activity in the form of culture solids. Using Solka-Floc as substrate, the 'difference sugar' level (total soluble sugar minus D-glucose) rapidly rose to the same relatively stable concentration under various hydrolysis conditions, which was independent of the total sugar and D-glucose concentrations. A rapid hydrolysis rate was observed initially during saccharification, followed by a much slower rate of sugar production. Repeated centrifugation of the reaction mixture and replacement of the supernatant with fresh enzyme solution resulted each time in the reinitiation of a rapid hydrolysis rate. Saccharifications using Avicel microcrystalline cellulose, acid-swollen cellulose, and cotton as substrates were also studied.

The stabilities and optima with respect to temperature and pH of the β-D-glucosidase, Avicelase, and carboxymethylcellulase (CMCase) activities of *Thermomonospora* sp., in the culture filtrate, culture whole broth, and filtrate after sonication of culture solids, are reported.[173] The Avicelase and carboxymethylcellulase activities occur in the extracellular culture fluid and have optima at about pH 7.0 and 5.9, and 65 and 70 °C, respectively. The CMCase activity is stable over 24 h at 60 °C, but declines by 50% in the same period at 65 °C. The Avicelase activity declines by 15% over 24 h at 55 °C, and by 50% at 60 °C. The highest pH studied (pH 7.3) was the most destabilizing for all three activities. The thermostable characteristics of the cellulases from *Thermomonospora* appear to make them suitable for commercial saccharification processes operating at elevated temperatures.

During the past few years, several reports have been published on the cellulolytic activity of *Thermomonospora curvata*, a thermophilic, cellulolytic actinomycete prevalent in municipal solid waste compost.[303] Thermophilic industrial fermentation processes currently appear attractive for microbiological reasons (prevention of pathogen survival and/or accumulation) and for engineering advantages, such as greater ease of cooling and of solid–liquid separation. Studies were therefore conducted on various cellulosic wastes to determine their potential for induction of cellulase synthesis in *T. curvata* and the extent of cellulose degradation under optimal culture conditions.

Trichoderma species can be cultured in stirred-tank fermentors on high (8%) cellulose concentrations without increasing the salt concentration of the medium when NH_4OH is used to control pH and as a nitrogen source.[304] Approximately 90% of the ammonia consumed by the organism can be added as NH_4OH. The advantage of using high concentrations of cellulose is that culture filtrates with greater cellulase activity are obtained. The advantage of a low salts medium is that unwanted solutes in the final enzyme preparation are reduced. The appearance of cellulase in the medium occurs later than net ammonia uptake so that only 20% of the final amount of cellulase has appeared when 80% of the maximum amount of ammonia has been consumed.

[302] J. D. Ferchak, B. Hagerdal, and E. K. Pye, *Biotechnol. Bioeng.*, 1980, **22**, 1527.
[303] F. J. Stutzenberger, *Biotechnol. Bioeng.*, 1979, **21**, 909.
[304] D. Sternberg and S. Dorval, *Biotechnol. Bioeng.*, 1979, **21**, 181.

The effect of lignin and some of its components on the production and activity of cellulase(s) of *Trichoderma reesii* have been studied.[305]

The formation and release of cellulolytic enzymes during growth of *Trichoderma reesei* on cellobiose and glycerol have been investigated.[306]

In an immunochemical approach to the cellolytic complex of *Trichoderma reesei* it was evident that the immunochemical technique is a powerful method for studying the cellulase complex and that the immunological identity found both among the *endo*-D-glucanase and the cellobiohydrolase components probably also reflects a functional identity.[307]

An article on the immunoelectrophoretic detection of cellulases describes the assessment of such enzymes in *Trichoderma reesei* by immunoreaction followed by determination of the enzymic activity.[308]

The multienzyme cellulase complex of *Trichoderma reesei* can be considered as being comprised of Avicelase, carboxymethylcellulase, and β-D-glucosidase, which together effectively solubilize the insoluble cellulose.[305] Cellulase production is enhanced by low pH. Investigations have shown that the production of a particular cellulase can be held to different levels by using lignin or its components. For example, by using ferulic acid, the β-D-glucosidase activity would be reduced to zero, whereas the partial activities of carboxymethylcellulase (21 U) and Avicelase (108 U) could be maintained. The Avicelase activity increased to 890 U at a 0.05% protocatechuic acid concentration compared to control values of 760 U. This means that by adding such components to a growing culture of *T. reesei*, one could preferentially produce a preparation rich in one enzyme and lacking another.

D-Glucanohydrolase from *Trichoderma reesei*, having a molecular weight of 52 000, was evaluated for kinetic properties with respect to cellobiose (see p. 276).[176]

Enzyme-stability studies for the cellulase of *Trichoderma reesei* have been reinvestigated under the conditions used for cellulose hydrolysis (pH 4.8, 50 °C, 24 h).[309] The cellobiohydrolase component as measured on Avicel is less stable than other enzymes of the cellulase complex, and is 60% inactivated by merthiolate (and other Hg compounds) under the above conditions. *endo*-(1→4)-β-D-Glucanase is much more stable, and more resistant to merthiolate and other compounds. Under unshaken conditions the Avicelase of the Rodgers strain C30 shows greater stability to heat than that of other available strains. Biocides must be selected not only for their ability to prevent contamination, but also for their compatibility with cellulases. Tetracyclin and chlorotetracyclin are inexpensive, effective in very low concentrations, have no harmful effect on the enzymes, and are compatible with the yeasts that subsequently grow on the sugar solutions to

[305] R. M. Vohra, C. K. Shirkot, S. Dhawan, and K. G. Gupta, *Biotechnol. Bioeng.*, 1980, **22**, 1497.

[306] M. P. Vaheri, M. E. O. Vaheri, and V. S. Kauppinen, *Eur. J. Appl. Microbiol. Biotechnol.*, 1979, **8**, 73.

[307] L. G. Fagerstam and L. G. Pettersson, *FEBS Lett.*, 1979, **98**, 363.

[308] M. Nummi, M.-L. Niku-Paavola, T.-M. Enari, and V. Raunio, *FEBS Lett.*, 1980, **113**, 164.

[309] E. T. Reese and M. Mandels, *Biotechnol. Bioeng.*, 1980, **22**, 323.

produce alcohol. Attempts have been made to stabilize the enzymes by chemical modification in such a way as to maintain their solubility. Glutaraldehyde treatment greatly increased the enzyme size, lowered the p*I* values, and gave a slight shift in the pH activity curve. There was, unfortunately, no increase in enzyme stability, and the activity of enzymes on solid celluloses was adversely affected. Shaking greatly reduced the hydrolysis of Avicel by *T. reesei* C 30 enzyme. The adverse effect was accompanied by a decrease in recoverable enzyme and protein.

In the studies of the '(1→4)-β-D-glucan cellobiohydrolases' of *Trichoderma reesei*, a new type of cellulolytic synergism was discovered.[310]

Trichoderma viride secretes a cellulase complex which is rich in β-D-glucosidase and therefore well suited for the saccharification of cellulosic materials, and this complex was investigated with respect to optimum conditions of reaction and enzyme stability.[177] Avicelase, carboxymethylcellulase, and β-D-glucosidase differed considerably in their physiochemical properties, therefore, as a compromise the conditions of hydrolysis were chosen to be 50 °C and pH 4.5. With the crude culture filtrate of *T. viride* a nearly pure D-glucose solution of 4% is reached from a 10% cellulose suspension, but wood pulp and newsprint are hydrolysed to a much smaller extent. With an enzyme concentrate up to 8%, D-glucose accumulated in the reaction fluid within 48 h giving a D-glucose : cellobiose ratio of 75 : 1; D-glucose was demonstrated to be the most potent inhibitor of total hydrolysis. The addition of D-glucose to the enzyme–substrate solution at zero time completely stopped its own formation, and cellobiose and reducing groups (oligosaccharides) accumulated. Removal of D-glucose by an ultrafiltration device, gave about 90% saccharification of cellulose to D-glucose in 48 h without any accumulation of cellobiose.

A procedure involving affinity chromatography on cross-linked cellulose was developed for separating enzymatic components of a cellulase complex from *Trichoderma viride*.[311] The *exo*-D-glucanases [(1→4)-β-D-glucan cellobiohydrolase and cellobiase] passed through the column in the equilibrating buffer, whereas the *endo*-D-glucanases were fixed in the same conditions. Five *endo*-(1→4)-β-D-glucanases were fractionated by elution with stepwise increases in the concentrations of carboxymethylcellulose added to the buffer. These *endo*-D-glucanases were differentiated by their substrate specificities and by their reaction pattern toward carboxymethylcellulose, cellulose, insoluble cellodextrins, and oligosaccharides.

The adsorption of cellulases from *Trichoderma viride* was studied during the hydrolysis of newspaper.[312] By measuring individual enzyme activities it was found that in the early stage of hydrolysis enzyme components showing C_xA were absorbed preferentially to those showing C_1A; afterwards this situation was inverted. Electrophoretic resolution of proteins in hydrolysates showed a continuous decrease of enzyme proteins in solution, and furthermore suggested that the enzymes once adsorbed remained immobilized on the substrate (even after extensive digestion). Experiments to cover the enzymes that had remained

[310] L. G. Fagerstam and L. G. Pettersson, *FEBS Lett.*, 1980, **119**, 97.
[311] M. Weber, M. J. Foglietti, and F. Percheron, *J. Chromatogr.*, 1980, **188**, 377.
[312] M. Castanon and C. R. Wilke, *Biotechnol. Bioeng.*, 1980, **22**, 1037.

in solution after typical hydrolysis showed a potential saving of enzyme of up to 40%.

19 Chitinases

The moulting fluid of the tobacco hornworm has chitinase activity which shows allosteric behaviour with chitin.[313] A parabolic curve is obtained on a double reciprocal plot of 1/v *vs.* 1/[S]; and a sigmoid curve results when v (2-acetamido-2-deoxy-D-glucose produced) is plotted against [S] (chitin concentration in terms of 2-acetamido-2-deoxy-D-glucose concentration. The Hill coefficient with insect chitin is 1.95 ± 0.08. Allostery of the chitinase enables the insect to exert additional control over the integrity of structure of its cuticle.

Two different enzymes, which cause the breakdown of nutritional chitin in the hunting spider *Cupiennius salei*, were purified to homogeneity and characterized.[77] One chitinase was enriched about 80-fold (molecular weight 48 000). Its action upon solubilized chitin yields *N,N'*-diacetylchitobiose (88%), trimers (5%), and monomers (7%) of 2-acetamido-2-deoxy-D-glucose, respectively. The enzyme has a pH-optimum around pH 7.2 and it liberates about 30 nmol of *N,N'*-diacetylchitobiose from chitin per mg protein per s. The enthalpy of activation is 9.5 kcal mol^{-1} at 30 °C. *N,N'*-Diacyclchitibiose, in turn, is cleaved by a specific β-D-2-acetamido-2-deoxyglucosidase (EC 3.2.1.30), which hydrolyses *N,N'*-diacetylchitobiose, the 4-methyl umbelliferyl β derivatives of 2-acetamido-2-deoxy-β-D-glucopyranose, 2-acetamido-2-deoxy-β-D-galactopyranose, and 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside at nearly equal rates (approx. 250 nkat per mg protein). The pH optima range around pH 5.4 for the chromogenic substrates and around pH 6.5 for *N,N'*-diacetylchitobiose. Heat stability for the enzyme is better at pH 6.5 than at pH 5.4; activity is not inhibited by any of the substrates used. β-D-2-Acetamido-2-deoxyglucosidase was purified about 160-fold (molecular weight 108 000) and is characterized by kinetic and thermodynamic parameters and inhibitors. Through inhibition studies with 2-acetamido-2-deoxy-D-glucono-1,5-lactone it could be shown that all activities pertain to only one enzyme bind to an identical active centre.

20 Chondroitinases

A 'mucopolysaccharidase' derived from a pathogenic strain of *Bacteroides distasonis* was isolated and purified by fractionation with cold acetone and ion-exchange chromatography on DEAE-cellulose at pH 8.0.[314] Three detectable enzyme activities from concentrated supernatant filtrates were obtained in a fraction precipitated by three volumes of cold acetone; these were DNAase-, hyaluronidase-, and chondroitinase-like activity. Separation of the DNAase was achieved by ion-exchange chromatography. Fractions designated as purified 'mucopolysaccharidase' contained both hyaluronidase- and chondroitinase-like activity.

[313] M. L. Bade and A. Stinson, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 349.
[314] W. Rudek and R. Haque, *J. Gen. Microbiol.*, 1980, **119**, 211.

21 Chondroitin ABC and AC Lyases

Chondroitin ABC and AC lyases split hexosaminidic linkages in galactosaminoglycans and hyaluronic acid.[315] Even-numbered oligosaccharides from hyaluronic acid with either D-glucuronic acid or 2-acetamido-2-deoxy-D-glucose in non-reducing position were used, prior to and after reduction with sodium borohydride, as substrates for chondroitin ABC and AC lyases. These substrates allowed elucidation of the effects of the nearest neighbourhood of the bond to be split on the action of the enzymes. The results indicate that chondroitin ABC lyase acts strictly as an endolyase towards hyaluronate and requires the presence of a disaccharide in both reducing and non-reducing positions of the *endo*-hexosaminidic bond to be split. None of the hexosaminidic bonds of the tetrasaccharide (5) is split by chondroitin ABC lyase. In contrast chondroitin AC lyase acts also as an *exo*-glycosidase towards hyaluronate and recognizes only the amino-sugar and the uronic acid residue that are linked *via* the 2-amino-2-deoxyhexopyranosyl bond which is split. Thus, the 2-acetamido-2-deoxy-D-glucose and D-glucuronic acid residues at both ends of a tetrasaccharide with the structure (5) are liberated.

β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→4)-D-GlcA

(5)

22 Dextranases

The regulation of dextranase synthesis of *Streptococcus mutans* has been investigated.[316] The paper reports on conditions of growth of *S. mutans* under which the production of D-glucosyltransferases was normal, whereas the release of dextranase could not be demonstrated.

23 β-D-Galactanases

An assay method, which employed the synthetic, chromogenic substrates β-D-Gal-(1→3)-β-D-Glc*N*Ac-1→$OC_6H_4NO_2$-*p*, β-D-Gal-(1→4)-β-D-Glc*N*Ac-1→$OC_6H_4NO_2$-*p*, and β-D-Gal-(1→6)-β-D-Glc*N*Ac-1→$OC_6H_4NO_2$-*p*, was developed for the rapid identification of the linkage specificity of *exo*-β-D-galactanases from any source *via* a coupled reaction with β-D-2-acetamido-2-deoxyhexosidase.[212]

A new strain of *Escherichia freundii* isolated from human faeces[317] releases *endo*-β-D-galactanase with lower levels of *exo*-glycosidases in the culture medium containing 0.3% of keratan sulphate. *endo*-β-D-Galactanase was purified by an improved purification procedure involving Amicon H1P10 hollow fibre filtration, QAE-Sephadex A-50, and CM-Sephadex C-50 chromatographies. The purified enzyme is completely free from proteases and *exo*-glycanases. The general properties of this enzyme are: molecular weight, 28 000; optimal pH 5.5

[315] H.-P. Ulrich, U. Klein, and K. von Figura, *Z. Phys. Chem.*, 1979, **360**, 1457.
[316] G. J. Walker, F. L. Murray, and J. G. Morrey-Jones, *FEBS Lett.*, 1980, **115**, 206.
[317] H. Nakagawa, T. Yamada, J. L. Chien, A. Gardas, M. Kitamikado, S. C. Li, and Y. T. Li, *J. Biol. Chem.*, 1980, **255**, 5955.

to 5.8; p*I*, pH 8.0. This enzyme hydrolysed keratan sulphates isolated from different sources to produce 3-*O*-2-acetamido-2-deoxy-D-glucopyranosyl 6-sulphate-D-galactose as the major products. In addition, the specificity of this enzyme toward various glycoconjugates was also studied.

24 *endo*-(1→3)-α-D-Glucanases

Lytic enzymes in the autolysis of *Schizophyllum commune* have been reported upon with particular reference to (1→3)-α-D-glucanase.[318]

25 *exo*-(1→3)-α-D-Glucanases

The hydrolysis (1→3)-α-D-glucosidic linkages in oligosaccharides and polysaccharides by *Cladosporium resinae exo*-(1→3)-α-D-glucanase, an enzyme which displays high activity towards nigerose but which is without any detectable action on water-insoluble mutan.[319] Hydrolysis patterns afforded by the enzyme are shown in Figures 3 and 4.

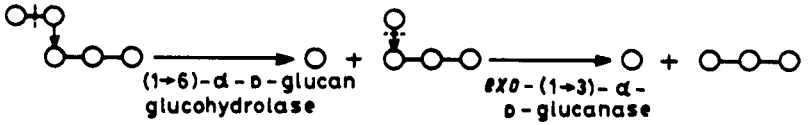


Figure 3 *Hydrolysis of the mixed-linkage pentasaccharide* sB_5 *by* S. mitis (1→6)-α-D-*glucan glucohydrolase, followed by* C. resinae *exo*-(1→3)-α-D-*glucanase.* ○, D-*Glucose residue;* ——, (1→6)-α-D-*glucosidic linkage;* ↓, (1→3)-α-D-*glucosidic linkage. Dotted lines drawn at right angles to a linkage indicate the point of hydrolysis*

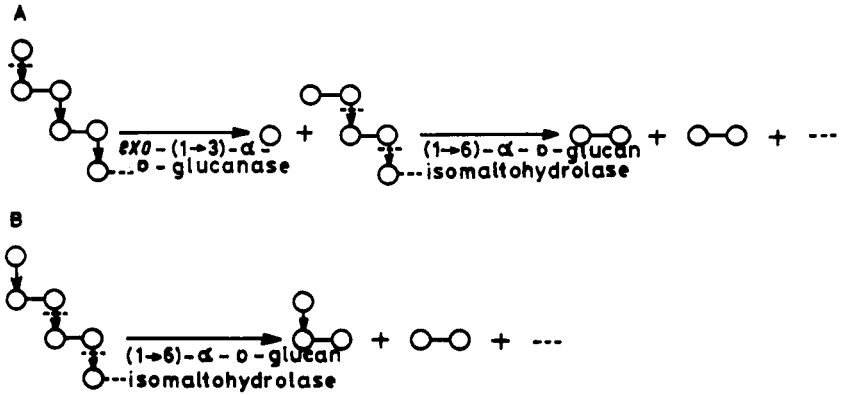


Figure 4 *Hydrolysis of* L. mesenteroides B-1355 *soluble dextran by* G_2*-dextranase: with (A) and without (B) prior treatment with exo-*(1→3)-α-D-*glucanase. A fragment of the structure proposed for a non-reducing terminal of the dextran is shown. The symbols are the same as in Figure 3*

[318] F. Reyes, R. Lahoz, and C. Vazquez, *Can. J. Microbiol.*, 1980, **26**, 1120.
[319] G. W. Walker and M. D. Hare, *Carbohydr. Res.*, 1979, **77**, 289.

26 *endo*-(1→3)-β-D-Glucanases

Ten species of marine invertebrates possessing the highest laminarinase activity have been investigated.[320] The following approaches in determining the type of action of partially purified (1→3)-β-D-glucanases: effect on modified substrates, comparative study of enzymolysis products by the Nelson's and D-glucose oxidase methods and stereochemistry of the enzymolysis products. Experimental results indicate that the examined marine invertebrates contain (1→3)-β-D-glucanases with *endo*-type activity; *exo*-enzymes are apparently absent.

Two purified pea (1→3)-β-D-glucanases (EC 3.2.1.6) hydrolyse laminaran (degree of polymerization 20), laminaridextrins (degree of polymerization 3–7), and their reduced ^{3}H-derivatives, (1→3)-β-D-glucans which are partially substituted (carboxymethylpachyman) or crystalline (curdlan), and mixed-linkage β-D-glucans.[321] Enzyme kinetics and product-formation indicate *endo*-hydrolyase activity with weak transglycosylase capacity. The enzymes do not hydrolyse β-D-glucopyranosidase, the (1→3)-linkage adjacent to the reducing end of chains, or cellulose and its derivatives. They degrade mixed-linkage β-D-glucans, in a manner similar to *Rhizopus arrhizus endo*-(1→3)-β-D-glucanase, to form the products expected from hydrolysis of linkages adjacent to (1→3)-linkages. With respect to action patterns, D-glucanase I (from apical growing tissue) differs from D-glucanase II (from basal maturing tissue) in several respects: (*a*) on a molar basis, I generates reducing groups from all substrates more rapidly than II; (*b*) lower laminaridextrins are hydrolysed by I at the non-reducing terminal linkage, whereas II preferentially hydrolyses internal linkages; (*c*) laminaran is hydrolysed to lower laminaridextrins by I more rapidly than II, but I takes longer than II to completely degrade laminarin chains; (*d*) the enzymes are differentially sensitive to different classes of non-competitive inhibitors. It is concluded that these β-D-glucanases differ in such a way that I preferentially continues to degrade fragments produced by *endo*-hydrolytic attack on long chains ('multiple attack' action pattern), whereas II hydrolyses internal linkages of the longest chains available ('multi-chain attack').

Two *endo*-(1→3)-β-D-glucanases are present in etiolated peas at opposite ends of the stem.[322] D-Glucanase I from subapical regions degrades substrates to a series of low molecular weight dextrins, and is most readily assayed reductometrically (*e.g.* as laminarinase). (1→3)-D-Glucanase II from basal regions preferentially hydrolyses internal linkages of long chains, and is most sensitively assayed viscometrically (*e.g.* as carboxymethylpachymanase). The activity of (1→3)-D-glucanase II but not I increases greatly near the apex in response to treatment of the tissue with auxin, and ethylene gas suppresses endogeneous activities and the auxin response, *i.e.* levels of these enzymes are under developmental controls which can be regulated. Different natural substrates for the two enzymes were identified primarily in tissue fractions soluble in hot water. Substrates for D-glucanase I are concentrated in apical regions, as is the enzyme itself, and those for D-glucanase II are in basal regions, implying that enzymes

[320] L. A. Elyakova and T. G. Shilova, *Comp. Biochem. Physiol. B*, 1979, **64**, 245.
[321] Y.-S. Wong and G. A. MacLachlan, *Biochim. Biophys. Acta*, 1979, **571**, 256.
[322] Y.-S. Wong and G. A. MacLachlan, *Plant Physiol.*, 1980, **65**, 222.

and substrates are normally in separate cellular compartments. Tissue sections stained with aniline blue for β-D-glucan show enhanced fluorescence in cell walls, and most of this can be removed either by hot water or the appropriate β-D-glucanase. The enzymes are not likely to function directly in promoting nutrition or growth in peas, but they could help, following secretion, to maintain channels for communication and translocation through cell walls.

Two buffer-soluble *endo*-(1→3)-β-D-glucanases (EC 3.2.1.6) have been purified to within 1% of electrophoretic homogeneity from etiolated *Pisum sativum* stem tissues.[323] Purified D-glucanase I and II differ in physical properties, such as electrophoretic mobility in sodium dodecyl sulphate polyacrylamide gels (M_r values were 22 000 and 37 000, respectively) and isoelectric focusing (p*I* values were 5.4 and 6.8, respectively). Although the enzymes have similar pH optima (5.5–6.0), K_m values for various substrates (0.6–7.4 mg ml^{-1}), and thermal inactivation profiles, they are localized in different tissues and they differ markedly in the rates with which they attack the internal linkages of long- *vs.* short-chain substrates. Glucanase I is concentrated in apical regions of the stem and is most effectively assayed reductometrically (as laminarinase), whereas glucanase II is localized in mature regions and is relatively more active in viscometric assays (as carboxymethylpachymanase).

The extracellular system of (1→3)-β-D-glucanases of *Alternaria tenuisima* and *Aspergillus vesicolor* has been studied.[324]

The occurrence of a (1→3)-β-D-glucanase in *Botrytis cinerca* has been reported.[325]

Motile actinomycetes capable of degrading walls of viable yeast cells were isolated from soil and identified as *Oerskovia xanthineolytica*.[326] A lytic assay based on susceptibility of enzyme-treated cells to osmotic shock was developed, and 10 of 15 strains of *O. xanthineolytica*, *O. turbata*, and non-motile *Oerskovia*-like organisms from other collections were found to possess yeast lytic activities. All lytic strains produced laminaranase and α-D-mannanase, but the amounts, determined by reducing group assays, were not proportional to the observed lytic activities. The *Oerskovia* isolates demonstrated chemotactic, predatory activity against various yeast strains and killed yeasts in mixed cultures. Of 15 carbon sources tested for production of lytic enzyme, purified yeast cell walls elicited the highest activity. D-Glucose repressed enzyme production and caused cells to remain in the microfilamentous and motile rod stages of the *Oerskovia* cell cycle. Crude lytic activity was optimal at pH 5.6 to 7.0 and inactivated by heating for 6 min at 50 °C. Partial purification by isoelectric focusing showed that all lytic activity was associated with four (1→3)-β-D-glucanases. The absence of protein disulphide reductase, β-D-2-acetamido-2-deoxyhexosidase, and D-phosphomannanase in crude preparations indicated that the principal enzyme

[323] Y.-S. Wong and G. A. MacLachlan, *Biochem. Biophys. Acta*, 1979, **571**, 244.
[324] V. Jirku, B. Kraznerova, and V. Krumphanzl, *Folia Microbiol.*, 1980, **25**, 24.
[325] D. Dubourdieu and P. Ribereau-Gayon, *C. R. Hebd. Seances Acad. Sci., Ser. D*, 1980, **290**, 25.
[326] J. W. Mann, T. W. Jeffries, and J. D. MacMillan, *Appl. Environ. Microbiol.*, 1978, **36**, 594.

responsible for yeast wall lysis was a (1→3)-β-D-glucanase that produced relatively little reducing sugar from yeast glucan.

Yeast lytic activity was purified from the culture supernatant of *Oerskovia xanthineolytica* grown on minimal medium with insoluble yeast glucan as the carbon source.[327] The lytic activity was found to consist of two synergistic enzyme activities, which copurified on carboxymethyl cellulose and Sephadex G-150, but were resolved on Bio-Gel P-150. The first component was a (1→3)-β-D-glucanase with a molecular weight of 55 000. The K_m for yeast glucan was 0.4 mg ml^{-1}: that for laminarin was 5.9 mg ml^{-1}. Hydrolysis of (1→3)-β-D-glucans was endolytic, yielding a mixture of products ranging from D-glucose to oligomers of 10 or more. The size distribution of products was pH dependent, smaller oligomers predominating at the lower pH. The D-glucanase was unable to lyse yeast cells without 2-mercaptoethanol or the second lytic component, an alkaline protease. Neither of these agents had any effect on the D-glucanase activity on polysaccharide substrates.

(1→3)-β-D-Glucanase and dimorphism in *Paraccidioides brasiliensis* are the subject of a study on the properties of this organism.[328]

27 *exo*-(1→6)-α-D-Glucanases

α-D-Glucosidases from *Candida tropicalis* and from rice convert 2,6-anhydro-1-deoxy-D-*gluco*-hept-1-enitol into 1-deoxy-α-D-*gluco*-heptulose (see p. 271).[157]

A crystallization procedure for *exo*-(1→6)-α-D-glucanase from *Arthrobacter globiformis* has been described.[329] The activity ratio of the enzyme on isomaltose, maltose, nigerose, and kojibiose was 100 : 3.9 : 0.03 : 0.006. The data reported suggests that the enzyme splits not only (1→6)-α-D-linkages but also other α-D-linkages of polysaccharides such as dextran and starch in the rates decreasing in the order shown on D-glucobiose.

28 *exo*-(1→6)-β-D-Glucanases

The β-D-glucosidase from sweet almonds and an *exo*-(1→6)-D-glucanase (glucodextranase) from *Arthrobacter globiformis* convert the substrate, 2,6-anhydro-1-deoxy-D-*gluco*-hept-1-enitol, into 1-deoxy-β-D-*gluco*-heptulose.[157] The work provides the first demonstration of the ability of an 'inverting' D-glucanase to create specific anomeric configuration *de novo* from a substrate lacking α or β configuration (see p. 271).

29 Glucoamylases

Glucoamylase acting on 0.1% starch solutions liberates D-glucose, the rate of formation of which is a measure for the enzyme activity.[330] It can be pursued

[327] J. H. Scott and R. Schekman, *J. Bacteriol.*, 1980, **142**, 414.
[328] A. Florez-Carreon, A. Gomez-Villanueva, and G. Sanblas, *J. Microbiol. Serol.*, 1979, **45**, 265.
[329] T. Ohya, H. Suzuki, and T. Sawai, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 203.
[330] H. Bartels, G. Rommele, and M. Bohmer, *Z. Anal. Chem.*, 1980, **302**, 127.

continuously with the aid of D-glucose dehydrogenase–NAD at 340 nm. Using a test set 15 analyses per hour may be performed with a standard deviation of 2–3%. The method may be automated.

The determination of D-glucosyltransferase activity in glucoamylase preparations has been reported.[331]

What seems to be a most remarkable outcome of a study of equilibrium relationships in the degradation of starch by a commercial glucoamylase, Amigase®, is that the equilibrium constants for the hydrolysis of the α-(1→4)- and α-(1→6)-linkages are rather different.[332] At equilibrium the isomaltose concentration is thus seen to be six times larger than that of maltose. The free enthalpy of hydrolysis of maltose was calculated to be -3500 J mol^{-1} at a unit concentration of reactants; the free enthalpy of hydrolysis of isomaltose was $+1400$ J mol^{-1}. The observed effect has important consequences for the industrial application of starch-degrading enzymes. In most of the enzyme preparations commonly used, (1→6)- and (1→4)-degrading activities are present; hence, this means that since the presence of a catalyst does not affect the thermodynamic equilibrium, and (1→6)- and (1→4)-forming activities will be present. From the present data it becomes apparent that, especially at high dry substance concentrations, the equilibrium isomaltose concentration will be much higher than that corresponding to the initial percentage of (1→6)-linkages present in native starch. Therefore if sufficient amounts of D-glucose are formed by the action of the (1→4)-splitting activity, the formation of (1→6)-linkages will become significant. This accounts for the well known observation of a transient peak in the D-glucose concentration on incubation of dextrins with amyloglucosidase. At long incubation times the glucose concentrations again decrease because of the reformation of mainly (1→6)-linked polymers. This paper, which provides an explanation for this phenomenon, may be helpful in the interpretation of the kinetic behaviour of the enzyme glucoamylase.

A kinetic approach to a detailed study of the thermal inactivation of an enzyme in the presence of a substrate having a stabilizing or destabilizing effect has been developed.[333] The method was used for a study of the kinetics of the thermal inactivation of soluble glucoamylase and of the enzyme immobilized on porous silica. It was found that binding of the substrate (maltose or maltodextrins) leads to an increase in the thermostability of glucoamylase, the stabilizing effect of the substrate being more pronounced relative to the soluble than to the immobilized enzyme (40-fold *vs.* 15-fold). The magnitude of the stabilization does not depend on the length of the substrate chain. The product of enzymatic hydrolysis, D-glucose, has a considerably smaller stabilizing effect. Stabilization by the substrate rather than immobilization of the enzyme on an inorganic carrier (three-fold increase in stability) plays the main role in the stabilization of glucoamylase; however, the overall thermostabilization of glucoamylase through immobilization and stabilization by the substrate has almost the same limit for the soluble and immobilized enzymes (40- to 50-fold).

[331] D. Kobrehel, *Biotechnol. Lett.*, 1979, **1**, 251.
[332] G. M. A. Van Beynum, J. A. Roels, and R. Van Tilburg, *Biotechnol. Bioeng.*, 1980, **22**, 643.
[333] A. A. Klesov and V. B. Gerasimas, *Biochemistry (USSR)*, 1979, **44**, 854.

An anti-(human renal α-D-glucosidase) antiserum human intestinal maltases was studied in immunodiffusion experiments.[136] An identity pattern was observed between renal neutral α-D-glucosidase and intestinal glucoamylase. Renal neutral α-D-glucosidase and intestinal glucoamylase were both completely precipitated by the antiserum, their maltase activity being only slightly inhibited in the antigen–antibody complex. From their molecular and immunological properties a large homology appears between human renal α-D-glucosidase and intestinal glucoamylase.

The distal intestine of suckling rats contains an anomalously high proportion of its brush-border membrane-typical enzymes in soluble or easily released form.[152] The results of a binding study with four forms of maltase–glucoamylase as a probe suggest that a portion of the soluble maltase–glucoamylase in the suckling rat intestine has a carbohydrate structure that differs from that of the membrane-bound enzyme. This difference may prevent or at least weaken attachment of the enzyme to the brush border membrane (see p. 269).

The nature of thermophilic amylolytic enzymes produced by a strain of *Aspergillus niger* was influenced by media composition.[262] While D-glucose, maltose, dextrin, and soluble starch induced the production of glucoamylase, pectin, and sodium polypectate almost exclusively induced the formation of α-amylase. Alkali ions suppressed the formation of glucoamylase (see p. 299).

The role of L-histidine side-chains in reactions catalysed by porcine pancreatic α-amylase, sweet potato β-amylase, and *Aspergillus niger* glucoamylase has been studied by using diethyl pyrocarbonate, a specific protein reagent.[244] Changes in the activity, binding affinity, and apparent kinetic parameters owing to ethoxycarbonylation have been determined. The role of exposed L-tyrosine side-chains in enzyme-catalysed reactions has also been studied for these amylases using *N*-acetylimidazole as the specific protein reagent.[245] The changes in activity, binding affinity ($\Delta k_{-1}/k_{+1}$), and kinetic parameters (K_m, k_2) owing to acetylation of the phenolic hydroxy-groups have been determined.

Aspergillus oryzae grown in wheat bran submerged culture produced three forms of glucoamylase, designated as glucoamylase I, II and III.[334] They were obtained in the ratio of 51:3:1. Glucoamylase I readily hydrolysed soluble starch to above 90% and had a strong debranching activity. Glucoamylase II and III hydrolysed soluble starch to about 75% and had weak debranching activity. Glucoamylase I was further purified by rechromatography, gel filtration, preparative isoelectric focusing on carrier ampholyte, and again gel filtration. Glucoamylase I was fairly stable at pH-range between 5.5 and 7.0 and temperature up to 40 °C. The limit of hydrolysis of soluble starch was 80% and the liberated product was only D-glucose.

Culture filtrates of *Cladosporium resinae* contain a mixture of enzymes including α-D-glucosidase, α-amylase, and two glucoamylases that are able to convert starch and pullulan efficiently into D-glucose (see p. 274).[165]

The production of glucoamylase from *Endomycopsis bispora* by different morphological types of the organism has been surveyed.[335] The influence of the

[334] B. C. Sama, T. Mitsue, and S. Ueda, *Starch*, 1979, **31**, 307.
[335] K. Winkler, F. Zickler, and H. Ruttloff, *Z. Allg. Mikrobiol.*, 1979, **19**, 577.

presence of fats, on the production of glucoamylase from *Endomycopsis bispora* has been studied.[336]

In an assessment of glucoamylase from *Endomycopsis bispora*, it was revealed that multiple forms of the enzyme are released during cultivation.[337]

Taka-amylase A, bacterial liquefying α-amylase, and glucoamylase from *Rhizopus niveus* were used in a study of hydrolysis of amylose (DP17) by various techniques in single- and mixed-amylase systems.[264] Theoretical treatment based on subsite theory was also successfully applied to the mixed amylase system (see p. 299).

Glucoamylase has been used in enzymically active immobilized forms obtained by the following routes: *via* glutaraldehyde cross-linking on(to) silica,[275] *via* immobilization porous silica,[333] and by a chelation process using titanium (IV)-treated gelatin capsules.[338]

30 Glycanases (Miscellaneous)

Eight cultivars of proso (*Panicum miliaceum*) millets showed β-amylase, protease, cellulase, and hemicellulase activities, with the exception of one sample that showed no hemicellulase activity.[278] Two of the cultivars were grown on different plots to permit study of the effects of different locations on enzyme levels (see p. 303).

31 Heparin and Heparan Sulphate Hydrolases

A heparan sulphate-degrading '*endo*-D-glucuronidase' was isolated from human placenta and partially purified by affinity chromatography on heparan sulphate–Sepharose 4B.[339] The *endo*-D-glucuronidase has a molecular weight of approximately 100 000 estimated by gel chromatography and a broad pH optimum between pH 4 and pH 6. Carboxyl-reduced heparan sulphate is not split by partially purified '*endo*-D-glucuronidase', but inhibits the action of that enzyme towards non-modified heparan sulphate. Low molecular weight heparan sulphate ($M_r \approx 3000$) is not attacked by the *endo*-D-glucuronidase. *N*-Desulphated heparan sulphate and heparin are only weak substrates. The amino-sugar adjacent to the D-glucuronic acid residue appearing at the reducing terminal of heparan sulphate fragments liberated by the '*endo*-D-glucuronidase' appears to be exclusively 2-acetamido-2-deoxy-D-glucose.

32 Hyaluronate Lyases

Hyaluronate lyase from *Propionibacterium acnes* has been purified 13 000-fold from the culture supernatant to homogeneity (as determined by polyacrylamide disc gel electrophoresis.)[340] The molecular weight of the purified enzyme was

[336] A. Taufez, K. Winkler, F. Zickler, and H. Ruttloff, *Z. Allg. Mikrobiol.*, 1979, **19**, 349.
[337] K. Winkler, H. Rutloff, and F. Zickler, *Z. Allg. Mikrobiol.*, 1979, **19**, 517.
[338] J. F. Kennedy and B. Kalogerakis, *Biochimie*, 1980, **62**, 549.
[339] U. Klein and K. Von Figura, *Z. Phys. Chem. (Leipzig)*, 1979, **360**, 1465.
[340] E. Ingham, K. T. Holland, G. Gowland, and W. J. Cunliffe, *J. Gen. Microbiol.*, 1979, **115**, 411.

85 110, as determined by gel filtration. The purified enzyme had a pH optimum at 6.4, was stable between pH 5.0 and 5.8 and was completely inactivated after 15 min at 50 °C. Preliminary studies suggested that the enzyme is active against chondroitin 4- and 6-sulphates, but not against dermatan sulphate. Analysis by paper chromatography of the reaction products from the degradation of hyaluronic acid by bacterial, testicular, and *P. acnes* enzymes suggested that the *P. acnes* enzyme is similar in its mode of action to other bacterial hyaluronate lyases. The enzyme from *P. acnes* may thus be tentatively classified as a hyaluronate lyase.

33 Hyaluronidases

A new micro-method for the assay of hyaluronidase activity has been described. The method utilizes chondroitin sulphate as a substrate.[341] The degraded products of chondroitin sulphate by hyaluronidase are determined by modified disc gel electrophoresis. The products are first concentrated into a single band in polyacrylamide gel, and then the band is stained with cetylpyridinium chloride. The absorbance of the band is proportional to the amount of the degraded products. The hyaluronidase activity is therefore linearly related to the absorbance. This procedure represents a sensitive method for the assay of hyaluronidase activity in the serum and urine.

A sensitive colorimetric assay for proteases and certain polysaccharidases is based on the digestion of proteoglycan from bovine nasal cartilage.[342] This high-molecular-weight substrate is trapped in the interstices of a polyacrylamide gel. The gel is then dispersed as small particles. Enzymes can diffuse into these particles and digestion products can diffuse out. Following digestion, the particles are centrifuged off and the digestion products in the supernatant are quantitated by reaction with the metachromatic dye 1,9-dimethylmethylene blue or by assay of their uronic acid content with carbazole reagent. Proteases from each of the four major classes can be quantitated at levels of 1–200 ng. The method is particularly suitable for the study of cartilage proteases that degrade matrix proteoglycans.

Commercial bovine testicular hyaluronidase preparations are contaminated by a small amount of protease activity, which is now partially inhibited by L-serine-protease inhibitors of pepstatin.[343] These protease inhibitors can be shown by agarose gel column chromatography to abolish or reduce hyaluronidase-induced degradation of bovine nasal cartilage proteoglycan subunit without affecting the ability of the enzyme to degrade chondroitin sulphate. In addition, immunodiffusion studies indicate that pretreatment of hyaluronidase with these protease inhibitors reduces or abolishes the ability of the enzyme to produce a second 'link-related' immunoprecipitin line upon digestion of link protein-containing proteoglycan fractions. Thus, the enhancement of immune reactivity and the unmasking of an additional antigen noted after digestion of cartilage

[341] S. S. Chen, D. S. Hsu, and P. Hoffman, *Clin. Chim. Acta*, 1979, **95**, 277.
[342] H. Nagase and J. F. Woessner, *Anal. Biochem.*, 1980, **107**, 385.
[343] H. D. Keiser and V. B. Hatcher, *Connect. Tissue Res.*, 1979, **6**, 229.

proteoglycan with testicular hyaluronidase are most likely owing to the exposure of additional antigenic sites or to the release of more highly immuno-reactive fragments by the contaminant proteases rather than to the action of hyaluronidase itself.

Primary and passaged cultures of chick embryo fibroblasts, prepared from 10-day embryonic skin and 12-day embryonic skeletal muscle, synthesize and secrete hyaluronidase at levels comparable to the tissues of origin.[344] The level of cell-associated hyaluronidase remains approximately constant during culture, while the enzyme accumulates extracellularly such that within a 48-h incubation period, levels of hyaluronidase in the medium are 2–4 times higher than that found associated with the cell layer. The cultures continue to produce hyaluronidase at approximately the same rate and distribution through as many as nine passages. In addition to the chick fibroblasts, various mammalian cells also produce hyaluronidase in culture. Both the cell- and medium-associated forms of the chick fibroblast hyaluronidase are '*endo*-glycosidases' of estimated molecular weight of 62 000, which preferentially cleave hyaluronate substrate at an acidic pH optimum. Neither the cell-associated nor secreted hyaluronidase are enzymatically active at neutral pH.

Treponema pallidum (Nichols strain) exhibited 'mucopolysaccharidase' activity; glycosaminoglycans were broken down more rapidly by viable treponemes than by heat-activated treponemes or membrane filtrates of treponemal suspensions.[345] Ouchterlony immunodiffusion demonstrated the occurrence of antibodies to the hyaluronidase-like enzyme within syphilitic sera. After intratesticular inoculation of 2×10^7 to 6×10^7 treponemes, these anti-mucopolysaccharidase antibodies were detected between 9 and 35 days' post-inoculation. In addition, glycosaminoglycans were present in the serum of infected animals 9 and 16 days' post-inoculation. Immune serum that contained antibodies to the mucopolysaccharidase restricted treponemal breakdown of glycosaminoglycans. It has been previously demonstrated that immune rabbit serum contains a factor that blocks attachment of *T. pallidum* (Nichols strain) to cultured mammalian cells. This factor was effectively absorbed by prior incubation with bovine hyaluronidase. It is postulated that *T. pallidum* attaches to glycosaminoglycans on the surface of cultured cells through the mucopolysaccharidase enzyme at the surface of the organisms. These findings are discussed in terms of the histopathogenesis of *T. pallidum* with applications to the healing immune response.

A 'mucopolysaccharidase' derived from a pathogenic strain of *Bacteroides distasonis* was isolated and purified by fractionation with cold acetone and ion-exchange chromatography on DEAE-cellulose pH 8.0 (see p. 311).[314]

A simple method is presented for removing protease(s) from commercial sources of *Streptomyces* hyaluronidase derived from *Streptomyces hyalurolyticus* using a column of insolubilized chicken ovomucoid.[346] The protease binds to ovomucoid; 91% of the hyaluronidase and no more than 0.7% of the protease passes through the column. A calcium chloride solution is used to

[344] R. W. Orkin and B. P. Toole, *J. Biol. Chem.*, 1980, **255**, 1036.
[345] T. J. Fitzgerald and R. C. Johnson, *Infect. Immun.*, 1979, **24**, 261.
[346] C. B. Caputo, J. Schrode, J. H. Kimura, and V. S. Hascall, *Anal. Biochem.*, 1980, **105**, 468.

remove the bound protease. Purified hyaluronidase cleaves the hyaluronic acid of proteoglycan aggregates without affecting the size of proteoglycan monomers. The ability of digested monomers to bind hyaluronic acid, however, is somewhat diminished.

34 Isoamylases

Isoamylase has been prepared by affinity chromatography of a commercial enzyme-preparation from a strain of Cytophaga (also known as a *Flavobacterium* or Polyangium).[347] The enzyme was not very stable, but the stability could be improved by calcium ions. The enzyme had a very low but significant activity on pullulan and on α-dextrins having maltosyl side-chains. The observation, which is contrary to previous reports, has been related to the specificity of isoamylase and other bacterial debranching-enzymes.

Crystalline isoamylase of *Pseudomonas amyloderamosa* was found to be contaminated with a trace of proteolytic enzyme.[348] This contaminant digested the isoamylase under neutral or alkaline conditions, especially in the presence of sodium dodecyl sulphate. A reliable molecular weight of the enzyme was obtained by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and by gel filtration on Sepharose®-6B in 6 M guanidinium chloride after heat inactivation of the contaminant. The molecular weight of the undegraded polypeptide chain of the isoamylase was about 9.0×10^4.

A report of a debranching enzyme of the isoamylase type, rather than the well known R-enzyme (pullulanase) type from potato (*Solanum tuberosum*) concludes that it is the first report of an isoamylase type debranching enzyme in the higher plants.[349]

35 Laminaranases

A lytic assay based on susceptibility of enzyme-treated yeast cells to osmotic shock was developed, and 10 of 15 strains of motile actinomycetes *Oerskovia xanthineolytica*, *O. turbata*, and non-motile *Oerskovia*-like organisms from other collections were found to possess yeast lytic activities.[326] All lytic strains produced laminaranase and α-D-mannanase, but the amounts, determined by reducing-group assays, were not proportional to the observed lytic activities (see p. 315).

Routes to the recovery of laminaranase and β-D-fructofuranosidase from industrial waste broths of baker's yeast have been investigated (see p. 250).[92]

36 Limit Dextrinases

A limit dextrinase has been purified 2700-fold from ungerminated peas (*Pisum sativum*) by affinity chromatography.[349a] The enzyme hydrolyses (1→6)-α-D-glucosidic linkages in α-limit dextrins containing at least one (1→4)-linked

[347] R. M. Evans, D. J. Manners, and J. R. Stark, *Carbohydr. Res.*, 1979, **76**, 203.
[348] A. Amemura, Y. Konishi, and T. Harada, *Biochem. Biophys. Acta*, 1980, **611**, 390.
[349] Y. Ishizaki, H. Tanicuchi, and M. Nakamura, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 2433.
[349a] D. Yellowlees, *Carbohydr. Res.*, 1980, **83**, 109.

α-D-glucose residue on either side of the susceptible linkage. The limit dextrinase also hydrolyses the polysaccharides amylopectin, amylopectin β-limit dextrin, glycogen β-limit dextrin, and pullulan, but has not activity towards glycogen.

37 Lysozymes

In a study of antibodies to synthetic antibody-combining sites it was shown that antibodies against a surface-simulation peptide with antibody-combining activity toward lysozyme antigenic site 3 react with lysozyme antibodies.[350]

A rapid and semi-automatic determination of lysozyme in biological fluids using kinetic analysis and turbidimetry is described and compared to other commonly used techniques.[351] The specificity of the method is satisfactory whereas that of clarification of a gel medium is apparently not. Normal values and standard errors for human plasma, urine, and leukocytes are given. A standardized expression of lysozyme activity is proposed and discussed. The importance of the means by which the blood sample is collected and prepared is underlined: plasma, decanted soon after collection, is preferable to serum.

A simple and rapid method is described for the removal of lysozyme from human whole salivary supernatant.[352] Saliva specimens were adsorbed with *Micrococcus lysodeikticus*. The saliva so treated was depleted of 95% of the lysozyme activity. Changes in total protein, lactoperoxidase, lactoferrin, immunoglobulin A, and the proportions of several anionic proteins were less than 10%. It is concluded that adsorption of saliva with *M. lysodeikticus* is a suitable procedure for the preparation of saliva that is selectively deficient in lysozyme.

The pH difference absorption spectra of human lysozyme were measured.[353] The difference spectra in the acidic region had a peak at 300 nm, as observed for hen and turkey lysozymes. The pH dependence curve of the extinction difference at 300 nm was well interpreted in terms of the pK values for the catalytic groups (3.4 for L-Asp-52 and 6.8 for L-Glu-35 at 0.1 ionic strength and 25 °C) determined from the pH dependence of the circular dichroism at 303.5 nm (S. Kuramitsu *et al.*, *J. Biochem.*, 1974, **76**, 671) and the fluorescence excited at 305 nm (S. Karumitsu *et al.*, *J. Biochem.*, 1978, **83**, 159). The difference spectra of human lysozyme in the alkaline pH region were characteristic of tyrosyl ionization. The perturbation of L-trypophyl residues, which had been observed for hen and turkey lysozymes (S. Kuramitsu and K. Hamaguchi, *J. Biochem.*, 1979, **85**, 443), was not observed for human lysozyme. On the basis of the pH dependence curves of the extinction difference at 245 and 295 nm, a rough estimate was made of the apparent pK values of the six L-tyrosyl residues as 9.2, 9.2, 10.5, 10.9, 12.4, and 12.5. A time-dependent spectral change observed above pH 11 was not due to the exposure of buried L-tyrosyl residues on alkali denaturation but was due mainly to disulphide cleavage and exposure of buried L-tryptophyl residues.

[350] M. Z. Atassi and S. Sakata, *Biochim. Biophys. Acta*, 1980, **624**, 573.
[351] J. Plouet and Y. Madec, *Clin. Chim. Acta*, 1979, **93**, 51.
[352] G. R. Germaine and L. M. Tellefson, *Infect. Immun.*, 1979, **26**, 991.
[353] S. Kuramitsu, K. Hamaguchi, S. Miwa, and K. Nakashima, *J. Biochem.* (*Tokyo*), 1980, **87**, 771.

Anti-(human lysozyme) immunoglobulin G has been used as a specific inhibitor in bactericidal and bacteriolytic reactions.[354] It was found that at low serum concentrations lysozyme was the primary bactericide active against *B. subtilis*. At appreciably higher serum concentrations, a lysozyme-independent bactericidal activity was also demonstrated.

Lysozymes from hen, duck II, and goose egg white and human lysozyme were compared at the level of their L-tryptophyl residues using the solvent perturbation technique and fluorescence emission in addition to two chemical modifications.[355] The three L-tryptophyl residues of goose lysozyme were found completely exposed to the solvent. The study of the situation of the aromatic residues of hen, duck II, and human lysozymes provided additional evidence that the three-dimensional structures of these enzymes are very similar, although an unexpected reactivity of L-tryptophan 28 of human lysozyme towards *N*-bromosuccinimide could be evidenced.

As part of a study of the 'whey' proteins of various mammals a comparison has been made of the α-lactalbumins and lysozymes of the kangaroo and horse.[356] In the milk of the red kangaroo (*Megaleia rufa*) there is only one α-lactalbumin and it occurs throughout lactation, but no lysozyme has been detected. There are two α-lactalbumins in the milk of the grey kangaroo (*Macropus giganteus*), one, designated α-lactalbumin Zone B, is present throughout lactation; the second, designated α-lactalbumin Zone A, is present only in late lactation. One lysozyme is also present. The milk of the horse (*Equus caballus*) contains one α-lactalbumin and at least one lysozyme. Partial amino-acid sequences are proposed from sequence determination and from analyses of tryptic peptides compared with the known sequences of other α-lactalbumins and lysozymes.

Saccharomyces cerevisiae (1→3)-β-D-glucan is a potent reticuloendothelial stimulant whose immunobiological activity is mediated, in part, by an increase in the number and function of macrophages.[357] In studying the role of D-glucan as a mediator of antibacterial activity, we attempted to ascertain the ability of D-glucan to modify the mortality of mice with experimentally induced Gram-positive bacteria, and to enhance antibacterial defences in rats as denoted by serum lysozyme and phagocytic activity. After intravenous administration of glucan, serum lysozyme concentrations were increased approximately seven-fold over control concentrations. The increase in serum lysozyme appeared to parallel the D-glucan-induced increase in phagocytosis and induced hyperplasia of macrophages. Prior treatment of mice with D-glucan significantly enhanced their survival when they were challenged systemically with *Staphylococcus aureus*. These studies indicate that D-glucan confers an enhanced state of host defence against bacterial infections.

[354] M. E. Selsted and R. J. Martinez, *Infect. Immun.*, 1978, **20**, 782.
[355] J. Perraudin, Y. Looze, J. Vincentelli, and J. Leonis, *Comp. Biochem. Physiol., Pt. B*, 1980, **65**, 127.
[356] K. Bell, H. A. McKensie, V. Muller, and D. C. Shaw, *Mol. Cell. Biochem.*, 1980, **29**, 3.
[357] P. L. Kokoshis, D. L. Williams, J. A. Cook, and N. R. DiLuzio, *Science*, 1978, **199**, 1340.

Lysozyme extracted from the egg white of tortoise, the first example of a reptilian lysozyme to have been purified, has been crystallized and its tertiary structure determined at low resolution by *X*-ray analysis.[358] This structure is shown to be closely homologous to that of hen egg white lysozyme. The crystals of tortoise egg white lysozyme contain a large proportion of liquid and the *X*-ray map shows that this forms large channels through the crystals into which the active sites of the enzyme molecules open. This indicates that tortoise lysozyme crystals may be suitable for low-temperature studies of true enzyme–substrate complexes.

Two crystal forms of lysozyme from the egg white of the embden goose (*Anser anser*) have been obtained, both of which are suitable for *X*-ray diffraction analysis.[359] The monoclinic form has space group $P2_1$ with cell dimensions $a = 38.3$ Å, $b = 65.7$ Å, $c = 45.2$ Å, $\beta = 116°$ and the triclinic form (space group $P1$) has cell dimensions of $a = 39.9$ Å, $b = 42.2$ Å, $c = 57.9$ Å and $\alpha = 98.8°$, $\beta = 102.5°$, $\gamma = 90.5°$.

In order to know whether or not the absence of the fourth isomer of hen egg lysozyme, containing three presumably native disulphide bonds and one open disulphide bond between L-cysteine-30 and L-cysteine-115, during air oxidation of reduced lysozyme (A. S. Acharya and H. Taniuchi, *J. Biol. Chem.*, 1976, **251**, 6934) is obligatory, the intermediate species formed from non-native lysozyme during thiol-dependent renaturation have been investigated under two different solution conditions.[360] In the first, non-native lysozyme, prepared by reshuffling of the disulphide bonds by incubation with 2-mercaptoethanol in the presence of 8 M urea, was incubated with an equimolar amount of reduced lysozyme at pH 8.0, 37 °C for 10 min, and then reacted with [1-^{14}C]iodoacetic acid. In the second, the same non-native lysozyme was incubated with 1 mM 2-mercaptoethanol at pH 8.0, 37 °C for 8 min, and reacted with [1-^{14}C]iodoacetic acid. The enzymically active, radioactive species formed in these systems were fractionated by gel filtration and ion-exchange chromatography and their tryptic peptides containing [1-^{14}C]carboxymethylcysteine were examined. Under the first conditions the fourth isomer was again not found, although the three other isomers containing three presumably native disulphide bonds and one open disulphide bond were present. On the other hand the fourth isomer as well as other isomers were clearly present under the second conditions. It follows that the formation of the fourth isomer is influenced by the solution conditions. From this it further follows that the absence of the fourth isomer under a set of conditions cannot be considered as a manifestation of the obligatory nature of the pathway for formation of the native disulphide bonds.

The acid–base titration curves of hen egg white lysozyme obtained by three groups (R. Sakakibara and K. Hanaguchi, *J. Biochem.*, 1968, **64**, 613; C. Tanford and R. Roxby, *Biochemistry*, 1972, **11**, 2192; W. Pfeil and P. L. Privalov, *Biophys.*

[358] R. Aschaffenburg, C. C. F. Blake, H. M. Dickie, S. K. Gayen, R. Keegan, and A. Sen, *Biochim. Biophys. Acta*, 1980, **625**, 64.
[359] M. G. Grutter, K. L. Rine, and B. W. Matthews, *J. Mol. Biol.*, 1979, **135**, 1029.
[360] A. S. Acharya and H. Taniuchi, *J. Biol. Chem.*, 1980, **255**, 1905.

Chem., 1976, **4**, 23) were analysed using the empirical formula of Linderstrøm-Lang.[361] Hen lysozyme has 32 ionizable groups including the α-amino- and α-carboxy-groups. Of the 21 groups other than the 11 arginyl residues, the pK values of 17 ionizable groups have been determined by various methods. Using these pK values, the pK values of the other four ionizable groups were estimated. The apparent pK values were obtained 2.0 ($pK_{int} = 3.4$), 2.1 ($pK_{int} = 3.5$), 2.5 ($pK_{int} = 3.8$), and 7.9 ($pK_{int} = 8.5$) at 0.1 ionic strength and 25 °C. The titration curves obtained by the above three groups were consistently explained in terms of the same set of pK_{int} values. The results obtained also showed that no buried and untitratable groups are present in the native lysozyme molecule.

Chemical changes in hen egg white lysozyme during heating at 150–250 °C for 20 min were investigated by means of i.r., e.s.r., and c.d. spectroscopies and gel permeation chromatography, and further a tryptic hydrolysate from the the lysozyme heated at 200 °C was analysed by ion-exchange chromatography.[362] At 150 °C, polymerization through disulphide linkages was observed, and at 180 °C, both polymerization and degradation occurred. When the temperature was raised to 200 °C, remarkable changes in the structure of lysozyme, such as cleavage and recombination of peptide bonds, occurred. Over 200 °C, polymerization and degradation occurred more violently.

The DC electrical conductivity of large single crystals of hen egg white lysozyme has been measured.[363] The samples were grown from aqueous solution and dried in air with silica gel. The temperature dependence of the conductivity obeyed the relation $\sigma = \sigma_0 \exp(-\Delta E/kT)$, with $\Delta E = 1.2$ eV. The ΔE value agreed with most of the previous results for various proteins in the form of lyophilized powder. On the other hand, $\log \sigma_0$, being between 7 and 11, was much larger than the previously reported values and differed among the samples. An irreversible decrease in σ_0, without affecting ΔE, was observed on heating the samples above 85 °C. It was shown that the set of results can be explained if the charge carriers responsible for the observed conduction are regarded as protons, originating from residual water molecules. Photoresponse of the samples to u.v. radiation between 305 and 315 nm was also observed. Reproducible and reliable results were obtained relatively easily in the present experiments, which is thought to be the main advantage of using single-crystal samples.

