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Amino-acids, Peptides, and Proteins

Volume 15

A Specialist Periodical Report

Amino-acids, Peptides, and Proteins

Volume 15

A Review of the Literature Published during 1982

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Preface

This fifteenth Report reviews papers that are relevant to the chemistry of amino-acids, peptides, and proteins published during 1982, except that for Inorganic Aspects (Chapter 5) the period dealt with includes 1981 as well. The format and style follow the pattern of previous volumes, but coverage of the Chemical Structure and Biological Activity of Hormones and Related Compounds (Chapter 5 in Volume 14) has been dropped. This reduction of the scope of the Report is clearly retrograde but was essential to hold down the size and cost. The need to cut back can be seen from the numbers of citations per volume: this was in the range 1500—2500 in the early Reports but has risen to 3500—4500 during recent years. Selecting a chapter to axe was difficult: it seemed best to relinquish the one that was most biological in emphasis (this being, nominally at least, a chemical Report) and for which it had in any case been difficult to recruit enough authors to cover all types of peptide regularly. This was done with great reluctance and is not in any way a reflection on the efforts of past contributors to the chapter, to whom we now express our thanks. Even after this major deletion the Report contains over 4300 citations. There is also a slight gap in the coverage we originally planned: it did not prove possible to cover Fluorescence this year, but it is hoped to remedy this with a two-year survey next time.

Balliol College, Oxford JOHN JONES
September 1983

Contents

Chapter 1 Amino-acids
By G. C. Barrett

1 Introduction 1
Textbooks and Reviews 1

2 Naturally Occurring Amino-acids 1
Occurrence of Known Amino-acids 1
New Natural Amino-acids 2
New Amino-acids from Hydrolysates 3

3 Synthesis of Amino-acids 4
General Methods 4
Asymmetric Synthesis 5
Prebiotic Synthesis of Amino-acids 6
Protein and Other Naturally Occurring Amino-acids 7
β- and Higher Homologous Amino-acids 11
α-Alkyl Analogues of Natural Amino-acids 11
Other Aliphatic, Alicyclic, and Saturated Heterocyclic α-Amino-acids 11
α-Halogenoalkyl Amino-acids 12
Aliphatic α-Amino-acids Carrying Side-chain Hydroxy and Alkoxy Groups 12
Aliphatic Amino-acids with Unsaturated Side Chains 12
Aromatic and Heteroaromatic Amino-acids 13
Amino-acids Containing Sulphur 13
Amino-acids Synthesized for the First Time 14
Labelled Amino-acids 14
Resolution of DL-Amino-acids 17

4 Physical and Stereochemical Studies of Amino-acids 19
Crystal Structures of Amino-acids and Their Derivatives 19
Nuclear Magnetic Resonance Spectrometry 20
Optical Rotatory Dispersion and Circular Dichroism 22
Mass Spectrometry 23
Other Physical Studies 23
Molecular-orbital Calculations 25

5 Chemical Studies of Amino-acids 26
Racemization 26
General Reactions 26

Specific Reactions of Natural Amino-acids and Their Derivatives 28
Non-enzymic Models of Biochemical Processes Involving Amino-acids 31
Effects of Electromagnetic Radiation on Amino-acids 31

6 Analytical Methods 32
Gas–Liquid Chromatography 32
Ion-exchange Chromatography 33
Thin-layer Chromatography 33
High-performance Liquid Chromatography 34
Fluorescence Methods 35
Determination of Specific Amino-acids 36

Chapter 2 Structural Investigations of Peptides and Proteins

IA: Protein Isolation and Characterization By M. D. Scawen, A. Atkinson, A. Electricwala, P. M. Hammond, and R. F. Sherwood

1 Introduction 37

2 Protein Isolation Methodology 37
Affinity Chromatography 37
General Comments 37
Coupling Techniques 47
Dye-affinity Chromatography 47
Hydrophobic Interaction Chromatography and Covalent Chromatography 53
Hydrophobic Interaction Chromatography 53
Covalent Chromatography 54
Immunoaffinity Chromatography 60
Metal Chelate Chromatography 60
Phase Partition 61
High-performance Liquid Chromatography 61
Other Chromatographic Techniques and Applications 66

3 Isolation of Specific Classes of Proteins 67
Membrane Proteins 67
Plasma Proteins 67
Proteins Involved with Coagulation and Fibrinolysis 80
Fibronectin 80
Complement and Associated Proteins 80
Other Plasma Proteins 80

4 Electrophoretic Techniques 81
Advances in Electrophoretic Techniques 81
One-dimensional Electrophoresis 81
Two-dimensional Electrophoresis 82
Isoelectric Focusing 83
Affinity and Immunoelectrophoresis 84
Isotachophoresis 84
Chromatofocusing 86
Protein Determination in Electrophoretic Gels 86
Protein Determination in Solutions and Suspensions 87

IB: Primary Structures, 1982
By J. Gagnon

Amino-acid Sequences Reported during 1982 88
Blood-clotting Proteins 88
Complement Proteins 88
Electron-transport Proteins 89
Enzymes 91
Globins 96
Histocompatibility Antigens and Antibodies 97
Histones 100
Hormones 100
Ribosomal Proteins 102
Structural Proteins 102
Toxins 103
Viral Proteins 103
Miscellaneous Peptides and Proteins 105

IC: Chemical Modification of Proteins
By R. Cassels and R. A. G. Smith

1 Introduction 111

2 Investigations of Known and Novel Reagents and Reactions 180
α-Dicarbonyl Compounds 180
Diethyl Pyrocarbonate 180
2-Chloromercuri-4-nitrophenol 181
N-(3-Pyrenyl)maleimide 181
Triazine Dyes 181
Ozone 182
Modification for Physical Techniques 182
Affinity Probes 184

Crosslinking Reagents 185
Affinity Labelling 186
Photoaffinity Labelling 188
Photocrosslinking 189
Mechanism-based Inhibitors 190

II: X-Ray Studies By W. D. Mercer

1 Introduction 191

2 Crystallographic Methods and Equipment 191
General Crystallographic Methods and Theory 191
Data Processing 196
Small-angle and Fibre Diffraction 196
Protein Crystallography 197
Structure Refinement 197
Protein Dynamics 198
Computer Graphics 198

3 Lectin and Concanavalin 198
Green-pea Lectin 198
Concanavalin A 199

4 Oxygen- and Electron-carrying Proteins 199
Myoglobin 199
Haemoglobin 199
Sickle Haemoglobin 200
Methydroxohaemerythrin 200
Haemocyanin 200
Cytochrome *c* 201
Cytochrome C_3 201
Cytochrome C_{551} 201
Ferredoxin 201

5 Ribonuclease and Lysozyme 201
Ribonuclease A 201
Ribonuclease T_1 202
Bacillus amyloliquefaciens Ribonuclease 202
Ribonuclease St 203
Hen Egg-white Lysozyme 203
Bacteriophage T_4 Lysozyme 203

6 Proteolytic Enzymes 203
Japanese Quail Ovomucoid 203
Trypsin/Trypsinogen 204

Elastase 204
Streptomyces griseus Protease B 204
Thermolysin 205
Carboxypeptidase A 205
Zn^{2+} G Peptidase 205
Penicillopepsin 206
Rhizopus chinensis Carboxyl Proteinase 206
Calotropin DI 206
Papain 206

7 Glycolytic Enzymes 206
Phosphorylase 206
L-Lactate Dehydrogenase 207
Alcohol Dehydrogenase 208

8 Hormones 208
Insulin 208

9 Other Globular Proteins 209
Dihydrofolate Reductase 209
Citrate Synthase 209
2-Keto-3-deoxy-6-phosphogluconate Aldolase 210
Aspartate Carbamoyltransferase 210
Catabolite Gene Activator Protein/*cro* Repressor 211
Superoxide Dismutase 211
Mellitin 212
Phospholipase A_2 212
Aspartate Aminotransferase 212
Glutathione Reductase 212
Elongation Factor G 213
Uteroglobin 213
Prothrombin 213
F_1 ATPase 213
Trimethylamine Dehydrogenase 213
MoFe Protein of Nitrogenase 214
L-Arabinose-binding Protein 214

10 tRNA-binding Proteins and tRNA 214
Methionyl-tRNA Synthetase 214
Tyrosyl-tRNA Synthetase 214
tRNA 215

11 Viruses 215
Satellite Tobacco Necrosis Virus 215
Tomato Bushy Stunt Virus 215
Southern-bean Mosaic Virus 216

Alfalfa Mosaic Virus Coat Protein 216
Polyoma Virus Capsid 216
Influenza Virus 216
Pf3 Bacteriophage 216
Pf1 Bacteriophage 216

12 Muscle and Muscle Proteins 217

13 Membranes and Membrane Proteins 217

14 Other Biological Structures 218
Ribosomes 218
Silk Fibroin 218
Intermediate Filaments 219
Photoreceptor Microvilli 219
Cytochrome C Oxidase 219
Cell-wall Glycoprotein 219
Bacterial-cell Envelope S-Layer 219
Phospholipids 219
Peptides and Polypeptides of Interest 219

15 Small-angle Scattering 221

16 Protein Conformations – Analysis and Predictions 222
Methods and Results of Structure Prediction 222
Analysis of Protein Structures 223
Graphics and Protein Structures 223
Protein Structural Elements 224

III: Conformation and Interaction of Peptides and Proteins in Solution Edited by R. H. Pain

1 Theoretical Aspects of Protein Conformation 225
Contributed by N. K. Rogers and M. J. E. Sternberg

Energy Calculations and Conformational Analysis 225
Water Structure and Electrostatics 226
Dynamics 227
Unfolding Mechanisms 228
Hydrogen Exchange 228
Analysis and Prediction of Secondary Structures 229
Analysis of Tertiary and Quaternary Structures 230
Prediction of Three-dimensional Structure 231
Protein Function 233
Conclusion 233

2 Stability and Folding of Proteins 233
Contributed by S. Craig

Stability 233
Effect of Disulphides 234
Effect of Individual Residues 234
Effect of Ligands 235
Solvent Effects 236
Intermediates in Protein Folding 237
Theories of Protein Folding 238
Proline Isomerization 238
Nucleation and Hydrophobic Cluster Model 238
Folding Pathways 239
Folding of Independent Domains and Protein Fragments 240

3 Circular Dichroism 240
Contributed by T. Brittain

General 240
Reviews 240
Instrumental 241
Theory 242
Small Molecules, Model Compounds, and Synthetic Polymers 243
Amino-acids and Derivatives 243
Dipeptides and Oligopeptides 245
Polypeptides 246
Proteins 247
Non-chromophoric Proteins 247
Chromophoric Proteins 252
Hormones 253
Added Extrinsic Chromophores 255
Nuclear Proteins 256

4 Magnetic Circular Dichroism 256
Contributed by T. Brittain

General and Model Systems 256
Proteins 257

5 Infrared and Raman Spectroscopy 259
Contributed by R. M. Stephens

Model Compounds 259
Model Calculations 259

Proteins 260
Albumin 260
Blood Proteins 261
Cytochromes 261
Enzymes 263
Gramicidin 263
Haemoglobin 263
Hormones 265
lac Repressor 265
Lysozyme 266
Riboflavin 266
Visual Pigments 267
Wheat Protein 269
DNA–Protein Interactions 269

6 N.M.R. Studies 270
Contributed by H. W. E. Rattle

Introduction 270
Amino-acids and Small Peptides 271
Amino-acids 271
Small Synthetic Peptides 272
Natural Peptides 274
Hormones 278
Enzymes 279
Oxidoreductases 279
Transferases 280
Hydrolases 282
Lyases, Isomerases, and Ligases 284
Other Proteins 285
Iron-containing Proteins 285
Copper Proteins 287
Calcium-binding Proteins 287
Muscle Proteins 288
Proteins Associated with Nucleic Acids 288
Glycoproteins 290
Proteins Associated with Lipids 290
Metallothioneins 291
Structural Proteins 292
Other Proteins 292

7 Mössbauer Spectroscopy 293
Contributed by D. P. E. Dickson

Haem Proteins 293
Iron–Sulphur Proteins 295
Iron-transport and -storage Proteins 296
Protein Dynamics 297

8 Protein Interactions 298
Contributed by D. J. Winzor, P. D. Jeffrey, and L. W. Nichol

Quaternary Organization 298
Overall Geometry and Conformational Changes 298
Subunit–Subunit Interactions and Allostery 299
Aspartate Transcarbamoylase 300
Self-associating Systems 301
Novel Methods of Analysis 301
Equilibria and Perturbations by Ligand 302
Actin Polymerization 304
Tubulin and Microtubules 305
Mixed Associations 306
Discrete-complex Formation 306
Multi-enzyme Complexes 308
Insulin Receptor Binding 309
Affinity Chromatography 309
Crosslinking Reactions 310
Chemically Induced Crosslinking 310
Macroassemblies Involving Self-association 311
Association of Two Reactants 311
Muscle-protein Interactions 312

Chapter 3 Peptide Synthesis
By I. J. Galpin, with Appendices compiled by C. M. Galpin

1 Introduction 315

2 Methods 316
Protective Groups 316
Established Methods of Amino-group Protection 316
New Methods of Amino-group Protection 318
Carboxyl Protection 321
Side-chain Protection 321
Formation of the Peptide Bond 327
Racemization 331
General Deprotection and Side Reactions during Synthesis 333
Repetitive Methods of Peptide Synthesis 335
Solid-phase Synthesis 335
Other Repetitive Methods 341
Polymeric Peptides 342
Enzyme-mediated Synthesis and Semisynthesis 344
Purification Methods 347

3 Syntheses 347

4 Appendix I: A List of Syntheses Reported in 1982 349
Natural Peptides, Proteins, and Partial Sequences 349
Sequential Oligo- and Poly-peptides 357
Enzyme Substrates and Inhibitors 358
Glycopeptides 359
Miscellaneous Peptides 360

5 Appendix II: Amino-acid Derivatives Useful in Synthesis 363
Coded Amino-acids 363
Other Amino-acids 366

6 Appendix III: Purification Methods 368
High-performance Liquid Chromatography 368
Gas–Liquid Chromatography 369
Other Chromatographic Methods 369

Chapter 4 Peptides with Structural Features not Typical of Proteins
By P. M. Hardy

1 Introduction 370

2 Cyclic Peptides 370
2,5-Dioxopiperazines (Cyclic Dipeptides) 370
Larger Cyclic Peptides 375

3 Cyclic Depsipeptides 384

4 Highly Modified Cyclic Peptides 389
Bicyclic β-Lactams 389
Monocyclic β-Lactams 392
Other Modified Cyclic Peptides 393

5 Linear Peptides 393
Peptides Containing α-Aminoisobutyric Acid 393
Peptides Containing Dehydroamino-acids 395
Other Dipeptide Derivatives 401
Other Larger Linear Peptides 405

6 Glycopeptides 408
Glycopeptide Antibiotics 408
Other Glycopeptides 412

Chapter 5 Metal Complexes of Amino-acids, Peptides, and Proteins

By R. W. Hay and K. B. Nolan

1 Introduction 414

2 Amino-acids 415
Synthetic and Spectroscopic Studies 415
Equilibrium Studies 426
Diffraction Studies 430
Stereochemistry and Stereoselectivity 433
Kinetics and Reactivity 435
Schiff Bases 437
General 439

3 Peptides 440
Complexes with Divalent Metal Ions 440
Complexes with Trivalent Metal Ions 447
Other Metal Complexes 450

4 Proteins 451
Haem-containing Proteins 451
Non-haem Iron-containing Proteins 456
Zinc-containing Proteins 457
Copper-containing Proteins 459
Other Metal–Protein Interactions 462

Abbreviations

Abbreviations for amino-acids and their use in the formulations of derivatives follow, with some exceptions, the various Recommendations of the I.U.P.A.C.–I.U.B. Commission on Biochemical Nomenclature, which have been reprinted in Volumes 4, 5, and 8 in this series.

Other abbreviations that have been used are listed here or are defined in the text and table footnotes.

Ac	acetyl
Acm	acetamidomethyl
Ad	adamantyl
Adoc	adamantyloxycarbonyl
Adpoc	1-(1-adamantyl)-1-methylethoxycarbonyl
AMP	adenosine 5′-monophosphate
Aoc	t-amyloxycarbonyl
Aox	4,5-dianisoyl-4-oxazolin-2-one amino-acid derivative
Asu	α-aminosuberic acid
Asx	aspartic acid or asparagine (not yet determined)
atm	atmosphere
ATP	adenosine 5′-triphosphate
Azoc	2-(4-phenylazophenyl)isopropyloxycarbonyl
Beoc	2-bromoethyloxycarbonyl
Boc	t-butoxycarbonyl
Bom	benzyloxymethyl
Bpoc	2-(4-biphenyl)isopropoxycarbonyl
Br.Bom	4-Br-benzyloxymethyl
BSA	bovine serum albumin
BTCO	1-benzotriazolylcarbonyl
Btm	benzylthiomethyl
Bu^t	t-butyl
Bzh	benzhydryl (diphenylmethyl)
$Bzh(OMe)_2$	4,4′-dimethoxybenzhydryl
Bzl	benzyl
Bzl(4-Cl)	4-chlorobenzyl
$Bzl(2,6\text{-}Cl_2)$	2,6-dichlorobenzyl
Bzl(4-CN)	4-cyanobenzyl
$Bzl(NO_2)$	4-nitrobenyzl
$Bzl(2\text{-}NO_2)$	2-nitrobenzyl
Bzl(OMe)	4-methoxybenzyl
Cac	9-carbazolylcarbonyl
c.d.	circular dichroism
Cha	cyclohexylamine

CLIP	adrenocorticotropic hormone (18—39)
Cm	carboxymethyl
Cmc	*S*-carboxymethylcysteine
CoA	coenzyme A
Cox	4,5-di-(4-chlorophenyl-4-oxazoline-2-one) derivative
Cpe	cyclopentyl
CPh_2Py	diphenyl-4-pyridylmethyl
DCCI	dicyclohexylcarbodi-imide
Dcha	dicyclohexylamine
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
Ddz	3,5-dimethoxy($\alpha\alpha$-dimethyl)benzyloxycarbonyl
Dha.NHEt	dehydroalanylethylamide
DHCH	1,2-dihydroxycyclohex-1,2-ylene
DMA	dimethylacetamide
Dmb	2,4-dimethoxybenzene sulphonyl
DMCBzl	dimethylcarbamoylbenzyl
DMCZ	dimethylcarbamoylbenzyloxycarbonyl
DMF	*NN*-dimethylformamide
Dmoc	1,3-dithian-2-yl-methoxycarbonyl
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
Dnp	2,4-dinitrophenyl
2,4-Dnps	2,4-dinitrosulphenyl
Dnpy	3,5-dinitro-1-(*p*-nitrophenyl)-4-pyridone
Dns	1-dimethylaminonaphthalene-5-sulphonyl (dansyl)
Dopa	3,4-dihydroxyphenylalanine
DP	degree of polymerization
DPA	diphenylacetyl
Dpp	diphenylphosphinoyl
Dppm	diphenyl-4-pyridylmethyl
DPtd	4,6-diphenylthieno[3,4-*d*][1,3]dioxal-2-one 5,5-dioxide
DTNB	5,5′-dithiobis-(2-nitrobenzoic acid)
Dts	dithiosuccinoyl
Ec	ethylcarbamoyl
edta	ethylenediaminetetra-acetate
EEDQ	2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline
e.i.–m.s.	electron-impact ionization mass spectrometry
En	ethylenediamine
e.n.d.o.r.	electron nuclear double resonance
e.p.r.	electron paramagnetic resonance
e.s.r.	electron spin resonance
Et	ethyl
f.a.b.	fast atom bombardment
Fal	hexafluorovaline
f.d.	field desorption
Fm	9-fluoroenylmethyl
Fmoc	9-fluoroenylmethoxycarbonyl

GABA	γ-aminobutyric acid
Gal	galactose
g.c.-m.s.	gas chromatograph–mass spectrometer combination
g.l.c.	gas–liquid chromatography
Glc	glucose
Glp	2-pyrrolidone-5-carboxylic acid
Glx	glutamic acid or glutamine (not yet determined)
GS	gramicidin S
GTP	guanosine 5′-triphosphate
HMPA	hexamethylphosphoric triamide
HOBt	hydroxybenzotriazole
h.p.l.c.	high-performance liquid chromatography
Iboc	isobornyloxycarbonyl
i.r.	infrared
LDA	lithium di-isopropylamide
Mal	maleoyl
Man	mannose
Mbh	4,4-dimethoxybenzyhydryl
Mbs	4-methoxybenzenesulphonyl
Mds	4-methoxy-2,6-dimethylbenzenesulphonyl
Me	methyl
Mea	mercaptoethylamine
$MePh_2Peoc$	2-methyldiphenylphosphinioethyloxycarbonyl
Mhoc	1-methylcyclohexylcarbonyl
Moc	methoxycarbonyl
Mpt	dimethylphosphothioyl
m.s.	mass spectrometry
Msc	2-(methylsulphonyl)ethoxycarbonyl
Mtb	2,4,6-trimethoxybenzene sulphonyl (mesitylene sulphonyl)
Mtc	2-methylthioethoxycarbonyl
MTM	methylthiomethyl
Mtr	2,3,6-trimethyl-4-methoxybenzenesulphonyl
NAD	nicotinamide-adenine dinucleotide
NCA	*N*-carboxyanhydride
Nma	maleimido
Nmps	4-methyl-2-nitrosulphenyl
n.m.r.	nuclear magnetic resonance
n.O.e.	nuclear Overhauser effect
Np	4-nitrophenyl
NPh	phthalimido
Nps	2-nitrophenylsulphenyl
Npys	3-nitropyridine-2-sulphenyl
Nsu	succinimido
OHFP	hexfluoroisopropyl ester
ONp	4-nitrophenyl ester
ONp(*o*)	2-nitrophenyl ester
ONp.Pic	2-nitro-4-(4-picolyloxycarbonyl)phenyl ester

ONSu	succinimido ester
OPcp	pentachlorophenyl ester
OPfp	pentafluorophenyl ester
OPic	4-picolyl ester
Opop	2-(phenacyloxy)phenyl ester
o.r.d.	optical rotatory dispersion
OTAT	thiazoline-2-thione ester
OTce	2,2,2-trichloroethyl ester
OTcp	2,4,5-trichlorophenyl ester
PAAC	isopropylidene aminoxycarbonyl
Pac	phenacyl
PAC	phenylacetyl
Pam	4-(hydroxymethyl)phenyl acetamidomethyl (resin)
PChd	3,5-di-t-butyl-4-oxo-1-phenyl-2,5-cyclohexadienyl
Peoc	2-triphenylphosphinioethyloxycarbonyl
Ph(SMe)	*p*-methylthiophenyl
Pic	4-picolyl
Picoc	4-picolyloxycarbonyl
Pipoc	piperidino-oxycarbonyl
Piv	pivaloyl
Pme	pentamethylbenzenesulphonyl
Pms	*p*-tolylmethylsulphonyl
Poc	cyclopentyloxycarbonyl
Poly(A)	poly(adenylic acid)
Ppoc	phenylisopropoxycarbonyl
Ppt	diphenylphosphinothioyl
Pth-Gly	the phenylthiohydantoin derived from glycine, *etc.*
py	pyridine
Pz	*p*-phenylazobenzyloxycarbonyl
SBu^t	t-butylthio
SCB	t-butyloxycarbonylsulphenyl
Scm	carboxymethylsulphenyl
SDS	sodium dodecyl sulphate
Spr^i	isopropylthio
Sub	5-dibenzosuberyl
Tac	toluene-*p*-sulphonylaminocarbonyl
tBa	t-butylammonium
TCBoc	2,2,2-trichloro-1,1-dimethylethoxycarbonyl
Tcp	2,4,5-trichlorophenyl
Tcroc	2-CF_3-chromonylmethylenecarbonyl
Tcrom	2-CF_3-chromonylmethyl
Tfa	trifluoroacetyl
Thp	tetrahydropyranyl
Tht	thiazolidine-2-thione
t.l.c.	thin-layer chromatography
Tmb	2,4,6-trimethylbenzyl
Tmeda	*NNN'N'*-tetramethylethylenediamine

Tmg	tetramethylguanidine
TMSE	2-trimethylsilylethoxycarbonyl
Tnps	2,4,6-trinitrophenylsulphenyl
Tos	toluene-*p*-sulphonyl
TRH	thyrotropin-releasing hormone
Troc	2,2,2-trichloroethoxycarbonyl
Trt	triphenylmethyl
Tse	2-(toluene-*p*-sulphonyl)ethyl
u.v.	ultraviolet
Xan	9-xanthydryl
Z	benzyloxycarbonyl
Z(2-Br)	2-bromobenzyloxycarbonyl
Z(OMe)	4-methoxybenzyloxycarbonyl
Ztf	1-benzyloxycarbonylamino-2,2,2-trifluoroethyl

1
Amino-acids

BY G. C. BARRETT

1 Introduction

The coverage given in this chapter draws mainly on the chemical literature, but also on the biochemical and biological literature where material relevant to the chemistry, occurrence, and analysis of amino-acids can be found. However, only brief coverage is given, as in previous years, of the distribution and biological roles of well known amino-acids.

Textbooks and Reviews.—Important new textbooks and symposium proceedings[1–4] cover non-protein amino- and imino-acids,[1] ammonia assimilation and amino-acid metabolism in plants,[2] and recent developments in amino-acid chemistry in the context of peptide and protein synthesis.[3,4] Reviews cover physiological roles for γ-aminobutyric acid (GABA)[5] and its β-hydroxy analogue,[6] crosslinking amino-acid residues in collagen,[7] and the history of the discovery of the existence of asparagine and glutamine residues in proteins.[8] Fowden has reviewed the recent literature for non-protein amino-acids.[9]

2 Naturally Occurring Amino-acids

Occurrence of Known Amino-acids.—This section is particularly concerned with the location of well-known amino-acids in unusual situations and of unusual amino-acids in a variety of sources.

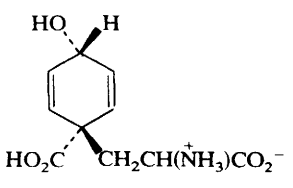
Methods for the isolation of proline and hydroxyproline from fossil bone have been described.[10] L-Canavanine isolated from *Canavalia gladiata* may be

[1] G. A. Rosenthal, 'Plant Nonprotein Amino- and Imino-acids: Biological, Biochemical, and Toxicological Properties', Academic Press, New York, 1982.
[2] B. J. Miflin and P. J. Lea, Encyclopaedia of Plant Physiology (New Series), 1982, Vol. 14A (Nucleic Acids and Proteins in Plants, Part I). p. 5.
[3] 'Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins', ed. B. Weinstein, Dekker, New York, 1982, Vol. 6.
[4] 'Peptides: Synthesis, Structure, Function', Proceedings of the 7th American Peptide Symposium, 1981, ed. D. H. Rich and E. Gross, Pierce Chemical Co., Rockford, Illinois, U.S.A., 1981.
[5] A. Santos-Ruiz, *Rev. Esp. Fisiol.*, 1982, **38,** 1.
[6] A. Mori, *Neurosciences (Kobe, Jpn.)*, 1982, **7,** 236.
[7] D. Fujimoto, *Seikagaku*, 1982, **54,** 314.
[8] C. Chibnall, *Trends Biochem. Sci. (Pers. Ed.)*, 1982, **7,** 191.
[9] L. Fowden in 'Biochemistry of Plants', ed. E. E. Conn, Academic Press, New York, *1981*, *Vol.* 7, p. 215; see also E. A. Bell, *ibid.*, p. 1.
[10] T. W. Stafford, R. C. Duhamel, C. V. Haynes, and K. Brendel, *Life Sci.*, 1982, **31,** 931.

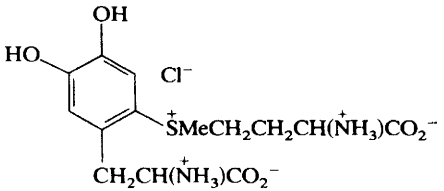
purified as its flavianic acid salt;[11] the pentacyanoammonioferrate positive spot seen in cellulose t.l.c. of extracts of alfalfa is histidine,[12] not canavanine as claimed earlier.

The simplest non-protein amino-acid, 2-aminobutanoic acid, has been located in mixed rumen ciliate protozoal culture media.[13] Other aliphatic α-amino-acids uncovered recently include L-*threo*-γ-hydroxycitrulline[14] and N^{δ}-benzoyl-γ-hydroxy-L-ornithine[15] from seeds of *Vicia pseudo-orubus*, N^{α}-(γ-glutamyl)-histidine, -ornithine, and -lysine from Shiitake mushroom (*Lentinus edodes*; the first report of the occurrence of these derivatives in mushrooms),[16] L-β-(1,4-cyclohexadienyl)-L-alanine from *Pseudomonas* 1–30,[17] and another 1,4-cyclohexadiene derivative, arogenic acid (1) from *Pseudomonas aureofaciens* as an intermediate in the biosynthesis of phenylalanine and tyrosine.[18]

The methionine adduct of dopa *o*-quinone, which forms during work-up of solutions of these amino-acids and therefore may appear in biological extracts, is proposed to possess structure (2).[19] The natural occurrence of *S*-methyl-L-cysteine and its sulphoxide has been reviewed.[20]



(1)



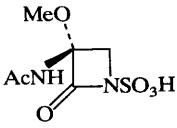
(2)

The possibilities for the existence of amino-acids and other important biochemicals on other planets have been reviewed.[21]

The archetypal β-amino-acid β-alanine has been found in mycelial cell walls of mature *Morchella esculenta*.[22]

New Natural Amino-acids.—*E*-2*S*-Amino-3-methyl-3-pentenoic acid is a new natural amino-acid, found in *Coniogramme intermedia*.[23] The β-lactam (3)[24] and the γ-lactone (4)[25] are cyclized *N*-acetyl-α-amino-acid derivatives isolated from bacterial cultures; (4) has little biological potency.

[11] R. Yang and D. Guo, *Huaxue Shiji*, 1982, 192 (*Chem. Abstr.*, 1982, **97**, 212 638).
[12] G. A. Rosenthal and D. L. Dahlman, *Experientia*, 1982, **38**, 1034.
[13] R. Onodera, K. Miura, and H. Fukuda, *J. Protozool.*, 1982, **29**, 122; R. Onodera and T. Ushijima, *ibid.*, p. 547.
[14] T. Miki and S. Hatanaka, *Phytochemistry*, 1982, **21**, 224.
[15] S. Makisumi, K. Mizusaki, S. Hatanaka, and N. Izumiya, *Phytochemistry*, 1982, **21**, 223.
[16] Y. Aoyagi, T. Sugahara, T. Hasegawa, and T. Suzuki, *Agric. Biol. Chem.*, 1982, **46**, 1939.
[17] N. Onishi, T. Watanabe, K. Izaki, and H. Takahashi, *J. Antibiot.*, 1982, **35**, 90.
[18] B. Keller, E. Keller, O. Salcher, and F. Lingens, *J. Gen. Microbiol.*, 1982, **128**, 1199.
[19] M. N. Gupta and P. J. Vithayathil, *Bioorg. Chem.*, 1982, **11**, 101.
[20] G. A. Maw, *Sulfur Rep.*, 1982, **2**, 1.
[21] B. Nagy, *Naturwissenschaften*, 1982, **69**, 301.
[22] M. E. Jacobs, *Comp. Biochem. Physiol. B*, 1982, **72**, 173.
[23] S. Hatanaka, Y. Murooka, K. Saito, Y. Ishida, and Y. Takeuchi, *Phytochemistry*, 1982, **21**, 453.
[24] W. L. Parker, W. H. Koster, C. M. Cimarusti, D. M. Floyd, W. C. Liu, and M. L. Rathnum, *J. Antibiot.*, 1982, **35**, 189.
[25] W. L. Parker, M. L. Rathnum, and W. C. Liu, *J. Antibiot.*, 1982, **35**, 900.



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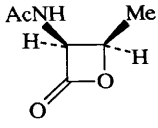
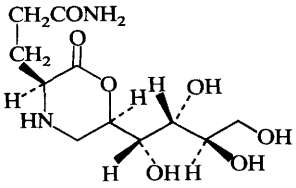
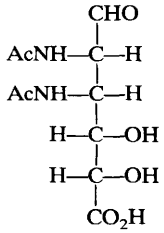


(4)

Re-investigation of agropine from crown-gall tumours (see also Vol. 10, p. 2) shows it to be N^2-(1′-deoxy-D-mannitol-1′-yl)-L-glutamine-1,2′-lactone (5).[26] The amino-glyconic acids as a class would be as well located among amino-acids as among amino-sugars. A compound of this type (6) occurs in *Pseudomonas aeruginosa* 170 005.[27]



(5)



(6)

New Amino-acids from Hydrolysates.—Unusual components of polypeptides and proteins are collected under this heading. The occurrence of *N*-trimethylalanine at the *N*-terminus of histone 2B from *Tetrahymena pyriformis* constitutes the first example of *N*-terminal blocking through methylation in the histone field.[28] Muscle myosin subfragment I has been shown by ^{1}H n.m.r. to carry the same *N*-terminal residue.[29]

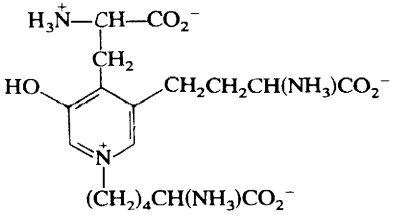
Crosslinking opportunities other than the disulphide grouping of cystine continue to stimulate a considerable amount of research effort, because of the importance of irreversible inter-chain reactions in the ageing process. The structure of pyridinoline (7), a crosslinking diamino-diacid from collagen (see also Vol. 13, p. 2), has been confirmed by f.a.b. mass spectrometry,[30] and the existence of deoxypyridinoline (the analogue of pyridinoline with H in place of the aliphatic hydroxy group) as a new crosslinking residue in collagen has been established by two research groups.[30,31]

'Isodityrosine', an oxidatively coupled dimer of tyrosine involving a diphenyl ether linkage, is a new phenolic crosslinking diamino-diacid found in hydrolysates of cell walls of many higher plants.[32]

[26] M. E. Tate, J. G. Ellis, A. Kerr, J. Tempe, K. E. Murray, and K. J. Shaw, *Carbohydr. Res.*, 1982, **104,** 105.
[27] Yu. A. Knivel, E. V. Vinogradov, A. S. Shashkov, B. A. Omitriev, and N. K. Kochetkov, *Carbohydr. Res.*, 1982, **104,** C4.
[28] M. Nomoto, Y. Kyogoku, and K. Iwai, *J. Biochem. (Tokyo)*, 1982, **92,** 1675.
[29] G. D. Henry, D. C. Dalgarno, G. Marcus, M. Scott, B. A. Levine, and I. P. Trayer, *FEBS Lett.*, 1982, **144,** 11.
[30] M. Barber, R. S. Bordoli, G. J. Elliott, D. Fujimoto, and J. Escott, *Biochem. Biophys. Res. Commun.*, 1982, **109,** 1041.
[31] T. Ogawa, T. Ono, M. Tsuda, and Y. Kawanishi, *Biochem. Biophys. Res. Commun.*, 1982, **107,** 1252.
[32] S. C. Fry, *Biochem. J.*, 1982, **204,** 449.

3 Synthesis of Amino-acids

General Methods.—Standard methods have been used for the synthesis of β-(3-pyrrolin-*N*-oxylyl)alanine (8) proposed as a paramagnetic amino-acid for use in peptide synthesis.[33] Alkylation of dimethyl acetylaminomalonate, diphenylmethylideneglycine ethyl ester, or diethyl malonate (followed by treatment with diphenylphosphoryl azide and benzyl alcohol to give the *N*-benzyloxycarbonyl amino-acid) was fully studied in this context.[33] Alkylation of acylamidomalonates continues to be widely used for the synthesis of α-amino-acids [homologues of 2-amino-5-(*p*-methoxyphenyl)pentanoic acid[34] and other examples mentioned later in this chapter, refs. 113, 115, 130, 131, 133, and 147]. Improvements have been achieved in the Schiff-base alkylation route,[35,36] where 73—94% yields of monoalkylation products were obtained in most cases using ion-pair extraction or catalytic liquid–liquid or solid–liquid phase-transfer techniques with ethyl *p*-chlorobenzylideneglycinate.[35] Yields of α-methyl-α-amino-acids were equally good in corresponding alkylation reactions of the alanine analogue.[36]



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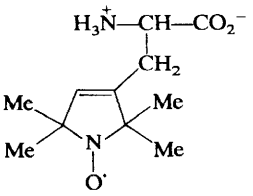


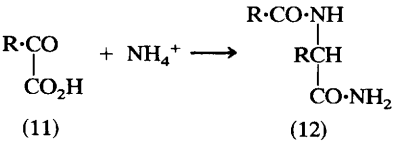
(8)

Alternative methods for the introduction of a nitrogen function adjacent to a carboxy group include amido-alkylation (for β-amino-acid synthesis $R^1{\cdot}CO{\cdot}NH{\cdot}CHR^2{\cdot}SO_2Tol$ has been proposed[37]), a racemization-free synthesis

(9) (10)

of *NN*-dialkylamino-acids (9) → (10),[38] and development of the biogenetically modelled amination of α-keto-acids in aqueous media,[39–41] which has led to

[33] L. Lex, K. Hideg, and H. O. Hankovszky, *Can. J. Chem.*, 1982, **60**, 1448.
[34] N. Kosui, M. Waki, T. Kato, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 918.
[35] L. Ghosez, J.-P. Antoine, E. Deffense, M. Navano, V. Libert, M. J. O'Donnell, and W. A. Brudu, *Tetrahedron Lett.*, 1982, **23**, 4255.
[36] M. J. O'Donnell, B. Le Clef, D. B. Rusterholz, L. Ghosez, J.-P. Antoine, and M. Navano, *Tetrahedron Lett.*, 1982, **23**, 4259.
[37] J. Morton, A. Rahim, and E. R. H. Walker, *Tetrahedron Lett.*, 1982, **23**, 4123.
[38] F. Effenberger, U. Burkard, and J. Willfahrt, *Angew. Chem.*, 1983, **95**, 50.
[39] F. Egami, Y. Makino, K. Sato, and M. Nishizawa, *Proc. Jpn. Acad. Sci., Ser. B*, 1981, **57**, 329.
[40] F. Egami, Y. Makino, M. Nishizawa, and K. Sato, *Nippon Kagaku Kaishi*, 1982, **56**, 537 (*Chem. Abstr.*, 1983, **97**, 198 523).
[41] H. Yanagawa, Y. Makino, K. Sato, M. Nishizawa, and F. Egami, *J. Biochem.* (*Tokyo*), 1982, **91**, 2087.



(11) (12)

the discovery of the novel reaction in which low yields (1—20%) of the corresponding *N*-(2-oxoalkanoyl)amino-acid amide (11) → (12) are formed. The α-amino-acids are easily obtained from these intermediates by acid hydrolysis.

Further exploration of amidocarbonylation routes to α-amino-acids (see Vol. 13, p. 4) has established that allylic alcohols react with acetamide in the presence of $Co_2(CO)_8$ and $HRh(CO)(PPh_3)_3$, reductive carbonylation with a 1:1 mixture of H_2 and CO at 100 atm at 110 °C in dioxane, giving good yields of *N*-acetylamino-acids (the allylic moiety is hydrogenated in this process).[42] Electrochemical reductive carboxylation of *N*-arylideneamines RN=CHAr and RN=CMeAr with CO_2 at a mercury cathode gives predominantly *C*-carboxylation products, and competing reduction of the double bond can be suppressed by increasing the water content of the medium.[43]

Synthesis of amino-acids and peptides exploiting 1,4-opening of β-lactams (see Vol. 13, p. 4) has been reviewed.[44] A forthcoming textbook[45] includes an exhaustive coverage of the synthesis of amino-acids.

A full account has been published of the synthesis of β-amino-acids from *N*-acetyl thioamides and $Ph_3P{=}CHCO_2Me$[46] followed by reduction of the resulting β-acetylaminoacrylate. A total synthesis of iturinic acid, $Me_2CH(CH_2)_8CH(\overset{+}{N}H_3)CH_2CO_2^-$, as its ethyl ester was included as an example of this efficient route.[46] The Reformatzky route to β-amino-acids[47] employing α-bromoalkanoic acids and Schiff bases gives moderate yields.

Most syntheses of amino-acids yield salts from which they may be recovered by passage through columns of crosslinked poly(4-vinylpyridine).[48] Basic amino-acids, however, elute as their mono-acid salts.

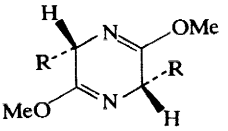
Asymmetric Synthesis.—All the papers encountered in the 1982 literature describe extensions of previously established principles. The general topic has been reviewed.[49]

Representative papers concerned with asymmetric hydrogenation[50,51] continue the use of chiral rhodium–phosphine complexes. Reductive amination of

[42] K. Hirai, Y. Takahashi, and I. Ojima, *Tetrahedron Lett.*, 1982, **23,** 2491.
[43] U. Hess and M. Ziebig, *Pharmazie*, 1982, **37,** 107.
[44] I. Ojima and N. Hatanaka, *Yuki Gosei Kagaku Kyokaishi*, 1982, **40,** 209 (*Chem. Abstr.*, 1982, **97,** 56 205).
[45] G. C. Barrett in 'The Chemistry and Biochemistry of the Amino-acids', ed. G. C. Barrett, Chapman and Hall, London, 1984, Chap. 5; C. N. C. Drey, *ibid.*, Chap. 3.
[46] M. Slopianka and A. Gassauer, *Liebigs Ann. Chem.*, 1981, 2258.
[47] M. Bellassoued, R. Arous-Chtara, and M. Gaudemar, *J. Organomet. Chem.*, 1982, **231,** 185.
[48] D. M. Jewett and R. L. Ehrenkaufer, *Anal. Biochem.*, 1982, **122,** 319.
[49] D. Hoppe, *Nachr. Chem., Tech. Lab.*, 1982, **30,** 782.
[50] N. Izumiya in 'Asymmetric Reactions and Processes in Chemistry', Am. Chem. Soc., Symp. Ser., No. 185, ed. E. L. Eliel and S. Otsuka, American Chemical Society, Washington, D.C., U.S.A., 1982, p. 272.
[51] J.-C. Paulin and H. B. Kagan, *J. Chem. Soc., Chem. Commun.*, 1982, 1261.

4-isopropylidene-2-methyloxazolin-5-ones using (*S*)-phenylethylamine gives *N*-acetyl-L-valine phenylethylamide in 44% enantiomeric excess.[52]

The asymmetric-alkylation approach also offers several alternative methodologies. Schiff bases $Ph_2C{=}NCH_2CO_2R$ (R = Me or Et) give up to 40% enantiomeric excess of the *S*-alanine derivatives after carbanion formation with $Pr^i{}_2NLi$ and methylation with a 1,2,5,6-di-isopropylidene-D-glucofuranose 3-methanesulphonate.[53] *N*-Benzylidene DL-phenylalanine methyl ester similarly underwent asymmetric methylation with methyl iodide in the presence of chiral lithium (*S*)-2-alkylpyrrolidines.[54] The chiral heterocycles (13) are masked Schiff bases and have been extensively studied[55] (see also Vol. 13, p. 5) in the context of asymmetric synthesis of α-amino-acids and their α-methyl analogues. Better than 95% stereoselectivity can be achieved through anion formation with BuLi, followed by alkylation with an alkyl or benzyl bromide.



(13)

Alkylation of chiral Schiff bases $RCMe{=}NCH_2CO_2^-$, where R is the (*S*)-*o*-*N*-(*N*-benzylpropylamino)phenyl grouping complexed to Cu^{2+}, gives predominantly (95%) *threo*-threonine in at least 97% optical purity when acetaldehyde is the other reactant.[56]

(−)-Menthyl isocyanoacetate $CNCH_2CO_2Men$ gives $H_2N(CH_2)_3CR(\overset{+}{N}H_3)$-$CO_2^-$ through successive alkylation with an alkyl iodide RI and acrylonitrile after anion formation with NaH, followed by acid hydrolysis.[57] Higher homologous amino-acids such as (3*S*,4*S*)- and (3*R*,4*S*)-$Me_2CHCH_2CH(\overset{+}{N}H_3)$-$CH(OH)CO_2^-$ were prepared in several steps from *N*-phthaloyl-L-leucyl chloride through condensation with (−)-menthyl t-butyl malonate and $NaBH_4$ reduction of the resulting β-oxo-ester.[57]

Full details of the enantioselective protonation of phenylglycine Schiff bases with a chiral acid leading to enantiomer excesses up to 70% have been published.[58] The preliminary communication describing this approach was discussed in Vol. 11, p. 16.

Prebiotic Synthesis of Amino-acids.—A number of papers mentioned elsewhere in this chapter have described new possibilities for the synthesis of

[52] G. V. Chel'tsova, E. I. Karpeiskaya, and E. I. Klabunovskii, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1981, 2350.

[53] P. Duhamel, J.-Y. Valnot, and J. J. Eddine, *Tetrahedron Lett.*, 1982, **23**, 2863.

[54] T. Yamashita, H. Mitsui, H. Watanabe, and N. Nakamura, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 961.

[55] (*a*) U. Schöllkopf, U. Groth, and W. Hartwig, *Liebigs Ann. Chem.*, 1982, 2407; (*b*) U. Schöllkopf, W. Hartwig, K. H. Posposchil, and H. Kehne, *Synthesis*, 1981, 966; U. Schöllkopf, U. Groth, K. O. Westphalen, and C. Deng, *ibid.*, p. 969; U. Groth, Y. Chiang, and U. Schöllkopf, *Liebigs Ann. Chem.*, 1982, 1756.

[56] Y. N. Belokon', I. E. Zel'tzer, M. G. Ryzhov, M. B. Saporovskaya, V. I. Bakhmutov, and V. M. Belikov, *J. Chem. Soc., Chem. Commun.*, 1982, 180.

[57] M. Kirihata, *Bull. Univ. Osaka Prefect., Ser. B*, 1981, **33**, 135 (*Chem. Abstr.*, 1982, **96**, 143 271).

[58] L. Duhamel and J. C. Plaquevent, *Bull. Soc. Chim. Fr.*, 1982, Part 2, 75.

amino-acids from simple starting materials under ambient conditions. Ammonia and glyoxylic acid yield *N*-oxalylglycine in aqueous solutions,[39–41,59] and u.v. irradiation of these solutions in the presence of an alkene with acetone as sensitizer gives aspartic acid, norvaline, valine, leucine, phenylalanine, and tyrosine.[59]

Simpler reactants such as CO_2[60,61] or methane[61,62] can be caused to react with nitrogen to yield amino-acids, using u.v. light or electric discharges as energy sources,[63] suggesting that the frozen surface of Titan, with its HCN–CH_4–N_2 atmosphere, could indeed have accumulated amino-acids.[61,62]

Glycine is converted into a mixture of seven aliphatic α-amino-acids at 200 °C in contact with N_2 and granite, basalt, or bentonite with or without $MnCO_3$ or Al_2O_3.[64] An entertaining abstract for a paper[65] describing the formation of amino-acids 'in systems not containing any source of N, utilizing compounds with antiseptic properties such as PhOH, resorcinol, *etc.*' hides the fact that the nitrogen molecule is the source of the amino groups in the products. The reaction is light-driven and not a bacterial process; the phenols are oxidized and water is cleaved by photolysis, to provide the energy to drive the (unlikely) reactions.[65]

Aqueous solutions of ammonium salts of dicarboxylic acids irradiated with ultra-short (picosecond) laser u.v. pulses gave the corresponding amino-dicarboxylic acids.[66]

Protein and Other Naturally Occurring Amino-acids.—There is space only for representative papers on production of protein α-amino-acids by fermentation (the formation of L-tryptophan in culture media of azaserine-resistant *Brevibacterium flavum* mutant[67] and of *Escherichia coli* offered L-serine and indole,[68] and L-lysine by *Brevibacterium lactofermentum* mutants[69]). The topic has been reviewed,[70–72] ref. 70 being taken from a volume containing numerous papers on the subject, and ref. 72 being narrower in its scope (L-dopa, L-cysteine, and D-*p*-hydroxyphenylglycine).

Methionine is biosynthesized from 5′-methylthioadenosine *via* 2-oxo-4-methylthiobutyric acid in rat liver.[73]

Coverage of the biosynthesis of the non-protein α-amino-acids is similarly selective. β-Pyrazolyl-L-alanine has 1,3-diaminopropane as precursor for the

[59] M. Nishizawa and F. Egami, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 2689.
[60] J. Gribbin, *New Sci.*, 1982, **94**, 413.
[61] M. Ishigami, M. Kinjo, K. Nagano, and Y. Hattori, *Origins Life*, 1982, **12**, 307.
[62] F. Raulin, D. Mourey, and G. Toupance, *Origins Life*, 1982, **12**, 267.
[63] A. R. Bossard, F. Faulin, D. Mourey, and G. Toupance, *J. Mol. Evol.*, 1982, **18**, 173.
[64] Ch. Ivanov and N. Slavcheva, *Dokl. Bolg. Akad. Nauk*, 1981, **34**, 1401.
[65] A. K. Sen, *J. Indian Chem. Soc.*, 1982, **59**, 476 (*Chem. Abstr.*, 1983, **97**, 163 440).
[66] V. S. Letokhov, Yu. A. Matveets, V. A. Semchishen, and E. V. Khoroshilova, *Appl. Phys. B*, 1981, **26**, 243.
[67] I. Shiio, S. Sugimoto, and K. Kawamura, *Agric. Biol. Chem.*, 1982, **46**, 1849.
[68] F. Wagner, S. Lang, W. G. Bang, K. D. Vorlop, and J. Klein, *Enzyme Eng.*, 1982, **6**, 251.
[69] M. E. Schonfeldt, and T. G. Watson, *S. Afr. Food. Rev.*, 1982, **9**, S111 (*Chem. Abstr.*, 1982, **97**, 90 343).
[70] Y. Minota, *Hakko to Kogyo*, 1982, **40**, 292.
[71] H. Enei, H. Shibai, and Y. Hirose, *Ann. Rep. Ferment. Processes*, 1982, **5**, 79.
[72] T. Yamamoto, *Kagaku Gijutsushi MOL*, 1982, **20**, 21 (*Chem. Abstr.*, 1982, **97**, 180 032).
[73] P. S. Backlund, C. P. Chang, and R. A. Smith, *J. Biol. Chem.*, 1982, **257**, 4196.

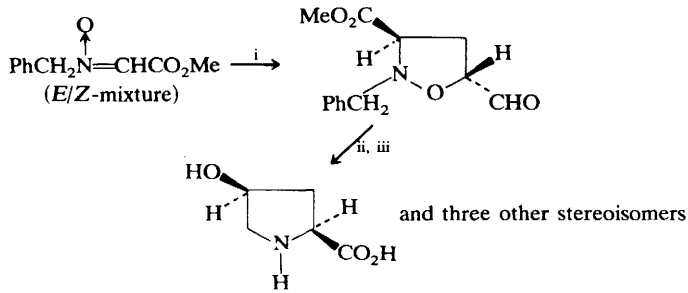
pyrazole moiety in cucumber seeds.[74] Biosynthesis of L-canavanine in jack bean (*Canavalia ensiformis*) has received further detailed study.[75]

Laboratory syntheses of amino-acids that occur in proteins or in other natural sources continue to attract the interest of academic and industrial research groups. Full details of the synthesis of glycine by ammonolysis of trichloroethylene[76] (Vol. 11, p. 9) and of DL-alanine by ammonolysis of 2-chloropropanoic acid in aqueous solution under pressure[77] (Vol. 14, p. 5) have now been published. By-products in the preparation of MeS-CH_2CH_2CHO from acrolein and methanethiol, for use in the Strecker synthesis of DL-methionine, have been shown to be oligomers $HO[CH(CH_2CH_2SMe)O]_n$ and aldol condensation products of the target aldehyde.[78]

Alternative syntheses have been reported for 4-hydroxy-DL-proline (Scheme 1),[79] L-α-amino-adipic acid from *N*-Boc-L-aspartic acid α-t-butyl ester (Scheme 2),[80] and L-dopa from L-glutamic acid (Scheme 3).[81]

As in two of the three preceding syntheses, cycloaddition offers increasingly attractive possibilities in synthesis; the approach has already been used in syntheses of the anti-tumour compound AT-125 ('acivicin'), and a further synthesis of this amino-acid (14) uses (*S*)-vinylglycine and chlorofulminic acid, ClNCO, from dichloroformaldoxime, $Cl_2C{=}NOH$, and $AgNO_3$.[82]

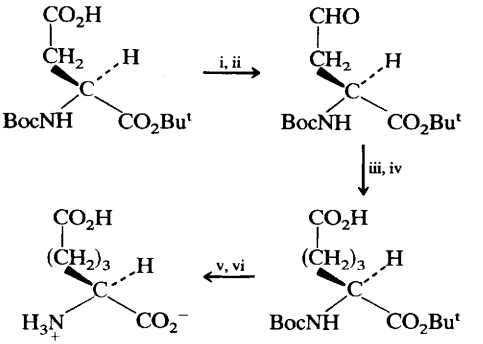
Aliphatic α-amino-acids for which syntheses have been reported recently include β-carboxy-L-aspartic acid. This is prepared by the reaction of $[(NH_3)_5CoO_2CCHO]^{2+}$ with $H_2C(CO_2Et)_2$ in DMSO, then dehydration, giving $[(NH_3)_5CoO_2CCH{=}C(CO_2Et)_2]^{2+}$;[83] the addition of NH_3 [through dissolution



Reagents: i, $CH_2{=}CHCHO$; ii, $H_2/Pd(OH)_2$; iii, hydrolysis

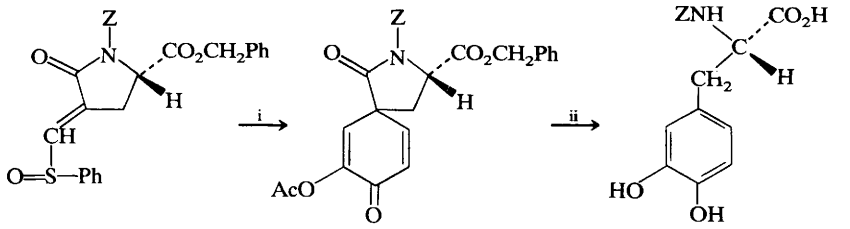
Scheme 1

[74] E. G. Brown, K. A. M. Flayeh, and J. R. Gallan, *Phytochemistry*, 1982, **21**, 863.
[75] G. A. Rosenthal, *Plant Physiol.*, 1982, **69**, 1066.
[76] M. Inoue and S. Enomoto, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 33.
[77] Y. Ogata and M. Inaishi, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3605.
[78] V. S. Balakin, B. S. Gorbunov, G. B. Zvegintseva, and L. S. Romanova, *Khim. Promst. (Moscow)*, 1982, 84 (*Chem. Abstr.*, 1982, **97**, 92 707).
[79] J. Hara, Y. Inouye, and H. Kakisawa, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3871.
[80] K. Ramsamy, R. K. Olsen, and T. Emery, *Synthesis*, 1982, 42.
[81] S. Danishefsky and T. A. Craig, *Tetrahedron*, 1981, **37**, 4081.
[82] P. A. Wade, M. K. Pillay, and S. M. Singh, *Tetrahedron Lett.*, 1982, **23**, 4563.
[83] N. E. Dixon and A. M. Sargeson, *J. Am. Chem. Soc.*, 1982, **104**, 6716.



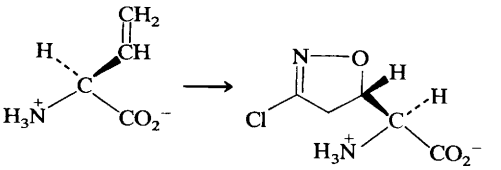
Reagents: i, EtO-COCl; ii, $NaBH_4$, CrO_3–py; iii, $Ph_3P{=}CHCO_2Et$; iv, Pd/C–H_2; v, saponification; vi, 6M HCl

Scheme 2



Reagents: i, $MeOCH{=}C(OAc)C(OSiMe_3){=}CH_2$ in refluxing xylene, 7 h, N_2; ii, hydrolysis

Scheme 3



(14)

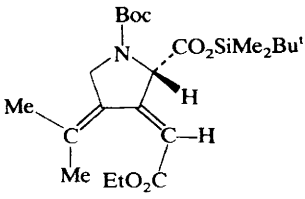
of the cobalt (III) complex in liquid ammonia] gives the malonate from which the target molecule is obtained through hydrolysis and resolution.[83] Stereoselective synthesis of δγ-dihydroxyisoleucine starts with Boc-glycine and $MeC{\equiv}CCH_2OH$, proceeds *via* stereoselective Claisen rearrangement of the *Z*-2-butenyl ester obtained from these reactants, and then *via* elaboration into the appropriate stereoisomer of $CH_2{=}CHCHMeCH(NHBoc)CO_2H$ and iodolactonization to give the lactone of the synthetic objective.[84]

Synthesis of hypusine from N^{α}-benzyloxycarbonyl-L-lysine benzyl ester, through treatment with (*R*)-$ZNHCH_2CH_2CH(OH)CH_2Br$ and deprotection,

[84] P. A. Bartlett, D. J. Tanzella, and J. F. Barstow, *Tetrahedron Lett.*, 1982, **23**, 619.

gave material identical in all respects with the natural compound, thus verifying its absolute configuration.[85] Synthesis of epimers of HO_2CCHMe-(*S*)-Arg-OH from D- or L-alanine and 5-acetylamino-2-bromopentanoic acid followed by conventional conversion of the resulting octopinic acids with $H_2NC(=NH)SMe$ into the octopines confirms the D-configuration of the alanine moiety of the natural (+)-octopine.[86] Similar approaches have verified the L,D-configuration for nopaline (from the crown-gall tumour of *Helianthus annus*) through synthesis from L-arginine and 2-oxoglutaric acid, separation, and assignments of configuration by enzymic methods.[87]

Heterocyclic syntheses in this area include an enantioselective synthesis of (−)-α-kainic acid from γ-ethyl-L-glutamate *via* (15), employing an elegant intramolecular thermal conversion into the protected product (16), proving unambiguously the absolute configuration at C-2.[88] Quisqualamine (17) has been synthesized through a multi-step route starting from ClCONCO and $HONHCH_2CH_2NHAc$.[89] The *N*-terminal amino-acid residue of the nikkomycins I, J, X, and Z, β-hydroxy-β-(5-hydroxy-2-pyridyl)valine, has been synthesized through a lengthy route starting from 6-methyl-3-pyridinol.[90] Of the four stereoisomers produced through this route, one was shown by ^1H n.m.r. and other methods to be identical with the natural amino-acid.[90]



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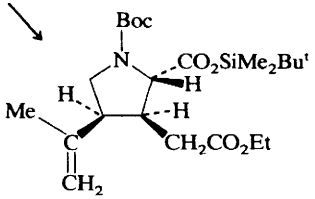

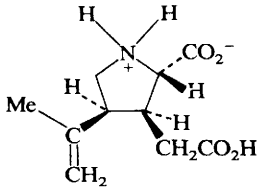

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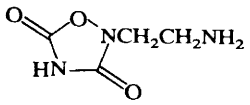
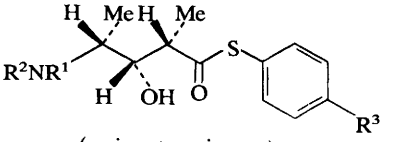


(17)

[85] T. Shiba, H. Akiyama, I. Umeda, S. Okada, and T. Wakamiya, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 899.
[86] K. Goto, M. Waki, N. Mitsuyasu, Y. Kitajima, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 261.
[87] S. Hatanaka, S. Atsumi, K. Furukawa, and Y. Ishida, *Phytochemistry*, 1982, **21**, 225.
[88] W. Oppolzer and K. Thirring, *J. Am. Chem. Soc.*, 1982, **104**, 1978.
[89] P. Dugenet, J. J. Yaouanc, and G. Sturtz, *Synthesis*, 1982, 781.
[90] W. Hass and W. A. Koenig, *Liebigs Ann. Chem.*, 1982, 1615.

β- and Higher Homologous Amino-acids.—A kinetic study of the reaction of acrylonitrile with aqueous ammonia, through which β-alanine is manufactured, has been reported.[91] The β-amino-acid (3*R*)-amino-(2*S*)-hydroxy-4-phenylbutanoic acid, present in bestatin, has been synthesized starting from Boc-D-phenylalanine 3,5-dimethylpyrazolyl ester, by reduction ($LiAlH_4$/THF) to Boc-D-phenylalaninal and then condensation with HCO_2Et followed by saponification and deprotection.[92] The synthesis of $ZNHCH_2CH_2COCO_2Me$ from $ZNHCH_2CH_2COCH_2S(O)Me$ through bromination (NBS) and methanolysis was followed by bakers' yeast reduction and saponification to give *N*-benzyloxycarbonyl-4-amino-2-hydroxybutanoic acid, a constituent of butirosin.[93] Another γ-amino-acid, this time from bleomycin, has been synthesized by aldol condensation of (*R*)-$R^1NR^2CHMeCHO$ with vinyloxyborane *cis*-$MeCH{=}C(OBR_2)SAr$; the resulting thiolester (18) (R^1 = H, R^2 = Z or Boc, R^3 = phthaloyl) gave the required product after conventional elaboration.[94]



(major stereoisomer)
(18)

α-Alkyl Analogues of Natural Amino-acids.—Candidates for inclusion in this section, whose interest stems largely from the search for enzyme inhibitors, are β-(4-hydroxyphenyl)-α-methylalanine and analogues, prepared from α-nitroalanine ethyl ester and the corresponding benzyl alcohol by condensation in the presence of Bu_4NCl and KF followed by hydrogenation[95] and α-difluoromethylornithine, prepared through the Strecker route as a potent ornithine decarboxylase inhibitor.[96] Other examples of the synthesis of these analogues are included in an earlier section of this chapter[53,54,57] and in later sections.

Other Aliphatic, Alicyclic, and Saturated Heterocyclic α-Amino-acids.—N^{α}-Protected L-2,3-diaminopropanoic acids have been prepared by Curtius rearrangement of corresponding aspartic acid derivatives.[97] L,L-2,5-Diaminoadipic acid has been obtained in the form of its piperidin-2-one by alkylation of $ZNHCH(CO_2H)CO_2Bu^t$ with L-homoserine lactone.[98]

[91] T. Saida and H. Michiki, *Kagaku Gijutsushi MOL*, 1982, **20**, 57 (*Chem. Abstr.*, 1982, **96**, 200 118).
[92] H. Kayahara, J. Kurita, and I. Tomida, *Shinshu Daigaku Nogakubu Kiyo*, 1981, **18**, 103 (*Chem. Abstr.*, 1982, **96**, 85 947).
[93] S. Iriuchijima and M. Ogawa, *Synthesis*, 1982, 41.
[94] M. Narita, M. Otsuka, S. Kabayashi, M. Ohno, Y. Umezawa, H. Morishima, S. Saito, T. Takita, and H. Umezawa, *Tetrahedron Lett.*, 1982, **23**, 525.
[95] B. Renger, *Arch. Pharm. (Weinheim)*, 1982, **315**, 472.
[96] J. E. Seely, H. Poso, and A. E. Pegg, *Biochem. J.*, 1982, **206**, 311.
[97] N. Noguchi, T. Kuroda, M. Hatanaka, and T. Ishimaru, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 633.
[98] D. S. Kemp and E. T. Sun, *Tetrahedron Lett.*, 1982, **23**, 3759.

(*E*)- and (*Z*)-1-amino-2-phenylcyclopropanecarboxylic acids have been prepared by building the cyclopropane ring on to 2-phenyl-4-benzylideneoxazolin-5-one using diazomethane.[99] (*RS*)-2-Cyclopropylglycine and D- and L-proline have been prepared from the 2-cyclopropyl-2-oxoethanoatocobalt(III) complex resulting from the reaction of 5-bromo-2-oxopentanoic acid with the aquapenta-amminecobalt(III) ion.[100] An improvement in the one-step route from L-lysine to L-pipecolic acid (L-piperidine-2-carboxylic acid) involves the use of $Na_2[Fe(CN)_5NO]$ in H_2O at pH 9.5.[101]

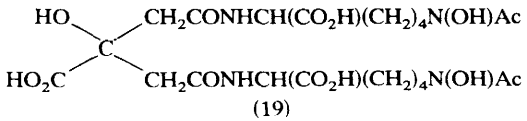
α-Halogenoalkyl Amino-acids.—β-Dibenzylamino-α-fluoroalkanoic acids were obtained from β-hydroxy-α-*NN*-dibenzylamino-acid benzyl esters by treatment with SF_3–NEt_3, *via* the corresponding *NN*-dibenzylaziridinium fluorides.[102]

threo- and *erythro*-β-fluoro-DL-aspartic acid and *threo*-β-fluoro-DL-asparagine, prepared as reported earlier (Vol. 12, p. 11), were assigned their configurations through *X*-ray crystal analysis;[103] tested for cytotoxicity, *threo*-β-fluoro-DL-aspartic acid possessed the greater potency.

α-Difluoromethylornithine is mentioned in the preceding section.[96]

Aliphatic α-Amino-acids Carrying Side-chain Hydroxy and Alkoxy Groups.—N^6-Acetyl-N^6-hydroxylysine, a constituent of aerobactin, has been synthesized from ε-hydroxy-L-norleucine *via* bromination (CBr_4/PPh_3) of its *N*-Boc methyl ester, which was treated with $AcNHOCH_2Ph$, then with anhydromethylenecitryl chloride after removal of the Boc group, giving a blocked form of the synthetic objective (19).[104]

Cleavage of *N*-benzyloxycarbonylaziridine carboxylic esters with alcohols gives substituted serines and threonines.[105]



(19)

Aliphatic Amino-acids with Unsaturated Side Chains.—'Dehydroamino-acids', or α-amino-αβ-alkenoic acids $H_3\overset{+}{N}C(=CR^1R^2)CO_2^-$, can be prepared through aminolysis of 4-alkylidene-oxazolin-5-ones, which are formed from the corresponding saturated α-amino-acids by treatment with dichloroacetic anhydride.[106] A new synthesis of *N*-acetyldehydroamino-acids involves treatment of α-azidoalkanoate esters with acetic anhydride in the presence of

[99] S. W. King, J. M. Riordan, E. M. Holt, and C. H. Stammer, *J. Org. Chem.*, 1982, **47**, 3270.
[100] P. J. Lawson, M. G. McCarthy, and A. M. Sargeson, *J. Am. Chem. Soc.*, 1982, **104**, 6710.
[101] L. Kisfaludy, F. Korenczki, and A. Katho, *Synthesis*, 1982, 163.
[102] L. Somekhkand and A. Shanzer, *J. Am. Chem. Soc.*, 1982, **104**, 5836.
[103] A. M. Stern, B. M. Foxman, A. H. Tashjian, and R. H. Abeles, *J. Med. Chem.*, 1982, **25**, 544.
[104] P. J. Maurer and M. J. Miller, *J. Am. Chem. Soc.*, 1982, **104**, 3096.
[105] K. Nakajima, M. Neya, S. Yamada, and K. Okawa, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 3049.
[106] D. J. Phelps and F. C. A. Gaeta, *Synthesis*, 1982, 234.

Re_2S_7.[107] Some of the *NN*-diacetyl homologue is also formed in this novel reaction.

$\beta\gamma$-Unsaturated amino-acids have received a good deal of attention, both for the development of new methodology and in extending the usefulness of known processes. Reduction of 5-(α-chloroalkyl)-2-substituted oxazoline-4-carboxylic esters with Zn, followed by hydrolysis, offers a new route to these compounds. The oxazolines are well known as intermediates for the synthesis of β-hydroxy-α-amino-acids[45] and are obtainable from ethyl isocyanoacetate and an α-chloroketone.[108]

Claisen rearrangement of allylic esters of *N*-acyl α-amino-acids using two equivalents of base gives the $\gamma\delta$-unsaturated α-amino-acids in moderate to good yields with a substantial degree of stereoselectivity.[84,109]

Competing routes to L-3,4-didehydroproline supplementing earlier reports involve either Chugaev elimination of 4-hydroxy-L-proline xanthate[110,111] or sulphoxide *syn*-elimination[112] for the selenoxide equivalent[113] from substrates prepared from 4-hydroxy-L-proline. The high regioselectivity in these reactions is notable, less than 10% of the 4,5-dehydro analogue being formed.

Aromatic and Heteroaromatic Amino-acids.—Conventional procedures have been employed in preparations of *p*-chlorophenylalanine (from *p*-chlorobenzyl bromide and diethyl acetamidomalonate),[113] benzoselenophen-3-ylglycine [by amidoalkylation with $ZNHCH(OH)CO_2H$],[114] 6-fluorotryptophan (from the indolylmethyl bromide and diethyl formamido- or acetamido-malonate),[115] and 4, 5-, 6-, and 7-azidotryptophans (from the corresponding indoles and tryptophan synthetase from *Neurospora crassa*).[116]

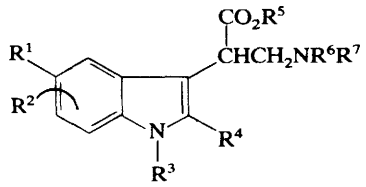
Amino-acids Containing Sulphur.—Electrochemical oxidation of dopa in the presence of cysteine gives mono- and di-cysteinyldopas (attack occurring at the phenolic moiety, giving yields 45, 12, and 8% for 5-, 2-, and 2,5-substitution, respectively).[117]

L-*S*-(2-Amino-2-carboxyethylsulphonyl)-L-cysteine (*alias* cysteine thiolsulphonate) gives *S*-sulpho-L-cysteine and L-alanine-3-sulphinic acid in high yields by treatment with aqueous sodium sulphite, offering convenient preparations of these compounds in view of the accessibility of the thiolsulphonate.[118]

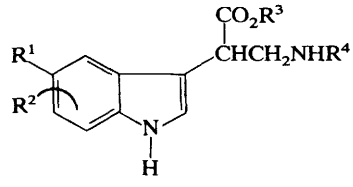
Addition of thiols to 4-methyleneglutamic acid has been used for the

[107] F. Effenberger and T. Beisswenger, *Angew. Chem.*, 1982, **94,** 210.
[108] F. Heinzer and D. Bellus, *Helv. Chim. Acta*, 1981, **64,** 2279.
[109] P. A. Bartlett and J. F. Barstow, *J. Org. Chem.*, 1982, **47,** 3933.
[110] J. R. Dormoy, B. Castro, G. Chappuis, U. S. Fritschi, and P. Grogg in 'Proceedings of the 16th European Peptide Symposium', ed. K. Brunfeldt, Scriptor, Copenhagen, 1981, p. 229.
[111] J. R. Dormoy, *Synthesis*, 1982, 753.
[112] H. Rueger and M. H. Benn, *Can. J. Chem.*, 1982, **60,** 2918.
[113] C. Sun and J. Zhang, *Huaxue Shiji*, 1981, **57,** 28 (*Chem. Abstr.*, 1982, **96,** 123 251).
[114] T. Sadeh, M. A. Davis, R. Gil, and U. Zoller, *J. Heterocycl. Chem.*, 1981, **18,** 1605.
[115] R. Yang and C. Ju, *Shengwu Huaxue Yu Shengwu Wuli Jinzhan*, 1981, **41,** 66 (*Chem. Abstr.*, 1982, **96,** 123 253).
[116] A. Saito and H. C. Rilling, *Prep. Biochem.*, 1981, **11,** 535.
[117] C. Hansson, *Experientia*, 1981, **37,** 1253.
[118] T. Ubuka, M. Kinuta, R. Akogi, S. Kiguchi, and M. Azumi, *Anal. Biochem.*, 1982, **126,** 273.

synthesis of *S*-(4-amino-2,4-dicarboxybutyl)cysteamine and *S*-(4-amino-2,4-dicarboxybutyl)cysteine.[119]



(20)



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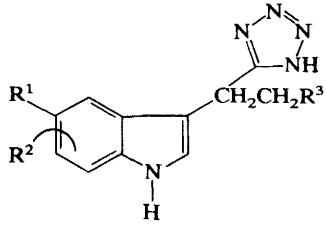


(22)

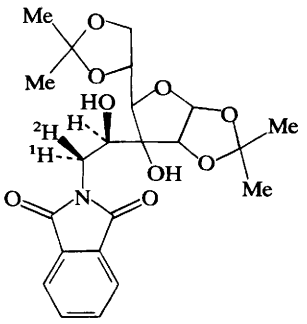
Amino-acids Synthesized for the First Time.—A series of typtophan analogues (20)—(22),[120] aminopiperidinecarboxylic acids related to nipecotic acid (prepared by starting from 5-aminonicotinic acid),[121] and GABA analogues [stereoisomers of *cis*-3-aminocyclohexanecarboxylic acid,[122] (*Z*)- and (*E*)-4-amino-3-(4-chlorophenyl)but-2-enoic acids[123]] include many new amino-acids. Preparations of other new amino-acids have been discussed elsewhere in this chapter.

Labelled Amino-acids.—Continuing the reflection of the high level of interest in the synthesis of isotopically labelled amino-acids by the relatively large amount of space devoted here, the volume of literature and its variety this year have, however, defeated the system used in previous volumes to give some sense of order to the coverage. The synthetic objectives described in the recent literature are all protein amino-acids (with one exception) and are covered in this section in order of increasing molecular complexity.

Chiral glycine enantiomers $H_3\dot{N}C^1H^2HCO_2^-$ have been synthesized from the hexulofuranose derived from D-glucose, as a chiral template, using reactions of its keto group, leading to phthalimidoacetaldehyde by $Pb(OAc)_4$ cleavage of (23).[124]

(3*R*, 4*S*)- and (3*R*, 4*R*)-valine-[4,4-^{2}H, ^{3}H] have been prepared by photolysis of the pyruvyl ester of (*S*)-(−)-$PhCH_2C\dot{H}MeC^2H_2OH$, leading to (*S*)-$PhCH_2CHMeC^2HO$ and thence to (1*R*, 2*R*)- and (1*S*, 2*R*)-alkanes

[119] G. K. Powell, H. C. Winter, and E. E. Dekker, *Biochem. Biophys. Res. Commun.*, 1982, **105**, 1361.
[120] M. E. Safdy, E. Kurchacova, R. N. Schut, H. Vidrio, and E. Hong, *J. Med. Chem.*, 1982, **25**, 723.
[121] P. Jacobsen, K. Schaumburg, J. J. Larsen, and P. Krogsgaard-Larsen, *Acta Chem. Scand., Ser. B*, 1981, **35**, 289.
[122] R. D. Allan, G. A. R. Johnston, and B. Twitchin, *Aust. J. Chem.*, 1981, **34**, 2231.
[123] R. D. Allan and H. Tran, *Aust. J. Chem.*, 1981, **34**, 2641.
[124] K. Kakinuma, N. Imamura, and Y. Saba, *Tetrahedron Lett.*, 1982, **23**, 1697.



(23)

$^3H^2HCHCHMeCH_2Ph$, from which the valine diastereoisomers were prepared through oxidation of the phenyl group to CO_2H, followed by other established stages.[125] Catalytic hydrogenation with H^3H, using Wilkinson's catalyst, showed unusual stereospecificity in leading to a 19:1 mixture of 2*SR*, 3*SR*, 4*RS*-[4-$^3H^2H$]-*N*-acetylvaline and its (3*RS*, 4*SR*)-diastereoisomer, indicating favoured 3-*re*,4-*si* attack on the *S*-component of (2*RS*)-(*E*)-[4-^2H]-2-acetylamino-3-methylbut-3-enoic acid.[126] (2*S*, 3*R*)-Serine-[3-$^2H_1$] and (2*S*, 3*S*)-serine-[2,3-$^2H_2$] have been prepared from the correspondingly labelled aspartic acids by Baeyer–Villiger oxidation of the derived *N*-trifluoroacetyl 2-amino-4-oxopentanoic acids.[127] (2*S*, 3*S*)- and (2*S*, 3*R*)-diastereoisomers, respectively, have been synthesized from (*E*)-$^2HCH{=}C^2HCO_2Me$ and (*Z*)-$^2HCH{=}CHCO_2Et$ *via* bromohydrins, treated with sodium azide followed by Pd-catalysed hydrogenation and hog-kidney acylase I resolution.[128]

L-Tryptophan-[1-^{13}C] has been prepared from DL-serine-[1-^{13}C] and indole using *Escherichia coli* and extended to tryptophan analogues through the use of substituted indoles.[129] [3-^{13}C,3-$^2H_2$]-Tryptophan is available through Mannich condensation of $^2H^{13}C^2HO$ with indole and Me_2NH, followed by condensation with diethyl formamidomalonate and resolution of the derived amino-acid as its *N*-chloroacetyl derivative.[130] Corresponding reactions with substituted indoles and with $^2HC^2HO$ gave other L-tryptophan analogues.[130] ^1H–^2H exchange of tryptophan with ^2H_2O, or hydrolysis of protected tryptophans with NaO^2H–^2H_2O, leads to [2-^2H]-DL-tryptophan.[131] Total synthesis of tryptophan from indole-3-carboxaldehyde by condensation with diethyl acetamidomalonate followed by catalysed addition of $^2H_2$ gave [2,3-$^2H_2$]-DL-tryptophan.[131] A general procedure for the preparation of $\alpha\beta$-deuteriated α-amino-acids giving almost quantitative exchange uses pyridoxal catalysis and reaction times of 2—8 days at 125 °C in ^2H_2O solution.[132]

[125] C. A. Townsend, A. S. Neese, and A. B. Theis, *J. Chem. Soc., Chem. Commun.*, 1982, 116.
[126] D. H. G. Crout, M. Lutstorf, P. J. Morgan, R. M. Adlington, J. E. Baldwin, and M. J. Crimmin, *J. Chem. Soc., Chem. Commun.*, 1981, 1175.
[127] D. Gani and D. W. Young, *J. Chem. Soc., Chem. Commun.*, 1982, 867.
[128] L. Slieker and S. J. Benkovic, *J. Labelled Compd. Radiopharm.*, 1982, **19**, 647.
[129] S. S. Yuan and A. M. Ajami, *Tetrahedron*, 1982, **38**, 2051.
[130] W. S. Saari, *J. Labelled Compd. Radiopharm.*, 1982, **19**, 389.
[131] E. Santaniello, M. Ravasi, and F. Astori, *J. Labelled Compd. Radiopharm.*, 1982, **19**, 611.
[132] D. M. Le Master and F. M. Richards, *J. Labelled Compd. Radiopharm.*, 1982, **19**, 639.

Labelled L-methionines described recently are the [methyl-^{13}C] and [3,4-$^{13}C_2$] compound and the [2,3,3-$^{3}H_3$] and [3,3-$^{2}H_2$] analogues of the [methyl-^{13}C] compound.[133] The ^{75}Se analogue of methionine has been synthesized from Me^{75}SeNa and 2-amino-4-bromobutanoic acid.[134]

Several papers describing the synthesis of ^{11}C-labelled amino-acids have appeared in the literature under review, continuing the modest flow of reports on this topic. There is some general interest in, as well as the specific appeal of, this work since the need to achieve good yields in a short time is a consequence of the short half-life of this isotope. L-[^{11}C]Glutamic acid labelled at the carboxy α- or γ-carbon atoms has been prepared by rapid enzyme-catalysed methods;[135] $^{11}CH_3I$ (from $^{11}CO_2$) has been used to prepare L-methionine-[methyl-^{11}C] (within 20 minutes)[136] and thence the *S*-adenosyl derivative by enzyme-catalysed condensation with ATP.[137] ^{11}C-Carboxy-labelled valine, leucine, and aminocyclopentanecarboxylic acid have been prepared from Na^{11}CN by rapid Bucherer–Strecker synthesis.[138]

^{15}N-Labelled amino-acids are readily available from $^{15}NH_4^+$ salts of carboxylic acids using enzymic methods[139] (L-[^{15}N]alanine from pyruvic acid and alanine dehydrogenase[140] and L-[^{15}N]aspartic acid from fumaric acid with immobilized *Escherichia coli B*, this amino-acid being a source of L-[^{15}N]alanine through the agency of immobilized *Pseudomonas dacunhae*[141]).

Alanine labelled with both ^{13}C and ^{15}N has been prepared from Ba$^{13}C_2$ and $^{15}NH_4Cl$ with NaCN through Strecker amination; exchange with $^{2}H_2O$ in the presence of glutamic–pyruvic transaminase and with $^{2}H_2{}^{18}O$ gave 80% and 71.4% of the heavy isotope analogues, respectively.[142]

^{14}C- and ^{3}H-labelled carnitines were prepared by methylation of GABA-$^{14}CO_2H$ with MeI followed by hydroxylation with butyrobetaine hydroxylase from bovine calf liver,[143] demethylation of which (using NaSPh in DMF) followed by methylation with $^{14}CH_3I$ gives the methyl-labelled L-compound.[144]

^{3}H-Labelling methods used for a wide range of amino-acids, catecholamines,

[133] D. C. Billington, B. T. Golding, M. J. Kebbell, I. K. Nassereddin, and I. M. Lockart, *J. Labelled Compd. Radiopharm.*, 1981, **18,** 1773.
[134] H. Yao and C. Dan, *Zhonghua Heyixue Zazhi*, 1982, **2,** 166 (*Chem. Abstr.*, 1982, **97,** 211 697).
[135] M. B. Cohen, L. Spotter, C. C. Chang, D. Behrendt, J. Cook, and N. S. Macdonald, *Int. J. Appl. Radiat. Isot.*, 1982, **33,** 613.
[136] J. Davis, Y. Yano, J. Cahoon, and T. F. Budinger, *Int. J. Appl. Radiat. Isot.*, 1982, **33,** 363.
[137] P. Gueguen, J. L. Morgat, M. Maziere, G. Berger, D. Comar, and M. Maman, *J. Labelled Compd. Radiopharm.*, 1982, **19,** 157.
[138] Y. Ye, R. Hua, Z. Zhou, and Y. Wang, *Nucl. Tech.*, 1981, 44.
[139] Z. E. Kahana and A. Lapidot in 'Stable Isotopes', Anal. Chem., Symp. Ser., Vol. 11, ed. H.-L. Schmidt, H. Förstel, and K. Heinzinger, Elsevier, Amsterdam, 1982, p. 747.
[140] A. Mocanu, G. Niac, A. Ivanhof, V. Gorun, N. Palibroda, E. Vargha, M. Bologa, and O. Barzu, *FEBS Lett.*, 1982, **143,** 153.
[141] Z. E. Kahana and A. Lapidot, *Anal. Biochem.*, 1982, **126,** 389.
[142] S. D. Dimitrijevich, M. D. Scanlon, and M. Anbar, *J. Labelled Compd. Radiopharm.*, 1982, **19,** 573.
[143] D. B. Goodfellow, C. L. Hoppel, and J. S. Turkaly, *J. Labelled Compd. Radiopharm.*, 1982, **19,** 365.
[144] S. T. Ingalls, C. L. Hoppel, and J. S. Turkaly, *J. Labelled Compd. Radiopharm.*, 1982, **19,** 535.

and alkaloids have been described,[145] and ^{13}C-enriched amino-acids have been reviewed.[146]

Resolution of DL-Amino-acids.—The major subdivisions of this topic have been delineated for some time and the recent literature, though increasingly extensive, can be covered efficiently under these headings.

Enzymic methods continue to be used in their classical form [$Ar(CH_2)_nCH(NHAc)CO_2H$ with Taka-acylase,[147,34] α-methyl-tryptophan or -phenylalanine esters with chymotrypsin,[148] *N*-acetyl derivatives of isotopically labelled serines with hog-kidney acylase I,[128] and *N*-phenylacetyl β-(N^1-uracilyl)alanine[149] and related derivatives of the nucleic acid bases[149,150] with penicillin amidase], although the use of immobilized enzymes or intact cells continues to increase. [1-^{14}C]-D-Leucine can be recovered from its DL-form in 83% yield through the action of immobilized L-amino-acid oxidase within 40 minutes, including the time taken for ion-exchange purification.[151] Chymotrypsin in aqueous solution encapsulated in a liquid membrane such as cyclohexane or C_{15} alkanes can effect 70% conversion of DL-phenylalanine methyl ester into L-phenylalanine within 15 minutes, transport through the membrane being mediated by pairing with quaternary ammonium ions.[152] The use of immobilized enzymes or intact cells in the amino-acid area has been reviewed.[153]

Chromatographic methods are being developed successfully into preparative-scale operations, and analytical techniques (covered in Section 6 of this chapter) are also being more widely studied in view of the importance in many areas of determining enantiomer ratios for amino-acid samples. Copper(II) complexation is a feature of several recent papers describing liquid chromatographic methods, using chiral eluents containing the copper(II) complexes of *N*-(toluene-*p*-sulphonyl)-L-phenylalanine and *N*-(toluene-*p*-sulphonyl)-D-phenylglycine over octadecylsilylated silica gel,[154] and similar use of an eluant containing the copper(II) complex of L-aspartyl-L-phenylalanine

[145] J. P. Bloxsidge, J. A. Elvidge, M. Gower, J. R. Jones, E. A. Evans, J. P. Kitcher, and D. C. Warrell, *J. Labelled Compd. Radiopharm.*, 1982, **18,** 1141.

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[147] N. Kosui, Y. Shimohigashi, M. Waki, T. Kato, and N. Izumiya, *Mem. Fac. Sci., Kyushu Univ., Ser. C*, 1981, **13,** 89 (*Chem. Abstr.*, 1982, **96,** 123 240).

[148] G. M. Anantharamaiah and R. W. Roeske, *Tetrahedron Lett.*, 1982, **23,** 3335.

[149] G. A. Korshunova, Yu. A. Semiletov, O. N. Ryabtseva, and Yu. P. Shvachkin, *Vestn. Mosk. Univ., Khim.*, 1982, **23,** 412 (*Chem. Abstr.*, 1983, **97,** 216 651).

[150] G. A. Korshunova, Yu. A. Semiletov, O. N. Ryabsteva, and Yu. P. Shvachkin, *Vestn. Mosk. Univ., Khim.*, 1982, **23,** 177 (*Chem. Abstr.*, 1983, **97,** 72 728).

[151] G. A. Digenis, R. Goto, J. E. Chaney, and O. Tamemasa, *J. Pharm. Sci.*, 1982, **71,** 818.

[152] T. Scheper, W. Halwachs, and K. Schuegerl, *Chem.-Ing.-Tech.*, 1982, **54,** 696.

[153] I. Chibata in 'Asymmetric Reactions and Processes in Chemistry', Am. Chem. Soc., Symp. Ser., No. 185, ed. E. L. Eliel and S. Otsuka, American Chemical Society, Washington, D.C., U.S.A., 1982, p. 195.

[154] N. Nimura, A. Toyoma, Y. Kasahara, and T. Kinoshita, *J. Chromatogr.*, 1982, **239,** 671.

methyl ester[155] or of *NN*-dimethyl-L-valine or *NN*-di-n-prolyl-L-alanine.[156] Elution with aqueous ammonia effects the resolution of DL-amino-acids over ion-exchange resins carrying imino(methanephosphonic) acid groups complexed to copper(II) through the imino groups.[157] Development of the use of chiral stationary phases continues, silica-bound formyl-L-valinamide being useful for the resolution of *N*-acetyl-DL-amino-acid alkylamides[158] and similar D-phenylglycine modified stationary phases offering convenient gram-scale resolution possibilities.[159] The patent literature[160] contains several recipes for the preparation of chiral supports. The affinity-chromatography approach has been used for the resolution of DL-tryptophan and its analogues over albumin-agarose, the D-enantiomer emerging first from the column.[161] In a newer variation of the chiral support approach, Dnp-amino-acid esters were separated over silica gel coated with the electron donor *P*-(+)-hexahelicene-7,7′-dicarboxylic acid (the L-enantiomer emerges first).[162] Preparative gas-chromatographic separation of volatile amino-acid derivatives over *N*-stearoyl-L-valine t-butylamide has been developed further (see Vol. 14, p. 17),[163] and new knowledge arising from studies of the influence of the structure of the perfluoroacyl group and the ester group on the retention characteristics of enantiomers of derivatized DL-amino-acids[164] and equivalent studies of the effects of the structure of the stearoyl-L-amino-acid t-butylamide on the resolution of *N*-trifluoroacetyl-DL-amino-acid isopropyl esters[165] (see also ref. 323) will assist further development of reliable preparative-scale techniques.

A forty-five page survey of the uses of h.p.l.c. and g.l.c. for resolution of racemates has appeared in a new treatise.[166]

The preferential crystallization route to resolution has been used with DL-amino-acids largely on a trial-and-error basis, but intensive studies of the effects of doping DL-amino-acid solutions with an L- or D-amino-acid of different structure[167—169] have led to greater insight into the process. The

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[157] W. Szczepaniak and W. Ciszewska, *Chromatographia*, 1982. **15**, 38 (*Chem. Abstr.*, 1983, **97**, 39 325).
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[159] W. H. Pirkle and J. M. Finn, *J. Org. Chem.*, 1982, **47**, 4037.
[160] D. W. House, U.S. Pat. 4 324 681 (*Chem. Abstr.*, 1983, **97**, 126 518).
[161] S. Allenmark, B. Bomgren, and H. Boren, *J. Chromatogr.*, 1982, **237**, 473.
[162] Y. H. Kim, A. Balan, A. Tishbee, and E. Gil-Av, *J. Chem. Soc., Chem. Commun.*, 1982, 1336.
[163] M. P. Zabokritskii, B. A. Rudenko, and V. P. Chizhkov, *Dokl. Akad. Nauk SSSR*, 1982, **263**, 1155.
[164] I. Abe, K. Izumi, S. Kuramoto, and S. Musha, *J. High Resolut. Chromatogr., Chromatogr. Commun.*, 1981, **4**, 549.
[165] S. C. Chang, R. Charles, and E. Gil-Av, *J. Chromatogr.*, 1982, **235**, 87.
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[167] L. Addadi, Z. Berkovitch-Yellin, N. Domb, E. Gati, M. Lahav, and L. Leiserowitz, *Nature (London)*, 1982, **296**, 21.
[168] L. Addadi, S. Weinstein, E. Gati, I. Weissbuch, and M. Lahav, *J. Am. Chem. Soc.*, 1982, **104**, 4610.
[169] L. Addadi, Z. Berkovitch-Yellin, I. Weissbuch, M. Lahav, L. Leiserowitz, and S. Weinstein, *J. Am. Chem. Soc.*, 1982, **104**, 2075.

dopant binds stereoselectively at the surface of the crystal of the enantiomer of the same configuration and causes gross physical differences in the crystal habit of this enantiomer compared with the appearance of crystals of the other enantiomer and differences in rate of growth of the two crystal types. The implication that absolute configuration can be assigned to enantiomers on this basis was also followed through and shown to be reliable.[168,169] Effects of degree of supersaturation and its control through the addition of acids or bases to the mother-liquor have been studied, leading to crops of crystals of better purity.[170] Details of a particular application of the preferential crystallization phenomenon to the resolution of DL-alanine as its toluene-*p*-sulphonate given in the abstract of a recent paper[171] are sufficiently complete to be followed without the need to resort to the original paper (which occupies seven pages). Either enantiomer may be obtained in 99.9% optical purity after recrystallization.

An example of resolution by crystallization of a diastereoisomeric derivative is included in the synthesis of β-carboxyaspartic acid,[83] the derivative being [(diethyl β-carboxyaspartato)tetramminecobalt(III)] perchlorate.

Further reports from Yamada's group describing the asymmetric transformation of *N*-acyl-DL-amino-acids,[172] combining preferential crystallization of one enantiomer with racemization by traces of acetic anhydride of the other enantiomer, include details of optimized procedures. Optical purities approaching 70% can be expected using an *N*-acyl group that is appropriate for a particular amino-acid.

The remaining topic represented in this section in previous volumes, enantioselective radiolysis of DL-amino-acids, receives a new stimulus with the report that a spin-polarized low-energy positron beam shows some discrimination for degradation of the enantiomers of DL-leucine.[173] It is predicted[173] that enhanced discrimination will be seen in β-irradiative degradation of DL-amino-acids containing heavier elements.

4 Physical and Stereochemical Studies of Amino-acids

Crystal Structures of Amino-acids and Their Derivatives.—The continuing theme of this section involves announcements of new *X*-ray and neutron-diffraction analyses and papers discussing the implications of previously gathered data.

DL-β-Carboxyaspartic acid adopts the dimeric packing mode in the solid state, with unusually strong hydrogen bonding.[174] (−)-Canavanine adopts the zwitterionic form with protonation on the α-amino group rather than on the

[170] S. Asai and S. Ikegami, *Ind. Eng. Chem. Fundam.*, 1982, **21**, 181.
[171] V. Feldnere, A. Vegnere, J. Vitals, and R. Udre, *Latv. PSR Zinat. Akad. Vestis, Kim. Ser.*, 1982, 310 (*Chem. Abstr.*, 1983, **97**, 110 364).
[172] C. Hongo, S. Yamada, and I. Chibata, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3286, 3291.
[173] D. W. Gidley, A. Rich, J. Van House, and P. W. Zitzewitz, *Nature (London)*, 1982, **297** 639; R. A. Hegstrom, *ibid.*, p. 643.
[174] B. Richey, M. R. Christy, R. C. Haltiwanger, T. H. Koch, and S. J. Gill, *Biochemistry*, 1982, **21**, 4819.

guanidino group;[175] the general behaviour of arginine salts as far as the specific interactions occurring between the guanidino group and the anions are concerned has been surveyed based on published crystal structures.[176]

S-Adenosyl-L-homocysteine[177] and blasticidin S hydrochloride pentahydrate[178] (a cytosine aminonucleoside acylated on the amino group by L-arginine) have received intensive conformational study by *X*-ray[177,178] and other physical and theoretical analytical methods.[177] 3-*N*-Oxalyl-L-2,3-diaminopropanoic acid (an example of a compound exhibiting crystal dimorphism) has been studied by i.r. spectrometry and potentiometric titration as well as by *X*-ray crystal analysis.[179]

Neutron-diffraction data (32 crystal structures) for amino-acids have been studied from the point of view of hydrogen-bond geometries displayed.[180] Of the 168 hydrogen bonds detected, 64 involved zwitterion groups $\overset{+}{N}H_3$ and CO_2^- and 18 were from $\overset{+}{N}H_3$ to sulphate or carbonyl groups, the majority (46) of these —N—H · · · O bonds being three-centred (bifurcated) and 9 being four-centred (trifurcated).

Statistical analysis of the disposition of side chains seen in *X*-ray structures of amino-acids has been used to derive limiting vicinal coupling constants for the staggered conformations.[181]

Nuclear Magnetic Resonance Spectrometry.—Major themes (conformational analysis,[177] acid–base characteristics) represented over the years in ^1H and ^{13}C n.m.r. studies of amino-acids are being supplemented increasingly by novel variations of the n.m.r. technique. Pioneering and specifically designed studies based on nuclei of higher atomic weight are also applied in the amino-acid area.

High salt concentrations in alkaline solutions of aspartic acid stabilize the two conformations involving *gauche* carboxy groups at the expense of the *anti* conformation.[182] Limiting component vicinal coupling constants for side-chain staggered rotamers of amino-acids have been calculated from statistical analysis of *X*-ray structures.[181]

Double-resonance ^1H n.m.r. has been used to determine solvent exchange rates catalysed by acids or bases of *E*- and *Z*-protons of *N*-acetylasparagine- and -glutamine-*N*-methylamides in water.[183] Relationships between the acidity

[175] A. Boyar and R. E. Marsh, *J. Am. Chem. Soc.*, 1982, **104,** 1995.
[176] D. M. Salunke and M. Vijayan, *Int. J. Pept. Protein Res.*, 1981, **18,** 348.
[177] T. Ishida, A. Tanaka, M. Inoue, T. Fujiwara, and K. Tomita, *J. Am. Chem. Soc.*, 1982, **104,** 7239.
[178] V. Swaminathan, J. L. Smith, M. Sundaralingam, C. Coutsogeorgopoulos, and G. Kartha, *Biochim. Biophys. Acta*, 1981, **655,** 335.
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[180] G. A. Jeffrey and H. Maluszynska, *Int. J. Biol. Macromol.*, 1982, **4,** 173; G. A. Jeffrey in 'Proceedings of the 1981 Meeting: Molecular Structure and Biological Activity', ed. J. F. Griffin and W. L. Duax, Elsevier, New York, 1982 (*Chem. Abstr.*, 1983, **98,** 1849).
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[182] G. Esposito, A. Donesi, and P. A. Temussi, *Adv. Mol. Relax. Interact. Processes*, 1982, **24,** 15.
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of *Z*- and *E*-isomers of *N*-nitroso-*N*-alkyl-α-amino-acids and their conformations have been established by measurement of ^{1}H n.m.r. spectra of solutions at varying pH values. *E*-Isomers are in many cases unable to adopt intramolecular hydrogen bonds between nitroso and carboxy groups, and they show stronger acid character.[184]

Proton spin–lattice relaxation for α-aminoisobutyric acid from 120 to 450 K reveals reorientation of amino and methyl groups, for which activation energies were estimated.[185]

A novel heteronuclear spin-echo method allowing assignment of features in a ^{1}H n.m.r. spectrum to both ^{12}C and ^{13}C species opens up interesting possibilities in mechanistic studies, illustrated in following the exchange of ^{13}C between alanine and pyruvic acid catalysed by alanine transferase.[186]

Interactions of the phenyl moiety of L-phenylalanine with 5′-AMP and poly(A) have been investigated by ^{2}H n.m.r., using the $^{2}H_5$-labelled amino-acid.[187]

Reassignment of indole C-5 and C-6 ^{13}C n.m.r. resonances suggested by Gribble and co-workers[188] has been confirmed through consideration of ^{13}C–^{13}C coupling constants observed for tryptophan-[3-$^{13}C_1$].[189] Broad-band proton-decoupled ^{13}C n.m.r. spectra of side-chain derivatives of lysine, serine, histidine, and cysteine were fully assigned to assist n.m.r. studies of crucial regions of enzymes.[190] *cis–trans* isomerism of *N*-acetyl *syn*- and *anti*-5-methylproline methylamide studied by ^{13}C n.m.r. reveals the influence of the steric effect of the 5-methyl group in destabilizing the *trans*-amide isomer but without altering the isomerization barrier.[191]

Many examples of uses of ^{13}C n.m.r. in biosynthetic studies employing ^{13}C-labelled substrates have been described in recent years, and an excellent example of complexities revealed in this way, supported by field-desorption mass spectrometry, is described for $^{13}CO_2$-feeding studies with *Spirulena maxima* and *Synechoccus cedrorum*.[192]

^{14}N nuclear quadrupole resonance data for fourteen *N*-acetylamino-acids show a positive correlation between electron density at N and the Taft inductive parameter σ*.[193] ^{15}N solid-state n.m.r. of histidine permits assignments to be made for isotropic and anisotropic chemical shifts for the imidazole-ring nitrogen atoms in various ionic forms,[194] giving a more precise

[184] B. Liberek, J. Clarkowski, K. Plucinska, and K. Stachowiak, *Org. Magn. Reson.*, 1982, **18,** 143.
[185] S. Idziak, J. Stankowski, and S. Guszczynski, *Bull. Acad. Pol. Sci., Ser. Sci. Chim.*, 1980, **28,** 691.
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[192] E. Bengsch, J.-P. Grivet, and H.-R. Schulten in 'Stable Isotopes', Anal. Chem., Symp. Ser., Vol. 11, ed. H.-L. Schmidt, H. Förstel, and K. Heinzinger, Elsevier, Amsterdam, 1982, p. 587.
[193] G. F. Sadiq, S. G. Greenbaum, and P. J. Bray, *Org. Magn. Reson.*, 1981, **17,** 191.
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description of tautomeric structure than can be obtained from ^{15}N n.m.r. data on solutions. Spin–lattice relaxation times T_1 and n.O.e. data for alanine, glutamic acid, and arginine in intracellular fluid can be related to the microviscosities of various environments and intermolecular associations between solutes in those environments.[195]

^{17}O n.m.r. of ^{17}O-enriched glycine, glutamic acid, and aspartic acid was particularly studied[196] as part of a study of a range of protein amino-acids.[196,197] High fields are needed to resolve resonances arising from the two carboxy groups of the amino-diacids, but the technique is capable of demonstrating, by concentration-dependence studies, that intramolecular association between the amino group and a side-chain carboxy group does not occur in these compounds.[196]

^{19}F n.m.r. of (+)- or (−)-perfluoro-2-propoxypropionyl amino-acids is a new variation of an established method for determination of enantiomer ratios.[198] Configurational assignments can be made to 2-amino-3-fluoroalkanoate esters by ^{19}F n.m.r., using the effect of complexation of the amino group by 18-crown-6.[199]

Optical Rotatory Dispersion and Circular Dichroism.—Established areas of interest in these techniques are represented in interpretation of c.d. data for Dnp derivatives of α-amino-acids and their β-methyl analogues,[200] where differences in c.d. spectra are associated with effects of the methyl substituent on rotamer populations, and for Dnp derivatives of β- and higher homologous amino-acid arylamides,[201] where characteristic exciton coupling between the aromatic groupings is shown only in those compounds where the amino group is attached to the chiral centre. *N*-Acetylamino-acid methylamides have been studied by c.d. spectrometry from the point of view of interactions between the amide groupings and an aromatic moiety in the side chain.[202] There were indications in the solvent and temperature dependence of c.d. spectra that changes in conformer populations could be discerned by this method.[202]

Novel chromophoric derivatives are represented in complexation of methionine or *S*-ethylcysteine with sodium tetrachloropalladate,[203] whose c.d. spectra are sufficiently responsive to enantiomeric purity to offer a sensitive racemization test for reactions involving these amino-acids. Familiar cobalt(II) and copper(II) complexes of threonine and isoleucine and their epimers have been studied from the point of view of stability in aqueous $NaNO_3$ (*allo* forms are less stable than the natural epimers), and c.d. spectra have been interpreted for the complexes.[204]

[195] K. Kanamoi, T. L. Legerton, R. L. Weiss, and J. D. Roberts, *Biochemistry*, 1982, **21**, 4916.
[196] I. P. Gerothanassis, R. Hunston, and J. Lauterwein, *Helv. Chim. Acta*, 1982, **65**, 1764, 1774.
[197] R. Hunston, I. P. Gerothanassis, and J. Lauterwein, *Org. Magn. Reson.*, 1982, **18**, 120.
[198] H. Kawa, F. Yamaguchi, and N. Ishikawa, *J. Fluorine Chem.*, 1982, **20**, 475.
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[201] M. Kawai and U. Nagai, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 1327.
[202] H. Matsuura, K. Hasegawa, and T. Miyazawa, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 1999.
[203] T. H. Lam, S. Fermandjian, and P. Fromageot, *J. Chim. Phys. Phys.-Chim. Biol.*, 1982, **79**, 101.
[204] N. Ivivic and V. Simeon, *J. Inorg. Nucl. Chem.*, 1981, **43**, 2581.

Further results from vibrational c.d. studies of amino-acids deal with deuteriated alanines.[205] Insight into the fundamental C—H stretching modes of alanine and the vibrational coupling between the Me—CH stretching modes has been gained by this study. The magnitude and direction of the displacements of the nuclei can be determined by this technique.

Mass Spectrometry.—Field-desorption mass spectra of 19 protected amino-acids have been reported.[206] In nearly all cases the molecular ion is the base peak. The f.d. technique is becoming more widely used and developed in new ways; voltages of around 1 kV applied to the emitter in liquid-ionization mass spectrometry of β-alanine accelerate the desorption of ions but not neutral molecules, and the base peak in this case is due to $M+H^+$ with the protonated dimer $2M+H^+$ particularly abundant.[207]

Secondary-ion mass spectra of phenylalanine have been compared with mass spectra obtained by the three more familiar ionization methods (electron impact, chemical ionization, and field desorption).[208] Bombardment by the primary-ion beam of a solid target (the amino-acid, or its hydrochloride deposited on graphite) produces the secondary ions with intensities determined by both the intensity and nature of the primary-ion beam, xenon being the most suitable.[208]

Laser-microprobe mass spectra of glycine and phenylalanine include quasi-molecular ions $M-1^-$ and $M+1^+$ as well as the decarboxylated molecular ion $M-45^+$.[209]

A more routine study (analytical applications in which the technique is used to support synthesis and structure determination are not included) deals with phenylalanine esters,[210] for which dioxopiperazine formation is an inevitable accompanying process. Trifluoroacetylation prior to mass-spectrometric study suppresses dioxopiperazine formation and increases the possibility of molecular-ion formation.[210]

Other Physical Studies.—The wide range of potentiometric, thermodynamic, and spectrometric studies featured in this section in previous volumes continues to be applied to amino-acids. Some of this seems routine but nevertheless valuable in many ways and occasionally necessary to correct previous erroneous information. For example,[175] p*K* values for canavanine and canaline have been correctly calculated from reconsideration of titration curves

[205] B. B. Lal, M. Diem, P. L. Polavarapu, M. Oboodi, T. B. Freedman, and L. A. Nafie, *J. Am. Chem. Soc.*, 1982, **104,** 3336; T. B. Freedman, M. Diem, P. D. Polavarapu, and L. A. Nafie, *ibid.*, p. 3343.

[206] D. F. Fraley, M. M. Bursey, D. H. Craig, and B. G. Goldsmith, *Eur. J. Mass Spectrom. Biochem., Med. Environ. Res.*, 1982, **2,** 13.

[207] M. Tsuchiya, T. Nonaka, T. Taira, and S. Tanaka, *Shitsuryo Bunseki*, 1982, **30,** 95 (*Chem. Abstr.*, 1982, **97,** 72 756).

[208] K. D. Klöppel in 'Recent Developments in Mass Spectrometry in Biochemistry, Medicine and Environmental Research', Anal. Chem., Symp. Ser., Vol. 7, ed. A. Frigerio, Elsevier, Amsterdam, 1981, p. 283.

[209] C. Schiller, K.-D. Kupka, and F. Hillenkamp in 'Recent Developments in Mass Spectrometry in Biochemistry, Medicine and Environmental Research', Anal. Chem., Symp. Ser., Vol. 7, ed. A. Frigerio, Elsevier, Amsterdam, 1981, p. 287.

[210] J. Das, *Indian J. Chem., Sect. B*, 1982, **21,** 71.

reported in 1935. Several other pK studies have been reported including DL-tryptophan in water-containing organic solvents[211] and a series of amino-acids in aqueous 2-propanol.[212] β-Carboxy-DL-aspartic acid shows pK_a 0.8, 2.5, 4.7, and 10.9 in water.[174] Revised pK_a values are reported for 3-*N*-oxalyl-L-2,3-diaminopropanoic acid.[179]

Conductimetric titrations of equimolar amounts of L-glutamic acid and L-histidine indicate greater interaction than between L-glutamic acid and D-histidine.[213] No specific interactions were found between L-glutamic acid and the other common basic amino-acids.[213]

Heats of stepwise dissociation of L-aspartic acid were calculated from calorimetric data determined at 15, 25, and 35 °C,[214] and similar studies have been undertaken for L-lysine monohydrochloride[215] and tyrosine derivatives.[216]

Osmotic coefficients of *N*-acetyl derivatives of glycinamide, alaninamide, and leucinamide and calorimetric data for these compounds in equimolal admixture with peptide homologues yield pairwise free-energy and enthalpy parameters.[217] Enthalpy-of-interaction coefficients have been measured calorimetrically for alanine, 2-aminobutanoic acid, norvaline, and norleucine with NaCl in aqueous solutions at 298.15 K.[218] Enthalpies of solution of a series of amino-acid hydrobromides in water–DMF mixtures at 298.15 K, measured calorimetrically, yield better measures of the hydrophobicity of the side chains than those based on transfer properties of amino-acids between immiscible phases.[219] An example of the latter approach has been described[220] for the partition of Dnp-amino-acids in buffered aqueous two-phase polymeric systems (ficoll and dextran). The penetration of leucine and norleucine from aqueous solutions into dimyristoylphosphatidylcholine monolayers has been followed by surface pressure measurements.[221] Ultrasound interferometry provides values of compressibility and solvation numbers of amino-acids in aqueous ethanol.[222] Zwitterion content of glycine in various aqueous media has been measured,[223] and the difference in average volumes of protein amino-acids (181 Å^2) compared with non-protein amino-acids (112 Å^2), in specific volumes (1.28 and 1.1 Å^3 dalton^{-1}, respectively), and in spectral-energy density (15 878 and 13 201 kcal g^{-1}, respectively) has been linked with a hypothesis accounting for the selection of amino-acids for incorporation into proteins.[224]

[211] R. S. Saxena and G. L. Sharma, *J. Indian Chem. Soc.*, 1982, **59,** 413.
[212] B. P. Dey, S. Dutta, and S. C. Lahiri, *Indian J. Chem., Sect. A*, 1982, **21,** 886.
[213] B. E. Akabue and P. Hemmes, *Adv. Mol. Relax. Interact. Processes*, 1982, **22,** 11.
[214] V. P. Vasil'ev, L. A. Kochergina, S. G. Iven'kova, and M. V. Kuturov, *Zh. Obshch. Khim.*, 1982, **52,** 1657.
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[216] T. Kiss and B. Toth, *Talanta*, 1982, **29,** 539.
[217] G. M. Blackburn, T. H. Lilley, and E. Walmsley, *J. Chem. Soc., Faraday Trans. 1*, 1982, **78,** 1641.
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[219] M. Booij and G. Somsen, *J. Chem. Soc., Faraday Trans. 1*, 1982, **78,** 2851.
[220] B. Yu. Zaslavskii, N. M. Mestechkina, L. M. Mikheeva, and S. V. Rogozhin, *J. Chromatogr.*, 1982, **240,** 21.
[221] M. Nakagaki and E. Okamura, *Bull. Chem. Soc. Jpn.*, 1982, **55,** 3381.
[222] A. K. Chattopadhyay and S. C. Lahiri, *Electrochim. Acta*, 1982, **27,** 269.
[223] G. Wada, E. Tamura, M. Okina, and M. Nakamura, *Bull. Chem. Soc. Jpn.*, 1982, **55,** 3064.
[224] V. A. Konyshev, *Vapr. Pitan.*, 1982, 24 (*Chem. Abstr.*, 1983, **98,** 1914).

Spectrometric techniques not covered in preceding sections include microwave spectrometry (glycine methyl ester carries an internal bifurcated hydrogen bond),[225] near-i.r. differential spectrophotometry (hydration numbers of amino-acids[226]), Raman spectrometry (L-histidine and its derivatives and imidazole analogues show a strong band at 1410 cm^{-1} in ^2H_2O characteristic of the *N*-deuteriated imidazolium ring[227]), and e.n.d.o.r.–e.s.r. spectrometry (*X*-irradiated crystals of L-alanine show evidence of movements of atoms from positions in the undamaged crystals[228] and stable-radical formation as a result of C—N cleavage,[229] while L-cysteine hydrochloride monohydrate undergoes decarboxylation through the same treatment[230]).

Electron diffraction indicates the extended conformation of glycine methyl ester to be the most stable.[231]

Molecular-orbital Calculations.—The hydration geometry of the glycine–water system and intermolecular interactions involved in two different models have been considered.[232] Other molecular-orbital calculations performed on glycine itself include gas-phase protonation by five potential protonating species (H^+, HeH^+, H_3^+, H, and H_2; the first of these is most strongly bound at any of the various points of attack)[233] and ordering of glycine orbitals accompanying conversion from the uncharged to the zwitterionic tautomer in the solid state.[234] A variety of physical parameters has been calculated for the aliphatic protein amino-acids by Boyd's force-field method,[235] and theoretical assessment of relationships between amino-acid structure and propensity towards the formation of supersaturated solutions has been reported.[236] Two possible solutions have been suggested for the mechanism by which one enantiomer is selected from a racemate to account for enantioselective metabolic processes.[237]

Conformational analysis of *N*-acetylglycine *N*-methylamide indicates the intramolecularly hydrogen-bonded form, involving the 7-membered ring structure rather than the 5-membered analogue, to be the most stable form.[238] A similar study for the corresponding derivatives of twenty natural amino-acids confirms both theoretical and experimental results from earlier investigations.[239]

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[233] L. R. Wright, R. F. Barkman, and A. M. Gabrielli, *J. Phys. Chem.*, 1982, **86,** 3951.
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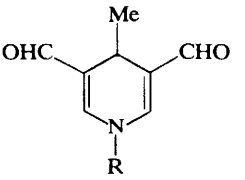
5 Chemical Studies of Amino-acids

Racemization.—As in previous volumes, the topic covers both mechanistic aspects and applications of D:L-ratios in fossil-dating.

Reviews have appeared covering results obtained from determinations of amino-acid D:L-ratios in dating relatively young fossils,[240] including racemization of residues in proteins,[241] in dating Quaternary molluscs, and in controversy associated with the technique.[242] A direct correlation between the ages assigned to samples from a 1800 year old yakusugi tree through measurement of their D:L-aspartic acid ratio and the ages assigned from tree-ring counting indicates close agreement, based on reasonable assumptions of average temperatures experienced by the tree.[243]

Mechanistic studies include OH^--catalysed epimerization of cobalt(III) complexes comprising amino-acids (aspartic acid, asparagine, or glutamic acid) and a chiral 3,7-diazanonane-1,9-diamine as ligands, in which the L-amino-acidato enantiomer is favoured,[244] and racemization rate constants of fifteen amino-acids under the standard protein-hydrolysis regime (110 °C, 6M HCl, in evacuated vacuum-sealed tubes),[245] variations in rate being related to electron-withdrawing character of side chains and to steric hindrance in the neighbourhood of the α-hydrogen atom.[245]

General Reactions.—Reactions at the amino group described in recent papers include important observations concerning reactions of lipid peroxides[246] or linoleic acid hydroperoxides[247] with amino-acids under physiological conditions. In the first of these studies, malondialdehyde, known to be a secondary product of lipid peroxidation, was shown to give fluorescent dihydropyridines (24) with a series of common amino-acids, though with cysteine reacting to



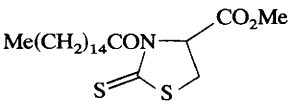
(24)

produce a different type of fluorescent compound.[246] The second study develops the hypothesis that fluorescent compounds formed from the hydroperoxides and glycine, lysine, arginine, histidine, and phenylalanine (but not

[240] S. Matsu'ura and N. Ueta, *Seikagaku*, 1982, **54**, 451.
[241] N. Fujii and K. Harada, *Kagaku No Ryoki*, 1982, **36**, 606.
[242] J. F. Wehmiller, *Quat. Sci. Rev.*, 1982, **1**, 83.
[243] I. Abe, K. Izumi, S. Kuramoto, and S. Musha, *Bunseki Kagaku*, 1982, **31**, 427 (*Chem. Abstr.*, 1982, **97**, 126 438).
[244] M. Yamaguchi, Y. Masui, M. Saburi, and S. Yoshikawa, *Inorg. Chem.*, 1982, **21**, 4138.
[245] R. Liardon and R. Jost, *Int. J. Pept. Protein Res.*, 1981, **18**, 500.
[246] J. M. C. Gutteridge, *Int. J. Biochem.*, 1982, **14**, 649; K. Kikugawa, Y. Machida, M. Kida, and T. Kurechi, *Chem. Pharm. Bull.*, 1981, **29**, 3003.
[247] H. Shimasaki, N. Ueta, and O. S. Privett, *Lipids*, 1982, **17**, 878.

from several other common amino-acids) may be an important pointer to irreversible reactions of proteins associated with the ageing process.[247] Familiar reactions which have been conducted with new or modified procedures include trifluoroacetylation of amino-acid esters with *N*-(trifluoroacetyl)nylon 6.6,[248] Schiff-base formation of amino-acids by transamination with benzophenone imine,[249] and preparation of alkanethiosulphenylcarbonylprolines.[250] Further studies of the condensation of amino-acids with formaldehyde[251] and glutaraldehyde[252] as a function of pH have been described (the ε-amino group of lysine is considerably more reactive than the α-amino group),[251] and more routine work is represented in kinetics of *N*-cyanoethylation,[253] *N*-(t-butoxycarbonyl)ation of tyrosines and 5-hydroxytryptophan (accompanied by *O*-t-butoxycarbonylation in all cases except 3-nitrotyrosine),[254] participation in the Mannich reaction,[255] and substitution of the amino-group by F using excess $NaNO_2$ in HF–pyridine.[256] The last-mentioned reaction leads to products of 1,2-shift at the phenyl moiety and corresponding insertion of the fluorine atom at C-3 when phenylalanines are involved (see also Vol. 13, p. 20), and the ratio of direct substitution products to rearrangement products was shown to be controlled by the concentration of HF in the reaction mixture.[256]

N-Acylation of amino-acid esters by the *N*-acylthiazolidinethione (25) yields an excess of the (*R*)-(−) form in the unreacted reagent when the amino-acid has the (*R*)-configuration, offering a novel Horeau-type procedure for the assignment of absolute configuration to chiral amines.[257]



(25)

N-Methylation of αβ-unsaturated α-amino-acids as their esters or dioxopiperazine derivatives can be effected with methyl iodide in the presence of sodium hydride.[258]

Reactions of amino-acids at the carboxy group featured in the recent literature include esterification of *Z*- or Boc derivatives with an alcohol using ethyl dimethylaminocarbodi-imide and 4-(dimethylamino)pyridine[259] and

[248] H. W. Tesch and R. C. Shulz, *Makromol. Chem.*, 1981, **182,** 2981.
[249] M. J. O'Donnell and R. L. Polt, *J. Org. Chem.*, 1982, **47,** 2663.
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[254] V. P. Pozdnev, *Khim. Prir. Soedin.*, 1982, 129.
[255] A. G. Agababyan, G. A. Gevorgyan, and O. L. Mndzhoyan, *Usp. Khim.*, 1982, **51,** 678.
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[258] C. G. Shin, Y. Sato, M. Hayakawa, M. Kondo, and J. Yoshimura, *Heterocycles*, 1981, **16,** 1573.
[259] M. K. Dhaon, R. K. Olsen, and K. Ramasamy, *J. Org. Chem.*, 1982, **47,** 1962.

effects of cycloamylose[260] and 2,2′-bipyridylpalladium(II)[261] in accelerating the hydrolysis of α-amino-acid esters. The conversion of amino-acid *p*-nitrophenyl esters into poly(amino-acid)s in aqueous solutions, catalysed by HCO_3^-, proceeds *via* Leuchs anhydrides (oxazolidine-2,5-diones).[262]

Reactions of amino-acids involving both amino and carboxy groups are covered in reviews of α-amino-acids in heterocyclic synthesis[263] and their use in asymmetric synthesis.[264] A mixture of eighteen common protein amino-acids refluxed during six weeks in an aqueous solution containing salts which might have been present in the oceans in prebiotic times is partly converted into mixtures of soluble polypeptides.[265]

Specific Reactions of Natural Amino-acids and Their Derivatives.—This section mostly covers reactions associated with the amino-acid side chains, but also includes work involving specific amino-acids that may be of more general character.

Thermal degradation of DL-glutamic acid gives 3,5,8,10-tetraketoperhydropyrrolo[*a*, *d*]pyrazine *via* pyroglutamic acid.[266] A convenient preparation of *N*-acylpyroglutamic acids[267] using conventional acyl chloride–triethylamine reagent systems and pyroglutamic acid as substrate involves mixed-anhydride formation followed by intramolecular *N*-acylation. γ-Esterification of aspartic and glutamic acids specifically involving the side-chain carboxy group can be accomplished without racemization *via* bis(amino-acidato)copper(II) complexes by benzylic halides.[268] Aspartyl *m*-chlorobenzoyl peroxide rearranges through a radical cage process with migration of the chiral centre, since the hydrogen atoms at the β-carbon atom were shown by labelling studies to undergo racemization.[269] Saponification of Boc-asparagine esters is accompanied by β-amide formation *via* the corresponding succinimide.[270] Decarboxylation kinetics of the new protein amino-acid β-carboxyaspartic acid[271] in 1M HCl and at pH 9.8 in 2M KOH have been described.[272] Carboxymethylenemalonic acid $HO_2CCH{=}C(CO_2H)_2$ is formed in substantial yield under the alkaline hydrolysis conditions, indicating that an alkaline hydrolysis procedure is not necessarily an unambiguous method by which the presence of this amino-acid in proteins can be demonstrated.[272]

Other aliphatic amino-acid chemistry reported recently continues familiar

[260] M. Yamamoto, H. Kobayashi, M. Kitayama, H. Nakaya, S. Tanaka, K. Naruchi, and K. Yamada, *Kogakubu Kenkyu Kohaku (Chiba Daigaku)*, 1981, **33**, 89 (*Chem. Abstr.*, 1982, **96**, 123 250).
[261] R. W. Hay and A. K. Basak, *J. Chem. Soc., Dalton Trans.*, 1982, 1819.
[262] A. Brack, *BioSystems*, 1982, **15**, 201.
[263] A. Kleemann, *Chem.-Ztg.*, 1982, **106**, 151.
[264] K. Drauz, A. Kleemann, and J. Martens, *Angew. Chem.*, 1982, **94**, 590.
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[268] W. A. R. Van Heeswijk, M. J. D. Eenink, and J. Feijen, *Synthesis*, 1982, 744.
[269] S. J. Field and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1982, 591.
[270] I. Schon, *Acta Chim. Acad. Sci. Hung.*, 1982, **109**, 219.
[271] M. R. Christy, R. M. Barkley, T. H. Koch, J. J. Van Buskirk, and W. M. Kirsch, *J. Am. Chem. Soc.*, 1981, **103**, 3935.
[272] M. R. Christy and T. H. Koch, *J. Am. Chem. Soc.*, 1982, **104**, 1771.

themes in assigning structures 1-ethyl-3,4-dehydropyrrolidine and 1-ethylpyrrole-2-aldehyde to the major reaction products from threonine and D-xylose heated in water for one hour at 150—160 °C,[273] while glycine and D-xylose give three blue pigments through reaction in slightly alkaline solutions.[274] Further results on oxidative processes undergone by aliphatic amino-acids include analytical possibilities for vanadyl compounds in 5M H_2SO_4 (1 mol proline reduces 4 mol VO_2^+ to give γ-aminobutyric acid and CO_2, but other aliphatic amino-acids do not react)[275] and the useful observation that chromium(III) oxide–pyridine gives reasonable yields of *N*-benzyloxycarbonyl oxamates $ZNH{\cdot}CO{\cdot}CO_2R$ from corresponding serine and threonine esters.[276] Unexpected ^3H loss through Chloramine-T degradation of hydroxyproline produced through metabolism of collagen containing [5-$^3H_1$-^{14}C]proline calls for a revised $^3H/^{14}C$ correction factor (1.68) when quantitative analysis of hydroxyproline is based on specific radioactivity data.[277]

^2H-Labelling studies indicate there to be no exchange of cyclopropane hydrogen atoms during the biogenesis of ethylene from 1-aminocyclopropane carboxylic acid.[278] Stereospecific conversion of 1-amino-2-ethylcyclopropanecarboxylic acid into 1-butene was established by plant-tissue studies using all four stereoisomers of the amino-acid.[279]

A practical separation of L-leucine from L-isoleucine based on the more rapid reaction of leucine with thionyl chloride in ethanol at 60 °C for one hour is accomplished by separating the resulting mixture of leucine ethyl ester hydrochloride from isoleucine hydrochloride.[280]

Weber continues the study of *N*-acetylcysteine (see Vol. 14, p. 24) for easily accomplished reactions that might have some significance in molecular evolution by showing that reaction with pyruvaldehyde at pH 7 in aqueous media containing imidazole gives good yields of *N*-acetyl-*S*-lactoylcysteine.[281] Other reactions involving sulphur-containing side chains include comparison of routes to *N*-trityl-L-homoserine (either from homoserine treated successively with Me_2SiCl_2 or Ph_2SiCl_2 and tritylation or from L-methionine by tritylation followed by methylation and displacement of the sulphonium grouping in OH^-)[282] and base-catalysed exchange behaviour of dehydromethionine.[283]

Aromatic and heteroaromatic side-chain reactions include a remarkably simple preparation of the phenylalanine–$Cr(CO)_3$ complexes through reaction

[273] T. Hara, E. Kubota, and H. Horita, *Chagyo Gijitsu Kenkyu*, 1982, 55 (*Chem. Abstr.*, 1983, **98**, 72 702).
[274] M. Miura and T. Gomyo, *Nippon Nogei Kagaku Kaishi*, 1982, **56**, 417 (*Chem. Abstr.*, 1983, **97**, 182 815).
[275] S. Klein, M. J. Waechter, and M. Hamon, *Analusis*, 1982, **10**, 120.
[276] A. V. Stachulski, *Tetrahedron Lett.*, 1982, **23**, 3789.
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[280] I. Kalnins, M. B. Andaburskaya, T. D. Shcheglova, and L. Krauja, *Prikl. Biokhim. Mikrobiol.*, 1981, **17**, 896.
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[283] D. C. Billington and B. T. Golding, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1283.

with $Cr(CO)_6$ in aqueous THF[284] and an important study of hydroxylation of phenylalanine by H_2O_2 at room temperature in the presence of iron–porphyrin complexes carrying pyridinium groups.[285] High stereoselectivity is observed[286] in the deacylation of *N*-acylphenylalanine *p*-nitrophenyl esters by bilayer vesicular systems containing *Z*-L-leucyl-L-histidine and a quaternary ammonium salt.[287] (−)-Homophenylalanine gives Boc-D-glutamic acid through $RuCl_3$–$NaIO_4$ degradation of its Boc derivative, thus establishing its absolute configuration.[288] Oxidation of tyrosine by ozone gives dopa and its oxidation products as well as *OO'*-dityrosine,[289] the first of these sequences (the conversion of dopa into dopachrome, the first stage of the melanin-forming process) having received detailed kinetic study leading to a revision of the Raper–Mason scheme for this multi-step process.[290] Dimerization through the indole α-position occurs when *N*-acetyltryptophan methyl ester is kept in trifluoroacetic acid solution during three hours at room temperature.[291] With N^{im}-formyltryptophan in liquid HF, cleavage of the protecting group is complete at 0 °C when ethane-1,2-dithiol is present.[292] Another aspect of reactivity of tryptophan derivatives studied in the chemical laboratory is catalytic transfer hydrogenation using HCO_2H–Pd (2,3-dihydrotryptophans are found as side-products),[293] while plant biochemical studies are represented in a study of L-tryptophan catabolism *via* kynurenic acid to 5-(2-carboxyethyl)-4,6-dihydroxypicolinic acid in papaverine-degrading *Nocardia* species.[294]

Further studies (see Vol. 14, p. 25) of π-benzyloxymethylhistidines as protected derivatives for racemization-free coupling to the carboxy group of this amino-acid demonstrate the security in this approach.[295] Exchange of the histidine imidazole C-2 hydrogen atom, as far as the role of metal catalysis is concerned, has been clarified and compared with the comparable process in nucleic acid bases.[296] Alternative routes to Boc-(N^{im}-trityl)histidine have been compared.[297]

Straightforward reactions applied to a variety of amino-acids, and differences accounted for, represent a useful area of study in support of peptide synthesis. A much faster reaction between proline and 1-fluoro-2,4-dinitrobenzene than seen with glycine in 30% aqueous DMSO is accounted for by NaOH catalysis applying only to the proline reaction in the complex-forming pre-equilibrium stage common to both reactions.[298]

[284] C. Sergheraert, J.-C. Brunet, and A. Tartar, *J. Chem. Soc., Chem. Commun.*, 1982, 1417.
[285] T. Shimidzu, T. Iyoda, and N. Kanda, *J. Chem. Soc., Chem. Commun.*, 1981, 1206.
[286] *cf.* K. Ohkubo, K. Sugahara, H. Ohta, K. Tokuda, and R. Ueoka, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 576.
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Non-enzymic Models of Biochemical Processes Involving Amino-acids.—This section, occasionally included in this chapter in previous volumes, usually covers observations of complexation phenomena of potential relevance to metabolic and other biochemical processes. Examples this year are the interaction seen between D-cycloserine and DNA from *E. coli* mutants,[299] pyridoxal phosphate–copper(II)-catalysed elimination of tryptophans [and its acceleration by poly(4-vinyl-*N*-dodecylpyridinium salts)],[300] and complexation by inclusion through the side chain of L-phenylalanine (but not L-tyrosine) into cyclohexaamylose.[301]

Effects of Electromagnetic Radiation on Amino-acids.—Photo-oxidation studies of tryptophan and its derivatives continue unabated, irradiation by u.v. or visible light leading to kynurenines[302–304] involving superoxide intermediates.[302] A comparison of dye-sensitized[303,304] with chemically generated singlet oxygen[304] reactions is included in this group of papers. Further results from the study of γ-irradiation of aqueous solutions of tyrosine and the correlation of the characteristic blue fluorescence with the formation of dityrosine have been reported.[305] Cleavage of the pyridinium ring of pyridinoline (7) as a result of u.v. photolysis, giving hydroxylysine, has been studied for its pH dependence.[306] A variation on experimental conditions generally used in this type of study has been applied to u.v. irradiation of lysine, producing glycine, alanine, threonine, and seven other ninhydrin-positive products, with degradation under nitrogen proceeding faster than under hydrogen.[307] Irradiation of tyrosine at 240—370 nm gives intensely absorbing initial products ($\lambda\lambda_{max}$ 260, 270 nm).[308] Effects of $HClO_4$ and HCO_2H on the radiolytic oxidation of cysteine solutions have been evaluated.[309]

Radical formation in photoionization of phenylalanine, tyrosine, or tryptophan in aqueous solution,[310] in photolysis at 340—380 nm or 280—320 nm of tryptophan and thymine (producing a thymine free radical through dissociation of the excited state of the tryptophan–thymine charge-transfer complex, or of products of reaction of solvated electrons with thymine, respectively),[311] and in radiolysis of *N*-acetylamino-acids in the solid state (yielding CO_2 and products of N–C^{α} cleavage)[312] has been studied by e.s.r.

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Tryptophan fluorescence[313—315] and phosphorescence[316] have been studied by the use of a new instrument capable of sub-nanosecond resolution[313] or by conventional methods particularly aimed at evaluating restricted molecular motion in cell membranes.[314,315]

Continuing studies of chiral discrimination in irradiation of D- or L-amino-acids generally reproduce the ambiguous conclusions arising from 0—11 MeV longitudinally polarized proton irradiation of solid L-leucine (1.1—1.7% racemization of D- or L-leucine accompanies 39—55% degradation)[317] and ^{90}Y β-irradiation of D- or L-alanine (greater relative radical formation in the D-enantiomer)[318] or β-irradiation of leucine (slight discrimination) and cysteine and tryptophan (no discrimination).[173]

6 Analytical Methods

Gas–Liquid Chromatography.—Clear advantages inherent in the technique of g.l.c. compared with some other separation methods outweigh the apparent drawbacks in the need to convert the amino-acid mixture into volatile derivatives. Procedures are well established for the preparation of *N*-trifluoroacetylamino-acid n-butyl esters,[319,320] s-butyl esters,[321] isobutyl esters,[322] isopropyl esters,[323] butylamides,[323] *N*-heptafluorobutyrylamino-acid isobutyl esters,[324] *N*-ethoxycarbonylamino-acid methyl esters,[325] oxazolidinones,[326] and (+)-3-methyl-2-butyl esters of *N*-methylamino-acids and their *N*-trimethylsilyl derivatives.[327] Objectives of these studies, often in combination with mass-spectrometric detection,[319—321] include the estimation of ^{15}N-abundance data,[319] determination of side-chain alkylated tyrosines and lysines,[320] separation of all isomers of five-carbon β-, γ-, and δ-aminoalkanoic acids,[321] determination of the imino-acids strombine and alanopine at ≥ 0.05 μg levels,[322] estimation of asparagine and glutamine,[325] and resolution of α-methyl-α-amino-acids[323] and *N*-methylamino-acids[327] either over chiral stationary phases[323] (see also refs. 163—166) or as diastereoisomeric derivative mixtures over coated capillaries.[327]

Determination of Kovat's retention indices for *N*-trimethylsilylamino-acids

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on fused-silica capillary columns coated with Carbowax or silicone oil has been reported.[328]

Ion-exchange Chromatography.—Leaving routine work aside, papers representative of continuing exploratory studies involve analysis at 10—100 picomole levels using *o*-phthaldialdehyde–mercaptoethanol fluorimetric estimation[329] and the virtues of D-glucosaminic acid as an early-eluting internal standard.[330]

Thin-layer Chromatography.—Considerable development of this technique still seems possible in the amino-acid field. Prior derivatization of an amino-acid mixture using 7-chloro-4-nitrobenzene-2-oxa-1,3-diazole followed by fluorimetric densitometry allows estimation of 3- and 4-hydroxyprolines[331] and of histidine and its 1- and 3-methyl derivatives[332] at 10—30 picomole levels. Even greater sensitivity accompanies the conversion of amino-acids into dimethylaminoazobenzenesulphonyl derivatives before t.l.c. (see also Vol. 14, p. 30).[333] Homocystine analysis using the silver nitroprusside spray reagent[334] is the subject of one of several papers concentrating on problems of t.l.c. analysis of sulphur-containing amino-acids (methionine and cystine,[335] cysteine and cystine[336]). Representative papers from the more routine areas of application deal with tryptophan and its metabolites[337] and two-dimensional t.l.c. of lysine and hydroxylysine in hydrolysed blood-serum proteins.[338]

Amino-acid derivatives receiving attention are *N*-acetylamino-acids (paper chromatography)[339] and Dnp-amino-acids (over-pressured t.l.c. using $CHCl_3$: CCl_4 : butanone : 1-propanol : methanol : acetic acid = 30:30:20:30:15:2).[340]

Assessments of improvements in techniques included in some of the preceding papers are supplemented by reports describing the separation of 35 amino-acids on Avicel F layers[341] and comparisons of silica gel, cellulose, and ion-exchange layers.[342]

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High-performance Liquid Chromatography.—This continues to be the growth area in amino-acid analysis. The general objectives, the establishment of acceptable separation parameters at maximum sensitivity, continue to provide challenges in a wide range of applications. Prior derivatization of amino-acid mixtures by dansylation[343–348] (down to 1—2 picomole levels[344]), 4-dimethylaminoazobenzene-4′-sulphonylation (down to 5—10 picomole levels,[349] but the limit is 458 picomole with hydroxyproline[350]), and reaction with 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole,[351] with fluorescamine,[347] and particularly with *o*-phthaldialdehyde and mercaptoethanol (a range of values down to less than 1 picomole[352])[352—360] has featured prominently in recent studies. Many of these studies have been conducted with representative amino-acid mixtures, but some have concentrated on specific amino-acids (lysine,[347] neurotransmitter amino-acids,[358] and proline and hydroxyproline[360]). Other studies concentrating on particular amino-acids include those on tryptophan and its metabolites,[361] branched-chain amino-acids,[362] iodinated tyrosines and analogues,[363] and specific derivative-formation methods (betaines after benzyl ester formation,[364] *S*-adenosylmethionine based on its reaction with dopamine to give 3-methoxytyramine,[365] and phenylalanine or tyrosine after conversion into *trans*-cinnamic acid and *p*-coumaric acid, respectively, by the action of phenylalanine–ammonia lyase[366]). The *S*-adenosylmethionine assay[365] is notable in permitting its estimation at 1 picomole levels using less than 1 mg adrenal tissue.

The general topic of picomole-level amino-acid analysis has been reviewed,[367] and the construction of an amino-acid analyser based on standard

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h.p.l.c. equipment using either ninhydrin or *o*-phthaldialdehyde down to 10 picomole levels, requiring less than 45 min for the analysis of protein hydrolysates, has been described.[368] Analogues of tetraiodothyroxine are useful as internal standards in quantitative amino-acid analysis by h.p.l.c.[369]

While phenylthiohydantoins remain the most widely studied amino-acid derivative by h.p.l.c.,[370—373] owing to the potential of this technique in support of peptide sequencing, other derivatives have also received attention. *N*-Acetylamino-acid *N*-methylamides have been used to determine capacity factors in reversed-phase h.p.l.c., and the relationship of these factors to side-chain hydrophobicity has been discussed.[374]

Electrochemical detection in amino-acid h.p.l.c. is being taken up in more laboratories, recent studies concentrating on 5-hydroxytryptophan (100 picogramme levels can be handled),[375] tryptophan itself,[376] and phenylalanine, tyrosine, and *m*-tyrosine.[377] The latter two papers include comparisons of fluorimetric and voltammetric estimations, showing the superiority of electrochemical detection.[376]

Preparative liquid-chromatographic separation of amino-acids and peptides on Amberlite XAD-4 (a polystyrene–divinylbenzene copolymeric reversed-phase adsorbent) allows the use of mixed solvents and acidic or basic solvents that cannot be used with silica and alkylated silicas.[378] Copper(II) ions may be added to the aqueous mobile phase in reversed-phase liquid chromatography to modify retention times of amino-acids through complexation.[379] The complexes show strong u.v. absorption, and this permits samples containing as little as 10 ng per 10 μl to be detected.

Fluorescence Methods.—Fluorimetry based on *o*-phthaldialdehyde reagent systems requires care in eliminating interference from impurity artefacts when the greatest sensitivity is sought.[380] Three- to five-fold higher values for histidine in urine are obtained using the fluorescamine procedure in place of the *o*-phthaldialdehyde method.[381] The fluorescent adduct of histidine with *o*-phthaldialdehyde, but not that with 3-methylhistidine, is destroyed by reaction with formaldehyde, permitting the estimation of the latter in the presence of the former.[382]

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Amperometric determination of amino-acids with a passivated copper electrode[383] and emission spectrometric assay of ^{15}N-labelled amino-acids[384] represent two areas where less-routine studies are being extended.

Determination of Specific Amino-acids.—This section covers quantitative analysis of specific amino-acids by modifications of general analytical methods. The high proportion of electrochemical procedures is notable.

Potentiometric determination of L-alanine, L-serine, tyrosine, and histidine at milligram levels using a copper(II)-sensitive electrode and copper(II) sulphate-containing electrolyte gives accurate results (error range 0.2—2.7%).[385] Immobilized L-tyrosine decarboxylase[386] or bacteria exercising the same function[387] have been employed in potentiometric assay for L-tyrosine. Amperometric titration using potassium iodate is advocated for estimations of cysteine and cystine.[388] Cyclic oxidative voltammetry of methionine[389] provides data in support of electrochemical studies.

Spectrophotometric assay of hydroxyproline in serum based on its oxidation to a red pyrrole dye,[390] Sakaguchi and Millon colorimetric procedures for arginine and tyrosine, respectively,[391] and assay of *N*-carbamoyl-β-alanine through spectrophotometry at 466 nm after reaction with antipyrine and diacetylmonoxime (Prescott–Jones method)[392] represent a much larger body of more routine work.

Enzymatic methods have been applied for the estimation of L-ornithine in serum, L-ornithine carbamoyltransferase effecting its conversion into citrulline, which is assayed colorimetrically after reaction with diacetyl semithiocarbazide.[393] Levels of L-canavanine in plants are determined by arginase-catalysed hydrolysis to canaline, whose amino-oxy functional group reacts quantitatively with pyridoxal 5′-phosphate (a process conveniently followed spectrophotometrically).[394]

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2

Structural Investigations of Peptides and Proteins

BY R. CASSELS, J. GAGNON, W. D. MERCER, R. H. PAIN, M. D. SCAWEN, R. A. G. SMITH, AND OTHERS

PART IA: Protein Isolation and Characterization *By M. D. Scawen, A. Atkinson, A. Electricwala, P. M. Hammond, and R. F. Sherwood*

1 Introduction

The last year has seen rather fewer papers describing the purification of proteins than previous years, although there are more applications reported from high-performance liquid chromatography; this is perhaps an indication of the growing acceptance of this technique, following the introduction of improved support phases.

The format of this report is similar to that of previous years and, again, the major emphasis has been on protein purifications employing affinity chromatography at some stage.

2 Protein Isolation Methodology

Affinity Chromatography.—*General Comments.* Affinity chromatography continues to be a major tool in the protein chemist's repertoire. Table 1 lists those proteins purified during 1982 in which affinity chromatography was employed at some stage.

In the majority of cases the protein of interest was adsorbed onto a ligand immobilized onto a suitable solid support. In two cases carbohydrate-specific proteins were purified by being directly adsorbed onto the dextran matrix of Sephadex. An endodextranase inhibitor from *Streptococcus mutans* bound specifically to Sephadex G-50[1] and a lectin from *Lathyrus sativus* bound specifically to Sephadex G-100.[2]

An interesting variation of affinity chromatography, which can greatly increase the specificity of a general ligand for a given enzyme, is to form a ternary complex with a specific, competitive inhibitor and the coenzyme. By using this approach alcohol dehydrogenase, aldehyde dehydrogenase, and lactate dehydrogenase could be specifically isolated from a rat-liver extract using NAD^+–agarose in the presence of pyrazole/NAD^+, chloral hydrate/NAD^+, and oxamate/NADH, respectively.[3]

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Table 1 *Proteins purified by affinity chromatography*

Protein	*Source*	*Matrix*	*Ligand*	*Eluant/Comment*	*Ref.*
Assimilatory nitrite reductase	*Hardeum vulgare* (barley)	Sepharose 4B	Ferredoxin	0—400 mM KCl gradient	15
*Sal*GI restriction endonuclease	*Streptomyces albus*	Sepharose 4B	Heparin	0—0.1M NaCl gradient	16
Lectin	*Secale cereale* (rye)	Agarose	*N*-Acetylglucosamine	0.1M *N*-acetylglucosamine	17
Lectin	*Hardeum vulgare* (barley)	Agarose	*N*-Acetylglucosamine	0.1M *N*-acetylglucosamine	17
Myosin light-chain kinase	Bovine carotid artery	Sepharose 4B	Calmodulin	Bind in presence of 0.5 mM Ca^{2+}. Elute with 200 mM NaCl, 7.5 mM EGTA	18
Lipoprotein lipase	*Salmo gairdneri* (rainbow trout)	Sepharose	Heparin	1.5M NaCl	19
Glutathione *S*-transferase	Bovine retina	Sepharose	Glutathione	Bind in 20 mM phosphate, pH 7.0. Elute with 50 mM Tris–HCl, pH 9.4	20
Lactate dehydrogenase B4	Rat tissues (various)	Sepharose Sepharose	(i) AMP (ii) Oxamate	0.2 mM NADH Bind in presence of 0.2 mM NADH. Elute in absence of NADH	21
Lectin	*Brachypodium sylvaticum* (brome grass)	Agarose	*N*-Acetylglucosamine	0.1M *N*-acetylglucosamine	22
Ca^{2+} transport ATPase	Human erythrocytes	Sepharose	Calmodulin	Batch-bind in presence of 0.1 mM Ca^{2+}. Pack into column and elute with 1 mM EGTA	23
Spermidine synthase	Rat prostate gland	AH-Sepharose	*S*-Adenosyl(5′)-3-	2.5 mM 5-adenosyl(5′)-3-methylthiopropylamine	24
Ubiquitin-activating enzyme	Rabbit reticulocytes	CH-Sepharose	Ubiquitin	Bind in presence of 2 mM ATP. Elute with 2 mM AMP, 0.4 mM pyrophosphate	25
Hexose-6-phosphate dehydrogenase	Rat liver	Sepharose	2′,5′-ADP	0.5 mM NADP	26

Haem-binding protein	Rabbit serum	Aminoethyl-agarose	Haemin	Decreasing pH gradient, pH 7.3—3.8	27
α_2-Macroglobulin	Human serum	CH-Sepharose	D-Tryptophan methyl ester	Chymotrypsin-bound and free α_2-macroglobulin separated by elution with 0.1M acetic acid or 40% (v/v) glycerol	28
Protein kinase	Wheatgerm	Sepharose	Phosphoryl acceptor (T-substrate)	0.1—0.3M KCl gradient	29
T-Protein component of glycine cleavage system	Chicken liver	Sepharose	Bovine serum albumin	0—0.3M NaCl gradient	30
Acetyl-CoA carboxylase	Chicken liver	Sepharose	Avidin	0.1 mM (+) biotin	31
cis-9, *trans*-11-octadecadienoate reductase	*Bacillus fibrosoluens*	Sepharose	Troloxquinone	65 mM phosphate, pH 7.2	32

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Table 1 *(cont.)*

Protein	*Source*	*Matrix*	*Ligand*	*Eluant/Comment*	*Ref.*
Cyclic nucleotide phosphodiesterase	Bovine adrenal glands or cardiac muscle	Sepharose	Cyclic GMP	10 mM cGMP	33
Lectin	*Limas flavus*	Sepharose	Bovine submaxillary mucin	10 mM *N*-acetylneuraminic acid	34
Steroid 12α-monooxygenase	Rat liver	Sepharose	Cholate	0.4% cholate, 0.05% Emulgen 913	35
Methyl transferase	*B. subtilis*	Sepharose	*S*-Adenosylhomocysteine	0—0.4 M NaCl gradient	36
Ca^{2+} protein	Bovine heart	Agarose	Phosphatidylserine	Bind in presence of 1 mM Ca^{2+}. Elute with 1M NaCl, 10 mM EDTA	37
Calmodulin-stimulated glycogensynthase kinase	Rabbit liver	Agarose	Calmodulin	Bind in 5 mM Ca^{2+}. Elute with 7 mM EGTA	38
HLA-DR antigens	Lymphoid cell line LG-2	Sepharose	*Lens culinaris* lectin	5% α-methyl mannoside	39
Transcriptional activator protein CII	Phage β	Agarose	Single-stranded DNA	0.1—1.0 M NaCl gradient	40
Glucosidase II	Rat liver	Sepharose	Concanavalin A	1 M α-methyl mannoside	41
UDP–*N*-acetylgalactosamine: globoside α-3-*N*-acetylgalacto-*S*-aminyltransferase	Canine spleen	Aminohexyl-Sepharose	Globotetraosyl ceramide	1 mM UDP	42
Phosphorylase kinase	Rat liver	Sepharose	Heparin	0.3 M NaCl	43
Malic enzymes	Canine intestinal mucosa	Sepharose	2′,5′-ADP and hexyl-NAD	0—0.22 M KCl gradient and 7 mM NAD	44
Sialyl transferases	Rat liver	Sepharose	CDP	0.1—1.0 M NaCl gradient followed by second CDP-Sepharose column eluted by 0—1 mM CDP gradient	45

Profilin	Bovine thyroid	Agarose	DNAase 1	3 M guanidinium chloride	46
Trypsin inhibitors	*Ascaris lumbricoides*	Sepharose	Trypsin	Batch-bind inhibitor. Elute with 0.2 M glycine, pH 1.75	47
Hexokinase	Yeast	CH-Sepharose	D-Glucosamine	0—3.75 mM ATP/Mg^{2+} gradient in buffer containing 1% glucose	48
Thymidylate synthase	Human L1210 cells	Sepharose	10-Formyl folate	Bind in presence of 50 μM dUMP. Elute by omitting dUMP from buffer	49
Malic enzyme	Pigeon liver	Agarose	N^6-(Aminohexyl) ADP	50 μM NADP	50
Phosphorylase *b*	Rabbit liver	Sepharose	AMP	10 mM AMP	51
Rennet	*M. miehei*	Aminohexyl-agarose	*N*-Acetylpepstatin	Bind at pH 5.5. Elute with 0.1 M acetic acid followed by 0.1 M Tris, pH 8.5	52
Rennet	*E. parasitica*	Aminohexyl-agarose	*N*-Isobutyl	Bind at pH 5.5. Elute with 0.1 M Tris, pH 8.5	52

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Table 1 *(cont.)*

Protein	*Source*	*Matrix*	*Ligand*	*Eluant/Comment*	*Ref.*
Amine oxidase	Bovine plasma	Sepharose	Concanavalin	0.3 M methyl α-D-glucopyranoside	53
Tryptophan 5-mono-oxygenase	Rat brain-stem	Agarose	2-Amino-4-hydroxy-6,7-dimethyltetra-hydropteridine	Bind at pH 7.6. Elute at pH 10.8	54
Tryptophan 3-mono-oxygenase	Rat adrenal	Sepharose	Heparin	250 mM $(NH_4)_2SO_4$, pH 8.5	55
Chymotrypsin-like enzyme	Sea urchin	Sepharose	Tryptophan methyl ester	Bind at pH 8.0 in 30 mM $CaCl_2$. Elute with 10 mM HCl	56
Uracil–DNA glycosylase	Carrot cells	Sepharose	Poly(rU)	10—500 mM phosphate gradient, pH 7.2	57
Tryptophan 5-mono-oxygenase	Mouse mastocytoma P815	Agarose	2-Amino-4-hydroxy-6,7-dimethyltetra-hydropteridine	25 mM Tris–acetate, pH 7.5, 50% ethylene glycol, 20 mM NaCl, 0.06% Tween 20	58
Cytochrome C oxidase	*Rhodopseudomonas sphaeroides*	Sepharose	Yeast cytochrome C	1 mg ml^{-1} horse-heart cytochrome C	59
Pyruvate dehydro-genase phospha-tase	Bovine kidney or heart	Sepharose	Pyruvate	Bind in presence of 0.12 M KCl, 0.2 mM $CaCl_2$. Elute with 0.2 mM EGTA	60
Acrosin inhibitor	Boar spermatozoa	Sepharose	Concanavalin A	Wash column with 0.1 M methyl α-D-glucopyranoside. Elute with 0.5 M sodium borate, pH 8.5	61
endo-Dextranase inhibitor	*Streptococcus mutans*	—	Sephadex G-50	Separate *endo*-dextranase from inhibitor protein on Sephadex G-50 eluted with buffer followed by 6 M guanidine hydrochloride to elute bound proteins	1
Ca^{2+} calmodulin tubulin kinase	Rat brain	Agarose	Calmodulin	Bind in 100 mM NaCl, 2 mM $MgCl_2$. Elute with 200 mM NaCl, 1 mM EDTA, 1 mM EGTA	62
Glycerol dehydrogenase	*Geotrichum candidium*	Sepharose	10-Carboxydecyl	5% propan-1-ol, 0.15 mM NAD	63

Myosin light-chain phosphate	Chicken gizzard	Sepharose	Phosphorylated myosin light chain	Bind in presence of 20 mM KCl, 10 mM magnesium acetate. Elute with 1 M KCl, 15 mM EDTA	64
Peroxidase	Calf thyroid	Sepharose	Glycyl-leucyl-tyrosine	Bind at pH 7.4. Elute at pH 8.5 or 9.8	65
Calmodulin	Rat liver	Agarose	Phenothiazine	Bind in presence of 1 mM Ca^{2+}. Elute with 0.3 M NaCl, 10 mM EGTA	66
Ferredoxin–NADP oxidoreductase	*Anabaena* sp	Agarose	ADP	0—0.6 M NaCl gradient	67
Histone-like protein	*E. coli*	Sepharose	DNA	0.25—0.6M KCl gradient	68
Kallikrein	Pig pancreas	Aminohexyl-Sepharose	Lysine	0—1.0 M NaCl gradient	69
Aminoglycoside 3-acetyltransferase	*Pseudomonas aeruginosa*	Sepharose	Kanamycin	0—1.2 M NaCl gradient	70

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Table 1 *(cont.)*

Protein	*Source*	*Matrix*	*Ligand*	*Eluant/Comment*	*Ref.*
N-Acetylglucosaminyltransferase	*Kluyveromyces lactis*	Sepharose	Concanavalin A	0.3 M methyl α-D-mannoside	71
Ornithine decarboxylase	Mouse kidney	Agarose	Pyridoxamine 5′-phosphate	10 μM pyridoxal 5′-phosphate	72
5′-Nucleotidase	Bovine rod	Sepharose	AMP	10 mM AMP	73
RNA polymerase II	Morris hepatoma 3924 A	Sepharose	Heparin	Bind in 0.25 M NH_4Cl. Elute with 0.8 M NH_4Cl	74
Folate-binding protein	Rat liver	Aminohexyl-Sepharose	5-Formyltetrahydropteroylglutamic acid	0—1.0 M KCl gradient followed by a 0—20 mM pteroyl glutamate gradient in 1.0 M KCl	75
Poly(ADP ribose) polymerase	Human lymphoid	Cellulose	DNA	0.2—1.5 M NaCl gradient	76
Pyruvate dehydrogenase phosphatase	Bovine heart	Sepharose	Dihydrolipoyl transacetylase	Bind in presence of 2 mM $CaCl_2$. Elute with 2 mM EGTA	77
Luciferase	*Vibrio larveyi* and others	Sepharose	2,2-Diphenyl propylamine	Bind in 0.1 M phosphate, 0.5 M NaCl, 0.5 M KCl, pH 8.5. Elute with 25 mM ethanolamine, 5 mM Tris, pH 9.1	78
Thioredoxin reductase	Rat liver	Sepharose	ADP	0.2 M potassium phosphate, pH 7.5	79
Lysozyme	Bacteriophage T_4	Agarose	C6-Muropeptide	1 M NaCl	80
Oxaloacetate decarboxylase	*Klebsiella aerogenes*	Sepharose	Avidin	0.15 M KCl, 0.05% Brij, 1.5 mM biotin	81
Glucoamylase	*Aspergilus niger*	Aminohexyl-Sepharose	Amylopectin	0.3 M MeCOONa, pH 5.5	82
Alkaline protease	Silkworm larva	Sepharose	Bowman–Birk inhibitor	0.2 M Tris–HCl, pH 8.0, 0.5 M NaCl, 2 M urea	83
Lectin	*Lathyrus sativus*		Sephadex G-100	0.1 M glucose	2
Protease (caldolysin)	*Thermus* T-351	Sepharose	Cbz-D-phenylalanine	Bind in 0.1 M Tris–acetate, pH 8.2. Elute with 0.1 M MeCOOH, 10 mM $CaCl_2$	84
Cysteine proteinase inhibitor	Human spleen	Sepharose	Papain	3 M KSCN	85

Phenol sulpho-transferase	Rat liver	Sepharose	*p*-Hydroxyphenyl	5—200 mM phosphate gradient, pH 7.0	86
Aldose reductase	Bovine, rat, or human lens	Aminohexyl-Sepharose	*p*-Nitrobenzaldehyde	0—0.1 M glyceraldehyde gradient followed by 0.05—0.1 M NaCl gradient	87
Peroxidation-inhibiting protein	Pig liver	Sepharose	Glutathionine–bromosulphophthalein	0.3 M KSCN	88
Ornithine decarboxylase	Rat liver	Sepharose	(i) Pyridoxamine phosphate	25 mM Tris–HCl, pH 7.2, 50 mM Tris–base gradient	89
			(ii) Heparin	0—0.3 M NaCl gradient	
			(iii) Pyridoxamine phosphate	0—0.2 mM pyridoxal phosphate gradient	
			(iv) Heparin	0—0.15 M NaCl gradient	

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Table 1 *(cont.)*

Protein	*Source*	*Matrix*	*Ligand*	*Eluant/Comment*	*Ref.*
Pyrroline-5-carboxylate reductase	Bovine retina	Sepharose	ADP	1 mM NADPH	90
Haemopexin	Pig serum	Aminohexyl-Sepharose	Haemin	0.5 M NaCl followed by 0.1 M citrate buffer, pH 4.0	91
Cytochrome *c* oxidase and cytochrome *c* reductase	Bovine-heart mitochondria	Thiol-Sepharose	Yeast cytochrome *c*	Oxidase and reductase separated by a 0—0.175 M NaCl gradient	92
Protein-synthesis initiation factor eIF-3	Rat liver	Cellulose	rRNA	0.1—0.5 M KCl gradient	93
Bacteriolytic enzyme	*Staphylococcus aureus*	Sepharose	Peptidoglycan	Bind at pH 7.0. Elute with 0.1 M glycine, pH 9.7, 1.8 M NaCl	94
ADP glucose synthetase	*Rhodopseudomonas sphaeroides*	Sepharose	AMP	0—0.1 M HEPES buffer, pH 7.0	95
Acyl-Co A carboxylase	*Mycobacterium tuberculosis*	Sepharose	Avidin	0—0.2 mM *d*-biotin gradient	96
Oxalate oxidase	Barley root	Agarose	Oxalate	50—500 mM KCl gradient	97

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Affinity elution from ion-exchanger material can occasionally be useful and has been used for the purification of rat-liver β-galactoside (α 2—6) sialyltransferase, which could be eluted from CM-cellulose with 0.3 mM CTP.[4]

Coupling Techniques. Cyanogen bromide continues to be the most frequently cited activation method, and further studies into the activation mechanisms of both dextran- and agarose-based supports have been carried out.[5]

Other coupling techniques have been used; for example haemin was coupled to aminoethyl-agarose using 1,1'-carbonyldi-imidazole in pure *NN*-dimethyl formamide as solvent.[6] This was found to give a higher and more reproducible yield of immobilized haemin for the purification of haem-binding proteins. One of the problems in synthesizing affinity matrices is to determine the concentration of immobilized ligand, and a method employing derivative-scanning spectrophotometry has been reported for the estimation of immobilized protein ligands.[7]

Dye-affinity Chromatogaphy. Affinity chromatography on immobilized dyes continues to be an important method for the purification of a wide variety of enzymes and proteins, as listed in Table 2. One area of application, the fractionation of serum protein, continues to receive extensive study in order that some of the minor components of serum can be isolated. The behaviour of α-1-proteinase inhibitor and α-1-acid-glycoprotein on Cibacron Blue-substituted Sephadex G-100 has been studied, with particular reference to ligand concentration and pH. Using gels with a high level of substitution it proved possible to isolate homogeneous α-1-acid glycoprotein in one step and homogeneous α-1-proteinase inhibitor in three steps.[8]

The behaviour of 27 plasma proteins on Cibacron Blue-substituted agarose columns eluted with salt gradients under a variety of conditions of pH has been studied. Four components, α_1-antitrypsin, caeruloplasmin, antithrombin III, and haemopexin, were enriched between 10- and 75-fold as a result of a single chromatographic step.[9,10]

Pregnancy-specific B_1-glycoprotein has been purified to homogeneity in 35% yield from retroplacental serum by sequential chromatography on three different dye ligands, Cibacron Blue F-3GA, Procion Turquoise MX-G, and Procion Red H-8BN. Besides being highly efficient, this procedure is also suitable for large-scale operation.[11]

Another example of the use of successive immobilized dye ligands has been the large-scale purification of 3-hydroxybutyrate dehydrogenase and malate dehydrogenase from *Rhodopseudomonas sphaeroides*. These two enzymes were initially separated by chromatography on Procion Red H-3B–Sepharose and

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Table 2 *Proteins purified by dye-affinity chromatography*

Protein	*Source*	*Matrix*	*Ligand*	*Eluant/Comment*	*Ref.*
Albumin peptic peptides	Human plasma	Agarose	Cibacron Blue F-3GA	0—4.0 M NaCl gradient	98
Lactate dehydrogenase A_4	Various rat tissues	Sepharose	(i) Cibacron Blue (ii) Oxamate	0.15 M NaCl, 10 M NADH. Bind in presence of 0.2 M NADH. Elute in absence of NADH	21
Aldehyde reductase	Ox kidney	Sepharose	Procion Orange MX-G	0—1 M KCl gradient	99
OXA-2 β-lactamase	*E. coli* J62	Sepharose	Cibacron Blue F-3GA	28 mM benzylpenicillin	100
Aldose reductase	Bovine lens	Agarose	Procion Red HE-3B	0.3 mM NADPH	101
Choline acetyltransferase	*Drosophila melanogaster*	Agarose	Procion Green HE-4BD	0.3 mM coenzyme A	102
Acyl-protein synthetase	*Photobacterium phosphoreum*	Sepharose	Cibacron Blue F-3GA	0.5 M NaSCN	103
Methylenetetrahydrofolate reductase	Porcine liver	Agarose	Cibacron Blue F-3GA	Batch-bind and wash with 5 mM NADH. Column elute with 2 M NaCl, 10% (v/v) glycerol	104
L-Aspartate oxidase	*E. coli*	Sepharose	Cibacron Blue F-3GA	0—0.4M KCl gradient	105
Protein kinase N1	Porcine-liver nuclei	Sepharose	Cibacron Blue F-3GA–dextran	0—3 mM ATP gradient in 0.4 M NaCl	106
5,10-Methylenetetrahydrofolate cyclohydrolase	*Clostridium formicoaceticum*	Sepharose	Cibacron Blue F-3GA	0.25 M KCl	107
DNA ligase	Bovine thymus	Sepharose	Cibacron Blue F-3GA	0.025—0.3 M phosphate gradient	108
Phosphoenolpyruvate carboxykinase	Chicken liver	Sepharose	Cibacron Blue F-3GA–dextran	1 mM ITP	109
Myosin phosphatase	Bovine aorta	Agarose	Procion Red HE-3B	1 M NaCl	110
Emulsin fucosidase	Almond	Sepharose	Cibacron Blue F-3GA	0.25 M NaCl	111
Lactate dehydrogenase	*Alcaligenes eutrophus*	Agarose Sepharose	Amicron Green A and Cibacron Blue F-3GA	0.25 M KCl, 0.5 mM NADH 0.75—0.75 M KCl gradient	112

Calmodulin-stimulated glycogen synthase kinase	Rabbit liver	Agarose	Cibacron Blue F-3GA–dextran	0.4 M NaCl	38
Glutamate synthase	*Saccharomyces cerevisiae*	Sepharose	Cibacron Blue F-3GA	0.1 mM NADH	113
6-Phosphogluconate dehydrogenase	Lambs liver	Agarose	Procion Red HE-3B	0—1 M KCl gradient	114
Glycerol-3-phosphate dehydrogenase	*Saccharomyces cerevisiae*	Sepharose	Cibacron Blue F-3GA	0.4 and 1.0 mM NADH	115
Pregnancy-specific B_1-glycoprotein	Human serum	Agarose	(i) Cibacron Blue F-3GA	pH gradient, pH 6.0—10.0	116
			(ii) Procion Turquoise MX-G	0.2 and 1.0 M KCl	
			(iii) Procion Red H-8BN	pH gradient, pH 6.0—10.0	

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Table 2 *(cont.)*

Protein	*Source*	*Matrix*	*Ligand*	*Eluant/Comment*	*Ref.*
Hexokinase	Pig heart	Sepharose	Procion Red H-8BN	1.8 mM ATP	117
RNA ligase	T_4 phage	Sepharose	Cibacron Blue F-3GA–dextran	(i) 0.4 M KCl (ii) 0—10 mM ATP gradient	118
UDP glucose: coniferylalcohol glucosyl transferase	Spruce (*Picea abies*)	Agarose	Procion Red HE-3B	0—2 M KCl gradient	119
4-Coumarate: CoA ligase	Spruce (*Picea abies*)	Sepharose	Cibacron Blue F-3GA	0.3—1.1 M Tris–HCl, pH 7.5, gradient	120
		Agarose	Procion Red HE-3B	0.1—0.9 M Tris–HCl, pH 7.5, gradient	120
Adenylate kinase	Chicken liver	Sepharose	Cibacron Blue F-3GA–dextran	(i) 3 mM ATP (ii) 0.1—1.5 M NaCl gradient	121
Enoyl-CoA reductases	Bovine liver	Agarose	Procion Red HE-3B	0—0.4 M KCl gradient used to separate 2-enoyl-CoA reductase from 2,4-dienoyl-CoA reductase	122
2,4-Dienoyl-CoA reductase	Bovine liver	Agarose	Procion Red HE-3B	5 mM NADP in 0.33 M KCl	122
Acetoacetyl-CoA synthetase	*Zoogloea ramigera*	Agarose	Procion Red HE-3B	0—0.5 M KCl gradient	123
Ferrochelatase	Bovine liver	Sepharose	Cibacron Blue F-3GA	Bind in 20 mM Tris–HCl, pH 8.0, 20% glycerol, 0.5 M NaCl, 1% Triton X-100, 1 mM dithiothreitol. Wash with above buffer containing 1.5 M NaCl and 0.2% Nonidet P-40. Elute enzyme with buffer containing 1.0 M NaCl and 1% sodium cholate.	124
NADH–dihydropteridine reductase	Liver	Agarose	Cibacron Blue F-3GA	0.1 mM NADH	125
DNA polymerase A	*Tetrahymena pyriformis*	Sepharose	Cibacron Blue F-3GA	0—0.4 M KCl, 30% glycerol gradient	126
5'-Nucleotidase	Bovine rod	Sepharose	Cibacron Blue F-3GA	Enzyme eluted in void volume	73

Myosin light-chain kinase	Bovine stomach	Agarose	Cibacron Blue F-3GA	0—1.5 M NaCl gradient	127
Haemoglobin	Human erythrocytes	Sepharose	Cibacron Blue F-3GA	(i) 0.1 M triethanolamine acetate, pH 8.0 (ii) 0.1 M triethanolamine acetate, 2 M NaCl	128
Malate dehydrogenase	*B. subtilis*, *B. caldotenax*, *T. aquaticus*, *E. coli*	Agarose	Procion Red HE-3B	All bound in 10 mM Tris–HCl, pH 7.2, or 10 mM phosphate, pH 7.2. Enzymes eluted with various combinations of KCl, L-malate, and NAD at pH 7.2	129
Phospholipase A	Rat liver	Agarose	Cibacron Blue F-3GA	0.2—1.0 M KCl gradient	130
Sepiapterin reductase	Rat erythrocytes	Agarose	Procion Red HE-3B	0.1 M KCl, 50 μM NADPH	131
Pyruvate kinase	Rat lung	Sepharose	Cibacron Blue F-3GA	Elute with 3 mM NAD to remove lactate dehydrogenase followed by 0.1 mM fructose 1,6-biphosphate and a 0—3M KCl gradient	132

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Table 2 *(cont.)*

Protein	*Source*	*Matrix*	*Ligand*	*Eluant/Comment*	*Ref.*
Calmodulin-deficient 3′:5′-cyclic nucleotide phosphodiesterase	Bovine heart	Agarose	Cibacron Blue F-3GA	1 μM cAMP in 1.5 M NaCl	133
Phosphofructokinase	*Bacillus subtilis*	Sepharose	Cibacron Blue F-3GA–dextran	0.1 mM ADP gradient in presence of 1 mM fructose 6-phosphate	13
3-Hydroxybutyrate dehydrogenase	*Rhodopseudomonas sphaeroides*	Sepharose	(i) Procion Red H-3B	(i) 1 M KCl	12
			(ii) Procion Blue MX-4GD	(ii) Wash column with 1 M KCl, elute enzyme with 2 mM NADH in 1 M KCl	
Malate dehydrogenase	*Rhodopseudomonas sphaeroides*	Sepharose	(i) Procion Red H-3B	(i) 1 M KCl containing 2 mM NADH	12
			(ii) Procion Blue MX-4GD	(ii) 0—0.7 M KCl gradient	
NAD^+ kinase	*Candida utilis*	Sepharose	Cibacron Blue F-3GA	40 mM NAD^+	134
Luciferase	Firefly	Sepharose	Cibacron Blue F-3GA	0.5 mM ATP	135

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purified separately by chromatography on Procion Blue MX-4GD–Sepharose.[12]

The potential power of this technique is demonstrated by the 1200-fold purification, and 24% recovery is achieved for phosphofructokinase from *Bacillus subtilis*. This enzyme was purified to homogeneity from a crude extract on a single column of Cibacron Blue–dextran–Sepharose eluted with ADP in the presence of 1 mM fructose 6-phosphate, forming a specific dead-end complex with the enzyme.[13]

A series of studies have shown that immobilized dyes can be chemically modified, by, for example, reductive cleavage with $Na_2S_2O_4$ or $NaBH_4$. Such modified dyes showed a weaker interaction with a range of test enzymes than did the unmodified dyes. This may be of value in increasing the recovery of strongly bound enzymes and adds yet another perturbation to the repertoire of dye-affinity chromatography.[14]

Hydrophobic Interaction Chromatography and Covalent Chromatography.—*Hydrophobic Interaction Chromatography.* Hydrophobic interaction chromatography, by which proteins are separated on the basis of differences in the strength of interaction between their hydrophobic regions and a stationary support matrix, has found widespread use in the protein-chemistry laboratory. Some degree of purification can be achieved with most proteins, since the majority of proteins have some regions that interact with a hydrophobic adsorbent.

Phenyl- and octyl-Sepharose remain the most widely used matrices, possibly owing to their commercial availability. New matrices, however, continue to be introduced. Schneider and Altendorf[136] describe a procedure for the isolation of the Fo portion of the ATP synthetase FiFo complex on deoxycholic acid-coupled poly(L-lysyl)-agarose. This matrix is used to bind the complex prior to dissociation. It has no reaction with water-soluble proteins such as the Fo moiety and so this can be eluted after dissociation. This advantage over other hydrophobic matrices such as phenyl- and octyl-Sepharose should find application in the separation of water-soluble and membrane proteins. A further report[137] details the use of Amberlite CG-50 as a hydrophobic resin. However, below pH 4.5, the carboxy group of the resin is undissociated and hydrophobically binds with the same group on proteins.

Adsorbed proteins can be eluted by an increase in pH; the carboxy group dissociates, ionic repulsion increases, and the enzyme material is released selectively. A major advantage of this resin is that it is extremely cheap compared to other hydrophobic matrices.

Poly(ethylene glycol)- and poly(vinyl alcohol)-substituted carbohydrate gels can be used as a means of mild hydrophobic chromatography.[138] Such ligands

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have an intermediate hydrophobicity and can be used where a weak bonding is desirable. They permit adequate bonding to the matrix but do not require 'severe' elution conditions.

A hydrophobic interaction matrix, phenyl-Sepharose, has been found useful for zinc-dependent affinity chromatography, and it has recently been applied to the purification of the Trp-containing S100b protein.[139] The S100b protein becomes highly hydrophobic on zinc binding and will bind to phenyl-Sepharose. It can be readily eluted with a chelating agent such as EDTA.

In a study on mannose-specific lectin activity of *E. coli* fimbriae, a less usual use has been found for octyl-Sepharose. It has been reported[140] that the ability to agglutinate guinea-pig erythrocytes in a mannose-sensitive manner correlates well with liability to hydrophobic interaction with octyl-Sepharose, giving a potential means of assay.

Schafer-Nielson and Rose[141] describe a procedure for the rapid separation of chromatin proteins (histones and non-histones) from nucleic acids. Mild conditions are used to bind the proteins, whilst the nucleic acids are not retained. Recovery is said to be dependent on the type of column used but is generally in excess of 80%.

In recent years, although the use of hydrophobic interaction chromatography has increased, there has been only a limited number of reports on the study of hydrophobic interaction. Recently, a study has been made of the hydrophobic interactions of α- and β-trypsin and α-chymotrypsin.[142] Satoh and Sato[143] describe a study of the purification of associated and unassociated forms of 1,4-glucan branching enzyme on hexylamine-Sepharose. The study is slightly unusual in that the enzyme is eluted from the hydrophobic column by an increase in ionic strength and not a decrease.

Table 3 lists examples from the literature of 1982 of protein materials isolated using hydrophobic interaction chromatography.

Covalent Chromatography. There continues to be a small number of reports on the use of covalent chromatography in protein purification. Thiol-Sepharose and thiopropyl-Sepharose continue to be the matrices of choice, although Schmelzer *et al.*[144] report the separation of sulphydryl oxidase from γ-glutamyltransferase on cysteinylsuccinamidopropyl-glass. Thiol-Sepharose was used to purify the selenoenzyme glutathione peroxidase, from bovine lens,[145] and thiopropyl-Sepharose was used to purify aldehyde dehydrogenase from mitochondria.[146] Thiopropyl-Sepharose has also been used in studies on a proteolytic enzyme from *Trypanosoma congolense.*[147]

A technique called 'covalent affinity' chromatography has been described,[148]

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Table 3 *Proteins purified by hydrophobic interaction chromatography**

Protein	*Source*	*Ligand/Matrix*	*Loading conditions*	*Elution conditions*	*Ref.*
ATP synthetase (F_1F_0)	*E. coli*	Deoxycholic acid–poly(L-lysine)-agarose	10 mM Tris–HCl, pH 8.0, 150 mM NaCl	Removal of F_1 by urea, selective elution of F_0 in presence of 10 mM taurodeoxycholate	136
Glucose oxidase Hyaluronidase Cholesterol oxidase	*A. niger* *Strep. hyalurolyticus* *Ps. fluorescens*	Amberlite CG-50	Various buffers at pH below 4.5	Increase in pH value	137
1,4-α-Glucan-branching enzyme	Rat liver	Hexylamine-Sepharose	10 mM Tris–HCl, pH 7.4, 15 mM MSH, 2 mM EDTA, 0.9% NaCl	NaCl gradient in loading buffer, 0.5—2.0 M	143
Polygalacturonase	Commercially prepared pectinase	Phenyl-Sepharose	High phosphate-buffer concentration	Decrease in ionic strength	149
Fructose phosphotransferase	*Rhodopseudomonas sphaeroides*	Hexylagarose	5 mM NaP, pH 7.5, 20% ethylene glycol	Gradient from 20—90% ethylene glycol	150
		Butylagarose	20 mM NaP, pH 7.5, 20% ethylene glycol	Gradient from 20—80% ethylene glycol	150
Fructosyltransferase	*Asparagus officinalis*	Octyl-Sepharose	10 mM KP, pH 6.5, 35% $(NH_4)_2SO_4$	10 mM KP, pH 6.5	151
Phospholipase D	*Streptomyces cinnamomeus*	Investigation of various aminoalkyl-substituted agaroses under various different conditions, for the chromatography of phospholipase D			152

* Abbreviations used in Table 3: DTT dithiothreitol, EDTA ethylenediaminetetra-acetic acid, KP potassium phosphate buffer, MSH β-mercaptoethanol, Na-CHO sodium cholate, NaP sodium phosphate buffer, Tris trishydroxymethylaminomethane.

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Table 3 *(cont.)*

Protein	*Source*	*Ligand/Matrix*	*Loading conditions*	*Elution conditions*	*Ref.*
Cytochrome P-448 component of benzo(a)pyrene hydroxylase	*Saccharomyces cerevisiae*	8-Amino-n-octyl-Sepharose	10 mM KP, pH 7.0, 0.3% Na-CHO, 1.0 mM EDTA	Loading buffer plus 0.1% Emulgen 911	153
Phosphatidyl-inositol-specific phospholipase *c*	Bovine platelets	Octyl-Sepharose	25 mM Tris–maleate, pH 6.9, plus 5 mM EGTA	Loading buffer plus 50% ethylene glycol	154
Collagen	Mollusca (various)	Phenyl-Sepharose	Not stated	Decreasing gradient of $(NH_4)_2SO_4$	155
Arylsulphatase C, estrone sulphatase	Sheep brain	Phenyl-Sepharose	10 mM KP, pH 7.5, 0.5 M $(NH_4)_2SO_4$	Loading buffer plus 5% Triton X-100	156
Calmodulin	Bovine brain	Phenyl- and octyl-Sepharose	50 mM Tris–HCl, pH 7.5, 5 mM $CaCl_2$	Loading buffer containing 1 mM EGTA in place of $CaCl_2$	157
Chlorophyllase	*Citrus unshiu*	Phenyl-Sepharose	100 mM phosphate, containing 3 M KCl	0—0.5% Triton X-100 gradient in loading buffer	158
Renin zymogen	Hog kidney	Octyl-Sepharose	20 mM phosphate, pH 6.5, 1 M $(NH_4)_2SO_4$	10 mM phosphate plus 50% ethylene glycol	159
Cytochrome P-450	Rat liver	8-Amino-octyl-Sepharose	10 mM KP, pH 7.25, 20% glycerol, 0.7% Na-CHO	Loading buffer containing Emulgen 913	160
Human interferon	Human lymphocyte culture	Alkylagarose	10 mM sodium acetate, pH 6.0, 0.5 M NaCl	Various binding studies carried out	161
Placental lactogen	Mouse placenta	Phenyl-Sepharose	100 mM NH_4HCO_3/NH_4OH, pH 9.0, 10 mM EDTA, 10 mM EGTA	5 mM sodium glycinate, 55% ethylene glycol	162
β-Galactosidase	Porcine spleen	Phenyl-Sepharose	10 mM NaP, pH 7.0, 0.1 M NaCl	Linear gradient from 20—90% ethylene glycol in loading buffer	163

Serum proteins (various)	Human serum	Phenyl-Sepharose	0.8 M $(NH_4)_2SO_4$, pH 7.0	Linear decreasing gradient of $(NH_4)_2SO_4$	164
Interleukin 2	Rat spleen	Phenyl-Sepharose	20 mM Tris–HCl, pH 7.2, plus 0.1% albumin	Loading buffer plus 1 M NaCl	165
Glycerol dehydrogenase	*Geotrichum candidum*	Octyl-Sepharose or 10-carboxy-decyl-Sepharose	20 mM acetate, pH 6.0	Loading buffer plus 0.5 M NaCl	166
D-Malic enzyme	*Pseudomonas fluorescens*	Octyl-Sepharose	10 mM phosphate, pH 7.4, 10 mM EDTA, 1 M $(NH_4)_2SO_4$	Linear decreasing gradient, 1 to 0 M $(NH_4)_2SO_4$ in loading buffer	167

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Table 3 *(cont.)*

Protein	*Source*	*Ligand/Matrix*	*Loading conditions*	*Elution conditions*	*Ref.*
L-Asparaginase	*Chlamydomonas*	Phenyl-Sepharose	50 mM KH_2PO_4/NaOH, pH 7.4, 4 M NaCl	Stepwise decrease from 4 to 1 M NaCl	168
Phosphoenol-pyruvate carboxylase	*E. coli*	Butyl-Sepharose Hexyl-Sepharose Octyl-Sepharose	50 mM Tris–H_2SO_4, pH 7.4	$(NH_4)_2SO_4$ gradient in loading buffer	169
C1 esterase inhibitor	Human plasma	Hexyl-Sepharose	40 mM NaP, pH 7.0, 0.4 M $(NH_4)_2SO_4$	Not retained	170
Immunoglobulins	Rabbit serum	Investigation of various ligands and binding conditions. Generally, elution was achieved using chaotropic agents			171
Pyruvate kinase	*Halobacterium cutirubrum*	Sepharose CL-4B	10 mM NaP, pH 6.5, 4M NaCl, 10 mM $MgCl_2$, 1 mM DTT	Not stated	172
Bacteriocin 28	*Clostridium perfringens*	Phenyl-Sepharose	10 mM phosphate, pH 6.8	Increasing concentration of ethylene glycol	173
Glycoprotein	Human erythrocytes	Phenyl-Sepharose	40 mM NaP, pH 7.4, Nonidet P-40, 0.3 M NaCl	Gradient with 40 mM NaP, pH 9.5, 1.0% Nonidet P-40, and 50 mM NaCl	174
Neural-cell adhesion protein	Chick-embryo brain	Octylagarose	1 mM Tris, pH 8.2, 200 mM EDTA, 1M KCl	75% ethylene glycol, 0.15 M KCl, 200 mM EDTA, 1 mM Tris, pH 8.2	175

NADH dehydrogenase	*Bacillus subtilis*	Octyl-Sepharose	10 mM KP, pH 7.0, 10 mM $(NH_4)_2SO_4$	Gradient from loading buffer to distilled water	176
Phospholipase A_2	Porcine ileum	Octyl-Sepharose	50 mM acetate, pH 5.0, 2 M NaCl, 20 mM $CaCl_2$	50 mM acetate, pH 5.0	177
Complement C_5	Human serum	Phenyl-Sepharose	150 mM NaCl, 20 mM EDTA, 50 mM ε-aminocaproic acid	50% glycerin in distilled water	178
Fibronectin	Human plasma	Octyl-Sepharose	Various buffers examined, elution with 0—2% Brij 35		179

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whereby ubiquitin-activating enzyme is purified using ubiquitin-Sepharose. The authors show that a thiolester intermediate is formed between the activating enzyme and the Sepharose-bound ubiquitin.

Immunoaffinity Chromatography.—The increase in the use of immunoaffinity chromatography for protein purification is clearly reflected by the increase in the number of references in the literature for 1982. Some of the proteins isolated by using this procedure are listed in Table 4.

The technique has the major advantage of separating copurifying contaminants that are difficult to remove by conventional procedures. For example, in the purification of Factor VIII,[180] plasma devoid of Factor VIII was used as a complex antigen. The antiserum raised was coupled to Sepharose and used as an immunosorbent to isolate Factor VIII for normal plasma. A similar procedure was also adopted for the purification of angiotensin I-converting enzyme,[181] and the technique has been loosely termed 'reversed immunoadsorption chromatography'.

Increasingly, monoclonal antibodies are used for the preparation of immunosorbent and for subsequent protein purification.[182—187] The antibodies produced by the specific hybridomas are homogeneous and directed against a specific antigenic determinant in the protein. For example, human plasminogen activator (HPA) was purified from urokinase using a monoclonal anti HPA IgG affinity column.[183]

In addition to exhibiting high specificity, the proteins can often be eluted from monoclonal antibody immunosorbents under relatively mild conditions, thereby preserving much of their biological activity. A novel approach has been the use of acetonitrile in a pH gradient as an eluant. This procedure was used to separate anti-human prolactin immunoglobulins into different antibody populations with varying immunoreactivity.[188]

Metal Chelate Chromatography.—Conventional metal chelate chromatography using zinc chelate agarose has been used in the purification of a phosphotyrosyl protein phosphatase from Ehrlich ascites tumour cells[203] and trypsin inhibitors from porcine colustrum.[204] This latter protein did not bind to the zinc chelate agarose, but a five-fold purification was achieved by the removal of other proteins.

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An unusual example of metal-ion-dependent chromatography is the purification of S100b protein from bovine brain on phenyl-Sepharose in the presence of Zn^{2+}. Contaminating proteins were eluted with Zn^{2+}-containing buffer and the S100b protein was eluted by EDTA.[205] The specific involvement of a metal ion, notably Ca^{2+}, has been used to advantage in the purification of a number of proteins. Pyruvate dehydrogenase phosphatase from bovine heart was purified by Ca^{2+}-dependent binding to immobilized dihydrolipoyl transacetylase, from which it was eluted with EDTA,[77] and a chymotrypsin-like enzyme from sea urchin was purified by utilizing its Ca^{2+}-dependent binding to immobilized tryptophan methyl ester at pH 8.0.[56]

Several calmodulin binding proteins have been purified using immobilized calmodulin: human-erythrocyte Ca^{2+} transport ATPase by a batch technique,[23] two myosin light-chain kinases from bovine carotid artery,[18] and a calmodulin-stimulated glycogen synthase kinase from rabbit liver.[38] In all cases binding was Ca^{2+}-dependent and elution was effected with EGTA.

Phase Partition.—Phase partition in aqueous systems continues to be a little-used technique for the purification of proteins. A report that purified dextran can be replaced by crude dextran, with only minor changes in the behaviour of two test enzymes, pullulanase and formate dehydrogenase, may broaden the appeal of the technique. The use of crude dextran entails a considerable reduction in cost.[206] Phase separation using poly(ethylene glycol) and dextran has been used as an initial step for the purification of RNA polymerase and glutamine synthetase from *E. coli*.[207]

Affinity partition, using Cibacron Blue F-3GA bound to poly(ethylene glycol), has been used for the large-scale purification of phosphofructokinase from baker's yeast. Crude extract was first fractionated with underivatized poly(ethylene glycol) and dextran. It was then extracted from the lower dextran phase into an upper Cibacron Blue–poly(ethylene glycol) phase. The enzyme was dissociated from the dye by the addition of solid potassium phosphates. These two steps gave a 58-fold purification of the enzyme starting from 1 kg baker's yeast.[208]

High-performance Liquid Chromatography.—The application of high-performance liquid chromatography (h.p.l.c.) to the separation, purification, and identification of proteins, peptides, and amino-acids is a field that is still developing. The sudden rise in publications in this area in this decade now warrants a separate section on the use of this technique in protein and peptide purification.

Two reviews[209,210] discuss the use of a variety of ion-exchange matrices including DEAE- and CM-glycophase-controlled pore glass with 100—530 Å

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Table 4 *Proteins purified by immunoaffinity chromatography*

Protein	*Source*	*Eluant/Comment*	*Ref.*
Neural-cell adhesion molecule (N-CAM)	Mouse/rat	Use of monoclonal antibody to chicken N-CAM. Elution with 50 mM diethylamine in phosphate/NaCl/Nonidet P-40/EDTA buffer, pH 11.8	182
Dipeptidylaminopeptidase IV	Human kidney	Enzyme purified 850-fold in 2 steps with 16.5% yield	189
Plasminogen activator	Human	Use of monoclonal antibodies for enzyme purification. Eluant: 0.1 M glycine hydrochloride, pH 2.5/05 M NaCl/0.1% Triton X-100	183
Glomerular basement membrane	Human	Three antigenic fractions isolated with elution buffer containing either NaCl, glycine, or NH_4SCN	190
Complement C6	Human	5 M guanidine hydrochloride for 3 h at 0—2 °C to dissociate antigen–antibody complex	191
Trypsin inhibitors	Corn/teosinte seeds	1 M propionic acid as eluant	192
IgA	Bovine colostrum	Use of specific anti bovine IgG antibodies to isolate IgA	193
Anti-(human prolactin) immunoglobulins	Sheep	Elution with 20% acetonitrile in a pH 7.0 to pH 2.0 gradient resulted in improved yield and immunoreactivity of IgG	188
Phytochrome	*Avena* sp.	Inhibition of proteolysis by immunoprecipitation method compared to immunoaffinity chromatography	194
IgA	Horse	—	195
C3b inactivator	Human	Use of monoclonal antibodies. Eluant: 0.14 M NaCl/0.05 M diethylamine/HCl, pH 11.5	184
Factor VIII	Human	Reverse immunoadsorption. Plasma, devoid of Factor VIII, was used as an antigen	180
Ligandin	Rat liver or testis	Elution with 0.1 M glycine, pH 3.0	196
Medium-size tumour antigen	Polyoma virus	Antibodies against synthetic peptide, corresponding to six *C*-terminal residues of antigen, were used for immunoaffinity column	197
5′-Nucleotidase	Rat liver	Use of monoclonal antibody immunoadsorbent. Elution with 50 mM diethylamine	185

Angiotensin I-converting enzyme	Human kidney	Reverse immunoadsorption. Antibodies to contaminant proteins were coupled to Sepharose 6B	181
Cyclic nucleotide phosphodiesterase	Bovine	Use of conformation-specific monoclonal antibody column	186
α-Fetoprotein	Human-cord serum	Eluant: 0.2 M Na_2CO_3, pH 11.6	198
Peroxidase	Peanut	Immunological non-identity with horse-radish or fungal enzyme	199
Urokinase	Human plasma	Elution with 0.1 M glycine/HCl, pH 2.2	200
Ferritin	*In vitro* protein-synthesizing system	Anti-ferritin-Sepharose column to detect antigen	201
Lysozymes	Human parotid	Elution with 0.2 M sodium acetate/HCl buffer, pH 1.8	202
Spine glycoprotein	*Schistosome mansoni*	Use of monoclonal antibody immunosorbent	187

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pore size and 300 Å polyethyleneimine (PEI) matrix with a binding capacity of 40 mg haemoglobin per g support; steric-exclusion supports for gel filtration of proteins from 15 000 to 100 000 daltons are also described, as are AMP affinity and antibody immunoaffinity h.p.l.c. matrices. The first review also describes the separation and measurement of various isoenzymes by h.p.l.c., including those of lactate dehydrogenase, creatine kinase, alkaline phosphatase, and hexokinase, and the separation of serum proteins on PEI anion exchangers including glycosylated and other haemoglobins.

Several matrices have been described or fully evaluated in the current year including 1000 Å and 4000 Å pore-size silica derivatized with PEI and crosslinked with butan-1,4-diol diglycidyl ether,[211] reverse d-phase matrices including octyl, octadecyl, cyanopropyl, and diphenyl bonded phases,[212] and large-pore silica silanized with n-alkylchlorosilanes[213] and 100 and 300 Å pore silanized silica,[214] the latter being excellent for the separation of proteins of greater than 15 000 daltons by gel permeation.

In the applications field h.p.l.c. matrices have often been substituted for conventional low-pressure matrices. Hence an anion-exchange h.p.l.c. matrix has been employed in place of DEAE-cellulose in the purification of rat-brain hexokinase with 67% recovery and a 4.5-fold purification to homogeneous product,[215] and a C_{18} μ-Bondapak column has been used, again in place of DEAE-cellulose, to purify α- and β-epidermal growth factors.[216] Similarly a C_{18} μ-Bondapak CN column with acetonitrile gradient followed by a μ-Bondapak CN column with propan-1-ol has been used in tandem to purify the transforming growth factor from murine sarcoma virus-transformed 3T3 cells, 430-fold to homogeneity.[217] Many other proteins have been purified or separated by h.p.l.c., including the following: luteinizing hormone-releasing hormone and thyrotropin-releasing hormone from human and bovine milk on reversed-phase RP-18;[218] trypsin and chymotrypsin purified free of each other on a variety of supports using acetonitrile in trifluoroacetic acid, at pH 2;[219] twelve angiotensins separated on a weak difunctional anion-exchange phase using an acetonitrile gradient in triethylammonium acetate,[220] apolipoprotein from the high-density lipoproteins of human serum on TSK SW3000 employing a mobile phase of tris-buffered urea or guanidinium chloride;[221] the glucoproteins peroxidase and glucose oxidase on concanavalin A–porous silica of 100—1000 Å pore size[222] and hepatic proliferation inhibitor to

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[219] K. Titani, T. Sasagawa, K. Resing, and K. A. Walsh, *Anal. Biochem.*, 1982, **123,** 408.
[220] M. Dizdaroglu, H. C. Krutzsch, and M. G. Simic, *Anal. Biochem.*, 1982, **125,** 190.
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[222] A. Borchert, P.-O. Larsson, and K. Mosbach, *J. Chromatogr.*, 1982, **244,** 49.

homogeneity by a linear sodium phosphate gradient on an anion-exchange matrix;[223] the antifreeze proteins from the larva of the beetle *Tenebrio molitor* on an I-125 gel-permeation column.[224] A micromethod for the purification of lysyl oxidase from tissue culture cells has been developed that employs affinity chromatography on an elastin–hydroxyethylmethacrylate gel followed by h.p.l.c. on a TSK SW3000 size-exclusion column.[225]

Two interesting separations have been described: firstly, the resolution of the 21 *E. coli* ribosomal proteins of the 30S subunit into 15 peaks using C_{18} μ-Bondapak with an acetonitrile gradient[226] (this can be used for both the purification and identification of the 30S ribosomal proteins) and, secondly, the separation of some of the proteins of human adenovirus 2.[227] In this case the closely property-related 10, 20, 21, 22, and 23 K proteins specified by the adenovirus 2 Elb coding region can be separated from a crude S100 cytoplasmic fraction, and from each other, by reversed-phase h.p.l.c. on a C_{18} column with a linear propan-1-ol gradient in pyridine formate or by NaCl gradient elution on an anion-exchange phase.

Three evaluation studies on protein separation were carried out in 1982. Firstly, a buffer system, comprising 80 mM sodium phosphate, pH 7.0, 0.32 M NaCl, and 20% ethanol, has been derived to minimize both ionic and hydrophobic interactions of proteins with the stationary phase on gel permeation chromatography.[228] Similarly the gel permeation of a synthetic mixture of proteins on I-60, I-125, and I-250 has been shown to be optimal at pH 3 with 4 M or 6 M guanidinium employed to prevent protein aggregation.[229] Thirdly, the behaviour of asymmetric proteins such as collagen and fibrinogen has been studied on a wide variety of different pore-size matrices.[230]

Reversed-phase chromatography, in an area that is becoming more applicable to proteins, and studies have been carried out to investigate some of the parameters that effect the behaviour of proteins on reversed-phase supports. Short-chain alkyl substituents, C_2—C_4, were shown to give higher recoveries of a variety of test proteins than the more commonly employed C_8 and C_{18} alkyl substituents. The optimum solvent was shown to be a propan-1-ol–trifluoroacetic acid mixture.[231] In another study, using insulin, the concentration of methanol as organic modifier was shown to be critical in achieving good separation and caused a ten-fold increase in the log K' value, whereas changes in salt concentration over the range 0.1—0.8 M or in pH over the range pH 2—4 had little effect.[232]

[223] J. B. McMahon and P. T. Iype, *J. Liq. Chromatogr.*, 1982, **5**, 751.
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[227] M. Green and K. H. Brackman, *Anal. Biochem.*, 1982, **124**, 209.
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[229] C. Lazure, M. Dennis, J. Rochemont, N. G. Seidah, and M. Chretien, *Anal. Biochem.*, 1982, **125**, 406.
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[231] M. J. O'Hare, M. W. Capp, E. C. Nice, N. H. C. Cooke, and B. G. Archer, *Anal. Biochem.*, 1982, **126**, 17.
[232] G. Vigh, Z. Varga-Puchony, J. Hlavay, and E. Papp-Hites, *J. Chromatogr.*, 1982, **236**, 51.

Many proteins exhibit non-ideal behaviour on h.p.l.c. size-exclusion columns, particularly at low ionic strength. In theory this can be exploited for the purification of proteins, and studies using myoglobin, lysozyme, and ovalbumin have shown that this is so.[233]

Polypeptides, both large and small, have been studied and separated on h.p.l.c.; for example conditions have been described for the separation of peptides on C_{18} columns with ammonium bicarbonate gradients,[234] the purification of peptides from tooth pulp on C_{18} in acetonitrile has been demonstrated,[235] and the reversed-phase separation of peptides for microsequence analysis using acetonitrile gradients in trifluoroacetic acid on C_8, C_{18}, and, for optimal separation, alkyl phenyl matrices has been described.[236] Two interesting systems have been reported: firstly, the separation of peptides from Bence-Jones protein on a 6 μm macroreticular styrene–divinyl benzene matrix at 70 °C with a linear gradient from water to ammonia, acetonitrile, propan-2-ol, pH 6.2,[237] and, secondly, the reversed-phase separation of peptides containing 5—51 amino-acids have been studied on octadecyl, hexyl, phenyl, and cyanopropyl stationary phases, both with and without secondary silanization (end-capping) of the stationary phase.[238] Comparisons of different mobile phases revealed that an aqueous buffer of low pH, high ionic strength, and containing alkylamine or alkylammonium compounds was the most effective eluant.

The second international congress on h.p.l.c. of proteins, peptides, and polynucleotides was held in Baltimore, U.S.A., in December 1982. This meeting dealt with many aspects of protein separation, both analytical and preparative, and described new matrices. The full publication of the meeting, in August 1983, is likely to be an invaluable source of data on new h.p.l.c.-based protein-purification technology.

Other Chromatographic Techniques and Applications.—Salting-out chromatography on agarose has been used for the purification of phenylalanyl-tRNA synthetase from hen-liver mitochondria. In this case advantage was taken of the ligand-induced solubility shift that occurs on adding $tRNA^{Phe}$ to the enzyme to achieve a 63-fold purification in three steps of salting-out and salting-out affinity chromatography.[239]

The rapid desalting of protein solutions without excessive dilution is a frequent problem in protein chemistry. A method combining ion exchange and gel filtration into a single column, by means of which proteins can be concentrated from a dilute solution and subsequently eluted and desalted at concentrations in excess of 10 mg ml^{-1}, has been described.[240]

[233] W. Kopaciewicz and F. E. Regnier, *Anal. Biochem.*, 1982, **126**, 8.
[234] D. R. Knighton, D. R. K. Harding, J. R. Napier, and W. S. Hancock, *J. Chromatogr.*, 1982, **249**, 193.
[235] H. E. May, F. S. Tanzer, G. H. Fridland, C. Wakelyn, and D. M. Desiderio, *J. Liq. Chromatogr.*, 1982, **5**, 2135.
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3 Isolation of Specific Classes of Proteins

Membrane Proteins.—Membrane proteins, particularly enzymes, play an important role in many physiological processes. A prime example of this is the distribution of electrolytes between intracellular fluid and plasma. The study of membrane proteins requires an initial dissociation of components of the membrane, preserving the enzymic, antigenic, or other activity of components. Generally, the dissociation step is carried out using a non-ionic detergent. The most commonly used are the neutral non-denaturing aryl polyoxyethylene ethers; Triton X-100 is a popular choice. These, however, absorb at 280 nm and are not effective in breaking protein–protein interactions. They form large micelles and have a low critical micelle concentration (as do the alkyl polyoxyethylene ethers such as Brij) and so their removal by dialysis is difficult. A new class of non-ionic detergents, suitable for membrane biochemistry, has recently been described.[241] These consist of *N*-D-gluco-*N*-methylalkanamide compounds and can be produced in high yield at very low cost. These detergents compare favourably with other commonly used non-ionic detergents; they have a high solubilizing power, are non-denaturing, and can be readily removed by dialysis. Another non-ionic dissociating agent that has recently been described is chloral hydrate (2,2,2-trichloroethane-1,1-diol), used to dissociate bovine-heart cytochrome C oxidase.[242] As chloral hydrate does not interfere with subsequent ion-exchange chromatography of the protein, the compound may find widespread use in the ion-exchange chromatographic study of membrane proteins.

Rivas *et al.*[240] during studies on the desalting of protein solutions have developed a method that is useful for the determination of the degree of detergent binding to membrane proteins. Although working with dodecyl dimethylamine *N*-oxide, the authors claim the procedure is suitable for any non-ionic detergent. The method consists of a chromatographic step, combining gel filtration on Sephadex G-25 with ion-exchange chromatography.

Although the study of a small number of microbial and plant membrane proteins has been reported, the major source of membrane protein continues to be animal tissues. A large number of the membrane proteins isolated during 1982 are from human sources, underlining the increasing clinical importance of the study of membrane proteins.

Table 5 lists the investigations of membrane proteins reported in the major journals during 1982.

Plasma Proteins.—A wide variety of proteins have been purified from plasma during 1982, and these are listed in Table 6. Most of these proteins are isolated by conventional ion-exchange chromatography and gel filtration. Increasingly affinity chromatography is being used as one of the steps in the purification procedure. This section on plasma proteins is broadly divided into four parts.

[241] J. E. K. Hildreth, *Biochem. J.*, 1982, **207**, 363.
[242] D. C. Griffin and M. Landon, *Biochem. J.*, 1982, **201**, 227.

Table 5 *Purification of membrane proteins**

Protein	*Source*	*Solubilizing conditions*	*Comments on purification, etc.*	*Ref.*
RNA polymerase	Cowpea mosaic virus	Magnesium depletion	DEAE-Sepharose (potassium acetate elution). Poly(U) Sepharose (potassium acetate–TGED elution). Glycerol gradient centrifugation	243
Glucosyl-transferase	Potato tuber	Raising ionic strength (0.1 M Tris/HCl, pH 7.4, stand at 0 °C for 72 h)	DEAE-cellulose (KCl elution)	244
NADH dehydrogenase	Ox heart	No details given	$(NH_4)_2SO_4$ fractionation. Sucrose gradient centrifugation	245
Xanthine oxidase	Bovine-milk/ mammary-epithelial cells	1% Triton X-100 in 1.5 M KCl	Electrophoretic studies made	246
Plasma-membrane polypeptides	*Trypanosoma brucei*	Membrane fractions were isolated by a variety of sonication and centrifugation steps. Fractions were then analysed by electrophoretic procedures and by chromatography on Con-A Sepharose		247
β-Adrenergic receptors	Turkey, pigeon, and frog erythrocytes	Each source material referenced to specific procedure	Each source has been compared by electrophoretic study	248
Dipeptidyl-aminopeptidase	Monkey brain	Triton X-100 in Tris/HCl, pH 7.7	DE-52 cellulose. AH-Sepharose	249
Ca^{2+} ATPase	Rat liver	Triton X-100	Sephadex G-200	250
5'-Nucleotidase	Bovine rods	Tris/HCl, pH 8.0, EGTA	5'-AMP Sepharose. Blue Sepharose. Sephacryl S-200	251
Adenosine deaminase-binding protein	Human placenta	DCHO/Triton X-100	Affinity chromatography on an adenosine deaminase affinity resin	252
Deoxyribonuclease	*Saccharomyces cerevisiae*	Triton X-100	Selective extraction	253
5'-Nucleotidase	Rat liver	Sulphobetaine 14	DEAE-cellulose. Ultrogel AcA-34. Immunoaffinity purification on monoclonal antibody–diazocellulose	254

α_1-Adrenergic receptor	Rat liver	Digitonin	Affinity chromatography on CP 57609-agarose (CP 57609 is an analogue of prazosin)	255
Mucin-like glycoprotein	Human-milk fat globule membranes	$MgCl_2$/guanidine hydrochloride	Sepharose CL-4B	256
Neutral proteinase	Rat muscle	Na-CHO	Characterization studies on Sepharose CL-6B	257
Oestrogen-dependent peroxidase	Rat uterus	CTAB	Sephacryl S-200. CM-cellulose	258

* Abbreviations used in Table 5: AcA acrylamide agarose, ADP adenosine diphosphate, AH aminohexyl, AMP adenosine monophosphate, ATP adenosine triphosphate, CH carboxyhexyl, CHAPS {3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate}, CM carboxymethyl, CoA coenzyme A, CP 57609 an analogue compound of prazosin, CTAB cetyltrimethylammonium bromide, DCCD dicyclohexylcarbodi-imide-reactive, DCHO sodium deoxycholate, DEAE, DE diethylaminoethyl, DTE dithioerythritol, EDTA ethylenediaminetetra-acetic acid, EGTA ethyleneglycol-bis-(β-aminoethylether)-*NNN'N'*-tetra-acetic acid, IgG immunoglobulin G, Na-CHO sodium cholate, p.a.g.e. polyacrylamide gel electrophoresis, PEG polyethylene glycol, QAE quaternary aminoethyl, SDS sodium dodecylsulphate, TGED 0.05 M Tris/acetic acid, pH 8.2, 25% (v/v) glycerol, 1 mM EDTA, 10 mM DTE.

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Table 5 (*cont.*)

Protein	*Source*	*Solubilizing conditions*	*Comments on purification*, etc.	*Ref.*
Glycophorin	Bovine	Lithium di-iodosalicylate, phenol extraction	Biogel A-1.5 M	259
Cyclic AMP-binding protein	Human erythrocytes	Triton X-100	DEAE-cellulose. Cyclic AMP Sepharose	260
Glucagon receptor	Rat liver	Lubrol-PX	Ultrogel AcA 22	261
Glycoprotein (various)	Porcine epidermis	DCHO	Concanavalin A Sepharose. Lentil lectin Sepharose	262
Vitelline membrane proteins	Hens' eggs	SDS	Gel filtration on Sephadex and electrophoretic studies	263
Glycoprotein (various)	Rat thymocytes and brain	DCHO	Affinity chromatography with MRC OX 2 antibody and gel filtration on Sephacryl S-300	264
Lactogen receptors	Rat liver	Triton X-100	Concanavalin A Sepharose. Sepharose 6B	265
β-Glucan oxidase	Ryegrass	Digitonin or octyl glucoside	DEAE-Sepharose	266
Cholesterol oxidase	*Nocardia rhodochrous*	Triton X-100	Gel filtration on Sephadex G-200	267
Cortical-membrane proteins (various)	*Gregarina blaberae*	Various	Isolation by discontinuous sucrose-gradient density centrifugation and electrophoretic study	268
Fibronectin-binding glycoprotein	Human platelets	Tris buffer + KBr	Fibronectin Sepharose	269
NADP adrenoxin reductase	Bovine adrenocortex	Na-CHO	$(NH_4)_2SO_4$ fractionation. DEAE-Sepharose. 2′,5′-ADP Sepharose	270
Pore protein	Rat liver	Triton X-100	Sucrose-gradient centrifugation. Differential centrifugation in presence of Triton. DEAE-Sepharose. CM-Sepharose	271
Ca^{2+}–Mg^{2+} ATPase	Rat brain	Triton X-100	Calmodulin Sepharose	272
D-Alanyl-D-alanine transpeptidase	*Streptomyces* strain K15	CTAB	Sephadex G-100. Ampicillin CH-Sepharose	273
Glycoprotein	Human-milk fat globule membrane	Selective extraction	Lectin affinity chromatography. Sephadex G-200	274

Ca^{2+}-transport ATPase	Human erythrocytes	DCHO	Calmodulin Sepharose	275
Plasma membrane proteins (various)	Human lymphocytes	DCHO	Phenylboronic acid agarose. Lentil lectin Sepharose	276
Chlorophyll–protein complexes	*Chlamydomonas reinhardii*	Triton or digitonin	Electrophoretic studies using lithium dodecyl sulphate	277
Glomerula basement membrane antigen	Human kidney	Collagenase digestion	Affinity chromatography using human anti-glomerular basement membrane autoantibodies	278
Calcium-binding protein IMCal	Rat intestine	Butan-1-ol or DCHO	Sephadex G-150. Hydroxyapatite	279
Glycoproteins (various)	Porcine lymph	DCHO	Ultrogel AcA 34. Lentil lectin Sepharose	280

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Table 5 *(cont.)*

Protein	*Source*	*Solubilizing conditions*	*Comments on purification, etc.*	*Ref.*
Cytochrome P-448 component of benzo(a)pyrene hydroxylase	*Saccharomyces cerevisiae*	DCHO plus Triton X-100	$(NH_4)_2SO_4$ fractionation. Amino-octyl-Sepharose. Hydroxyapatite. CM-Sephadex C-50. DEAE Sephacel	153
Human growth-hormone-binding sites	Rabbit kidney	Triton X-100	Gel chromatography on Sepharose CL-6B as part of characterization study	281
IgG receptor	Human granulocytes	Triton X-100	Preliminary electrophoretic study	282
Casein kinase	Bovine mammary glands	Triton X-100	Sepharose CL-4B	283
Acyl-CoA cholesterol acyltransferase	Rat liver	Triton X-100	Fractionation with PEG 6000	284
DCCD-reactive proteolipid	*Bacillus subtilis*	Extraction with $CHCl_3$: MeOH	Sephadex G-50. DEAE-cellulose	285
Cytochrome b_5	*Tetrahymena pyriformis*	Triton X-100 plus Na-CHO	DEAE-cellulose. $(NH_4)_2SO_4$ fractionation. Sephadex G-100. DEAE-cellulose. Sephadex G-100	286
33 kDa protein	Spinach chloroplast	NaP buffer extraction of thylakoid membranes	DEAE-Sephadex CL-6B. Hydroxyapatite	287
Glycophorin	Rabbit erythrocytes	Lithium di-iodosalicylate	DEAE-cellulose. Biogel A-1.5 m. DEAE-cellulose	288
Xanthine oxidase	Bovine milk	Extraction with butan-1-ol	$(NH_4)_2SO_4$ fractionation. Preparative electrofocusing. Concanavalin A agarose	289
Ca^{2+}–Mg^{2+} ATPase modulator protein	Human erythrocytes	EGTA	Sephacryl S-300	290
Glycoprotein IIb–IIIa	Human platelets	EGTA	Sepharose 4B. *Tab*-Protein A Sepharose	291
3-Hydroxy-3-methyl glutaryl-Co A reductase	Human liver	Buffer extraction with glycerol	$(NH_4)_2SO_4$ fractionation, agarose hexane-hydroxymethylglutaryl CoA affinity chromatography	292
Opiate-binding site	Mammalian brain	Glyco-DCHO, digitonin	Wheatgerm agglutinin agarose	293

Polynoidin	Scale worms	Triton X-100	Biogel A-5 m. Sephadex G-25. DEAE-cellulose	294
β-Endorphin-binding protein	Rat brain	CHAPS	Sepharose 6B. Differential centrifugation	295
Cytochrome oxidase	*Pseudomonas aeruginosa*	Triton X-100	DEAE-cellulose (repeated)	296
Phospholipid acyl-hydrolase	*Saccharomyces cerevisiae*	Na-CHO	Acetone precipitation. $(NH_4)_2SO_4$ fractionation. Sephadex G-200	297
Fodrin kinase	Rat brain	Triton X-100	DE-52 cellulose. Sephacryl S-300	298
Penicillin-binding protein 3	*Streptococcus pneumoniae*	Triton X-100	Phosphocellulose. DEAE-Sephacel. Chromatofocusing. Blue Sepharose. DEAE-Sephacel	299

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Table 5 *(cont.)*

Protein	*Source*	*Solubilizing conditions*	*Comments on purification, etc.*	*Ref.*
Steroid-binding protein	*Pseudomonas testosteroni*	Formation of spheroplasts, followed by selective buffer washes	DEAE-cellulose. Sephadex G-100. CM-cellulose. Hydroxyapatite. Phosphocellulose	300
Cylic AMP-binding protein	Human erythrocytes	Triton X-100	DEAE-cellulose. Cyclic AMP affinity column	301
Yolk-sac membrane Fc receptor	Rabbit foetus	Buffer solubilized by the use of Nonidet P-40	Biogel A-1.5 m. Concanavalin A Sepharose	302
ATPase	*Zea mays*	DCHO	Preliminary studies carried out with SDS/p.a.g.e.	303
Lens fibre proteins	Bovine-eye lens	Urea and EDTA extractions	Examination using electrophoresis	304
Phosphatidyl-serine synthetase	*Clostridium perfringens*	Triton X-100	Affinity chromatography on cytidine 5'-diphosphate diacylglycerol Sepharose	305
Complement Clq inhibitor	Human lymphocytes	Triton X-100	Clq-Sepharose. QAE-Sephadex	306
Glycoprotein	Human erythrocyte	Butanol extraction	Butanol extraction. DEAE-Sephacel. Hydroxy apatite. Phenyl-Sepharose. Trypan Blue Sepharose	174
Phosphotyrosyl-protein phosphatase	TCRC-2 cell line	Nonidet P-40	Wheatgerm lectin Sepharose. Histone Sepharose	307
Collagen α1(I) receptor	Human platelets	Triton X-100 or DCHO	A-5 m agarose. α1-Sepharose. Collagen fibril Sepharose	308
Neural-cell adhesion protein	Chick-embryo brain	Nonidet P-40	DEAE-cellulose. Octylagarose	175

Proteases IV and V	*Escherichia coli*	Emulphogen	$(NH_4)_2SO_4$ fractionation. DEAE-cellulose. Ultrogel AcA 34. Hydroxyapatite	309
Phospholamban	Bovine heart	DCHO	Sephadex G-75	310
Lipoprotein-receptor glycoprotein	Bovine adrenal	Triton X-100	DEAE-cellulose. Low-density lipoprotein Sepharose	311
Cofactor for thrombin activation of protein C	Rabbit lung	Triton X-100	Di-isopropylphosphorothrombin agarose	312
NADH dehydrogenase	*Bacillus subtilis*	Triton X-100	Octyl-Sepharose	176
Phosphatidyl-inisotol synthase	Soybeans	Brij W-1	Glycerol gradient centrifugation	313

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Table 6 *Purification of plasma proteins**

Protein	*Source*	*Major procedures*				*Ref.*
		Affinity chromatography	*Ion exchange*	*Gel filtration*	*Other*	
Proteins involved with coagulation and fibrinolysis						
Factor VIII	Human	Immunoaffinity chromatography				180
Urokinase	Human	Immunoaffinity chromatography				200
Fibrinogen	Rat		CM-cellulose DEAE-cellulose			326
Antithrombin III	Human				Preparative i.e.f.	323
Heparin Cofactor II	Human	Heparin Sepharose	DEAE-Sepharose	Sephadex G-150		327
Fibronectin						
Fibronectin	Human	Gelatin Sepharose				314
Fibronectin-like protein	Human	Gelatin Sepharose				315
Adhesive protein	Human	Protein A Sepharose Affigel Blue			SDS-p.a.g.e.	316
Complement and associated proteins						
C2	Guinea pig	'Aged' CNBr-activated Sepharose 4B	CM-Sephadex C-50 DEAE-Sepharose		$CaCl_2$ ppt. $(NH_4)_2SO_4$ ppt.	317
C2	Human	C4b-Sepharose	CM-52 cellulose		$(NH_4)_2SO_4$ ppt.	318
C3b	Human, rabbit, bovine	Factor H Sepharose	DEAE-Sepharose		PEG ppt.	328
C3b inactivator	Human	Immunoaffinity chromatography			H.p.l.c. on TSK 3000 SW	321

C3 and C5	Human	Phenyl-Sepharose	DEAE-Sephacel	Sephacryl S-300		319
C6	Human	Immunoaffinity chromatography				320
C1 esterase inhibitor	Human	Hexyl-Sepharose	DEAE-cellulose		PEG ppt.	329
Complement control protein (Factor H)	Human	Lysine Sepharose	DEAE-Sephadex A50 DEAE-Sepharose CL-6B	Sepharose 6B HA-Ultrogel	PEG ppt.	330
Other plasma proteins						
Apoliprotein F	Human	Anti ApoF-Sepharose Anti ApoA-I-Sepharose	CM-cellulose			331
Apolipoprotein AI	Human				Preparative i.e.f.	324
Apoliprotein B	Human			Biogel A5 m	Ultracentrifugation	332
Apolipoproteins	Rat				SDS-glycerol-p.a.g.e.	333
C-Apolipoproteins	Human				H.p.l.c.	332

* Abbreviations used in Table 6: h.p.l.c. high-pressure liquid chromatography, i.e.f. isoelectric focusing. PEG polyethylene glycol, SDS-p.a.g.e. sodium dodecyl sulphase–polyacrylamide gel electrophoresis.

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Table 6 *(cont.)*

Protein	*Source*	*Major procedures*				*Ref.*
		Affinity chromatography	*Ion exchange*	*Gel filtration*	*Other*	
HDL	Human			Sephacryl-200		334
HDL fractions	Human				Ultracentrifugation h.p.l.c. on TSKG 3000 SW	335
LDL peptides	Human			Sephadex G-75	Ultracentrifugation	336
Haptoglobin	Rat		DEAE-Sephadex A-50	Sephadex G-150		337
Hyaluronidase	Human				$(NH_4)_2SO_4$ ppt., chromatofocusing	338
Iron-binding fragments of transferrin	Rabbit	Blue Sepharose	DEAE-Sephadex A-50			339
α_2-Macroglobulin	Human		DEAE-cellulose DE-52		Dextran sulphate and $CaCl_2$ ppt.	340
α_2-Macroglobulin	Plaice			Ultrogel AcA 34		341
α_2-HS glycoprotein	Human	Blue Sepharose	DEAE-Sephadex, DEAE-cellulose	Sephadex G-200	$(NH_4)_2SO_4$ ppt., hydroxyapatite	342
α_1-Antitrypsin	Horse	Cibacron Blue agarose	DEAE-cellulose	Sephadex G-200		343
Thiol protease inhibitors	Rat		DEAE-Sephacel	Sephadex G-75, Sephadex G-150	$(NH_4)_2SO_4$ ppt.	344
Immunoglobulin A	Horse	Immunoaffinity chromatography				195
Sugar peptide compounds	Bovine				Dialysis, electrophoresis	345
Hepatic lipase	Dog	Heparin Sepharose			$(NH_4)_2SO_4$ ppt.	346
γ-Glutamyl transferase	Human	Immunoaffinity chromatography				347

Plasma proteins	Human	Cibacron Blue F-3GA				348
Spermidine oxidase	Human	Cadaverine Sepharose		Biogel A15	Hydroxyapatite	349
Riboflavin carrier protein	Human	Riboflavin AH-Sepharose			Preparative gel electrophoresis	325
HMW kininogen	Bovine		DEAE-Sephadex A-50 CM-Sephadex G-50	Sephadex G-150		350
Thymidine triphosphate nucleotidohydrolase	Human	DEAE-Affigel Blue	DEAE-cellulose DE-52, phosphocellulose		$(NH_4)_2SO_4$ ppt., hydroxyapatite, electrofocusing	351
Haemopexin	Pig	Haemin AH-Sepharose	DEAE-Sepharose		Rivanol ppt.	352

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[347] Z. O. Echetebu and D. W. Moss, *Enzyme*, 1982, **27,** 9.
[348] E. Gianazza and P. Arnaud, *Biochem. J.*, 1982, **203,** 637.
[349] W. A. Gahl, A. M. Vale, and H. C. Pitot, *Biochem. J.*, 1982, **201,** 161.
[350] T. Shimada, T. Sugo, H. Kato, and S. Iwanaga, *J. Biochem. (Tokyo)*, 1982, **92,** 679.
[351] N. Dahlmann, *Biochemistry*, 1982, **21,** 6634.
[352] R. Majuri, *Biochim. Biophys. Acta*, 1982, **719,** 53.

Proteins Involved with Coagulation and Fibrinolysis. This part deals with several proteins and enzymes involved with the formation and dissolution of blood clots. Although most of the factors involved in the coagulation cascade are now recognized, active research centres around the purification and mechanism of action of some of the labile factors. For example, Factor VIII was purified 9000-fold by gel filtration and immunoaffinity chromatography using immunoglobulins isolated from a rabbit immunized with the plasma of a patient devoid of Factor VIII as a ligand.[180]

The search for a suitably potent plasminogen activator continues, and recently the isolation and characterization of an activator, with urokinase-like activity, from human plasma have been described.[200]

Fibronectin. A novel procedure has been described for the isolation of plasma fibronectin.[314] It is claimed that particulate gelatin coupled to Sepharose 4B is a superior affinity matrix over melted gelatin Sepharose and is able to purify fibronectin in high yield using glucose as an eluant. Apart from fibronectin, other proteins have been isolated from serum with cell attachment and functional characteristic similar to those of fibronectin.[315,316]

Complement and Associated Proteins. Several complement proteins and their subfractions from different animal sources have been purified and studied in detail.

Complement component C2 from guinea-pig serum was purified to homogeneity on 'aged' CNBr-Sepharose.[317] The purified protein was a single polypeptide chain with an apparent molecular weight of 102 000. This result was similar to that from human C2,[318] showing a high degree of homology among these proteins. Salihi *et al.*[319] isolated C3 and C5 with 41% and 20% yield, respectively, from human serum by hydrophobic chromatography on phenyl-Sepharose. C3 was loosely bound to the resin whereas C5 bound firmly and was eluted with 50% glycerol. The contaminant proteins C4bp and Factor H in C5 were separated by gel filtration on Sephacryl S-300.[319] Immunoaffinity chromatography has been used for the purification of C6[320] and C3b inactivator[321] proteins, and their biochemical properties have been described.

Other Plasma Proteins. This section deals with various proteins, enzymes, inhibitors, lipoproteins, *etc.* that have been isolated from plasma but not categorized in any of the above three sections. This list continues to grow as some of the minor protein components previously difficult to separate by column chromatography have now been resolved by the use of more sensitive

[314] P. P. Agin and T. K. Gartner, *Biochim. Biophys. Acta*, 1982, **716,** 443.
[315] J. K. Czop, J. L. Kadish, and K. F. Austen, *J. Immunol.*, 1982, **129,** 163.
[316] E. G. Hayman, E. Engvall, E. A. Hearn, D. Barnes, M. Pierschbacher, and E. Ruoslahti, *J. Biol. Chem.*, 1982, **95,** 20.
[317] M. A. Kerr and J. Gagnon, *Biochem. J.*, 1982, **205,** 59.
[318] N. M. Thielens, M. B. Villiers, A. Reboul, C. L. Villiers, and M. G. Coulomb, *FEBS Lett.*, 1982, **141,** 19.
[319] A. A. Salihi, J. Ripoche, L. Pruvost, and M. Fontaine, *FEBS Lett.*, 1982, **150,** 238.
[320] W. P. Kolb, L. M. Kolb, and J. R. Savary, *Biochemistry*, 1982, **21,** 294.
[321] L. M. Hsiung, A. N. Barclay, M. R. Brandon, E. Sim, and R. R. Porter, *Biochem. J.*, 1982, **203,** 293.

techniques like high-pressure liquid chromatography (h.p.l.c.), isoelectric focusing (i.e.f.), and gel electrophoresis. For example, lipoprotein C has been separated by column chromatography into three fractions, CI, CII, and CIII. Each of these fractions has now been further resolved by the use of h.p.l.c.[322] Similarly affinity chromatography is widely used for plasmid protein purification owing to its high resolving power.

Some of these methods have been used on a preparative scale for the purification of milligram amounts of protein.[323—325] For example, Ap-I has been separated from serum in a single preparation i.e.f. step with 75% yield.[324]

4 Electrophoretic Techniques

The extensive and varied use of electrophoretic techniques for protein separation and characterization can be appreciated by reference to the conference proceedings of 'Electrophoresis '81'.[353] This publication is not only a comprehensive guide to the most recent theories and techniques of electrophoresis but also illustrates the wide-ranging biochemical and clinical applications. Perhaps the most significant advances have been in the improvement in one- and two-dimensional techniques to allow complex protein mixtures, particularly from body tissues and fluids, to be analysed in more detail and, following on from that, the amino-acid analysis of proteins directly isolated from gels following electrophoretic separation. The latter technique has become possible as the amounts of protein required for amino-acid analysis and sequencing become less, and it is recognized in a series of papers related to the elucidation of enzyme structure.[354]

Advances in Electrophoretic Techniques.—The following subsections will describe advances in the techniques of electrophoresis as applied to the preparation and characterization of proteins.

One-dimensional Electrophoresis. As part of a continuing reappraisal of the nature of electrophoretic separation, a mathematical model has been adapted for computer simulations and used to predict the characteristic behaviour of a variety of electrophoretic techniques using a knowledge of chemical equilibria and physical transport phenomena.[355] The model seeks to unify the recognized forms of electrophoretic process, which include zone and moving-boundary electrophoresis, isotachophoresis, isoelectric focusing, and electrodialysis. For those less inclined to address the theoretical aspects, Chrambach and Jovin[356]

[322] P. Schwandt, W. O. Richter, and P. Weisweiler, *J. Chromatogr.*, 1982, **225,** 185.
[323] G. Murano, M. Miller-Anderson, and L. Williams, *Thromb. Res.*, 1982, **24,** 489.
[324] P. Forgez and M. J. Chapman, *J. Biochem. Biophys. Methods*, 1982, **6,** 283.
[325] C. V. R. Murthy and P. R. Adiga, *Biochem. Int.*, 1982, **5,** 289.
[353] 'Electrophoresis '81, Advanced Methods – Biochemical and Clinical Applications', ed. R. C. Allen and P. Arnaud, Walter de Gruyter, 1981.
[354] 'Methods in Enzymology, 91, Enzyme Structure, Part I', ed. C. H. W. Hirs and S. N. Timasheff, Academic Press, 1983.
[355] M. Bier, O. A. Paulsinski, R. A. Mosher, and D. A. Saville, *Science*, 1983, ***219,*** *1281.*
[356] A. Chrambach and T. M. Jovin, *Electrophoresis*, 1983, **4,** 190.

have presented a simplified explanation of moving-boundary electrophoresis and described in detail nineteen working buffer systems for electrophoresis in acid through to alkaline pH conditions. In a similar, practical approach ten continuous electrophoresis buffers, covering the pH range 3.8—10.2, have been described for the separation of proteins,[357] and the same author has used acidic electrophoretic conditions to separate two previously undetected variants of glutamic-pyruvic transaminase.[358]

An agarose–polyacrylamide gradient gel has been used for studying chemically crosslinked proteins.[359] The high resolving power in the molecular-weight range 10 000–600 000 daltons claimed for this gel enables analysis of a wide size range of component proteins when the crosslinked complexes are cleaved. A 6 M urea–polyacrylamide gel run under acidic conditions (pH 3.6) has been used as a highly dissociating medium for the separation of prolamins.[360] These very insoluble cereal storage proteins have previously only been separated effectively using urea–starch gels; the acrylamide-based gel has the benefit of greater reproducibility and enables all the alcohol-soluble proteins of cereals to be analysed on the same gel. A new gel electrophoretic system for the separation of small peptides and proteins (200—100 000 daltons) utilizes a volatile buffer, triethylamine/formic acid, pH 11.7, and protein reaction with a covalently binding, fluorescent NH_2 reagent, 1,3,6-trisulphonylpyrene 8-isothiocyanate.[361] Under such conditions the strongly negatively charged proteins migrate according to molecular weight and, being fluorescent, can be detected without fixing or staining. Extracted proteins are easily freed from salts by evaporation and are readily available for amino-acid analysis or sequencing.

Two-dimensional Electrophoresis. There has been a continuing trend towards the use of high-resolution, ultra-thin-layer, two-dimensional gels, particularly for the analysis of genetic-disease markers. A comprehensive review of the latest two-dimensional methodological procedures[362] is complemented by some general criteria laid down for its application to the development of clinical laboratory tests.[363] To take full advantage of the advances in electrophoretic resolution, several computer-based data-analysis systems have been described.[364—369] The application of two-dimensional electrophoresis to

[357] T. McLellan, *Anal. Biochem.*, 1982, **126**, 94.
[358] T. McLellan, *Am. J. Hum. Genet.*, 1982, **34**, 623.
[359] D. F. Warren, M. A. Naughton, and L. M. Fink, *Anal. Biochem.*, 1982, **121**, 331.
[360] M. Lauriere and J. Mosse, *Anal. Biochem.*, 1982, **122**, 20.
[361] A. Tsugita, S. Sasada, R. van den Broek, and J. J. Scheffler, *Eur. J. Biochem.*, 1982, **124**, 171.
[362] M. J. Dunn and A. H. M. Burghes, *Electrophoresis*, 1983, **4**, 97.
[363] D. S. Young and R. P. Tracy, *Electrophoresis*, 1983, **4**, 117.
[364] P. Tarroux, *Electrophoresis*, 1983, **4**, 63.
[365] P. F. Lemkin, *Electrophoresis*, 1983, **4**, 71.
[366] P. F. Lemkin and L. E. Lipkin in 'Electrophoresis '81', ed. R. C. Allen and P. Arnaud, Walter de Gruyter (Hawthorne), 1981, p. 401.
[367] P. A. Jansson, L. B. Grim, J. G. Elias, E. A. Bagley, and K. K. Lonberg-Holm, *Electrophoresis*, 1983, **4**, 82.
[368] C. N. Mariash, S. Seelig, and J. H. Oppenheimer, *Anal. Biochem.*, 1982, **121**, 388.
[369] J. Taylor, N. L. Anderson, and N. G. Anderson in 'Electrophoresis '81', ed. R. C. Allen and P. Arnaud, Walter de Gruyter (Hawthorne), 1981, p. 383.

the study of specific human diseases can be illustrated by reference to Duchenne muscular dystrophy,[370,371] abnormal fibrinogens,[372] cell-surface membrane proteins from leukaemic cells,[373] and abnormal immunoglobulins.[374]

The other recent and major application of two-dimensional electrophoresis has been as a micropreparative step prior to the peptide mapping, amino-acid analysis, and sequencing of proteins. Proteins separated by SDS-p.a.g.e. have been chemically[375] and enzymatically[376] cleaved whilst retained within a gel piece, and the products of cleavage were subsequently separated by a second electrophoretic step. Amino-acid analysis of proteins excised from two-dimensional gels has become possible with the ever improving sensitivity of modern analysers. Polypeptide subunits resolved on gels may be fixed, stained, and then extracted and hydrolysed for amino-acid analysis.[377,378] A method has also been described that attempts to measure the amino-acid composition of ^{14}C- and ^{35}S-labelled proteins by computerized microdensitometry of two-dimensional gels.[379] In the second part of their review on two-dimensional electrophoresis, on analysis and application, Dunn and Burghes[380] point out that sequencing of proteins excised from gels may not be far away, with the latest gas-phase sequencers capable of working with as little as 5 picomoles of protein.

With over one thousand references in 1982 in which two-dimensional electrophoresis was employed the technique seems certain to continue as an important aid for protein characterization.

Isoelectric Focusing. The theory, methodology, and applications of isoelectric focusing (i.e.f.) are described in a new book in the series on laboratory techniques in biochemistry and molecular biology.[381] Two trends in the development of i.e.f. can be identified as further attempts to move away from the use of ampholytes to form pH gradients by using poly buffer systems[382—384] and from the use of immobilized buffering groups to generate the pH gradient.[385,386] The latter technique uses buffering groups covalently linked to a

[370] A. H. M. Burghes, M. J. Dunn, H. E. Statham, and V. Dubowitz, *Electrophoresis*, 1982, **3**, 177.
[371] A. H. M. Burghes, M. J. Dunn, H. E. Statham, and V. Dubowitz, *Electrophoresis*, 1982, **3**, 185.
[372] F. Brosstad, B. Tiege, B. Olaisen, K. Gravem, H. C. Godal, and H. Stormorken in 'Fibrinogen', ed. F. Haverkate, A. Henschen, W. Nieuwenhuizen, and P. W. Straub, Walter de Gruyter (Hawthorne), 1983, p. 145.
[373] R. L. Felsted and S. K. Gupta, *J. Biol. Chem.*, 1982, **257**, 13 211.
[374] 'Physiology of Immunoglobulins', ed. S. E. Ritzmann, Alan R. Liss Inc., New York, 1982.
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[376] P. Tijssen and E. Kurstak, *Anal. Biochem.*, 1983, **128**, 26.
[377] C. de Jong, G. J. Hughes, E. van Wieringen, and K. J. Wilson, *J. Chromatogr.*, 1982, **241**, 361.
[378] W. E. Brown and G. C. Howard, *Methods Enzymol.*, 1983, **91**, 36.
[379] G. I. Latter, E. Metz, S. Burbeck, and J. Leavitt, *Electrophoresis*, 1983, **4**, 122.
[380] M. J. Dunn and A. H. M. Burghess, *Electrophoresis*, 1983, **4**, 173.
[381] P. G. Righetti, 'Isoelectric focusing', Elsevier Biomedical Press, Amsterdam, 1983.
[382] L. M. Hjelmeland and A. Chrambach, *Electrophoresis*, 1983, **4**, 20.
[383] Z. Bukas, l. M. Hjelmland, and A. Chrambach, *Electrophoresis*, 1983, **4**, 27.
[384] A. Pekkala-Flagan and D. E. Comings, *Anal. Biochem.*, 1982, **122**, 295.
[385] B. Bjellquist, K. Ek, P. G. Righetti, E. Gianazza, A. Gorg, R. Westermeier, and W. Postel. *J. Biochem. Biophys. Methods*, 1982, **6**, 317.
[386] G. Dossi, F. Celentano, E. Gianazza, and P. G. Righetti, *J. Biochem. Biophys. Methods*, 1983, **7**, 123.

matrix that is effectively used as an anti-connective medium. The advantages claimed over carrier ampholytes are greater gradient stability with time, narrow pH gradients can be formed, higher loading capacity and resolution, uniform conductivity, and buffering capacity and abolition of cathodic drift. The method has been used to analyse genetic variations within the human Gc system, related to the vitamin D-binding protein of human plasma[387] and genetic variants of the human protease inhibitor α_1-antitrypsin.[388] Classification of genetic variants of α_1-antitrypsin by more traditional i.e.f. in thin layers of agarose has also been described.[389,390]

Preparative i.e.f. in layers of granulated gels is attractive given the high resolution attainable, but it appears limited at present to laboratory-scale quantities (up to 1 gram protein) because of the heat generated when using thicker gels to accommodate increased protein loading.[391] Several proteins have been reported as purified by preparative i.e.f., for example apolipoprotein A1,[392] brain-fibroblast growth factor,[393] and epidermal growth factor.[394]

Affinity and Immunoelectrophoresis. The preparation of affinity gels for affinity electrophoresis of lectins (immobilized *p*-aminophenyl glycosides) and ribonuclease (immobilized uridine 3',5'-diphosphate 5'-*p*-aminobenzamidine) has been described,[395] and the kinetics of protein:ligand complex formation and dissociation reactions have been studied.[396] Concanavalin A and other lectins have been used in electrophoretic systems, including two-dimensional electrophoresis, to characterize a number of glycoproteins by virtue of their affinity for the carbohydrate chain.[397,398,399]

Crossed immunoelectrophoresis has remained a method of choice for studying protein interactions, notably of blood proteins such as Factor VIII-related antigen[400] and Factor IX.[401] It has also been used, along with other immunoelectrochemical techniques, for the analysis of membrane proteins, particularly envelope proteins of viruses[402] and mycoplasmas.[403]

Isotachophoresis.—Preparative flat-bed isotachophoresis in granulated gels has been used to isolate cat-liver cytosolic glutathione S transferase in 100 mg

[387] H. Cleve, W. Patutschnick, W. Postel, J. Wesser, and A. Gorg, *Electrophoresis*, 1982, **3**, 342.
[388] A. Gorg, W. Postel, J. Weser, S. Weidinger, W. Patutschnick, and H. Cleve, *Electrophoresis*, 1983, **4**, 153.
[389] G. J. Buffone, B. J. Stennis, and C. M. Schmibor, *Clin. Chem.*, 1983, **29**, 328.
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[392] P. Forgez and M. J. Chapman, *J. Biochem. Biophys. Methods*, 1982, **6**, 283.
[393] D. Gospodarnadez, Ge-Mung Lut, and J. Cheng, *J. Biol. Chem.*, 1982, **257**, 12 266.
[394] B. E. Magun, S. R. Planck, L. M. Matrisian, and J. S. Finch, *Biochem. Biophys. Res. Commun.*, 1982, **108**, 299.
[395] V. Horejsi, M. Ticha, P. Tichy, and A. Moly, *Anal. Biochem.*, 1982, **125**, 358.
[396] V. Matousek and V. Horejsi, *J. Chromatogr.*, 1982, **245**, 271.
[397] T. C. Boghansen and J. Hau, *Acta Histochem.*, 1982, **71**, 47.
[398] G. L. E. Koch and M. J. Smith, *Eur. J. Biochem.*, 1982, **128**, 107.
[399] J. R. Pink, D. Hoessli, A. Tartakoff, and R. Hooghe, *Mol. Immunol.*, 1983, **20**, 491.
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[401] A. Itzuka and T. Nagao, *Br. J. Haematol.*, 1983, **53**, 687.
[402] 'Immunochemical Analysis of Membrane Proteins', ed. O. J. Bjerrum, Elsevier Biomedical Press, Amsterdam, 1983.
[403] 'Methods in Mycoplasmology 1', ed. S. Razin and J. G. Tully, Academic Press, New York, 1983.

Table 7 *Separation of proteins by chromatofocusing*

Protein	*Source*		*Ref.*
Apolipoproteins	*Human*	Blood	410, 411
Hyaluronidase		Blood	412
Haemoglobins		Blood	413
'Kashmir' albumin		Blood	414
Interleukin-2		Blood	415
γ-Crystallin		Eye lens	416
Sphinogmyelinase		Placenta	417
Enolases		Brain	418
Tonin (serine protease)	*Rat*	Submandibular gland	419
Fibrinogen D and E		Blood	420
Phosphatylinositol-phosphodiesterase		Liver and kidney	421
Trypsin inhibitors	*Swine*	Colostrum	422
β-Lactamases	*Escherichia coli*		423, 424
β-Lactamases	*Pseudomonas aeruginosa*		425
Cytochrome C_2	*Rhodopseudomonas viridis*		426
Glucan hydrolases	*Trichoderma viride*		427
Hexokinase	*Saccharomyces cerevisiae*		428

[410] P. Weisweiler and P. Schwandt, *Clin. Chim. Acta*, 1982, **124,** 45.
[411] M. S. Jauhiainen, M. V. L. Ilkka, I. M. Pentilla, and E. V. Puhakainen, *Clin. Chim. Acta*, 1982, **122,** 85.
[412] M. Fenger, *J. Chromatogr.*, 1982, **240,** 173.
[413] N. M. Alexander and W. E. Neeley, *J. Chromatogr.*, 1982, **230,** 137.
[414] C. R. Tillyer, B. Dowding, and A. L. Tarnoky, *IRCS Med. Sci.: Biochem.*, 1982, **10,** 636.
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[416] H. Bloemendal, G. Groenewoud, M. Versteeg, M. Kibbelaar, and G. Berbers, *Exp. Eye Res.*, 1983, **36,** 537.
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[418] A. Schmizu, F. Suzuki, and K. Kato, *Biochim. Biophys. Acta*, 1982, **717,** 348.
[419] E. S. P. Cheng and B. J. Morris, *Anal. Biochem.*, 1982, **126,** 295.
[420] I. Kalvaria, A. V. Corrigall, and R. E. Kirsch, *Thromb. Res.*, 1983, **29,** 459.
[421] K. Hirasawa, R. F. Irvine, and R. M. C. Dawson, *Biochem. Biophys. Res. Commun.*, 1982, **107,** 533.
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[424] R. G. Werner and A. M. Erne, *FEMS Microbiol. Lett.*, 1983, **17,** 211.
[425] B. L. Toth-Martinez, S. Gal, and L. Kiss, *J. Chromatogr.*, 1983, **262,** 379.
[426] W. Welte, H. Hudig, T. Wacker, and W. Kreutz, *J. Chromatogr.*, 1983, **259,** 341.
[427] D. A. Thomas, J. R. Stark, and G. H. Palmer, *Carbohydr. Res.*, 1983, **114,** 343.
[428] E. Kopetzki and K.-D. Entian, *Anal. Biochem.*, 1982, **121,** 181.

quantity[404] and Fc binding serum proteins.[405] It has also continued to find application for assessing the purity of reaction products during peptide synthesis, for example the synthesis of Ala-Glu and Tyr-Lys as soluble, stable replacements for Glu/Tyr deficiency in chronic uremia.[406]

Chromatofocusing.—The emergence of chromatofocusing can be guaged by the rapidly expanding list of proteins separated using this principle (Table 7). Chromatofocusing has been recently reviewed as a preparative procedure[407] and compared to i.e.f. for the recovery and analysis of proteins from complex biological fluids.[408] The technique has been adapted to h.p.l.c. to provide more rapid separation (30 min) and found capable of detecting heterogeneity in protein preparations, which was not demonstrated by reversed-phase or size-exclusion chromatography.[409]

Protein Determination in Electrophoretic Gels.—Proteins may be determined directly in gels, following electrophoresis, by general and specific staining methods or be removed from the gel for further analysis or preparative procedures.