A preliminary analysis is presented of whether the isotropic temperature factors derived from refinement of the tetragonal crystal structure of hen egg white lysozyme at 2 Å resolution can be interpreted in terms of molecular motion.[364] If the contributions to the temperature factors from experimental errors, crystal disorder, and imperfections of the molecular model are neglected, the apparent thermal motion is found to be compatible with the pair of molecules that have the strongest interactions in the crystal either moving as a rigid-body with liberation about a common axis, or vibrating in an intramolecular mode with the principal amplitudes radial to this axis. There is only weak

[361] S. Kuramitsu and K. Hamaguchi, *J. Biochem.* (*Tokyo*), 1980, **87**, 1215.
[362] F. Hayase, H. Kato, and M. Fujimaki, *Agric. Biol. Chem.* (*Jpn.*), 1975, **39**, 1255.
[363] M. Ataka and S. Tanaka, *Biopolymers*, 1980, **19**, 669.
[364] M. J. E. Sternberg, D. E. P. Grace, and D. C. Phillips, *J. Mol. Biol.*, 1979, **130**, 231.

evidence for hinge-bending, *i.e.* that the two lobes of lysozyme vibrate so that their separation varies. Side-chains that are exposed to the solvent and some segments of the main polypeptide chain (including residues 70–73, 82–84, 101–103) have greater apparent thermal motion than the remainder of the molecule. An appendix considers the location of instantaneous axes in the hinge-bending model.[365]

By using the antigenic structure of lysozyme determined in this Reporter's laboratory and the *X*-ray co-ordinates, the closest-atom distances between each of the residues in the three antigenic sites and all the other amino-acids of the lysozyme molecule have been calculated.[366] These calculations enabled one to identify the nearest neighbours to each of the site residues. Thus the immediate environment of each site residue is described. For the three antigenic sites there is a total of 71 neighbouring residues. The effects of evolutionary amino-acid substitutions in site-neighbouring residues on the binding capacity of protein-binding sites in general and on protein antigenic sites in particular are discussed. These together with the direct replacements in site residues, will account for the major effects. However, the limitations of this treatment are stressed. The smaller effects on antigenic sites of replacements at once-removed and even at more distant locations, which, when they become cumulative, could be considerable, are brought to attention, together with any influences of conformational readjustments that can take place as a result of evolutionary amino-acid replacements.

The binding constants of 4-methylumbelliferyl β-D-glycosides of *N*-acetylchitobiose and *N*-acetylchitotriose [(Glc*N*Ac)$_2$MeU] and [(Glc*N*Ac)$_3$MeU] to hen lysozyme were determined by measuring changes in the fluorescence at 375 nm.[367] It was shown that (Glc*N*Ac)$_2$MeU and (Glc*N*Ac)$_3$MeU bind mainly at subsites B, C, and D, and A, B, C, and D, respectively, with the terminal MeU group bound at subsite D. The rate of hydrolysis of (GlcNAc)$_3$MeU catalysed by hen and turkey lysozymes was determined at 0.1 ionic strength and 42 °C in the pH range of 2 to 8. The release of 4-methylumbelliferone was followed fluorimetrically. The pH dependences of k_{cat}, k_{cat}/K_m, and K_m were analysed assuming that non-productive binding occurs competitively and that the molecular species with ionized L-Asp^{52} and protonated L-Glu^{35} is active. Comparison of the pH dependences of the kinetic constants for hen lysozyme with those for turkey lysozyme, in which L-Asp^{101} of hen lysozyme is replaced by glycine, made it possible to determine the p*K* values of L-Asp,^{52} L-Glu,^{35} and L-Asp.^{101} The p*K* values of L-Asp^{52} and L-Glu^{35} were 3.60 and 6.20, respectively, for hen and turkey lysozymes and 3.95 and 6.55, respectively, for their non-productive complexes. The p*K* value for L-Asp^{101} of hen lysozyme was 4.20 for the free enzyme, 3.30 for the non-productive complex, and 3.95 for the productive complex. These p*K* values, except for the p*K* value of L-Asp^{52} of the non-productive complex, are in excellent agreement with those determined by spectroscopic methods, and demonstrates that lysozyme-catalysed hydrolysis

[365] R. Diamond, *J. Mol. Biol.*, 1979, **130**, 252.
[366] M. Z. Atassi and A. L. Kazim, *Biochem. J.*, 1980, **187**, 163.
[367] Y. Yang and K. Hamaguchi, *J. Biochem. (Tokyo)*, 1980, **87**, 1003.

can be fully explained in terms of the proposal based on the *X*-ray data with regard to the participation of L-Asp[52] and L-Glu.[35]

The interactions of methyl 2-acetamido-2-deoxy-β-D-glucopyranosides, di-*N*-acetyl chitobiose, and tri-*N*-acetylchitotriose with hen egg white lysozyme in which L-Trp[62] is modified to kynurenine (Kyn[62]–lysozyme) were studied by measuring the changes in the c.d. band and fluorescence of the kynurenine at various pH values.[368] The pH profiles of the binding constants of these saccharides of Kyn[62]–lysozyme were very similar to those to intact lysozyme, although the binding constants were lower for the modified lysozyme than for intact lysozyme. The p*K* values of L-Asp,[52], L-Glu,[35] L-Asp,[48] and L-Asp[101] in Kyn[62]–lysozyme and in its complexes with the glycoside and disaccharide (Glc*N*Ac)$_2$ were in agreement with those of intact lysozyme and its complexes. The p*K* values of L-Asp[52] and L-Glu[35] in the modified lysozyme–(GlcNAc)$_3$ complex were also in agreement with those of the complex with intact lysozyme, but the p*K* shift of L-Asp[101] was smaller for Kyn[62]–lysozyme than for intact lysozyme. The significance of the decreased binding constants to Kyn[62]–lysozyme is discussed.

In a study on the binding of 2-acetamido-2-deoxy-D-glucose and chitobiose to hen egg white lysozyme the crystallization of the enzyme at 40 °C and 50 °C in the presence of the small molecular weight molecules was examined.[369] It was concluded that the molecular area of lysozyme involved in its enzymic mechanism appears flexible and seems to 'rigidify' on binding certain substrates or inhibitors such as chitobiose.

The reaction of hen egg white lysozyme with chitotriose and chitohexase has been investigated at temperatures to below 100 °C by using aqueous methanol and dimethyl sulphoxide cryosolvents.[370] Initial investigations involving the effects of increasing cosolvent concentration on the catalytic and structural properties of the enzyme indicated that both methanol and dimethyl sulphoxide cryosolvents, at subzero temperatures, had no adverse effects on lysozyme. Time-dependent changes in the fluorescence emission of the enzyme, under non-turnover conditions, permitted the detection of two intermediates in the reaction with the trimer and three intermediates in the case of the hexamer. The fastest reaction observed for both substrates was complete within minutes at −90 °C and is attributed to initial substrate binding, followed by rapid isomerization to form a 'loose' enzyme–substrate complex. The subsequent, slower reactions correspond to successive isomerizations of enzyme–substrate complex to 'tighter' complexes. From dye binding displacement reactions, and comparison of dissociation constants, it is concluded that the predominant mode of binding for the hexamer at subzero temperature is productive. Rate and dissociation constants were determined for the observed reactions as a function of temperature, pH, and cosolvent concentration. Overall the reaction pathway in methanol cryosolvents at subzero temperatures appears to be very similar to

[368] K. Teshima, S. Kiramitsu, K. Hamaguchi, F. Sakiyama, K. Mizuno, and N. Yamasaki, *J. Biochem. (Tokyo)*, 1980, **87**, 1015.

[369] J. Berthou, A. Lifchitz, J. Saint-Blancard, and P. Jolles, *FEBS Lett.*, 1979, **108**, 10.

[370] A. L. Fink, R. Homer, and J. P. Weber, *Biochemistry*, 1980, **19**, 811.

that determined from investigations in aqueous solution at ambient temperatures using a variety of rapid reaction techniques. Conditions necessary to accumulate and stabilize each of the observed intermediates have been determined, thus permitting further studies to delve more deeply into the nature of the intermediates.

The thermal and guanidinium chloride denaturation of lysozyme has been investigated at various concentrations of tri-*N*-acetylchitotriose [(Glc*N*Ac)$_3$], a trisaccharide which binds specifically at the active site of native lysozyme.[371] The presence of (Glc*N*Ac)$_3$ leads to a readily observable stabilization of the protein to thermal and guanidinium chloride denaturation. An analysis of guanidinium chloride denaturation curves shows that the stability of lysozyme is increased by 495 cal mol^{-1} by the presence of 3×10^{-4} M (Glc*N*Ac)$_3$. This corresponds to an increase in the stability of lysozyme of 385 and 1275 cal mol^{-1}. These results are in excellent agreement with predictions based on an equation derived by Schellman, *Biopolymers*, 1975, **14**, 999, to take into account the effect of ligand binding on the melting temperature of a protein:

$$T_{1/2} = \frac{TT_0R}{\Delta H_D} \ln(1+K_B[S]) \qquad (1)$$

where T and T_0 are $T_{1/2}$ values in the presence and absence of (Glc*N*Ac)$_3$, ΔH_D is the enthalpy of denaturation in the presence of (Glc*N*Ac)$_3$, K_B is the equilibrium constant for the binding of (Glc*N*Ac)$_3$ to lysozyme, and [S] is the free concentration of (Glc*N*Ac)$_3$. Thus, the increased stability of an enzyme in the presence of its substrate, coenzyme, or any small molecule that it binds specifically results because binding to the native state shifts the unfolding equilibrium and decreases the concentration of unfolded states of the enzyme. It is suggested that this may be a more important factor than substrate-induced conformational changes in accounting for the decreased rates of protein catabolism frequently observed *in vivo* at elevated substrate concentrations.

Rates of lysis by lysozyme of synchronously dividing cells of *Bacillus subtilis* rose in concert with the stepwise increase in cell numbers.[372] Asynchronously growing cells showed no periodicities in sensitivity; rates of lysis reflected the exponential increase in culture density.

The cleavage of cell-wall tetrasaccharide, the (1→4)-linked dimer of the basic repeating disaccharide 2-acetamido-2-deoxy-β-D-glucopyranosyl (1→4)-*N*-acetyl-D-muramic acid, by lysozyme has been studied at various concentrations of lysozyme and over long time ranges.[373] A theoretical analysis of the kinetic results indicates that direct hydrolysis of the tetrasaccharide by binding in subsites CDEF of the active sites of lysozyme is significant at long times relative to the transglycosylation pathway. The binding constant for tetrasaccharide in CDEF is shown to be 10^3 times larger than that determined on the basis of an analysis of kinetic data over a more restricted range of times and concentrations.

[371] C. N. Pace and T. McGrath, *J. Biol. Chem.*, 1980, **255**, 3862.
[372] C. Edwards, *J. Gen. Microbiol.*, 1980, **119**, 277.
[373] K. Bockelheide, S. L. Patt, G. Weisz, J. H. Baldo, and B. D. Sykes, *Can. J. Biochem.*, 1979, **57**, 785.

Clostridium perfringens and isolated walls of this organism autolysed rapidly when incubated in buffer at pH 7.0 with the release of free-reducing groups but no *N*-terminal amino-acids (see p. 287).[215]

A 6 Å resolution electron-density map has been calculated for a bacterial lysozyme produced by *Streptomyces erythraeus*.[374] This lysozyme differs from the vertebrate lysozyme in its size, amino-acid composition, and specificity. The structure was determined by the method of isomorphous replacement. Three heavy-atom derivatives were obtained by soaking crystals of the lysozyme in $HgCl_2$, K_2PtCl_4, and $UO_2(NO_3)_2 6H_2O$. The resulting electron-density map clearly shows the molecular boundary. The molecule is ellipsoidal in shape with average dimensions 50 Å × 35 Å × 35 Å. High-resolution analysis and sequence analysis of the molecule are in progress.

38 Mannanases (Miscellaneous)

Extraction and fractionation of the β-D-mannanases in germinating lucerne (alfalfa) seeds produced four fractions if extracts were stored prior to chromatography.[375] However, when extracts were applied rapidly to DEAE-cellulose, one of the fractions of high-molecular weight (separated subsequently by gel chromatography) was not detected, indicating that it was formed after maceration. On storage, the amount of the lower-molecular-weight, unbound fraction from chromatography on DEAE-cellulose did not increase relative to that bound. During germination, the bound and unbound fractions both increased initially and then decreased, with only small changes in the ratio of each. The results suggest that three of the β-D-mannanase fractions are formed *in vivo* prior to maceration.

Lytic strains of *Oerskovia xanthineolytica*, *O. turbata*, and non-motile *Oerskovia*-like organisms produced laminaranase and α-D-mannanase, but the amounts, determined by reducing group assays, were not proportional to the observed lytic activities (see p. 315).[326]

D-Mannan-hydrolysing enzymes of yeasts and yeast-like organisms have been studied.[376]

39 Pectate, Pectin, and Poly-D-galacturonate Lyases

A rapid, sensitive slab gel electrophoretic method for the study of pectic enzymes is described.[377] By incorporating pectin in acrylamide gels and subsequent staining with ruthenium red, simultaneous detection of esterases, glycosidases, and poly-D-galacturonate lyases was possible due to their differing staining reactions. Application of the method to the study of the thermal stability of enzymes is described. Approximate isoelectric points of the enzymes could be determined from their positions in the gel.

[374] R. Sarma, S. Harada, N. Tanaka, M. Kakudo, S. Hara, and T. Ikenaka, *J. Biochem. (Tokyo)*, 1979, **86**, 1765.
[375] N. K. Matheson, D. M. Small, and L. Copeland, *Carbohydr. Res.*, 1980, **82**, 325.
[376] J. Augustin, J. Zamek, L. Kuniak, and A. Kockovakatochvilova, *Folia Microbiol.*, 1980, **25**, 301.
[377] R. H. Cruickshank and G. C. Wade, *Anal. Biochem.*, 1980, **107**, 177.

The characterizations of intracellular poly-D-galacturonate lyases from *Klebsiella oxytoca*, *Yersinia enterolytica*, and *Erwinia chrysanthemi* have been reported.[378]

A poly-D-galacturonate lyase from the culture fluid of *Lachnospira multiparus* was purified about 20-fold.[379] The optimum pH and temperature for enzyme activity were 8.0 and 40 °C. The enzyme required Ca^{2+} and was inhibited by H_4 e.d.t.a.; it preferred poly-D-galacturonate as substrate, cleaving (1→4)-glycosidic linkages randomly to form unsaturated galacturonates, mainly the unsaturated digalacturonate. Some properties of the crude and purified enzyme preparations are described.

An unspecialized strain of *Rhizoctonia solani* (FC895) produced *in vitro* seven poly-D-galacturonase isoenzymes (four major and three minor peaks), which were separated by isoelectric focusing over a narrow pH range.[380] The same isoenzyme pattern was detected when hypocotyls of cauliflower seedlings inoculated with the fungus were used as the enzyme source. A specialized strain (FC1241) produced two isoenzymes (one major and one minor peak). The four major isoenzymes of FC895 and the major isoenzyme of FC1241 had the same pH optimum and mode of action of sodium polypectate.

40 Poly-D-galacturonases

A rapid, sensitive slab gel electrophoretic method for the study of pectic enzymes including poly-D-galacturonase is described (see p. 330).[377]

A comparative study of extracellular and 'surface' poly-D-galacturonases produced *in vitro* by *Colletotrichum lindermuthianum* has been reported.[381]

Three forms of poly-D-galacturonase from *Saccharomyces fragilis* (*Kluyveromyces fragilis*) were separated by column chromatography and repeated preparative disc electrophoresis.[382] Each enzyme was almost homogeneous as judged by polyacrylamide gel electrofocusing and disc electrophoresis. Enzymes I and II were similar but enzyme III was different from enzymes I and II in isoelectric point. The three enzymes resembled one another in their enzymic action on pectic acid and in their other properties. All the three enzymes showed macerating activity toward the potato and carrot tissues.

Poly-D-galacturonase has been used in enzymically active immobilized form prepared by a covalent binding to hydroxalkylmethacrylate gels.[383]

41 *exo*-Poly-D-galacturonases

An *exo*-poly-D-galacturonase is produced by *Lachnospira multiparus*.[379]

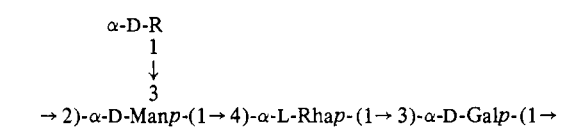
[378] S. T. Bagley and M. P. Starr, *Curr. Microbiol.*, 1979, **2**, 381.
[379] M. Wojciechowicz, K. Heinrichova, and A. Ziolecki, *J. Gen. Microbiol.*, 1980, **117**, 193.
[380] A. Scala, L. Camardella, F. Scala, and F. Cervone, *J. Gen. Microbiol.*, 1980, **116**, 207.
[381] D. Cantenys and J.-P. Barthe, *C. R. Hebd. Seances Acad. Sci. Ser. D*, 1979, **289**, 1183.
[382] J. Lim, Y. Yamasaki, Y. Suzuki, and J. Ozawa, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 473.
[383] L. Rexova-Benkova, M. Mrackova, and K. Babor, *Collect. Czech. Chem. Commun.*, 1980, **45**, 163.

42 Pullulanases

Enzymic digestion of raw-starch granules takes place much more slowly than does the breakdown of soluble or gelatinized starch.[253] Many effects have been made to define the factors that affect starch granule enzymolysis, but as yet it has not proved possible to demonstrate efficient granule-breakdown *in vitro* by any amylase. Consideration has been given to the possibility that the process requires the synergistic action of two or more enzymes. Indeed, the suggestion was made many years ago that a hypothetical 'raw-starch factor' is involved in the process, and this possibility has not yet been eliminated, although the nature of the so-called raw-starch factor has not been identified. It has generally been assumed that such a factor would be enzymic in nature, and increasing attention has recently been paid to the possibility that two or more amylolytic enzymes acting together may function synergistically in raw-starch digestion. In view of the observation that debranching enzymes may assist amylolysis of barley-starch granules, this possibility has been further investigated. Results show that pullulanase (NG) has little effect on the α-amylolysis of waxy-maize, starch granules, but stimulates β-amylase action greatly.

43 *endo*-Rhamnanases

Twelve bacteriophages lysing only smooth *Salmonella typhimurium* strains were shown to have similar morphology, *i.e.* an icosahedral head to which a short, non-contractile tail carrying six spikes was attached.[384] All phages degraded their lipopolysaccharide (LPS) receptors as shown by their ability to cleave off D-[^{14}C]galactosyl-containing oligosaccharides from *S. typhimurium* cells labelled in their LPS. The oligosaccharides inhibited the α-D-galactosyl-specific *Bandeiraea simplicifolia* lectin agglutination of human type B erythrocytes, indicating that all 12 phage glycanases were of *endo*-rhamnanase specificity, *i.e.* hydrolysed the α-L-rhamnopyranosyl-(1→3)-D-galactopyranosyl-linkage in the *S. typhimurium* *O*-polysaccharide chain. Two of the phages, 28B and 36, were studied in more detail. Whereas the phage 28B glycanase hydrolysed the *S. typhimurium* lipopolysaccharides into dodeca- and octa-saccharides, the phage 36 glycanase in addition cleaved off tetrasaccharides. Both phage enzymes hydrolysed the *O*-polysaccharide chains of lipopolysaccharides from *Salmonella* belonging to sero-groups A, B, and D1, which are built up of tetrasaccharide-repeating units (6) identical except for the nature of the 3,6-dideoxyhexopyranosyl group (R).



(6) R = 3,6-dideoxyhexopyranosyl group

[384] S. B. Svenson, J. Lonngren, N. Carlin, and A. A. Lindberg, *J. Virol.*, 1979, **32**, 583.

The phage 28B and 36 *endo*-rhamnanases hydrolysed also a lipopolysaccharide from which the 3,6-dideoxyhexosyl substituents had previously been hydrolysed off. However, neither of the enzymes was active on lipopolysaccharide preparations in which the C-2–C-3 bond of the L-rhamnopyranosyl ring had been opened by periodate oxidation. D-Glucosylation at O-6 of the D-galactopyranosyl residues in the *S. typhimurium* lipopolysaccharides was found to be incompatible with hydrolysis by both enzymes. However, in a lipopolysaccharide glucosylated at O-4 of the D-galactopyranosyl residues, the adjacent α-L-rhamnopyranosyl linkages were found to be preferentially cleaved.

44 Sucrose α-D-Glucohydrolases and 'Sucrase–Isomaltases'

A continuous optical method for the assay of D-glucose-releasing hydrolases, α-D-glucosidase, αα-trehalase, β-D-fructofuranosidase, is reported (see p. 247).[82]

In answer to the question 'does sucrase–isomaltase always exist as a complex in human intestine?' evidence for the *in vivo* existence of free, active isomaltose in addition to the sucrase–isomaltase complex in human proximal jejunum but not in ileum.[385]

The effect of the pesticides phosalone and lindane on the activity of some dipeptidases and disaccharidases including sucrase α-D-glucohydrolase (sucrase) in rat intestinal mucosa has been studied.[112]

45 αα-Trehalases

A continuous optical method for the assay of D-glucose-releasing hydrolases, α-D-glucosidase, αα-trehalase, β-D-fructofuranosidase, is reported (see p. 247).[82]

The effect of the pesticides phosalone and lindane on the activity of some dipeptidases and disaccharidases including αα-trehalase in rat intestinal mucosa has been studied (see p. 258).[112]

Hydrolysis of αα-trehalose by trehalase prepared from the flesh fly *Sarcophagar barbata* has been shown to proceed with inversion of configuration at C-1 of one of the two D-glucose molecules released.[386]

Continuous and discontinuous coupled fluorometric assays, which couple trehalose hydrolysis to peroxidation of the fluorogenic compounds eugenol (4-allyl-2-methoxyphenol) or 4-hydroxyphenylacetic acid using D-glucose oxidase and peroxidase as ancillary enzymes, have been developed for the measurement of αα-trehalase activity from the cellular slime mould, *Dictyostelium discoideum*.[387] With these methods, product formation was linear with time and the coupled reaction rate was directly proportional to the level of enzyme assayed. The validity of both the discontinuous and continuous fluorometric assays was confirmed by comparative studies with discontinuous spectrophotometric assays for D-glucose. The high level of sensitivity achieved with these assays makes them ideally well suited for: (i) elucidation of the regulatory

[385] H. Skovbjerg, H. Sjostrom, and O. Noren, *FEBS Lett.*, 1979, **108**, 399.
[386] K. H. Clifford, *J. Chem. Soc., Chem. Commun.*, 1979, 136.
[387] K. A. Killick, *Anal. Biochem.*, 1980, **105**, 291.

mechanisms underlying the dramatic changes in αα-trehalase activity that occur during spore germination and cellular aggregation in *Dictyostelium*, and (ii) characterization studies involving electrophoresis or isoelectric focusing of αα-trehalase in solid matrices in which enzymatic activity is measured either quantitatively in gel eluates or qualitatively in the *in situ* localization of the enzyme histochemically.

46 *endo*-(1→4)-β-D-Xylanases

The mode of action of the extracellular *endo*-(1→4)-β-D-xylanase produced by *Trametes hirsuta* on a (4-*O*-methyl-D-glucurono)-D-xylan and a modified, essentially neutral D-xylan from white willow (*Salix alba* L.) has been studied.[388] Xylotetraose and xylohexaose, together with aldotetraouronic and aldohexaouronic acids, were the main products. The acidic oligosaccharides had a 4-*O*-methyl-D-glucopyranosyluronic acid group attached to the non-reducing D-xylopyranosyl end-group. The action pattern of the xylanase corresponds to that of a typical *endo*-enzyme that acts more readily in the middle of chain, and the specific region of its action appears to involve five D-xylopyranosyl residues. The products of the enzymic treatment of the D-xylan have revealed a regular distribution of the 4-*O*-methyl-D-glucopyranosyluronic acid groups attached to the D-xylan backbone.

endo-(1→4)-β-D-Xylanase from the culture filtrate of a mushroom, *Termitomyces clypeatus*, has been purified to a single protein band on disc gel electrophoresis at pH 9.5, and has a molecular weight of approx. 9.0×10^4.[389] It also acts on amylopectin L-arabino-D-xylan and L-arabino-D-galactan. The enzyme shows maximum activity on (1→4)-β-D-xylan at pH 5.5 and at 55 °C and is fairly stable between pH 3 and 10 and temperatures up to 60 °C. Hg^{2+} is the most potent inhibitor, whereas Fe^{2+}, Ag^{+}, iodoacetate, and phosphate moderately inhibit the enzyme activity.

(1→4)-β-D-Xylanase has been detected in both cell-free extracts and culture fluids of the yeast *Cryptococcus albidus* var. *aerius* grown on D-glucose as the only carbon source.[390] Mild acid treatment of whole cells proved that the enzyme was extracellularly located. The activity remained almost completely linked to the wall after cell breakage, only being liberated in the presence of salt at high concentration. After release, the enzyme became very unstable and so has been characterized *in situ* in 'permeabilized' cells. The maximum production took place at the beginning of the exponential growth phase. The optimum pH and temperature for activity were 5.0 and 40 °C, respectively. The enzyme degraded D-xylan and D-xylo-oligosides by an *endo*-splitting mechanism giving xylobiose, xylotriose, and D-xylose as the main end-products. Activation energy and kinetic constants for D-xylan degradation were determined. Several metal

[388] M. Kubackova, S. Karacsonyi, L. Bilisics, and R. Toman, *Carbohydr. Res.*, 1979, **76**, 177.
[389] A. K. Ghosh, P. C. Banerjee, and S. Sengupta, *Biochem. Biophys. Acta*, 1980, **612**, E84, 143.
[390] V. Notario, T. G. Villa, and J. R. Villanueva, *J. Gen. Microbiol.*, 1979, **114**, 415.

ions such as Ag^+ and Hg^{2+} inhibited the enzyme. The possible function of this *endo*-D-xylanase in *Cr. albidus* var. *aerius* is discussed.

47 Xylanases (Miscellaneous)

Five different D-xylanases and β-D-xylosidase in the culture medium of *Aspergillus niger* have been purified to homogeneity from 13- to 52-fold by a procedure of gel and hydroxylapatite chromatography.[208] The strain was isolated from soil of the African equatorial forest. Gel chromatography of the purified enzymes indicated that three of the xylanases have molecular weights of 31 000 and the other two D-xylanases have molecular weights of 50 000.

48 Carbohydrate Isomerases

D-Glucose Isomerases. – The production of D-glucose isomerase by different strains of *Streptomyces* has been investigated.[391]

The simultaneous production of D-glucose isomerase and a cellulase complex by *Streptomyces flavogriseus* has been reported.[301]

A genetic improvement of D-glucose isomerase production in *Streptomyces nigrificans* has been effected by u.v. irradiation.[392]

D-Glucose isomerase has been used in enzymically active immobilized forms obtained: glutaraldehyde linking with collagen fibrils and by the same procedure that coimmobilized with D-glucose oxidase.[393]

49 Carbohydrate Oxidases

D-Galactose Oxidases. – A study of the co-ordination chemistry of three chemically modified forms of the copper(II)-containing enzyme, D-galactose oxidase, has been carried out.[394] Alkylation of one unique histidine residue by iodoacetamide yields in inactive enzyme that does not bind CN^- or F^-, a property characteristic of the native enzyme. Oxidation by *N*-bromosuccinimide in 5 mM acetate buffer produces in inactive enzyme that binds F^- and CN^- in a manner very similar to that of the unmodified enzyme. However, after oxidation in 100 mM acetate buffer, CN^- causes a chemical reduction and F^- interacts with the copper(II) ion only weakly. Apparently, the different ionic strengths of 5 and 100 mM acetate buffer allow two and three L-tryptophans, respectively, to be oxidized. Taken together, these results suggest that different factors affect anion and substrate binding and further suggest that different sites at the one copper(II) centre take part in these two processes. The $[Fe(CN)_6]^{3-}$ ion interacts with all modified forms and the unmodified enzyme to produce similar changes in the

[391] K. Demnerova, S. Pospisil, O. Valentova, and J. Kas, *Biotechnol. Lett.*, 1979, **1**, 293.
[392] K. Demnerova, S. Pospisil, M. Blumaverova, and J. Kas, *Biotechnol. Lett.*, 1979, **1**, 299.
[393] S. Gondo, M. Morishita, and T. Osaki, *Biotechnol. Bioeng.*, 1980, **22**, 1287.
[394] M. E. Winkler and R. D. Bereman, *J. Am. Chem. Soc.*, 1980, **102**, 6244.

optical spectra. However, the e.s.r. spectrum does not disappear with either of the modified forms as has been observed for the unmodified D-galactose oxidase. This represents definitive evidence that the unique optical changes are not due to the presence of Cu^{III} in galactose oxidase. The additional observation of organic radical signals in the e.s.r. spectra of the modified forms in the presence of $Fe(CN)_6^{3-}$ suggest that the loss of the copper e.s.r. signal in the native enzyme under similar conditions may be caused by magnetically coupled copper(II) radical pair or perhaps a copper(II)–ferricyanide dimer pair.

Exposure of D-galactose-containing glycosphingolipids and certain glycoproteins to D-galactose oxidase followed by treatment with sodium borotritide has been used to label specifically the terminal D-galactose and 2-acetamido-2-deoxy-D-galactose moieties of these glycoconjugates.[395] This labelling procedure was shown to be ineffective with a lipid isolated from rat testis, 1-*O*-palmityl-2-*O*-palmitoyl-3β-D-galactosyl-glycerol, under conditions which resulted in excellent labelling by this procedure. It was shown that neither 1-*O*-palmityl-2-*O*-palmitoyl-3β-(D-galactosyl 3′-sulphate)-glycerol nor D-galactosylsulphate-ceramide ('classical sulphatide') was labelled by this procedure indicating that sulphate substitution of the D-galactose inhibits the action of D-galactose oxidase. The labelling of D-galactosyl-ceramide was not inhibited in the presence of the D-galactoglycerolipid, D-galacto-sulphate-glycerolipid, or sulphatide. The property of the lipid aglycone responsible for inhibiting D-galactose oxidase action remains to be determined.

Labelling of intact human erythrocytes with D-galactose oxidase–sodium [$^3H_4$]borohydride resulted in the incorporation of radioactivity into the monosaccharide transporter.[396] When the purified labelled protein was subjected to sodium dodecyl sulphate–gel electrophoresis, the peak of radioactivity migrated more slowly than the peak of Coomassie Blue-staining material. *endo*-β-D-Galactanase treatment of the purified labelled transporter led to partial loss of the label, sharpening of the electrophoretic profile, and a change in the apparent molecular mass of the polypeptide from 5.5×10^4 to 4.6×10^4. Approximately 50% of the transporter bound to a column of *Ricinus communis* agglutinin I-agarose. These findings demonstrate that the transporter is heterogeneously glycosylated and, in conjunction with other data, show that it is a transmembrane protein and probably a source of erythroglycan.

Methods for growing the fungus, *Dactylium dendroides*, and for purifying the secreted protein, D-galactose oxidase, are described. Fungal growth takes 1 week.[397] Enzyme purification takes 2 days and yields 70 mg homogeneous enzyme per 25 litres' growth medium.

D-Glucose Oxidases. – The cytochemical localization of D-glucose oxidase in the peroxisomes of *Aspergillus niger* has been described.[398]

D-Glucose oxidase has been used in enzymically active immobilized forms obtained by the following routes: reaction with collagen activated by an acyl-

[395] C. A. Lingwood, *Can. J. Biochem.*, 1979, **57**, 1138.
[396] F. R. Gorga, S. A. Baldwin, and G. E. Lienhard, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 955.
[397] P. Tressel and D. J. Kosman, *Anal. Biochem.*, 1980, **105**, 150.
[398] J. P. van Dijken and M. Veerhuis, *Eur. J. Appl. Microbiol. Biotechnol.*, 1980, **9**, 275.

azide activation process,[399] glutaraldehyde linking with collagen fibrils and by the same procedure but coimmobilized with D-glucose isomerase,[393] *etc.*[400]

50 Carbohydrate Transferases

Cyclomaltodextrin D-Glucanotransferases. – The structural features of the sugars required as an acceptor of cyclodextrin glycosyltransferases from *Bacillus megaterium* and *B. macerans* were investigated using 22 kinds of monosaccharides and their derivatives.[401] It is concluded on the basis of the experimental results that the requirement for an acceptor of the intermolecular transfer reaction catalysed by this enzyme is the pyranose structure having the same configurations of the free C-2, C-3, and C-4 hydroxy-groups as in D-glucopyranose. The reaction products of the *B. megaterium* enzyme from starch and 2-deoxy-D-glucose, a poor acceptor, were analysed by paper chromatography. The main product was isolated and identified by the chemical and enzymatic methods as 4-*O*-α-D-glucopyranosyl-2-deoxy-D-glucose. The products contained a series of ordinary malto-oligosaccharides besides a series of malto-oligosaccharides each terminated by a single 4-*O*-α-D-glucosyl-2-deoxy-D-glucose residue at the reducing end.

An extracellular cyclodextrin glucanotransferase from *Bacillus macerans* was purified to homogeneity by adsorption on starch, ammonium sulphate fractionation, column chromatography on DEAE-cellulose, and gel filtration on Sephadex® G-100.[402] The enzyme had a molecular weight of 67 000 and consisted of one polypeptide chain. The isoelectric point was pH 5.4. Temperature and pH optima were 60 °C and 5.4–5.8, respectively. The purified enzyme was quite stable at 50 °C (pH 6.0), but lost 80% of its activity at 60 °C for 30 min (pH 6.0). Prolonged digestion by trypsin did not affect the catalytic properties of the enzyme. The K_m for starch was 5.7 mg ml^{-1}.

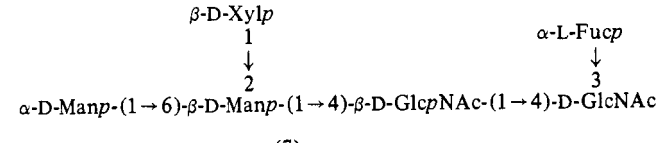
The transfer reactions, particularly the cyclization reaction, catalysed by the cyclodextrin D-glucanotransferase from *Klebsiella pneumoniae* were studied with (1→4)-α-D-glucopyranosyl chains (DP 16).[403] The initial rate of the cyclization reaction with substrate concentrations from 1 up to 16 mM indicated a V of 6.2 kcat kg^{-1} of protein and a molar catalytic activity of 421.6 kcat mol^{-1} of enzyme. K_m was found to be 1.03 mM. In addition to the cyclization, cyclodextrin D-glucanotransferase simultaneously catalysed a disproportionation of the substrate, yielding shorter malto-oligosaccharides and (1→4)-α-D-glucopyranosyl chains, which were significantly longer than the substrate itself. Cyclomaltohexaose and cyclomalto-octaose were accumulated in the course of longer incubation. They arose mainly from coupling reactions with the initially formed cyclomaltohexaose and corresponding disproportionation of these transfer products. The extremely low formation rates of the higher cyclodextrins point to a 'mistake' of the enzyme, when cyclizing to cyclomaltohexaose and cyclomalto-octaose.

[399] P. R. Coulet, R. Sternberg, and D. R. Thevenot, *Biochim. Biophys. Acta*, 1970, **612**, 317.
[400] V. Sahai and K. Das, *Indian J. Biochem. Biophys.*, 1980, **17**, 287.
[401] S. Kitahara, S. Okada, and T. Fukui, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 2369.
[402] A. Stavin and P. E. Granum, *Carbohydr. Res.*, 1979, **75**, 243.
[403] H. Bender, *Carbohydr. Res.*, 1980, **78**, 133.

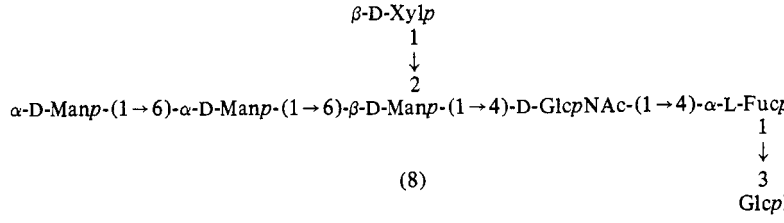
The time course of the (1→4)-α-D-glucopyranosyltransfer reactions catalysed by the cyclodextrin D-glucanotransferase from *Klebsiella pneumoniae* was studied with several commercial amyloses, potato starch, and amylopectin, respectively.[404] Amyloses were poor substrates for the cyclization reaction. In the initial phase of the transfer reactions, the cyclodextrin D-glucanotransferase catalysed a rapid shortening of the amylose chains. The rates of this shortening reaction was significantly accelerated by addition of malto-oligosaccharides. Maximum rate of cyclohexa-amylose formation was reached with amylose chains sufficiently short ($<Glc_{100}$) for the cyclization reaction. Cyclomaltohexaose was formed with maximum rate from amyloses containing amylopectin impurities in the initial phase of the transfer reactions, suggesting that the non-reducing ends of the outer amylopectin chains serve as acceptors for the disproportionation of the amylose. Accordingly, water-soluble, high-molecular-weight products containing higher percentages of lengthened outer chains were obtained from potato starch or amylopectin. In the course of the transfer reactions only traces of smaller malto-oligosaccharides were detected chromatographically.

51 Glycopeptide-linkage Hydrolases

4-L-Aspartyl-β-D-glucosylamine Amidohydrolases. – Asparagine-linked oligosaccharides of stem bromelain glycopeptides were quantitatively released by digestion with the almond glycopeptidase which cleaves N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl) hydrogen L-asparaginate linkages in glycopeptides with oligopeptide moieties.[405] The primary structures of the two oligosaccharide components (D-Man)$_3$(D-Xyl)$_1$(L-Fuc)$_1$(D-GlcNAc)$_2$, (7) and (D-Man)$_2$(D-Xyl)$_1$-(L-Fuc)$_1$(D-GlcNAc)$_2$, (8) were elucidated.



(7)



(8)

52 Nucleases

Exonucleases. – Exonucleases from *Crotalus adamanteus* venom has only L-threonine as *N*-terminal amino-acid residue.[406] It was examined for its amino-

[404] H. Bender, *Carbohydr. Res.*, 1980, **78**, 147.
[405] H. Ishihara, N. Takahashi, S. Oguri, and S. Tejima, *J. Biol. Chem.*, 1979, **254**, 10 715.
[406] L. B. Dolapchiev, R. A. Vassiveva, and K. S. Koumanov, *Biochim. Biophys. Acta*, 1980, **622**, 331.

acid composition, -SH and S-S groups. It has no free -SH groups and seven S-S bonds. The analysis of the carbohydrate residues in the enzyme proves that it is a glycoprotein. It contains neutral sugars (9.2%), amino-sugars (1.9%), and 10 neuraminic acid residues per molecule.

53 Phosphatases

Alkaline Phosphatases. – Alkaline phosphatase was partially purified from human milk and its antigenic, functional, and structural properties were characterized.[407] By immunochemical and enzymic criteria, the enzyme resembled the alkaline phosphatase isoenzyme found in human liver. Two-dimensional electrophoretic analysis showed that the milk enzyme differed from the liver both in subunit molecular weight and in isoelectric point. These differences were shown to result from variation in neuraminic acid content.

The non-specific alkaline phosphatase of yeast (*Saccharomyces* strain 1710) has been purified by ion-exchange, hydrophobic, and affinity chromatography.[408] This vacuolar enzyme has a molecular weight of 130 000 and is composed of subunits (probably of 66 000 molecular weight). It also has a small quantity of covalently associated carbohydrate; hydrolysis yielded D-mannose and 2-amino-2-deoxy-D-glucose. The *endo*-β-D-2-acetamido-2-deoxyglucanase of *Streptomyces plicatus* released carbohydrate indicating that the latter was attached to protein through an N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl) hydrogen L-asparaginate glycopeptide bond.

54 Proteinases

Proteinase I from the cellular slime mould *Dictyostelium discoideum* is conjugated with phosphoryl moieties and a characterization of the moieties has now been reported.[409] Essentially all of the phosphate associated with purified enzyme was released as a sugar phosphate during mild alkaline hydrolysis. The sugar-phosphate was isolated from alkaline hydrolysates of proteinase I by gel filtration and was identified as 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate. In separate experiments, involving acid hydrolysis of proteinase I, it was shown that enzyme-phosphate could also be isolated as *O*-phosphorylserine. Based on the recovery of *O*-phosphorylserine from acid hydrolysates, it was concluded that the majority of the 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate residues in the proteinase were esterified to peptidyl serines through phosphoester bonds.

55 Sulphatases

Arylsulphatases A and B. – The activity of the glycosidase, 'arylsulphatase', was assayed in serum samples using the 4-methylumbelliferyl substrates and found to be independent of sex and age (see p. 239).[47]

[407] T. A. Hamilton, S. Z. Gornicki, and H. H. Sussman, *Biochem. J.*, 1977, **177**, 197.
[408] H. R. Onishi, J. S. Tkacz, and J. O. Campen, *J. Biol. Chem.*, 1979, **254**, 11943.
[409] G. L. Gustafson and L. A. Milner, *J. Biol. Chem.*, 1980, **255**, 7208.

In a study of eight glycoside hydrolases in serum samples from 72 cystic fibrosis patients, 85 cystic fibrosis parents, and 34 healthy and diseases controls, did not show any significant change (see p. 241).[54]

Optimal assay conditions, the levels of activities in normal adults and children, and the stability of the activity on storage at −20 °C or 4 °C are reported for arylsulphatase A (see p. 243).[61]

Chondroitin 4-Sulphate Sulphatases. – Evidence is presented indicating that three sulphatase activities towards UDP-2-acetamido-2-deoxy-D-galactose 4-sulphate, nitrocatechol sulphate, and chondroitin 4-sulphate are functions of the same hen oviduct enzyme.[410] Using chondroitin [^{35}S]sulphate from chick embryo cartilage, it is shown that hydrolysis of ester sulphate by this enzyme is limited to 4-sulphate groups occurring in the non-reducing terminal 2-acetamido-2-deoxy-D-galactose 4-sulphate, and 2-acetamido-2-deoxy-D-galactose 4,6-bis-sulphate residues.

Glycosulphatases (Miscellaneous). – A glycosulphatase present in the soluble fraction of disrupted *Pseudomonas carrageenovora* has been purified 500-fold by gel filtration on Sephacryl S-200 and ion-exchange chromatography on DEAE-Sepharose CL-6B.[411] By dodecylsulphate polyacrylamide gel electrophoresis the enzyme is practically homogeneous and has a molecular weight of 55 000. Conditions of optimal sodium chloride concentration and pH at 25 °C were 0.25–0.50 mol dm^{-3} and pH 7.0, respectively. The purified enzyme was inhibited by inorganic phosphate. Preparation is described of neocarrabiose 4-*O*-[^{35}S]-sulphate and *neocarratetraose* 4-*O*-[^{35}S]sulphate from labelled *Chondrus crispus*. The purified glycosulphatase is active against both these substrates although only one of the two sulphate esters in the tetrasaccharide is hydrolysed. Analysis of the reaction products was by gel filtration, electrophoresis, and ^{13}C nuclear magnetic resonance spectroscopy. The results are consistent with the products of desulphation being, respectively, neocarrabiose and neocarratetraose 4-*O*-monosulphate with the sulphate ester proximal to the reducing end [3,6-anhydro-*O*-α-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-3,6-anhydro-*O*-α-D-galactopyranosyl-(1→3)-D-galactose 4-*O*-sulphate].

UDP-2-acetamido-2-deoxy-D-galactose 4-Sulphate Sulphatases. – Evidence is presented indicating that three sulphatase activities towards UDP-acetamido-2-deoxy-D-galactose 4-sulphate, nitrocatechol sulphate, and chondroitin 4-sulphate are functions of the same hen oviduct enzyme (see above).[410]

56 Miscellaneous Enzymes

Dextransucrases. – Dextransucrase has been produced for fermentation of *Leuconostoc mesenteroides* with and without continuous sucrose addition to improve enzyme production.[412] The enzyme preparation has been concentrated

[410] M. Tsuji, Y. Nakanishi, H. Habuchi, K. Ishihara, and S. Suzuki, *Biochim. Biophys. Acta*, 1980, **612**, 373.
[411] M. W. McLean and F. B. Williamson, *Eur. J. Biochem.*, 1979, **101**, 487.
[412] A. Lopez and P. Monsan, *Biochimie*, 1980, **62**, 323.

from the fermentation broth by ultrafiltration and purified by gel permeation chromatography on Ultrogel. The specific activity of the dextran sucrase was greatly enhanced by calcium chloride addition to the purified enzyme.

Enzymically active immobilized forms of dextranases have been obtained by covalent coupling on to an amino-porous silica support activated with glutaraldehyde.[412]

α-Lactalbumins. – As part of a study of the 'whey' proteins of various mammals, a comparison has been made of the α-lactalbumins and lysozymes of the kangaroo and horse.[356] In the milk of the red kangaroo (*Megaleia rufa*) there is only one α-lactalbumin and it occurs throughout lactation, but no lysozyme has been detected. There are two α-lactalbumins in the milk of the grey kangaroo (*Macropus giganteus*): one, designated α-lactalbumin Zone B, is present throughout lactation; the second, designated α-lactalbumin Zone A, is present only in late lactation. One lysozyme is also present. The milk of the horse (*Equus caballus*) contains one α-lactalbumin and at least one lysozyme. Partial amino-acid sequences are proposed from sequence determination and from analyses of tryptic peptides compared with the known sequences of other α-lactalbumins and lysozymes.

Levansucrase. – A macromolecular complex including an inactive and protease-sensitive form of levansucrase was isolated from phenol treatment of *Bacillus subtilis*.[413] This complex has a weak affinity for hydroxyapatite. Its molecular weight is evaluated at 300 000 this levansucrase form may be activated either by Triton X-100, above its critical micellar concentration, or by phospholipase C. The enzyme form associated with the complex is quite different from the exocellular levansucrase, which is protease-insensitive and detergent-insensitive, shows high affinity for hydroxyapatite and has a molecular weight of 54 000. Triton X-100 disaggregates this complex. The active levansucrase released becomes protease-insensitive and has the same apparent sedimentation coefficient as the exocellular enzyme. Possible relationships between the existence of this trapped complex and the secretion process of levansucrase are discussed.

Pectinesterases. – A rapid, sensitive slab gel electrophoretic method for the study of pectic enzymes is described.[377] By incorporating pectin in acrylamide gels and subsequent staining with ruthenium red, simultaneous detection of pectinesterases was possibly due to their differing staining reactions. Application of the method to the study of the thermal stability of enzymes is described. Approximate isoelectric points of the enzymes could be determined from their positions in the gel.

Peroxidases. – The fact that purification of human 'myeloperoxidase' can be achieved *via* affinity chromatography of agarose-immobilized concanavalin A indicates that the enzyme molecule is a glycoprotein.[414]

[413] M.-F. Petit-Glatron, R. Chambert, and M. Steinmetz, *Eur. J. Biochem.*, 1980, **103**, 189.

[414] D. P. Merrill, *Prep. Biochem.*, 1980, **10**, 133.

Index of Enzymes Referred to in Chapter 6[a]

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β-D-2-Acetamido-2-deoxy-galactosidase	2-Acetamido-2-deoxy-β-D-galactoside acetamidodeoxy-galactohydrolase	3.2.1.53	237
endo-β-D-2-Acetamido-2-deoxyglucanase	D-Mannosyl-glycoprotein-(1→4)-*N*-acetamidodeoxy-β-D-glycohydrolase (see also Chitinase, E.C. 3.2.1.14)	3.2.1.96	287
β-D-2-Acetamido-2-deoxy-glucosidase	2-Acetamido-2-deoxy-β-D-glucoside acetamidodeoxy-glucohydrolase	3.2.1.30	237
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exo-(1→3)-α-D-Glucanase	1,3-α-D-Glucan 3-glucohydrolase	3.2.1.84	313
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Glucoamylase	1,4-α-D-Glucan glucohydrolase	3.2.1.3	316
D-Glucose isomerase	*see* D-Xylose isomerase E.C. 5.3.1.5	[5.3.1.18]	335
α-D-Glucosidase	α-D-Glucoside glucohydrolase	3.2.1.20	264
β-D-Glucosidase	β-D-Glucoside glucohydrolase	3.2.1.21	264
β-D-Glucuronidase	β-D-Glucuronide glucuronoso-hydrolase	3.2.1.31	277
Glycanases (miscellaneous)			319
Glycopeptide-linkage hydrolases			338
Glycosulphatases (miscellaneous)			340
Heparan sulphate hydrolase			319
Heparin hydrolase		[3.2.1.19]	319
Hyaluronate lyase	Hyaluronate lyase	4.2.2.1	319
Hyaluronidase	Hyaluronate 4-glycano-hydrolase	3.2.1.35	320
α-L-Iduronidase	α-L-Iduronide iduronohydrolase	3.2.1.76	280

Isoamylase	Glycogen 6-glucanohydrolase	3.2.1.68	322
α-Lactalbumin			341
Laminaranase		(3.2.1.6 & 3.2.1.39)	322
Limit dextrinase		(3.2.1.10 & 3.2.1.41)	322
Levansucrase	Sucrose: 2,6-β-D-fructan 6-β-D-fructosyltransferase	2.4.1.10	341
Lysozyme	Mucopeptide-*N*-acetylmuramoylhydrolase	3.2.1.17	323
D-Mannanases (miscellaneous)			330
β-D-Mannanase			330
α-D-Mannosidase	α-D-Mannoside mannohydrolase	3.2.1.24	280
Neuraminidase	Acylneuraminyl hydrolase	3.2.1.18	283
Nucleases			338
Pectate lyase	Poly(1,4-α-D-galacturonide) lyase	4.2.2.2	330
Pectin lyase	Poly(methoxygalacturonide) lyase	4.2.2.10	330
Pectinesterase	Pectin pectylhydrolase	3.1.1.11	341
Peroxidase			341
Phosphatase			339
Poly-D-galacturonase	Poly(1,4-α-D-galacturonide) glycanohydrolase	3.2.1.15	331
exo-Poly-D-galacturonase	Poly(1,4-α-D-galacturonide) galacturonohydrolase	3.2.1.67	331
Poly-D-galacturonate lyase			330
Proteinase			339
Pullulanase	Pullulan 6-glucanohydrolase	3.2.1.41	332
endo-Rhamnase			332
Sialidase[b]			283
Sucrose-isomaltase			333
Sucrose α-D-glucohydrolase	Sucrose α-D-glucohydrolase	3.2.1.48	333
Sulphatase			339
αα-Trehalase	αα-Trehalose glucohydrolase	3.2.1.28	333
UDP-2-acetamido-2-deoxy-D-galactose 4-sulphate sulphatase			340
endo-(1→4)-β-D-Xylanase	1,4-β-D-Xylan xylanohydrolase	3.2.1.8	334
Xylanases (miscellaneous)			335
D-Xylose isomerase	D-Xylose ketol isomerase	5.3.1.5	335
β-D-Xylosidase		[3.2.1.37]	287

[a] See Introduction (Part II, Chapter 1, p. 1).

[b] Sialidase is a term generally used to include neuraminidase and unspecified acylneuraminyl hydrolases. Since there is little evidence for the existence of acylneuraminyl hydrolases that do not cleave 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonyl residues, use of the term neuraminidase is preferred.

7
Glycolipids and Gangliosides

BY I. M. MORRISON

1 Introduction

Glycoconjugate research has been extensively reviewed in a two volume publication which is the record of a symposium held in 1977. The articles on glycolipids and gangliosides are too numerous to mention individually.[1] Likewise several articles relevant to this chapter are included in the latest volume of 'Methods in Carbohydrate Chemistry'.[2] The inhibition of lipid-linked saccharide formation by antibiotics is reviewed in a book on the mechanisms of saccharide polymerization and depolymerization.[3] Chapters on glycolipids are included in two of the volumes of the multivolume treatise on the biochemistry of plants.[4] The role of glycolipids in the assembly of polymers in eukaryotes is considered in a book on polysaccharide metabolism,[5] and that role in saliva and dental caries has also been reviewed.[6]

2 Analytical and General Methods

Several papers have appeared on the high-performance liquid chromatography of glycolipids. D-Glucosyl-, lactosyl-, and trihexosyl-ceramides, globoside, and hematoside have been separated into molecular species on a reversed-phase column as their *O*-acetyl *N*-4-nitrobenzoyl derivatives.[7] This procedure has since been improved so that 1 nmol of glycolipid can be detected and has been used for the analysis of neutral glycolipids from human erythrocytes.[8] Neutral glycosphingolipids have been separated as their per-*O*-benzoyl derivatives on silica gel.[9] These authors used benzoic acid anhydride as the benzoylating reagent and

[1] 'Glycoconjugate Research', ed. J. D. Gregory and R. W. Jeanloz, Academic Press, New York, 1979, Vol. 1 and 2.
[2] 'Methods in Carbohydrate Chemistry', ed. R. L. Whistler and J. N. BeMiller, Academic Press, New York, 1980, Vol. 8.
[3] 'Mechanism of Saccharide Polymerization and Depolymerization', J. J. Marshall, Academic Press, New York, 1980.
[4] 'The Biochemistry of Plants, Vol. 3, Carbohydrates - Structure and Function', ed. J. Preiss, Academic Press, New York, 1980.
[5] 'Polysaccharide Metabolism', R. W. Stoddart, Croon-Helm Ltd, London, 1981.
[6] 'Saliva and Dental Caries', ed. I. Kleinberg, S. A. Ellison, and I. D. Mandel, Information Retrieval Incorporated, Arlington, 1979.
[7] A. Suzuki, S. Handa, and T. Yamakawa, *J. Biochem. (Jpn.)*, 1977, **82**, 1185.
[8] A. Suzuki, S. K. Kundu, and D. M. Marcus, *J. Lipid Res.*, 1980, **21**, 473.
[9] S. K. Gross and R. H. McCluer, *Anal. Biochem.*, 1980, **102**, 429.

N,N-dimethyl-4-aminopyridine as the catalyst. This method has been extended to monosialogangliosides and as little as 50 pmol can be quantified.[10]

^{2}H N.m.r. spectroscopy has been used to study the cerebroside–phospholipid bilayers.[11] The interior of the bilayer exhibited uniformly increasing orientational order as the concentration of cerebroside increased, whereas the surface of the bilayer became disordered. The extent of disorder was dependent on the cerebroside. Homonuclear *J*-resolved two-dimensional ^{1}H n.m.r. spectroscopy has been applied to D-glucosylceramide.[12] In comparison with the one-dimensional spectrum, the possible advantage of this technique when applied to blood-group-active glycosphingolipids was discussed based on preliminary results on a pentaosylceramide.

Permethylated derivates of up to pentaosylceramides have been analysed by direct inlet ammonia chemical-ionization mass spectrometry.[13] Fragment ions produced by loss of methanol from the protonated molecular ion were observed in all spectra and ions due to the cleavage of glycosidic bonds were major ones under these conditions. The presence of hydroxy-fatty acids could also be determined.

Highly purified anti-D-galactocerebroside antibodies have been applied to the analysis of lipid–lipid and lipid–protein interactions.[14] The results suggest that the physicochemical properties of lipids and especially the lipid–lipid interaction in the hydrophobic region of the lipid matrix plays an important role in the antigen–antibody–complement reaction.

The synthesis of glycolipids has been reviewed.[15] A method has been described for preparing a new class of model glycolipids.[16] Aldobionic acids are linked to an alkylamine through an amide bond and can be prepared in large quantities. These glycolipids precipitate specifically with lectins and precipitation occurs at glycolipid concentrations just above their critical micelle concentration. Alkyl β-D-glucopyranosides, where the alkyl chains are from 8–12 carbon units long, have been synthesized and shown to have mild detergent properties.[17] The physical properties of the products have been established and it was shown that they are suitable for membrane research. A simpler synthesis of alkylglycoside detergents has been published in which their effects on the kinetic and physical properties of cytochrome *c* oxidase (EC 1.9.3.1) has indicated that they are superior to other nonionic detergents for studying membrane proteins.[18]

An improved method for the covalent attachment of glycosphingolipids to solid supports or macromolecules is based on the oxidation of the olefinic bond

[10] E. G. Bremer, S. K. Gross, and R. H. McCluer, *J. Lipid Res.*, 1979, **20**, 1028.
[11] L. J. Neuringer, B. Sears, and F. B. Jungalwala, *CRC Crit. Rev. Biochem.*, 1979, **7**, 325.
[12] A. Yamada, J. Dabrowski, P. Hanfland, and H. Egge, *Biochim. Biophys. Acta*, 1980, **618**, 473.
[13] T. Ariga, T. Murata, M. Oshima, M. Maezawa, and T. Miyatake, *J. Lipid Res.*, 1980, **21**, 879.
[14] T. Uchida and Y. Nagai, *J. Biochem. (Jpn.)*, 1980, **87**, 1843.
[15] R. Gigg, *Chem. Phys. Lipids*, 1980, **26**, 287.
[16] T. J. Williams, N. R. Plessas, I. J. Goldstein, and J. Lönngren, *Arch. Biochem. Biophys.*, 1979, **195**, 145.
[17] W. J. De Grip and P. H. M. Bovee-Geurts, *Chem. Phys. Lipids*, 1979, **23**, 321.
[18] P. Rosevear, T. VanAken, J. Baxter, and S. Ferguson-Miller, *Biochemistry*, 1980, **19**, 4108.

on the sphingosine moiety.[19] The products can then be coupled to the solid support or other macromolecule and these have been shown to be useful reagents for various studies in cell biology and immunology. Other studies have involved the oxidation of the olefinic bond in the ceramide moiety of peracetylated glycolipids.[20] The method of oxidation and subsequent further reactions have been used to modify the glycolipids in various ways. Among the possible uses of these modified products are structural studies on the oligosaccharide fragments which are easily released from the modified glycolipids.

The synthesis, purification, and characterization of two types of spin-labelled glycosyl carrier lipids and shorter chain isoprenols have been described.[21] Both types of probes could be incorporated into phospholipid bilayers where each became orientated at, or very close to, the water–hydrocarbon interface. The electron-spin resonance spectra of the phosphodiesters were dependent on the matrix. The transbilayer diffusion rates and self-association of the above lipids have been determined in model phospholipid membranes.[22] The slow transposition rates of the species in either small or large unilamellar phosphatidylcholine vesicles argues against their proposed role in the transbilayer portage of sugar units during polysaccharide and glycoprotein assembly.