A comparison of dyes available for staining protein bands in gels[429] still favours Coomassie Brilliant Blue R, although no stain is applicable for all conditions of p.a.g.e. and i.e.f. Crosslinking of basic proteins and small polypeptides to polyacrylamide gels using formaldehyde prior to staining can improve detection, and a combined staining technique based on Coomassie Blue and silver staining following crosslinking has been used for histone and ribosomal proteins.[430] Carrier ampholytes will react with stains and are also fixed by formaldehyde, which has limited the technique for i.e.f. gels, but ampholytes will remain soluble in 40% methanol and 4% formaldehyde containing Coomassie Blue, leaving only polypeptides to precipitate and stain.[431]

Numerous modifications of the silver-stain technique for proteins have been aimed at improving sensitivity and reducing background staining in polyacrylamide[432–437] and agarose[438] and on nitrocellulose paper.[439] The enhancement of silver staining following glutaraldehyde treatment of gels has been explained as being due to oxidation of the aldehyde groups by silver ions resulting in

[404] R. V. Battersby and C. J. Holloway, *Electrophoresis*, 1982, **3**, 275.
[405] E. M. Nicolalsen and C.-H. Brogren, *J. Immunol. Methods*, 1982, **54**, 165.
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[432] R. R. Burk, M. Eschenbruch, P. Leuthard, and G. Steck, *Methods Enzymol.*, 1983, **91**, 247.
[433] M. Eschenbruch and R. R. Burk, *Anal. Biochem.*, 1982, **125**, 96.
[434] M. Porro, S. Vim, G. Antoni, and M. Saletti, *Anal. Biochem.*, 1982, **127**, 316.
[435] G. G. Giulian, R. L. Moss, and M. Greaser, *Anal. Biochem.*, 1983, **129**, 277.
[436] J. Guevara, jun., D. A. Johnston, L. S. Ramagali, B. A. Martin, S. Capetillo, and L. V. Rodriguez, *Electrophoresis*, 1982, **3**, 197.
[437] B. A. Perret, R. Feliz, M. Furlan, and E. A. Beck, *Anal. Biochem.*, 1983, **131**, 46.
[438] E. M. Willoughby and A. Lambert, *Anal. Biochem.*, 1983, **130**, 353.
[439] K. C. L. Yuen, T. K. Johnson, R. E. Denell, and R. A. Consigli, *Anal. Biochem.*, 1982, **126**, 398.

metallic silver depositions within the gel that act as nucleation sites for additional silver localization in the protein bands.[440] This is consistent with the reaction of aldehydes, in general, with ammoniacal silver (Tollens reagent).

Protein-blotting techniques have been comprehensively reviewed,[441] and nitrocellulose is still the most widely used immobilized matrix, whether protein is transferred by diffusion,[442,443] mass method, or electroelution.[444,445] A new positively charged nylon membrane has been evaluated as an immobilizing matrix and found to have greater capacity for protein binding and be more sensitive to antibody or lectin overlay.[446] Transfer to nitrocellulose followed by overlay has been used for detection of glycoproteins using concanavalin A and horseradish peroxidase[447,448] and proteins in general by first reacting them with 2,4-dinitrofluorobenzene, then with antibody to dinitrophenol, and finally peroxidase–antiperoxidase reagents to visualize.[449] The method is approximately 100 times more sensitive than Coomassie Blue or Amido Black stains.

Stained and destained proteins have been electrophoretically eluted from gel slices into agarose by the use of a discontinuous buffer system[450] and by retention in a discontinuous conductivity gradient.[451] A gel eluter for the collection of small volumes containing high concentrations of proteins from slab gels has also been described.[452]

Protein Determination in Solutions and Suspensions.—The Folin–phenol, biuret, u.v. absorption, and *o*-phthalaldehyde (OPA) fluorescence methods have been compared.[453] The Folin–phenol assay is still the recommended general laboratory procedure, with the OPA fluorescence assay as the most sensitive; less than 10 ng protein can be quantitated with hydrolysed protein. The Coomassie Blue dye-binding assay has been adopted for direct measurement of protein in cell suspensions by the addition of Triton X-100 or NaOH prior to the dye,[454] and the biuret assay has been modified for use with homogenates of fatty tissues including heart, liver, kidney, and brain.[455]

Agarose-immobilized proteins have been determined by formation of Cu^{2+}–protein complexes and reaction of the Cu^{2+} ions with diethyldithiocarbamate to form a dark yellow colour.[456] A spectrophometric non-destructive method for determining the protein density of affinity gels has also been described.[457]

[440] A. S. Dion and A. A. Pomenti, *Anal. Biochem.*, 1983, **129,** 490.
[441] J. M. Gershoni and G. E. Palade, *Anal. Biochem.*, 1983, **131,** 1.
[442] A. M. Aubertin, l. Tondre, C. Lopez, G. Obert, and A. Kirn, *Anal. Biochem.*, 1983, **131,** 127.
[443] M. P. Reinhardt and D. Malamud, *Anal. Biochem.*, 1982, **123,** 229.
[444] M. W. Hunkapiller, E. Lujan, F. Ostrander, and L. E. Mood, *Methods Enzymol.*, 1983, **91,** 227.
[445] W. Lin and M. Kasamatsu, *Anal. Biochem.*, 1983, **128,** 302.
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PART IB: Primary Structures, 1982 *By J. Gagnon*

The purpose of this section is to present a comprehensive list of amino-acid sequences reported during 1982 (Table). The primary structures cited are those derived from automated and manual methods of protein-sequence determination. Amino-acid sequences deduced from nucleotide sequences but not directly determined have not been included.

A collection of papers presented at the IVth International Conference on Methods in Protein Sequence Analysis (Brookhaven National Laboratory, September 1981) has been published.[1] Many aspects of protein sequencing are covered and the new technology is well reviewed.

Table *Amino-acid sequences reported during* 1982

Protein	*Origin*	*Comments*	*Ref.*
Blood-clotting Proteins			
Antithrombin	Chicken	*N*-Terminal sequence, 19 residues	2
Fibronectin	Bovine	Sequences of eight CNBr fragments, 502 residues	3
Fibronectin	Bovine	Sequence of a sulphydryl-containing peptide, 27 residues	4
Fibronectin	Human	Sequence of the cell attachment domain, 108 residues	5
Fibronectin	Human	*N*-Terminal sequences of DNA-, heparin-, and gelatin-binding tryptic fragments	6
Fibronectin	Human	Localization of a site interacting with platelet receptor, 27 residues	7
Protein C	Bovine	Complete sequences of light and heavy chains, 155 and 260 residues, respectively	8, 9
Protein Z	Bovine	Sequence of vitamin K-dependent part of the protein, 43 residues	10
Complement Proteins			
C_{1q}	Human	Complete sequences of A and B chains, 224 and 226 residues, respectively	11

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
C_{1r}	Human	*N*-Terminal sequences of eight CNBr fragments of the catalytic chain	12
C_2	Guinea pig	*N*-Terminal sequences of fragments C_{2a} and C_{2b}, 28 and 26 residues, respectively	13
C_3	Human	*N*-Terminal sequence of a fragment generated by Factor I cleavage, 27 residues	14
C_3	Human	Sequence around the internal thioester bond, 49 residues	15
C_4	Mouse	Partial *N*-terminal sequences of the three subunits	16
C_4	Human	Partial *N*-terminal sequences of polymorphic tryptic peptides	17
C_{4a}	Bovine	Complete sequence of the anaphylatoxin, 77 residues	18
C_4 binding protein	Human	*N*-Terminal sequence, 36 residues	19
C_6 and C_7	Human	Partial *N*-terminal sequences	20
C_9	Human	*N*-Terminal sequence of a hydrophobic domain	21
Factor B	Human	*N*-Terminal sequences of nine CNBr fragments	22
Factor H	Human	*N*-Terminal sequence, 17 residues	23
Electron-transport Proteins			
Cytochrome b_5	Rat	Complete sequence, 133 residues	24
Cytochrome C_1	Bovine	Complete sequence, 241 residues	25, 26
Cytochrome C_3	*Desulfovibrio desulfuricans*	*N*-Terminal sequence, 15 residues	27

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Cytochrome C_{550}	*Nitrobacter agilis*	Complete sequence, 109 residues	28
Ferredoxin	*Synechocystis* 6714	Complete sequence, 96 residues	29
Ferredoxin	*Chlorogloeopsis fritschii*	Complete sequence, 98 residues	30
Ferredoxin	Fern	Complete sequence, 95 residues	31
Ferredoxin	*Methanosarcina barkeri*	*N*-Terminal sequence, 20 residues	32
Ferredoxin I	*Desulfovibrio africanus*	Complete sequence, 61 residues	33
Ferredoxins I and II	*Nostoc* strain MAC	Complete sequences of both proteins, 98 residues each	34
Ferredoxin (3Fe:3S)	*Methanosarcina barkeri*	Complete sequence, 59 residues	35
Ferredoxin (4Fe:4S)	*Clostridium thermoaceticum*	Complete sequence, 63 residues	36
Flavocytochrome b_2	Baker's yeast	Sequences of some Cys-containing peptides	37
Haemocyanins	Arthropod	Sequences around the presumptive active site of three proteins	38
Haemocyanin	Spiny lobster	*N*-Terminal sequences of fragments from limited proteolysis	39
Hydrophobic component of chloroplast coupling factor	Spinach	*N*-Terminal sequence, 27 residues	40

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Enzymes			
Aldehyde dehydrogenase	Human	Sequence around a reactive Cys residue, 53 residues	41
D-Amino-acid oxidase	Porcine	Complete sequence, 347 residues	42—44
cAMP-dependent protein kinase	Bovine	Sequence around phosphorylated residues of subunit R_{II}	45
cAMP-dependent protein kinase	Bovine	Determination of an unusual blocked *N*-terminal sequence	46
cAMP-dependent protein kinase I	Porcine	Partial sequence of regulatory subunit	47
Angiotensin-converting enzyme	Rabbit	*N*- and *C*-terminal sequences of lung and testis isoenzymes	48
Aspartate transcarbamylase	*E. coli*	Sequence around three active-site Lys residues	49
Ca^{2+}- and Mg^{2+}-dependent ATPase	Rabbit, lobster	Identification of fluorescein-labelled peptides	50
ATPase (F_1F_0)	Bovine	Sequence of a tryptic peptide, 40 residues	51
Carbonic anhydrase	Tammar wallaby	Partial sequences of CNBr peptides, some 50 residues	52
Catabolic dehydroquinase	*Neurospora crassa*	Partial sequences of enzymes purified from *N. crassa* and *E. coli*	53
Catalase	Bovine	Complete sequences of liver enzyme and partial sequence of erythrocyte enzyme, 506 and 493 residues, respectively	54
Catalase	Human	Partial sequence of the erythrocyte enzyme	55

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Cathapsin B	Bovine	Complete sequence of the light chain, 47 residues	56
Citrate synthase	Porcine	Complete sequence, 437 residues	57
γ-Cystathionase	Rat	Identification of the Lys active-site residue	58
Cytochrome oxidase	Bovine	Complete sequence of polypeptide VIa, 98 residues	59
Cytochrome oxidase	*Saccharomyces cerevisiae*	Complete sequence of subunit VI, 108 residues	60
Cytochrome P450	*Pseudomonas putida*	Complete sequence, 412 residues	61—63
Cytochrome P450	Rabbit	*N*- and *C*-terminal sequences of form 3a	64
Cytochrome P450	Rabbit	Partial sequence of isozyme 2	65
Cytochrome P450 (phenobarbital induced)	Rabbit	Partial sequence, 90% of total structure	66
Cytochrome P450	Rat	*N*-Terminal sequences of isoenzymes RLM3 and RLM5	67
Cytochrome P450	Rat	*N*-Terminal sequences of isozymes PB-4 and PB-5	68
Cytochrome P450d	Rat	*N*-Terminal sequence, 30 residues	69
2-Deoxyribose 5-phosphate aldolase	*E. coli*	*N*- and *C*-terminal sequences	70
Dihydrofolate reductase	Bovine	Complete sequence, 186 residues	71

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Diol dehydratase	*Klebsiella pneumoniae*	*N*-Terminal sequences of four subunits, 40 residues each	72
DNA G primase	*E. coli*	*N*-Terminal sequence	73
DNA polymerase I	*E. coli*	*N*- and *C*-terminal sequences	74
Epoxide hydrolase	Human	*N*-Terminal sequence, 19 residues	75
Fructose 1,6-bisphosphate aldolase	*Drosophila melanogaster*	Sequence around the substrate-binding active-site Lys, 103 residues	76
Fructose 1,6-bisphosphate aldolase	*Staphylococcus aureus* and *epidermidis*	Sequences around the active site of the two enzymes, 26 residues each	77
Fructose 1,6-bisphosphatase	Rabbit	Sequence of a large CNBr fragment, 84 residues	78
Fructose 1,6-bisphosphatases	Rabbit, rat, chicken, and snake	Partial sequences of the *S*-peptides from four enzymes	79
Fructose 1,6-bisphosphatase	Rabbit	Localization of active-site Lys residue	80
Glucose 6-phosphate dehydrogenase	*Leuconostoc mesenteroide*	Sequence around an essential Lys residue	81
Glutathione reductase	Human	Sequences of NADPH and interface domains, 136 and 114 residues, respectively	82
Glutathione peroxidase	Rat	Sequence around the active-site selenocysteine, 46 residues	83
sn-Glycerol 3-phosphate dehydrogenase	*Drosophila melanogaster*	Sequences of the *C*-terminal tryptic peptides from the two major isoenzymes	84

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Glycogen synthase	Rabbit	Sequences around the phosphorylation sites	85,86
cGMP-dependent protein kinase	Bovine	Sequence around the ATP-binding site, 37 residues	87
Histidine decarboxylase	*Lactobacillus* 30a	Complete sequence of the β-chain, 81 residues, comparison with mutant 3	88
p-Hydroxybenzoate hydroxylase	*Pseudomonas fluorescens*	Complete sequence, 394 residues	89
Hypoxanthine–guanine phosphoribosyltransferase	Human	Complete sequence	90, 91
Invertases p60 and p62	*Saccharomyces cerevisiae*	Partial sequences of leader peptides	92
β-Lactamase	*Pseudomonas aeruginosa*	Sequence around the active-site Ser, 14 residues	93
Leucine aminopeptidase	Bovine	Complete sequence, 478 residues	94
Lipase	Porcine	Determination of disulphide bridges and sulphydryl groups	95
Lipoamide dehydrogenase	Porcine	Sequences of nine tryptic peptides	96
Lipoamide dehydrogenase	*Bacillus stearothermophilus*	Sequence around the active site, 16 residues	97
Lysozymes	Pekin duck	Complete sequences of 3 enzymes, 129 residues each	98
Lysozyme	Ostrich	Complete sequence, 185 residues	99
Met-tRNA synthetase	*E. coli*	Partial sequence, 84% of total structure	100

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
NADPH-cytochrome P450 reductase	Rabbit	*N*-Terminal sequence, 69 residues	101
Nitrogenase iron protein	*Azotobacter vinelandii*	Complete sequence, 289 residues	102
Pancreatic peptidases	Canine	Partial sequences of leader peptides from six enzymes	103
Phosphoglucose isomerase	Porcine	Sequences of two pyridoxal 5'-phosphate-labelled peptides	104
Phospholipase A	Sea snake	Complete sequences of three enzymes, I, III, and IV, 118 residues each	105
Phospholipase A2	Crotalus atrox	Complete sequence, 122 residues	106
Phospholipase A2	Human	*N*-terminal sequence, 40 residues	107
Phospholipase A2	Porcine	*N*-Terminal sequence, 48 residues	108
Phosphorylase	Potato	Sequence around the active site	109
Phosphotransferase system, enzyme I	*Salmonella typhimurium*	*N*-Terminal sequence, 17 residues	110
Phosphotransferase system, phosphocarrier protein	*Salmonella typhimurium*	Complete sequence, 84 residues	111, 112
Prepepsinogen	Rat	Partial *N*-terminal sequence	113
Prochymosin	Cat	*N*-Terminal sequence, 55 residues	114
Renin	Mouse	Complete sequences of light and heavy chains, 48 and 288 residues, respectively	115, 116
Ribonuclease	*Aspergillus saitoi*	Complete sequence, 106 residues	117

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Ribonucleases	African porcupine and casiragua	Complete sequences of both enzymes, 128 residues each	118
Ribulose bisphosphate carboxylase oxygenase	*Rhodospirillum rubrum*	Sequence of pyridoxal 5′-phosphate-binding peptide, 20 residues; sequences of CNBr fragments, 339 of 475 residues	119, 120
Sucrase–isomaltase	Rat	*N*-Terminal sequence of the precursor	121
Sucrase–isomaltase	Rabbit	Sequence of *N*-terminal membranous segment, 32 residues	122
Streptokinase	*Streptococcus*	Complete sequence, 415 residues	123
Threonine dehydratase	*E. coli* and *Salmonella typhimurium*	*N*-Terminal sequences of both proteins, 25 residues each	124
Thymidylate synthetase	*Lactobacillus casei*	Localization of the folylpolyglutamate-binding sites	125
Tyrosinase	*Neurospora crassa*	Complete sequence, 407 residues	126—128
Urokinase	Human	Complete sequences of light and heavy chains from low- and high-molecular-weight forms	129—132
Globins			
Erythrocruonin AIII	Earthworm	Complete sequence, 157 residues	133
Erythrocruonin	*Tylorrhynchus heterochaetus*	Complete sequence of the smallest chain, 139 residues	134

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Haemoglobin	Armadillo	Complete sequence of the α-chain	135
Haemoglobin	Asiatic ass and mountain zebra	Complete sequences of α- and β-chains from both sources	136
Haemoglobin	Bar-headed goose	Complete sequences of α- and β-chains	137
Haemoglobin	Canada goose and mute swan	Complete sequences of α- and β-chains from both sources	138
Haemoglobin	Egyptian fruit bat	Complete sequences of α- and β-chains	139
Haemoglobin	Goat and sheep	Complete sequences of both γ-chains	140
Haemoglobin	Indian elephant	Complete sequences of α- and β-chains	141
Haemoglobin	Pheasant	Complete sequences of αA-, αD-, and β-chains	142
Haemoglobin	White rhinoceros	Complete sequences of α- and β-chains	143
Haemoglobin E	Chicken	Complete sequence of the ε-chain, partial sequence of the αE-chain	144
Haemoglobin M	Chicken	Complete sequence of the β-like chain, partial sequence of the α-like chain	145
Haemoglobin (monomeric)	Bull frog	Complete sequence, 132 residues	146
Histocompatibility Antigens and Antibodies			
Antiazobenzene-arsonate Ab	Mouse	Sequences of V-region of L-chains of 10K44-7A1 and 10K26-12A1	147
Antigalactan Ab	Mouse	Sequences of V-regions from ten κ-chains	148

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Anti-Glu-Ala-Tyr polymer Ab	Mouse (BALB/c)	*N*-Terminal sequences of H- and L-chains from five monoclonal anti GAT Ab, 43 residues each	149
Antilysozyme Ab	Mouse	*N*-Terminal sequences of H- and L-chains, 25 residues each	150
Bence-Jones proteins	Human	*N*-Terminal sequences of two proteins, some 50 residues	151
Bence-Jones protein (MeV)	Human	Sequence of V-region, 108 residues	152
Bence-Jones protein (Weir)	Human	Complete sequence	153
Disease protein (HCD)	Human	Partial sequence of leader peptide	154
H-2K^{k}	Mouse	*N*-Terminal sequence, 28 residues	155
HLA-A2	Human	Comparative partial sequences of variants M7 and DR1	156
HLA-A28 and -A2	Human	Partial sequences of A28 and A2, 96% and 90% of total structures, respectively	157
HLA-DR	Human	*N*-Terminal sequences of α- and β-chains	158—160
HLA-DW2/DR2	Human	*N*-Terminal sequence, 179 residues	161
IgA (Cryoimmunoglobulin Nzu)	Human	Partial sequence of a CNBr peptide from the VH-domain	162
IgD	Human	Complete sequence of δ H-chain, 512 residues	163

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
IgD	Human	Sequences around seven carbohydrate moieties of the hinge region	164
IgG	Lagomorph	Sequence of CH2-domain of hare IgG, partial sequences of CH2-domains from other hares, cottontail rabbit, and pika	165
IgG	Rat	*N*-Terminal sequences of leader peptides of three L-chains	166
IgG	Rabbit	Partial sequence of the C-domain of a κ-like chain	167
IgM	Mouse	Complete sequence of the μ-chain, 576 residues	168
β_2-Microglobulin	Bovine	Complete sequence, 98 residues	169
β_2-Microglobulin	Mouse	Sequences of two allelic variants from different strains	170
β_2-Microglobulin	Turkey	*N*-Terminal sequence, 7 residues	171
Monoclonal Ab, 2S1.3	Mouse	Complete sequence of L-chain	172
Myeloma proteins binding inulin	Mouse (BALB/c)	Sequences of VL- and VH-domains of W3082 and J606	173
Myeloma proteins	Mouse (BALB/c)	*N*-Terminal sequences of 14 V-regions	174
Myeloma proteins (IgGκh and IgGκn)	Human	*N*-Terminal sequences of two L-chains, 25 residues each	175
Myeloma protein (IgM)	Mouse (MOPC 104E)	Sequences around five glycosylation sites	176

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Secretory component (SC) (IgA and IgM associated)	Human	Partial *N*-terminal sequence	177
Class I histocompatibility Ag and β_2-microglobulin	Miniature swine	*N*-Terminal sequences of three SLA heavy chains and the light chain	178
Histones			
Chromatin	Bovine	Localization of various phosphorylation sites	179
H1	Sea urchin	Sequence of first 171 residues	180
H1	Sea urchin	Partial sequence, 91 residues	181
H2A	Cuttle-fish	Complete sequence, 124 residues	182
H2B	Chicken	Complete sequence, 125 residues	183
H2B	*Tetrahymena*	Complete sequence, 119 residues	184
H3	*Saccharomyces cerevisiae*	Complete sequence, 135 residues	185
H4	Human	Partial sequence	186
Hormones			
Anterior pituitary polypeptide	Porcine	*N*-Terminal sequence, 50 residues	187
Corticotropin-like peptide	Salmon	Complete sequence, 24 residues	188
Dynorphin	Porcine	Complete sequence, 32 residues	189
[Met]-enkephalin-containing peptide (5300 Mr)	Bovine	Complete sequence, 50 residues	190

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Enkephalin-containing peptide (18200 Mr)	Bovine	Complete sequence, 165 residues	191
Follicle-stimulating hormone	Human	Localization of three of the six disulphide bonds of the β-subunit	192
Glicentin-related peptide	Porcine	Complete sequence, 30 residues	193
Glucagon-37	Porcine	Complete sequence, 37 residues	194
Growth-hormone-releasing factor	Human	Complete sequence, 40 residues	195
Insulin	Carp	Complete sequences of A- and B-chains, 21 and 31 residues, respectively	196
β-Lipotropin	Human	*N*-Terminal sequence	197, 198
MSEL-neurophysin	Human	Complete sequence, 95 residues	199
Neuropeptide γ	Porcine	Complete sequence, 36 residues	200
Nerve growth factor	Bovine	Partial *N*-terminal sequence, 22 residues	201
Opioid peptides (8600 and 12600 Mr)		Sequence of two related Met-enkephalin-containing peptides	202
Peptide γγ	Porcine	Complete sequence, 36 residues	203
Progastricsin	Human	Sequence of the 'pro' part, 43 residues	204
Pro-opiomelanocortin	Porcine	Sequence of a *C*-terminal extension	205

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Rimorphin	Bovine	Sequence of the tridecapeptide	206
Thymosin $\beta 4$ (hormone-like)	Calf	Complete sequence, 43 residues	207
Ribosomal Proteins			
Acidic phosphoprotein P_2	Rat	Complete sequence, 111 residues	208
eL12'/eL12'-P	*Artemia salina*	Complete sequence, 108 residues	209
L2	*E. coli*	Complete sequence, 272 residues	210
L9	*E. coli*	Complete sequence, 149 residues	211
L19	*E. coli*	Complete sequence, 127 residues	212
S1	*E. coli*	Complete sequence, 557 residues	213, 214
SA1 (L7/L12-type)	*Streptomyces griseus*	Complete sequence, 125 residues	215
YS4, YS6, YS11, YS15, YS16, YS22, YL10, and YL31	*Saccharomyces cerevisiae*	*N*-Terminal sequences of eight proteins	216
Structural Proteins			
Elastin precursor	Sheep	*N*-Terminal sequences of the precursor and of tropoelastin, 30 and 20 residues, respectively	217
Collagen $\alpha 1$(I)	Chicken	Sequence of CNBr peptide CB8, 279 residues, completion of the helical portion	218
Collagen type IV	Mouse	Partial sequence of fragment P1	219

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Toxins			
β-Bungarotoxins $\beta_2, \beta_3, \beta_4, \beta_5$	*Bungarus multicinctus*	Complete sequences of A- and B-chains, 120 and 60 residues, respectively, of four toxins	220, 221
Conotoxin MI	Marine snail	Complete sequence, 14 residues	222
Enterotoxin (heat-stable)	*E. coli*	Complete sequence by f.a.b. mass spectrometry and carboxypeptidase, 19 residues	223
Neurotoxins	Sea snake	Complete sequences of two long-chain toxins, 69 residues each	224
Protein CM-2	Puff adder	Complete sequence, 82 residues	225
Taipoxin	Australian snake taipan	Complete sequences of α- and β_1-subunits, 119 and 118 residues, respectively	226, 227
Toxin (host-specific)	*Helminthrosporium carbonum*	Complete structure of the cyclic tetrapeptide	228
Toxin V	*Anemonia sulcate*	Complete sequence, 46 residues	229
Viral Proteins			
Coat protein	Cowpea chlorotic mottle virus	Near-complete sequence, 190 protein	230
Coat protein	Southern bean mosaic virus	Sequence of the C subunit, 260 residues	231
Envelope glycoprotein precursor (PE2)	Sindbis virus	*N*-Terminal sequence	232
Gene A2 protein	Bacteriophage T5	*N*-Terminal sequence, 19 residues	233

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Gene III protein	Bacteriophage f1	Partial sequence	234
Glycoprotein gp71A	Friend murine leukaemia virus	Complete sequence, 445 residues, eight glycosylation sites	235
Haemagglutinin	Fowl plague virus	Sequence around the cleavage site	236
Hexon capsid protein	Adenovirus type 5	Partial sequence	237
Major internal protein (P24)	Human T-cell leukaemia virus	*N*-Terminal sequence, 25 residues	238
Neuraminidase	Influenza virus, asian strain A/Tokyo/3/67	Near-complete sequence of pronase-released heads	239
Neuraminidase	Influenza virus, type A	Partial sequence	240
P21 transforming protein	Murine sarcoma virus	Localization of a phosphorylated Thr residue	241
P60 transforming protein	Rous sarcoma virus	Sequence around phosphorylated Tyr residue	242
Polypeptides P3-6a and P3-6b	Polio virus	Partial *N*-terminal sequences	243
Proteins P12	Mouse leukaemia viruses	Complete sequences of R-MuLV and M-MuLV proteins, 84 residues each	244
Surface antigen	Hepatitis B	Partial sequences of proteins from subtypes adw and ayw	245
Tail protein	Bacteriophage P22	*N*- and *C*-terminal sequences	246
Terminal protein precursor	Adenovirus	Partial sequence	247
Virion glycoproteins RE1 and RE2	Sindbis virus	*N*-Terminal sequences	248

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Viral protein (11000 Mr)	Adenovirus	Partial *N*-terminal sequence	249
Miscellaneous Peptides and Proteins			
Acetyl choline receptor	*Electrophorus electricus*	*N*-Terminal sequence of α-subunit, 35 residues	250
Acetyl choline receptor	*Electrophorus electricus*	*N*-Terminal sequences of four subunits, 24 residues each	251
Actin	Rabbit	Identification of myosin-binding sites	252
ADP/ATP carrier	Bovine	Complete sequence, 297 residues	253
cAMP receptor protein	*E. coli*	*N*- and *C*-terminal sequences	254
Amyloid-related apoprotein (apoSAA$_1$)	Human	Complete sequences of two species of protein, 104 residues each	255
Anion-transport protein	Human	Partial sequence around the anion-binding domain	256
Anti-bacterial proteins	Cecropia moth	Complete sequence of cecropin D, 37 residues, near-complete sequences of cecropin C, E, and F	257
Anti-bacterial protein	Chinese oak silk moth	Complete sequence of cecropin B and D, 37 and 36 residues, respectively	258
Anti-tumour proteins	*Aspergillus*	Partial sequences of three proteins, some 50 residues each	259
Apoferritin	Human	Complete sequence, 174 residues	260
Apoliprotein A-I	Dog	Complete sequence, 232 residues	261

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Apolipoproteins A-I and A-IV	Rat	*N*-Terminal sequences of precursor forms	262, 263
Apolipoprotein E	Human	Complete sequence, 299 residues	264
Basic proline-rich proteins	Human	Complete sequence of IB-9, 61 residues, and partial sequence of IB-6	265
Basic proline-rich protein	Human	Complete sequence of P-E, 61 residues	266
Calmodulin	Human	Complete sequence, 148 residues	267
β-Casein	Horse	*N*- and *C*-terminal sequences	268
Colicin E1	*E. coli*	Localization of a fragment active in membrane depolarization	269
Complex-forming glycoprotein	Human	Sequences around three Cys residues	270
Contact-site A glycoprotein	*Dictyostelium discoideum*	*N*-Terminal sequence, 27 residues	271
C-Reactive protein	Rabbit	Complete sequence, 186 residues	272
C-Reactive protein and serum amyloid P component	Plaice	*N*-Terminal sequences of CRP and SAP, 30 and 20 residues, respectively	273
γ-Crystallins	Mouse	Partial *N*-terminal sequences of four polypeptides	274
Crystallin S_{III}	Squid	*N*-Terminal sequence, 36 residues	275
Dentin	Rat	*N*-Terminal sequences of fraction γ2 and γ3, 15 residues each	276

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
DNA-binding protein I	*E. coli*	*N*-Terminal sequence, 40 residues	277
DNA-binding proteins (53 000 Mr)	Human and mouse transformed cell lines	*N*-Terminal sequences of four proteins	278
Elongation factor G	*E. coli*	Complete sequence, 701 residues	279
Elongation factor Tu	*E. coli*	Sequence of fragment B, 203 residues	280
Epidermal growth-factor-binding protein	Mouse	Partial sequence, 108 residues	281
Favin	Fava beans	Complete sequence of the β-chain, 182 residues, partial *N*-terminal sequence of precursor	282, 283
Fimbrial protein CFA1	*E. coli*	Complete sequence, 147 residues	284
Flagellins	*Caulobacter crescentus*	Sequences of peptides from Fla A and Fla B	285
High-mobility-group 14 protein (HMG14)	Bovine	Localization of phosphorylation sites	286
Histidine-rich glycoprotein	Human	*N*-Terminal sequence, 22 residues	287
α2-HS-glycoprotein	Human	*N*-Terminal sequence, 31 residues	288
Interferon α	Human	Near-complete sequence	289
α-Lactalbumin	Rat	Complete sequence, 140 residues	290
Lactotransferrin	Human	Sequence of *C*-terminal CNBr peptide, 88 residues	291
Largomycin	*Streptomyces pluricolorescens*	*N*- and *C*-terminal sequences, 32 and 4 residues, respectively	292

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Lectin	*Lathyrus sativus*	*N*-Terminal sequences of heavy and light subunits, 25 residues each	293
Mannose/Glucose-specific lectin	*Vicia cracca*	Sequence of small subunit, 53 residues	294
Metallothioneins 1 and 2	Crab	Complete sequences of both proteins, 58 and 57 residues, respectively	295
Metallothionein	Rat	*N*-Terminal sequence of three polymorphic forms, 25 residues each	296
Myosin	Chicken	Complete sequence of the L-1 light chain, 190 residues	297
Myosin	Rabbit	Sequences around the active Lys residues	298
Normal fecal antigen-1 (NFA-1)	Human	*N*-Terminal sequence, 10 residues	299
Nucleolar protein C23	Rat	*N*-Terminal sequence, 9 residues	300
Osteocalcin	Old world monkey	Complete sequence, 49 residues, by gas chromatography–mass spectrometry	301
Outer-membrane protein	*E. coli*	Sequence of leader peptide, 22 residues	302
Outer-membrane protein I	*E. coli*	Complete sequence, 340 residues	303
Ovotransferrin	Chicken	Partial sequence, 605 of 705 residues	304
P2 myelin protein	Human	Complete sequence, 131 residues	305
P2 myelin protein	Rabbit	Complete sequence, 131 residues	306
P7 myelin proteolipid	Rat	Partial sequence	307

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
α-Parvalbumin	Frog	Complete sequence of pI = 4.88 component, 109 residues	308
Parvalbumin	Rat	Complete sequence, 109 residues	309
Penicillin-binding proteins	*E. coli*	*N*-Terminal sequences of PBP 5 and 6, 28 and 25 residues, respectively	310
Pilin	*Klebsiella pneumonia*	*N*-Terminal sequence, 25 residues	311
Pre-α-lactalbumin	Porcine	*N*-Terminal sequence	312
Pre-α-lactalbumin	Rabbit	*N*-Terminal sequence	313
Proline-rich phosphoglycoprotein	Cynomolgus monkey	Complete sequence, 42 residues	314
Prostatic-binding protein	Rat	Complete sequence of component C1, 88 residues	315
Protamine	Boar	*N*- and *C*-terminal sequences	316
α-Protein	Rat	Complete sequence of component I, 88 residues	317
Protein HC	Human	*N*- and *C*-terminal sequences	318
Protein phosphatase inhibitor-1	Rabbit	Complete sequence, 165 residues	319, 320
α_1-Proteinase inhibitor	Human	Localization of three glycosylation sites	321
Proteinase inhibitors (low mol. weight)	Potato	Complete sequences of a chymotrypsin and a trypsin inhibitor, 52 and 51 residues, respectively	322
Proteolipid (lipophilin)	Bovine	Near-complete sequence	323—325

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Proteolipid (lipophilin)	Bovine	Sequence of a hydrophobic tryptic peptide	326
Reaction-centre protein	*Rhodopseudomonas sphaeroides*	*N*-Terminal sequences of L, M, and H subunits, 25—28 residues	327
Seed-storage protein	Castor beans	Complete sequences of the small and large subunits, 34 and 61 residues, respectively	328
Serum amyloid A proteins	Human	*N*-Terminal sequences of four SAA proteins, some 20 residues each	329
Skin calcium-binding protein (S-CaBP)	Rat	Sequence of a tryptic peptide	330
Sialoglycoprotein D (glycophorin *c*)	Human	*N*-Terminal sequence, 47 residues	331
Speract	*Strongylocentrotus purpuratus*	Sequence of the decapeptide	332
Thymosins $\beta 8$ and $\beta 9$	Calf	Complete sequences of $\beta 8$ and $\beta 9$, 39 and 41 residues, respectively	333
γ-Trace (basic microprotein)	Human	Complete sequence, 120 residues	334
Transferrin	Human	Complete sequence, 678 residues	335
Transferrin	Rabbit	Partial sequences of *N*- and *C*-terminal iron-binding fragments	336
Trypsin inhibitor	Barley	*N*-Terminal sequence	337
Ubiquitin-like protein	Insect	Complete sequence, 74 residues	338
Variant surface glycoprotein (VSG 117)	*Trypanosoma brucei*	Complete sequence, 470 residues	339

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Table *(cont.)*

Protein	*Origin*	*Comments*	*Ref.*
Vicilin(seed-storage protein)	Pea	Complete sequence of a sub-unit, 124 residues	340
Whey phospho-protein	Rat	Partial *N*-terminal sequences of the *in vivo* and *in vitro* Wp-protein	341
Z-Protein	Rat	Complete sequence, 127 residues	342

PART IC: Chemical Modification of Proteins *By R. Cassels and R. A. G. Smith*

1 Introduction

As in previous volumes of this Specialist Periodical Report, it has been necessary to summarize developments in the fertile field of protein chemical modification in table form. Although a thorough coverage of this topic has been attempted, we have omitted routine uses of well established reagents even where (as with glutaraldehyde) the detailed chemistry is frequently unclear. In addition, solid-phase immobilization techniques, radiolabelling procedures, modifications of prosthetic groups or protein-bound carbohydrate, and protein–protein couplings for ELISA have been excluded unless they utilize or demonstrate novel features of reagent or protein reactivity.

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Table *Chemical modification of proteins**

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Abrin, globulin (anti mouse lymphocyte)	Horse	SPDP, chlorambucil	Lys, Cys Lys	Cytotoxic immunoconjugates	1
Acetylcholinesterase	*Torpedo electricus*	1-Pyrenebutyl, ethyl phosphofluoridate 1-Pyrenebutyl phosphodichloridate	Ser	Fluorescence study of enzyme 'ageing'	2
Acetylcholinesterase	Monkey, rat, and chicken brain	DFP	Ser	Species variation of IC_{50}	3
Acetylcholinesterase	Electric eel	CMC		Irreversible inhibitor	4
Acetylcholinesterase		1-Bromopinacolone		Active-site-directed covalent inhibitor	5
Acetylcholinesterase	Bovine erythrocytes	Diethyl *p*-nitrophenyl phosphate	Ser	Kinetics of inhibition identify isoenzymes	6
	Electrophorus electricus	DFP, *NN'*-di-isopropylphosphorodiamidic fluoride			
Acetylcholine receptors	Embryonic and adult chicken muscle	Bromo[^{3}H]acetylcholine	Cys	Comparison of α-subunits from various sources	7
Acetylcholine receptor, nicotinic	*Musca domestica*	4-(*N*-Maleimido)benzyltrimethylammonium iodide		Affinity label	8
Acetyl-CoA carboxylase	Rat liver	5'-*p*-Fluorosulphonylbenzoyladenosine		Identification of AMP- and ATP-binding sites	9
Acid proteinases	Human gastric mucosa and stomach carcinoma	DEP		Possible activation of pepsinogen II	10
Acrosin inhibitor	Boar spermatozoa	2,3-Butanedione 2,3-Cyclohexanedione	Arg	Active-site characterization	11
Actin	Rabbit muscle	NEM (Iodoacetamido)tetramethylrhodamine	Lys-373	Polymerization accelerated	12
		7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole	Lys-372		

Actin	Rabbit muscle	IAEDANS		Complex formation with sub-fragment 1 and heavy meromyosin studies	13
Actin	Rabbit muscle	(Iodoacetamido)tetramethyl-rhodamine	(—SH)	Polymerization studied by fluorescence photobleaching recovery	14
F-Actin	Rabbit muscle	*N*-(Iodoacetyl)-*N*-(5-sulpho-1-naphthyl)ethylenediamine	(—SH)	Actin–myosin interaction	15
F-Actin	Muscle	Glutaraldehyde		Effect on muscle contraction	16
G-Actin	Rabbit muscle	Tetranitromethane	Tyr	N.m.r. study	17
G-Actin	Rabbit sketetal muscle	IAEDANS	Cys-373	Mg^{2+}-induced conformational change	18

* Abbreviations for reagents used in the Table: BNPS 2-nitrophenylsulphenylbromide, CMC 1-cyclohexyl-3-(2-morpholinyl-4-ethyl)carbodi-imide metho-*p*-toluenesulphonate, DCCI *NN'*-dicyclohexylcarbodi-imide, DFP di-isopropyl phosphofluoridate, DTNB 5,5'-dithiobis-(2-nitrobenzoic acid), EDC 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide, FDNB 1-fluoro-2,4-dinitrobenzene, FITC fluorescein isothiocyanate, IAEDANS *N*-iodoacetyl-*N'*-(1-naphthyl-5-sulphonic acid) ethylene diamine, NEM *N*-ethylmaleimide, SPDP *N*-succinimidyl 3-(2-pyridyldithio)propionate, TLCK N^{α}-(*p*-toluenesulphonyl)lysine chloromethylketone, TNBS 2,4,6-trinitrobenzenesulphonic acid.

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
F-Actin/myosin sub-fragment 1	Rabbit	Various bis-esters		Crosslinking increases Mg^{2+} ATPase activity	19
Actin/myosin	Rabbit muscle	EDC	$—CO_2H/NH_2$	Zero-length crosslinking	20
Acyl-carrier protein	*Escherichia coli* K12	*N*-Acylimidazoles		Effects of acyl chain length on hydrodynamic stability	21
Acyl-CoA dehydrogenase	Pig kidney	Cyclohexane-1,2-dione	Arg	Arg in active centre but not essential	22
Adenotoxin reductase	Bovine adrenal cortex	α-Dicarbonyl reagents	Arg	Ten out of 33 Arg modified	23
Adenosine deaminase	Calf-intestine mucosa	CNBr-activated soluble dextran	Lys ε-NH_2	Specific activity reduced; K_m increased	24
S-Adenosylhomocysteinase	Rat liver	Iodoacetamide	—SH	Two essential SH	25
S-Adenosylhomocysteinase		2′-Deoxyadenosine		Products of adenine elimination bind tightly but non-covalently	26
S–Adenosylhomocysteine hydrolase	Rat liver	Adenosine		Inactivation *in vitro* of enzyme from deaminase-inhibitor-treated animals	27
Adenylate cyclase	Rabbit heart	2′,3′-Dialdehyde ATP/$NaBH_4$	$—NH_2$	Interaction with the membrane	28
Adenylate cyclase	*Bordella pertussis*	Azido-[^{125}I]calmodulin		77 K subunit labelled	29
Adenylate cyclase	Turkey erythrocyte	[^{125}I]-*p*-Azidobenzylcarazolol		β-Adrenergic antagonist photoaffinity label	30
ADP/ATP carrier, membrane bound	Beef-heart mitochondria	Pyridoxal 5′-phosphate	Lys-146, -48, -106	'Inside–outside' differentiation	31
ADP glucose synthetase	*E. coli*	Cyclohexane-1,2-dione	Arg	Arg involved in allosteric activation	32
ADP receptor	Human blood platelets	2-Azidoadenosine [β-^{32}P]-diphosphate *p*-Mercuribenzenesulphonate		Binding to adenylate cyclase inhibitor	33
ADP/ATP translocator protein	Beef-heart mitochondria	*N*-[^{3}H]NEM	—SH	30 000-M_r protein labelled	34

ADP/ATP translocator protein	Beef-heart mitochondria	NEM	—SH	Change in —SH reactivity probes protein reorientation in membrane	35
α_2-Adrenergic receptor	Human platelet	*p*-Isothiocyanatoclonidine	$—NH_2$	Affinity label	36
α_2-Adrenergic receptor protein	Rat brain	Isothiocyanatoclonidine	(—SH, $—NH_2$)	Synthesis of affinity probe	37
β-Adrenergic receptor	Frog erythrocyte membrane	*N*-Succinimidyl-6-(4′-azido-2′-nitrophenylamino)hexanoate		Bifunctional photoactive crosslinker	38
β-Adrenergic receptor	*Rana pipiens*	^{125}I-*p*-Azidobenzylcarazolol		Photoaffinity label	39

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
β-Adrenergic receptor	Rat lung	NEM	—SH	Effects on ^{3}H-hydroxybenzylisoproterenol binding	40
β-Adrenergic receptor	Turkey erythrocyte membrane	(4-Azidobenzimidyl)-3,3-dimethyl-6-hydroxy-7-(2-cyano-3-iodoindol-4-yloxy)-1,4-diazaheptane and 4-azidobenzoyl analogue		Photoaffinity labels	41
β-Adrenergic receptor	Turkey, pigeon, and frog erythrocyte membranes	Iodoazidobenzylpindolol		Photoaffinity label	42
Agglutinin	*Arachis hypognea*	Iodine	Tyr	Effect on lactose binding	43
Agglutinin	*Ricinus communis*	Various reagents	—NH_2, Trp, Arg, —CO_2H, Tyr	Involvement of residues in sugar binding	44
Alanine-neochymotrypsinogen		Acetic anhydride	α,ε-NH_2	Studies on *N*-terminal Ala-149	45
Ala-tRNA synthetase	*Escherichia coli*	5-Bromouridine	(Cys)	Michael addition leads to dead-end complex	46
Albumin	Human serum	Sulphanilamide		Protein–protein crosslinking induced	47
Albumin	Human serum	3,3′,4′,5-Tetrachlorosalicylanilide		Photoactivation depends on chlorosalicyl moiety	48
Albumin	Human	*N*-Succinimidyl-6-(4′-azido-2′-nitrophenylamino)hexanoate, *N*-hydroxysuccinimidyl-4-azidobenzoate		Anti (crosslinked) antibodies raised for isolation of labelled ligand–receptor complexes	49
Albumin	Bovine serum	ACTH–thioglycine/Ag^+/*N*-hydroxysuccinimide		Protein–protein coupling *via* —COSH grouping	50

Albumin	Bovine serum	FDNB	Lys	Changes in physical properties studied	51
Albumin	Human serum	Laevulinic acid ($0^{2',3'}$-adenosine acetal)/EDC		*Cis*-diol haptenization method	52
Albumin	Bovine serum	Various fluorescein and rhodamine derivatives	($—NH_2$)	Fluorescence triplet probes	53
Albumin	Bovine serum	2-Nitro-4-sulphophenyl esters	(Lys)	Water-soluble active esters	54
Albumin	Bovine serum	Diazotized [4-aminobenzoyl-Gln'] serum thymic factor		Immunogenic conjugate	55
Albumin	Bovine serum	Diazotized *p*-aminophenyl-diethyl phosphate	Tyr, His	Protein conjugate for immunoassay	56
Albumin	Bovine serum	Succinic anhydride, daunorubicin/EDC	Lys	Reversible drug-carrier conjugate	57
Albumin	Human serum	Cortisol, 16α-hydroxyestrone	Lys, ε-NH_2	*Via* Schiff base	58

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Albumin	Rat serum	Iodoacetamide	Cys	Modification in perfused liver	59
Alcohol dehydrogenase	Horse liver	Procion Blue MX-R	Cys-174	Specific reaction in hydrophobic pocket	60
Alcohol dehydrogenase and others	Yeast	Benzoylacrylic acid derivatives	Cys	Selective α,β-unsaturated inactivators	61
Alcohol dehydrogenase	Yeast	3-Diazopyridine 1-N^6-ethenoadenose dinucleotide	(—SH)	Four sulphydryls modified	62
Alcohol dehydrogenase	Yeast	3-Chloroacetyl pyridine adenine dinucleoside		Label lost from inactivated enzyme	63
Aldehyde dehydrogenase	Sheep liver	Disulphiram, 2,2′-dithiopyridine	Cys	SH groups not necessarily essential for catalysis	64
Aldehyde dehydrogenase	Human erythrocyte	Disulphiram	Cys	Irreversible inhibition	65
Aldehyde dehydrogenase isoenzyme E_1	Human liver	Iodoacetamide	Cys-30	Disulphiram blocks selective alkylation	66
Aldehyde dehydrogenase	Human liver	^{14}C-Disulphiram	Cys	No incorporation of label during inactivation	67
Aldolase	Rabbit muscle	4-Chloro-7-nitrobenzofurazan	Cys-237	Neighbouring-group participation	68
Aldose reductase	Human brain	4-Hydroxymercuribenzoate Iodoacetate	Cys Cys	Characterization studies	69
Aldose reductase	Bovine lens	Phenylglyoxal, DEP, thiol reagents	Arg, His, Cys	His and Arg near nucleotide site	70
Alginate lyase	*Turbo cornutus*	Various	Cys, Trp, Lys	Essential Trp, Lys	71
Alkaline phosphatase	Bovine intestine	Methyl-4-azidobenzoimidate		Photoaffinity labelling	72
Alkaline protease	*B. subtilis var. DY*	PMSF, dansyl fluoride	Ser	C.d. and fluorescence characterization	73
Alkaline proteinase	Guinea-pig lymphoid cells	DFP and others	Ser, Cys	Characterization	74

D-Amino-acid oxidase	Pig kidney	1-Fluoro-2,4-dinitrobenzene	Tyr-17, Lys-5	Mutually exclusive modification of two active-site residues	75
Amino-acid models		Formaldehyde/sodium cyanoborohydride	$-NH_2$	*N*-Cyanomethyl amines formed	76
Aminoacyl-tRNA synthetases	Sheep liver	Oxidized initiator $tRNA^{Met}$	Lys	Schiff base formed: one tRNA per enzyme molecule	77
4-Aminobutyrate aminotransferase	Porcine brain	P^1P^2-Bis-(5′-pyridoxal) diphosphate/$NaBH_4$	Lys	Approx. two Lys per dimer blocked	78
γ-Aminobutyric acid–α-ketoglutate aminotransferase	Rat and mouse brain	5-Fluoro-4-oxopentanoic acid		Enzyme-activated irreversible inhibition	79

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Aminopeptidase	*Aeromonas* sp.	Butane-2,3-dione and other reagents	5Trp, 3—4Tyr, 2His, 2Arg	Photochemical modification not Arg-specific	80
Aminopeptidase	*Aeromonas proteolytica*	Tetranitromethane and others	Tyr, His, $-CO_2H$	Active-site probes	81
Aminopeptidase	Pig small-intestine microvillus	Dimethyl-3,3′-dithiobispropionimidate	$-NH_2$	Subunit structure investigation	82
cAMP-binding protein	Bovine-brain microtubule	8-Azido-cAMP		Tubulins not labelled	83
cAMP-binding proteins	Human platelets	8-Azido-[^{32}P]cAMP		Photoaffinity labelling	84
cAMP-binding proteins	*Neurospora crassa*	8-Azido-cAMP		Identification of regulatory subunit	85
cAMP-binding proteins	Rabbit gastric gland	8-Azidoadenosine 3′,5-monophosphate		Identification of 58 k and 48 k proteins	86
cAMP-dependent protein kinase	Rabbit muscle	DTNB and analogues	Cys	Reporter thiol in catalytic subunit	87, 88
cAMP-dependent protein kinase	Bovine heart	7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole	Cys	Two Cys modified in catalytic subunit	89
cAMP-dependent protein kinase	Bovine heart	Leu-Arg-Arg-Ala-Cys-(3-nitro-2-pyridinesulphenyl)-Leu-Gly AcLeu-Arg-Arg-Ala-Cys-(3-nitro-2-pyridinesulphenyl)-Leu-GlyOEt	Cys-198	Affinity labelling of active-site SH	90
cAMP-dependent protein kinase	Calf cerebral cortex	8-Azidoadenosine 3′:5′-[^{32}P]-monophosphate		Molecular characterization	91
cAMP-dependent protein kinases R_I and R_{II}	F9 embryonic carcinoma cells	8-Azido-[^{32}P]cAMP		Quantification of R_I and R_{II} in subcellular fractions	92
cAMP-dependent protein kinase	3T3-L1 fibroblasts	8-Azidoadenosine 3′:5′-monophosphate		Quantification in cell extracts	93

cAMP-dependent protein kinase	*Kluyveromyces fragilis*	8-Azido-[^{32}P]cAMP		Photoaffinity labelling	94
cAMP-dependent protein kinase	Porcine heart	8-Azidoadenosine 3′:5′-monophosphate	Tyr	Role of 8-azido-cAMP site in holoenzyme dissociation	95
cAMP-dependent protein kinase	Porcine skeletal muscle	Iodoacetic acid	Cys	Two of seven Cys alkylated only after reduction	96
cAMP-dependent protein kinase	Rat thyroid	8-Azido-cAMP		Identification of enzymes by SDS-p.a.g.e.	97
cAMP-dependent protein kinase, type 1	Y1 mouse adrenocortical tumour-cell mutants	8-Azido-[^{32}P]cAMP		Study of regulatory subunit	98

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
cAMP-receptor	Human T lymphocyte	8-Azido-[^{32}P]cAMP		Photoaffinity labelling	99
Amylase A	*Aspergillus oryzae*	DEP	His	Catalytic role of His	100
Amylase A	*Aspergillus oryzae*	Dimethyl-(2-hydroxy-5-nitrobenzyl)-sulphonium bromide	Trp	Trp location	101
Angiotensin I-converting enzyme	Bovine lung	*p*-[*NN*-Bis(chloroethyl)amino]-phenylbutyric acid and proline derivative	—CO_2H	Irreversible inhibition due to ester formation in active site	102
Angiotensin I-converting enzyme	Human kidney	Methyl acetimidate Methyl *p*-hydroxybenzimidate Methyl 4-mercaptobutyrimidate	—NH_2	Active-site Lys	103
Angiotensin II/angiotensin II receptor	Rat adrenal membrane	Disuccinimidyl suberate	—NH_2	Receptor characterization	104
Anion-transport protein	Human erythrocytes	2,6-Di-iodo-4-sulphophenyl isothiocyanate		Partial sequence of inhibitor-binding peptide	105
Anti-DNP antibody	Mouse	2,4-Dinitrophenyl ester of derivatized Ficoll	(Lys)	Multivalent polymer affinity label for constructing immune complexes model	106
Antithrombin	Bovine	Heparin/nitrous acid	—NH_2	Conjugation *via* 2,5-D-anhydromannose —CHO	107
Antithrombin III	Human plasma	Tolylene-2,4-di-isothiocyanate	—NH_2	Coupled to NH_2-enriched heparin	108
α-Antitrypsin fragments	Human	*N*-(7-Dimethylamino-4-methylcoumarinyl)maleimide	Cys	Fluorescent detection of Cys in peptide maps	109
Antiviral protein	Pokeweed	Various reagents	Lys, Tyr, His, Arg	Effects on function	110
Anthranilate synthase	*Neurospora crassa*	6-Diazo-5-oxo-L-norleucine azaserine		Inactivation substrate-dependent	111
Anthranilate synthase	*Serratia marcescens*	Pyridoxal 5′-phosphate, NaCN	Lys (Cys)	Labelling of glutamine-binding site	112

Apolipoprotein A-I	Human serum	5-Dansyl chloride		Fluorescence polarization probe for Stokes radius	113,114
Apolipoprotein B	Human plasma	Iodoacetamide	—SH	Blockade of exchange polymerization	115
Apolipoprotein C-III	Human plasma	(i) Tetranitromethane (ii) Sodium hydrosulphite (iii) 5-Dansyl chloride	Tyr	Fluorescence probe for vesicle interaction	116
Apomyoglobin	Tuna	1,5-AEDANS	Cys	Single Cys fluorescence labelled	117
Arginase	Rat liver	DFP, PMSF	Ser	Ser in substrate-binding site	118
Arylamidase	Rat skeletal muscle	4-Hydroxymercuribenzoate	—SH	Inactivation	119
Arylhydroxamic acid *N*,*O*-Acyltransferase	Hamster liver	*N*-Hydroxy-2-acetamidofluorene *N*-Hydroxy-4-acetamido-biphenyl		Inactivation *via* arylnitrenium ions	120

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Arylhydroxamic acid *N*,*O*-Acyltransferase	Hamster liver	*N*-Hydroxy-*N*-phenylacetamides		Electrophilic species released from enzyme	121
Asialofetuin		(i) [^{14}C]- or [^{3}H]-formaldehyde/$NaCNBH_3$ (ii) Iodomethane/pH 11	Lys	Metabolic label	122
Asialothyroglobulin (27S)	Bovine thyroid gland	Succinic anhydride, TNBS Tetranitromethane, *N*-acetylimidazole	Lys Tyr	Effects on membrane binding	123
L-Asparaginase	*E. coli*	Succinic anhydride, acetic anhydride *NN*-Dimethyl-1,3-propanediamine/carbodi-imide D-Glucuronic acid/$NaCNBH_3$	Lys Lys	Effects on plasma clearance rates	124
Aspartase	*E. coli*	L-Aspartic-β-semialdehyde	—SH	Essential Cys	125
Aspartate aminotransferase	Pig heart	Serine-*O*-sulphate		Aldol condensation product of pyruvate and PLP isolated	126
Aspartate aminotransferase	Pig heart	Pyridoxal 5′-sulphate *S-trans*-Carboxyethenyl pyridoxal	Lys	Fluorescent lysine modifications	127
Aspartate aminotransferase	Chicken-heart mitochondrion	Phenylglyoxal	Arg-292	Binding site for substrate's distal—CO_2H	128
Aspartate aminotransferase	Pig heart	Cyclohexane-1,2-dione and other α-dicarbonyls	Arg	Essential Arg	129
Aspartate aminotransferase isoenzymes	Pig heart	4′-*N*-(2,4-Dinitro-5-fluorophenyl)pyridoxamine 5′-phosphate	Lys-258	Study of enzyme–coenzyme interaction	130
Aspartate apoaminotransferase	Porcine heart	Pyridoxal 5′-phosphate	—NH_2	Calorimetric study	131
Aspartate transcarbamoylase	*E. coli*	D- and L-bromosuccinate	Lys-83, -84, and -224	Partial modification of three active-site lysines	132
Aspartate transcarbamoylase	*E. coli*	Glutaraldehyde		Subunit assembly	133

ATPase	Beef-heart mitochondrion	7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole	Tyr	Inactivation	134
ATPase	Beef-heart mitochondrion	DCCI	—CO_2H	Activity inhibited at 1 mole DCCI per mole F_o	135
ATPase	Beef-liver mitochondrion	Ethenoadenosine triphosphate dialdehyde derivative		Conformational changes	136
ATPase	Rabbit sarcoplasmic reticulum	*N*-(1-Oxy-2,2,6,6-tetramethyl-4-piperidinyl) iodoacetamide	—SH	Two conformational states of active site	137
ATPase	Beef-heart mitochondria	Periodate-oxidized ATP		β-Elimination of triphosphate identified	138
ATPase	Rabbit sarcoplasmic reticulum	Fluorescein 5′-isothiocyanate		Fluorescence enhancement by vanadate	139
ATPase inhibitor peptide	Rat-liver mitochondrion	*N*-Hydroxysuccinimidyl *p*-[^{14}C]-azidobenzoate	—NH_2	Study of ATPase complex	140

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
ATPase inhibitor protein, IF_1/ATPase F_1	Bovine-heart mitochondria	Methyl 4-azidobenzimidate Various		Independent inhibition of complex V	141
ATPase, oligomycin-sensitive	Beef-heart mitochondrion	Dibromobimane	Cys	Fluorescent crosslinker	142
Ca^{2+}-ATPase	Rabbit skeletal muscle	Fluorescein isothiocyanate	$—NH_2$	1 mol mol^{-1} inactivates	143
$(Ca^{2+} + Mg^{2+})$-ATPase	Human erythrocyte membrane	*N*-(4-Azido-2-nitrophenyl)-2-aminoethylsulphonate		Photolabelling	144
Ca^{2+},Mg^{2+}-activated ATPase	*E. coli*	2,3-Dialdehyde ATP		ATP- and ADP-binding sites in normal and mutant enzymes	145
ATPase chains	Rabbit sarcoplasmic reticulum	IAEDANS, 6-(iodoacetamido)-fluorescein (IAF)	(—SH)	Chain-interaction study	146
F_1-ATPase	Beef heart	Dansyl chloride		Fluorescence labelling	147
F_1-ATPase	*E. coli*	DCCI	$—CO_2H$	One of three β-subunits modified	148
F_1-ATPase	Beef-heart mitochondrion	Pyridoxal 5′-phosphate Phenylglyoxal DCCI 4-Chloro-7-nitrobenzofurazan	$—NH_2$ Arg? $—CO_2H$ Tyr	Multiple nucleotide-binding sites	149
F_1-ATPase	*E. coli*	[^{14}C]DCCI	Glu	Identification of modified residue	150
F_1-ATPase	*E. coli*	2′-(5-Dimethylamino-naphthalene-1-sulphonyl)-amino-2′-deoxy-ATP		Fluorescence studies	151
F_1-ATPase	Rat-liver mitochondrion	3′-*O*-(4-Benzoyl)benzoyl adenosine-5′-triphosphate	$—CH_2—$	Photoactivatable affinity probe	152
F_1-ATPase	*Saccharomyces cerevisiae*	5′-*p*-[^{3}H]Fluorosulphonylbenzoyl adenosine	Tyr	90% inactivation	153
BF_1-ATPase	*E. coli*	Various		Essential Arg, Lys, and carboxyl identified	154

Na^+,K^+-ATPase	Pig-kidney medulla	Ethylmercurythiosalicylate	—SH	Reversible blocking	155
Na^+,K^+-ATPase	Pig outer renal medulla	[^{125}I]Iodonaphthylazide [^{3}H]-*N*-Ouabain-*N'*-(2-nitro-4-azidophenyl)ethylenediamine		Interior and exterior labelling of cell surface membrane	156
Na^+,K^+-ATPase	Canine kidney	Diazotized *p*-[^{35}S]sulphanilic acid		Labelling of hydrophilic surface	157
Na^+,K^+-ATPase	Rat-kidney outer medulla	6-[(3-Carboxy-4-nitrophenyl)-thiol]-9-β-D-ribofuranosyl-purine 5'-triphosphate	(Tyr-OH)	Affinity labelling	158
Na^+,K^+-ATPase	Dog kidney	Fluorescein isothiocyanate		Probe for conformational changes	159
ATP-dependent deoxyribonuclease	*Micrococcus luteus*	Butane-2,3-dione	Arg	Active-site Arg	160
$F_0 \cdot F_1$-ATP synthase	*Rhodospirillum rubrum*	4-Chloro-7-nitrobenzofurazan DCCI	—CO_2H	Modification of β-subunit	161

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Bacterio-opsin	*Halobacterium halobium*	*m*-Diazirinophenyl retinal	Ser-193 Glu-194	Photocrosslinking: topology	162
Bacteriorhodopsin	*Halobacterium halobium*	EDC/amines	—CO_2H	Effects on photocyclic and electric response	163
Bacteriorhodopsin	*Halobacterium halobium*	Methylacetimidate, then diazotized sulphanilic acid	Lys Tyr-64	Lys prevented from reacting in second step	164
Bacteriorhodopsin, chymotryptic fragment C2		H·CHO/$NaBH_4$	Lys-30, -40, -41	Function unimpaired	165
Band 3 protein	Human erythrocytes	Formaldehyde/borohydride	Lys	Two outward-facing residues modified	166
Band 3 protein	Human erythrocytes	4,4′-Di-isothiocyano-stilbene-2,2′-disulphonic acid		C-Transition related to band 3 denaturation	167
Bile-salt-binding polypeptides	Rat hepatocyte plasma membrane	Bile salt azo derivatives		Identification of polypeptides	168
Biogenic amine transporter	Bovine adrenal medulla	4-Azido-3-nitrophenylazo-(5-hydroxytryptamine)		Photoaffinity label	169
α-Bungarotoxin/Membrane receptor	*Bungarus* sp./Chicken retina membrane	*N*-Succinimidyl-6-(4-azidonitrophenylamino)hexanoate		Identification of receptor polypeptides	170
β-Bungarotoxin	*Bungarus multicinctus*	*N*-Succinimidyl-[2,3-^{3}H]-propionate	Lys?	Brain synapse-binding study	171
α-Bungarotoxin	*Bungarus multicinctus*	Tetranitromethane	Tyr	Two Tyrs nitrated	172
Butyryl-CoA dehydrogenase	*Megasphaera elsdenii*	(3-Pentynoyl) pantetheine, (3-chloro-3-butenoyl) pantetheine	Glu	Allene reacts with γ-carboxyl	173
C1	Human plasma	EDC	—CO_2H	Crosslinking for e.m.	174
C3b	Human	[^{14}C]Methylamine [^{3}H]Iodoacetic acid	(Glu) Cys	Reactive thiol ester	175
C4	Human	Methylamine, hydrazine	Thioester	Conformational and functional changes studied	176
C4	Human serum	Primary alkyl amines	Glu; thioester	Inactivation studies	177

C6	Human	Various		Essential thiol and active-site serine identified	178
C9	Human serum	12-(4-Azido-2-nitrophenoxy)-stearoyl, [1-^{14}C]glucosamine		Photolabelling of hydrophobic domain	179
Calcitonin receptor	Human breast cancer cell T47D	β-(γ-Glutamyl)-*N*-ethyl-4-azido-2-nitroaniline calcitonin/$h\nu$		Novel linkage of N_3 to Gln-γ-carboxamide	180
Calmodulin/phospholamban	Carcine cardiac sarcoplasmic reticulum	Dithiobis-(succinimidyl propionate)	$-NH_2$	Receptor characterization	181
γ-Carboxyglutamic acid-containing peptides	Various	Morpholine/formaldehyde	Gla	Mannich base gives γ-methylene glutamic acid	182

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Carboxylase/oxygenase	*Rhodospirillum rubrum*	2-*N*-Chloroamino-2-deoxypentitol 1,5-bis-phosphate	Met	Active-site Met selectively oxidized	183
Carboxylic ester hydrolase	Human pancreas	DFP	Ser	Determination of groups essential for activity	184
		Diethyl *p*-nitrophenyl phosphate	Ser		
		DEP	9 His		
		N-Ethyl-5-phenylisoxazolium-3′-sulphonate	35—36 —CO_2H		
Carboxypeptidase A		Diazotized *p*-arsanilic acid	Tyr-248	^{15}N n.m.r. study	185
R61 DD-carboxypeptidase	Streptomyces	Monobactams	Ser	Binding similar to that of penicillins	186
Carboxypeptidase B	Porcine	Phenylglyoxal	Arg	Effects of Arg modification on Tyr residues	187
Casein		(i) Alkali treatment (ii) 2-Mercaptoethylpyridine	Cys Dehydroalanine residues	Forms *S*-β-(2-pyridylethyl)-L-cysteine	188
Cathepsins B, H, and L	Human liver	L-*trans*-Epoxysuccinyl-leucylamido-(4-guanidino)butane and analogues	Cys	Oxirane less reactive to low-mol.-wt. thiols	189
Citrate lyase	*Klebsiella aerogenes*	*p*-Azidobenzoyl-CoA		Labelling of β-subunit	190
Cholera toxin	*Vibrio cholerae*	4-Chloro-7-nitrobenzofuran	$-NH_2$	Probe bound to subunit A detects binding of ganglioside to B	191
Chromatin protein	Mouse Ehrlich ascites tumour cell	*NN′*-Bis-(9-acridinyl)-4-aza-4-(4-azidobenzoyl)-1,7-diaminoheptane dihydrochloride and similar reagents		Photolabels only DNA-associated proteins. H1, H2A/H2B, and H3 labelled	192
Chromosomal proteins	Duck erythrocyte	2-Mercaptoacetate	Lys	Forms ε-*N*-mercaptoacetyllysine	193
Chymotrypsin	Bovine	4-(Trifluoromethyl)-α-bromoacetanilide	Met-192	^{19}F n.m.r. reporter group	194

α-Chymotrypsin	Bovine	Various *N*-acylimidazoles	Ser	Kinetics of acylation/deacylation studied	195
Chymotrypsin	Bovine	*p*-Toluene [$^2H_7$]sulphonyl fluoride and other ^{13}C- and D-labelled derivatives	Ser	Active-site motion studied by n.m.r.	196
Chymotrypsin	Bovine	Isatoic anhydride	Ser	Anthraniloyl-enzyme formed	197
Chymotrypsin	Bovine	PMSF, FITC	Ser	Synthesis of anhydrotrypsin	198
Chymotrypsin	Bovine	PMSF	Ser	Modification rate-constant determination method	199
		TLCK	His		
α-Chymotrypsin		Haloenol lactones:		Irreversible enzyme inactivation *via* internal haloketone attack on acyl-enzyme	200
		2-Phenyl-4(*E*)-(1-bromoethylidene)tetrahydro-2-furanone	Ser		
		2-Phenyl-4(*E*)-iodomethylidene-tetrahydro-2-furanone			

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Chymotrypsin-like enzyme	Sea urchin (*Hemicentrotus pulcherrimus*)	PMSF, TPCK	Ser	Characterization of enzyme	201
Chymotrypsinogen	Bovine	(i) Ethylenediamine + EDC (ii) *O*-Methylisourea	$-CO_2H$	Superguanidination gives less stable protein	202
Citrate lyase	*Klebsiella aerogenes*	3-Fluorocitrate		Citrate synthase and aconitase not inactivated	203
Citrate lyase	*Rhodopseudomonas gelatinosa*	Various bisimidates	$-NH_2$	Subunit analysis by cross-linking and immunoelectron microscopy	204
Clathrin-associated proteins		Dimethyl suberimidate, dimethyl 3,3′-dithiobis-(propionimidate)	$-NH_2$	Characterization	205
CoA transferase intermediate	Porcine heart	Methyl mercaptopropionate	$-CO_2H$	Active-site thiol ester	206
Colipase A	Pig	Tetranitromethane	Tyr-53(?)	Inter- and intra-molecular crosslinks	207
Concanavalin A		*N*-(Oxysuccinimide) esters of: 4-Azidosalicylic acid *N*-(4-Azidosalicyl)-6-aminocaproic acid 4-Azidobenzoyl glycyl tyrosine	Lys	Photocrosslinkers radioiodinated	208
Concanavalin A		1-Pyreneacrylic acid *N*-oxysuccinimide ester	(Lys)	Fluorescence characterization	209
Concanavalin A and other lectins	*Ricinus communis*, human and rat hepatocytes	^{125}I-labelled arylazide glycopeptides		Highly specific photoactivatable site-specific reagents	210
Coupling factor I	Spinach chloroplast thylakoid membrane	2-Azido-ADP		Tight nucleotide-binding site	211
Creatine kinase	Rabbit muscle	IAEDANS and other thiol reagents	Cys	Refolding of denatured enzyme studied	212

Creatine kinase	Rabbit muscle	γ-(*p*-Azidoanilide)-1-N^6-etheno-adenosine-triphosphate		Both subunits labelled	213
Creatine kinase III	Rabbit muscle	2-Chloromercuri-4-nitrophenol	—SH	Two Cys in active site	214
Crotoxin	*Crotalus durissus terrificus*	*p*-Bromophenacyl bromide	His	Irreversible inactivation of component B	215
α-Crystallin	Bovine lens	DTNB, 6-(4′-maleimidyl-anilino)naphthalene-2-sulphonic acid, IAEDANS	—SH	Probes of subunit association	216
Cutinase	*Fusarium solani*	Various	His, Ser, $-CO_2H$	Identification as serine hydrolase	217
γ-Cystathionase	Rat liver	βββ-Trifluoroalanine	Lys	Product is *N*-ε-glycyl-lysine	218

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Cytochalasin B-binding	Human erythrocyte, chicken fibroblast, and rat adipocyte	[^{3}H]Cytochalasin B		Photolabelling	219
Cytochrome b_5	Bovine	Dansyl chloride/deoxycholate	(Lys)	Fluorescence probe of phospholipid interactions	220
Cytochrome b_2	*Hansenula anomala*	Acrylamide	Cys?	High concentrations of acrylamide needed	221
Cytochrome b_6/f-complex	Spinach chloroplast	2,3-Dimethyl-5-(4′-{3-(4-azido-2-nitroanilino)-[2′,3′-^{3}H]-propionoxy}-n-butyl)-1,4-benzoquinone		Photoaffinity label	222
Iso-1-cytochrome C	Yeast	*N*-(4-Azidophenylthio)-phthalimide	—SH	Cleavable *S*-4-azidophenylthio derivative	223
Cytochrome C	Horse heart	*N*-[1-^{13}C]Acetylimidazole	Tyr, Lys	^{13}C n.m.r. study	224
Cytochrome C	Horse heart	*N*-[1-^{13}C]Acetylimidazole	Tyr	Different Tyr reactivities aid n.m.r. assignment	225
Cytochrome C oxidase	Beef-heart mitochondrion	Iodoacetamide	Cys-115	Probe for orientation in membranes	226
Cytochrome C oxidase		1-Myristoyl, 2—12-amino-(4-*N*-3-nitro-1-azidophenyl)dodecanoyl *sn*-glycero-3-[^{14}C]phosphocholine	His-22, Phe-23, His-26	Localization of lipid-binding domain	227
		1-Palmitoyl, 2-(2-azido-4-nitro)-benzoyl *sn*-glycero-3-[^{3}H]-phosphocholine	Thr-27, Met-29		
Cytochrome C/cytochrome C oxidase	Horse/beef heart	EDC, 1-ethyl-3-[3-(trimethylamino)propyl] carbodi-imide	—NH_2/—CO_2H	Subunit II of oxidase linked to C	228
Cytochrome C peroxidase	Bakers' yeast	EDC	—CO_2H	Four to five non-haem —CO_2H of 48 modified	229

Cytochrome P450	*Pseudomonas putida*	2-Bromoacetamido-4-nitro-phenol	Cys	Cys-355 fast-reacting	230
Cytochrome P450	Rat liver	Various cyclopropylamines		Evidence for cation-radical intermediates	231
Cytochrome P450	Rat liver	Chloramphenicol		Protein–ester linkage cleaved by NH_2OH	232
Cytochrome P450	Rat-liver microsome	Dithiobis-(succinimidyl propionate)	$-NH_2$	Two-dimensional electrophoresis studies	233
Cytochrome P450	Rat-liver microsome	Halothane metabolites		Anaerobic inactivation	234
Deoxycytidylate deaminase	*E. coli*	Thymidine triphosphate/u.v.		Reversible photolabelling	235
Deoxyribonuclease	Bovine pancreas	*p*-Nitrobenzenesulphonyl fluoride	Tyr-51, -62, -73, -145, -172	Mild specific reagent	236

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref*
Deoxyribonuclease I/ lysozyme, papain	Ox pancreas, egg white, *Carica papaya*	Dimethyl suberimidate	—NH_2	Effect on response of enzyme activity to ionic strength	237
Dihydrofolate reductase	*Lactobacillus casei*	Iodoacetyl derivative of methotrexate		Slow covalent labelling	238
Dihydrofolate reductase	Chicken liver	Various organomercurials	Cys	Ten-fold increase in activity	239
Dihydrofolate reductase	*Lactobacillus casei*	DEP	His	Six to seven His modified, one to two essentially	240
Dihydrofolate reductase	*Lactobacillus casei*	Tetranitromethane, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole	Tyr	Inactivated for steric reasons	241
DNA-dependent RNA-polymerase	*E. coli*	cAdenosine-5′-trimetaphosphate	His	Affinity reagent	242
Dopamine β-hydroxylase	Bovine adrenal	β-Chlorophenethylamine		Intermediacy of α-aminoacetophenone	243
E apoprotein	Human plasma	Cysteamine	Cys	Gives positively charged Lys analogue	244
Ecdysterone receptor	*Drosophila melanogaster*	Ecdysterone/*hv*		Photolabelling; 15% yield	245
Elastase and other proteases	Human leucocyte and others	Substituted benzoxazinones, quinazolines, and anthranilates	Ser	New reversible specific elastase inhibitors	246
Elastase and others	Human leucocyte and others	Various 2-fluoroacyl-arylsulphonyl fluorides	Ser	Potent specific irreversible elastase inhibitors	247
Elongation factor G 70-S ribosome	*E. coli* MRE 600	Dimethyl-4,9-diaza-5,8-dioxo-6,7-dihydroxydodecanebisimidate	—NH_2	EF-G-binding site located using bifunctional cleavable crosslinker	248
Elongation factor Tu	*E. coli*	*N*-Tosyl-L-PheAla-chloromethane	Cys-81	Role of Cys-81 in tRNA binding	249
Enkephalinase	Mouse striatum	Azidothiorphan		Localization studies	250
Enniatin synthetase	*Fusarium oxysporum*	Various thiol-reactive agents	—SH	Two functionally distinct thiols in active centre	251

Enolase	Yeast	Tetranitromethane	Tyr	Metal-binding site obstructed	252
Enterotoxin A	*Staphylococcus*	Bromoacetic acid	His	Toxic site His modified	253
Epidermal growth-factor receptor	A 431 cell membrane	5′-*p*-Fluorosulphonylbenzoyl adenosine		Identification of receptor	254
Epidermal growth-factor membrane receptor	Mouse	Aminobenzyloxymethyl-paper, diazotized		Immunoautoradiographic detection	255
Epoxide hydrolase	Mouse cytosol	Various chalcone oxides	Cys	Possibly slow-turnover substrates	256
Erabutoxins a, b, c	*Laticauda semifasciata*	Acetyl anhydride, *N*-succinimidyl propionate	Lys	Isoelectric-focusing study	257
Fab fragment of IgG	Rabbit	*N*-[4-(*p*-Maleimidophenyl)-butyryl]phosphatidyl-ethanolamine	—SH	Irreversible coupling to vesicles for targetting	258

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Fab dimer/ferritin	Rabbit	Glutaraldehyde	$-NH_2$	Proteoglycan electron microscopy	259
Factor XI, XIa	Human	Succinic anhydride Phenylglyoxal hydrate Ethylenediamine/EDC	Lys Arg $-CO_2H$	Role of charged groups	260
Fatty-acid synthase	Mammary gland	*S*-(4-Bromo-2,3-dioxobutyl)-CoA	—SH	—SH essential for condensing partial reaction	261
Fatty-acid synthetase	Chicken liver	1,3-Dibromo-2-propanone	—SH	Cysteine and pantatheine crosslinked in homodimer	262
Fatty-acid synthetase	Pigeon liver	*S*-(4-Bromo-2,3-dioxobutyl)-CoA	Cys, 4′-phosphopantatheine	Affinity label	263
Fatty-acid synthetase	*Brevibacterium ammoniagens*	Dimethyl suberimidate	Lys	Subunit structure by e.m.	264
Fatty-acid synthetase	Pigeon liver	DTNB	—SH	Intramolecular pantatheino-Cys link	265
Fatty-acid synthetase	Pigeon liver	(i) Pyridoxal 5′-phosphate, (ii) NaB^3H_4	Lys, ε-NH_2	Enoyl reductase activity inactivated	266
Fibrin	Bovine	2-Methoxy-2,4-diphenyl-3(2H)-furanone		Fluorescent substrate for proteases	267
Fibrinogen receptor/fibrinogen	Human platelet, human plasma	Methyl 4-azidobenzoimidate	$-NH_2$	Photoreactive heterobifunctional crosslinker	268
Fibronectin (and others)	Human plasma	EDC/Glycine ethyl ester	$-CO_2H$	Enhanced fibronectin binding to modified proteins	269
Fibronectin	Human plasma	Succinic anhydride Phenylglyoxal EDC/glycine ethyl ester	Lys Arg $-CO_2H$	Arg and Lys in interaction with gelatin	270
Fibronectin	Human plasma	3-[(2-Nitro-4-azidophenyl)-2-aminoethyldithiol]-*N*-succinimidyl propionate	Cys	Photoactivatable, heterobifunctional, reversible crosslinker	271

Flavocytochrome b_2	Bakers' yeast	Bromopyruvate	Cys	Location of active-site Cys	272
Flavocytochrome b_2	Bakers' yeast	2-Keto-3-butynoate	Cys	Highly specific for Cys at neutral pH	273
Folate transport system	*Lactobacillus casei*	Folic acid/EDC	Lys	*O*-Acyl isourea derivative of folate	274
Fructose 1,6-bi-phosphatase	Porcine kidney	Pyridoxal 5'-phosphate/$NaBH_4$ DTNB	Lys Cys	Effects on sugar binding	275
Fructose 1,6-bi-phosphatase	*Ceratatis capitata*	Tetranitromethane	Tyr	One essential, two inessential Tyr per subunit	276
β-Galactosidase	*E. coli*	Iodine	Tyr-253	Inactivation on mono-iodination	277
Glucagon	Pig	Methyl acetimidate (*N*-Hydroxysuccinimido)biotin	Lys-12 α-NH_2	Hormone receptor visualization using fluoroescein–avidin	278

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Glucagon	Porcine	Dimethylsulphoxide/HCl	Trp-25, Met-27	Oxindoylylalanine and Met sulphoxide produced	279
Glucagon		Citraconic anhydride	His-1 α-NH_2, Lys-12 ε-NH_2	α-NH_2 restored by 10 mM HCl/40 °C, ε-NH_2 by pH 4.0/R.T.	280
Glucagon	Pig	Iodine/chloramine-T	Met-27	Effect on receptor binding	281
Glucoamylase	*Aspergillus saitoi*	*N*-Bromosuccinimide	Trp	Studies on Trp residues	282
Glucoamylase	*Aspergillus saitoi*	CMC	—CO_2H	Characterization	283
β-Glucocerebrosidase	Human placenta	Trimannosyldilysine/EDC	—NH_2	8—9 mol of Man_3Lys_2/67 000 mol. wt. subunit	284
Glucocorticoid receptor	Murine mammary cytoplasm	Phenylglyoxal and other dicarbonyls	Arg	Arg in binding site	285
D-Glucose transporter	Human erythrocyte	[^{3}H]Cytochalasin B/$h\nu$		Photoaffinity labelling	286
Glucose transporter	Human erythrocyte	[^{3}H]Cytochalasin B		Natural photoaffinity ligand	287
Glucose transport protein		[^{3}H]Cytochalasin B		Photoaffinity label identifies protein	288
Glucose 6-phosphate translocase	Rat-liver microsome	4,4′-Di-isothiocyano-1,2-diphenyl[^{3}H]ethane-2,2′-disulphonic acid	—NH_2		289
Glucose transport proteins	Human erythrocytes	*N*-Maleoylmethionine sulphone	—SH	Membrane impermeant reagent	290
α-Glucosidase	Human liver	*p*-Aminophenyl-β-D-galactopyranoside/EDC	—CO_2H	Retention of activity	291
β-Glucosidase/casein Sepharose 4B		Glutaraldehyde, *m*-maleimidobenzyl *N*-hydroxysuccinimide ester	—NH_2	New proteinase assay	292
β-Glucuronidase	Murine urine	*N*-Ethyl-*S*-phenylisoxazolium-3′-sulphonate	—CO_2H	Essential residues	293
		Tetranitromethane	Tyr		
		Succinic anhydride and others	Lys		

Glutamate dehydrogenase	Bovine liver	5′-[(*p*-Fluorosulphonyl)-benzoyl]-1-N^6-etheno-adenosine		Modification without inactivation	294
Glutamate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase	Bovine liver, yeast	Pyrene maleimide	Cys-115 Cys-149	Specific fluorescent labelling	295
Glutamate semialdehyde dehydrogenase	*E. coli* CSH52 pLC44-11	*p*-Chloromercuribenzoate Dimethyl suberimidate	—SH $—NH_2$	Characterization of structure and function	296
Glutamate synthase	*Saccharomyces cerevisiae*	Homocysteine sulphonamide	—SH	Transition-state inhibitor	297

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Glutaminase (phosphate dependent)	Rat kidney	6-Diazo-5-oxo-L-[6-^{14}C]-norleucine		Turnover *versus* labelling	298
Glutamine phosphoribosyl pyrophosphate amidotransferase	*E. coli* pur F	[6-^{14}C]Diazo-5-oxa-norleucine	Cys-1	Affinity analogue of glutamine	299
Glutamine synthetase	*E. coli*	Methionine sulphoximine, ethionine sulphoximine, 3-amino-3-carboxytetrahydrothiopyran sulphoximine		ATP-dependent inactivation 'freezes' conformations	300
γ-Glutamylcysteine synthetase	Rat kidney	Cystamine-Sepharose	—SH	Active-site affinity probe	301
γ-Glutamyltransferase	Rat pancreas	6-Diazo-5-oxo-L-norleucine 5-Iodoacetamidofluorescein	Ser or Thr Met?	Characterization	302
γ-Glutamyltranspeptidase	Rat kidney	*p*-Diazohippurate *p*-Diazobenzoate		Subunit crosslinking produced	303
γ-Glutamyltranspeptidase	Rat kidney	3-Trifluoromethyl-3-(*m*-[^{125}I]-iodophenyl)diazirine		Labels hydrophobic domain	304
Glutathione reductase	Yeast	Benzyl selenosulphate *p*-Nitrobenzyl selenosulphate	—SH	Intermediate selenosulphides	305
Glutathione reductase	Human erythrocyte	Butane-2,3-dione, cyclohexane-1,2-dione, phenylglyoxal	Arg	Pyridine nucleotide-binding sites contain Arg	306
Glutathione transferase B (ligandin)	Rat-liver cytosol	*NN*-Dimethyl-3′-methyl-4-aminoazobenzene	Cys?	Characterization of subunits' genetic origin	307
Glyceraldehyde-3-phosphate dehydrogenase	*Bacillus stearothermophilus*, sturgeon	3-Chloroacetylpyridine–adenine dinucleotide	Cys-149	Affinity labelling	308
Glyceraldehyde-3-phosphate dehydrogenase	*Palinurus vulgaris* and pig	β-(2-Furyl)acryloyl phosphate		'Half-of-the-sites' substrate analogue gives chromophoric acyl-enzyme in crystal form	309

D-Glyceraldehyde-3-phosphate dehydrogenase	Porcine muscle	Iodoacetamide	Cys	Reaction rates in crystal similar for all four subunits	310
Glyceraldehyde-3-phosphate dehydrogenase	Rat muscle	Butane[2,3-^{14}C]dione	Arg	Arg-134 essential	311
Glyceraldehyde-3-phosphate dehydrogenase	Rabbit muscle	Various thiol reagents	Cys-149	Activity loss does not parallel modification	312
Glyceraldehyde-3-phosphate dehydrogenase	Rabbit muscle	Octamethylene bis(methane-[^{35}S]thiosulphonate)	Cys-149 Cys-281	Intersubunit crosslinks	313
Glyceraldehyde-3-phosphate dehydrogenase	Yeast	Iodoacetate, iodoacetamide	Cys-149	Effects on binding and fluorescence of Auramine *O*	314

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Glycerol-3-phosphate dehydrogenase	Rabbit muscle	Pyridoxal 5′-phosphate/$NaBH_4$	Lys	Label at NADH-binding site	315
Glycophorin A	Human erythrocytes	Photoreactive phospholipids [^{14}C]Dicyclohexylcarbodi-imide	Glu-70	Delineation of region embedded in vesicle membranes	316
Glyoxalase II and others		Glutathione derivatives		Photoaffinity labels for GSH-utilizing systems	317
cGMP-binding proteins	*Drosophila*	[^{32}P]cGMP		Direct photoaffinity labelling	318
cGMP-dependent protein kinase	Bovine lung	5′-*p*-Fluorosulphonyl[^{14}C]-benzoyladenosine	Lys	Determination of active-site sequence	319
Gonadatropin-releasing hormone antagonist		Ethylene glycol bis-(succinimidyl)succinate	Lys	Dimer can act as agonist	320
Gonadotropin-releasing hormone receptor	Rat ovarian granulosa cell	[N^{ϵ}-Azidobenzoyl-D-Lys^{6}]-GRH/*hν*		Photoaffinity labelling	321
Gonadotropin-releasing hormone receptor	Rat pituitary membrane	[Azidobenzoyl-D-Lys^{6}]-GRH		Photoaffinity labelling	322
Gonadotropin	Human chorionic	Succinic anhydride Cyclohexane-1,2-dione	Lys Arg	Activity reduced	323
Gramicidin S synthetase 1	*Bacillus brevis*	Phenylglyoxal	Arg	Single essential Arg	324
Gramicidin S synthetase 2	*Bacillus brevis*	Phenylglyoxal	Arg	Role of Arg in ATP binding	325
Isoleucyl tRNA synthetase	*E. coli*				
Growth hormone	Human	Disuccinimidyl suberate	$-NH_2$	Analysis of complex	326
Growth-hormone receptor	Rabbit liver				
Haemagglutinin A	*Helix pomatia*	Fluorescein isothiocyanate		T-Lymphocyte mapping	327

Haemoglobin	Human erythrocyte	*N*-Iodoacetylaminoethyl-5-naphthalene-1-sulphonate	Cys-β93	Probe for ligand effects on structure	328
Haemoglobin	Human	Acetic anhydride DTNB	Val-1α Val-1β	pK and reactivity *via* competitive labelling	329
Haemoglobin	Human	D-Glucose-6-phosphate	α-NH_2	Keto–amine bond in β-chains	330
Haemoglobin	Human erythrocyte	4-Isothiocyanatobenzoic acid 4-Isothiocyanatobenzene-sulphonamide	Val-1	Effect on chloride binding	331
Haemoglobin	Human erythrocyte	[^{3}H]Pyridoxal/$NaBH_4$	Val-α1 α-NH_2	Schiff-base formation	332
Haemoglobin	Human erythrocyte	21 thiol reagents	Cys-β93	Effects related to sterically restricted access and negative charge shielding	333

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Haemoglobin	Human erythrocyte	Various crosslinkers		E.m. studies on whole cells	334
Haemoglobin A	Human erythrocyte	Cyanate	*N*-Terminal Val	Contribution of *N*-termini to total buffering power	335
Haemoglobin A	Human erythrocyte	Cystine dimethyl ester, cystine diethyl ester, iodoacetamide	Cys-β93	Decrease in pK of deoxy Bohr-effect group	336
Haemoglobin A	Human erythrocyte	4,4′-Dithiodipyridine	Cys-β93	Reactivity as function of degree of O_2 saturation	337
Haemoglobin A	Human	Glucose	α-NH_2	Formation of Schiff base *via* labile intermediate	338
Haemoglobin A	Human erythrocyte	Glycineamide/trypsin	Arg-α141	Reversible enzymatic peptide formation	339
Haemoglobin A	Human erythrocyte	Pyridoxal 5′-phosphate	Val-β1, Lys-β82	Major product (Val-1-PLP)$_2$Hb	340
Haemoglobin S	Human	Acetaldehyde	Lys, Tyr, α-NH_2	Increased solubility and O_2 affinity	341
Haemoglobin S	Human erythrocyte	Bis-(3,5-dibromosalicyl)fumarate	Lys-82β_1, Lys-82β_2	Increases solubility of deoxy form	342
Haemoglobin S	Human	Dimethyl adipimidate	Lys-40α, Lys-127α(?)	O_2 affinity and solubility increased	343
Haemoglobin S	Human	Dimethyl adipimidate	Lys	Intermolecular (β^s–β^s) and intramolecular (α–β^s) cross-linking	344
Hexokinase	Rat brain	Phenylglyoxal	Arg	Four slow-reacting Arg essential	345
Hexokinase	Yeast	Cibacron Blue F3G-A/metal ions		Bivalent metal ions accelerate inactivation	346
Hexokinase/protein kinase	Ehrlich ascites cells	2-Isobutyl 3-oxo-4-chloro-2-butyl carbonate	(—SH)	Glycolysis inhibitor	347
Hexokinase	Ascites tumour cell	Dimethyl suberimidate	—NH_2	Functional role of Lys	348
Hexose transport protein	Chicken embryo fibroblast	[^{3}H]Cytochalasin B/$h\nu$		Photoaffinity label	349

High-density lipoprotein	Human serum	Various azido-palmitic, -oleic, and -linoleic acids		Photocrosslinking	350
High-mol.-wt. (138—340k) glycoproteins	Chinese-hamster ovary	Isethionyl 3-(*N*-2,4-dinitrophenyl)aminopropionimidate	$-NH_2$	Membrane-impermeable immunoreactive cell-surface probe	351
Histidine decarboxylase	*Lactobacillus* 30a	5,5′-Dithiobis-(2-nitrobenzoic acid)	—SH	Non-essential —SH: CN^- reverses inactivation	352
Histidine decarboxylase	Rat gastric mucosa	2-Hydroxy-5-nitrobenzyl bromide	(Trp)	Characterization of activity	353
Histones	Goose, chicken	Succinic anhydride	Lys	Incomplete succinylation and electrophoresis gives molecular size	354

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Histone H3	Chicken erythrocyte	Fluorescein disulphide	—SH	Reversible fluorophore label	355
Histone H3	Chicken erythrocyte	*N*-[*p*-(2-Benzimidazolyl-phenyl)]maleimide	Cys-110	Super-reactivity reduced by removing highly basic portions of H3	356
Hormone-binding proteins	Rat-liver nuclei	Thyroxine/*hν* 3,5,3′-Tri-iodothyronine/*hν*		Photoaffinity labelling by underivatized hormones	357
p-Hydroxybenzoate hydroxylase	*Pseudomonas desmolytica*	DEP	His	Essential His	358
p-Hydroxybenzoate hydroxylase	*Pseudomonas fluorescens*	DEP	His	Two essential His	359
D-β-Hydroxybutyrate apodehydrogenase	Rat-liver submitochondrial vesicles	α-Dicarbonyl reagents	Arg	Arg in binding site	360
D-β-Hydroxybutyrate dehydrogenase	Rat-liver mitochondrion	α-Dicarbonyl reagents	Arg	Study of reagents' membrane permeability	361
3β,20α-Hydroxysteroid oxidoreductase	Calf blood	19-Nortestosterone 17-bromoacetate		3β,20α dual activity at active site	362
IgA (myeloma 315)		Tetranitromethane	Tyr-34$_L$ and -33$_H$	N.m.r. studies of nitrated protein	363
IgE	Mouse	Fluorescein 5′-isothiocyanate		Distribution of F_c receptors in cell-membrane complex	364
IgG	Rabbit	Aminobutylethylisoluminol isothiocyanate	(—NH_2)	Chemiluminescent label for immunoassay	365
IgG	Rabbit	[6-(*N*-4-Aminobutyl)-*N*-ethyl]-amino-2,3-dihydrophthalazine-1,4-dione and EDC	—CO_2H	Chemiluminescent conjugate	366
IgG	Mouse	1-(*p*-Benzyldiazonium)EDTA and others	(Tyr)	Chelate labels for tumour imaging	367
IgG	Rabbit	Various	Various	C1q-binding site identified	368
IgG	Rabbit	Acetic anhydride	Lys-322	Confirms C1q-receptor site	369

IgG (anti-CEA)	Goat serum	*p*-{1,2-Dicarba-*closo*-[1-^{3}H]-dodecaboran(12)-2-yl}-benzenediazonium ion		Boron labelling for neutron-capture therapy	370
IgG, liposomes	Mouse	SPDP	—NH_2, —SH	Liposome targetting	371
IgG, Sendai virus particles	Human	SPDP	—NH_2, —SH		372
IgG fraction	Human serum	DTNB	—SH, S—S	Disulphide exchange with labile protein S—S	373
IgG fragments	Sheep	Monobromobimane	—SH	Uses —SH from reduced $(Fab)_2$	374

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Immunoglobulins/ protein A–Sepharose 4B		Dimethyl pimelimidate	—NH_2	Immunoaffinity matrix	375
Immunoglobulins	Mouse, rabbit	(4-Hydroxy-3-iodo-5-nitrophenyl)acetic acid, oxysuccinimide ester (4-Hydroxy-3-nitrophenyl)-acetic acid, oxysuccinimide ester		Hapten-self Ig complexes	376
Initiation factor 2	*E. coli* MRE 600	Fluorescein isothiocyanate	—NH_2	Fluorescence studies of binding to 30S ribosomal subunits	377
Insulin	Beef	(i) Methylsulphonylethoxycarbonyl *N*-hydroxysuccinimide ester (ii) Dicarboxylic acid bis-(*p*-nitrophenyl) esters	—NH_2	Preparation of dimers	378
Insulin	Bovine	Dithiothreitol	Disulphide	Enthalpy of reduction measured	379
Insulin	Porcine	3-[4-(*N*-Biotinyl-6-aminocaproyloxy)phenyl] propionic acid *N*-succinimide ester	Lys	Cleavable biotin/avidin handle	380
Insulin derivative	Pig	Hydroxysuccinimide esters of carboxyl-substituted biotin analogues	—NH_2	Hormone derivatives for affinity chromatography	381
Insulin receptor	Rat-liver membranes	DEP	His	His required for insulin binding	382
Insulin receptor	Rat adipocyte	$N^{\varepsilon B29}$- and $N^{\varepsilon R1}$-(Azidobenzoyl) insulins		Subunit structure identified	383
Insulin receptor/insulin	3T3-L1 adipocytes	Disuccinimidyl suberate	—NH_2	Receptor identification	384
Insulin receptor/insulin	Rat adipocyte	Disuccinimidyl suberate	—NH_2	Role of —S—S— in receptor	385

[D-Ala^{A1}]Insulin	Beef	Bis-(4-nitrophenyl)adipate		Dimer preparation	386
Insulins I and II/nitrocellulose paper	Rat pancreas	*N*-Hydroxysuccinimidyl *p*-azidobenzoate	—NH_2	Immobilization for immunoelectrophoresis	387
Insulin receptor	Rat adipocyte	Ten crosslinking reagents	—NH_2, —SH	Intra- and inter-molecular crosslinking	388
Insulin receptor	Rat adipocyte	(2-Nitro-4-azidophenylacetyl^{B2})-des-Phe^{B1}-insulin		Photoaffinity labelling	389
Insulin (^{125}I)/insulin receptor	Human lymphoblastoid cell IM-9	Disuccinimidyl suberimidate	—NH_2	Study of receptor subunit structure	390
Insulin receptor/insulin	Rat-heart myocyte	Disuccinimidyl suberate	—NH_2	Receptor characterization	391
Insulin receptor	Rat hepatocyte	[2-Nitro-4-azidophenylacetyl^{B2}]-des-Phe^{B1}-insulin		Photoaffinity labelling	392

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Insulin receptor	Rat pancreatic islet	EDC	$-CO_2H$	Insulin binding reduced	393
		1,3-Difluoro-4,6-dinitrobenzene, tetranitromethane	Tyr, Cys		
Insulin-like growth-factor receptor	Chicken-embryo fibroblast	Disuccinimidyl suberate	$-NH_2$	Receptor characterization	394
Insulin-like growth-factors I and II receptors/insulin-like growth factors I and II	Various human and rat membranes and cells	Disuccinimidyl suberate			395
Interferon	Rabbit and mouse fibroblast	Phe-halomethyl ketone	His	Hydrophobic site?	396
Interferon α-2/interferon receptor	Human leukocyte, human cells	Dithiobis-(succinimidyl propionate)	$-NH_2$	Receptor characterization	397
		Disuccinimidyl suberate			
Isocitrate dehydrogenase (DPN-dependent)	Porcine heart	3-Bromo-2-ketoglutarate, 3-ene-2-ketoglutarate	Cys	Subunit function	398
		Glycine ethyl ester/1-cyclohexyl-3-(2-morpholinoethyl)-carbodi-imide	Glu, Asp		
		Cyanate	Lys		
Isocitrate dehydrogenase	Pig heart	(*RS*)-3-Bromo-2-ketoglutarate		Substrate and inactivator	399
Isocitrate dehydrogenase	Pig heart	3,4-Didehydro-2-ketoglutarate		Decomposition product of 3-bromo compound	400
Juvenile-hormone-binding proteins	*Leucophaea maderae*	10,11-Epoxyfarnesyl diazoacetate		Photoaffinity labelling	401
Juvenile-hormone-synthesizing enzymes	Insects	Allatoxins		Oxidative activation to quinone methide	402
Kinases	Various	Various iodoacetyl derivatives of ATP		*Exo*-active-site-directed reagents	403

Lac carrier protein	*E. coli*	DEP, Rose Bengal/light	His	Specific inactivation of lactose transport	404
β-Lactamase	*S. albus* G, *Actino-madura* R39	Clavulanate	Ser and others	Branched reaction pathways studied	405
β-Lactamase	*S. albus* G, *Actino madura* R39	β-Iodopenicillanate	Ser and others	Turnover gives dihydro-thiazine	406
β-Lactamases	*P. aeruginosa*, *E. coli*	Cloxacillin	Ser	Stable acyl-enzyme isolated	407
β-Lactamase	*E. coli*	Olivanic acids	(Ser)	Acyl-enzyme tautomerization	408
β-Lactamase	*Bacillus cereus*	Dansyl-penicillin	Ser	Covalent intermediate trapped at low pH	409
β-Lactamase	*Bacillus cereus*, *E. coli*	6-β-(Trifluoromethane sulphonyl)amidopenicillanic acid sulphone		Potent inhibitor	410

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
β-Lactamase I	*Bacillus cereus*	Phenylglyoxal	Arg	Eight Arg modified	411
β-Lactamase (R_{TEM}), β-lactamase I	*E. coli, Bacillus cereus*	(i) Tetranitromethane (ii) Sodium dithionite (iii) Carbonyl di-imidazole *N*-(2,2,2-trifluoroethoxy-carbonyl)imidazole	Tyr-105 —NO_2-Tyr-105 —NH_2-Tyr-105	Produces benzoxazolinoyl-alanine residue	412
Lactate dehydrogenase	Pig heart	Tetranitromethane	Tyr-237	Reporter group for slow structural changes	413
L-Lactate dehydrogenase	*Lactobacillus casei, Lactobacillus curvatus*	Dimethyl-(2-hydroxy-5-nitrobenzyl)sulphonium bromide	Trp	Probe for effector-induced conformational changes	414
Lactate dehydrogenase M4	Porcine	*O*-(4-Diazo-3,5-di[^{125}I]iodobenzoyl)sucrose	(Tyr)	Endocytosis study	415
Lactoperoxidase	Bovine milk	Butane-2,3-dione/*hν* and other diketones	Trp, Met, Arg, Tyr, Asp, Ser, Glu, His	Sensitized photochemical inactivation not specific for Arg	416
Lactoperoxidase	Bovine milk	Various α-dicarbonyls/*hν*		Light exclusion necessary for specific Arg modification	417
Lactoperoxidase	Bovine milk	Diazotized sulphanilate	Tyr, His	His not essential	418
Large-T protein	Polyoma and SV40 viruses	Periodate-oxidized [α-^{32}P]ATP	—NH_2	Ribose moiety of ATP cleaved and oxidized	419
Lectin (anti-B specific)	*Streptomyces* sp.	NBS	Trp	Two residues modified	420
Lipase	*Geotrichum candidum*	EDC, CMC	—CO_2H	EDC modifies 9-CO_2H, CDC-4	421
Lipase, colipase	Pancreas	Dimethylsuberimidate	—NH_2	Possible lipid-binding-site induction	422
15-Lipoxygenase	Soybean	14,15-Dehydroarachidonic acid		Possible pentadienyl free-radical mechanism	423

Liver plasma membranes	Rat	2-[(2-Nitro-4-azidophenyl)-sulphenyl]-Trp^{25}-glucagon		Photoactivated irreversible activation of adenylase cyclase	424
Low-density lipoprotein	Human	Cyclohexane-1,2-dione	Arg	Modification decreases cell uptake	425
Low-density lipoprotein	Human plasma	Cyclohexane-1,2-dione	(Arg)	Liver-binding studies	426
Low-density lipoprotein	Human plasma	Cyclohexane-1,2-dione	Arg	Clearance delayed by abolition of receptor binding	427
Low-density lipoprotein	Human plasma	Formaldehyde/$NaBH_4$, sucrose	Lys	Catabolism studies	428
Low-density lipoprotein	Human serum	*N*-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-maleimide		Spin labelling	429

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Low-molecular-weight phospho-carrier protein	*Salmonella typhi-murium*	3-[(2-Bromoacetimido)-methyl]-2,2,5,5-tetra-methyl-1-pyrrolidinyloxyl	Met-1	E.s.r. probe	430
Low-molecular-weight phospho-carrier protein	*Salmonella typhi-murium*	(*N*-Iodoacetylamino-ethyl)-5-naphthyl-amino-1-sulphonate	Met-1	Fluorescence probe	431
Lysozyme	Hen egg-white	*N*-Bromosuccinimide	Trp-62	Enzymic activity	432
Lysozyme	Hen egg-white	[^{13}C]Formaldehyde/NaCNBH$_3$	ε-NH$_2$, α-NH$_2$	N.m.r. assignments	433
Lysozyme	Hen egg	Mercaptoethanol and methyl *p*-nitrobenzenesulphonate	Disulphide	Effects of partial reduction studied	434
Lysozyme	Hen egg	Ethyleneimine	Carboxyl	2-Aminoethyl esters formed	435
Lysozyme	Hen egg	*exo-cis*-3,6-*endo*-Epoxy-4,5-*cis*-epoxyhexahydrophthalic anhydride	(Lys)	Versatile reversible hetero-bifunctional crosslinker	436
Lysozyme	Hen egg	Tetranitromethane	Tyr	N.m.r. study of two modified Tyr	437
Lysozyme	Hen egg	Ozone	Trp-62	Specific oxidation at low O$_3$ concs.	438
α_2-Macroglobulin	Human	Simple amines	Thioester	Conformational changes studied	439
α_2-Macroglobulin	Human	[^{14}C]Methylamine 2,4-Dinitrophenyl thiocyanate	Thioester Cys	Thioester cleavage related to conformational change	440
α_2-Macroglobulin	Human	[^{14}C]Methylamine		Kinetic study	441
α_2-Macroglobulin-like protein	*Pleuronectes platessa*	Methylamine	Thioester?	Low-molecular-weight homologue of human protein	442
Malate dehydrogenase	Porcine heart mitochondrion	Iodoacetamide	His (active centre)	Study of hybrid-modified dimer	443
Malate dehydrogenase	Pig and chicken cytoplasm and mitochondrion	Methyl acetimidate, methyl-4-hydroxy-3-nitrobenzimidate, acetylimidazole	Lys	Characterization	444

Malate thiokinase		Methoxycarbonyl-CoA disulphide	—SH	Half-of-the-sites reactivity	445
Malic enzyme	Pigeon liver	Periodate-oxidized NADP	Lys	Essential Lys	446
Malonyl-CoA decarboxylase	Goose uropygial gland	Acetic anhydride	Lys	Specific acylation: essential Lys	447
α-Melanotropin	Synthetic	2-Nitro-4-azidophenylsulphenyl chloride	Trp-9 Lys-11	Reaction at Lys as well as at Trp	448
Membrane proteins	Bovine striatum	[^{3}H]Chlorpromazine		Photocrosslinking	449
Membrane proteins	*Halobacterium halobium*	5-Isothiocyanato-1-naphthalene azide, *p*-azidophenylisothiocyanate	—NH_2	New photoactivatable hydrophobic heterobifunctional crosslinking reagents	450

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Membrane proteins	Bovine brain	Lysergic acid diethylamide/u.v.		Irreversible photoaffinity labelling	451
Membrane proteins	Human erythrocyte	4-Maleimide-2,2,6,6-tetramethylpiperidino-oxyl	—SH	E.s.r. conformational studies	452
Membrane proteins	Rat-liver mitochondria	Various maleimides	—SH	Role of —SH in permeability	453
Membrane proteins	Human erythrocytes	Periodate + 2-(4-aminophenyl)-[1-^{14}C]ethylamine + $NaCNBH_3$ Disuccinimidyl 3,3′-dithiobis-(propionate)		Membrane topology study	454
Membrane proteins	Human erythrocytes	Bis-(sulphosuccinimidyl)-suberate, 3,3′-dithiobis-(sulphosuccinimidyl propionate)		Water-soluble crosslinkers	455
Membrane proteins	Rat adipocyte	4-Azidobenzoyl calmodulin		Saturatable labelling	456
Mercuric reductase	*Pseudomonas aeruginosa* pA 09501	DTNB	—SH	Presence of redox-active disulphide	457
Meromyosin	Rat muscle	Mixed anhydrides of substituted benzoic acids and mononucleotides		Variety of phosphorylating agents used	458
Meromyosin/subfragment 1	Rat muscle	Mixed anhydride of AMP and mesitylene carboxylic acid		Phosphorylation not acylation	459
Microtubule protein	Bovine brain	Formaldehyde/$NaCNBH_3$	—NH_2	Essential —NH_2 in α-chain	460
Mucor rennin	*Mucor pusillus*	Methylene Blue/*hν*	His	Essential His (photo-oxidation)	461
Muscarinic receptors	Rat adenohypophysis	*N*-Methyl-4-piperidylazidobenzilate		Photoaffinity label	462
Muscarinic receptor	Rat cerebral cortex	*N*-Methyl-4-piperidylbenzilate/*hν*		Powerful photoaffinity probe	463
Myoglobin	*Applysia brasiliana*	Isothiocyanate spin label	—NH_2	Temperature-induced conformation changes	464

Myosin	Chicken gizzard	FDNB	Cys	Light and heavy chains modified: activity restored by thiolysis	465
Myosin	Sheep-heart atrium and ventricle	*N*-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyl)iodoacetamide, *N*-ethylmaleimide	Cys	Structural differences near fast-reacting SH-1 in two isozymes (e.s.r.)	466
Myosin alkali light-chain subunits A1 and A2	Vertebrate skeletal muscle	Tetranitromethane	Tyr	Affects reassociation to heavy chains	467
Myosin R-LC	*Aequipecten irradians*	*N*-Hydroxysuccinimidyl-4-azido-benzoate	Lys	Heterobifunctional photocrosslinkers	468
	Mercenaria mercenaria Rabbit	*p*-Azidophenacyl bromide	Cys		
Myosin S-1	Rabbit skeletal muscle	*N*-Ethylmaleimide		Extensive modification	469

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Myosin S-1	Rabbit skeletal muscle	*N*-(1-Oxyl-2,2,6,6-tetra-methyl-4-piperidinyl)iodo-acetamide	—SH	E.s.r. studies	470
Myosin subfragment 1	Canine cardiac muscle	CMC, methyl *p*-toluene sulphonate/nitrotyrosine ethyl ester	$—CO_2H$	Single essential $—CO_2H$	471
Myosin subfragment 1	Rabbit skeletal muscle	5′-*p*-Fluorosulphonyl benzoyl adenosine	—SH	Forms internal —S—S—	472
Myosin subfragment 1	Rabbit skeletal muscle	*NN′*-*p*-Phenylene dimaleimide and other reagents	—SH	Reagents of varying length	473
Myosin subfragment 1	Rabbit	TNBS IAEDANS	Lys Cys	Group interactions studied	474
Myosin subfragment 1	Rabbit muscle	*N*-[7-(Dimethylamino)-4-methyl-3-coumarinyl]-maleimide	—SH	Zero-length crosslinking to actin	475
		EDC	$—CO_2H/—NH_2$		
Myosin subfragment 1		*N*-[4-(Trifluoromethyl)phenyl]-iodoacetamide	—SH	^{19}F n.m.r. reporter study	476
Myosin subfragment 1		5-(Iodoacetamido)fluorescein		Binding of MgATP studied	477
NAD glycohydrolase	*Bungarus fasciatus*	Pentane-2,4-dione *N*-Ethyl-5-phenyl-isoxazolium-3′-sulphonate	Lys $—CO_2H$	Lys, $—CO_2H$ essential for activity	478
7S nerve growth factor	Mouse	NBS	Trp	One essential Trp modified	479
Neurophysin	Bovine	Cyclohexanedione	Arg	Arg-8 function	480
Neurophysin	Human and bovine posterior pituitary tissue	L-[Methyl-^{3}H]Met-L-Tyr-*p*-azido-L-Phe amide	Tyr-49	Tyr-49 close to high-affinity hormone-binding site	481
Neutral protease	*Streptomyces naraensis*	DEP	His	Activity regeneration by NH_2OH	482

Nucleosome core proteins	Chromatin	*N*-(2,2,5,5-Tetramethyl-3-carbonylpyrrolidinyl-1-oxy)-imidazole	Tyr	Spin-label study	483
Nucleosome core particle	Chicken erythrocytes	TNBS Butane-2,3-dione	Lys Arg	Arg essential to DNA folding in particle	484
Oestradiol 17β dehydrogenase	Human placenta	12β-(Bromoacetoxy)-4-oestrene-3,17-dione, 16α-(bromo-acetoxy)-1,3,5(10)-oestra-triene-3,17β-diol 3-methyl ether	His	Two His labelled in active centre	485
Oestradiol 17β dehydrogenase	Human placenta	17β-[1(*R*)-1-Hydroxy-2-propynyl]androst-4-en-3-one, 17β-[1-oxo-2-propynyl]-androst-4-en-3-one		Irreversible affinity alkylators	486
Oestrogen receptor	Rabbit-uterus cytoplasm	3-Hydroxy-17β-(*p*-nitrophenyl-dithio)-1,3,5(10)-oestratriene	Cys	New affinity label	487

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Oestrogen synthetase (aromatase)	Human placenta	19,19-Difluoroandrost-4-ene, 3,17-dione		Acyl fluoride postulated	488
Oestrogen synthetase	Human placenta	(17α-Ethynyl-19-nortestosterone)		Cofactor required for inactivation	489
Opiate receptor	Rat brain	Diazoamide and aryl azide derivatives of fentanyl		Some irreversible attachment of ^{3}H label	490
Opiate receptor	Mouse-brain synaptosome	Morphinone	Cys	Analgesic effect of morphine blocked	491
Opiate receptor	Rat-brain membrane	2-Nitro-4-azidophenyl-D-Ala-Leu-enkephalin derivatives		Photoaffinity labelling	492
Opiate receptor	Rat-brain synaptosome	DEP, Rose Bengal/*hv*	His	His at ligand-binding site	493
Opiate receptor	Rat synaptosomal membrane	DTNB and other disulphides	—SH	Effect of reagent charge on agonist binding	494
Ornithine decarboxylase	Hepatoma cell and variant	[^{14}C]Difluoromethylornithine		Specific irreversible inhibitor	495
Ornithine decarboxylase	Mouse kidney	α[S-^{14}C]Difluoromethylornithine		Autoradiographic location of enzyme *in vivo*	496
Ornithine decarboxylase	Rat, mouse	α-Difluoromethyl[5-^{14}C]-ornithine		Quantitation of enzyme in tissues	497
Ornithine decarboxylase	*E. coli* *P. aeruginosa*	α-Fluoromethylornithine α-Difluoromethyl arginine		Inhibition of bacterial growth	498
Ornithine decarboxylase	*E. coli*	DL-α-Monofluoromethyl-putrescine		Specific to *E. coli* enzyme	499
Ornithine decarboxylase	Rat liver	α-[5-^{14}C]Difluoromethyl-ornithine		Two active sites found	500
Ovotransferrin		Bromopyruvate	Anion-binding site	*Via* ternary complex including Fe	501
5-Oxo-L-prolinase	Rat kidney	DTNB, NEM, 5′-*p*-fluorosulphonyl benzoyl adenosine and inosine	Cys	Role of —SH	502

Papain		2-(*N'*-Acetyl-L-phenylalanyl-amino)ethyl 2'-pyridyl disulphide	Cys-25	S_2-Subsite-specific two-protonic-state probe	503
Papain	*Papaya carica*	Methyl-3-nitro-2-pyridyl disulphide Methyl-2-pyridyl disulphide	—SH	Methanethiolation by disulphide interchange	504
Papain	*Papaya carica*	(1-Oxyl-2,2,5,5-tetramethyl-Δ^3-pyrroline-3-methyl)methane thiosulphonate	—SH	Thiol-selective spin label	505
Papain		Various thionoesters	Cys	Resonance Raman study of dithioacyl enzymes	506
Papain/ficin	*Papaya carica/Ficus glabrata*	Chloroacetate	Cys	pH dependence of alkylation	507
Papaya peptidase A	*Papaya carica*	2,2'-Dipyridyl disulphide, 4-chloro-7-nitrobenzofurazan	Cys	Reactivity probes	508

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Parathyroid hormone receptor/parathyroid hormone	Canine renal plasma membrane	2-Nitro-5-azidophenylsulphenyl chloride		Mol. wt. 60 000 component identified	509
Penicillin-binding proteins	*Bacillus megaterium*	[^{3}H]Benzylpenicillin		SDS-p.a.g.e./fluorography fingerprints cell state	510
Pepsin	Hog stomach mucosa	*p*-Diazobenzenesulphonic acid and others		Photoirradiation damages pepsin	511
Pepsin-like enzyme	Human gastric mucosa	DEP	(His)	Activation of zymogen	512
Pepsinogen	Chicken	3-{[(2,5-Dioxo-1-pyrrolinyl)-oxy]-carbonyl}-2,5-dihydro-2,2,5,5-tetramethyl-1 H-pyrrolyl-1-oxy		Activation of spin-labelled zymogen	513
Peptide chain termination release factor ribosomal proteins	*E. coli*	Dimethyl suberimidate	$-NH_2$	Identification of ribosomal binding domain	514
Peptide transporter	*Saccharomyces cerevisiae*	Leucyl-leucyl-4-azido-2-nitrophenyl-alanine, leucyl *p*-nitroanilide		Classical and direct photoaffinity labelling	515
Phenol sulphotransferase	Rat liver	Phenylglyoxal NEM Ribonucleotide 2′,3′-dialdehydes	Arg Cys Lys	Dialdehydes affinity label	516
Phenylalanyl-tRNA synthetase	Yeast	3′-Oxidized $tRNA^{Phe}$	Lys, ε-NH_2	Schiff-base formation and reduction	517
Phosphofructokinase	Rat erythrocytes and reticulocytes	Dimethyl suberimidate, dimethyl 3,3′-dithiobispropionimidate	Lys	Modification of allosteric properties	518
6-Phosphogluconate dehydrogenase	*Candida utilis*	Acetyl phosphate, 1,3-diphosphoglycerate, carbamoyl phosphate	His?	Non-enzymatic phosphorylation	519

Phosphoglucase isomerase	Porcine muscle	Pyridoxal 5′-phosphate/$NaBH_4$	Lys	Active-site sequencing	520
Phosphoglycerate kinase	Rat muscle	DTNB, iodoacetate	—SH	One fast-reacting —SH	521
Phospholipase A_2	*Agkistrodon halys blomhoffi*	Sodium cyanate, formaldehyde/ sodium cyanoborohydride	Lys ε-NH_2	Role of lysine in phospho-lipolysis	522
Phospholipase A_2	*Crotalus atrox* venom	Iodoacetamide	Met-10	Sulphonium derivative has en-hanced activity	523
Phospholipase A_2	*Naja naja siamensis* and others	*p*-Bromophenacyl bromide	His-48	Effect of group with p*K* 8.55	524
Phosphorylase	Potato	*P′P″*-Bis-(5′-pyridoxal) diphos-phate	Lys	Crosslinking	525
Phosphorylase *a*	Rabbit skeletal muscle	Dimethyl suberimidate	—NH_2	Effect of ligands on tetramer dissociation	526

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Phosphorylase *a* and *b*	Rabbit muscle	CNBr-activated maltoligosaccharides		Blockade of glycogen binding	527
Phosphorylase *b*	Rabbit liver and skeletal muscle	8-[*m*-(*m*-Fluorosulphonylbenzamido)benzylthio]adenine		Activator-site characterization	528
Phosphorylase kinase	Rabbit skeletal muscle	8-[γ-^{32}P]Azidoadenosine 5′-triphosphate and 2′,3′-dialdehyde derivative		Photoaffinity labelling	529
Phosphorylase kinase	Rabbit muscle	Dimethyl suberimidate	Lys	Types of subunit contact delineated	530
Phosphorylase kinase	Rabbit skeletal muscle	5′-(*p*-Fluorosulphonylbenzoyl)-adenosine		Affinity labelling	531
Photophosphorylation coupling factor	Spinach	Eosin isothiocyanate		Labelling dependent on proton motive force	532
Plasmin	Human	4-Amidinophenyl esters of substituted benzoic acids	Ser	Stabilized acyl enzymes	533
Plasminogen	Human	Cyclohexane-1,2-dione	Arg	Lys-Sepharose affinity abolished in isolated kringle 4, not in intact protein	534
Plasminogen	Human	(DFP)	Ser	Isolation and cleavage of DIP-zymogen	535
Plasminogen kringle 4	Human plasma	Cyclohexane-1,2-dione EDC	Arg-70 Asp-56	Residues essential for ligand binding	536
Plastocyanin	Spinach	EDC/ethylene diamine	—CO_2H	Large shift in redox potential	537
Platelet-derived growth-factor receptor/platelet-derived growth factor	Various cells	Ethylene glycol bis-(succinimidyl succinate), disuccinimidyl succinate	—NH_2	Receptor identification	538
Polypeptides	*Rhodopseudomonas capsulata* photosynthetic membrane	Dithiobis-(succinimidyl propionate)	—NH_2	Topographical study	539

Prealbumin	Human	*N*-(Ethyl-2-diazomalonyl)-3,5,3′-triodothyronine, *N*-(ethyl-2-diazomalonyl)-thyroxine		Some carbene-independent photolabelling	540
Proinsulin-converting enzyme	Rat islet of Langerhans secretory granule	L-Ala-L-Lys-L-Arg chloromethyl ketone	(His)	Thiol protease	541
Prolactin/membrane receptor	Rat	*N*-Hydroxysuccinimidyl azidobenzoate	(—NH_2)	Photoactivated crosslinking	542
Propapain	*Papaya carica*	Iodo[^{14}C]acetamide, *N*-iodoacetylaminoethyl-5-naphthylamine-1-sulphonic acid	—SH	Propain may be thiol–disulphide interchange isomer of papain	543
Prostaglandin-synthesis enzymes	Rat microsomes	11,12-Dehydroarachidonic acid		5,6-Dehydro compound also inhibits leukotriene synthesis	544

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Protease	*Schistosoma mansoni*	Various	Various	Identification of serine protease	545
Proteases IV and V	*E. coli*	Various amino-acid chloromethyl ketones		Characterization of function	546
Protein/RNA	Rat-liver ribosomes	EDC		Protein–DNA crosslinks	547
Protein kinase	Chloroplast thylakoid membrane	NEM and others	Cys	Adenine nucleotides prevent —SH modification	548
Prothrombin	Bovine plasma	Dithiobis-(succinimidyl propionate)	—NH_2	Dimerization study	549
Prothrombin	Human	110 °C	Gla	Decarboxylation of Gla to Glu	550
Purine nucleoside phosphorylase	Human erythrocytes	6-Chloro-9-(3,4-dioxopentyl)-purine	Arg	Arg-directed purine derivatives	551
		9-(3,4-Dioxopentyl)hypoxanthine	Arg		
P_2-Purinergic receptor	Guinea pig	Arylazidoaminopropionyl ATP		Photoaffinity labelling	552
P_2-Purinergic receptor	Guinea-pig vas deferens	Arylazidoaminopropionyl ATP		Photoaffinity-labelling mechanism	553
Purothionin	Wheat flour	Acetic and succinic anhydrides	—NH_2	Role of Lys and Tyr	554
		Tetranitromethane	Tyr		
Pyroglutamyl peptidase	*Bacillus amyloliquefaciens*	*N*-Carbobenzoxy-L-pyroglutamyl diazomethyl ketone	Cys?	Characterization as selective inhibitor	555
Pyruvate dehydrogenase	*Bacillus stearothermophilus*	Various bis(imidoesters)		Crosslinking reversed with methylamine	556
Pyruvate dehydrogenase complex	*E. coli*	*O*-Phenylene bismaleimide	—SH	Lipoate acetyltransferase components crosslinked	557
Pyruvate kinase	Rabbit muscle	ADP-dialdehyde	Lys(?)	Forms morpholine-like product *via* reversible complex	558
Pyruvate kinase	Rabbit muscle	5′-*p*-Fluorosulphonylbenzoyl adenosine and guanosine	—SH	Three reactions, two thiol-sensitive	559
Pyruvate oxidase	*E. coli*	Phenylglyoxal, butane-2,3-dione	Arg	Arg role in thiamin pyrophosphate binding	560

Pyruvate oxidase	*E. coli*	NEM	Cys	Active-site Cys	561
Retinol-binding protein	Human plasma	β-[9-^{3}H]Ionyl bromoacetate β-[11-^{3}H]Ionylidene-ethyl bromoacetate [15-^{3}H]Retinyl bromoacetate		Labelling of retinol binding site	562
Rhodanese	Bovine liver	FDNB/dithiothreitol	Hydro-disulphide (—SSH)	2,4-Dinitrothiophenol released on reduction	563
Rhodopsin	Bovine	3-Diazoacetoxyretinal		Synthesis and binding study	564
Riboflavin-binding protein	Hen egg-white	EDC and CMC	15 —CO_2H	Single essential —CO_2H	565

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Riboflavin-binding protein	Hen egg-white	2-Mercaptoethanol Dithiothreitol	Disulphide bridges (8)	Role of S—S bonds in flavin binding	566
Ribonuclease A	Bovine	6,6′-Diselenobis-(3-nitrobenzoic acid)	—SH	Formation of seleno derivatives for ^{77}Se n.m.r.	567
Ribonuclease A		Dimethylsuberimidate		Dimer inhibits peroxidase endocytosis	568
Ribonuclease	*Bacillus intermedius*	DEP	His	One His gives two monoethoxyformylated species	569
Ribonuclease T_1		Ozone	Trp-59	Role of Trp residue	570
Ribonuclease A/ ribonuclease inhibitor complex	Human placenta	Methyl acetimidate	Lys	Difference labelling identifies Lys in binding site	571
Ribonucleotide reductase	Mouse lymphosarcoma cell	[^{32}P]dATP/u.v.		Direct photoaffinity labelling of allosteric site	572
Ribosomes	*E. coli*	*p*-Azido[^{3}H]puromycin		Suppression of non-specific labelling	573, 574
Ribosomes	*E. coli*	4-(4-Formylphenoxy)-butyrimidate and fluorescein hydrazines	(Lys)	Proteins L10 and L7/12 labelled	575
		N-[7-(Dimethylamino)-4-methylcoumarinyl]maleimide	(—SH)		
Ribosomes	*Saccharomyces cerevisiae* Y166	Dimethylmaleic anhydride	—NH_2	—NH_2 regenerated at pH 6	576
Ribosome 50S subunit	*E. coli*	Phenylglyoxal	Arg	Arg implicated in aminoacyl-tRNA binding	577
Ribosomal 50S subunit	*E. coli* CL13	[^{3}H]Puromycin		Immunoelectron microscopic binding-site localization	578
Ribulose biphosphate carboxylase/oxygenase	*Rhodospirillum rubrum* *Spicacia oleracea*	DEP	His	One essential His, partial recovery of activity with NH_2OH	579

Ribulose biphosphate carboxylase/oxygenase	*Rhodospirillum rubrum*	2-Bromoacetylaminopentitol 1,5-biphosphate	Met	Active-site residue identification	580
Ricin	*Ricinus communis*	Disuccinimidyl suberate	$-NH_2$	A- and B-chains linked	581
Ricin A-chain/anti mouse IgG	Rabbit	SPDP	Lys/Cys	Selective killing of leukaemia cells	582
Ricin/anti-thy 1.1 antibody	*Ricinus communis*	*N*-Succinimidyl (4-azidophenyldithio)propionate	(Lys, Cys)	Antibody–toxin conjugate for protein-synthesis inhibition kinetics	583
Ricin A-chain/IgG [Fab′ and $F(ab')_2$]	*Ricinus communis*	SPDP and others	$-SH$, $-NH_2$	Cytotoxic conjugates	584

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Ricin/monoclonal antibody	Rabbit	Chloroambucil *N*-oxysuccinimidyl ester		Immunotoxin synthesis	585
RNA polymerase	*E. coli*	*N*-Trichloromethylthiocyclohex-4-ene-1,2-dicarboximide (captan)	Probably thiol	Reaction with DNA-binding site	586
RNA polymerase σ-subunit	*E. coli*	TNBS	Lys	Five Lys modified to abolish activity	587
S100 protein	Bovine brain	1-Azidonaphthalene-5-sulphonate		Fluorescence probe of protein unfolding	588
S100 protein	Bovine brain	1-Azidonaphthalene-5-sulphonate		Fluorescence probe for phosphatidyl-choline-vesicle binding	589
Scrapie agent	(Hamster)	[^{14}C]DEP	His	Protein nature of agent confirmed	590
Serine hydroxymethyltransferase	Rabbit-liver cytosol	Methyl methanethiosulphonate	4 Cys	Enzymatic role of —SH	591
Serine proteases	Various	*p*-Guanidinobenzoic acid esters of fluorescein	Ser	Fluorescent active-site titrants	592
Serine proteases	Various	[^{3}H]DFP	Ser	Identification in complex systems by autoradiography	593
Serotonin carrier protein	Guinea-pig platelets and brain synaptosomes	Azidoimipramine		Two synaptosomal and four platelet proteins labelled	594
Somatomedin receptor/somatomedin	Human placental membrane	Disuccinimidyl suberate	—NH_2	Receptor characterization	595
Steroid receptor	*Xenopus laevis* oocyte plasma membrane	17,21-Dimethyl-19-norpregn-4,9-diene-3,20-dione		M_r 110 000 protein identified	596
Subtilisin		4-(4′-Aminophenylazo)phenylarsonic acid, 4-(4′-dimethylaminophenylazo)phenylarsonic acid	Ser-221?	Resonance Raman spectroscopic studies	597

Succinyl-CoA synthetase	*E. coli*	Dansyl chloride		Both subunits labelled	598
Sucrase–isomaltase complex	Rabbit brush border	3-Trifluoromethyl-3-(*m*-[^{125}I]-iodophenyl) diazirine		Hydrophobic region of isomaltase modified	599
Submitochondrial membranes	Bovine heart	3-Azido-9-{[14-(diethylamino)-1-methylbutyl]-amino}-7-methoxyacridine		Comparison of energized and non-energized membranes	600
Superoxide dismutase	Bovine erythrocytes	Succinic anhydride	(Lys)	Reduced subunit interactions	601
Superoxide dismutase (Cu, Zn)	Bovine erythrocytes	Cyanate	Lys	Enzymatic role of Lys	602
Superoxide dismutases (Cu, Zn)	Bovine, yeast	Phenylglyoxal	Arg	Changes Cu^{II} co-ordination geometry	603

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Surface antigen	Hepatitis B virus	Pyrenesulphonyl azide		Photoactivatable hydrophobic probe	604
Surface antigen	Murine epidermal cell	IAEDANS	Cys	Cytotoxicity haptenic reagent	605
Synaptic vesicle proteins	Bovine cerebral cortex	Dimethyl adipimidate	Lys	Selectivity of crosslinking greater at lower reagent concentration	606
Thick filament proteins	*Rana pipiens*	Dimethylsuberimidate and others	Lys	Effects on Ca^{2+}-induced structural changes	607
Thiolase 1	Porcine heart	5-Mercurio-*N*-dansyl-L-cysteine	—SH	Fluorescent thiol reagent	608
Thiosulphate sulphurtransferase	Bovine liver	Iodoacetic acid	Cys-247	Affinity analogue destroys activity	609
Thylakoid membrane proteins	Spinach	[^{14}C]DCCI/[^{14}C]glycine ethyl ester or [^{3}H]serotonin	—CO_2H	Some proteins show increased labelling in the light	610
Thymidylate synthetase	*Lactobacillus casei*	Pteroylheptaglutamate/EDC	Lys-51, -58	Active ester labels both subunits	611
Thymidylate synthetase	*Lactobacillus casei*	FDNB 1,5-Difluoro-2,4-dinitrobenzene	Cys Lys, Ser	Crosslinking of Cys and Ser	612
Thymidylate synthetase	*Lactobacillus casei*	Butane-2,3-dione	Arg	Active-site residue modified	613
Thyroglobulin	Human thyroid	Iodine	Tyr	Formation of thyroid hormone: I may cleave peptide bonds	614
Thyroid-hormone receptor	Rat pituitary GH_1 cell	*N*-2-Diazo-3,3,3-trifluoropropionyl-3,5,3′-*tri*-iodo-L-thyronine/u.v.		Photoaffinity label	615
Thyrotropin/thyrotropin receptor	Bovine pituitary, porcine and human thyroid	Disuccinimidyl suberate	—NH_2	Receptor isolation	616
α-Toxin	*Naja naja siamensis*	Fluorescein isothiocyanate	Lys-23	Probe for receptor-induced conformational changes	617

Transcortin	Human	Tetranitromethane	Tyr	Tyr involved in cortisol binding	618
Transferrin	Human serum	Acetic anhydride	$-NH_2$	Differential kinetic labelling	619
Transforming growth-factor receptor, TGF	Human A431 carcinoma cells and normal rat-kidney fibroblasts	Disuccinimidyl suberate, hydroxysuccinimidyl *p*-azidobenzoate		Affinity crosslinking	620
L,D-Transhydrogenase	*Rhodopseudomonas spheroides*	*p*-Hydroxymercuribenzoic acid	Cys	Buried Cys residues	621
Translational elongation factor G	*E. coli*	Pyridoxal 5′-phosphate/$NaBH_4$	Lys	One Lys essential for GTP binding	622
		Butane-2,3-dione	Arg		
3,3′,5-Tri-iodo-L-thyronine receptor	GH_3 rat-pituitary tumour cells	*N*-Bromoacetyl-3,3′,5-tri-iodo-L-thyronine		Affinity labelling	623

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Troponin α-tropomyosin	Rabbit cardiac and skeletal muscle	*N*-(4-Azidobenzoyl-[2-^{3}H]glycyl)-*S*-(2-thiopyridyl)cysteine	Cys-190	Heterobifunctional photoaffinity probe	624
Troponin	Rabbit skeletal muscle	*N*-(1-Anilinonaphthyl-4)-maleimide		TN–TNC binding (fluorescence	625
Troponin 1	Rabbit	*N*-Dansylaziridine		Calmodulin interaction studied	626
Troponin 1	Rabbit fast skeletal muscle	[^{3}H]Acetic anhydride	Lys	Competitive labelling	627
Troponins I, T, and C	Rabbit skeletal muscle	*N*-(4-Azidobenzoyl-[2-^{3}H]-glycyl)-*S*-(2-thiopyridyl)-cysteine	Cys-48, -64	Subunit interactions	628
Troponin C	Rabbit	Dansylaziridine 5-(Iodoacetamido)eosin	Met-25 Cys-98	Energy-transfer study	629
Troponin and troponin I/C, I/T complexes	Rabbit skeletal muscle	Iodoacetamide	Cys	Proximity of Cys to interaction sites	630
Trypsin	Bovine	Dansyl Gly/Ala/Phe esters of 4-amidinophenol	Ser-195	Preparation of fluorescent acyltrypsins	631
Tryptophan 5-monooxygenase	Rat-brain stem	Dimethyl suberimidate	—NH_2	Confirms tetrameric structure	632
Tryptophanyl-tRNA synthetase	*Bacillus stearothermophilus*	Brown MX-5BR		Triazine-dye affinity label	633
Tubulin	Bovine brain	*NN'*-Ethylenebis(iodoacetamide)	—SH	—SH critical for microtubule assembly	634
Tubulin	Lamb brain	2-Hydroxy-5-nitrobenzyl bromide	Trp	Colchicine binding abolished	635
Tubulin	Porcine brain	Bisimidates	Lys ε-NH_2	—NH_2 3 Å apart in dimer	636
Tubulin	Chick	Monobromo(trimethylammonio)bimane	—SH	Fluorescent microtubule assembly studied	637

Tubulin	Pig brain	Potassium [^{14}C]cyanate	Lys	Inhibition of microtubule assembly	638
β_2-Tubulin	Bovine brain and others	*NN'*-Ethylenebis(iodoacetamide)	—SH	Lesser reactivity towards crosslinker than β_1-form	639
Tuftsin receptor	Mouse peritoneal macrophage	2-Nitro-4-azidophenylsulphonyl chloride	Trp	Preparation of tuftsin analogue as receptor probe	640
Turnip crinkle virus	(*Brassica chinensis*)	Dimethyl suberimidate		Protein subunit gives dimers	641
Tyrosine 3-monooxygenase	Rat adrenal gland	Dimethyl suberimidate	$-NH_2$	Confirms tetrameric structure	642
Tyrosine protein kinase	Ehrlich ascites	Various chloromethyl ketones 2-*O*-(Isobutoxycarbonyl)lactate chloromethyl ketone			643

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Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Ubiquinol–cytochrome C oxidoreductase complex	Beef heart	DEP		Reversible modification	644
Ubiquitin-activating enzyme	Rabbit reticulocyte	Iodoacetamide	Cys	Active-site Cys modified, preventing thiol-ester formation	645
Urease	Jackbean	Ammonium 4-chloro-7-sulphobenzofuran	Cys	Thiol-specific fluorogenic reagent	646
Uricase	*Candida utilis*	DTNB, *O*-iodosobenzoate	Cys	Characterization of —SH groups	647
Urokinase	Human urine	Cyanuric fluoride	Tyr	Study of Tyr ionization	648
Urokinases	Human	Dansyl fluoride DNS-Gly-Nle-Lys-CH_2Cl	Ser His	Active-site-directed fluorescent probes	649
Xanthine oxidase	Milk	FDNB	Lys	Exposed and buried residues	650
Xanthine oxidoreductase	Rat liver	2-Iodosobenzoic acid Disulphiram and others	—SH	One —SH essential for NAD^+ utilization	651
D-Xylose-binding protein	*E. coli K-12*	NBS	Trp	Reduces D-xylose binding	652
Von Willebrand factor	Human plasma	TNBS	Lys	Six to seven Lys modified	653
Various proteins		Ozone	Tyr	*OO'*-Dityrosine formation	654
Various proteins		Succinic anhydride	$-NH_2$	Effects of reversing amino-group charge	655
Various proteins		1,3,6-Trisulphonylpyrene 8-isothiocyanate	$-NH_2$	Strongly acidic fluorescent label for p.a.g.e.	656
Various proteins	L cells	ω-(*m*-Diazirinophenoxy) derivatives of fatty acids		Photoreactive fatty acids	657
Various proteins	Various	9-Azidoacridine		Probes aromatic and nucleotide binding sites	658
Various proteins	Various	Various *N*-chloroamines	His, Tyr Cys	Chloroamines formed from HOCl	659

Various proteins	Various	Various amine boranes/formaldehyde	Lys	Alternative reductive-alkylation methods	660
Various proteins	Various	*cis*-Dichlorodiamine platinum(II)	Met	Reactivity studied by h.p.l.c.	661
Various proteins	Various	Various α-diketones and $h\nu$		O_2-Dependent photosensitized inactivation can occur	662
Various proteins	Various			Review of chemistry of protein denaturation	663

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2 Investigations of Known and Novel Reagents and Reactions

α-Dicarbonyl Compounds.—2,3-Butanedione and similar compounds have been widely used as reagents specific for active-site Arg residues. However, Mäkinen and co-workers have demonstrated[664] that, when reacted under u.v. or visible light (depending on the reagent), lactoperoxidase is modified at His, Tyr, Asp, Ser, Glu, and probably Trp residues as well as Arg and that enzyme inactivation can occur regardless of the function of the Arg residues. Similarly, *Aeromonas* aminopeptidase did not react with 2,3-butanedione in the dark,[665] but under visible illumination five Trp residues, three to four Tyr, two His, and two Arg were modified. Furthermore, the enzyme was inactivated by methylglyoxal and 2,3-pentanedione in u.v. light. The α-dicarbonyl moiety acts as a photon absorber, giving rise to non-specific type-II singlet photo-oxygenation. Thus, substituted histidines, *e.g.* 1-methyl-His, and other free-radical scavengers or singlet oxygen quenchers can be used as specific protectors against α-dicarbonyl-sensitized photochemical inactivation.

2,3-Butanedione, 2,3-pentanedione, and 1-phenyl-1,2-propanedione cause significant inactivation over remarkably wide wavelength ranges (u.v. and visible), suggesting that in modifications with these reagents virtually all illumination[666] should be avoided.

Thus, to ensure Arg-specific modification, reactions should be carried out in the dark, in the presence of scavengers, or using reagents without photochemical effects in the visible region, and the modified protein should be subjected to spectral analysis and an examination of susceptible residues.

These findings are summarized in a review[667] (52 references).

Diethyl Pyrocarbonate.—Two groups of workers have modified *p*-hydroxybenzoate hydrolase with diethyl pyrocarbonate. In the first study[668] the inhibition of the enzyme was correlated with the modification of a single His that could be protected by NADPH. Side reactions were reduced by keeping the pH below 7 and using a low concentration of DEP (0.07—0.5 mM), although approximately 1 mol of Tyr mol^{-1} enzyme was modified and was not regenerated by treatment with hydroxylamine. No Cys residues were modified. 3 mols of His per mol of enzyme were modified. The second study,[669] also using DEP at pH 7, found four out of nine His residues modified, with the accompanying complete loss of activity reversible by H_2NOH. NADPH itself was not modified, but the effects on other residues were not investigated. Both studies conclude that a His residue is located in the NADPH binding site, and also find that above pH 7 the rate of modification of the enzyme by DEP is greatly increased, probably owing to reaction with residues other than His, for example Tyr.

[664] K. K. Mäkinen and P. L. Mäkinen, *Eur. J. Biochem.*, 1982, **123**, 171.
[665] K. K. Mäkinen, P. L. Mäkinen, S. H. Wilkes, M. E. Bayliss, and J. M. Prescott, *J. Biol. Chem.*, 1982, **257**, 1765.
[666] K. K. Mäkinen and P. L. Mäkinen, *FEBS Lett.*, 1982, **137**, 276.
[667] K. K. Mäkinen and P. L. Mäkinen, *Photochem. Photobiol.*, 1982, **35**, 761.
[668] H. Shoun and T. Beppu, *J. Biol. Chem.*, 1982, **257**, 3422.
[669] R. A. Wijnandi and F. Müller, *Biochemistry*, 1982, **21**, 6639.

The variable degree of recovery of insulin-binding activity obtained from the modified insulin receptor[670] by H_2NOH treatment has been attributed to the irreversible formation of bis(ethoxycarbonyl)imidazole. In dihydrofolate reductase,[671] while no modification of Tyr was detectable by monitoring A_{280}, the modification of His (followed at 242 nm, where *N*-carbethoxyhistidine absorbs) was only 50% reversible by H_2NOH.

2-Chloromercuri-4-nitrophenol.—Creatine kinase III[672] reacted with this compound giving incorporation of 2 mol Hg (mol enzyme subunit)$^{-1}$ in a biphasic reaction. Reaction occurred very rapidly ($k \sim 10^6$ dm^3 mol^{-1} s^{-1}) with the Cys susceptible (independently) to iodoacetamide and DTNB then slowly ($k \sim$ 450 dm^3 mol^{-1} s^{-1}) with a second and non-essential Cys. The authors conclude that two Cys residues may be juxtaposed in the active site, and caution must therefore be exercised in defining the catalytically essential nature of Cys by reaction with labile ligands such as 2-chloromercuri-4-nitrophenol.

***N*-(3-Pyrenyl)maleimide.**—This reagent gave 'half-of-the-sites' reactivity[673] with glutamate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase. The hydrophobic region of the molecule causes low solubility in aqueous buffers, but its selectivity towards hydrophobic areas in proteins was illustrated in this case by the identification of a single fluorescent area in the tryptic peptide map.

Triazine Dyes.—Cibacron Blue F3G-A irreversibly and quantitatively inactivated yeast hexokinase ($K_d = 250\ \mu$mol dm^{-3}). The inactivation was promoted by metal ions (in the order $Zn^{2+} > Cu^{2+} > Ni^{2+} > Mn^{2+}$), which formed ternary complexes with the enzyme and the dye. Almost complete protection against modification was given by 1 mM ATP. These results[674] parallel closely the reaction of Procion Green H-4G with yeast hexokinase (see Volume 14), but the smaller Cibacron Blue probably binds only at the nucleotide site, rather than occupying the sugar site as well.

Procion Blue MX-R, a dichlorotriazinyl structural analogue of Cibacron Blue F39-A, caused a 90% activity loss in horse-liver alcohol dehydrogenase[675] after 30 min incubation at pH 8.5 and 37 °C with incorporation of 1 mol dye (mol 40 K subunit)$^{-1}$ at Cys-174. No other Cys residues were affected.

Tryptophan, ATP, ADP, and GTP all protect tryptophanyl-tRNA-synthetase[676] from inactivation by Brown MX-5BR, which is large enough to occupy both parts of the active site ($K_d = 6.7\ \mu$mol dm^{-3}).

[670] P. F. Pilch, *Biochemistry*, 1982, **21**, 5638.
[671] H. H. Daron and J. L. Aull, *Biochemistry*, 1982, **21**, 737.
[672] A. H. Fawcett, A. I. Keto, P. Mackerras, S. E. Hamilton, and B. Zerner, *Biochem. Biophys. Res. Commun.*, 1982, **107**, 302.
[673] M. A. Jacobson and R. F. Colman, *Biochemistry*, 1982, **21**, 2177.
[674] P. Hughes, R. F. Sherwood, and C. R. Lowe, *Biochem. J.*, 1982, **205**, 453.
[675] D. A. P. Small, C. R. Lowe, A. Atkinson, and C. J. Bruton, *Eur. J. Biochem.*, 1982, **128**, 119.
[676] J. E. C. McArdell, A. Atkinson, and C. J. Bruton, *Eur. J. Biochem.*, 1982, **125**, 361.

Ozone.—In spite of the great reactivity of O_3, high specificity can be achieved, as demonstrated[677] by the oxidation of Trp-62 in lysozyme. In H_2O or neutral phosphate, with a flow rate of 50 nmol O_3 min^{-1} and 5 mg ml^{-1} lysozyme, stoicheiometric inactivation by modification of Trp-62 occurred. With a flow rate of 50 nmol O_3 min^{-1}, three to four Trp residues were oxidized, with a small degree of Met oxidation. These results contrast with those of previous studies that found Trp-108 and -111 to oxidize before Trp-62. The stoicheiometry and specificity depend on the conditions used.

When reacted with spectrin, insulin, glucagon, or ribonuclease, ozone induced non-disulphide crosslinks[678] owing to formation of *OO'*-dityrosine. With galactose oxidase, *OO'*-dityrosine formation accompanied an eight-fold increase in activity. In spectrin, which has 44 Tyr per monomer, only 0.5 nmol of *OO'*-dityrosine was formed per nmol of spectrin after 2 min reaction with O_3.

Modification for Physical Techniques.—A protease assay using fluorescent labelling[679] has been reported. 2-Methoxy-2,4-diphenyl-3(2H)furanone is used to label either fibrin or casein, and fluorescent peptides are assayed after proteolysis by the test enzyme and removal of intact substrate either by filtration or centrifugation (in the case of fibrin) or acid precipitation (for casein). The fluorescent substrates can be prepared in advance and stored for several weeks, and the sensitivity (nanogram quantities of trypsin, chymotrypsin, and elastase) is similar to that for radioactive substrates. The development of fluorescent inverse acylating agents for trypsin[680] has been reported: following acylation by dansyl-Gly, D-Ala, or D-Phe esters of *p*-amidinophenol, deacylation gives approximately 100% recovery of activity. Ageing-induced conformational changes in organophosphorus-inhibited acetylcholinesterase have been studied using novel pyrene organophosphates[681] as fluorescence probes. 1-Pyrene butyl ethyl phosphoro-chloridate and -dichloridate react rapidly and specifically to give stoicheiometric conjugates, non-aged in the case of the former and aged in the case of the latter according to the criterion of reactivation by pyridine-2-aldoxime methyl iodide. Difluorescein disulphide and fluorescein cysteamine disulphide, new, fully reversible, thiol-specific reagents, have been used[682] to investigate histone–histone and histone–DNA interactions. The fluorescence yield of the fluorescein diol is low, so reactions can be followed by measuring the decrease in intensity.

A review (106 references) covering the fluorescent labelling of cytoskeletal proteins[683] and associated techniques for *in vivo* testing has been published.

The problems associated with the use of ^{125}I in radioimmunoassay (safety, short shelf-life, need for long counting times, impossibility of a homogeneous assay

[677] M. M. Dooley and J. B. Mudd, *Arch. Biochem. Biophys.*, 1982, **218**, 459.
[678] H. Verweij, K. Christianse, and J. Van Steveninck, *Biochim. Biophys. Acta*, 1982, **701**, 180.
[679] R. Wiesner and W. Troll, *Anal. Biochem.*, 1982, **121**, 290.
[680] T. Fujioka, K. Tanizawa, and Y. Kanaoka, *Chem. Pharm. Bull.*, 1982, **30**, 230.
[681] G. Amitai, Y. Ashani, A. Gafni, and I. Silman, *Biochemistry*, 1982, **21**, 2060.
[682] E. Wingender and A. Arellano, *Anal. Biochem.*, 1982, **127**, 351.
[683] T. E. Kreis and W. Birchmeier, *Int. Rev. Cytol.*, 1982, **75**, 209.

system) have been overcome[684] by the use of a new chemiluminescent label (an isothiocyanate derivative of aminobutylethylisoluminol) to modify protein antigens and antibodies. The labelled proteins (0.2 mol label mol^{-1} IgG) gave detectable counts for 10^{-17}—10^{-16} mol within a few seconds. A similar approach for a solid-phase immunoassay[685] has been described, in which IgG reacts with [6-(*N*-4-aminobutyl)-*N*-ethyl]amino-2,3-dihydrophthalazine-1,4-dione/EDC to give an assay sensitivity of 1 ng.

Two new modifications with potential in cancer treatment have been published. In the first, boron-labelled antibodies were prepared[686] by coupling the *p*-{1,2-dicarba-*closo*-1-[^{3}H]dodecaboran(12)-2-yl} benzene diazonium ion to a carcinoembryonic antigen IgG. The method is based upon the release (following thermal neutron absorption) of α- and $^4_3$Li particles with a maximum range in tissues of $<10\ \mu$M and energies too small for extensive tissue damage. Labelling overnight at 4 °C and pH 6.5 leaves the immunoreactivity of IgG unaffected at DBD:IgG ratios of up to 20:1 and results in the incorporation of approximately 5 carbaboranes per molecule, estimated by azo-dye formation. With a possible 8×10^5 atoms of boron per tumour cell, disintegrations on the cell-surface membrane could form the basis for increased lethality relative to other means of irradiation. The second method[687] allows tumour imaging with radioactive metal chelates conjugated to monoclonal antibodies. High-resolution γ-camera images of 200—300 mg tumours in mice have been produced by labelling leukaemia-cell-specific MCAs with chelates such as 1-(*p*-carboxymethoxybenzyl)EDTA/DCCI and the carboxycarbonic anhydride of DTPA. With a 250-fold reagent excess, these reagents gave 0.9 mol and 0.5 mol, respectively, of chelate per mol IgG. The targetting of a wide variety of radioisotopes is possible, *e.g.* ^{67}Ga, ^{111}In, or ^{99}Tc for imaging and ^{47}Sc for killing. Unlike iodine isotopes, detachment of the chelate from the protein leads to immediate excretion.

A novel specific ^{19}F n.m.r. label, *N*-(4-trifluoromethyl)phenyl iodoacetamide, has been used[688] to label the reactive SHl of myosin subfragment 1. The n.m.r. spectrum has a single resonance of line width 110 Hz, with a chemical shift sensitive to temperature, pH, ionic strength, and active-site binding nucleotides. The reagent is prepared from iodoacetic acid and α,α,α-trifluorotoluidine and modifies 1 SH per mol protein after reaction in 1.5-fold excess at pH 7.9 in the dark, at 0 °C, for 10 min. The modification[689] of α-chymotrypsin by 4-(trifluoromethyl)-α-bromoacetanilide, however, is less specific: although 1 mol CF_3 mol^{-1} enzyme is incorporated, three products are possible because two sites (Met-192 and a second residue, not Met-192 or Ser-195) are modified. Two major ^{19}F n.m.r. signals are seen, with the

[684] A. Patel, M. S. Morton, J. S. Woodhead, M. E. T. Ryall, F. McCapra, and A. K. Campbell, *Biochem. Soc. Trans.*, 1982, **10**, 224.
[685] P. J. Cheng, I. Hemnilä, and T. Lövgren, *J. Immunol. Methods*, 1982, **48**, 159.
[686] E. Mizusawa, H. L. Dahlman, S. J. Bennett, D. M. Goldenberg, and M. F. Hawthorne, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79**, 3011.
[687] D. A. Scheinberg, M. Strand, and O. A. Gansow, *Science*, 1982, **215**, 1511.
[688] J. W. Shriver and B. D. Sykes, *Biochemistry*, 1982, **21**, 3022.
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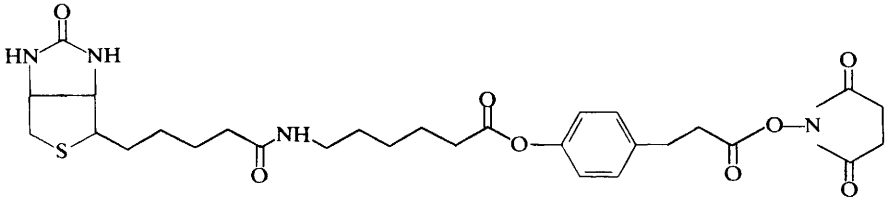
conclusion that reagents of the type $ArCOCH_2Br$ are not always specific for Met-192 or Ser-195.

6,6′-Diselenobis-(3-nitrobenzoic acid) is a new Se analogue of Ellman's reagent and has been used to modify ribonuclease A and lysozyme[690] for ^{77}Se n.m.r. (in 8M urea), after reductive cleavage of —Se—Se— bonds. The released selenoate has $\varepsilon_{432} = 10^4\ dm^3\ mol^{-1}\ cm^{-1}$.

For spin-labelling proteins, (1-oxyl-2,2,5,5-tetramethyl-Δ^3-pyrroline-3-methyl)methanethiosulphonate[691] is a more specific and faster reagent than maleimide spin labels. Thus, Cys-25 in papain was modified in 5 min at pH 4.5, with 0.5—3.5 mol label mol^{-1} papain, and the loss of activity was fully reversed by dithiothreitol. The reagent's minimal rotational freedom provides high sensitivity to the conformation of the thiol site.

Finally, a new p.a.g.e. system has been developed[692] for peptides and proteins of mol. wt. 200—100 000. The volatile buffer system (triethylamine/formic acid, pH 11.7) contains 1,3,6-trisulphonyl-pyrene-8-isothiocyanate, which adds a strongly negative charge to proteins and increases their solubility, allowing migration according to molecular weight and easier and more efficient extraction. After evaporation to remove salts, the proteins are ready for sequencing, Edman degradation, *etc.* Because the protein bands are fluorescent, no fixing or staining is needed; this is particularly useful for small peptides, and the sensitivity is five times greater than that of Coomassie Blue, with 0.05—5 μg of protein being detectable. However, the reaction is irreversible, the protein is inactivated, and the bands are less sharp than in the Laemmli system.

Affinity Probes.—Several interesting applications of the biotin–avidin interaction ($K_d \sim 10^{-15}$ mol dm^{-3}) have been published this year. For example, the *N*-hydroxysuccinimide ester of 3-[4-(*N*-biotinyl-6-aminocaproyloxy)phenyl]-propionic acid (1) has been synthesized[693] for isolation of the insulin-receptor complex. Retrieval of the modified complex (1—NH_2 modified after ~5 h incubation with a nine-fold excess of reagent over insulin) is achieved by absorption on immobilized avidin at physiological pH, followed by cleavage of the phenyl ester linkage under conditions (*e.g.* 1M H_2NOH,



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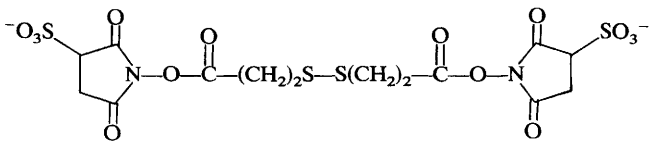
[690] N. P. Luthra, R. C. Costello, J. D. Odom, and R. B. Dunlap, *J. Biol. Chem.*, 1982, **257**, 1142.
[691] L. J. Berliner, J. Grunvald, H. O. Hankovsky, and K. Hideg, *Anal. Biochem.*, 1982, **119**, 450.
[692] A. Tsugita, S. Sasada, R. Van den Brock, and J. J. Scheffler, *Eur. J. Biochem.*, 1982, **124**, 171.
[693] C. A. Mouton, D. Pang, C. V. Natraj, and J. A. Shafer, *Arch. Biochem. Biophys.*, 1982, **218**, 101.

pH 7) that neither denature the proteins nor result in peptide cleavage but that give >90% release after 40 min at 25 °C. This approach circumvents the problem of releasing the tightly bound biotinyl complex from the immobilized avidin, but Hofmann *et al.*[694] chose to weaken the biotin–avidin interaction instead by modifying the biotin, although insulin itself provides an advantageous steric impediment to the interaction. Thus, while $t_{1/2}$ for the avidin–biotin complex is 200 days, that for the dethiobiotin–succinoylavidin complex is 11 hours. $N^{\alpha}1^{\beta}$-6-(Dethiobiotinylamido)hexyl-insulin was eluted from succinoylavidin-Sepharose by 20 mM biotin with 50—60% recovery. It is stressed, however, that this method may not be as useful for other peptide hormones. The 'handle' technique illustrated by these two papers therefore seems promising for the isolation of the insulin receptor under mild conditions. A similar application[695] is the use of *N*-biotinyl-N^{ϵ}-acetimidoglucagon to locate the glucagon receptor on Chinese hamster ovary cells by fluorescence microscopy, after incubation with fluorescein-labelled avidin.

A novel[696] cell-surface-reactive membrane-impermeable immunoreactive probe, isethionyl 3-(*N*-2,4-dinitrophenyl)aminopropionimidate, which does not markedly alter the net charge on proteins, has been synthesized. Soluble in aqueous buffers at pH 7 and above, it is stable in solution at pH 8 and 25 °C with a $t_{1/2}$ of 57 min. It reacts with bovine serum albumin at pH 8 at a rate similar to that of TNBS, the substituted protein being recognized by anti-DNP antibody. It has also been reacted with Chinese hamster ovary cells (9×10^7 —NH_2 groups modified per cell at 10 mM IDNPAP, pH 8, 30 min, 3 °C).

Crosslinking Reagents.—Many established crosslinkers are being used routinely for probing subunit topology,[697,698] identifying receptors,[699,700] and delivering drugs.[701,702] However, new reagents continue to appear, for example[703] 3,3′-dithiobis(sulphosuccinimidyl propionate) (DTSSP) (2) and bis(sulphosuc-



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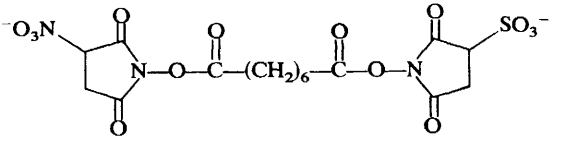
[702] Y. Masuho, K. Kishida, M. Saito, N. Urnenoto, and T. Hara, *J. Biochem.* (*Tokyo*), 1982, **91**, 1583.
[703] J. V. Staros, *Biochemistry*, 1982, **21**, 3950.



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cinimidyl) suberate (BS^3) (3). These hydrophilic membrane-impermeable crosslinkers give better yields than di-isethionyl-3,3'-dithiobis(propionimidate) in model reactions with aldolase and give a sharper cut-off between the tetramer and higher oligomers.

In contrast to alkyl imidates, DTSSP and BS^3 abolish the charge of modified $-NH_2$ groups. The use of di-isethionyl-3,3'-dithiobis(propionimidate) and 3,3'-dithiobis(sulphosuccinimidyl propionate) has been reviewed.[704]

The identification of ribosomal proteins bound to elongation factor G has been achieved[705] using the cleavable crosslinker dimethyl-4,9-diazo-5,8-dioxo-6,7-dihydroxydodecanebis(imidate).

Affinity Labelling.—Halomethyl ketones of amino-acids and peptides have continued to be widely used as histidine-directed affinity labels, even for proteins without obvious amino-acid binding functions. Thus, N^{α}-tosyl-L-phenylalanine chloromethyl ketone inactivated rabbit and mouse fibroblast interferons[706] by a mechanism proposed to involve binding to a hydrophobic pocket (the corresponding lysine derivative was not an inactivator). Similarly, the bromomethyl ketone of Boc-L-leucine was a fairly specific inactivator of a tumour-derived protein kinase.[707] A variety of N^6 or 8-(ω-iodoacetamidoalkyl) derivatives of ATP have been synthesized and used as '*exo* site' inhibitors of various kinases.[708] Certain of these compounds were isoenzyme- or species-specific. 3-Chloroacetyl pyridine adenine dinucleotide has been used to label both glyceraldehyde-3-phosphate dehydrogenase[709] and yeast alcohol dehydrogenase;[710] in the latter case loss of label from the inactivated enzyme was observed. The nitrogen mustard chlorambucil and its proline derivative have been shown to inactivate angiotensin-converting enzyme with formation of a nucleophile-sensitive bond attributed to alkylation of an essential Asp or Glu.[711]

Several interesting applications of thiol-specific reagents have been described. 6-(3-Carboxy-4-nitrophenyl)thiol-9-β-D-ribofuranosyl purine 5'-triphosphate is a poor substrate for Na^+,K^+-dependent ATPase at pH 7.4 but becomes an irreversible inactivator at pH 8.5. However, the inactivation was not ascribed to attack by an essential thiol (at the 6-position of the purine analogue) but rather to tyrosine OH.[712] A specific disulphide interchange

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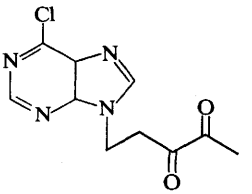
reagent, 3-hydroxy-17β-(*p*-nitrophenyldithio)-1,3,5(10)-oestratriene, was used to label a thiol in the oestrogen receptor of rabbit nucleus.[713] A mixed disulphide of a phenylalanine derivative and 2-thiopyridine has also been used as a subsite-specific two-protonic-state probe of papain.[714]

Among affinity labels based on acylation mechanisms, 5′-*p*-fluorosulphonyl benzoyl adenosine has found wide use but does not always react as an acylating agent. The reagent inactivated myosin subfragment 1 without incorporation of the radiolabelled compound. The proposed mechanism was formation of a thiosulphonate by reaction with one protein thiol, followed by displacement with another thiol group and production of a disulphide bond and (presumably) the sulphinate analogue.[715] Similar reactions may be involved in the inactivation of pyruvate kinase by this and related reagents.[716] Russian workers have continued to explore the modifications of meromyosin by mixed anhydrides of mononucleotides and substituted benzoic acids.[717] These reagents appear to transfer the phosphoryl rather than the benzoyl moiety to the protein.[718] c-Adenosine-5′-trimetaphosphate has also been used as a phosphorylating affinity label for DNA-dependent RNA polymerase.[719]

Periodate-oxidized nucleotides have been widely used as affinity labels either with or without concomitant reduction by borohydride ion. However, the dialdehyde derivatives are unstable and their reactions with proteins poorly characterized. Periodate-oxidized ATP has been shown to undergo β-elimination of tripolyphosphate ion leading to an adenine-containing dialdehyde derivative that may itself react with proteins, albeit less specifically than the parent compound.[720] This reaction may cause serious problems if ^{32}P incorporation is being used as a measure of labelling. Periodate-oxidized derivatives of fluorescent[721] and photoaffinity[722] ATP analogues as well as oxidized tRNA[723] have been reported.

A novel type of arginine-directed affinity label based on α-diketones such as (4) has been shown to be much more effective at inactivating purine nucleoside



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phosphorylase than butanedione.[724] The widespread occurrence of essential arginine in enzymes may make this a fruitful approach to new affinity labels.

Reviews have appeared on the affinity labelling of glucocorticoid receptors[725,726] and on the use of deamination reactions.[727]

Photoaffinity Labelling.—This topic has continued to burgeon, with a large number of diverse applications of established reagents such as 8-azido-cAMP being reported. Photoaffinity labelling of cyclic nucleotide-dependent protein kinases has been reviewed,[728] and three reviews of photolabelling in lipid–protein systems have appeared.[729,730,731] There have been few reports of new types of photoreactive ligand, and the promise of α-diazophosphonic acids was not fulfilled because the latter reagents were found to be hydrolysed to PO_3^{2-} and diazo compounds.[732] However, diazomalonyl derivatives of thyronine and thyroxine have been used in photolabelling studies of human prealbumin,[733] and thyroid hormone receptor has been labelled with a stabilized 2-diazo-3,3,3-trifluoropropionyl derivative of triodothyronine.[734] Diazirines have continued to find applications in selected cases where acid stability and/or carbene reactivity is required. *m*-Diazirinophenyl retinal has been used to study the topology of bacterio-opsin[735] where the identified sites of attachment (Ser, Glu) suggested pathways other than pure carbene insertion. 3-Trifluoromethyl-3-*m*-iodophenyl diazirine was used for hydrophobic site labelling of glutamyl transpeptidase[736] and sucrose isomaltase complex.[737] ω-(*m*-Diazirinophenoxy) derivatives of fatty acids have also been used to label lipid contact regions of intrinsic membrane proteins.[738] However, the majority of 'hydrophobic' photoaffinity-labelling studies have used aryl azides, particularly 2-nitro-4-azidophenyl derivatives of fatty acids or phospholipids[739,740,741] or azidonaphthalene derivates.[742,743] Aryl azide analogues of β-adrenergic antagonists have been used to label adenylate cyclase[744] and the β-adrenergic

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receptor itself.[745–748] Opiate receptors and related targets have been probed using azidothiorphan (encephalinase location in mouse brain),[749] an enkephalin derivative itself,[750] and fentanyl analogues.[751] The use of *p*-azido-[^{3}H]puromycin to map *E. coli* ribosomes has been improved by measures to reduce non-specific labelling.[752,753] 9-Azidoacridine has been reported as a versatile probe of hydrophobic and nucleotide binding sites.[754] Among the numerous azido nucleotide analogues, the *N*-(arylazido)aminopropionyl ATP analogues have been used to label purinergic receptors.[755,756]

Reversible direct photolabelling of deoxycytidylate deaminase by thymidine triphosphate has been reported.[757]

Photocrosslinking.—*N*-(4-Azidophenylthio)phthalimide has been described as a new sulphydryl-specific, cleavable, heterobifunctional photoactivated cross-linking reagent,[758] and two reports have dealt with the use of a similar reagent, *N*-(4-azidobenzoyl-[2-^{3}H]glycol)-*S*-(2-thiopyridyl)cysteine, to cross-link troponin subunits[759] and troponin/tropomyosin.[760] Several reports have described the use of proteins or peptides derivatized with the tryptophan-selective reagent 2-nitro-4-azidophenylsulphenyl chloride. However, in α-melanotropin this reagent reacts with Lys as well as Trp.[761] The range of reagents available for hydrophobic photolabelling has been extended with the synthesis of 5-isothiocyanato-1-naphthalene azide and *p*-azidophenylisothiocyanate. Bacteriorhodopsin or erythrocyte band 3 proteins labelled with these reagents and photolysed in membranes or vesicles gave homopolymers of the proteins.[762] Photochemical-crosslinking studies of lipid–protein interactions have been reviewed.[763] *N*-Oxysuccinimide esters of azidosalicyl

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acid derivatives have been used as non-cleavable photoactivated heterobifunctional reagents that can readily be radioiodinated.[764]

Mechanism-based Inhibitors.—The design and application of suicide substrates are areas where the interaction of protein chemistry and mechanistic enzymology continues to be fruitful. Two general[765,766] and three specialist (aromatase,[767] monoamine oxidase,[768] and penicillins[769]) reviews have appeared. In the case of aromatase, further reports of inactivation by alkyne[770] and fluorinated[771] substrate analogues have appeared. The inactivation of ornithine decarboxylase by di- and mono-fluoromethylornithine[772—775] as well as fluoromethyl analogues of arginine[775] and putrescine[776] has assumed importance because of the implications of altered polyamine synthesis for tumour and bacterial growth. Two alkyne analogues of arachidonic acid have been synthesized;[777] the 11,12-dehydroarachidonic acid inhibits prostaglandin synthesis, and the 5,6-dehydro analogue also inhibits the generation of leukotrienes.

Liver aryl hydroxamic acid *N,O*-acyltransferases are inactivated by a variety of *N*-hydroxyamides in a process thought to involve elimination and generation of arylnitreniun ions.[778,779] In one case, an electrophilic species was apparently released from the generating enzyme.[779] The inactivation of juvenile hormone synthesizing enzymes by allotoxins has been ascribed to generation of an enzyme-bound quinone methide by partial oxidation.[780] This type of reactive intermediate has not been widely exploited in the design of suicide substrates. In the penicillin field, further studies have identified the branched reaction pathways involved in the inactivation of β-lactamases by clavulanate[781] and β-iodopenicillanate.[782] A stabilized acyl-enzyme has been shown to be important in the action of cloxacillin on β-lactamases,[783] and

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interconvertible acyl-enzyme forms have been identified in the olivanic acid–β-lactamase complex.[784] Most suicide substrates cause inactivation by partition of the enzyme-bound intermediate between normal turnover and a (usually minor) covalent reaction with the enzyme. An unusual example of this mechanistic branching occurs in the case of *E. coli* Ala-tRNA synthetase and 5-bromouridine,[785] where Michael addition of enzyme nucleophile to C-6 of the substrate is usually followed by breakdown to 5-bromouracil, ribose, and free enzyme (*i.e.* a hydrolytic 'normal' substrate reaction). A minor pathway leading to a dead-end complex was identified and apparently involved essential thiols of the enzyme.

PART II: X-Ray Studies *By W. D. Mercer*

1 Introduction

This year's report on *X*-ray structure determinations follows closely the pattern set last year. Preliminary crystallization reports are contained in the Table, while structure determinations are covered in more detail in the various sections. Once again the diversity of structures being studied by *X*-ray and neutron-diffraction methods is reflected in the section entitled 'Other Globular Proteins', and the use of computer graphics systems is showing increasing applications, both during structure determinations for the fitting of electron-density maps and also for structure analyses. The use of colour pseudo-space-filling graphics shows how powerful these techniques can be when examining molecular-surface interactions.

Of the structure determinations reported in 1982 a new family of ribonucleases, dihydrofolate reductase from several species, and aminoacyl-tRNA synthetases are of note. Several virus structures have been reported or improved, and the finding that the polyoma virus capsid pentamers can form both pentavalent and hexavalent morphological units is of interest.

Once again pressure of space has led to the coverage of amino-acids and peptides being curtailed. Readers are referred to the Cambridge Structure Data Bank for this information.

2 Crystallographic Methods and Equipment

General Crystallographic Methods and Theory.—A news report[1] has appeared to confirm the existence of a functional *X*-ray laser.

The most recent modifications to the SIR program[2] and the MAGEX procedure[3] have been reported.

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Table *Preliminary crystallization reports*

Protein	*Source*	*Space group*	*Cell dimensions*			
			a/nm	*b*/nm	*c*/nm	β/deg
Anti-T lectin	Peanut	$P2_12_12$	12.93	12.69	7.69	—
Sulphite reductase (haemoprotein subunit)	*E. coli*	$P2_12_12_1$	6.97	7.74	8.88	—
Lactate dehydrogenase	*Bacillus stearothermophilus*	$P6_122$ or $P6_522$	8.7	8.7	35.8	—
Lactate dehydrogenase + NADH + fructose 1,6-bisphosphate	*Bacillus stearothermophilus*	$P6_322$	29.0	29.0	14.6	—
Renin	Mouse	$P2_1$	9.64	10.44	7.74	101.2
HPr protein from phosphoenolpyruvate:sugar phosphotransferase	*E. coli*	$C222$	3.27	4.26	10.56	—
β-Crystallin	Bovine	$C222_1$	15.42	16.52	7.84	—
Exotoxin A	*Pseudomonas aeruginosa*	$P2_1$	6.06	10.02	5.98	98.6
Resolvase		$P6_222$ or $P6_422$	5.97	5.97	16.94	—
Glyceraldehyde-3-phosphate dehydrogenase	*Bacillus coagulans*	$C222_1$	9.56	13.72	13.19	—
Flavocytochrome C_{552}	*Chromatium vinosum*	$C2$	16.43	8.48	10.80	106.9
Ribose-binding protein	*Salmonella typhimurium*	$P2_1$	6.44	6.06	6.28	91.25
DNA-binding protein	Bacteriophage T4	$P1$	6.77	6.78	6.60	α = 101.6 β = 107.0 γ = 105.2
Poliovirus type I	—	$P2_12_12$	32.4	35.9	38.1	—
Isoamylase	*Pseudomonas*	$P2_12_12_1$	13.79	5.29	15.12	—
α-Lactalbumin	Bovine	$P3_121$ or $P3_221$	5.74	5.74	7.50	—
		$P622$	9.40	9.40	6.71	—
		$P321$	9.37	9.37	6.69	—
α-Lactalbumin	Human	$P2_12_12$	3.36	6.99	4.73	—
Deoxyribonuclease I	Bovine pancreas	$C2$	13.16	5.49	3.84	91.4
Ribonuclease A[1]	Pancreas	$C2$	7.34	3.35	10.22	104
Ribonuclease B[1]	Pancreas	$P2_1$	5.14	7.56	3.10	108
Diphtheria toxin	—	$P1$	7.08	7.07	6.53	α = 95.2 β = 91.3 γ = 99.7
Diphtheria toxin	—	$P3_112$ or $P3_212$	9.79	9.79	10.03	—

Mol. wt. and no. of subunits	*Mol. wt. of asymmetric unit*	V_m/ $nm^3\ dalton^{-1}$ $\times 10^3$	*Precipitant*	pH	*Ref.*
110 000 (4)	110 000	2.87	PEG6000[a]	7.0	*b*
220 000 (4)	55 000	2.20	PEG6000	7.8	*c*
136 000 (4)	68 000	2.80	Ammonium sulphate	—	*d*
136 000 (4)	272 000	3.30	Ammonium sulphate	>7.8	*d*
38 000 (1)	152 000	2.51	PEG6000	—	*e*
9017 (1)	9017	2.04	Lithium sulphate	3.7	*f*
46 400 (2)	92 800	2.69	MPD[g]	~7.0	*h*
~68 000	68 000	2.64	PEG6000	7.3	*i*
21 000 (1)	21 000	2.20	Ammonium sulphate	7.5	*j*
145 000 (4)	72 500	2.98	Sodium citrate	6.8	*k*
72 000 (3)	144 000	2.50	Ammonium sulphate	7.3	*l*
29 000 (1)	58 000	2.11	PEG4000	6.5	*m*
25 000 (1)	75 000	—	PEG6000	8.0	*n*
—	—	—	Low ionic strength	7.0	*o*
95 000 (1)	95 000	2.90	Ammonium sulphate	3.5	*p*
14 300 (1)	14 300	2.47	Ammonium sulphate	6.5	*q*
14 300 (1)	14 300	2.95	Ammonium sulphate	6.5	*q*
14 300 (1)	28 600	2.95	Ammonium sulphate	6.5	*q*
14 300 (1)	14 300	1.92	Ammonium sulphate	6.5	*r*
31 000 (1)	31 000	2.20	PEG6000	6.6	*s*
13 500 (1)	27 000	2.23	PEG4000	—	*u*
13 500 (1)	27 000	1.98	PEG4000	—	*u*
62 000 (1)	124 000	2.50	PEG6000[v] + 2M NaCl	8.0	*w*
62 000 (1)	62 000	2.24	Potassium tartrate	9.0	*x*

Table (*cont.*)

Protein	*Source*	*Space group*	*Cell dimensions*			
			a/nm	*b*/nm	*c*/nm	β/deg
Chloroperoxidase	*Caldariomyces fumago*	$C222_1$	15.11	5.79	10.27	—
Glycosylated ribonuclease	Bovine pancreas	$P2_12_12_1$	5.92	5.60	8.10	—
Aconitase	Pig heart	$P2_12_12$	17.35	7.22	7.28	—
Cytochrome $P450_{CAM}$	*Pseudomonas putida*	$P2_12_12$	17.4	6.4	3.9	—
		$P4_122$ or $P4_322$	6.42	6.42	25.5	—
		$P2_12_12_1$	10.85	10.44	3.64	—
Fructose 1,6-phosphate aldolase	*Drosophila melanogaster*	$P2_12_12_1$	8.63	11.57	15.14	—
5S rRNA	*Thermus thermophilus*	$P3_121$ or $P3_221$	9.9	9.9	36.0	—
Agglutinin	Peanut	$P2_12_12$	13.0	12.7	7.8	—
Phosphoribosyl-anthranilate isomerase-indole-3-glycerol phosphate synthase	*E. coli*	$P4_1$ or $P4_3$	10.47	10.47	6.77	—
		$P4_12_12$ or $P4_32_12$	6.97	6.97	24.07	—
Colipase A^{gg}	Horse	$I222$	7.3	7.5	3.2	—
Avidin	Hen egg-white	$P2_12_12$	7.47	8.00	4.31	—
MoFe proteins of nitrogenase	*Clostridium pasteurianum*	$P2_1$	6.99	15.10	12.20	110.3
	Azotobacter vinelandii	$P2_1$	15.39	7.29	20.82	105.0
Phaseolin	*Phaseolus vulgaris*	$P1$	6.66	6.66	6.66	$\alpha = \beta = \gamma = 90$
Histones $(H3–H4)_2$	Calf thymus	$P6_1$ or $P6_5$	8.1	8.1	10.00	—

(*a*) Polyethylene glycol; the number refers to the average molecular weight. (*b*) D. M. Salunke, M. Islam Khan, A. Surolia, and M. Vijayan, *J. Mol. Biol.*, 1982, **154,** 177. (*c*) D. E. McRee and D. C. Richardson, *J. Mol. Biol.*, 1982, **154,** 179. (*d*) H.-P. Schär, H. Zuber, and M. G. Rossmann, *J. Mol. Biol.*, 1982, **154,** 349. (*e*) J. P. Mornon, E. Surcouf, J. Berthou, P. Corvol, and S. Foote, *J. Mol. Biol.*, 1982, **155,** 539 (*f*) L. T. J. Delbaere, L. M. Bruse, and E. B. Waygood, *J. Mol. Biol.*, 1982, **157,** 161. (*g*) 2-Methylpentan-2,4-diol. (*h*) C. Slingsby, L. R. Miller, and G. A. M. Berbers, *J. Mol. Biol.*, 1982, **157,** 191. (*i*) R. J. Collier and D. B. McKay, *J. Mol. Biol.*, 1982, **157,** 413. (*j*) P. C. Weber, D. L. Ollis, W. R. Bebrin, S. S. Abdel-Meguid, and T. A. Steitz, *J. Mol. Biol.*, 1982, **157,** 689. (*k*) B. Lee, J. P. Griffith, C. H. Park, R. I. Sheldon, J. McLinden, A. L. Murdock, and R. E. Amelunxen, *J. Mol. Biol.*, 1982, **158,** 153. (*l*) F. R. Salemme, *J. Mol. Biol.*, 1982, **159,** 551. (*m*) S. Mowbray and G. A. Petsko, *J. Mol. Biol.*, 1982, **160,** 545. (*n*) D. B. McKay and K. R. Williams, *J. Mol. Biol.*, 1982, **160,** 659. (*o*) J. M. Hogle. *J. Mol. Biol.*, 1982, **160,** 663. (*p*) M. Sato, Y. Hato, Y. Ii, K. Miki, N. Kasai, N. Tanaka, and T. Harada. *J. Mol. Biol.*, 1982, **160,** 669. (*q*) R. E. Fenna, *J. Mol. Biol.*, 1982, **161,** 203. (*r*) R. E. Fenna, *J. Mol. Biol.*, 1982, **161,** 211. (*s*) D. Suck, *J. Mol. Biol.*, 1982, **162,** 511. (*t*) Also includes data for complexes with deoxyadenosine polymers. (*u*) G. D. Brayer and A. McPherson, *J. Biol. Chem.*, 1982, **257,** 3359. (*v*) The crystallization procedure used is a novel one using both

Mol. wt. and no. of subunits	*Mol. wt. of asymmetric unit*	V_m/ nm^3 $dalton^{-1}$ $\times 10^3$	*Precipitant*	pH	*Ref.*
40 000 (1)	40 000	2.80	PEG6000	6.2	*y*
14 000 (1)	28 000	2.28	PEG6000	6.2	*z*
66 000 (1)	66 000	3.47	Ammonium sulphate	7.0	*aa*
45 000 (1)	45 000	2.40	Ammonium sulphate	7.0	*bb*
45 000 (1)	45 000	2.90	PEG6000	7.4	*bb*
45 000 (1)	45 000	2.30	Ammonium sulphate	7.0	*bb*
158 000 (4)	158 000	2.36	PEG6000	7.8	*cc*
—	—	—	MPD	7.0	*dd*
98 000 (4)	98 000	3.3	PEG	7.0	*ee*
49 500 (1)	49 500	3.75	Ammonium sulphate	7.5	*ff*
49 500 (1)	49 500	2.95	PEG1000	6.8	*ff*
10 000 (1)	10 000	2.19	Isopropanol	3.8	*hh*
62 400 (4)	31 200	2.06	PEG6000	6.0	*ii*
200 000 (2+2)	220 000	2.74	PEG6000	7.5	*jj*
250 000 (2+2)	500 000	2.26	PEG6000	7.5	*jj*
150 000 (3)	150 000	1.97	Ammonium sulphate	—	*kk*
43 000 (2+2)	43 000	2.20	PEG6000	7.5	*ll*

polyethylene glycol and high salt concentration. (*w*) R. J. Collier, E. M. Westbrook, D. B. McKay, and D. Eisenberg, *J. Biol. Chem.*, 1982, **257,** 5283. (x) B. McKeever and R. Sarma, *J. Biol. Chem.*, 1982, **257,** 6923. (y) B. Rubin, J. van Middlesworth, K. Thomas, and L. Hager, *J. Biol. Chem.*, 1982, **257,** 7768. (z) B. Rubin, V. Carperos, and E. Kezar, *J. Biol. Chem.*, 1982, **257,** 8896. (*aa*) A. H. Robbins, C. D. Stout, D. Piszkiewicz, O. Gawron, C.-S. Yoo, B.-C. Wang, and M. Sax, *J. Biol. Chem.*, 1982, **257,** 9061. (*bb*) T. L. Poulos, M. Perez, and G. C. Wagner, *J. Biol. Chem.*, 1982, **257,** 10 427. (*cc*) O. Brenner-Holzach and J. D. G. Smit, *J. Biol. Chem.*, 1982, **257,** 11 747. (*dd*) K. Morikawa, M. Kawakami, and S. Takemura, *FEBS Lett.*, 1982, **145,** 194. (*ee*) K. W. Olsen and R. L. Miller, *FEBS Lett.*, 1982, **145**, 303. (*ff*) J. L. White, M. G. Grütter, E. Wilson, C. Thaller, G. C. Ford, J. D. G. Smit, J. N. Jansonius and K. Kirschner, *FEBS Lett.*, 1982, **148,** 87. (gg) Also includes data for pig colipase crystal forms and a large range of horse colipase crystal forms. (*hh*) M. Pierrot, J.-P. Astier, M. Astier, M. Charles, and J. Drenth, *Eur. J. Biochem.*, 1982, **123,** 347. (*ii*) E. Pinn, A. Pähler, W. Saenger, G. A. Petsko, and N. M. Green, *Eur. J. Biochem.*, 1982, **123,** 545. (*jj*) M. S. Weininger and L. E. Mortenson, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79,** 378. (*kk*) S. Johnson, G. Grayson, L. Robinson, R. Chahade, and A. McPherson, *Biochemistry*, 1982, **21,** 4839. (*ll*) E. Lattman, R. Burlingame, C. Hatch, and E. N. Moudrianakis, *Science*, 1982, **216,** 1016.

Subramanian and Hall[4,5,6] have described techniques for the normalizing of structure factors. The choice of scaling function is shown to be crucial,[4] and the best function has been determined. Methods for obtaining the most reliable value of the overall temperature factor from Wilson plots have been considered,[5] and ways of estimating the expected errors have been described.[6]

Podjarny and Faerman[7] have described a new determinantal theory for solving the phase problem incorporating stereochemical information, and Chang[8] has considered experimental methods for *X*-ray phase determination using multiple diffraction.

Hauptman has described the theory[9] behind the integration of the techniques of direct methods and isomorphous replacement, and Hauptman and co-workers[10] have reported the first applications of the technique. The theory behind the integration of anomalous dispersion and direct methods has also been reported.[11]

Cascarano and co-workers[12] have shown how even the lighter anomalous scatterers can be located by means of a two-wavelength technique. Arndt and co-workers[13] have shown how synchrotron radiation with a wavelength near the absorption edge of an anomalous scatterer can be used to determine phases.

Data Processing.—Rees[14] has derived a general theory for the *X*-ray intensity statistics of crystals twinned by merohedry, and Oatley and French[15] have described an improved profile-fitting method for the analysis of diffractometer intensity data. The possible uses of the method, both off-line and on-line, are discussed.

Rossmann and Henderson[16] have described the use of the molecular-replacement method to determine the phases for bacteriorhodopsin to 0.6 nm resolution. Potential further uses of the technique are discussed.

Bhat and Blow[17] have described an efficient computer procedure for finding regions of continuous electron density in electron-density maps. The procedure has been applied to the structure determination of tyrosyl-tRNA synthetase.

Small-angle and Fibre Diffraction.—A neutron-diffraction data-collection system using a position-sensitive multidetector has been described.[18] The deriva-

[4] V. Subramanian and S. R. Hall, *Acta Crystallogr., Sect. A*, 1982, **38**, 577.
[5] S. R. Hall and V. Subramanian, *Acta Crystallogr., Sect. A*, 1982, **38**, 590.
[6] S. R. Hall and V. Subramanian, *Acta Crystallogr., Sect. A*, 1982, **38**, 598.
[7] A. D. Podjarny and C. Faerman, *Acta Crystallogr., Sect. A*, 1982, **38**, 401.
[8] S.-L. Chang, *Acta Crystallogr., Sect. A*, 1982, **38**, 516.
[9] H. Hauptman, *Acta Crystallogr., Sect. A*, 1982, **38**, 289.
[10] H. Hauptman, S. Potter, and C. M. Weeks, *Acta Crystallogr., Sect. A*, 1982, **38**, 294.
[11] H. Hauptman, *Acta Crystallogr., Sect. A*, 1982, **38**, 632.
[12] G. Cascarano, C. Giacovazzo, A. F. Peerdeman, and J. Kroon, *Acta Crystallogr., Sect. A*, 1982, **38**, 710.
[13] U. V. Arndt, T. J. Greenhough, J. R. Helliwell, J. A. K. Howard, S. A. Rule, and A. W. Thompson, *Nature (London)*, 1982, **298**, 835.
[14] D. C. Rees, *Acta Crystallogr., Sect. A*, 1982, **38**, 201.
[15] S. Oatley and S. French, *Acta Crystallogr., Sect. A*, 1982, **38**, 537.
[16] M. G. Rossmann and R. Henderson, *Acta Crystallogr., Sect. A*, 1982, **38**, 13.
[17] T. N. Bhat and D. M. Blow, *Acta Crystallogr., Sect. A*, 1982, **38**, 21.
[18] M. Roth and A. Lewit-Bentley, *Acta Crystallogr., Sect. A*, 1982, **38**, 670.

tion of the necessary equations and some typical examples of measured data are included.

A technique for the direct structure analysis from small-angle *X*-ray diffraction patterns has been reported.[19] Examples of its use, including bacteriophage T7, are given.

Stubbs and Makowski[20] have described a method for the co-ordinated use of isomorphous replacement and layer-line splitting in the phasing of fibre-diffraction data. The application in the structure determination of tobacco mosaic virus at 0.67 nm resolution is presented.

Baumstark and co-workers[21] have reported a procedure for the evaluation of small-angle scattering diagrams from polydispersed-particle suspensions, and Egelman and de Rosier[22] have considered the Fourier transform of actin and other helically disordered systems.

Protein Crystallography.—van Wendel de Joode and Zeelen[23] have reviewed how single-crystal *X*-ray structure analysis can be of use in medicine and pharmaceutical chemistry.

Bott and co-workers[24] have described how isoelectric focusing has been used to remove heterogeneity in three different proteins. This improved purity has led to bigger crystals that diffract more strongly. The general applicability of this technique is discussed.

Structure Refinement.—Collins[25] has shown how information theory can be used to improve electron-density images from imperfect data by iterative entropy maximization.

Scheringer[26] has given a treatment of equations of constraint in least-squares refinement, and Jameson[27] has described a technique for structure refinement using data from a twinned crystal.

Wlodawer and Hendrickson[28] have reported a procedure for the joint refinement of macromolecular structures using *X*-ray and neutron-diffraction data. A refinement of ribonuclease A using neutron data to 0.28 nm spacings and *X*-ray data to 0.20 nm spacings is described.

Olthof and Schenk[29] have described the application of their phase-extension technique to metmyoglobin at 0.2 nm resolution. They show that the procedure conserves enantiomorphs and can be used to improve the resolution.

Agard and Stroud[30] have described how averaging the electron density of non-crystallographically related molecules can be used for improvement of the

[19] D. I. Svergun, L. A. Feigin, and B. M. Schedrin, *Acta Crystallogr., Sect. A*, 1982, **38,** 827.
[20] G. Stubbs and L. Makowski, *Acta Crystallogr., Sect. A*, 1982, **38,** 417.
[21] M. Baumstark, W. Welte, and W. Kreutz, *Acta Crystallogr., Sect. A*, 1982, **38,** 835
[22] E. H. Egelman and D. J. de Rosier, *Acta Crystallogr., Sect. A*, 1982, **38,** 796.
[23] M. D. van Wendel de Joode and F. J. Zeelan, *Recl. Trav. Chim. Pays-Bas*, 1982, **101,** 81.
[24] R. R. Bott, M. A. Navia, and J. L. Smith, *J. Biol. Chem.*, 1982, **257,** 9883.
[25] D. M. Collins, *Nature, (London)*, 1982, **298,** 49.
[26] C. Scheringer, *Acta Crystallogr., Sect. A*, 1982, **38,** 618.
[27] G. B. Jameson, *Acta Crystallogr., Sect. A*, 1982, **38,** 817.
[28] A. Wlodawer and W. A. Hendrickson, *Acta Crystallogr., Sect. A*, 1982, **38,** 239.
[29] G. J. Olthof and H. Schenk, *Acta Crystallogr., Sect. A*, 1982, **38,** 117.
[30] D. A. Agard and R. M. Stroud, *Acta Crystallogr., Sect. A*, 1982, **38,** 186.

electron-density image and for subsequent phase refinement. The application of a rapid method for this averaging is described for the α-bungarotoxin structure.

Derewenda and co-workers[31] have discussed the evaluation of root-mean-square errors in protein co-ordinates and shown that values based on various reciprocal-space residuals are in good agreement with each other.

Protein Dynamics.—van Gunsteren and Berendsen[32] have described computer-simulation techniques for the study of molecular dynamics, and Ponnuswamy and Bhaskaran[33] have examined ways of determining the internal fluctuations in globular proteins.

Kossiakoff[34] has reported a new approach using neutron diffraction and hydrogen exchange to study the size and nature of conformational fluctuations in trypsin. The model that best fits the data suggests localized disruption of the secondary structure involving the breaking of a small number of hydrogen bonds.

van Gunsteren and Karplus[35] have reported a protein-dynamics study of bovine pancreatic trypsin inhibitor. They have compared the protein in solution and in the crystalline state and shown that in the solid state more side chains are specifically fixed in their positions.

Computer Graphics.—A photographic method has been described[36] that allows the determination of the co-ordinates of a molecular model from two photographs taken from arbitrary directions. An accuracy of 0.03 nm is claimed and the method has been used on the structure analysis of cytochrome C_3.

Whitted[37] has reviewed some of the recent advances in computer graphics and shows some examples of the state of the art.

Lesk and Hardman[38] have described computer programs capable of drawing stereo pairs of protein molecules. These stereo pairs consist of schematic diagrams with α-helices represented as cylinders and β-sheets as arrows.

3 Lectins and Concanavalin

Green-pea Lectin.—The crystal structure of the mitogenic lectin from the green pea has been determined at 0.6 nm resolution.[39] Data from a native

[31] Z. S. Derewenda, A. M. Brzozowski, A. Stepien, and M. J. Grabowski, *Acta Crystallogr., Sect. A*, **38**, 432.
[32] W. F. Van Gunsteren and H. J. C. Berendsen, *Biochem. Soc. Trans.*, 1982, **10**, 301.
[33] P. K. Ponnuswamy and R. Bhaskaran, *Int. J. Pept. Protein Res.*, 1982, **19**, 549.
[34] A. A. Kossiakoff, *Nature (London)*, 1982, **296**, 713.
[35] W. F. van Gunsteren and M. Karplus, *Biochemistry*, 1982, **21**, 2259.
[36] Y. Iga, M. Kusunoki, N. Yasuoka, and M. Kakudo, *Acta Crystallogr., Sect. B*, 1982, **38**, 2181.
[37] T. Whitted, *Science*, 1982, **215**, 767.
[38] A. M. Lesk and K. D. Hardman, *Science*, 1982, **216**, 539.
[39] E. J. Meehan, jun., J. McDuffie, H. Einspahr, C. E. Bugg, and F. L. Suddath, *J. Biol. Chem.*, 1982, **257**, 13 278.

crystal and a uranyl heavy-atom derivative crystal were collected by diffractometer, and an electron-density map was calculated using phases determined by isomorphous-replacement and anomalous-dispersion contributions. The molecule is a peanut-shaped dimer and is similar to the concanavalin A dimer. The pea lectin dimer may well contain a dimer-wide contiguous pleated sheet as is seen in the concanavalin A dimer.

Concanavalin A.—A refinement of the structure of concanavalin A at 0.175 nm resolution has been described.[40] A fast Fourier least-squares refinement procedure has been used and has given an R-factor of 0.167 for 22 042 structure factors. The refinement has allowed the manganese- and calcium-binding sites to be seen more clearly, and both ions are seen to be pseudo-octahedral. Average bond lengths for the five Mn^{2+}—O and the seven Ca^{2+}—O bonds are 0.228 nm and 0.245 nm, respectively. For the Mn^{2+} ion this is 0.01 nm longer than that seen for the Mn^{II} hexa-aquoion and at the high end of the range seen for Mn^{2+}—O complexes. This may well have repercussions for the use of manganese in n.m.r. studies of metalloproteins. The improved structure also confirms the non-proline *cis* peptide bond immediately adjacent to the Ca^{2+}, and the carbohydrate-binding sites have been located.

4 Oxygen- and Electron-carrying Proteins

Myoglobin.—Hartmann and co-workers[41] have determined the structure of sperm-whale metmyoglobin at 80 K to a resolution of 0.2 nm. The overall structure is similar to that of the protein at 300 K except that the volume is smaller and the structure is more rigid. However, even at 80 K there is evidence for the existence of conformational substates.

The binding of imidazole to ferric Aplysia and sperm-whale myoglobins has been studied by difference Fourier analysis.[42] The imidazole binds to the iron distal sites in both proteins despite the absence of the distal histidine in the Aplysia protein. In the sperm-whale myoglobin the distal histidine is displaced by the exogenous imidazole and leads to structural changes in the region of the haem. In both proteins the exogenous imidazole ring is approximately perpendicular to the haem plane and points towards the external part of the haem crevice.

Haemoglobin.—An X-ray analysis of the iron–oxygen bond in human oxyhaemoglobin has been described[43] and has shown that the FeOO bond angle is 156°. This value is intermediate between the angles seen in the 'picket fence' complex and oxyerythrocruorin. This position of the N^{ε} of histidine E7 in the α-subunit suggests that it forms a hydrogen bond to the bound oxygen as has

[40] K. D. Hardman, R. C. Agarwal, and M. J. Freiser, *J. Mol. Biol.*, 1982, **157**, 69.
[41] H. Hartmann, F. Parak, W. Steigemann, G. A. Petsko, D. Ringe Ponzi, and H. Frauenfelder, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79**, 4967.
[42] M. Bolognesi, E. Cannillo, P. Ascenzi, G. M. Giacometti, A. Merli, and M. Brunori, *J. Mol. Biol.*, 1982, **158**, 305.
[43] B. Shaanan, *Nature* (*London*), 1982, **296**, 683.

been seen for oxymyoglobin. In the β-subunit this bond length is longer, suggesting that any such hydrogen bond would be weaker.

The structure of cobalt-substituted human deoxyhaemoglobin has been determined at 0.25 nm resolution and compared to the native ferrous form.[44] The distance between the metal and the haem plane is 0.033 nm for the cobalt derivative and 0.056 nm for the native form, and the metal-to-histidine F8N$^{\epsilon}$ bond length is 0.01—0.02 nm longer for the cobalt form. The distance between the histidine N$^{\epsilon}$ and the mean haem plane is unaltered and there are no significant changes in the structure of the protein.

Perutz and Brunori[45] have described model-building studies that explain the low oxygen affinity of many fish haemoglobins. A stereochemical model for the binding of the allosteric effectors, ATP and GTP, is also proposed.

Sickle Haemoglobin.—A low-resolution study of a second monoclinic crystal form of deoxygenated sickle haemoglobin has been reported.[46] This shows that the interconversion of the two forms depends on a shift along only one of the axes that form the double filament.

Vassar and co-workers[47] have shown that crystal growth of sickle haemoglobin in acidic polyethylene glycol is initiated by fibre formation, these fibres then converting to larger units, macrofibres, within several hours. These macrofibres then align and fuse, and it seems that these fibrous forms are a metastable state whose ultimate fate is to crystallize.

Methydroxohaemerythrin.—Stenkamp and co-workers[48] have described in detail the refinement of methydroxohaemerythrin at 0.2 nm resolution. Since the intention was to obtain more detailed information on the binuclear iron complex, these atoms together with all side chains previously thought to be complexed to the iron atoms were initially left out of the refinement. The fact that the crystallographic asymmetric unit contains four independent subunits has been used to check the various refinement steps.

Haemocyanin.—The 0.5 nm resolution structure of haemocyanin from the spiny lobster has been reported.[49] The hexameric molecule has 32 point symmetry and the 0.5 nm resolution map clearly shows the shape of the hexamer and the subunit boundaries. The subunits are positioned at the corners of an antiprism, the molecule appearing hexagonal, 12 nm diameter, when viewed along the three-fold axis and rectangular, 9.0 nm × 12.0 nm, when viewed along the two-fold axis. Each subunit makes extensive contacts with three other subunits and possibly a weaker contact with a fourth subunit.

[44] G. Fermi, M. F. Perutz, L. C. Dickinson, and J. C. W. Chien, *J. Mol. Biol.*, 1982, **155**, 495.
[45] M. F. Perutz and M. Brunori, *Nature (London)*, 1982, **299**, 421.
[46] L. S. Rosen and B. Magdoff-Fairchild, *J. Mol. Biol.*, 1982, **157**, 181.
[47] R. J. Vassar, M. J. Potel, and R. Josephs, *J. Mol. Biol.*, 1982, **157**, 395.
[48] R. E. Stenkamp, L. C. Sieker, and L. H. Jensen, *Acta Crystallogr., Sect. B*, 1982, **38**, 784.
[49] E. J. M. van Schaick, W. G. Schutter, W. P. J. Gaykema, A. M. H. Schepman, and W. G. J. Hol, *J. Mol. Biol.*, 1982, **158**, 457.

Lamy and co-workers[50] have examined the structure of the 48 subunit haemocyanin from horseshoe crab by electron microscopy.

Cytochrome *c*.—Koppenol and Margoliash[51] have described the asymmetric distribution of charges on the surface of horse cytochrome *c*.

Cytochrome C_3.—The crystal structure of cytochrome C_3 from *Desulphovibrio desulphuricans* has been determined at 0.25 nm resolution.[52] The molecule is folded into two domains with one haem in each with the other two haems lying in the groove that divides the molecule. The core of the protein is the compact four-haem cluster, which has a fairly high degree of exposure to solvent. The arrangement of haem groups suggests that electron transfer may occur through direct haem-group contacts, either by the overlapping system of π-orbitals or through mediation by intervening side chains or both.

Cytochrome C_{551}.—The refinements of the ferri and ferro forms of cytochrome C_{551} from *Pseudomonas aeruginosa* have been described.[53] The oxidized and reduced forms have almost identical tertiary structures with the largest differences along the two edges of the haem crevice. The structure of cytochrome C_{551} is compared in a detailed way with tuna cytochrome C, and the implications of the differences seen for the functioning of cytochrome C_{551} are discussed.

Ferredoxin.—A crystallographic refinement of ferredoxin from *Azobacter vinelandii* has been performed at 0.2 nm resolution.[54] The refinement has located 344 water molecules out of over 700 in the asymmetric unit. The geometries of the 3Fe and 4Fe centres have also been revealed in detail. The 3Fe–3S centre is liganded to five cysteinyl sulphur atoms and a solvent oxygen (water or hydroxyl) with distorted tetrahedral co-ordination at each iron centre. The 4Fe–4S cluster is liganded to 4 cysteinyl sulphur atoms and is very similar in geometry to the (4Fe–4S)$4S_\gamma$ clusters seen in high-potential iron protein and *Peptococcus ferredoxin*. The paper discusses the structure of the protein and the protein environments of the iron–sulphur sites in detail.

5 Ribonuclease and Lysozyme

Ribonuclease A.—The structure of bovine pancreatic ribonuclease A has been refined[55] at 0.2 nm resolution using a restrained-parameter least-squares procedure. The final R-factor is 0.159 with the main-chain atoms determined with

[50] J. Lamy, P.-Y. Sizaret, J. Frank, A. Verschoor, R. Feldmann, and J. Bonaventura, *Biochemistry*, 1982, **21**, 6825.
[51] W. H. Koppenol and E. Margoliash, *J. Biol. Chem.*, 1982, **257**, 4426.
[52] M. Pierrot, R. Haser, M. Frey, F. Payan, and J.-P. Astier, *J. Biol. Chem.*, 1982, **257**, 14 341.
[53] Y. Matsuura, T. Takano, and R. E. Dickerson, *J. Mol. Biol.*, 1982, **156**, 389.
[54] D. Ghosh, S. O'Donnell, W. Furey, jun., A. H. Robbins, and C. D. Stout, *J. Mol. Biol.*, 1982, **158**, 73.
[55] A. Wlodawer, R. Bott, and L. Sjölin, *J. Biol. Chem.*, 1982, **257**, 1325.

an estimated accuracy of 0.017 nm. The final model also includes a phosphate ion in the active site and 176 water molecules, many with partial occupancy. All bond lengths and angles are very close to ideal values, and the model is in very good agreement with the final difference Fourier maps.

The two active-site histidines, His-12 and His-119, form hydrogen bonds to the phosphate ion. His-119 also hydrogen-bonds to the carboxyl group of aspartate-121, and His-12 hydrogen-bonds to the carbonyl of threonine-45. The active-site region is very similar to that of ribonuclease S, and the root-mean-square discrepancy for this region is 0.06 nm. For the parts of the structure common to ribonuclease A and S, residues 1—16 and 24—123, the root-mean-square discrepancy is 0.106 nm.

Borkakoti and co-workers[56] have reported a least-squares refinement of ribonuclease A at 0.145 nm resolution with a final R-factor of 0.26 for the 19 238 reflections. Many minor corrections to the secondary structure have been made, and 79 water molecules have been located in the first co-ordination sphere. A sulphate ion has been located in the active site, and alternative sites have been found for the histidine-119 side chain with occupancies of 0.80 and 0.20. Extensive disorder for side chains on residues 35—39 is also seen. The root-mean-square deviation in atomic position between the starting and final model is 0.11 nm with some shifts of 0.7 to 0.8 nm, where side chains were relocated.

A hydrogen-exchange study has been reported for ribonuclease A[57] and together with neutron diffraction has allowed a determination of the degree of exchange occurring at the hydrogens. Most protected peptide amide hydrogens were involved in hydrogen bonds with main-chain carbonyl oxygens.

Ribonuclease T_1.—The structure of ribonuclease T_1 complexed with 2′-guanylic acid has been described.[58] The enzyme is folded into an α-helix of 4.5 turns covered by a four-stranded antiparallel β-sheet. The strict specificity for guanine arises from hydrogen bonds from the main-chain peptide groups to the O-6 and N-1 hydrogen of the guanine together with stacking of tyrosine-45 on the guanine. The geometry of the active site has also shown the residues involved in catalysis and suggested a mechanism for the catalytic step.

***Bacillus amyloliquefaciens* Ribonuclease.**—The crystal structure of the ribonuclease from *Bacillus amyloliquefaciens* has been reported[59] at 0.25 nm resolution. The electron-density map was readily interpretable and reveals that the molecule consists of a large central β-sheet, five-stranded, and two α-helices. This structure is very different from that of bovine pancreatic ribonucleases and in addition the residues forming the active site do not come from analogous regions of the structure.

[56] N. Borkakoti, D. S. Moss, and R. A. Palmer, *Acta Crystallogr., Sect. B*, 1982, **38**, 2210.
[57] A. Wlodawer and L. Sjölin, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79**, 1418.
[58] U. Heinemann and W. Saenger, *Nature (London)*, 1982, **299**, 27.
[59] Y. Mauguen, R. W. Hartley, E. J. Dodson, G. G. Dodson, G. Bricogne, C. Chothia, and A. Jack, *Nature (London)*, 1982, **297**, 162.

Ribonuclease St.—Another member of the Bacillus family of ribonucleases, Ribonuclease St, an enzyme from *Streptomyces erythreus*, has had its structure determined at 0.25 nm resolution.[60] Again it is seen that the enzyme shows little similarity to bovine pancreatic ribonuclease both in terms of protein folding and in terms of the active-site geometry. The active site, however, does show some similarity to the *Bacillus amyloliquefaciens* enzyme.

Hen Egg-white Lysozyme.—The crystal structures of an orthorhombic and a monoclinic form of hen egg-white lysozyme have been determined at 0.6 nm resolution.[61] In the monoclonic crystal at this resolution the molecules appear identical to the tetragonal form of the crystals and the molecular packing occludes the active sites. In the orthorhombic crystals the molecules are again identical to the tetragonal form but only sugar-binding sites A and B are blocked, the lower part of the active-site cleft being accessible. Neither of these crystal forms can be used for low-temperature studies of substrate binding.

Viscoelasticity measurements on crystals of hen egg-white lysozyme have been reported[62] and the data can be explained on the assumption that the molecule consists of two rigid domains connected by a flexible link. Binding of the inhibitor, *N*-acetyl-D-glucosamine, leads to an increase of about 40% in the interdomain rigidity.

Bacteriophage T_4 Lysozyme.—Grutter and Matthews[63] have examined the structure of a mutant form of T_4 lysozyme with a single amino-acid substitution 2.5 nm from the active site. The change, the replacement of glutamate-128 by a lysine residue, causes very little change in the structure of the lysozyme molecule, although the enzyme has only 4% of the catalytic activity of the native enzyme. The results suggest that glutamate-128 participates directly in substrate binding and/or catalysis. This glutamate residue is located on the *C*-terminal domain, an area that has no counterpart in the hen egg-white lysozyme molecule. It is suggested that the role of this *C*-terminal domain is to bind the peptide crosslink that connects neighbouring saccharide strands in the *E. coli* cell wall.

6 Proteolytic Enzymes

Japanese Quail Ovomucoid.—The structure of the third domain of Japanese quail ovomucoid, a Kazal-type inhibitor, has been crystallographically refined with energy constraints.[64] The hydrogen-bonding pattern and the amino-acid

[60] K. T. Nakamura, K. Iwahashi, Y. Yamamoto, Y. Iitaka, N. Yoshida, and Y. Mitsui, *Nature (London)*, 1982, **299**, 564.
[61] P. J. Artymiuk, C. C. F. Blake, D. W. Rice, and K. S. Wilson, *Acta Crystallogr., Sect. B*, 1982, **38**, 778.
[62] T. Ya. Morozova and V. N. Morozov, *J. Mol. Biol.*, 1982, **157**, 173.
[63] M. G. Grütter and B. W. Matthews, *J. Mol. Biol.*, 1982, **154**, 525.
[64] E. Papamokos, E. Weber, W. Bode, R. Huber, M. W. Empie, I. Kato, and M. Laskowski, jun., *J. Mol. Biol.*, 1982, **158**, 515.

variation in the Kazal family show a high degree of structure and sequence conservation. The conformation of the reactive-site loop is similar to those of basic pancreatic trypsin inhibitor, *Streptomyces subtilisin* inhibitor, and soybean trypsin inhibitor. A computer graphics system has been used to model complexes of the ovomucoid with trypsin, chymotrypsin, and elastase. Detailed interactions are proposed for the enzyme–inhibitor complex.

Trypsin/Trypsinogen.—The crystal structure of trypsinogen mercurated at the Cys-191–Cys-200 disulphide has been analysed[65] in its free form and bound to pancreatic trypsin inhibitor in the presence of the dipeptide Ile-Val. The mercurated disulphide is located in the mobile activation domain of the molecule, and in the derivatized trypsinogen molecule the single mercury atom is not detectable. In the trypsinogen–PTI complex the mercury atom is well defined, and these results suggest thermal or static disorder for the activation domain. Structure determinations at 173 and 103 K show that only the region near the *N*-terminus becomes more ordered, suggesting that the activation domain exhibits predominantly static disorder.

The crystal structure of trypsinogen complexed with pancreatic secretory trypsin inhibitor (Kazal type) has been reported at 0.18 nm resolution.[66] The structure of the complex and 162 water molecules has been refined to an R-factor of 0.195 for the 26 341 reflections. The inhibitor binds at nine sites on the contact area and has a conformation very similar to that of the quail ovomucoid. The trypsinogen part of the complex resembles the trypsin structure, but two segments of the activation domain adopt a different conformation. These detailed differences are described fully.

Elastase.—The binding of a trifluoroacetyl dipeptide anilide inhibitor to porcine pancreatic elastase has been determined[67] at 0.25 nm resolution by difference Fourier techniques. The inhibitor, a lysyl-alanine derivative, binds with the trifluoroacetyl group at the S_1 subsite in the active centre. The dipeptide anilide binds at sites close to the S_1' to S_3' substrates with the dipeptide forming a parallel pleated sheet with the protein backbone chain. This is very different from the binding of *N*-acetylated short peptide inhibitors to elastase.

***Streptomyces griseus* Protease B.**—The structure of protease B bound to the third domain of the ovomucoid inhibitor from turkey has been determined at 0.18 nm resolution.[68] The structure has been refined by a restrained-parameter least-squares technique and shows that the carbonyl carbon atom of the reactive bond between Leu-18 and Glu-19 lies 0.271 nm away from the

[65] J. Walter, W. Steigemann, T. P. Singh, H. Bartunik, W. Bode, and R. Huber, *Acta Crystallogr., Sect. B*, 1982, **38**, 1462.
[66] M. Bolognesi, G. Gatti, E. Menegatti, M. Guarneri, M. Marquart, E. Papamokos, and R. Huber, *J. Mol. Biol.*, 1982, **162**, 839.
[67] D. L. Hughes, L. C. Sieker, J. Bieth, and J.-L. Dimicoli, *J. Mol. Biol.*, 1982, **162**, 645.
[68] M. Fujinaga, R. J. Read, A. Sielecki, W. Ardelt, M. Laskowski, jun., and M. N. G. James, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79**, 4868.

Ser-195 side-chain oxygen. This distance is 0.05 nm shorter than a normal van der Waals contact. Unlike the trypsin–pancreatic trypsin inhibitor complex the Leu–Glu bond is not distorted away from planarity towards a pyramidal configuration.

Thermolysin.—The structure of thermolysin has been refined[69] to a nominal resolution of 0.16 nm and to a final R-factor of 0.213 for the 34 671 reflections. The refined structure has root-mean-square deviations of 0.0021 nm from ideal bond lengths and 2.9° from ideal bond angles. The final model includes 173 solvent molecules, seven of which are buried in the molecule. Unusual features seen in the structure include a *cis*-proline, a γ-turn, and a single turn of left-handed helix. The analysis also confirms that the structure does not contain any unusual features that specifically confer the thermostability on the protein.

The structure of thermolysin complexed with a mercaptan inhibitor has been determined,[70] and the complex serves as a model for the inhibition of zinc peptidases by substrate-analogue mercaptans. The inhibitor binds with the sulphur atom, presumably in the anionic form, tetrahedrally co-ordinated to the zinc and displacing a water molecule which is bound to the native protein. Such binding had been predicted on general chemical grounds and inferred from spectroscopic studies.

Carboxypeptidase A.—A refined crystal structure has been reported for the complex of carboxypeptidase with the 39-residue inhibitor protein from potatoes.[71] The inhibitor structure contains a core of three disulphide bridges and one turn of 3_{10} helix. The carboxy-terminal glycine of the inhibitor is cleaved from the inhibitor and remains trapped in the back of the active-site pocket. Several interactions between the inhibitor and residues of the protein resemble possible features of substrate binding, but not all these interactions would be available to ester substrates. With the exception of some of the inhibitor-binding residues the carboxypeptidase A protein structure is very similar to that of the unliganded form, which was solved from a totally different crystal form.

Zn^{2+} G Peptidase.—The structure of a zinc-containing penicillin-resistant D-alanyl-D-alanine peptidase from *Streptomyces albus* has been determined at 0.25 nm resolution.[72] The enzyme consists of two globular domains with a single polypeptide chain link. The *N*-terminal domain contains three α-helices and one disulphide bridge and the *C*-terminal domain contains three α-helices, a five-stranded β-sheet, two disulphide bridges, and the zinc ion. The zinc ion is ligated by three histidine residues and is located in a cleft. The active-centre

[69] M. A. Holmes and B. W. Matthews, *J. Mol. Biol.*, 1982, **160,** 623.
[70] A. F. Monzingo and B. W. Matthews, *Biochemistry*, 1982, **21,** 3390.
[71] D. C. Rees and W. N. Lipscomb, *J. Mol. Biol.*, 1982, **160,** 475.
[72] O. Dideberg, P. Charlier, G. Dive, B. Joris, J. M. Frère, and J. M. Ghuysen, *Nature* (*London*), 1982, **299,** 469.

geometry suggests that the mechanism of action may be related to that of the other zinc carboxypeptidases.

Penicillopepsin.—The binding of an esterified tripeptide fragment of pepstatin to the aspartyl proteinase penicillopepsin has been described at 0.18 nm resolution.[73] As well as clearly seeing the inhibitor-binding mode, a substantial enzyme conformational change is seen. A β-loop of residues (71—83) moves, providing confirmation of the importance of enzyme flexibility in the aspartyl proteinase mechanism. The binding mode seen is similar to that predicted for the binding of good substrates to penicillopepsin.

***Rhizopus chinensis* Carboxyl Proteinase.**—Bott and co-workers[74] have examined the binding of pepstatin to *Rhizopus* carboxyl proteinase at 0.25 nm resolution. The results suggest that the statine residue of the inhibitor approaches an analogue of the catalytic transition state. As with penicillopepsin[73] the β-flap is seen to fold down onto statine-4 of the inhibitor. The detailed binding features have been extended to a model for substrate binding and the transition-state complex. This model has led the authors to suggest a mechanism for the action of carboxyl proteinases.

Calotropin DI.—The structure of calotropin DI, a sulphydryl proteinase from the madar plant, has been determined at 0.32 nm resolution.[75] The protein chain is folded into two distinct lobes, one composed mainly of α-helices, the other composed of an all-antiparallel β-sheet. This overall molecular architecture resembles that found in papain and actinidin. Several amino-acid side chains in the active site have been identified and show a similar arrangement to those seen in papain and actinidin. Subtle changes in the folding of the first 18 *N*-terminal residues explain the altered reactivity of calotropin DI to synthetic substrates that are hydrolysed by papain and actinidin.

Papain.—The effects of substitution of phenyl hippurates on the structure–function relationship of papain hydrolysis have been studied[76] by combining the results of *X*-ray crystallography, computer graphics, and kinetic studies.

7 Glycolytic Enzymes

Phosphorylase.—Sprang and co-workers[77] have reported studies on the nucleoside inhibitor site of phosphorylase *a*. They have examined the binding of adenine, caffeine, adenosine, inosine, ATP, and FMN by difference Fourier

[73] M. N. G. James, A. Sielecki, F. Salituro, D. H. Rich, and T. Hofmann, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79**, 6137.
[74] R. Bott, E. Subramanian, and D. R. Davies, *Biochemistry*, 1982, **21**, 6956.
[75] U. Heinemann, G. P. Pal, R. Hilgenfeld, and W. Saenger, *J. Mol. Biol.*, 1982, **161**, 591.
[76] R. N. Smith, C. Hansch, K. H. Kim, B. Omiya, G. Fukumura, C. D. Selassie, P. Y. C. Jow, J. M. Blaney, and R. Langridge, *Arch. Biochem. Biophys.*, 1982, **215**, 319.
[77] S. Sprang, R. Fletterick, M. Stern, D. Yang, N. Madsen, and J. Sturtevant, *Biochemistry*, 1982, **21**, 2036.

analysis and have shown that an intercalative complex is formed with the heterocyclic ring system stacked between the side chains of a phenylalanine residue Phe (-285) and a tyrosine residue (Tyr-612). When so bound the inhibitor stabilizes the same conformation of residues 282—286 which binds α-D-glucose, an inhibitor that shows synergism with the purine ligands. No other significant hydrogen-bonded contacts are made, and the polar groups of the ligands are solvated at the surface of the protein. The binding of the inhibitors has also been studied by kinetic and calorimetric techniques.

The binding of α-D-glucose to phosphorylase *a* has been studied[78] by crystallographic methods. The inhibitor binds at the catalytic site located at the junction of the *N*- and *C*-terminal domains. The active-site residues, located on flexible loops of the polypeptide chain at the domain interface, form at least five well defined hydrogen bonds with the bound glucose. Model-building studies show that changes in chirality or substitution at any of the glucose hydroxyl groups can abolish or greatly reduce the binding of the inhibitor. These changes lead to a reduction in hydrogen-bonding ability, steric conflicts in the active site, or both. The structural differences of the binding in the T- and R-forms of the enzyme have been examined and the effects of substrate analogues on binding are discussed.

The structural changes associated with activation of phosphorylase have been examined by crystallographic and kinetic studies.[79] The use of a substrate analogue, glucose cyclic 1,2-phosphate, has shown that its binding results in a partial activation. This leads to changes in the crystal-lattice constants, and in difference Fouriers considerable structural rearrangements are seen to occur. One such change, an order-to-disorder transition for the hairpin loop (residues 282—286) at the entrance to the active site, leads to a substrate phosphate-binding pocket appearing at the active site and increases accessibility to oligosaccharides. The phosphorus atom of the glucose cyclic 1,2-phosphate lies 0.68 nm away from the pyridoxal phosphate phosphorus atom. Possible catalytic mechanisms involving a catalytic function for the pyridoxal phosphate are discussed.

Goldsmith and co-workers[80] have determined the structure of the glucose heptamer, maltoheptase, at 0.25 nm resolution by difference Fourier analysis of its complex with phosphorylase *a*. It is a left-handed helical structure with 6.5 glucose residues per turn and a rise per residue of 0.24 m.

L-Lactate Dehydrogenase.—The low-resolution structure of L-lactate dehydrogenase from *Lactobacillus casei* has been determined.[81] The protein, an allosteric enzyme, has been crystallized as a complex with the activators fructose-1,6-bisphosphate and cobalt ions. The molecular two-fold axes have been located and all the tetramers in the unit cell have 222 symmetry. Comparison with the dogfish enzyme has allowed the enzyme's orientation to

[78] S. R. Sprang, E. J. Goldsmith, R. J. Fletterick, S. G. Withers, and N. B. Madsen, *Biochemistry*, 1982, **21**, 5364.
[79] S. G. Withers, N. B. Madsen, S. R. Sprang, and R. J. Fletterick, *Biochemistry*, 1982, **21**, 5372.
[80] E. Goldsmith, S. Sprang, and R. Fletterick, *J. Mol. Biol.*, 1982, **156**, 411.
[81] M. Buehner, H.-J. Hecht, R. Hensel, and U. Mayr, *J. Mol. Biol.*, 1982, **162**, 819.

be determined and has shown that the structures are similar enough to allow a structure determination to be carried out by the molecular-replacement method. The structure at 0.5 nm resolution shows that the *L. casei* enzyme lacks the *N*-terminal arm and the active-site loop is involved in intermolecular contacts.

Alcohol Dehydrogenase.—Eklund and co-workers[82] have determined the structures of the complexes of liver alcohol dehydrogenase with pyrazole, NAD^+ + pyrazole, and NAD^+ + 4-iodopyrazole. The pyrazole binary complex crystals are isomorphous with the native crystals and the pyrazole binds to the active-site zinc atom in a manner analogous to that of imidazole. The crystals of the ternary complex are isomorphous with the alcohol dehydrogenase–NADH–dimethyl sulphoxide complex. One of the pyrazole nitrogens binds to the active-centre zinc atom and the other nitrogen is 0.2 nm away from the C-4 atom of the nicotinamide. The iodine atom of the 4-iodopyrazole lies in the hydrophobic substrate cleft. The effects of substitutions on the pyrazole ring are discussed in terms of their effects on the inhibition properties of the analogues.

The binding of 1,4,5,6-tetrahydronicotinamide adenine dinucleotide and *trans*-4-(*N*,*N*-dimethylamino)cinnamaldehyde to alcohol dehydrogenase has been described.[83] Two crystal modifications have been examined, an orthorhombic form, with the coenzyme analogue bound to the apoenzyme conformation, and a triclinic form, with coenzyme and substrate bound to the holoenzyme conformation. The analysis shows that the aldehyde molecule is directly liganded to the metal atom in the triclinic ternary complex.

Crystal-structure analysis of alcohol dehydrogenase complexed with NAD^+ and *p*-bromobenzyl alcohol has been reported at 0.29 nm resolution.[84] Both subunits of the dimer bind substrate and coenzyme in the same manner, and the bromophenyl group is accommodated in a large hydrophobic pocket. The alcohol oxygen is directly liganded to the catalytic zinc atom, the zinc being four-co-ordinated with no room for a water molecule to make the zinc five-co-ordinate. The observed structure is probably a non-productive complex that could rapidly become productive by a rotation of the alcohol. A model for this productive complex readily explains the stereospecificity of hydride transfer observed for ethanol.

8 Hormones

Insulin.—The crystal structure of bovine insulin lacking the phenylalanine residue at position 1 of the B-chain has been determined at 0.25 nm resolution.[85] The crystals are nearly isomorphous with the 2-zinc porcine insulin structure, and this has been used in the phasing of the *X*-ray data. Fast Fourier

[82] H. Eklund, J.-P. Samana, and L. Wallen, *Biochemistry*, 1982, **21**, 4858.
[83] E. Cedergren-Zeppezauer, J.-P. Samama, and H. Eklund, *Biochemistry*, 1982, **21**, 4895.
[84] H. Eklund, B. V. Plapp, J.-P. Samama, and C.-I. Branden, *J. Biol. Chem.*, 1982, **257**, 14 349.
[85] G. D. Smith, W. L. Duax, E. J. Dodson, G. G. Dodson, R. A. G. de Graaf, and C. D. Reynolds, *Acta Crystallogr., Sect. B*, 1982, **38**, 3028.

refinement and the use of the computer program MODELFIT have given a residual of 0.18 for the 2128 structure factor.

The removal of the B1 phenylalanine residue allows almost free rotation around the C^{β}–C^{γ} bond of tyrosine residue A14. This increase in mobility explains the discrepancies in the calculated and observed circular-dichroism spectra.

9 Other Globular Proteins

Dihydrofolate Reductase.—The crystal structures of *E. coli* dihydrofolate reductase with methotrexate and *Lactobacillus casei* dihydrofolate reductase with NADPH and methotrexate have been extended to 0.17 nm resolution.[86] Both enzymes have a very similar structure, and several interesting features, including a *cis* linkage at Gly-97 to Gly-98, have appeared during the refinement. Detailed changes in methotrexate binding have appeared and include the involvement of a fixed water molecule, and a model for the binding of the true substrate is discussed.

The binding of NADPH has also been examined[87] in the refined structure. Several structurally conserved water molecules are seen to be involved in the binding, and three oxygen atoms from the enzyme lie in the plane of the pyridine ring and may possibly help in stabilizing a C-4 carbonium ion in the transition state.

The structure of chicken-liver dihydrofolate reductase complexed with NADPH and the inhibitor phenyltriazine has been determined at 0.29 nm resolution.[88] The overall molecular shape is the same as that of the bacterial enzymes, and the majority of the extra residues are located at three loops far from the active site. The triazine ring of the inhibitor binds in a position analogous to the pyrimidine portion of methotrexate, while the inhibitor's phenyl ring occupies a position analogous to the pyrazine and C-9–N-10 portion of methotrexate. NADPH binding is very similar to that seen with the bacterial enzymes. The movements associated with the binding of the inhibitor have been examined by difference Fourier analysis, and it is seen that two residues, Glu-30 and Tyr-31, move by more than 0.3 nm.

Dauber and co-workers[89] have examined the structure, energetics, and dynamics of ligand binding to dihydrofolate reductase.

Citrate Synthase.—The crystallographic refinements of two different forms of citrate synthase at 0.27 and 0.17 nm resolution have been reported.[90] The enzyme is seen to have substantially different conformations in the two crystal forms, and it seems that a structural change is required for enzymatic activity.

[86] J. T. Bolin, D. J. Filman, D. A. Matthews, R. C. Hamlin, and J. Kraut, *J. Biol. Chem.*, 1982, **257,** 13 650.
[87] D. J. Filman, J. T. Bolin, D. A. Matthews, and J. Kraut, *J. Biol. Chem.*, 1982, **257,** 13 663.
[88] K. W. Volz, D. A. Matthews, R. A. Alden, S. T. Freer, C. Hansch, B. T. Kaufman, and J. Kraut, *J. Biol. Chem.*, 1982, **257,** 2528.
[89] P. Dauber, D. J. Osgusthorpe, and A. T. Hagler, *Biochem. Soc. Trans.*, 1982, **10,** 312.
[90] S. Remington, G. Wiegand, and R. Huber, *J. Mol. Biol.*, 1982, **158,** 111.

The molecule is a dimer and the overall structure is all α-helix with 40 helices in total in the dimer. Many of these helices are either kinked or smoothly bent over large angles. Several of the helices show an unusual antiparallel packing. Each subunit is composed of two domains, one large, one small, and in the tetragonal crystal forms the two domains have a deep cleft between them. In the monoclinic crystal form an 18° rotation of the small domain has closed the cleft.

Substrate binding has been examined, and the citrate- and CoA-binding sites are found to be composed of residues from both subunits.

2-Keto-3-deoxy-6-phosphogluconate Aldolase.—The structure of 2-keto-3-deoxy-6-phosphogluconate aldolase has been extended to 0.28 nm resolution,[91] and all 125 residues have now been located. The molecule is folded as an eight-stranded α/β barrel, and the interfaces of the two forms of crystallographic trimers have been examined. The active site has been located near the Schiff base forming lysine residue, but a supposedly catalytic glutamate residue is found to be 2.5 nm away.

The folding of 2-keto-3-deoxy-6-phosphogluconate aldolase has been compared with triose phosphate isomerase and pyruvate kinase.[92] Results seem at first to suggest convergent evolution to a structure of great stability since there seems to be no orientational preference when the structures are compared. However, all three enzymes activate a C—H bond adjacent to a carbonyl group and their active sites correspond to the f-strand:F-helix region of the barrel. This might suggest divergent evolution from a common precursor.

Aspartate Carbamoyltransferase.—The crystal structures of native and CTP-bound aspartate carbamoyltransferase have been refined at 0.30 and 0.28 nm resolution, respectively.[93] The overall structure of the enzyme is the same in the two forms, but large differences are seen in the area of CTP binding to the regulatory chain. A ten-residue segment of the catalytic chain, well ordered in the native form, is found to be disordered when CTP is bound. Each catalytic monomer makes contact with three catalytic chains and two regulatory momomers, and each regulatory monomer makes contact with one other regulatory chain and two catalytic chains. The authors examine the interface regions in detail and discuss known chemical modifications and mutations in the light of the structure.

The binding modes of CTP, ATP, 5-bromo-CTP, 8-bromo-GTP, formycin A 5′-triphosphate, 3-N^6-etheno-ATP, phosphate/carbamoyl-D,L-aspartate, and pyrophosphate to the catalytic and regulatory chains of aspartate carbamoyl transferase have been examined at 0.3 nm resolution.[94] Pyrophosphate and phosphate penetrate deeply into the phosphate crevice of the catalytic

[91] I. M. Mavridis, M. H. Matada, A. Tulinsky, and L. Lebioda, *J. Mol. Biol.*, 1982, **162,** 419.
[92] L. Lebioda, M. H. Matada, A. Tulinsky, and I. M. Mavridis, *J. Mol. Biol.*, 1982, **162,** 445.
[93] R. B. Honzatko, J. L. Crawford, H. L. Monaco, J. E. Ladner, B. F. P. Edwards, D. R. Evans, S. G. Warren, D. C. Wiley, R. C. Ladner, and W. N. Lipscomb, *J. Mol. Biol.*, 1982, **160,** 219.
[94] R. B. Honzatko and W. N. Lipscomb, *J. Mol. Biol.*, 1982, **160,** 265.

chain, whereas the non-brominated nucleotides bind with their β- and γ-phosphates at an exposed part of the cleft. The brominated nucleotides bind at the same place, but all three phosphates and the ribose moiety interact with the protein.

Binding to the regulatory chain is also seen to be in two modes. ATP and CTP bind to nearly the same site on the allosteric domain and in similar conformations. The effector 8-bromo-CTP binds at a site that does not overlap the ATP site.

Binding studies[95] of Gd^{3+}–ATP, Al^{3+}–ATP, and Gd^{3+}–CTP show the nucleotide complexes to be located at the allosteric effector domain of the enzyme and bound in conformations similar to those of the metal-free nucleotides. No binding of nucleotides is observed in the phosphate crevice, but a single gadolinium ion binds in the active-site region.

Catabolite Gene Activator Protein/*cro* Repressor.—The 0.29 nm resolution electron-density map of *E. coli* catabolite gene activator protein has been interpreted in light of the amino-acid sequence.[96] Each subunit of the dimer is composed of two domains, the larger *N*-terminal domain binding cyclic AMP and forming the subunit interface. Binding of the cAMP has been described in detail and may alter the relative orientation of the two subunits. This in turn would change the structure of the DNA-binding site and the protein may bind to left-handed B-DNA

Steitz and co-workers[97] have described the similarity between the DNA-binding regions of catabolite gene activator protein and the *cro* repressor protein from bacteriophage λ. A structural unit of two consecutive helices is very similar in the two structures and it is the second helix of the unit which is proposed to bind to the major groove of DNA.

Ohlendorf and co-workers[98] have considered the interaction of *cro* repressor with DNA and shown how recognition appears to occur *via* multidentate hydrogen bonds between the protein and the base-pair atoms in the DNA major groove.

Weber and co-workers[99] have shown that the cAMP-binding domains of the regulatory subunit of cAMP-dependent protein kinase and the catabolite gene activator protein are homologous.

Superoxide Dismutase.—The structure of bovine erythrocyte Cu,Zn superoxide dismutase has been determined at 0.2 nm resolution.[100] All four crystallographically independent subunits have been fitted and were refined to a residual R-factor of 0.255 with a root-mean-square deviation of 0.003 nm from ideal

[95] R. B. Honzatko and W. N. Lipscomb, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79,** 7171.
[96] D. B. McKay, I. T. Weber, and T. A. Steitz, *J. Biol. Chem.*, 1982, **257,** 9518.
[97] T. A. Steitz, D. H. Ohlendorf, D. B. McKay, W. F. Anderson, and B. W. Matthews, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79,** 3097.
[98] D. H. Ohlendorf, W. F. Anderson, R. E. Fisher, Y. Takeda, and B. W. Matthews, *Nature (London)*, 1982, **298,** 718.
[99] I. T. Weber, K. Takio, K. Titani, and T. A. Steitz, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79,** 7679.
[100] J. A. Trainer, E. D. Getzoff, K. M. Beem, J. S. Richardson, and D. C. Richardson, *J. Mol. Biol.*, 1982, **160,** 181.

bond lengths. Each subunit is composed of eight antiparallel β-strands formed into a flattened cylinder with three external loops. The largest loop contains a disulphide bridge and a zinc-binding region. The active-site Cu^{2+} and Zn^{2+} ions lie 0.63 nm apart at the bottom of a long channel with the zinc buried but the copper accessible to the solvent. The imidazole of His-61 forms a bridge between the copper and zinc ions and is coplanar with them. Detailed analysis of the metal-ion-binding sites is presented.

Mellitin.—Mellitin, the principal component of bee venom, has had its structure determined at 0.28 nm resolution[101] followed by refinement at 0.20 nm resolution. The tetrameric molecule has one crystallographic dyad axis but also shows non-crystallographic dyads giving the molecule 222 symmetry.

The structure interpretation[102] shows that each chain is composed of two α-helical segments with an overall bent rod shape. The tetramer is arranged with polar residues on the surface and the interior formed almost entirely by apolar groups. Possible mechanisms leading to the polymerization of mellitin are discussed.

Phospholipase A_2.—The structure of prophospholipase A_2 has been reported at 0.3 nm resolution.[103] The structure was determined using the rotation function and has been refined to an R-factor of 0.27. The position of the calcium ion, which had been left out of the calculations, has been determined. The first ten residues of prophospholipase, seven of which are removed on activation, are seen to be disordered, as is the loop 62—73, which is well located in the phospholipase A_2 structure. These differences may explain the large variation in activity for the two forms.

Aspartate Aminotransferase.—The structure of the complex of 2-oxoglutarate with chicken-heart cytosolic aspartate aminotransferase at 0.32 nm resolution has been reported.[104] The enzyme is an α/β protein with 15 α-helices and 13 β-strands per subunit. The *N*- and *C*-termini are closely associated and form a small domain. The pyridoxal phosphate molecule has been located, and the lysine residue that forms the aldimine bond to the coenzyme has also been found. The 2-oxoglutarate is located near the coenzyme and between the side chains of two arginine residues.

Glutathione Reductase.—The binding of FAD to dimeric glutathione reductase has been reported,[105] and the FAD is seen to bind in an elongated conformation with the flavin portion in the centre of one subunit and the adenine portion extending to the surface. The FMN portion is totally buried while the AMP portion is partly accessible to the solvent. The NADPH

[101] T. C. Terwilliger and D. Eisenberg, *J. Biol. Chem.*, 1982, **257,** 6010.
[102] T. C. Terwilliger and D. Eisenberg, *J. Biol. Chem.*, 1982, **257,** 6016
[103] B. W. Dijkstra, G. J. H. van Nes, K. H. Kalk, N. P. Brandenburg, W. G. J. Hol, and J. Drenth, *Acta Crystallogr., Sect. B*, 1982, **38,** 793.
[104] E. G. Harutyunyan, V. N. Malashkevich, S. S. Terysan, V. M. Kochkina, Yu. M. Torchinsky, and A. E. Braunstein, *FEBS Lett.*, 1982, **138,** 113.
[105] G. E. Schulz, R. H. Schirmer, and E. F. Pai, *J. Mol. Biol.*, 1982, **160,** 287.

substrate passes its reduction equivalents to the *re* face of the flavin ring, which at its *si* face passes them to the redoxactive cysteine bridge. The FAD phosphates are not bound by positively charged side chains, but the electron density suggests that a cation is involved in giving electroneutrality. Amino-acid sequence similarities with three other FAD-binding proteins are discussed.

Elongation Factor G.—Crystals of the *N*- and *C*-terminal fragments of elongation factor G from *E. coli* have been reported.[106] The two fragments have molecular weights of 49 000 and 25 000, respectively, and both crystal forms have been characterized.

Uteroglobin.—The structure of two dimers of rabbit uteroglobin have been elucidated[107] using the molecular-replacement method. The work has been performed in two laboratories, and the molecular folding is seen to be the same in all the crystal forms.