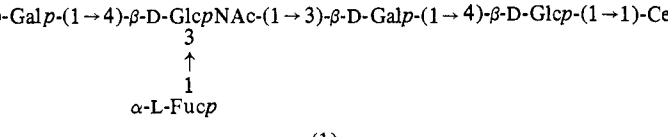
The activation of the lysosomal hydrolysis of complex glycolipids by non-enzymic proteins has been reviewed.[23] Another review has discussed the organization of glycolipids and glycoproteins in cellular membranes.[24] This review also discussed the role of cell-surface receptors in intercellular interactions, cell transformation and cell cycle regulation, the Golgi apparatus, and cell membrane composition as well as giving a model of intercellular recognition.

The recent availability of the model glycolipids, *N*-alkylaldobionamides, has allowed the systematic investigation of glycolipid-specific agglutination of lipid vesicles by concanavalin A and *Ricinus communis* agglutinin 1 to be studied.[25] Concanavalin A agglutinated negatively charged α-D-glucosylglycolipid-containing vesicles which was abolished both by methyl α-D-mannopyranoside and by elevated ionic strengths. The *R. communis* lectin agglutinated both neutral and negatively charged vesicles containing α-D-galactosyl glycolipids. This lectin required the presence of Ca^{2+} ions and its action could be reversed by methyl α-D-galactopyranoside.

3 Animal Glycolipids

The pentaosylceramide (1) has been shown to be the most common neutral glycolipid containing more than two sugar residues found in human brain.[26] It is the first report of the presence of L-fucose in such a glycolipid.

[19] W. W. Young, jun., R. A. Laine, and S. Hakomori, *J. Lipid Res.*, 1979, **20**, 275.
[20] D. L. MacDonald, L. M. Patt, and S. Hakomori, *J. Lipid Res.*, 1980, **21**, 642.
[21] M. A. McCloskey and F. A. Troy, *Biochemistry*, 1980, **19**, 2056.
[22] M. A. McCloskey and F. A. Troy, *Biochemistry*, 1980, **19**, 2061.
[23] K. Sandhoff and E. Conzelmann, *Trends Biochem. Sci.*, 1979, **4**, 231.
[24] S. Yu. Tumanova, *Biochem. (USSR) (Engl. Trans.)*, 1978, **43**, 307.
[25] R. Y. Hampton, R. W. Holz, and J. Goldstein, *J. Biol. Chem.*, 1980, **255**, 6766.
[26] M. T. Vanier, J. E. Månsson, and L. Svennerholm, *FEBS Lett.*, 1980, **112**, 70.



(1)

The concentration of D-galactosylceramide from cerebral and cerebellar white matter of a patient who had died from Krabbes disease, which is considered to be caused by a deficiency of D-galactosylceramide-β-D-galactosidase, was reduced to 10–20% of that in controls.[27] The ceramide (1) concentration was increased 10–100-fold. The present results suggest that the above deficiency hypothesis is incorrect and that the disease is caused by an accumulation of D-galactosylsphingosine (psychosine), which reaches toxic levels and kills the oligodendroglial cells. The disease is therefore considered to be a psychosine lipidosis.

The use of radiolabelled glycolipid-polymers has overcome the difficulty for detecting glycosphingolipids in radioimmunoassays.[28] The isoantigenic expression of Forssman glycolipid in human gastro-intestinal tissue was reported and 1 ng of Forssman glycolipid can be detected in plasma membrane preparations.

The glycolipid pattern of stomach tissue of a human with the rare blood-group A, p has been determined.[29] p-Erythrocytes lack both the P^k and P glycolipids, and P^k erythrocytes lack the P glycolipids but have five times the concentration of P^k glycolipids. p-Fibroblasts cannot synthesize P^k and P glycolipids. However, there may be differences between cells in their biosynthetic ability in the same way as is known for Lewis substances.

The α-D-2-acetamido-2-deoxy-galactosidase (EC 3.2.1.49) from human liver required sodium taurocholate to solubilize the glycolipids before hydrolysing globotriglycosyl- and globopentaglycosyl-ceramide.[30] The kinetics of hydrolysis of the glycolipids were very different from those of the derived oligosaccharides. The failure of the enzyme to prevent the accumulation of globotriglycosylceramide in Fabry's disease suggests that its role *in vivo* is the hydrolysis of glycoconjugates containing non-reducing terminal 2-acetamido-2-deoxy-D-galactopyranosyl group.[31]

The thermotropic behaviour of aqueous dispersions of D-glucocerebroside from Gaucher's spleen has been investigated by differential scanning calorimetry.[32] Two distinct phase changes were observed. The low-temperature transition was exothermic and involved a transformation of a metastable gel phase to a more highly ordered gel conformation. The high-temperature transition was associated with the main gel–liquid crystalline transition and was not rapidly reversible.

[27] L. Svennerholm, M.-T. Vanier, and J. E. Månsson, *J. Lipid Res.*, 1980, **21**, 53.
[28] W. W. Young, J. W. Regimbal, and S. Hakomori, *J. Immunol. Methods*, 1979, **28**, 59.
[29] M. E. Breimer, B. Cedergren, K.-A. Karlsson, K. Nilson, and B. E. Samuelson, *FEBS Lett.*, 1980, **118**, 209.
[30] K. J. Dean and C. C. Sweeley, *J. Biol. Chem.*, 1979, **254**, 9994.
[31] K. J. Dean and C. C. Sweeley, *J. Biol. Chem.*, 1979, **254**, 10001.
[32] E. Freire, D. Bach, M. Correa-Freire, I. Miller, and Y. Barenholz, *Biochemistry*, 1980, **19**, 3662.

A radioimmunoassay has been developed to measure Le^b-active glycolipids in human plasma,[33] by use of an antiserum from a goat immunized with the Le^b blood-group hapten, lacto-*N*-difucohexaose 1, conjugated to poly(L-lysine). About 30% of the total Le^b-active glycolipid in whole blood is associated with the erythrocytes and the rest is found in plasma.

A correlation between the P blood group and the relative quantities of neutral glycosphingolipids has been demonstrated using an h.p.l.c. technique.[34] Erythrocytes from P_1 individuals contained more globotriaosylceramide and less lactosylceramide than did erythrocytes from P_2 individuals. These results suggest that a new phenotype exists in the P blood-group system which has further implications regarding the biosynthesis of P blood-group glycolipids. The erythrocytes of a patient agglutinated less readily than normal cells with various P, and P P^k sera, and exhibited a decreased capacity to absorb these antibodies.[35] An analysis of the erythrocyte glycosphingolipids showed that the globoside (P-antigen) content was $<25\%$ normal and the trihexosylceramide (P^k-antigen) content was only 30–40% of normal. The ganglioside contents were 4–6 times normal. These serological and chemical features suggest a new phenotype in the P blood-group system.

The carbohydrate chains of both polyglycosylceramides and polyglycosylpeptides have been shown to be unbranched in i-erythrocytes.[36] The total ABH blood-group activity of human erythrocytes is made up from glycosphingolipids (*ca.* 5%), polyglycosylceramides (10–15%), alkali-labile conjugates (5–15%), and alkali-stable glycoproteins (65–75%).[37]

The glycosphingolipid receptor for *Escherichia coli* when attaching to human urinary tract epithelial cells and when agglutinating human erythrocytes has been chemically identified as a globotetraosylceramide.[38]

A major component of the non-acid glycosphingolipid fraction from meconium of a blood-group O individual has been identified, by conventional techniques, as (2).[39] This glycolipid has long been thought of as a precursor substance of Lewis- and ABH-active glycolipids.

$$\beta\text{-D-Gal}p\text{-}(1\rightarrow3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1\rightarrow3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow4)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow1)\text{-Cer}$$

(2)

[33] T. E. Rohr, D. F. Smith, D. A. Zopf, and V. Ginsburg, *Arch. Biochem. Biophys.*, 1980, **199**, 265.
[34] K. S. Fletcher, E. G. Bremer, and G. A. Schwarting, *J. Biol. Chem.*, 1979, **254**, 11196.
[35] S. K. Kundu, S. M. Steane, J. E. C. Bloom, and D. M. Marcus, *Vox Sanguinis*, 1978, **35**, 160.
[36] E. Zdebska, A. Magnuska, G. Kuśnierz, H. Seyfried, and J. Kościelak, *FEBS Lett.*, 1980, **120**, 33.
[37] Z. Wilczynska, H. Miller-Podroza, and J. Kościelak, *FEBS Lett.*, 1980, **112**, 227.
[38] H. Leffler and C. Svanborg Edén, *FEMS Microbiol. Lett.*, 1980, **8**, 127.
[39] K. A. Karlsson and G. Larson, *J. Biol. Chem.*, 1979, **254**, 9311.

$$\alpha\text{-D-Gal}p\text{NAc-}(1\rightarrow3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow4)\text{-D-Glc}$$
$$\begin{matrix} & & 2 & & \\ & & \uparrow & & \\ & & 1 & & \\ & \alpha\text{-L-Fuc}p & & & \end{matrix}$$

(3)

The tetrasaccharide (3) has been isolated and characterized from the urine of a blood-group A-specific patient.[40] It is possible that the substituents on the D-galactose residue may be reversed. The urine of a child with fucosidosis was found to contain 4–8-fold more Le^a activity than a control.[41] Le^b and H activities were also present but no A activity. The Le^b and H activities may originate from an Le^a precursor in spite of the non-secretory type of patient.

D-Galactose and 2-acetamido-2-deoxy-D-glucose were selectively taken up, in the presence of D-glucose, by human peripheral lymphocytes either in the normal state or stimulated by phytohaemagglutinin or concanavalin A.[42] However, the incorporation into normal cells falls off quicker than in stimulated cells. The major glycosphingolipid was lactosylceramide, which increased at a faster rate than the total glycosphingolipid. Stimulation by the lectins increased the incorporation of D-galactose into II^3-*N*-acetyl-neuraminosyl-lactosylceramide by *ca.* 15-fold but had no effect on more complex gangliosides.

The accessibility of D-galactosylceramides in myelin from the central nervous system to probe reagents has been investigated.[43] Half of the D-galactose residues could be attacked by D-galactose oxidase, whereas more than 90% of these residues could be attacked by periodate ions in isolated myelins although the extent of attack in myelin lipids is not as good.

The glycolipids from human milk fat globule membranes contain more monohexosylceramide than lactosylceramide and there is seven times more D-galactosylceramide than D-glucosylceramide.[44] A further difference between the two monohexosylceramides is that the D-galactose derivative contains hydroxy-fatty acids, whereas the D-glucosyl derivatives contain only non-hydroxy-fatty acids.

D-Mannose and 2-acetamido-2-deoxy-D-glucose have been incorporated into fibroblasts from a patient with mannosidosis and the incorporation could be partially prevented by the presence of an α-D-mannosidase.[45] The major products were three oligosaccharides, structures (4)–(6).

$$\alpha\text{-D-Man}p\text{-}(1\rightarrow3)\text{-}\beta\text{-D-Man}p\text{-}(1\rightarrow4)\text{-D-Glc}p\text{NAc}$$

(4)

[40] C. Derappe, A. Lundblad, L. Messeter, and S. Svensson, *FEBS Lett.*, 1980, **119**, 177.
[41] M.-J. Tchilian, M. Lemonnier, J. Font, R. Bourrillon, J. Cartron, and J. Bamberger, *C. R. Hebd. Seances Acad. Sci., Ser. D*, 1979, **289**, 193.
[42] C. Vakirtzi-Lemonias, G. P. Evangelatos, V. M. Kapoulas, and G. M. Levis, *Eur. J. Biochem.*, 1980, **109**, 541.
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[44] J. Bouhours and D. Bouhours, *Biochem. Biophys. Res. Commun.*, 1979, **585**, 1217.
[45] M. A. Chester, B. Hultberg, N. E. Nordén, and L. Szabo, *Biochim. Biophys. Acta*, 1980, **627**, 244.

α-D-Man*p*-(1→2)-α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-D-Glc*p*NAc

(5)

α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-D-Glc*p*NAc

(6)

In contrast to human erythrocytes and platelets, the major neutral glycolipids in human neutrophils are glycosphingolipids of the lactoneo-type with smaller amounts of the galactosyl-type.[46] Although only about 10% of the total, neutrophils are the most readily available source of lactoneotriaosylceramide. Six neutral glycolipids were isolated and their structures confirmed by the usual method. None of these glycolipids had novel structures.

Synthetic glycolipids containing an α-D-mannoside group linked by a hydrophilic spacer arm to cholesterol have been incorporated into bovine erythrocytes by exchange from glycolipid-containing liposomes.[47] Although bovine erythrocytes are not themselves susceptible to concanavalin A-mediated agglutination, the incorporated glycolipids could be agglutinated if the distance between the sugar and cholesterol moieties was *ca.* 2.6×10^{-9} m. Aggregation could be reversed by methyl α-D-mannopyranoside and did not occur with β-D-galactose-containing glycolipids. Two amphipathic proteins (mol. wt *ca.* 4×10^3) have been obtained respectively from ethanol and chloroform–methanol extracts of bovine erythrocytes.[48] These proteins have an affinity for gangliophilin and one has strong Paul–Bunnell antigen activity. They will directly cross-link with globoside or will link in directly through antiglobosides antibodies to the neighbouring globoside-associated protein.

The D-mannosyl residue from GDP-D-mannose can be transferred to dolichyl-pyrophosphate di-*N*-acetylchitobiose to give the equivalent trisaccharide derivative by microsomes from calf pancreas.[49]

A new sulphated glycosphingolipid (7) has been isolated from hog gastric mucosa and characterized by conventional techniques including the use of specific glycoside hydrolases.[50] The structure of a complex fucolipid, containing twenty sugar residues, from the same source and exhibiting blood-group (A + H) activity has been investigated.[51] The results suggest that the molecule has four non-reducing termini, one of which bears the disaccharide (8), two bear blood-

β-D-Glc*p*NAc6SO_3-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p*-(1→1)-Cer

(7)

β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-

(8)

[46] B. A. Macher and J. C. Klock, *J. Biol. Chem.*, 1980, **255**, 2092.
[47] R. R. Rando, J. Slama, and F. W. Bangerter, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 2510.
[48] C. A. Lingwood, S. Hakomori, and J. H. Li, *FEBS Lett.*, 1980, **112**, 265.
[49] A. Herscovics, C. D. Warren, B. Bugge, and R. W. Jeanloz, *FEBS Lett.*, 1980, **120**, 271.
[50] A. Slomiany, B. L. Slomiany, and C. Annese, *Eur. J. Biochem.*, 1980, **109**, 471.
[51] B. L. Slomiany, A. Slomiany, N. I. Galicki, and K. Kojima, *Eur. J. Biochem.*, 1980, **113**, 27.

group A determinants and the other bears blood-group H determinant. A proposed structure for the inner core was also presented.

The glycolipid content in the mitochondrial lipids from mucosa of pig, rabbit, and frog have been determined as 7.0, 5.6, and 10.5%, respectively, of the total lipid fractions.[52]

The antibiotic, showdomycin, inhibited the synthesis of D-glucosyl- and D-mannosyl-phosphoryl-dolichol as well as lipid-linked oligosaccharide by enzyme preparations from pig aorta.[53] However the same antibiotic stimulated the incorporation of D-glucose residues into lipid-linked sugars in yeast membrane preparations. A soluble D-mannosyltransferase was obtained, on detergent treatment of a pig aorta particulate enzyme preparation, which catalysed the transfer of D-mannose residues from GDP-D-mannose to form a heptasaccharide-lipid provided an acceptor lipid was present.[54] This acceptor lipid could have either a tri- or a penta-saccharide component. The lipid could be partially degraded by α-D-mannosidase.

Studies on the composition of the glycosphingolipids from porcine plasma have indicated that the composition may be subject to considerable intrinsic, probably genetic, variability.[55] In addition to 4 major species previously reported, significant amounts of D-galactosylceramide and di-D-galactosylceramide were found.

D-Mannose from GDP-D-mannose has been incorporated into endogenous lipid and protein acceptors to give D-mannosyl-phosphosyl-polyprenol by microsomes from sheep lung.[56] There was no precursor–product relationship.

The glycosphingolipid patterns in a line of kidney cells from African green monkeys infected with herpes virus has been compared with that of normal cells.[57] The neutral glycolipids accounted for 80% of the total. Herpes simplex virus type I infection resulted in a three- to four-fold increase in D-galactose incorporation into D-glucosylceramide and a decrease in D-galactose incorporation into higher glycolipids.

Soluble fractions have been isolated from liver, proximal intestine, and adrenal gland homogenates from adult hamsters, which have sulphotransferase activity against glycochenodeoxycholic acid.[58] Female hamsters had a higher activity than male ones and the reaction was inhibited by glycolithocholic and chenodeoxycholic acids.

The lipid and lysosomal enzyme levels in tissues from a strain of mice afflicted with an autosomal recessive neuroviscereal storage disorder have been determined and both sphingomyelinase and D-glucocerebrosidase activities in the affected animals were consistently diminished.[59] The activities in new-born mice

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[53] M. S. Kang, J. P. Spencer, and A. D. Elbein, *J. Biol. Chem.*, 1979, **254**, 10037.
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were clearly attenuated over controls. D-glucocerebroside and lactosylceramide accumulated in tissue, whereas the gangliosides G_{M_2} and G_{M_3} accumulated in brain tissue, and G_{M_3} and asialo-G_{M_2} were stored in the liver.

Mitochondria from mice liver have the ability to incorporate D-glucose from UDP-D-glucose into two products.[60] These have been tentatively identified as dolichyl-D-glucosylphosphate and D-glucosylceramide. The biosynthesis of both is inhibited by UDP but stimulated by divalent cations.

Splenic lymphocyte preparations from several strains of mice have been cultivated in the presence of lectins, lipopolysaccharide, or lipoprotein and these mitogens stimulated the incorporation of D-galactose into acid-insoluble cell material.[61] There were differences between B and T cells both in their neutral glycolipid and ganglioside fractions. The lipopolysaccharide and lipoprotein had a greater effect on the neutral glycolipids.

Mouse neurotumour cell lines can synthesize specific glycosphingolipids independently of the type of tissue culture conditions employed.[62] The G26 glioma tumour cell lines synthesized D-galactosyl sulphate ceramide, which is not associated with neuroblastoma or astrocytoma cell lines, as well as G_{M_3} and other glycolipids. The synthesis of this glycolipid was not enhanced by either dibutyrylcyclicAMP or choleratoxin. The incorporation of sulphate was enhanced by the presence of hydrocortisone.[63] Cortisone had a small effect but other steroids had no effect.

Fluorescence-labelled trinitrophenylated stearoylated dextrans have been used as controllable analogues of cell-membrane proteins on 3T3 mouse fibroblast plasma membranes.[64] At concentrations of 10^2 to 3×10^3 molecules μm^{-2}, homogeneous fluorescence was obtained. Cross-linking with antibodies stopped diffusion. This antibody inhibition of diffusion did not affect the diffusion of a lipid probe of fluorescein isothiocyanate-labelled membrane proteins.

Exposure of rats to methylmercuric chloride or diethylmercury had no effect on brain weight or myelin content but caused a reduction in the D-galactolipid levels.[65] The major change was in the fatty acid component of the cerebrosides.

Significant differences in the concentration of individual glycero-D-glucolipids between rat stomach antrum, body, and fore-stomach have been observed.[66] There was a 5 times overall increase in D-glucolipid in the antral portion over the fore stomach. The level of sulphated glycero-D-glucolipid in the antrum was even higher and it is postulated that these lipids may be involved in the protection of the gastric mucosa of the antrum since that is the most acidic portion of the stomach. The structure of the antral glycero-D-glucolipid has been characterized as (9) by the usual methods.

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[66] B. L. Slomiany, N. I. Galicki, K. Kohima, and A. Slomiany, *Eur. J. Biochem.*, 1980, **111**, 259.

α-D-Glc*p*6SO_3-(1→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→3)-1(3)-*O*-alkyl-2-*O*-acylglycerol

(9)

α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer
2
↑
1
α-L-Fuc*p*

(10)

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer

(11)

A novel blood-group B-active tetraglycosylceramide (10) has been isolated from rat large intestine.[67] It is probably only present in epithelial cells and is absent from the small intestine. A novel blood-group H-active triglycosylceramide (11) has been isolated from rat small intestine and is exclusive to epithelial cells.[68] The analysis of the L-fucolipids from the small intestine of rats has demonstrated that human blood-group A-positive and -negative strains exist.[69] A-type fucolipids were found in one strain and H-type in the other. These correspond to human A- and O-activities and this is the first report of A-negative strains. Examination of the total glycosphingolipid fraction from rat small intestine has shown more than 30 fractions of mono- to dodeca-glycosylceramides.[70] Several of these were hitherto unknown oligohexosylceramides containing 4, 5, 6, and 7 sugar residues and a tetraglycosylceramide with blood-group A-determinants.

The donation of D-mannose residues from D-mannosylretinyl phosphate to glycoconjugate receptors in rat liver has been studied.[71] The sub-unit molecular weight of the D-mannose acceptors has been further characterized and compared with those generated by D-mannosyldolichyl phosphate. Conditions have been developed to achieve a controlled transfer of either dolichol phosphate or retinol phosphate from rat liver liposomes to microsomal vesicles.[72] Vitamin A takes its place in some D-mannosylation reactions of glycoproteins which form a different pathway from the one involving dolichol as the lipid intermediate. The incorporation of 2-acetamido-2-deoxy-D-glucose into prothrombin and glycolipid in rat liver was inhibited by warfarin.[73] The kinetics of inhibition were similar. The glycosylation of L-asparagine residues in proteins is known to require a D-glucose-containing dolichyldiphosphate oligosaccharide. Derivatives containing 1, 2, and 3 D-glucose residues were found in rat liver but only that containing the three D-glucose residues could be used as a donor in the transfer to protein.[74]

Lithocholic, chenodeoxycholic, and deoxycholic acids, but not cholic acid or the glycine or taurine conjugates of these acids, can act as receptors for D-glucuronic acid in a reaction catalysed by an agarose-bound UDP-glucuronsyltransferase from rat liver.[75] The use of the bound enzyme allows the synthesis of these bile acid conjugates for kinetic studies in man and other animals.

D-Mannosyldolichylmonophosphate and dolichyldiphosphate oligosaccharides have been synthesized by rat spleen lymphocytes from GDP-D-mannose and UDP-2-acetamido-2-deoxy-D-glucose.[76] These products are either used for glycosylation of proteins or are degraded. In resting lymphocytes, degradation to

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[68] M. E. Breimer, G. C. Hansson, K.-A. Karlsson, and H. Leffler, *Biochim. Biophys. Acta*, 1980, **617**, 85.
[69] M. E. Breimer, G. C. Hansson, K.-A. Karlsson, and H. Leffler, *FEBS Lett.*, 1980, **114**, 51.
[70] M. E. Breimer, G. C. Hansson, K.-A. Karlsson, and H. Leffler, *Biochem. Biophys. Res. Commun.*, 1980, **95**, 416.
[71] W. Sasak and L. M. De Luca, *FEBS Lett.*, 1980, **114**, 313.
[72] J. Frot-Coutaz, R. Letoublon, and R. Got, *FEBS Lett.*, 1979, **107**, 375.
[73] R. G. Meeks and D. Couri, *Biochim. Biophys. Acta*, 1980, **630**, 238.
[74] R. J. Staneloni, R. A. Ugalde, and L. F. Leloir, *Eur. J. Biochem.*, 1980, **105**, 275.
[75] P. Czygan, A. Stiehl, and M. Senn, *Biochim. Biophys. Acta*, 1980, **619**, 228.
[76] R. Cacan, B. Hoflack, and A. Verbert, *Eur. J. Biochem.*, 1980, **106**, 473.

phospho-oligosaccharides and oligosaccharides is the major reaction, being caused by the enzymes alkaline phosphatase and *endo*-D-2-acetamido-2-deoxyglucanase H. Similar experiments using lymphocytes and lymphocyte homogenates have produced qualitatively and quantitatively different products.[77] The differences between the lipid intermediates exclude the possibility that intracellular glycosyltransferases could account for the glycosyltransferase activities observed with whole lymphocyte suspensions. The authors concluded that *ecto*-glycosyltransferases involved in the dolichol cycle are present at the outer surface of lymphocytes.

D-Glucosylceramide has been transferred from liposomes to red cells in rats and the reverse reaction has also been observed.[78] Both processes were activated by a preparation from bovine spleen and the transfer agent was characterized as a protein.

Isolated rat hepatocytes, when incubated with exogenous dolichol, caused the dolichol to be phosphorylated, become enriched in the endoplasmic reticulum and there were D-mannosylated.[79]

The migration-inhibition factor activity with rat macrophages was inhibited by the glycolipid (12) but not by cytolipin R, haematoside, or the blood-group B-active glyclipid (13).[80] Inhibition of the activity was proportional to the concentration of (12).

α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer
2
↑
1
α-L-Fuc*p*

(12)

α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer
2
↑
1
α-L-Fuc*p*

(13)

Glycosphingolipids have been isolated from guinea-pig gastric mucosa and the neutral ones were found to consist of mono-, di-, tri-, and penta-glycosylceramides.[81] The gangliosides G_{M4}, G_{M3}, G_{D3} were also found. None of the glycolipids contained 2-acetamido-2-deoxy-D-glucose and L-fucose residues together.

The water-soluble glycolipids have been isolated from guinea-pig macrophage and shown to contain the glycolipid receptor for the migration-inhibitory

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factor.[82] Enhancement was achieved only by the acidic fraction and this could be abrogated by removal of a terminal L-fucose residue.

A high incidence of tumour regression in guinea-pig hepatocellular carcinomas has been observed by treatment with muramyl dipeptides and trehalose dimycolate as an oil in water emulsion.[83]

Antibodies against the three neutral glycosphingolipids, Forssman glycolipid, globoside I, and asialo-G_{M_2}, have been elicited in rabbits.[84] Three glycolipids with the same non-reducing terminal 2-acetamido-2-deoxy-D-galactose residues were discriminated with IgG antibodies whereas IgM antibodies showed cross-activity.

Dolichyldiphosphoryloligosaccharide–protein oligosaccharyltransferase has been isolated from hen oviduct rough endoplasmic reticulum and purified.[85] The enzyme requires divalent cations, has a complete dependence on exogenously supplied protein acceptors and oligosaccharyldiphosphoryldolichol and a pH optimum of 7–7.5. Chick embryo cells, treated with 2-deoxy-2-fluoro-D-glucose cannot complete the formation of lipid-linked oligosaccharides.[86] In contrast to 2-deoxy-D-glucose, 2-deoxy-2-fluoro-D-glucose was not incorporated into lipid-linked oligosaccharides.

Major differences have been observed between spontaneous and chemically induced transplantable murine T and B lymphoblastoid tumours in the distribution patterns of both neutral glycosphingolipids and gangliosides.[87] The distribution patterns were determined by high-performance t.l.c. and the differences were both quantitative and qualitative.

The coliphage M13 coat protein in synthetic bilayer membranes can be labelled by using 12-(4-azido-2-nitrophenoxy)-stearoyl-2-acetamido-2-deoxy-D-glucose.[88] The probe preferentially labels the protein over the lipid. The results indicated that protein labelling can be affected specifically from within the hydrocarbon millieu of a model membrane system.

4 Animal Gangliosides

A critical study has been made of the alditol acetate method for quantitating small amounts of hexoses and 2-acetamido-2-deoxy-hexoses in gangliosides and optimum conditions have been recommended.[89] The authors favour the use of a glycolipid as an external standard.

Some gangliosides have been observed to sediment in the ultracentrifuge as oligomeric aggregates, independent of the concentration, in the range 10^{-3}–

[82] T. J. Higgins, D. Y. Lui, H. G. Remold, and J. R. David, *Biochem. Biophys. Res. Commun.*, 1980, **93**, 1259.
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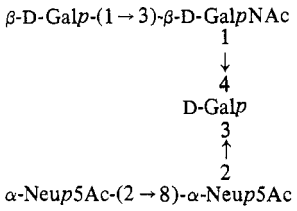
[88] V. W. Hu and B. J. Wisnieski, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5460.
[89] L. A. Torello, A. J. Yates, and D. K. Thompson, *J. Chromatogr.*, 1980, **202**, 195.

10^{-9} M.[90] The sedimentation coefficients were lowered in dimethylsulphoxide–water mixtures and no aggregation occurred in 1 M urea. Ganglioside analogues with two hydrocarbon chains instead of the ceramide moiety showed similar sedimentation behaviour as the native compound.

Micellar dispersions of pure di- and tri-neuraminosylgangliosides are hydrolysed at slow rates by bacterial neuraminidases but the rates are increased considerably on addition of phospholipid.[91] The authors suggest that the rate of hydrolysis is affected predominantly by interactions between the carbohydrate chains of ganglioside molecules. The data also confirmed that ganglioside metabolism can best be studied when the ganglioside is part of biological or model membranes.

A review has considered glycolipids as indicators of tumourigenesis where progressive alterations have been observed in neoplasm ganglioside metabolism and serum neuraminic acid levels during chemically induced carcinogenesis.[92]

Fragment C of tetanus toxin appears to retain most of the determinants of the toxin molecule as determined by interactions with neural or thyroid membranes and with gangliosides.[93] It interacts equally well as the intact toxin whether the gangliosides are in aqueous suspension or imbedded in liposomes or whether examined as a function of ganglioside concentration or individual ganglioside specificity. In contrast, fragment B has none of the determinants required for receptor interactions in membrane-binding assays. Using cholera toxin and polystyrene-adsorbed gangliosides as a model system, a tentative identification has been made of the receptor structure for tetanus toxin.[94] From the data obtained using a wide range of gangliosides it is considered that the natural binding structure for the tetanus toxin has the sequence (14), which occurs in the G_{1b} gangliosides and in the oligosaccharide chains of certain cell-membrane glycoproteins.



(14)

Fibroblasts incorporate mono-, di-, and tri-neuraminosylgangliosides in response to attack by the heat-labile enterotoxin from *Escherichia coli*.[95] The mononeuraminosylganglioside was most effective and the cells responded with

[90] W. Mraz, G. Schwarzmann, J. Sattler, T. Momoi, J. Delbrügge, B. Seeman, and H. Wiegandt, *Z. Physiol. Chem.*, 1980, **361**, 177.
[91] B. Cestaro, Y. Barenholz, and S. Gatt, *Biochemistry*, 1980, **19**, 615.
[92] D. J. Morré and T. M. Kloppel, *J. Supramol. Struct.*, 1978, **9**, 157.
[93] N. P. Morris, E. Consiglio, L. D. Kohn, W. H. Habig, M. C. Hardegree, and T. B. Helting, *J. Biol. Chem.*, 1980, **255**, 6071.
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[95] J. Moss, S. Garrison, P. H. Fishman, and S. H. Richardson, *J. Clin. Invest.*, 1979, **64**, 381.

an increase in the adenosine 3′,5′-phosphate content. Other studies suggest that both glycolipids and glycoproteins with a G_{M_2}-type structure were possible erythrocyte receptors for the two haemagglutinins of enterotoxigenic *E. coli*.[96]

The effect of gangliosides on *Clostridium botulinum* type A neurotoxin was examined by determining the concentration of gangliosides necessary to detoxify 50% of the toxin.[97] By far the most effective was $G_{T_{1b}}$ and further results have confirmed that the location and the number of neuraminic acid residues in gangliosides are of significance in the detoxification.

The ganglioside G_{D_3} in the presence of lecithin enhanced the binding of serotonin to protein.[98] Other gangliosides gave lower or negative responses. It was suggested that the ganglioside may regulate the concentration of biogenic amines in the synapse.

An improved yield of gangliosides was obtained from human brain by extraction with a chloroform–methanol–water mixture.[99] Further purification by phase partition and dialysis gave a purer preparation than obtained by other methods. The technique has been applied to the gangliosides of forebrain in infancy and old age. The total ganglioside mixture could then be separated into mono-, di-, tri-, tetra-, and penta-neuraminosyl-gangliosides on the anion-exchange resin, Spherosil®–diethylaminoethyl–dextran.[100] This resin had a higher binding capacity, showed lower unspecific adsorption effects and gave a better separation of the higher gangliosides from human infant forebrain and cerebellum than other anion-exchange resins. An improved method for the extraction and identification of human brain gangliosides has used dialysis against Carbowax or Millipore filter cones for concentration.[101] The method was suitable for post-mortem or biopsy samples.

A review on the structure and subcellular distribution of gangliosides in the brain indicates that they are localized at the nerve endings.[102]

Two isomers of brain tetraneuraminosylgangliosides have been isolated and partially characterized.[103] That from human, bovine, and chicken tissue was $IV^3(Neu5Ac)_2II^3(Neu5Ac)_2$tetraosylceramide, whereas that from codfish was $IV^3(Neu5Ac)II^3(Neu5Ac)_3$tetraosylceramide. The full structure of this ganglioside from human brain has been established by conventional techniques, including neuraminidase treatment, as (15).[104] Evidence has been presented to show that there exists, in human brain tissue, a ganglioside in which the neuraminic acid residues contain a carboxy-group that is bound in a structure that is alkali-labile and reducible with borohydride ion.[105] This evidence is based on the

[96] A. Faris, M. Lindahl, and T. Wadström, *FEMS Microbiol. Lett.*, 1980, **7**, 265.
[97] M. Kitamura, M. Iwamori, and Y. Nagai, *Biochim. Biophys. Acta*, 1980, **628**, 328.
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[99] L. Svennerholm and P. Fredman, *Biochim. Biophys. Acta*, 1980, **617**, 97.
[100] P. Fredman, O. Nilsson, J.-L. Tayot, and L. Svennerholm, *Biochim. Biophys. Acta*, 1980, **618**, 42.
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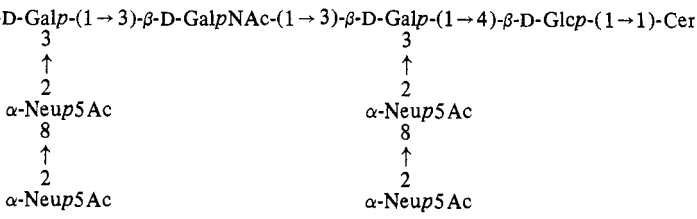
[104] P. Fredman, J.-E. Månsson, L. Svennerholm, K. A. Karlsson, I. Pascher, and B. E. Samuelsson, *FEBS Lett.*, 1980, **110**, 80.
[105] S. K. Gross, M. A. Williams, and R. H. McCluer, *J. Neurochem.*, 1980, **34**, 1351.



(15)

reactions whereby a ganglioside with an internal ester, on reduction and acidic hydrolysis will form a 5-acetamido-5-deoxy-nonulopyranoside and an anhydro-sugar derivative. About seven times the amount of nonulopyranoside was obtained from a reduced untreated fraction than was obtained from an alkali-treated one.

Four different glycolipid : glycosyltransferase activities involved in the biosynthesis of gangliosides have been tested in a Simian virus 40-transformed glial cell culture derived from the cerebrum of a foetus with Tay-Sachs disease.[106] The presence of CMP-*N*-acetylneuraminic acid : neolactotetraosylceramide neuraminosyltransferase activity suggested a probable pathway for the synthesis of neuraminosylneolactotetraosylceramide in addition to a specific blockage of G_{M1a} ganglioside synthesis from G_{M2} in these transformed cells.

The ganglioside content and composition of 25 human gliomas have been determined and the concentration of total ganglioside was decreased below normal in nearly all tumours.[107] There was a higher concentration of the simpler gangliosides but a lowering of the polyneuraminosylganglioside content. For example G_{D3} accounted for 20% of the lipid-bound neuraminic acid in astrocytoma grade IV cells compared with 4–5% in normal cells.

The gangliosides G_{D3} and G_{M1} have been coupled to protein *via* their carboxy-group, antisera were raised and purified by affinity chromatography.[108] The antibodies to G_{D3} were highly specific and did not cross-react with G_{M3}, lactosyl ceramide, or other glycolipids, whereas those antibodies to G_{M1} cross-reacted with asialo-G_{M1}, G_{D1b}, and to a lesser extent with G_{M2} and asialo-G_{M2}. The product obtained by periodate oxidation of the neuraminic acid residue of G_{M1} would also cross-react.

The activator protein for the degradation of glycolipids G_{M2} and G_{A2} by β-D-2-acetamido-2-deoxy-galactosidase A has been purified and its physical properties determined.[109] The activator did not enhance the hydrolysis of G_{M2} and G_{A2} by β-D-2-acetamido-2-deoxy-galactosidase B. The probable mode of action was to form a lipid–protein complex that is able to present the gangliosides in the correct form to the enzyme. Two distinct activator proteins have been isolated simultaneously from human liver for the hydrolysis of G_{M1} by

[106] M. Basu, K. A. Presper, S. Basu, L. M. Hoffman, and S. E. Brooks, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4270.
[107] T. D. Traylor and E. L. Hogan, *J. Neurochem.*, 1980, **34**, 126.
[108] S. K. Kundu, D. M. Marcus, and R. W. Veh, *J. Neurochem.*, 1980, **34**, 184.
[109] E. Conzelmann and K. Sandhoff, *Z. Physiol. Chem.*, 1979, **360**, 1837.

β-D-galactosidase and G_{M_2} by β-D-2-acetamido-2-deoxy-galactosidase A, respectively.[110] Each activator had little effect on the other reaction.

The selective spin-labelling of neuraminic acid residues in glycolipids and glycoproteins has been used as a new method to study cell-surface interactions in human erythrocyte membranes.[111]

The total concentration of gangliosides in human palatal oral epithelium was 75 nmol g^{-1} fresh weight with mono- and di-neuraminosyl-gangliosides account for 96% of the total.[112] G_{D_3} (24%) was the major ganglioside. The oral epithelial gangliosides may contribute to the barrier function of the oral mucosa.

A radioassay for G_{M_1} concentrations in cerebrospinal fluid has been developed which uses its affinity with cholera enterotoxin.[113] The method agrees with the densitometric technique and can detect 2.5 ng ml^{-1}.

The membrane-bound β-D-glucosylceramidase : β-D-glucosidase of normal cultured human skin behaves as an acidic protein with only traces of other β-D-glucosidase activities.[114] The residual β-D-glucosidase in cells from enzyme-deficient donors with types I and II D-glucosylceramidosis (Gaucher's disease) displays similar characteristics as one of the trace enzymes from normal cells. At late confluency, cultured skin fibroblasts from patients with G_{M_1} gangliosidosis type I showed a 15-fold increase in G_{M_1} content over normal cells, whereas patients with Tay–Sachs and Sandhoff's diseases showed a 50-fold and 30-fold, respectively, increase in G_{M_2}.[115] The analysis of accumulating lipid is therefore of great importance in the diagnosis of gangliosidosis.

The metabolism of 4-*O*-methyl-*N*-acetylneuraminic acid has been studied.[116] The K_m for incorporation of this synthetic neuraminic acid into fetuin by microsomal fractions of equine liver is five times that for *N*-acetylneuraminic acid. The product reacts similarly to the natural substrate on treatment with acid or fowl-plague virus neuraminidase but is resistant to *Vibrio cholerae* neuraminidase.

The major haematoside of equine erythrocyte gangliosides, *N*-glycoloyl-neuraminosyl-lactosylceramide, has been synthesized in a labelled form by mild periodate ion oxidation and reduction with [^{3}H]borohydride ion.[117] The above haematoside was confirmed as the major component of the ganglioside fraction from equine erythrocytes but smaller quantities of the 4-*O*-acetylneuraminosyl derivative were also found.[118] In contrast, bovine erythrocytes also had characteristic patterns but these could be divided into four types on the basis of their ganglioside composition.

[110] S. C. Li, T. Nakamura, A. Ogamo, and Y. T. Li, *J. Biol. Chem.*, 1979, **254**, 10 592.
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[112] U. Lekholm and L. Svennerholm, *Arch. Oral. Biol.*, 1979, **24**, 47.
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[114] O. T. Mueller and A. Rosenberg, *J. Biol. Chem.*, 1979, **254**, 3521.
[115] R. K. Pullakat, H. Reha, and N. G. Beratis, *Biochem. Biophys. Res. Commun.*, 1980, **92**, 149.
[116] J.-M. Beau and R. Schauer, *Eur. J. Biochem.*, 1980, **106**, 531.
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[118] S. Hamanaka, S. Handa, J. Inoue, A. Hasegawa, and T. Yamakawa, *J. Biochem. (Jpn.)*, 1980, **87**, 639.

Binding of choleragen to liposomes containing G_{M_1} from bovine brain was highly specific, rapid, and not readily reversible.[119] Only small amounts of toxin would bind to liposomes containing $G_{D_{1b}}$, G_{A_1}, and G_{M_2}. Thus the interaction of choleragen with G_{M_1}-containing model membranes mimicked the characteristics and specificity noted with biological membranes. The ganglioside G_{M_1} from bovine brain was hydrolysed to G_{A_1} and neuraminic acid by Sendai virus neuraminidase even in the absence of surfactants, whereas the neuraminidases from Newcastle disease and influenza A viruses had little or no effect under the same conditions.[120] In the absence of a surfactant, the neuraminidase from Sendai virus was more efficient than that from *Arthobacter ureafaciens* in hydrolysing G_{M_1}.

The gangliosides of calf thymus and of lymphocytes from blood, lymph nodes, lymph, and spleen of normal and leukaemic cows have been investigated to determine whether a relationship exists between the ganglioside composition and the maturity of the lymphocytes.[121] *N*-Glycoloylneuraminosyl-lactosylceramide accounted for 97% of the ganglioside fraction from all normal peripheral lymphocytes studied but the ganglioside spectrum from the thymus was far more complex. The ganglioside pattern for leukaemic peripheral lymphocytes was also complex and resembled that from the thymus. The authors concluded that the structure and composition of lymphocyte gangliosides become more simple during maturation and ageing.

Gangliosidosis fibroblasts were able to assimilate bovine testicular β-D-galactosidase and bovine liver β-D-glucuronidase at 90- and 464-times the assimilation rate of horseradish peroxidase.[122] The assimilation was not affected by each other so they occurred at different sites. The binding of G_{M_1} to bovine serum albumin has been studied using the absorption and fluorescence properties of the protein chromophores.[123] Two forms of the G_{M_1}-protein complex exist that differ in their molecular weight. Each complex has a ratio of one ganglioside micelle per albumin polypeptide chain. One complex is slowly converted to the other complex, which is a dimer of the first.

The glycolipid composition of whole cells and plasma membranes from two canine kidney cell lines have been studied.[124] The glycolipids were increased in the plasma membrane but neuraminosylglycolipids more complex than the Tay–Sachs ganglioside were not found. SV40 Virus cells contained less complex lipids (haematoside).

The antigenic determinants of the gangliosides $G_{D_{1a}}$, $G_{D_{1b}}$, G_{M_1}, G_{M_3}, and G_{T_1} from caprine brain grey matter were examined with rabbit antisera and by a

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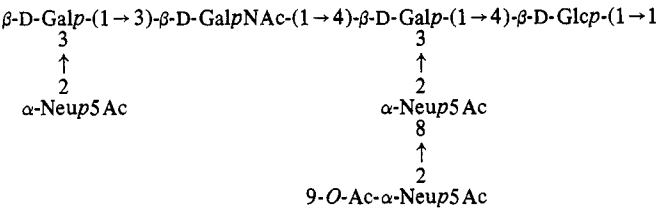
D-glucose-liposome test.[125] The immunological cross-reactions indicated that the terminal D-galactose residue and not the *N*-acetylneuraminic acid residue was immunodominant.

High amounts of the polar ganglioside G_{T_1} were found in brain tissue of normothermic hamsters relative to that found in mice.[126] The brain gangliosides from hibernating hamsters had an even more polar content. The authors suggest that the provision of more polar gangliosides at lower environmental temperatures might provide the neuronal membranes with a more efficient method for complexing Ca^{2+} ion. The parent DC-3F cell line from chinese hamster cells had its full complement of gangliosides from haematoside to dineuraminosylgangliosides but an actinomycin-D-resistant subline contained only haematoside.[127]

Acidic glycolipids from guinea-pig macrophages enhance the response of macrophages to migration inhibition factor, suggesting a role of glycolipid receptors for this lymphocyte mediator.[128] Neuraminidase treatment of the glycolipids resulted in a loss of their biological activity, which remained intact on β-D-galactosidase treatment.

Four major gangliosides have been found in both mouse and rat foetal brain.[129] G_{M_1} was the major mono-neuraminosylganglioside but at the early stages G_{D_3} was dominant over G_{M_1}. Thy-1 active glycolipids have been isolated from murine brain and lymphoma cell lines and characterized by radiolabelling, t.l.c., and an immune-response plaque-forming cell assay.[130] One active compound was detected in a mixture of fifteen gangliosides. A novel ganglioside, containing an alkali-labile linkage, has been isolated from mouse brain.[131] The complete structure of the ganglioside (16) has been determined by conventional methods and it can be considered as a derivative of $G_{T_{1b}}$.



(16)

[125] S. Saha and B. H. Ghosh, *Indian J. Biochem. Biophys.*, 1977, **14**, 350.
[126] R. Hilbig and H. Rahmann, *Comp. Biochem. Physiol.*, 1979, **62**, 527.
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[128] D. Y. Liu, K. D. Petschek, H. G. Remold, and J. R. David, *J. Immunol.*, 1980, **124**, 2042.
[129] L. N. Irwin, D. B. Michael, and C. C. Irwin, *J. Neurochem.*, 1980, **34**, 1527.
[130] K. P. Kato, T. J. Wang, and J. Esselmann, *J. Immunol.*, 1979, **123**, 1977.
[131] R. Ghidoni, S. Sonnino, G. Tettamanti, N. Baumann, G. Reuter, and R. Schauer, *J. Biol. Chem.*, 1980, **255**, 6990.

The ganglioside patterns of the milk fat globule membrane and the murine mammary tumour virus isolated from the milk of infected mice are very similar except that the milk fat globule membrane contained a low ganglioside homologue, probably G_{M_2}.[132] A common and dominant component was a dineuraminosylganglioside, which had the same neutral glycolipid component as $G_{D_{1a}}$, but both neuraminic acid residues possessed the susceptibility to neuraminidase treatment typical of terminal residues.

Several derivatives of the ganglioside G_{M_1} have been prepared and tested as receptors for choleragen in G_{M_1}-deficient transformed mouse fibroblasts and rat glial C6 cells.[133] G_{M_1} in which the *N*-acetyl groups of the neuraminic acid residue was replaced by an *N*-glycoloyl group and G_{M_1} oxidized by either D-galactose oxidase or periodate ion were as effective as G_{M_1} in increasing the sensitivity of the cells to the toxin. When G_{M_1} was modified by replacing the fatty acid with an acetyl group, the responsiveness of both cell types to the toxin was enhanced. Thus, although the oligosaccharide moiety of G_{M_1} contains the binding determinants for choleragen, the lipid portion can modulate the toxin–membrane interactions necessary for activation of adenylate cyclase. Murine type I interferon from fibroblasts is inhibited in both its antiviral and antigrowth activities when gangliosides are added to cell cultures and this is related to the fact that this type of interferon binds strongly to ganglioside affinity columns.[134] The type II interferon does not bind to ganglioside affinity columns and gangliosides have no effect on its antiviral and antigrowth activities. Preadipocytes of the mouse cell line 3TC-L1 have been shown to differentiate into cells with the enzymatic and morphological characteristics of adipocytes.[135] The total cellular ganglioside content falls on differentiation. G_{M_3} and $G_{D_{1a}}$ constitute the majority of gangliosides in both cell types with the choleragen receptor G_{M_1} being less than 5%. Differentiation brings about a 75–85% reduction in G_{M_1}. Non-differentiating 3TC-C2 control cells do not exhibit changes in the total ganglioside content, G_{M_1} content, or the choleragen binding capacity that are observed with 3T3-L1 cells. The BCG-induced natural killer cell activity of murine peritoneal exudate cells has been abolished by preincubation with anti-*N*-tetraosylceramide but not with other anti-glycolipid antibodies.[136] In contrast the cytotoxic activity of alloimmune T cells was not affected by anti-*N*-tetraosylceramide antiserum. This *N*-tetraosylceramide (asialo-G_{M_1}) display may be characteristic of natural killer cell populations and aid in the isolation of this population of cytotoxic cells.

The incorporation of 2-amino-2-deoxy-D-glucose and D-galactose into the gangliosides G_{M_2}, G_{M_3}, and $G_{D_{1a}}$ in an opiate receptor-positive clonal mouse neuroblastoma cell line N4TG1 and a mouse neuroblastoma X rat glioma hybrid clonal cell line NG108-15 has been inhibited by β-endorphin, enkephalin

[132] C. Gosselin-Rey, L. Gosselin, C.-M. Calberg-Bacq, C. François, S. Kozma, P. M. Osterrieth, and G. van Dessel, *Eur. J. Biochem.*, 1980, **107**, 25.
[133] P. H. Fishman, T. Pacuska, B. Holm, and J. Moss, *J. Biol. Chem.*, 1980, **255**, 7657.
[134] H. Ankel, C. Krishnamurti, F. Besancon, S. Stefanos, and E. Falcoff, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 2528.
[135] b. C. Reed, J. Moss, P. H. Fishman, and M. D. Lane, *J. Biol. Chem.*, 1980, **255**, 1711.
[136] W. W. Young, S. I. Hakomori, J. M. Durdik, and C. S. Henney, *J. Immunol.*, 1980, **124**, 199.

morphine and laevophanol.[137] Exposure of the cells to agents that raise the cyclic AMP levels overcome this inhibition caused by the opiates.

The interaction of G_{M1} ganglioside with rat brain neuronal membranes followed biphasic kinetics with a break at $0.07–0.08 \times 10^{-6}$ M G_{M1}.[138] Below the concentration of the break, the binding process was irreversible, and above the break there was a rapid exchange. It was postulated that hydrophobic interactions took place below the break, whereas above the break the G_{M1} interacts as a micelle.

Cultures of the dorsal root or the superior cervical ganglia of rats can incorporate 2-amino-2-deoxy-D-glucose into glycolipids.[139] The major gangliosides produced were G_{Q1}, G_T, G_{D1b}, G_{D1a}, and G_{M1} with some other material produced possessing mobility between mono- and di-neuraminosylgangliosides. Incubations *in vitro* of gangliotetraosylceramide with CMP-*N*-acetylneuraminic acid in the presence of young rat brain homogenate or the homogenates of a large variety of cultured cell lines resulted, in all cases, in the formation of G_{M1b} (17) with no trace of the naturally occurring G_{M1a}.[140] In addition some cells synthesized a dineuraminosylganglioside (G_{D1}), which differed from G_{D1c} and G_{D1b} in bearing only one substituent at O-3 of the terminal D-galactose residue. Both neuraminic acid groups in G_{D1} were degraded by periodate ion oxidation to a 7-carbon fragment confirming lack of substitution at O-8. G_{M1b} could not be detected as a natural product in rat brain. Gangliosides have been found to inhibit the action of adenylate cyclase and cyclic AMP phosphodiesterase in rat brain homogenates and synaptosomes.[141] Intracerebral injection of 250 μg of suramin in rats caused a small increase in glycosaminoglycan content but a large increase in the concentration of G_{M2}, G_{M3}, and G_{D3} in the treated region of the brain.[142] Suramin-treated rats may be useful animal models for mucopolysaccharidosis.

Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(1→4)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer

(17)

Cell fractions from rat kidney have been isolated and assayed for their ability to synthesize possible intermediates in the biosynthesis of gangliosides.[143] Activity for the initial glycosylation of ceramide was present in all fractions studied. Activities for further glycosylation reactions were largely found in the Golgi fraction so it was concluded that this site was involved in the modification of several glycolipids, which are destined to form cell-membrane components as well as modifying glycoprotein for secretion.

[137] G. Dawson, R. McLawhon, and R. J. Miller, *J. Biol. Chem.*, 1980, **255**, 129.
[138] G. Toffano, D. Benvegwu, A. C. Bonetti, L. Facci, A. Leon, P. Orlando, R. Ghidoni, and G. Tettamanti, *J. Neurochem.*, 1980, **35**, 861.
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[141] J. R. Lovely, N. M. Butt, and S. A. Saeed, *Biochem. Soc. Trans.*, 1980, **8**, 128.
[142] B. Constantopoulos, S. Rees, B. G. Cragg, J. A. Barranger, and R. O. Brady, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 3700.
[143] B. Fleischer, *J. Supramol. Struct.*, 1977, **7**, 79.

Ganglioside G_{D3} synthetase (CMP-*N*-acetylneuraminic acid : G_{M3} ganglioside neuraminosyltransferase) has been concentrated *ca.* 100-fold relative to total homogenates, in the Golgi apparatus fractions from rat liver.[144] The enzyme could not be dissociated from membranes. It appeared to be a glycoprotein since the activity bound to concanavalin A-Sepharose was eluted with increasing concentrations of methyl α-D-mannopyranoside. The enzyme was further characterized with respect to the regulation of activity by nucleotides and compared in this regard with other neuraminosyltransferases of ganglioside biosynthesis.[145] It has been suggested that gangliosides can regulate the glycosylation of endogenous membrane proteins in rat liver from a study of the incorporation of radiolabelled sugars.[146] The incorporation was stimulated by lactosylceramide and G_{M2} but inhibited by more complex gangliosides. The effects on the glycosylation of an exogenous acceptor, ovomucoid, were slight by comparison. None of the gangliosides tested had any effect on the neuraminosyl transfer from CMP--*N*-acetylneuraminic acid.

Butyrate-treated AH7974 type rat ascites hepatoma cells had a 2–3-fold deviation of their CMP-*N*-acetylneuraminic acid : lactosylceramide neuraminosyltransferase activity for the formation of G_{M3} over untreated cells.[147] The AH7974F free type cells had no such activity. Four glycosyltransferase activities involved in glycolipid synthesis were reduced by butyrate treatment.

The binding of Ca^{2+} ions to single ganglioside species, their free reducing neuraminosyl-oligosaccharide and ganglioside mixtures from chicken brain has been investigated by ion-sensitive electrodes.[148] Unlike the neuraminosyl-oligosaccharides and free *N*-acetylneuraminic acid, gangliosides possessed different modes of binding for Ca^{2+} ions depending on the total concentration of Ca^{2+} ions. Ca^{2+} ions could be released from the Ca^{2+}–ganglioside complexes by acetylcholine and Na^+, Li^+, and K^+ ions.

A neuraminosyltransferase activity that catalyses the synthesis of G_{T1a} from G_{D1a} and CMP-*N*-acetylneuraminic acid has been demonstrated in a particulate fraction from chicken brain.[149] The enzyme could also catalyse the synthesis of G_{D1a} from G_{M1} with a lower activity. Other properties of the enzyme were determined.

Two 2-amino-2-deoxy-D-glucose-containing gangliosides, one containing a pentasaccharide and the other a heptasaccharide fragment have been purified from the ganglioside fraction of chicken skeletal muscle.[150] The structures (18) and (19) were confirmed by accepted methods.

The variability of the gangliosides content of fish brain has been demonstrated in a table containing the detail from eighteen fish species.[151]

[144] C. M. Eppler, D. J. Morré, and T. W. Keenan, *Biochim. Biophys. Acta*, 1980, **619**, 318.
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[146] W. D. Merritt and D. J. Morré, *Biochim. Biophys. Acta*, 1980, **620**, 261.
[147] T. Taki, Y. Hirabayashi, R. Kondo, and K. Kojima, *J. Biochem. (Jpn.)*, 1979, **86**, 1395.
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[149] H. C. Yohe and R. K. Yu, *J. Biol. Chem.*, 1980, **255**, 608.
[150] J.-L. Chien and E. L. Hogan, *Biochim. Biophys. Acta*, 1980, **620**, 454.
[151] R. Hilbig and H. Rahmann, *J. Neurochem.*, 1980, **34**, 236.

α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer

(18)

α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer

(19)

5 Plant Glycolipids

The biosynthesis of acyl lipids in plant tissues has been reviewed.[152]

An attempt has been made to isolate neuraminic acid-containing glycolipids from etiolated hypocotyls of soybeans using methods used for isolating gangliosides from rat liver.[153] The maximum amount recovered was <0.021 nM g^{-1} fresh weight or 1:100 000 of that found in rat liver. It was concluded that this plant tissue lacked gangliosides. Developing pea (*Pisum sativum*) cotyledons incorporate radioactivity *in vivo* from D-mannose and 2-amino-deoxy-D-glucose into glycolipids.[154] Different lipid species are labelled, including neutral, ionic-nonacidic, and acidic lipids. The acidic lipids are similar to the polyisoprenoid lipid intermediates formed *in vitro* by pea cotyledons. The distribution of radioactivity in the D-galactosylglycerol and di-D-galactosylglycerol fractions of field bean (*Vicia faba*) on feeding labelled carbon dioxide has been determined.[155] It is suggested that mono-D-galactosyldiacylglycerol is formed from highly unsaturated diacylglycerols. There are at least two pools of mono-D-galactosyldiacylglycerol, differing in their degree of substitution of the fatty acids, from which di-D-galactosyldiacylglycerol is formed. A crude organelle preparation from germinating castor bean (*Ricinus communis*) endosperm has been shown to catalyse the incorporation of D-mannose from GDP-D-mannose into acid-labile D-mannolipid.[156] The most rapidly synthesized is a D-mannosyl-phosphoryl-polyisoprenol, whereas the more polar lipids formed contained oligosaccharide. The presence of lipid- and protein-bound sugars in the major organelle fractions from the same tissue has been established.[157] Detailed fractionation of the organelles and their lipid components have shown that, although 2-acetamido-2-deoxy-D-glucose and D-mannose residues, the inner core sugars, were found in all fractions, differences were noted in the organellar distribution of peripheral sugar residues such as L-fucose, L-arabinose, and D-xylose.

Glycolipids accounted for 13.2% of the total lipid fraction in carrot roots.[158] The mono-D-galactosyldiglycerides are the major component with lesser amounts of di-D-galactosyldiglycerides.

The kinetics of the incorporation of linoleate and linolenate into the mono-D-galactosyldiacylglycerol fraction of maize leaves has suggested that there is a transfer of linoleate from phosphatidylcholine to the glycolipid.[159] The initial rate of accumulation of linolenate into mono-D-galactosyldiacylglycerol was very similar to the rate of desaturation of linoleate and the results suggest that the desaturation of linoleate occurs while associated with mono-D-galactosyldiacylglycerol.

The glycolipid content of developing brown rice (*Oryza sativa*) has been shown to accumulate only up to eight days after flowering, which was a shorter

[152] J. L. Harwood, *Progr. Lipid Res.*, 1979, **18**, 55.
[153] J. M. Cherry, T. J. Buckhout, and D. J. Morré, *Experientia*, 1978, **34**, 1433.
[154] S. K. Browder and L. Beevers, *Plant Physiol.*, 1980, **65**, 924.
[155] J. P. Williams, *Biochim. Biophys. Acta*, 1980, **618**, 461.
[156] R. B. Mellor and J. M. Lord, *Planta*, 1979, **146**, 91.
[157] R. B. Mellor, T. Krusius, and J. M. Lord, *Plant Physiol.*, 1980, **65**, 1073.
[158] J. Soimajarvi and R. R. Linko, *J. Agric. Food Chem.*, 1979, **27**, 1279.
[159] J. C. Hawke and P. K. Stumpf, *Arch. Biochem. Biophys.*, 1980, **203**, 296.

β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-1-sitosterol

(20)

β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-1-sitosterol

(21)

period than the neutral lipids.[160] The sterylglycosides (20) and (21) have been characterized from rice bran.[161] It is possible that similar oligosaccharides are attached to campestrol and stigmasterol.

Storage of wheat flour for five years has resulted in a reduction in the mono-D-galactosyldiacylglyceride and di-D-galactosyldiacylglyceride content.[162] The glycolipid content of the non-starch endosperm of four varieties of wheat account for 20–40%.[163] This is a much greater amount than is found in the starch fraction (1–7%), the germ (0–3.5%), or the aleurone layer (2–10%). New methods have been described for the quantitative analysis of lipid classes, including glycolipids, in cereal grains and similar tissues.[164] The estimated errors for major lipid classes are ±1.5%, whereas minor lipid classes have errors of ±5%.

Spinach leaves have been labelled with [^{14}C]carbon dioxide, and the chloroplasts removed and separated into envelope and thylakoids with a view to establishing whether the envelope plays a dominant role in D-galactolipid biosynthesis and whether there is export of D-galactolipid from envelopes to thylakoids.[165] Since the thylakoid lipid always contained more label than the envelope counterparts, it was concluded that lipid export was too fast to be followed by the techniques used. In spinach chloroplast envelope membranes, the mono-D-galactosyldiacylglycerols could be separated into several distinct and non-mixing pools.[166] Molecules made *de novo* with unsaturated fatty acids are efficient substrates for D-galactosylation *in vivo*. After isolation, envelopes incorporated UDP-D-galactose into completely different patterns of D-galactolipids and molecular species pointing to changed properties of this membrane system or to a loss of regulatory factors. The profile of the hydrolysis of lipids in thylakoid membranes from spinach chloroplasts has suggested that ionic lipids are less accessible to lipolytic acyl hydrolase than D-galactolipids.[167] The composition of molecular species of lipids from chloroplast thylakoid membranes of spinach (*Spinacia oleracea*), soybean (*Glycine max*), rice (*Oryza sativa*), and maize (*Zea mays*) have been determined.[168] There were no significant differences between the species. In the mono-D-galactosyldiacylglycerol fraction, the 1 linolenoyl/2 linolenoyl species was the dominant species, whereas there were equal amounts of the 1 linolenoyl/2 linolenoyl and 1 palmitoyl/2 linolenoyl in the di-D-galactosyldiacylglycerol fraction.

[160] N. H. Choudhury and B. O. Juliano, *Phytochemistry*, 1980, **19**, 1063.
[161] H. Ohnishi and Y. Fujino, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 333.
[162] M. J. Warwick, W. H. H. Farrington, and G. Shearer, *J. Sci. Food Agric.*, 1979, **30**, 1131.
[163] K. D. Hargin and W. R. Morrison, *J. Sci. Food Agric.*, 1980, **31**, 877.
[164] W. R. Morrison, S. L. Tan, and K. D. Hargin, *J. Sci. Food Agric.*, 1980, **31**, 329.
[165] J. Joyard, R. Douce, H. P. Siebertz, and E. Heinz, *Eur. J. Biochem.*, 1980, **108**, 171.
[166] H. P. Siebertz, E. Heinz, J. Joyard, and R. Douce, *Eur. J. Biochem.*, 1980, **108**, 177.
[167] A. Rawyler and P.-A. Siegenthaler, *Eur. J. Biochem.*, 1980, **110**, 179.
[168] M. Nishihara, K. Yokota, and M. Kito, *Biochim. Biophys. Acta*, 1980, **617**, 12.

D-Galactolipids have been shown to be poorly represented in the lipid fraction of oak galls.[169]

6 Microbial and Miscellaneous Glycolipids

Membrane-derived oligosaccharides are a novel class of D-glucose-containing oligosaccharides found in the cell envelope of *Escherichia coli* and other Gram negative organisms. Previous work has shown that these oligosaccharides contain *sn*-1-glycerophosphate and smaller amounts of phosphoethanolamine. The structure of the parent oligosaccharide has now been studied by conventional techniques.[170] The oligosaccharides contain 10–12 D-glucopyranosidic residues per molecule, with both (1→2) and (1→6) bonds present. They are highly branched structures with four non-reducing termini per molecule and their low specific rotation suggests that the glycosidic bonds are predominantly in the β-D-configuration.

The carbohydrate chain of the D-glucosylpyrophosphate lipid, already shown to be formed by preparations of *Acetobacter xylinum*, has been shown to be elongated first by a D-glucose residue from UDP-D-glucose and then by a D-mannose residue from GDP-D-mannose to give the lipid (22) whose carbohydrate structure was confirmed by the usual techniques.[171] The prenol probably has an allylic structure. The possible role of these glycolipids in the synthesis of *exo*-polysaccharides is considered.

Polyprenyl diphosphate saccharides have been synthesized by enzyme preparations of *Rhizobium meliloti* from UDP-D-glucose.[172] The saccharide components were confirmed as D-glucose and β-D-glucosyl-(1→3)-D-galactose.

Of the total lipid fraction from the extremely halophilic bacterium *Halobacterium marismortui*, 86% was polar lipid and each component was based on 2,3-di-*O*-phytanyl-*sn*-glycerol.[173] One glycolipid (23) was characterized and another partially characterized. In contrast with other organisms of this family, *H. marismortui* did not contain any sulphated glycolipid but compensated by having a high content of phosphatidylglycerosulphate.

β-D-Man*p*-(1→3)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1-pyrophosphateprenol)

(22)

1-*O*-[β-D-Glc*p*-(1→6)-α-D-Man*p*-(1→2)-α-D-Glc*p*]-2,3,di-*O*-phytanyl-*sn*-glycerol

(23)

[169] L. Rey, J. P. Dubacq, and A. Tremolieres, *Phytochemistry*, 1980, **19**, 2569.
[170] J. E. Schneider, V. Reinhold, M. K. Rumley, and E. P. Kennedy, *J. Biol. Chem.*, 1979, **254**, 10135.
[171] R. O. Couso, L. Ielpi, R. C. Garćia, and M. A. Dankery, *Arch. Biochem. Biophys.*, 1980, **204**, 434.
[172] M. E. Tolmasky, R. J. Staneloni, R. A. Ugalde, and L. F. Leloir, *Arch. Biochem. Biophys.*, 1980, **203**, 358.
[173] R. W. Evans, S. C. Kushwaha, and M. Kates, *Biochim. Biophys. Acta*, 1980, **619**, 533.

A glycolipid-containing hopane, 1-*O*-(2-acylamido-2-deoxy-β-D-glucopyranosyl)-2,3,4-tetrahydroxypentane-29-hopane, has been isolated from the acidophilic, thermophilic *Bacillus acidocaldarius*.[174] This novel glycolipid has a cholesterol-like function in membranes.

Esterification of the lipoteichoic acid in *Stachylococcus aureus* with L-alanine residues has been shown to affect the biological properties of the lipopolysaccharide.[175] Amongst the properties affected are the lipoteichoic acid-carrier activity and a reduced inhibitory effect of autolytic enzymes.

The lipopolysaccharide from *Thermoplasma acidophilum* is a linear polysaccharide with the structure (24) as established by Smith degradation, methylation, acetolysis, and chromium(VI) oxide oxidation analyses.[176]

It has been shown that both the structure and method of binding of bacterial lipopolysaccharides affects the resistance of Gram negative bacteria to purified bacterial leukocyte proteins.[177]

A novel phosphoglycolipid (25) and a trihexosyldiacylglycerol (26) have been found in the lipid fraction of the wall-less saprophytic organism, *Acholeplasma granularum*.[178] It has been shown that the polar lipid composition of a related organism, *A. laidlawii*, is regulated as a response to environmental changes.[179] In particular, the ratio between the dominating mono-D-glucosyldiglyceride and di-D-glucosyldiglyceride is extensively changed. These changes affect the geometry of the membrane lipids. The response of the organism to the environmental changes is required on the basis of molecular shape and for the cell to maintain its optimal membrane stability.

Methods have been investigated for the extraction of the inositol-containing phospholipids of *Saccharomyces cerevisiae* and *Neurospora crassa*.[180] Slightly alkaline conditions were required to remove the very polar phosphoinositol-containing sphingolipids from these organisms.

The two membrane-bound enzymes catalysing the transfer of lipid-linked D-mannose residues to protein in *Saccharomyces cerevisiae* have been solubilized by detergent treatment.[181] The one transferring D-mannose residues to dolichylphosphate was purified 140-fold, whereas that transferring the D-mannose residue to the nascent protein was purified 380-fold. A D-mannoprotein from the cell wall served as acceptor for the second enzyme. Some physical properties of those enzymes have been recorded. A new membrane preparation from *S. cerevisiae* has been developed for the synthesis of large oligosaccharide lipids.[182] With GDP-D-mannose, the major product had the carbohydrate com-

[174] K. Poralla, E. Kannenberg, and A. Blume, *FEBS Lett.*, 1980, **113**, 107.
[175] W. Fischer and P. Rösel, *FEBS Lett.*, 1980, **119**, 224.
[176] P. F. Smith, *Biochim. Biophys. Acta*, 1980, **619**, 367.
[177] J. Weiss, S. Beckerditequagliata, and P. Elsbach, *J. Clin. Invest.*, 1980, **65**, 619.
[178] P. F. Smith, K. R. Patel, and A. J. N. Al-Shammari, *Biochim. Biophys. Acta*, 1980, **617**, 419.
[179] Å. Wieslander, A. Christiansson, L. Rilfors, and G. Lindblom, *Biochemistry*, 1980, **19**, 3650.
[180] B. A. Hanson and R. L. Lester, *J. Lipid Res.*, 1980, **21**, 309.
[181] P. Babczinski, A. Haselbeck, and W. Tanner, *Eur. J. Biochem.*, 1980, **105**, 509.
[182] L. Lehle, *Eur. J. Biochem.*, 1980, **109**, 589.

[α-D-Man*p*-(1→2)-α-D-Man*p*-(1→4)-α-D-Man*p*-(1→3)]$_8$-α-D-Glc*p*-(1→1)-diglycerol tetraether

(24)

[β-D-Glc*p*-(1→1)-X-glycerol-3-*O*-][D-glyceraldehyde-3-*O*-][1,2-di-acyl-X-glycerol-3-α-D-Glc*p*-(1→2)-α-D-Glc*p*-6-*O*-]phosphate

(25)

β-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→1)-diacylglycerol

(26)

position, (D-Man)$_9$(D-GlcNAc)$_2$. On further incubation with UDP-D-glucose, three oligosaccharides, (D-Glc)$_{1-3}$(D-Man)$_9$(D-GlcNAc)$_2$ were identified. It is suggested that the immediate precursor in the synthesis of the core region, (D-Man)$_{12-17}$(D-GlcNAc)$_2$, of yeast mannoprotein is the D-glucose-containing lipid oligosaccharide (D-Glc)$_3$(D-Man)$_9$(D-GlcNAc)$_2$. That is, only part of what has been defined as inner core is built up on the lipid carrier. After transfer to the protein, the oligosaccharide is modified by excision of the D-glucose residues and subsequent further D-mannosylation to give the required oligosaccharide chains of native mannoproteins. The phosphoglycolipid antibiotic, diumycin, has been found to inhibit the formation of dolichylphosphate D-mannose in *S. cerevisiae* as well as transfer of oligosaccharide to protein.[183] The inhibitory action of diumycin does not seem to be competitive with respect to dolichylphosphate.

Two new glycolipids, schizonellin A (27) and B (28) have been isolated from cultures of the smut fungus *Schizonella melanogramma*.[184] These glycolipids are active against Gram positive bacteria as well as some Gram negative organisms and fungi. They also have a strong haemolytic action on bovine erythrocytes.

The glycoprotein, cutinase, has been induced in *Fusarium solani* by cutin hydrolysates.[185] The induced cells possess an enzyme system which catalyses the transfer of D-mannosyl residues into glycolipid but not into oligosaccharide lipid. Electrophoresis of products suggest that cutinase is one of the glycosylation products *in vitro*.

2-*O*-Ac,3-*O*-Acyl-β-D-Man*p*-(1→4)-erythritol

(27)

2,6-di-*O*-Ac,3-*O*-Acyl-β-D-Man*p*-(1→4)-erythritol

(28)

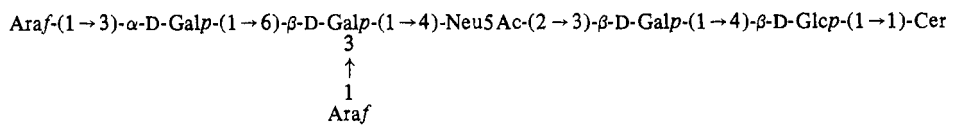
Extraction of lipids from fresh and frozen cells of *Candida bogoriensis* has shown the presence of a steryl glucoside, which was identified as β-D-glucopyranosyl cholesterol.[186] Density-gradient centrifugation of cell homogenates showed only one layer with this glucoside and sterol D-glucosyltransferase activity was also found in this layer.

Reduction of the environmental temperature for growing the blue-green alga *Anabaena variabilis* has resulted in the desaturation of the palmitic acid residues to palmitolenic acid in the D-galactosyldiacylglycerol fraction.[187] Lipid synthesis was also suppressed. A new major osmoregulatory solute in blue-green alga has been identified as α-D-glucosylglycerol.[188]

[183] P. Babczinski, *Eur. J. Biochem.*, 1980, **112**, 53.
[184] G. Deml, T. Anke, F. Oberwinkler, B. M. Gianetti, and W. Steglich, *Phytochemistry*, 1980, **19**, 83.
[185] C. L. Soliday and P. E. Kolattukudy, *Arch. Biochem. Biophys.*, 1979, **197**, 367.
[186] T. Kastelic-Suhadolc, *Biochim. Biophys. Acta*, 1980, **620**, 322.
[187] N. Sato and N. Murata, *Biochim. Biophys. Acta*, 1980, **619**, 353.
[188] M. A. MacKay, L. J. Borowitzka, and R. S. Norton, *Proc. Aust. Biochem. Soc.*, 1980, **13**, 130.

The principal glycolipids from the membranes of somatic cells and gonidia of *Volvox carteri* have been identified as sulphoquinovosyldiglyceride and mono- and di-D-galactosyldiglyceride.[189] In another green alga, *Fritschiella tuberosa*, the end of linear growth has been demonstrated to coincide with a reduction in the glycolipid content.[190] The reduction in glycolipid content continued during the stationary phase.

An unusual neuraminosylglycolipid (29) has been isolated from the hepatopancreas of the starfish *Patira pectinifera*. Some of the proposed linkages have not been definitively confirmed.[191]



(29)

A novel sphingophosphonoglycolipid has been isolated from the skin of the marine gastropod *Aplysia kuroda*.[192] The lipid was a bis(2-aminoethylphosphono)pentaosylceramide where the oligosaccharide contained one mole each of D-glucose, 3-*O*-methyl-D-galactose, and 2-amino-2-deoxy-D-galactose, and two moles of D-galactose.

The D-mannolipid participating in the glycosyltransferase reaction of schistosome has been partially purified.[193] The lipid acceptor has several characteristics of a phosphorylated polyisoprenoid and appears to be synthesized by these parasites at relatively slow rates.

[189] K. R. Moseley and G. A. Thompson, jun., *Plant Physiol.*, 1980, **65**, 260.
[190] M. Wettern, *Phytochemistry*, 1980, **19**, 513.
[191] G. P. Smirnova and N. K. Kochetkov, *Biochim. Biophys. Acta*, 1980, **618**, 486.
[192] S. Araki, Y. Komai, and M. Satake, *J. Biochem. (Jpn.)*, 1980, **87**, 503.
[193] F. D. Rumjanek, *Comp. Biochem. Physiol.*, 1980, **65**, 345.

8

Chemical Synthesis and Modification of Oligosaccharides, Polysaccharides, Glycoproteins, Enzymes, and Glycolipids

C. M. STURGEON

1 Synthesis of Polysaccharides, Oligosaccharides, Glycoproteins, Glycopeptides, and Glycolipids

Polysaccharides. – The direct polycondensation of D-cellobiose and dicarboxylic acid chlorides has been achieved without blocking of excess hydroxy-groups.[1] Optimal reaction conditions were determined and some properties of the products were described. Novel polyamide esters containing 2-acetamido-2-deoxy-D-glucose residues in the main chain have been synthesized by direct polycondensation of the unblocked sugar with dicarboxylic acid chlorides.[2] A synthetic α-(1→3)-L-rhamnan (d.p. 30) has been prepared by polycondensation of 4-*O*-acetyl-3-*O*-trityl-1,2-*O*-*exo*-cyanoethylidene L-rhamnopyranoside with a triphenylmethylium perchlorate catalyst.[3]

The synthesis of amylose block co- and ter-polymers has been described.[4] Fully acetylated amylose (amylose triacetate) was hydrolysed to yield a hydroxy-terminated amylose triacetate (HATA) oligomer, which was reacted with di-isocyanates to produce block copolymers. Prepolymers of hydroxy-terminated amylose acetate oligomer and diphenylmethane di-isocyanate or 2,4-toluene di-isocyanate were reacted with both hydroxy-terminated polybutadiene and poly(propylene glycol) to form block terpolymers. Products were readily deacetylated to yield amylose block co- and ter-polymers, formation of which was demonstrated by intrinsic viscosity increases, gel-permeation chromatography results, and i.r. and n.m.r. spectroscopic studies.

1,6-Anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose has been polymerized using a phosphorous pentafluoride catalyst.[5] Debenzylation of the product yielded a synthetic linear dextran, lamellar single crystals of which were grown for electron and *X*-ray powder diffraction studies.

Oligosaccharides. – 2-Methoxypropene converts carbohydrates into cyclic isopropylidene acetals under kinetic control, yielding partially protected sugars of

[1] K. Kurita, N. Hirakawa, and Y. Iwakura, *J. Polym. Sci., Polym. Chem. Ed.*, 1980, **18**, 365.
[2] K. Kurita, K. Miyajima, T. Sannan, and Y. Iwakura, *J. Polym. Sci., Polym. Chem. Ed.*, 1980, **18**, 359.
[3] N. K. Kochetkov and N. N. Malysheva, *Tetrahedron Lett.*, 1980, **21**, 3093.
[4] M. M. Lynn, V. T. Stannett, and R. D. Gilbert, *J. Polym. Sci., Polym. Chem. Ed.*, 1980, **18**, 1967.
[5] H. Chanzy, C. Guizard, and A. Sarko, *Int. J. Biol. Macromol.*, 1980, **2**, 149.

value in synthesis.[6] The efficient one-step conversion of a glycoside into a 1-thioglycoside has been reported by treatment of the glycoside or saccharide derivative with trimethyl[methyl(or phenyl)thio] silane in the presence of zinc iodide and tetrabutylammonium iodide.[7] The mild conditions required commend the method for structural studies of antibiotics, oligosaccharides, and biopolymers containing sugars. Glycosides have been prepared in exceptionally fast reactions involving metal-ion-promoted activation of the sugar moiety with silver or mercury(I) nitrate.[8]

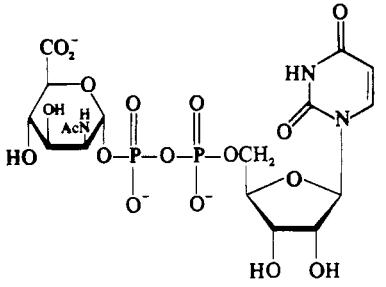
Ethyl vinyl ether and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose have been reacted in the presence of mercury(I) acetate and toluene-4-sulphonic acid catalysts to yield the acetylated vinyl, 1-ethoxy-ethyl, and 1-ethoxy-but-3-enyl glycosides in various proportions.[9]

A general method of synthesis of β-D-mannopyranosides by glycosidation of C-1 has been reported.[10] Several novel aryl and alkyl D-mannopyranosides and 1-thio-D-mannopyranosides have been synthesized for evaluation of insulin-like and insulin-antagonistic properties.[11] The preparation of ω-substituted alkyl 1-thio-α-D-mannopyranosides for the same purpose has also been reported.[12] Several 1-*N*-substituted derivatives [haloacetyl-, glycyl-, (dimethyl)amino-acetyl-, azidoacetyl-, trifluoroacetyl-, and trifluoromethylsulphonyl-] of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosylamine have been synthesized as potential metabolic inhibitors of cellular-membrane glycoconjugates.[13] Sodium 4-retinamidophenyl β-D-glucopyranosiduronate and sodium 2-retinamidoethyl β-D-glucopyranosiduronate have been synthesized as potential chemotherapeutic agents for the treatment of bladder cancer.[14]

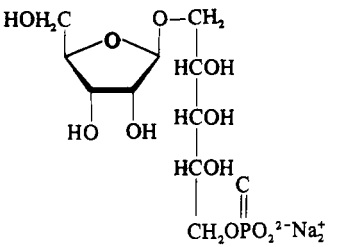
A rapid and efficient route for the synthesis of the biosynthetically important nucleotide sugar (1) has been reported.[15] The ribofuranosylribitol phosphate monomer (2), part of an antigen elaborated by *Haemophilus influenzae* type b that causes meningitis in children, has been synthesized for immunological studies.[16] The preparation of novel mono- and di-saccharide glycals for the synthesis of oligodeoxy-oligosaccharides has been described.[17]

Syntheses of 4,6-diacetamido-4,6-dideoxy-α-D-galactopyranosyl α-D-glucopyranoside, 4-amino-4,6-dideoxy-6-fluoro-α-D-galactopyranosyl α-D-glucopyranoside, and a derivative of 4,6-diacetamido-4,6-dideoxy-α-D-glucopyranosyl α-D-glucopyranoside *via* nucleophilic displacement reactions of appropriate

[6] E. Fanton, J. Gelas, and D. Horton, *J. Chem. Soc., Chem. Commun.*, 1980, 21.
[7] S. Hanessian and Y. Guindon, *Carbohydr. Res.*, 1980, **86**, C3.
[8] S. Hanessian, C. Bacquet, and N. Lehong, *Carbohydr. Res.*, 1980, **80**, C17.
[9] H. M. Dettinger, J. Lehmann, and K. Wallenfels, *Carbohydr. Res.*, 1980, **87**, 63.
[10] V. K. Srivastava and C. Schuerch, *Carbohydr. Res.*, 1980, **79**, C13.
[11] P. L. Durette and T. Y. Shen, *Carbohydr. Res.*, 1980, **81**, 261.
[12] P. L. Durette and T. Y. Shen, *Carbohydr. Res.*, 1980, **83**, 178.
[13] B. Paul, R. J. Bernacki, and W. Korytnyk, *Carbohydr. Res.*, 1980, **80**, 99.
[14] M. I. Dawson and P. D. Hobbs, *Carbohydr. Res.*, 1980, **85**, 121.
[15] T. Yamazaki, C. D. Warren, A. Herscovics, and R. W. Jeanloz, *Carbohydr. Res.*, 1980, **79**, C9.
[16] P. J. Garegg and B. Samuelsson, *Carbohydr. Res.*, 1980, **86**, 293.
[17] J. Thiem, P. Ossowski, and J. Schwentner, *Chem. Ber.*, 1980, **113**, 955.



(1)

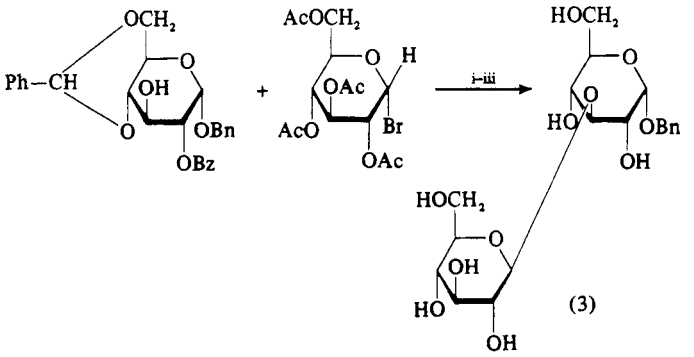


(2)

sulphonic esters or chlorodeoxy-derivatives have been reported.[18] The synthesis of the symmetrical derivative 4-amino-4,6-dideoxy-6-fluoro-α-D-galactopyranosyl 4-amino-4,6-dideoxy-6-fluoro-α-D-galactopyranoside was also described.

The syntheses of benzyl α- and β-sophorosides (benzyl 2-*O*-β-D-glucopyranosyl-α- and -β-D-glucopyranosides) and of benzyl α-laminaribioside (benzyl 3-*O*-β-D-glucopyranosyl-β-D-glucopyranoside) [(3), Scheme 1] have been reported.[19] Two methods of linking racemic 2,3-dideoxy-pent-2-enopyranosid-4-ulose (4) to suitably protected monosaccharide derivatives have been compared.[20] One method is based on the use of the diethyl azodicarboxylate–triphenylphosphine–mercury(I) halide glycosylating system; the other involves the facile displacement of a hemiacetal acyloxy group in the presence of tin(IV) chloride.

Syntheses of methyl β-L-rhamnopyranoside, 6-*O*-β-L-rhamnopyranosyl-D-glucose and -D-galactose, and 4-*O*-β-L-rhamnopyranosyl-L-rhamnose using 4-*O*-acetyl-2,3-*O*-carbonyl-α-L-rhamnopyranosyl bromide as glycosylating

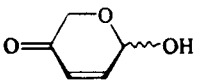


Reagents: i, silver trifluoromethane sulphonate–1,1,3,3-tetramethylurea–CH_2Cl_2; ii, NaOMe–MeOH; iii, $MeCO_2H$

Scheme 1

[18] A. F. Hadfield, L. Hough, and A. C. Richardson, *Carbohydr. Res.*, 1980, **80**, 123.
[19] K. Takeo, *Carbohydr. Res.*, 1980, **86**, 151.
[20] G. Grynkiewicz, *Carbohydr. Res.*, 1980, **80**, 53.

reagent have been described.[21] The readily accessible 4-*O*-benzoyl-2,3-*O*-cyclohexylidene-α-L-rhamnopyranosyl bromide (5) has been shown to be an effective intermediate for the synthesis of β-L-rhamnopyranosides in good yield.[22] Condensation of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide with methyl 4-*O*-acetyl-α-L-rhamnopyranoside gives two products, which have been separated chromatographically on silica gel and deblocked, yielding 2-*O*- and 3-*O*-α-L-rhamnosyl-L-rhamnose.[23]



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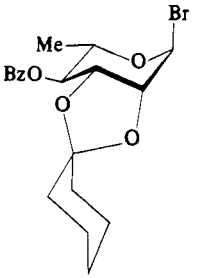
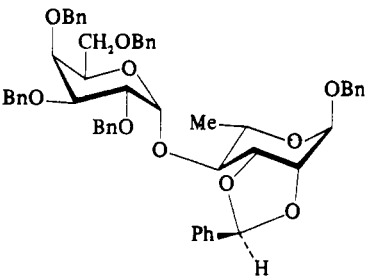


(5)

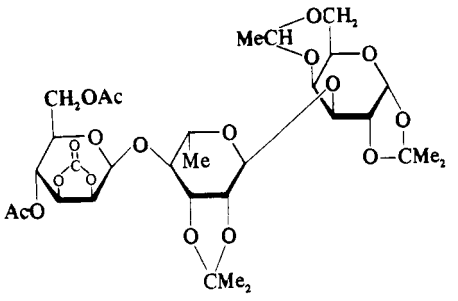
Dioxolane-type benzylidene acetals have been used as temporary protecting groups in the synthesis of 4-*O*-α-D-galactopyranosyl-L-rhamnose and 4-*O*-α-D-galactopyranosyl-2-*O*-β-D-glucopyranosyl-L-rhamnose.[24] The halide ion-catalysed reaction of benzyl *exo*-2,3-*O*-benzylidene-α-L-rhamnopyranoside with tetra-*O*-benzyl-α-D-galactopyranosyl bromide yielded the intermediate (6), which could be converted into the disaccharide or, after stereoselective hydrogenolysis of the *exo*-benzylidene group, reacted with tetra-*O*-acetyl-α-D-glucopyranosyl bromide and deblocked to yield the trisaccharide.



(6)

[21] L. V. Backinowsky, N. F. Balan, A. S. Shashkov, and N. K. Kochetkov, *Carbohydr. Res.*, 1980, **84**, 225.
[22] T. Iversen and D. R. Bundle, *Carbohydr. Res.*, 1980, **84**, C13.
[23] D. Schwarzenbach and R. W. Jeanloz, *Carbohydr. Res.*, 1980, **81**, 323.
[24] P. Fügedi, A. Lipták, P. Nánási, and A. Neszmélyi, *Carbohydr. Res.*, 1980, **80**, 233.

Koenigs-Knorr glycosylation of 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-3-*O*-(2,3-*O*-isopropylidene-α-L-rhamnopyranosyl)-α-D-galactopyranose by 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide, as well as Helferich glycosylation of the former compound using tetra-*O*-acetyl α-D-mannopyranosyl and -α-D-glucopyranosyl bromides proceeds smoothly to give high yields of trisaccharide derivatives (7)–(9).[25] Following transformation of these derivatives into their α-deca-acetates using a new and efficient procedure, Zemplén deacetylation



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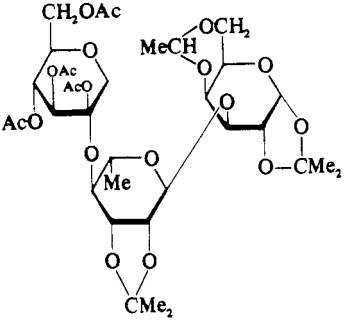

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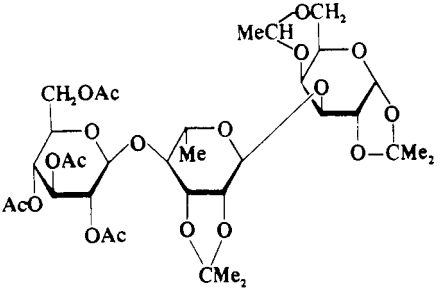


(9)

afforded *O*-β-D-mannopyranosyl-, *O*-α-D-mannopyranosyl-, and *O*-β-D-glucopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→3)-D-galactoses.

The synthesis and characterization of methyl 6-*O*-β-D-galactopyranosyl-β-D-galactopyranoside and methyl *O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside have been reported using 6-*O*-acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-galactopyranosyl chloride as starting material.[26] A facile synthesis of 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-galactose has been reported.[27] Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-galacto-

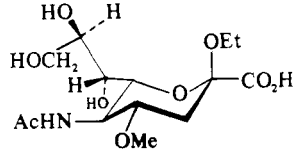
[25] V. I. Betaneli, M. V. Ovchinnikov, L. V. Backinowsky, and N. K. Kochetkov, *Carbohydr. Res.*, 1980, **84**, 211.
[26] V. K. Srivastava, S. J. Sondheimer, and C. Schuerch, *Carbohydr. Res.*, 1980, **86**, 203.
[27] S. S. Rana, J. J. Barlow, and K. L. Matta, *Carbohydr. Res.*, 1980, **84**, 353.

pyranoside was prepared and then reacted with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in the presence of silver triflate and tetra-*N*-methylurea, which appears to be essential for successful coupling of a glycosyl halide with 'aglycones' having a 6-*O*-benzyl group. Following deblocking the required disaccharide was obtained in 75% yield.

Improved syntheses of 2-deoxy-2-iodo-α-D-linked disaccharides have been reported using silver imidazolate–zinc chloride or silver imidazolate–mercury(II) chloride to promote glycosylation.[28] The preparation of the 6-aminohexyl glycosides of a series of disaccharides having the *O*-β-D-galactopyranosylated 2-acetamido-2-deoxy-β-D-glucopyranose sequence has been reported.[29] D-Galactosylation of appropriately protected 6-(benzyloxycarbonylamino)-hexyl 2-acetamido-2-deoxy-β-D-glucopyranosides yielded the desired glycosides with this sequence and containing (1→3)-, (1→4), and (1→6)-linkages. The methyl glycoside of 2,3,6-trideoxy-3-dimethylamino-4-*O*-(2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)-L-*lyxo*-hexopyranose, a disaccharide occurring in anti-tumour anthracycline antibiotics, has been prepared, as have the methyl and benzyl glycosides of the corresponding 2,3,6-trideoxy-3-acetamido-4-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl derivative.[30]

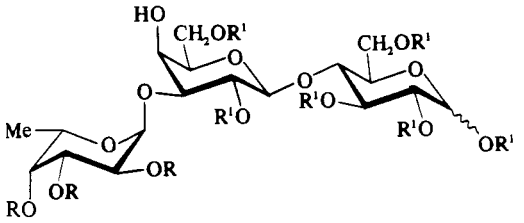
The 4-methylumbelliferyl α-ketoside of *N*-acetylneuraminic acid has been synthesized by reacting the sodium salt of 4-methylumbelliferone with the 2-chloro-2-deoxy-derivative of peracetylated methyl *N*-acetylneuraminate.[31] The synthesis of a neuraminic acid ketoside by an intramolecular oxymercuration–demercuration reaction has been reported.[32] Mercuration of ethyl 5-acetamido-3,5-dideoxy-2-*O*-ethyl-4-*O*-methyl-D-*glycero*-D-*galacto*-nonulosonate in a nonhydroxylic solvent yields, after demercuration, the ethyl glycoside (10).

Condensation of 1,2,3,6-,2′,6′-hexa-*O*-acetyl-α,β-lactose with 2,3,4-tri-*O*-benzyl L-fucopyranosyl bromide yields the α-(1″→3′)-linked protected trisaccharide (11), which, after deblocking, gave *O*-α-L-fucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose (3′-*O*-α-L-fucopyranosyl-lactose).[33] The formation of the product obtained on acetonation of lactose at elevated temperature to provide the required acetal intermediate for the synthesis was found to be thermodynamically controlled.



(10)

[28] P. J. Garegg and B. Samuelsson, *Carbohydr. Res.*, 1980, **84**, C1.
[29] J. Vernon, D. Roseman, and Y. C. Lee, *Carbohydr. Res.*, 1980, **82**, 59.
[30] J. Boivin, C. Monneret, and M. Païs, *Tetrahedron Lett.*, 1980, **21**, 2413.
[31] R. T. Lee, Y. C. Lee, G. H. Thomas, L. W. Reynolds, and Y. Uchida, *Anal. Biochem.*, 1980, **101**, 166.
[32] J. M. Beau, R. Schauer, J. Haverkamp, L. Dorland, J. F. G. Vliegenthart, and P. Sinay, *Carbohydr. Res.*, 1980, **82**, 125.
[33] H. H. Baer and S. A. Abbas, *Carbohydr. Res.*, 1980, **84**, 53.



R = Bn, R^1 = Ac

(11)

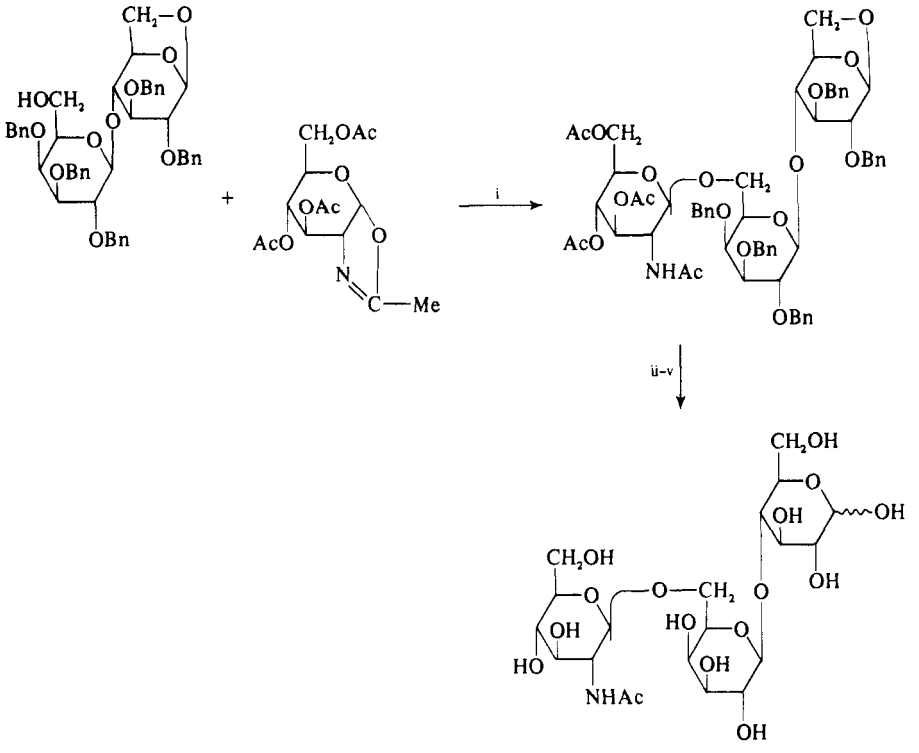
As part of a program of synthesis of oligosaccharides in human milk, *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose (6′-*N*-acetylglucosaminyl-lactose) has been prepared.[34] The synthetic route involves condensation of a 1,6-anhydro-β-lactose derivative having an unprotected hydroxy group at the C-6′ position with the acetylated oxazoline of 2-acetamido-2-deoxy-D-glucopyranose, and subsequent removal of the protecting groups (Scheme 2). Using the same approach lacto-*N*-tetraose [*O*-β-D-galactopyranosyl-(1→3)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose] has also been synthesized.[35]

1,2,3,6,2′,3′-Hexa-*O*-acetyl-β-lactose has been condensed with tri-*O*-acetyl-α-L-fucopyranosyl bromide under Koenigs-Knorr conditions.[36] Concomitant [3′→6′]-acetyl migration occurred and the L-fucopyranosyl group became attached β-glycosidically in position 3′. Deblocking of the product yielded *O*-α-L-fucopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose (6′-*O*-α-L-fucopyranosyl-lactose), a compound of potential use in investigation of the specificity of combining sites on lectins and on antibodies.

The reactions of 6′-chloro-6′-deoxyraffinose deca-acetate with various nucleophilic anions (Br^-, I^-, N_3^-) to yield the corresponding 6′-substituted raffinoses have been investigated.[37] Numerous reactions of these compounds yielding mono- and di-substituted derivates of raffinose (*e.g.* 3′,6′-anhydro- and 3′,6′-3″,6″-dianhydroraffinose, and 6′,6″-diazido-6′,6″-dideoxyraffinose) were reported.

The syntheses of three oligosaccharide phosphates (12)–(14), which are fragments of the biosynthetic intermediates of *Salmonella O*-specific polysaccharides, have been reported.[38] The conditions of the MacDonald procedure were found to be satisfactory for introduction of the phosphate group into the oligosaccharides, which contain glycosidic bonds that are relatively labile towards acid hydrolysis.

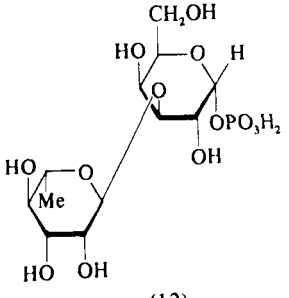
[34] H. Matsuda, H. Ishihara, and S. Tejima, *Chem. Pharm. Bull.*, 1979, **27**, 2564.
[35] T. Takamura, T. Chiba, H. Ishihara, and S. Tejima, *Chem. Pharm. Bull. (Jpn.)*, 1980, **28**, 1804.
[36] H. H. Baer and S. A. Abbas, *Carbohydr. Res.*, 1980, **83**, 146.
[37] L. Hough, A. C. Richardson, and M. A. Salam, *Carbohydr. Res.*, 1980, **80**, 117.
[38] L. L. Danilov, M. F. Troitzky, N. S. Utkina, V. N. Shibaev, and N. K. Kochetkov, *Carbohydr. Res.*, 1980, **87**, 146.



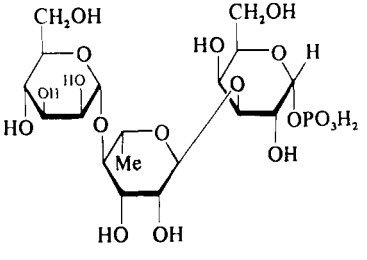
Reagents: i, nitromethane–toluene–4-toluene sulphonic acid, 95 °C, 1 h; ii, Pd–H_2; iii, Ac_2O–py; iv, Ac_2O–AcOH–H_2SO_4; v, $\bar{O}Me$ Na^+

Scheme 2

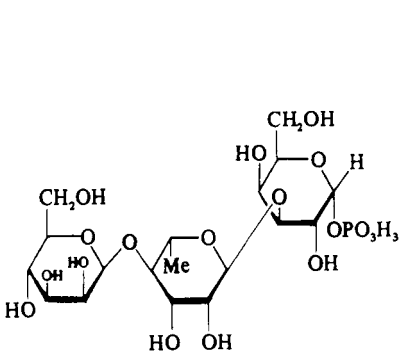
The trisaccharide *O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (15) has been synthesized by preparing *O*-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose, converting the product into an oxazoline, and using this to glycosylate



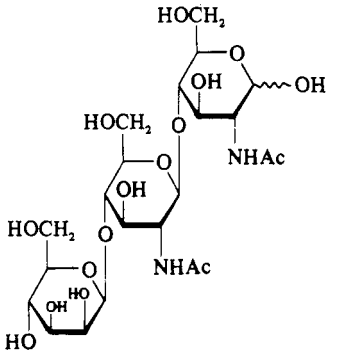
(12)



(13)



(14)



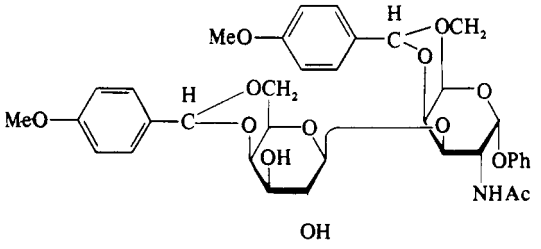
(15)

a suitably protected chitobiose derivative of 2-acetamido-2-deoxy-D-glucose.[39] The chemical synthesis of (15), which occurs in the 'core' saccharide region of most *N*-glycoproteins, is important as it provides a pure reference compound for the structural investigation of glycoproteins and lipid intermediates. An alternative synthesis has been reported: a derivative of *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (di-*N*-acetylchitobiose) was prepared and coupled to a β-D-mannopyranosyl group at *O*-4′.[40] This route provides intermediates particularly suitable for conversion to a trisaccharide phosphate for eventual synthesis of dolichyl pyrophosphate 'lipid intermediates' involved in the biosynthesis of *N*-glycoproteins.

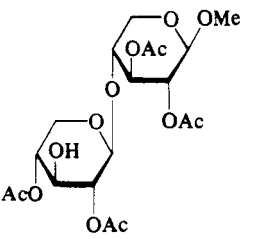
Zemplén deacetylation of phenyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside yields (16), which has been selectively benzoylated to give the 3′-benzoate.[41] On halide ion-catalysed condensation with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide, followed by deblocking, phenyl *O*-α-L-fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside was obtained.

The stepwise synthesis of methyl 4-*O*-[3-*O*-(β-D-xylopyranosyl)-β-D-xylopyranosyl]-β-D-xylopyranoside (17) has been reported.[42] The key intermediate (18) was obtained by condensing 2,3-di-*O*-acetyl-β-D-xylopyranoside with 2,4-di-*O*-acetyl-3-*O*-benzyl-α-D-xylopyranosyl bromide and separating and crystallizing the α-D- and β-D-anomeric forms. The reaction of (18) with 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide followed by deacetylation yielded the crystalline glycosides (17) and (19), which differ only in the stereochemistry of the interglycosidic linkage at the non-reducing end of the molecules.

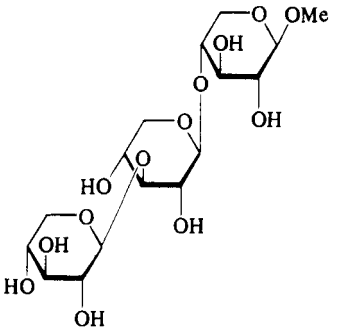
[39] C. D. Warren, C. Augé, M. L. Laver, S. Suzuki, D. Power, and R. W. Jeanloz, *Carbohydr. Res.*, 1980, **82**, 71.
[40] C. Augé, C. D. Warren, R. W. Jeanloz, M. Kiso, and L. Anderson, *Carbohydr. Res.*, 1980, **82**, 85.
[41] S. S. Rana, J. J. Barlow, and K. L. Matta, *Carbohydr. Res.*, 1980, **87**, 99.
[42] P. Kováč and J. Hirsch, *Carbohydr. Res.*, 1980, **79**, 303.



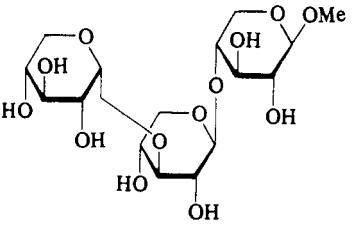
(16)



(18)



(17)



(19)

Part (20) of the repeating unit in the *O*-specific side-chains of *Salmonella* lipopolysaccharides belonging to sero-groups A, B, and D has been synthesized.[43] The 4-trifluoroacetamidophenyl group is readily hydrolysed to a 4-aminophenyl group, which can be used for the formation of a linkage to protein moieties.

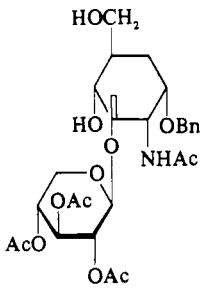
2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-3-*O*-(β-D-xylopyranosyl)-D-glucopyranose (3-*O*-β-D-xylopyranosyl-*N*-acetyl-lactosamine) has been prepared from benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside.[44] Coupling of the latter with 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide followed by debenzylidation yields the key intermediate (21) in the procedure. Preferential etherification and esterification of (21) were investigated. Selective benzylation of (21) afforded the 6-*O*-benzoate, which on condensation with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide followed by deblocking afforded the desired branched trisaccharide.

The immunochemical determination of the structure of dextrans has been extended by preparation of a series of branched trisaccharides (22)–(24) corresponding to the three possible branch points in natural dextrans.[45] Three *O*-allyl-di-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-1-*O*-tosyl-D-glucopyranose derivatives were coupled with 2-[4-(4-toluenesulphonamido)phenyl]ethanol to give, after decarbanilation and deallylation, the corresponding 2,3-, 2,4-, and 3,4-di-*O*-

[43] P. J. Garegg, H. Hultberg, and C. Lindberg, *Carbohydr. Res.*, 1980, **83**, 157.
[44] S. Oguri, H. Ishihara, and S. Tejima, *Chem. Pharm. Bull. (Jpn.)*, 1980, **28**, 35.
[45] R. Eby and C. Schuerch, *Carbohydr. Res.*, 1980, **79**, 53.

α-D-Gal*p*-(1 → 2)-α-D-Man*p*-(1 → 4)-α-L-Rha*p*-(1 → 0)-C_6H_4-(4-NHCOCF$_3$)

(20)



(21)

α-D-Glc*p*-(1 → 6)-[α-D-Glc*p*-(1 → 2)]-α-D-Glc*p*

(22)

α-D-Glc*p*-(1 → 6)-[α-D-Glc*p*-(1 → 3)]-α-D-Glc*p*

(23)

α-D-Glc*p*-(1 → 6)-[α-D-Glc*p*-(1 → 4)]-α-D-Glc*p*

(24)

benzyl-α-D-glucopyranosides. Reaction of the diols with two equivalents of 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-1-*O*-tosyl-D-glucopyranose gave protected forms of the required branched trisaccharides having an α-D-glucopyranosyl group at O-6 and one at O-2, O-3, or O-4.

The 8-methoxycarbonyloctyl glycoside of the tetrasaccharide hapten *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-D-glucopyranose (25) and the trisaccharide glycoside 8-methoxy-carbonyloctyl *O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-D-glucopyranoside have been synthesized by sequential Koenigs–Knorr reactions from monosaccharide units.[46] The tetrasaccharide (25) represents the complete skeletal repeating unit of *Shigella flexneri* sero-group Y lipopolysaccharide. Both oligosaccharide haptens are functionalized for covalent attachment to protein, cell surfaces, and solid supports.

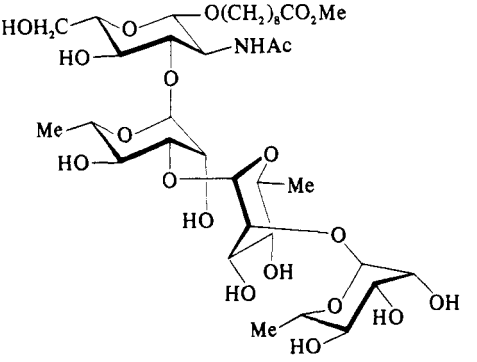
The tetrasaccharide (26), the repeating unit of the *O*-specific polysaccharide chain of the lipopolysaccharide from *Salmonella muenster* and *S. minneapolis*, has been obtained by glycosylation of benzyl 2,6-di-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-benzoyl-α-D-glucopyranosyl)-β-D-galactopyranoside with 3-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-β-L-rhamnopyranose-1,2-

[46] D. R. Bundle and S. Josephson, *Carbohydr. Res.*, 1980, **80**, 75.

(methylorthoacetate) followed by removal of protecting groups.[47] Following a similar procedure the pentasaccharide (27), the repeating unit of the *O*-specific polysaccharide chain of the lipopolysaccharide from *S. strasbourg*, has also been prepared.[48]

The stepwise synthesis of an aldopentaouronic acid derivative (28) related to branched (4-*O*-methyl-D-glucurono)-D-xylans has been reported.[49] The product contains all the basic structural features of branched hardwood (4-*O*-methyl-D-glucurono)-D-xylan.

Silver trifluoromethanesulphonate-promoted condensation of 3,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide with benzyl 3,6-di-*O*-benzyl-α-D-mannopyranoside and benzyl 2,4-di-*O*-benzyl-3,6-di-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-



(25)

β-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-D-Gal*p*
4
↑
1
α-D-Glc*p*

(26)

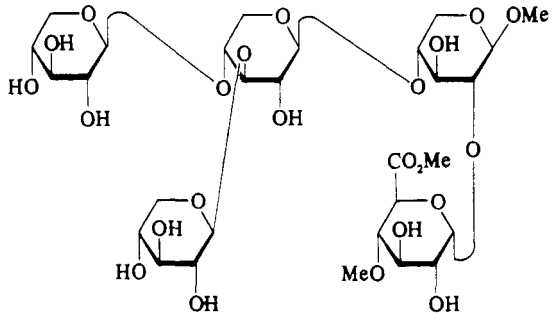
α-Tyv-(1→3)-β-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-D-Gal*p*
4
↑
1
α-D-Glc*p*

(27) Tyv = tyvelose

[47] N. K. Kochetkov, V. I. Torgov, N. N. Malysheva, A. S. Shashkov, and E. M. Klimov, *Tetrahedron*, 1980, **36**, 1227.

[48] N. K. Kochetkov, V. I. Torgov, N. N. Malysheva, and A. S. Shashkov, *Tetrahedron*, 1980, **36**, 1099.

[49] P. Kováč, J. Hirsch, V. Kováčik, and P. Kočiš, *Carbohydr. Res.*, 1980, **85**, 41.



(28)

α-D-mannopyranoside gave a protected pentasaccharide and a protected hepta-saccharide in good yield.[50] After deblocking, the free penta- (29) and hepta- (30) saccharides were obtained. These form part of the complex-type carbohydrate portion of glycoproteins.

D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-β-D-Man*p*
2
↑
1
β-D-Glc*p*NAc
4
↑
1
β-D-Gal*p*

(29)

D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→2)-β-D-Man*p*-(1→3)-α-D-Man*p*
6
↑
1
D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→2)-β-D-Man*p*

(30)

Glycopeptides and Glycosylated Amino-acids. – The total regio- and stereo-controlled synthesis has been reported of a small glycopeptide (31) proposed to be part of the structure of a glomerular basement membrane glycopeptide having nephritogenic activity.[51]

A new synthesis of acylated glycosylamides (β-D-galactopyranosyl-β-D-galacto-pyranosyl-, 2-acetamido-2-deoxy-β-D-glucopyranosyl-, β-lactosyl-, and di-*N*-acetyl-β-chitobiosyl- derivatives of 4-*N*-glycosyl-L-asparagine) by use of the triethylamine-catalysed reaction of acyl-glycosyl isothiocyanates with carboxylic

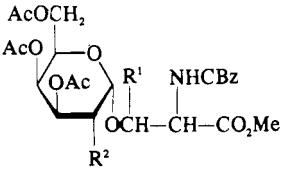
[50] J. Arnarp and J. Lönngren, *J. Chem. Soc., Chem. Commun.*, 1980, 1000.
[51] T. Ogawa, S. Nakabayashi, and S. Shibata, *Carbohydr. Res.*, 1980, **86**, C7.

acids has been described.[52] The scheme of formation of the products was discussed.

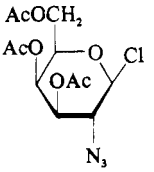
The synthesis of derivatives (32) and (33) of O^3-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine or -L-threonine by a convenient new procedure has been reported.[53] The key intermediate, 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl chloride (34), was prepared and condensed with the methyl ester of *N*-(benzyloxycarbonyl)-L-serine or -L-threonine to yield, after deblocking, the desired derivatives.

α-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1-L-Asn

(31)



(32) R^1 = H; R^2 = NHAc
(33) R^1 = Me; R^2 = NH_2



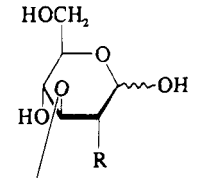
(34)

Three glycopeptides that span the amino-acid sequence 17–23 of bovine pancreatic deoxyribonuclease A and contain a 2-acetamido-2-deoxy-D-glucose residue have been synthesized.[54] 2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl-L-seryl)-L-aspart-1-oyl-(4-nitrobenzyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine, 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl-L-seryl)-L-aspart-1-oyl-(L-alanine methyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine, and 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-benzyloxycarbonyl-L-aspart-1-oyl-(L-alanyl-L-threonyl-L-leucyl-L-alanyl-L-serine 4-nitrobenzyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine were prepared.

The synthesis of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (35) and some carbohydrate analogues (36)–(38) have been described and immunoadjuvant activities of the four compounds examined.[55] Compound (36) had stronger activity than (35), whereas the activity of (37) was comparable to that of (35), and the 2-deoxy-D-*arabino*-hexose analogue (38) did not show any adjuvant activity. In another study (35) and 13 new analogues were synthesized by the conventional organic chemical procedure using dicyclohexylcarbodi-imide-*N*-hydroxy-5-norbornene-2,3-dicarboximide as a coupling agent.[56] The immunoadjuvant activities of the derivatives were investigated and results indicate that

[52] A. Y. Khorlin, S. E. Zurabyan, and R. G. Macharadze, *Carbohydr. Res.*, 1980, **85**, 201.
[53] B. Ferrari and A. A. Pavia, *Carbohydr. Res.*, 1980, **79**, C1.
[54] H. G. Garg and R. W. Jeanloz, *Carbohydr. Res.*, 1980, **86**, 59.
[55] M. Kiso, Y. Kaneda, H. Okumura, A. Hasegawa, I. Azuma, and Y. Yamamura, *Carbohydr. Res.*, 1980, **79**, C17.
[56] S. Kobayashi, T. Fukuda, H. Yukimasa, M. Fujino, I. Azuma, and Y. Yamamura, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 2570.

the presence of an α-amino-acid adjacent to the lactic acid moiety in (35) is important for high biological activity, the L-valine analogue having the most favourable orientation. The anomeric configurations of (35) and several of its derivatives have been established in a ^{13}C n.m.r. study.[57] Results are in agreement with those obtained by h.p.l.c.



(35) R = NHAc
(36) R = $NH_3^+Cl^-$
(37) R = OH
(38) R = H

A general and convenient synthesis of 2-acetamido-2-deoxy-β-D-glucosyl-(1→4)-*N*-acetyl-muramoyl-L-alanyl-D-isoglutamine has been reported using an improved oxazoline method.[58]

Various 6-amino- and 6-(acylamino)-6-deoxy-carbohydrate analogues of *N*-acetylmuramyl-L-(alanyl, valyl, or seryl)-D-isoglutamine have been synthesized starting from benzyl 2-acetamido-6-azido-3-*O*-(D-1-carboxyethyl)-2,6-dideoxy-4-*O*-tetrahydropyranyl-α-D-glucopyranoside.[59] Biological activities of the derivatives were determined.

Carbohydrate analogues of (35), including 2-acetamido-2,6-dideoxy-3-*O*-(D-2-propionyl-L-alanyl-D-isoglutaminyl)-D-glucopyranose, 2-acetamido-6-amino-2,6-dideoxy-3-*O*-(D-2-propionyl-L-alanyl-D-isoglutaminyl)-D-glucopyranose, and 2-acetamido-2-deoxy-3-*O*-(D-2-propionyl-L-alanyl-D-isoglutaminyl)-D-xylopyranose, have been synthesized.[60] Their immunoadjuvant activities were determined to clarify the structural requirements for biological activity.

Syntheses of a series of 4-*O*-(2-acetamido-2-deoxy-β-D-glucosyl)-6-*O*-acyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine derivatives with potential anti-tumour activity have been reported.[61] The compounds were prepared either by acylation of a disaccharide dipeptide derivative or by acylation of a disaccharide followed by coupling with the dipeptide moiety.

[57] T. D. J. Halls, M. S. Raju, E. Wenkert, M. Zuber, P. Lefrancier, and E. Lederer, *Carbohydr. Res.*, 1980, **81**, 173.
[58] M. Kiso, Y. Kaneda, R. Shimizu, and A. Hasegawa, *Carbohydr. Res.*, 1980, **83**, C8.
[59] A. Hasegawa, H. Okumura, M. Kiso, I. Azuma, and Y. Yamamura, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 1309.
[60] A. Hasegawa, H. Okumura, M. Kiso, I. Azuma, and Y. Yamamura, *Agric. Biol. Chem. (Jpn.)*, 1980, **44**, 1301.
[61] M. Inage, M. Imoto, Y. Kambayashi, S. Kusumoto, and T. Shiba, *Tetrahedron Lett.*, 1980, **21**, 3767.