Prothrombin.—The low-resolution structure of fragment 1 (residues 1—156) from bovine prothrombin has been determined.[108] At 0.4 nm resolution the molecule is seen to have dimensions of 5.4 nm × 3.9 nm × 3.2 nm, and the molecule appears to have no secondary structure at all. The protein is composed of two domains separated by a cleft, the smaller domain containing the ten γ-carboxyglutamic acids. Accurate identification of the calcium- and carbohydrate-binding sites is not yet possible.

F_1 ATPase.—The structure of the soluble portion of mitochondrial ATP-synthesizing complex (F_1 ATPase) has been determined at 0.9 nm resolution.[109] The molecule appears to be composed of two equal halves, each formed from three regions of equal size, these regions forming a distorted hexagonal or octahedral arrangement. How these results correlate to the proposed subunit composition of the complex is discussed together with an explanation for the complicated binding data of F_1 ATPase.

Triethylamine Dehydrogenase.—Trimethylamine dehydrogenase containing $[4Fe–4S]^{2+}$ centre and covalently bound FMN has been crystallized using a macroseeding technique.[110] An anomalous-scattering difference Patterson has located the position of the two $[4Fe–4S]^{2+}$ centres, and a self-rotation function has indicated that the molecule has a non-crystallographic two-fold axis relating the two subunits.

[106] L. S. Reshetnikova, M. B. Garber, N. P. Fomenkova, S. V. Nikonov, and Y. N. Chirgadze, *J. Mol. Biol.*, 1982, **160,** 127.
[107] M. Buehner, A. Lifchitz, R. Balla, and J. P. Mornon, *J. Mol. Biol.*, 1982, **159,** 353.
[108] G. Olsson, L. Anderson, O. Lindqvist, L. Sjölin, S. Magnusson, T. E. Petersen, and L. Sottrup-Jensen, *FEBS Lett.*, 1982, **145,** 317.
[109] L. M. Amzel, M. McKinney, P. Narayanan, and P. L. Pedersen, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79,** 5852.
[110] L. W. Lim, F. S. Mathews, and D. J. Steenkamp, *J. Mol. Biol.*, 1982, **162,** 869.

MoFe Protein of Nitrogenase.—*X*-Ray diffraction data to 0.24 nm resolution have been collected for native crystals of the MoFe protein of nitrogenase from *Clostridium pasteurianum*.[111] A 0.6 nm resolution rotation function has located the crystallographic dyad and the pseudo-mutually perpendicular dyads relating the α and β subunits in the $\alpha_2\beta_2$ tetramer. At low resolution therefore the protein has 222 symmetry.

L-Arabinose-binding Protein.—Calculations have shown[112] that the proposed structural changes in the hinge-bending model for L-arabinose-binding protein involve only small changes in the protein's internal energy. Solvation-energy changes are significantly larger than the internal-energy changes. These observations fit in with previous structural studies.

10 tRNA-binding Proteins and tRNA

Methionyl-tRNA Synthetase.—The crystal structure of a monomeric fragment of methionyl-tRNA synthetase from *E. coli* has been reported at 0.25 nm resolution.[113] The proteolytic treatment leaves a monomeric fragment that has full specificity for methionine and $tRNA^{Met}$ and has unimpaired activity in both the activation and aminoacylation reactions.

The molecule is elongated, 9.0 nm × 5.2 nm × 4.4 nm, and contains several helices. The overall structure is biglobular, the first globule containing domains I and II and the second globule being composed of domain III. Domain I consists of a five-stranded, parallel, pleated sheet with helices connecting the strands, and domain II, inserted between sheet strands three and four, has a less ordered structure. Domain III, the *C*-terminal part of the polypeptide chain, contains 57% of the residues in α-helices. The two globules, linked by a single chain crossing, are separated by a large cleft.

The *N*-terminal domain contains a nucleotide-binding fold similar to that seen in the dehydrogenases, suggesting that the aminoacyl-tRNA synthetases may be members of the nucleotide-binding family. Proposed binding sites for ATP, methionine, and $tRNA^{Met}$ are described.

Tyrosyl-tRNA Synthetase.—A reinterpretation of the structure of tyrosyl-tRNA synthetase from *Bacillus stearothermophilus* has been reported.[114] The subunit structure consists of an *N*-terminal α/β domain composed of five helices and a region of 99 residues at the *C*-terminus that appear to be disordered. The reinterpretation has revealed that five of the six strands in the α/β domain are topologically identical to the first five strands of the NAD^+-binding domain of the dehydrogenases, three of the strands forming a mononucleotide-binding fold.

[111] T. Yamane, M. S. Weininger, L. E. Mortenson, and M. G. Rossmann, *J. Biol. Chem.*, 1982, **257**, 1221.
[112] B. Mao, M. R. Pear, J. A. McCammon, and F. A. Quiocho, *J. Biol. Chem.*, 1982, **257**, 1131.
[113] C. Zelwer, J. L. Risler, and S. Bruie, *J. Mol. Biol.*, 1982, **155**, 63.
[114] T. N. Bhat, D. M. Blow, P. Brick, and J. Nyborg, *J. Mol. Biol.*, 1982, **158**, 699.

The location of the binding of tyrosyl adenylate shows that the adenine lies in a position similar to the nicotinamide position in the dehydrogenases. The tyrosine moiety lies in a pocket at one side of the sheet in an orientation quite different from that in any part of the coenzyme in dehydrogenases.

Barker and Winter[115] have analysed the conservation of cysteine and histidine in the structures of tyrosyl- and methionyl-tRNA synthetases and have found a region of homology containing residues that may be directly involved in the catalytic step.

tRNA.—A diagonal-plot analysis has been described for tRNA[116] and clearly allows the identification of medium- and long-range interactions involved in the various structural domains.

11 Viruses

Satellite Tobacco Necrosis Virus.—The crystal structure of Satellite tobacco necrosis virus (STNV) has been determined at 0.3 nm resolution.[117] Phases were calculated using a single isomorphous heavy-atom derivative and the 60-fold non-crystallographic symmetry in the virus particle. These phases were used to calculate the electron-density map, which was interpreted with the aid of a computer graphics system. The subunit is composed of two four-stranded antiparallel β-sheets that form a β-roll structure while the *N*-terminal region forms an α-helix. Three such α-helices, from three subunits, pack together at the particle's three-fold axes where they extend into the RNA region of the virus. The topology of the backbone and the conformation of the subunit are clearly similar to those seen for tomato bushy stunt virus (TBSV) and for Southern-bean mosaic virus (SBMV). However, STNV is assembled in a $T = 1$ structure and structures involved in RNA binding in TBSV and SBMV are used in subunit–subunit interactions in STNV. In the icosahedrally averaged structure no RNA is yet visible, but the protein surface facing the RNA contains many hydrophilic residues, especially lysine and arginine residues.

Tomato Bushy Stunt Virus.—Robinson and Harrison[118] have reported the structure of the expanded state of tomato bushy stunt virus at 0.8 nm resolution. The results show that in the expansion of the particle the tertiary structure of the subunit domains is preserved and the conformational changes involved are restricted to localized flexible regions. A branched opening, 8 nm long and large enough for a 2 nm diameter sphere to pass through, appears at each of the 60 faces after expansion. The translations and rotations of the subunits necessary for the expansion are detailed, and possible functions of the expansion are discussed.

[115] D. G. Barker and G. Winter, *FEBS Lett.*, 1982, **145,** 191.
[116] R. Malathi and N. Yathindra, *Biochem. J.*, 1982, **205,** 457.
[117] L. Liljas, T. Unge, T. A. Jones, K. Fridborg, S. Lövgren, U. Skoglund, and B. Strandberg, *J. Mol. Biol.*, 1982, **159,** 93.
[118] I. K. Robinson and S. C. Harrison, *Nature (London)*, 1982, **297,** 563.

Southern-bean Mosaic Virus.—The amino-acid sequence of Southern-bean mosaic virus coat protein has been determined and its relation to the three-dimensional structure of the virus particle examined.[119] The calcium-binding site is seen to lie on a quasi-threefold axis, and the calcium ion is liganded by the carboxyl side chains of glutamate residues. The regions facing the RNA are seen to contain large numbers of arginine and lysine residues while the internal residues of the β-barrel are extremely hydrophobic.

Alfalfa Mosaic Virus Coat Protein.—The collection of *X*-ray diffraction data to 0.45 nm resolution for $T = 1$ alfalfa mosaic virus protein aggregates crystallized as hexagonal crystals has been reported.[120] The orientation of the particles in the unit cell has been determined using a rotation-function analysis, and packing considerations show that the particles may have small protrusions along their two-fold axes.

Polyoma Virus Capsid.—A model for the structure of the polyoma virus capsid has been proposed[121] based on *X*-ray data to 2.25 nm resolution. Trial phases were calculated from models for the structure based on electron micrographs and low-angle *X*-ray diffraction measurements and were then refined by comparing calculated and observed structure factors. The results show, unexpectedly, that the hexavalent and pentavalent morphological units both consist of pentamers of protein subunits showing that specificity of bonding is not conserved among the subunits in the icosahedrally symmetric capsid.

Influenza Virus.—Webster and co-workers[122] have examined the molecular mechanisms of variation in influenza virus and related sequence changes to the known structure of the haemagglutinin molecule.

Pf3 Bacteriophage.—Fibre-diffraction data have been reported[123] for the filamentous bacteriophage Pf3, which crystallizes on a hexagonal net whose dimensions vary with the relative humidity. The data are consistent with the particle having 27_5 helix symmetry with an axial rise of 0.277 nm in the dry state.

Pf1 Bacteriophage.—Fibre diffraction and electron microscopy have been used[124] to investigate a complex intermediate between replication and assembly of the bacteriophage. This structure is flexible in solution but in oriented dry fibres it forms a regular helix of 4.5 nm pitch with 6.0 dimeric protein units per turn. The results imply that conformational changes are required of the

[119] M. A. Hermodson, C. C. Abad-Zapatero, S. S. Abdel-Meguid, S. Pundak, M. G. Rossmann, and J. H. Tremaine, *Virology*, 1982, **119,** 133.
[120] S. S. Abdel-Meguid, K. Fukuyama, and M. G. Rossmann, *Acta Crystallogr., Sect. B*, 1982, **38,** 2004.
[121] I. Rayment, T. S. Baker, D. L. D. Caspar, and W. T. Murakami, *Nature (London)*, 1982, **295,** 110.
[122] R. G. Webster, W. G. Laver, G. M. Air, and G. C. Schild, *Nature (London)*, 1982, **296,** 115.
[123] C. Peterson, W. T. Winter, G. W. Dalack, and L. A. Day, *J. Mol. Biol.*, 1982, **162,** 877.
[124] G. G. Kneale, R. Freeman, and D. A. Marvin, *J. Mol. Biol.*, 1982, **156,** 279.

DNA as it is transferred from the double-stranded form to the replication-assembly complex and subsequently to the virion.

12 Muscle and Muscle Proteins

Studies of low-angle diffraction patterns from frog sartorius muscle have been described.[125] Using synchrotron radiation, a time resolution of a few milliseconds has been achieved allowing the various stages of the contractile cycle to be examined from the viewpoints of both tension development and *X*-ray pattern.

Lowy and Poulsen[126] have also used synchrotron radiation to examine the *X*-ray diffraction pattern from contracting molluscan unstriated muscle with a time resolution of 0.5—1 second. These studies have allowed a model to be proposed for the contractile cycle that accounts for the observed diffraction patterns.

Amos and co-workers[127] have used three-dimensional image reconstruction from electron micrographs to show that S-1 is a comma-shaped molecule, the head of which interacts with F-actin near the groove between the two strands of monomers.

McLachlan and Karn[128] have shown that the amino-acid sequence of the rod portion of nematode myosin is highly repetitive and has the characteristics of an α-helical coiled coil. The molecular surface shows alternate patches of positive and negative charges, and interactions of these clusters on adjacent molecules could account for the observed spacings of the myosin cross-bridges in muscle.

13 Membranes and Membrane Proteins

Ovchinnikov[129] has reviewed the structure–function relationships in rhodopsin and bacteriorhodopsin.

The preparation of two-dimensional crystals of rhodopsin has been reported,[130] and a structural analysis has shown that the rhodopsin molecules are associated as dimers, 2.0—2.5 nm wide and 7.0—8.0 nm long.

Gruner and co-workers[131] have reported an *X*-ray diffraction analysis of isolated bovine rod outer-segment disks during dehydration.

An *X*-ray analysis of a contracted form of the trigonal purple membrane of *Halobacterium halobium* has been described.[132] The cell dimensions reduce from 6.27 to 5.95 nm and the *X*-ray intensities differ totally. Chemical analysis

[125] H. E. Huxley, A. R. Faruqi, M. Kress, J. Bordas, and M. H. J. Koch, *J. Mol. Biol.*, 1982, **158,** 637.
[126] J. Lowy and F. R. Poulsen, *Nature (London)*, 1982, **299,** 308.
[127] L. A. Amos, H. E. Huxley, K. C. Holmes, R. S. Goody, and K. A. Taylor, *Nature (London)*, 1982, **299,** 467.
[128] A. D. McLachlan and J. Karn, *Nature (London)*, 1982, **299,** 226.
[129] Yu. A. Ovchinnikov, *FEBS Lett.*, 1982, **148,** 179.
[130] J. M. Corless, D. R. McCaslin, and B. L. Scott, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79,** 1116.
[131] S. M. Gruner, D. T. Barry, and G. T. Reynolds, *Biochim. Biophys. Acta*, 1982, **690,** 187.
[132] R. Henderson, J. S. Jubb, and M. G. Rossmann, *J. Mol. Biol.*, 1982, **154,** 501.

has shown a reduction in the lipid content of this form. The tighter packing appears to result from a 10° rotation of the molecules relative to the native form.

The crystallization of photosynthetic reaction centres from *Rhodopseudomonas viridis* has been published,[133] and the crystals were shown to diffract to 0.25 nm spacings.

Miller[134] has described the determination of the three-dimensional structure of the photosynthetic membranes from *Rhodopseudomonas viridis* by electron microscopy. This reveals a large central structure protruding from both sides of the membrane surrounded by six smaller centres of mass.

Sadler and Worcester[135] have studied oriented photosynthetic membranes from *Euglena gracilis* by neutron-diffraction analysis. Orientation was achieved either by centrifugation and controlled drying or by the application of magnetic fields.

The observation of a resonance effect in *X*-ray diffraction analysis of biological membranes has been described,[136] and the use of the technique to locate intrinsic metal ions in membranes has been reported.[137]

Murthy and Worthington[138] have presented improved *X*-ray diffraction data from dry nerve myelin.

14 Other Biological Structures

Ribosomes.—Kühlbrandt and Unwin[139] have used electron microscopy and image processing to analyse the structure of lizard oocyte ribosomes. Using gold-thioglucose the whole ribosome is seen in outline while the use of glucose shows predominantly the RNA. The RNA forms a dense central core with the protein forming most of the ribosomal surface.

Wittmann and co-workers[140] have reported the preparation of three-dimensional crystals of *E. coli* ribosomes, and Clark and co-workers[141] have produced crystalline sheets of the 50S ribosomal subunits from *E. coli*.

Silk Fibroin.—Lamellar, chain-folded single crystals and crystal aggregates of *Bombyx mori* silk fibroin and various alanine/glycine copolypeptides with a β-structure have been shown to be helically twisted.[142] The twisted crystals provide morphological evidence for the favoured twist sense of β-sheets in globular proteins.

[133] H. Michel, *J. Mol. Biol.*, 1982, **158,** 567.
[134] K. R. Miller, *Nature (London)*, 1982, **300,** 53.
[135] D. M. Sadler and D. L. Worcester, *J. Mol. Biol.*, 1982, **159,** 467.
[136] J. Stamatoff, P. Eisenberger, J. K. Blasie, J. M. Pachence, A. Tavormina, M. Erecinska, P. L. Dutton, and G. Brown, *Biochim. Biophys. Acta*, 1982, **679,** 177.
[137] J. K. Blasie, J. M. Pachence, A. Tavormina, M. Erecinska, P. L. Dutton, J. Stamatoff, P. Eisenberger, and G. Brown, *Biochim. Biophys. Acta*, 1982, **679,** 188.
[138] N. S. Murthy and C. R. Worthington, *Biochim. Biophys. Acta*, 1982, **689,** 451.
[139] W. Kühlbrandt and P. N. T. Unwin, *J. Mol. Biol.*, 1982, **156,** 431.
[140] H. G. Wittmann, J. Müssig, J. Piefke, H. S. Gewitz, H. J. Rheinberger, and A. Yonath, *FEBS Lett.*, 1982, **146,** 217.
[141] M. W. Clark, K. Leonard, and J. A. Lake, *Science*, 1982, **216,** 999.
[142] B. Lotz, A. Gonthier-Vassal, A. Brack, and J. Magoshi, *J. Mol. Biol.*, 1982, **156,** 345.

Intermediate Filaments.—Intermediate-sized filaments reconstituted from purified desmin, epidermal keratin, and the 68 000 molecular weight neurofilament protein have been shown to have a longitudinal periodicity of about 21 nm.[143]

Photoreceptor Microvilli.—*X*-Ray diffraction patterns have been recorded from fixed squid retinas.[144] A weak diffuse band has been assigned to rhodopsin molecules ordered in the plane of the membrane, and an arc at $(8.5\ \text{nm})^{-1}$ is attributed to a cytoplasmic or extracellular structure.

Cytochrome C Oxidase.—Fuller and co-workers[145] have described a method for preparing two-dimensional crystals of beef-heart cytochrome C oxidase.

The structure of cytochrome C oxidase vesicle crystals has been analysed at 2 nm resolution.[146] On the side of the membrane corresponding to the cytoplasmic face of the inner mitochondrial membrane the oxidase molecule protrudes 5 nm into solution. About half the mass of the protein is in this domain, which contains the cytochrome C-binding site. On the other side of the membrane the protein protrudes less than 2 nm.

Using media of different density the cytochrome C oxidase monomer is seen to be Y-shaped.[147] The stem of the Y protrudes 5 nm into solution and the monomers are compactly paired as dimers.

Cell-wall Glycoprotein.—The three-dimensional structure of the crystalline glycoprotein cell-wall layer from the alga *Lobomonas piriformis* has been determined at 2.0 nm resolution.[148] The cell-wall layer is seen to consist of crystalline plates, the centres and edges of which display distinctly different but isomorphous structures.

Bacterial-cell Envelope S-Layer.—The outermost layer of the thermophilic bacterium *Sulpholobus acidocaldarius*, the S-layer, consists of a two-dimensional array of protein molecules. The structure of this layer has been determined,[149] and the external surface is seen to be fairly smooth. In contrast the cellular side is sculpted with large cavities and protruding pedestals.

Phospholipids.—Büldt and de Haas[150] have reported neutron- and *X*-ray diffraction studies on sn-3- and sn-2-phospholipids.

Peptides and Polypeptides of Interest.—The crystal structures of the *C*-terminal tetrapeptide amide of gastrin,[151] the 23—26 fragment of human

[143] D. Henderson, N. Geisler, and K. Weber, *J. Mol. Biol.*, 1982, **155,** 173.
[144] H. R. Saibil, *J. Mol. Biol.*, 1982, **158,** 435.
[145] S. D. Fuller, R. A. Capaldi, and R. Henderson, *Biochemistry*, 1982, **21,** 2525.
[146] J. F. Deatherage, R. Henderson, and R. A. Capaldi, *J. Mol. Biol.*, 1982, **158,** 487.
[147] J. F. Deatherage, R. Henderson, and R. A. Capaldi, *J. Mol. Biol.*, 1982, **158,** 501.
[148] P. J. Shaw and G. J. Hills, *J. Mol. Biol.*, 1982, **162,** 459.
[149] K. A. Taylor, J. F. Deatherage, and L. A. Amos, *Nature (London)*, 1982, **299,** 840.
[150] G. Büldt and G. H. de Haas, *J. Mol. Biol.*, 1982, **158,** 55.
[151] W. B. T. Cruse, E. Egert, M. A. Viswamitra, and O. Kennard, *Acta Crystallogr., Sect. B*, 1982, **38,** 1758.

adrenocorticotropic hormone,[152] and the *C*-terminal pentapeptide of substance P[153] have been determined.

The crystal structure of a highly active analogue of thyrotropin-releasing hormone has been reported,[154] as have the structures of three crystalline pentapeptide fragments of suzukacillin, a membrane-channel-forming polypeptide.[155]

The crystal structure of alamethicin has been described[156] showing that the conformation is largely α-helical with a bend in the helix axis at a proline residue. Starting from this crystal structure models for the voltage-gated ion channel have been proposed.

Dock and Moras[157] have determined the structure of a chiral macrocyclic tetracarboxamide, which has allowed them to provide a model for a membrane potassium channel.

Resonance Raman spectroscopy and *X*-ray crystallographic studies of dithioester compounds have been used to obtain details of the structure of acylpapain intermediates.[158]

The orientation of fibres in collagen has been measured by *X*-ray diffraction, and the effects of bending and torsion have been examined.[159]

The structures of several peptides with interesting structural features have been reported. Unusual intramolecular hydrogen bonding in cycloamanide A has been described,[160] and Bavoso and co-workers[161] have reported the structure of a hairpin-shaped hexapeptide stabilized by multiple intramolecular hydrogen bonds.

Barnes and van der Helm[162] have described the structures of two cyclic hexapeptides, and Bandekar and co-workers[163] have examined the conformations of cyclized dipeptide models for specific types of β-bends. Chiang and Karle[164] have determined the structure of the synthetic cyclic tetrapeptide Ala-Pro-Phe-Pro and, although the synthesis was designed to yield the LLLL isomer, the crystal contains a 1:1 mixture of the LLLL and LLDL isomers.

[152] G. Précigoux, S. Geoffre, M. Hospital, and F. Leroy, *Acta Crystallogr., Sect. B*, 1982, **38,** 2172.
[153] M. Cotrait and M. Hospital, *Biochem. Biophys. Res. Commun.*, 1982, **109,** 1123.
[154] B. Stensland and S. Castensson, *J. Mol. Biol.*, 1982, **161,** 257.
[155] A. K. Francis, C. R. Pulla Rao, M. Iqbal, R. Nagaraj, M. Vijayan, and P. Balaram, *Biochem. Biophys. Res. Commun.*, 1982, **106,** 1240.
[156] R. O. Fox, jun. and F. M. Richards, *Nature (London)*, 1982, **300,** 325.
[157] J.-P. Behr, J.-M. Lehn, A.-C. Dock, and D. Moras, *Nature (London)*, 1982, **295,** 526.
[158] C. P. Huber, Y. Ozaki, D. H. Pliura, A. C. Storer, and P. R. Carey, *Biochemistry*, 1982, **21,** 3109.
[159] J. A. Klein and D. W. L. Hukins, *Biochem. Biophys. Acta*, 1982, **719,** 98.
[160] C. C. Chiang, I. L. Karle, and Th. Wieland, *Int. J. Pept. Protein Res.*, 1982, **20,** 414.
[161] A. Bavoso, E. Benedetti, B. DiBlasio, V. Pavone, C. Pedone, G. P. Lorenzi, and V. Muri-Valle, *Biochem. Biophys. Res. Commun.*, 1982, **107,** 910.
[162] C. L. Barnes and D. van der Helm, *Acta Crystallogr., Sect. B*, 1982, **38,** 2589.
[163] J. Bandekar, D. J. Evans, S. Krimm, S. J. Leach, S. Lee, J. R. McQuie, E. Minasian, G. Némethy, M. S. Pottle, H. A. Scheraga, E. R. Stimson, and R. W. Woody, *Int. J. Pept. Protein Res.*, 1982, **19,** 187.
[164] C. C. Chiang and I. L. Karle, *Int. J. Pept. Protein Res.*, 1982, **20,** 133.

15 Small-angle Scattering

Small-angle *X*-ray scattering studies have been reported for L-leucine dehydrogenase[165] and the complex between trypsin and α_2-macroglobulin.[166] This latter complex has also been studied by electron microscopy.[167]

The overall shape of the *E. coli lac* repressor has been examined[168] by low-angle scattering and shown to be elongated with the operator DNA-binding domains located at both ends. Low-angle studies on the *lac* repressor subunits have also been reported.[169]

The structure of DNA-dependent RNA polymerase has been examined at low ionic strength by small-angle *X*-ray scattering.[170] Under these conditions the molecules aggregate to form the active holoenzyme.

Small-angle neutron-scattering studies have been described[171] for the mitochondrial ADP/ATP-carrier protein in detergent solution, and low-angle *X*-ray scattering has been used[172] to study a complex between yeast $tRNA^{Phe}$ and two molecules of *E. coli* $tRNA^{Glu}$.

Inelastic neutron-scattering analysis has been used[173] to confirm the view that glucose binding leads to a stiffening of the hexokinase structure. Small-angle neutron-scattering studies have been described for earthworm haemoglobin[174] and Bence-Jones protein Mcg.[175] Similar studies have been reported for the complex of initiator $tRNA^{Met}$ with trypsin-modified methionyl-tRNA synthetase[176] and for *E. coli* tyrosyl-tRNA synthetase and its interaction with $tRNA^{Tyr}$.[177]

Neutron-scattering studies of reconstituted complexes of fd DNA and gene 5 protein have shown[178] that the DNA is near the centre of the complex and have allowed[179] models for the high-mobility group 14 core nucleosome complex to be proposed.

Neutron-diffraction studies[180] and synchrotron *X*-ray diffraction studies[181]

[165] Y. Hiragi, K. Soda, and T. Ohshima, *Makromol. Chem.*, 1982, **183,** 745.
[166] B. Branegard, R. Österberg, and B. Sjöberg, *Eur. J. Biochem.*, 1982, **122,** 663.
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[168] D. B. McKay, C. A. Pickover, and T. A. Steitz, *J. Mol. Biol.*, 1982, **156,** 175.
[169] I. Pilz, E. Schwarz, N. Wade-Jardetzky, R. P. Bray, and O. Jardetzky, *FEBS Lett.*, 1982, **144,** 247.
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[171] M. R. Block, G. Zaccai, G. J. M. Lauquin, and P. V. Vignais, *Biochem. Biophys. Res. Commun.*, 1982, **109,** 471.
[172] L. Nilsson, R. Rigler, and P. Laggner, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79,** 5891.
[173] B. Jacrot, S. Cusack, A. J. Dianoux, and D. M. Engelman, *Nature (London)*, 1982, **300,** 84.
[174] P. Martel, B. M. Powell, O. H. Kapp, and S. N. Vinogradov, *Biochem. Biophys. Acta*, 1982, **709,** 134.
[175] M. Schiffer, F. J. Stevens, F. A. Westholm, S. S. Kim, and R. D. Carlson, *Biochemistry*, 1982, **21,** 2874.
[176] P. Dessen, G. Fayat, G. Zaccai, and S. Blanquet, *J. Mol. Biol.*, 1982, **154,** 603.
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[180] G. F. Elliott, Z. Sayers, and P. A. Timmins, *J. Mol. Biol.*, 1982, **155,** 389.
[181] Z. Sayers, M. H. J. Koch, S. B. Whitburn, K. M. Meek, G. F. Elliott, and A. Harmsen, *J. Mol. Biol.*, 1982, **160,** 593.

of bovine corneal stroma have been reported, and neutron diffraction has been used to study rat-liver interphase nuclei.[182] Small-angle neutron scattering has also been used to study bovine casein micelles and submicelles.[183]

16 Protein Conformations – Analysis and Predictions

Methods and Results of Structure Prediction.—Robson[184] has reviewed some of the techniques available for the prediction of protein structures, and Sternberg and co-workers[185] have described an approach to structure prediction based on the combinational docking of secondary structural elements. Finney[186] has reviewed the Monte Carlo techniques available in structure analysis, and Palau and co-workers[187] have reported prediction methods for secondary structure. Their analysis suggests that the nearest-neighbour-prediction techniques have reached the limit of their accuracy.

Argos and Palau[188] have analysed amino-acid distributions in 44 proteins and have suggested possible improvements in the accuracy of prediction techniques. Paul[189] has discussed a theory for structure prediction based on treating the protein as a polyelectrolyte and then forming a compact structure that places ionized groups at the surface. Cid and co-workers[190] have approached structure prediction by considering hydrophobicity profiles for the protein.

Busetta and Hospital[191] have analysed two prediction methods when applied to 38 proteins of known structure. They show that predictions are better for proteins with a single type of secondary structure and that prior knowledge of the protein type improves the prediction. Busetta and Barrans[192] have estimated the different contributions to a protein's free energy by analysis of known structures. These contributions can then be allowed for in structure predictions.

Kubota and co-workers[193] have analysed the correspondence of homologies in primary and secondary structures of proteins.

Zimmermann and co-workers[194] have reported a conformational-energy analysis of melanostatin, and Montecucchi and Gozzini[195] have described a structure for sauvagine, a 40-residue, biologically active polypeptide from a frog.

A structural model for maize zein proteins has been reported,[196] and a

[182] K. Ibel, *J. Mol. Biol.*, 1982, **160,** 77.
[183] P. H. Stothart and D. J. Cebula, *J. Mol. Biol.*, 1982, **160,** 391.
[184] B. Robson, *Biochem. Soc. Trans.*, 1982, **10,** 297.
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[186] J. L. Finney, *Biochem. Soc. Trans.*, 1982, **10,** 305.
[187] J. Palau, P. Argos, and P. Puigdomenech, *Int. J. Pept. Protein Res.*, 1982, **19,** 394.
[188] P. Argos and J. Palau, *Int. J. Pept. Protein Res.*, 1982, **19,** 380.
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[191] B. Busetta and M. Hospital, *Biochim. Biophys. Acta*, 1982, **701,** 111.
[192] B. Busetta and Y. Barrans, *Biochem. Biophys. Acta*, 1982, **709,** 73.
[193] Y. Kubota, K. Nishikawa, S. Takahashi, and T. Ooi, *Biochim. Biophys. Acta*, 1982, **701,** 242.
[194] S. S. Zimmerman, R. Baum, and H. A. Scheraga, *Int. J. Pept. Protein Res.*, 1982, **19,** 143.
[195] P. C. Montecucchi and L. Gozzini, *Int. J. Pept. Protein Res.*, 1982, **20,** 139.
[196] P. Argos, K. Pedersen, M. D. Marks, and B. A. Larkins, *J. Biol. Chem.*, 1982, **257,** 9984.

proposed α-helical super-secondary structure associated with protein-DNA recognition has been described.[197]

Pincus and Klausner[198] have published a prediction for the sixteen-residue leader sequence of murine K light chain, and Akeroyd and co-workers[199] have reported a prediction for the structure of phosphatidyl-choline-transfer protein. Secondary-structure predictions for silk-moth chorion proteins have been published.[200]

Analysis of Protein Structures.—McLachlan[201] has reported a very fast way of superposing two sets of atomic co-ordinates, and the calculation can be performed fast enough for it to be used in on-line graphics systems. Sippl[202] has described a method for a similar rapid comparison of structures that can run on minicomputers. Ramakrishnan and Soman[203] have reported a new algorithm for the identification of α-helices, extended structures, and bends using the virtual-bond approach.

Chothia and Lesk have described the evolution of the small copper-containing β-sheet proteins plastocyanin and azurin[204] and have analysed the β-sheet packing in the immunoglobulin domain.[205] Chou and co-workers[206] have analysed the minimum energies of parallel and antiparallel β-sheets and examined how the right-handed twist of the sheet comes about.

Kyte and Doolittle[207] have reported a simple method for displaying the hydropathic character of a protein, and Cohen and co-workers[208] have analysed the packing of α-helices and β-sheets in six α/β proteins. The results are used to provide a tertiary-structure prediction algorithm. Chothia and Janin[209] have described the orthogonal packing of β-sheets from both a theoretical and an observational point of view. This packing is compared with the aligned packing class.

Graphics and Protein Structures.—Dearing[210] has considered the use of computer graphics and other techniques in the study of protein–substrate interactions, and Kröhn[211] has described the use of computer graphics in the design of peptide analogues. Heritage[212] has discussed the role of computers and graphics in pesticide design.

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[204] C. Chothia and A. M. Lesk, *J. Mol. Biol.*, 1982, **160,** 309.
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[210] A. Dearing, *Biochem. Soc. Trans.*, 1982, **10,** 307.
[211] A. Kröhn, *Biochemistry. Soc. Trans.*, 1982, **10,** 309.
[212] K. J. Heritage, *Biochem. Soc. Trans.*, 1982, **10,** 310.

Kuntz and co-workers[213] have reported an algorithm for examining feasible ligand–receptor interactions, and Furie and co-workers[214] have described the use of computer graphics to generate models of blood-coagulation proteins.

Weiner and co-workers[215] have published an analysis of the electrostatic-potential molecular surfaces of proteins using a colour graphics system. The approach shows predictive promise for drug design.

Milner-White[216] has described a computer-graphical method for defining the interacting interfaces of oligomeric proteins.

Protein Structural Elements.—A partially disordered system that shows flip-flop hydrogen bonding has been described,[217] and Sawyer and James[218] have reported how carboxylate–carboxylate interactions may help to stabilize protein structures.

Gellatly and Finney[219] have described a procedure for the calculation of protein volumes, and new patterns of polypeptide-chain hydrogen bonding have been found in glycylglycylglycine.[220]

An analysis of protein structures has revealed[221] side-chain characteristic main-chain conformations for the 20 amino-acids, and de Leeuw and Altona[222] have determined constants for the calculation of side-chain conformations in amino-acids.

Warwicker and Watson[223] have reported a calculation of the electric potential in the active site of proteins due to the α-helix dipole. They have shown that in phosphoglyceromutase the α-helical structure could stabilize negatively charged substrates in the active-centre cleft.

The surroundings of 170 phenylalanine side chains in 28 proteins have been analysed,[224] and the study reveals a preferred interaction between the rings' edges and oxygen atoms.

Sheridan and co-workers[225] have considered the α-helical dipole and how it may stabilize the 4-α-helix proteins. Cytochrome C', haemerythrin, myohaemerythrin, cytochrome b_{562}, and T4 lysozyme domains were examined.

Thornton and Chakauya[226] have analysed the conformations at the terminal regions of proteins and shown a preference for β-sheet and α-helix at the

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[225] R. P. Sheridan, R. M. Levy, and F. R. Salemme, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79,** 4545.
[226] J. M. Thornton and B. L. Chakauya, *Nature (London)*, 1982, **298,** 296.

N- and *C*-termini, respectively. Eisenberg and co-workers[227] have described the helical hydrophobic moment, a way of defining the amphiphilicity of α-helices.

PART III: Conformation and Interaction of Peptides and Proteins in Solution

Edited by R. H. Pain, with contributions by T. Brittain, S. Craig, D. P. E. Dickson, P. D. Jeffrey, L. W. Nichol, H. W. E. Rattle, N. K. Rogers, R. M. Stephens, M. J. E. Sternberg, and D. J. Winzor

1 Theoretical Aspects of Protein Conformation

Contributed by N. K. Rogers and M. J. E. Sternberg

We present a survey of the literature until the end of February 1983, which may be supplemented by the Ramachandran Festschrift[1] and the book 'Computing in Biological Sciences'.[2]

Energy Calculations and Conformational Analysis.—With the exception of the hydrophobic effect, there has been little further development of accurate energy functions this year. Israelachvili and Pashley[3] carried out experiments on the macroscopic hydrophobic forces between two cylinders and have extrapolated their results to the molecular level, giving an analytical form for the hydrophobic interaction between two spherical molecules. The need for more precise potential functions was illustrated by Islam and Neidle,[4] who used a variety of functions in the conformational analysis of a DNA-binding drug.

Classical conformational analysis on model systems has continued,[5–7] and in particular relaxation of the bond lengths and peptide planarity[8,9] expands the conformationally allowed regions. Specific peptides have been studied; they include acetylcholine,[10] substance P,[11] melanostatin,[12] gastrin,[13] and cyclic calcium-transport peptides.[14] For collagen,[15] diffraction patterns were calculated from a predicted structure.

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[15] V. G. Tumanyan and N. G. Esipova, *Biopolymers*, 1982, **21,** 475.

Quantum-mechanical studies have been used in conformational analysis[16—18] and increasingly in the functional analysis of active sites. Serine proteases,[19] carboxypeptidases,[20] trypsin,[21] and ferredoxins[22] have all been examined. Of particular interest was a quantum-mechanical analysis by Thomas *et al.*[23] of oxygen environments around aromatic rings, which was found to be in good agreement with known structures.

Conformational analysis has been extensively used to consider enzyme–substrate interactions using the known geometry of the binding sites. Penicillins and penicillinases,[24,25] carbohydrates and glycoproteins,[26] and ligand binding to thermolysin,[27] methyltransferase,[28] and staphylococcal nuclease[29] have all been studied in this way. Blaney *et al.*[30] have carried out an analysis of the binding of thyroid hormone analogues to prealbumin and have attempted to calculate differences in the solvation energies.

Water Structure and Electrostatics.—Interest in electrostatics has been high this year[31] and applications have been diverse. Progress has been made in continuum-dielectric formalisms that can be used to explain some of the hydrophobic behaviour of the centre of proteins,[32] and Warwicker and Watson have developed an algorithm to calculate the electrostatic potential of a system with any distribution of charges and dielectrics.[33] This could be used to modify the Tanford–Kirkwood model[34—36] for pK calculations, removing the geometric constraints. The visual representation of electric fields has also been improved with the use of colour graphics.[37]

Warshel[38] has developed his method of microscopic dielectrics, which is based on permanent and induced dipoles associated with each protein atom

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[38] A. Warshel, *Acc. Chem. Res.*, 1981, **14**, 284.

and water molecule. Goodfellow[39] has continued Monte Carlo simulations of water and found considerable non-pair additive and co-operative effects. The solvent structure and particularly the counter-ion distribution have been studied by Clementi *et al.*[40] The uncertainty in modelling the dielectric is reflected in the use of uniform,[41] cavity,[32–36] distance–dependent,[37] and microdielectric[38] methods. A calibration of these methods with an experimental system is clearly needed.

Bone and Pethig[42] have continued their experimental work on the conductivity and enzymic activity of lysozyme with increased hydration. The role of proton–electron displacements in catalysis has also been considered by Resslen.[43] The functional effect of the distribution of charged residues in cytochrome *c* has been discussed by Koppenol and Margoliash[44] and by others in relation to molecular orientation[45] and to the mechanism by which genome regulating proteins find their target sites.[46] The structure of alamethicin has suggested a way in which it could act as a voltage gated ion channel.[47] Payens[48] posed the question 'Why are enzymes so large?', which Chou *et al.*[49] went a long way to answer, partly in terms of electrostatics.

McLachlan and co-workers[50,51] have observed periodic charge distributions along filamentous proteins and suggested applications in muscle contraction.[51] Eden *et al.*[52] have examined the change in compressibility of cytochrome in different states of oxidation by the thermodynamic relationship between the compressibility and the velocity of sound.

Dynamics.—Molecular dynamics have been the object of experimental and theoretical studies with an emphasis on correlation between the two approaches. Comparisons have been made with experimental data from fluorescence depolarization spectroscopy,[53] Mössbauer spectroscopy,[54] and in particular crystallographic temperature factors measured over a very wide temperature range.[55] Further to these, Karplus and co-workers[56,57] have explored ways

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of examining the pressure dependence of tyrosine ring motions and predicting reaction rates from dynamic simulations.

Developments in calculating techniques and in the underlying theory have continued. van Gunsteren and Karplus[58,59] have investigated the effects of various solvent models and the constraining of bond lengths and angles in the simulation of bovine pancreatic trypsin inhibitor (BPTI). If these constraints may be applied, then computing time could be drastically reduced. The harmonic approximation has been examined,[60] and the behaviour of correlation functions[61,62] with time has also been studied.

BPTI has continued as the most favoured system, because of its manageable size, but work has also been carried out on hinge bending in arabinose-binding protein,[63] and model calculations on the dynamics of α-helices have been done over a wide temperature range[64] and have been correlated with temperature factors. More mechanical models have been used for both the α-helix[65] and entire proteins.[66] The stability of proteins has also received some scrutiny from dynamical arguments.[67]

Unfolding Mechanisms.—Scheraga and co-workers[68] have continued their helix-coil analysis for homopolymers, whilst Van *et al.*[69] have weighted helix-coil parameters for given residues according to their adjacent residue types. Theoretical studies using Ising models on α-helix and β-sheet denaturation have been carried out by Marchi and co-workers.[70,71] Pefferkorn *et al.*[72] have examined helix-coil parameters at an interface and applied the results to membrane-bound proteins. Theoretical folding pathways have been considered,[73] and Nishio *et al.*[74] have followed thermal denaturation of general polymers using photon correlation spectroscopy.

Hydrogen Exchange.—Hydrogen exchange is a particularly suitable system for theoretical analysis because it is readily measurable by experiment. Two effects are especially involved in determining the rate of hydrogen exchange: dynamics and the local unfolding and the local charge environment in which the exchanging group finds itself. Neutron scattering[75] and n.m.r.[76] have

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followed the amide exchange rates in the β-sheet of BPTI, and conclusions have been drawn about the dynamics of β-sheets by Salemme,[77] who suggests co-operative motions.

The electrostatic effects have been studied by quantum mechanics[78] and titration experiments[79] and by the use of model peptides.[80] Isotope exchange has also been studied by random walk calculations.[81]

Analysis and Prediction of Secondary Structures.—The relationship between local sequence and local structure has continued to be examined.[82–86] This year several new approaches have been developed to compare secondary-structure predictions.[87–90] In addition, the existing methods have been applied to a variety of proteins.[91—101]

Examinations of known protein structures have revealed several features including the occurrence of asparagines in disallowed conformations,[82] the presence of the γ-helix,[83] and the orientation of peptide planes.[84] Thornton and Chakauya[85] have analysed the distribution of α-helical, β-strand, and coil conformations at the *N*- and *C*-termini of globular proteins and have found that often an α-helix occurs at the *C*-terminus. Balasubramanian and Raghunathan[86] have also analysed the occurrence of regular secondary structures in proteins.

A method of improving the prediction of secondary structures in proteins has been developed by Taylor and Thornton.[87] Their approach considers proteins formed primarily from β–α–β units and locates a template that is used to predict this unit. This method leads to an improvement of 7.5% in the prediction. Several other workers[88,89] have explored the accuracy of secondary-structure prediction and suggested possible improvements such as the consideration of hydrophobic residues. Eisenberg *et al.*[90] have quantified the spatial distribution of hydrophobic side chains in an α-helix to quantify the amphilicity of the structure and thereby identify membrane-bound α-helices.

Existing methods of prediction have been applied to a variety of proteins including various histones,[91—93] sea-anemone toxins,[94] maize zein proteins,[95]

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silkmoth chorion proteins,[96] phosphatidylcholine-transfer proteins,[97] and chicken ovomucoid.[98] More general surveys that use these predictive methods have been applied to nucleic acid-binding proteins[99] and to membrane-bound proteins.[100] An intriguing use of secondary-structure prediction methods has been reported by De Grado *et al.*,[101] who predicted an α-helical region in α-, β-, and γ-interferons and thereby located a sequence resemblance between γ-interferon and α- and β-interferons.

Analysis of Tertiary and Quaternary Structures.—The complexity of the three-dimensional structure of a protein is reflected by the variety of the types of analyses performed. Most of the work can be divided into studies on the following: methods of representing and comparing the atomic co-ordinates,[102—108] the location of residue types in the globular protein,[109—112] the conformation and packing of α-helices and β-strands,[113—120] and the location of intron–exon junctions.[121,122]

Phillips[123] originally proposed the distance plot to represent in two dimensions the interatomic separation of all pairs of Cα-atoms in a globular protein. This year Sippl[102] has explored the use of such plots to compare the structures of different proteins and showed how both short- and long-range similarities in conformation can be detected. Braun[103] has used a similar two-dimensional plot to represent handedness in protein structures. Several workers[104,105] have

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[103] W. Braun, *J. Mol. Biol.*, 1983, **163,** 613.
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[115] C. Chothia, *J. Mol. Biol.*, 1983, **163,** 107.
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[119] L. Lebioda, M. H. Hatada, A. Tulinsky, and I. M. Mavridis, *J. Mol. Biol.*, 1982, **162,** 445.
[120] O. B. Ptitsyn in ref. 1, p. 49.
[121] D. C. Phillips, M. J. E. Sternberg, and B. J. Sutton in 'Evolution from Molecules to Men', ed. D. S. Bendall, Cambridge University Press, Cambridge, 1983, p. 145.
[122] C. S. Craik, S. Spring, R. Fletterick, and W. J. Rutter, *Nature (London)*, 1982, **299,** 180.
[123] D. C. Phillips in 'British Biochemistry Past and Present', ed. T. W. Goodwin, Academic Press, London, 1970, p. 11.

explored the use of differential geometry to represent the fold of globular proteins. The more traditional method of comparing structures involves a least-squares minimization of the sum of distances between pairs of superposed atoms, and improved algorithms for this procedure have been reported.[106,107]

The distribution of residue types has been explored by several workers.[109—112] One interesting study was by Prabhakaran and Ponnuswamy,[109] who started by computing best-fitting ellipsoids and surface areas to investigate shape anisotropy.

The overall fold of proteins can often be expressed in terms of the packing of α-helices and β-strands. One important feature of observed β-sheets in globular proteins is that they generally have a right-handed twist[124] as one views along a β-strand. Scheraga and co-workers[113,114] have used energy calculations to explore the cause of the observed twist. They suggest that non-bonded interactions of side-chain groups with main-chain atoms are responsible for the preferred right-handedness. Chothia[115] has quantified the relationship between successive main-chain θ, ψ-angles that is required to form a strongly twisted sheet. The packing of two β-sheets in a variety of arrangements has been explored. Chothia and Janin[116] considered orthogonal packing in which the β-strand directions are about 90° to each other. Lesk and Chothia[117,118] considered the sequence and structural changes in different immunoglobulin domains and in plastocyanin and azurin. Another structural motif that has been examined[119] is the 8-fold α/β barrel of 2-keto-3-deoxy-6-phosphogluconate aldolase that was compared with the barrels in triose phosphate isomerase and pyruvate kinase. In contrast to these studies that try to relate the structure of a protein to its sequence, Ptitsyn[120] has shown how random sequences may have secondary structures and topologies similar to those of real proteins.

Last year we reported the study by Go[125] on the correlation between boundaries in a distance plot that considers distant Cα–Cα separations and the locations of intron–exon junctions in haemoglobin. This year Phillips *et al.*[121] have shown that such a distance plot identifies a segment of polypeptide chain that starts near the middle of a protein, goes to the surface, and returns to the middle. Such a segment was termed a 'sector', and a correlation between the locations of sectors and intron–exon boundaries was demonstrated for hen egg-white lysozyme. However, Rutter and co-workers[122] have shown that the locations of intron–exon boundaries in several proteins map to amino-acid residues located at the protein surface. At present the number of proteins whose three-dimensional structure and gene sequence are known is limited, so a clearer idea of the relationships between protein conformations and intron–exon boundaries must await further experimental work.

Prediction of Three-dimensional Structure.—This year there has been an emphasis both on the use of existing methods to predict unknown

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[125] M. Go, *Nature (London)*, 1981, **291,** 90.

structures[126—137] and on the development of new approaches.[138—147] In addition the pathway of folding continues to be modelled.[148,149]

The prediction of an unknown protein structure based on its sequence similarity to a molecule of known conformation has been pursued by several workers.[126—132] Recent analyses, *e.g.* refs. 150—152, of the structure of DNA-binding proteins and their interactions with DNA have provided rules for several predictions.[126,127] For example Matthews *et al.*[126] have proposed a structure for the DNA-binding region of the *lac* repressor from its homology with the *cro* repressor. Other studies have considered blood-coagulation factors and thrombin,[129] sugar-binding proteins,[130] the eye-lens protein,[131] and the major histocompatibility antigens.[132] Secondary-structure prediction followed by stereochemical arguments have led to the structures of α- and β-interferons,[133] the chromophore-binding domain of ovine rhodopsin,[134] and polymerase protein of the influenza virus.[135] Conformational-energy calculations were used by Scheraga and co-workers to predict the structure of the membrane-bound protein of melittin.[136] Model building was used by Lim[137] to suggest a novel structure for collagen.

Various new methods[138—147] are being explored for tertiary-structure prediction. Busetta and Barans[138] have used empirical parameters to predict the topology of all-α- and all-β-proteins. The use of distance constraints has been

[126] B. W. Matthews, D. H. Ohlendorf, W. F. Anderson, and Y. Takeda, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79,** 1428.
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explored by several workers.[139–140] A recent interest has been in the prediction of exposed regions of the polypeptide chain with the aim of locating antigenic determinants.[141–144]

The pathway of folding has been modelled by several workers.[148,149] Weaver[148] has incorporated the stabilities of the intermediate states into the diffusion-collision model. Kano and Go use a two-dimensional lattice model of a protein and obtain relaxation times for both folding and unfolding.

Protein Function.—A major motivation for the study of protein structure is to obtain a deeper understanding of the relationship between conformation and activity. Both classical[153,154] and quantum-mechanical[155,156] energy calculations have been directed to this goal, and sometimes these studies are aided by sophisticated computer graphics.[157]

Scheraga and Pincus[153] have computed the conformational energies of complexes of alternating copolymers of *N*-acetylglucosamine and *N*-acetylmuramic acid with an egg-white lysozyme. De Coen *et al.*[154] have modelled peptide substrates and inhibitors of serine peptidases. Quantum-mechanical calculations have been applied to high-potential iron–sulphur proteins[155] and to catalase.[156] The development of colour computer graphics, at San Francisco, has encouraged studies on macromolecular-ligand interactions.[157,30] Kuntz *et al.* report a method to explore geometrically feasible alignments of receptors of known structures. Blaney *et al.*[30] used computer graphics to model the thyroxine–prealbumin complex and then predicted and measured the relative binding affinities of four previously untested thyroid hormone analogues.

Conclusion.—The trend this year has been away from the further development of potential-energy functions and towards empirical methods of secondary-structure prediction. Electrostatics have continued to play an important role, but careful calibration of the model is required.

2 Stability and Folding of Proteins

Contributed by S. Craig

This field is reviewed in a book by Ghélis and Yon.[158]

Stability.—A general correlation between the thermostability of proteins and their resistance to proteolysis has been noted.[159] The thermal stability of cytochrome P450,[160] type I and type III collagen from rat skin,[161] and

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[161] C. C. Danielson, *Biochem. J.*, 1982, **203,** 323.

cytochrome P448 of *Saccharomyces cerevisae*[162] has been investigated. Thermal-denaturation studies have been carried out on human somatotropin, human choriomammotropin, ovine prolactin,[163] ovomucoid,[164] and genetic variants of the Kunitz soybean trypsin inhibitor.[165] An investigation of the dynamic states of the three methionyl residues of streptomyces subtilisin inhibitor[166] has shown the hydrophobic core of the protein to be very stable with a $T_m > 90$ °C and a slow equilibrium between native and denatured states. The free energies of stabilization for RNase A, lysozyme, α-lactalbumin, and myoglobin have been estimated as 7.3 ± 0.2, 8.9 ± 0.1, 4.3 ± 0.1, and 7.9 ± 0.2 kcal mol^{-1}, respectively.[167] Thermally induced conformational changes have been observed in cytochrome *b*5,[168] human immunoglobulin G cryoglobulin,[169] and an analogue of the repeat pentapeptide of tropoelastin *cyclo*(L-Val-L-Pro-Gly-L-Val-Gly)$_2$.[170]

Effects of Disulphides. The enthalpy and heat-capacity changes during the reduction of insulin indicate that the conformational change in insulin associated with the reduction of the three disulphide bonds is thermodynamically of the same nature as protein thermal denaturation.[171] Chicken egg-white lysozyme has been reduced to various levels in the absence of denaturant, and the results are consistent with disulphide bonds being important in the formation and maintenance of secondary structure.[172] The relative stabilities of intermediates in the regeneration of reduced ribonuclease A with glutathiones appear to determine which pathway of regeneration is followed.[173]

Effect of Individual Residues. Hollecker and Creighton have investigated the effect on protein stability of converting the amino groups of β-lactoglobulins A and B, cytochrome *c*, and ribonuclease to acidic groups.[174] The φ and ψ angles of the twenty natural amino-acids for 38 different globular protein crystals have been used to study the main-chain conformation characteristics of the side chains.[175] The thermal stability of the triple-helix conformation of procollagen and collagen is found not to be a simple function of the hydroxyproline and imino-acid content of the protein;[176] however, the heat stability of this

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conformation in collagen model peptide $(Pro\text{-}Pro\text{-}Gly)_{10}$ increases with enzymic incorporation of hydroxyproline residues.[177]

In cytochrome *c*, Phe-10 and Tyr-97 are close together in the structure, as are Phe-46 and Tyr-48 and Tyr-67 and the haem group, and it is concluded that two aromatic rings adjacent to each other produce a rigid local structure that could act as an anchor around which the protein folds.[178] An Asp-86-to-Gly substitution makes the red-cell carbonic anhydrase I hiroshima-1 variant easier to inactivate in guanidine hydrochloride than the ordinary enzyme; the substitution also reduces the efficiency of refolding.[179] The reciprocal interactions of the haem iron and proximal histidine residues of haemoglobin regulate the presence of a special α-helical domain.[180] The fast-reacting sulphydryl group of rat-muscle phosphoglycerate kinase is necessary for maintenance of secondary structure.[181] Protonated Ala-149 in α-chymotrypsin is the cause of the hypersensitivity of the enzyme to urea denaturation at pH 7.5—10.[182] The specific contributions of each residue to the stability of the conformation of substance P *C*-terminal pentapeptide have been calculated.[183] Chemically deglycosylated ovine pituitary lutropin is found to be relatively more heat stable than the native lutropin,[184] contrary to expectations.[185]

Effect of Ligands. The *lac* repressor headpiece is stabilized to thermal denaturation by the presence of NaCl,[186] and the heat stability and structure of the λ *tof* repressor protein in solution have been investigated in relation to the binding ability of DNA.[187] Myoglobin is stabilized by 2—3 kcal mol^{-1} to thermal denaturation with cyanide and azide as ligands,[188] while the effect of external ligands on the acid denaturation of myoglobin has been studied.[189] Two different pathways of acid unfolding of myoglobin have been noted,[190] ligand binding in only one pathway being responsible. Ca^{2+} stabilizes the secondary structure of DNase I to some extent against guanidine hydrochloride denaturation.[191]

The binding of thyroxine to thyroxine-binding globulin is found to stabilize it to acid and guanidine hydrochloride denaturation.[192] The effects of collagen binding on fibronectin conformation[193] and ligand effects on the self-

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association of rabbit-muscle phosphofructokinase[194] have been studied. A haem-dependent domain is present in haemoglobin β-subunits.[195]

The effect of phospholipid structure on the thermal stability of rhodopsin[196] and the stabilization of phosphofructokinase by methylamine[197] have been studied. Conformational changes have been observed in pepsin when pepstatin binds.[198] The two major domains of band 3 protein have been found to be independent of each other with regard to their stability, indicating minimum interaction between them.[199]

Solvent Effects. The effect of dimethyl sulphoxide and its homologues on the thermal stability of lysozyme has been studied,[200] and the enthalpy of denaturation showed a complex dependence on solvent composition. The tetradecapeptide Thr-His-Thr-Asn-Ile-Ser-Glu-Ser-His-Pro-Asn-Ala-Thr-Phe is disordered in aqueous buffer but in 10% dimethyl sulphoxide is found to adopt an ordered structure.[201] Pig-heart mitochondrial malate dehydrogenase is found to be stabilized by high ionic strength and high enzyme concentrations.[202]

Addition of dodecyl sulphate to the binding subunit of cholera toxin in aqueous buffer causes the protein to undergo a transition from mainly β-sheet to mainly α-helix conformation.[203] The denaturation and renaturation of bacteriorhodopsin have been investigated in detergents and lipid-detergent mixtures.[204] The effects of detergent, pH, and temperature on the conformation of the enterotoxin from *Clostridium perfringens* have been studied.[205] The effects of pH on the conformation of subtilisin DY[206] and fibronectin[189] and on the stability of the ligand-binding site of bovine-heart myoglobin carbonyl[207] have been investigated. Acid, base, and guanidine have been used in the reversible denaturation of *Aequorea* green fluorescent protein.[208] Low pH has been used to study the N—F transition of bovine plasma albumin[209] and the unfolding of ferricytochrome *c*.[210] The denaturation of α- and β-trypsin at alkali and neutral pH in the presence and absence of Ca^{2+} has been studied.[211]

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Low concentrations of ethanol and methanol are found to stabilize methaemoglobin; however, high concentrations of the solvents and all concentrations of iso- and n-propanol have a destabilizing effect.[212] Methanol-stabilized intermediates in the thermal unfolding of ribonuclease B have been studied,[213] and a very fast relaxation in the unfolding of ribonuclease A has been found to be dependent on solvent viscosity.[214]

Intermediates in Protein Folding.—The role of intermediates in protein folding has been reviewed by Kim and Baldwin.[215] Native pepsinogen has been shown to be in rapid equilibrium with the initial unfolded form, proline isomerization subsequently producing slowly refolding forms. These partially folded forms do not lie on the direct folding pathway but are trapped by their having proline isomers that differ from those in the native state.[216] The presence of wrong proline isomers does not inhibit the overall folding of pepsinogen but prevents the close packing of the structural elements into the native form.[217] A non-interacting globule-coil model has been used to predict the conformational characteristics of rare folding-pathway intermediates for ribonuclease A, lysozyme, apomyoglobin, trypsin inhibitor, and other proteins.[218]

A short α-helix involving His-12 in ribonuclease A c-peptide carboxylate[219] and ribonuclease A[220] has been found to be stable enough to act as a folding intermediate. The fast and slow folding forms of unfolded ribonuclease A are found to differ in their tyrosine fluorescence.[221] Intermediates in the unfolding[213] and folding [222] of ribonuclease A at low temperature in aqueous methanol have been investigated. The rate-limiting steps in the regeneration of reduced ribonuclease A have been studied.[173] The first active molecules of ribonuclease produced from regeneration of the reduced protein are only partially oxidized. They have a near-native conformation that is reached without being trapped in the wrong structure by too many incorrect disulphide bonds.[223]

A highly populated stable intermediate in the unfolding of bovine carbonic anhydrase has been observed, and kinetic data indicate that it is on the folding pathway.[224] Conformations of intermediates trapped during the folding of black-mamba venom toxins I and K have been investigated using circular dichroism.[225] Results from the refolding of rabbit creatine kinase suggest that

[212] A. Cupare, D. Giacomazza, and L. Cordone, *Biopolymers*, 1982, **21,** 1081.
[213] R. C. Biringer and A. L. Fink, *J. Mol. Biol.*, 1982, **160,** 87.
[214] T. Y. Tsong, *Biochemistry*, 1982, **21,** 1493.
[215] P. S. Kim and R. L. Baldwin, *Ann. Rev. Biochem.*, 1982, **51,** 459.
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[219] P. S. Kim, A. Bierzynski, and R. L. Baldwin, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79,** 2470.
[220] A. Bierzynski and R. L. Baldwin, *J. Mol. Biol.*, 1982, **162,** 173.
[221] A. Rehage and F. X. Schmid, *Biochemistry*, 1982, **21,** 1499.
[222] R. C. Biringer and A. L. Fink, *Biochemistry*, 1982, **21,** 4748.
[223] A. T. Garel and J.-R. Garel, *J. Biol. Chem.*, 1982, **257,** 4031.
[224] R. W. Henkens, B. B. Kitchell, S. C. Lottich, P. J. Stein, and T. J. Williams, *Biochemistry*, 1982, **21,** 5918.
[225] M. Hollecker, T. E. Creighton, and M. Gabriel, *Biochimie*, 1981, **63,** 835.

there is no significant accumulation of folded but inactive intermediates.[226] An intermediate in the subunit folding and assembly of bacteriophage P22 tail-spike endorhamnosidase has been isolated.[227] Two pathways of unfolding for the active three-fragment complex of horse cytochrome *c* have been observed at different temperatures, one pathway going *via* the major folding intermediate (1—25) H. (56—104), which consists of fragment (56—104) and the ferrohaem fragment H(1—25); the other pathway does not involve this intermediate.[228] The guanidine hydrochloride-induced unfolding of the α-subunit of tryptophan synthetase is a stepwise process with the α_2 domain unfolding first.[229]

Theories of Protein Folding.—The folding and association of proteins are reviewed.[230]

Proline Isomerization. The refolding of the constant fragment of the immunoglobulin light chain[231] and of the corresponding reduced constant fragment[232] has been studied. In both cases fast and slow refolding forms of the unfolded protein have been observed, and they appear to interconvert by proline isomerization. Proline isomerization is likely to account for the observed slow and fast refolding forms of unfolded swine pepsinogen.[216] The rate of reactivation of lactate dehydrogenase after guanidine hydrochloride denaturation is independent of proline isomerization.[233]

The proline isomerization in unfolded ribonuclease A is found to be independent of temperature,[234] and results from ribonuclease fragments suggest that proline may be in a *cis* or *trans* conformation for folding as either permits formation of the β-bend necessary for a compact β-sheet structure to form in the hydrophobic initiation site.[235] The difference in fluorescence observed between the fast and slow refolding forms of unfolded ribonuclease A is thought to be caused by proline peptide bond isomerization during the $U_F \rightleftharpoons U_S$ reaction,[221] and further evidence for this comes from the c.d. spectra, which show that U_F and U_S are identical in their state of unfolding as far as secondary structure is concerned.[236]

Nucleation and Hydrophobic Cluster Model. A hypothesis of protein folding *in vivo* in which folding proceeds through prefolded peptide segments of three to fourteen amino-acids has been proposed.[237] The three-species model of refolding $U_1 \rightleftharpoons U_2 \rightleftharpoons N$ established for ribonuclease A is shown to be valid for the

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[231] Y. Goto and K. Hamaguchi, *J. Mol. Biol.*, 1982, **156,** 891.
[232] Y. Goto and K. Hamaguchi, *J. Mol. Biol.*, 1982, **156,** 911.
[233] G. Zettlmeissel, R. Rudolph and R. Jaenicke, *Eur. J. Biochem.*, 1982, **125,** 605.
[234] F. X. Schmid, *Eur. J. Biochem.*, 1982, **128,** 77.
[235] E. R. Stimson, G. T. Montelione, Y. C. Meinwald, R. K. E. Rudolph, and H. A. Scheraga, *Biochemistry*, 1982, **21,** 5252.
[236] F. X. Schmid, *FEBS Lett.*, 1982, **139,** 190.
[237] A. Galat, *Int. J. Biochem.*, 1982, **14,** 883.

folding of hen egg-white lysozyme, and results suggest that the direct-folding process is nucleation controlled.[238] Results presented indicate that the folding of ribonuclease A is not nucleation controlled in the sense that it has not got a rate-limiting nucleation step,[213] and the first structure in refolding appears to involve secondary structure rather than hydrophobic clusters. A model of ribonuclease A refolding has been proposed that involves the formation of units of secondary structure with exposed hydrophobic regions, which then pack together by hydrophobic interactions.[222] Results presented[239] for ribonuclease favour a sequential mechanism of folding, with the rate of folding being determined by the stability of a structural precursor. The folding transitions of ribonuclease have been studied using amide circular dichroism.[240] The two latter papers are discussed further.[241]

Helix-dipole interactions are shown to be important in stabilizing and in the folding of four α-helical proteins,[242] as the short inter-helical connections would be entropically favourable for electrostatic association of sequentially adjacent helices. Co-operative motions observed in protein β-sheets are thought to be relevant to protein folding, as the motions suggest a highly co-operative pathway for β-sheet folding.[243]

Folding Pathways.—The *in vitro* assembly of active catalytic subunits of aspartate transcarbamylase from unfolded polypeptide chains has been studied.[244] The refolding of bovine neochymotrypsinogen[245] and the reactivation of yeast superoxide dismutase[246] have been investigated. The small proteins L29 and L30 of *Escherichia coli* ribosomes are suggested as potentially interesting models for the study of protein folding.[247] The β-subunit of chorionic gonadotropin can only refold from the reduced state after deglycosylation, while the α-subunit can refold in the glycosylated or deglycosylated state.[248]

The rate-determining steps in the refolding and reconstitution of porcine skeletal-muscle lactate dehydrogenase have been examined.[249] In the regeneration of the active pyruvate dehydrogenase complex the reshuffling of reassembled subunits is suggested to be the rate-determining step.[250] The ordered structure present in $LiClO_4$-denatured ribonuclease A plays no role in determining the folding pathway,[251] suggesting that the ordered structures in

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[239] A. M. Labhardt, *J. Mol. Biol.*, 1982, **157,** 357.
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[243] F. R. Salemme, *Nature (London)*, 1982, **299,** 754.
[244] D. L. Burns and H. K. Schachmann, *J. Biol. Chem.*, 1982, **257,** 8638.
[245] C. T. Duda and A. Light, *J. Biol. Chem.*, 1982, **257,** 9866.
[246] L. D. Arnold and J. R. Lepok, *FEBS Lett.*, 1982, **146,** 302.
[247] A. J. Gudkov, S. Yu. Veryaminov, J. Behlke, and V. N. Bushuev, *FEBS Lett.*, 1982, **141,** 254.
[248] J. M. Goverman, T. F. Parsons, and J. G. Pierce, *J. Biol. Chem.*, 1982, **257,** 15 059.
[249] G. Zettlmeissl, R. Rudolph, and R. Jaenicke, *Biochemistry*, 1982, **21,** 3946.
[250] R. Jaenicke and R. N. Perham, *Biochemistry*, 1982, **21,** 3378.
[251] J. B. Denton, Y. Konishi, and H. A. Scheraga, *Biochemistry*, 1982, **21,** 5155.

ribonuclease A do not all have an equivalent influence on the folding pathways. The thioester bond in the α-chain of the fourth component of human complement is thought to play a crucial role in the folding of the molecule.[252] The pathway of regeneration of ribonuclease A from reduced protein varies according to the stabilities of the oxidized intermediates.[173] The short α-helix involving His-12 in ribonuclease A, which acts as a folding intermediate, is consistent with the framework model.[220]

Folding of Independent Domains and Protein Fragments.—The carboxy-terminal fragment 206—316 of thermolysin has been studied;[253,254] it can fold into a stable configuration with the thermodynamic properties of a small globular protein. A three-domain structure has been put forward for high-molecular-weight high-mobility-group non-histone proteins.[255] Domains in haemoglobin[180,195] and band 3 protein[199] have been investigated. The structure of apomyoglobin is thought to be due to the interaction of two or more distinct structural units representing the product of independent folding processes.[256]

Denaturation and renaturation of the $F_c(t)$ and pFc′ fragments of human immunoglobulin G[257] have been carried out, and the folding of fragments of cytochrome c has been compared to the folding of the intact protein.[258] The secondary structure properties of mouse immunoglobulin A have been examined, and the difference in stability of mouse and human immunoglobulin A to guanidine hydrochloride denaturation is thought to be due to the unusual C_H1 domain of the mouse protein.[259] The unfolding of the α_1- and α_2-domains of the α-subunit of tryptophan synthetase has been carried out with guanidine hydrochloride.[229]

3 Circular Dichroism

Contributed by T. Brittain

Over the past year many hundreds of papers have appeared concerning the use of circular dichroism (c.d.), predominantly for the estimation of protein secondary structure. This review is therefore selective in its coverage, whilst every attempt has been made to maintain the scope of the material previously reviewed.

General.—*Reviews.* As in previous years, reviews have appeared concerned with the general field of conformational analysis of optically active biopolymers.[260,261] The use of c.d. as a detection method in rapid-reaction studies has

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[253] C. Vita, A. Fontana, and I. M. Chaiken, *Biochemistry*, 1982, **21**, 2016.
[254] C. Vita and A. Fontana, *Biochemistry*, 1982, **21**, 5196.
[255] G. R. Reeck, P. J. Isackson, and D. C. Teller, *Nature (London)*, 1982, **300**, 76.
[256] G. Collona, C. Balestrieri, E. Bismuto, L. Servillo, and G. Irace, *Biochemistry*, 1982, **21**, 212.
[257] A. Sumi and K. Hamaguchi, *J. Biochem. (Tokyo)*, 1982, **92**, 823.
[258] G. R. Parr and H. Taniuchi, *J. Biol. Chem.*, 1982, **257**, 10 103.
[259] N. M. Young and R. E. Williams, *Int. J. Pept. Protein Res.*, 1982, **19**, 243.
[260] A. G. Sukhomudrenko, *Spectrosk. Mol. Krist. Mater., Resp. Shk.-Semin. 4th*, 1979, **2**, 202.
[261] F. Ciardelli, M. Aglielto, C. Carlini, E. Chiellini, and R. Solaro, *Pure Appl. Chem.*, 1982, **54**, 521.

been reviewed. Equipment is now available for the detection of changes of 10^{-5} absorption units at 1 ms resolution, making it especially effective in studies of stereochemical relationships within enzyme substrate complexes.[262] The application of fluorescence-detected c.d. to studies on proteins has also been reviewed.[263]

Instrumental. Two different approaches to c.d. measurements in scattering systems have been reported. The first has shown that c.d. parameters may be determined by photoacoustic methods in which the dependence of the magnitude of the photoacoustic signal on the ellipticity of the impinging light is detected.[264] In the second case it has been shown that in thick suspensions, owing to the non-applicability of Lambert's law, Grosjean–Legrand c.d. instrumentation is not appropriate. In these systems the addition of a plate of opal glass to the optics of a conventional instrument allows the molar c.d. to be measured in multiple-scattering situations when a beam of polarized light is incident on the scattering medium.[265,266]

Instrumentation for the simultaneous measurement of absorption and c.d. spectra has been described in two independent reports. In one case a computer-controlled spectrometer capable of simultaneous measurements in the 190—700 nm region has been described. Absorbance is measured in this instrument by determining the log ratio of the intensity of the deviated extraordinary beam emergent from a MgF_2 Rochon polarizer, and the undeviated ordinary beam is passed through the sample. C.d. measurement is made by means of quarter-wave modulation of the plane-polarized undeviated beam from the Rochon polarizer, using photoelastic modulation at 50 kHz.[267] Simultaneous measurement is also possible using a synchrotron spectrometer. In this case a constant current is maintained to the detector during a scan, and from the log of the photomultiplier gain and various wavelength-dependent system parameters it is possible to obtain simultaneously a pseudo-absorption spectrum as well as a c.d. spectrum. A true absorption spectrum is obtained only after the contributions of a blank sample have been subtracted.[268]

It is now possible to observe vibrational effects on protein secondary structure using a spectropolarimeter equipped with a 2450 MHz microwave waveguide. Using this equipment it has been shown that high levels of microwave radiation (600 mW g^{-1}) induce a decrease in α-helix in spectrin as a result of both thermal vibration and strain on intramolecular hydrogen bonds, which normally maintain secondary structures.[269]

Instrumentation necessary for double-modulation Fourier-transform i.r. c.d.

[262] P. M. Bayley, *U.V. Spectrom. Group Bull.*, 1981, **9,** 91.
[263] E. W. Lobenstein, *Diss. Abstr. Int. B*, 1981, **42,** 1860.
[264] G. S. Mityurich, *Dokl. Akad. Nauk BSSR*, 1982, **26,** 414.
[265] Y. Taniguichi and Y. Shimura, *Bull. Chem. Soc. Jpn.*, 1982, **55,** 754.
[266] Y. Tamaguchi and Y. Shimura, *Bull. Chem. Soc. Jpn.*, 1982, **55,** 2847.
[267] R. W. Mason, *Anal. Chem.*, 1982, **54,** 646.
[268] J. C. Sutherland, P. C. Keck, K. P. Griffin, and P. Z. Takacs, *Nucl. Instrum. Methods Phys. Res.*, 1982, **195,** 375.
[269] M. J. Ortner, M. J. Galvin, C. F. Chignell, and D. I. McRee, *Cell. Biophys.*, 1981, **3,** 335.

has been produced. The performance of this apparatus has been evaluated and compared with that of a conventional dispersive instrument.[270]

Theory. Reasonable agreement has been achieved between theoretical prediction and observed c.d. parameters for adenylate kinase. Using an origin-independent matrix formalism, including essentially all the electronic transitions occurring at wavelengths >185 nm, a linear dielectric function was employed to evaluate the intertransition coupling potentials.[271] Absorption and c.d. lineshapes of a molecular dimer (composed of two identical subunits) have been derived using approximate analytical expressions in the strong electronic-coupling limit, giving excellent agreement between theory and observations.[272]

Calculations have been made of side-chain conformation effects on the π–π^* absorption and c.d. spectra of general helices and polypeptide β-structures. In the general helix case variations of side-chain conformation only slightly affect c.d. in the backbone region but produce $\leqslant$10 nm band shifts in other regions. The angular dependence of the principal 200 nm band shows that variations of χ' within the stable range should have little effect on the π–π^* c.d. spectra of α-helical polypeptides containing a non-chromophoric substituent on C^{β}.[273] The dipole-interaction model, including interactions amongst all atoms, when applied to poly(Gly), poly(Ala), and poly(Val) in the β_P (parallel) and β_A (antiparallel) pleated-sheet structure predicts the c.d. spectrum to be very sensitive to side-chain structure and conformation. This theory, however, predicts insufficient rotational strength to account for the observed c.d. for poly(Gly) and poly(Ala) β-structures in uniform planar lattices. The predictions for poly(Val) show close approximation to experimental values when χ' is near $-60°$ for the β_A structure and near 140° for the β_P structure.[274] A modified factor analysis of the circular dichroism spectra of cyclic dipeptides, *N*-acetyl amino-acid methylamides, and ribosomal proteins has been reported.[275]

On the basis of transient dichroic ratio measurements on bacteriorhodopsin in the visible absorption region, the strong exciton coupling mode, used previously to explain the visible c.d. of the purple membrane, has been rejected. A time-dependent analysis of the exciton interaction and consideration of the coupling strength have led to a redefinition of the interaction between retinals in the purple membrane as weak or very weak, as defined by Foerster.[276]

A new Gaussian-type sum rule has been devised for ellipticity, which, whilst reducing to the usual type of sum rule in certain limits, contains a Gaussian factor that serves to improve convergence.[277]

[270] L. A. Nafie, E. D. Lipp, and C. G. Zimba, *Proc. SPIE-Int. Soc. Opt.*, 1981, **289**, 457.
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[274] J. Applequist, *Biopolymers*, 1982, **21**, 779.
[275] P. Pancoska and K. Blaha, *Commun. Czech. Pol. Colloq. Chem. Thermodyn. Phys. Org. Chem. 2nd*, 1982, 2980, 223.
[276] R. E. Godfrey, *Biophys. J.*, 1982, **38**, 1.
[277] I. Kimel, *Phys. Rev. B: Condens. Matter*, 1982, **25**, 6561.

Vibrational c.d. intensities have been calculated for some deuteriated isotopomers of Ala by the use of the fixed-partial-charge model, and an analysis of two overtones that uses Fermi resonance methods has yielded a revised set of frequencies for the fundamentals. These in turn lead to a refined Urey–Bradley description of these modes.[278]

The effects of errors introduced by constrained analysis on protein c.d. have been investigated together with the role they may play in causing analyses to fail for some proteins. The effects of operational and experimental sources of error on the calculation of protein secondary structure have also been studied.[279]

Small Molecules, Model Compounds, and Synthetic Polymers.—*Amino-acids and Derivatives.* As in most of the recent years the majority of studies utilizing c.d. to investigate amino-acid systems have involved the use of metal-ion complexes. A number of papers have appeared concerned with cobalt complexes of amino-acids. Complexes of the general type $[Co(en)_2L]^{2+}$ and $[Co(en)L_2]^+$ [HL = *S*-Asp(Asn) or *S*-Glu] have been prepared and their sterochemistry has been investigated. Hydrogen bonding in *trans*-(*O*)-$[Co(en)(Asn)_2]^+$ has been identified from c.d. measurements in a range of different solvents.[280] Induced c.d. has been measured in racemic Co^{III} complexes for $[Co(\beta\text{-Ala})(en)_2]^{2+}$ and $[Co(Gly)(en)_2]^{3+}$ (β-Ala = β-alaninato, Gly = glycinato) when they were dissolved in aqueous D-tartrate^{2-} solution at room temperature. A single negative c.d. band was observed in the 400—500 nm region. C.d. bands were attributed to differences in the interaction between the Λ- and Δ-enantiomers with D-tartrate^{2-}.[281] The c.d. spectra of the resolved complexes $[Co(CN)_2L_2]^-$ (HL = Gly or Ala) have been measured and compared with those of $(-)_{588}$-$[Co(CN)_2(C_2O_4)(en)]^-$. The optical activity of the *cis,cis,cis*-$[Co(C)_2(N)_2(O)_2]^-$ type is concluded to arise from the arrangement of the donor atoms, the non-ligated atoms making only minor contributions.[282] The configurations of a number of isomers of $(-)_{589}$-*cis*-(NO_2)-*trans*-(NH_2)(D-Ala) dinitrotrimethylenediamine cobalt(III) have been assigned by use of c.d. measurements.[283] The c.d. spectra and stereoselectivity in the formation of the diastereoisomers of $[Co(phen)_2(S\text{-am})]^{2+}$ [phen = 1,10-phenanthroline, am = an α-amino-acid anion of Gly, (*S*)-Ala, (*S*)-Phe, (*S*)-Leu, (2*S*, 3*S*)-isoLeu, or (*S*)-Val] have been described and compared with those of $[Co(en)_2(S\text{-am})]^{2+}$.[284]

Tri-μ-oxy-triaquohexakis-(L-amino-acid) tri-iron(III), which contains the $[Fe_3O]^{7+}$ unit, has been proposed as a model for the ferritin Fe core. The c.d. of these complexes is due mainly to the interaction of the charge-transfer

[278] B. B. Lal, M. Diem, P. L. Polavarapu, M. Oboodi, T. B. Freedman, and L. A. Nafie, *J. Am. Chem. Soc.*, 1982, **104**, 3336.
[279] J. P. Hennessey and W. C. Johnson, *Anal. Biochem.*, 1982, **125**, 177.
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[281] C. E. Oh, D. H. Kang, and G. C. Shin, *Taehan Hwahakhoe Chi*, 1981, **25**, 306.
[282] S. Fujinami and M. Shibata, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 2939.
[283] M. J. Malinar, R. Herak, M. B. Celap, N. Pavlovic, S. Milic, and D. Stojanov, *Glas. Hem. Drus., Beograd*, 1981, **46**, 303.
[284] A. Tatehata, *Inorg. Chem.*, 1982, **21**, 2496.

manifold with the asymmetric carbon atom of the amino-acids. It is not possible simply to relate the sign of the c.d. extrema to any specific molecular structure. Extrema for five L-amino-acids with aliphatic side chains occur at the same wavelengths in each case: 218(+), 265(−), 267(+), 311(+), 378(−), 475(+), 538(+), and 620(+) nm. Complexes using the D-amino-acids exhibit inverted spectra.[285] From the c.d. spectra and by analogy with $CoLL_2$ (L_2 = L-amino-acidate ion) it appears that Fe^{II} mixed chelates of the form $FeLL_1$ [H_2L = *NN'*-bis(salicylidene)ethylenediamine, HL_1 = L-Ala, L-Val, or L-Phe] preferentially form the Λ-*cis*-β-(*fac*) isomer.[286]

Ternary complexes of the form [Pd(L-His)(L-aa)]·$2H_2O$ (L-aa = Asn, Gln, Ser, Thr, or homo-Ser) exhibit negative and positive c.d. peaks at 307—328 and ~300 nm, respectively. Whereas the magnitude of the observed ellipticity is simply given by additivity for Pd(L-His)(Q) (HQ = Gly, L-Ala, or L-Val) and Pd(L-HisOMe)(L-aa), the magnitudes for Pd(L-His)(L-aa) deviate significantly from those estimated by simple additivity. This latter anomaly appears to arise from ligand–ligand interactions between the carboxylato group of His and the OH or NH_2 group of the L-aa in solution.[287] A racemization test for Met and *S*-ethylcysteine has been proposed, based on the observation that the enantiomers of these amino-acids when complexed with sodium tetrachloropalladate display Cotton effects of opposite sign.[288]

The mode of co-ordination of His to Cu^{II} in Cu^{II} complexes of Schiff bases derived from pyridoxal, salicylaldehyde, or pyruvic acid and His, HisOMe, and amino-acids with non-polar side chains has been investigated by c.d. spectroscopy. The His residues show a marked tendency to bind Cu^{II} through chelate-ring types complementary to those of the fused-carbonyl type. Thus in complexes derived from pyridoxal the His binds Gly-like, whereas in those derived from pyruvic acid the binding is histamine-like. The conformations of the co-ordinated Schiff-base ligands were deduced from c.d. spectra and discussed in relation to vitamin B_6 model reactions.[289] The complexes of Cu^{II} with L-Asp^{2-} and L-Pro^{-} show positive charge-transfer bands in their c.d. spectra at 230 nm and negative bands at 270 nm. The effects of the addition of non-chiral ligands to these complexes were observed in the charge-transfer region.[290]

C.d. studies on $Cs[PtLCl_2]$ (L = *N*-methyl-L-hydroxy-Pro or L-Pro) show that the cyclic imino-acid possesses the *S*-configuration.[291]

Mixed Zn^{II} complexes with 1,10-phenanthroline and L-Ala, L- and D-Ser, L-Thr, L-Pro, or L- and D-Val show exciton splitting at 268 nm, the intensity of which is dependent on the amino-acid ligated.[292]

The c.d. spectra of *N*-DNP-*O*-benzoyl derivatives (DNP = 2,4-dinitrophenyl) of L-Ser, L-Thr, and L-*allo*-Thr and the DNP derivatives of

[285] R. N. Puri, R. O. Asplund, and W. F. Tucker, *Inorg. Chem. Acta*, 1982, **66**, 7.
[286] M. Nakamura, T. Itoh, H. Okawa, and S. Kida, *J. Inorg. Nucl. Chem.*, 1981, **43**, 2281.
[287] A. Odeni and O. Yamauchi, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3773.
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[290] S. Bunel, C. Ibarra, M. Rodriguez, A. Urbina, and C. A. Bunton, *J. Inorg. Nucl. Chem.*, 1981, **43**, 871.
[291] L. Kh. Minacheva, O. P. Slyudkin, M. A. Porai-Moshits, and T. S. Khodashova, *Koord. Khim.*, 1982, **8**, 557.
[292] S. Bunel, G. Larrazabal, and A. Decinti, *J. Inorg. Nucl. Chem.*, 1981, **43**, 2781.

L-Phe and (βR)- and (βS)-β-Me-L-Phe have been recorded. The observed effect of Me substitution at the β-position was explained by the change in population of the rotamer g^- ($\chi' = -60°$), which is responsible for the negative Cotton effect at 400 nm. The negative sign of the ellipticity at 400 nm of the DNP derivatives described by the DNP-aromatic rule is also due to contributions from the g^- rotamer.[293]

A simple and fast micromethod has been described for the detection of optical purity of small amounts of amino-acids (0.1—1 $\mu g\,ml^{-1}$). The amino-acids are reacted with fluorescamine to yield the pyrrolinone-type chromophores, which then exhibit c.d. bands at 400—300 nm with a much larger associated rotational strength than that observed for the free amino-acids in the visible region.[294]

A comparison of experimental c.d. values for *N*-acetyl-L-Ala-*N*-methylamide and *N*-acetyl-L-Ser-*N*-methylamide with those predicted by theoretical calculations shows that many of the conformational-energy calculations previously reported yield reasonable values for those molecules in non-polar, but not in polar, media.[295]

Dipeptides and Oligopeptides. In relatively non-polar solvents, such as fluoroalcohols, the chemotactic peptide HCO-Met-Leu-Phe-OH shows a folded conformation, whilst in H_2SO_4 it remains intact but displays a c.d. spectrum typical of a highly solvated and totally disordered peptide.[296] Studies of H-Gly-$(Pro)_n$-OH ($n = 2$—5) in water as a function of pH, temperature, and added salts show the presence of a helical polyPro-like structure when $n = 3$.[297] Valinomycin forms a 2:1 sandwich complex with Ca^{2+} at Ca^{2+}:valinomycin ratios of <0.5 in acetonitrile. At high Ca^{2+} concentrations the protein takes up a conformation similar to that seen for the free protein in polar solvents. Possible conformations for the sandwich complex are proposed.[298] The c.d. spectra of the short-lived mononuclear superoxo-Co^{III} complex of bleomycin together with its decay product, the dinuclear μ-peroxo-Co^{III} complex, and its stabilized DNA complex have been reported.[299]

Membrane-protein interactions have been studied using model peptide-membrane mimetic media. Several hydrophobic peptides solubilized in aqueous sodium dodecyl sulphate micelle systems show solvation similar to bulk MeOH but with decreased conformational freedom. Strongly hydrophilic peptides solubilized in non-polar reversed micelles are located in small H_2O pools in close association with the surfactant head groups. This interfacial region has a distinct conformational impact on the peptides.[300]

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[294] V. Toome and B. Wegrzynski, *Anal. Lett.*, 1981, **14,** 1725.
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[299] A. Garnier-Suillerot, J. P. Albertini, and L. Tosi, *Biochim. Biophys. Res. Commun.*, 1981, **102,** 499.
[300] L. M. Gierasch, J. E. Lacy, K. F. Thompson, and A. L. Rockwell, *Biophys. J.*, 1982, **37,** 275.

Exciton chirality theory has been used to analyse the spectra of DNP-Leu-X-Pro-Val-*p*-NA (*p*-NA = *p*-$NO_2C_6H_4NH$, X = D-Ala, Gly, or Ala) and DNP-Gly-X_1-X_2-Gly-*p*-NA (X_1 = D-Ala, X_2 = Pro; X_1 = Pro, X_2 = Asn, Gly, Ala, Gln, or D-Ala). The spectra were classified by the sign of the bands at 305 and 350 nm into two groups, both having β-turn conformation. The intensity of the 350 nm band correlates well with the β-turn preference, and some generalized relationships have been proposed connecting sequence and conformation.[301]

It has been shown that c.d. can be used to estimate the contribution of various β-bend types in a related series of compounds such as *cyclo*-(L-Ala-L-Ala-ε-aminocaproyl) and *cyclo*-(L-Ala-D-Ala-ε-aminocaproyl) cyclic dipeptides.[302]

The formation of a Pd^{II}–thioether bond in *S*-Me-L-Cys-containing peptides considerably enriches the u.v. region of the c.d. spectrum where $S \rightarrow Pd^{II}$ charge transfer as well as intrasulphur transitions are observed.[303] The absolute configurations of the optical isomers of $[Co^{III}L_2]^-$ (H_2L = glycyl-glycine, -β-alanine, -L-alanine, or -L-leucine, β-alanyl-glycine or -L-alanine, L-alanyl-glycine or -β-alanine, L-leucylglycine, or L-prolylglycine) and [Co(Gly-Gly)(L-Pro-Gly)]$^-$ have been determined on the basis of their c.d. patterns in the ligand-transition spectral region.[304] The c.d. spectra of [*N*-(carboxymethyl)-L-β-(2-pyridyl)-α-Ala](aa)Co^{III} (aa = Gly, D,L-Ala, D-Thr, D,L-Val, or D-Asp) and [*N*-(carboxymethyl)-L-His](aa)Co^{III} have been resolved into two contributions, one from the optically active amino-acidate ring and the other from the rest of the molecule.[305,306]

Polypeptides. The conformation of poly(α-L-Asp) has been investigated on a sample that showed no β-bonds. The polypeptide passes through a conformational change induced by changes in the degree of ionization, which leads to the precipitation of a probable helical form.[307]

The low-molecular-weight form of poly[*S*-(carboxymethyl)-L-cysteine], unlike the high-molecular-weight form, produces β-structures by intermolecular association and not by chain folding. The low-molecular-weight β-structure is formed at 0.004 M and remains stable on dilution to 8×10^{-5} M. In 19 mM $NaClO_4$ at pH 4.5 the residual ellipticity at 200 nm changes from 24 000 to 40 000 deg cm^2 $dmol^{-1}$ over the range $(0.5—1) \times 10^{-5}$ M. At $< 5 \times 10^{-5}$ M an intramolecular β-structure without aggregation is proposed.[308] In hexafluoro-2-propanol poly{*S*-[*C*3-(hydroxypropyl)carbamoylmethyl]-L-cysteine} takes on a random-coil conformation exhibiting negative Cotton effects in the far-u.v. region. In D_2O the high-molecular-weight polymer is mostly in the β-form, assuming a perfect β-structure in mixed alcohol–D_2O mixture of 13:87 ratio.

[301] K. Sato, M. Kawai, and U. Nagai, *Biopolymers*, 1981, **20**, 1921.
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[303] B. Decock-le Reverend, C. Loucheux, T. Kowalik, and H. Kozlowski, *Inorg. Chim. Acta*, 1982, **66**, 205.
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[308] H. Maeda, K. Kadono, and S. Ikeda, *Macromolecules*, 1982, **15**, 822.

At lower ratios of alcohol the polymer is almost randomly coiled. Thus a double solvent-induced β-coil transition occurs in alcohol–D_2O mixtures. This behaviour is interpreted in terms of the properties of hexafluoro-2-propanol.[309] The reaction of a similar polymer, poly(*S*-carboxyethyl-L-cysteine), with Acridine Orange has also been reported. At pH > 7 no c.d. is induced on addition of the dye, whilst at pH ≤ 7, where the polymer is in the β-form, optical activity is induced in the visible and near-u.v. region. Spectral analysis suggests the dye is present as the dimer at neutral pH and as the monomer at lower pH. A dissymmetric structure of the complexes is presented in which the dye cations bind to the polymer side chains in such a way that the longer axes of the molecular planes form a two-fold, right-handed helix along the extended polymer chain.[310]

A film cast from the solution of a copolymer of methionine *S*-oxide with Met or Ala showed a new trough between 205 and 210 nm in its c.d. spectrum. The film exhibits features of both a random coil and an α-helix. On immersion in water the helix becomes disordered as the film dissolves.[311]

C.d. data have shown that poly[Glu(OMe)-Glu(OMe)-Lac] in THF and poly[Lys(CO_2CH_2Ph)-Glu(OMe)-Lac] in CF_3CH_2OH form stable right-handed α-helices at or below room temperature, but these helices can be denatured thermally. The helical stability was not influenced by the nature of the side chain or solvent, the hydrogen-bonding pattern of the polydepsipeptides being the main factor affecting conformational stability.[312]

The conformations of synthetic fragments of bacteriorhodopsin in various solvents have been studied. The heptapeptide Boc-Leu-Val-Pro-Ala-Ile-Ala-Phe-OBzl (Boc = t-butoxycarbonyl) in hexafluoroisopropanol exhibits c.d. behaviour consistent with the presence of a helical structure. In hexane the heptapeptide and the pentapeptide Boc-Pro-Ala-Ile-Ala-Phe-OBzl assume a β-like structure. In dimyristoylphosphatidylcholine vesicles other differences are highlighted, and the heptapeptide exhibits β-structure whilst the pentapeptide and the tripeptide Boc-Ile-Ala-Phe-OBzl are helical.[313]

Proteins.—*Non-chromophoric Proteins.* Measurements on bovine-lens crystallins show some variation in β-structure content, but this is believed to be of little biological significance. The βH^- and γ-crystallins show ellipticity at 235 nm probably originating from the interaction between peptide amide and aromatic amino-acid transitions. Differences in the 250—360 nm region are assigned to differences in μ–μ coupling between aromatic amino-acids. In the near-u.v. region there was no apparent contribution from disulphide bonds, although c.d. in this region showed clearly that the tryptophan residues in γ-crystallins are mostly buried, whilst the tyrosine residues in both β-crystallins are in relatively polar environments.[314] In another report it was

[309] T. Tomiyama and S. Ikeda, *Polym. J. (Tokyo)*, 1981, **13**, 1111.
[310] S. Ikeda, T. Yoshida, and T. Imae, *Biopolymers*, 1981, **20**, 2395.
[311] S. Aiba, N. Minoura, and Y. Fujiwara, *Makromol. Chem.*, 1982, **183**, 133.
[312] G. Wouters, R. Katakai, W. J. Becktel, and M. Goodman, *Macromolecules*, 1982, **15**, 31.
[313] T. Sugihara, E. R. Blout, and B. A. Wallace, *Biochemistry*, 1982, **21**, 3444.
[314] J. N. Liang and B. Chakrabarti, *Biochemistry*, 1982, **21**, 1847.

shown that the c.d. of calf-lens γ-crystallins in the 240—289 nm region closely parallels the Phe content of the three subtypes.[315]

Three papers have appeared concerned with the properties of the three epidermolytic toxins A, B, and C from *Staphylococcus aureus*. The far-u.v. c.d. of toxins A and B suggests the presence of 9% helix and 46% β-structure in both cases. Trifluoroethanol perturbation caused an initial increase in ellipticity at 205 nm followed by a change characteristic of $\beta \rightarrow \alpha$ transition. The intense near-u.v. spectra have been interpreted as arising from unusually rigid molecules with slightly different aromatic side-chain arrangements.[316] In a separate report protein A has been analysed as containing 31% α-helix, 13% β-helix, and 56% random coil. The *N*-terminus of the molecule is composed to 4Fc binding units each containing two antiparallel α-helices connected by random coil; the *C*-terminus is mainly random coil.[317] In a Gaussian analysis of the near-u.v. region of toxins A, B, and C the spectra were resolved into two components. The rotational strength associated with toxin A, however, was significantly different from that observed for B and C. The Gaussian curves were identified as arising from two Phe bonds (the ^1L_b transition O—O and $0+930\ cm^{-1}$), four Tyr bands (the ^1L_b transition, the $800\ cm^{-1}$ primary vibronic progression) with the weakest of these overlapping a Phe band, and one Trp band (^1L_b). The low intensity of the Tyr bands suggests that these residues are buried. The Trp contribution is positive for the A toxin but negative for the B and C toxins. Toxin B shows a positive intensity of $\sim 9000\ deg\ cm^2\ dmol^{-1}$ near 273 nm from a disulphide bond.[318]

α_2-Antiplasmin shows 16% α-helix, 18% β-structure, and 66% random coil. Near-u.v. studies showed that antiplasmin binds to the fragment of plasminogen consisting of the three *N*-terminal triple-loop structures and in so doing undergoes a conformational change.[319]

The agglutinin anti-A_{HP} from *Helix pomatia* shows a positive Cotton effect at 219.5 nm probably due to Trp. This effect increases in intensity subsequent to the addition of *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, raffinose, and melibiose, so allowing the determination of the affinity between the agglutinin and various ligands.[320]

The antibacterial peptides cecropins A and B from *Hyalophora cecropia* show largely random-coil conformations. However, in hydrophobic solvents cecropin A becomes largely helical. The B peptide becomes ~20% helical when incorporated into phosphatidyl choline-diacetyl liposomes. It is proposed that residues 1—11 form an amphipathic helix with charged residues on one side and the hydrophobic residues on the other.[321]

IgG complexes possessing rheumatoid factor activity (RF) have been found to have abnormal near-u.v. c.d. while the monomeric IgG presents a normal

[315] I. Kabasawa and E. Sakave, *Nippon Ganka Gakkai Zasshi*, 1982, **86**, 562.
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[318] L. Spero, *Biochim. Biophys. Acta*, 1981, **671**, 193.
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[320] I. Ishiyama, T. Yoshii, and A. Takatsu, *Dtsch. Gesundheitswes.*, 1981, **36**, 1600.
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spectrum. Far-u.v. data show that IgG found in RF complexes differs considerably from normal IgG either in the complex or after dissociation.[322]

Least-squares curve fitting of the c.d. spectrum of wool keratin *S*-carboxymethylated low-S protein (SCMKA) yields satisfactory results only when β-turn contributions are considered. In this case the protein appears to contain 52—54% α-helix and little β-structure. The α-content is not significantly altered by the addition of PrOH, although at high concentration SCMKA undergoes an α → random thermal transition at ~50 °C. The protecting groups have little effect on the protein conformation in water.[323]

A high-molecular-weight pea-leaf protein similar to the groE protein of *E. coli* has been reported to contain 47% α, 13% β, and 16% β-turn structure.[324] The seed globins from various *Cucurbita* species undergo considerable conformational change on raising the pH of solution from 6 to 10, consistent with an α-helix to random-coil transition.[325]

The binding of either Ca^{2+}, Mg^{2+}, or Ni^{2+} to a single site in yeast enolase leads to identical changes in the protein tertiary structure. Mg^{2+} appears to have no effect on secondary structure. Substrate binding to the enzyme nearly reverses the changes induced by the metal ions. Ni^{2+} and Co^{2+} appear to bind in octahedral or distorted-octahedral environments. The catalytic and inhibitory sites of the enzyme appear to have very little c.d. activity.[326]

Human parotid saliva proline-rich glycoprotein shows ~60% of polyproline II helix whether in the native form or as tryptic peptides or their deglycosylated homologues, in good agreement with prediction from data on amino-acid sequence.[327]

The effects of SDS on the conformation of lysozyme, the mitogens from *Phytolacca americana* (fractions Pa2 and Pa4), lectin from *Wisteria floribunda*, ovine lutropin, a Bence-Jones protein, and $histone_{H2B}$ have been investigated. Whereas the helical content of lysozyme is slightly raised by SDS, in the case of the Bence–Jones protein and lectin it increased from near zero to 25—30% and in the case of the histone to 48%. No helix formation was apparent in the mitogens or lutropin. Both hydrophobic and electrostatic interactions occur in reconstructive denaturation, and the newly formed helices are stabilized by hydrophobic shielding by the alkyl chains of the alkyl sulphate. The reorganization of secondary structure is favoured by (i) the presence of helicogenic amino-acids, (ii) the availability of positively charged side chains for interaction with the dodecyl ion, (iii) the absence of surplus surface negative charge, and (iv) the absence of extensive disulphide crosslinking.[328]

Phototoxicity in erythrocyte membranes has been studied by means of c.d. Illumination of erythrocyte ghosts in the presence of a photosensitizer leads to

[322] U. Alkner, M. Uesson, U. B. Hansson, and J. Glans, *Mol. Immunol.*, 1982, **19,** 21.
[323] T. Amiya, K. Kajiwara, T. Mujamoto, and H. Inagaki, *Int. J. Biol. Macromol.*, 1982, **4,** 165.
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[326] K. M. Collins and J. M. Brewer, *J. Inorg. Biochem.*, 1982, **17,** 15.
[327] J. P. Aubert, N. Porchet, A. Boersma, M. H. Loucheux-Lefebvre, and P. Degand, *Biochim. Biophys. Acta*, 1982, **704,** 361.
[328] B. Jirgensons, *J. Protein Chem.*, 1982, **1,** 71.

a 50% reduction in helical content of the membrane proteins over a period of 75 min.[329]

Structural and denaturation studies on cathepsin D from bovine spleen show marked similarities with pepsin, indicating the possible existence of a precursor of the protein or an endogenous inhibitor complex.[330] The u.v. c.d. of pinellin, a plant protein obtained from the rhisome *Pinellia*, shows an unusual single negative extremum at 226 nm and two positive peaks at 295 and 286 nm together with a negative peak at 248 nm. The addition of guanidine–HCl causes a decrease of ellipticity of these bands with a transition midpoint at 2.75M. Refolding of the protein followed at 226 nm is biphasic, the slow step having a first-order rate constant of 0.144 min^{-1}.[331]

Acid induces an isomerization and expansion of bovine plasma albumin identified by a decrease in the ellipticities monitored at 208, 222, 262, and 268 nm. In the initial part of the transition the ellipticity in the 295—300 nm region increases in a manner that exactly correlates with the increase of the rotational relaxation time of the Trp side chains.[332] The conformation of acid and related forms of human and bovine α-lactalbumins shows compact structures with secondary structure similar to that of native α-lactalbumin, although their tertiary structure shows a slow fluctuation.[333]

The bee-venom melittin shows a concentration-dependent pH transition from a largely random structure to a largely α-helical structure at high pH. The acetylated or succinylated protein shows 60% α-helix at neutral or low pH whilst the acylated protein forms more stable oligomers than the native protein.[334]

Stopped-flow measurements have shown that δ-chymotrypsin adopts a more helical conformation when mixed with SDS. The rate of change of conformation is strongly dependent on SDS concentration. In the region 5—12mM SDS the rate increases sharply. At >5mM SDS the native conformation is changed to another conformation without passing through the reaction intermediate observed at <5mM SDS.[335] The binding of rat plasma α_1-proteinase inhibitor ($\alpha_1 I_3$) to chymotrypsin produces structural changes in the inhibitor resulting from partial proteolysis. Binding occurs with a 1:1 stoicheiometry, although at higher concentrations of inhibitor a slow change of the c.d. spectrum is associated with partial proteolysis of free inhibitor. Addition of human $\alpha_1 I_3$ leads to the formation of an enzymically inactive ternary complex.[336]

Avian pancreatic polypeptide shows a strong rotational band in the near u.v. associated with paired Tyr-21 residues in the dimer. Analysis of the far-u.v.

[329] M. J. Ortner and C. S. Paschall, *Photobiochem. Photobiophys.*, 1982, **4**, 79.
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[331] Q. Xu and Z. Lu, *Shengwu Huaxue Yu Shengwu Wuli Xuebao*, 1981, **13**, 379.
[332] M. Sogami, S. Era, S. Nagaoka, and H. Inouye, *Int. J. Pept. Protein Res.*, 1982, **19**, 263.
[333] D. A. Dolgikh, R. I. Gilmanshin, E. V. Brazhnikov, V. E. Bychkova, G. V. Semisotnov, Y. S. Venyaminov, and O. B. Ptitsyn, *FEBS Lett.*, 1981, **136**, 311.
[334] J. Bello, H. R. Bello, and E. Granados, *Biochemistry*, 1982, **21**, 461.
[335] K. Takeda, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 1335.
[336] F. Esnar, F. Gauthier, and J. C. Maurizot, *Biochimie*, 1981, **63**, 767.

region indicates a predominance of α-helix and the presence in solution of significant β-structure not observed in the *X*-ray structure.[337]

A change in the secondary structure of *Propionibacterium shermanii* methylmalonyl-CoA transcarboxylase resulting from quaternary interactions was traced to interactions among the six polypeptides that make up the 12 S_H subunit. Association to form the hexameric 12 S_H subunit leads to an increase of 19% α-helix and a concomitant 8% decrease in antiparallel β-sheet and 7% in random coil in the secondary structure of the subunits.[338]

C.d. spectra have shown that both cytoplasmic and mitochondrial aspartate animotransferases form identical initial complexes with the coenzyme derivative 4′-*N*-(2,4-dinitro-5-fluorophenyl)pyridoxamine 5′-phosphate. Further reaction, however, leads to the irreversible labelling of the active site of the cytoplasmic enzyme and regeneration of the mitochondrial enzyme. By monitoring changes in the c.d. of the mitochondrial enzyme, initial kinetic characterization of the cleavage of the coenzyme to form pyridoxal 5′-phosphate was possible.[339]

Twenty-one individual proteins from the 30S subunit of *E. coli* ribosomes have been studied in the 190—240 nm region. Almost all the proteins (except S12, S17, S18, and S19) show a highly ordered secondary structure. 240—310 nm spectra indicate that the aromatic amino-acid side chains are fixed in the tertiary structure of the proteins. A number of characteristic bands in the far-u.v. region have been suggested as markers of native character for ribosomal proteins.[340]

The fast-refolding (U_F) and slow-refolding (U_S) forms of RNase A show essentially identical secondary structures. In the equilibrium mixtures of the two species, produced on long-term unfolding, it appears that both forms are completely unfolded with *cis–trans* isomerization about the proline peptide bonds being responsible for the formation of the U_S species. Differences in the ellipticity of the two forms may arise from local changes around Tyr residues caused by proline isomerization.[341]

The inner histone complex of chicken erythrocytes dissociates at pH 6 into $(H3–H4)_2$ tetramers and H2A–H2B dimers with little change in α-helical content as monitored at 222 nm. Below pH 4.0 the histones become partially unfolded, lose specific secondary and tertiary structure, and undergo non-specific aggregation.[342] In a separate study the c.d. characteristics of chromatosomes, nucleosome cores, and subnucleosomes have been measured. Analyses of histone contributions to the far-u.v. c.d. of subnucleosomes showed that H2A–H2B pairs and H3–H4 pairs in nucleosomes are nearly 50% α-helical and that their helix content is not dependent on nucleosome integrity.[343]

Changes in the molar ellipticity, at 220 nm, of bovine pancreatic DNase I, induced by the addition of guanidine–HCl, show a co-operative transition with

[337] W. Strassburger, U. Glatter, A. Wollmer, J. Fleischauer, D. A. Mercola, T. L. Blundell, I. Glover, J. E. Pitts, I. J. Tickle, and S. P. Wood, *FEBS Lett.*, 1982, **139,** 295.
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[339] D. Carotti, F. Riva, R. Santucci, F. Ascoli, and P. Fasella, *Eur. J. Biochem.*, 1982, **124,** 589.
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[342] A. P. Butler and D. E. Olins, *Biochim. Biophys. Acta*, 1982, **698,** 199.
[343] A. J. Mencke and R. L. Rill, *Biochemistry*, 1982, **21,** 4362.

a midpoint near 2M at temperatures ranging from 12 to 36 °C. 1mM Ca^{2+} appears to stabilize the protein slightly towards denaturation. The free energy for denaturation is 9.3 kcal mol^{-1} and 8.9 kcal mol^{-1} at 25 °C in the presence and absence of 1mM Ca^{2+}, respectively. The *N*-terminal half of DNase I appears β-rich while the *C*-terminal half is α-rich.[344]

The acidic ribosomal proteins eL12 of *Artemia salina*, BL9 of *Bacillus subtilis*, and MA1 of *Micrococcus lysodeikticus* are all dimers in solution with a high degree of ordered secondary structure and thermostability as judged by c.d. measurements.[345] The use of optical-activity measurements in the elucidation of structure–function relationships in muscle-protein systems has been discussed.[346]

Chromophoric Proteins. A number of papers have appeared concerned with the study of chlorophyll–protein complexes. Isolated protein–chlorophyll complexes retain their c.d. characteristics down to −250 °C but tend to show a blue shift and some loss of intensity. Photosystem (PS) I complex showed c.d. activity independent of which detergent was used in its preparation. Partial resolution of chloroplast fragments into contributions from PSI, antenna complexes, and PSII has been achieved.[347] A distinct c.d. band has been observed for chlorophyll *b* even at room temperature in the light-harvesting chlorophyll *a*/*b*–protein complex (LHC) isolated from *Vicia faba*. This feature indicates an asymmetric environment for chlorophyll *b* similar to that seen in whole chloroplasts. The chlorophyll *a* peaks at 673 (+) and 685 (−) nm seen at room temperature exhibit fine structure at cryogenic temperatures, with bands at 667 (+) and 675 (+) nm. Some differences in c.d. activity are seen in various LHC preparations, which are assumed to originate from various contributions of pigment–pigment and pigment–protein interactions and of Cotton-effect characteristics of pigments in cholesteric liquid-crystal-like structures.[348] Complexes of chlorophillide *a* and myoglobin with molecular weights of $>10^5$ show c.d. spectra that are highly red-shifted, non-conservative, and very intense and very similar to those of reaction-centre complexes from purple photosynthetic bacteria. At 710 nm the c.d. of one of these complexes has a higher rotational strength than any previously recorded for chlorophyll.[349]

Four chlorophyll–protein complexes (CP) have been isolated from chloroplasts using Deriphat 160. Complexes 2 and 3 show a c.d. pattern identical to that previously reported for CP2. Complexes 2, 3, and 4 all show a c.d. component of a split-exciton type with extrema at 660 (−) and 680 (+) nm together with evidence of disorganized chlorophyll. A barley mutant lacking chlorophyll *b* showed only complexes 1 and 4. In this case complex 4 showed a c.d. spectrum

[344] N. Okabe, E. Fujita, and K. Tomita, *Biochim. Biophys. Acta*, 1982, **700**, 165.
[345] A. T. Gudkov, S. Y. Venyaminov, R. Amons, W. Moeller, and T. Itoh, *FEBS Lett.*, 1981, **136**, 235.
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[349] R. M. Pearlstein, R. C. Davis, and S. L. Ditson, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79**, 400.

of a much less disorganized chlorophyll site, which could be generated by subtracting the c.d. of CP1 from that of photoactive mutant chloroplast fragments or the c.d. of complexes CP1 and CP2 from pea chloroplast fragments. Deriphat appears to preserve, at least partially, a new type of chlorophyll–protein complex.[350]

The photoinduced c.d. spectrum of P700 of chlorophyll–albumin complexes shows bands at 689 and 700 nm, previously described, together with a negative band at 465 nm and a positive band at 715—800 nm assigned to the P700 radical cation. The values of the exciton splitting of the P700 dimer for the Qy- and Bx-, By-transitions and the spectral shifts of the band centres are typical of chlorophyll aggregated.[351]

C.d. difference spectroscopy has been used successfully to identify the tight complex formation between iron–sulphur proteins and flavoproteins from spinach chloroplast and beef adrenal cortex electron-transfer systems.[352]

The u.v. and low-temperature c.d. have been recorded for rhodopsin and squid lumirhodopsin solubilized in 50mM acetyl glucoside.[353,354]

Uteroferrin exhibits two conservative visible c.d. bands indicative of exciton splitting of a tyrosinate-to-Fe charge-transfer band. Additional features in the near u.v. may result from Fe co-ordination to other Tyr residues. Changes in the near-u.v. and visible c.d. between pink and purple forms of uteroferrin, subsequent to mercaptoethanol reduction, are consistent with a proposed reorientation of Tyr residues ligated to iron. Reduction does not lead to any significant changes in the aromatic region, suggesting that the un-co-ordinated Tyr and Trp reside in a stable environment. Conversion from the purple to the pink form by mercaptoethanol probably results from S—S cleavage in the vicinity of the Fe site accompanied by a 40% decrease in the protein's overall α-helical content.[355]

Bands in the near and far u.v. together with those at >300 nm have been detailed and correlated with specific structural organizations of haemoglobin.[356]

Hormones.—Studies on [Ala^{B24}, Thr^{B30}], [Ala^{B25}, Thr^{B30}], and [Ala^{B26}, Thr^{B30}] bovine insulins and the corresponding Ala → Leu analogues showed no apparent correlation between decreased biological activity and conformational change in solution.[357] The conformations of insulin, des-B-chain *C*-terminal pentapeptide, des-B-chain *C*-terminal octapeptide, and chymotryptic fragment I peptide show similar backbone structure. The analogues contain an α-helix,

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[351] V. V. Shubin, T. V. Epimovskaya, and N. V. Karapetyan, *Zh. Fiz. Khim.*, 1981, **55,** 2916.
[352] H. Hasumi, S. Nakamura, K. Kunimasa, H. Yoshizumi, J. H. Parcells, and T. Kimura, *J. Biochem.* (*Tokyo*), 1982, **91,** 135.
[353] T. Yoshvzawa and Y. Schichida, *Methods Enzymol.*, 1982, **81,** 634.
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[355] B. C. Antanaitis, T. Strekas, and P. Aisen, *J. Biol. Chem.*, 1982, **257,** 3766.
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but in a more relaxed form than the native molecule, and a structure embedded in the I peptide contributes to the spectrum of insulin.[358]

The conformation of Boc-Aib-Gly-Phe-Met-NH_2 (Aib = $NHCMe_2CO$), a Met-enkephalin analogue, has been found to be a β-turn with Aib-Gly as the corner residues. The Aib-Aib and Gly-Aib analogues have incipient 3_{10} helical structures. The Gly-Gly and Gly-Phe forms of enkephalin have energetically low β-turn conformation leading to solvent-dependent conformational transitions.[359]

Lutropin undergoes a thermal conformational transition accompanied by dissociation into biologically inactive subunits. Organic solvents such as 1,4-dioxane, dimethyl sulphoxide, formamide, and 2,2,2-trifluoroethanol all accelerate the thermal transition, which shows multi-phasic kinetics.[360]

Anionic detergents such as SDS, phosphatidyl glycerol, and L-α-phosphatidic acid induce helix formation in glucagon, secretin, and vasoactive intestinal peptide. Helix formation is maximal in the receptor-binding section of the proteins, namely residues 13—20.[361]

Substitution at position 5 in angiotensin with Val, Leu, Ala, or Gly alters the interaction between Tyr^4 and His^6. This in turn increases the kink in the peptide backbone at this site. These findings have been related to previously measured biological activities of these analogues.[362]

The diastereoisomeric oxytocin analogues [1-*hemi*-D-Cys]-, [2-D-Tyr]-, and [5-D-Asp]-oxytocin have overall backbone conformations similar to that of oxytocin itself but different side-chain orientations. In contrast the 1-penicillamine analogues [1-penicillamine-4Thr]- and [1-penicillamine-2Phe,4Thr]-oxytocin have backbone and disulphide conformations that are different from those of oxytocin. The potency of the analogues has been related to side-chain orientation.[363] In aqueous solution synthetic, non-glycosylated human choriogonadotropin carbonyl terminal peptides of the β-subunit show no evidence for the formation of α- or β-structure but in 90% trifluoroethanol show some β-turns. Disulphide-linked dimers show evidence of stabilization of particular skewness of the disulphide dihedral angle, depending on the location of the S—S bond. The Phe^{115} of the β-subunit contributes to the c.d. activity above 250 nm.[364]

Pig relaxin and insulin show similar c.d. spectra, although the separated chains of insulin and relaxin show significant differences. The *S*-sulpho forms of insulin A- and B-chains and *S*-sulpho relaxin A-chain appear largely unordered, whereas the *S*-sulfo relaxin B-chain has >90% β-structure. The relaxin B-chains become largely unordered when cleaved from the six *C*-terminal residues. Significant interactions and conformational changes are

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[361] R. M. Robinson, E. W. Blakeney, and W. L. Mattice, *Biopolymers*, 1982, **21**, 1217.
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[363] V. J. Hruby, H. I. Mosberg, J. W. Fox, and A. T. Tu, *J. Biol. Chem.*, 1982, **367**, 4916.
[364] D. Puett, R. J. Ryan, and V. C. Stevens, *Int. J. Pept. Protein Res.*, 1982, **19**, 506.

observed between oxidized A- and B-chains of insulin and relaxin. Recombination of native relaxin peptides is dependent on whether the reduced chains are separated or not prior to reoxidation.[365] Evidence has been obtained that mouse prolactin may undergo modification during secretion. The overall conformation of prolactin is similar in both the storage and secreted forms. However, whereas the storage form exhibits c.d. activity associated with its two Trp residues, the secreted form shows no Trp c.d.[366]

Phosphatidylcholine induces partial helix formation in human gastrin I at neutral pH but phosphatidylserine does not, unless the Glu residues are protonated. Reduced somatostatin and substance P are partially helical in phosphatidylserine but not in phosphatidylcholine. Both lipids induce helix formation in glucagon. The polypeptides thus form helices only when uncharged or of opposite charge to the lipid polar head group. 5-Ile-angiotensin I and synthetic δ-sleep-inducing peptide remain unordered in lipid solution.[367] β-Lipotropin, β-MSH, and corticotropin are in left-handed helical conformations of the poly-L-Pro type in aqueous solution. The reversible melting of this conformation is non-co-operative. The activity of these hormones has been correlated with the conformation of separate fragments of the protein chain.[368]

Added Extrinsic Chromophores.—The effect of lipids on the novel c.d. activity of lutein–ovalbumin complexes has been studied. On a molar basis the effect of lipids was in the order oleate > linoleate > lecithin with laurate having no effect at all. A wide range of suggestions have been made to account for these observations.[369] Although no appreciable change in secondary structure was observed when riboflavin bound to riboflavin-binding protein at neutral pH, marked changes in the aromatic region were observed at pH 3.7. It is suggested that riboflavin binds to the protein in an aromatic-rich cleft.[370] Flavoproteins have been modelled by FMN binding to poly(L-Lys) and poly(L-His). Binding in the range pH 3—11 suggests that FMN interacts with the proteins *via* hydrophobic as well as ionic bonding and is further influenced by the involvement of the ribityl side chain of FMN. Small changes in the environmental conditions of the interacting molecules modify their mode of interaction considerably.[371]

Complex formation in a 1 : 1 mixture of NADPH-adrenodoxin reductase and adrenodoxin resulted in the appearance of a distinct difference c.d. spectrum, with a broad trough at 500—600 nm and extrema at 452, 383, and 350 nm. Monitoring binding at 452 nm allowed an estimation of the dissociation constant for the complex of 4.56×10^{-7}M. The addition of sodium chloride to the complex in solution produced changes not attributable to simple dissociation of the complex.[372] The binding constants of egg-white lysozyme with

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[372] H. Sakamoto, Y. Ichikawa, T. Yamano, and T. Takagi, *J. Biochem.* (Tokyo), 1981, **90**, 1445.

sulphonic acid-containing phenylaronaphthol dyes have been measured over a range of pH and ionic strength. The c.d. of the dyes changes owing to electronic perturbations of Trp when they bind to the protein probably at Lys[33].[373] C.d. data suggest that Acridine Orange binds to the acid α-helical form of poly(α-L-Glu) in such a manner that the long molecular axes of the dye form a right-handed super-helix around the peptide-core α-helix. No such complex formation is possible at pH > 7.[374]

Nuclear Proteins.—Cleavage of polypeptide fragments from Sendai virus ribonucleoprotein has been found to cause alterations in the protein secondary structure but to leave the morphology unaffected.[375] Although structural predictions of the structure of protamines from various species suggest a common globular character, which reconciles some of the existing models with some experimental data, incompatible results have still been obtained from c.d., *X*-ray diffraction, and electron microscopy.[376]

4 Magnetic Circular Dichroism

Contributed by T. Brittain

Although of wide-ranging application over the past year the literature covering the use of m.c.d. has been overwhelmingly dominated by studies on iron-containing proteins in general and haem proteins in particular.