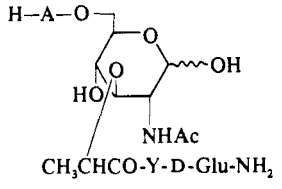
Incorporation of an amino-acid into the 6-position of the muramic acid moiety of muramyl dipeptide has been achieved, thus providing an effective means for the synthesis of various new derivatives.[62] Six new muramyl dipeptide derivatives have been synthesized *via* *O*-aminoacyl intermediates (39) and their immunological activities determined.

The synthesis of the *O*-(2-*O*-α-D-glucopyranosyl)-β-D-galactopyranoside of optically pure δ-hydroxy-L-lysyl-glycine, which forms an essential part of the glomerular basement membrane, has been reported for the first time.[63] The use of the levulinyl group as a protecting group for *O*-2 of the D-galactose favours formation of a β-linkage with the dipeptide and enables extension, by the specific removal of levulinate in the presence of the acetyl groups, of the D-galactose derivative (40) in the 2-direction with the D-glucose derivative (41). Functionalization of the anomeric centre of D-glucose with an *N*-methylacetimidyl group facilitates formation of the required α-interglycosidic linkage between the D-galactose and D-glucose moieties.

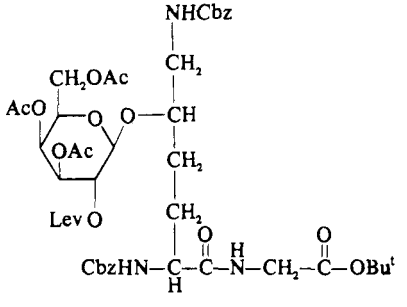
Novel analogues of glycopeptides having ω-amino-alkanoic acids or heterocyclic groups linked to D-mannose through an amide linkage have been prepared.[64]

Three 2,3,4,6-tetra-*O*-benzyl-1-*O*-(*N*-benzyloxycarbonyltripeptidyl)-D-glucopyranosides (42)–(44) have been synthesized from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside and the active esters of the appropriate *N*-protected tripeptides in the presence of imidazole.[65] The anomeric mixtures were resolved and the α- and β-anomers characterized.

The fully protected 1-esters of D-glucopyranuronic acids (45) and (46) have been prepared as anomeric mixtures by the imidazole-promoted reaction of benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate with 1-benzyl-4-pentachlorophenyl *N*-benzyloxycarbonyl-L-aspartate and 1-benzyl-5-pentachlorophenyl *N*-



(39) A, Y = amino-acid residues



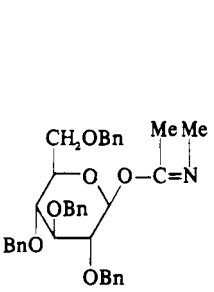
(40) Lev = levulinate [= $MeOC(CH)_2CO-$]

[62] S. Kobayashi, T. Fukuda, H. Yukimasa, I. Imada, M. Fujino, I. Azuma, and Y. Yamamura, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 2917.

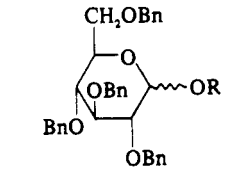
[63] H. J. Koeners, C. Schattenkerk, J. Verhoeven, and J. H. van Boom, *Tetrahedron Lett.*, 1980, **21**, 2373.

[64] M. M. Ponpipom, R. L. Bugianesi, and T. Y. Shen, *Carbohydr. Res.*, 1980, **82**, 141.

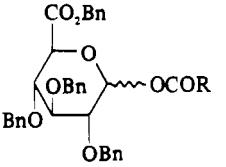
[65] S. Valenteković and D. Keglević, *Carbohydr. Res.*, 1980, **82**, 31.



(41)



(42) R = Cbz-Gly-Gly-Gly
(43) R = Cbz-L-Phe-Gly-Gly
(44) R = Cbz-Gly-Gly-L-Phe



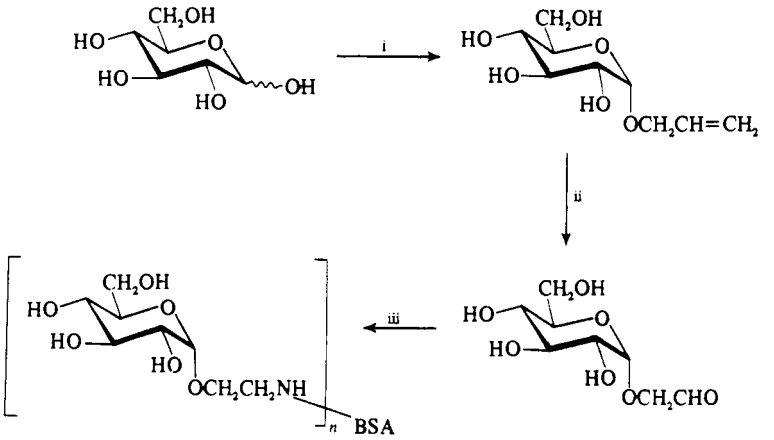
(45) R = CH_2–CH(NHCbz)–CO_2Bn

(46) R = CH_2–CH_2–CH(NHCbz)–CO_2Bn

benzyloxycarbonyl-L-glutamate, respectively.[66] The anomers were separated and characterized.

Glycoproteins. – The preparation and applications of synthetic glycoproteins have been reviewed.[67]

Several model glycoproteins have been prepared by coupling sugars and oligosaccharides of known structure to bovine serum albumin. One such procedure, involving coupling of alkenyl glycosides to proteins by reductive ozonolysis followed by reductive amination, is shown in Scheme 3.[68] This general method is advantageous in that each of the individual reactions is well documented and their incorporation into the reaction scheme thus does not require development of an extensive array of 'control' experiments.

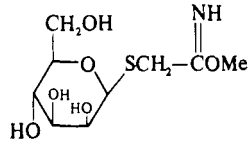


Reagents: i, vinyl alcohol; ii, O_3–MeOH–Me_2S; iii, bovine serum albumin (BSA)–$NaCNBH_3$

Scheme 3

[66] D. Ljevaković and D. Keglević, *Carbohydr. Res.*, 1980, **86**, 43.
[67] C. P. Stowell and Y. C. Lee, *Adv. Carbohydr. Chem. Biochem.*, 1980, **37**, 225.
[68] M. A. Bernstein and L. D. Hall, *Carbohydr. Res.*, 1980, **78**, C1.

D-Mannosyl-[^{125}I]-bovine serum albumin has been prepared by reacting albumin with the 2-imino-2-methoxyethyl-1-thio-glycoside (47).[69] Chloroquine- and ammonium ion-inhibition of receptor-mediated endocytosis of the glyco-conjugate by macrophages was then investigated.



(47)

2-Imino-2-methoxyethyl 1-thioglycosides of β-D-galactose, 6-*O*-methyl-β-D-galactose, α-L-arabinose, β-D-fucose, β-L-fucose, β-D-glucose, β-D-xylose, β-D-allose, α-D-mannose, 2-acetamido-2-deoxy-β-D-glucose, 2-acetamido-2-deoxy-β-D-galactose, 2-deoxy-β-D-*arabino*-hexose, and 3-*O*-methyl β-D-glucose have been prepared and used for the preparation of some new neoglycoproteins by amidination of bovine serum albumin.[70] The reaction was complete in a few hours at pH 7–10, and the extent of amidination could be controlled by altering the ratio of imido ester to protein amino group. The binding of these synthetic amidinino-neoglycoproteins to rabbit liver membranes has also been reported.[71]

Neoglycoproteins containing thioglycosides having an ω-aldehydo group in the aglycone (*e.g.* *N*-[*S*-(β-D-galactopyranosyl)thioacetyl]amino-acetaldehyde) have been attached to bovine serum albumin by reductive amination using sodium cyanoborohydride.[72] The neoglycoproteins are good inhibitors of asialo-α_1-acid glycoprotein binding to rabbit liver plasma membrane.

Synthetic muramyldipeptide has been conjugated to bovine and human serum albumins in carbodi-imide-mediated reactions, and a 4-isothiocyanatophenyl derivative of the compound has been conjugated to keyhole limpet haemocyanin and to human serum albumin using thiophosgene.[73] The resulting conjugates were used successfully as antigens for induction of antibodies against muramyl dipeptide.

Oligosaccharides (22)–(24) have been converted into the isothiocyanate derivatives with thiophosgene and coupled to bovine serum albumin, yielding conjugates that can be used as artificial antigens for production of antisera specific for each of the branch points in dextrans.[45] β-(4-Aminophenyl)ethylamine derivatives of sialo-oligosaccharides have been coupled to proteins *via* the phenylisothiocyanate intermediates under conditions preserving labile sugar linkages.[74] The conjugates were used to elicit antibodies against the sialyloligosaccharides.

[69] C. Tietze, P. Schlesinger, and P. Stahl, *Biochem. Biophys. Res. Commun.*, 1980, **93**, 1.
[70] C. P. Stowell and Y. C. Lee, *Biochemistry*, 1980, **19**, 4899.
[71] C. P. Stowell, R. T. Lee, and Y. C. Lee, *Biochemistry*, 1980, **19**, 4904.
[72] R. T. Lee and Y. C. Lee, *Biochemistry*, 1980, **19**, 156.
[73] C. M. Reichert, C. Carelli, M. Jolivet, F. Audibert, P. Lefrancier, and L. Chedid, *Mol. Immunol.*, 1980, **17**, 357.
[74] D. F. Smith and V. Ginsburg, *J. Biol. Chem.*, 1980, **255**, 55.

A stable biologically active glycosylated derivative of insulin has been prepared by treatment of insulin with maltose in the presence of sodium cyanoborohydride.[75]

Radioactively labelled D-glucose from several manufacturers contains radioactive impurities of unknown structure that bind covalently to proteins (*e.g.* collagen, fibronectin, basic myelin protein, bovine serum albumin, and haemoglobin) and thus simulate nonenzymatic glycosylation.[76] Radiolabelled sucrose covalently linked to protein has been used in a device for quantifying degradation of plasma proteins catabolized by lysosomal mechanisms.[77, 78]

Glycolipids. – A facile preparation of *N*-fatty acylated 2-amino-2-deoxy-β-D-glucopyranosides, which are key intermediates for the synthesis of various complex glycolipids, has been reported.[79]

Use of a 1,3-dichloro-1,1,3,3-tetra-isopropyldisiloxane protecting group has facilitated the synthesis of a naturally occurring phosphatidyl-α-D-glucosyldiacylglycerol *via* a phosphotriester intermediate (Scheme 4).[80] The presence of the unsaturated fatty acid in the molecule to be synthesized excludes, at the final stage of the synthesis, the use of the commonly applied benzyl protecting group.

A convenient method for large-scale preparation of stearoyl 1-[^{14}C]sulphogalactosyl-sphingosine has been developed.[81] A lysosulphatide intermediate was prepared by alkaline hydrolysis of cerebroside sulphate and then reacted with 1-[^{14}C]stearic acid through the acyl chloride procedure.

2 Modification of Polysaccharides and Oligosaccharides and Uses of Modified Polysaccharides and Oligosaccharides

The use of polysaccharide matrices for attachment of ligands continues to expand for applications in affinity chromatography and immunoadsorption techniques. The quantitation and purification of antibodies and antigens using immunoadsorbents prepared from agarose or cellulose have been reviewed.[82] Biomedical applications of synthetic derivatives of polysaccharides have been described.[83] Summaries of recent literature on molecular immobilization and

[75] M. Brownlee and A. Cerami, *Science*, 1979, **206**, 1190.
[76] B. Trüeb, C. G. Holenstein, R. W. Fischer, and K. H. Winterhalter, *J. Biol. Chem.*, 1980, **255**, 6717.
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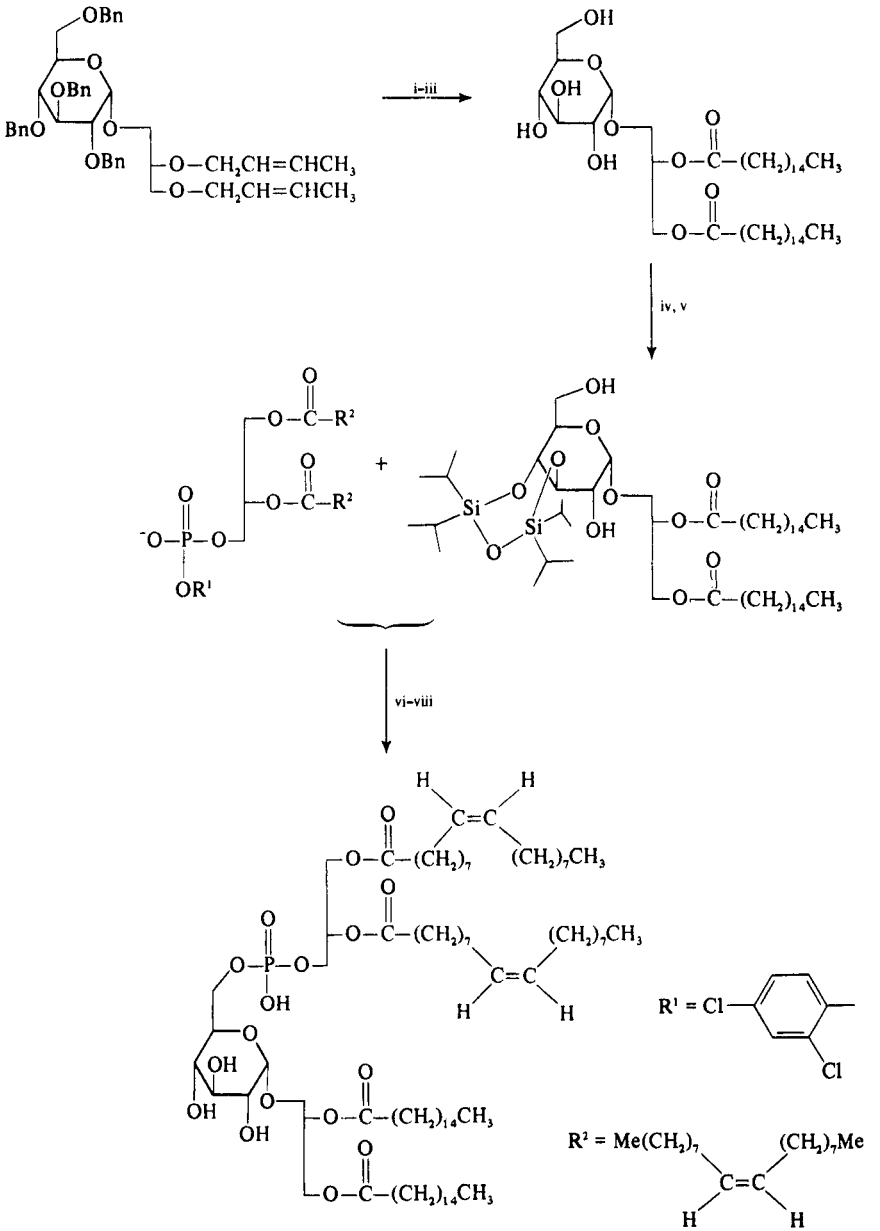
[78] R. C. Pittman, S. R. Green, A. D. Attie, and D. Steinberg, *J. Biol. Chem.*, 1979, **254**, 10 562.
[79] M. Kiso, H. Nishiguchi, and A. Hasegawa, *Carbohydr. Res.*, 1980, **81**, C13.
[80] C. A. A. van Boeckel and J. H. van Boom, *Tetrahedron Lett.*, 1980, **21**, 3705.
[81] G. Dubois, B. Zalc, F. Le Saux, and N. Baumann, *Anal. Biochem.*, 1980, **102**, 313.
[82] R. S. Nezlin, *Pure Appl. Chem.*, 1979, **51**, 1421.
[83] 'Biomedical Polymers - Polymeric Materials and Pharmaceuticals for Biomedical Use', ed. E. P. Goldberg and A. Nakajima, Academic Press, New York, 1980.



Reagents: i, $K^+Bu^tO^-$; ii, $Me(CH_2)_{14}COCl$; iii, H_2–Pd; iv, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane–py; v, H^+–DMF; vi, 2,4,6-tri-isopropylbenzenesulphonyl-3-nitro-1,2,4-triazolide–py; vii, *N,N,N′,N′*-tetramethylguanidinium *syn*-4-nitrobenzaldoximate–THF; viii, tetrabutylammonium fluoride–THF–py.HCl

Scheme 4

bioaffinity phenomena, much of which involves use of polysaccharide matrices, are being published at regular intervals.[84-87]

By using a bound spectral polarity-detecting label, the solvation power of the solvent near the polymeric carbohydrate matrix of some polar chromatographic media intended for the separation of biopolymers in aqueous solutions has been investigated.[88] With highly polar materials such as cellulose and cross-linked polydextran gels, the solvation power of water molecules is reduced and the polarity detected in the vicinity of the sorbent is much lower than that of water. Several carbohydrate derivatives were compared with respect to the magnitude of the difference between the polarity of the microenvironment of sorbents and the polarity of water.

Methods for introducing isonitrile functional groups into polymeric carbohydrate derivatives have been described.[89] Proteins and low molecular weight ligands were bound covalently to these materials by four-component reactions involving the isonitrile function on the polymer, amino-, or carboxy-groups on the ligand, an aldehyde group, and a complementary fourth component.

A micromethod for quantitation of sialoglycoconjugate immobilization on insoluble supports has been developed.[90]

Some theoretical aspects of the desorption of a column-bound protein by elution with its biospecific ligand have been considered in cases where the protein–ligand complex has a diminished but finite affinity for the adsorbent.[91]

Polysaccharides continue to be extremely important as support media in the field of affinity chromatography.[92] The preparation of imidazole-containing matrices for affinity chromatography of haemoproteins has been described.[93] The affinity chromatographic purification of glycosidases[94] and neuraminidases[95] has been reviewed. The use of affinity chromatography in the industrial ethanol fractionation of human plasma has been reported.[96] The application of affinity chromatography and hydrophobic chromatography to the purification of serum albumin has been described.[97] Immobilized lectins have been used for the purification of membrane glycoproteins,[98] and for the isolation of cell-

[84] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Tech.*, 1980, **2**, 66.
[85] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Tech.*, 1980, **2**, 155.
[86] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Tech.*, 1980, **2**, 244.
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[88] E. Brynda, P. Štrop, F. Mikeš, and J. Kálal, *J. Chromatogr.*, 1980, **196**, 39.
[89] L. Goldstein, *Biochimie*, 1980, **62**, 401.
[90] A. P. Corfield, T. L. Parker, and R. Schauer, *Anal. Biochem.*, 1979, **100**, 221.
[91] R. J. Yon, *Biochem. J.*, 1980, **185**, 211.
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[97] M. J. Harvey, in 'Methods of Plasma Protein Fractionation', ed. J. M. Curling, Academic Press, London, 1980, p. 189.
[98] R. Lotan and G. O. Nicolson, *Biochim. Biophys. Acta*, 1979, **559**, 329.

surface proteins of Novikoff tumour cells.[99] Nucleotides immobilized *via* different parts of the molecule have been applied to the purification of NAD^+-dependent dehydrogenases.[100] The separation of neurons and glial cells by affinity methods has been described.[101]

The use of affinity electrophoresis continues to expand. The technique has been applied to studies of the interactions between L-lactate dehydrogenase isoenzymes and immobilized adenosine monophosphate,[102–104] and between lectins and carbohydrates.[105, 106]

The concanavalin A reactivity pattern of human amniotic fluid α-fetoprotein has been examined by crossed affinoimmunoelectrophoresis, in an attempt to develop a definite test for neural tube defects.[107]

The association constants for interaction of BALB/c and NZB myeloma anti-D-fructan antibodies with high molecular levans and some low molecular weight compounds (rye-grass levan, inulin, sucrose, and D-fructo-oligosaccharides) have been determined by affinity electrophoresis.[108]

A new technique for desorbing substances tightly bound to affinity gels involves flat-bed electrophoretic desorption by isoelectric focusing.[109]

Agaroses. – Unmodified agarose has been used for the purification of a D-galactose-binding lectin from the haemolymph of *Sarcophaga peregrina*.[110] One form of modeccin, the toxin from *Adenia digitata*, can be purified by affinity chromatography on unmodified agarose, to which it binds.[111] A second form of modeccin binds only to agarose pre-treated with acid to expose D-galactose units. Acid-treated agarose has also provided an affinity matrix for purification of a lectin from *Hura crepitans* seeds.[112]

Alkyl and aromatic derivatives of agarose continue to be widely applied in preparative techniques involving hydrophobic chromatography and for enzyme immobilization. A new method has been developed for the quantitative determination of alkylamino-side-chains covalently coupled to agarose.[113] Bound alkylamino-groups are reacted with 2-hydroxy-1-naphthaldehyde and then treated with benzylamine to form a soluble Schiff's base, which can be deter-

[99] J. R. Glenney and E. F. Walborg, *J. Supramol. Struct.*, 1979, **11**, 493.
[100] C. R. Lowe, I, P. Trayer, and H. R. Trayer, *Methods Enzymol.*, 1980, **66**, 192.
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[104] K. Nakamura, A. Kuwahara, and K. Takeo, *J. Chromatogr.*, 1980, **196**, 85.
[105] R. Turková, M. Tichá, and J. Kocourek, *J. Chromatogr.*, 1980, **192**, 408.
[106] V. Čeřovský, M. Tichá, V. Hořejši, and J. Kocourek, *J. Biochem. Biophys. Methods*, 1980, **3**, 163.
[107] B. Norgaard-Pedersen, K. Toftager-Larsen, J. Philip, and P. Hendersson, *Clin. Genet.*, 1980, **17**, 355.
[108] S. Sugii and E. A. Kabat, *Carohydr. Res.*, 1980, **82**, 113.
[109] L. A. Haff, M. Lasky, and A. Manrique, *J. Biochem. Biophys. Methods*, 1979, **1**, 275.
[110] H. Komano, D. Mizuno, and S. Natori, *J. Biol. Chem.*, 1980, **255**, 2919.
[111] L. Barbieri, M. Zamboni, L. Montanaro, S. Sperti, and F. Stirpe, *Biochem. J.*, 1980, **185**, 203.
[112] A. Falasca, C. Franceschi, C. A. Rossi, and F. Stirpe, *Biochim. Biophys. Acta*, 1980, **632**, 95.
[113] P. Lustenberger, P. Formstecher, and M. Dautrevaux, *J. Chromatogr.*, 1980, **193**, 451.

mined spectrophotometrically. The method was shown to be superior to that involving 2,4,6-trinitrobenzene sulphonic acid.

Butylamine-agarose has been used for the purification by hydrophobic chromatography of leader (signal) peptidase from *Escherichia coli*.[114] The P2 primary alkylsulphohydrolase of the detergent-degrading bacterium *Pseudomonas* C12B has also been purified on butylamine-agarose.[115]

Diphtheria toxin has been purified and its fragments separated by hydrophobic chromatography on butylamine-, hexylamine-, and octylamine-agarose.[116]

Rat liver phosphoprotein phosphatase has been purified on hexylamine-agarose.[117] The same matrix has been used successfully for hydrophobic chromatographic isolation of rat renal renin,[118] and for affinity chromatographic purification of amine oxidase.[119]

Octylamine derivatives of agarose have been used for purification of β-D-galactosidase from *Aspergillus niger*,[120] for isolation of uroporphyrinogen I synthase from human erythrocytes,[121] and for purification of a completely inactive renin from hog kidney extract.[122]

Hydrophobic interaction chromatography utilizing phenyl-agarose has been applied to the isolation of human erythrocyte uroporphyrinogen I synthetase,[116] to the purification of rat kidney transamidinase,[123] to the purification of nucleoside diphosphatase from rat liver microsomes,[124] to the large-scale preparation of human fibroblast interferon,[125] to the purification of an enzyme with lysophospholipase activity from rat intestinal mucosa,[126] and to the isolation of guanylate cyclase from bovine brain.[127]

The application of amino-alkyl agaroses to immobilization of flavin derivatives for use in affinity chromatography has been reviewed.[128]

The chromatographic behaviour of poly(adenylic acid) homopolynucleotides on several matrices, including octyl, phenyl, and ethanolamine derivatives of agarose, has been investigated.[129] By using different experimental conditions it was possible to evaluate the contribution to the interaction of electrostatic, hydrophobic, hydrogen-bonding, and/or charge-transfer effects.

[114] C. Zwizinski and W. Wickner, *J. Biol. Chem.*, 1980, **255**, 7973.
[115] J. M. Cloves, K. S. Dodgson, G. F. White, and J. W. Fitzgerald, *Biochem. J.*, 1980, **185**, 23.
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[120] F. Widmer and J. L. Leuba, *Eur. J. Biochem.*, 1979, **100**, 559.
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The merits of hydrophobic interaction chromatography on octyl-agarose as a method for the separation of carrier ampholytes from peptides have been investigated.[130] *K* Values for the association of octyl groups with small model peptides and with carrier ampholytes were determined as a function of ionic strength and pH. Separation of a somatomedin-containing peptide mixture into seven fragments by hydrophobic chromatography on octyl-agarose was reported.

The hydrophobic interaction of β-D-galactosidase and agarose substituted with 3,3′-diaminodipropylamine has been studied in both batch and column experiments.[131] The equilibrium and the binding rate constants were determined for different phosphate buffer concentrations.

A new method has been described for the immobilization of enzymes and other proteins on hydrophobic gels.[132] The enzyme (alcohol dehydrogenase, asparaginase, or trypsin) was pre-treated with dodecyl-, octyl-, or hexyl-aldehyde to introduce several hydrophobic groups, which were then available for multiple hydrophobic interactions with octyl- or phenyl-agarose. The binding, although by adsorption, is very strong: enzyme cannot be removed with 1 M potassium chloride, 50% (v/v) ethylene glycol, or 0.5% v/v Triton® X-100.

Cross-linked agarose has been reacted with (3-aminopropyl)triethoxysilane, resulting in the incorporation of amino-groups into the agarose in high yield.[133] The introduced amino-groups can be reacted readily with proteins using glutaraldehyde as mediator.

Cyclic imidocarbonate derivatives of agarose continue to be used widely for the immobilization of biologically active macromolecules and for affinity chromatography. The reaction of a cyclic imidocarbonate group with a protein is presumed to occur by nucleophilic attack of an amino-group on the *trans*-imidocarbonate ring to give either an isourea or an *N*-substituted imidocarbonate, or an *N*-substituted carbonate (see Vol. 13, p. 592).

The cyanogen bromide used to activate polysaccharide carriers for covalent binding of biologically active substances should not contain cyanide ions, as has been recently discussed.[134]

A technique has been developed to prevent leakage of proteins immobilized on agarose by conventional methods such as the cyanogen bromide procedure.[135] Treatment of the immobilized protein with glutaraldehyde has been shown to decrease leakage of antibody or enzyme protein from the matrix to undetectable levels without significantly affecting biological activity.

The construction and operation of a device for cyanogen bromide activation of agarose gels have been described.[136] The main advantages associated with use of the apparatus are its simplicity, the speed of reaction, and the reduction of free vapours of cyanogen bromide.

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Recent references to the preparation of active immobilized forms of enzymes (for use as immobilized enzymes) and immunologically active macromolecules (for use as immunoadsorbents) and of various affinants (for use as affinity chromatography materials) by such methods are summarized in Tables 1,[137-162] 2,[163-200] and 3,[201-322] respectively. These derivatives are listed according to the uses prescribed since, for example, the insoluble form of an enzyme used for affinity chromatography may not be enzymically active.

Table 1 *Use of agarose cyclic imidocarbonates for the preparation of active immobilized enzymes*

Enzyme coupled to agarose cyclic imidocarbonate	*EC No.*	*Ref.*
Alcohol dehydrogenase	1.1.1.1	137, 138
Alkaline phosphatase	3.1.3.1	139, 140
Amine oxidase	1.4.3.4	119, 141
Carboxypeptidase Y	–	142
Catalase	1.11.1.6	119
Chymotrypsin	3.4.21.1	140, 143
2',3'-Cyclic nucleotide 3'-phosphohydrolase	–	144
Deoxyribonuclease I	3.1.21.1	145
Dextranase	3.2.1.11	140
Fatty acid synthetase	–	146
β-D-Galactosidase	3.2.1.23	147
D-Glucose dehydrogenase	1.1.1.47	140
L-Glutamate dehydrogenase	1.4.1.2	148, 149
L-Glutaminase	3.5.1.2	150
D-Glyceraldehyde-phosphate dehydrogenase	1.2.1.12	151-154
Hydrogenase	1.18.3.1	155
20β-Hydroxysteroid dehydrogenase	1.1.1.53	156
Lactate dehydrogenase	1.1.1.27	137, 140, 157
Lactoperoxidase	–	135
Malate dehydrogenase	1.1.1.37	140
Phosphotransferase	–	158
Transketolase	2.2.1.1	159
Trypsin	3.4.21.4	143, 160, 161
UDP-D-glucuronosyltransferase	2.4.1.17	162

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Table 2 *Use of agarose cyclic imidocarbonates for the preparation of immunoadsorbents*

Immunologically active component coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Anti-(acetylcholine receptor) antibody	Purification of nicotinic acetylcholine receptors	163
Anti-(rat adipocyte plasma membrane proteins) antibody	Purification of rat intrinsic membrane proteins	164
Anti-(rat serum albumin) antibody	Removal of albumin during purification of rat α-fetoprotein	165
Anti-(aminopeptidase) antibody	Removal of aminopeptidase during purification of sucrase–isomaltase	166
Anti-(barley β-amylase) antibody	Purification of barley β-amylase	167
Anti-(baboon lung angiotensin 1-converting enzyme) antibody	Large-scale purification of angiotensin 1-converting enzyme from human plasma	168
Anti-(L-aspartate aminopeptidase) antibody	Purification of L-aspartate aminopeptidase	169
Anti-(blood-group A or B or O) antibody	Demonstration of association of blood-group A-determinants with carcinoembryonic antigen	170
Anti-(human breast neoantigens) antibody[a]	Purification of breast-specific neoantigens	171
Anti-(catalase) antibody	Separation of catalase from L-phenylalanine hydroxylase	172
Anti-(human colon neoantigens) antibody[a]	Purification of colon-specific neoantigens	171
Anti-(2′,3′-cyclic nucleotide 3′-phosphohydrolase) antibody	Purification of 2′,3′-cyclic nucleotide 3′-phosphohydrolase	144
Anti-(*d*H-2^b histocompatibility antigens) antibody	Purification of a detergent solubilized form of H-2^b histocompatibility antigen from RBL-5 tumour cells	173
Anti-(factor VIII) antibody	Purification of human factor VIII	174
	Study of structure and function of factor VIII complexes	175
Anti-(β-D-glucuronidase) antibody $F(ab')_2$ fragments	Purification of urinary β-D-glucuronidase from mice treated with androgens	176
Anti-(human plasma group-specific component) antibody	Purification of an α_1-anti-trypsin-like material from normal human liver	177
Anti-(hepatitis B surface antigen) antibody	Removal of hepatitis B surface antigen from blood and blood products	178
Anti-(herpes virus type 1 glycoprotein) antibody	Purification of herpes virus type 1 glycoprotein	179
Anti-(hisB enzyme from *Salmonella typhimurium*) antibody	Purification of a bifunctional core of the hisB enzyme from *S. typhimurium*	180
Anti-(human immunoglobulin G) antibody	Purification of human leukocyte interferon	181

Table 2 *(cont.)*

Immunologically active component coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
	Study of the reactions and non-specific adsorptive properties of agarose cyclic imidocarbonate	182
Anti-(human immunoglobulin G subclasses 1 and 2) antibody	Isolation of subclasses of human immunoglobulin G	183
Anti-(normal human serum) antibody[a]	Removal of contaminants during purification of organ-specific neoantigens	171
Anti-(rat glandular kallikrein) antibody	Purification of rat submandibular kallikreins	184
Anti-(human α_2-macroglobulin) antibody	Quantitative immunoimmobilization of α_2-macroglobulin–β-trypsin complex	185
Anti-(human β_2-microglobulin) antibody	Isolation of plasma-membrane components from cultured human pancreatic cells	186
Anti-(nitrotyrosine) antibody	Purification of a nitrotyrosyl peptide obtained from 3-phosphoglycerate kinase	187
Anti-(*Schistosoma mansoni* soluble egg antigens) antibody	Purification of *S. mansoni* soluble egg antigens	188
Anti-thrombin antibody	Fractionation of heparin	189, 190
Anti-(bovine thyroid-stimulating hormone) antibody	Purification of thyroid-stimulating hormone from rat	191
Anti-(human transferrin) antibody	Purification of the transferrin receptor from human placenta	192
Immunoglobulin G	Development of a method for stabilizing proteins immobilized on agarose	135
	Purification of C_{1q}, a subcomponent of complement	193
Lipoic acid	Purification of antibodies directed against biotin	194
Oestradiol 17β-glutathione	Separation of antigen–enzyme–antibody complex from antigen–enzyme in enzyme immunoassays for human placental lactogen and progesterone	195
Protein A	Indirect immunoprecipitation technique for recovery of labelled NADP-glutamate dehydrogenase from *Chlorella* cell homogenates	149
	Removal of contaminants during purification of organ-specific neoantigens	171
	Detection of enzyme–immunoglobulin G complexes in human serum	196

Table 2 *(cont.)*

Immunologically active component coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
	Separation of human immunoglobulins 1, 2, and 4 by pH-gradient elution	197
	Removal of immunoglobulin G from sera prior to testing for rubella-specific immunoglobulin M	198
	Purification of insulin–receptor complexes from adipocyte plasma membranes	199
	Purification of immunoglobulin G from anti-(2-DT-diaphorase) antibody	200
	Purification of anti-(factor VIII) immunoglobulin G	174
Thrombin	Purification of antibodies against human (thrombin–anti-thrombin) complex	190
Thyroid-stimulating hormone	Purification of anti-(thyroid-stimulating hormone) antibody	191
Transferrin	Purification of the transferrin receptor from human placenta	192
Urinary protein (from patients with metastatic colonic cancer)[a]	Purification of anti-(urinary protein) antibody	171

[a] See Scheme 5b (52).

Table 3 *Use of agarose cyclic imidocarbonates for the preparation of affinity chromatography materials without linkage extension*

Ligand or affinant coupled to agarose cyclo imidocarbonate	*Use of product*	*Ref.*
L-Alanine	Purification of nucleosidase diphosphatase	201
Albumin	Affinity chromatography of soluble transferrin receptors	202
	Purification of lipoteichoic acid from *Streptococcus pyogenes*	203
4-Aminobenzylsuccinic acid	Purification of carboxypeptidase from *Streptomyces griseus*	204
6-Aminohexanoyl-L-alanyl-L-alanyl-D-leucylamide	Affinity chromatography of subtilisin	205
2-(6-Aminohexanoyl)amino-2-deoxy-D-glucose	Purification of 2-acetamido-2-deoxy-D-glucose kinase from rat	206
2-(6-Aminohexanoyl)-lacto-*N*-fucopentaosylamine	Purification of α-L-fucosidase I	207
2-(6-Aminohexanoyl)amino-2-deoxy-L-fucose	Purification of α-L-fucosidase from liver of a fucosidosis patient	208
	Purification of two forms of human α-L-fucosidase	209

Table 3 *(cont.)*

Ligand or affinant coupled to agarose cyclo imidocarbonate	*Use of product*	*Ref.*
	Purification of α-L-fucosidase from controls and patients with I-cell disease	210
	Affinity chromatography of α-L-fucosidase from human liver	211
2-(6-Aminohexanoyl)amino-2-deoxy-β-D-galactose	Affinity chromatography of peanut agglutinin	212
6-Aminohexanoyl *N*-methylacridinium chloride	Purification of eel and erythrocyte acetylcholinesterases	213
6-Aminohexanoyl-D-tryptophan	Affinity chromatography of chymotrypsin	214
N^6-(6-Aminohexyl)adenosine 3′,5′-diphosphate	Purification of glutamate synthetase from *Escherichia coli*	215
	Purification of malic enzyme from bovine heart mitochondria	216
	Purification of bovine liver NADH-cytochrome *b* reductase	217
	Purification of bovine adrenodoxin reductase	218
	Purification of avian liver 3-hydroxy-3-methylglutaryl coenzyme A lyase	219
	Purification of succinic semialdehyde reductase from rat brain	220
	Affinity chromatography of rat liver aldehyde reductase	221
N^6-(6-Aminohexyl)adenosine 5′-phosphate	Purification of aldehyde dehydrogenase from bovine liver	222
	Purification of folylpoly(γ-glutamate) synthetase from *Corynebacterium*	223
	Purification of human placental adenosine kinase	224
	Purification of rat heart adenosine kinase	225
	Removal of other dehydrogenases during purification of L-fucose dehydrogenase	226
8-(6-Aminohexyl)inosine 5′-monophosphate	Purification of inosine 5′-phosphate dehydrogenase	227
4-Aminophenyl 2-amino-2-deoxy-D-glucopyranoside	Purification of galactosyltransferase isoenzymes I and II	228
4-(4-Aminophenylazo)phenylarsonic acid	Purification of hen alkaline phosphatases	229
4-Aminophenyl 1-thio-β-D-galactopyranoside	Purification of a human hepatic lectin	230
L-Arginine	Purification of L-arginine aminopeptidase from human foetal liver	231
Asialofetuin	Affinity chromatography of lactose-binding proteins from chicken	232

Table 3 *(cont.)*

Ligand or affinant coupled to agarose cyclo imidocarbonate	*Use of product*	*Ref.*
	Purification of a lectin from *Bothrops atrox* venom	233
Asialo-α_1-acid glycoprotein	Purification of a human hepatic lectin	230
Avidin	Purification of human liver propionyl-coenzyme A carboxylase	234
Blue Dextran®	Purification of human interferon	181
	Purification of aldehyde dehydrogenase from bovine liver	222
	Affinity chromatography of D-glyceraldehyde 3-phosphate dehydrogenase	235
	Purification of cytoplasmic valyl-tRNA synthetase from *Euglena gracilis*	236
	Purification of rat kidney 9-hydroxy-prostaglandin dehydrogenase	237
Calmodulin	Purification of (Ca^{2+}-Mg^{2+})-ATPase from human erythrocyte membranes	238, 239
Carboxypeptidase inhibitor (potato)	Purification of pancreatic carboxy-peptidases A and B	240
Concanavalin A	Identification of transformed liver cell colonies	241
	Purification of a murine melanoma-associated antigen	242
	Affinity chromatography of isolectins from *Dolichos biflorus*	243
	Purification of D-glucose transport system from the adipocyte plasma membrane	244
	Affinity chromatography of incomplete intracellular forms of intestinal surface membrane sucrase–isomaltase	245
	Purification of human fibroblast interferon	246
	Purification of herpes simplex virus tumour-associated antigen from human kidney carcinoma	247
	Affinity chromatography of lysosomal enzymes from mucolipidosis patients	248
	Purification of CMP-*N*-acetyl-neuraminic acid : G_{M_3} ganglioside sialyltransferase (G_{D_3} synthetase)	249
	Purification of Na,K-ATPase from rectal glands of spiny dogfish	250
	Purification of an acid phosphatase from *Drosophila melanogaster*	251
	Affinity chromatography of human leukocyte interferon components	252

Table 3 *(cont.)*

Ligand or affinant coupled to agarose cyclo imidocarbonate	*Use of product*	*Ref.*
Concanavalin A *(cont.)*	Purification of antigenic fractions from mycelial fungi	253
	Development of a method for efficient and selective recovery of membrane glycoproteins from immobilized concanavalin A	254
	Purification of antigens of *Fasciola hepatica* adult worms	255
	Affinity chromatography of α-D-mannosidase from human plasma	256
	Purification of acid β-D-galactosidase from feline liver	257
	Purification of rat α-fetoprotein	258
	Affinity chromatography of cathepsin D isoenzymes from porcine spleen	259
	Purification of dipeptidyl peptidase IV from human submaxillary gland	260
	Purification of acid phosphatase from rat Dunning prostatic adenocarcinoma	261
	Purification of a preneoplastic antigen in liver carcinogenesis	262
	Purification of sphingolipid β-D-galactosidases from human placenta	263
	Determination of levels of β-D-fructofuranosidase produced by a mutant strain of *Saccharomyces cerevisiae*	264
	Purification of heparosan *N*-sulphate D-glucuronosyl 5-epimerase	265
	Affinity chromatography of lobster axon plasma membrane proteins	266
	Purification of gonadotrophins	267
	Purification of myeloperoxidase from human blood	268
	Purification of human fibroblast interferon	125
	Purification of α-lactalbumin from mouse milk	269
	Purification of nucleoside diphosphatase from rat liver microsomes	124
	Purification of Tora-bean (*Phaseolus vulgaris*) lectin	270
	Affinity chromatography of antigenic components of detergent-solubilized adipocyte intrinsic membrane proteins	164
	Affinity chromatography of large glycopeptides of human teratocarcinoma-derived cells	271

Table 3 *(cont.)*

Ligand or affinant coupled to agarose cyclo imidocarbonate	*Use of product*	*Ref.*
Concanavalin A *(cont.)*	Purification of a tartrate-sensitive splenic acid phosphatase from patients with Gaucher's disease	272
	Purification of cholinesterase from chicken egg yolk	273
	Purification of tumour-specific transplantation antigens from induced murine sarcomas	274
	Affinity chromatography of gelonin, an inhibitor of protein synthesis	275
	Purification of α-D-2-acetamido-2-deoxy-galactosidase from porcine liver	276
	Affinity chromatography of platelet glycoproteins	277
	Purification of aspartate tRNAs	278
	Purification of alkaline phosphatases from hen egg yolk	279
Deoxyribonucleic acid	Affinity chromatography of rat liver chromatin	279
2-Di(6-aminohexanoyl)amino-2-deoxy-D-glucitol	Affinity chromatography of glycoside hydrolases	280
2-Di(6-aminohexanoyl)amino-2-deoxy-D-glucose	Affinity chromatography of glycoside hydrolases	280
4-Di(6-aminohexanoyl)aminophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside	Affinity chromatography of glycoside hydrolases	280
4-Di(6-aminohexanoyl)aminophenyl-β-D-glucopyranoside	Affinity chromatography of glycoside hydrolases	280
Fetuin	Affinity chromatographic purification of lectin-like molecules on murine thymocytes and spherocytes	281
	Purification of potato lectin	282
Fibrin	Affinity chromatography of fibronectin	283
	Purification of cold-insoluble globulin from rat plasma	284
Fibrinogen	Affinity chromatography of platelet proteins	285
Folate-binding protein	Affinity chromatography of urinary folates	286
Gelatin	Purification of fibronectin and some of its tryptic fragments	287
GDP-Hexanolamine	Purification of β-D-galactoside α-(1 → 2)-fucosyltransferase from porcine submaxillary gland	288
Haemoglobin	Purification of human renal renin	289
Haptoglobin	Purification of haemoglobin	290
Helix pomatia agglutinin	Purification of pure B and T cell populations	291

Table 3 *(cont.)*

Ligand or affinant coupled to agarose cyclo imidocarbonate	*Use of product*	*Ref.*
Heparin	Affinity chromatography of native and chemically modified anti-(thrombin III) antibody	292
	Purification of a heparin-neutralizing protein from rabbit platelets	293
	Preparation of human plasma depleted of anti-(thrombin III) antibody	294
	Removal of cholesterol from blood	295
	Affinity chromatography of laminin, a basement membrane glycoprotein	296
	Affinity chromatography of rat plasma lipoproteins	297
	Affinity chromatography subfractionation of human high-density lipoproteins	298
Histone	Purification of rat liver phosphoprotein phosphatase	117
	Affinity chromatography of non-histone chromatin proteins from pig liver and kidney	299
Homoarginine	Purification of a transamidinase from *Lathyrus sativus* seedlings	300
Lactoferrin	Affinity chromatography of transferrin receptors from rat kidney cell membrane	202
Lens culinaris phytohaemagglutinin	Removal of contaminating membrane glycoproteins during purification of P-glycoprotein from Chinese hamster ovary cell mutants	301
	Purification of tumour-specific transplantation antigens from induced murine sarcomas	274
O-Lipopolysaccharide (*Salmonella johannesburg*)	Affinity chromatography of *endo*-glycosidase activity of phage $\theta 1(40)$	302
Naja naja siamensis neurotoxin	Purification of the acetylcholine receptor from *Torpedo californica* electric organ excitable membranes	303
Neuraminic acid derivatives (α-ketosides)	Affinity chromatography of neuraminidase activity in influenza virus	304
α-Neurotoxin	Purification of acetylcholine receptors from *Torpedo californica*	163
Norleucine	Purification of D-galactosyltransferase isoenzymes I and II	228
	Purification of histone acetylase A from calf thymus tissues	305
Oestradiol 7α-decamethylene derivative	Purification of calf uterine cytosol oestradiol receptor	306

Table 3 *(cont.)*

Ligand or affinant coupled to agarose cyclo imidocarbonate	*Use of product*	*Ref.*
Ovomucoid	Affinity chromatographic removal of proteases from hyaluronidase preparations from *Streptomyces hyalurolyticus*	307
Pisum sativum agglutinin	Removal of contaminating membrane glycoproteins during purification of P-glycoprotein from Chinese hamster ovary cell mutants	301
Poly(adenylic acid)	Purification of a poly(uridylic acid)-specific *exo*-ribonuclease from calf thymus	308
Poly(guanylic acid)	Purification of two ribonucleases from barley wheat	309
Poly(L-lysine)	Quantitative removal of heparin from plasma and other aqueous solutions	310
Poly(uridylic acid)	Purification of murein transglycosylase from *Escherichia coli*	311
	Affinity chromatography of poly-(adenylic acid)-containing cellular and viral RNAs	312
Protease inhibitor V (soybean)	Purification of trypsin, chymotrypsin, and kallikrein	143
Ricinus communis agglutinin	Removal of contaminating membrane glycoproteins during purification of P-glycoprotein from Chinese hamster ovary cell mutants	301
	Purification of tumour-specific transplantation antigens from induced murine sarcomas	274
	Investigation of glycoproteins in purified myelin	313
Soybean trypsin inhibitor	Removal of proteases during purification of AMP-dependent protein kinases	314
	Affinity chromatography of trypsins and chymotrypsins	315
	Affinity chromatography of trypsins	316
Thyroglobulin	Removal of phytohaemagglutinin from supernatants of cultured lymphocytes	317
Transferrin	Affinity chromatography of soluble transferrin receptors	202
Tridacnin	Purification of the L-arabino-D-galactan from *Gladiolus* stigma	318
Tubulin	Purification of calmodulin from *Tetrahymena pyriformis*	319
2-Vinyl-4-[3′-(*N*-3″-aminopropyl)-acrylaminodeuteroporphyrin	Purification of ferrochelatase	320

Table 3 *(cont.)*

Ligand or affinant coupled to agarose cyclo imidocarbonate	*Use of product*	*Ref.*
Wheat germ agglutinin	Affinity chromatography of antigenic components of detergent-solubilized adipocyte intrinsic membrane proteins	164
	Purification of gamma protein from HeLa cells	321
	Affinity chromatography of cardiac sarcolemmal vesicles	322
	Purification of tumour-specific transplantation antigens from induced murine sarcomas	274

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Agarose derivatives have been used in various novel procedures applying the principles of affinity chromatography. The use of affinity chromatography in an immunoassay system for rapid separation of antigen–enzyme complexes from antigen–enzyme–antibody complexes has been described.[195] In an enzyme immunoassay for human placental lactogen and progesterone where Δ5,3-keto-steroid isomerase is used as the marker enzyme, oestradiol 17β-glutathione immobilized on agarose retains the antigen–enzyme complexes.

A double-selection method for isolating active enzyme molecules using substrate-analogue affinity chromatography and elution with transition-state analogues has been reported.[214] Native chymotrypsin and inactive [^{3}H]deoxychymotrypsin were applied together to a column of immobilized D-tryptophan methyl ester, on which both enzyme forms were retained. Catalytically active enzyme could be selectively desorbed with chymostatin, a peptide aldehyde, leaving catalytically inactive deoxychymotrypsin bound to the substrate analogue affinity column. This technique may afford a simple and general method for separating enzymes and other catalysts according to their molecular turnover numbers.

Tetrameric avidin has been coupled to agarose cyclic imidocarbonate and then treated with guanidinium chloride to form monomeric avidin-agarose.[234]

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Following treatment with biotin to saturate biotin-binding sites, the affinity matrix could be used for the purification of human liver propionyl-coenzyme A carboxylase.

The interaction of poly(adenylic acid)-containing cellular and viral ribonucleic acids with poly(uridylic acid) immobilized on agarose has been investigated.[312] The specificity, sensitivity, selectivity, and affinity of binding were studied in detail.

γ-t-Butyl esters of γ-L-glutamyl peptides have been immobilized by reaction with agarose cyclic imidocarbonate and applied to the purification of folylpolyglutamate endopeptidase from chicken intestine.[323]

Agarose activated with cyanogen bromide has been used for the preparation of polymeric biospecific adsorbents for binding of blood components,[324] and for the immobilization of sequence-specific endonucleases.[325]

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DNS-derivatization of the acid-hydrolysed and unhydrolysed amine derivatives arising from the reaction of agarose activated with cyanogen bromide and reacted with *N*-α-acetyl-L-ornithine has shown that L-arginine is produced.[182] This confirms that the amino–agarose reaction product is an *O*-substituted isourea. Solutions of mixed amino-acids and mixed peptides were loaded serially on to an immunoadsorbent prepared by coupling sheep anti-(human immunoglobulin G) antibody to agarose activated with cyanogen bromide. Results obtained after subjecting the column to a number of successive adsorption–desorption cycles suggest that both ionic and non-ionic interactions are involved in the adsorption process, and may explain some of the undesirable non-specific adsorption phenomena often observed for immunoadsorbents.

Direct attachment of an affinity ligand or enzyme to agarose cyclic imidocarbonate has often proved to be unrewarding since the approach of the counter molecule to the ligand or enzyme may be sterically impeded. By inserting a covalent bridge between the matrix and the ligand, this difficulty can be overcome. Bridging is also used when functional groups on the required ligand do not react with those on the matrix, since a new type of functionality can be introduced. Modifications used recently to provide bridges in the preparation of affinity chromatography matrices, *etc.*, from agarose cyclic imidocarbonate are shown in Schemes 5a, b and Table 4.[326–383] Other derivatives of agarose cyclic imidocarbonate used for miscellaneous purposes are listed in Table 5.[384–405]

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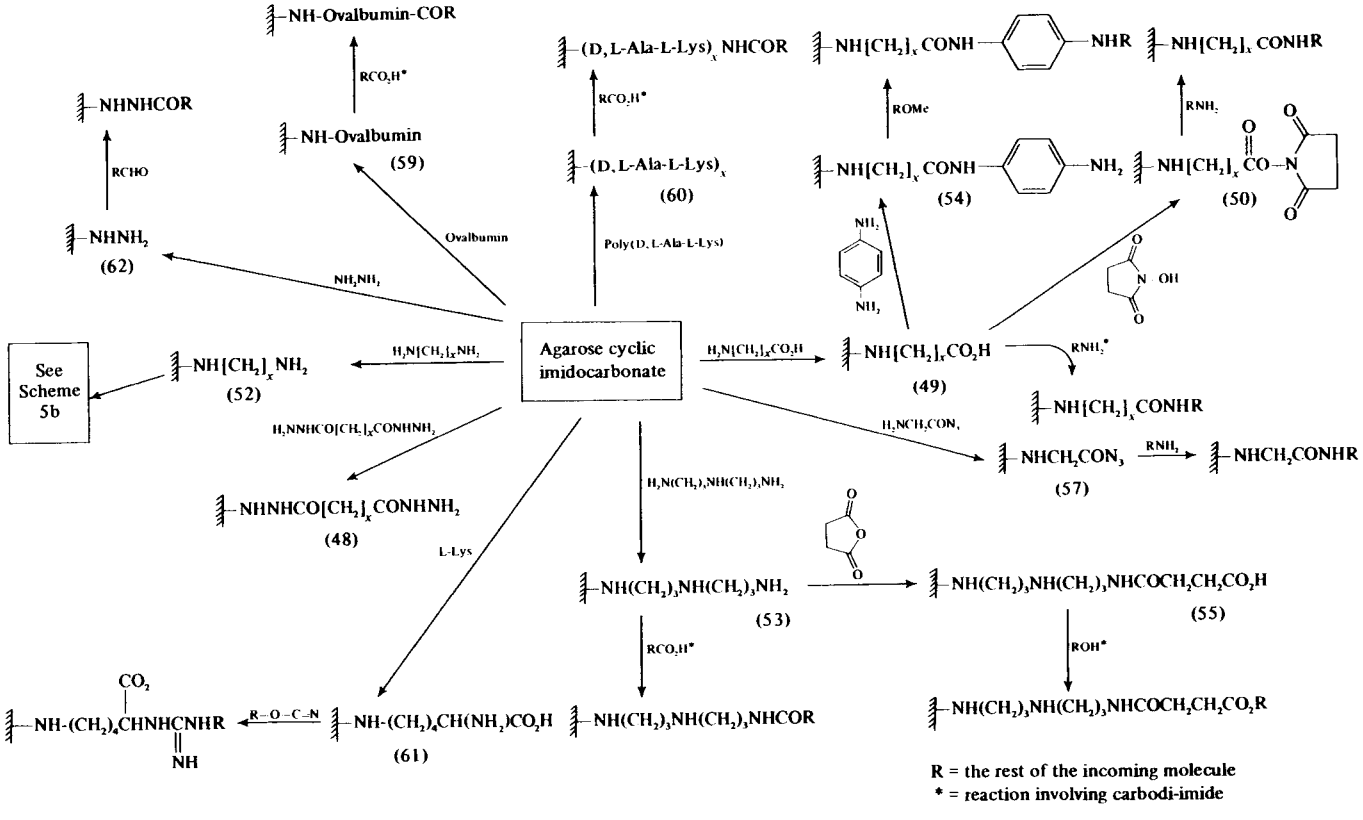
NH-Ovalbumin-COR
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(59)
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RCO2H*
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Poly(D,L-Ala-L-Lys)
NH[CH2]x CONH NHR
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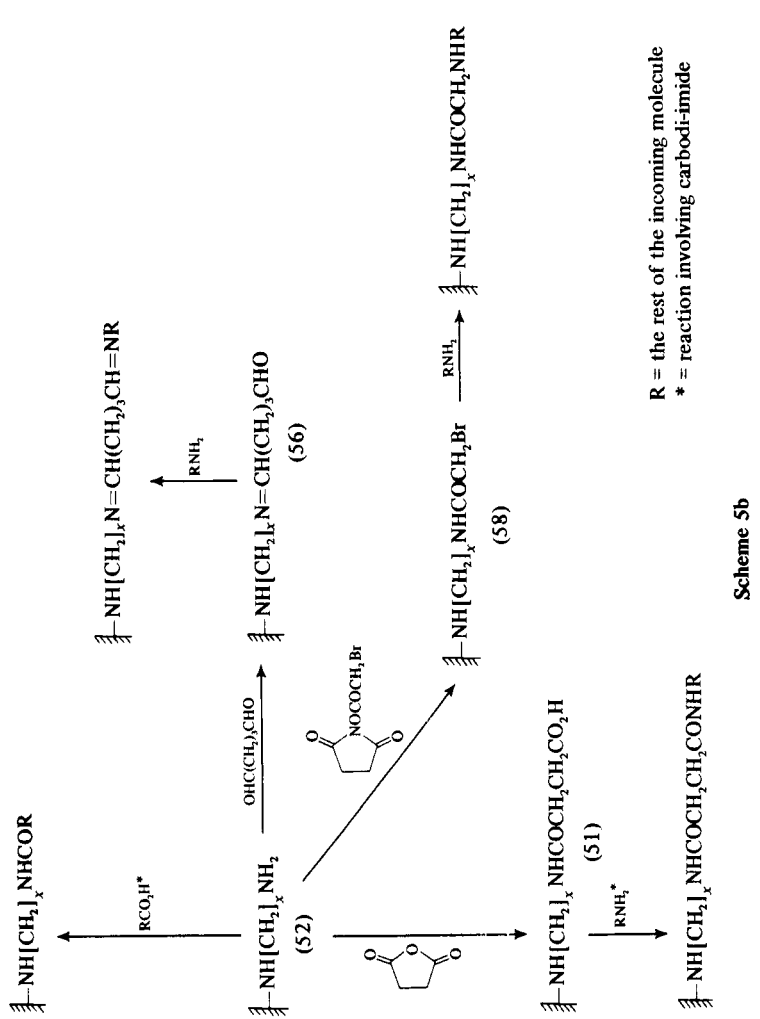
NH2NH2
Agarose cyclic imidocarbonate
H2N[CH2]xCO2H
NH[CH2]xCO2H
(49)
RNH2*
NH[CH2]x CONHR
See Scheme 5b
NH[CH2]x NH2
(52)
H2N[CH2]xNH2
H2NNHCO[CH2]xCONHNH2
NHNHCO[CH2]xCONHNH2
(48)
H2NCH2CON3
NHCH2CON3
RNH2
NHCH2CONHR
(57)
H2N(CH2)3NH(CH2)3NH2
L-Lys
NH(CH2)3NH(CH2)3NH2
(53)
NH(CH2)3NH(CH2)3NHCOCH2CH2CO2H
(55)
RCO2H*
ROH*
NH(CH2)3NH(CH2)3NHCOR
NH(CH2)3NH(CH2)3NHCOCH2CH2CO2R
CO2
NH-(CH2)4CHNHCNHR
NH
R-O-C-N
NH-(CH2)4CH(NH2)CO2H
(61)
R = the rest of the incoming molecule
* = reaction involving carbodi-imide

Scheme 5a


NH[CH2]xNHCOR
RCO2H*
NH[CH2]xNH2
(52)
OHC(CH2)3CHO
NH[CH2]xN=CH(CH2)3CHO
(56)
RNH2
NH[CH2]xN=CH(CH2)3CH=NR
NOCOCH2Br
NH[CH2]xNHCOCH2Br
(58)
RNH2
NH[CH2]xNHCOCH2NHR
NH[CH2]xNHCOCH2CO2H
(51)
RNH2*
NH[CH2]xNHCOCH2CH2CONHR
R = the rest of the incoming molecule
* = reaction involving carbodi-imide

Scheme 5b

Table 4 *Use of agarose cyclic imidocarbonate for the preparation of affinity chromatography materials* via *linkage extension*

Species coupled to agarose cyclic imidocarbonate	*Agarose intermediate*[a]	*Use of product*	*Ref.*
Adenosine triphosphate, periodate-oxidized	48	Affinity chromatography of rat liver glucocorticoid receptor	326
L-Alanyl-L-alanyl-D-leucylamide	49	Purification of subtilisin BPN'	205
4-Amino-DL-benzylsuccinic acid, *N*-hydroxysuccinimide derivative	50	Affinity chromatography of carboxypeptidase A and chemically modified derivatives thereof	327
4-Aminobenzyl 1-thio-2-acetamido-2-deoxy-β-D-glucopyranoside	51	Purification of *Bandeiraea simplicifolia* isolectins	328
2-Amino-2-deoxy-D-glucose	49	Purification of hexokinases from *Drosophila melanogaster*	329
8-(6-Aminohexyl)amino-adenosine triphosphate	49	Purification of hexokinases from *Drosophila melanogaster*	329
6-Aminohexyl 1-thio-2-acetamido-2-deoxy-β-D-glucopyranoside	49	Purification of β-D-2-acetamido-2-deoxyhexosidases A and B	264
6-Aminohexyl 1-thio-β-D-galactoside	49	Removal of β-D-galactosidase during purification of β-D-2-acetamido-2-deoxyhexosidases A and B	264
4-Aminophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside	49	Purification of *endo*-β-D-2-acetamido-2-deoxygalactanase	330
4-Aminophenyl β-D-galactopyranoside	51	Purification of *Bandeiraea simplicifolia* isolectins	328
4-Aminophenyl β-D-maltoside	49	Purification of a neutral α-glucosidase from horse kidney	331
4-Aminophenyl 1-thio-β-D-galactopyranoside	49	Purification of sphingolipid β-D-galactosidases from human placenta	263
4-Aminophenylthymidine 3'-phosphate	49	Purification of human liver thymidine kinase	332
8-(3-Aminopropylamino)adenosine	49	Purification of *S*-adenosyl-L-homocysteine hydrolase from rat liver	333
3-Aminopyridine adenine dinucleotide	49	Purification of hexokinases from *Drosophila melanogaster*	329

Table 4 *(cont.)*

Species coupled to agarose cyclic imidocarbonate	*Agarose intermediate*[a]	*Use of product*	*Ref.*
Ampicillin	49	Purification of penicillin-binding proteins 1a and 1b from *Escherichia coli*	334
N-(4-Androsten-3-on-17β-oxycarbonyl)-6-aminohexoic acid	48	Purification of the steroid-1-dehydrogenase from *Nocardia opaca*	335
D-Arginine	49	Purification of pancreatic carboxypeptidases A and B	240
Benzylamine	49	Purification of firefly luciferase	336
Bilirubin	52	Affinity chromatography of solubilized liver plasma membrane-binding proteins	337
N-t-Butoxyl-amastatin	52	Purification of aminopeptidase A from pig kidney	338
Cadaverine	49	Separation of putrescine oxidase from spermidine oxidase in foetal bovine serum	339
9-(5-Carboxypentylamino)acridine isobutyric anhydride	52	Purification of acetylcholinesterase from *Electrophorus electricus* electric organ	340
Cephalexin	49	Purification of penicillin-binding protein 3 from *Escherichia coli*	334
Cholate	53	Purification of a bile salt-activated lipase from human milk whey	341
Cholic acid	52	Affinity chromatographic removal of serum albumin during purification procedures	342
	52	Purification of two cholic acid-binding proteins from rat liver cytosol	343
Choline	53	Purification of choline dehydrogenase from rat liver mitochondria	344
2,4-Diamino-5-(3,5-dimethoxy-4-amino)benzyl pyrimidine	49	Affinity chromatography of bacterial dihydrofolate reductases	345
cis-3,4-Diamino-2-tetrahydrothiophenevaleric acid sulphate	52	Affinity chromatographic retrieval of streptavidin	346
4,5-Dimethoxy-1,2-benzoquinone	54	Affinity chromatography of menadione reductase	347
6,7-Dimethyl-5,6,7,8-tetrahydropterin	55	Purification of rat liver phenylalanine hydroxylase	348

Endotoxin 56
2′,3′-Epoxypropyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D- glucopyranoside 52
Folic acid 52
10-Formyl-5,8-dideazafolate 52
Glycogen 48
Guanosine diphosphate, periodate-oxidized 48
Guanosine monophosphate, periodate-oxidized 52
Guanosine triphosphate 48
Haematin 52
Haematoporphyrin 52
Heparin, *O*-desulphated 52
H1 Histones (calf thymus chromatin) 57
Hyaluronic acid 56
Iminobiotin 52
Kinetin riboside, periodate-oxidized 52
Lipotrophins 48
L-Lysine 58
Methotrexate 52
Purification of an endotoxin-binding protein from *Limulus polyphemus* membranes 349
Purification of an α-D-glucosidase from thick embryo 350
Purification of carbon monoxide dehydrogenase from *Clostridium thermoaceticum* 351
Purification of mammalian tumour thymidylate synthetase 352
Affinity chromatography of glycogen-binding components of rat tissues 353
Affinity chromatography of rat liver succinyl coenzyme A synthetase 354
Purification of two deoxyribonucleases from *Aspergillus niger* 355
Purification of phosphoenolpyruvate carboxykinase 356
Purification of guanine nucleotide regulatory protein from turkey erythrocytes 357
Purification of haem-binding proteins (apoglobin, albumin, and haemopexin) 358
Purification of haem-binding proteins (apoglobin, albumin, and haemopexin) 358
Purification of heparosan *N*-sulphate D-glucuronosyl 5-epimerase 265
Purification of super-coiled PM2 deoxyribonucleic acid 359
Purification of brain glycoprotein NsA3 360
Affinity chromatographic retrieval of streptavidin from culture broth of *Streptomyces avidinii* 361
Purification of a cytokinin-binding protein from wheat germ 362
Purification from rat brain of a novel enzyme which converts β-endorphin into methionine-enkephalin 363
Purification of L-lysine 6-aminotransferase 364
Purification of folate-binding protein from human milk 365, 286

Table 4 *(cont.)*

Species coupled to agarose cyclic imidocarbonate	*Agarose intermediate*[a]	*Use of product*	*Ref.*
	52	Purification of porcine liver dihydrofolate reductase	366
	52	Purification of bacterial dihydrofolate reductases; comparison of the efficacy of different gels for the purification	345
Nicotinamide adenine dinucleotide	18	Purification of nicotinamide deamidase from growth medium of cultured neuroblastoma cells	367
	18	Affinity chromatography of rabbit muscle D-fructose-1,6-biphosphate aldolase	368
	52	Affinity chromatography of *Sn*-glycerol-3-phosphate dehydrogenase	369
Nicotinamide adenine dinucleotide, periodate-oxidized	48	Affinity chromatography of *Sn*-glycerol-3-phosphate dehydrogenase	369
Nicotinamide adenine dinucleotide phosphate	52	Purification of malic enzyme from bovine heart mitochondria	216
Nicotinamide adenine dinucleotide phosphate	48	Purification of 8-hydroxy-5-deazaflavin-dependent $NADP^+$ reductase from *Methanococcus vannielii*	370
17β-Oestradiol 17-hemisuccinic acid	59	Purification of nuclear oestrogen receptor from chicken liver	371
Oestradiol, 7α-decamethylene and 7α-trimethylene derivatives	60	Affinity chromatography of calf-uterine cytosol oestradiol receptor	306
Pepstatin (treated with *N*-hydroxysuccinimide)	52	Purification of a completely inactive renin from hog kidney	122
	52	Purification of cathepsin D from porcine spleen	259
	52	Purification of human renal renin	289
	52	Affinity chromatography of cathepsins D from normal human organs and some tumours	372
Phenylbutenolide acetic acid	52	Purification of rat heart cyclic nucleotide phosphodiesterase	373

S3 Pneumococcal capsular polysaccharide, cyanogen bromide-activated	61
Porphobilinogen	52
Pyridoxal hydrochloride	52
Pyridoxal 5′-phosphate	52
Ribosomal ribonucleic acid, periodate-oxidized	48
Transfer ribonucleic acid, periodate-oxidized	62
	48
Soybean trypsin inhibitor	49
Tetrahydromethotrexate	52
4-Toluic acid	52
N,*N*,*N*-Trimethylammonium bromide	52
Urinary protein (from colonic cancer patients)	52
Vancomycin	49

[a] See Schemes 5a and 5b, pp. 415, 416.

Purification of antibodies to the capsular carbohydrate of Type III pneumococci	374
Purification of uroporphyrinogen I synthetase from *Chlorella regularis*	375
Purification of rat brain pyridoxal kinase	376
Purification of rat brain pyridoxamine 5-phosphate oxidase	376
Affinity chromatographic identification of rat liver ribosomal proteins that bind to *Escherichia coli* RNA	377
Purification of phenylalanine tRNA synthetase from soybean cotyledon	378
Purification of rat liver ribosomal proteins that bind to elongator and initiator RNA	379
Purification of an acrosin-like enzyme from sea urchin sperm	380
Purification of thymidylate synthetase	381
Purification of toluene dioxygenase from *Pseudomonas putida*	382
Purification of cholinesterase from hen's egg yolk	273
Purification of rabbit antibodies specific for the urinary protein components	171
Affinity chromatography of murein precursors	383

Table 5 *Use of agarose cyclic imidocarbonates for the preparation of miscellaneous derivatives*

Species coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Axinella polypoides lectin	Comparative structural study of snail (*Helix pomatia*) galactans and sponge (*Axinella polypoides*) lectins	384
Bandeiraea simplicifolia lectin I	Resolution of structurally homologous sugar nucleotides and oligosaccharides	385
Blood-group (A + H) substance	Investigation of carbohydrate-binding activity of a lectin-like glycoprotein from *Dolichos biflorus*	386
Blue Dextran®	Study of the interaction of tRNA-synthetases with Blue Dextran; assignment of the Blue Dextran binding site on the synthetases	387
	Investigation of the structure–function relationships of *Neurospora crassa* pyruvate kinase	388
	Investigation of the binding of Cibacron Blue F3GA to the flavin and NADH-binding sites in cytochrome b_5 reductase	389
Cobalamin[a]	Affinity chromatographic investigation of the interactions of *Klebsiella pneumoniae* diol dehydrase with the coenzyme	390
Collagen	Investigation of the interactions between bovine cornea proteoglycans and collagen	391
	Investigation of interactions between collagen, glycosaminoglycans, and fibronectin	392
Concanavalin A	Study of lectin–carbohydrate interactions using a competitive enzyme inhibition assay	393
	Investigation of effects of detergents on the binding of hepatoma cell-surface D-[^{3}H]galactoproteins to immobilized concanavalin A	394
	Support for the immobilization of phosphodiesterase	395
Fibronectin	Investigation of interactions between collagen, glycosaminoglycans, and fibronectin	392
Group C streptococci[b]	Biochemical characterization of human Fc receptor material from lymphoblastoid cell lines	396
Heparin	Immobilization of rabbit reticulocytes which were then used to incorporate labelled amino-acids into proteins	397
	Investigation of reactions of covalently immobilized heparin with thrombin or Factor Xa in the presence and absence of anti-(thrombin III) antibody	398
	Development of a colorimetric method for assay of heparin content in immobilized heparin preparations	399
Hyaluronate[a]	Development of a solid-phase radioassay for hyaluronate	400

Table 5 *(cont.)*

Species coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Lens culinaris lectin	Study of culture density and age-dependent interactions of 3T3 and SV3T3 cells with *Lens culinaris* lectin	401
Neurophysins I and II	Investigation of the interactions of the bound molecule with oxytocin and vasopressin	402
Peanut agglutinin	Study of the reactivity of human lymphoid and lymphoblastoid cells with the lectin	403
Pyridoxal 5'-phosphate[a]	Immobilization of nucleoside diphosphatase at its allosteric site	404
Ricinus communis lectin	Investigation of effects of detergents on the binding of hepatoma cell surface D-[^{3}H]-galactoglycoproteins to immobilized *R. communis* lectin	394
Ricinis communis toxin	Determination of the critical density of immobilized toxin at which glutaraldehyde-mediated protein–protein monoconjugation with lysozyme occurs	405

[a] See Scheme 5b (52).
[b] See Scheme 5a (61).

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A simple method for measuring colorimetrically the amount of heparin covalently coupled to agarose has been developed.[399] The method involves monitoring the dye depletion in the supernatant as toluidine blue is adsorbed on to the immobilized heparin.

Very efficient separations of structurally homologous sugar nucleotides and oligosaccharides have been achieved using *Bandeiraea simplicifolia* lectin I immobilized on agarose.[385] UDP-D-glucose and UDP-D-galactose, for example, could be separated by as much as three column volumes using the matrix, as could the corresponding 2-acetamido-2-deoxy-derivatives. The immobilized preparation was also used successfully to resolve the alditols of lactose and

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melibiose, to determine the radiochemical purity of UDP-D-galactose and UDP-2-acetamido-2-deoxy-D-galactose preparations, and to determine the relative proportions of UDP-2-acetamido-2-deoxy-D-glucose and UDP-2-acetamido-2-deoxy-D-galactose in the sugar mixture isolated from cultured cells.

Nitroxide spin-labels have been attached to cyanogen bromide-activated agarose directly and through oligoglycines and ω-amino-carboxylic acids of different lengths.[406] The homogeneities of the carbohydrate environments of the immobilized labels were investigated by measuring dipolar interactions between nitroxides as a function of solvation and of spin dilution with a diamagnetic analogue, as well as by electron exchange between the nitroxides and paramagnetic metal ions in solution. Results showed that an ideal spacer length for affinity separations is 12 atoms, which is in good agreement with data from a number of affinity separations.

Commercially available Affi-gel® supports, agarose beaded gel included in a polyacrylamide support having different spacer arm lengths, have been used for the preparation of affinity matrices. The spacers are anchored by stable ether linkages. Affi-gel 601, an affinity gel containing a boronate group, has been used for the pre-fractionation and concentration of plasma nucleosides before injection in h.p.l.c. systems, thus greatly increasing sensitivity as well as selectivity.[407] The normal human plasma levels of inosine and adenosine have been reliably measured in all samples examined using the gel. An organomercurial-agarose derivative, Affi-gel 501, has been used for the affinity chromatographic recovery from solution of histones H1, H2B, H3, and the high mobility group protein HMG-1, following their phosphorylation.[408]

Highly sensitive assays for proteinases have been developed using immobilized luminogenic substrates.[409] The substrates, L-Ala-L-Ala-L-Ala-L-Phe-isoluminol and L-Ala-L-Phe-L-Pro-L-Arg-isoluminol, were immobilized by carbodi-imide-mediated reaction with Affi-gel 10. Affi-gel 10 has also been used for the immobilization of human growth hormone.[410,411] A non-primate growth hormone receptor was purified successfully on the affinity matrix. (4-Aminophenyl) β-D-lactoside immobilized on Affi-gel 10 has been used in a study of the binding properties of peanut agglutinin.[412] Ovine follitrophin coupled to Affi-gel 10 has been applied to the purification of follitrophin receptors from calf testes.[413]

Peroxidase immobilized on Affi-gel 10 has been used in the development of a simple device for the spectrophotometric assay of immobilized enzymes.[414]

Benzoquinone-activated agarose has provided a satisfactory matrix for the immobilization of phosphotransferase.[158]

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D-Galactose has been reacted with agarose activated with divinylsulphone and applied to the large-scale production of a lectin from Sunn Hemp seeds (*Crotalaria juncea*).[415] Calmodulin immobilized on the same matrix has provided a suitable affinity system for the isolation of a calmodulin-dependent cyclic nucleotide phosphodiesterase from bovine brain.[416]

Agarose has been activated with divinylsulphone, treated with 4-nitrobenzyl alcohol, reduced, and then diazotized prior to reaction with immunoglobulin G.[417] The immunoadsorbent was used for purification of subcomponents $C_{1\bar{q}}$, $C_{1\bar{r}}$ and $C_{1\bar{s}}$ of the first component of complement from Cohn Fraction I.

6-Aminopenicillanic acid has been immobilized on carboxymethyl- and succinylamino(2-hydroxy)propyl-derivatives of agarose for use in the preparation of penicillin-binding proteins from *Escherichia coli*.[418] The second agarose derivative was prepared by treating agarose with epibromohydrin to form the amino(2-hydroxy)propyl ether derivative, which was then succinylated. Sulphobromophthalein has been immobilized on epichlorohydrin-activated agarose and used for affinity chromatography of liver plasma membrane proteins.[337]

Agarose into which epoxy-groups have been introduced by reaction with compounds such as diglycidyl ether or 1,4-bis(2,3-epoxypropoxy)butane have been used to prepare affinity chromatography matrices. Soybean agglutinin has been isolated on a matrix containing 2-acetamido-2-deoxy-D-galactose.[243] Immobilized L-alanyl-L-alanyl-L-alanyl-4-nitroanilide has been used in a one-step purification of human leukocyte elastase by biospecific affinity chromatography at sub-zero temperatures.[419]

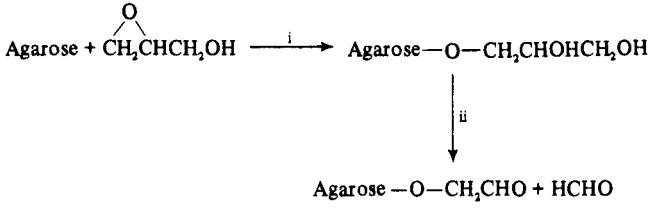
Epoxy-activated agarose has been used for the immobilization of cyclomaltohexaose for the purification of a limit dextrinase from ungerminated peas,[420] and for immobilization of 2,4-diamino-5-(3,5-dimethoxy-4-hydroxy)benzyl pyrimidine for affinity chromatographic purification of bacterial dihydrofolate reductases.[345] δ-*N*-(Phosphonacetyl)-L-ornithine has been reacted with epoxy-activated agarose through the α-amino group and used for the isolation of ornithine transcarbamylase from rat liver.[421] The affinity matrix could be used for longer than 12 months with no detectable loss of activity. Polyethylene glycol coupled to agarose through the mediation of bis-oxiran has been used for the separation of human and rabbit peripheral granulocytes, lymphocytes, and erythrocytes.[422] Diaminoethyloxiran-agarose has been applied to the immobilization of 3-oxo-17β-hydroxy-5α-androstan-17α-(6-hexanoic acid) for use in the purification of sex steroid binding protein from human serum.[423]

[415] B. Ersson, *Biotechnol. Bioeng.*, 1980, **22**, 79.
[416] R. K. Sharma, T. H. Wang, E. Wirch, and J. H. Wang, *J. Biol. Chem.*, 1980, **255**, 5916.
[417] D. H. Bing, J. M. Andrews, K. M. Morris, E. Cole, and V. Irish, *Prep. Biochem.*, 1980, **10**, 269.
[418] H. Amanuma and J. L. Strominger, *J. Biol. Chem.*, 1980, **255**, 11173.
[419] K. K. Andersson, C. Balny, P. Douzou, and J. G. Bieth, *J. Chromatogr.*, 1980, **192**, 236.
[420] D. Yellowlees, *Carbohydr. Res.*, 1980, **83**, 109.
[421] N. J. Hoogenraad, T. M. Sutherland, and G. J. Howlett, *Anal. Biochem.*, 1980, **101**, 97.
[422] U. Matsumoto and Y. Shibusawa, *J. Chromatogr.*, 1980, **187**, 351.
[423] P. H. Pétra and J. Lewis, *Anal. Biochem.*, 1980, **105**, 165.

Zinc chelate chromatography has been applied successfully to the purification of human serum inter-α-trypsin inhibitor.[424] The matrix required was prepared by treating epoxy-activated agarose with iminodiacetic acid and then equilibrating the matrix with aqueous zinc chloride to obtain the zinc chelate form.

A simple procedure has been reported for the conversion of epoxy-activated agarose into amino-derivatives using ammonia solution.[425] The amino-derivatives were succinylated and then activated with *N*-hydroxysuccinimide using mild conditions. The adsorbents thus obtained have no charged group in the linkage region between the ligand and agarose, thus reducing the nonspecific adsorption, and the ether and amide bonds formed are stable even in an alkaline medium. *Lens culinaris* lectin was immobilized using the matrix and applied to the affinity chromatography of solubilized red blood cell membranes.

A gel matrix that can be used in sequence to separate proteins and then immobilize them has been prepared by etherifying agarose with glycidol and subsequently oxidizing with periodate (Scheme 6).[426] The matrix provides an inert support equivalent to agarose for the separation of proteins at neutral or acidic pH, but rapidly adsorbs proteins through formation of stable alkylamine linkages on exposure to alkali or to concentrated sodium cyanoborohydride. The possibility of arraying proteins electrophoretically using such matrices is of potential importance in the analysis of complex mixtures.



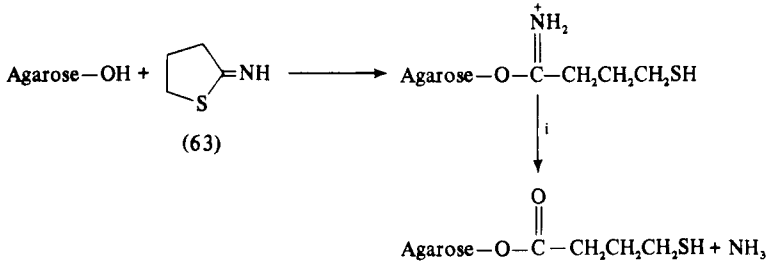
Reagents: i, NaOH; ii, $NaIO_4$

Scheme 6

Agarose treated with iminodiacetic acid to form the bis(carboxymethyl)amino derivative and then equilibrated with Cu^{2+} has been used for the purification of human fibroblast and leukocyte interferons.[246]

2-Iminothiolane (63) has been shown to react with hydroxy-groups, and can thus be used to introduce thiol groups into polysaccharides (Scheme 7).[427] By performing the reaction in the presence of 4,4′-dithiodipyridine, agarose derivatives containing 4-dithiopyridyl groups have been prepared. These activated derivatives couple easily *via* intermolecular disulphide bond formation with proteins containing thiol or 4-dithiopyridyl groups, as has been demonstrated

[424] J. P. Salier, J. P. Martin, P. Lambin, H. McPhee, and K. Hochstrasser, *Anal. Biochem.*, 1980, **109**, 273.
[425] I. Matsumoto, N. Seno, A. M. Golovtchenko-Matsumoto, and T. Osawa, *J. Biochem. (Jpn.)*, 1980, **87**, 535.
[426] J. R. Shainoff, *Biochem. Biophys. Res. Commun.*, 1980, **95**, 690.
[427] A. C. Alagon and T. P. King, *Biochemistry*, 1980, **19**, 4341.



Reagent: i, H_2O

Scheme 7

for ragweed antigen E. The resulting polysaccharide–protein conjugates have good stability and may be used in affinity chromatography and for the purification of proteins containing thiol groups.

Periodate-oxidized agarose treated with L-histidine and then reacted with 4-diazobenzylphosphonic acid has provided a matrix for affinity chromatographic purification of human and bovine alkaline phosphatases.[428]

Lipopolysaccharides from *Salmonella minnesota* have been immobilized on polyacrylic hydrazide-derivatives of agarose and applied to the purification of lipopolysaccharide-binding proteins from mouse lymphocytes.[429]

Thiopropyl-agarose has been used for the purification of mercurated ribonucleic acid.[430] More than 85% of mercuriated rat liver ribonucleic acid became bound to the column, to which less than 0.5% of nonmercuriated material became bound.

Thiol-activated agarose has been applied to the purifications of cytochrome *c* oxidase from *Saccharomyces cerevisiae*,[431] of rat liver iodothyronine deiodinases,[432] and of human fibroblast interferon.[433]

Anti-(guinea-pig immunoglobulin G) antibody for use as second antibody in enzyme immunoassay has been immobilized on activated thiol-agarose by the thiol–disulphide interchange reaction.[434]

Several affinity ligands and proteins have been coupled covalently by nucleophilic displacement to agarose preparations substituted with 4-toluenesulphonyl chloride.[435] N^6-(6-Aminohexyl)adenosine monophosphate immobilized by this method was applied to the affinity chromatography of lactate dehydrogenase, and immobilized soybean trypsin inhibitor has been used for the purification of

[428] E. Mössner, M. Boll, and G. Pfleiderer, *Z. Physiol. Chem.*, 1980, **361**, 543.
[429] K. Yokoyama, J. Mashimo, N. Kasai, T. Terao, and T. Osawa, *Z. Physiol. Chem.*, 1979, **360**, 587.
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[431] K. Bill, R. P. Casey, C. Broger, and A. Azzi, *FEBS Lett.*, 1980, **120**, 248.
[432] D. Fekkes, E. Van Overmeeren, G. Hennemann, and T. J. Visser, *Biochim. Biophys. Acta*, 1980, **613**, 41.
[433] O. A. Senussi, T. Cartwright, and P. Thompson, *Arch. Virol.*, 1979, **62**, 323.
[434] Y. Umeda, F. Suzuki, A. Kosaka, and K. Kato, *Clin. Chim. Acta*, 1980, **107**, 267.
[435] K. Nilsson and K. Mosbach, *Eur. J. Biochem.*, 1980, **112**, 397.

trypsin. Active derivatives of peroxidase and alcohol dehydrogenase were also prepared.

Triazinyl dyes (usually Cibacron Blue F3GA) react with agarose *via* the chlorine atoms on the triazine ring to form matrices with considerable potential for preparative and analytical application.[436] The immobilized dyes have been successfully applied to several purifications including those of glycerokinase from *Bacillus stearothermophilus*,[437] human interferon,[438] 6-phosphofructokinase from *Escherichia coli* K-12,[439] isocitrate dehydrogenase from *Mytilus edulis*,[440] alcohol dehydrogenase from *Drosophila* sp.,[441] murein transglycosylase from *E. coli*,[311] alcohol dehydrogenase from tomato,[442] β-lactamase from *Streptomyces cellulosae*,[443] serine hydroxymethyltransferases from monkey liver[444] and mung bean,[445] splenic acid phosphatase from patients with Gaucher's disease,[272] succinic semialdehyde reductases from rat brain,[220] T4 deoxyribonucleic acid ligase,[446] and aldehyde reductase from rat liver.[221] Cibacron Blue F3GA coupled to agarose has also been used for the affinity chromatographic separation of two nonidentical subunits of ribonucleotide reductase from calf thymus.[447]

Cibacron Blue F3GA immobilized on Affi-gel® ('Affi-gel Blue') has been applied to the purification of glutamate dehydrogenase from *Candida utilis*,[448] 15-hydroxyprostaglandin dehydrogenase from porcine kidney,[449] and adenosine triphosphate diphosphohydrolase from pig pancreas.[450]

The use of Cibacron Blue F3GA and 21 related 'Procion' dyes immobilized on agarose for the affinity chromatographic purification of dihydrofolate reductase from Walker 256 carcinoma has been documented.[451] The capacity and selectivity of the matrices were found to be greatest when the incorporation of dye into the matrix was low. The application of several Procion dyes, each immobilized on agarose, to the purification of inosine 5′-monophosphate dehydrogenase from *Escherichia coli* has also been investigated.[452]

The interaction of Cibacron Blue-agarose with several serum albumins has been studied.[453] Although all albumins investigated bound to the adsorbent, human serum albumin bound to a far greater extent than the others. From the

[436] C. R. Lowe, D. A. P. Small, and A. Atkinson, *Int. J. Biochem.*, 1980, **13**, 33.
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[439] K. N. Ewings and H. W. Doelle, *Aust. J. Biol. Sci.*, 1980, **34**, 125.
[440] E. J. H. Head, *Eur. J. Biochem.*, 1980, **111**, 575.
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[442] M. Nicolas and J. Crouzet, *Phytochemistry*, 1980, **19**, 15.
[443] H. Ogawara and S. Horikawa, *J. Antibiot.*, 1979, **32**, 120.
[444] K. S. Ramesh and N. A. Rao, *Biochem. J.*, 1980, **187**, 623.
[445] D. N. Rao and N. A. Rao, *Biochim. Biophys. Acta*, 1980, **92**, 1166.
[446] M. Sugiura, *Anal. Biochem.*, 1980, **108**, 227.
[447] L. Thelander, S. Eriksson, and M. Åkerman, *J. Biol. Chem.*, 1980, **255**, 7426.
[448] B. A. Hemmings, *J. Biol. Chem.*, 1980, **255**, 7925.
[449] D. T. Y. Kung-Chao and H. H. Tai, *Biochim. Biophys. Acta*, 1980, **614**, 1.
[450] D. Lebel, G. G. Poirier, S. Phaneuf, P. St. Jean, J. F. Laliberte, and A. R. Beaudoin, *J. Biol. Chem.*, 1980, **255**, 1227.
[451] S. J. Johnson, K. J. Stevenson, and V. S. Gupta, *Can. J. Biochem.*, 1980, **58**, 1252.
[452] C. R. Lowe, M. Hans, N. Spihey, and W. T. Drabble, *Anal. Biochem.*, 1980, **104**, 23.
[453] R. J. Leatherbarrow and P. D. G. Dean, *Biochem. J.*, 1980, **189**, 27.

results of competitive binding experiments it is suggested that the interaction between the dye and human serum albumin involves the bilirubin-binding sites on the protein, which is not the case for the other albumins.

Optimal conditions have been determined for the electrophoretic desorption of human serum albumin from Cibacron Blue F3GA immobilized on agarose.[454] Advantages of the desorption method include the mild conditions, high yield, and small desorbate volume.

The application of copolymerized agarose–polyacrylamide gels as the support for immobilizing Cibacron Blue F3GA has been demonstrated for the analytical electrophoresis of proteins possessing an affinity for the dye, using bovine serum albumin as a model protein.[455] Optimal conditions are described for preparing gels with suitable mechanical strengths and which allow rapid electrophoresis of proteins. The ease of preparation of the matrices recommends their use for various quantitative analytical investigations.

Chemical features of agarose derivatization with trichloro-*sym*-triazine have recently been described.[456] The reactions of dichloro-*sym*-triazinyl-agarose and 2-methoxy-4,6-dichloro-*sym*-triazinyl-agarose with diamino-derivatives of ethane, propane, butane, and hexane were investigated.

Trichloro-*sym*-triazine-activated agarose has been reacted with 4-amino-phenylethylamine, diazotized, and used to immobilize heparin.[457] The product was applied to the affinity purification of mouse anti-thrombin antibodies. Anti-thrombin antibodies, albumin, fibrinogen, and thrombin have been immobilized separately by reaction with triazine-activated agarose for use in studies on the interactions of the four molecules.[458] 6-Hydroxy-9-(4-aminobenzyl)-purine immobilized on triazine-activated agarose has been applied to the affinity chromatographic purification of purine nucleoside phosphorylase from human red cells.[459]

Amylopectins. – Amylopectin treated with dibutyl tin dilaurate and fluorescein isothiocyanate has been used to detect the location of λ receptor protein (for which the conjugate has an affinity) in the outer membrane of *Escherichia coli* cells.[460]

Controlled activity pesticide systems have been prepared by reacting amylopectin with isocyanate derivatives of 4-amino(1,1-dimethylethyl)-3-(methylthio)-(1,2,4)-triazin-5(4*H*)-one ('metribuzin') or acid chloride derivatives of 2,4-phenoxyacetic acid ('2,4-D') and 2,2-dichloropropionic acid (dalpon).[461]

[454] M. R. A. Morgan, E. George, and P. D. G. Dean, *Anal. Biochem.*, 1980, **105**, 1.
[455] S. J. Johnson, E. C. Metcalf, and P. D. G. Dean, *Anal. Biochem.*, 1980, **109**, 63.
[456] L. T. Hodgins and M. Levy, *J. Chromatogr.*, 1980, **202**, 381.
[457] T. H. Finlay, V. Troll, and L. T. Hodgins, *Anal. Biochem.*, 1980, **108**, 354.
[458] M. O. Longas, W. S. Ferguson, and T. H. Finlay, *Arch. Biochem. Biophys.*, 1980, **200**, 595.
[459] W. R. A. Osborne, *J. Biol. Chem.*, 1980, **255**, 7089.
[460] T. Fererci, M. Schwetorat, S. Ullrich, and J. Vilmart, *J. Bacteriol.*, 1980, **142**, 521.
[461] C. L. McCormick and D. K. Lichatowich, *J. Polym. Sci., Polym. Lett. Ed.*, 1979, **17**, 479.

Amyloses. – 2,3-Di-*O*-acetylamylose has been treated with 4-toluenesulphonyl chloride in pyridine to yield products that were converted into 2,3-di-*O*-acetyl-6-deoxy-6-iodoamylose and thence into 6-deoxy-amylose.[462] A random substitution pattern was demonstrated after analysis of hydrolysis products obtained on digestion of 6-deoxy-amylose with *Bacillus subtilis* α-amylase.

Degradation of amylose with α-amylase from *Bacillus subtilis* under suitable conditions produces mainly maltohexaose, maltoheptaose, and maltotriose.[463] These have been used for the preparation of aldonolactones by hypoiodite oxidation and electrolytic oxidation. By treatment with 1,2-diaminoethane, the aldonolactones can be converted into *N*-(2-aminoethyl)aldonamides. Both species are active as primers for phosphorylase.

A new amylose acid ion-exchanger, carboxymethylamylose, has been tested for its ability to separate the proteins extracted from carp gonads (*Cyprinus carpio*).[464] The new product features a resolution capacity similar to that of the cellulose analogue. Best results were obtained when discontinuous saline gradients were used for elution.

Amylose has been carboxymethylated to increase solubility before dyeing with Remazol Brilliant Blue R.[465] The product, a new chromogenic substrate for α-amylase, is either soluble or can be readily dispersed to form fine gelatinous suspensions. Assays based on the use of this substrate are sensitive and highly specific for α-amylase.

The modes of action of human pancreatic, human salivary, and porcine pancreatic amylases on Cibacron Blue F3GA-amylose have been compared.[466] Introduction of dye substituents into the amylose molecule did not alter the substrate characteristics of amylose toward human serum amylase.

Amylose cross-linked with epichlorohydrin to form an affinity chromatographic substrate has been used to demonstrate that precursor maltose-binding protein synthesized *in vitro* is active in binding maltose.[467]

Controlled-activity pesticide systems have been prepared by reacting amylose with pesticide derivatives (*cf.* amylopectin, p. 430).[461]

Celluloses. – Water-soluble enzymes have been adsorbed on to a column of unmodified cellulose in the presence of high concentrations of salts (*e.g.* ammonium sulphate, sodium formate).[468] The adsorbed enzymes were chromatographically eluted by decreasing the salt concentration. The principal attractive force between cellulose and the protein molecule was suggested to be that of hydrogen bonding.

Cellulose gel particles of spherical or irregular shape have been prepared by dispersing cellulose–aqueous calcium thiocyanate solution or gel in organic

[462] C. E. Weill, M. Kaminsky, and J. Hardenbergh, *Carbohydr. Res.*, 1980, **84**, 307.
[463] W. N. Emmerling and B. Pfannemüller, *Carbohydr. Res.*, 1980, **86**, 321.
[464] M. Serban and O. Gozia, *Rev. Roum. Biochim.*, 1980, **17**, 83.
[465] B. V. McCleary, *Carbohydr. Res.*, 1980, **86**, 97.
[466] B. Klein and J. A. Foreman, *Clin. Chem.*, 1980, **26**, 250.
[467] T. Ferenci and L. L. Randall, *J. Biol. Chem.*, 1979, **254**, 9979.
[468] T. Fujita, Y. Suzuki, J. Yamauti, I. Takagahara, K. Fujii, J. Yamashita, and T. Horio, *J. Biochem. (Jpn.)*, 1980, **87**, 89.

solvents and then regenerating the cellulose with methanol.[469] The gel thus prepared had high porosity and sufficient rigidity to be used for gel filtration of large solutes. The cellulose gel had similar low adsorptivity and chemical stability, but higher thermal resistance and remarkably improved mechanical strength, when compared with conventional macroporous gels such as agarose or cross-linked dextran.

A technique for acetylation of cellulose in a homogeneous solution *via* its trinitrite ester has been described using acetic anhydride with a pyridine catalyst.[470] Depending on the reaction conditions used, different degrees of substitution were obtained. In acetates of low degree of substitution, the hydroxy-groups appeared to have been esterified selectively at C-2 in the anhydro-D-glucose units of the cellulose molecules.

Cellulose acetate has been used successfully for the liquid chromatographic separation of several enantiomeric mixtures.[471] A theoretical treatment allowing calculation of percentage purity is proposed.

Chymotrypsin[472] and L-phenylalanine ammonia-lyase[473] have been immobilized by entrapment in cellulose acetate. Different solvent systems have been investigated for the immobilization in cellulose acetate beads of *Actinoplanes missouriensis*, *Kluyveromyces fragilis*, or *Saccharomyces cerevisiae* cells.[474] Thermophylic microbial cells (*Caldariella acidophila*) have also been immobilized by co-casting into cellulose acetate membranes.[475]

4-Aminocarbanilated derivatives of cellulose have been investigated as matrices for the immobilization of β-D-galactosidase and other enzymes.[476] The polymers contain numerous surface amino-groups which are available for coupling. The preparation of 4-aminobenzyl derivatives of cellulose and their utilization for enzyme immobilization have been discussed.[477]

Benzoylated diethylaminoethylcellulose has been applied successfully to the fractionation of renatured or hybridized deoxyribonucleic acid molecules, as well as to the determination of single-stranded structures in deoxyribonucleic acid.[478]

Cellulose *trans*-2,3-carbonate has been shown to be an effective matrix for the immobilization of antigens and antibodies.[479] The coupling reaction can be performed using mild conditions that preserve immunological reactivity.

[469] S. Kuga, *J. Chromatogr.*, 1980, **195**, 221.
[470] P. Månsson and L. Westfelt, *Cell. Chem. Technol.*, 1980, **14**, 13.
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[472] N. R. Kil'deeva, N. I. Larinova, N. F. Kazanskaya, and A. D. Virnik, *Biochemistry (USSR)*, 1980, **45**, 433.
[473] W. Marconi, F. Bartoli, R. Gianna, F. Morisi, and G. Spotorno, *Biochimie*, 1980, **62**, 575.
[474] P. Linko, K. Poutanen, L. Weckstrom, and Y. Y. Linko, *Biochimie*, 1980, **62**, 387.
[475] M. De Rosa, A. Gambacorta, E. Esposito, E. Drioli, and S. Gaeta, *Biochimie*, 1980, **62**, 517.
[476] C. G. Beddows, R. A. Mirauer, and J. T. Guthrie, *Biotechnol. Bioeng.*, 1980, **22**, 311.
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[478] L. S. Popov, *Biochemistry (USSR)*, 1979, **44**, 1435.
[479] J. F. Kennedy, D. Catty, and P. A. Keep, *Int. J. Biol. Macromol.*, 1980, **2**, 137.

Statistical methods of experimental planning have been used to correlate reaction parameters with the degree of substitution of carboxymethylated cellulose derivatives.[480] Reaction conditions were optimized on the basis of the statistical models.

The use of cellulose derivatives as supports for biologically active compounds has been discussed with particular reference to antibiotics and enzymes.[481] Acid chloride derivatives of carboxymethylcellulose were used for immobilization of glucoamylase (amyloglucosidase), as were cellulose carbonate derivatives.

Some parameters of the reaction between chloroacetic acid and cellulose have been investigated.[482] The product structures of the chloroacetylcellulose derivatives, which were insoluble in water and in organic solvents, were determined by i.r. spectroscopy.

Cellulose activated with cyanogen bromide has been used for the immobilization of goat anti-(rabbit immunoglobulin G) antibody for use in an enzyme immunoassay for pancreatic glucagon.[483] Anti-(carcinoembryonic antigen) antibody has been covalently coupled to cellulose paper discs previously reacted with cyanogen bromide.[484] Several neuraminic acid-containing glycoproteins and oligosaccharides have been immobilized by reaction with cellulose which had been activated with cyanogen bromide, treated with hexanedioic acid dihydrazide and diazotized.[90] The immobilized preparations were used in the development of a micromethod for quantitation of sialoglycoconjugates coupled to insoluble supports.

An improved Zeisel-gas chromatographic technique for determination of molar substitution in cellulose ether derivatives has been reported.[485] Hexanedioic acid was used to catalyse hydriodic acid cleavage of substituted alkoxy-groups to their corresponding alkyl iodides. *In situ* xylene extraction of the alkyl iodides in a sealed tube allows determination of methoxy-, ethoxy-, hydroxyethoxy- or hydroxypropoxy-substitution in mixed or homogeneous cellulose ethers.

Deoxyribonucleic acid adsorbed on cellulose has been applied to the affinity chromatographic purification of histone acetylase A from calf thymus tissues.[305]

The modification of cellulose with 2,3-epoxypropyldiethylamine has been reported.[486]

In a study of the extracellular cellulolytic enzyme complex produced by *Clostridium thermocellum*, trinitrophenyl carboxymethylcellulose, a chromogenic derivative of carboxymethylcellulose, has been used as a substrate for determination of cellulase activity.[487]

[480] N. Olary, S. Staimberg, D. Bonteh, and N. Asaudel, *Cell. Chem. Technol.*, 1980, **14**, 713.

[481] C. Simionescu, D. Dumitriu, V. Bulacovschi, and V. I. Popa, *J. Polym. Sci., Polym. Symp. Ed.*, 1979, No. 66, 171.

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[485] K. L. Hodges, W. E. Kester, D. L. Wiederrich, and J. A. Grover, *Anal. Chem.*, 1979, **51**, 2172.

[486] M. Antal and L. Kuniak, *Cell. Chem. Technol.*, 1980, **14**, 845.

[487] A. Shinmyo, D. V. Garcia-Martinez, and A. L. Demain, *J. Appl. Biochem.*, 1979, **1**, 202.

The influence of alkali and available-water concentrations on substituent distributions after heterogeneous ethoxylation of cellulose has been discussed.[488] A computer simulation of a stochastic process was used to calculate distribution-profiles at various molar substitution intervals of hydroxyethylation. The distribution profile for a given simulation was then fitted with experimental data to determine relative ratios of hydroxy-group reactivity. This combined approach provides a technique superior to those previously used to determine relative reactivities.

Mitomycin C has been microencapsulated in ethylcellulose by coacervation in the presence of polyethylene and cyclohexane.[489] The chemotherapeutic potential of the modified drug was investigated in dogs. Microcapsules containing ethyl-cellulose and poly(lactic acid) have been used to prepare derivatives of sulphamethizole, 5-fluorouracil, and doxorubicin.[490] The sustained release properties of the microencapsulated drugs were investigated.

Cellulose has been esterified, treated with hydrazine, diazotized, and then coupled with 4-aminophenyl boric acid to yield a boronate–cellulose matrix with a 1 nm-long spacer arm.[491] This matrix is useful for the separation of aminoacyl-transfer ribonucleic acids and *O*-methylribose nucleosides, and for separation as a group of transfer ribonucleic acids containing the base queuine.

The properties of derivatives prepared by grafting cellulose with vinyl polymers [poly(methyl methacrylate), poly(ethyl acrylate), poly(acrylonitrile), and poly(acrylic acid)] have been investigated.[492] The i.r. spectra of the products are reported, together with their stabilities to acid or alkali and their water retention values.

A detailed study has been reported concerning the grafting of ethyl acrylate on to cellulose using Fenton's reagent as initiator in the presence of various additives.[493] The reactivities of vinyl acetate and ethyl acetate in Fenton's reagent-initiated grafting on to cellulose were compared, and the percentage of grafting and efficiency of reaction were determined as functions of several reaction variables.

The formation and behaviour of photo- and mechano-induced free radicals in cellulose have been studied by e.s.r. spectroscopy.[494] The capability of these free radicals to initiate graft copolymerization reactions was demonstrated. Mechano-radicals were found to react more efficiently and rapidly with oxygen and methyl methacrylate than photoinduced free radicals, and a higher degree of grafting efficiency was obtained from cellulose treated mechanically.

[488] J. E. Glass, A. M. Buettner, R. G. Lowther, C. S. Young, and L. A. Cosby, *Carbohydr. Res.*, 1980, **84**, 245.
[489] T. Kato, R. Nemoto, H. Mori, and I. Kumagai, *Cancer*, 1980, **46**, 14.
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[494] D. N. S. Hon, *J. Polym. Sci., Polym. Chem. Ed.*, 1980, **18**, 1857.

A new method of preparation of fluoro-containing graft copolymers of cellulose has been developed in order to produce modified cellulose fibres with good oil- and water-proof properties.[495] The copolymers contain *N*-methylol methacrylamide groups.

The chemical modification of cellulose by solid-phase reaction with glyoxal has been investigated.[496] Glyoxalation occurs chiefly on the primary hydroxy-groups of cellulose. Quantitative characteristics of the rigidity and stability of cellulose films and fibres after reaction with glyoxal were established.

Several methods have been developed for the preparation of fibrous cellulose ionites using sodium vinylsulphonate, itaconic acid, aniline, or formaldehyde.[497] Reaction conditions were established and the ion-exchange properties of the cellulose ionites were investigated under static and dynamic conditions.

α-Amylase and trypsin have been immobilized on *S*-methyldithiocarbonate and dithiobis(thioformate) derivatives of cellulose.[498]

2,4-Dichloro-6-(X)-*sym*-triazines [where X = 5-nitro-2-furaldehyde hydrazone, 3-(5-nitro-2-furyl)acrolein hydrazone, or 5-nitro-furfurylidenacetone hydrazone] have been condensed with cellulose to yield the appropriate 2,4-oxycelluloso-*sym*-triazine derivatives.[499] These compounds have shown good antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Candida albicans*, *Aspergillus niger*, *Penicillium cyclopium*, and *Rhizopus oryzae*.

The cross-linking of linter cellulose powder with epichlorohydrin under different reaction conditions has been investigated.[500] Relationships between structural characteristics (extent of cross-linking, number of dihydroxypropane side-chains, lattice type, and morphological structure) and some macroporous properties (solubility, swelling in water and aqueous sodium hydroxide, and water vapour sorption) were studied.

Cellulose cross-linked with epichlorohydrin has been used for the affinity chromatographic separation of components of the cellulase complex from *Trichoderma viride*.[501]

The introduction of isonitrile functional groups on to cellulose backbones has been described and the immobilization of a number of enzymes on these supports reported.[89]

[495] J. P. Dzjdanova, Z. A. Rogovin, L. S. Sletkina, F. A. Baybikov, and A. V. Kashkin, *Cell. Chem. Technol.*, 1980, **14**, 623.

[496] G. A. Petropavlovsky, E. I. Larina, and I. T. Borisova, *Cell. Chem. Technol.*, 1980, **14**, 683.

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[501] M. Weber, M. J. Foglietti, and F. Percheron, *J. Chromatogr.*, 1980, **188**, 377.

The characterization of cellulose ethers and cellulose mixed ethers by means of g.c. has been described using methylhydroxyethylcellulose as an example.[502]

Burst-forming units from the blood of eight normal adults have been grown in methylcellulose culture.[503]

Denatured deoxyribonucleic acids immobilized on nitrocellulose papers at elevated temperature have been used in a method for identifying 5-methylcytosine.[504] A chromatographic technique for isolating bacterial plasmid deoxyribonucleic acid using an agarose column connected to a nitrocellulose column has been described.[505] The one-step procedure allows separation of the deoxyribonucleic acid at a concentration suitable for direct electron microscopy.

Oligo-(dT)-cellulose has been used for affinity chromatography of poly-(adenylic acid)-containing ribonucleoproteins.[506] A study of the interaction of poly(adenylic acid) with different absorbents for affinity chromatography of nucleic acids has included investigation of the use of oligo-dT- and -MN-cellulose derivatives.[129] Using different experimental conditions it was possible to evaluate the contribution of electrostatic, hydrophobic, hydrogen-bonding, and/or charge-transfer interactions to the binding of nucleic acid and affinity matrix.

Controlled-activity pesticide systems have been prepared by reacting cellulose with pesticide derivatives (*cf.* amylopectin, p. 430.[461]

Type II hexokinase has been purified from rat skeletal muscle by affinity elution chromatography on cellulose phosphate.[507] Using the effector molecule D-glucose 6-phosphate as the eluting ligand, over 400-fold purification could be achieved in the affinity elution step alone. By specific elution with substrate (D-fructose 1,6-bisphosphate) and allosteric effector (adenosine monophosphate), human liver D-fructose 1,6-bisphosphatase has been purified successfully using cellulose phosphate.[508]

The reaction of cellulose phosphonate and *N*-vinyl-2-pyrrolidone has been investigated.[509] The cellulose derivative has improved thermal stability and flame-retardant properties.

Carboxymethylcellulose has been partially depolymerized with cellulase and then dyed with Remazol Brilliant Blue R or Remazol Brilliant Black B.[465] The modified cellulose has been used as a sensitive chromogenic substrate for the assay of (1→4)-β-D-glucanase. Remazol Brilliant Blue R immobilized on cellulose has been used for the affinity chromatography of rat liver lactate dehydrogenase.[510]

Succinylaminodecylcellulose has been reacted with ampicillin forming an affinity matrix, which has been used successfully for the purification of two

[502] P. Friese, *Z. Anal. Chem.*, 1980, **303**, 279.
[503] H. Rochant, U. Testa, W. Vainchenker, A. Henri, M. Titeux, H. Tonthat, and F. Feuilhade, *C. R. Hebd. Seances Acad. Sci.*, 1980, **291**, 229.
[504] H. Sano, H. D. Royer, and R. Sager, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 3581.
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[507] S. S. Qadri and J. S. Easterby, *Anal. Biochem.*, 1980, **105**, 299.
[508] A. Dzugaj and M. Kochman, *Biochim. Biophys. Acta*, 1980, **614**, 407.
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carboxypeptidases/transpeptidases with different penicillin sensitivities from *Proteus mirabilis*.[511]

The position and degree of ester sulphation of natural cellulose sulphate have been determined by ^{13}C n.m.r. spectroscopy.[512] Some new cellulose sulphate derivatives have been described and their applications discussed.[513]

Chitin and Chitosans. – Unmodified chitin has been used for the affinity chromatographic purification of human salivary lysozyme.[514] The lectin activity from *Entamoeba histolytica* trophozoites has been purified on columns of chitin.[515]

Methods for substantially enhancing the metal-chelating performance of both chitin and chitosan have been described.[516]

Subtilisin, trypsin, and *Streptomyces griseus* neutral proteinase ('Pronase') have been immobilized by glutaraldehyde-mediated reaction with chitosan.[517]

Fully acylated chitosan derivatives that are soluble in organic solvents have been prepared by boiling chitosan with dodecanoyl chloride, tetradecanoyl chloride, or hexanoyl chloride.[518] Evaporation of the chloroform solvent from solutions of these poly(acyl)chitosans left a rubber-like thin film.

N-Acylchitosan gels having acyl groups $CO(CH_2)_nMe$, where $n = 0$–4, have been prepared and used for hydrophobic chromatographic purification of phosphorylase.[519]

Two novel methods for the determination of the degree of *N*-acetylation of chitosan have been described.[520] By determining the amount of periodate consumed on oxidizing the modified chitosan the percentage of de-*N*-acetylated groups can be calculated, as units containing acetamido-groups are resistant to oxidation. Some over-oxidation may occur, however, so results may be high. Better results may be obtained using an i.r. spectroscopic technique in which the concentration of *N*-acetyl groups is obtained by measuring the absorbance of the amide I band at 1655 cm^{-1}, using the hydroxy-group absorption band at 3450 cm^{-1} as internal reference standard.

N-Formyl, *N*-chloroacetyl, *N*-glycyl, *N*-isobutyryl, and *N*-pentanoyl derivatives of chitosan have been prepared.[521] The effects of *N*-substitution and the physical form of the products on the rate of hydrolysis by *Streptomyces griseus* chitinase were investigated.

A study has been made of the gelation behaviour of chitosan solutions on reaction with acyl anhydrides.[522] The effect on the time to onset of gelation of

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[512] S. Hunt and T. N. Huckerby, *Int. J. Biol. Macromol.*, 1980, **2**, 389.
[513] R. G. Schweiger, *Carbohydr. Res.*, 1979, **70**, 185.
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[517] J. L. Leuba and F. Widmer, *Biotechnol. Lett.*, 1979, **1**, 109.
[518] S. Fujii, H. Kumagai, and M. Noda, *Carbohydr. Res.*, 1980, **83**, 389.
[519] T. Baba, R. Yamaguchi, Y. Arai, and T. Itoh, *Carbohydr. Res.*, 1980, **86**, 161.
[520] G. K. Moore and G. A. F. Roberts, *Int. J. Biol. Macromol.*, 1980, **2**, 115.
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such factors as the nature and concentration of the acyl anhydride, the chitosan concentration, the temperature, and the nature of the cosolvent has been examined. Results indicate that gelation occurs because of decreased solubility of the polymer molecules due to the increased extent of *N*-acylation. The degree of *N*-acylation required to initiate gelation depends on the molecular weight of the acyl anhydride and on the concentration of chitosan, but is independent of temperature.[523] A mechanism has been proposed for the gelation process.

Waste mycelia of *Aspergillus niger* from a citric acid production plant have been treated with boiling alkali to obtain an insoluble chitosan–glucan complex whose i.r., e.s.r., and *X*-ray diffraction spectra have been determined.[524] Several transition and post-transition-metal ions are chelated and collected by the complex in higher yield than by animal chitosan.

Cyclomalto-oligosaccharides. – Initial results of crystallographic studies of the structure of hydrated cyclomalto-octaose have been presented.[525] The 'cage-type' structure obtained for the hydrated molecule has been compared with the 'channel-type' structure obtained for the hydrated cyclomalto-octoase–n-propanol complex.[526]

The structural chemistry of linear cyclomaltohexaose–polyiodide complexes has been investigated by determining the *X*-ray crystallographic structures of (64) and (65).[527] With metal iodides in aqueous solution 'channel-type' complexes are observed. In these, cyclomaltohexaose molecules stack like coins in a roll, and polyiodide chains are embedded within the tubular cavity. These complexes may serve as models for the amylose–iodine complex.

$$(\text{Cyclomaltohexaose})_2\text{-}LiI_3\cdot I_2\cdot 8H_2O$$

(64)

$$(\text{Cyclomaltohexaose})_2\text{-}Cd_{0.5}\cdot I_5\cdot 27H_2O$$

(65)

The preparation and characterization of triamino-per-*O*-methyl-cyclomaltohexaose has been reported.[528] This trisubstituted cyclomaltohexaose is the first reported derivative of intermediate substitution number for which the positions of substitution are established.

Triamino-per-*O*-methyl-cyclomaltohexaose has been studied as a potential host molecule that would catalyse a simple chemical reaction by specific stabilization of its transition state.[529] The chosen 'guest' molecule was a phosphate monoester.

[523] G. K. Moore and G. A. F. Roberts, *Int. J. Biol. Macromol.*, 1980, **2**, 78.
[524] R. A. A. Muzzarelli, F. Tanfani, and G. Scarpini, *Biotechnol. Bioeng.*, 1980, **22**, 885.
[525] K. Lindner and W. Saenger, *Biochem. Biophys. Res. Commun.*, 1980, **92**, 933.
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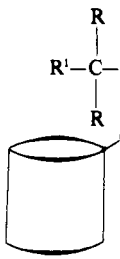
[529] J. Boger and J. R. Knowles, *J. Am. Chem. Soc.*, 1979, **101**, 7631.

Formation constants for binary complexes of cyclomaltohexaose and 4-nitrophenol/4-nitrophenolate have been determined by means of a novel acid–base spectrophotometric analysis.[530]

Cyclomaltoheptaose 6-monotosylate has been modified by reaction with mercaptans, and rigidly capped cyclomaltoheptaose has also been prepared.[531] The reactivity of the modified cyclomalto-oligosaccharides was compared with that of the native molecule for the hydrolysis of 3- and 4-nitro- and methyl phenyl acetates.

The synthesis of cyclomaltoheptaose–dihydronicotinamide, which has a nicotinamide moiety coupled to a cyclomalto-oligosaccharide molecule, has been described.[532] The compound has a dihydropyridine ring at the open side of the cyclomaltoheptaose cavity and can reduce a substrate within the cavity, thus simulating a dehydrogenase.

By reaction of cyclomaltoheptaose 6-monotosylate with alkyl mercaptans, 6-deoxy-6-alkylthio-cyclomaltoheptaose (66)–(68) have been prepared.[533] Studies of the hydrolyses of 3- and 4-substituted phenyl acetates showed that the 3-selectivity effect occurred with (66), whereas none was observed with (68). This variation in selectivity was attributed to a change in the catalytic rate constant caused by the substituent on cyclomaltoheptaose.



(66) R = H, R^1 = OH
(67) R = H, R^1 = Me
(68) R = R^1 = Me

The selectivity of cyclomalto-oligosaccharide bisimidazole ribonuclease mimics has been reversed by changing the catalyst geometry.[534] Even modest catalytic rate enhancements were shown to combine with suppression of the uncatalysed reactions to lead to highly selective chemical processes.

Pyridoxal–pyridoxamine coenzymes have been linked to cyclomaltoheptaose, producing an artificial enzyme-like system, which has good substrate specificity in transaminations and which can produce some chiral induction.[535] The selectivity of the modified cyclomaltoheptaose was compared with pyridoxamine alone and with pyridoxamine mixed with cyclomaltoheptaose. Results demonstrated that the modified compound shows selectivity and rate acceleration and is thus a good 'first generation' artificial transaminase.

[530] R. I. Gelb, L. M. Schwartz, B. Cardelino, and D. A. Laufer, *Anal. Biochem.*, 1980, **103**, 362.
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[533] K. Fujita, A. Shinoda, and T. Imoto, *Tetrahedron Lett.*, 1980, **21**, 1541.
[534] R. Breslow, P. Bovy, and C. L. Hersh, *J. Am. Chem. Soc.*, 1980, **102**, 2115.
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Regiospecifically bifunctionalized cyclomalto-oligosaccharide has been demonstrated to provide the first successful model for carbonic anhydrase.[536]

The exploitation of the inclusion properties of cyclomalto-oligosaccharides in liquid chromatography has been described.[537] The use of cyclomalto-oligosaccharide inclusion compounds in research and industry has also been reviewed.[538]

A structural study of the inclusion compounds of tolbutamide and chlorpropamide with cyclomaltoheptaose in aqueous solution has been reported.[539] Data from ^{1}H n.m.r. and ^{13}C n.m.r. experiments suggest that the phenyl moiety of each drug is included in the cyclomalto-oligosaccharide cavity chiefly by hydrophobic interactions, and that the primary hydroxy-side of the cyclomalto-oligosaccharide molecule was tightly associated with each drug.