General and Model Systems.—An advanced magneto-optical technique that allows simultaneous detection of Kerr rotation and reflectance m.c.d. using a piezobirefringent modulator has been described. The wavelength region covered can be extended from 300 to 2500 nm.[377] Both theoretical MO calculations and experimental observations have been combined to produce a general theory describing the m.c.d. band sign patterns for haem derivatives. The band patterns for substituted and unsubstituted haems and chlorins have been explained in terms of symmetry effects on the orbital-energy differences between the two highest occupied and the two lowest unoccupied molecular orbitals of the porphyrins and chlorins involved. This work holds the possibility of identifying the nature and symmetry of haem and chlorin sites in hitherto unidentified metallo proteins.[378-380] Comparison of the m.c.d. spectra of the O_2 complex of mercaptide haem with those obtained for cytochrome P450 reveals

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thiolate co-ordination in the reduced high-spin native protein and its oxygenated forms.[381] Spectra have been recorded for catalase and its model porphyrin compounds, and the co-ordination number of deuteriohaemin bisimidazole has been determined for the high- and low-spin forms.[382,383]

Charge-transfer bands at 230 nm of varying intensity have been identified in Pd^{II}–thioether peptide complexes. The Faraday effect for methionyl-containing peptides appears to follow a simple sum rule for the contributions of individual Pd–Met complexes.[384] Further studies have shown that the m.c.d. activity of the Pd–thioether complexes falls into two classes dependent on the nature of the chemical structure of the ligand. It is suggested that Pd may complex with a thioether inside a linear moiety, as is the case for L-Met-containing peptides, or else in an intracyclic fashion. The intensity of the charge-transfer bands associated with each of these types of complex is well correlated with their retention times on h.p.l.c.[385]

Proteins.—Cytochrome *c* oxidase has been the protein of interest in a number of studies. It has been shown that the cytochrome *a* site of the enzyme may be well modelled, in m.c.d. terms at least, in both oxidation states by the bis-1-methylimidazole complex of haem *a*. The 1,2-dimethylimidazole complex of haem *a* appears to be a good model for the oxygen-reducing cytochrome a_3 site of the enzyme.[386] The removal of copper from cytochrome *c* oxidase either by cyanide or bathocuproine shifts the 420 nm m.c.d. band of the oxidized form of the protein to lower wavelength whilst increasing the amplitude of the spectrum. In the reduced form the normal m.c.d. peak at 446 nm is red shifted and substantially decreased in amplitude. These m.c.d. observations of conformational change are supported by both c.d. and e.p.r. measurements on the reduced NO complex, which suggest that copper depletion leads to a rearrangement of the axial ligands to Fe in cytochrome a_3. These results emphasize the close structural association of Cu and Fe in this enzyme.[387] Ultra-low-temperature m.c.d. of the photolysed products of the CO complexes of haemoglobin, myoglobin, and cytochrome *c* oxidase has been reported. Low-temperature m.c.d. shows that all of the haem proteins contain high-spin ($S = 2$) iron in the deoxy form, which becomes low spin on combination with CO. On photolysis of the CO complexes, at low temperature, new unligated species are formed. In the cases of haemoglobin and myoglobin the new species differ considerably in their m.c.d. activity from the normal deoxy species. In the case of cytochrome oxidase, however, the photolysed species is indistinguishable from the unliganded species and moreover is indistinguishable from the normal deoxymyoglobin spectrum. This

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finding is attributed to the absence of any significant structural change in the cytochrome oxidase molecule when CO becomes bound to the haem a_3 iron.[388]

The near-i.r. m.c.d. of myeloperoxidase shows a band at 1000 nm similar to that seen in other high-spin haem proteins. On addition of CN^- the band moves to 1500 nm, a position typical of low-spin haem. Measurements in the 350—700 nm region strongly suggest that the haem in this protein is closely related to a ring of the chlorin type.[389]

Investigation of the absorption e.p.r. and m.c.d. characteristics of a range of cytochrome P450–CAM-ligand complexes, utilizing all of the possible protein ligands, has led to the identification of the natural ligand *trans* to the cysteinate in the native enzyme. All the evidence indicates an O atom donor ligand, most probably an alcohol or amide.[390] A 23% decrease in the intensity of the Soret-band m.c.d. of cytochrome P450 LM2 was observed when it was reconstituted with NADPH cytochrome P450 reductase in phospholipid vesicles. A further decrease of 7% was observed when cytochrome b_5 was subsequently added. These findings confirm that protein–protein association occurs between these partners in membrane-type systems. These observations have been combined with electron-flow studies in a proposed mechanism of coupled reversible association reactions in the native membrane.[391]

Low-temperature m.c.d. of the reduced Mo–Fe (molybdoferredoxin) protein of nitrogenase from *Klebsiela pneumoniae* is dominated by its paramagnetic Mo–Fe centre. Oxidation of the protein reveals the presence of the Fe–S clusters, and the two types of centre may be distinguished by their distinct paramagnetic behaviour. The m.c.d. spectra and magnetization curves should provide good criteria for assessing the validity of models for these complex metal centres present in nitrogenase.[392] The m.c.d. characteristics of the iron–sulphur centres of proteins have been reviewed and discussed.[393]

Theoretical calculations and experimental observations have shown the electronic effects of the binding of diatomic molecules such as CO, NO, and O_2 on the m.c.d. characteristics of the haems of haemoglobin, its α- and β-chains, and myoglobin.[394] Using the predictions of Keegan *et al.*[379] it has been possible through the use of m.c.d. band patterns to substantiate the previous suggestion that sulphaemoglobin contains as its iron-binding centre a pseudochlorin.[395]

The m.c.d. spectra of hepatic Bi–Zn–metallothioneins are dominated by transitions from the Zn–thiolate centre. Renal Bi–Cu–metallothionein 2 on the other hand exhibits spectra resulting from the mixing of two contributions from the Bi– and Cu–thiolate-binding sites, respectively.[396]

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5 Infrared and Raman Spectroscopy

Contributed by R. M. Stephens

Model Compounds.—Laser Raman spectroscopy has been used to investigate the amide frequencies of three peptides known to contain β-turn structure. These studies concluded that the amide I band for type III β-turn structure appears in the range 1665—1667 cm^{-1} and the amide III band at 1265—1286 cm^{-1}.[397] Conformational studies of poly-α-L-aspartic acid in which β-bands appeared to be absent showed that a precipitated form, induced by changes in the degree of ionization, was almost fully helical.[398] The solution Raman spectra of H-$(Ala)_3$-OH (I), H-Ala-D-Ala-Ala-OH (II), and H-$(Ala)_2$-D-Ala-OH (III) were very similar, indicating that these three diastereomeric alanyl tripeptides had similar conformations in solution. The solid-phase spectrum of (I) indicated an antiparallel β-pleated sheet structure, that of (II) an α-helical structure, and that of (III) a random non-planar structure. The different structures of (I) and (III) were attributed to different lattice energies caused by different packing of the molecules in the crystal.[399] A solvent and concentration study of three *cis*-lactams in the near-i.r. region resulted in partial revision of amide-group combination band assignments for δ-valerolactam and ε-caprolactam, permitting differentiation between the strained-ring and larger-ring *cis*-lactams. The near-i.r. spectra of the lactams were characterized by overtone and combination bands attributable to the non-H-bonded amide grouping. Conditions favouring interamide association resulted in the disappearance of bands without concomitant replacement by distinctive bands assignable to H-bonded molecules. The spectral behaviour of *trans* open-chain secondary amides, *e.g.* AcNHMe and BzNHMe, showed clearly defined contributions from $2\nu_{NH}$ and (ν_{NH} + amide II) plus other combination modes assignable to H-bonded molecules.[400]

Model Calculations.—The normal modes of various isotopic species of polyglycine I in the antiparallel-chain rippled-sheet structure were calculated as a test of the force field for the parent polypeptide. Small modifications made to a previous force field resulted in a general improvement of predicted frequencies, particularly of CH_2 wag and CH_2 twist modes, and a very satisfactory accounting of the i.r. bands of the isotopic molecules.[401] The i.r. spectra of *N*-deuteriated β-poly-(L-alanine) were recorded and used, together with an improved transferable force field for polyglycine I, to make a significant improvement in the force field and band assignments of β-poly-(L-alanine). Assignment of the unperturbed N—D stretch frequency confirmed previous analyses, indicating that the H bond in this antiparallel-chain pleated-sheet structure is stronger than that in the antiparallel-chain rippled-sheet

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[401] A. M. Dwivedi and S. Krimm, *Macromolecules*, 1982, **15**, 177.

structure of polyglycine I.[402] The complete vibrational frequency assignments for alanine, alanine-*C*-d_1, alanine-*C*-d_3, alanine-*C*-d_4, and alanine-*N*-d_3 in aqueous solutions have been reported. The assignments are based primarily on solution-phase Raman spectra using results from i.r. and Raman solid-phase spectra, a Urey–Bradley normal-co-ordinate analysis and data from existing literature also being taken into account. The new spectra have led to a self-consistent assignment. With the exception of a few modes where skeletal stretching and rocking motions are strongly mixed, the observed spectra may be interpreted in terms of group frequencies and predominantly local motions. In this respect these results differ from previous investigations, which postulate extensive vibrational coupling between all parts of the molecule.[403]

Proteins.—*Albumin.* The Raman spectra of complexes of bovine serum albumin (BSA) and of detergents such as SDS and tetradecyltrimethylammonium bromide (TTAB) revealed that the population of the *gauche*, *gauche*, and *trans* conformation about the C—S—S—C group increased with an increase in the concentration of both detergents. When *SH*-blocked BSA was complexed with TTAB, a skeletal vibration at 540 cm^{-1} was assigned to the *trans*, *gauche*, and *trans* conformation about the C—S—S—C group. The local environment change of tyrosine residues was also observed upon complexing.[404] I.r. spectra of BSA during hydration were measured between 4000 and 1000 cm^{-1} at 10—90% relative humidities. Hydration changes were reflected in changes in the amide A, B, I, and II bands. At low humidities the A and B bands of BSA were at 3300 and 3067 cm^{-1}, respectively, and they both shifted by about 5 cm^{-1} to a higher frequency at 90% relative humidity. As expected, the absorption intensity of these two bands also increased with increased hydration. The amide I band at 1665 cm^{-1} did not shift on hydration changes but broadened with the appearance of a shoulder at 1635 cm^{-1} at 90% relative humidity. However, the amide II band occurred at 1539 and 1598 cm^{-1} for low and high humidities, respectively.[405] Fourier-transform (FT) i.r. spectroscopy has been applied to the study of protein interactions with surfaces. In addition the various spectroscopic techniques and methods used to interpret absorbed protein spectra were discussed. These included transmission and attenuated total-reflection (ATR) experiments with aqueous solutions of both single proteins and protein mixtures. Also included are spectral subfractions, spectral derivation, and spectral deconvolution. Use of these techniques showed that in transmission studies of albumins a conformation change occurred when the protein concentration reached ~3% (wt./vol.) in saline. Spectral evidence also indicated the possibility of H-bonded polymerization of albumin as concentration increased. Studies of albumin–fibrinogen mixtures allowed to

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[404] K. Aoki, H. Okabayashi, S. Maezawa, T. Mizuno, M. Murata, and K. Hiramatsu, *Biochim. Biophys. Acta*, 1982, **703**, 11.
[405] Z. Zhang and S. Li, *Shengwu Huaxue Yu Shengwu Wuli Jinzhan*, 1982, **44**, 33.

adsorb completely onto a surface illustrated that albumin adsorbed first, which was followed by fibrinogen adsorption and displacement of albumin.[406]

Blood Proteins. Adsorption and desorption kinetics of whole blood proteins on various surfaces such as germanium have been followed using FT i.r. The slopes of the desorption curves were reproducible, and high time resolution allows subtle changes to be followed in the adsorbed film as it undergoes desorption. One of the major advantages of the method is the confirmation of transient features.[407] An *ex vivo* FT i.r./ATR experiment to study blood-protein adsorption involved the use of live dogs in which blood was pumped through an arterial–venous shunt to the ATR cell and back into the animal. The results of these live-dog experiments were compared to results obtained using donated whole blood. The experiments demonstrated that FT i.r. can be used to study aqueous physiological flowing solutions in real time with the sensitivity necessary to detect minor changes.[408] Differences in the protein conformation and composition in human platelet membrane compared with those in human erythrocyte membrane have been revealed using i.r. and laser Raman spectroscopy. It was suggested that fibrinogen and not albumin was responsible for some of the differences in the protein composition of the two membranes.[409]

Cytochromes. An intermediate redox state O-cytochrome *c* at alkaline pH, generated on rapid reduction by sodium dithionite, has been observed by resonance Raman (RR) spectroscopy in combination with the continuous-flow technique. The RR spectrum of the intermediate state is reported for excitation in both the (α, β) and the Soret optical-absorption band. The spectra of the intermediate state are more like those of the stable reduced form rather than those of the stable oxidized form. For excitation at 514.5 nm, the most prominent indication of an intermediate state is the frequency shift from 1562 cm^{-1} in the stable oxidized state through 1535 cm^{-1} in the intermediate state to 1544 cm^{-1} in the stable reduced state. The intermediate species is interpreted as the state in which the haem Fe is reduced but the protein remains in the oxidized-state conformation with methionine-80 displaced as sixth ligand to the haem Fe, before relaxing to the conformation of the stable reduced state with methionine-80 returned as sixth ligand.[410] The oxygenated and carbonyl species of cytochrome *o* from *Vitreoscilla* have been investigated using i.r. spectroscopy. The first evidence of oxygen bound to a terminal oxidase (ν_{O-O} at 1134 cm^{-1}) was presented, a finding consistent with either oxygenyl or O_2^- bonding. The carbonyl species exhibits a C—O band at 1964 cm^{-1} and an unusual sensitivity of band intensity to temperature. These i.r. parameters indicate binding features for cytochrome *o* that are different

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[407] M. R. Gendreau, *Appl. Spectrosc.*, 1982, **36,** 47.
[408] R. J. Jakobsen, S. Winters, and R. M. Gendreau, *Proc. SPIE-Int. Soc. Opt. Eng.*, 1981, **289,** 469.
[409] R. Cataliotti, P. Gresele, P. Speranzini, G. Paliani, and G. G. Nenci, *Stud. Biophys.*, 1982, **88,** 159.
[410] M. Forster, R. E. Hester, B. Cartling, and R. Wilbrandt, *Biophys. J.*, 1982, **38,** 111.

from those of other haemoproteins.[411] RR spectra are reported for reduced horse-heart cytochrome *c* at pH 7—13.6 as well as for the pH 13.6 derivatives of reduced cytochrome *c* with CN^- and CO, using dye-laser excitation at 550 nm, the peak of the *Q*(O—O) visible absorption for reduced cytochrome *c*. In marked contrast to the similarity of the visible absorption spectra, the RR spectra at pH 7 and 13.6 are dramatically different in the low-frequency region below $\sim 500\ cm^{-1}$. Above pH 12 the very detailed low-frequency spectrum of reduced cytochrome *c* is replaced by one with very few bands. The low-frequency spectrum at pH 7 may be caused by Raman scattering of fundamental vibrations that involve motion of the pyrrole substituents and that are intensified by symmetry lowering caused by the asymmetric haem-substituent substitution pattern. This asymmetry is unusually effective in this case because of the tight binding of the protein and covalent attachment of the haem of thioether links to cysteine residues. This situation no longer exists above pH 13, where the protein structure is more relaxed and the symmetry of the haem is effectively higher.[412]

Dynamic interactions of carbon monoxide with a_3 Fe and Cu_B in cytochrome *c* oxidase in beef-heart mitochondria at temperatures between 10 and 280 K have been observed using FT i.r. spectroscopy. CO bound to cytochrome a_3 Fe absorbs near $1963\ cm^{-1}$ with minor bands at lower frequencies. Photolysis at low temperatures transfers CO to Cu_B, with the major component near $2062\ cm^{-1}$ and a minor one at $2043\ cm^{-1}$. Vibrational assignments are made by comparison with haem and Cu carbonyls, by frequency dependence of all bands on the isotopic mass of CO and by similar behaviour of major and minor components with photolysis and relaxation kinetics as a function of temperature. Reformation of a_3 FeCO after photolysis is an apparent first-order process at temperatures <210 K with a distribution of rate constants. The kinetics are well described by a power law, and Arrhenius behaviour is followed between 140 and 180 K. The major component of a_3 FeCO shows a narrow absorption band ($\Delta\nu = 2.4\ cm^{-1}$) whereas that of Cu_BCO shows a broader absorption band ($\Delta\nu = 6\ cm^{-1}$). These data indicate that in the reduced CO complex a_3 FeCO is in highly ordered non-polar surroundings sufficiently separated from Cu_B not to be perturbed by the motion of the latter, whereas Cu_BCO is in less ordered, more flexible surroundings.[413] The RR spectra of *P. aeruginosa* cytochrome oxidase (I) are reported for oxidized and reduced (I) and for reduced (I) after addition of CN^-. The spectra of reduced (I) obtained at 413.1 and 457.9 nm excitation are attributed to the haem *c* and *d* entities, respectively. The haem *d* spectrum indicates reduced chromophore symmetry and compares with those of Cu^{2+} chlorin and isochlorin model compounds. The spectrum is altered significantly by the presence of CN^-, which binds to the haem *d* moiety and converts the five-co-ordination of the imidazole ligand to a six-co-ordinate low-spin configuration. The interpretation of CN^--induced changes is difficult. No large electronic interactions between haems *c* and *d* are expected as CN^- binding does not affect the haem *c* spectrum of reduced (I).

[411] M. G. Choc, D. A. Webster, and W. S. Caughey, *J. Biol. Chem.*, 1982, **257**, 865.
[412] W. G. Valance and T. C. Strekas, *J. Phys. Chem.*, 1982, **86**, 1804.
[413] F. G. Fiamingo, R. A. Altschuld, P. P. Moh, and J. O. Alben, *J. Biol. Chem.*, 1982, **257**, 1639.

The spectrum of oxidized (I) obtained with 457.9 nm excitation shows Raman action modes comparable in number to those of reduced (I) but with changes in both position and intensity. In the oxidized enzyme the group giving rise to the mode at 1726 cm^{-1} interacts strongly with the chlorin electronic system, as in the case of reduced (I).[414]

Enzymes. Two reaction intermediates of D-amino-acid oxidase with substrate analogues have been studied using RR techniques. The intermediates studied were in the aerobic oxidative reaction of the enzyme with β-cyano-D-alanine (I) and the other in the reverse reductive reaction of the enzyme with chloropyruvate and NH_4^+. Both intermediates are characterized with the charge-transfer absorption bands in the long-wavelength region extending beyond 600 nm. The RR spectra of the two intermediates excited at 488.0 and 514.5 nm were those of oxidized flavin (FAD) consistent with the previous assumption that FAD is involved with these reaction intermediates. Relatively simple RR spectra were obtained for those intermediates with excitation at 632.8 nm, which is within the region of the charge-transfer bands. The charge-transfer interaction probably involves the N-5–C-4a region extending to the C-10a–C-1–C-2 region of isoalloxazine nucleus. The Raman line at 1657 cm^{-1} for the intermediate with chloropyruvate and NH_4^+ was assigned to C=N of an imino-acid from the isotopic frequency shift of ^{15}N substitution. This assignment involves an imino-acid, α-imino-β-chloropropionate.[415]

Gramicidin. Various *N*-deuteriated gramicidin S analogues have been synthesized, and i.r. spectroscopy has been used to examine residue-specific peptide-group vibrations. There are two pairs of equivalent intramolecular hydrogen bonds involving Leu-NH and Val-NH. Hydrogen bonding accounts entirely for the apparent kinetic barrier to proton exchange for Leu-NH, while for Val-NH <30% is of steric origin. Fermi resonance was observed in the amide A′ band, and ν_{ND} (Val) exhibits the stronger coupling to the deformation modes. These results represent the first selective characterization of intramolecular H bonds in a heteropolypeptide in solution.[416]

Haemoglobin. The Fe-histidine stretching mode in deoxyhaemoglobin displays a large change in frequency and width upon lowering the temperature from 300 to 10 K. The temperature dependence of the data indicates the presence of dynamic processes. The dynamics of this mode in frozen haemoglobin (Hb) can be qualitatively and quantitatively described as a vibrational dephasing *via* anharmonic coupling to other vibrations of the haem–imidazole system.[417] Quaternary-structure-induced differences in the RR spectra of the haem in both the high- and low-frequency regions were detected in a variety of

[414] Y. Ching, M. R. Ondrias, D. L. Rousseau, B. B. Muhoberac, and D. C. Wharton, *FEBS Lett.*, 1982, **138**, 239.
[415] R. Miura, Y. Nishina, K. Shiga, H. Tojo, H. Watari, Y. Miyake, and T. Tamano, *J. Biochem.* (*Tokyo*), 1982, **91**, 837.
[416] E. M. Krauss and S. I. Chan, *J. Am. Chem. Soc.*, 1982, **104**, 1824.
[417] M. R. Ondrias, D. L. Rousseau, and S. R. Simon, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79**, 1511.

human Hb. These differences may be the result of (i) changes in the amino-acid sequence, induced by genetic and chemical modifications, and (ii) alterations in the quaternary structure. For samples in solution in buffer of low ionic strength, differences in the 1357 cm^{-1} line correlated with differences in the 216 cm^{-1} line (the Fe–histidine stretching mode). For samples in solutions containing inorganic phosphate and for frozen samples this correlation is not found. Thus, changes in the Fe–histidine bond do not necessarily result in an alteration of the electronic structure of the porphyrin. The quaternary-structure-induced changes in the vibrational modes associated with the haem group demonstrate that there is extensive communication between the haem and the globin, which is relevant to the models for the energetics of co-operativity.[418] Raman studies of the Fe–histidine stretching mode in transient species of carp ligand-free Hb (deoxyHb) and oxyHb, generated by photolysis at 10 ns of carbonylHb under constant oxygen flow, indicated that ligand binding caused a change in the Fe–histidine stretching mode in the photolysed species relative to that of the corresponding deoxy species. For both the deoxy and photolysed species of Hb the R state had higher frequencies than the T state. These frequencies for deoxyHb-T, photolysed Hb-T, deoxyHb-R, and photolysed Hb-R were 214, 215, 217, and 225 cm^{-1}, respectively. The haem proximal environment is very nearly identical for O_2- and CO-bound Hb. This structure-sensitive degree of freedom, which is qualitatively similar to that observed with human HbA, was related to the kinetic constants that contribute to co-operativity by comparing yields of geminate recombination.[419]

Picosecond time-resolved RR spectroscopy was used to study the intermediate product in the photodeligation of human oxyHb. The picosecond difference spectrum between oxyHb and the photolysed intermediate showed resonance maxima at 1540 and 1590 cm^{-1}, which is 10—15 cm^{-1} lower than those observed for deoxyHb. The band at 1590 cm^{-1} was depolarized, whereas the band at ~1540 cm^{-1} consisted of two components, a depolarized one at 1538 cm^{-1} and an anomalously polarized one at 1550 cm^{-1}. The 1590 and 1538 cm^{-1} bands may come from the ν_{10} and ν_{11} porphyrin modes of an electronically excited high-spin haem. The possibility that the photolysis intermediate of oxyHb observed is the electronically excited deoxyHb with substantial π–π^* character is discussed.[420] Raman intensity measurements for the Fe—O_2 stretching band of oxyHb were used to construct an excitation profile, which shows that resonance enhancement occurs mainly *via* the B and O transitions; no contribution was detectable from an out-of-plane charge-transfer transition. Direct coupling of the νFe—O_2 to the porphyrin π–π^* transitions was explained on the basis of competition between the π^* orbitals of porphyrin and O_2 for Fe *d*-electrons. The RR spectrum of nitrosylmyoglobin at pH 8.4 was due solely to six-co-ordinate haem–NO, but lowering the pH to 5.8 converted the RR spectrum to one characteristic of five-co-ordinate haem–NO, consistent with Fe–imidazole dissociation *via* protonation. The Fe—NO

[418] M. R. Ondrias, D. L. Rousseau, J. A. Shelnutt, and S. R. Simon, *Biochemistry*, 1982, **21**, 3428.
[419] J. M. Friedman, R. A. Stepnoski, and R. W. Noble, *FEBS Lett.*, 1982, **146**, 278.
[420] J. Terner, T. G. Spiro, D. F. Voss, C. Paddock, and R. B. Miles, *Springer Ser. Chem. Phys.*, 1982, **23**, 327.

stretching frequencies were as expected at 553 and 596 cm^{-1} for the high- and low-pH forms, but the low-pH form showed an additional ^{15}NO-sensitive band at 573 cm^{-1}, which was assigned to Fe—N—O bending in the five-co-ordinate complex. The RR spectrum of oxymyoglobin showed a shoulder at ~270 cm^{-1}, which shifted down by ~3 cm^{-1} upon $^{18}O_2$ substitution, and it was suggested to contain the Fe–imidazole stretching mode.[421] Measurements of the depolarization ratio (DPR) of the four resonant Raman lines at 1375, 1506, 1583, and 1638 cm^{-1} were made on human oxyHb for all exciting wavelengths of an argon iron laser. There was a significant variation in the DPR in the wavelength region between the Soret and β-bands. The dispersion curves have a different shape for the four Raman lines. Measurements of the DPR at three pH values (6.3, 7.4, and 8.0) and two Hb concentrations (1.2×10^{-4}M and 5×10^{-5}M) show that the concentration and pH value change the dispersion curve in a similar way. The DPR of the 1375-Raman line at 496.5 nm excitation wavelength was measured for different concentrations in the range 4×10^{-4} to 4×10^{-5}M. With decreasing concentration the DPR increased. This effect is caused by the dimerization of the tetrameric oxyHb molecule. The dispersion curves are interpreted in terms of mode mixing of the molecular vibrations of the haem group due to symmetry lowering of its environment. This symmetry change is induced by interactions with the solvent with charged and polar groups on the surface of the globular protein.[422]

Hormones. The secondary structures of β-lipotropin, β-MSH, and corticotropin have been studied using c.d. and i.r. spectroscopy. The polypeptide chains in aqueous solution are in left-handed helix conformations of the poly-L-proline type. The reversible melting process of the left-handed conformation when heated in aqueous solution is non-co-operative and upon cooling the left-handed structure is stabilized. The functional activity of the hormone was correlated with the conformation of separate fragments of the hormone chain.[423]

lac *Repressor.* The secondary structure of the headpiece of the lactose repressor was shown to contain approximately 50% helical content, depending on the ionic strength. The decomposition of the i.r. spectrum into a sum of Gaussian curves reveals clearly the absence of a vibrational band around 1630 cm^{-1}, thus excluding the presence of a multi-stranded β-pleated sheet. The only β-structure compatible with the i.r. results seems to be a two-stranded antiparallel β-sheet, as judged from results on the β-sheet model compound gramicidin S. The unusually strong intensity of the amide I′ band favours the existence of such a structure. If the results are compared with several secondary-structure predictions, part of the helical residues should be located between leucine-45 and arginine-35 and a two-stranded β-sheet structure should be situated in the *N*-terminal part of the headpiece.[424]

[421] M. A. Walters and T. G. Spiro, *Biochemistry*, 1982, **21,** 6989.
[422] R. Schweitzer, W. Dreybrodt, A. Mayer, and S. El Naggar, *J. Raman Spectrosc.*, 1982, **13,** 139.
[423] A. A. Makarov, V. M. Lobachov, Yy. A. Pankov, and N. G. Esipova, *Biofizika*, 1981, **26,** 941.
[424] M. Schnarr and J. G. Maurizot, *Eur. J. Biochem.*, 1982, **128,** 515.

Lysozyme. Two nitrated tyrosines in hen egg-white lysozyme have been monitored separately and simultaneously, using RR spectroscopy. The intensity of the NO_2 symmetrical stretching mode was used to determine that the pK_a values of the nitrated moieties were 6.76 and 6.52, respectively. The frequency of this mode for each residue (1340 and 1328 cm^{-1}) was compared to that of model systems. The high-frequency band was characteristic of a nitrotyrosine residue in an exposed aqueous environment (residue 23), whereas the low-frequency band indicated a hydrophobic and H-bonded site for the second nitrotyrosine (residue 20 or 53).[425]

Riboflavin. The perturbation of the Raman spectrum of 8-mercaptoflavin (I) on binding to the apoenzymes of L-lactate oxidase (II), glucose oxidase, and Old Yellow Enzyme, proteins that stabilize the quinonoid form of the flavin, was studied and compared to that of riboflavin. Although the Raman spectra of the two protein-bound flavins were very different, the three highest wavenumbers of the (II)–(I) complex occurred at 1614, 1536, and 1504 cm^{-1}, and these were equated with those of riboflavin at 1631, 1582, and 1547 cm^{-1} on the basis that the Raman spectra of flavins with electron-donating substituents at position 8 have their three highest wavenumber peaks at positions between those of riboflavin and quinonoid flavin. The data are applicable to the electron distribution of the quinonoid form of (I).[426] RR spectroscopy on various ionic species of 8-mercaptoriboflavin (IA) in aqueous solution shows large vibrational spectral changes upon ionization of the 8-SH group. These changes and the superposition of the RR excitation profile and the visible spectrum for the 8-*S*-species indicate that there is a substantial amount of the quinoid thioketone resonance form presence in aqueous solutions of 8-*S*-(IA). RR spectra of (IA) bound to riboflavin-binding protein confirm the conclusion from visible spectra that (IA) binds to the protein in the 8-SH protonated form. However, there are changes in the 1250 cm^{-1} region of the RR spectrum upon binding aqueous (IA) to the protein. The 1257 cm^{-1} band in aqueous solution moves to 1248 cm^{-1} on the protein binding. This shift is also observed in DMSO solutions of (IA). As the 1257 cm^{-1} band shifts upon formation of the N-3 deuterioriboflavin, the lowering of a δ(NH) mode at N-3 is interpreted to mean that there is weaker H-bonding between flavin N-3 and riboflavin-binding protein than between flavin and H_2O.[427]

The effect of H-bonding between flavin and different polar solvents (H_2O, DMSO, MeCN) has been studied in detail. There were several RR spectral changes observed between aqueous flavin species and flavin in a non-H-bonding solvent. Band II shifted to a higher frequency, band IX disappeared or shifted to a lower frequency, band X shifted to a lower frequency, and the intensities of bands III and IV changed. Bands X and IX shifted on deuteriation in D_2O and therefore involve δ(NH) at N-3 of the flavin. The RR spectra of three flavoproteins were also investigated and, when compared to an aqueous solution of FAD, acyl-CoA dehydrogenase (IB) showed a change in

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[426] L. M. Schopfer and M. D. Morris, *Dev. Biochem.*, 1982, **21**, 447.
[427] J. Schmidt, M. Y. Lee, and J. T. McFarland, *Arch. Biochem. Biophys.*, 1982, **215**, 22.

band IX consisting of the disappearance or shift to lower frequency of this band. This change was identical to the difference between the RR spectrum of riboflavin in 67% DMSO–33% H_2O when compared to that of an aqueous solution of riboflavin. The RR spectrum of FAD of acyl-CoA oxidase (IIB) differed from that of aqueous FAD by a shift of band II to higher frequency, the shift of band X to lower frequency, and the shift or disappearance of band IX. These changes were identical to those observed upon dissolving riboflavin in a non-H-bonding solvent. In addition band VI was split in (IIB). The RR spectrum of the FAD of glutathione reductase (IIIB) was quite similar to that of aqueous FAD except that a new band was present between bands IX and X. Comparison of the flavoprotein RR spectra with the RR spectra of free flavins in solvents of varying H-bonding ability indicated that FAD at the active site of (IIIB) was strongly H-bonded. These RR investigations served as a basis for a unique structure–function study of two enzymes, with different activities for the same substrates.[428] A normal mode analysis of the in-plane vibrations of lumiflavin was performed by using the flavin complexed with riboflavin-binding protein. RR spectra of the complex revealed 13 bands. Bands I, II, and X, at 1631, 1584, and 1252 cm^{-1}, respectively, were prominent. Band II demonstrated the significance in the lumiflavin isoalloxazine of an N-5=C-4a–C-10a=N-1 conjunction pathway. Band X frequency increases on N-3 deuteriation, which is apparently due to a decrease in C=O bending and not greatly due to N-3—H bending. The characteristics of analogous band X of *Clostridium* MP flavodoxin and the D-amino-acid oxidase–benzoate complex are discussed. In glucose oxidase this band is missing. Thus, the H-bond pattern for these proteins appears to be different.[429]

Visual Pigments. Bacteriorhodopsin (BR), the protein of the purple membrane of *Halobacterium*, involves a retinylidene Schiff base as its photochemical-active chromophore. Resonance Raman spectra, which selectively probe the retinylidene Schiff-base chromophore, were obtained from aqueous suspensions of the purple membrane. In the unphotolysed chromophore of BR, the Schiff N carries a proton whose reversible release during the photoinduced cyclic process is essential for the function of the membrane as a proton pump. It is expected that during the process the Schiff-base group undergoes conformational and/or conflgurational changes. The vibrational modes of $=C_{14}H—C_{15}H=NH^+—$ of the retinylidene Schiff-base chromophore can be assigned on the basis of deuterium exchange at C_{15} and replacement of the Schiff-base proton by a deuteron. RR spectra of BR_{603}, a chromophore with an additional double bond in the retinal ring (3-dehydroretinal) but an identical terminal group, were used in the analysis and various types of H-bonding modes identified. It was concluded that the intensities and frequencies of such modes reflect the conformation of the terminal group. A comparison of the RR spectra of BR_{520} (all-*trans*) and the 13-*cis* component BR_{548} from the dark-adapted purple membrane revealed that the conformation of the protonated

[428] J. Schmidt, P. T. Coudron, A. W. Thompson, K. L. Watters, and J. T. McFarland, *Biochemistry*, 1983, **22,** 76.
[429] T. G. Spiro, W. D. Bowman, P. K. Dutta, and M. J. Benecky, *Dev. Biochem.*, 1982, **21,** 554.

Schiff base is significantly different in these two chromophores. This is ascribed to variable interactions with the protein. The relation between RR spectral data and the conformational structure of the terminal group is discussed.[430]

The RR spectrum of the primary photoproduct K of bacteriorhodopsin has been obtained using a novel low-temperature spinning-sample technique. Purple membrane at 77 K is illuminated with spatially separated active (pump) and probe laser beams. The 514 nm pump beam produces a photostationary steady-state mixture of bacteriorhodopsin and K. This mixture is then rotated through the red-probe (676 nm) beam, which selectively enhances the Raman scattering from K. The essential advantage of the successive pump and probe technique is that it prevents the fluorescence excited by the pump beam from masking the red-probe Raman scattering. K exhibits strong Raman lines at 1516, 1294, 1194, 1012, 957, and 811 cm^{-1}. The effects of C_{15} deuteriation on K lines correlate well with those seen in 13-*cis* model compounds, indicating that K has a 13-*cis* chromophore. However, the presence of unusually strong low-wavenumber lines at 811 and 957 cm^{-1}, attributable to H out-of-plane wags, indicates that the protein holds the chromophore in a distorted conformation after *trans–cis* isomerization.[431] The analysis of the energy storage, RR spectra, molecular structure, and pathways of formation of bathorhodopsin has been achieved using the simulation of the protein constraint by an effective steric potential, assuming a limited relaxation of the protein cavity during isomerization time. A reasonable estimate of the protein flexibility confines the isomerization pathway in a unique way and simulates the trapping of bathorhodopsin as a strained-type intermediate. Adjusting the single parameter that represents the protein rigidity to stimulate the energy storage and RR lines of bathorhodopsin leads to a quite unique prediction of its structure and explains the difference in quantum yield for the bathorhodopsin from rhodopsin and isorhodopsin.[432]

Empirical assignments of most of the Raman lines observed between 600 and 1700 cm^{-1} from all *trans*-retinal and several deuteriated derivatives are reported. A modified Urey–Bradley force field, transferred from model polyenes and polyenals, was refined to reproduce satisfactorily the frequencies and deuterium shifts observed in the spectra. From this analysis, simple rules are formulated that describe the coupling between out-of-plane wagging modes of *trans*-vinyl H's and deuterons. In addition, the distribution of Raman intensity in the 1100—1400 cm^{-1} region of the retinal spectrum results from vibrational mixing of the C_9 and C_{13} Me stretches with the C—C stretches and the CCH bends of the conjugated chain. The assignment of the observed spectral lines of retinal to specific vibrations and the formulation of a reliable force field provide a basis for predicting the effects of structural and environmental perturbations on the Raman spectrum of the retinylidene chromophore. Thus, the expected changes caused by Schiff-base formation and

[430] G. Massig, M. Stockburger, W. Gaertner, D. Oesterhelt, and P. Towner, *J. Raman Spectrosc.*, 1982, **12**, 287.
[431] M. Braiman and R. Mathies, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79**, 403.
[432] A. Warshel and N. Barboy, *J. Am. Chem. Soc.*, 1982, **104**, 1469.

by protonation of the Schiff base were discussed.[433] Fourier-transform i.r. (FT i.r.) difference spectroscopy was used to examine the state of protonation of the retinylidene Schiff base in the first intermediates of the photocycle of bacteriorhodopsin (BR), BR 570, and K. RR spectroscopy provides evidence that BR 570 is protonated, but these results have been questioned. FT i.r. difference spectral changes in the BR 570-to-K transition clearly indicate that BR 570 contains a protonated Schiff base. In contrast, the K intermediate displays a Schiff base that is altered but is still associated to some degree with a proton. Because the low-temperature FT i.r. difference spectrum of BR 570 and K is similar to recently reported low-temperature RR spectra of BR 570 and K, most, but not all, vibrational changes in the BR 570-to-K transition of the chromophore could be assigned. A simple model of the first step in the photocycle, which involves a movement of the Schiff-base proton away from a counter-ion, is consistent with the data.[434] An introduction and overview of the use of RR spectroscopy of rhodopsin and bacteriorhodopsin including future trends has been produced by Lewis.[435]

Wheat Protein. The techniques of near-i.r. reflectance spectroscopy (n.i.r.s.) and dye-binding (d.b.) have been assessed in attempting to evaluate the protein content in oat groats. The 584 samples ranging from 9.0 to 26.4% protein were subdivided into 37 standard samples to develop the calibration equations and 547 independent samples. Measurements using the d.b. method indicated that the precision was less for high-protein samples than for low- or medium-protein samples. With the n.i.r.s. method, precision was not related to protein level. In conclusion it was felt that either n.i.r.s. or d.b. studies are satisfactory techniques for estimating the protein content of oat groats in a breeding programme.[436]

DNA–Protein Interactions. The RR spectrum of native DNA was obtained by using excitation at 257 nm. The spectral lines are assigned to the different nucleotide bases that provide the resonance effect. The interactions of basic peptides (arginine Me ester, lysine Me ester, arginylarginine) were investigated using excitation at 300 and 257 nm. Both arginine Me ester and arginyl-arginine recognize the A–T base pairs in the large and narrow grooves of DNA. The DNA–lysine Me ester interaction is probably non-specific.[437] RR spectra of complexes between DNA and histone have shown that the basic residues selectively modify the environment of certain DNA bases: lysine modifies the G–C bases and arginine the A–T bases, whether the residues are alone or included in a protein. This selectivity of interaction allowed confirmation of the particular role played by H4 in the structure of nucleosomes and the important role of arginine residues in this interaction.[438] The visible and Raman spectra of actinomycin and actinomycin–DNA complexes in H_2O have been analysed.

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[435] A. Lewis, *Methods Enzymol.*, 1982, **88,** 561.
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[437] A. Laigle, L. Chinsky, and P. Y. Turpin, *Nucleic Acids Res.*, 1982, **10,** 1070.
[438] A. Laigle, L. Chinsky, P. Y. Turpin, J. Liquier, and E. Taillandier, *Biochimie*, 1981, **63,** 831.

The vibrational structure of the lowest excited electrónic state of the complex has been evaluated, as has the mechanism of the molecular interaction.[439]

6 N.M.R. Studies

Contributed by H. W. E. Rattle

Introduction.—The number of papers dealing with applications of n.m.r. to proteins and related molecules increases seemingly without limit, and reviewers of the field have been correspondingly busy. Reviews of work on specific proteins are listed in the appropriate sections of this article, but more general collections may be mentioned here: 'Biochemical Structure Determination by NMR',[440] 'Biological Magnetic Resonance, Volume 4',[441] and 'Conformation of Biological Molecules – New Results from NMR'.[442] The first of these presents state-of-the-art material on eight problems currently under investigation by n.m.r., including myoglobins, metallothionein, parvalbumins, and other calcium-binding proteins. The second covers spin-labelling in disease, ^{113}Cd spectroscopy, photo-CIDNP studies of proteins, and ring-current applications, while the last provides a broader survey of conformational studies with a good collection of references up to the end of 1980. A detailed review of magnetic-resonance work on the active sites of proteins has been produced.[443] One technique finding increasing application in this field is the isotopic labelling with ^{18}O and ^{17}O of phosphates involved in enzymic interactions, and this has been described and reviewed.[444] Workers engaged in labelling studies may also be interested in a strategy for uniform ^{15}N labelling of both nucleic acids and proteins for subsequent solid-state n.m.r.[445] and in a paper on the use of special strains of *E. coli* to produce specifically ^{13}C-labelled amino-acids for subsequent biosynthetic incorporation into proteins.[446] The characteristics of ^{13}C-labelled peptides are discussed[447] with particular reference to the relation between information content and labelling pattern.

Theoretical calculations have always played an important role in the interpretation of n.m.r. spectra, and they are steadily becoming more sophisticated and more valuable. The application of ring-current calculations,[448] theories and techniques for studying the internal dynamics of proteins,[449] and the theory and applications of the transferred nuclear Overhauser effect for the study of small ligands bound to proteins[450] have been reviewed. The development of

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two-dimensional spectroscopy has brought the term 'connectivity' into our vocabulary; connectivities between amide and α-protons in peptides and proteins may be established by selective population transfer in combination with the Redfield (2-1-4-1-2) pulse sequence.[451] Two-dimensional correlated spectroscopy (c.o.s.y.) may be used for the unequivocal assignment of histidine residues.[452] The 'normal' protein-proton spectrum may be simplified by a related technique, in which the summation of spectra obtained with different spin-echo delay times eliminates signals from all even multiplets and collapses odd multiplets such as triplets into single lines.[453] Of course, when J values are accessible, they are very valuable in the analysis of protein spectra; recent papers investigate the limiting coupling constants for side-chain rotamers,[454] the conformational dependence of the vicinal-proton coupling constant for the C_α—C_β bond in peptides,[455] and the importance of solvent interactions in the values of the five-bond [H—C_α—C(O)—N—C_α—H] coupling constant in the peptide moiety.[456]

Amino-acids and Small Peptides.—*Amino-acids.* As always, the mainstream of amino-acid studies concentrates on their use as simple systems for the testing of new techniques or theories. Cross-relaxation effects in the photo-CIDNP spectra of *N*-acetyltyrosine and *N*-acetyltryptophan are used, for example,[457] to assess the possibilities for observing population transfer between amino-acids in proteins. Trials of the methods and effects of isotopic labelling have been reported using deuterium in phenylalanine[458] and ^{17}O in glycine, alanine, and glutamic and aspartic acids,[459] while the more familiar ^{13}C labelling, this time biosynthetically accomplished in *Spirulena maxima* and *Synechococcus cedrorum*, was shown to be neither random nor statistical.[460] Carbon–carbon coupling constants are reported for labelled tryptophan[461] and ^{13}C–^{15}N vicinal coupling constants for a number of other amino-acids.[462] The further development of ^{15}N labelling as a usable technique is also exemplified in studies of the stereospecificity of the polymerization of DL-leucine and α-OMe-DL-glutamic acid anhydrides[463] and of the acid–base and tautomeric equilibria in solid histidine.[464] Even closer to our ultimate biological goals is the use of ^{15}N relaxation times and nuclear Overhauser enhancement to probe

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the intracellular environment in intact *Neurospora crassa*, yielding microviscosity data unobtainable by any other technique.[465] More conventional conformational studies have been reported for 5-adenoyl-L-homocysteine[466] and for the 5-*cis* and 5-*trans* isomerism in a number of acylproline analogues.[467] The relaxation times of ^{13}C nuclei as a probe of proline-ring conformations have been discussed.[468] High-salt solvent conditions can induce conformational changes in aspartate, stabilizing the conformers with *gauche* carboxylates at the expense of *trans* conformers.[469] Other amino-acid studies reported recently include primary-amide hydrogen exchange rates,[470] the acidity–conformation relation in a number of *N*-nitroso-*N*-alkyl-α-amino acids,[471] kinetics of aldolation and β-elimination of some β-hydroxyl aromatic amino-acids,[472] the reaction of formaldehyde with the amino groups of alanine and lysine,[473] and the interaction of glycine with water and ions.[474]

Small Synthetic Peptides. The stereoselectivity of oligopeptide syntheses can be slightly affected by the solvent and activating agents,[475] as shown in the formation of di- and tri-peptides. Conformational and dynamic studies of Ala-Trp and Gly-His show that their internal motions are slow compared to overall tumbling,[476] while a type II β-turn was detected in Me_3CCO-Pro-Aib-NHMe in solution (Aib = α-aminoisobutyric acid)[477] in line with *X*-ray studies of the crystalline form. Comparison has also been made between the crystal and solution conformations of (Ac-Asp-α-Abu).[478] The *N*-terminal tripeptide of human serum albumin, Asp-Ala-His, shows a marked preference for binding to Zn^{II} rather than to Pr^{III},[479] which may or may not account, as claimed, for the ability of HSA to bind transition-metal ions in the presence of Ca^{2+}. An interesting n.m.r. study at high pressure of some dipeptides reveals differences in the activation volume for amide rotation if proline is one of the residues.[480] This will clearly add to the influence that proline has on conformational rearrangements within proteins; the steric effects of proline on an antecedent alanine residue are reported[481] to result in the predominant

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conformation always being the one in which the β-methyl of alanine eclipses its carbonyl group. β-Turn stability in the tripeptide Ac-Pro-Gly-X-OH varied as X = Leu > Ala > Ile, Gly > Phe.[482] Rates of proline *cis–trans* isomerization in oligopeptides have also been determined.[483] Other work reported recently on small synthetic peptides includes application of the INEPT polarization transfer method for the determination of ^{15}N relaxation parameters,[484] the nature and relative stabilities of monomeric and dimeric species of the octapeptide (L-Val-D-Val)$_4$,[485] the structure in solution of Z-Cys-Pro-Leu-Cys-OMe and its complex with iron,[486] and the effect of solvent and pH on ^{13}C chemical shifts in derivatized amino-acids and tripeptides.[487] The ^{13}C n.m.r. spectra of solid carnosine, a dipeptide (Ala-His) found in muscle, are greatly enhanced in intensity without serious loss of resolution by the introduction of cobalt chloride into the powdered sample; it appears that this provides a general way of improving spectra obtained, using cross-polarization and magic-angle spinning, from solid peptide samples.[488] Similar solid-state n.m.r. techniques were used to determine conformation-dependent ^{13}C shifts in polyvaline, polyisoleucine, and polyleucine in the α-helical and β-sheet forms.[489] Analysis of the ^{13}C spectra of polyaspartic acid samples prepared by hydrolysis of polysuccinimide under various conditions revealed a random distribution of α- and β-bonds in all samples,[490] while the stereoselectivity of polymerization of DL-valine and DL-leucine monomers, also investigated by ^{13}C resonance, revealed the expected preference for isotactic sequences but with no isotactic block longer than 6 units.[491] From the same laboratory come ^{15}N results,[492] showing that separate signals are detected from the central residue in each of the four possible triads L-L-L, L-D-L, L-L-D, and D-L-L. An interesting amphiphilic block copolypeptide with hydrophilic termini and a hydrophobic central block altered the liquid crystal → gel phase transition in a deuterium-labelled dipalmitoyl-phosphatidylcholine membrane.[493]

Turning now to cyclic peptides, we find a sequence of papers dealing with proline-containing molecules, including ^{13}C and ^{15}N studies of *cyclo*-(Pro-Phe-Gly-Phe-Gly),[494] solid-state ^{13}C[495] and solution proton[496] investigations of

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cyclotriproline, and two-dimensional spectroscopy[497] of the conformational equilibrium of *cyclo*-(Pro-NBGly$_2$) (NBGly = *O*-nitrobenzylglycine). A cyclic analogue of the proline-containing repeat pentapeptide of tropoelastin, *cyclo*-(Val-Pro-Gly-Val-Gly)$_2$, shows temperature-dependent conformational behaviour, at low temperature combining a β-turn with a ten-membered H-bonded ring similar to a 3_{10} helix, but at higher temperature assuming an antiparallel β-pleated sheet similar to that of gramicidin S.[498] The modelling of β-bends has occupied other workers: *cyclo*-(Gly-Cys-Gly)$_3$ triply bridged by 1,3,5-tris(thiomethyl)benzene forms a structure consisting of three β-bends,[499] while a comparison[500] between *cyclo*-(L-Ala-L-Ala-ε-aminocaproyl) and *cyclo*-(L-Ala-D-Ala-ε-aminocaproyl) reveals that the first exists as types I and III and the second as type II β-bends. Other studies of cyclic peptides include the synthesis, conformation, and interaction with small molecules of some bis(cyclic dipeptides),[501] the isomerization of azobenzene-containing cyclic oligosarcosine,[502] and a conformational analysis of the Cys-Pro-Val-Cys loop closed by a disulphide bridge as a model for small disulphide loops in proteins.[503] The possibility of suitably designed cyclic peptides acting as metal-binding agents is investigated using peptides with acidic side chains[504] such as *cyclo*-(Glu-Glu) and *cyclo*-(Glu-Pro)$_2$ and also[505] the cyclic octapeptides *cyclo*-(Phe-Pro)$_4$, *cyclo*-(Leu-Pro)$_4$, and *cyclo*-(Lys-Z-Pro)$_4$. In other investigations[506,507] specifically directed to the binding of Ca^{2+} ions by cyclic octapeptides the metal ion was co-ordinated in a central binding cavity to four carbonyl oxygen atoms in a coplanar arrangement. Binding of the calcium stabilized the octapeptide to a single conformation. A model for the zinc-binding site of carbonic anhydrase has been produced in the form of *cyclo*-(Gly-His-Gly-His-Gly-His-Gly); the Zn^{2+} ion binds all three imidazole side chains.[508]

Natural Peptides. The membrane channels formed by gramicidin A have been investigated by a series of specific ^{13}C-label experiments.[509] Two symmetrically related binding sites for Na^+ and Tl^+ ions were detected, centred at the Trp carbonyls, and separated by 23 Å, with all three tryptophan residues (9, 11, and 13) combining in the ion co-ordination. Thallium-205 resonance[510] also reveals two binding sites for gramicidin in trifluoroethanol but[511] only one site

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when the gramicidin was incorporated in micelles. Intramolecular hydrogen bonding in gramicidin S has been studied by n.m.r./i.r. following selective deuteriation[512] and by proton and ^{15}N n.m.r. of the ornithine side chain,[513] indicating the presence of intramolecular hydrogen bonds between ornithine $N_\delta H_3^+$ and D-phenylalanine carbonyl groups. Hydrogen bonds were also delineated in gramicidin S in spin-label relaxation-enhancement experiments using 1-oxy-2,2,6,6-tetramethylpiperidine in dimethylsulphoxide solvent;[514] gramicidin S analogues $Pro^{4,4'}$-$Ala^{5,5'}$ and $Pro^{4,4'}$-$Asn^{5,5'}$ in the same solvent show less propensity to form normal hydrogen-bond patterns than they do in aqueous solution. Since the action of the native molecule is initiated by interaction with phospholipid membranes, this may explain the antibiotic inactivity of the analogues.[515] Successful application to the librational motions of gramicidin S of a theory of ^{13}C relaxation behaviour[516] and the binding of Li^+ ions to gramicidin S and valinomycin[517] are reported. Valinomycin in acetonitrile forms two types of complex with Ca^{2+} ions, a 2:1 (peptide–ion–peptide) sandwich and an equimolar complex; the significance of these is discussed.[518] Only a few years ago, gramicidin and valinomycin would have been the only peptide antibiotics discussed in n.m.r. studies, but now the range has broadened considerably. Two-dimensional spectroscopy of siomycin[519] has permitted assignment of the ^{13}C spectrum directly from the known proton shifts, and the complete structure of the antibiotic glycopeptide ristomycin A is reported,[520] as are the structures of cirratiomycin A and B[521] and the conformation of triostin A.[522] The microdynamics of molecular motion in the cyclic hexadepsipeptide pristinamycin 1_A have been determined by ^{13}C relaxation measurements,[523] and the parts of cephalosporin molecules engaged in interactions with human serum albumin have been identified by high-resolution n.m.r.[524] A general ^1H resonance study of the cyclic hexadepsipeptide antibiotic beauvericin[525] revealed that it has different conformations in polar and non-polar solvents but that complexation with ions in aqueous solution made it adopt the conformation found in non-polar solvents. Non-polar solvents were used in studies[526,527] of synthetic fragments of suzukacillin, a membrane

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channel-forming polypeptide, showing them to adopt 3_{10} helical structures. Suzukacillin is very rich in α-aminoisobutyric acid (Aib), and model peptide studies on fragments of bradykinin suggested that substitution of Aib for Pro might lead to a 3_{10} structure in this molecule, too. However ^{1}H resonance measurements[528] of the whole substituted molecule, while indicating several conformations involving β-turns, failed to reveal any 3_{10} helix. β-Turns were detected in des-Arg^9 bradykinin in DMSO and water,[529] although other studies at 600 MHz[530] indicated that the molecule is in rapid equilibrium among many conformers with no persistent structural features at all in aqueous solution.

Endorphins and enkephalins continue to excite considerable interest among experimenters. Photo-CIDNP experiments on human β-endorphin[531] showed that the mobility and accessibility to solvent of tyrosines-1 and -27 were severely restricted on binding to lipid micelles, while the local conformation of the Tyr-Gly-Gly-Phe segment of 5-Met-enkephalin was shown[532] to be maintained in β-endorphin. A conformational transition in Met-enkephalin from an equilibrium between unfolded conformations in aqueous solution to a folded structure in non-polar solvents has been found,[533] and a theoretical analysis reinforces the picture of a number of alternative conformations in water.[534] Enkephalin has been cyclized[535] and its complexes with Cu^{2+}[536] and Al^{3+} have also been studied by n.m.r.[537] N.m.r. studies were reported in 1982 on a number of other biologically important small peptides, including the cyclic pentapeptides malformin A[538] and viscumamide,[539] the antineoplastic agent dolastatin 3,[540] and some biotin-containing peptides.[541] It has also been established[542] that the agent responsible for binding methylmercury in human erythrocytes is glutathione, and in a further use of ^{1}H and ^{13}C n.m.r. for identification it has been established that ferribactin, a siderochrome (iron-chelating peptide) from *Pseudomonas fluorescens*, is a nonapeptide that contains two residues each of lysine and N^6-formyl-N^6-hydroxyornithine.[543]

The neurotoxins of snake venoms continue, too, to provide interesting challenges in n.m.r. Small but important differences between the crystal and

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X-ray structures of α-cobrotoxin have been reported,[544] while differences between long and short neurotoxins in terms of the rigidity of the three-strand β-sheet that contains the active residues have been revealed by hydrogen–deuterium exchange studies.[545] The results correlate well with the different kinetic properties of the long and short neurotoxins. The dynamics of erabutoxin have been measured *via* relaxation times of methyl groups, using both ^{1}H[546] and ^{13}C[547] resonance; the results agree well on the regions of the molecule that show restricted motion, while still being consistent with the idea of flexible and dynamic structures for the proteins. Slow interconversion ($2.5\ s^{-1}$) between two conformations of toxin B from *Naja naja* is found at the midpoint of a pH-induced conformational transition; the rate rises to some $600\ s^{-1}$ at 60 °C.[548] ^{19}F labelling of neurotoxin II, also from *Naja naja*, has been used to determine a number of intramolecular distances and stands up well to comparison with the *X*-ray structure;[549] it was subsequently possible[550] to bind spin-labelled derivatives of the molecule to purified acetylcholine receptor protein, and also[551] to demonstrate the presence in solution of a β-structure in the central loop of the molecule with a β-turn at residues 31—34.

Like neurotoxins and enkephalins, the peptide inhibitors of enzymes offer a particular interest in conformational studies because of their very explicit dependence on shape for activity. Many of them also have the advantages of being very stable in solution and of being an appropriate size for n.m.r. studies. A good example is the basic pancreatic trypsin inhibitor (BPTI), for which[552] two-dimensional studies of amide-proton exchange rates revealed rates for 38 of the 53 backbone amides. The data included exchange rates for a number of amide protons near the protein surface that could not be correlated readily with the apparently accessible surface areas indicated by the crystal structure. A number of amide exchange rates are also reported,[553] and the dynamics of the molecule have been investigated in terms of their effect on ^{1}H[554] and ^{13}C[555] resonances. The stability of BPTI as related to electrostatic interactions was discussed[556] following employment of the Tanford–Kirkwood electrostatic theory in the evaluation of p*K* values obtained *via* ^{13}C spectroscopy: the total

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electrostatic free energy of the molecule is stabilizing at neutral pH despite the substantial net positive charge borne by the molecule. CIDNP studies of the tyrosines of BPTI indicate a major loss of solvent accessibility on binding to trypsin and chymotrypsin and their zymogens.[557] Assignments of the proton spectrum of trypsin inhibitor E from *Dendroaspis polylepis* are reported following two-dimensional spectroscopy at 500 MHz;[558] the protons of virtually all 59 residues were assigned, using only the known sequence and the n.m.r. data. Assignment of three methionyl carbonyl carbon resonances in the *Streptomyces* subtilisin inhibitor required double labelling using ^{13}C and ^{15}N;[559] once assigned, their dynamics were investigated over a wide range of temperatures.[560] ^{2}H labelling was used in measurements on the tryptophan-86 residue of the same protein;[561] the local conformation round the residue was stable up to pH 11.5 and up to 85 °C at pH 7. Finally, in a study of pepstatin[562] clear evidence was adduced for a tetrahedral intermediate in the binding of pepstatin to pepsin. Difference spectroscopy utilizing protonated and partly deuteriated pepstatin bound to pepsin points the way to a potentially useful method for simplifying the spectra of large-molecular-weight complexes.[563]

Hormones. The orientation of the asparagine side chain in the oxytocin analogue 2-alanine oxytocin has been deduced from proton coupling constants.[564] Another analogue, D-glutamine[4] oxytocin, seems from ^{13}C measurements to have a very similar conformation to the native molecule, but it exhibits greatly reduced activity.[565] A selenium derivative of oxytocin has been used to investigate the disulphide-bridge region of the molecule,[566] and structural studies of oxytocin antagonists such as 1-penicillamine oxytocin have been reported,[567,568] in addition to studies of the binding of the hormone to neurophysin.[569,570]

The hypothalamic hormone somatostatin is a cyclic peptide that exists in a number of conformers in solution. Energy calculations were used[571] to predict a number of low-energy conformations from which those compatible with n.m.r. data could be selected – an interesting approach, which finds an echo in

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a paper on melanostatin from another laboratory.[572] Experiments on somatostatin analogues[573] appear to show stacking between Phe[6] and Phe[11] as a stabilizing factor in the structure. Once the ring structure is opened, the resulting acyclic precursor appears to settle into a stable β-turn/β-sheet conformation;[574] the conformations of side chains in the native peptide, with some reassignments, have been determined at 500 to 600 MHz.[575]

L-His-L-Pro interconverts between the s-*cis* and s-*trans* rotational isomers of the amide bond with an average rate constant of about 2.0 ks^{-1}; the same residues, at positions 6 and 7 in angiotensin II, interconvert at least 70 times faster.[576] Conformation–activity relationships in a substituted angiotensin are reported,[577] as are the effects of lanthanide shift reagents on the spectra of angiotensin and (Glu[4]) oxytocin.[578] Two molecules related to each other and very widely distributed, thymopoietin and ubiquitin, are going to be of great interest; the pentapeptides that may apparently be associated with the active sites of these molecules, respectively (Arg-Lys-Asp-Val-Tyr) and (Tyr-Asn-Ile-Glu-Lys), have been subjected to considerable n.m.r. experimentation, particularly in their associations with lanthanides.[579–581] Other hormone fragments studied include nine model peptides of the insulin A-chain (by ^{15}N resonance in natural abundance)[582] and the *C*-terminal fragment 21—28 of vasoactive intestinal peptide.[583]

Enzymes.—*Oxidoreductases.* A novel method involving proton–proton transferred nuclear Overhauser enhancements has been used to investigate the conformation of NAD bound to alcohol dehydrogenases;[584] the conformation of the adenosine and nicotinamide ribose was 3′ *endo* of the N type. A tentative design for the hydrophobic pocket of the substrate-binding site of aldehyde reductase I containing two anion-binding sites has been proposed following binding of NAD-*P*-2-oxodiacid adducts as n.m.r. probes.[585] The possibilities of using ^{19}F as a probe for dehydrogenase mechanisms are explored in a series of papers in which fluorinated substrates and inhibitors

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were employed.[586–588] Dehydrogenase activity in an intact cell system has been monitored by p.m.r. measurements of bulk isotope exchange in the cells,[589] and the role of the essential histidine in the activity of lipoamide dehydrogenase has been elucidated through monitoring its signal following photoinactivation of the enzyme in the presence of Rose Bengal.[590] A novel system of active-site coupling through highly mobile peptide chains in the multienzyme pyruvate dehydrogenase complex from *Bacillus stearothermophilus* appears likely following monitoring of chain mobility through partial proteolysis of the complex.[591,592] In dihydrofolate reductase, labelling with [γ-^{13}C] tryptophan permitted a number of partial assignments to be made, and subsequent measurements suggest different modes of binding for different ligands;[593] the dihydrofolate–folate–NADP complex has been shown by ^{1}H and ^{13}C n.m.r.[594] to exist in three interconverting conformational states that occur in different proportions at different pH values, with the ionizable group responsible for the change not being one of the seven histidine residues. Strong isotope effects on the methylene–methyl interconversion catalysed by methylenetetrahydrofolate reductase from pig liver are reported.[595] Contact shifts due to the high-spin non-haem iron atom in catechol dioxygenases were used[596] to show a monodentate catecholate configuration in catechol 1,2-dioxygenase from *P. arvilla* and a chelated catecholate structure in protocatechuate 3,4-dioxygenase from *P. aeruginosa*, while in lipoxygenase I[597] it has been shown that the Fe is definitely in a high-spin state. Proton-resonance measurements of the molybdoferredoxin of nitrogenase from *Klebsiella pneumoniae* showed[598] that metal-binding sites, detected through relaxation enhancement by Mn^{2+} ions, are essential for the enzymic function of the nitrogenase; the spin states of a similar MoFe protein from *A. vinelandii* appear to be two M centres ($S = \frac{3}{2}$) and four P centres ($S = 0$) in the native states and diamagnetic M centres with $S = \frac{5}{2}$ P centres in the oxidized form.[599]

Transferases. ^{13}C labelling work on thymidylate synthetase from *Lactobacillus casei*[600] suggests that the active-site arginyl residue has a ^{13}C resonance at 156.9 p.p.m. The acid–base catalysis of α-glucan phosphorylases has been

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discussed,[601] and ^{31}P resonance has been used to monitor the production of ribose 1-phosphate from orthophosphate by nucleoside phosphorylase,[602] to study the activation of glycogen phosphorylase,[603] and, *via* ^{18}O–^{16}O isotope shifts of the phosphorus resonance, to observe the scissile bond of purine nucleoside phosphorylases;[604] apparently there is no phosphoryl enzyme intermediate in the reaction. The enzyme-catalysed formation of *S*-adenosyl methionine has been shown[605] to occur with inversion of the configuration at the C-5′ of ATP. Proton n.m.r.[606] shows a strong interaction between succinate and native cytosolic aspartate aminotransferase, but not such specific interaction with enzyme modified at a single arginine residue; a detailed ^{31}P resonance study of a similar enzyme from mitochondria has been described.[607]

Isotope shifts using specifically labelled adenosine 5′-[$\gamma(s)$-^{16}O, ^{17}O, ^{18}O]triphosphate have been used to determine the stereochemical course of phosphoryl transfer catalysed by yeast hexokinase[608] and glucokinase from rat liver,[609] in both cases suggesting an in-line mechanism. The histidines[610] and monovalent cation sites[611] of pyruvate kinase have also been discussed. The ternary complex ^{31}P spectrum of halibut muscle 3-phosphoglycerate kinase can be accounted for entirely on the basis of the various binary complex spectra, there being no evidence therefore for any substantial involvement of phosphoenzyme intermediates.[612] Magnesium resonance has been used to investigate the binding of Mg^{2+}–ADP and Mg^{2+}–ATP to creatine kinase,[613] suggesting that the cation in the ternary complex was not in the fast-exchange state, while the paramagnetic effects of Cr–ADP have been used[614] to deduce that metal-ion co-ordination of the transferable phosphoryl group precedes phosphoryl transfer and is a requirement of the creatine kinase reaction; similar experiments are also reported for adenylate kinase.[615] Hill plots of histidine titrations have been used to show that the n.m.r. signals of two histidines in arginine kinase are affected by the same three titratable groups;[616] histidine titrations and the lack of pH-dependent structural isomerization of human-muscle adenylate kinase have been discussed.[617] Interactions of RNA polymerase with substrate have been studied by ^{31}P

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n.m.r.[618] and paramagnetic substitution;[619] in the latter study, with Co^{2+} substituted for one of the two Zn^{2+} ions of the enzyme, direct metal–ATP co-ordination was demonstrated. The stereochemical course of nucleotidyl transfer catalysed by T7-induced DNA polymerase[620] and that of the 3′–5′ exonuclease activity of T4 polymerase are reported.[621]

Hydrolases. All known phospholipase A_2 molecules have Glu-4 and Phe-5 in their sequences. A series[622] of modified proteins was used to show that both Tyr-5 and norleucine-4-substituted enzymes were inactivated, but for different reasons, the former being due to a distortion of the catalytic site and the latter to loss of a binding site for micelles. Evidence from ^{31}P seems to show that cobra venom phospholipase A_2 has an activator site separate from its catalytic site.[623] ^{18}O isotope shifts demonstrated[624] that acid hydrolysis of α-D-ribofuranose-1-[^{18}O]phosphate cleaved the C—O bond, while both acid and alkaline phosphatases cleaved the O—P bond. ^{113}Cd resonance[625] was employed in a most interesting study of the dimeric alkaline phosphatase, showing that in the absence of sufficient metal ions metal will migrate from one monomer to the other in order to permit binding of phosphate to it, giving a half-of-sites reactivity.

The ready availability, reasonable size, and extensive earlier literature of lysozyme make it a continuing subject for a number of researchers. It has been used as a 'typical globular protein' for studies of relaxation dispersion in the crystalline[626] and lyophilized powder[627] forms, and relaxation studies in solid lysozyme showed[628—630] that the main source of relaxation was methyl-group rotation but with other contributions from slow motions and groups with exchangeable protons. Relaxation[631] and denaturation[632] of lysozyme in solution have also been discussed. The indole NH proton resonances in the tryptophan residues of lysozyme seem to exchange for solvent deuterium by two different mechanisms with different activation energies;[633] assignments of a number of protons from residues in the β-sheet region of lysozyme have been made,[634] and assignments of *N*-methyl resonances in ^{13}C reductively methy-

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lated lysozyme are also reported.[635] Another 'golden oldie' for the n.m.r. spectroscopist, RNAse A, appears these days to be popular largely for the light it can cast on the folding and unfolding mechanisms of proteins. Current reports include proton relaxation during unfolding,[636] the equilibrium between *cis*- and *trans*-proline conformers in fragments of RNAse A,[637] observation of methanol-stabilized intermediates in the unfolding process,[638,639] salt-bridge stabilization of the helix formed by the isolated *C*-peptide (residues 1—13) of RNase A,[640] and the existence of a purine-ligand-induced conformational change in the active site of the enzyme, revealed through perturbations in the titration behaviour of histidines 12, 48, and 119.[641]

Replacement of the Zn^{2+} at the activation site of bovine-lens leucine aminopeptidase by Mn^{2+} has permitted elucidation of some aspects of the action of the inhibitor *N*-(leucyl)-*O*-aminobenzenesulphonate,[642] while similar substitutions, this time with Co^{2+}, in carboxypeptidase A permitted both inhibition by β-phenylpropionate[643] and the catalytic role of the metal ion[644] to be investigated. In both cases one metal-bound water molecule was affected. ^{15}N resonance of carboxypeptidase A selectively enriched with ^{15}N enabled experimenters[645] to establish that β-phenylpropionate can successfully compete with an azo N-atom as the H-bond acceptor of the phenolic proton of tyrosine-248, which is complexed in its azo form to the catalytically essential Zn^{2+} ion.

The comparison between *X*-ray and n.m.r. studies of the serine proteases has been reviewed, in English[646] and in Japanese.[647] Peptide models for the active site have been prepared for serine proteases in general[648] and for α-chymotrypsin in particular.[649] Both proton and ^{19}F resonance studies[650] show the formation of a hemiacetal between the free aldehyde of *N*-acetyl-DL-*p*-fluorophenyl alaninal and the active-site serine residue of chymotrypsin. Binding of 4-(trifluoromethyl)-α-bromoacetanilide to the enzyme is also reported, involving alkylation of the Met-192 residue,[651] while tosylchymotrypsin, labelled with ^{2}H or ^{13}C in the tosyl group, has been used to demonstrate[652]

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that the local structure of the active site is rather loose, in that the tosyl group moves freely and is in a solvent-rich environment. The active sites of other proteases have also been studied: the very low-field proton resonance characteristic of the H-bond between imidazolium and aspartate groups of the Ser,His,Asp catalytic triad of serine proteinases was not found in subtilisin,[653] while molecular-orbital calculations on the His-57–Asp-102 couple in β-trypsin agreed with n.m.r. experiments in not supporting an earlier charge-relay mechanism proposed for the enzyme.[654] The active-site histidine[655] and catalytic mechanism[656] of α-lytic protease have also been discussed. Other hydrolase enzymes reported in 1982 include thermolysin,[657] Mucor rennin,[658] papain at low temperatures,[659] and the conformations and conformational changes of pepsin on binding the potent peptide inhibitor pepstatin.[660,661]

Lyases, Isomerases, and Ligases. A structural model has been proposed[662] for the active site of chicken-liver mitochondrial phosphoenolpyruvate, which was shown to have one binding site for Mn^{2+}. The kinetics of threonine aldolase reactions have been followed using a model system.[663] Rates of carbon dioxide/carbonate exchange catalysed by human carbonic anhydrase I are reported following ^{13}C studies,[664] and the interaction of sulphate with the enzyme has also been investigated;[665] a CO_2 hydration activity for Mn^{2+}-substituted carbonic anhydrase B of some 7% of that of the native Zn^{2+} enzyme has been found in a series of experiments that also show a direct binding of HCO_3^- to the metal ion, while CO_2 is much more weakly attached to the enzyme.[666] Manganese substitution was also used in studies of the binding of glutathione to glyoxalase I;[667] two water molecules are bound in the co-ordination sphere of the metal, and one of them is displaced on attachment of the glutathione, the remaining water being implicated[668] in the catalytic step.

Some studies of steroid isomerase, including histidine titration and the detection of some unusually mobile residues in the chain, form our only Class 5 protein study this year.[669] Hardly better represented are the ligases: the active-site phosphohistidine of succinyl-CoA synthetase from *E. coli* has been

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observed through its ^{31}P resonance, showing that the P atom is rigidly held and that the phosphoryl group was in the monoanionic form at pH 7.25.[670] The same workers also detected the existence of two phosphorylated intermediates in catalysis by the enzyme, leading to a detailed model for the catalysis.[671] Rates of synthesis of various dinucleoside tri- or tetra-phosphates by *E. coli* lysyl-tRNA synthetase were monitored by ^{31}P and ^{1}H spectroscopy; considerable enhancement of the rate occurred on addition of 150μM $ZnCl_2$ to the reaction mixture.[672]

Other Proteins.—*Iron-containing Proteins.* A series of crosslinked mixed-valency hybrid haemoglobins (Hb) has been prepared from derivatives of Hb C and human normal adult Hb; the spectral changes of these are not concerted on ligation, implying that a simple two-state model is inadequate and that intermediate structures may exist during the co-operative oxygenation of Hb.[673] The surface histidines of Hb have been titrated[674] with a number of assignments being made using modified Hb molecules, and their relaxation behaviour has been followed.[675] A number of the corresponding surface histidines in haemoglobin S (sickle haemoglobin) have pK values that differ from those in the normal molecule, in particular indicating that the *N*- and *C*-terminal regions of the sickle molecule are altered.[676] Methods for identifying Hb S have been described.[677] The proximal histidines of haemoglobin form an effective probe of the haem pocket and have been used in a comparison between variant Hb molecules;[678] the influence of quaternary structure on iron–histidine binding is reflected in the hyperfine-shifted resonances of exchangeable imidazole NH protons, although a detailed analysis of the contributing factors is not yet possible.[679] Other recent studies involving haemoglobin include methylmercury binding,[680] diffusion coefficients measured by pulsed-field gradient n.m.r.,[681,682] and the interactions between haemoglobin and 2,3-diphosphoglycerate,[683] inositol hexaphosphate,[684] and model membranes.[685] The first report of subunit specificity in mono-oxygenase-like activity in tetrameric haemoglobin has been reported.[686]

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Among the recent work involving myoglobins, we may note ^{129}Xe resonance measurements of xenon binding,[687] high-pressure studies,[688] a review of the motions of aliphatic residues,[689] and model studies of the electronic state of the haem group.[690] Quadrupole-echo methods[691] have been used to determine haem structure in magnetically ordered microcrystals of ferrimyoglobin, a new and potentially valuable technique. Small chemical-shift differences are found between haem resonances from different components of soybean leghaemoglobin, possibly indicating substitutions among the haem contact residues.[692]

A review of n.m.r. studies on low-spin cytochromes has been published.[693] Models of the cytochromes *b*, in which the haem group is not covalently bound to the protein, have been proposed following unsymmetrical phenyl substitution[694] and temperature dependence of proton isotopic shifts.[695] Cytochrome P450 is technically a cytochrome *b*, although relaxation studies[696] indicate that acetanilide binds to it in a specific complex not found with cytochrome b_5. The dihaem cytochrome cd_2 from *Pseudomonas aeruginosa*, which acts as a nitrite reductase, appears from ^{15}N resonance to have a weak interaction with NO_2^- ion;[697] its proton spectrum indicates a structural transition with a pK of 5.8, although not many resonances are resolvable since the molecular weight is 120000.[698] Comparison of the structures of several variant cytochromes *c* in which tyrosine residues were substituted variously by leucine or phenylalanine indicated[699] that the effects on the structure were minimal; acetylation experiments[700,701] showed widely differing reactivities of the tyrosines, with acetylation at Tyr-74 leading to conformational change in the molecule. Comparative studies of the haem environment in a number of cytochromes c_{553} have been reported,[702] as have the electron-transfer reactivity, monitored by n.m.r. and photochemical methods, of cytochrome *c*[703] and the magnetic susceptibility of ferricytochrome *c*.[704] Electron-transfer mechanisms in the tetrahaem cytochrome c_3 of *Desulphovibrio vulgaris* have been analysed by a series of saturation transfer experiments, taking into account all

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16 redox states of the protein.[705] The binding of iron hexacyanide[706,707] and platinum complexes[708] to cytochrome *c* assists in *X*-ray structure determination and proton-resonance assignments.

The geometry of the complexes between horseradish peroxidase and aromatic substrates has been elucidated with a modified n.m.r. spectrometer,[709] and the axial imidazole in the reduced enzyme has been shown, in contrast to the interpretation of other spectroscopic data, not to be deprotonated.[710]

Some sophisticated new double-resonance and spin-echo techniques have been applied to ferredoxin from *Anabaena variabilis*, resulting in the assignment of a number of ^{1}H and ^{13}C resonances.[711,712] Ferredoxins are also the subjects of electronic-structure calculations,[713] pH-dependence studies,[714] and electronic spin–lattice relaxation measurements.[715] A $g' = 1.74$ e.p.r. signal, severely reduced by phosphate binding, in the iron-containing bovine-spleen purple acid phosphatase, has been reported.[716]

Copper Proteins. The molecular motion of methionine-121, one of the Cu ligands of azurin, increases with pH and temperature, indicating a lengthening and perhaps breaking of the Cu—S bond. This correlates with redox inactivation of the molecule and with deprotonation of the histidine-35 copper ligand; the coupling between methionine motion and histidine deprotonation has also been discussed.[717] No major structural changes are observed when the Cu of azurin is replaced with nickel;[718] these studies strengthen the case for the central role of His-35. ^{23}Na and ^{43}Ca resonance have been used to investigate sodium- and calcium-binding sites on haemocyanin.[719]

Calcium-binding Proteins. ^{43}Ca resonance has been used to delineate the calcium-binding sites of calmodulin,[720,721] and calmodulin, parvalbumin, and troponin C[722] ^{1}H resonance showed a number of conformational changes induced in calmodulin by Ca^{2+} binding.[723] Detailed studies on synthetic

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analogues of the high-affinity site III of rabbit skeletal troponin C[724] and on cleavage fragments of troponin C-containing single Ca^{2+}-binding sites[725] have been performed and comparisons between rabbit and pike troponin C published.[726] The phylogenetic division of parvalbumins into two classes, α and β, is supported by comparative ^{113}Cd and ^{1}H measurements;[727] in other studies of parvalbumin, the principal axis of the magnetic-susceptibility tensor of bound ytterbium was determined as a necessary precursor to detailed lanthanide-shift measurements.[728]

Muscle Proteins. N.m.r. evidence for a short hinge region in the myosin rod takes the form of the observation that less than 4% of the fragment gives resonances consonant with random-coil structures;[729] similar conclusions were drawn by other workers,[730] who found sharp resonances corresponding to <25 residues per chain in rabbit long S2 myosin fragments. The S2 'head' subfragment appears to exist in an equilibrium between two conformational states, with the one that predominates at low temperatures being identified with the state obtained by binding Mg–ADP.[731] A sharp resonance in the spectrum of fast twitch muscle S1 has been assigned to α-*N*-trimethylalanine at the *N*-terminal blocking group of the myosin light chain A1. The signal broadened on binding actin, indicating immobilization.[732] Further studies[733] using shift and broadening probes identified labile regions in different parts of the headgroup that were differentially constrained on actin binding. The high-affinity metal-binding site on G-actin appears to be less than 10 Å from the ATP-binding site;[734] the effect of thorium (Th^{4+}) on the formation of crystalline actin tubes is reported.[735] Also published in 1982 were reports of interactions between troponin C and CNBr-cleaved fragments of troponin I[736] and of proton-resonance evidence for flexibility in microtubule-associated proteins.[737]

Proteins Associated with Nucleic Acids. Earlier n.m.r. reports on the existence of unique globular domains in histones H1 and H5 have been supported by recent microcalorimetric data[738] and (for H1) by ^{13}C resonance in both

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aqueous and 2-chloroethanol solution;[739] a method for precise elimination of the *N*-terminal domain of H1 prior to n.m.r. experiments has been reported,[740] as have methods for deuteriation of histones of *Physarum polycephalum*, monitored by deuterium resonance.[741] *N*-Trimethylalanine has been identified as the blocked *N*-terminal residue of histone H2B from *Tetrahymena pyriformis*,[742] and a study[743] of the binding of acetylated peptides of histone H4 to DNA supports the view that acetylation *in vivo* lifts the *N*-terminal region of this histone off the DNA and thereby permits or initiates structural changes in chromatin. Relaxation studies of clupeine, the protamine extracted from herring sperm, reveal the molecules as being essentially extended in aqueous solution, with side-chain flexibility whose phosphate dependence differs from fraction to fraction.[744]

A comparison, using ^{31}P resonance, of protein–RNA interactions in a variety of systems, including ribosomes, polysomal mRNA, and RNA viruses, revealed[745] a wide range of relaxation and NOE effects. Clearly the protein–RNA complexation differs widely between the complexes. A series of studies of ribosomal proteins indicate[746] considerable independent mobility of protein L7/L12 *in situ* on the ribosome and compact globular structures for proteins L11,[747] L29 and L30,[748,749] and S4, S7, S8, S15, and S16.[750]

A method likely to become of increasing importance in structural investigations is the generation of altered versions of the protein, each with a known single amino-acid substitution, by genetic-manipulation techniques. The specific assignments resulting from the application of this method to *lac* repressor[751] permitted comparison between the interaction of the repressor with *lac* operon and other DNA, showing that the *N*-terminal region is capable of recognizing the operon sequence.[752] The relation between conformational changes and DNA-binding activity of λ *tof* repressor protein has also been investigated.[753]

Photo-oxidation of *E. coli* initiation factor 3 inactivates the protein and is shown by a number of methods including n.m.r. to be due to the selective loss

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of His-139,[754] which participates in the binding of the initiation factor to the 30 S ribosomal subunit. The stereochemical course of GTPase activity[755] and the structure of the GDP–Me^{2+} complex[756] of the elongation factor of *E. coli* have also been investigated by n.m.r. methods, both studies using oxygen isotopic substitution.

^{31}P resonance measurements on the binding of oligonucleotides to the gene 5 protein of phage fd reflect a specific binding site for the 5′-phosphate dianion;[757] binding of oligonucleotides is found[758] to affect two tyrosine and one phenylalanine residue in the protein. For the coat protein of fd phage, solid-state n.m.r. reveals a rigid backbone[759] with some flipping of aromatic side-chain rings.[760] The main part of the isolated coat protein of alfalfa mosaic virus[761] is also rather rigid but with a flexible *N*-terminal of about 36 residues; no such flexible region has been detected in ^{13}C resonances of southern-bean mosaic virus, whose only sharp peaks seem to come from the side chains of surface residues on the coat protein.[762]

Glycoproteins. Space precludes discussion of a number of studies that were concerned with the structure and conformation of the polysaccharide components of glycoproteins. However, it has been shown by energy calculations[763] that the conformation of glycosylated β-turns consistently found by n.m.r., with the amide proton of the glycoside amide bond nearly *trans* to the anomeric proton of the sugar, in fact lies very close in energy to the *cis* form. The carbohydrate–protein linkage has been studied for glycoserines,[764] *N*-acetylmuramyl-L-alanyl-D-isoglutamine,[765] and phenylalanylglucopyranoside enantiomers.[766]

Proteins Associated with Lipids. Reports on protein–lipid interactions tend often to concentrate on the effect on lipid order and conformation and thus fall outside the scope of this review, although it can be a grey area: where do lipids immobilized by proteins end and protein–lipid complexes begin? Starting strictly in the field of peptide conformation, 400 MHz proton n.m.r. of peptidolipin[767] indicated that the heptameric peptide moiety forms a γ-turn around the central Pro residue. The conformations of a number of hyd-

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rophobic peptides have been investigated in membrane-mimetic environments,[768] as have a series of synthetic fragments of bacteriorhodopsin.[769] The structure of melittin has been determined by *X*-ray diffraction and related to n.m.r. studies, particularly with regard to folding of the monomer into the tetrameric form;[770] two-dimensional n.m.r. has been used to determine the conformation and orientation of melittin at the lipid–water interface,[771] and the interaction between melittin and lipids has also been discussed.[772—774]

The two methionine residues, Met-8 and Met-81, of glycophorin have been used as probes into the structure of the protein, revealing possible metastable states.[775—777] Lateral diffusion of glycophorin and other proteins in bilayers has been discussed,[778] as has the effect of glycophorin on lipid order.[779—781]

Proton n.m.r. evidence for secondary and tertiary structure in myelin basic protein has been reported,[782] as have the effects of lipid interactions on the spectrum of the protein;[783] differential broadening of the resonance from Met-20 relative to lines from near the protein termini was attributed to motional restriction on binding to the micelle. Also discussed have been the crystalline lipovitellin–phosvitin complex,[784] human high-density lipoproteins,[785] phospholipid binding to cytochrome oxidase,[786] and the environment and mobility of hydrophobic and hydrophilic regions in ^{19}F-labelled coat protein from phage M13 in micelles and vesicles.[787]

Metallothioneins. A series of $^{113}Cd^{2+}$ studies on the structure of metallothioneins, sulphur-rich metal-binding proteins, has been reviewed.[788] Recent additions to the series provide unambiguous evidence[789] for a two-domain structure in rat-liver metallothionein containing separate three- and four-metal

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clusters, while in the mud crab *S. serrata* the two clusters are both identical to the mammalian three-metal site.[790] Four- and three-metal sites were also found for human metallothionein[791] and selectively for copper[792] at the three-metal 'B' site in the calf protein. (See also ref. 793.) E.p.r. and n.m.r. studies[794] indicate that rat-liver Cu–metallothionein is very susceptible to oxidation, with 18 titratable cysteines in anaerobically prepared protein reducing to 1—12 in metallothionein prepared in the presence of air.

Structural Proteins. The temperature dependence of the proton spectrum of hydrated collagen is reported,[795] as are molecular motion in collagen fibrils measured by solid-state n.m.r.[796] and the molecular mechanism of mineralization of collagen monitored by ^{13}C n.m.r. of the model polypeptide $(Pro\text{-}Pro\text{-}Gly)_n$.[797] Earlier n.m.r. studies indicating a mobile contact region between collagen molecules were supported by measurements on collagen fibrils labelled with deuteriated leucine;[798] both conformations determined by *X*-ray diffraction for the leucine side chain are found, and they interconvert at rates that are proportional to temperature. Gelatin-gel formation[799] and the temperature dependence of molecular mobility in gelatine solutions[800] have also been investigated, and so has molecular motion in cellulose, pectin, and bean cell walls.[801] It has proved possible[802] to monitor directly by ^{13}C resonance the synthesis of silk fibroin in the silk glands of the silkworm *Bombyx mori.*

Other Proteins. Proton n.m.r. studies of thionins of known sequence from barley and wheat[803] have revealed features of their secondary and tertiary structures similar to those of crambin, a related hydrophobic protein from *Crambe abyssinica*; the methyl-proton spectrum of crambin has also been analysed.[804] The structure and mobilities of wheat gliadins, components of gluten, are also reported,[805,806] the gliadins apparently being much more tightly folded than the glutenin components.

In our final section we list studies of proteins that have found no ready home in the foregoing but are no less important for that: comparisons of HPr

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proteins from different micro-organisms,[807] titration, exchange, and assignments of the *E. coli* adenosine cyclic 3′,5′-phosphate receptor protein,[808] cyclic nucleotide binding of the *E. coli* adenosine cyclic 3′,5′-phosphate receptor protein,[809] the binding of Na^+ ions to bovine Gla protein,[810] ligand binding in the *E. coli* L-arabinose-binding protein,[811] ^{31}P studies of flavodoxin from *Azotobacter vinelandii*,[812] folding of kringle fragment 1 of human plasminogen,[813] association of β-lactoglobulin A,[814] ^{31}P studies of bovine β-casein,[815] the histidines of chicken ovomucoid,[816] the phosphoserine[817] and galactose[818] of hen egg-white ovalbumin, the tryptophan of human serum albumin by optically detected magnetic resonance,[819] self-diffusion of bovine serum albumin using pulsed-gradient spin-echo techniques,[820] divalent metal binding to bovine serum albumin,[821] deuterium resonance of water in immobilized protein systems,[822] and freeze-thawing hysteresis in biological systems.[823]

7 Mössbauer Spectroscopy

Contributed by D. P. E. Dickson

During 1982 work has been reported on haem proteins, iron–sulphur proteins, and iron-transport and -storage proteins. There has also been a continuation of studies involving the use of Mössbauer spectroscopy to investigate protein dynamics. All of the above work has been of iron-containing systems and has used the ^{57}Fe Mössbauer nuclide. In addition there has been one investigation using ^{119}Sn.

Cusack *et al.*[824] have synthesized eleven novel tin(II) and tin(IV) complexes of sulphur-containing amino-acids. Their Mössbauer spectra show that there is bonding between the tin atom and the nitrogen atom of the amino-acid.

Haem Proteins.—The work on haem proteins falls into three main categories: (i) the investigation of the simpler haem proteins and their reaction products, (ii) the observation of unusual spin and valence states of iron in haem proteins, enzymes, and model systems, and (iii) the analysis of complex enzyme systems containing haem centres.

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There have been a number of attempts in the past to observe differences between the Mössbauer parameters of the ferrous ion in the α- and β-subunits of oxyhaemoglobin and deoxyhaemoglobin. A recent careful analysis of the Mössbauer spectra[825] has revealed small differences, which indicate that there is an inequivalence of the iron atom between the two subunits. A Mössbauer study[826] of the properties of the products of the reaction of haems with the superoxide ion, O_2^-, has shown that they are high-spin ferric with a similar structure to peroxo complexes of high-spin ferric porphyrins.

Levy *et al.*[827] have made low-temperature Mössbauer measurements on paramagnetic horse methaemoglobin, which taken with e.p.r. measurements demonstrate the existence of a low-spin ferrous state of haemoglobin that is not identical to the typical hydroxymethaemoglobin low-spin ferrous state. The amount of this species increased when samples were gradually cooled rather than being quenched in liquid nitrogen. This state is formed both from aquomethaemoglobin and hydroxymethaemoglobin and can even be formed from the normal high-spin and low-spin species at 210 K where the water is already frozen. These results are interesting in terms of conformational equilibria in the region of the haem pocket. The data are explained in terms of an energy barrier between the different conformational states.

The intermediate-spin ($S = \frac{3}{2}$) ferric state is unusual, but it is thought to have been observed in certain haem proteins and enzymes. Mössbauer spectroscopic studies[828] suggest that a pure intermediate-spin ferric state exists in a porphyrin complex with a vinylidene group inserted into an iron–nitrogen bond. This spin state appears to occur in both solid-state and frozen-solution samples, and this is the first time that it has been observed in both forms. The origin of the unusual state lies in the highly distorted geometry around the iron atom. The intermediate-spin ($S = 1$) state of Fe^{IV} is thought to occur in reaction products of the haem enzyme peroxidase. Simonneaux *et al.*[829] have presented evidence obtained from Mössbauer spectra that similar states occur in certain thermally unstable air-sensitive iron–porphyrin complexes.

The nitrite reductase from *Thiobacillus denitrificans* has been identified as a haem protein, and Mössbauer spectra obtained by Huynh *et al.*[830] show that the haem *c* : haem *d* ratio is 1 : 1. In the oxidized form both haems are low-spin ferric with characteristic Mössbauer parameters. In the reduced form both haems are ferrous, with the *d* haem giving the characteristically large chemical shift of a high-spin ferrous iron atom, while the parameters of the *c* haem indicate that it remains in the low-spin configuration.

The enzyme cytochrome oxidase from the bacterium *Thermus thermophilus* is a haem protein, cytochrome c_1aa_3. Mössbauer spectra have been

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obtained[831] from samples fully reduced, fully oxidized, and fully oxidized and complexed with CN^-. The cytochromes *a* and c_1 yield spectra quite similar to those reported for cytochromes *c* and b_5. In the oxidized state the Mössbauer data indicate non-interacting low-spin ferric haems, whereas the *a* and c_1 sites of the reduced enzyme are typical of low-spin ferrous haemochromes. Reduced cytochrome a_3 is high-spin ferrous with parameters like those for deoxymyoglobin. With the addition of CN^- to the oxidized enzyme the a_3 site gives a quadrupole-split doublet spectrum with parameters typical of low-spin ferric haem–CN^- complexes. The low-temperature Mössbauer spectra show that the electronic ground state of the a_3–CN^- complex has integer spin, suggesting a coupling between the low-spin ferric haem ($S = \frac{1}{2}$) to Cu^{2+} ($S = \frac{1}{2}$) to yield an $S = 1$ ground state. A sample from a different preparation yielded different spectra. Neither spectra had properties consistent with the widely accepted spin-coupling model. Copper–protoporphyrin–iron(III) complexes could provide possible models for the active site of bovine cytochrome *c* oxidase, which is thought to contain a high-spin ferric iron atom coupled antiferromagnetically to Cu^{2+} to give an $S = 2$ unit. Mössbauer spectra of such complexes indicate that the copper atom is close enough to the iron atom to affect the Mössbauer quadrupole splittings.[832] The Mössbauer spectra show the presence of high-spin ferric iron as in the enzyme, with similar chemical shifts but significantly different quadrupole splittings.

The enzyme hydroxylamine oxidoreductase from *Nitrosomonas europaea* is a complex protein ($M_r = 220\,000$) with an $(\alpha\beta)_3$ structure. Mössbauer spectra obtained by Lipscomb *et al.*[833] from samples in both the oxidized and reduced states indicate that it contains seven *c*-type haems and about one unusual group termed P-460. The Mössbauer data suggest that P-460 contains one iron atom per $\alpha\beta$ subunit and that this iron atom is in a haem group.

The nature of the active centres in photosynthetic systems is of considerable interest. Petrouleas and Diner[834] have obtained Mössbauer spectra of intact and separated Photosystem-II particles of *Chlomydomonas reinhardti*. The data indicate the presence of at least two iron components. One is low-spin ferric and probably arises from cytochrome b_{559}, while the other is light sensitive and is attributed to a ferrous–quinone complex.

Iron–Sulphur Proteins.—In recent years many of the Mössbauer spectroscopic studies of iron–sulphur proteins have involved proteins containing [3Fe–3S] clusters. The Mössbauer spectra of these clusters are characteristic, which has been an important factor in their discovery and classification. Mössbauer spectra obtained from the ferredoxin from *Methanosarcina barkeri*[835] indicate

[831] T. A. Kent, E. Münck, W. R. Dunham, W. F. Filler, K. L. Findling, T. Yoshida, and J. A. Fee, *J. Biol. Chem.*, 1982, **257**, 12 489.
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that it contains [3Fe–3S] clusters and, taken together with e.p.r. data, show that there are no other iron-containing species present.

The interpretation of the Mössbauer spectra of [3Fe–3S] clusters in various biological systems, such as ferredoxin II from *Desulfovibrio gigas*, leads to a spin-coupling model for the cluster.[836] This leads to the conclusion that the enzyme sulphite reductase from *Escherichia coli* contains a sirohaem exchange coupled to a [4Fe–4S] cluster at its active site, and not a [3Fe–3S] cluster.

There is a growing body of evidence that under suitable conditions interconversions can take place between [3Fe–3S] and [4Fe–4S] clusters in iron–sulphur proteins. Moura *et al.*[837] have used Mössbauer spectroscopy to show that, when *D. gigas* ferredoxin II, which normally contains [3Fe–3S] clusters, is reconstituted with excess (5 iron atoms per molecule) iron, [4Fe–4S] clusters are formed, with 3:1 inequivalent iron sites in the oxidized case and 2:2 inequivalent iron sites in the reduced case. Further treatment of this form with $Fe(CN)_6^{3-}$ leads to the formation of typical [3Fe–3S] clusters. Incubation of ferredoxin II with ^{57}Fe leads to the formation of [4Fe–4S] clusters, with the extra iron atom going onto particular iron sites. Bell *et al.*[838] have presented Mössbauer spectroscopic evidence for the conversion of the [4Fe–4S] clusters in the four-iron ferredoxin from *Bacillus stearothermophilus* into [3Fe–3S] clusters following treatment with potassium ferricyanide.

Various enzyme systems are thought to contain iron–sulphur clusters. Mössbauer spectroscopy can be useful in identifying them, as [1Fe], [2Fe–2S], [3Fe–3S], and [4Fe–4S] clusters have characteristically different Mössbauer spectra. The Mössbauer spectra of the mung-bean trypsin inhibitor[839] show two quadrupole-split doublets. The major doublet component has Mössbauer parameters that are typical of a [4Fe–4S] cluster. Mössbauer data obtained by Krüger *et al.*[840] indicate that hydrogenases I and II from *Desulfovibrio desulfuricans* contain two [4Fe–4S] clusters together with one [3Fe–3S] cluster.

There has been considerable interest in the presence and role of iron–sulphur clusters in photosynthetic membranes. The Mössbauer spectra of isolated chromatophores of *Rhodopseudomonas spheroides* between 4.2 and 300 K show the presence of a symmetric quadrupole-split doublet with parameters similar to those of bacterial ferredoxins.[841] Reduction of these chromatophores with sodium dithionite gives a high-spin ferrous doublet and magnetic broadening at 4.2 K.

Iron-transport and -storage Proteins.—The iron-transport protein transferrin contains two iron sites, but recent Mössbauer data[842] indicate that these two sites are equivalent.

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[842] S. Smit, B. Leijnse, and A. M. Van der Kraan, *J. Inorg. Biochem.*, 1981, **15,** 329.

The iron-storage protein ferritin is found in higher animals, and a closely similar protein, sometimes known as phytoferritin, occurs in plants. A somewhat different iron-storage protein has been found in bacteria and has been designated as bacterioferritin.

Mössbauer spectra have been obtained by Goodman *et al.*[843] from samples of plant material from duckweed, stocks, soybean, and pea. The spectra obtained at low temperatures and in the presence of applied magnetic fields show that the iron is present in the ferric form and most of it is in an antiferromagnetically ordered state. The data are consistent with the presence of ferritin, but with iron cores of less than 1.5 nm, compared with the values of 6—7 nm normally found in mammalian ferritin.

A comparison of the Mössbauer spectra of horse-spleen ferritin with those of the possible model compounds serum iron salt and iron phosphate indicates that the growth of the iron cores in ferritin is a chemical process.[844]

The Mössbauer spectra of normal and virus-infected cultured chick-embryo fibroblasts and rat glioma cells have been obtained between 0.08 and 318 K.[845] Iron in both ferritin-like and bacterioferritin-like forms was found in various relative amounts, suggesting a close relationship between the two iron-storage materials. The bacterioferritin-like iron was found to be predominantly membrane bound. At temperatures above 260 K wide lines were observed in the spectra, with different linewidths for normal and virus-infected samples. This was interpreted to indicate different effective viscosities for the iron-containing particles in the two cases.

Mössbauer spectroscopy has been used to investigate the distribution of iron in rat organs and its localization in liver subcellular fractions.[846] Following a daily injection of ^{57}Fe–sucrose into the tail veins of the rats for six days, spectra were obtained from samples of blood, spleen, liver, and liver subcellular fractions. The spectra of the blood samples are consistent with the presence of haemoglobin, while spectral components corresponding to the presence of ferritin are found in the spleen, liver, and liver subcellular fraction samples.

Protein Dynamics.—Mössbauer spectroscopy can yield important information concerning protein dynamics as a result of the sensitivity of the Mössbauer effect to any motion of the Mössbauer nucleus.

Information on the dynamics of metmyoglobin has resulted from a further analysis of Mössbauer data on the temperature dependence of the mean-square displacement of the haem iron.[847] The magnitudes of the smallest mean-square displacements observed at 80 K indicate that intramolecular motions can be frozen out to a surprisingly large degree, although the fact that there are still some significant mean-square displacements points to the existence of conformational substates.

[843] B. A. Goodman, P. C. DeKock, and J. D. Rush, *J. Plant Nutr.*, 1982, **5,** 345.
[844] L. Chen, S. Lian, T. Huang, S. Jiang, X. Yang, M. Wang, and Y. Gu, *Fenzi Kexue Xuebao*, 1982, **2,** 25.
[845] E. R. Bauminger, S. G. Cohen, S. Ofer, and U. Bachrach, *Biochim. Biophys. Acta*, 1982, **720,** 133.
[846] B. K. Semin, A. A. Novakova, A. Yu Aleksandrov, I. I. Ivanov, A. B. Rubin, and R. N. Kuzmin, *Biochim. Biophys. Acta*, 1982, **715,** 52.
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Parak *et al.*[848] have presented further data on the protein dynamics of deoxymyoglobin crystals, involving interpretation of the data in terms of fluctuations between slightly different conformational states. The technique of monitoring protein dynamics by means of Rayleigh scattering of Mössbauer radiation has been applied to metmyoglobin[849] and has shown that there are large intramolecular movements.

8 Protein Interactions

Contributed by D. J. Winzor, P. D. Jeffrey, and L. W. Nichol

The arrangement of material this year reflects the emphasis in the literature on the use of established procedures and theories for the detection and quantitative characterization of different types of interactions involving particular protein systems. These range from intramolecular rearrangements, through self-associations, to heterogeneous associations, particular attention being given in the latter categories to the formation of intricate and extensive assemblies, such as microtubules and multi-enzyme complexes. Nevertheless, attention has been given to basic principles, as in a review on the folding and association of proteins,[850] and to theoretical developments, especially in the areas of affinity chromatography, reversible crosslinking reactions, and the consideration of thermodynamic non-ideality effects. Mention is made of these advances in developing the general theme of progressively increasing complexity in the patterns of specific protein interactions.

Quaternary Organization.—*Overall Geometry and Conformational Changes.* Harding and Rowe[851] examined the problems associated with the interpretation of the solution structures of protein molecules in terms of the several functions that have been formulated. They concluded that, provided the molecules may be regarded as ellipsoids of revolution, the ratio of the sedimentation concentration regression coefficient to the intrinsic viscosity (the R function) is the preferred index for the estimation of axial ratios. The use of the R function calculated using the hydrodynamic parameters of ovalbumin revealed that a hen's egg (axial ratio 1.5 : 1) is a plausible model for the shape of the molecule.[852] A new potential source of error in the assessment of frictional coefficients of macromolecules was identified by Crossley and coworkers,[853] who found anomalous temperature dependence of the quantity by quasi-elastic light scattering.

Conformational changes arising from different types of interactions in proteins have been studied by a variety of techniques. The course of events in the pressure-induced dissociation of the tetramer of pig-heart lactic dehydrogenase

[848] F. Parak, E. W. Knapp, and D. Kucheida, *J. Mol. Biol.*, 1982, **161**, 177.
[849] Yu. F. Krupyanskii, F. Parak, V. I. Gol'danskii, R. L. Mössbauer, E. E. Gaubman, H. Engelmann, and I. P. Suzdalev, *Z. Naturforsch., Teil C*, 1982, **37**, 57.
[850] R. Jaenicke, *Biophys. Struct. Mech.*, 1982, **8**, 231.
[851] S. E. Harding and A. J. Rowe, *Int. J. Biol. Macromol.*, 1982, **4**, 160.
[852] S. E. Harding, *Int. J. Biol. Macromol.*, 1981, **3**, 398.
[853] J. M. Crossley, S. P. Spraggs, J. M. Creeth, N. Noble, and J. Slack, *Biopolymers*, 1982, **21**, 233.

has been explained as an initial dissociation to monomer followed by a monomer conformational change, both processes being accompanied by a negative volume change.[854] Spectral studies of the furylacryloyl derivative of the tetrameric enzyme glyceraldehyde-3-phosphate dehydrogenase have shown that, even in the crystal, it can assume the interconvertible conformations characteristic of the acyl enzyme in solution.[855] Thus this system could provide a good model for crystallographic studies of the molecular basis of half-site reactivity and the effector role played by NAD^+ in catalysis. Small-angle *X*-ray scattering experiments showed that the binding of L-arabinose causes the radius of gyration of L-arabinose-binding protein of *E. coli* to decrease, apparently by the closing of a cleft between two lobes of the protein.[856] A change in tertiary structure was also reported for another protein, human thyroxine-binding globulin, which becomes more compact upon binding thyroxine.[857] Changes in secondary structure were detected by spectral methods for bovine-brain S-100b protein[858] and transcarboxylase.[859] The former shows a decrease in helical content in the presence of calcium, while an increase in helix content accompanies the association that forms the central cylindrical subunit in the latter protein. A conformational change accompanying the binding of adenosine(5′)pentaphospho(5′)adenosine to human-muscle adenylate kinase was also indicated by a ^1H n.m.r. study.[860]

Subunit–Subunit Interactions and Allostery. It was shown that the pyridine moiety of the NAD coenzymes affects the co-operative behaviour in the tetrameric enzyme glyceraldehyde-3-phosphate dehydrogenase,[861] but evidence is still being sought to confirm that the catalytic sites mutually interact.[862] Significant effects of metal ions upon reassociation of the negatively co-operative dimeric enzyme alkaline phosphatase were interpreted in terms of metal-ion stabilization of the monomeric and dimeric structures.[863] A study of the binding of pyridoxal 5′-phosphate to dimeric aspartate aminotransferase showed that strong interactions between enzyme subunits result in weak negative co-operativity at alkaline pH and positive co-operativity at acid pH.[864] Kadenbach and Merle[865] provided a useful analysis of the current state of knowledge of the subunit composition and function of individual subunits of cytochrome *c* oxidase from higher eukaryotes.

[854] K. Müller, H.-D. Lüdemann, and R. Jaenicke, *Biophys. Chem.*, 1981, **14,** 101.
[855] A. Mozzarelli, R. Berni, G. L. Rossi, M. Vas, F. Bartha, and T. Keleti, *J. Biol. Chem.*, 1982, **257,** 6739.
[856] M. E. Newcomer, B. A. Lewis, and F. A. Quiocho, *J. Biol. Chem.*, 1981, **256,** 13 218.
[857] S. Grimaldi, H. Edelhoch, and J. Robbins, *Biochemistry*, 1982, **21,** 145.
[858] R. S. Mani, B. E. Boyes, and C. M. Kay, *Biochemistry*, 1982, **21,** 2607.
[859] J. P. Hennessey, jun., W. C. Johnson, jun., C. Bahler, and H. G. Wood, *Biochemistry*, 1982, **21,** 642.
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[862] J. W. Cardon and P. D. Boyer, *J. Biol. Chem.*, 1982, **257,** 7615.
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[864] M. Arrio-Dupont and D. Vergé, *J. Mol. Biol.*, 1982, **157,** 383.
[865] B. Kadenbach and P. Merle, *FEBS Lett.*, 1981, **135,** 1.

A multi-authored review[866] on the allosteric properties of haemoglobin was complemented by several new studies, including investigations on the effects of specifically bound chloride ions on dimer–tetramer assembly,[867] the linkage between carbon dioxide and four-step oxygen binding,[868] and the effect of pH on the oxygen affinity of the protein.[869] In the latter work the interesting conclusion was reached that protons affect oxygen binding directly rather than through effects on the quaternary-state equilibrium. Another study involved a thorough analysis in thermodynamic terms of the Perutz mechanism, which, it was argued, was not an appropriate description for the molecular processes involving haemoglobin.[870] A theoretical analysis was also made of the kinetics of oxygen binding, which accounts successfully for the experimental characteristics of the process.[871] Several papers on arthropod haemocyanins were published during the year, the emphasis being on the role of the different subunits in controlling the assembly of the oligomers[872,873] and their location within the final structure.[874,875] A comprehensive review of the current picture of both the molluscan and arthropodan haemocyanins was presented by van Holde and Miller.[876] There is growing interest in the crystallins, the proteins of the eye lens. Two studies employed spectral methods to identify structural differences in α-, β-, and γ-crystallins such as differences in β-structure, hydrophobic regions, and sulphydryl accessibility.[877,878] Siezen and Shaw[879] compared lens proteins from the squid, S-crystallins, with vertebrate crystallins and found similarities in quaternary structure and sequence between the S-crystallins and vertebrate β-crystallins.

Aspartate Transcarbamoylase. Four connected papers[880—883] dealt with the assembly and subunit interactions of this complex protein. In the first two, the assembly of the catalytic trimer from folded monomers and unfolded polypeptide chains was shown to be consistent with an assembly mechanism involving rapid collapse of randomly coiled chains to give partially folded monomers,

[866] 'Methods in Enzymology', ed. E. Antonini, L. Rossi-Bernardi, and E. Chiancone, Academic Press, New York, 1981, Vol. 76.
[867] M. A. Flanagan, G. K. Ackers, J. B. Matthew, G. I. H. Hanania, and F. R. N. Gurd, *Biochemistry*, 1981, **20,** 7439.
[868] K. Imaizumi, K. Imai, and I. Tyuma, *J. Mol. Biol.*, 1982, **159,** 703.
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[875] P.-Y. Sizaret, J. Frank, J. Lamy, J. Weill, and J. N. Lamy, *Eur. J. Biochem.*, 1982, **127,** 501.
[876] K. E. van Holde and K. I. Miller, *Q. Rev. Biophys.*, 1982, **15,** 1.
[877] J. N. Liang and B. Chakrabarti, *Biochemistry*, 1982, **21,** 1847.
[878] U. P. Andley, J. N. Liang, and B. Chakrabarti, *Biochemistry*, 1982, **21,** 1853.
[879] R. J. Siezen and D. C. Shaw, *Biochim. Biophys. Acta*, 1982, **704,** 304.
[880] D. L. Burns and H. K. Schachman, *J. Biol. Chem.*, 1982, **257,** 8638.
[881] D. L. Burns and H. K. Schachman, *J. Biol. Chem.*, 1982, **257,** 8648.
[882] D. L. Burns and H. K. Schachman, *J. Biol. Chem.*, 1982, **257,** 12 214.
[883] S. Subramani and H. K. Schachman, *J. Biol. Chem.*, 1982, **257,** 12 219.

followed by a slow conformational change producing competent monomers, which then associate rapidly *via* dimers to form stable, active trimers. The significance of various intersubunit interactions was also investigated. It was found, firstly, in experiments with the trimeric catalytic subunits that substrates cause a marked strengthening of bonds within this entity and hence these interactions are likely to be important to the co-operativity of the enzyme.[882] Secondly, a model accounting for the characteristics of the ligand-promoted decrease in the strength of the bonds between the catalytic and regulatory subunits was presented.[883] Tauc and co-workers[884] formulated a model that stipulates homotropic and heterotropic co-operative interactions between catalytic sites of the enzyme promoted by effectors in terms of a 'primary effect', exerted site by site, and a 'secondary effect', mediated by substrate.

In a very interesting crystallographic study, the structure of the complex between *N*-(phosphonacetyl)-L-aspartate and aspartate transcarbamoylase was determined at 3.5 Å resolution.[885] This complex provides a model for the gross structural changes accompanying substrate activation of the enzyme. It was found that rotations of the catalytic trimers around the three-fold axis and of a regulatory dimer around its two-fold axis, plus some expansion of the structure along axes, considerably enlarge the central cavity of the molecule and allow increased accessibility to it from the exterior when substrate is bound.[885] A calculation of the low-angle *X*-ray scattering curve expected at pH 5.8 from these results with that obtained experimentally at pH 8.3 indicates that the structural changes determined in the three-dimensional *X*-ray analysis reflect those occurring under conditions where the enzyme is active.[886]

Self-associating Systems.—*Novel Methods of Analysis.* In addition to studies of specific self-associating protein systems there have been several methodological developments. Availability of two-phase aqueous systems has prompted re-investigation of the simulated behaviour of self-associating solutes in counter-current distribution.[887] A second theoretical study has been concerned with the effect of the membrane compression wave on the light-scattering spectrum of a protein undergoing reversible dimerization.[888] Xu and Weber[889] have addressed the problem of protein dimerization for which the apparent equilibrium constant is a function of the degree of dissociation: the concept of time-averaged chemical potential is used in conjunction with a model in which monomer and dimer undergo structural changes at rates that are slow in comparison to those of association and dissociation. There have also been refinements of existing methodologies. For example, the use of non-linear least-squares techniques has been discussed in relation to improving the

[884] P. Tauc, C. Leconti, D. Kerbiriou, L. Thiry, and G. Hervé, *J. Mol. Biol.*, 1982, **155,** 155.
[885] J. E. Ladner, J. P. Kitchell, R. B. Honzatko, H. M. Ke, K. W. Volz, A. J. Kalb (Gilboa), R. C. Ladner, and W. N. Lipscomb, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79,** 3125.
[886] R. B. Altman, J. E. Ladner, and W. N. Lipscomb, *Biochem. Biophys. Res. Commun.*, 1982, **108,** 592.
[887] L. Backman, *J. Chromatogr.*, 1982, **237,** 185.
[888] S. L. Whittenburg, *J. Theor. Biol.*, 1982, **96,** 151.
[889] G.-J. Xu and G. Weber, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79,** 5268.

analysis of sedimentation equilibrium[890] and sedimentation velocity[891] distributions for self-associating macromolecular systems. Halvorson[892] has described the technique of significant factor analysis for defining the number of species present in an interacting mixture.

The study of concentrated solutions has also attracted attention, particularly in regard to consideration of thermodynamic non-ideality in terms of excluded volume. By this means it was shown[893] that the exclusion of low concentrations of polyethylene glycol by albumin can be explained by the simple excluded-volume model but that the precipitation of proteins by polyethylene glycol is seemingly more complex. The self-association of myoglobin has been detected[894] by comparing the concentration dependence of its apparent molecular weight with that of haemoglobin and showing that the observed dependence of the molecular-weight ratio is inconsistent with that predicted on the basis of excluded volumes of non-associating solutes. Of interest in relation to the prediction of solute activity coefficients in terms of excluded volumes is the extension of the hard-sphere model to allow for electrostatic repulsion between charged molecules, which is manifested as an increase in the radius of the effective hard-spherical particle representing the protein.[895] To this end the recent report[896] of a simple method of protein-charge estimation is timely, since knowledge of protein valence is required for such calculations of the effective radius.

Equilibria and Perturbations by Ligand. The high-pressure dissociation of porcine lactate dehydrogenase has been shown to reflect a two-state tetramer–monomer equilibrium, characterized by a volume change, $\Delta V_{diss.}$, of -420 ml mol^{-1},[897] whereas a favourable entropy change provides the energy for the isodesmic self-association of the S5 protein from the *E. coli* 30 S ribosomal subunit.[898] Sedimentation equilibrium results obtained with C-phycocyanin are best described by the coexistence in equilibrium of monomeric, trimeric, and hexameric forms, the existence of which has also been detected by sedimentation velocity experiments in a band-forming centrepiece.[899] The association of bovine SH-κ-casein is characterized by a critical micelle concentration that decreases with increasing ionic strength, the degree of polymerization being 30 and independent of ionic strength between 0.1 M and 1.0 M.[900] High-concentration active-enzyme centrifugation has been used to detect three new active polymeric forms of pig-kidney phosphofructokinase,[901] and isoelectric focusing has been used to detect a range of oligomeric

[890] M. L. Johnson, J. J. Correia, D. A. Yphantis, and H. R. Halvorson, *Biophys. J.*, 1981, **36,** 575.
[891] G. P. Todd and R. H. Haschemeyer, *Proc. Natl. Acad. Sci. U.S.A.*, 1981, **78,** 6739.
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[893] D. H. Atha and K. C. Ingham, *J. Biol. Chem.*, 1981, **256,** 12 108.
[894] A. P. Minton and M. S. Lewis, *Biophys. Chem.*, 1981, **14,** 317.
[895] A. P. Minton and H. Edelhoch, *Biopolymers*, 1982, **21,** 451.
[896] C. L. Ford and D. J. Winzor, *Biochim. Biophys. Acta*, 1982, **703,** 109.
[897] K. Müller, H.-D. Lüdemann, and R. Jaenicke, *Biophys. Chem.*, 1982, **16,** 1.
[898] S. H. Tindall and K. C. Aune, *Arch. Biochem. Biophys.*, 1982, **214,** 516.
[899] C. Huang and D. S. Berns, *Biochemistry*, 1981, **20,** 7016.
[900] H. J. Vreeman, J. A. Brinkhuis, and C. A. van der Spek, *Biophys. Chem.*, 1981, **14,** 185.
[901] C. S. Johnson and W. C. Deal, jun., *J. Biol. Chem.*, 1982, **257,** 913.

forms of pig-heart NAD-specific isocitrate dehydrogenase.[902] Other studies of protein self-association have demonstrated the existence of a dimer–tetramer equilibrium in solutions of pig-heart fumarase,[903] the reversible dissociation of the first component of human complement,[904] the self-association of yeast *exo*-β-glucanases,[905] and the reversible interconversion of monomeric and polymeric forms of 2',3'-cyclic nucleotide 3'-phosphodiesterase.[906]

The feasibility of protein self-association providing a means of metabolic control is amply demonstrated by a study of rabbit-muscle phosphofructokinase, the simplest mode of association for which is $M \rightleftarrows M_2 \rightleftarrows M_4 \rightleftarrows M_{16}$ on the basis of sedimentation velocity studies.[907] Whereas the presence of activators such as ADP and phosphate favours the formation of tetramers, the inhibitor citrate favours the formation of dimers.[907] In a separate study of this enzyme, physiologically significant concentrations of methylamine derivatives are shown to enhance not only enzymic stability but also the state of aggregation of phosphofructokinase.[908] Sedimentation equilibrium has been used to obtain quantitative information on the interplay between protein self-association and ligand binding for several systems, such studies having shown, for example, that the binding of zinc ions to human carbon monoxyhaemoglobin causes dissociation into $\alpha\beta$ dimers.[909] Likewise, reversible dissociation of dimeric concanavalin A is effected by 2-propanol.[910] For rabbit-muscle troponin C, a monomer–dimer system, the association constant is increased some seven-fold by the presence of calcium ions (0.1mM).[911] Enhanced association is also observed with myelin basic protein in the presence of detergents and lipids, the effect of lysophosphatidylcholine being to increase not only the dimerization constant but also the isodesmic constant for the indefinite self-association of dimers.[912] The aggregation equilibria of *E. coli* RNA polymerase core and holoenzyme (core enzyme plus σ-factor) have been studied by sedimentation velocity and light scattering.[913,914] At concentrations of sodium chloride less than 0.2 M the core aggregates to a tetramer in the absence of $MgCl_2$ and to an octamer in its presence, the holoenzyme forming dimers in the presence or absence of $MgCl_2$; substitution of iodide for chloride suppresses aggregation, whereas fluoride enhances the association phenomena.[913] Other examples of ligand-induced self-association include the reassociation of dissociated formyltetrahydrofolate synthetase to its enzymically active tetrameric state by mono-

[902] S. Hayman and R. F. Colman, *Arch. Biochem. Biophys.*, 1982, **218,** 492.
[903] S. Beeckmans and L. Kanarek, *Int. J. Biochem.*, 1982, **14,** 971.
[904] R. J. Ziccardi and J. Tschopp, *Biochem. Biophys. Res. Commun.*, 1982, **107,** 618.
[905] A. Sánchez, A. R. Nebreda, and T. G. Villa, *FEBS Lett.*, 1982, **145,** 213.
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[907] L. K. Hesterberg and J. C. Lee, *Biochemistry*, 1982, **21,** 216.
[908] S. C. Hand and G. N. Somero, *J. Biol. Chem.*, 1982, **257,** 734.
[909] R. D. Gray and W. L. Dean, *Arch. Biochem. Biophys.*, 1982, **217,** 202.
[910] A. J. Sophianopoulos and J. A. Sophianopoulos, *Arch. Biochem. Biophys.*, 1982, **217,** 751.
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[912] R. Smith, *Biochemistry*, 1982, **21,** 2697.
[913] S. L. Shaner, D. M. Piatt, C. G. Wensley, H. Yu, R. R. Burgess, and M. T. Record, jun., *Biochemistry*, 1982, **21,** 5539.
[914] H. Heumann, P. Stöckel, and R. May, *FEBS Lett.*, 1982, **148,** 91.

valent cations[915] and the aggregation of fibrinogen by metal ions[916] and of human-urine β-galactosidase by galactose.[917]

In contrast to all of the above examples, which have entailed the detection of preferential ligand binding by observing displacement of monomer–polymer equilibria, direct evidence of preferential interaction has been obtained for the binding of oxygen to sickle-cell haemoglobin.[918] The binding of oxygen to the gel (polymer) phase is non-co-operative, the association constant being about three times smaller than that for normal haemoglobin in the low-affinity T quaternary state. In another study entailing a different methodological approach, a theoretical analysis of the kinetics of 'hysteretic' allosteric enzymes has been made in terms of a model in which the interconversion between dimeric and monomeric states of an enzyme is much slower than the binding of ligand to equivalent and independent sites on each oligomeric form.[919] Graphical methods are used to analyse the relaxation kinetics of this dissociating enzyme system for a range of association rate constants, and also for situations in which only one enzymic form binds ligand.