The '50% effective' doses of five barbiturate–cyclomaltohexaose complexes on oral administration to mice have been compared with those of the free barbiturates.[540] In all cases the effective dose was significantly lower for the complexed barbiturate.

Cyclomaltoheptaose inclusion complexes of 25 flavouring substances have been prepared.[541] The crystalline complexes contained 8–13% included substance, as determined by g.l.c. Complex formation was proved by *X*-ray diffraction and stability tests. The possible uses of such complexes in the food industry are discussed.

Ground mixtures of aspirin, benzoic acid, and 4-acetoxy-diphenyl have been prepared with tri-*O*-methyl-cyclomaltoheptaose.[542] The mixtures were studied by *X*-ray diffraction and i.r. spectroscopy. Results indicated that the drug molecules were monomolecularly dispersed in the cyclomalto-oligosaccharide mixtures.

Dextrans. – The chromatographic properties of columns of cross-linked dextran gels have been discussed,[543,544] and the effect of particle-size distribution on column efficiency has been described.[545]

By using a bound spectral polarity-detecting label, the solvation power of the solvent near the cross-linked dextran matrix has been investigated.[88]

The use of water-soluble biospecific polymers, including dextran, for the purification of proteins has been reviewed.[546]

[536] I. Tabushi, Y. Kuroda, and A. Mochizuki, *J. Am. Chem. Soc.*, 1980, **102**, 1152.
[537] E. Smolková-Keulemansová and S. Krýsl, *J. Chromatogr.*, 1980, **184**, 347.
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[543] J. M. Sosa, *Anal. Chem.*, 1980, **52**, 910.
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[545] K. Nakanishi, S. Yamamoto, R. Matsuno, and T. Kamikubo, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 1943.
[546] P. Hubert and E. Dellacherie, *J. Chromatogr.*, 1980, **184**, 325.

Three human parotid α-amylase isoenzymes have been separated by chromatography on Sephadex® G-75.[547] The dextran α-1,2-debranching enzyme from *Flavobacterium* sp. M-73 has been purified by affinity chromatography on Sephadex G-150.[548]

4-Aminobenzamidine has been coupled to periodate-oxidized dextran directly and through glycine, 6-aminohexanoic acid, or 12-aminododecanoic acid spacer ligands.[549] The strength of the interaction of trypsin with the immobilized 4-aminobenzamidine derivatives was determined by affinity electrophoresis.

The internal water of swollen Sephadex® gels can dissolve appreciable amounts of the water-insoluble dyes azobenzene and dimethylaminoazobenzene.[550] Since the solubilities of the dyes were proportional to the fourth power of the concentration of the dextran chain in swollen Sephadex gels, it can be assumed that the dyes are accommodated in co-operatively hydrated water contained in unit cells made up of four dextran chains.

Haemoglobin has been alkylated by reaction with *N*-bromoacetylaminoethylaminodextran.[551]

Hydrazinolysed derivatives of carboxymethyl-Sephadex® have been prepared and converted to the azide form before coupling with D-arginine and D-phenylalanine derivatives.[552] The products were applied to the affinity chromatography of carboxypeptidase B, for which they proved to be effective and specific adsorbents. This method of preparing activated cross-linked dextran should find further application in affinity chromatography as the adsorbents have stable amide linkages and retain the favourable physical properties of Sephadex®.

Soluble dextrans (mol. wt. 1×10^4, 4×10^4, 7×10^4, and 2×10^6) have been carboxymethylated, coupled with *N*,*N*′-bis(3-aminopropyl)piperazine in a carbodi-imide-mediated reaction, and then acylated with the *N*-hydroxysuccinimide ester of 3-carboxy-6-nitrophenyl β-D-galactopyranoside.[553] The enzyme derivative was used in the development of a homogeneous enzyme immunoassay method for protein determination.

Cyanogen bromide-activated derivatives of cross-linked dextran have been used for the immobilization of collagen and denatured collagen.[554] The products were used in an investigation of the kinetics of binding of labelled fibronectin to native and denatured collagen.

Sephacryl® S-200, a copolymer of dextran and polyacrylamide, has been activated with cyanogen bromide and reacted with cobalamin.[555] Following adsorption of transcobalamin II on to the modified beads the conjugate

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[550] M. Janado, K. Takenaka, J. Nakamori, and Y. Yano, *J. Biochem. (Jpn.)*, 1980, **87**, 57.
[551] S. C. Tam and J. T. F. Wong, *Can. J. Biochem.*, 1980, **58**, 732.
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[555] D. W. Jacobsen, Y. D. Montejano, K. S. Vitols, and F. M. Huennekens, *Blood*, 1980, **55**, 160.

adsorbed, and enabled detection of, murine leukaemia cells containing receptors for the cobalamin–transcobalamin II complex.

Diethylaminoethyl derivatives of cross-linked dextran have been used suspended in a nutrient solution in an improved large-scale culture method for diploid cells, which produces viral vaccines.[556]

The interaction of neutral polysaccharides with membrane models has been studied by determining the binding of fluoresceinyl-thiocarbamoyl-dextran to phosphatidylcholine multilamellar liposomes.[557] The amount of derivatized dextran bound to liposomes increased with temperature and showed two sharp changes at temperatures near the phase transitions of synthetic phosphatidylcholine multilamellar liposomes.

The application of 'dextran–magnetite', a suspension of dextran-coated ferromagnetic iron oxide, as a relaxation agent in the measurement of erythrocyte water exchange using pulsed n.m.r. spectroscopy has been demonstrated.[558] Results compared favourably with those obtained using conventional methods.

A general method that involves use of iron–dextran–antibody conjugates for simultaneous staining of two components in high resolution immuno-electron microscopy has been reported.[559]

Sephadex® has been sulphated by anhydrous reaction with chlorosulphonic acid and successfully applied to the isolation of human plasma-coagulation Factors II, IX, and X.[560] These species were readily adsorbed to the matrix and could be eluted with a sodium chloride gradient. The derivatized beads were found to have a high degree of sulphate esterification and when hydrated were stable for more than two years in buffered solution at neutral pH.

Sulphopropyl-Sephadex® has been applied to purification of a tartrate-resistant acid phosphatase from the spleen of a patient with Gaucher's disease.[561] The matrix has also been used successfully for purification of the H blood-group β-D-galactoside α-(1,2)-fucosyltransferase from porcine submaxillary gland.[288]

Blue Dextran® (dextran coupled with the dye Cibacron Blue F3GA) has been used as a substrate for dextranase in order to demonstrate the validity of a new general procedure for quantitation of enzymes degrading macromolecular substrates.[562]

Preparation of Blue Dextran® immobilized on agarose continue to be used widely as affinity supports for the purification of numerous molecular species. These have recently included human leucocyte interferon,[181] 4-*N*-trimethylaminobutyraldehyde dehydrogenase,[222] D-glyceraldehyde phosphate dehydrogenase,[235] cytoplasmic valyl-transfer ribonucleic acid synthetase,[236] 9-hydroxyprostaglandin dehydrogenase,[237] yeast L-arginyl- and L-aspartyl-transfer ribo-

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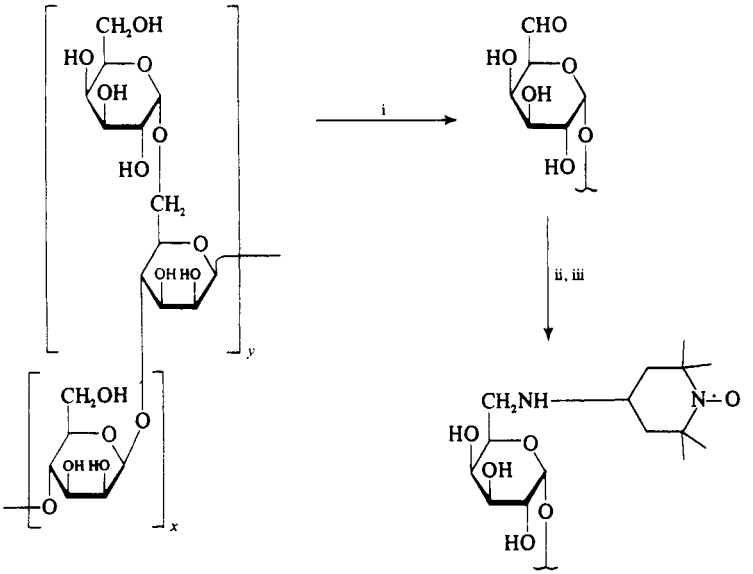
nucleic acid synthetases,[387] *Neurospora crassa* pyruvate synthetase,[388] cytochrome b_5 reductase,[389] and H3-specific nucleohistone kinase.[563]

D-Fructans. – Inulin, a (2→1)-β-D-fructan, has been carboxymethylated and then coupled to *Brucella abortus* cells in a carbodi-imide-mediated reaction.[564] The conjugate was used as immunogen for production of anti-inulin antibodies in mice.

D-Galacto-D-mannans. – A high-yielding, specific method for the chemical derivatization of D-galactose-containing polysaccharides has been described.[565] D-Galactose oxidase is used to introduce, at C-5 of the D-galactopyranosyl groups, an aldehyde group whose reductive amination, using any primary or secondary amine and sodium cyanoborohydride, affords a polymer bearing a substituent of choice on C-5. The general sequence of reactions is summarized in Scheme 8, which represents the spin-labelling of guaran.

Guar gum preparations have been cross-linked with epichlorohydrin to yield gel filtration media, which have been applied to the separation of proteins of mol. wt. 5×10^3 to 7×10^4.[566]



Reagents: i, D-galactose oxidase–catalase; ii, 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl–sodium cyanoborohydride; iii, dialysis or ethanol precipitation

Scheme 8

[563] C. B. Shoemaker and R. Chalkley, *J. Biol. Chem.*, 1980, **255**, 11 048.
[564] C. C. Chien, *Infect. Immun.*, 1980, **27**, 746.
[565] L. D. Hall and M. Yalpani, *Carbohydr. Res.*, 1980, **81**, C10.
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Glycosaminoglycans. – Interactions between the carbohydrate chains of hyaluronic acid and chondroitin sulphate have been investigated, using immobilized glycosaminoglycans.[567] Chondroitin 4- and 6-sulphates, hyaluronic acid, dermatan sulphate, and heparin were coupled to Dowex® AG50 (sulphonamide form) in carbodi-imide-mediated reactions.

Surfaces prepared from different sulphated polysaccharides and a polyethylene surface chemically modified with sulphate groups have been examined with respect to their interactions with thrombin.[568] Different fractions of heparin, heparan sulphate, dermatan sulphate, chondroitin sulphate, and dextran sulphate were used. All the modified surfaces adsorbed thrombin, which retained its activity towards both fibrinogen and a synthetic chromogenic substrate.

A semi-synthetic 'heparin-analogue' has been characterized as a polysulphated chondroitin (mol. wt. 1.0×10^4) with four *O*-sulphate groups per disaccharide.[569]

Lipopolysaccharides. – Lipopolysaccharides have been immobilized by reaction with polyacrylic hydrazide-agarose and used to isolate membrane proteins with binding sites for lipopolysaccharides.[429] *Salmonella johannesburg O*-polysaccharide immobilized on agarose cyclic imidocarbonate has been used to purify phage ϕ1(40).[302]

Lipoteichoic Acids. – The periodate oxidation and chromium(III) chloride coupling methods have been compared for the sensitization of indicator erythrocytes with staphylococcal lipoteichoic acid for the detection of specific antibody.[570] Erythrocytes sensitized with periodate-activated lipoteichoic acid were found to be superior.

D-Mannans. – D-Mannan has been conjugated with tyramine and then iodinated with ^{125}I for use in a radioimmunoassay for measurement of serum anti-(D-mannan) antibodies.[571]

Miscellaneous Oligosaccharides and Glycolipids. – The partial benzoylation of methyl β-kojibioside and methyl β-sophoroside has been reported.[572]

'Pseudo cord-factors', in which the 6-hydroxy-groups of the trehalose core have been transformed into carboxy-groups, have been prepared.[573] Lipid substituents are attached to the carboxy-groups of the disaccharide, either by ester or amide linkages.

A ^3H-labelled monosulphated trisaccharide having 2-acetamido-2-deoxy-D-glucose 6-sulphate at the non-reducing terminus has been prepared from heparan sulphate and used as a substrate for 2-acetamido-2-deoxy-D-glucose 6-sulphate sulphatase.[574]

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Two sialyloligosaccharides isolated from the urine of sialidosis patients have been reduced with tritiated potassium borohydride solution.[575] Neuraminidase activities determined using the modified oligosaccharides (69), (70) as substrates confirm the hypothesis that a specific defect of α-(2→6)-neuraminidase activity is a dominant factor in sialidosis B.

The experimental product ratios for *Bacillus amyloliquefaciens* α-amylase acting on reducing end-labelled ^{14}C-maltodextrins ranging in chain length from 3–10 units have been reported.[576]

The specificity of α-D-galactosidase towards some 4-deoxy-analogues of methyl α-D-galactopyranoside has been examined.[577] The effect of replacing the ring oxygen atom by a sulphur atom was also examined, using methyl 5-thio-α-D-galactopyranosides.

4-Aminophenyl β-D-galactopyranoside has been immobilized on Bio-Gel® P100 for use as an affinity matrix for the purification of a lung lectin from rat and human sources.[578]

Phlorizin, a β-glycoside present in apple tree roots, has been cross-linked with urea and formaldehyde to serve as an affinity matrix for purification of concanavalin A and lentil lectin.[579]

2-Amino-2-deoxy-D-glucose has been coupled to agarose *via* an *N*,*N*-bis(3-deoxy-2-hydroxypropyl)-6-aminohexanoic spacer arm.[580] The matrix provides a biospecific, oil-soluble coated support for purification of concanavalin A.

The ^{13}C n.m.r. spectroscopic chemical shifts and spin–lattice relaxation times of D-galactosylsphingosine derivatives in C^2HCl_3–$C^2H_3O^2H$ have been reported.[581]

Gangliosides G_{D_3} and G_{M_1} have been treated with *N*-hydroxysuccinimide in the presence of a carbodi-imide mediator to form activated esters, which were then coupled to native or methylated human serum albumin.[582] The conjugates were used to raise anti-ganglioside antibodies, which were isolated by chromatography on ganglioside–aminopropyl silica gel columns.

Galactocerebroside has been derivatized for use as the ligand for affinity chromatography (Scheme 9).[583] The immobilized glycolipid provided an immunoadsorbent for isolation of anti-glycolipid antibodies. Such antibodies have also been purified using gangliosides activated with *N*-hydroxysuccinimide and coupled directly to aminopropyl silica gel.[584]

Pectic and other Polyuronic Acids. – Conditions for obtaining ^{14}C-acetylated pectic acid with high specific activity have been reported.[585] The acetylated

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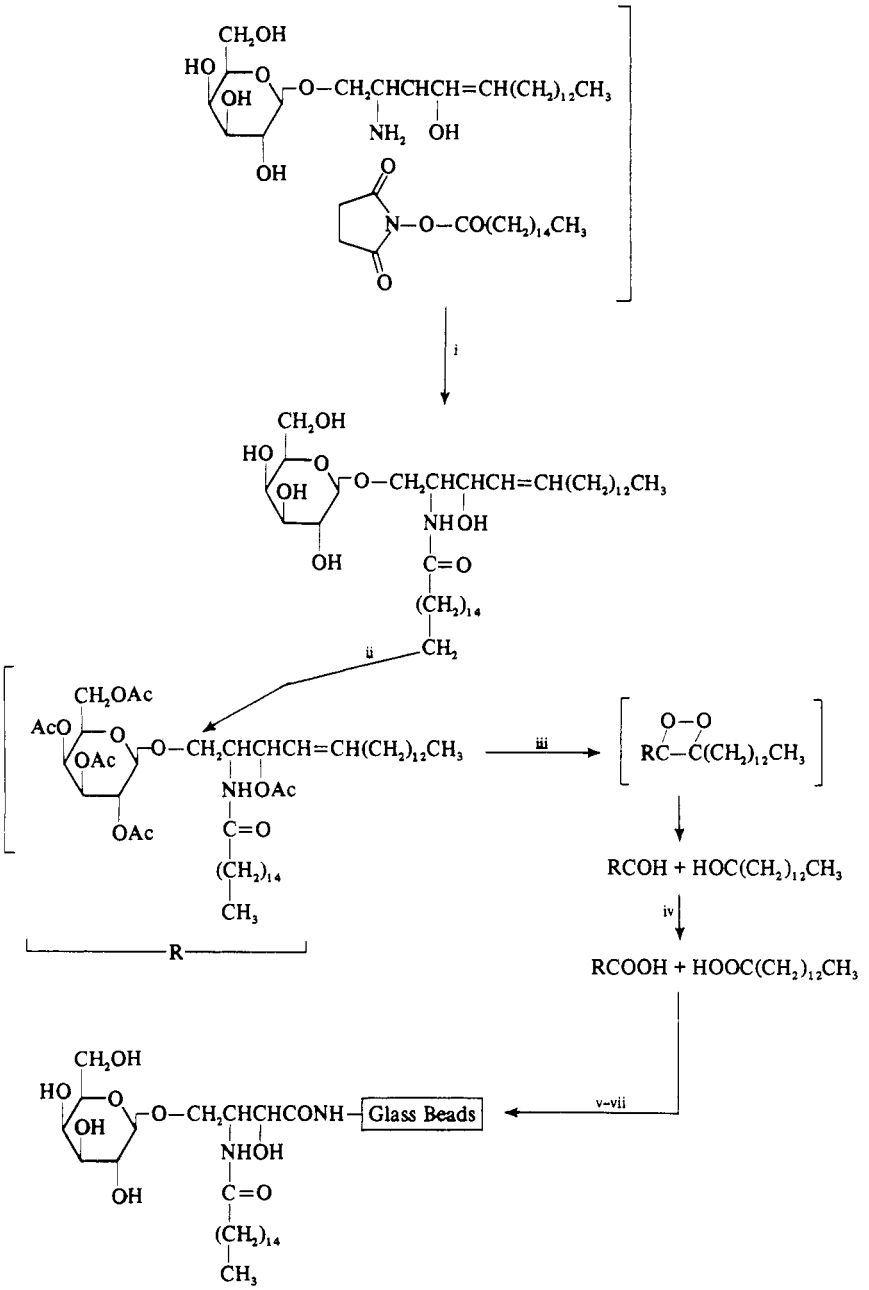
α-Neu*p*NAc-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-D-Glc*p*NAc-itol-1-^{3}H

(69)

α-Neu*p*NAc-(2 → 6)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-D-Glc*p*NAc-itol-1-^{3}H

(70)



Reagents: i, THF; ii, Ac_2O-py; iii, O_3-pinacolone; iv, H_2O_2-AcOH; v, *N'*,*N'*-carbodiimidazole-amino-alkyl glass beads-THF; vi, Ac_2O; vii, NaOH-MeOH

Scheme 9

pectic acid provides a substrate for the deacetylase associated with VII, VIII, and VIIII phage particles.

The production and properties of ethyl esters of sunflower pectic acid have been described.[586]

Sodium pectate preparations cross-linked with different amounts of epichlorohydrin have been applied to the affinity chromatographic separation of two pectinesterase enzymes from orange.[587] The binding characteristics of the enzymes were dependent on the degree of cross-linking of the affinity matrix.

Red blood cells have been co-immobilized in an alginate network and stored for five weeks or more in isotonic sodium chloride solution containing nutrients.[588] The cells can be released by dissolving the matrix with citrate. Both immobilized and released cells retain their bioconcave shape over the storage period. Measurements indicated that immobilization preserves cellular function, which is lost in cells stored conventionally in suspension under comparable conditions.

Protoplasts have been immobilized by entrapment in calcium alginate gels.[589]

Starch. – Results of a scanning electron microscopic study of starch granules with or without α-amylase attack have been reported.[590]

The mechanism of the interaction between starch and fatty acid esters has been studied in frozen starch noodles.[591] The effects of fatty acid esters on properties (*e.g.* viscosity, adhesive force, iodine-affinity) of the starch paste were examined.

Grafting of poly(vinylacetate) on to starch using benzoyl peroxide and azobisisobutyronitrile as initiators has been reported.[592] The relative reactivities of the two initiators were determined and the percentage of grafting expressed as a function of different reaction variables.

L-Fucose has been immobilized on starch by epichlorohydrin-cross-linking and the product was used for the purification of an L-fucose-binding lectin from *Ulex europaeus*.[593]

3 Modification of Glycoproteins and Uses of Modified Glycoproteins

Rhodamine- and fluorescein-containing hydrazides have been synthesized and used for fluorescent labelling of glycoconjugates on cell surfaces or in solution.[594] The glycoconjugates were oxidized with sodium periodate or D-galactose oxidase to form an aldehyde group, which reacts with the hydrazide. Cell-surface neur-

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aminic acid and D-galactose residues on thymocytes and hematodes as well as glycoproteins and gangliosides in solution have been fluoresceinylated by this method, which has many potential applications.

By carbodi-imide-mediated coupling of horse spleen ferritin with excess of bishydrazides, novel covalent electron-dense reagents for the ultrastructural localization of glycoconjugates have been prepared.[595] The reagent has been applied to the localization of periodate-oxidized neuraminic acid residues on erythrocyte cell surfaces.

Albumins. – The interaction of several serum albumins with Cibacron Blue F3GA has been studied.[453] The strong nature of the interaction between the dye and human serum albumin is attributed to involvement of the bilirubin-binding sites on the protein.

Approximately 20 citraconyl groups per monomer of protein have been introduced into human serum albumin using citraconic anhydride.[596] The apparent Stokes radius increased but there was no significant change in helix content as measured by o.r.d. Citraconylation prevented the precipitation of albumin on heating, and significantly slowed the rate of formation of soluble aggregates, even though a large number of citraconyl groups were lost during the heating.

Terrylitin and trypsin, two proteolytic enzymes, have been modified by polyaldehyde-mediated bonding to human serum albumin.[597] The hydrolysis of fibrin and fibrinogen by the modified enzymes was investigated. The fibrinolytic activity of the modified enzymes was found to be higher than that of the native proteins.

Antibodies. – Anti-(human α_2-macroglobulin) antibody has been treated with α-*N*-(4-tosyl)-L-lysine chloromethyl ketone hydrochloride prior to immobilization on agarose.[185]

Anti-(thrombin III) antibody has been chemically modified with dimethyl(2-hydroxy-5-nitrobenzyl)sulphonium bromide, a tryptophan-directed reagent.[292] One hydroxynitrobenzyl moiety was introduced per molecule of anti-(thrombin III) antibody. Unlike native anti-(thrombin III) antibody, the modified protein does not bind to a heparin-agarose affinity column. The results indicate that the integrity of a specific L-tryptophan residue is critical to the binding of heparin to anti-(thrombin III). Results obtained after modification of anti-(thrombin) antibody with *N*-bromosuccinimide also suggest that one or more L-tryptophan residues are involved in binding of the antibody to heparin.[598]

Immunoglobulins have been linked to peroxidase, D-glucose oxidase, penicillinase, and β-D-galactosidase using *N*,*N*′-(oxydimethylene)dimaleimide.[599]

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Human anti-(measles) immunoglobulin G has been covalently modified with periodate-oxidized peroxidase.[600] The interactions of the immunoenzyme complex with immobilized anti-(human immunoglobulin G) antibody were investigated.

Antibody raised against rabbit antibody Fc fragments has been coupled to β-D-galactosidase using 3-maleimidobenzoyl-*N*-hydroxysuccinimide.[601] The antibody was also reacted with periodate-activated peroxidase. The ability of the two enzyme–antibody conjugates to detect specific anti-(human growth hormone) antibodies was compared.

Microencapsulated antibodies have been used in a radioimmunoassay method for cortisol.[602]

Hormonal Glycoproteins. – Human follicle-stimulating hormone, dansylated human follicle-stimulating hormone, and human follicle-stimulating hormone α- and β-subunits have been individually conjugated by photoactivation to an azidobenzoyl derivative of a glycopeptide isolated from fetuin.[603] Human follicle-stimulating hormone–α-subunit conjugate, when recombined with untreated β-subunit, showed up to 50% increase in the biological activity as compared to the control preparation. When the conjugated human follicle-stimulating hormone β-subunit was combined with untreated α-subunit there was little change in biological activity. These experiments demonstrate that the photoactivation procedure, although random and site-nonspecific in nature, provides a potential means of attachment of glycopeptides to the protein moiety with concomitant enhancement of its hormonal activity.

Absorption spectra for the complexes formed between β-lactoglobulin and vitamin A have been reported.[604]

Bovine thyroglobulin has been succinylated and then coupled to β-ecdysone *via* a carbodi-imide-mediated reaction in the presence of the acylation catalyst 4-dimethylaminopyridine.[605] This procedure reverses the standard strategy for synthesizing ester linkages in hapten–protein conjugates and should have widespread applicability for the preparation of conjugates for immunological work.

Phytohaemagglutinins. – A specific elevation of cell-associated α-D-mannosidase has been observed in human skin fibroblasts cultured with concanavalin A.[606] It was concluded that the elevation of this enzyme activity resulted from a specific binding to the cell surface mediated by concanavalin A.

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Co^{III} has been stoicheiometrically incorporated into concanavalin A.[607] The modified protein still possesses a binding site for an additional divalent transition-metal ion, which, together with Ca^{II}, can induce the sugar-binding ability. No hydrogen peroxide oxidation of Co^{II} occurs with demetallized concanavalin A activated with Ca^{II} and Co^{II} unless Co^{II} is presented in stoicheiometric excess. Evidence is presented that suggests that kinetically stable Co^{III} is bound to a completely different location from the thermodynamically stable Co^{II} protein site.

Bleaching of fluorescein-conjugated concanavalin A-labelled plasma membranes with 488 nm laser light has been reported to lead to some broadening and loss of protein bands and the appearance of high molecular weight material, indicating membrane protein cross-linking.[608] Irradiation of whole cells labelled with fluoresceinated concanavalin A leads to cell death. The effects observed are dependent upon total light exposure and concentration of modified concanavalin A.

Gelonin, a new inhibitor of protein synthesis, has been linked by a disulphide bridge to concanavalin A.[275] Native gelonin appears to lack the ability to bind to cell surfaces and enter intact cells. However, when coupled to concanavalin A, which is capable of binding to cells, gelonin is rendered toxic to intact cells.

Peanut agglutinin has been acylated with a new hetero-bifunctional, photosensitive cross-linking reagent, *N*-[4-(4-azidophenylazo)benzoyl]-3-aminopropyl-*N'*-oxysuccinimide ester.[212] The lectin derivative binds specifically and reversibly to neuraminidase-treated human erythrocyte ghosts, and upon irradiation covalent attachment of over 35% of the bound lectin occurs. The affinity cross-linked ghosts were solubilized in deoxycholate, immunoprecipitated with anti-(peanut agglutinin) antibody, and analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

The binding of acetyl, citraconyl, glycinyl, homoarginyl, *N*-bromosuccinimide, and nitro- derivatives of *Phaseolus vulgaris* phytohaemagglutinin to pig spleen lymphocytes has been assessed.[609] The mitogenic activity of the acetyl derivative was impaired, but the other derivatives retained the same activity as the native phytohaemagglutinin.

Transferrins. – Periodate inactivation of ovotransferrin and human serum transferrin has been reported.[610] Extensive inactivation of both glycoproteins was observed when approximately four L-tyrosine residues had been destroyed. Amino-acid analysis and 360 MHz ^1H n.m.r. spectroscopic studies confirmed that L-tyrosine is the only amino-acid residue rapidly oxidized. The correlation of loss of L-tyrosine with the loss of iron-binding activity suggests strongly that the L-tyrosines involved are those that function as ligands to metal ions bound to

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the protein. Periodate oxidation was shown to cause local structural changes in ovotransferrin, but oxidation does not grossly alter the protein conformation.

Immobilized Derivatives of Glycoproteins. – The modification of glycoproteins to yield biologically active immobilized derivatives continues to attract attention. These derivatives and their uses are summarized in Table 6.[611-626]

A micromethod for quantitation of sialoglycoconjugate immobilization on insoluble supports has been reported.[90] Mild acidic hydrolysis of the affinity preparation releases glycosidically bound neuraminic acid, which is immediately isolated on Dowex® resins and assayed colourimetrically.

Glutaraldehyde-cross-linking of immunoglobulin G previously reacted with agarose cyclic imidocarbonate reduces leakage of the antibody from the matrix to undetectable levels without noticeably affecting antigen-binding activity.[135]

The quantitation and purification of antibodies and antigens using immunoadsorbents have been reviewed.[82]

Antigens immobilized by coating on polystyrene tubes have been used to develop a prototype automated system for enzyme-linked immunosorbent assays.[627] One operator can process 900 samples per day. The application of the system to toxoplasmosis serodiagnosis was studied.

The use of lectin affinity chromatography in the purification of membrane glycoproteins has been reviewed.[98] Lectin affinity chromatography of cell-surface proteins of Novikoff tumour cells has been discussed.[99] The interaction of immobilized lectins and H-Z antigens has been described.[628]

Soybean trypsin inhibitor immobilized on agarose has been used to isolate and concentrate proteases quantitatively from both dilute solutions and pancreatic tissue.[626] The proteases bound to the adsorbent were then layered on focusing gel plates at a pH value permitting desorption by H^+-ions. This sample application procedure permitted simultaneous desorption and focusing of the enzymes.

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Table 6 *Modification of glycoproteins by coupling to insoluble matrices and other macromolecules*

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
α_1-Acid glycoprotein, and glycopeptides derived therefrom	Reaction with cyanogen bromide derivatives of agarose, cellulose and cross-linked dextran	Development of a micromethod for quantitation of immobilized neuraminic acid in sialoglycoconjugates	90
Albumin	Emulsion polymerization in biodegradable microspheres of poly(acryldextran)	Investigation of a new matrix for immobilization	611
	Reaction with epoxy-activated acrylic beads	Investigation of the characteristics of the support matrix for immobilization	612
	Reaction with *sym*-triazine-activated agarose	Purification of thrombin	458
	Reaction with agarose cyclic imidocarbonate	Isolation of lipoteichoic acid	203
	Glutaraldehyde-mediated coimmobilization with catalase and D-glucose oxidase	Part of enzyme electrode for measurement of D-glucose	613
	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of soluble transferrin receptors	202
Anti-(acetylcholine receptor) antibody	Reaction with agarose cyclic imidocarbonate	Purification of nicotinic acetylcholine receptors	163
Anti-(rat adipocyte membrane proteins) antibody	Reaction with agarose cyclic imidocarbonate	Purification of rat intrinsic membrane proteins	164
Anti-(rat serum albumin) antibody	Reaction with agarose cyclic imidocarbonate	Removal of albumin during purification of rat α-foetoprotein	165
Anti-(α-foetoprotein) antibody	Entrapment in polyacrylamide gel	Immunoadsorptive purification of mouse α-foetoprotein	614
Anti-(aminopeptidase) antibody	Reaction with agarose cyclic imidocarbonate	Removal of aminopeptidase during purification of sucrase–isomaltase	166
Anti-(barley β-amylase) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive/purification of barley β-amylase	167

Table 6 (*cont.*)

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
Anti-(baboon lung angiotensin 1-converting enzyme) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of angiotensin 1-converting enzyme on a large-scale	168
Anti-(L-aspartate aminopeptidase) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of L-aspartate aminopeptidase	169
Anti-(rabbit b4 immunoglobulin) antibody	Reaction with cellulose trans-2,3-carbonate	Immunoadsorptive isolation of b4(κ) immunoglobulin G	479
Anti-(blood-group A or B or O) antibody	Reaction with agarose cyclic imidocarbonate	Demonstration of association of blood-group A-determinants with carcinoembryonic antigen	170
Anti-(*human breast neoantigens*) antibody	Reaction with agarose cyclic imidocarbonate provided with linkage extension (52)	Immunoadsorptive purification of breast-specific neoantigens	171
Anti-($C_{1\bar{r}}$ sub-component of complement) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive removal of $C_{1\bar{s}}$ during purification of $C_{1\bar{r}}$	417
Anti-($C_{1\bar{s}}$ sub-component of complement) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive removal of $C_{1\bar{r}}$ during purification of $C_{1\bar{s}}$	417
Anti-(carcinoembryonic antigen) antibody	Reaction with cellulose cyclic imidocarbonate	Investigation of the binding of carcinoembryonic antigen with anti-(carcinoembryonic antigen) antibody	484
Anti-(catalase) antibody	Reaction with agarose cyclic imidocarbonate	Separation of catalase from phenylalanine hydroxylase	172
Anti-(human colon neoantigens) antibody	Reaction with agarose cyclic imidocarbonate provided with linkage extension (52)	Immunoadsorptive purification of colon-specific neoantigens	171
Anti-(2′,3′-cyclic nucleotide 3′-phosphohydrolase) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of 2′,3′-cyclic nucleotide 3′-phosphohydrolase	144
Anti-(*d*H-2^b histocompatibility antigens) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of H-2^b histocompatibility antigen	173

Anti-(factor VIII) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of human factor VIII	174
	Reaction with agarose cyclic imidocarbonate	Study of structure and function of factor VIII complexes	175
Anti-(β-D-glucuronidase) antibody $F(ab')_2$ fragments	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of urinary β-D-glucuronidase from mice	176
Anti-(human plasma group-specific component) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of an α_1-antitrypsin-like material from liver	177
Anti-(hepatitis B surface-antigen) antibody	Reaction with agarose cyclic imidocarbonate	Removal of hepatitis B surface-antigen from blood and blood products	178
Anti-(herpes virus type 1 glycoprotein) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of herpes virus type 1 glycoprotein	179
Anti-(hisB enzyme from *Salmonella typhimurium*) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of a bifunctional core of the hisB enzyme from *S. typhimurium*	180
Anti-(human immunoglobulin E) antibody	Glutaraldehyde- or carbodi-imide-mediated reaction with nylon balls or powder	Immobilized antibodies for use in enzyme immunoassays for immunoglobulin E	615
Anti-(human immunoglobulin G) antibody	Glutaraldehyde-mediated reaction with Silochrome®	Study of the interaction of immobilized antibodies with immunoglobulin G-peroxidase complexes	600
Anti-(guinea-pig immunoglobulin G) antibody	Reaction with activated thiol-agarose	Immobilized second antibodies for use in enzyme immunoassays for insulin	434
Anti-(human immunoglobulin G subclasses 1 and 2) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive isolation of subclasses of human immunoglobulin G	183
Anti-(rat glandular kallikrein) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive isolation of rat submandibular kallikreins	184
Anti-(human immunoglobulin G) antibody	Reaction with agarose cyclic imidocarbonate	Purification of human leukocyte interferon	181
	Reaction with agarose cyclic imidocarbonate	Study of the reactions and non-specific adsorptive properties of agarose cyclic imidocarbonate	182
Anti-(normal human serum) antibody	Reaction with agarose cyclic imidocarbonate provided with linkage extension (52)	Removal of contaminants during purification of organ-specific neoantigens	171

Table 6 (*cont.*)

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
Anti-(human α_2-macroglobulin) antibody	Reaction with agarose cyclic imidocarbonate	Quantitative immunoimmobilization of α_2-macroglobulin–β-trypsin complex	185
Anti-(human β_2-microglobulin) antibody	Reaction with agarose cyclic imidocarbonate	Isolation of plasma membrane components from cultured human pancreatic cells	186
Anti-nitrotyrosine antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of a nitrotyrosyl peptide obtained from 3-phosphoglycerate kinase	187
Anti-(pancreatic antigen) antibody	Glutaraldehyde-mediated cross-linking of the antibody	Immunoadsorptive purification of a human pancreas-specific protein	616
Anti-(phosphoenolpyruvate carboxylase) antibody	Glutaraldehyde-mediated reaction with Ultrogel®	Immunoadsorptive purification of phosphoenolpyruvate carboxylase	617
Anti-(*Schistosoma mansoni* soluble egg antigens) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of *S. mansoni* soluble egg antigens	188
Anti-(sucrase–isomaltase) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of sucrase–isomaltase from pig intestine	166
Anti-thrombin antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of anti-(thrombin–antithrombin complex) antibody; fractionation of heparin	190
	Reaction with agarose cyclic imidocarbonate	Fractionation of heparin	189
	Reaction with *sym*-triazine-activated agarose	Immunoadsorptive purification of thrombin	458
	Co-immobilized with heparin by reaction with agarose, polyvinylalcohol, polyhydroxyethylmethacrylate or silicone-coated nylon previously activated with cyanogen bromide	Examination of potential of immobilized preparation for provision of anticoagulant activity to biomedical materials	618

Anti-(bovine thyroid-stimulating hormone) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of thyroid-stimulating hormone from rat	191
Anti-(human transferrin) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of the transferrin receptor from human placenta	192
Apotransferrin	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of transferrin receptors from rat kidney cell membrane	202
Asialo-α_1-acid glycoprotein	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of a human hepatic lectin	230
Asialofetuin	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of lactose-binding proteins from adult chicken	232
	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of a lectin from *Bothrops atrox*	233
	Entrapment by cocasting in latex hydrazide spheres	Probes for influenza virus neuraminidase in infected cells	619
Axinella polypoides lectin	Reaction with agarose cyclic imidocarbonate	Comparative structural study of snail (*Helix pomatia*) galactans and sponge (*Axinella polypoides*) lectins	384
Bandeiraea simplicifolia lectin I	Reaction with agarose cyclic imidocarbonate	Resolution of structurally homologous sugar nucleotides and oligosaccharides	385
Blood-group (A + H) substance	Copolymerization with the *N*-carboxy-anhydride of L-leucine	Affinity chromatographic isolation of a lectin from *Dolichos biflorus*	243
	Copolymerization with the *N*-carboxy-anhydride of L-leucine	Affinity chromatographic purification of a lectin from tomato (*Lycopersicon esculentum*)	620
	Copolymerization with the *N*-carboxy-anhydride of L-leucine	Affinity chromatographic purification of a lectin from *Euonymus europaeus*	621
	Copolymerization with the *N*-carboxy-anhydride of L-leucine	Affinity chromatographic purification of a lectin from *Sophora japonica*	622
	Ethyl chloroformate-mediated copolymerization with bovine serum albumin	Affinity chromatography of blood-group A-specific lectin from *Vicia cracca*	623
Collagen	Reaction with agarose cyclic imidocarbonate	Investigation of the interactions between bovine cornea proteoglycans and collagen	391

Table 6 (*cont.*)

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
	Reaction with agarose cyclic imidocarbonate	Investigation of interactions with several glycosaminoglycans	392
Collagen, denatured collagen	Reaction with Sephadex® cyclic imidocarbonate	Investigation of the kinetics of binding of fibronectin to collagen	554
Concanavalin A	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of sucrase–isomaltase from pig small intestine	166
		Affinity chromatographic purification of an α_1-antitrypsin-like material from normal human liver	177
		Affinity chromatographic purification of liver α-L-fucosidase from a fucosidosis patient	208
		Identification of transformed liver cell colonies	241
		Affinity chromatographic purification of a murine melanoma-associated antigen	242
		Affinity chromatography of isolectins from *Dolichos biflorus*	243
		Affinity chromatographic purification of the D-glucose transport system from adipocyte plasma membrane	244
		Affinity chromatography of incomplete intracellular forms of intestinal surface membrane sucrase–isomaltase	245
		Affinity chromatographic purification of human fibroblast interferon	246
		Affinity chromatographic purification of herpes simplex virus tumour-associated antigen from human kidney carcinoma	247
		Affinity chromatography of lysosomal enzymes from mucolipidosis patients	248

Affinity chromatographic purification of CMP-*N*-acetylneuraminic acid: G_{M_3} ganglioside neuraminyltransferase (G_{D_3} synthetase)	249
Affinity chromatographic purification of Na,K-adenosine triphosphatase from rectal glands of spiny dogfish	250
Affinity chromatographic purification of an acid phosphatase from *Drosophila melanogaster*	251
Affinity chromatography of human leukocyte interferon components	252
Affinity chromatographic purification of antigenic fractions from mycelial fungi	253
Development of method for efficient and selective recovery of membrane glycoproteins from immobilized concanavalin A	254
Affinity chromatographic purification of antigens of *Fasciola hepatica* adult worms	255
Affinity chromatography of α-D-mannosidase from human plasma	256
Affinity chromatographic purification of acid β-D-galactosidase from feline liver	257
Affinity chromatographic purification of rat α-foetoprotein	258
Affinity chromatography of cathepsin D isoenzymes from porcine spleen	259
Affinity chromatographic purification of dipeptidyl peptidase IV from human submaxillary gland	260
Affinity chromatographic purification of acid phosphatase from rat Dunning prostatic adenocarcinoma	261

Table 6 (*cont.*)

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
Concanavalin A (*cont.*)	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of a preneoplastic antigen in liver carcinogenesis	262
		Affinity chromatographic purification of sphingolipid β-D-galactosidases from human placenta	263
		Determination of levels of β-D-fructofuranosidase produced by a mutant strain of *Saccharomyces cerevisiae*	264
		Affinity chromatographic purification of heparosan *N*-sulphate D-glucuronosyl 5-epimerase	265
		Affinity chromatography of lobster axon plasma membrane proteins	266
		Affinity chromatographic purification of gonadotrophins	267
		Affinity chromatographic purification of myeloperoxidase from human blood	268
		Affinity chromatographic purification of human fibroblast interferon	125
		Affinity chromatographic purification of α-lactalbumin from mouse milk	269
		Affinity chromatographic purification of nucleoside diphosphatase from rat liver microsomes	124
		Affinity chromatographic purification of Tora-bean (*Phaseolus vulgaris*) lectin	270
		Affinity chromatography of antigenic components of detergent-solubilized adipocyte intrinsic membrane proteins	164

	Affinity chromatography of large glycopeptides of human teratocarcinoma-derived cells	271
	Affinity chromatographic purification of a tartrate-sensitive splenic acid phosphatase from patients with Gaucher's disease	272
	Affinity chromatographic purification of cholinesterase from chicken egg yolk	273
	Affinity chromatographic purification of tumour-specific transplantation antigens from induced murine sarcomas	274
	Affinity chromatography of gelonin, an inhibitor of protein synthesis	275
	Affinity chromatographic purification of α-D-2-acetamido-2-deoxy-galactosidase from porcine liver	276
	Affinity chromatography of platelet glycoproteins	277
	Affinity chromatographic purification of aspartate transfer ribonucleic acids	278
	Affinity chromatographic purification of alkaline phosphatases from hen's egg yolk	229
	Study of lectin–carbohydrate interactions using a competitive enzyme inhibition assay	393
	Investigation of effects of detergents on the binding of hepatoma cell-surface D-[^{3}H]galactoglycoproteins to immobilized concanavalin A	394
	Support for the immobilization of phosphodiesterase	395
Erythrocytes, trypsin-treated	Affinity chromatographic purification of a lectin from tomato (*Lycopersicon esculentum*)	624
Unmodified		

Table 6 (*cont.*)

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
Fetuin	Reaction with cyanogen bromide derivatives of agarose, cellulose, and cross-linked dextran	Development of a micromethod for quantitation of immobilized neuraminic acid in sialoglycoconjugates	90
	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of lectin-like molecules on murine thymocytes and splenocytes	281
	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of potato lectin	282
	Entrapment by cocasting in latex hydrazide spheres	Probes for influenza virus neuraminidase in infected cells	619
Fibrinogen	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of platelet proteins	285
	Reaction with *sym*-triazine-activated agarose	Investigation of the interaction of fibrinogen and heparin	458
	Entrapment in polyacrylamide gel	Substrate for modified proteolytic enzymes	597
	Reaction with *N*-hydroxysuccinimide-derivatized beads of poly(acrylonitrile)	Development of an assay for detection of defective platelets	625
Fibronectin	Reaction with agarose cyclic imidocarbonate	Investigation of interactions between fibronectin, gelatin, and glycosaminoglycans	392
Follitrophin	Reaction with Affi-gel® 10	Affinity chromatographic purification of folitrophin receptors from calf testes	413
Human growth hormone	Reaction with Affi-gel® 10	Affinity chromatographic purification of a non-primate growth-hormone receptor	410, 411
Haptoglobin	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of haemoglobin	290
Helix pomatia agglutinin	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of B and T lymphocytes	291

Immunoglobulin G	Reaction with agarose cyclic imidocarbonate	Model system for investigation of method of stabilizing proteins immobilized on agarose by glutaraldehyde-cross-linking	135
	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of C_{1q}, a subcomponent of the first component of complement	193
	Reaction with agarose previously activated with divinylsulphone, treated with 4-nitrobenzyl alcohol, and diazotized	Immunoadsorptive purification of subcomponents of complement	417
	Emulsion polymerization in biodegradable microspheres of poly(acryloyldextran)	Investigation of a new matrix for immobilization	611
	Reaction with epoxy-activated acrylic beads	Investigation of characteristics of a new matrix for immobilization	612
Lactoferrin	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of transferrin receptors from rat kidney cell membrane	202
Lens culinaris phytohaemagglutinin	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of H-2^b histocompatibility antigen	173
	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of *tumour-specific transplantation antigens* from induced murine sarcomas	274
	Reaction with agarose cyclic imidocarbonate	Removal of contaminating membrane glycoproteins during purification of P-glycoprotein from Chinese hamster ovary cell mutants	301
	Reaction with agarose cyclic imidocarbonate	Study of culture density and age-dependent interactions of 3T3 and SV3T3 cells with *Lens culinaris* lectin	401
	Reaction with *N*-hydroxysuccinimide derivative of epoxy-activated agarose pre-reacted with ammonia and succinic anhydride	Affinity chromatography of solubilized human red blood cell membrane	425

Table 6 (*cont.*)

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
Ovomucoid	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic removal of proteases from hyaluronidase preparations	307
Peanut agglutinin	Reaction with agarose cyclic imidocarbonate	Study of the reactivity of human lymphoid and lymphoblastoid cells with the lectin	403
Pisum sativum lectin	Reaction with agarose cyclic imidocarbonate	Removal of contaminating membrane glycoproteins during purification of P-glycoprotein from Chinese hamster ovary cell mutants	301
Ricinus communis agglutinin	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of tumour-specific transplantation antigens from induced murine sarcomas	274
	Reaction with agarose cyclic imidocarbonate	Removal of contaminating membrane glycoproteins during purification of P-glycoprotein from Chinese hamster ovary cell mutants	301
	Reaction with agarose cyclic imidocarbonate	Investigation of glycoproteins in purified myelin	313
	Reaction with agarose cyclic imidocarbonate	Investigation of effects of detergents on binding of hepatoma cell-surface [^{3}H]D-galactoglycoproteins to immobilized *R. communis*	394
Ricinus communis toxin	Reaction with agarose cyclic imidocarbonate	Determination of the critical density of immobilized toxin below which protein–protein monoconjugation occurs using lysozyme and glutaraldehyde-cross-linking	405
Soybean trypsin inhibitor	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic concentration of and isolation of proteases	626
	Reaction with agarose activated with 4-toluenesulphonyl chloride	Affinity chromatographic purification of trypsin	435

	Reaction with agarose cyclic imidocarbonate provided with linkage extension (50)	Affinity chromatographic purification of an acrosin-like enzyme from sea urchin	380
	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of trypsins and chymotrypsins	315
	Reaction with agarose cyclic imidocarbonate	Removal of proteases during purification of AMP-dependent protein kinases	314
Thyroglobulin	Reaction with agarose cyclic imidocarbonate	Removal of phytohaemagglutinin from supernatants of lymphocytes cultured with phytohaemagglutinin	317
Thyroid-stimulating hormone	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of anti-(thyroid-stimulating hormone) antibody	191
Transferrin	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of the transferrin receptor from human placenta	192
	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of soluble transferrin receptors	202
Tridacnin	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of the arabinogalactan from *Gladiolus* stigma	318
Wheat germ agglutinin	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of antigenic components of detergent-solubilized adipocyte intrinsic membrane proteins	164
	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of cathepsin D isoenzymes	259
	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of tumour-specific transplantation antigens from induced murine sarcomas	274
	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of gamma protein from HeLa cells	321
	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of cardiac sarcolemmal vesicles	322

Albumin immobilized in spherical polyacrylamide gel microparticles has been used in a study of the binding of drugs (diazepam, digitoxin, and warfarin) to albumin.[629] Three separate discrete binding sites on albumin were demonstrated. The method has wide applicability for determination of the capacity of drugs and other compounds to affect binding of radioactively labelled markers.

4 Modification of Enzymes and Uses of Modified Enzymes

β-D-2-Acetamido-2-deoxyhexosidases. – Placental β-D-2-acetamido-2-deoxyhexosidase A and the α-subunits of the same enzyme from human kidney have been cross-linked with glutaraldehyde.[630] The modified enzymes were used successfully to elicit antibodies to the enzymes in rabbits.

β-Amylases. – Soybean β-amylase has been modified by reaction with sulphydryl reagents.[631] Two sulphydryl groups out of a total of four in the native enzyme reacted with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), iodoacetamide, or monoiodoacetate at high ionic strength, resulting in inactivation of the enzyme. At low ionic strength only one sulphydryl group was accessible to DTNB, and the enzyme remained active. Maltose and cyclomaltohexaose protect the essential sulphydryl group from modification.

α-L-Fucosidases. – Two non-identical subunits (mol. wt. 5.9×10^4 and 5.4×10^4) of human liver α-L-fucosidase have been demonstrated after reduction and *S*-carboxymethylation of the enzyme.[632]

D-Galactose Oxidase. – The effects of anions and ferricyanide on the Cu^{II} binding site of three chemically modified forms of D-galactose oxidase have been studied.[633] Results suggested that different factors affect binding of anions and binding of substrates, and that different sites at the one Cu^{II} centre are involved in the two processes.

β-D-Galactosidases. – ^{14}C-Carboxymethylation of cysteine residues has been reported for wild-type and mutant forms of β-D-galactosidase from *Escherichia coli*.[634] The extent of modification and its effects on biological activity, antibody elicitation, physical stability, and labelling of particular residues of or by the enzyme were described.

β-D-Galactosidase has been conjugated to antibodies raised against rabbit Fc fragments.[601] Conjugation was effected using 3-maleimido-benzoyl-*N*-hydroxysuccinimide ester. *N*,*N*′-(Oxydimethylene) dimaleimide has also been used successfully for covalently coupling β-D-galactosidase to immunoglobulin molecules.[599]

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D-Glucose Oxidase. – Acidification of a solution of D-glucose oxidase in acidified glycerol has yielded apo-D-glucose oxidase with low residual enzyme activity.[635] On further treatment with charcoal an inactive apo-enzyme preparation was obtained. The apo-enzyme has been used to develop a convenient and rapid method for amperometric measurement of flavin adenine dinucleotide at extremely low concentrations (10^{-12} M).

Lysozyme. – A reductive modification of L-tryptophan residues in hen egg white lysozyme has been performed in trifluoroacetic acid with pyridine-borane.[636] Six L-tryptophan residues were fully reduced to the corresponding 2,3-dihydro-compounds without any detectable change in the enzyme molecule, but enzymic activity was abolished in the reduced lysozyme.

The titration curves of native lysozyme and a modified derivative containing an ester linkage between glutamic acid-35 and δ-hydroxytryptophan-108 have been compared.[637] Native lysozyme contained one more ionizable group with pK 6.1 than did the modified enzyme. This result indicates that glutamic acid-35 has a pK of 6.1, that the state of ionization of glutamic acid-35 has little effect upon the dissociation behaviour of other groups, and that, since the modified polysaccharide has much less fluctuation due to the intramolecular cross-link protein fluctuation has little effect on the titration curves.

Natural abundance ^{13}C n.m.r. spectroscopy has been applied to a study of the carboxymethylation of histidine-15 of hen egg white lysozyme upon treatment with iodoacetate.[638] The chemical modification was shown not to affect significantly the conformation of the protein.

α-D-Mannosidase. – The chemical modification of L-tryptophan residues of *Phaseolus vulgaris* α-D-mannosidase using *N*-bromosuccinimide or 2-hydroxy-5-nitro-benzyl bromide has been reported.[639]

Peroxidases. – The oxidation of native horse radish peroxidase to Compound II by photochemically generated inorganic free radicals (SO_4^{2-}, Cl_2^-, and Br_2^-) has been studied.[640] The rates of accumulation of Compound II for different rates of free-radical generation and for different reagent concentrations were measured.

Peroxidase has been activated by treatment with fluorodinitrobenzene and periodate and then covalently attached to the outer surface of liposomes.[641] The enzyme is located on the external surface of the liposome membrane and may be used for morphological study of coated liposomes and their interactions with cells. Minimal noncovalent attachment of the enzyme to the liposomes was observed.

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Phosphatases. – Alkaline phosphatase and insulin have been separately activated with methyl 4-mercaptobutyrimidate.[642] The modified insulin was further reacted with 2,2′-pyridine disulphide and then coupled with the activated alkaline phosphatase *via* a disulphide exchange reaction. The product was used in an enzyme immunoassay for insulin.

Immobilized Derivatives of Enzymes. – Several reviews concerning the present and future applications of immobilized enzymes have appeared.[643–647] The proceedings of a conference on the future of enzyme engineering development include reports of applications of immobilized enzymes.[648] The book highlights those areas of enzyme engineering showing the greatest promise for future practical exploitation and emphasizes the industrial and biomedical importance of immobilized enzymes. Recent developments and future directions of enzyme technology in Japan have been reviewed.[649]

The use of immobilized enzymes in evaluating subunit interactions has been described.[650] Potential application of immobilized enzyme derivatives in enzyme therapy of genetic disease has been discussed.[651]

Requirements of immobilized enzyme engineering unique to the food and beverage industry have been highlighted and potential applications of immobilized carbohydrases, proteases, and other enzymes in food processing have been described.[652] Recent commercial and research developments in the field are also discussed.

The uses of immobilized enzymes in analytical chemistry have been comprehensively reviewed.[653–655] In another review the principles of enzyme immobilization have been summarized and the application of enzyme electrodes, enzyme thermistors, and enzyme reactors in bioanalysis have been described.[656] The advantages and shortcomings associated with analytical devices based on immobilized enzymes are identified, and ways of minimizing the disadvantages are suggested.

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In a recent book the strengths and weaknesses of several immobilized enzyme techniques have been systematically assessed, and a theoretical framework for development of analytical immobilized enzyme systems has been established.[657] The authors are particularly concerned with such applications in analytical clinical chemistry.

A comparison of four commercial instruments that measure D-glucose using immobilized reagents has been published.[658] The biamperometric determination of ethanol, lactate, and glycerol using immobilized enzymes in flow streams has been reported.[659]

A survey of the applications of enzyme electrode probes has been presented.[660] Probes for the analysis of more than 40 different substrates and 25 different enzymes are described. Some of the basic properties of enzyme electrodes are described.

Polymeric matrices suitable for the immobilization of enzymes and other biologically active molecules have been discussed in two reviews.[661,662] The use of chitin and chitosan for immobilization of enzymes has been described.[663]

A review has appeared concerning the use of magnetic supports for immobilizing enzymes and preparing bioaffinity adsorbents.[664] The immobilization of enzymes on titanium-activated inorganic supports has been reviewed.[665] The Tate and Lyle Carbohydrate Chemistry Award Lecture, concerning transition-metal oxide chelates of carbohydrate-directed macromolecules, has appeared.[666] Enzyme immobilization by condensation copolymerization into cross-linked polyacrylamide gels has been discussed.[667] The preparation of 4-aminobenzylcellulose and its utilization for enzyme immobilization have been described.[477]

The immobilization of glycoenzymes through their carbohydrate side-chains has been reported.[668]

Several reviews concerning individual immobilized enzyme systems have appeared. These include a review of the preparation and properties of immobilized sequence specific endonucleases,[325] a discussion of the use of immobilized β-D-galactosidase for the continuous hydrolysis of lactose in milk systems,[669] a

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review of the enzymatic saccharification of cellulose in membrane reactors,[670] and a review of the properties and applications of immobilized sulphydryl oxidase preparations.[671]

Ultrafiltration of raw sewage has been achieved using multiple enzyme systems immobilized by adsorption on non-cellulosic ultrafiltration membranes.[672]

The kinetics of immobilized enzyme systems have been described.[673] External diffusion effects on the kinetic constants of immobilized enzyme systems[674] and diffusional limitations in liquid-phase reactions catalysed by enzymes immobilized on porous supports[675] have been discussed. In another theoretical treatment the effects of intraparticle diffusion on a single pellet of immobilized enzyme have been analysed in order to predict the existence of multiple steady states for any given kinetic scheme.[676]

A simple mathematical model has been developed to describe the behaviour of enzymes immobilized within an artificial membrane with a homogeneous distribution of the active sites.[677]

A pH-dependent deactivation model has been developed for immobilized and soluble enzymes in which the rate of enzyme decay is assumed to be proportional to the concentration of the active enzyme.[678] The model developed applies reasonably well to pH-stability data previously reported.

Theoretical treatments concerning the behaviour of immobilized enzymes in packed-bed and other reactors have been published.[679,680] Kinetic studies of a membrane enzyme reactor have been described.[681] Unstirred plane membrane ultrafiltration cells have been used as enzymatic reactor units and a theoretical treatment of their behaviour has been presented.[682]

The use of immobilized enzymes in continuous-flow analysis has been described.[683]

Interest in the technology and future applications of immobilized cells continues to expand.[644,684,685]

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The use of immobilized microbes has been reviewed.[686] Reviews have appeared concerning methods of immobilization of microbial cells,[687] preparation of immobilized microbial cells for use in fermentation processes,[688] entrapment of microbial cells in cellulose beads,[689] and immobilization of microbial cells on metal hydroxides.[690]

The preparation of immobilized animal cells has been described.[691]

Photocross-linkable resin prepolymers and urethane prepolymers have been used for the immobilization by entrapment of enzymes, microbial cells, and organelles.[692] Protein foam structures bearing cells and organelles have been produced by glutaraldehyde-mediated cocross-linking with serum albumin at sub-zero temperatures.[693]

The application of fibre-entrapped whole microbial cells to the hydrolysis of urea has been described.[694]

A theoretical treatment explaining the kinetics of gas production by immobilized micro-organisms has been presented.[695]

Many preparations of immobilized enzymes and cells and of enzymes coupled to other macromolecules to give water-soluble or -insoluble forms have been reported during the past year. These derivatives and their uses are summarized in Table 7.[696-864] Immobilized enzymes are referred to in this Table, whether they are enzymically active or not. Immobilized cells are included in this Table as most of them retain some enzymic activity after immobilization.

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Table 7 *Modification of enzymes and cells by coupling to insoluble matrices and other macromolecules*

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
Acid phosphatase	3.1.3.2	Entrapment within layers of polymerized human serum albumin	Multilayer immobilized enzyme for use in an ultrafiltration cell	696
Actinoplanes missouriensis cells	–	Entrapment in cellulose acetate beads	Living immobilized cells with D-glucose isomerase activity	474
Alcaligenes eutrophus cells	–	Entrapment in alginate gel	Living immobilized cells with hydrogenase activity for the regeneration of coenzymes	697
Alcohol dehydrogenase	1.1.1.1	Reaction with agarose cyclic imidocarbonate	Investigation of the interaction between subunits of the immobilized oligomeric enzyme	137
		Reaction with agarose cyclic imidocarbonate	Enzyme electrode for the determination of ethanol	138
		Reaction with octyl- or phenyl-agarose pre-treated with dodecyl-, octyl-, or hexyl-aldehyde	Validation of a new method of protein immobilization using reductive alkylation with hydrophobic aldehydes	132
		Reaction with 4-toluenesulphonyl chloride-derivatized agarose	Active immobilized enzyme; demonstration of new method of enzyme immobilization	435
		Reaction with pre-derivatized nylon tubing	Active immobilized enzyme; investigation of the flow kinetics of the system for the reaction between ethanol and NAD	698
		Reaction with pre-derivatized nylon tubing	Active immobilized enzyme; investigation of the effect of temperature on the immobilized enzyme in flow systems; determination of activation energies	699

		Glutaraldehyde-mediated cross-linking with albumin to form a membrane	Active immobilized enzyme; investigation of the feasibility of cofactor regeneration with an electron carrier	700
		Glutaraldehyde-mediated reaction with aminoethyl-cellulose; reaction with *sym*-triazine-activated cellulose; reaction with agarose cyclic imido-carbonate	Affinity chromatography of NAD	701
Aldehyde dehydrogenase	1.2.1.3	Co-immobilization with urate oxidase on nylon pre-activated with triethyloxonium-tetrafluoro-borate, copolymerized with polyethyleneimine and glutaraldehyde-cross-linked	Stable nylon tube enzyme reactor for the automated determination of uric acid in serum	702
		Reaction with agarose cyclic imidocarbonate using low levels of cyanogen bromide to favour attach-ment of the dimeric enzyme through a single covalent linkage	Investigation of functional and structural properties of immobi-lized subunits of the enzyme	139
		Reaction with agarose cyclic imidocarbonate	Investigation of effects of diffu-sional limitation on the activity of immobilized enzymes	140
Amine oxidase	1.4.3.4	Co-casting with collagen followed by glutaraldehyde-cross-linking	Enzyme electrode for deter-mination of monoamines in freshness testing of meat	703
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme used in column form for assay of polyamines	141
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme	119
Aminoacylase	3.5.1.14	Reaction with hydrazine-activated poly-γ-methyl-D-glutamate glass beads	Active immobilized enzyme	704
α-Amylase	3.2.1.1	Graft copolymerization of vinylated enzyme with cellulose, agarose, starch, or cross-linked dextran matrices	Investigation of a new coupling method and the properties of the active immobilized enzymes	705
		Reaction with *S*-methyldithiocarbonate and dithiobis(thioformate) derivatives of cellulose	Investigation of the properties of the active immobilized enzyme	498

Table 7 (*cont.*)

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Glutaraldehyde-mediated reaction with alkylamine-silica	Active immobilized enzyme used in recirculated differential batch reactors for the production of high-maltose syrups	706
		Reaction with cyanogen bromide-activated hydroxy-alkylmethacrylate gel (Spheron®)	Active immobilized enzyme	707
		Reaction with polyphenolic resins prepared by copolymerization of hydroquinone and formaldehyde or glutaraldehyde	Investigation of new matrices for the preparation of active immobilized enzymes	708
		Reaction with Ti^{IV} oxide coated magnetic iron oxide	Study of the properties of the active immobilized enzyme	709
β-Amylase	3.2.1.2	Glutaraldehyde-mediated reaction with alkylamine-silica	Active immobilized enzyme used in recirculated differential batch reactors for the production of high-maltose syrups	706
		Reaction with cyanogen bromide-activated hydroxy-alkylmethacrylate gel (Spheron®)	Active immobilized enzyme	707
		Reaction with diazotized 4-aminobenzyl-cellulose	Mixed with immobilized pullulanase for continuous conversion of starch to maltose	710
Arginosuccinate lyase Arginosuccinate synthetase	4.3.2.1 6.3.4.5	Entrapment, with other enzymes of the urea cycle, into a matrix of fibrin formed by proteolytic digestion of fibrinogen	Active immobilized enzymes comprising the urea cycle	711
Arthrobacter simplex cells	–	Copolymerization with water-miscible urethane prepolymers	Living immobilized cells for conversion of hydrocortisone to prednisolone	712
Arthrobacter X4 cells	–	Entrapment in gelatin followed by glutaraldehyde-cross-linking	Living immobilized cells with xanthine oxidase activity;	713

			study of application of the living immobilized cells for organic synthesis	
L-Asparaginase	3.5.1.1	Reaction with octyl- or phenyl-agarose pre-treated with dodecyl-, octyl-, or hexyl-aldehyde	Validation of a new method of protein immobilization using reductive alkylation with hydrophobic aldehydes	132
Aspartate aminotransferase	2.6.1.1	Reaction with acyl azide-derivatized collagen membranes	Active immobilized enzyme; comparison of denaturation of soluble and immobilized enzymes	714
Azotobacter vinelandii cells	–	Adsorption on cationic or anionic ion-exchange resins; reaction with cellulose activated with cyanuric chloride	Living immobilized cells for use in microbial reactors	715
Bacillus sp. (thermophilic) cells	–	Entrapment in polyacrylamide gel	Living immobilized cells with β-D-galactosidase activity	716
Bacillus sp. cells	–	Entrapment in polyacrylamide gel	Living immobilized cells for continuous production of bacitracin	717
Bacillus sp. (alkalophilic) cells	–	Entrapment in polyacrylamide gel	Living immobilized cells for preparation of phenylglycine-related D-amino-acids	718
Bacillus stearothermophilus cells	–	Glutaraldehyde-mediated reaction with diethyl-aminoethyl-cellulose	Living immobilized cells with β-D-galactosidase activity	719
Brevibacterium ammoniagenes cells	–	Entrapment in polyacrylamide gel	Living immobilized cells with fumarase activity	720
Brevibacterium flavum cells	–	Entrapment in κ-carrageenan	Living immobilized cells for continuous production of L-malic acid	721
Caldariella acidophila cells	–	Co-casting into cellulose acetate membranes	Living immobilized cells with β-D-galactosidase activity	475
Candida lipolytica cells	–	Entrapment in polyacrylamide gel	Living immobilized cells	722

Table 7 (*cont.*)

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Entrapment in polyacrylamide gel	Living immobilized cells for continuous synthesis of citric acid	723
Candida tropicalis cells	–	Copolymerization with a photocross-linkable resin prepolymer or with a urethane prepolymer	Living immobilized cells with adenylate kinase activity	724
		Adsorption onto a ceramic support	Investigation of adsorption as a method of cell immobilization	725
Carbonate dehydratase	4.2.1.1	Emulsion copolymerization with acrylamide and acryldextran	Investigation of properties of active immobilized enzyme	611
Carboxypeptidase Y	–	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme for α-carboxyl deblocking in peptide synthesis	142
Catalase	1.11.1.6	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme	119
		Emulsion copolymerization with acrylamide and acryldextran	Investigation of properties of active immobilized enzyme	611
		Glutaraldehyde-mediated copolymerization with D-glucose oxidase and albumin	Enzyme electrode for determination of D-glucose	613
		Entrapment in polyacrylamide gel; coimmobilized with D-glucose oxidase by encapsulation in cellulose acetate	Direct measurement of oxygen concentration gradient around particles of immobilized enzyme	726
		Glutaraldehyde-mediated cross-linking with D-glucose oxidase on to particles of inactive protein	Oxidation of D-glucose in D-glucose–D-fructose mixtures; removal of oxygen from beer	727
		Cocasting with peroxidase or peroxidase and D-glucose oxidase with gelatin into membranes	Enzyme electrodes for determination of peroxidase substrates	728
		Glutaraldehyde-cross-linking on to polyethyleneimine-coated glass microbeads	Active immobilized enzyme	729

		Entrapment in photosensitive polymers of 2-chloroethylvinyl ether, *N*-vinyl pyrrolidone, and stilbazole	Active immobilized enzyme; development of new method of enzyme immobilization	730
Catechol 1,2-dioxygenase	1.13.11.1	Glutaraldehyde-cross-linked with or without albumin; reaction with agarose cyclic imidocarbonate	Enzyme electrode for determination of catechol	731
Cellulase	3.2.1.4	Entrapment in polyacrylamide gel	Part of bienzyme electrode system for determination of D-glucose released from cellulase substrates	732
		Glutaraldehyde-mediated reaction with silanized Spherosil®; adsorption on Duolite® S-761 resin	Active immobilized enzyme for use in enzyme reactors	733
Chloroplasts		Glutaraldehyde-cross-linking with albumin at subzero temperatures	Scanning and transmission electron microscopic studies of the immobilized cells	693
		Entrapment in agar gel	Immobilized photochemical system for regenerating NADPH from NAD	734
		Copolymerization with albumin and 2-hydroxyethyl acrylate	Living immobilized cells; investigation of thermal stability of living immobilized cells and method of immobilization	735
Choline oxidase	1.1.3.17	Glutaraldehyde-mediated reaction with partially aminated polyacrylonitrile membranes	Enzyme electrode for the amperometric determination of choline	736
Chromatophores	–	Glutaraldehyde-cross-linking with albumin at subzero temperatures	Scanning and transmission electron microscopic studies of the immobilized cells	693
Chymotrypsin	3.4.21.2	Reaction with agarose cyclic imidocarbonate	Study of association–dissociation phenomena in proteins	737
		Reaction with agarose cyclic imidocarbonate	Investigation of effects of diffusional limitation on the activity of immobilized enzymes	140
		Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of protease inhibitor V from soybean	143

Table 7 (*cont.*)

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Graft copolymerization of vinylated enzyme with cellulose, agarose, starch, or cross-linked dextran matrices	Investigation of a new coupling method and of the properties of the active immobilized enzymes	705
		Reaction with poly(*N*-substituted acrylamide) gels	Active immobilized enzyme	738
		Reaction with polymers containing isonitrile functional groups	Active immobilized enzyme; development of new methods of enzyme immobilization	89
		Glutaraldehyde-mediated reaction with (3-aminopropyl)-triethoxysilane-treated agarose	Active immobilized enzyme	133
		Entrapment in cellulose acetate films	Active immobilized enzyme	739
		Reaction with activated poly(vinyl alcohol) matrices	Active immobilized enzyme	740
		Entrapment in polyacrylamide gel	Investigation of thermal denaturation of active immobilized enzyme	161
		Reaction with hydrazine-activated poly-γ-methyl-D-glutamate glass beads	Active immobilized enzyme	704
		Reaction with glass beads pre-treated with pyridine 4-carboxyaldehyde and then activated with cyanogen bromide	Active immobilized enzyme	741
		Reaction with Sephadex® activated with cyanogen bromide	Study of effects of organic solvents on catalysis by immobilized enzymes	742
		Following acryloylation of the enzyme, entrapment in polyacrylamide granules	Study of properties of the active immobilized enzyme	743
		Following *cis*-cinnamoylation of the enzyme, microencapsulation into semi-permeable nylon membranes	Photosensitive immobilized enzyme system for determination of light signals	744
		Following protection of the enzyme with a synthetic macromolecular inhibitor, reaction with Acaprosuc® (an insoluble cross-linked polyacrylic gel)	Active immobilized enzyme	745

Clostridium cells	–	Formaldehyde-mediated cross-linking with gelatin; entrapment in polyacrylamide gel	Living immobilized cells for stereospecific hydrogenation reactions	746
Clostridium acetobutyricum cells	–	Entrapment in alginate gel	Living immobilized cells for solvent production	747
		Entrapment in alginate gel	Living immobilized cells for continuous production of butan-1-ol and propan-2-ol	748
		Entrapment in agar gel	Living immobilized cells for continuous production of H_2 from alcohol factory waste waters	749
		Entrapment in polyacrylamide gel, agar gel membranes, or collagen membranes	Living immobilized cells; investigation of behaviour of released hydrogen in gel membrane	750
Corynebacterium simplex cells	–	Cocasting with collagen followed by glutaraldehyde-mediated cross-linking	Living immobilized cells for production of prednisolone from hydrocortisone	751
Curvularia lunata spores	–	Entrapment in polyacrylamide granules or in calcium alginate beads	Following germination of the spores, living immobilized cells for the conversion of cortexolone to cortisol	752
Cytochrome P450	–	Entrapment in gelatin membranes; co-immobilized with D-glucose oxidase by the same method	Enzyme electrode for determination of cytochrome P450 substrates	753
Deoxyribonuclease I	3.1.21.1	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of actin	145
Dextranase	3.2.1.11	Reaction with porous Ti^{IV} oxide particles pre-coated with diazotized 1,3-diaminobenzene	Active immobilized enzyme employed in reactor-separator for hydrolysis of macromolecular carbohydrates	754
		Reaction with agarose cyclic imidocarbonate	Investigation of effects of diffusional limitation on the activity of immobilized enzymes	140

Table 7 (*cont.*)

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Glutaraldehyde-mediated reaction with zirconia-coated alkylamine glass	Active immobilized enzyme	755
Dextransucrase	2.4.1.5	Glutaraldehyde-mediated reaction with alkylamine silica	Study of properties of active immobilized enzyme	756
		Reaction with glutaraldehyde-activated Spherosil®	Investigation of properties of active immobilized enzyme	757
Dipeptidase	3.4.13.11	Entrapment, with other enzymes of the urea cycle, into a matrix of fibrin formed by proteolytic digestion of fibrinogen	Active immobilized enzymes comprising the urea cycle	711
Enoate reductase	–	Reaction with glutaraldehyde-activated controlled pore glass; co-immobilized with formate dehydrogenase by the same method	Study of properties of the active immobilized enzyme	746
Erythrocytes	–	Glutaraldehyde-cross-linking with albumin at sub-zero temperatures	Scanning and transmission electron microscopic studies of the immobilized cells	693
		Entrapment in alginate gel	Development of a new method of storage for erythrocytes	588
Escherichia coli cells	–	Entrapment on nylon mesh between silicone rubber and cellophane membranes	Potentiometric microbial electrode for determination of L-glutamic acid	758
		Entrapment in κ-carrageenan gel	Living immobilized cells for continuous glutathione production	759
		Entrapment in κ-carrageenan gel followed by glutaraldehyde treatment in the presence of 1,6-diaminohexane	Living immobilized cells for continuous glutathione production	760
		Entrapment in κ-carrageenan gel with or without locust bean gum, followed by glutaraldehyde treatment with or without 1,6-diaminohexane	Living immobilized cells for production of L-aspartic acid	761

		Entrapment in polyacrylamide gel in the presence of L-aspartic acid	Kinetic study of aspartate aminotransferase reaction catalysed by free and immobilized *E. coli* cells	762
		Entrapment in polyacrylamide gel	Thermal stability studies of aspartate ammonium lyase reaction catalysed by free and immobilized *E. coli* cells	763
Fatty acid synthetase	–	Reaction with agarose cyclic imidocarbonate	Immunoadsorptive purification of rabbit anti-(fatty acid synthetase) antibody	146
Flavokinase	2.7.1.26	Carbodi-imide-mediated reaction with aminoalkyl-agarose beads	Affinity chromatographic preparation of flavin 5'-phosphates	764
Formate dehydrogenase	1.2.1.2	Reaction with glutaraldehyde-activated controlled pore glass; co-immobilized with enoate reductase by the same method	Study of properties of the active immobilized enzyme	746
β-D-Fructofuranosidase	3.2.1.26	Entrapment in polyacrylamide gel	Development of a theoretical method for prediction of elution profiles from immobilized enzyme columns	765
		Entrapment in photosensitive polymers of 2-chloroethyl vinyl ether, *N*-vinyl pyrrolidone, and stilbazole	Active immobilized enzyme; development of new method of enzyme immobilization	730
		Reaction with porous glass coated with ZrO_2	Enzyme thermistor for control of sucrose concentration in a continuously operating model fermenter	766
		Entrapment in polyacrylamide gel	Part of bienzyme electrode system for determination of D-glucose released from cellulase substrates	732
		Glutaraldehyde-mediated reaction with alkylamine-glass; adsorption on to cation- or anion-exchange resins; carbodi-imide-mediated reaction with cation-exchange resin	Active immobilized enzyme; study of properties of immobilized and soluble enzymes	767, 768

Table 7 (*cont.*)

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Adsorption on anion-exchange resin	Study of diffusional limitations in liquid-phase reactions catalysed by enzymes immobilized on porous supports	675
D-Galactose oxidase	1.1.3.9	Modification of a commercially available D-glucose analyser by substituting D-galactose oxidase for D-glucose oxidase	Analysis of D-galactose in neonates; comparison of method with a modified homogeneous enzymic method	769
β-D-Galactosidase	3.2.1.23	Reaction with 4-aminocarbanilated derivatives of cellulose activated by treatment with sodium nitrite, *N*-hydroxysuccinimide, or glutaraldehyde	Investigation of new polymeric matrix for enzyme immobilization	476
		Reaction with epoxy-activated agarose	Active immobilized enzyme for use in enzyme reactors	612
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; removal of D-galactose from glycolipids and glycoproteins	147
		Co-immobilized with *Saccharomyces cerevisiae* cells	Fermentation of cellobiose (produced by *S. cerevisaie* cells) to ethanol	770
endo-D-Galacturonanase		Reaction with Spheron® activated with cyanogen bromide	Study of the properties of the active immobilized enzyme	771
β-D-Glucanase		Adsorption on to Duolite® S-761 resin	Active immobilized enzyme	736
Glucoamylase	3.2.1.3	Glutaraldehyde-mediated reaction with alkylamine-silica	Active immobilized enzyme used in recirculated differential batch reactors for production of high-maltose syrups	706
		Reaction with polyphenolic resins prepared by polymerization of hydroquinone and formaldehyde or glutaraldehyde	Investigation of new matrices for the preparation of active immobilized enzymes	708

		Co-casting with collagen followed by glutaraldehyde-cross-linking	Active immobilized enzyme for hydrolysis of soluble starch	772
		Entrapment in photosensitive polymers of 2-chloroethyl vinyl ether, *N*-vinyl pyrrolidone, and stilbazole	Active immobilized enzyme; development of new method of enzyme immobilization	730
		Reaction with diazotized 5-aminosalicylic acid bonded to a TiO_2 film deposited on glass beads	Active immobilized enzyme; optimization of a new method for immobilization of enzymes	773
		Reaction with titanium–urea, –acrylamide, –citric acid, or –lactose complexes; reaction with Ti^{IV} chloride	Active immobilized enzyme	774
		Glutaraldehyde-mediated reaction with silylated porous silica gel	Study of thermal stabilization of glucoamylase	775
		Glutaraldehyde-mediated reaction with ZrO_2-coated silanized glass	Study of effects of pore-fusion limitation on dextrin hydrolysis by the active immobilized enzyme	776
		Adsorption on to Duolite® S–761 resin	Active immobilized enzyme	733
		Entrapment in polyacrylamide gel followed by adsorption on SP-Sephadex®	Study of thermal stability of the active immobilized enzyme	777
		Entrapment in polyacrylamide gel	Study of thermal stability of the active immobilized enzyme	778
		Entrapment in polyacrylamide gel; glutaraldehyde-mediated reaction with silk	Part of bienzyme electrode system for determination of D-glucose released from starch substrates	732
		Glutaraldehyde-mediated reaction with bovine serum albumin on silk	Enzyme electrode for determination of starch hydrolysis products	728
Glucose dehydrogenase	1.1.1.47	Entrapment in polyacrylamide gel	Part of bienzyme electrode system for determination of NAD^+	732
		Co-casting with D-glucose oxidase in gelatin membranes	Enzyme electrode for determination of NAD^+	728
		Reaction with agarose cyclic imidocarbonate	Study of effects of diffusional limitation on the activity of the immobilized enzyme	140

Table 7 (*cont.*)

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
D-Glucose oxidase	1.1.3.4	Reaction with 4-aminocarbanilated cellulose derivatives activated by reaction with sodium nitrite, *N*-hydroxysuccinimide, or glutaraldehyde	Investigation of new polymeric matrix for enzyme immobilization	476
		Carbodi-imide-mediated reaction with carbon electrodes	System for the electrochemical regeneration of oxygen	779
		Reaction with acyl-azide derivatized collagen films	Electrochemical study of reactions at interfaces of the active immobilized enzyme–collagen membranes	780
		Graft copolymerization of vinylated enzyme with cellulose, agarose, starch, or cross-linked dextran matrices	Investigation of a new coupling method and of the properties of the active immobilized enzymes	705
		Concanavalin A-mediated binding to the cell wall of *Saccharomyces cerevisiae* cells	Immobilized multienzyme complex continuous conversion of sucrose to D-fructose and D-gluconic acid	781
		Glutaraldehyde-mediated cross-linking with catalase on to particles of insolubilized inactive protein	D-Glucose oxidation in D-glucose–D-fructose mixtures; oxygen removal from beer	727
		Radiation-induced copolymerization of methyl methacrylate or hydroxyethyl acrylate at low temperature inside poly(vinyl chloride) tubes and on polyethylene films	Active immobilized enzyme for medical applications	782
		Carbodi-imide-mediated reaction with graphitic oxide on Teflon®; glutaraldehyde-mediated reaction with a platinum disc pre-reacted with allylamine; reaction with carbon paste	Rotating disc enzyme electrodes for kinetic studies of biocatalysis; characterization of the immobilized enzyme layer	783
		Co-immobilized with catalase by encapsulation in cellulose acetate	Direct measurement of oxygen concentration gradient around particles of immobilized enzyme	726

Reaction with Ti^{IV} oxide	Active immobilized enzyme; development of new method of enzyme immobilization	784
Glutaraldehyde-mediated cocross-linking with bovine serum albumin into an artificial proteic membrane	Used in conjunction with immobilized peroxidase in cytochemical visualization of local concentrations of metabolites	785
Reaction with activated poly(vinyl alcohol) matrices	Active immobilized enzyme	740
Reaction with hydrazine-activated poly(γ-methyl-D-glutamate) glass beads	Active immobilized enzyme	704
Co-immobilized with peroxidase by reaction with alkylamine glass	Active immobilized enzyme; incorporated into the flow system of an autoanalyser for the continuous determination of serum D-glucose	786
Glutaraldehyde-, *sym*-triazine-, or dimethylsuberimidate-mediated reaction with oxidized polyethylene beads pre-derivatized with 1,2-diaminoethane	Active immobilized enzyme; comparison of different immobilization methods	787
Entrapment in polyacrylamide gel	Part of bienzyme electrode system for determination of products of soluble enzyme reactions	732
Co-casting in gelatin membranes with glucoamylase, β-D-fructofuranosidase, β-D-glucosidase (maltase), hexokinase, D-glucose dehydrogenase, catalase, or peroxidase	Enzyme electrodes for determination of products of reactions catalysed by the co-immobilized enzymes	728
Co-immobilized with cytochrome P450 by entrapment in gelatin membranes	Enzyme electrode for determination of cytochrome P450 substrates	753
Paraformaldehyde-mediated reaction with the tip of a Clark-type oxygen electrode	Rate-determination method for analysis of D-glucose	788

Table 7 (*cont.*)

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Glutaraldehyde-mediated cross-linking with polyethyleneimine-coated glass microbeads	Active immobilized enzyme	729
		Glutaraldehyde-mediated copolymerization with catalase and albumin	Enzyme electrode for determination of D-glucose	613
		Co-casting with collagen into a membrane followed by glutaraldehyde-cross-linking; co-immobilization with D-glucose phosphate isomerase by the same method	Immobilized enzyme sensor for determination of D-glucose	789
D-Glucose phosphate isomerase	5.3.1.9	Adsorption of a polystyrene-sulphonate cation-exchanger equilibrated with Ti^{4+}, Zr^{4+}, and V^{+} ions, followed by glutaraldehyde-cross-linking	Active immobilized enzyme for determination in a compact bed reactor	790
		Reaction with polyphenolic resins prepared by copolymerization of hydroquinone and formaldehyde or glutaraldehyde	Investigation of new matrices for the preparation of active immobilized enzymes	708
		Co-immobilized with D-glucose oxidase by co-casting with collagen into a membrane followed by glutaraldehyde-cross-linking	Immobilized enzyme sensor for determination of D-glucose	789
β-D-Glucosidase	3.2.1.21	Co-immobilized with *Saccharomyces cerevisiae* cells by entrapment in alginate gels	Fermentation of cellobiose (produced by *S. cerevisiae* cells)	790
		Adsorption on Amberlite® DP-1 cation-exchange resin	Active immobilized enzyme for conversion of cellobiose to D-glucose in packed bed and fluidized bed reactors	791
D-Glucose 6-phosphate dehydrogenase	1.1.1.49	Co-immobilized with hexokinase on the inner walls of plastic tubing	Continuous flow analysis of D-glucose in serum	792
		Reaction with activated poly(vinyl alcohol) matrices	Active immobilized enzyme	740
β-D-Glucuronidase	3.2.1.31	Glutaraldehyde-mediated reaction with hornblende or porous glass; reaction with Enzacryl-TiO®	Active immobilized enzyme	793

L-Glutamate dehydrogenase	1.4.1.2	Reaction with agarose cyclic imidocarbonate	Synthesis of L-[^{13}N]amino-acids in high yield and purity	148
L-Glutamate dehydrogenase ($NADP^+$)	1.4.1.4	Reaction with agarose cyclic imidocarbonate	Immunoadsorbent purification of antibodies to the enzyme	149
L-Glutaminase	3.5.1.2	Reaction with agarose cyclic imidocarbonate	Study of properties of active immobilized enzyme	150
Glyceraldehyde phosphate dehydrogenase	1.2.1.12	Reaction with agarose cyclic imidocarbonate followed by treatment with dithiothreitol to form dimers	Affinity chromatographic purification of soluble dimers of the homologous enzyme from mammalian sources	151
		Reaction with agarose cyclic imidocarbonate	Investigation of subunit interactions of the immobilized native monomers of the enzyme	152, 153
		Reaction with agarose cyclic imidocarbonate	Investigation of the reactivity of the immobilized enzyme	154
Glycerol dehydrogenase	1.1.1.6	Reaction with alkylated nylon; glutaraldehyde mediated cross-linking with a nylon-polyethyleneimine copolymer	Immobilized enzyme reactor integrated into the flow system of an autoanalyser for routine estimation of glycerol	794
			Immobilized enzyme reactor integrated into the flow system of an autoanalyser for routine estimation of triglycerides (lipolytic enzymes added in solution)	795
Glycogen phosphorylase	–	Entrapment in polyacrylamide gels	Development of an activity staining method for (1→4)-α-D-glucan branching enzyme	796
Hansenula polymorpha cells	–	Entrapment in polyacrylamide gels	Living immobilized cells with methanol oxidase activity	797
Hexokinase	2.7.1.1	Co-immobilized with D-glucose phosphate on the inner walls of plastic tubing	Continuous flow analysis of D-glucose in serum	792

Table 7 (*cont.*)

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Co-casting with D-glucose oxidase in gelatin membranes	Enzyme electrode for determination of adenosine triphosphate	728
		Entrapment in polyacrylamide gel	Part of bienzyme electrode system for determination of adenosine triphosphate	732
Hydrogenase (*Chromatium vinosum*)	1.18.3.1	Reaction with agarose cyclic imidocarbonate; adsorption on to, or glutaraldehyde-mediated coupling with, ω-aminoalkyl-agaroses	Study of thermal stability of solubilized, membrane-bound, and artificially immobilized forms of the enzyme	155
Hydrogenase (*Desulfovibrio gigas*)	1.18.3.1	Reaction with glutaraldehyde-activated aliphatic amino-Spherosil®; reaction with diazotized aromatic amino-Spherosil®	Active immobilized enzyme	798
20β-Hydroxysteroid dehydrogenase	1.1.1.53	Reaction with agarose cyclic imidocarbonate	Study of the renaturation and urea-induced denaturation of the active immobilized enzyme	156
Inorganic pyrophosphatase	3.6.1.1	Entrapment, with other enzymes of the urea cycle, into a matrix of fibrin formed by proteolytic digestion of fibrinogen	Active immobilized enzymes comprising the urea cycle	711
Klebsiella pneumoniae cells	–	Glutaraldehyde-mediated cocasting with collagen	Living immobilized cells from nitrogen fixation in batch recycle or continuous reaction reactors	799
Kluyvera citrophila cells	–	Entrapment in polyacrylamide gel	Living immobilized cells for continuous production of ampicillin	800
Kluyveromyces fragilis cells	–	Entrapment within cellulose and cellulose acetate beads	Living immobilized cells with β-D-galactosidase activity	474

Kluyveromyces lactis cells	–	Glutaraldehyde-mediated reaction with polyphenyleneoxide polymers	Living immobilized cells with β-D-galactosidase activity; investigation of new support for cell immobilization	801
Lactate dehydrogenase	1.1.1.27	Reaction with agarose cyclic imidocarbonate	Study of effects of diffusional limitation on the activity of the immobilized enzyme	140
		Reaction with agarose cyclic imidocarbonate	Investigation of the interactions between subunits of the immobilized oligomeric enzyme	137
		Reaction with agarose cyclic imidocarbonate; reaction with Sephadex® cyclic imidocarbonate	Development of a new system for semi-automated evaluation of enzyme kinetic parameters in enzyme reactions	157
		Reaction with *sym*-triazine-activated cellulose; reaction with agarose cyclic imidocarbonate	Affinity chromatography on NAD	701
		Glutaraldehyde-mediated reaction with nylon tubing pre-reacted with benzidine *via* a carbodi-imide-mediated reaction	Study of the effects of temperature and pH on the kinetics of the active immobilized enzyme	802
Lactobacillus sp. cells	–	Heat treatment of crude cell extract	Living immobilized cells; used in batch processes for conversions of corn and bagasse hydrolysates to D-fructose	803
Lactoperoxidase	–	Adsorption on polystyrene tissue culture flasks	Labelling of L cell proteins involved in adhesion to polystyrene	804
		Reaction with agarose cyclic imidocarbonate	Model system for investigation of method of stabilizing proteins immobilized on agarose by glutaraldehyde-cross-linking	135
Lipoprotein lipase	3.1.1.34	Carbodi-imide-mediated reaction with carboxyhexyl-agarose	Study of interaction of the immobilized enzyme with apolipoproteins and heparin	805

Table 7 (*cont.*)

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
Luciferase	–	Reaction with agarose cyclic imidocarbonate	Study of binding of immobilized luciferase and soluble flavin reductase	806
		Reaction with activated agarose	Investigation of the role of sulphydryl groups in catalysis by and inactivation of soluble and immobilized luciferase	807
		Reaction with activated polysaccharide matrices	Study of changes in the pH dependence of the catalytic activity and stability of the enzyme after immobilization	808
		Reaction with agarose or cellophane film	Analytical system for the determination of very low concentrations of adenosine triphosphate; used in assays for adenosine triphosphate-converting enzymes	809
Lysozyme	3.2.1.17	Glutaraldehyde-cross-linking of the soluble enzyme	Study of the relationship between kinetic properties of the enzyme and its molecular conformation	810
Malate dehydrogenase	1.1.1.37	Reaction with agarose cyclic imidocarbonate	Study of effects of diffusional limitation on the activity of the immobilized enzyme	140
Methanogenic microbial cells	–	Co-casting with agar gel; copolymerization with acrylamide; glutaraldehyde-cross-linking with collagen	Living immobilized cells for continuous production of methane	811
Nitrifying bacterial cells	–	Adsorption on a millipore filter	Living immobilized cells; microbial sensor for con-	812

			tinuous determination of ammonia	
Nocardia erythropolis cells	–	Entrapment in agar gel or polyacrylamide gels	Microbial membrane electrode for determination of cholesterol	813
Nocardia opaca cells	–	Entrapment in polyacrylamide gels	Microbial membrane electrode for assay of steroid hormones	814
Nocardia rhodocrous cells	–	Copolymerization with hydrophobic and hydrophilic urethane resin prepolymers	Living immobilized cells with steroid Δ^1-dehydrogenase and 17β-hydroxysteroid dehydrogenase activity	815
Nonphosphorylating electron transport particles	–	Entrapment in agar gel	Living immobilized cells; used in electrochemical device for determination of either NADH or succinate	816
L-Ornithine carbamoyltransferase	2.1.3.3	Entrapment, with other enzymes of the urea cycle, into a matrix of fibrin formed by proteolytic digestion of fibrinogen	Active immobilized enzymes comprising the urea cycle	711
Papain	3.4.22.2	Reaction with 4-aminocarbanilated derivatives of cellulose activated by treatment with sodium nitrite, *N*-hydroxysuccinimide, or glutaraldehyde	Investigation of new polymeric matrices for enzyme immobilization	476
		Glutaraldehyde-mediated reaction with Biogel P-100®	Depolymerization of tissue proteins during purification of smallpox virus	817
		Reaction with hydrous titanium(IV) oxide	Active immobilized enzyme for chill-proofing of beer	818
		Reaction with titanium(IV)-activated glass fibre paper	Active immobilized enzyme for chill-proofing of beer	819
		Adsorption on hydrous titanium(IV) oxide	Active immobilized enzyme; development of a new method of enzyme immobilization	820

Table 7 (*cont.*)

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Reaction with titanium(IV) oxide or cellulose supports pre-coated with diazotized 1,3-diaminobenzene	Active immobilized enzyme	821
Penicillin acylase		Reaction with Amberlite XAD-7® beads	Active immobilized enzyme for the deacylation of benzylpenicillin	822
		Reaction with Amberlite XAD-7® beads	Determination of penetration of the immobilized enzyme into the support using a staining method	823
		Glutaraldehyde-mediated cross-linking on to carboxymethylcellulose or Amberlite XAD-7® beads	Study of properties of active immobilized enzyme and comparison with those of the soluble enzyme	824
Penicillin amidase	3.5.1.11	Modification of the enzyme with acrolein followed by entrapment in polyacrylamide gel	Active immobilized enzyme for synthesis of β-lactam antibiotics	825
		Entrapment in polyacrylamide gel	Active immobilized enzyme	826
Pepsin	3.4.23.1	Reaction with 4-aminocarbanilated derivatives of cellulose activated by treatment with sodium nitrite, *N*-hydroxysuccinimide, or glutaraldehyde	Investigation of new polymeric matrix for enzyme immobilization	476
Peroxidase	1.11.1.7	Co-casting in gelatin membranes with catalase or with catalase and D-glucose oxidase	Enzyme electrodes for determination of peroxidase substrates	728
		Graft copolymerization of vinylated enzyme with cellulose, agarose, or cross-linked dextran matrices	Investigation of a new coupling method; study of properties of the active immobilized enzymes	705

		Radiation-induced copolymerization of methyl methacrylate or hydroxyethyl acrylate at low temperature inside poly(vinyl chloride) tubes and on polyethylene films	Active immobilized enzyme for medical applications	782
		Glutaraldehyde-mediated cocross-linking with bovine serum albumin into an artificial proteic membrane	Used in conjunction with immobilized D-glucose oxidase in a method for cytochemical visualization of local concentrations of metabolites	785
		Reaction with hydrazine-activated poly-γ-methyl-D-glutamate glass beads	Active immobilized enzyme	704
		Co-immobilized with D-glucose oxidase by reaction with alkylamine glass	Active immobilized enzyme; incorporated into the flow system of an autoanalyser for the continuous determination of serum D-glucose	786
		Reaction with 4-toluenesulphonyl chloride-derivatized agarose	Active immobilized enzyme; demonstration of a new method of enzyme immobilization	435
		Reaction with Affi-gel® 10	Assessment of a simple device for the spectrophotometric assay of immobilized enzymes	414
Phenol 2-mono-oxygenase	1.14.13.7	Entrapment in polyacrylamide gel; adsorption on to DEAE-Sephadex® or DEAE-Biogel®; carbodi-imide-mediated reaction with aminohexyl-agarose	Enzyme electrode for quantitative determination of phenol at micromolar concentrations	827
L-Phenylalanine ammonia-lyase	4.3.1.5	Entrapment in cellulose acetate fibres made haemocompatible by blending with a platelet anti-aggregating agent	Active immobilized enzyme; removal of phenylalanine and tyrosine from blood or food of phenylketonuria patients	473
Phosphodiesterase	3.1.4.18	Adsorption on to concanavalin A immobilized on agarose	Active immobilized enzyme used in oligonucleotide analysis	395

Table 7 (*cont.*).

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
Phosphotransferase	–	Reaction with agarose cyclic imidocarbonate; reaction with benzoquinone-activated agarose; adsorption on to glass; reaction with titanium (IV) chloride-, carboxyl-, or alkylamine-derivatized glass	Active immobilized enzyme; comparison of methods of immobilization	158
Proteus mirabilis cells	–	Formaldehyde-mediated cocross-linking with gelatin	Living immobilized cells for stereospecific hydrogenation reactions	746
Protoplasts	–	Entrapment in alginate gel	Living immobilized cells	589
Pseudomonas dacunhae cells	–	Entrapment in κ-carrageenan gel, followed by glutaraldehyde cross-linking	Living immobilized cells for continuous production of L-alanine from L-aspartic acid	828
Pseudomonas denitrificans cells	–	Entrapment in alginate gel	Living immobilized cells; denitrification of drinking water in batch and column processes	829
Pullulanase	3.2.1.41	Complexation with tannic acid followed by adsorption on to triethylaminoethylcellulose	Active immobilized enzyme; used in column form for conversion of pullulan to maltotriose	710
Pyruvate dehydrogenase	4.1.1.1	Reaction with aminomethyl derivatives of polystyrene	Study of changes in the functional behaviour of the active immobilized enzyme arising from the electrostatic interaction of the cationic carrier polymer and the anionic substrate	830

Rhodopseudomonas capsulata chromatophores	–	Entrapment in alginate gel	Living immobilized cells; improvement of the physical and biochemical properties of the gel beads by using barium as gel-inducing agent	831
Saccharomyces carlsbergensis cells	–	Entrapment in κ-carrageenan gel	Living immobilized cells; continuous production of ethanol in batch and column processes	832
Saccharomyces cerevisiae cells	–	Entrapment in polyacrylamide or agarose gel; cross-linking with glutaraldehyde; reaction with agarose cyclic imidocarbonate	Living immobilized cells with β-D-fructofuranosidase activity; comparison of methods of immobilizing cells	833
		Entrapment in polyacrylamide gel	Immobilized multienzyme complex used in a continuous-flow stirred tank reactor for conversion of sucrose to fructose and gluconic acid	781
		Entrapment in polyacrylamide gel	Study of properties of living immobilized cells	834
		Adsorption on cationic or anionic ion-exchange resins; reaction with cellulose activated with cyanuric chloride	Living immobilized cells for use in microbial reactors	715
		Unspecified reaction with five carriers	Living immobilized cells for fermentation of molasses	835
		Co-casting with gelatin followed by cross-linking with formaldehyde	Living immobilized cells with β-D-fructofuranosidase activity	836
		Entrapment in polyacrylamide gel	Living immobilized cells with alcohol dehydrogenase activity	837
		Co-immobilized with β-D-glucosidase or β-D-galactosidase by entrapment in alginate gels	Living immobilized cells; microbial conversion of non-metabolizable substrates	770
		Coprecipitation, with different amounts of iron oxide, in alginate gel	Study of the properties of the magnetic living immobilized cells	838

Table 7 (*cont.*)

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Entrapment in cellulose acetate beads	Living immobilized cells with β-D-fructofuranosidase activity	474
		Circulation through beech wood chips	Living immobilized cells	839
		Reaction with glutaraldehyde-activated ceramic Raschig rings pre-coated with gelatin	Living immobilized cells; used in reactors for production of ethanol from D-glucose	840, 841
Serratia marcescens cells	–	Entrapment in κ-carrageenan gel	Living immobilized cells for the continuous production of L-isoleucine	842
Spheroplasts	–	Glutaraldehyde-cross-linking with albumin at sub-zero temperatures	Scanning and transmission electron microscopic studies of the immobilized cells	693
α-Steroid dehydrogenase	–	Glutaraldehyde-mediated cross-linking with albumin to form a membrane	Active immobilized enzyme; investigation of the feasibility of co-factor regeneration with an electron carrier	700
Streptomyces sp. cells	–	Entrapment in gelatin followed by glutaraldehyde-cross-linking	Living immobilized cells with D-glucose isomerase activity	843
Streptomyces flavogriseus cells	–	Adsorption on to benzyl-DEAE-, DEAE- or TEAE-cellulose	Study of properties of the living immobilized cells	844
Streptomyces griseus neutral proteinase ('Pronase')	3.4.24.4	Glutaraldehyde-mediated reaction with chitosan	Active immobilized enzyme	517
Subtilisin	3.4.21.14	Glutaraldehyde-mediated reaction with chitosan	Active immobilized enzyme	517
Thrombin	3.4.21.5	Reaction with *sym*-triazine-activated agarose	Affinity chromatography of heparin	458
		Reaction with agarose cyclic imidocarbonate	Affinity chromatography of	189

Thrombin–anti-thrombin complex	–	Reaction with agarose cyclic imidocarbonate	Immunoadsorbent purification of antibody raised against the complex	190
Thylakoids	–	Glutaraldehyde-cross-linking with albumin at sub-zero temperatures	Living immobilized cells; adenosine triphosphate regeneration in photosystems I and II	845
		Glutaraldehyde-cross-linking with albumin at sub-zero temperatures	Scanning and transmission electron microscopic studies of the immobilized cells	693
Transketolase	2.2.1.1	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; study of the properties of the immobilized subunits	159
Triacylglycerol-lipase	3.1.1.3	Carbodi-imide-mediated reaction with 6-amino-hexanoyl-agarose; glutaraldehyde-mediated reaction with silanized glass beads or Dowex® MWA-1 resin; reaction with activated Duolite® A-7	Active immobilized enzyme; alteration of foaming properties of yolk-containing egg white	846
Trichosporon brassicae cells	–	Adsorption on a porous acetyl-cellulose membrane	Living immobilized cells; microbial sensor for the continuous determination of volatile compounds	812
Trypsin	3.4.21.4	Reaction with 4-aminocarbanilated derivatives of cellulose activated by treatment with sodium nitrite, *N*-hydroxysuccinimide, or glutaraldehyde	Investigation of new polymeric matrix for enzyme immobilization	476
		Carbodi-imide-mediated reaction with porous glass or organic macroporous carriers containing carboxyl groups	Active immobilized enzyme; model system for study of inhomogeneous distribution of fixed enzymes inside carriers	847
		Reaction with *S*-methyldithiocarbonate and dithiobis(thioformate) derivatives of cellulose	Investigation of the properties of the active immobilized enzyme	498
		Reaction with polymers containing isonitrile functional groups	Active immobilized enzyme; development of new methods of enzyme immobilization	89

Table 7 (*cont.*)

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Glutaraldehyde-mediated reaction with chitosan	Active immobilized enzyme	517
		Reaction with agarose cyclic imidocarbonate	Affinity chromatography of protease inhibitor V from soybean	143
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; study of the thermal stability of the immobilized enzyme	161
		Reaction with hydrazine-activated poly(γ-methyl-D-glutamate) glass beads	Active immobilized enzyme	704
		Adsorption onto molecular sieves	Study of effects of external mass transfer on activation energy of substrate hydrolysis by the active immobilized enzyme	848
		Reaction with Tygon® tubing	Study of protein hydrolysis using the active immobilized enzyme	849, 850
		Reaction with polythiol pre-heated with 4-vinyl pyridine and then treated with cyanogen bromide	Active immobilized enzyme; development of new methods of enzyme immobilization	741
		Glutaraldehyde-mediated reaction with sand in the presence of 3-aminopropyltriethoxysilane, 1,1,6,6-tetraminohexane, 1,6-diaminohexane, or melamine, or alone	Comparison of properties of the preparations of active immobilized enzymes	851
		Glutaraldehyde-mediated reaction with sand pre-treated with 3-aminopropyltriethoxysilane	Active immobilized enzyme	852
		Glutaraldehyde-mediated reaction with macroporous Silochrome®	Study of the catalytic properties of the immobilized enzyme in a flow-through reactor	853

		Reaction with agarose cyclic imidocarbonate	Affinity chromatography of a protease inhibitor from raji grains	160
		Carbodi-imide-mediated reaction with succinamido-propyl-glass beads	Development of a differential microrecirculation reactor for kinetic analysis of soluble and immobilized enzymes	854
		Reaction with octyl- or phenyl-agarose pre-treated with dodecyl-, octyl-, or hexyl-aldehyde	Validation of a new method of protein immobilization using reductive alkylation with hydrophobic aldehydes	132
Urate oxidase	1.7.3.3	Reaction with polymers containing isonitrile functional groups	Active immobilized enzyme; development of new methods of enzyme immobilization	89
		Co-immobilization with aldehyde dehydrogenase on nylon pre-activated with triethyloxonium-tetrafluoroborate, copolymerized with polyethyleneimine and glutaraldehyde-cross-linked	Stable nylon tube enzyme reactor for the automated determination of uric acid in serum	702
		Glutaraldehyde-mediated reaction with controlled-pore glass tubing	Active immobilized enzyme for continuous flow analysis of uric acid in biological fluids	855
		Entrapment in hollow fibres made of poly(vinyl alcohol) and cellulose acetate	Active immobilized enzyme for use in hollow-fibre reactors; study of the kinetics of the reactors	856
		Reaction with film or fibre of poly(methylglutamate)-acyl azides or with beads coated with the polymer	Active immobilized enzyme; development of new method of enzyme immobilization	857
		Reaction with poly-γ-methyl-D-glutamate glass beads pre-treated with hydrazine	Active immobilized enzyme	704
Urease	3.5.1.5	Glutaraldehyde-mediated reaction with alkylamine glass beads	Routine analysis of serum urea	858
		Entrapment in hollow fibres made of poly(vinyl alcohol) and cellulose acetate	Active immobilized enzyme for use in hollow-fibre reactors; study of the kinetics of the reactor systems	856

Table 7 (*cont.*)

Enzyme or cell	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Reaction with poly-γ-methyl-D-glutamate glass beads pre-treated with hydrazine	Active immobilized enzyme	704
		Reaction with film or fibre of poly(methyl-glutamate)acyl azides or with beads coated with the polymer	Active immobilized enzyme; development of new method of enzyme immobilization	857
		Entrapment with magnetite in polyacrylamide gel	Assessment of the performance of an immobilized enzyme reactor utilizing a revolving magnetic field	859
UDP-glucuronosyltransferase	2.4.1.17	Reaction with agarose cyclic imidocarbonate	Synthesis of bile acid glucuronides	162
Urokinase	3.4.21.31	Radiation-induced copolymerization of methyl methacrylate or hydroxyethyl acrylate at low temperature inside poly(vinyl chloride) tubes and on polyethylene films	Active immobilized enzyme for medical applications	782
		Reaction with human red cell membranes, using 4,4′-di-isothiocyano-2,2′-stilbene disulphonate as mediator	Active immobilized enzyme	860
Xanthine oxidase	1.2.3.2	Glutaraldehyde cross-linking of milk, milk and gelatin, or commercially available xanthine oxidase	Active immobilized enzyme for use in organic syntheses	861
endo-(1→3)-β-D-Xylanase	3.2.1.32	Adsorption on to Duolite® S-761 resin	Active immobilized enzyme for use in enzyme reactors	733
exo-(1→4)-β-D-Xylosidase	3.2.1.37	Reaction with titanium(IV) chloride-activated alumina; reaction with glutaraldehyde-activated alkylamine porous silica	Active immobilized enzyme; comparison of different immobilization methods	862
		Reaction with titanium(IV) chloride-activated alumina; reaction with glutaraldehyde-activated alkylamine	Study of properties of the active immobilized enzyme	863
Yeast mitochondria	–	Copolymerization with a photocross-linkable resin prepolymer or a urethane prepolymer	Living immobilized cells with adenylate kinase activity	864

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Some aspects of immobilized glycoside and polysaccharide hydrolases, carbohydrate isomerases, and carbohydrate oxidases, *etc.*, are worthy of comment.

The thermal stabilization of glucoamylase by attachment to an insoluble support has been studied by several groups. Both soluble and immobilized forms of the enzyme have increased thermal stability in the presence of substrate.[775] The overall thermostabilization of glucoamylase by immobilization and by substrate stabilization has almost the same limit for both soluble and immobilized enzyme (*i.e.* 40–50-fold). The thermal stability of glucoamylase entrapped in polyacrylamide gel has been shown to be slightly enhanced relative to the soluble enzyme.[777,778]

The effects of pore diffusion limitation on starch dextrin hydrolysis by immobilized glucoamylase have been investigated.[776] When dextrin is hydrolysed by the immobilized enzyme preparation use of a larger molecular weight substrate leads to lower overall rates of hydrolysis (as compared with the soluble enzyme) owing to slow substrate diffusion, the maltose concentration during the reaction is lower owing to slow diffusion of reaction intermediates, and the maximum D-glucose concentration observed is lower owing to the slow diffusion of the monosaccharide.

The results of a kinetic study of the thermal stabilization of β-D-fructofuranosidase after immobilization suggest that ionic bonding of the enzyme to the support gives a more loosely deformed enzyme preparation than does covalent bonding to the support.[768]

Glycogen phosphorylase has been immobilized by entrapment in running gels prior to electrophoresis.[796] The immobilized enzyme catalyses the co-operative synthesis of glycogen with (1→4)-α-D-glucan branching enzyme in the absence of primer glycogen. Newly synthesized glycogen is visible as dense white bands,

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which can be detected visually or by densitometry, thus providing an activity staining method for the enzyme.

A cytochemical method has been used to visualize the existence of local concentrations of metabolites within an immobilized bienzyme system.[785] D-Glucose oxidase and peroxidase were co-immobilized by glutaraldehyde-mediated cross-linking with albumin into an artificial proteic membrane. From experimental kinetic parameters, theoretical concentration profiles could be calculated by computer using numerical analysis methods.

The use of immobilized enzymes in analysis continues to attract much attention. Several new systems for the measurement of D-glucose in particular have been developed. D-Glucose oxidase and peroxidase, co-immobilized by reaction with alkylamine glass, have been incorporated into the flow system of an autoanalyser I for the continuous determination of D-glucose in serum.[786] D-Glucose 6-phosphate dehydrogenase and hexokinase, co-immobilized on the inner walls of plastic tubing, have also been applied to the continuous flow analysis of D-glucose.[792]

A commercially available D-glucose analyser has been modified by substituting immobilized D-galactose oxidase for immobilized D-glucose oxidase in the system.[769] The modified analyser was used for the analysis of D-galactose in neonates. Only 0.025 ml of serum was required for each determination. Results were compared with those obtained using a modified homogeneous enzymic method.

A rate-determination method for the analysis of D-glucose has been developed using D-glucose oxidase immobilized by paraformaldehyde-mediated coupling of the gel-entrapped enzyme over the tip of an oxygen electrode.[788] The method compares favourably with the solution-enzyme method and has several advantages over that method, *viz*, simplicity, economic use of enzyme, and linearity over a greater concentration range.

Based on a D-glucose oxidase sensor for the determination of D-glucose, several D-glucose oxidase-bienzyme electrodes have been developed.[732] Enzymes producing D-glucose by hydrolysis of saccharides (glucoamylase, β-D-fructofuranosidase, and cellulase) and enzymes that consume D-glucose in reactions they catalyse (hexokinase, D-glucose dehydrogenase) have been co-immobilized with D-glucose oxidase. The use of the bienzyme systems was demonstrated for concentration measurements (blood D-glucose, maltose, adenosine triphosphate, nicotinamide adenine dinucleotide, starch) and enzyme activity measurements (α-amylase, adenosine triphosphatase, lactate dehydrogenase).

The analytical determination of substrate concentrations using bienzyme electrode systems based on a D-glucose sensor has also been reported for the following enzyme pairs: D-glucose oxidase–glucoamylase; D-glucose oxidase–β-D-fructofuranosidase; D-glucose oxidase–maltase (β-D-glucosidase); D-glucose oxidase–hexokinase; D-glucose dehydrogenase–D-glucose oxidase; and catalase–peroxidase.[728] D-Glucose oxidase, peroxidase, and catalase were also co-immobilized and applied to the analytical determination of substrates for peroxidase.

Catalase, D-glucose oxidase, and albumin have been co-immobilized around a platinum screen or a platinum–iridium wire in the development of an enzyme

electrode for determination of D-glucose.[613] The potentiometric enzyme electrodes prepared may have sufficient specificity for D-glucose to enable development of a continuous *in vivo* D-glucose sensor.

Using several different preparations of immobilized D-glucose oxidase, rotating disc enzyme electrodes have been developed and evaluated in terms of kinetic performance and immobilized enzyme layer characterization.[783] The effects of the disc surface and the immobilization reactions were discussed in terms of the properties of the enzyme electrode. By varying the electrode rotation speed, effects of substrate mass transport on enzymatic catalysis could be investigated. The rotating disc enzyme electrode has proved to be advantageous for studies of surface catalysis because mass transport to the catalytic layer is well defined. The hydrolysis of soluble starch by a rotating disc of immobilized glucoamylase has also been investigated.[772]

By co-immobilizing D-glucose oxidase with cytochrome P450 in the preparation of an enzyme electrode for the determination of cytochrome P450 substrates, the addition of expensive nicotinamide adenine dinucleotide to each sample has been avoided.[753] The co-factor in the two-enzyme system can be replaced by enzymatically generated hydrogen peroxide.

Co-immobilization of D-glucose isomerase with D-glucose oxidase in an enzyme sensor for D-glucose has been shown to improve the sensitivity and stability of the sensor.[789]

Direct measurement of the thickness of the unstirred diffusion layer outside immobilized biocatalysts has been achieved using preparations of co-immobilized catalase and D-glucose oxidase.[726]

Immobilized β-D-fructofuranosidase has been used in an enzyme thermistor to control the sucrose concentration in a continuously operating model fermenter containing immobilized yeast cells and converting sucrose to ethanol.[766]

The properties of α-amylase immobilized on hydrous titanium(IV) oxide-coated magnetic iron oxide have been reported and compared with the previously reported properties of the soluble form of the enzyme.[709] The action pattern in the hydrolysis of soluble starch was found to be unaffected by immobilization. The magnetic recovery of the immobilized enzyme from suspension was complete.

The use of carbohydrases in immobilized enzyme reactors for the conversion of carbohydrate substrates continues to attract much attention. Mixtures of immobilized glucoamylase and immobilized β-amylase or α-amylase have been compared for their efficacy in the production (in recirculated differential batch reactors) of high-maltose syrups from corn starch.[706] Mixtures containing fungal α-amylase gave higher maximum amounts of maltose than did those with β-amylase.

A mixture of immobilized pullulanase and immobilized β-amylase has been used for the continuous conversion of starch into maltose.[710] The presence of contaminating α-amylase activity in the pullulanase preparation ensured complete reaction. The immobilized pullulanase preparation was also used in column form for the conversion of pullulan to maltotriose.

The use of immobilized dextranase in combination with molecular permeation chromatography for the concomitant degradation of dextran and the separation of products of high and low molecular weight has been described.[754]

Following immobilization of β-D-galactosidase on epoxy-activated acrylic beads, the kinetic parameters of the immobilized enzyme have been investigated in stirred batch, continuous stirred tank, and packed bed reactors.[612]

The feasibility of using a mixture of immobilized D-glucose oxidase and catalase industrially for oxidation of D-glucose in D-glucose–D-fructose mixtures and for removal of oxygen from beer has been investigated.[727] Immobilized D-glucose phosphate isomerase has been used in a compact bed reactor for isomerization of D-glucose.[790] Cellobiose has been converted to D-glucose using immobilized β-D-glucosidase preparations in packed bed and fluidized bed reactors.[791]

The preparation and study of the properties of living immobilized cells, many of which are well suited to industrial applications, have resulted in numerous publications in the last year. Many of the immobilized cells have enzymic activity. The immobilized cell preparations which have been characterized as having carbohydrase activity include β-D-fructofuranosidase activity in immobilized *Saccharomyces cerevisiae* cells,[474,833,836] β-D-galactosidase activity in immobilized *Bacillus* sp. cells,[716] *Bacillus stearothermophilus* cells,[719] *Caldariella acidophila* cells,[475] *Kluyveromyces fragilis* cells,[474] and *K. lactis* cells,[801] and D-glucose phosphate isomerase activity in immobilized cells of *Actinoplanes missouriensis*,[474] *Lactobacillus* sp.,[803] and *Streptomyces* sp.[843]

Saccharomyces cerevisiae cells have been immobilized by coprecipitation, with different concentrations of iron oxide, in calcium alginate gel.[838] The magnetic living immobilized cells can be readily recovered from the reaction media. Inclusion of magnetic material in the preparation appeared to have no adverse effect on the yeast cells.

A multienzyme complex consisting of β-D-fructofuranosidase, D-glucose oxidase, and catalase has been reconstituted by binding D-glucose oxidase, in a concanavalin A-mediated reaction, to the cell walls of *Saccharomyces cerevisiae* cells.[781] The cell flocculate obtained was stabilized by entrapment in polyacrylamide gel. The immobilized multienzyme complex was used in a continuous-flow stirred tank reactor system for the conversion of sucrose to D-fructose and D-gluconic acid. The system showed no loss in efficiency after 20 days of continuous operation.

Enzymes have been co-immobilized with micro-organisms for the microbial conversion of non-metabolizable substrates.[770] The enzyme hydrolyses a substrate that the micro-organism cannot metabolize in order to provide a metabolizable substrate for the organism. Thus *Saccharomyces cerevisiae* cells co-immobilized with β-D-glucosidase can complete the fermentation of cellobiose to ethanol, and *S. cerevisiae* cells co-immobilized with β-D-galactosidase convert whey permeate to ethanol.

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