Actin Polymerization. This process involves basically indefinite self-association of the nucleated helical polymerization type, but it is evidently complicated in its detailed mechanism. Fluorescence photobleaching recovery has proven a valuable method for unravelling the complexities of the polymerization and subsequent gelation.[920] Wegner and Neuhaus[921] showed that in the head-to-tail polymerization high concentrations of divalent cations cause association and dissociation reactions to occur mainly at different ends of the filament, whereas at low concentrations or in the presence of potassium ions these reactions take place at each end with similar frequencies. An analysis of the kinetics of the polymerization showed that spontaneous fragmentation of actin filaments occurs, its extent depending upon solution parameters.[922] Some interesting biological aspects of the polymerization of actin were explored in a study of the thermodynamics of the process in fourteen vertebrate species ranging from desert lizards to deep-sea fish.[923] The findings suggested that conservation of the ability of actin to polymerize under different conditions of temperature and pressure is achieved by alterations in the types of bonds involved and by changes in the energy costs of altering the actin conformation during the assembly process.

A variety of compounds are known to affect the extent of actin polymerization, an example being provided by profilin, which was shown to bind to monomeric actin to give an inert complex thereby perturbing the coexisting equilibrium reactions of the polymerization process.[924] It was shown that

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[916] F. S. Steven, M. M. Griffin, B. S. Brown, and T. P. Hulley, *Int. J. Biol. Macromol.*, 1982, **4**, 367.
[917] E. Paschke, R. Niemann, G. Strecker, and H. Kresse, *Biochim. Biophys. Acta*, 1982, **704**, 134.
[918] H. R. Sunshine, J. Hofrichter, F. A. Ferrone, and W. A. Eaton, *J. Mol. Biol.*, 1982, **158**, 251.
[919] S. V. Klinov and B. I. Kurganov, *J. Theor. Biol.*, 1982, **98**, 73.
[920] J. F. Tait and C. Frieden, *Biochemistry*, 1982, **21**, 3666.
[921] A. Wegner and J.-M. Neuhaus, *J. Mol. Biol.*, 1981, **153**, 681.
[922] A. Wegner and P. Savko, *Biochemistry*, 1982, **21**, 1909.
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[924] F. Markey, H. Larsson, K. Weber, and U. Lindberg, *Biochim. Biophys. Acta*, 1982, **704**, 43.

deoxyribonuclease I inhibits the filament assembly at specific ends of F-actin.[925] Other mixed associations affecting actin-filament assembly, in non-muscle cells, have been studied. Attention has been given to the identification of actin-containing complexes that bind cytochalasins, the ability of these structures to stimulate actin-filament assembly *in vitro*, and the inhibition of the assembly process by a class of proteins with cytochalasin-like activity.[926] Two groups of workers[927,928] concentrated their attention on the interactions of α-actinin and vinculin with actin. The former[927] believe that the proteins interact sideways with actin filaments and affect their interactions with cell membranes by structural rearrangement rather than direct involvement. The latter[928] showed that calcium affects the interaction of both α-actinin and vinculin (from HeLa cells) with actin. Koteliansky and co-workers[929] found that filamin from chicken gizzard is actually a more effective stimulator of actin polymerization than is α-actinin, and they speculate that this protein may therefore be very important in microfilament formation in non-muscle cells.

Tubulin and Microtubules. Ligands that inhibit the assembly of tubulin were discussed in several reports.[930—934] It was shown, for example, that the 1:1 complex between tubulin and colchicine, involving tryptophan residues,[931] in the presence of GTP has similar assembly properties to those of tubulin itself.[932] However, a sedimentation equilibrium study of the colchicine–tubulin $\alpha\beta$ dimer at low temperature showed that it dissociates less readily than does the unliganded dimer.[930] A related altered conformation of tubulin was postulated as being responsible for the colchicine poisoning of microtubule assembly.[930] The drug griseofulvin, which disrupts the mitotic apparatus *in vivo* and inhibits microtubule formation *in vitro*, apparently acts directly and stoicheiometrically with the tubulin dimer.[933] Another reagent, 1-fluoro-2,4-dinitrobenzene, can inhibit assembly completely by incorporation of between one and two dinitrophenyl groups to form dinitrophenyltubulin.[934]

Tubulin interactions with other proteins have also claimed attention, with chromatographic determination of a dissociation constant of 4μM being reported for the calmodulin–tubulin dimer system,[935] and with the finding that G-actin also binds to the protein in solution.[936] At a higher level of structural organization, the binding of dynein 21 S ATPase to microtubule doublets has

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[927] B. M. Jockusch and G. Isenberg, *Cold Spring Harbor Symp. Quant. Biol.*, 1982, **46,** 613.
[928] K. Burridge and J. R. Feramisco, *Cold Spring Harbor Symp. Quant. Biol.*, 1982, **46,** 587.
[929] V. E. Koteliansky, V. P. Shirinsky, G. N. Gneushev, and V. N. Smirnov, *FEBS Lett.*, 1981, **136,** 98.
[930] H. W. Detrich, tert., R. C. Williams, jun., and L. Wilson, *Biochemistry*, 1982, **21,** 2392.
[931] R. B. Maccioni and N. W. Seeds, *Biochem. Biophys. Res. Commun.*, 1982, **108,** 896.
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[935] H. Kumagai, E. Nishida, and H. Sakai, *J. Biochem.* (*Tokyo*), 1982, **91,** 1329.
[936] A. B. Verkhovsky, I. G. Surgucheva, V. I. Gelfand, and V. A. Rosenblat, *FEBS Lett.*, 1981, **135,** 290.

been discussed.[937] The roles of GDP and GTP in the assembly–disassembly process were explored by several groups[938–941] with the cautionary note sounded that the properties of this process may depend on the method of isolating the protein.[938] Farell and Jordan[939] also advanced a warning with their conclusion that it is premature to use microtubule structural-polarity assignments as a basis for predicting microtubule kinetic polarity *in vivo*. Sedimentation equilibrium was used to identify 18S and 30S oligomers in glycerol-cycled bovine-brain microtubule protein.[942] These species are apparently mainly determined by the content of microtubule-associated proteins (MAPs). MAPI alone is able to promote tubulin assembly in microtubules,[943] while the effects on the assembly process of dimethyl sulphoxide,[944] cobalt,[945] and nickel[946] were also studied.

With regard to network formation, viscometry was employed to conclude that porcine-brain tubulin can associate with neurofilaments to produce three-dimensional networks *in vitro*.[947] The larger picture gained further texture by the immunofluorescence experiments of Brenner and Brinkley,[948] which indicate that centrioles and pericentriolar material, collectively known as the centrosome, serve as templates for the initiation and assembly of specific microtubule arrays in cells and thus constitute the primary microtubule organizing centres of interphase cells.

Mixed Associations.—*Discrete-complex Formation.* Determination of thermodynamic-association constants using fluorescence polarization for the 1 : 1 complex formation between α_1-protease inhibitor (α_1-antitrypsin) and two derivatives of chymotrypsin modified at the active site has implicated serine as an important residue in the strong association of the inhibitor to the unmodified enzyme.[949] This inhibitor is normally present in the serum at a concentration level of approximately 0.13%, but lower levels are found in certain individuals prone to chronic obstructive pulmonary disease likely as a result of elastolysis in the lung. The relevance of such studies of protease-inhibitor reactions is evident, and other examples are to be found in work on the interaction of urinary trypsin inhibitor with bovine trypsin,[950] of antithrombin with pancreatic trypsin,[951] and of porcine serum and colostrum protease

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[950] M. Tominaga, H. Takeda, and M. Muramatu, *J. Biochem. (Tokyo)*, 1982, **91**, 1391.
[951] R. F. Wong, T.-L. Chang, and R. D. Feinman, *Biochemistry*, 1982, **21**, 6.

inhibitors with trypsin, chymotrypsin, and elastase.[952] In the same area, both intrinsic protein fluorescence[953] and small-angle *X*-ray scattering[954] have established that α_2-macroglobulin binds maximally two molecules of trypsin to form a complex with radius of gyration 7.1 nm. Since both α_1-antitrypsin and α_2-macroglobulin are physiologically important inhibitors in the serum, investigation of competitive equilibria between them and trypsin[955] is particularly noteworthy.

There are interactions involving enzymes other than protease inhibition that contribute to control responses. Illustrations are provided by the finding of significant interactions between NADPH-cytochrome *c* reductase, haem oxygenase, and biliverdin reductase,[956] three enzymes involved in the catalytic conversion of haem to bilirubin, and by the demonstration that 1,3-diphosphoglycerate tightly bound to 3-phosphoglycerate kinase is the substrate for glyceraldehyde-3-phosphate dehydrogenase in a direct-transfer reaction *via* an enzyme–substrate–enzyme complex in the glycolytic pathway.[957] Moreover, thermodynamic characterization has been provided for the association of lumazine protein, with bacterial luciferase employing the decay of the lumazine emission anisotropy,[958] and of the binding of human carbonic anhydrase II to CO-haemoglobin; interestingly, the latter study[959] utilized a counter-current distribution technique, the theory for which, as applied to interacting systems, has been extensively formulated in the past but little used.

Interest has continued in the increasing number of interactions involving the cytochromes, emphasis being given to association of cytochrome *c* with cytochrome *c* oxidase[960] and with cytochrome b_5,[961] which in a separate study[962] was shown to bind to each subunit of methaemoglobin by complementary electrostatic interaction. Work has also continued on lectin interactions, with evidence for the binding of concanavalin A to transferrin[963] and for the mixed association of two lectins from the single plant source *Vicia cracca*.[964] Moreover, we find progress in the understanding of the function of fibronectin, both in terms of the hypothesis that circulatory fibronectin in binding tightly to modified (denatured) proteins may mediate their uptake by the reticuloendothelial system[965] and that fibronectin in the extracellular matrix may influence cell shape, mobility, and physiological function by direct interaction with the cytoskeletal constituents, vinculin, α-actinin, tropomyosin, and myosin.[966]

[952] B. G. Ohlsson, B. R. Weström, and B. W. Karlsson, *Biochim. Biophys. Acta*, 1982, **705**, 357.
[953] D. L. Straight and P. A. McKee, *Biochemistry*, 1982, **21**, 4550.
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[955] K. Beatty, J. Travis, and J. Bieth, *Biochim. Biophys. Acta*, 1982, **704**, 221.
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[958] A. J. W. G. Visser and J. Lee, *Biochemistry*, 1982, **21**, 2218.
[959] L. Backman, *Eur. J. Biochem.*, 1981, **120**, 257.
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[962] M. R. Mauk and A. G. Mauk, *Biochemistry*, 1982, **21**, 4730.
[963] J.-P. Kerckaert and B. Bayard, *Biochem. Biophys. Res. Commun.*, 1982, **105**, 1023.
[964] C. M. Baumann and H. Rüdiger, *FEBS Lett.*, 1981, **136**, 279.
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[966] V. E. Koteliansky, H. N. Gneushev, M. A. Glukhova, A. S. Shartava, and V. N. Smirnov, *FEBS Lett.*, 1982, **143**, 168.

A noteworthy feature of several (but not all) of the papers cited in this section is the reporting of values for apparent equilibrium constants governing the mixed associations. This reassuring trend is well exemplified in an article dealing with several types of interaction involving bovine prothrombin and fragments of it produced by specific catalysed proteolysis.[967] Thus, a dimerization constant of 10^{10} M^{-1} was found for one fragment (NH_2-terminal residues 1—39), a heterogeneous association constant of 10^9 M^{-1} for the complex formation of this fragment with that comprising the NH_2-terminal 156 amino-acid residues (fragment 1), and a value of 10^8 M^{-1} for the constant governing the interaction between fragment 1—39 and an antibody raised against fragment 1. The work[967] in developing the hypothesis that a hydrophobic region of prothrombin containing at least the NH_2-terminal 39 residues is specifically involved in the dimerization reaction and in the binding of prothrombin to phospholipid vesicles is a relevant contribution to the understanding of one step in the regulation of blood coagulation.

Multi-enzyme Complexes. Studies of the reassociation of the pyruvate dehydrogenase complex of *E. coli* suggested that the initial binding of E1 (pyruvate dehydrogenase) subunits to the E2E3 (dihydrolipoamide transacetylase–dihydrolipoamide dehydrogenase) subcomplex can lead to the production of a metastable complex that slowly rearranges to a more stable form.[968] In the same study the binding of one E1 dimer to an E2E3 protomer was shown to be consonant with a limiting polypeptide-chain stoicheiometry of 2:1:1 for the components E1:E2:E3. The kinetics of the reactivation of the complex from *B. stearothermophilus* indicated that reshuffling of the reassembled subunits, not regeneration of E2 or E3, was rate-determining in the generation of activity, the *in vitro* assembly being a spontaneous process.[969] A new reversible crosslinking procedure utilizing bis(imidoesters) was applied to this complicated assembly and shown to have considerable promise for elucidating subunit arrangement in multi-enzyme complexes.[970] Spectral studies of the interaction of the pyruvate dehydrogenase component with the coenzyme thiamine pyrophosphate were interpreted in terms of the presence of two active centres of equal catalytic efficiency in the molecule.[971]

Sumrada and Cooper[972] concluded that urea degradation in yeast is carried out by a protein composed of identical multi-functional subunits rather than a complex of different enzymes. In contrast, the three RNA processing enzymes in *E. coli* were shown likely to exist as a complex.[973] Dang and co-workers[974] provided a useful summary of the state of knowledge of the complexes of aminoacyl-tRNA synthetases in eukaryotes in their compilation of physicochemical data from a variety of sources.

[967] D. A. Madar, M. M. Sarasua, H. C. Marsh, L. G. Pedersen, K. E. Gottschalk, R. G. Hiskey, and K. A. Koehler, *J. Biol. Chem.*, 1982, **257**, 1836.
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[974] C. V. Dang, D. L. Johnson, and D. C. H. Yang, *FEBS Lett.*, 1982, **142**, 1.

Insulin Receptor Binding. The complexities of this interaction continue to be elucidated by a number of different approaches. Corin and Donner[975] used kinetic studies to suggest a model with two interconvertible classes of receptor binding sites rather than co-operative interactions between sites, while Clark and Harrison[976] and Pilch[977] directed their attention to the types of reactive groups on the receptor that might be involved. The former workers found evidence for a covalent linkage *via* disulphide–sulphydryl interchange. The latter study with the histidine-specific reagent diethyl pyrocarbonate suggested that a histidine residue is required. Gould and co-workers[978] contributed to future studies of the properties of the insulin receptor by the discovery that it can be successfully solubilized by the dialysable detergent octyl β-glucoside. An examination of the properties of insulin and its receptor led to the suggestion that the self-association behaviour of insulin could explain many of the observations, including the phenomenon of receptor clustering on the membrane surface and the characteristics of the binding response.[979]

Affinity Chromatography. The relative infancy of affinity chromatography as a method for studying interactions is evident from the fact that there are virtually as many investigations concerned with methodological developments as there are reports of its application to experimental systems. Affinity chromatography on phenathiazine-Sepharose has been used to show that bovine-brain calmodulin, rabbit-muscle troponin C, and bovine-brain S100b protein bind to the antipsychotic drug in a calcium-dependent manner.[980] In similar vein, the binding of rabbit-muscle troponin T and several of its fragments to tropomyosin immobilized on Sepharose has been used to provide evidence for a two-site model of troponin attachment to tropomyosin.[981] The interplay between self-association of neurophysin and its interaction with oxytocin and vasopressin has been assessed by quantitative affinity chromatography on agarose to which the tripeptide Met-Tyr-Phe had been covalently attached;[982] the results are consistent with the well established concept that neurophysin II is a reversibly dimerizing system and that both neuropeptide and tripeptide analogues are bound preferentially by dimer. A partition-equilibrium study of the interaction between glyceraldehyde-3-phosphate dehydrogenase and aldolase using NAD-Sepharose as affinity matrix has also been reported.[983]

The scope of quantitative affinity chromatography for studying interactions has been extended considerably by a theoretical study of the consequences of an interplay between ligand binding and reversible adsorption of a multi-valent

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[976] S. Clark and L. C. Harrison, *J. Biol. Chem.*, 1982, **257,** 12 239.
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[982] S. Angal and I. M. Chaiken, *Biochemistry*, 1982, **21,** 1574.
[983] M. Kálmán and L. Boross, *Biochim. Biophys. Acta*, 1982, **704,** 272.

macromolecular solute.[984] Explicit expressions are derived for a range of combinations of interactions wherein the ligand interacts with either the solute or the matrix and hence perturbs the solute–matrix equilibria. These relationships, which permit quantitative interpretation of a wider range of affinity chromatography experiments designed to elucidate thermodynamic parameters, are also considered in relation to their relevance to metabolite-induced changes in the subcellular distribution of proteins and enzymes.[984] Whereas previous treatment of quantitative affinity chromatography has been based on partition-equilibrium concepts, a theory of column chromatography has been developed that considers chemical kinetics and mass-transfer kinetics in experiments where there is competition between matrix-bound and free ligand for the partitioning macromolecular solute.[985] This more general theory of quantitative affinity chromatography identifies the constraints on rate constants that are imposed by application of the earlier expressions based on equilibrium considerations; it also extends, in principle, its potential to include the evaluation of rate constants as well as thermodynamic parameters.

A novel experimental development is the report of affinity chromatography with a mobile zone of affinity ligand instead of a column of immobilized ligand.[986] The experiment is conducted on a conventional gel-chromatography column, a zone of the slower-migrating reactant (B) being placed on the column before application of a zone of faster reactant (A). Interaction between the two reactants during coincidence of the two migrating zones leads to distortion of the elution profiles because of the different migration rate of the AB complex. The consequent change in the elution volume of slow constituent may be interpreted in terms of the equilibrium constant for the reaction $A+B \rightleftarrows AB$, as demonstrated in a study of the interaction between single-strand DNA and ribonuclease.[986]

Crosslinking Reactions.—*Chemically Induced Crosslinking.* Crosslinking reagents have been used to probe subunit contact interactions, as in a study of the crosslinking of phosphorylase kinase with dimethylsuberimidate,[987] and to form covalently linked protein polymers,[988,989] for example symmetric and asymmetric dimers of insulin linked *via* specific amino groups of the monomers.[988] The major thrust in this area, however, has been the use of heterobifunctional reagents to crosslink proteins of dissimilar kind, examples being provided by the linkage of rabbit skeletal troponin and α-tropomyosin[990] and of yeast cytochrome *c* and beef-heart cytochrome *c* oxidase.[991] A typical advance in this type of endeavour was the development of the radioactively labelled reagent 3-[(2-nitro-4-azidophenyl)-2-aminoethyldithio]-*N*-succinimidyl propionate, which may be covalently linked to one

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[985] H. W. Hethcote and C. de Lisi, *J. Chromatogr.*, 1982, **248**, 183.
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protein in the dark and forms the bridge with the second protein on photolysis.[992] The authors of the latter work,[992] which used the crosslinking of gelatin and fibronectin as a model, pointed to the potential of identifying protein–protein and other interactions at the cell surface by reduction of the crosslink that effects transfer of the radioactive label to the second acceptor.

Macroassemblies Involving Self-association. It is well known that, without the addition of any synthetic crosslinking reagent, several proteins self-associate in a variety of patterns to form linear chains, helices, ellipsoids, or extensive three-dimensional networks by crosslinking of molecules of the same kind. The protein from tobacco mosaic virus provides an example where Raman spectroscopy has established that the distribution of the four tyrosine residues per molecule among hydrogen-bonding states is different for the rod-shaped (microscopically helical) form and the disc, composed of protein molecules.[993] Flagellin A and B are two other proteins capable of self-association to form filamentous structures, but in this instance it has been shown that both are conjointly necessary for the formation of complete flagella.[994] The nucleated-helical polymerizations of actin and of tubulin, discussed earlier, may also be viewed as crosslinking reactions, as may the polymerization of fibrin.[995—997] In this area it has been confirmed that release of fibrinopeptide A from fibrinogen is the necessary prerequisite for association;[998] the study compared light-scattering results of solutions in which fibrinogen was activated by reptilase, which releases fibrinopeptide A, and by thrombin, which releases both fibrinopeptides A and B. The two types of sites formed by thrombin action, together with sites thought already to be present on fibrinogen, ensure that the fibrin monomer is multi-functional, capable of complex formation with fibrinogen[999] and of self-crosslinkage in end-to-end, staggered overlap and lateral-aggregation patterns.[998,1000] Work on plasmin-catalysed fibrinolysis also appeared with the demonstration of the reversible crosslinking of α_2-plasmin inhibitor to fibrinogen by plasma transglutaminase.[1001]

Association of Two Reactants. Theoretical work has been extended on the crosslinking of a multi-valent acceptor by a bivalent ligand to form an equilibrium mixture of complexes comprising networks of alternating acceptor and ligand molecules.[1002] This work, relevant for example to a model antigen–antibody system, gives explicit expressions for the evaluation of interaction

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[1002] L. W. Nichol, M. J. Sculley, and D. J. Winzor, *J. Theor. Biol.*, 1982, **96,** 723.

parameters from a combination of precipitin and radioimmunoassy experiments and discusses for the first time the implications of ligand self-interaction. It was shown that dilution of such a crosslinking mixture by solvent may result in the appearance of a precipitate, provided a ligand self-interaction (such as dimerization) jointly operates with the array of crosslinking reactions.[1002] Since such theoretical developments and several formulations on which they are based neglect the heterogeneity of naturally occurring antibody populations, it is timely to note the extensive work in progress on the preparation and interactions of monoclonal antibodies. An illustrative set of papers[1003—1006] concerns studies, including radioimmunoassays, on a series of monoclonal antibodies directed against different functional domains of fibronectin; many other acceptors, however, have been employed in similar work, including tubulin,[1007] cytoskeletal proteins,[1008,1009] fibrinogen,[1010] and a variety of enzymes.[1011—1015]

The numerical simulation of multi-modal reaction boundaries pertaining to the sedimentation velocity of systems in which a macromolecular equilibrium association is mediated by a ligand has been performed by Cann.[1016] It is noted, however, that a ligand bridge may not necessarily be involved in such reactions, which may instead proceed by a ligand-induced conformational change of the macromolecule. In a less theoretical vein, we find reports of the crosslinking of F-actin filaments by fodrin,[1017] an axonally transported protein of molecular weight 930000, and fimbrin,[1018] a cytoskeletal protein of molecular weight 68000. In contrast, when protamine was added to F-actin it caused breakage of the filaments.[1019] The reaction of protamine with G-actin, however, resulted in several forms of macroassembly depending on the constituent molar ratio of addition and the ionic strength.[1019]

Muscle-protein Interactions.—Of the multitude of interactions that occur between muscle proteins, those between myosin and actin (thin filament) have attracted most attention. For reasons of solubility, myosin has generally been

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[1014] M. L. Dao, B. C. Johnson, and P. E. Hartman, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79**, 2860.
[1015] M. Nakane and T. Deguchi, *FEBS Lett.*, 1982, **140**, 89.
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[1017] J. R. Glenney, jun., P. Glenney, and K. Weber, *J. Biol. Chem.*, 1982, **257**, 9781.
[1018] A. Bretscher, *Proc. Natl. Acad. Sci. U.S.A.*, 1981, **78**, 6849.
[1019] E. Grazi, E. Magri, and I. Pasquali-Ronchetti, *Biochem. J.*, 1982, **205**, 31.

replaced by one of its proteolytic fragments, either the double-headed fragment (heavy meromyosin) or the single-headed subfragment 1. Comparative studies of the binding of myosin and heavy meromyosin to actin have yielded essentially the same binding constant and the same co-operative responses in the presence of tropomyosin–troponin complex,[1020] thereby confirming the assumption that the remainder of the myosin molecule (light meromyosin) is not involved in the interaction with binding sites on actin.

Rate and equilibrium constants for the interaction of myosin subfragment 1 with F-actin in the presence of ADP have been determined from pressure-perturbation studies on the basis of non-co-operative binding.[1021] However, the absence of nucleotide results in binding that is co-operative and dependent on filament concentration, being weaker at higher concentrations of F-actin.[1022] In this regard, changes in the fluorescence anisotropy of labelled F-actin upon addition of myosin subfragments in the absence of nucleoside phosphate have been attributed to conformational changes within the actin filament as the result of its interaction with myosin heads.[1023] It is also possibly significant that nucleotide-dependent self-association has been reported for myosin subfragment 1 obtained from skeletal[1024] and cardiac muscle,[1025] an ATP-induced conformational change rather than a change in state of aggregation, having been demonstrated for myosin and heavy meromyosin derived from chicken gizzard.[1026]

Of greater physiological interest is the interaction of myosin with regulated actin, *i.e.* the F-actin–tropomyosin–troponin complex. From the experimental viewpoint there has been further substantiation[1027—1029] of the original finding[1030] that the extent of binding of myosin subfragment 1 exhibits a calcium-sensitive, sigmoidal dependence on its free concentration. However, uncertainty surrounds the interpretation that should be placed on these observations, which have traditionally been interpreted in terms of models based on isomerization of the actin filament. In a reappraisal of the original results,[1030] it has been shown[1031] that the sigmoidal binding curve is consistent with a steric blocking model of muscle relaxation in which the interaction of tropomyosin with the myosin-binding sites of the filament is considered to be an equilibrium process rather than an all-or-none phenomenon. Although kinetic studies on the actin activation of heavy meromyosin ATPase activity are considered[1032] to refute the concept of competition between myosin and tropomyosin for the same actin sites, interpretation of the binding data in terms of the steric

[1020] L. E. Greene, *FEBS Lett.*, 1982, **139,** 233.
[1021] M. A. Geeves and H. Gutfreund, *FEBS Lett.*, 1982, **140,** 11.
[1022] H. Yoshimura and K. Mihashi, *J. Biochem.* (*Tokyo*), 1982, **92,** 497.
[1023] M. Miki, P. Wahl, and J.-C. Auchet, *Biochemistry*, 1982, **21,** 3661.
[1024] J. E. Morel and M. Garrigos, *Biochemistry*, 1982, **21,** 2679.
[1025] D. P. Flamig and M. A. Cusanovich, *Biochemistry*, 1981, **20,** 6760.
[1026] H. Suzuki, T. Kamata, H. Onishi, and S. Watanabe, *J. Biochem.* (*Tokyo*), 1982, **91,** 1699.
[1027] A. Inoue and Y. Tonomura, *J. Biochem.* (*Tokyo*), 1982, **91,** 1231.
[1028] H. Nagashima and S. Asakura, *J. Mol. Biol.*, 1982, **155,** 409.
[1029] J. N. Murray, M. K. Knox, C. E. Trueblood, and A. Weber, *Biochemistry*, 1982, **21,** 906.
[1030] L. E. Greene and E. Eisenberg, *Proc. Natl. Acad. Sci. U.S.A.*, 1980, **77,** 2616.
[1031] D. J. Winzor, L. D. Ward, and L. W. Nichol, *Arch. Biochem. Biophys.*, 1982, **217,** 397.
[1032] A. Sobieszek, *J. Mol. Biol.*, 1982, **157,** 275.

blocking model does have the advantage that the experimentally observed[1030] dependence of the binding curve upon filament concentration is predicted,[1031] models based on filament isomerization being deficient in this regard. The interactions of myosin and tropomyosin with actin filaments involve the binding of multi-valent ligands to a linear array of sites, an undeniably complicated problem that has been the subject of two general theoretical studies[1033,1034] in terms of several different models that also predict co-operativity of binding.

Another muscle-protein reaction to have been the subject of several investigations is that between smooth-muscle myosin light-chain kinase and calmodulin. Fluorescence studies have established that phosphorylation of the kinase causes a 500-fold decrease in the association constant for the formation of a 1:1 complex between these two proteins.[1035] Smooth-muscle myosin light-chain kinase also binds to actin,[1036,1037] but there is seemingly some uncertainty about the interdependence of the two reactions. Whereas one study finds that the binding to actin is unaffected by the presence of Ca^{2+} and calmodulin,[1036] there is also a report[1037] that calmodulin and F-actin compete for a smooth-muscle protein with myosin light-chain kinase activity, the interactions with calmodulin and actin being predominant in the presence and absence, respectively, of Ca^{2+}. Extreme Ca^{2+} sensitivity is also observed in the interaction between calmodulin and troponin I, the strength of which is increased some 4500-fold by the presence of Ca^{2+}.[1038] Other systems that have been investigated include the binding of creatine kinase to myosin and myosin subfragments[1039] and the formation of complexes between the C-, I-, and T-subunits of bovine cardiac troponin.[1040]

Acknowledgement.—The authors are indebted to Elisabeth A. Owen for assistance with the literature search.

[1033] T. L. Hill, *Biophys. Chem.*, 1981, **14**, 31.
[1034] T. Tsuchiya and A. Szabo, *Biopolymers*, 1982, **21**, 979.
[1035] D. A. Malencik, S. R. Anderson, J. L. Bohnert, and Y. Shalitin, *Biochemistry*, 1982, **21**, 4031.
[1036] R. Dabrowska, S. Hinkins, M. P. Walsh, and D. J. Hartshorne, *Biochem. Biophys. Res. Commun.*, 1982, **107**, 1524.
[1037] K. Sobue, K. Morimoto, K. Kanda, K. Fukunaga, E. Miyamoto, and S. Kakiuchi, *Biochem. Int.*, 1982, **5**, 503.
[1038] C. H. Keller, B. B. Olwin, D. C. La Porte, and D. R. Storm, *Biochemistry*, 1982, **21**, 156.
[1039] R. S. Mani and C. M. Kay, *Int. J. Biochem.*, 1981, **13**, 1197.
[1040] D. M. Byers and C. M. Kay, *FEBS Lett.*, 1982, **148**, 12.

3
Peptide Synthesis

BY I. J. GALPIN
Appendices compiled by
C. M. GALPIN

1 Introduction

Over the past year peptide synthesis has continued to flourish, although no major solution syntheses have been reported. Considerable attention has been turned towards protecting-group chemistry, and a historical review covering the fifty-year period since the advent of the benzyloxycarbonyl protecting group has been published.[1] Solid-phase methodology continues to be the most popular approach and both polystyrene and polyamide supports have been used frequently.

One interesting development has been the use of solid-phase synthesis to prepare small fragments of the foot-and-mouth virus protein.[2] These peptides were then used to immunize guinea pigs and thus induce the raising of antibodies that would neutralize the foot-and-mouth virus protein. Such a method obviously has wider possibility and may well provide the basis for future vaccine production.[3]

The synthesis of large polypeptides still presents major problems, by both the solid-phase method and solution techniques. Comments on the difficulties involved in the synthesis of such large peptides by solution methods have appeared in two papers,[4,5] one of which[5] is concerned with the problems encountered in the total synthesis of ribonuclease A.

Characterization of large synthetic peptides has always been difficult. However, the advent of fast atom bombardment mass spectroscopy has provided a valuable analytical tool. This technique, which allows the mass spectrum of underivatized peptides to be recorded, has recently been utilized in the characterization of insulin. ACTH, and melitin.[6] Certainly, in the future fast atom bombardment will increasingly add complementary information to that provided by other analytical methods.

[1] J. S. Fruton, *Trends Biochem. Sci.*, 1982, **7**, 37.
[2] J. L. Bittle, R. A. Houghten, H. Alexander, T. M. Shinnick, D. J. Rowlands, and F. Brown, *Nature (London)*, 1982, **298**, 30.
[3] J. Beale, *Nature (London)*, 1982, **298**, 14.
[4] O. M. Vol'Pina, I. I. Mikhaleva, and V. T. Ivanov, *Bioorg. Khim.*, 1982, **8**, 5.
[5] H. Yajima and N. Fujii, *Kagaku No Ryoiki*, 1982, **36**, 296.
[6] M. Barber, R. S. Bordoli, G. J. Elliott, R. D. Sedgwick, A. N. Tyler, and B. N. Green, *J. Chem. Soc., Chem. Commun.*, 1982, 936.

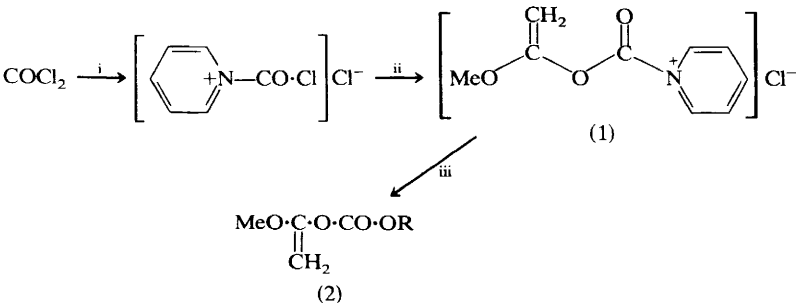
Conference proceedings have been published for the 1981 Japanese Symposium held in Nagoya[7] and for the 3rd U.S.S.R.–F.R.G. Symposium.[8]

Several useful texts, including the fourth volume of 'The Peptides'[9] and Pettit's 'Synthetic Peptides', Volume 6, have been published.[10] Volume 6 of 'Chemistry and Biology of Amino Acids, Peptides and Proteins' has appeared,[11] as has a monograph on β-endorphin.[12] Also, a teaching text on polypeptide and protein structure[13] has been published. An excellent volume entitled 'Perspectives in Peptide Chemistry',[14] which is dedicated to Professor Robert Schwyzer on his sixtieth birthday, covers many aspects of peptide synthesis and contains major works from authors representing seventeen countries.

As in previous years, the format of this chapter follows that established previously. New synthetic methods including protecting-group and coupling methodology are discussed in detail, whereas routine syntheses employing established methods are in the main presented in the Appendices at the end of the chapter. Similarly, data for new derivatives useful in synthesis and purification methods are presented in the Appendix.

2 Methods

Protective Groups.—*Established Methods of Amino-group Protection.* Although the methods for the introduction of the tert-butoxycarbonyl and



Reagents: i, Pyridine; ii, $Hg(CH_2{\cdot}CO_2Me)_2$; iii ROH (R = Bu^t, Ph·CH_2, or $Me_3Si{\cdot}CH_2CH_2$)

Scheme 1

[7] 'Peptide Chemistry 1981, Proceedings of the 19th Symposium on Peptide Chemistry, Nagoya, Japan, 1981', ed. T. Shiori, Peptide Institute, Protein Research Foundation, 1982.

[8] 'Chemistry of Peptides and Proteins, Proceedings of the 3rd U.S.S.R.–F.R.G. Symposium', ed. W. Woelter, E. Wunsch, Yu. Ovchinikov, and V. Ivanov, de Gruyter, Berlin, 1982, Vol. 1.

[9] 'The Peptides, Analysis, Synthesis, Biology', ed. E. Gross and J. Meienhofer, Academic Press, New York, 1981, Vol. 4 (Modern techniques of conformational, structural and configurational analysis).

[10] G. R. Pettit, 'Synthetic Peptides', Elsevier, Amsterdam, Netherlands, 1982, Vol. 6.

[11] 'Chemistry and Biochemistry of Amino Acids, Peptides and Proteins', ed. B. Weinstein, Marcel Dekker, New York, 1982, Vol 6.

[12] 'Hormonal Proteins and Peptides', ed C. H. Li, Academic Press, New York, 1982, Vol. 10 (β-Endorphin).

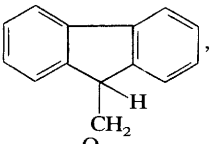
[13] A. G. Walton, 'Polypeptides and Protein Structure', Elsevier, New York, 1981.

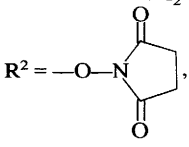
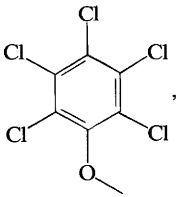
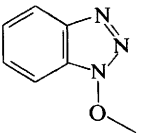
[14] 'Perspectives in Peptide Chemistry', ed. A. Eberle, R. Geiger, and T. Wieland, Karger, Basel, 1981.

benzyloxycarbonyl protecting groups are well established, new methods continue to be developed. Alkylmethoxyvinyl carbonates of the general structure (2) shown in Scheme 1 have been prepared from the corresponding pyridinium carbonates.[15] These alkylmethoxyvinyl carbonates have been used as a means of introducing the tert-butoxycarbonyl, benzyloxycarbonyl, and β-(trimethylsilyl)ethoxycarbonyl protecting groups, the appropriate carbonate being prepared by reaction of the pyridinium carbonate (1) with the corresponding alcohol. Reactions with an amino-acid in dioxan–water mixtures gave a quantitative yield of the *N*-protected derivative.

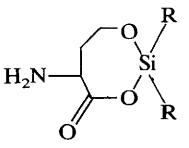
Related activated carbonates (3) have also been prepared.[16] These carbonates have been used to introduce the Fmoc, Troc, and Z protecting groups into O-unprotected hydroxyl-containing amino-acids. In the Fmoc case the succinimidyl carbonate was the most efficient for introducing the protecting group when serine, threonine, or tyrosine was examined.

$$R^1{\cdot}O{\cdot}CO{\cdot}R^2$$

(3) $R^1 =$  , $Ph{\cdot}CH_2{-}$, or $Cl_3C{\cdot}CH_2{-}$

$R^2 =$  ,  , or 

The introduction of *N*-trityl protection has been improved by using intermediate protection with a silyl group for the protection of hydroxyl or carboxyl groups.[17] The method uses either trimethylsilylchloride dimethyldichlorosilane, or diphenyldichlorosilane for this intermediate protection. The silylated amino-acid is then treated with trityl chloride and triethylamine in order to form the *N*-trityl derivative, after which methanol is added to decompose the intermediate silyl ester, or ether. The use of dichlorosilanes[18] to prepare a derivative (4) from homoserine allowed an efficient preparation of



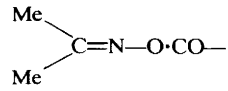
(4) R = Me or Ph

[15] Y. Kita, J. Haruta, H. Yasuda, K. Fukunaga, Y. Shirouchi, and Y. Tamura, *J. Org. Chem.*, 1982, **47,** 2697.
[16] A. Paquet, *Can. J. Chem.*, 1982, **60,** 976.
[17] K. Barlos, D. Papaioannou, and D. Theodoropoulos, *J. Org. Chem.*, 1982, **47,** 1324.
[18] K. Barlos and D. Theodoropoulos, *Z. Naturforsch., Teil B*, 1982, **37,** 886.

N-tritylhomoserine after hydrolysis of the intermediate silyl ether/ester. In the case of homoserine, problems with lactonization are frequently encountered when attempting to prepare the *N*-trityl derivatives without intermediate protection.

Although *N*-formyl groups are not frequently used for amino protection in peptide synthesis, the introduction of a formyl group at the end of a synthesis is frequently required in order to produce biologically active peptides, for which the straightforward use of *p*-nitrophenyl and 2,4,5-trichlorophenyl formate in formylation has been reported.[19] Comparison has also been made between the use of *p*-nitrophenyl formate and formylation using acetic anhydride–formic acid, apamin, MCD peptide, and peptide 401.[20] The kinetics of the reaction of these reagents with both α-amino and side-chain amino groups were studied. Using formic acid and a low concentration of acetic anhydride, monoformylation of peptide 401 (MCD peptide) was achieved. This means of formylation gave rise to N^{α}-formyl derivatives, rather than the formyl derivative of the lysine-ε-amino function. Similarly, apamin was also formylated on the α-amino group when these reaction conditions were used. However, when *p*-nitrophenyl formate was used at a pH of 9.5 (5 equivalents), both peptide 401 and apamin gave derivatives that were formylated in the lysine side chain rather than at the α-amino group.

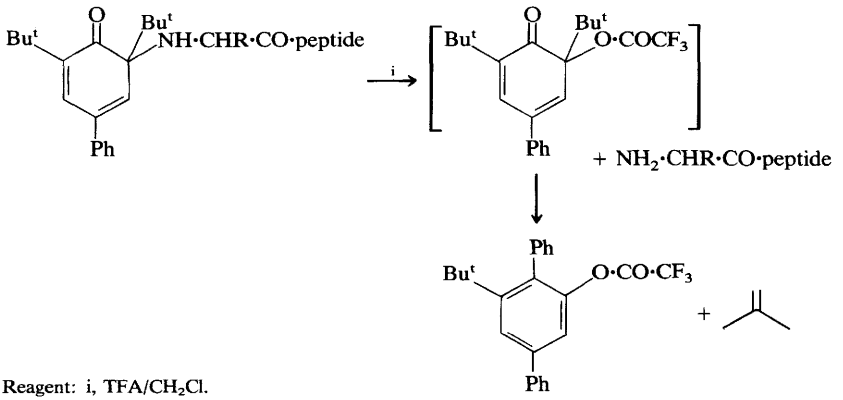
New Methods of Amino-group Protection. A wide range of new amino protecting groups has evolved in the past year, and it has been claimed that ^{13}C n.m.r. spectroscopy is particularly useful in studying amino protecting groups when acid lability is being considered, as the rate of acidolytic cleavage has been correlated with ^{13}C parameters for a variety of relatively new protecting groups.[21] The isopropylidine aminoxycarbonyl (PAAC) protecting group has been developed for the protection of amino functions.[22] The protecting group (5) may be introduced using the nitrophenyl or hydroxysuccinimidyl mixed carbonate or the corresponding chloroformate. This protection is removed by catalytic hydrogenolysis and is stable to trifluoroacetic acid and formic acid. The PAAC group was used for the preparation of lysine vasopressin and gastrin (11—17) using the DCCI/HOBt coupling method.



(5)

The 3,5-di-tert-butyl-4-oxo-l-phenyl-2,5-cyclohexadienyl (PChd) protecting group has been suggested for use in peptide synthesis.[23] This group is introduced by anodic oxidation of 2,6-di-t-butyl-4-phenol in the presence of amino-acid esters. The group is stable to base but may be cleaved by 10—50%

[19] J. Matinez and J. Laur, *Synthesis*, 1982, 893.
[20] C. E. Dempsey, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2625.
[21] W. Fuchs, H. Kalbacher, and W. Voelter, *Org. Magn. Reson.*, 1981, **17**, 157.
[22] D. Gillessen and A. Trzeciak, *Dev. Endocrinol.*, 1981, **13**, 37.
[23] M. H. Khalifa, G. Jung, and A. Rieker, *Liebigs Ann. Chem.*, 1982, 1068.

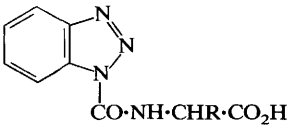


Reagent: i, TFA/CH_2Cl.

Scheme 2

trifluoroacetic acid in dichloromethane, according to the route outlined in Scheme 2. The PChd group is also cleaved by catalytic hydrogenolysis. The utility of the protecting group was demonstrated by the assembly of the (14—20) fragment of human lymphoblastoid interferon, couplings being mediated once again by the DCCI/HOBt method.

Reaction of 1-benzotriazolyl carbonyl chloride with amino-acids yields the BTCO amino-acid derivative (6).[24] Although formally fulfilling the role of amino protecting group, the BTCO group provides a useful route to hydantoins and to peptides containing a urea linkage.[25]



(6)

Many sulphonyl-based amino protecting groups have been described in the past year. The 4-methoxy-2,3,6-trimethylbenzenesulphonyl protecting group (Mtr) has been proposed for both α-amino and side-chain amino protection.[26] The Mtr group is labile to 0.15—0.3 M methane sulphonic acid in trifluoroacetic acid–thioanisol (9 : 1), being cleaved in 1—2 hours at 20 °C. It is resistant to hydrogenolysis and to both TFA and HCl. This new protecting group, which can be used for the synthesis of methionine-containing peptides, has been used to prepare mastopyran-X and chicken-gastrin-releasing peptide (c. GRP).

(Alkyldithio)carbonyl groups (7) have been examined for amino protection.[27] The corresponding chlorocarbonyl disulphide is prepared by reaction of

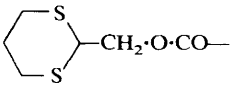
R·S·S·CO—

(7) R = Ph, cyclohexyl, or Bu^t

[24] I. Butula, B. Zorc, and V. Vela, *Croat. Chem. Acta*, 1981, **54**, 435.
[25] B. Zorc and I. Butula, *Croat. Chem. Acta*, 1981, **54**, 441.
[26] M. Fujino, M. Wakimasu, and C. Kitada, *J. Chem. Soc., Chem. Commun.*, 1982, 445.
[27] E. Wuensch, L. Moroder, R. Nyfeler, and E. Jaeger, *Hoppe-Seyler's Z. Physiol. Chem.*, 1982, **363**, 197.

chlorocarbonylsulphenyl chloride with the appropriate thiol. The amino-acid derivative is then prepared by reaction of the carbonyl disulphide (7) with the t-butyl- or trimethylsilyl-amino-acid ester. Direct acylation, using Schotten–Baumann conditions, is not possible as the alkaline conditions used in this reaction lead to decomposition of the chlorocarbonyl disulphide. The corresponding butyl esters are then cleaved by treatment with TFA and the trimethylsilyl esters by treatment with ice water. The protecting group with R equal to phenyl was found to be particularly labile, giving rise to urea derivatives; thus it did not provide adequate amino protection. The t-butyl and cyclohexyl derivatives are stable to acid but are rapidly decomposed by alkali. Quantitative removal of the (cyclohexyl)dithiocarbonyl protection is best achieved by reductive cleavage, using a thiol in the presence of triethylamine, or by trialkyl phosphine in the presence of *p*-toluene sulphonic acid. Coupling of alkyldithiocarbonyl-protected amino-acids may be achieved using the mixed-anhydride or DCCI method, although in the latter case HONSu has to be added in order to suppress the formation of impurities. Hydroxysuccinamide or nitrophenyl active esters may also be used, but salt coupling methods cannot be adopted as the sodium hydroxide or triethylamine gives rise to cleavage of the alkyldithiocarbonyl protecting group. The use of carbonyl disulphides for the protection of the amino group of proline has been investigated in detail.[28] The kinetics of the thiolysis of a range of alkyldithiocarbonyl protecting groups using thiophenol and triethylamine have been examined. The kinetics showed that the thiolysis varied considerably with the nature of the alkyl group, the $t_{\frac{1}{2}}$ (minutes) being 0.5 for R = methyl, 0.7 for R = ethyl, and 26.5 for R = isopropyl. The correct selection of the appropriate alkyl group therefore provides a protection for proline using alkyldithiocarbonyl protection, which is compatible with the dithiasuccinoyl protecting group.[29]

The use of 1,3-dithian-2-yl-methoxycarbonyl (Dmoc) protecting group (8) has been investigated.[30,31] The protecting group, which is introduced using the corresponding mixed nitrophenyl carbonate, is stable to TFA in dichloromethane but may be removed by a two-step deprotection procedure. The first stage is oxidation to the corresponding bis-sulphone, using peracetic acid in dichloromethane, followed by treatment with dimethylamine, which liberates the free amino group. The use of this protecting group was demonstrated by the synthesis of several small peptides. The synthesis of *N,N*-bis(phenylthiomethyl)- and *N,N*-bis(diphenylarsenomethyl)-amino-acid derivatives has been reported,[32] although their application to peptide synthesis is not discussed in detail.



(8)

[28] G. Barany, *Int. J. Pept. Protein Res.*, 1982, **19**, 321.
[29] G. Barany and R. B. Merrifield, *J. Am. Chem. Soc.*, 1977, **99**, 7363.
[30] R. Barthels and H. Kunz, *Angew. Chem., Int. Ed. Engl. (Suppl.)*, 1982, 702.
[31] H. Kunz and R. Barthels, *Chem. Ber.*, 1982, **115**, 833.
[32] M. G. Voronkov, *Z. Chem.*, 1981, **21**, 403.

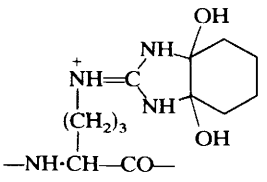
Carboxyl Protection. Relatively little attention has been focused upon the protection of the carboxyl group of amino-acids. However, following the report last year[33] that considerable racemization was observed when dimethylaminopyridine was used to catalyse esterification reactions, the problem has been investigated in more detail.[34] When Boc-Phe-OH was esterified with methanol or benzyl alcohol in the presence of DCCI and 4-dimethylaminopyridine, 36% racemization was observed. However, when Nps-Phe-OH was esterified in a similar manner, no racemization was detected. Also, when Nps-Phe-OH was coupled to a hydroxymethylated copolystyrene–2% divinylbenzene, using DCCI and 4-dimethylaminopyridine, no racemization was observed. Other studies[35] using *N*-protected amino-acids, the water-soluble carbodi-imide ethyl-3-(3-dimethylaminopropyl)carbodi-imide hydrochloride, and 0.1—0.5 equivalents of 4-dimethylaminopyridine in dichloromethane showed that the tendency to racemization is dependent on both the nature of the protected amino-acid and the esterifying alcohol, although in many cases no racemization was observed. Preparation of Boc-Ser(Bzl)-OMe and Boc-Cys(Bzl)-OMe gave rise to 2—3% racemization, and Boc-Asp(OBzl)-OH or Boc-Glu(OBzl)-OH gave extensive racemization on esterification. The tendency to racemization was also dependent on the nature of the alcohol following the order $Bu^tOH > Bzl\text{-}OH > EtOH > MeOH$. Also, less racemization was observed when a short reaction time was used or the concentration of dimethylaminopyridine was reduced to 0.1 equivalents. The method, which allows the preparation of t-butyl esters, may prove to be of considerable use if the degree of racemization can be minimized or indeed eliminated.

Side-chain Protection. The use of cyclopentyloxycarbonyl for the protection of the N^{ϵ}-amino function of lysine has been examined.[36] The group, which is a little more stable than benzyloxycarbonyl, was used in the synthesis of an equine β-melanotropin using solid-phase methodology. On HF deprotection, side products were observed that were due to the incomplete removal of the cyclopentyloxycarbonyl protection, although the side products could be eliminated by recycling of the partially deprotected material.

The decomposition of amino-acid copper complexes with H_2S is not particularly efficient and therefore new methods of decomposing such complexes have been investigated.[37] It was found that thioacetamide at an alkaline pH was highly effective for decomposing the copper salts of Lys(Z), Orn(Z), or Tyr(Bzl). This new method gave between 50 and 90% yields for the ornithine and lysine cases but was not optimized for the tyrosine derivatives.

Protection of the guanidine function of arginine remains as one of the most serious problems in side-chain protection, and a review examining the range of protection available has been published.[38] The use of arginine with the

[33] E. Atherton, N. L. Benoiton, E. Brown, R. C. Sheppard, and B. J. Williams, *J. Chem. Soc., Chem. Commun.*, 1981, 336.
[34] B. Neises, T. Andries, and W. Steglich, *J. Chem. Soc., Chem. Commun.*, 1982, 1132.
[35] M. K. Dhaon, R. K. Olsen, and K. Ramasamy, *J. Org. Chem.*, 1982, **47**, 1962.
[36] *J. Izdebski, D.* Yamashiro, T. B. Ng, and C. H. Li, *Int. J. Pept. Protein Res.*, 1982, **19**, 327.
[37] U. F. Taylor, D. F. Dyckes, and J. R. Cox, jun., *Int. J. Pept. Protein Res.*, 1982, **19**, 158.
[38] Z. Prochazka, K. Jost, and K. Blaha, *Chem. Listy*, 1981, **75**, 699.

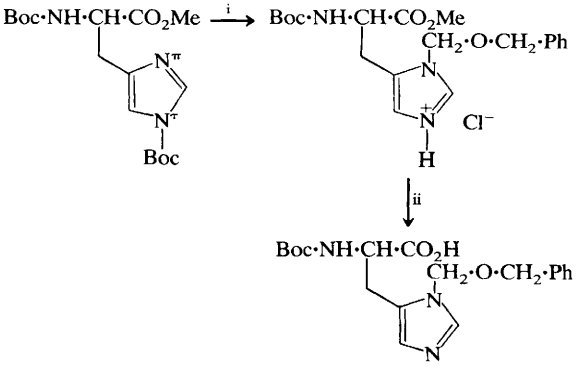


(9)

guanidine function protected as the 1,2-dihydroxycyclohex-1,2-ylene derivative (9) has been reported.[39] The protecting group is introduced by reacting arginine in borate buffer with 1,2-cyclohexanedione, and the resulting 1,2-diol found in the protecting group is stabilized by interaction with borate. Coupling using active esters, mixed anhydrides, or the DCCI/HOBt method may be used, and lactam formation or acylation of the hydroxyl groups is not observed. Removal of the protecting group may be achieved by treatment with hydroxylamine in water between pH 8 and 8.5, the conditions of removal being rather variable and prone to influence by steric factors. A synthesis of thynnine ZI is at present under investigation.[40] This peptide contains 22 arginine residues out of a total of 34 residues. Synthesis of three fragments containing ornithine in place of arginine has been described, and ultimately the free ornithine peptide will be amidinated to give the arginine peptide; however, to date this conversion has not been discussed.

The 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr) protecting group mentioned above[26] has been used for the protection of the imidazole function of histidine[41] and for the protection of the side-chain amino function of lysine. The protecting group is removed from amino groups by treatment with methane sulphonic acid in TFA in the presence of thioanisole but is removed from the imidazole nitrogen by treatment with trifluoroacetic acid in the presence of dimethylsulphide or by treatment with *N*-hydroxybenzotriazole. It was found to be more stable than the corresponding *p*-toluenesulphonyl or methoxybenzenesulphonyl groups and proved to be useful in solid-phase synthesis. The precise position of the Mtr group is not specified, but presumably it blocks the τ-nitrogen. A full paper has now appeared describing the development and use of the π-benzyloxymethyl protecting group.[42] Initial attempts were made to reduce the availability of the π-nitrogen lone pair by the introduction of bromine at the 2 and 5 positions of the imidazole ring. This protection could be removed at the end of a synthesis by hydrogenolysis using a rhodium catalyst, and racemization during coupling was reduced but not totally eliminated. Blockade of the π-nitrogen with a phenacyl group as proposed earlier was found to be unsatisfactory, but it remained clear that blocking of the π-nitrogen was essential if racemization were to be prevented. As an alternative, the benzyloxymethyl (Bom) protecting group was investigated. This was introduced by the route shown in Scheme 3. The group is

[39] U. Hevelke, J. Foehles, J. Knott, and H. Zahn, *Monatsh. Chem.*, 1982, **113**, 457.
[40] F. Marchiori, G. Borin, D. Stivanello, V. Moretto, and G. Chessa, *Hoppe-Seyler's Z. Physiol. Chem.*, 1982, **363**, 1483.
[41] M. Wakimasu, C. Kitada, and M. Fujino, *Chem. Pharm. Bull.*, 1982, **30**, 2766.
[42] T. Brown, J. H. Jones, and J. D. Richards, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1553.



Reagents: i, $PhCH_2{\cdot}O{\cdot}CH_2{\cdot}Cl/CH_2Cl_2$; ii, NaOH (aq).

Scheme 3

stable to base, TFA, and carboxylic acids but is readily cleaved by HBr in TFA or by catalytic hydrogenolysis. Its use was demonstrated in a solid-phase synthesis of [Ile^5]-angiotensin II, which confirmed that the new protecting group was compatible with the most generally used method of solid-phase synthesis, which utilizes t-butyl-based protection for α-amino functions and benzyl-based protection for side chains.

Sulphonyl-based protection has also been used for blocking the indole nitrogen of tryptophan.[43] 2,4,6-Trimethoxybenzenesulphonyl and 4-methoxy-2,3,6-trimethylbenzenesulphonyl protecting groups were studied. The derivatives are both stable to TFA but may be removed using HF–anisole–1,2-ethanedithiol or by treatment with methane sulphonic acid. Both derivatives were produced by reaction of Boc-Trp-OBzl with the appropriate sulphonyl chloride in the presence of sodium hydride, and it was found that the 4-methoxy-2,3,6-trimethylbenzenesulphonyl derivative was more susceptible to acid cleavage than the trimethoxybenzenesulphonyl derivatives.

The most popular protecting group for the indole function of tryptophan is the formyl group. The N^{in}-formyl group may be removed by treatment with HF at 0 °C when ethane-1,2-dithiol or butane-1,4-dithiol is present.[44] Deformylation can be monitored by u.v. spectroscopy as tryptophan itself has a λ_{max} at 280 nm whereas formyl tryptophan absorbs at 300 nm. Monitoring by this technique showed that cleavage was complete in ten minutes at 0 °C, when HF–anisole–ethanedithiol (85:10:5) was used. An alternative deprotection procedure has been established that uses the mixture of HF–dimethylsulphide–*p*-thiocresol–*p*-cresol in the ratio (25:65:5:5).[45] The N^{in}-formyl derivative was shown to be stable to HF alone, or to the action of methanesulphonic acid, but was removed by nucleophiles or aqueous base. The mixture developed for the removal of formyl protection mentioned above includes *p*-cresol as it promotes resin swelling and also acts as a scavenger. Using this mixture no

[43] T. Fukuda, M. Wakimasu, S. Kobayashi, and M. Fujino, *Chem. Pharm. Bull.*, 1982, **30,** 2825.
[44] G. R. Matsueda, *Int. J. Pept. Protein Res.*, 1982, **20,** 26.
[45] W. F. Heath, J. P. Tam, and R. B. Merrifield, *J. Chem. Soc., Chem. Commun.*, 1982, 896.

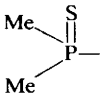
butylation was observed when a cation source was present. In this deprotection medium, the HF concentration is relatively low, and in order to achieve complete deprotection of both N^{in}-formyl tryptophan and methionine sulphoxide it was found that after treatment with the above mixture at 0 °C for two hours a second treatment with HF, in which the concentration was increased to 90%, was required for one hour. The alternative route using HF–anisole (9:1) followed by basic deformylation and thiolytic cleavage of the sulphoxide gave a more complex range of products. The utility of this deprotection regime was demonstrated in a synthesis of the gastrin pentapeptide amide.

An improved method for the preparation of γ-glutamyl esters and β-aspartyl esters using an intermediate copper complex has been developed.[46] Generally, lower yields are observed when the copper complex of glutamic acid or aspartic acid is alkylated, as large amounts of water are required in order to maintain solubility using dimethylformamide–water. However, when tetramethylguanidine is used as a cation in place of sodium or lithium, the water content can be reduced to 10%. The highly soluble tetramethylguanidine salt can be used directly, and a wide range of esters has been produced. Although the method has been used to prepare various benzyl esters, t-butyl esters are not mentioned. After alkylation, the free esters of glutamic or aspartic acid are liberated by treatment with EDTA sodium salt.

Aspartyl-β-phenacyl esters have been used in solid-phase synthesis.[47,48] In the first paper it is made clear that α,β rearrangement can easily occur on removal of the phenacyl group with 1 M sodium thiophenoxide in DMF. Under these conditions the intermediate aminosuccinyl derivative is readily formed, which gives rise to the β-peptide on ring opening. However, using selenophenol under neutral conditions it was possible to remove the β-phenacyl group from aspartic acid.[48] If the conditions were made basic, however, formation of the aminosuccinyl derivative again occurred. Under these conditions no benzyl ester cleavage was observed; however, the resin benzyl ester linkage could be cleaved using sodium selenophenoxide.

Cyclopentyl esters have also been used in solid-phase synthesis to minimize α,β rearrangement on deprotection.[49] In a synthesis of human β-endorphin (1:9) aspartic and glutamic cyclopentyl esters were used, along with tyrosine cyclopentyl ether. The derivatives, which are stable to TFA over 72 hours, are completely removed by treatment with HF in 8 minutes at 0 °C.

The dimethylphosphinothioyl (Mpt) protecting group (10) has also been used for the protection of the phenolic function of tyrosine.[50] Reaction of Mpt-Cl

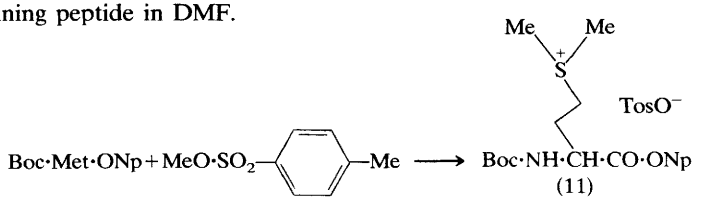


(10)

[46] W. A. R. Van Heeswijk, M. J. D. Eenink, and J. Feijen, *Synthesis*, 1982, 744.
[47] P. Gaudreau, J. L. Morell, and E. Gross, *Int. J. Pept. Protein Res.*, 1982, **19**, 280.
[48] J. L. Morell, P. Gaudreau, and E. Gross, *Int. J. Pept. Protein Res.*, 1982, **19**, 487.
[49] D. Yamashiro, R. Garzia, R. G. Hammonds, jun., and C. H. Li, *Int. J. Pept. Protein Res.*, 1982, **19**, 284.
[50] M. Ueki and T. Inazu, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 204.

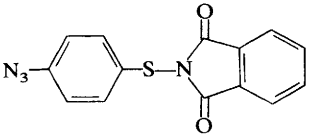
with tyrosine under Schotten–Baumann conditions gives the *N,O*-bis-Mpt derivative. The *N*-Mpt protection is readily cleaved by treatment with 0.2 M HCl, in dichloromethane, leaving the *O*-Mpt derivative as product. Alternatively the *O*-Mpt protection may be cleaved by treatment with 1 M sodium hydroxide. The utility of the method was demonstrated by the synthesis of [D-Ala^2]-enkephalin. Comments on the difficulty of achieving complete butylation of the Z-Tyr-OMe under acid-catalysed conditions have been mentioned,[51] and it was claimed that only 70% of the required product could be obtained even following chromatography.

The possibility of protecting the thioether function of methionine as a sulphonium salt has been investigated.[52] Boc-Met-ONp was reacted with methyl-*p*-toluenesulphonate according to Scheme 4 to give the sulphonium salt (11). Methane-*p*-toluenesulphonate can also be used to convert a methionine residue into the methylsulphonium salt *in situ* in a peptide chain. This protection remains intact during aminolysis and is stable to treatment with TFA or diethylamine. Treatment with HBr in acetic acid exchanged the anion from tosylate to bromide, and hydrogenolysis in the presence of palladium resulted in decomposition. The methionine sulphonium derivative is readily reconverted to methionine by thiolysis with mercaptoethanol. This protecting group has the advantage that it increases the solubility of the methionine-containing peptide in DMF.



Scheme 4

Protection of the thiol function of cysteine continues to be a major problem area in peptide synthesis, and considerable efforts have been made in the last year to develop new protecting groups for this residue. The *S*-9-fluoromethyl derivative of cysteine has been prepared[53] by reaction of 9-fluorenylmethylchloride with cysteine. The protecting group is resistant to acid and to catalytic hydrogenolysis in the presence of palladium but is cleaved by the action of ammonia in methanol or by treatment with 20% piperidine in DMF. Preparation of Boc-Cys(Fm)-ONp provided a derivative that was used in the synthesis of small peptides. The phthalimide derivative (12) has been



(12)

[51] H. P. C. Driessen, H. Bloemendal, W. W. De Jong, and G. I. Tesser, *Int. J. Pept. Protein Res.*, 1982, **20**, 289.
[52] M. Bodanszky and M. A. Bednarek, *Int. J. Pept. Protein Res.*, 1982, **20**, 408.
[53] M. Bodanszky and M. A. Bednarek, *Int. J. Pept. Protein Res.*, 1982, **20**, 434.

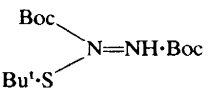
used to prepare *S*-azidophenylthio derivatives. The group has not been used in synthesis so far, but it has been used to block the thiol function of cysteine in reduced proteins. The azidophenylthio group permits crosslinking after photoactivation and may be removed (if necessary) by cleavage with dithiothreitol.[54]

$Ph_2{\cdot}PO{\cdot}CN$ (13) $Ph_2{\cdot}PS{\cdot}CN$ (14)

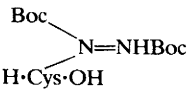
Diphenylphosphinyl (13) and diphenylthiophosphinyl cyanides (14) have been found to react selectively with cysteine derivatives having a free amino group.[55] This protection may be removed by unsolvated fluoride using either tetrabutylammonium or caesium fluorides in an appropriate organic solvent. In contrast, the diphenylphosphinyl or diphenylthiophosphinyl chlorides are N-selective; thus cysteine methyl ester hydrochloride undergoes reaction at the amino function when the chlorides are used, but with the corresponding cyanide reaction occurs on sulphur.

Di-t-butyldiazodicarboxylate (15) has been used for the activation of thiol groups in the preparation of *S*-t-butylcysteine and a new activated cysteine derivative.[56,57] The reagent (15) reacts with thiols such as t-butylmercaptan in the presence of a catalytic amount of sodium methoxide to give the stable compound (16), which may act as a t-butylthio carrier. Reaction of compound (16) with cysteine in water allows a new method of preparation for *S*-t-butylcysteine. Alternatively, if cysteine is the thiol in the initial reaction, then the activated cysteine derivative (17) is produced.[56] This activated sulpheno hydrazide can then be used as one component in the formation of a non-symmetrical cysteine peptide. The applicability of this method was demonstrated by the formation of several intermediate-size peptides containing cysteine.

$Bu^t{\cdot}O{\cdot}CO{\cdot}N{=}N{\cdot}CO{\cdot}OBu^t$ (15)



(16)

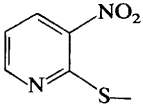


(17)

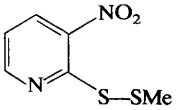
Use of the 3-nitropyridinesulphenyl (Npys) (18) group has been demonstrated in the preparation of several non-symmetrical cysteine peptides. Originally, the group was introduced by reaction of a free cysteine SH with Npys-Cl; however, it has been shown that reaction of Npys-Cl with several other *S*-substituted cysteine derivatives permits exchange of the existing group for the Npys group. Thus, *S*-*p*-methoxybenzyl, *S*-t-butyl, *S*-trityl, and *S*-Acm derivatives are converted to the corresponding Npys derivatives by treatment with Npys-Cl in dichloromethane. In contrast, the *S*-benzyl group does not react with Npys-Cl but may be converted to the Npys derivative by reaction with Npys-Br in trifluoroethanol or in trifluoroethanol and dichloromethane mixtures. The

[54] R. B. Moreland, P. K. Smith, E. K. Fujimoto, and M. E. Dockter, *Anal. Biochem.*, 1982, **121**, 321.
[55] L. Horner, R. Gehring, and H. Lindel, *Phosphorus Sulfur*, 1981, **11**, 349.
[56] E. Wuensch and S. Romani, *Hoppe-Seyler's Z. Physiol. Chem.*, 1982, **363**, 449.
[57] E. Wuensch, L. Moroder, and S. Romani, *Hoppe-Seyler's Z. Physiol. Chem.*, 1982, **363**, 1461.

group was used to prepare [Lys[8]]-vasopressin,[58,59] and syntheses showed the protecting groups to be stable to TFA, 88% formic acid, and HF–anisole (9:1); however, the presence of a thiol rapidly brought about cleavage of the protecting group. The *S*-Npys protecting group may readily be removed by 0.1 M HCl in dioxan or by treatment with triphenylphosphine under neutral conditions. The Npys group is stable in the presence of dimethyl sulphide, which acts as a scavenger but is rapidly removed by treatment with dithiothreitol at pH 8.5.



(18)



(19)

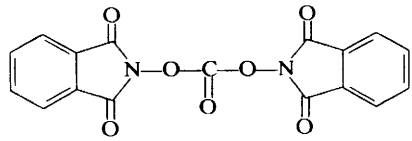
The disulphide (19) has recently been utilized for the introduction of the thiomethyl group.[60] Thus compound (19) reacts readily with thiols or cysteine residues to give an *S*-thiomethyl derivative. This reaction thus allows an efficient spectrophotometric assay of free thiol groups by estimation of the liberated 3-nitro-2-pyridone. It is claimed that the simpler methyl-2-pyridyl disulphide is more useful in this assay for thiol groups as the thiopyridone is more stable than the corresponding 3-nitro derivative.

Formation of the Peptide Bond.—Dicyclohexylcarbodi-imide probably remains the most popular of all the coupling reagents, and a review of the general aspects of carbodi-imide chemistry including those that are relevant to peptide synthesis has appeared.[61] The mixed-anhydride method also remains popular, and the extent of racemization in mixed-anhydride synthesis has been examined using the coupling of Tfa-Pro-Val-OH to H-Pro-OMe as a test case,[62] the effects of solvent, temperature, and additives such as zinc chloride being investigated. Use of dimethylphosphinothioyl (Mpt mixed anhydrides) has been examined.[63] These are prepared from reaction of a suitably *N*-protected amino-acid with the corresponding chloride, and it is claimed that side-chain hydroxyl groups do not require protection. Mpt-Trp-OMpt has been isolated in high yield (92%), and in this case wrong-way opening of the non-symmetrical anhydride was estimated at 0.6%. Preformed Mpt mixed anhydrides were also used in solid-phase synthesis using a standard polystyrene resin.

Diphthalimidocarbonate (20) has been used for the preparation of *N*-hydroxyphthalimide active esters.[64] The reagent (20) is reacted with *N*-protected amino-acids in the presence of triethylamine to give the active ester.

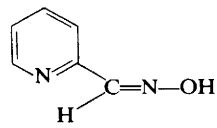
[58] R. Matsueda, S. Higashida, R. J. Ridge, and G. R. Matsueda, *Chem. Lett.*, 1982, 921.
[59] R. J. Ridge, G. R. Matsueda, E. Haber, and R. Matsueda, *Int. J. Pept. Protein Res.*, 1982, **19**, 490.
[60] T. Kimura, R. Matsueda, Y. Nakagawa, and E. T. Kaiser, *Anal. Biochem.*, 1982, **122**, 274.
[61] A. Williams and I. T. Ibrahim, *Chem. Rev.*, 1981, **81**, 589.
[62] E. Berger, K. Neubert, H. Bang, and H.-D. Jakubke, *Z. Chem.*, 1982, **22**, 379.
[63] M. Ueki and T. Inazu, *Chem. Lett.*, 1982, 45.
[64] K. Kurita and H. Imajo, *J. Org. Chem.*, 1982, **47**, 4584.

In THF the reaction is slow, but the addition of triethylamine increases the rate of reaction considerably.



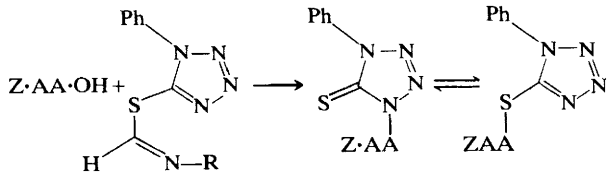
(20)

The oxime (21) has also been used to form active esters.[65] In this case the active ester is prepared by reacting the *N*-hydroxy compound (21) with the *N*-protected amino-acid in the presence of DCCI, using THF or dichloromethane as solvent.



(21)

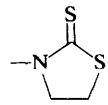
Z and Boc amino-acids have also been reacted with the compound (22), in the manner shown in Scheme 5.[66] The activated species was found to be a mixture of the *N*- and *S*-acyl compounds as indicated in Scheme 5, and coupling with an amino-acid methyl ester gave dipeptide in yields between 60 and 80% with no trace of racemization being observed by the Weygand test.



(22)

Scheme 5

The thiazolidine-2-thiones[67] (23) have been used for the synthesis of chicken-gastrin-releasing peptide;[68] these derivatives were used for the assembly of fragments, but the combination of fragments was carried out by either the azide method or by using DCCI/HOBt.



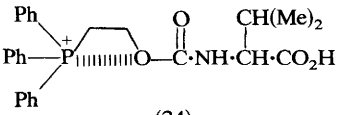
(23)

[65] T. Plucinski and G. Kupryszewski, *Pol. J. Chem.*, 1981, **55,** 573.
[66] U. Schmidt and M. Dietsche, *Angew. Chem., Int. Ed. Engl.*, 1982, **21,** 143.
[67] H. Yajima, K. Akaji, Y. Hirota, and N. Fujii, *Chem. Pharm. Bull.*, 1980, **28,** 3140.
[68] K. Akaji, N. Fujii, H. Yajima, M. Moriga, A. Takagi, K. Mizuta, M. Noguchi, and T. J. McDonald, *Int. J. Pept. Protein Res.*, 1982, **20,** 276.

In order to cut out base-catalysed side reactions in couplings it has been proposed that the use of tertiary amines in couplings should be eliminated,[69] as normally tertiary amines are added to the coupling mixture to liberate the free amino component from the corresponding salt. Thus, if weak acid salts were employed, the use of tertiary bases might be avoided. However, as carboxylic acid salts are unsuitable as they may be activated by coupling agents, pentachlorophenol, 2,4-dinitrophenol, and hydroxybenzotriazole were investigated. These acidic compounds could be used for salt formation and may be used to cleave the Bpoc, Nps, and trityl protecting groups. The results showed that use of HOBt brought about quantitative cleavage of these protecting groups, giving the corresponding HOBt salt. This was then acylated with either symmetrical anhydride, mixed anhydride, or active ester, without the addition of tertiary amines. The HOBt salt may also be obtained by hydrogenolysis of benzyloxycarbonyl protection in the presence of HOBt or by thiolysis of Nps in the presence of HOBt. Alternatively, acetate may be displaced by HOBt to give the corresponding HOBt salt. Thus, when using these HOBt salts, the amino component remains sufficiently nucleophilic to be acylated using the acylating reagents mentioned above.

The coupling of thiol peptides has again been mentioned,[70,71] and in both cases coupling of the free-thiol peptide was achieved using silver nitrate in the presence of *N*-hydroxysuccinamide.

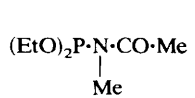
The acid chloride method, which usually gives rise to massive racemization during coupling, has been used under rather special circumstances to couple sterically hindered amino-acids.[72,73] When the 2-phosphonioethoxycarbonyl protecting group was used to block the amino function of valine, the corresponding acid chloride could be prepared by treatment with oxalyl chloride and coupled to *N*-methyl-leucine t-butyl ester without any evidence of racemization. It is proposed that the positively charged phosphorous atom of the protecting group interacts with the urethane oxygen in the manner indicated in (24), thus preventing oxazalone formation. It also seems possible that interaction with the urethane carbonyl forming a six-membered ring could be an alternative explanation. If the results using the 2-phosphonioethoxycarbonyl protecting group prove to be general, then considerable use could be made of the acid chloride method in cases where a high degree of activation is required.



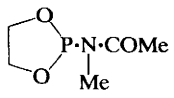
(24)

Several coupling reagents containing phosphorous have been reported. The *N*-acylphosphoramidites (25) and (26) have been used as condensing agents.[74]

[69] M. Bodanszky, M. A. Bednarek, and A. Bodanszky, *Int. J. Pept. Protein Res.*, 1982, **20,** 387.
[70] J. Blake, J. Hagman, and J. Ramachandran, *Int. J. Pept. Protein Res.*, 1982, **20,** 97.
[71] J. Blake, K. K. Hines, and C. H. Li, *Int. J. Pept. Protein Res.*, 1982, **20,** 429.
[72] H.-H. Bechtolsheimer and H. Kunz, *Angew. Chem., Int. Ed. Engl.*, 1982, **21,** 630.
[73] H. Kunz and H.-H. Bechtolsheimer, *Liebigs Ann. Chem.*, 1982, 2068.
[74] W. Kawanobe, K. Yamaguchi, S. Nakahama, and N. Yamazaki, *Chem. Lett.*, 1982, 825.

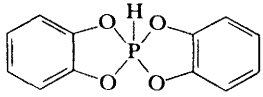


(25)

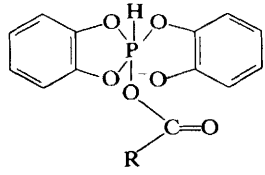


(26)

The yields and optical purity of products obtained by this method are rather variable, but the Young test showed no racemization when the temperature was carefully controlled. The reagent 2,2'-*spiro*-bis-(1,3,2-benzodioxaphosphole) (27)[75] has been utilized as a coupling reagent. The compound, which is prepared by reaction of catechol with phosphorous trichloride, was shown by the Izumiya test to give 3.2% racemization when diethylaminoethylpolystyrene was used as base. However, when HONSu was used as an additive, racemization was reduced to less than 0.1%. It is postulated that the active ester (28) is formed rapidly but that this active ester is not prone to racemization.

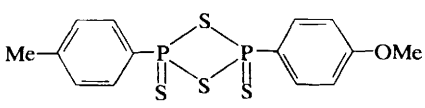


(27)

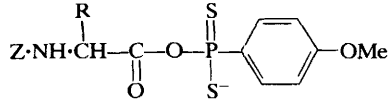


(28)

2,4-Bis-(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetene-2,4-disulphide *LR* (29) has also been employed as a coupling reagent.[76] It is claimed that at 0 °C using 2 equivalents of triethylamine and one equivalent of an amino-acid methyl ester coupling occurs without racemization. The intermediacy of a mixed anhydride (30) is invoked, and no additives are needed in order to suppress racemization.



(29)



(30)

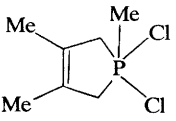
Details relating to the use of 1,3,4-trimethyl-Δ-3-phospholene-1,1-dichloride (31) in peptide synthesis were inaccuractely reported in Volume 11 of this series (page 335).[77,78] Coupling yields using this reagent in fact range from 70 to 100% except when serine and tyrosine are present with an unprotected hydroxyl group, in which case the yield falls to between 30 and 50%. Although the Young test shows some racemization, it appears that syntheses using Z and Boc amino-acids give quite satisfactory results. The production of water-soluble by-products when using this coupling reagent is also advantageous.

[75] J. I. G. Cadogan, I. Gosney, D. Randles, S. Yaslak, and R. P. Ambler, *J. Chem. Soc., Chem. Commun.*, 1982, 298.

[76] U. Pedersen, M. Thorsen, E.-E. A. M. Khrisy, K. Clausen, and S.-O. Lawesson, *Tetrahedron*, 1982, **38**, 3267.

[77] E. Vilkas, M. Vilkas, and J. Sainton, *Tetrahedron Lett.*, 1978, 2933.

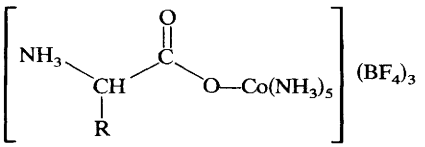
[78] E. Vilkas, M. Vilkas, and J. Sainton, *Nouv. J. Chem.*, 1978, **2**, 307.



(31)

Stannic chloride, dibutyldichlorostannane, and the tin(IV) compound (32) have been used as catalysts in the coupling of Z-Gly-ONp with glycine butyl ester.[79] Tetrabutylstannane was found to be inactive, and clearly the tin compound is required to be bifunctional as well as containing one or more electron-withdrawing groups. Peptide-bond formation in the presence of metal-ion protecting groups has also received some attention.[80,81] The amino-acid cobalt complex (33) may be coupled with Boc amino-acid active esters or Boc amino-acid symmetrical anhydrides. The resulting Boc dipeptide bound to cobalt may be removed by treatment with sodium borohydride for one minute or by treatment with NaSH without loss of chirality of the amino-acids. Alternatively the Boc group may be removed with 95% TFA over 30 minutes to give the free cobalt-bound dipeptide, which can then be used in further couplings with an appropriate acylating species. Using this technique leucine- and methionine-enkephalin have been synthesized with no diastereoisomers being observed.

$Bu_2Sn(OBzl)_2$
(32)



(33)

Racemization.—For many years it was assumed that the azide method was completely free of all racemization, but for some time it has been apparent that racemization can occur during azide coupling. The chiral stability during the preparation of hydrazides and coupling by the azide procedure has therefore been investigated in detail.[82] Various protected dipeptidehydrazides (34) were

$$Z{\cdot}Gly{\cdot}X{\cdot}NH{\cdot}NH_2$$
(34) X = Ala, Leu, Ile, Phe, or Val

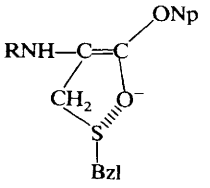
prepared, using either hydrazinolysis of the methyl ester, DCCI/HOBt coupling of hydrazine with the corresponding dipeptide acid, or reaction of the 2,4-disubstituted-5-(4H)-oxazalone with hydrazine. The resulting hydrazide was then converted to the corresponding azide and coupled with lysine benzyl ester prior to examination on the ion-exchange column of an amino-acid analyser. When *N*-methylmorpholine was used as the base in the azide coupling, less

[79] N. M. Olenik, I. P. Garkusha-Bozhko, and L. M. Litvinenko, *Ukr. Khim. Zh.* (*Russ. Ed.*), 1981, **47**, 723.
[80] S. S. Isied, J. Lyon, and A. Vassilian, *Int. J. Pept. Protein Res.*, 1982, **19**, 354.
[81] S. S. Isied, A. Vassilian, and J. M. Lyon, *J. Am. Chem. Soc.*, 1982, **104**, 3910.
[82] N. L. Benoiton, K. Kuroda, and F. M. F. Chen., *Int. J. Pept. Protein Res.*, 1982, **20**, 81.

racemization was observed in DMF than in dichloromethane or ethyl acetate. It was concluded that reaction of hydrazine with the oxazalone was the simplest and most efficient means of preparing the hydrazide and that a water-soluble carbodi-imide in combination with HOBt and hydrazine was advantageous when compared with a similar system using dicyclohexylcarbodi-imide. Using the methyl ester route, most cases gave less than 1% racemization, and frequently the level of racemization was approximately 0.1%. The case where X = alanine appeared to be exceptional, with a greater degree of racemization being observed.

An equation based on extra thermodynamic considerations has been proposed[83] for the prediction of the racemization that might be expected in a fragment coupling; the equation correlates racemization with the primary structure. The measurement of racemization in 13 reference reactions was examined by n.m.r. spectroscopy, and use of the derived equation permitted prediction of the racemization to be anticipated in a particular fragment coupling. A good fit between the calculated and found extent of racemization was observed, although at present the method has only been applied to those amino-acids with simple side chains.

The high degree of racemization encountered when coupling with cysteine dipeptide esters has been investigated in Kovacs' laboratory.[84] The rates of racemization of Z-Gly-Cys(Bzl)-ONp and Z-Cys-(Bzl)-ONp were found to be virtually identical. In this case an oxazalone is not implicated in the racemization, but an enol (35) in which the sulphur *d*-orbitals overlap with the *p*-orbitals of oxygen is believed to be involved. Stabilization in this manner is held to be more plausible than the *S*-α-carbon interaction previously proposed,[85a] which involves a three-membered ring. However, this argument suffers badly under Occeanis razor since there is at least one analogy for the three-membered interaction in a simple non-amino-acid structure where no other stabilization seems feasible. Anteunis *et al.*[85b] also support the three-membered interpretation.

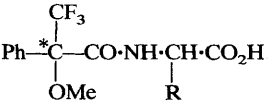


(35)

Amino-acid derivatives (36) have been used as a basis for a new racemization test that utilizes ^{19}F n.m.r.[86] Both *R* and *S* isomers have been examined, and the case where R = methyl appears to be the most useful compound for study. When the compound (36) is coupled to phenylalanine-methyl ester the

[83] D. Le Nguyen, J. R. Dormoy, B. Castro, and D. Prevot, *Tetrahedron*, 1981, **37**, 4229.
[84] J. Kovacs and Y. Hsieh, *J. Org. Chem.*, 1982, **47**, 4996.
[85a] M. Barber, J. H. Jones, and M. J. Witty, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2425.
[85b] M. J. O. Anteunis, F. Borreman, D. Wante, and R. Schrooten, *Tetrahedron Lett.*, 1981, **22**, 3101.
[86] W. Breuer and I. Ugi, *J. Chem. Res.*, 1982, 271.

^{19}F signal of the CF_3 group can be monitored as the diastereoisomers have considerably different ^{19}F chemical shifts. The method was used to examine racemization in many standard cases, and as anticipated the azide, the DPPA method, the DCCI/HONSu method, and the DCCI/HOBt method all showed less than 3% racemization, whereas with DCCI alone 75% racemization was observed. Unfortunately, the limit of sensitivity of this method is approximately 3%.



(36) *R or S configuration

Many h.p.l.c. and g.c. methods for the separation of diastereoisomers have been reported, and these are tabulated in the section of this chapter that deals with purification. A detailed survey of racemization during coupling has been carried out,[87] using h.p.l.c. to monitor the coupling between Z-Phe-Val-OH and H-Pro-OBu^t. Using this system a detection limit of 0.01% could be achieved. As anticipated, diethylphosphorocyanidate and diphenylphosphorazidate gave low racemization, and in both cases excess triethylamine must be avoided. The additives HONSu, HOBt, and HONB all decreased the racemization that was seen with DCCI, and also the racemization in the triphenylphosphine/dipyridylsulphide method was considerably reduced by the addition of additives. Carbonic mixed anhydrides gave an increased level of racemization, whereas the azide method in general gave only slight racemization.

General Deprotection and Side Reactions during Synthesis.—The removal of benzyl-based protecting groups by sodium in liquid ammonia is known to give rise to many side reactions. The nature of these side reactions has recently been investigated in some detail,[88] and it has been found that in order to remove the benzyl protection completely only the theoretical amount of sodium in liquid ammonia should be used rather than the amount required to give a persistent blue colour. The paper lists the following deleterious side reactions.

(i) The formation of hydantoins from amino-acid amides.
(ii) The reduction of primary amides such as the side chain of asparagine to the corresponding alcohol. Thus Boc-asparagine is converted to Boc-homoserine in 25% yield.
(iii) Deamination and transpeptidation are observed. Thus Z-Asn-Gly-NH_2 gives 20% of H-Asn-Gly-NH_2 and 20% of a mixture of α- and β-aspartyl glycine amide.

The paper emphasizes that these side reactions are generally only observed when a considerable excess of sodium is present.

Catalytic transfer hydrogenation of peptides containing methionine has been

[87] S. Takuma, Y. Hamada, and T. Shioiri, *Chem. Pharm. Bull.*, 1982, **30**, 3147.
[88] I. Schoen, T. Szirtes, and T. Ueberhard, *J. Chem. Soc., Chem. Commun.*, 1982, 639.

demonstrated by synthesis of methionine-enkephalin using transfer hydrogenation to remove the benzyloxycarbonyl group.[89] In this work *cis*-decalin and cyclohexene were found to be good hydrogen donors whereas *trans*-decalin and tetralin were not as good. It was found that cyclohexene was in fact the best hydrogen donor, as when *cis*-decalin was used the benzyloxycarbonyl group could not be removed from Z-Cys(Bzl)-OH and *O*-benzyl groups were also difficult to remove. It appears that tryptophan is particularly susceptible to reduction during transfer hydrogenation, using palladium and formic acid.[90] When Ac-Trp-OEt was subjected to the conditions used for removal of the nitro group from nitroargiñine (five-hour treatment), the 2,3-dihydrotryptophan derivative was isolated in 30% yield. However, if the reaction time is kept to the ten minutes required for the removal of a benzyloxycarbonyl group, then tryptophan remains unaffected.

The hydrogenolytic debenzylation of sulphur-containing peptides has been investigated using Palladium Black.[91] It was found that hydrogenolysis of *S*-benzylcysteine in the presence of Palladium Black leads to desulphurization yielding inhibiting thiols and alanine. This inhibitory effect may be countered by adding BF_3 etherate, and under these conditions *S*-benzyl is quantitatively removed from cysteine.

Anhydrous hydrogen fluoride is used by many workers for the removal of side-chain benzyl protecting groups, particularly in solid-phase synthesis. The deprotection medium HF–anisole–thioanisole–*o*-cresol is frequently used,[92,93] and, where methionine has been protected as its sulphoxide, reduction with mercaptoethanol may be used to reliberate the free methionine.[93] Alternatively cleavage of side-chain benzyl protection and reduction of methionine sulphoxide to methionine may be achieved by deprotecting with HF in the presence of 2-mercaptopyridine.[94] In an impressive synthesis of human parathyroid hormones (1—84)[95] the final deprotection with HF was carried out at 0 °C for one hour in a mixture of anisole, dimethylsulphide, and ethanedithiol. Thus, under carefully controlled conditions HF is certainly a good means of total deprotection.

Trifluoromethanesulphonic acid–trifluoroacetic acid–thioanisole is becoming increasingly used for the removal of benzyl- and sulphonyl-based protecting groups, especially by Japanese workers.[96—102] Generally, the deprotection is

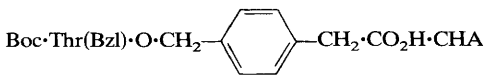
[89] Y. Okada and N. Ohta, *Chem. Pharm. Bull.*, 1982, **30,** 581.
[90] Y. Kikugawa and M. Kashimura, *Chem. Pharm. Bull.*, 1982, **30,** 3386.
[91] G. Losse, H. U. Stiehl, and B. Schwenzer, *Int. J. Pept. Protein Res.*, 1982, **19,** 114.
[92] T. Abiko and H. Sekino, *Chem. Pharm. Bull.*, 1982, **30,** 3271.
[93] T. Abiko, I. Onodera, and H. Sekino, *Chem. Pharm. Bull.*, 1982, **30,** 2604.
[94] D. Yamashiro, *Int. J. Pept. Protein Res.*, 1982, **20,** 63.
[95] T. Kimura, T. Morikawa, M. Takai, and S. Sakakibara, *J. Chem. Soc., Chem. Commun.*, 1982, 340.
[96] S. Shimamura, K. Yasumura, K. Okamoto, K. Miyata, A. Tanaka, M. Nakamura, K. Akaji, and H. Yajima, *Chem. Pharm. Bull.*, 1982, **30,** 2433.
[97] M. Wakimasu, C. Kitada, and M. Fujino, *Chem. Pharm. Bull.*, 1982, **30,** 2364.
[98] S. Funakoshi and H. Yajima, *Chem. Pharm. Bull.*, 1982, **30,** 1697.
[99] K. Yasumura, K. Okamoto, and H. Yajima, *Chem. Pharm. Bull.*, 1982, **30,** 3970.
[100] K. Akaji, N. Fujii, H. Yajima, and D. Pearson, *Chem. Pharm. Bull.*, 1982, **30,** 349.
[101] S. Funakoshi, N. Fujii, H. Yajima, C. Shigeno, I. Yamamoto, R. Morita, and K. Torizuka, *Chem. Pharm. Bull.*, 1982, **30,** 1706.
[102] H. Yajima, Y. Minamitake, S. Funakoshi, I. Katayama, N. Fujii, T. Segawa, Y. Nakata, T. Yasuhara, and T. Nakajima, *Chem. Pharm. Bull.*, 1982, **30,** 344.

achieved in approximately one hour, and in cases where methionine is present as its sulphoxide treatment with dithiothreitol[101] or mercaptoethanol[102] has been used to convert the sulphoxide back to give the corresponding methionine peptide. On occasion *m*-cresol[103] and skatole[104] have been added to the deprotection medium to act as scavengers. From the wide usage of the methanesulphonic acid–TFA–thioanisole method it appears that this method of deprotection is now an established alternative to the use of anhydrous hydrofluoric acid.

Repetitive Methods for Peptide Synthesis.—*Solid-phase Synthesis.* A rather general review covering the use of functionalized polymers in organic synthesis has appeared.[105] The review discusses solid-phase peptide synthesis and covers use of polymeric reagents.

Although the original chloromethyl Merrifield resin is still widely used, the oxymethylphenylacetamidomethyl (PAM) resin has become more popular. Its use has been demonstrated in syntheses of human γ-lipotropin[106] and cecropin-A (1—33).[107] In both these syntheses tryptophan was protected as the N^{in}-formyl derivative, and cyclopentyl[106] and cyclohexyl[107] esters were used for the protection of the side-chain ester function of aspartic acid. These esters have also been used for side-chain protection of both aspartic acid and glumatic acid in syntheses of endorphin analogues[108] and a myeloma protein M603 fragment.[109] In the synthesis of the M603 fragment the peptide was linked to the bromopropionyl resin using an α-methylphenacyl ester, the ester linkage on this occasion being cleaved by photolysis to give an 89% yield. A bromo polymer was also used in the synthesis of ostrich β-endorphin.[110] A double-headed analogue of β-endorphin has also been prepared using a bromomethyl polymer.[111]

In the cecropin synthesis[107] the first residue Boc-Thr(Bzl)-OH was initially esterified to (4-bromomethyl)phenyl acetic acid, giving the phenacyl ester (37) as the cyclohexylamine salt. This was then coupled to the resin by DCCI coupling. Further residues were added in a double cycle, the first coupling being carried out using a symmetrical anhydride; this was followed by a second coupling using the Boc-protected amino-acid derivative and DCCI in the presence of HOBt. 50% TFA in the presence of ethanedithiol was routinely



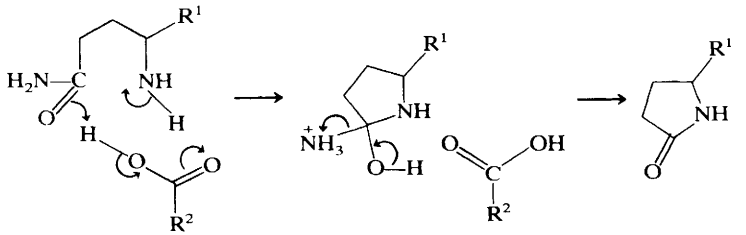
(37)

[103] K. Yasumura, K. Okamoto, S. Shimamura, M. Nakamura, K. Odaguchi, A. Tanaka, and H. Yajima, *Chem. Pharm. Bull.*, 1982, **30**, 866.
[104] K. Okamoto, K. Yasumura, N. Yamamura, S. Shimamura, K. Miyata, A. Tanaka, M. Nakamura, H. Kawauchi, and H. Yajima, *Chem. Pharm. Bull.*, 1982, **30**, 2595.
[105] A Akelah and D. C. Sherrington, *Chem. Rev.*, 1981, **81**, 557.
[106] J. Izdebski, D. Yamashiro, C. H. Li, and G. Viti, *Int. J. Pept. Protein Res.*, 1982, **20**, 87.
[107] R. B. Merrifield, L. D. Vizioli, and H. G. Boman, *Biochemistry*, 1982, **21**, 5020.
[108] R. Garzia, D. Yamashiro, C. H. Li, and P. Nicolas, *Int. J. Pept. Protein Res.*, 1982, **20**, 194.
[109] R. D. Dimarchi, J. P. Tam, and R. B. Merrifield, *Int. J. Pept. Protein Res.*, 1982, **19**, 270.
[110] D. Yamashiro, R. G. Hammonds, jun., and C. H. Li, *Int. J. Pept. Protein Res.*, 1982, **19**, 251.
[111] D. Yamashiro, C. H. Li, P. Nicolas, and R. G. Hammonds, jun., *Int. J. Pept. Protein Res.*, 1982, **19**, 348.

used for the removal of Boc protection. When coupling Boc-Arg(Tos)-OH only the DCCI coupling was used in order to avoid lactam formation. On several occasions a third coupling was required in order to achieve complete acylation. The completeness of the acylation was monitored using a new ninhydrin method and resulted in an average yield of 99.8% being achieved for each coupling cycle.

In the immunoglobulin fragment synthesis mentioned above[109] it was noted that when Boc-Glu(Bzl) was the terminal residue a pyrrolidone (pyroglutamyl) residue was formed at the amino terminus. This was suppressed by using the cyclohexyl ester for the protection of the glutamic acid side chain. This inhibition of the cyclization was attributed to steric hindrance by the side-chain protecting group preventing attack by the α-amino function.

A detailed investigation into the formation of pyrrolidone carboxylic acid derivatives as mentioned above has been carried out.[112] It was found that the formation of such residues was catalysed by weak acid but was not catalysed by strong acid. Thus, in DCCI couplings the presence of a free Boc-amino-acid (a weak acid) caused some termination by pyroglutamyl formation. One very satisfactory way of avoiding this was found to be the use of preformed symmetrical anhydride in DMF. Thus, when a resin-bound peptide with a free amino-terminal glutamine was treated with Boc-Ile-OH in the presence of dichloromethane 10% formation of pyrrolidone carboxylic acid derivative was observed. TFA (0.5%) was found to cause significant pyroglutamyl formation; also, as the TFA concentration increased the extent of pyroglutamyl formation decreased. Thus, in the removal of Bpoc protection using trifluoroacetic acid it is important to remove the TFA very rapidly in order to minimize pyroglutamyl formation. A mechanism that is facilitated by carboxylate groups is proposed (Scheme 6).



Scheme 6

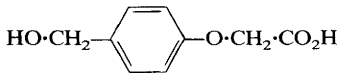
Many methods of coupling are used in solid-phase peptide synthesis, although the majority of workers use DCCI either alone or in symmetrical anhydride formation. Di-isopropyl carbodi-imide in combination with hydroxybenzotriazole has also been used.[113] In both this type of coupling and the DCCI/HOBt mediated coupling mentioned above[109] acylation is assumed to proceed *via* the hydroxybenzotriazole active ester. Coupling using aromatic

[112] R. D. Dimarchi, J. P. Tam, S. B. H. Kent, and R. B. Merrifield, *Int. J. Pept. Protein Res.*, 1982, **19**, 88.

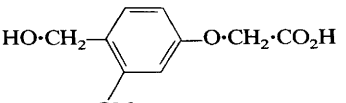
[113] B. R. Clark, J. Dattilo, and D. Pearson, *Int. J. Pept. Protein Res.*, 1982, **19**, 448.

active esters in the presence of HOBt has also been reported.[114] This method relies on the catalytic effect of the HOBt to increase the rate of reaction of the aromatic active ester. The method was used in solid-phase syntheses of [Lys^8]- and [Arg^8]-vasopressin and oxytocin.[115]

Polydimethylacrylamide-based resins have become more widely used, and linkage agents for use with this type of resin or indeed polystyrene-type resins have been investigated.[116,117] 4-Hydroxymethylphenoxyacetic acid (38) and 3-methoxy-4-hydroxymethylphenoxyacetic acid (39) were examined. The peptides bound to both linkage agents could be cleaved from the resin by treatment with TFA, although with the linkage agent (39) much greater acid lability was observed, the ester linkage being cleaved by 1% TFA in dichloromethane. With the linkage agent (39), however, care must be exercised in acidic cleavage as the differential between cleavage of t-butyl-based side-chain protection and cleavage from the resin is not very large. In spite of this slight problem, the resin has a particular advantage in that it allows cleavage of protected fragments from the resin, which in turn allows purification and subsequent use in further synthesis. Synthesis of gastrin fragments was used to illustrate the utility of the method, employing the Fmoc protecting group for α-amino functions, with side-chain protection being effected using t-butyl-based protection.



(38)



(39)

Fmoc protection was also used in a synthesis of LHRH that was carried out on a hydroxymethylphenyloxymethyl resin.[118] The fragments (1—6) and (7—10) of LHRH were prepared on the hydroxymethylphenyloxymethyl resin, being cleaved from the resin prior to purification by treatment with TFA. After purification on LH20 and preparative h.p.l.c., the two fragments were assembled on an α-aminobenzyl resin, giving the total sequence of LHRH. HF treatment then yielded the final product.

A *p*-benzyloxybenzylamine resin has been used in the synthesis of chicken and porcine vasointestinal peptide.[119,120] Assembly of the peptide was again carried out using Fmoc symmetrical anhydrides, although the original acylation of the resin was carried out by the DCCI/HOBt method. The Kaiser test was used to monitor the completeness of the coupling, and when incomplete coupling was observed acetic anhydride was used for termination of failure

[114] K. M. Sivanandaiah and S. Gurusiddappa, *Indian J. Chem., Sect. B*, 1981, **20,** 1061.
[115] K. M. Sivanandaiah and S. Gurusiddappa, *Indian J. Chem., Sect. B*, 1982, **21,** 139.
[116] R. C. Sheppard and B. J. Williams, *J. Chem. Soc., Chem. Commun.*, 1982, 587.
[117] R. C. Sheppard and B. J. Williams. *Int. J. Pept. Protein Res.*, 1982, **20,** 451.
[118] E. Pedroso, A. Grandas, M. A. Saralegui, E. Giralt, C. Granier, and J. Van Rietschoten, *Tetrahedron*, 1982, **38,** 1183.
[119] R. Colombo, *Int. J. Pept. Protein Res.*, 1982, **19,** 71.
[120] R. Colombo, *Experientia*, 1982, **38,** 773.

sequences. Treatment with 60% TFA in dichloromethane in the presence of anisole and thiophenol yielded the peptide amide product.

The phenolic polyacryloylmorpholine-based gel-phase support has been used for the synthesis of several peptides.[121—123] The syntheses used a phenolic support that is claimed to be superior to the benzyl ester resin, the first residue being attached by coupling with di-isopropylcarbodi-imide in the presence of 4-dimethylaminopyridine. The syntheses utilized Boc for α-amino protection and benzyl for side-chain protection, the Boc group being removed by treatment with BF_3 etherate in the presence of benzyl alcohol. Subsequent addition of amino-acids was carried out by treatment with the Boc amino-acid hydroxybenzotriazolyl active ester, which was formed by preactivation with di-isopropylcarbodi-imide and hydroxybenzotriazole, the coupling being carried out in dimethylacetamide. Cleavage from the resin could readily be achieved by transesterification with dimethylaminoethanol, by hydrogen peroxide-catalysed cleavage at pH 10.5, or by hydrogenolysis. Using hydrazinolysis it was possible to liberate the peptide hydrazides that could be purified and used in subsequent fragment couplings. Catalytic-transfer hydrogenation using Palladium Black with formic acid as the hydrogen donor was ultimately used to cleave side-chain benzyl protection.[123,124] When the resin linkage is through a benzyl ester, rather than through a phenyl ester, the catalytic-transfer hydrogenation method was used to cleave the linkage to the resin at the same time as cleaving the side protection.

Transesterification catalysed by $Ti(OPr^i)_4$ in the presence of methanol has been used for removal of peptides from standard Merrifield resins.[125] This catalyst, which on this occasion was used for forming a methyl ester by transesterification, may also be used in solution to prepare benzyl or isopropyl esters.

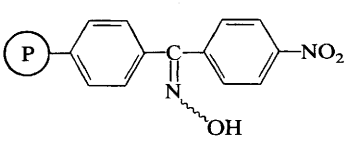
The mechanism of HF cleavage and of concomitant reduction of methionine sulphoxide to methionine during HF cleavage has been investigated in detail.[126,127] The reduction of methionine sulphoxide is quantitative when a low concentration of HF in dimethyl sulphide is used (1 : 3 v/v), and this low concentration of HF is also capable of cleaving most benzyl-based protecting groups; HF–thioanisole was found to be less satisfactory. This work[126,127] shows that when HF is in a low concentration an S_N2 mechanism predominates, in contrast to the S_N1 reaction that predominates at higher HF concentrations. For this reason carbo-cation intermediates are not generated when a low concentration of HF is used. In the presence of thiols both the sulphoxide protection and the N^{in}-formyl protection used for tryptophan are removed. When this deprotection method is used, a second stage employing higher

[121] R. Epton, G. Marr, P. W. Small, and G. A. Willmore, *Int. J. Biol. Macromol.*, 1982, **4**, 62.
[122] R. Epton, P. Goddard, S. J. Hocart, G. Marr, and D. Hudson, *Int. J. Biol. Macromol.*, 1982, **4**, 233.
[123] M. Buckle, R. Epton, G. Marr, P. W. Small, and D. Hudson, *Int. J. Biol. Macromol.*, 1982, **4**, 275.
[124] R. Epton, J. Hocart, G. Marr, and P. W. Small, *Polymer*, 1982, **23**, 1685.
[125] H. Rehwinkel and W. Steglich, *Synthesis*, 1982, 826.
[126] J. P. Tam, W. F. Heath, and R. B. Merrifield, *Tetrahedron Lett.*, 1982, **23**, 2939.
[127] J. P. Tam, W. F. Heath, and R. B. Merifield, *Tetrahedron Lett.*, 1982, **23**, 4435.

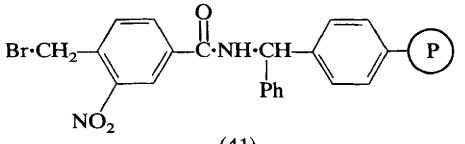
concentrations of HF is used to cleave both the peptidyl–resin bond and the tosyl group from arginine.

Resin cleavage using methane sulphonic acid and TFA has also been investigated,[128] and it was found that this system was as effective as trifluoromethane sulphonic acid–TFA at cleaving the peptidyl–resin linkage. Solid-phase synthesis of Leu^5-enkephalin using DCCI/HOBt for coupling with *N*-tritylamino protection has been carried out.[129] In this synthesis the trityl protection was removed by 10% trichloroacetic acid in dichloromethane, and at the end of the synthesis the peptidyl–resin linkage was cleaved by treatment with HBr in TFA over 90 minutes.[129]

Several new resins have been proposed for use in synthesis. Polymer-bound oximes (40) have been used for the solid-phase synthesis of a melitin analogue.[130] This resin may be used for the assembly of fragments and permits peptide fragment condensation when activated by the addition of acetic acid. Direct cleavage by aminolysis may also be achieved without racemization. The resin (41) has been prepared and used in a synthesis of apamin.[131] The (1—6) protected fragment was assembled on the resin by the symmetrical anhydride method; cleavage of the fragment could be achieved either by photolysis or by hydrazinolysis. This protected fragment was then coupled with the resin-bound (7—18) portion, using either DCCI/HOBt or azide methods. Total deprotection was then carried out using HF and anisole, giving the tetra-Acm apamin; oxidative deprotection giving apamin was then carried out.



(40)



(41)

A macroporous resin containing styrene and radiation grafted onto teflon has been claimed to be superior to the basic chloromethyl resin.[132,133] The polymer, which has high capacity and mechanical strength, contains a bromomethyl group through which the peptidyl–resin linkage is established by reaction with a Boc amino-acid caesium salt or by use of the Pcp ester in the presence of HOBt. At the end of the synthesis, cleavage may be achieved by the use of HBr in the presence of TFA and anisole, regenerating the bromomethyl polymer, which can then be recycled. The use of the resin was demonstrated by a synthesis of the (57—69) fragment of human haemoglobin

[128] J. W. Van Nispen, J. P. Polderdijk, W. P. A. Janssen, and H. M. Greven, *Recl. Trav. Chim.*, 1981, **100,** 435.

[129] P. Cordopatis, D. Papaioannou, and D. Theodoropoulos, *Dev. Endocrinol.*, 1981, **13,** 63.

[130] W. F. DeGrado and E. T. Kaiser, *J. Org. Chem.*, 1982, **47,** 3258.

[131] E. Giralt, F. Albericio, E. Pedroso, C. Granier, and J. Van Rietschoten, *Tetrahedron*, 1982, **38,** 1193.

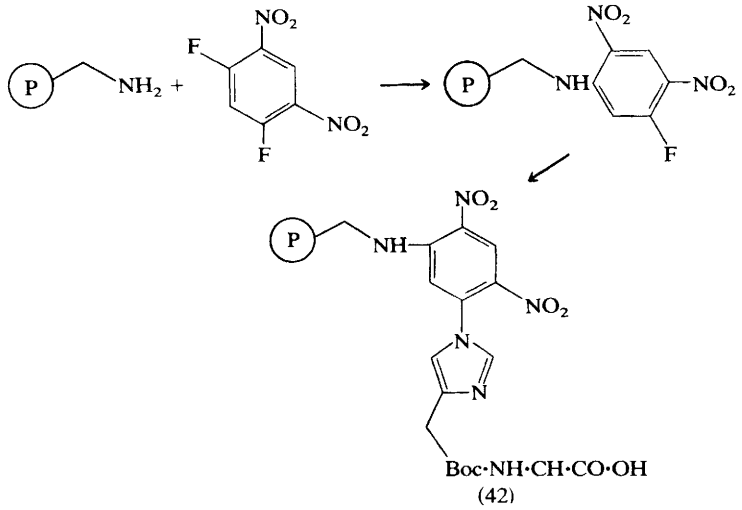
[132] M. V. Sidorova, G. A. Zheltukhina, E. I. Filippovich, M. B. Shishova, and R. P. Evstigneeva, *J. Gen. Chem. U.S.S.R.*, 1982, **51,** (Part 2), 2247; *Zh. Obshch. Khim.*, 1982, **51,** 2605.

[133] M. V. Sidorova, G. A. Zheltukhina, U. O. Kalei, G. I. Aukone, E. I. Filippovich, and R. P. Estigneeva, *J. Gen. Chem. U.S.S.R.*, 1982, **52,** (Part 2), 1047; *Zh. Obshch. Khim.*, 1982, **51,** 1194.

β-chain. The use of Sephadex LH20 as a solid-phase support has also been mentioned.[134] In this case up to 45% of the hydroxyl sites on the polymer may be acylated using a Boc amino-acid nitrophenyl ester in the presence of imidazole. Synthesis using Boc amino-acids can then be carried out in the normal way.

Porous silica beads internally coated with a new copolymer prepared from *N*-[2-(4-acetoxyphenyl)ethyl]acrylamide and *N*-[3-(triethoxysilyl)propyl]acrylamide have been described.[135] The resulting phenolic polymer has been acylated with Boc-Gly-OH in the presence of dimethylaminopyridine and DCCI; unreacted phenolic hydroxyl groups were then blocked by acetylation prior to synthesis. Peptide synthesis was carried out using DCCI/HOBt coupling, and deprotection was mediated by HCl in benzyl alcohol. Direct functionalization of a styrene–1% divinylbenzene copolymer to give a phenolic polymer has been reported.[136] Bromination in carbon tetrachloride in the presence of a trace of iodine and bromine gives a bromo derivative that may be lithiated using butyl-lithium. Treatment with t-butyl hydroperoxide under argon gave the free phenolic polymer. The phenolic content was estimated by a radiochemical method using [^{14}C]acetic anhydride.

Resin linkage using side-chain attachment has also been discussed. An aminomethyl copolystyrene–divinylbenzene resin was coupled with 1,3-difluoro-4,6-dinitrobenzene as in Scheme 7.[137] The fluorodinitrobenzene resin was then coupled with Boc-His-OH, thus blocking the imidazole function. The resin-bound histidine (42) was then coupled by the DCCI/HOBt method to H-Gly-OBzl. The Boc group was then removed and Boc-His(Dnp)-Gly-OH

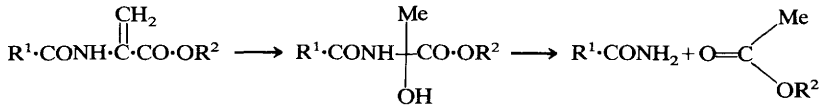


(42)

Scheme 7

[134] U. O. Kalei, N. N. Podgornova, N. K. Zentosova, N. Yu. Kozhevnikova, and G. P. Vlasor, *J. Gen. Chem. U.S.S.R.*, 1982, **51**, (Part 2), 2414; *Zh. Obshch. Khim.*, 1982, **51**, 2799.
[135] R. Epton, G. Marr, and R. G. Ridley, *Polymer*, 1982, **23**, 306.
[136] R. Epton, G. Marr, and P. W. Small, *Polymer*, 1981, **22**, 842.
[137] S. S. Isied, C. G. Kuehn, J. M. Lyon, and R. B. Merrifield, *J. Am. Chem. Soc.*, 1982, **104**, 2632.

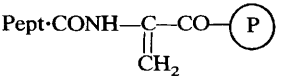
added; after Boc deprotection a second mole of Boc-His(Dnp)-Gly-OH was added. Cyclization was then carried out on the solid support, and cleavage using a thiol in DMF gave an 85% yield of *cyclo*-(His-Gly)$_3$.



(43) (44) (45)

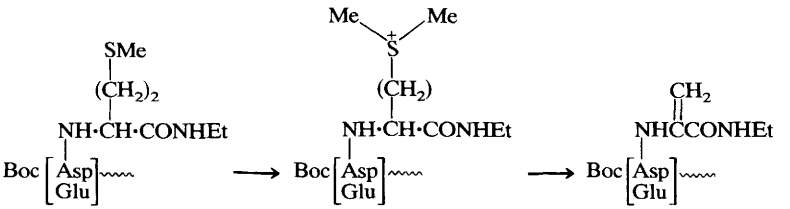
Scheme 8

The use of the dehydroalanine residue as an amide protecting group and/or a resin linkage has been reported.[138] Thus, the dehydroalanyl peptide (43) on treatment with water gives the intermediate (44), which decomposes to the peptide amide (45), as in Scheme 8. This may be achieved by treatment with 1 M HCl in the presence of glacial acetic acid with 3 equivalents of water. Similarly, the method may be used to prepare a peptidyl–resin linkage (46);



(46)

once again, this linkage undergoes cleavage on treatment with dilute acid. The method has been used for side-chain and main-chain protection in a synthesis of oxytocin. In this synthesis the dehydroalanine was masked until a late stage by incorporating methionine ethyl amide as the side-chain derivative of aspartic or glutamic acid (see Scheme 9). Treatment with methane-fluorosulphonic acid gave the corresponding sulphonium salt (47), which on treatment with sodium hydroxide gives the dehydroalanyl derivative (48).



(47) (48)

Reagents: i, $MeSO_3F$; ii, NaOH.

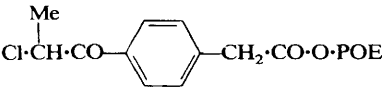
Scheme 9

Other Repetitive Methods. Several syntheses using the liquid-phase method have been reported, and both ^{13}C[139] and ^{1}H[140] n.m.r. spectroscopies have been used to monitor the build-up of peptide on the polyethylene glycol support. The formation of α-helix and β-pleated sheet could be observed, and it was

[138] K. Noda, D. Gazis, and E. Gross, *Int. J. Pept. Protein Res.*, 1982, **19**, 413.
[139] W. Schoknecht, K. Albert, G. Jung, and E. Bayer, *Liebigs Ann. Chem.*, 1982, 1514.
[140] A. A. Ribeiro, R. P. Saltman, M. Goodman, and M. Mutter, *Biopolymers*, 1982, **21**, 2225.

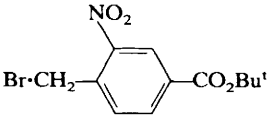
found from c.d. measurements[139] that α-helical structure was observed at the hexapeptide level.

The photolabile support (49)[141] has been used for a synthesis of the (32—36) fragment of thymopoietin II. The first residue, Boc-Tyr(Bzl)-OH, was initially attached as its ethyldi-isopropylammonium salt. However, it was found that a more efficient esterification could be achieved if the resin and esterifying acid were mixed in the presence of potassium fluoride, the reaction being carried out for 40 hours at 50 °C. Couplings were carried out by a standard DCCI method, and intermediate deprotections were carried out using TFA in dichloromethane. At the end of the synthesis the protected peptide was released by photolysis at 350 nm using DMF as solvent.



(49)

The use of compound (50) in the preparation of a linkage agent in liquid-phase synthesis has been described.[142] The compound was coupled to a Boc-amino-acid caesium salt, then deprotected with TFA and the amino function reblocked with Boc-azide prior to reaction with glacial polyethylene glycol.



(50)

A rapid method known as the 'hold in solution' method[143] has been described. Initial acylation of an amino-acid benzyl ester is carried out in 1,2-dichloroethane using a water-soluble carbodi-imide and HOBt. The organic layer is then washed and the Boc group, which is used for the protection of the amino function, is removed by acidolysis, following the addition of HCl in dioxane. Neutralization was carried out, then followed by washing prior to the next acylation. The reactions were checked by t.l.c., and the washing cycles consisted of extraction with 0.1 M HCl, 0.5 M sodium carbonate, and water. When emulsions formed, centrifugation was used to break the emulsion. The advantages are claimed to be that the method is fast and allows easy characterization of intermediates, whilst not requiring a large excess of the acylating species or special apparatus.

Polymeric Peptides.—The majority of polypeptides prepared are listed in Appendix I. Polymerization of *N*-carboxyanhydrides[144—146] and thiocarboxyanhydrides[147] has been studied. Generally low stereospecificity is

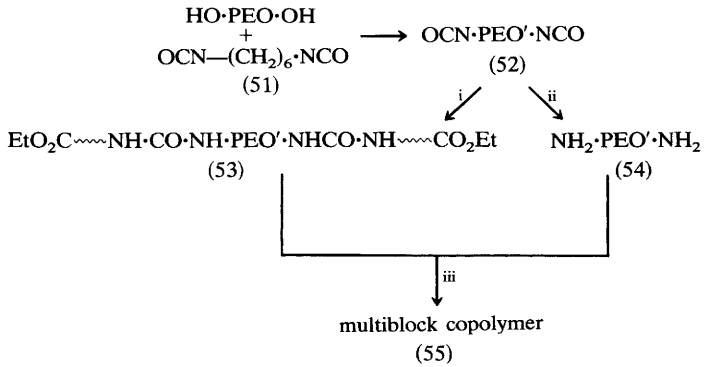
[141] F. S. Tjoeng and G. A. Heavner, *Tetrahedron Lett.*, 1982, **23,** 4439.
[142] B. Hemmasi, W. Stueber, and E. Bayer, *Hoppe-Seyler's Z. Physiol. Chem.*, 1982, **363,** 701.
[143] S. Nozaki and I. Muramatsu, *Bull. Chem. Soc. Jpn.*, 1982, **55,** 2165.
[144] H. R. Kricheldorf and W. E. Hull, *Biopolymers*, 1982, **21,** 1635.
[145] M. Amouval-Ruderman and H. Sekiguchi, *Polym. Bull.*, 1981, **6,** 69.
[146] M. Ova and T. Takahashi, *J. Polym. Sci. Polym. Chem.*, 1982, **20,** 529.
[147] H. R. Kricheldorf and T. Mang, *Makromol. Chem.*, 1982, **183,** 2113.

observed when racemic amino-acid *N*-carboxyanhydrides are used,[144] and ^{15}N n.m.r. showed that the stereo blocks are not usually larger than four monomer units. Benzylamine, triethylamine, or potassium t-butoxide were used to initiate the polymerization.[144] Initiation with chiral quaternary ammonium acetates[145] and *N*-butylamine[146] was also examined. Polymerization using the thiocarboxyanhydrides[147] was found to show low stereoselectivity, and isotactic blocks were not generally larger than hexapeptides when valine and leucine were studied. As in one of the previous papers[144] the polymerization was studied using ^{1}H, ^{13}C, and ^{15}N n.m.r.[147]

Direct polymerization of histidine or N^{im}-benzylhistidine with diphenylphosphoryl azide[148] has been reported. A suspension polymerization in the presence of diphenylphosphoryl azide and triethylamine indicated that imidazole protection was not required for the preparation of poly-L-histidine. A photoresponsive polymer formed by coupling polybutanoic acid derivatives with aminostilbene has also been reported.[149]

Monodispersed poly-γ-benzylglutamates have been prepared by the fragment condensation method.[150] Poly(Glu-OBzl) compounds ($n = 4$, 8, 16, 32, 64, or 128) have been prepared by fragment condensation, starting from Nps-Glu(OBzl)-ONSu. Salt coupling gave the dipeptide, which was then recycled to provide all the higher homologues. Purification of the protected fragments was carried out on LH20, and the deprotected peptide was purified on Sephadex G50. It was found[151] that β-structure was first observed at the tetrapeptide level and that α-helical structure was first seen when the peptide contained between 6 and 8 residues.

Multi-block copolymers have been prepared from oligo(ethylene oxide) and oligopeptide fragments using chymotrypsin for the formation of amide bonds.[152] Synthesis of the multi-block copolymer (55) was carried out by the route shown in Scheme 10. The bis-isocyanate was condensed with



Reagents: i, NH_2~~CO_2Et (peptide ethylester); ii, H_2/Pd/Bzl·OH; iii, Chymotrypsin.

Scheme 10

[148] T. Naruse, B. Nakajima, A. Tsutsumi, and N. Nishi, *Polym. J.*, 1981, **13,** 1151.
[149] A. Fissi, J. L. Houben, N. Rosato, S. Lopes, O. Pieroni, and F. Ciardelli, *Makromol. Chem. Rapid Commun.*, 1982, **3,** 29.
[150] T. Ozaki and A. Shoji, *Makromol. Chem. Rapid Commun.*, 1982, **3,** 157.
[151] T. Ozaki, A. Shoji, and M. Furukawa, *Makromol. Chem.*, 1982, **183,** 771.
[152] A. Leonhardt, F. Gutzler, and G. Wegner, *Makromol. Chem. Rapid Commun.*, 1982, **3,** 461.

polyethylene oxide under controlled conditions to give the modified bis-isocyanate (52). This was then either converted to the peptide derivative (53) or hydrogenolysed to give the bis-amine (54). The two components (53) and (54) were then polymerized in aqueous DMSO using chymotrypsin as catalyst; gel filtration was used to isolate the products.

Enzyme-mediated Synthesis and Semisynthesis.—The use of enzymes for the formation of peptide bonds seems now to have become firmly established, to the extent that it provides a viable alternative to chemical methods of synthesis. Two reviews have been published covering a variety of enzymes and substrates,[153,154] and the thermodynamic aspects of enzyme-mediated peptide synthesis have been considered in detail.[155]

The specificity of pepsin-catalysed amide-bond formation has been examined[156] using the coupling of Z-Phe-OH or Z-Gly-Phe-OH with H-Leu-OBu^t or H-Leu-NHPh. H.p.l.c. techniques were used to analyse the reaction, and between 95 and 100% yields were achieved. It was concluded that K_{syn} depends upon the $pK_{a'}$ of the carboxylic acid and the amine component and that 1,4-butanediol shifts the pK_a of the carboxylic acid to a higher apparent value, thus increasing K_{syn}. Precipitation of product was not rate determining, and ionic strength did not affect the rate of reaction. Detailed knowledge of the hydrolytic reaction is important, and often transpeptidation may be observed.

The possibility of using nucleophiles bound to an insoluble support in enzyme-mediated peptide synthesis has been investigated.[157] Thermolysin, α-chymotrypsin, and papain have been used to couple carboxyl components to a silica-supported leucine amide. It was found that a spacer of 21 bonds (7 amino-acid residues) was required in order to achieve efficient coupling. This spacer is required in order to allow the coupling residue (leucine) to approach the active site of the enzyme. It was anticipated that soluble supports such as polyethylene glycol would eliminate this problem.

Extensive use of enzymic coupling has been reported in a synthesis of dynorphin (1—8)[158] and in the preparation of the *C*-terminal octapeptide of CCK.[159] The dynorphin (1—8) synthesis used α-chymotrypsin, trypsin, and papain in the assembly of the constituent peptides. Using α-chymotrypsin, the yield varied between 52 and 72%; with trypsin it was 65% and with papain it ranged between 71 and 80%. Phenylhydrazides were used for carboxyl protection, being removed by treatment with ferric chloride or *N*-bromosuccinimide. In the work on the CCK octapeptide[159] phenylhydrazides were again used for carboxyl protection. Similar enzymes were employed, although on this occasion thermolysin was also used. The final combination of the (1—4) and (5—8) fragments was carried out using DCCI/HOBt, and sulphation of tyrosine-2 was

[153] H. D. Jakubke and P. Kuhl, *Pharmazie*, 1982, **37**, 89.
[154] K. Morihara and T. Oka, *Kagaku No Ryoiki*, 1982, **36**, 444.
[155] D. D. Petkov, *J. Theor. Biol.*, 1982, **98**, 419.
[156] H. Bozler, S. I. Wayne, and J. S. Fruton, *Int. J. Pept. Protein Res.*, 1982, **20**, 102.
[157] A. Koennecke, S. Dettlaff, and H. D. Jakubke, *Monatsh. Chem.*, 1982, **113**, 331.
[158] W. Kullmann, *J. Org. Chem.*, 1982, **47**, 5300.
[159] W. Kullmann, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79**, 2840.

carried out using pyridine–SO_3 after removal of benzyl protecting group by transfer hydrogenation, using cyclohexadiene and Palladium Black. These two syntheses served to illustrate that enzyme-mediated coupling can be used to replace conventional chemical coupling agents when the sequence of the peptide undergoing synthesis contains residues that are appropriate to the specificity of the enzymes available.

Formation of peptide hydrazides by enzyme-catalysed reaction of hydrazine or a protected hydrazide has been carried out.[160—162] Terminal arginine may be converted into its corresponding hydrazide by treatment with hydrazine in the presence of trypsin; this may be carried out either during tryptic cleavage or after isolation of the product from tryptic digestion. Similar results have also been obtained by reaction with chymotrypsin, elastase, and subtilisin. No side-chain protection is required, and butane-1,4-diol and glycerol appear to be suitable cosolvents, although the hydrazide did not form when DMF was used as solvent. Thin-layer chromatography appears to be of considerable use in characterization of products.[162] Peptide hydrazides produced in this way are particularly useful in semisynthesis when further couplings are to be carried out by the azide method.

[Ile^{13}, Hse^{20}]-ribonuclease A has been prepared by a semisynthetic method.[163] The natural peptide was cleaved at methionine-20 by cyanogen bromide. [Ile^{13}, Met^{20}]-*S*-peptide-Gly_2-OH was then prepared by standard Merrifield solid-phase synthesis. HF deprotection and purification gave the extended *S*-peptide. Digestion with cyanogen bromide removed the diglycyl fragment from the *C*-terminus and liberated the homoserine lactone at position 20; this was subsequently coupled with bovine *S*-protein at pH 7.5, using phosphate buffer. H.p.l.c. was used to monitor the formation of the semisynthetic ribonuclease A.

(1—15)/(21—124) ribonuclease A [des(16—20) ribononuclease A] has been prepared.[164] The (1—10) and (11—15) *S*-peptide fragments were mixed in the presence of clostripain as a catalyst. Stereospecific condensation was achieved by adding (21—124) ribonuclease A, which acts as a trap for (1—15) but not for the (1—10) or (11—15) fragments. This resulted in the formation of a (1—15)/(21—124) non-covalent complex of ribonuclease A.

[Nle^4, Tyr^5]- and [Nle^8]-bovine pancreatic phospholipases A2 have been prepared.[165] Cyanogen bromide cleavage of ε-acetimidated bovine phospholipase A2 gave the (9—123) fragment. This was then coupled with Boc-Nle-ONSu or Boc-Met-ONSu to give the (8—123) fragments. The (1—7) portions were synthesized by solid-phase techniques and then coupled with the Boc-(8—123) portion after TFA cleavage of the latter. Coupling of the Boc-(1—7)-OH was carried out by the isobutylchloroformate mixed-anhydride

[160] R. M. L. Jones and R. E. Offord, *Biochem. J.*, 1982, **203,** 125.
[161] S. Yagisawa, *J. Biochem. (Tokyo)*, 1982, **89,** 491.
[162] W. Kullmann, *J. Liq. Chromatogr.*, 1981, **4,** 1947.
[163] P. Hoogerhout and K. E. T. Kerling, *Recl. Trav. Chim.*, 1982, **101,** 246.
[164] G. A. Homandberg, A. Komoriya, and I. M. Chaiken, *Biochemistry*, 1982, **21,** 3385.
[165] G. J. M. Van Scharrenburg, W. C. Puijk, M. R. Egmond, P. H. Van Der Schaft, G. H. De Haas, and A. J. Slotboom, *Biochemistry*, 1982, **21,** 1345.

method as glycine was *C*-terminal. After deprotection the various enzyme analogues were obtained.

A semisynthetic approach to sperm-whale myoglobin by fragment condensation has been reported.[166] 3-Bromo-2-(2-nitrophenylsulphenyl)skatole was reacted with acetimidyl-myoglobin after haem removal. This cleaved the tryptophan residue, giving a (15—153) fragment. Thus after reduction with mercaptoethanol and coupling with Nps-Trp-ONSu the protected (14—153) fragment was obtained. Boc-(6—13)-ONSu and Boc-(1—5)-ONSu were sequentially added, and after deprotection the haem was reinserted, giving [ε-Lys[19]]-acetimidyl-myoglobin in 4% overall yield. This demonstrates the feasibility of analogue synthesis for myoglobin.

Insulin analogues have continued to be a major target of semisynthesis.[167–170] The preparation of citraconyl-insulins by reaction with citraconic anhydride has been studied.[167] At pH 8.5 Al citraconyl-insulin is obtained in 41% yield, whereas at pH 7 Al,Bl-dicitraconyl-insulin is isolated in a yield of 39%. After treatment with excess citraconic anhydride at a pH of 5.6, partial deblocking was achieved by reducing the pH to 5. After 30 minutes Al,Bl-dicitraconyl-insulin was the major product, whereas after 17 hours Bl-citraconyl-insulin was obtained in 40% yield. The rate of deblocking between pH 3.5 and 5 follows the order B29 > Al > Bl. Thus, the side-chain derivative is relatively labile.

[Leu^{B24}]- and [Leu^{B25}]-insulin analogues were prepared by trypsin-mediated semisynthesis.[168] Synthetic B(23—30) was coupled to material obtained by tryptic digestion of native insulin.

A partially protected des-Ala^{B30}-insulin B-chain disulphide has been prepared.[169] The B-chain di-*S*-sulphonate was treated with carboxypeptidase A at pH 9.5 to remove the *C*-terminal residue. Reduction with mercaptoethanol and subsequent oxidation with iodine gave the B-chain disulphide. This was then esterified with methanol and BF_3 and subsequently subjected to selective tryptic hydrolysis at pH 5.7 with trypsin. Under these conditions the α-ester is hydrolysed, leaving the side-chain ester functions on residues B13 and B21 intact. Reaction with Msc-ONSu then gave the corresponding B $\alpha^{B1},\varepsilon^{B29}$ derivative. The B-chain was then selectively activated with isobutyl chloroformate, and the dipeptide H-Thr-Arg-OH was added. Alkaline removal of the Msc group at pH 13 then gave a 30% overall yield of the required derivative.

The semisynthesis of a shortened open-chain model of proinsulin has been described.[170] The proinsulin model (56) was prepared from a modified A-chain bisdisulphide (57). The modified A-chain was obtained by a combination of stepwise and fragment condensations to give the A-chain bisdisulphide. Coupling of (57) with the des-B30 derivative mentioned above[169] by the mixed-anhydride method gave 29% combination. Deprotection and chromatography then gave the open-chain proinsulin model hexa-*S*-sulphonate (56).

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[167] V. K. Naithani and H. G. Gattner, *Hoppe-Seyler's Z. Physiol. Chem.*, 1982, **363**, 1443.
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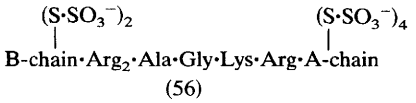
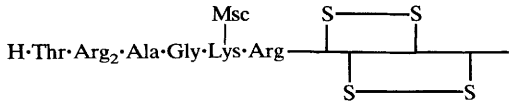
[169] E. E. Büllesbach, E. W. Schmitt, and H. G. Gattner, *Int. J. Pept. Protein Res.*, 1982, **20**, 207.
[170] E. E. Büllesbach, *Tetrahedron Lett.*, 1982, **23**, 1877.



(56)



(57)

Purification Methods.—A number of papers concerned with the chromatography of protected and deprotected peptides have been published. Also, a large number of papers dealing with h.p.l.c. and the separation of peptide diastereomers has been reported. Owing to the large number of papers in this category the details are collected together and presented in Appendix III.

3 Syntheses

A large number of peptides have been prepared by both solution- and solid-phase methods. The details of the majority of the syntheses will not be discussed, and reference is only provided in the form of the tables given in Section 4, Appendix I.

Much interest continues to be shown in endorphin peptides.[49,108,110,111,171] In this area the need for the rapid production of analogues for physiological testing has brought wide usage of solid-phase synthesis. Syntheses of the 17-residue sequence of dynorphin have been published.[43,97] The solution synthesis[97] that used the fragment condensation approach employed DCCI and HONB, giving a 41% overall yield of pure product as indicated by t.l.c. and h.p.l.c. The 4-methoxy-2,6-dimethylbenzene-sulphonyl group was used for the protection of arginine, and 0.15 M methane-sulphonic acid in trifluoroacetic acid–thioanisole (9:1) was used for total deprotection.

The (1—55) sequence of human γ-lipotropin has been prepared by a solid-phase synthesis, employing an oxymethylphenylacetamidomethyl resin.[106] Interesting features of this synthesis were the use of cyclopentyl protection for the side chain of aspartic acid and the use of N^{in}-formyl tryptophan and methionine sulphoxide protection. Protection of these two amino-acids is at present being more widely used in solid-phase synthesis.

A solution synthesis of γ_3-melanotropin[96] has been carried out again, using methionine sulphoxide and arginine with sulphonyl-based protection. The solution synthesis employed a fragment-coupling approach, and total deprotection using trifluoromethane-sulphonic acid–TFA-thioanisole gave the 27-residue peptide.

Work on human parathyroid hormone[95,98,101] has culminated in the total solution synthesis of the 85-residue hormone. The solution synthesis[95] utilized maximal protection, the tosyl group being used to protect both arginine and

[171] D. Yamashiro, P. Nicolas, and C. H. Li, *Int. J. Pept. Protein Res.*, 1982, **20**, 43.

histidine, with benzyl-based protection being used for other side-chain functional groups. Thirteen fragments were assembled, each with its carboxyl terminus protected as a phenacyl ester, the phenacyl group being removed by treatment with zinc in acetic acid. Fragment couplings were achieved using a water-soluble carbodi-imide in the presence of hydroxybenzotriazole; the major fragments (1—22), (23—38), (39—68), and (69—84) were coupled, extending from the carboxyl terminus to give 2.4 g of the fully protected (1—84) sequence. HF deprotection in the presence of anisole, dimethyl sulphide, and ethanedithiol gave a product that was purified by CMC chromatography, gel filtration on Sephadex G50, and h.p.l.c., giving a homogeneous product as characterized by analytical h.p.l.c. and SDS gel electrophoresis, which is reasonably comparable in activity with the W.H.O. bovine PTH(1—84) standard.

Sulphonyl-based protecting groups have also been widely used in the synthesis of gastrin-releasing peptide and mastopyran-X.[26,41,68] Such protecting groups have been used both for α-amino protection[26] and for side-chain protection.

Solid-phase syntheses of chicken[119] and porcine[120] VIP have been reported. Both syntheses utilized the base-labile Fmoc protecting group for α-amino functions with side-chain protection being t-butyl based. The related peptide porcine PHI[172] has also been prepared. This synthesis was, however, carried out by solution methods using the combination of six fragments to produce the full 27-residue peptide. Nps and benzyloxycarbonyl were used for α-amino protection, and side-chain protection was butyl based. The fragments were combined using DCCI in combination with HONSu or HOBt, and the purity of the product was demonstrated by h.p.l.c. and t.l.c. The optical integrity was checked by a procedure using a D-amino-acid oxidase and a g.c. method using the chiral phase chirosil-val. These methods showed that only racemization associated with 6 M acid hydrolysis had occurred and that no racemization had occurred during synthesis.

A careful synthesis of the antibacterial peptide cecropin A (1—33) has been described,[107] again using a solid-phase method. The preparation of this peptide was carried out by the route outlined above in the section dealing with solid-phase synthesis, and various methods were used to confirm the structure. These included gel filtration, h.p.l.c., amino-acid analysis, and sequencing of the resin-bound peptide.

Studies on a synthetic bicyclic fragment of soya bean Bowman–Birk inhibitor (9—24)[173] and on a 13-residue peptide designed to resemble the primary binding site of basic pancreatic trypsin inhibitor[174] have been published. The Bowman–Birk inhibitor[173] was prepared by solution synthesis utilizing Cys(MeOBzl) at positions 9 and 24 and Cys(Acm) at positions 14 and 22. After the appropriate fragment condensations side-chain protection and the methoxybenzyl sulphur protection were removed by treatment with HF; oxidation with $K_3Fe(CN)_6$ then gave the bis-Acm peptide. The second disulphide

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[173] N. Nishino and N. Izumiya, *Biochim. Biophys. Acta*, 1982, **708**, 233.

link was then formed by treatment with iodine. In the work on the BPTI inhibitor-binding site[174] a solid-phase synthesis was carried out and once again Acm was used for sulphur protection. After cleavage from the resin the bis-Acm peptide was oxidized with iodine in acetic acid to give the product disulphide.

4 Appendix I: A List of Syntheses Reported in 1982

The syntheses are listed under the name of the peptide or protein to which they relate, as in previous years.

Peptide	*Ref.*
Natural Peptides, Proteins, and Partial Sequences	
Actinomycin	
[Sar[1]]-actinomycin D	175
Adrenocorticotropic hormone (ACTH)	
Ostrich ACTH	103
[Lys[17,18]]-ACTH (11—18)	176
ACTH (1—24)	177
Salmon CLIP, [ACTH (18—39)]	99
ACTH (4—7) analogues	178
[^{14}C]Acetyl-ACTH (4—7)	179
ACTH-Gly-SH	70
Allergen M	
Allergen M (88—103)	180
AM toxin	
AM toxin	181
AM toxin III	182
Angiotensin	
Angiotensin II	42, 143
[Cys[5,10]]-angiotensinogen (5—14)	183
Anthopleurin A	
Anthopleurin A	44
Apamin	
Apamin	131
Formyl-apamin	20
Aspartame	
Aspartame	184

[174] J. P. Kitchell and D. F. Dyckes, *Biochim. Biophys. Acta*, 1982, **701,** 149.
[175] K. Nakajima, T. Tanaka, M. Neya, and K. Okawa, *Bull. Chem. Soc. Jpn.*, 1982, **55,** 3237.
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[184] F. J. Vinick and S. Jung, *Tetrahedron Lett.*, 1982, **23,** 1315.

Peptide	*Ref.*
Bacteriorhodopsin	
Bacteriorhodopsin fragment	185
Bleomycin	
Bleomycin A2	186, 187
Deglycobleomycin	188
Bombesin	
Bombesin	43
Bombesin	189
Bombesin analogues	190
Bradykinin	
Bradykinin analogues	191
Des-[Pro^2]-bradykinin	192
Affinity-labelled bradykinin analogue	193
Cardiotoxin	
A modified cardiotoxin	194
β-Casomorphin	
β-Casomorphin	195
β-Casomorphin-5	196
Cecropin	
Cecropin A (1—33)	107
Chemoattractant peptides	
Chemoattractant peptides	19
Chemoattractant peptide related to For-Met-Leu-Phe-OH	197
Chlamydocin	
Ala^4-chlamydocin	198
Cholecystokinin-pancreozymin (CCK-PZ)	
CCK-PZ (30—33)	47
CCK-PZ octapeptide	159
[β-Asp^{32}]-CCK-PZ (27—33)	199
Chorionic gonadotropin (CG)	
β-Subunit of human CG	200

[185] T. Sugihara, E. R. Blout, and B. A. Wallace, *Biochemistry*, 1982, **21,** 3444.
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Peptide	*Ref.*
Corticotropin-releasing factor (CRF)	
Ovine CRF	201
Cyl-2	
[Leu^4]-, [D-Leu^4]-, and [Pro^3, D-Leu^4]-Cyl-2	202
Delta-sleep-inducing peptide (DSIP)	
DSIP	143
Dermorphin	
Dermorphin	203
Deomorphin analogues	204
Destraxin	
Destraxin	181
Distamycin	
Distamycin A analogue	205, 206
Dynorphin	
Dynorphin	43, 97
Dynorphin (1—8)	158
Tritiated dynorphin (1—17)	207
Endorphin	
β-Endorphin	12
Ostrich β-endorphin	110
[Trp^{27}]-β-endorphin	208
[D-Ala^2]-, [Gln^8]-, and [D-Ala^2, Gln^8]-human β-endorphin (1—9)	49
[D-Ala^2]-, [Gln^8]-, and [D-Ala^2, Gln^8]-human β-endorphin (1—17)	209
[Arg^{24,28,29}]- and [Arg^{9,19,24,28,29}]-human β-endorphin (1—28)	210
[Gln^8]- (1—28) and [D-Ala^2, Gln^8]- (1—28) human β-endorphin	108
[Gln^{31}]-β-endorphin, position 8 analogues	171
β-Endorphinyl-Gly-SH	71
Double-headed analogue of β-endorphin	111
Enkephalin	
Enkephalin	81, 126, 162
Leucine-enkephalin	129
Leucine-enkephalin analogues containing $NH_2(CH_2)_nCO_2H$ ($n = 2$—5)	211
Methionine-enkephalin	212
Enkephalin analogue	63, 65
Enkephalin analogue, H-Tyr-aminovaleryl-Phe-Met-OH	213
Enkephalin analogues, H-Tyr-D-Met(O)-Gly-Phe-$(NH)_2$COEt	214
[D-Ala^2]-enkephalin	50

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Peptide	*Ref.*
Enkephalin analogues containing α-amino-n-butyric acid	215
Enkephalin-ol analogues	216
Enkephalin analogues, H-Tyr-D-Ala-Gly-Phe-$(NH)_2$COR	217
Enkephalin analogues, H-Tyr-D-Ala-Gly-Phe-$(NH)_2$COEt	218
[D-Ala^2]-enkephalinamide/hydrazide	219
Modified enkephalins containing *N*-acylated Gly-Gly-OH	220
[Penicillamine^2, Cys^5]-enkephalinamide	221
Cyclic enkephalin analogues	222
Dimeric enkephalins	223
Dimeric enkephalins, (H-Tyr-D-Ala-Gly-Phe-$NHCH_2)_2CH_2$	224
Enkephalin isosters	225
Dehydroenkephalins	226
[D-Ala^2, 5-leucinol]-enkephalin	227
[Dehydro-Ala^2, Leu^5]-enkephalin	228
Erythromycin	
$(Ala)_n$ attached to erythromycin (n = 1—3)	229
FK156, immunoactive peptide	
Synthesis of FK156	230
Immunostimulating peptide, FK156	231
Foot-and-mouth disease virus protein	
Foot-and-mouth disease virus VP1 (141—160) and (200—213)	2
Gastrin	
Human gastrin 1	93
Gastrin fragments	116
Gastrin tetrapeptide	48
Gastrin (11—17)	22
Gastrin (22—30) analogue	232
Pentagastrin	45, 126

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Peptide	*Ref.*
[Boc-^{14}C]-pentagastrin	233
Gastrin-releasing peptide (GRP)	
GRP	26, 189
[GRP] analogues	190
Chicken GRP	41, 68
Glutathione	
N-Acyl-glutathione	234
Gramicidin	
Synthesis of [1-^{13}C-D-Leu^{12,14}]-gramicidin A	235
[D-Ser$^{4,4'}$]-gramicidin S	236
Haemoglobin	
Human haemoglobin β-chain (57—69)	132
Insulin	
[Leu^{B24}]- and [Leu^{B25}]-insulin	168
[D-Arg^{B22}]-porcine insulin	237
[Trp^{A1}]- and [D-Trp^{A1}]-bovine insulin	238
$N^{\alpha A1}$, $N^{\varepsilon B29}$-Diaminosuberoyl-[D-Ala$_2$^{A1,B1}]-insulin	239
Citraconyl-insulin	167
Covalently linked insulin dimers	240
Partially protected des-(Ala^{B30})-insulin B-chain disulphide	169
Insulin B (13—20)	139
Hybrid insulins with modified B-chain	241
Human insulin B-chain (9—14) and (15—20)	242
Human insulin B-chain (1—8)	243
Open-chain proinsulin model	170
Luteinizing-hormone-releasing hormone (LHRH)	
LHRH	118
[^{3}H]LHRH	244
LHRH analogue	43
[Aza-Gly^{10}]-LHRH analogues	245
[Ac-Thr^1, D-Phe^2, D-Trp^{3,6}]-LHRH (antagonist)	246
Hydrophobic, superagonist [D-amino-acid^6]-LHRH analogues	247

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Peptide	*Ref.*
LHRH prohormone model	248
Lipotropin	
Human γ-lipotropin	106
Lysozyme	
Lysozyme fragment	160
Hen egg-white lysozyme (38—54)	249
Malformin	
[Ile^3, Val^5]-malformin (allomalformin)	250
Mastopyran	
Mastopyran X	41
Mastopyran X	26
Mast-cell degranulating peptide (MCD)	20
Formyl-MCD	
Melanotropin	
Equine β-melanotropin	36
Bovine γ_3-MSH	96
Salmon α-MSH II	104
α-Melanotropin analogues	251
[Nle^4]-α-MSH (1—10)	51
[Cys^{4,10}]-α-MSH disulphide	252
Photoreactive derivatives of α-melanotropin	253
Myeloma I_g	
Myeloma I_g H-V (43—55)	109
Myoglobin	
Myoglobin	166
Neurotensin	
Acetyl-neurotensin (8—13)	254
Neurophysin	
Photoaffinity-labelled neurophysin	255
Plasminogen	
Plasminogen active-site nonapeptide disulphide	256
Oxytocin	
Oxytocin	115, 138
[Malamidic acid^5]-oxytocin	257
[D-Gln^4]-oxytocin	258
[t-Leu^8]-oxytocin	259
[Aminoacetone^9]- and des-(Gly^9)-[*p*-aminobenzamide^8]-oxytocin	260

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Peptide	*Ref.*
Oxytocin analogues with high, specific natriuretic activity	261
PHI	
PHI	172
Polystes kinin	
Polystes kinin (4—12)	176
Parathyroid hormone (PTH)	
Human PTH (1—84)	95
PTH analogues	262
Human PTH (1—34)	263
Human PTH (1—38)	101
Human PTH (19—38)	98
Human PTH (32—43) and (43—55)	264
Phospholipase A_2	
[Nle^4, Tyr^5, Nle^8]-phospholipase A_2	165
Physalaemin	
[Lys^5, Thr^6]-physalaemin	102
Protohaemin	
Peptide derivatives of protohaemin IX	265
Ribonuclease (RNase)	
RNase	5, 266, 267
[Ile^{13}, Hse^{20}]-*S*-peptide lactone	163
[Ile^{13}, Hse^{20}]-RNase A	163
(1—15)/(21—124) RNase	164
Somatostatin	
Somatostatin	268
[Nle^8]-somatostatin-28	269
[*NN'*-Dimethyl-Asn^5]-somatostatin	270
Somatostatin analogue, *cyclo*-(Abu-Asn-Phe_2-D-Trp-Lys-Thr-Phe)	271
Substance P	
Substance P	272
Substance P (7—11)	273
Substance P analogues	274
Substance P (5—11) analogues containing 5-aminovaleric acid	275

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Peptide	*Ref.*
Substance P antagonists	276
Substance P, C-terminal heptapeptide amide analogues	277
[D-Pro^2, D-Trp^{7,9}]-substance P	278
[Phe$(N_3)^{7,8}$, Nle^{11}]-substance P	279
[4-^{3}H-Phe^8]-substance P	280
Thienamycin	
Thienamycin precursor	281
Thymic peptides	
[Dab^3]- and [Asn^5, Gln^9]-serum thymic factor	282
[^{125}I]-Labelled derivatives of thymic factor	283
Thymopoietin II (32—49) analogue	92
Thymopoietin II (32—36)	141
Des-acetyl-thymosin α	284
Thynnine	
Thynnine Z1 ornithine analogue fragments	40
Thyrotropin-releasing hormone (TRH)	
TRH analogues	285
[^{3}H]TRH	286
Triostin A	
Des-*N*-tetramethyltriostin A	287
Tuftsin	
Tuftsin analogues	288
Uraemic hexapeptide	
Uraemic hexapeptide	289
Urotensin	
Urotensin II	100, 113
Vasointestinal peptide (VIP)	
Chicken VIP	119
Porcine VIP	120
Vasopressin	
Tritiated vasopressin analogues	290
[t-Leu^8]-vasopressin	259
[Lys^8]-vasopressin	59
Lysine-vasopressin	22
[Lys^8]- and [Arg^8]-vasopressin	114

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Peptide	*Ref.*
Arginine-vasopressin antagonists	291
[D-Gln[4]]-vasopressin	258
Selective antagonists of the vasopressor response to arginine-vasopressin	292
Selective antagonists of the antidiuretic response to arginine-vasopressin	293
Zizyphin	
9,10-Dihydrozizyphin G	294
Sequential Oligo- and Poly-peptides	
Ac-$(Aib)_n$-N^1-methylamides ($n = 1$, 2, or 3)	295
Z-$(Aib)_n$-OBu^t ($n = 3$—5)	296
Poly-β-alanine	297
H-$(Ala\text{-}Lys\text{-}Gly)_{10}$-Phe-OH	298
Poly[Asp(OBzl), Lys(Z)]	146
Polydepsipeptides	299
Poly-Glu-(stilbene)	149
Poly-Glu-$(OBzl)_n$ ($n = 4$, 8, 16, 32, 64, or 128)	150
Nps-$[Glu(OBzl)]_n$-OBzl ($n = 2$—24)	151
H-$(Gly\text{-}Pro)_n$-OH ($n = 2$—5)	300
Poly-His and poly-His-(Bzl)	148
Homo-oligoprolines	301
H-$(Hyp\text{-}Pro\text{-}Gly)_{10}$-OH	302
H-$(Ile\text{-}Lys\text{-}Gly)_n$-Phe-OH ($n = 3$—5)	298
H-$(Lys\text{-}Ala_2)_n$-OH ($n = 10$—34)	303
Poly-(Lys_3-DOPA) and poly-(DOPA-Lys_n) ($n = 1$—3)	304
Poly-(ε-Lys) derivatives	305
Peptide block copolymers	306
Poly-amino-acids as epoxidation catalysts	307
Polyethylene oxide/peptide block copolymer	152
Polyvinyl/polyamino-acid graft copolymer	308

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Peptide	*Ref.*
Enzyme Substrates and Inhibitors	
Amastatin analogue	309
Angiotensin-converting enzyme (ACE) inhibitor	310—314
ACE inhibitor containing dehydrophenylalanine	315
ACE inhibitor, 5-benzamido-4-oxo-6-phenylhexanoyl-Pro-OH	316
Bovine-lung angiotensin I-converting enzyme inhibitor	317
Bowman-Birk (9—24)/(14—22) disulphide	173
Bowman-Birk inhibitor	267
Thiopeptide as a substrate for cadmium carboxypeptidase	318
Carboxypeptidase A, thioamide substrate	319
α-Chymotrypsin/subtilisin substrate	320
Cysteine proteinase inhibitor	321
Fluorogenic substrate for cystine aminopeptidase	322
Leukocyte elastase inhibitor	323
Chromogenic substrates for human leukocyte elastase	324
Enkephalinase inhibitor	325—327
Peptide, folate inhibitors	328
Chromogenic substrates for human fibrinolytic proteinase (SFP)	324
S-Formyl-glutathione, substrate for bacterial formate dehydrogenase	329
Bovine gastricsin substrate	330
Substrate for cyclic nucleotide independent histone kinase	331
Isopenicillin synthetase inhibitor	332

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Peptide	*Ref.*
Substrate for oxoprolinase	333
Keto analogue of pepstatin	334
Pepstatinyl-Gly-Lys_2OH	335
Procollagen C-proteinase inhibitor (H-Tyr_2-Arg-Ala-Asp_2-Ala-OH)	336
Human renin inhibitors	337
Renin inhibitor, L-α-hydroxyisovaleryl-Leu-Val-Phe-OMe	338
Renin inhibitors, H-Leu_2-Val-Phe-OMe analogues with Leu replaced by 3-amino-2-hydroxy-5-methylhexanoic acid	339
Serine proteinase inhibitors	340
Serine proteinase inhibitors	341
Statine	342
Synthetic thrombin substrate	343
Fluorogenic peptide substrate for thrombin	344
Thrombin inhibitor	345
Thrombin inhibitor	346
Thrombin, plasmin, and urokinase inhibitors	347
Thrombin substrate	348
Thrombin substrates, Tos-X-Arg-OMe [X = (Me)Val or Sar]	349
Thirteen-residue binding-site model for bovine pancreatic trypsin inhibitor	174
Glycopeptides	
N-Acetylmuramylalanyl-D-isoglutamine analogues	350
N-Acetylmuramyl dipeptides	351
Amino-acid glucopyranosyl esters	352
Glucosaminic acid peptides	353
Glycopeptides	354, 355
Glycopeptide haptens	356

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Peptide	*Ref.*
N^{α}-Glycosyl-gastrin-related peptides	357
Glycopeptides containing the *N*-acetylglucosaminyl-(β1—4)-*N*-acetylmuramyl disaccharide unit	358
Immunoadjuvant peptide, NAM-Ala-D-Ala-D-Glu-NH_2 analogue	359
Immunoadjuvant peptide	360—362
Muramyl dipeptide analogues	363, 364
Muramyl dipeptides, deoxyanalogues	365

Miscellaneous Peptides

Ac-dehydroPhe-Phe-OMe	366
Ac-L-(U-^{14}C)-Leu-Leu-Arg-H	367
Ac-Met-Lys-(biotinyl)-Met_2-OMe	368
Active-site model of α-chymotrypsin, cyclic disulphide nonapeptide	369
Unsymmetrical cystine peptides	56
Aib peptides	370
Peptides containing β-alanine	371
Peptides containing 1-aminocyclopropane-1-carboxylic acid	372
Peptides containing 4-amino- and 4-hydroxy-cyclohexane-1,1-dicarboxylic acid	373
Peptides containing β-aminobutyric acid	374
γ-Aminobutyric acid analogues of restricted conformation	375
γ-Aminobutyric acid analogues of restricted conformation	376
Peptides of 2-aziridine carboxylic acid	377, 378
Peptides containing carbazoloquinones	379
Diastereoisomeric Ala_3 peptides	380

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Peptide	*Ref.*
α-Alkylated β,γ-unsaturated α-amino-acids	381
Analgesic peptide, H-Asn-Ala-Gly-Ala-OH	382
Analogues of *cyclo*-(Tyr-Arg)	383
H-Asp-Ala-His-NH_2	384
Asp-/Glu-phosphoric acid analogues	385
Boc-Cys-Pro-Val-Cys-NHMe disulphide	386
γ-Carboxyglutamic acid-containing peptides	387
Bicyclic nonapeptide	388
Bis(cyclic dipeptides)	389
SS′-Bis[*cyclo*-(Gly-Cys-Cys-Sar-Pro)]	390
cyclo-(Dehydroamino-acid-Gly)	391
cyclo-(D-Pro-Pro_2)$_n$ ($n = 1$—5)	392
cyclo-[Glu(amino-acid)-His]	393
cyclo-[(Sar)$_n$-azobenzene-(Sar)$_n$-CO-C_2H_4CO]	394
cyclo-[Trp-Sar_2-Lys(Dns)-Pro]	395
Cytotoxic peptide derivatives	396
Cytotoxic 26-peptide	130
Dehydroaspartic acid peptides	397
Dehydrodipeptides	398
Dehydropeptides	377
Dehydropeptides	378
Dehydrotripeptides	399
α,β-Dehydrotryptophan derivatives	400
(*Z*, *Z*) and (*Z*, *E*) geometrical isomers of dehydrodipeptides	401
Peptides containing 4,4-difluoro- and 4-keto-L-proline	402
Diketopiperazines	403
3,6-Dinitro-1:8-naphthaloyl dipeptides	404

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Peptide	*Ref.*
Fatty-acid derivatives of N-[N^{α}-(γ-D-Glu)-Lys]-D-Ala	405
D,L-*O*-Fluorophenylalanine derivatives	406
α-Fluoro-β-amino-acids	407
D,L-β-Fluoro-Asp and -Asn derivatives	408
GABA analogues, *cis*-3-aminocyclohexane-carboxylic acids	409
GABA analogues, (*Z*)- and (*E*)-4-amino-3-(4-chlorophenyl)but-2-enoic acids	410
Glp-Gly-Arg-*p*-NO_2 anilide	411
Glp-His-Gly-OH	412
N^2-(γ-D-Glu)-*meso*-(2L),(2'D)-diaminopimelic acid derivatives	413
Growth-promoting factor analogues containing lysine	414
Hydrolysis catalysts, Asp-β-Ala-Gly-Ser-β-Ala-Gly-His-β-Ala-Gly	415
Lactam-bridged dipeptides	416
Lys-Orn-methotrexate analogues	417
Lys and Orn polypeptides with pending pyrimidine bases	418
Twelve-membered macrocyclic peptide thiolactone	419
[^{2}H]-Methionine and [^{13}C]-methionine derivatives	420
N-Methylated *O*-benzyl N^{α}-alkoxycarbonyl α-amino-acid hydroxamates	421
Ribosides of L-methyl DOPA	422
Monoacyl 2-alkyl gem-diamines	423
α-Amino-acids containing nitroxide-free radical function	424
Nucleotidyl amino-acids and peptides	425
Oligonucleopeptides containing β-(uracilyl-N^1) and β-(adenylyl-N^9) blocks	426
2'(3')-*O*-Aminoacyltriribonucleoside diphosphates	427
Opioid 7-peptide	135
Peptide aldehydes	428
Peptide alkaloids	429

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Peptide	*Refs.*
Peptide hormones	430
Peptide sweeteners, Tfa-Asp-anilide derivatives	431
Phage-inactivating bifunctional Lys derivatives	432
Phosphonodipeptides	433
Phosphonodipeptides	434
Pivaloyl-Pro-Aib-NHMe	435
Piv-Pro-D-Pro-OMe	436
[^{2}H]Serine derivatives	437
Decapeptide with characteristics of a human spermatozoal antigen	438
Tetrahydrofolate analogues	439
Tetrapeptides containing alanine and proline	440
Tetra- and hexa-peptides containing Pro-Gly-Gly-Pro and Aib-Pro sequences	441
Thiazoline amino-acid derivatives	442
Peptides containing thioasparagine	443
[^{13}C]- and [^{2}H]-labelled tryptophan derivatives	444
Tryptophan analogues as antihypertensive agents	445
[^{2}H]Tryptophan derivatives	446
Visnagin-9-sulphonylamino-acid dipeptides	447

5 Appendix II: Amino-acid Derivatives Useful in Synthesis

The list of derivatives is divided into two groups: those of the coded amino-acids and those of other amino-acids.

Compound	*M.p./°C*	$[\alpha]_D$*[1]	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
Coded Amino-acids					
Alanine					
Bu^t-S-S-CO-Ala-OH	112—113	−19.5	2	MeOH	27
Bu^t-S-S-CO-Ala-ONSu	121—122	−57.9 −69.4*[2]	1	Dioxan	27

*[1] $[\alpha]_D$ measurements were made in the range 18—29 °C. *[2] Measured at $\lambda = 546$ nm.

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Compound	*M.p./°C*	$[\alpha]_D$*1	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
cHex-S-S-CO-Ala-OH	97—98	−35.0 −42.1*2	2	MeOH	27
cHex-S-S-CO-Ala-ONSu	118—119	−75.0 −89.7*2	1	DMF	27
Dmoc-Ala-OH	166—168	−0.72	0.9	MeOH	31
PChd-Ala-OH	181—183	—	—	—	23
PChd-Ala-OMe	76	—	—	—	23
Arginine					
Boc-Arg(DHCH)-OH	—	+3.1	1	DMF	39
Z-Arg(DHCH)-OH	—	−3.2	1	50% HOAc	39
Z-Arg(DHCH)-OH-HCl	—	−8.3	1	DMF	39
Z-Arg(DHCH)-ONSu-HCl	—	−9.9	1	DMF	39
Aspartic acid					
Boc-Asp(Dha-NHEt)-OH	139—142	−7.8	1	EtOH	138
Z-Asp(ONp)-OBu^t	77—79	−27.9	1.08	DMF	199
Cysteine					
Boc-Cys(Me)-NHEt	102—103	−21.2	1	EtOH	138
Z-Cys(Bzl)-OBzl	66—67	−41.8	1	HOAc	91
Boc-Cys(Fm)-ONp	170—172	−22	1.3	DMF+ 1% HOAc	53
Z-Cys(SEt)-OH	73—76	−118.8	1	MeOH	91
Glutamic acid					
Boc-Glu(Dha-NHEt)-OH	124—126	−7.3	1	EtOH	138
Boc-Glu(OCpe)-OH	79—80	−9.9	4	MeOH	49
Glycine					
Dmoc-Gly-OH	163	—	—	—	31
Mtr-Gly-OH	149—152	—	—	—	41
PChd-Gly-OH	167—168	—	—	—	23
PChd-Gly-OMe	73—74	—	—	—	23
Histidine					
N^{α},N^{τ}-Boc-His(Bom)-OH	50	+93.9	1.0	$CHCl_3$	42
N^{α},N^{π}-Boc-His(Bom)-OMe-HCl	152	−19.1	1.0	MeOH	42
N^{α},N^{τ}-Boc-His(Boc)-OMe	96	+25.6	1.0	CCl_4	42
N^{α},N^{π}-Boc-His(Bom)-OH	155	+6.9	0.5	MeOH	42
N^{α},N^{π}-Boc-His(Bom)-OMe	103	−10.8	0.55	MeOH	42
Boc-His(Mtr)-OH-DCHA	136—137	+18.8	1	MeOH	41
H-His(Mtr)-OH	162—164	−27.1	1	AcOH	41
Z-His(2,5-Br_2)-OH	92—94	−1.1	1.0	MeOH	42
Z-His(Mtr)-OH-DCHA	149—150	+14.7	0.9	MeOH	41
Z-His(2,5-Br_2)-OMe	65—69	−6.7	1.0	MeOH	42
Isoleucine					
Dmoc-Ile-OH	143	−0.84	1	MeOH	31
Mtr-Ile-OH-CHA	189—190	+13.6	1.1	MeOH	26
Mtr-Ile-OH-CHA	189—190	+13.6	1.1	MeOH	41
PChd-Ile-OH	92—94	—	—	—	23
PChd-Ile-OEt	65—66	—	—	—	23
PChd-Ile-OMe	99—101	—	—	—	23
Leucine					
Boc-Leu-NH-NH-Troc	Oil	−21.6	1	DMF	92
Dmoc-Leu-OH	148—150	−7.6	1	MeOH	31
PChd-Leu-OH	131—133	—	—	—	23
PChd-Leu-OBzl	86—87	—	—	—	23
PChd-Leu-OEt	72—73	—	—	—	23
ZLeu-NPh	110—111	−33.3	1	AcOH	64

Compound	*M.p./°C*	$[\alpha]_D$*1	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
Lysine					
Boc-Lys(2Cl-Z)-ONp	88—90	−14.5	1	MeOH	304
Boc-Lys(Mtr)-OH-DCHA	169—170	+8.6	0.9	MeOH	26,41
Boc-Lys(Poc)-OH-DCHA	126—128	+7.2	3.0	$CHCl_3$	36
cHex-S-S-CO-Lys(Boc)-ONp	68—70	−39.4 −47.7*2	1	Dioxane	27
$Cl^-H_2^+$-Lys(2Cl-Z)-ONp	154—156	+18.0	1	EtOH	304
H-Lys(Mtr)-OH	228—230	+1.1	0.9	MeOH	26,41
H-Lys(Poc)-OH	243—246	+13	2.0	80% HOAc	36
Z-Lys(Mtr)-OH-DCHA	164—165	+5.8	0.9	MeOH	26,41
Z(OMe)-Lys(Z)-Tht	85—88	−78.0	0.8	THF	68
Methionine					
Boc-Met(Me^+)-ONp-$TosO^-$	159—161	−30	1	DMF 1% HOAc	52
cHex-S-S-CO-Met-ONp	94—95	−50.6 −6.3*2	1	Dioxane	27
Z(OMe)-Met(O)-OMe	81—82	−41.8	1	MeOH	101
Phenylalanine					
Boc-Phe-OPic	92—94	−28.0	0.99	DMF	191
Bu^t-S-S-CO-Phe-ONSu	117—118	−87.0 −104.1*2	1	Dioxane	27
cHex-S-S-CO-Phe-OH	131—132	−31.5 −37.9*2	2	MeOH	27
cHex-S-S-CO-Phe-ONSu	129—130	−48.3 −57.8*2	1	Dioxane	27
Dmoc-Phe-OH	165	+23.9	1	MeOH	31
Fmoc-Phe-OH	175—177	+37.6	0.75	$CHCl_3$	280
Z-Phe-NPh	108—109	−44.2	1	AcOH	64
Z(OMe)-Phe-NH_2	180—182	−17.4	0.9	DMF	263
Proline					
cHex-S-S-CO-Pro-OH	166—168	−84.15 −103.5*2	1	MeOH	27
Dmoc-Pro-OH	133—134	−37.7	1	MeOH	31
PChd-Pro-OBzl	144—147	—	—	—	23
PChd-Pro-OH	81—82	—	—	—	23
Z-Pro-NPh	104—106	−82.1	1	AcOH	64
Serine					
Dmoc-Ser-OH	170—171	+8.2	1	MeOH	31
Fmoc-Ser-OMe	128	+4.0	1	EtOAc	16
Troc-Ser-OH	115	+11.7	1	EtOAc	16
Threonine					
Fmoc-Thr-OH-DCHA	165	+9.8	1	DMF	16
Tryptophan					
Boc-Trp(Dmb)-OH	100	−9.43	0.5	DMF	43
Boc-Trp(Mbs)-OH	83—84	−28.1	0.5	DMF	43
Boc-Trp(Mtb)-OH	82—84	−15.4	0.5	DMF	43
cHex-S-S-CO-Trp-OH	183 dec.	−53.9 −65.1*2	1	MeOH	27
Boc-Trp(Mtr)-OH	88—90	−24.8	0.5	DMF	43
cHex-S-S-CO-Trp-ONSu	142—143	−88.8 −106.8*2	1	Dioxane	27
Dmoc-Trp-OH	163—165	+11.8	1	MeOH	31
H-Trp(Mbs)-OH	218—220	−34.8	0.5	DMF	43
H-Trp(Tos)-OH	227	−29.2	0.5	HOAc	43
Msc-Trp-OTcp	190	−36.6	1.3	DMF	448

Compound	*M.p./°C*	$[\alpha]_D$*[1]	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
Trt-Trp(Mbs)-OBzl	96	+43.1	0.5	DMF	43
Trt-Trp(Tos)-OBzl	101—103	+47.6	0.5	DMF	43
Tyrosine					
Boc-Tyr(Cpe)-OH	109—110	+17.0	2	MeOH	49
$Cl^-H_2^+$-Tyr(Mpt)-OEt	154—156.5	−15.0	1	EtOH	50
Dmoc-Tyr-OH	121	+26.7	1	MeOH	31
Fmoc-Tyr-OBzl	152	−11.3	1	EtOAc	16
Fmoc-Tyr-OMe	126—127	+3.4	1	EtOAc	16
Mpt-Tyr-OEt	68—69	−15.0	1	EtOH	50
Mpt-Tyr(Mpt)-OEt	79—82	−12.5	1	EtOH	50
Mpt-Tyr(Mpt)-OH-DCHA	169—170	−15.0	1	EtOH	50
Mps-Tyr(Et)-OH-DCHA	163—165	+4.9	0.2	DMF	261
Tfa-Tyr(Bzl)-OBzl	83—84	−15.0	1.0	MeOH	383
Troc-Tyr-OBzl	112	−9.3	1	EtOAc	16
Valine					
Dmoc-Val-OH	162—163	−3.0	1	MeOH	31
Fmoc-Val-OH	144	+4.6	1	EtOAc	16
Fmoc-Val-OMe	95	−5.0	1	EtOAc	16
PChd-Val-OEt	77—78	—	—	—	23
PChd-Val-OH	113—114	—	—	—	23
Troc-Val-OH	85	−4.1	1	EtOH	16
Other Amino-acids					
L-α-Aminoadipic acid (Aad)					
Boc-Aad(OH)-OBu^t	Oil	+5.8	2	$CHCl_3$	449
Boc-Aad(OEt)-OBu^t	Oil	+7.3	1.5	$CHCl_3$	449
Z-Aad-OBzl	87—89	−13.3	2	Acetone	450
1-Aminocyclopropane-1-carboxylic acid (Acc)					
$Cl^-H_2^+$-Acc-ONp	181—184	—	—	—	372
Tos-$O^-H_2^+$-Acc-OTmb	187—189	—	—	—	372
Nps-Acc-ONp	157—158	—	—	—	372
Aminoisobutyric acid (Aib)					
PChd-Aib-OH	169—171	—	—	—	23
PChd-Aib-OEt	88—89	—	—	—	23
Arginine					
Z-Arg(NO_2)-ol	134—136	−17.0	1.0	DMF	191
Diaminobutyric acid (Dab)					
Tos-L-Dab(Boc)-OH	136—140	−47.1	2	1M NaOH	222
Tos-D-Dab(Boc)-OH	139—141	+48	2	1 M NaOH	222
Dehydroaspartic acid (Dha)					
Boc-Dha-$(OH)_2$	118—120	—	—	—	397
Boc-Dha-$(OMe)_2$	Oil	—	—	—	397
Z-Dha-$(OMe)_2$	Oil	—	—	—	397
L-2,3-Diaminopropionoic acid (Dpr)					
Tos-D-Dpr(Boc)-OH	123—124	+68.5	4.0	1 M NaOH	222
Troc-Dpr-OH	179—180	−15.5	0.7	1 M NaOH	451
Troc-Dpr[Z(OMe)]-OH-DCHA	190—191	+3.7	0.9	MeOH	451
Z-Dpr-OH	213—214	−9.5	0.4	1 M NaOH	451

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Compound	*M.p./°C*	$[\alpha]_D$*[1]	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
Z-Dpr[Z(OMe)]-OH	145—147	−13.7	0.76	MeOH	451
Z-Dpr[Z(OMe)]-OH-DCHA	178—180	+4.1	0.8	MeOH	451
L-β-3,4-Dihydroxyphenyl-α-alanine(Dopa)					
$Cl^-H_2^+$-Dopa(Me_2)-ONp	204	+26.0	1	MeOH	304
L-Nps-Dopa(Me_2)-OH-DCHA	157	+36.6	1	MeOH	304
L-Nps-Dopa(Me_2)-ONp	130	−93.8	1	EtOAc	304
Homoarginine (Har)					
Boc-Har(NO_2)-OBzl	—	−10.0	0.6	DMF	191
$Cl^-H_2^+$-Har(HCl)-OMe	118—120	+34.0	1.0	MeOH	383
Homoserine					
Trt-Hse-OH-DCHA	141—142	−16.5	2	MeOH	18
β-Hydroxyaspartic acid (Hya)					
erythro-Boc-D,L-Hya-($OMe)_2$	Oil	—	—	—	397
erythro-Z-D,L-Hya-($OMe)_2$	Oil	—	—	—	397
threo-Boc-D-Hya-($OMe)_2$	63—65	−26.8	2.6	EtOAc	397
threo-Boc-L-Hya-($OMe)_2$	61—65	−28.6	2.9	EtOAc	397
threo-Z-D,L-Hya-($OMe)_2$	115—116	—	—	—	397
ε-Hydroxynorleucine (Hnl)					
L-Boc-Hnl-OH	112—113	−6.36	7.3	MeOH	452
D,L-Z-Hnl-OH	111—112	—	—	—	452
D-Lysine					
Tos-D-Lys(Z)-OH	119—121	+41	2.4	1 M NaOH	222
D-Ornithine					
Tos-D-Orn(Z)-OH	118—120	+39.5	2	1 M NaOH	222
Phenylalanine					
D-$Cl^-H_2^+$-Phe(α-Me)-OMe	112—115	−23.6	8	1 M HCl	453
Boc-Phe(4-guanidino)-OEt	131—133	−21.1	0.8	MeOH	383
Boc-Phe(4-NO_2)-OEt	61	−20.0	1.0	MeOH	383
Nps-Phe(4-Et)-OH	108—111	+45.3	0.15	DMF	261
Nps-Phe(4-Et)-OH-DCHA	170—173	+17.2	0.13	DMF	261
Nps-Phe(4-Me)-OH-DCHA	176—178	+11.0	0.2	DMF	261
Nps-Phe(4-Me)-OTcp	127—133	−25.0	0.2	DMF	261
Nps-Phe(4-NMe_2)-DCHA	159—163	+49.0	0.19	DMF	261
Nps-Phe(4-NMe_2)-OTcp	113—117	+21.7	0.11	DMF	261
Nps-Phe(4-NHZ)-OH-DCHA	170—174	+30.1	0.19	DMF	261
Nps-Phe(4-NHZ)-OTcp	54—57	−5.9	0.2	DMF	261
Nps-Phe(4-NO_2)-OH-DCHA	190—193	+13.1	0.06	DMF	261
Piperazic acid (Pip)					
Boc-Pip-OH	123—124	+63.26	0.98	AcOH	454
Boc-Pip-OPcp	136—138	—	—	—	454
Boc-Pip-OPfp	Oil	—	—	—	454
$Cl^-H_2^+$-Pip-OMe	171—172	−7.5	2	MeOH	202

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Compound	*M.p./°C*	$[\alpha]_D$*[1]	*Conc./g 100 cm^{-3}*	*Solvent*	*Ref.*
Proline					
Z-dehydro-Pro-OMe	Oil	—	—	—	455
H(4,4-F_2)-Pro-OH	244—248 dec.	−34.5	0.6	H_2O	402
Z(4,4-F_2)-Pro-OBzl	48—50	—	—	—	402
Z(4-keto)-Pro-OBzl	56.5—57.5	—	—	—	402
Boc(4-keto)-Pro-OH	155—157	+15.0	0.7	$CHCl_3$	402
Thioasparagine (Tan)					
Z-Tan-OBzl	107—108.5	−19.6	—	—	443
Z-Tan-OH	124—125	−39.1	—	—	443
Tryptophan					
L-Cl$^-$$H_2^+$-Trp(α-Me)-OMe	159	+27.8	9.9	MeOH	453
D-Cl$^-$$H_2^+$-Trp(α-Me)-OMe	160	−28.3	9.9	MeOH	453
Tyrosine					
Boc(allyl)-Tyr-OH-DCHA	138—140	−29.1	0.9	MeOH	383

6 Appendix III: Purification Methods

Methods for the purification of protected peptides and proteins are given; the list also includes reference to the purification of free peptides and separation of diastereoisomers.

Technique	*Ref.*
High-performance Liquid Chromatography	
Separation of peptide and protein mixtures	456, 457
Ion-pairing effects in reversed-phase h.p.l.c.	458, 459
Prediction of retention times on h.p.l.c.	460
Mobile and stationary phases for h.p.l.c.	461
Fluorescent precolumn labelling of amino-acids	462
Precolumn derivatization	463
H.p.l.c. of ACTH (1—24)	177
Separation of H-Leu-Dopa-OH diastereoisomers	464
Insulin purification by reversed-phase h.p.l.c.	465, 466
Determination of Cys and Met	467
Analysis of [^{125}I]CCK derivatives	468
Separation of dipeptide conformers of proline	469
Separation of dimethylaminoazobenzene sulphonyl (DABS) amino-acids	470

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Technique	*Ref.*
Separation of dansylamino-acids	471, 472
Amino-acid analysis	473, 474
PTH derivatives	475
Glucopyranosyl- and arabinopyranosyl-amino-acid derivatives	476
Chiral eluents for enantiomer separation	477—479
Use of Cu^{II} complexes	480—482
Separation of diastereoisomers	483
Gas–Liquid Chromatography	
Separation of derivatized amino-acids on a chiral column	484—488
Separation of dipeptide diastereoisomers	489
G.c. of amino-acid oxazolidinones	490
G.c. of *N*-methylamino-acid diastereoisomers	491
G.c.–m.s. of derivatized amino-acids	492, 493
Other Chromatographic Methods	
Fluorimetric amino-acid analysis	494
Enantiomer separation on an immobilized-protein stationary phase	495
Enantiomer separation by zwitterion-pair chromatography	496
Affinity chromatography of aminopeptidases	497
Relative hydrophoticity estimation by partition chromatography	498
Molecular-weight estimation by size-exclusion chromatography	499
H.p.t.l.c. on RP8 and RP18	500
H.p.t.l.c. of oligopeptides using ion pairing	501

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4

Peptides with Structural Features not Typical of Proteins

BY P. M. HARDY

1 Introduction

The general remarks made in the last report apply also to this presentation. The synthesis of peptides containing dehydroamino-acid residues has been a particularly active field in the year under review, and such compounds have been given a separate section for the first time. The number of papers falling in the area covered by this chapter has increased by about 10 percent in 1982 compared to the previous reports compiled by this reviewer, reflecting current interest in the synthesis of modified peptides in general. Once again, in order to make the best use of the space available, references to earlier work are given in only a few instances; the 1982 references cited normally provide ready access to these.

Two useful reviews of the mass spectrometry of modified peptides have appeared. The first details in particular the usefulness of fast atom bombardment, using the peptaibophol antibiotics and the marine-derived cyclic depsipeptides as examples,[1] while the second compares field desorption and secondary-ion mass-spectra techniques for such diverse examples as phosphoramidon, blasticidin S, chymostatin, and antipain.[2]

2 Cyclic Peptides

2,5-Dioxopiperazines (Cyclic Dipeptides).—Two novel non-cyclol alkaloids, *N*-(D-lysergyl-L-valyl)-*cyclo*-(L-valyl-D-prolyl) (1a) and an analogue in which L-leucine replaces L-valine (1b), have been isolated from the field ergot. The L-phenylalanine analogue (1c) was earlier characterized in 1973.[3] An isomer of the siderophore triornicin (see last year's report) has been isolated from the same source, *Epicoccus purpurascans*. This compound, isotriornicin (2), differs only in that the acetyl and (*E*)-5-hydroxy-3-methyl-2-pentenoyl hydroxamate groups are interchanged.[4] Bipolarimide (3), found in cultures of *Bipolaris sorokiniana*, is probably formed from *cyclo*-(Phe-Phe) through epoxidation and

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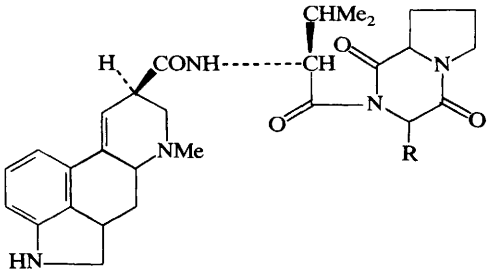
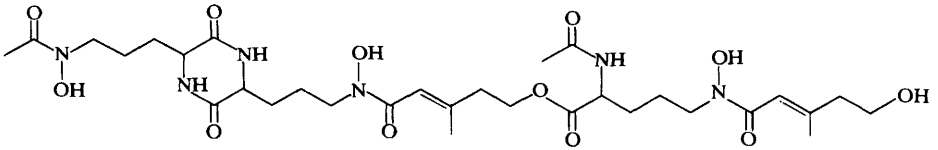
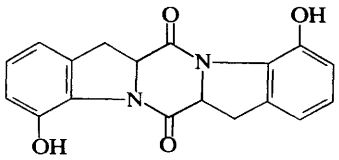


(1) a; R = $CHMe_2$
b; R = CH_2CHMe_2
c; R = CH_2Ph

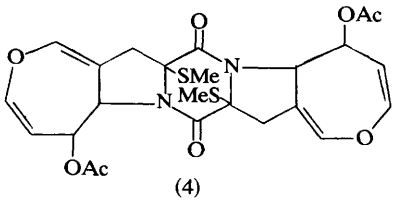


(2)



(3)

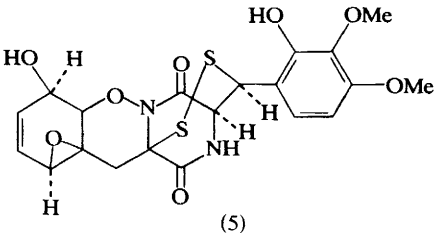
cyclization. The structure is based on *X*-ray work, which showed a molecule consisting of two identical halves, with strong intramolecular O—H · · · O hydrogen bonds. Bipolarimide has an interesting biosynthetic resemblance to bisdethiobis(methylthio)acetylaranotin (4).[5] Phenylalanine anhydride itself has



(4)

been isolated from *Gliocladium virens*, and it may be the synthetic precursor also of its novel co-metabolite gliovirin (5). The structure of this antibiotic, selectively active against members of the Oomycetes, was also determined by *X*-ray analysis. It closely resembles the L,L-isomer of *cyclo*-(Phe-Phe) in its ^{13}C n.m.r. spectrum, being clearly distinct from the L,D-isomer.[6]

[5] C. M. Maes, P. S. Steyn, P. H. van Rooyen, and C. J. Rabie, *J. Chem. Soc., Chem. Commun.*, 1982, 350.
[6] R. D. Stipanovic and C. R. Howell, *J. Antibiot.*, 1982, **35**, 1326.



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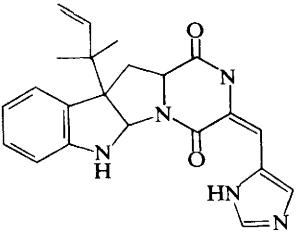
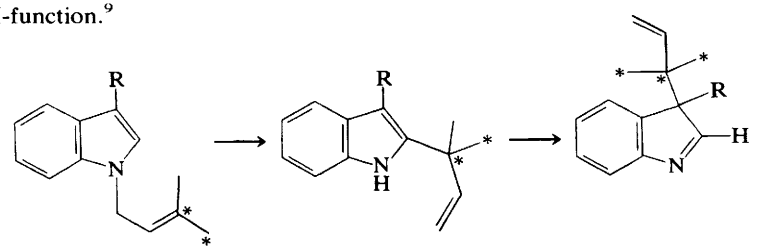


(6)

An investigation of [1,2-$^{13}C_2$]-acetate and [2,3-$^{13}C_2$]-mevalonic acid lactone incorporation into roquefortine (6), the natural neurotoxin contaminant of blue-vein cheese, suggests that the most likely method of incorporation of the isopentenyl group is as shown in Scheme 1, loss of the regiospecific integrity of the label occurring in the second step.[7] An extraction procedure for isolating *cyclo*-(His-Pro) from human blood has been developed. This dioxopiperazine is derived from TRH by the action of pyroglutamate aminopeptidase. Radioimmunoassay measurement after extraction showed higher concentrations in hypothyroid patients than in normal or hyperthyroid patients.[8] The distribution of *cyclo*-(His-Pro)-like immunoreactivity in the rat gastrointestinal tract suggests that this cyclic dipeptide may be involved in regulating rat GI-function.[9]

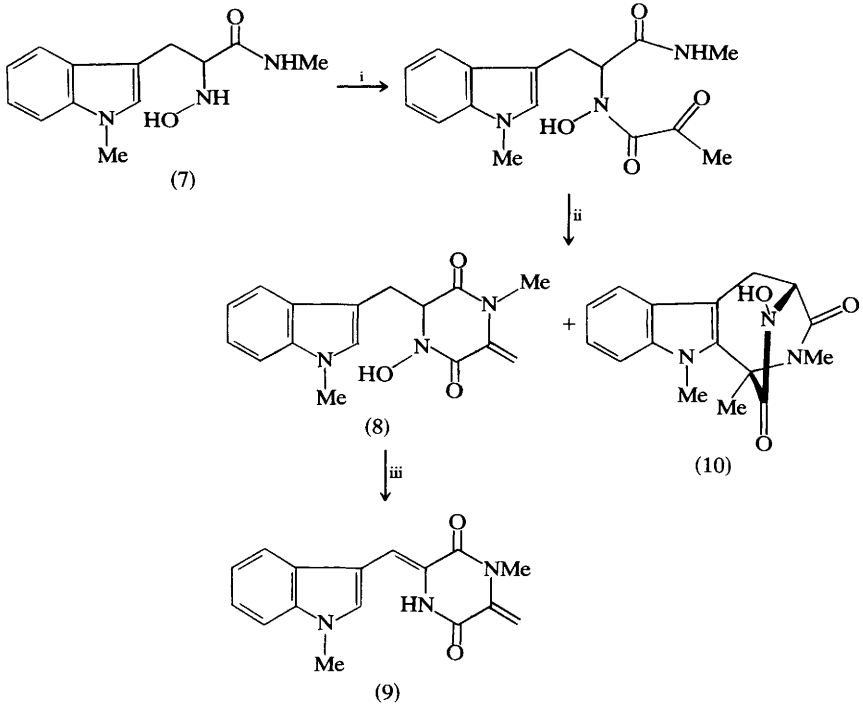


* indicates ^{13}C atoms

Scheme 1

Three papers concern the synthesis of dioxopiperazines. In one, *N*-hydroxytryptophan has been used in the preparation of oxidized dioxopiperazines related to natural products. Acylation of α-(hydroxyamino)-β-(*N*-methylindol-3-yl)-*N*-methylpropanamide (7) with pyruvoyl chloride followed by treatment with trifluoroacetic acid formed two products (Scheme 2), one of which (8) can be readily converted to a neoechinulin analogue (9). Formation of the tetracyclic product (10) can be rationalized as involving the generation of a carbenium ion by protonation of the exomethylene double bond followed by intramolecular alkylation of the indole ring. Prolonged treatment of (8) with trifluoroacetic acid gives (10) in quantitative yield.[10] A

[7] C. P. Gorst-Allman, P. S. Steyn, and R. Vleggar, *J. Chem. Soc., Chem. Commun.*, 1982, 652.
[8] M. Mari, T. Mallik, C. Prasad, and J. F. Wilber, *Biochem. Biophys. Res. Commun.*, 1982, **109**, 541.
[9] M. Mori, J. Pegues, C. Prasad, R. M. Edwards, and J. F. Wilber, *Biochem. Biophys. Res. Commun.*, 1982, **109**, 982.
[10] H. C. J. Ottenheim, R. Plate, J. H. Noordik, and J. D. M. Herscheid, *J. Org. Chem.*, 1982, **47**, 2147.



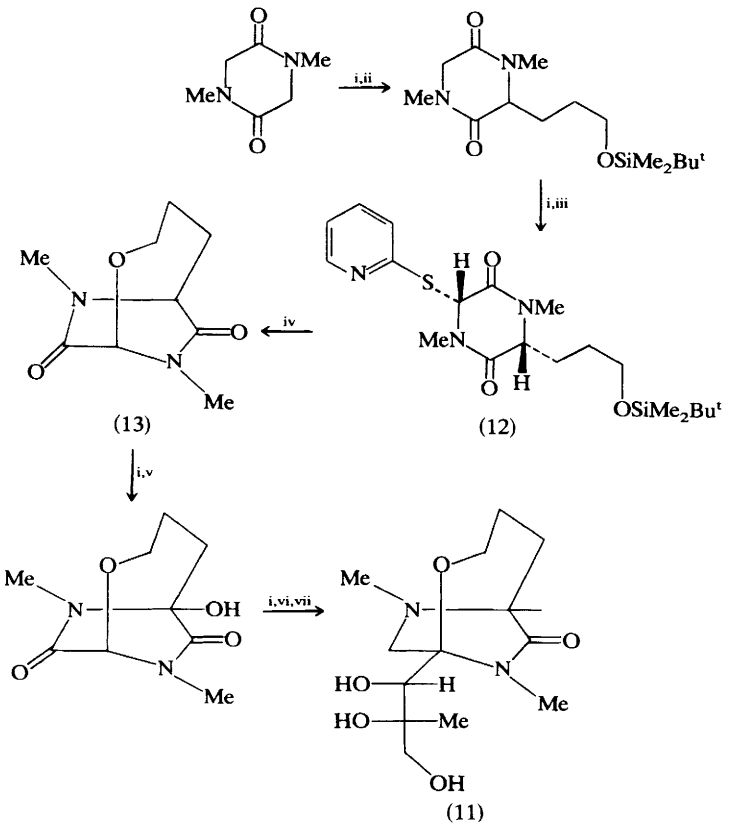
Reagents: i, MeCOCOCl; ii, CF_3CO_2H; iii, tosyl chloride, pyridine.

Scheme 2

number of approaches to the synthesis of bicyclomycin, which has an interesting profile of antibacterial activity and low toxicity, were outlined in the last report. A new and efficient reaction allows an overall 'three-pot' synthesis of bicyclic piperazinediones under mild conditions in high yield. This has been demonstrated in the preparation of *NN'*-dimethyl-4-desmethylenebicyclomycin (11) (Scheme 3). The key step is the metal-mediated intramolecular cyclization of a pyridyl thioether (12); the bicyclic product (13) was obtained in 90—99% isolated yield in 2—3 min at 25 °C. In the last aldol step of the sequence the C-6 hydroxyl was effectively protected as the lithium alkoxide.[11] Finally, a naphthalene analogue of *cyclo*-(Gly-Phe) (14) has been prepared (Scheme 4). The shielding of the heterocyclic-ring methylene protons by the aromatic ring is less dependent upon temperature in this analogue, supporting the proposal that the reduction of shielding in *cyclo*-(Gly-Phe) with increase in temperature is caused by the enhancement of aromatic-ring torsional vibrations. Replacement of the benzene ring by naphthalene dampens these vibrations.[12]

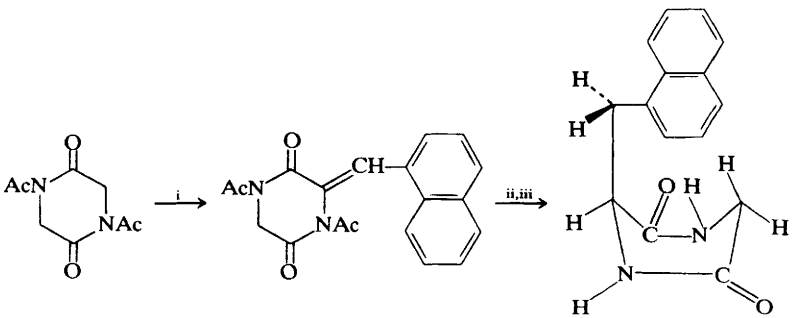
[11] R. M. Williams, O. P. Anderson, R. W. Armstrong, J. Josey, H. Meyers, and C. Eriksson, *J. Am. Chem. Soc.*, 1982, **104**, 6092.

[12] I. J. Frigerio, I. D. Rae, and M. G. Wong, *Aust. J. Chem.*, 1982, **35**, 1609.



Reagents: i, $LiNPr^i_2$, THF, −78 °C; ii, $I(CH_2)_3OSiMe_2Bu^t$; iii, 2,2′-dipyridyl disulphide; iv, $PhMgClO_4$ (2 eq.), THF, 25 °C; v, oxodiperoxymolybdenum–hexamethylphosphorotriamide–pyridine; vi. ; vii, H_2SO_4 MeOH.

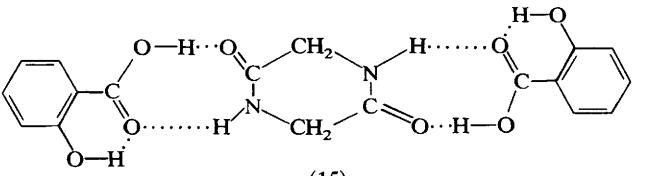
Scheme 3



Reagents: i, naphthalene-1-carbaldehyde, $KOBu^t$, DMF; ii, Pd/C, EtOH–HOAc; iii, hydrazine hydrate, EtOH.

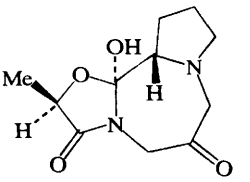
Scheme 4

The crystal structure of *cyclo*-(Met-Gly) (CMG) shows a nearly planar ring with the side chain folded on it. The sulphur atom is the primary site for metal co-ordination in complexes of the type $[M(CMG)_2Cl_2]$ (M = Pt or Pd), [Au(CMG)Cl], and $[Hg(CMG)Cl_2]$. There is some evidence also of additional co-ordination through deprotonated amide nitrogens.[13] *X*-Ray analysis of *cyclo*-(Aib_2) and *cyclo*-(Aib-Ile) shows small but significant deviations of the rings from planarity. The former adopts a slight chair conformation and the latter a slight boat.[14] In the crystal of the 2,5-piperazinedione–salicylic acid 1:2 complex the heterocyclic ring is nearly planar, with the carboxyl group of salicylic acid forming hydrogen bonds with both the NH and CO groups of the amide bond (15).[15]



(15)

Attempts have been made to prepare stable tetrahedral intermediates (cyclols) from β-alanine-containing precursors. *N*-(*N*-Z-β-Ala)-Phe-Pro-ONp (Z = *N*-benzyloxycarbonyl, ONp = *p*-nitrophenyl ester) on cyclization gave *N*-(*N*-Z-β-Ala)-*cyclo*-(Phe-Pro), although Z-(*N*-Z-Ala)-β-Pro-ONp and *N*-[*N*-(*R*)-(α-hydroxyisovaleryl)-β-Ala]-Pro-ONp gave the corresponding anhydrocyclols. This confirms that cyclols containing two condensed six-membered rings are not isolable even under mild cyclization conditions in favourable systems. The first example of a ten-membered *cyclo*-depsitripeptide has been synthesized (16) by acylation of *cyclo*-(β-Ala-Pro) with α-benzyloxypropionyl chloride followed by hydrogenolytic removal of the *O*-benzyl group.[16]

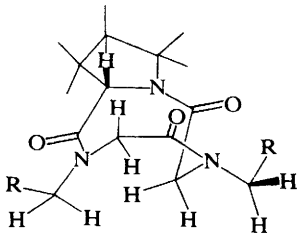


(16)

Larger Cyclic Peptides.—The conformations and biological activity of cyclic peptides have been reviewed.[17] Cyclic tripeptides containing proline continue to be a focus of conformational studies. *X*-Ray analysis shows *cyclo*-(*N*-Bzl-Gly-Pro_2), like *cyclo*-(Pro_3) and *cyclo*-(*N*-Bzl-Gly_2-Pro), to have a crown conformation. One of the three peptide bonds is not quite planar, and the

[13] M. Bressan, R. Ettorre, F. Marchiori, and G. Valle, *Int. J. Pept. Protein Res.*, 1982, **19**, 402.
[14] K. Suguna, S. Ramakumar, N. Shamala, B. V. Venkataram Prasad, and P. Balaram, *Biopolymers*, 1982, **21**, 1847.
[15] K. I. Varughese and G. Kartha, *Acta Crystallogr., Sect. B*, 1982, **38**, 301.
[16] F. Pinner, G. Zanotti, and G. Lucente, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1311.
[17] H. Kessler, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 512.

proline nitrogen atoms have a small amount of pyramidal character.[18] Solid-state ^{13}C n.m.r. spectra of the LLL- and LLD-stereoisomers of *cyclo*-(Pro_3) do not show the signals expected for two independent molecular forms as earlier shown in *X*-ray work.[19] Conformational analysis of the LLD-isomer by 270 and 500 MHz one- and two-dimensional ^1H n.m.r. indicates a twisted-boat backbone conformation.[20] Mononuclear two-dimensional spin-echo-correlated n.m.r. combined with two-dimensional exchange spectroscopy shows that in $CDCl_3$ *cyclo*-(Pro-NBGly$_2$) (NB = *N*-*o*-nitrobenzyl) exists in a 20% boat (17):90% crown (18) equilibrium.[21] The LL- and LD-stereoisomers of *cyclo*-(Ala$_2$-ε-aminocaproyl) have been prepared, and coupling constants obtained from their ^1H n.m.r. spectra are consistent with the dihedral angles of the computed lowest-energy β-bend conformations of types I + III and II, respectively. The LL-form associates in DMSO, but its diastereoisomer does not.[22] Examination of *cyclo*-(β-Ala)$_3$ by *X*-ray and n.m.r., supplemented by molecular-mechanics calculations, suggests that the molecule has a range of local minima close in energy to the global minimum. The crystal structure agrees with the presence of molecules in several different conformations, as does the ambiguous ^1H n.m.r. spectrum.[23]



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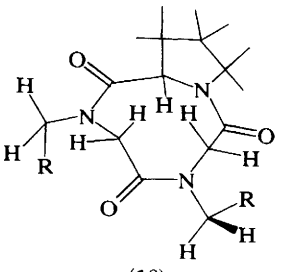
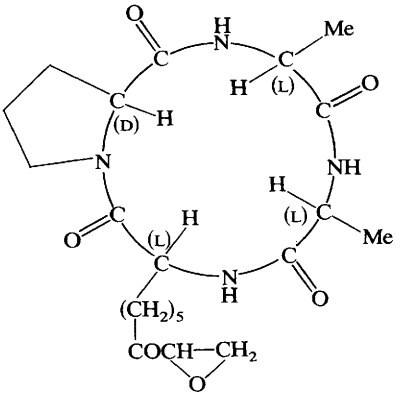


(18)

Sequence assignment on the basis of fragmentation during electron-impact ionization–mass spectrometry (e.i.–m.s.) indicated the structure of the host specific toxin (HC toxin) from *Helminthosporium carbonum* to be *cyclo*-[(2-amino-9,10-epoxy-8-oxodecanoyl)-alanyl-alanyl-prolyl].[24] However, further

[18] J. W. Bats and H. Fuess, *Acta Crystallogr., Sect. B*, 1982, **38**, 1004.
[19] H. Kessler, W. Bermel, and H. Forster, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 689.
[20] H. Kessler, W. Bermel, A. Friedrich, G. Krack, and W. E. Hull, *J. Am. Chem. Soc.*, 1982, **104**, 6297.
[21] H. Kessler, R. Schuck, and R. Siegmeier, *J. Am. Chem. Soc.*, 1982, **104**, 4486.
[22] J. Bandekar, D. J. Evans, S. Krimm, S. J. Leach, S. Lee, J. R. McQuie, E. Minasian, G. Nemethy, M. S. Pottle, H. A. Scheraga, E. R. Stimson, and R. W. Woody, *Int. J. Pept. Protein Res.*, 1982, **19**, 187.
[23] D. N. J. White, C. Morrow, P. J. Cox, C. N. C. Drey, and J. Lowbridge, *J. Chem. Soc., Perkin Trans. 2*, 1982, 239.
[24] J. M. Leisch, C. C. Sweeley, G. D. Stahfeld, M. S. Anderson, D. J. Weber, and R. P. Scheffer, *Tetrahedron*, 1982, **38**, 45.

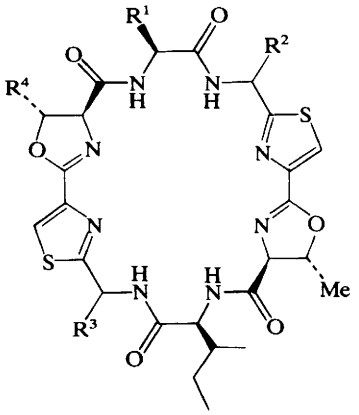


(19)

examination using f.a.b.[25] and g.c.–m.s.[26] techniques shows that in fact proline is on the other side of the epoxy-amino-acid (19), highlighting the sometimes misleading nature of e.i.–m.s. results as rearrangements can occur. The unusual component of HC toxin, 2-amino-9,10-epoxy-8-oxodecanoic acid (Aoe), also occurs in the phytotoxic cyclic tetrapeptide Cyl-2, which has the sequence *cyclo*-[D-Tyr(Me)-Ile-Pip-Aoe]. Three analogues in which the Aoe residue is replaced, [Leu^4]-, [D-Leu^4]-, and [Pro^3-D-Leu^4]-Cyl-2, have now been prepared by cyclization of 1-succinimidyl esters. While the ^{1}H n.m.r. spectrum of the [Leu^4] analogue was similar to that of Cyl-2, the other two showed a different pattern.[27] A search has been made to try and find the optimal conditions for preparing [Ala^4]-chlamydocin, *i.e. cyclo*-(Aib-Phe-D-Pro-Ala). All four possible linear tetrapeptide 1-succinimidyl esters have been prepared and cyclized. Three of the sequences gave only 2—3% yields of cyclic tetrapeptide, but Ala-Aib-Phe-D-Pro-OSu gave a 44% yield. The results, although not readily explicable, open the way to the synthesis of chlamydocin itself.[28]

The *X*-ray structure of *cyclo*-(Ala-Pro-Phe-Pro) has been reported. Although the synthesis was designed (no details are given) to produce only the LLLL-isomer, the unit cell proved to contain the LLDL-diastereoisomer as well. Both molecules have a *cis–trans–cis–trans* backbone conformation; in the all-L isomer, however, there is an approximately two-fold rotation axis whereas the LLDL-form is totally asymmetric.[29] In contrast to e.i.–m.s., f.a.b.–m.s. of the cyclic tetrapeptide tentoxin gives a (protonated) molecular ion and fragment ions that allow the sequence to be determined.[30] Three cyclic tetrapeptides,

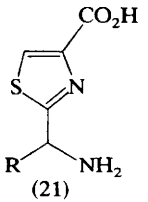
[25] M. L. Gross, D. McCrery, F. Crow, K. B. Tomer, M. R. Pope, L. M. Cinfetti, H. W. Knoche, J. M. Daly, and L. D. Dunkle, *Tetrahedron Lett.*, 1982, **23,** 5381.
[26] J. D. Walton and E. D. Earle, *Biochem. Biophys. Res. Commun.*, 1982, **107,** 785.
[27] A. Yasutake, H. Aoyagi, I. Sada, T. Kato, and N. Izumiya, *Int. J. Pept. Protein Res.*, 1982, **20,** 246.
[28] J. Postuszak, J. H. Gardner, J. Singh, and D. H. Rich, *J. Org. Chem.*, 1982, **47,** 2982.
[29] C. C. Chiang and I. L. Karle, *Int. J. Pept. Protein Res.*, 1982, **20,** 133.
[30] J. L. Aubagnac, F. M. Devienne, and R. Combarieu, *Tetrahedron Lett.*, 1982, **23,** 5263.



Patellamide	R^1	R^2	R^3	R^4
A	EtMeCH	Me_2CH	Me_2CH	H
B	Me_2CHCH_2	$PhCH_2$	Me	Me
C	Me_2CH	$PhCH_2$	Me	Me

(20)

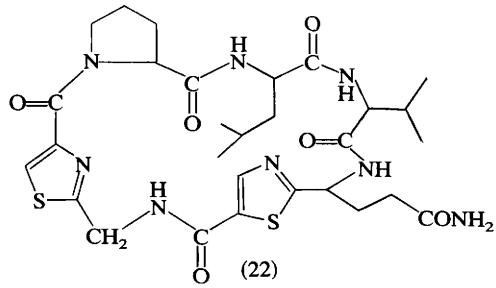
patellamides A, B, and C (20), have been isolated from the tunicate *Lissoclinum patella* (from which ulicylamide and ulithiacylamide were earlier obtained). All contain an unusual fused oxazoline-thiazole unit, which degrades on 6 M HCl hydrolysis to give the thiazole amino-acid (21) and either threonine or serine, depending on R^4. The patellamides are cytotoxic to, for example, murine leukaemia cells.[31]



(21)

The Indian Ocean sea hare *Dolabella auricularia* has proved to be a productive source of anti-cancer compounds, the ninth of which has proved to be the cyclic pentapeptide *cyclo*-[Pro-Leu-Val-(Gln)Thz-(Gly)Thz] (22). As only 1 mg of (non-crystalline) compound was isolated, the configurations of the amino-acids have not been established with certainty. The alternative structure *cyclo*-[Val-Leu-Pro-(Gly)Thz-(Gln)Thz] cannot be ruled out on the evidence, but it is felt to be less likely by biosynthetic precedent.[32] [Ile^3-Val^5]-malformin (*allo*-malformin) (23) has been synthesized. It proved identical to malformin (24) with respect to biological activity, c.d., o.r.d., and h.p.l.c. It differs slightly,

[31] C. M. Ireland, A. R. Durso, R. A. Newman, and M. P. Hacker, *J. Org. Chem.*, 1982, **47**, 1807.
[32] G. R. Pettit, Y. Kamano, P. Brown, D. Crust, M. Inoue, and C. L. Herald, *J. Am. Chem. Soc.*, 1982, **104**, 905.



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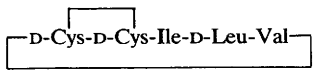

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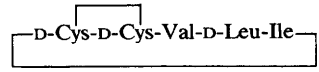
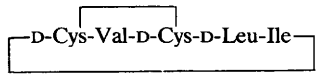


(24)

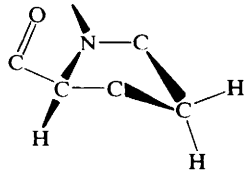
however, in its R_f value on t.l.c., which enabled its absence in natural malformin to be established. However, natural malformin was found to contain [Val^5]-malformin; this impurity is thought to have given rise to the Val-Cys dipeptide on partial hydrolysis during the original sequence determination, leading to the erroneous identification of malformin as (25).[33] The actinomycin-related cyclic pentapeptide *cyclo*-(Thr-D-Val-Pro-Sar-MeAla) has been prepared, and an *X*-ray crystal analysis shows the peptide bonds to be *trans* and shows the presence of a Sar-MeAla β-turn and a possible (long) $4 \rightarrow 1$ (Thr $\rightarrow$ Pro) hydrogen bond. N.m.r. studies are compatible with a similar ring conformation in solution.[34]



(25)

Three diastereoisomers of a cyclic pentapeptide *cyclo*-(Phe-Trp-Lys-Thr-Pro), which contains the active sequence of somatostatin bridged by a proline residue, have been examined by n.O.e. and two-dimensional spin-echo-correlated n.m.r. spectroscopy. The LDLLL-isomer has a *cis*-Thr-Pro bond with a Lys-Thr-Pro-Phe βVI-turn and a Phe-Trp-Lys γ-turn. The LDLDL- and LLLDL-isomers adopt a different conformation with a *trans* Thr-Pro bond that may have either a β,γ-arrangement (βI: Thr-Pro-Phe-Trp; γ: Trp-Lys-Thr) or a γ,γ-arrangement, the two D-residues having internally oriented NH groups.[35] A complete analysis of the 600 MHz ^{1}H n.m.r. spectrum of the proline residues in *cyclo*-(D-Phe-Pro-Gly-D-Ala-Pro) and *cyclo*-(Gly-Pro-Gly-D-Ala-Pro) has been carried out. Results indicate that each compound contains one proline ring in the Ramachandran A form (26) and one in the Ramachandran

[33] M. Bodanszky, M. A. Bednarok, A. E. Yiotakis, and R. W. Curtis, *Int. J. Pept. Protein Res.*, 1982, **20**, 16.
[34] A. B. Mauger, O. A. Stuart, R. J. Highet, and J. V. Silverton, *J. Am. Chem. Soc.*, 1982, **104**, 174.
[35] H. Kessler and V. Eiermann, *Tetrahedron Lett.*, 1982, **23**, 4689.



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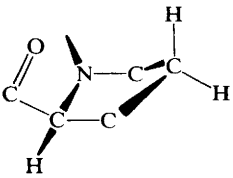
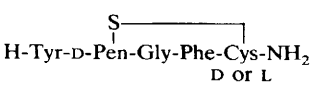
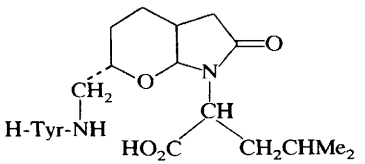


(27)

B form (27).[36] *cyclo*-(Pro-Phe-Gly-Phe-Gly) and *cyclo*-(Pro-Phe-Gly-Phe-Gly)$_2$ and four specifically deuteriated derivatives have been prepared and studied by n.m.r. spectroscopy. The cyclic pentapeptide appears to adopt a β,γ-conformation with two intramolecular hydrogen bonds, but the cyclic decapeptide results are consistent with a conformation similar to a β-pleated sheet with four transannular H-bonds. The smaller ring preferentially binds Li^+ to Na^+ in CD_3CN. It does not bind K^+. By contrast, the larger ring forms a (weak) complex only with K^+.[37] The D-penicillamine-containing cyclic enkephalin analogues (28) have high biological activity but exhibit a preference for δ-receptors over μ-receptors, an unusual binding for enkephalinamide derivatives.[38] An enkephalin analogue fixed in a β-bend conformation (29) has the same order of activity on guinea-pig ileum as morphine,[39] while enkephalin in which the Tyr-Gly amide bond is replaced by an alkene link shows full biological activity.[40] The protected pentapeptide Boc-Tan-Cys(PMB)-Pro-Leu-Gly-NH_2 (Tan = thioasparagine, PMB = *p*-methoxybenzyl) was prepared from the corresponding β-cyanoalanine precursor by treatment with hydrogen sulphide and ammonia. No racemization occurred during thioamide formation, but attempts to remove the Boc group lead to some decomposition of the thioamide group.[41]



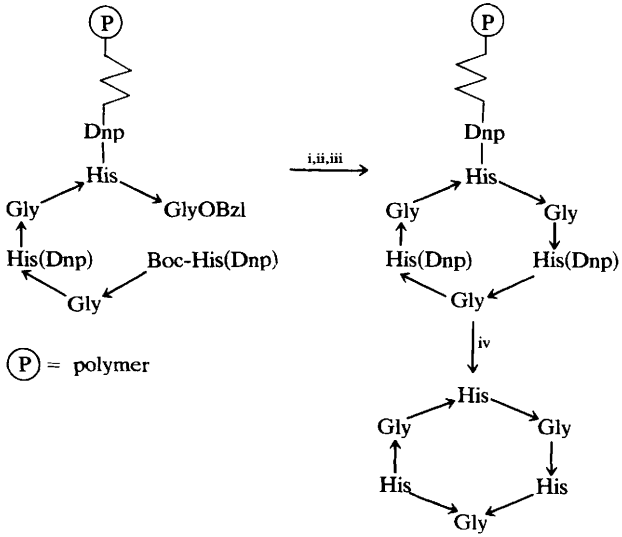
(28)



(29)

Continuing on to cyclic hexapeptides, *cyclo*-(Gly-His)$_3$ has been prepared by a solid-phase synthesis using (aminomethyl)copoly(styrene–1% divinylbenzene). This polymer was treated with excess 1,5-difluoro-2,4-dinitrobenzene and then with Boc-histidine. The polymer-bound histidine was then built up

[36] A. C. Bach, A. A. Bothner-By, and L. M. Gierasch, *J. Am. Chem. Soc.*, 1982, **104**, 572.
[37] H. Kessler, W. Hehlein, and R. Schuck, *J. Am. Chem. Soc.*, 1982, **104**, 453.
[38] H. I. Mosburg, R. Hurst, V. J. Hruby, J. J. Galliga, T. F. Burks, K. Gee, and H. I. Yamamura, *Biochem. Biophys. Res. Commun.*, 1982, **106**, 506.
[39] J. L. Krstenansky, R. L. Baranowski, and B. L. Currie, *Biochem. Biophys. Res. Commun.*, 1982, **109**, 1368.
[40] M. D. Hann, P. G. Sammes, P. D. Kennewell, and J. B. Taylor, *J. Chem. Soc., Perkin Trans. 1*, 1982, 307.
[41] H. Sancii and A. F. Spatola, *Tetrahedron Lett.*, 1982, **23**, 149.



Reagents: i, HBr–HOAc, CF_3CO_2H–CH_2Cl_2; ii, pyridine; iii, pyridine–toluene–EEDQ; iv, RSH, DMF (O_2 free).

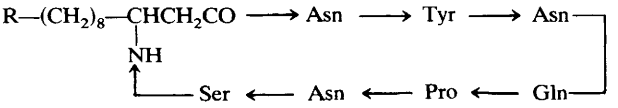
Scheme 5

into a linear hexapeptide, which was cyclized as in Scheme 5. The crude yield of *cyclo*-(Gly-His)$_3$ was 85%, reduced to 42% after h.p.l.c. No linear or oligomeric cyclic peptides could be detected in the crude product.[42] The conformation of five cyclic hexapeptides of the constitution *cyclo*-(Phe-Phe-Trp-Lys-Thr-Phe) in which the chirality of the Trp and two of the Phe residues is varied has been investigated by difference n.O.e. and two-dimensional spin-echo-correlated n.m.r. A conformation containing two β-turns seems likely in four of the five peptides. In *cyclo*-(D-Phe-Phe-Trp-Lys-Thr-Phe), however, the NH protons of two adjacent amino-acids are inwardly oriented.[43] *X*-Ray analysis of *cyclo*-(Phe-D-Leu-Gly-Phe-Leu-Gly)·4H_2O and *cyclo*-(Phe-D-Leu-Gly-D-Phe-Leu-Gly)·2H_2O shows both peptides to adopt a common conformation of two β-turns linked by extended glycyl residues, but the former has only one of the two possible transannular hydrogen bonds and the latter none. Instead, four of the amide carbonyl groups are involved in very strong H-bonds with the two water molecules.[44] The crystal structure of *cyclo*-(Gly-Pro-Pro-Gly-Pro-Pro)·3H_2O shows the presence of one transannular H-bond. The molecule is asymmetric, one half of the molecule containing a *cis*-Gly-Pro and a *cis*-Pro-Pro link in consecutive positions while the other half has a type I β-turn encompassing two *trans*-configured proline residues.[45] *X*-Ray examination also reveals that the natural cyclic hexapeptide cycloamanide A, *cyclo*-(Val-Phe-Phe-Ala-Gly-Pro), has a single transannular H-bond of an unusual

[42] S. S. Isied, C. G. Kuchi, J. M. Lyon, and R. B. Merrifield, *J. Am. Chem. Soc.*, 1982, **104**, 2632.
[43] H. Kessler, M. Bernd, and I. Damm, *Tetrahedron Lett.*, 1982, **23**, 4865.
[44] C. L. Barnes and D. van der Helm, *Acta Crystallogr., Sect. B*, 1982, **38**, 2589.
[45] M. Csugler, K. Sasvari, and M. Hollosi, *J. Am. Chem. Soc.*, 1982, **104**, 4465.

(4 → 1) type bridging the Phe-Ala sequence. Even though the chirality is LL, the $\phi\psi$ values are characteristic of a DL-β-bend of type II'.[46]

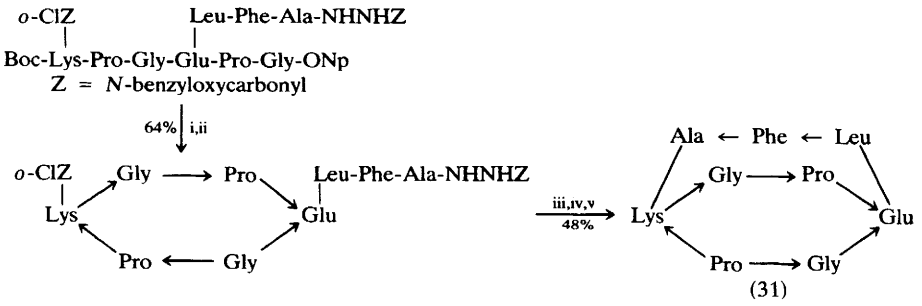
The structures of the β-amino-acid components of three of the iturin A group of antibiotics have been revised (30). As the structures of the β-amino-acids of iturin C, mycosubtilin, and bacillomycins L and D were determined by



Iturin		
A1	Et	[Me_2CH / $MeCH_2CHMe$] previous assignments
A2	Pr^n	
A3	EtCHMe	
A4	Me_2CHCH_2	} new components
A5	Bu^n	
A6	$Me_2CHCH_2CH_2$	
A7	$Me(CH_2)_4$	

(30)

comparison with iturin A, these may also have to be revised. The structures of five minor components of iturin A have also been elucidated (30).[47] The calcium complexes of two synthetic cyclic octapeptides *cyclo*-[Glu(γ-OBzl)-Sar-Gly-(*N*-R)-Gly]$_2$ (R = hexyl or cyclohexyl) have been studied by 360 MHz ^{1}H n.m.r. A similar conformation is proposed for both compounds, although the cyclohexyl derivative is a superior cation-transport substance. They are thought to form octahedral-type complexes, the calcium ion being bound by the four coplanar carbonyls of the (all-*trans*) Glu-Sar and Gly-(*N*-R)-Gly peptide bonds. Restriction of the rotation of the Glu side chain occurs on ion binding.[48] A bicycle nonapeptide (31) has been synthesized (Scheme 6) as a



(31)

Reagents: i, CF_3CO_2H; ii, $(Pr^i)_2NEt$, DMF; iii, HBr, HOAc; iv, HCl, $NaNO_2$; v, DMF, $(Pr^i)_2NEt$.

Scheme 6

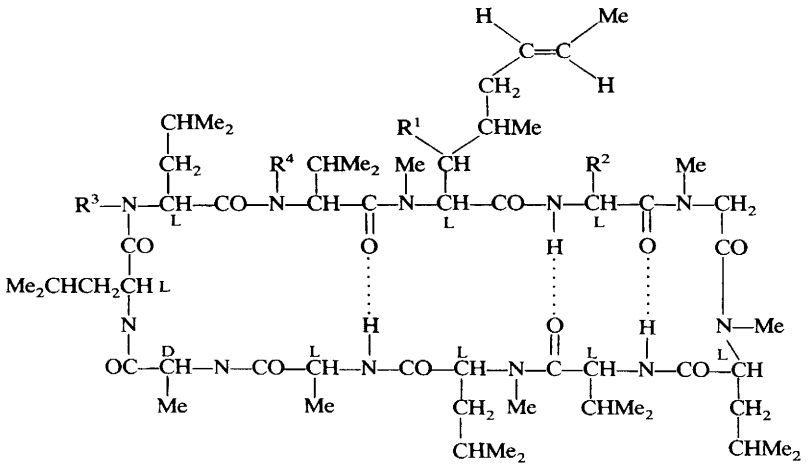
[46] C. C. Chiang, I. L. Karle, and Th. Wieland, *Int. J. Pept. Protein Res.*, 1982, **20**, 414.
[47] A. Isogai, S. Takayama, S. Murakoshi, and A. Suzuki, *Tetrahedron Lett.*, 1982, **23**, 3065.
[48] D. W. Hughes and C. M. Deber, *Biopolymers*, 1982, **21**, 169.

model topologically similar to the bicyclic cryptates for investigating metal-ion interactions. Initial c.d. measurements in acetonitrile indicate that it binds Zn^{II} in a 2:1 peptide:Zn^{II} ratio.[49]

Gramicidin S (GS) is featured in five papers. Three analogues, [D-Ser(Bzl)$^{4,4'}$]-GS, [D-Ser$^{4,4'}$]-GS, and [Orn(Boc)$^{2,2'}$-D-Ser$^{4,4'}$]-GS, have been prepared. The first of these showed high anti-bacterial activity, equal to that of GS itself. On treatment of the analogue containing four substitutions with *NN'*-dicyclohexylcarbodi-imide and cuprous chloride one residue of D-serine was converted to dehydroalanine. Catalytic hydrogenation of this product (Palladium Black, MeOH, 25 °C) gave 96.2% chiral induction to product D-alanine.[50] Mono- and di-formyl gramicidin S show 50% and 5%, respectively, of the biological activity of their parent. The monoformyl derivative forms an aggregate that is more stable under acidic than under neutral or alkaline conditions. The tendency of formyl peptides to aggregate has been noted earlier with gramicidin A and the polymycins.[51] Variably *N*-deuteriated GS derivatives have been prepared by exchange and their isotopic compositions determined by n.m.r.; difference i.r. spectroscopy in DMSO solution of these derivatives afforded isolation of residue-specific peptide-group vibrations. This novel combination of methods unambiguously demonstrates two pairs of equivalent intramolecular H-bonds, with donor groups provided by the NH's of Val and Leu.[52] The enhancement of spin–lattice relaxation rates per mole of added paramagnetic free radical (nitroxide) has been examined in GS. An increase in all proton relaxation rates (not just those in the amides) was observed, the enhancement being greater with those protons being most exposed to solvent. Provided no specific nitroxide–molecule complexes exist, the method may be generally useful for evaluating the relative solvent exposure of protons.[53] A theory relating ^{13}C n.m.r. relaxation parameters to molecular motions at three levels has been described and successfully tested on GS, giving the rates of internal librational motions.[54]

The structures of cyclosporins A to D, neutral cyclic [11]-peptides, were determined in 1976—77. New minor components from the same source, *Tolypocladium inflatum* (formerly called *Trichoderma polysporum*), have now been sequenced (32).[55] A series of cyclic oligopeptides *cyclo*-(D-Pro-Pro$_2$)$_n$ ($n = 1$—8) have been obtained by cyclization of the three possible diastereoisomeric triproline 2,4,5-trichlorophenyl esters. The proportion of the various ring sizes in the product depended markedly on the position of the D-residue in the linear sequence (see Table).[56]

[49] J. C. Tolle, M. A. Staples, and E. R. Blout, *J. Am. Chem. Soc.*, 1982, **104**, 6883.
[50] S. Ando, H. Aoyagi, M. Waki, T. Kato, N. Izumiya, K. Okomato, and M. Kondo, *Tetrahedron Lett.*, 1982, **23**, 2195.
[51] B. R. Srinavasa, *J. Antibiot.*, 1982, **35**, 571.
[52] E. M. Krauss and S. I. Chan, *J. Am. Chem. Soc.*, 1982, **104**, 1824.
[53] N. Niccolai, G. Valensin, C. Rossi, and W. A. Gibbons, *J. Am. Chem. Soc.*, 1982, **104**, 1534.
[54] O. W. Howarth and L. Y. Lian, *J. Chem. Soc., Perkin Trans. 2*, 1982, 263.
[55] R. Traber, H.-R. Loosli, H. Hofmann, M. Kuln, and A. von Wartburg, *Helv. Chim. Acta*, 1982, **65**, 1655.
[56] M. Rothe and W. Mästle, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 220.



Cyclosporin	R^1	R^2	R^3	R^4	K
A	OH	Et	Me	Me	L
E	OH	Et	Me	H	L
F	H	Et	Me	Me	L
G	OH	Pr	Me	Me	L
H	OH	Et	Me	Me	D
I	OH	Pr^i	H	Me	L

(32)

Table

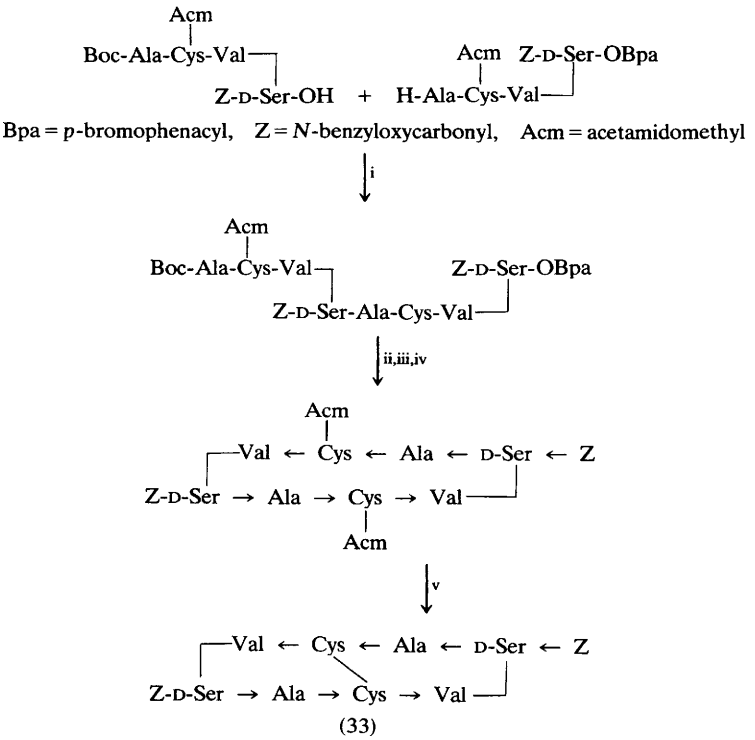
Starting sequence	DOP*/%	*Yield*/% $n=1$	$n=2$	$n=3$	$n=4$	$n=5$
		(*a*) $c \ll 0.001$ mol l^{-1} in pyridine				
DLL	1	78.3	2.5			
LDL	1.6	3.2	51.7			
LLD	20.1	30.8	3.5			
		(*b*) $c = 0.1$ mol l^{-1} in DMF/NEt_3				
DLL	1	5.5	4.1	22.0	11.6	5.7
LDL	—	—	70.5	4.6	3.2	<1
LLD	5.4	4.9	29.0	17.2	7.1	4.7

* DOP = *N*-terminal dioxopiperazine cleavage.

3 Cyclic Depsipeptides

A synthesis of a precursor (33) of des-*N*-tetramethyltriostin A (TANDEM) (Scheme 7) has been reported; it circumvents the racemization observed at *C*-terminal alanine in an earlier synthesis by carrying out fragment coupling and cyclization with Z-D-serine at *C*-terminal positions. Even so, the product appeared to have undergone 4—10% racemization of alanine residues. This is thought, however, to be occurring on acid hydrolysis of the peptide to the component amino-acids.[57] The crystal structure of TANDEM itself shows a

[57] M. K. Dhaon, J. P. Gardner, and R. K. Olsen, *Tetrahedron*, 1982, **38**, 57.



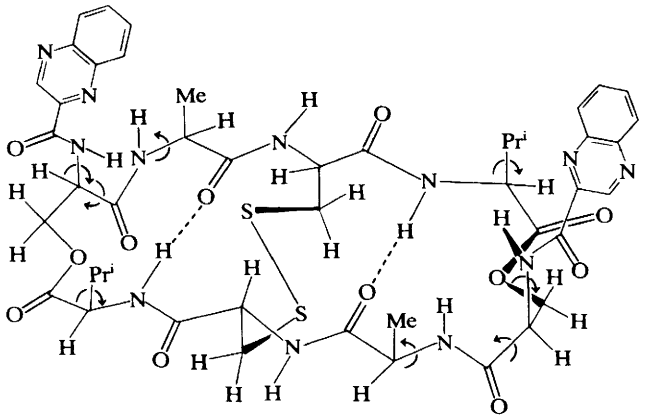
(33)

Reagents: i, Me_2CHCH_2OCOCl, *N*-methylmorpholine; ii, Zn, HOAc; iii, CF_3CO_2H; iv, 1-hydroxybenzotriazole, 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide; v, I_2, MeOH.

Scheme 7

somewhat asymmetric peptide ring with the disulphide cross-bridge lying on the opposite side with respect to the quinoxaline chromophores. Although the two points of attachment of the chromophores are 12 Å apart, the perpendicular distance between the quinoxaline rings is only about 6.5 Å. In this conformation the molecule could not participate in two-base-pair-sandwich intercalation as this requires a 10 Å separation between the quinoxaline rings.[58] The solution conformation of TANDEM has been examined by ^1H n.m.r. The results underline the P-helical nature of the cross-bridge, and overall the conformation deduced (34; the curved arrows denote conformational mobility) resembles that determined by *X*-ray. A similar solution study on a TANDEM analogue in which L-serine replaces D-serine shows a close resemblance except that the positions and orientations of the serine α-protons and the NHCO-quinoxaline residues have been interchanged. It is clear that this analogue does not bind to DNA because the quinoxaline chromophores lie close to the plane

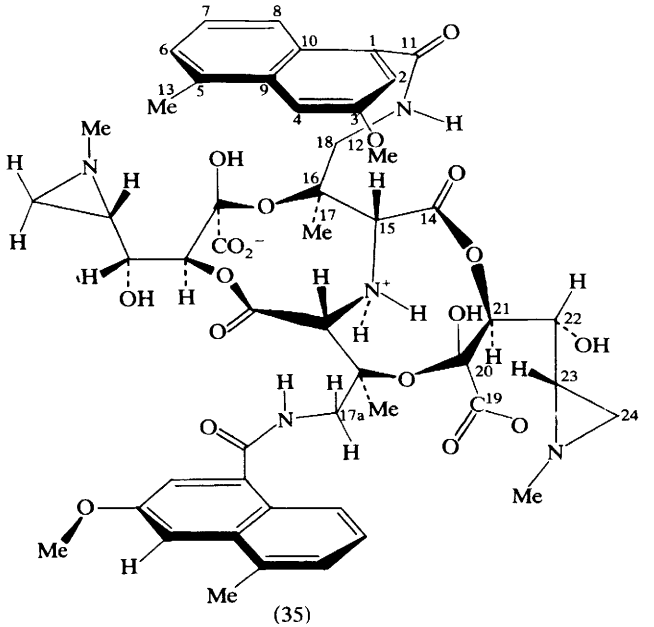
[58] M. B. Hossain, D. van der Helm, R. K. Olsen, P. G. Jones, G. M. Sheldrick, E. Egert, O. Kennard, M. J. Waring, and M. A. Viswamitra, *J. Am. Chem. Soc.*, 1982, **104**, 3401.



(34)

of the depsipeptide ring.[59] The ^{1}H n.m.r. spectra of antibiotics of the quinoxaline group have been the subject of a further discussion.[60]

Another bridged 'cyclodepsipeptide' of curious structure, carzinophilin A from *Streptomyces sahachiroi*, has yielded up its secrets and is now established as a dimer (35) that possesses two-fold symmetry. Hydrolysis with 5% NH_4OH



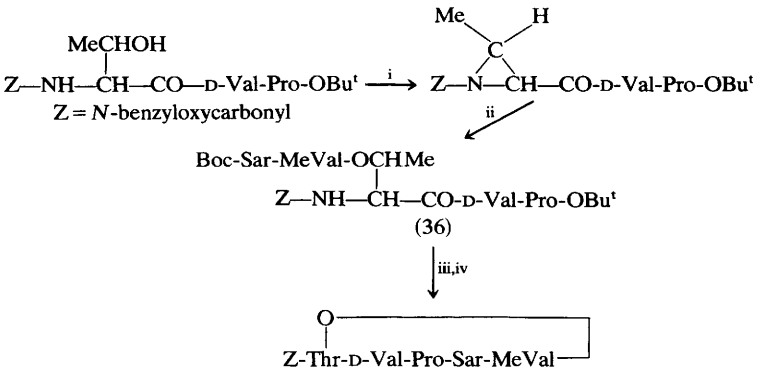
(35)

[59] E. Hyde, J. R. Kalman, D. H. Williams, D. G. Reid, and R. K. Olsen, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1041.

[60] M. P. Williamson, D. Gavreau, D. H. Williams, and M. J. Waring, *J. Antibiots.*, 1982, **35**, 62.

followed by 20% HCl gives (*SS*-)-*erythro*-4-amino-2,3-dihydroxy-3-methylbutanoic acid, glycine (by a retro-aldol cleavage between C-15 and C-16), 3-methoxy-5-methylnapthalene-1-carboxylic acid, and 2,4-diamino-3-hydroxy-3-methylbutanoic acid. The conformation depicted indicates a similarity to the bis-intercalating anti-tumour antibiotics, suggesting its mode of action. The presence of two aziridine groups in close proximity to the chromophores also accounts for the observed specifically acid-promoted interstrand crosslinking of DNA by this antibiotic.[61] A cyclotetradepsipeptide with the sequence of the phytotoxin AM-toxin III has been synthesized. The dehydroalanine residue was elaborated after cyclization by Hofmann degradation of a 2,3-diaminopropionic acid residue. The synthetic product, *cyclo*-(Δ-Ala-Ala-Hmp-Ahp), was identical to natural material in physical properties and its necrotic activity in apple leaves. Two analogues in which tyrosine and *O*-methyltyrosine replaced Ahp were also prepared, but only showed weak biological activity.[62]

A total synthesis of actinomycin D and its $[Ser^1]_2$ analogue has been reported. In this synthesis the key *O*-peptide intermediate (36) was generated by the ring opening of an aziridine by a carboxyl group (Scheme 8).[63] Actinomycin D labelled with ^{15}N at all twelve nitrogen atoms has been prepared by

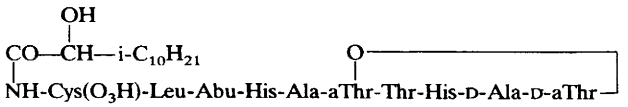


Reagents: i, diethylazodicarboxylate, Ph_3P; ii, Boc-Sar-MeVal-OH, CH_2Cl_2, 50 °C, 7 days; iii, CF_3CO_2H: iv, *NN'*-dicyclohexylcarbodi-imide, 1-hydroxybenzotriazole.

Scheme 8

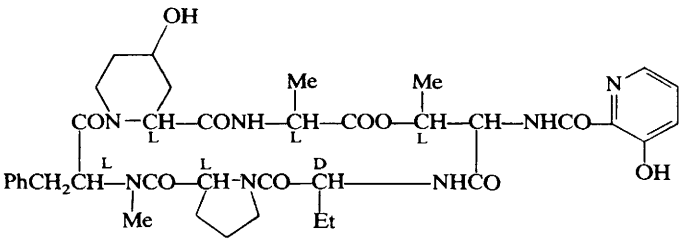
incubation of *Streptomyces parvulus* with [^{15}N]glutamic acid. The ^{15}N n.m.r. spectrum of the product suggests a model for the solution conformation with different H-bonding interactions in the monomer formed in organic solvents and the dimer formed in aqueous solution.[64] Imacidin C, the main peptide

[61] J. C. Lown and C. C. Hanstock, *J. Am. Chem. Soc.*, 1982, **104**, 3213.
[62] T. Kanmera and N. Izumiya, *Int. J. Pept. Protein Res.*, 1982, **19**, 79.
[63] K. Nakajima, T. Tanaka, M. Neya, and K. Okawa, *Bull. Chem. Soc., Jpn.*, 1982, **55**, 3237.
[64] R. H. Shafer, J. V. Formica, C. Delfini, S. C. Brown, and P. A. Mirau, *Biochemistry*, 1982, **21**, 6496.



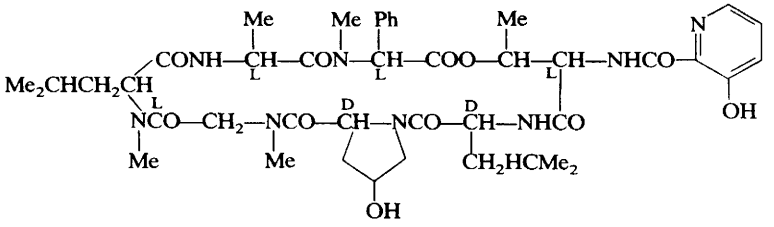
(37)

antibiotic from *Streptomyces olivaceus*, has been identified as an (*R*)-β-hydroxyisotridecanoic acid decapeptide lactone (37). The amino-acid configurations were determined enzymatically and by gas chromatography on a chiral stationary phase as *N*-trifluoroacetyl isopropyl esters.[65] Two new peptide lactones of the streptogramin family, virginiamycin S_5 (38) and viridogrisein II



(38)

(39), have been sequenced. In the former, alanine and 4-hydroxypipecolic acid replace the phenylglycine and 4-oxopipecolic acid found in the main component of the virginiamycins (S_1), and in the latter *N*-methyl-leucine replaces the *N*-β-dimethyl-leucine found in viridogrisein I.[66]

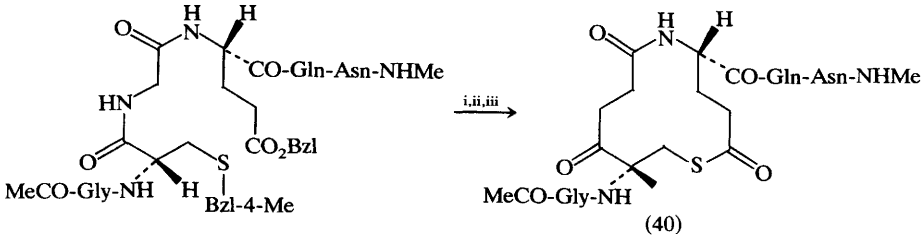


(39)

An *X*-ray analysis of the crystal structure of a valinomycin–sodium picrate complex shows a water molecule in the region within the valinomycin usually occupied by K^+. The Na^+ was external to the macrocycle, bonding to the three carbonyl oxygens adjacent to the lactyl residue and to the water molecule. By comparison to K^+, Na^+ is more weakly bound to the depsipeptide and more strongly to the picrate, explaining the greater effects of picrate-like anions on the valinomycin-catalysed membrane transport of Na^+ compared to K^+.[67] Solution studies on valinomycin in the presence of Li^+ indicate that a weak 1:1 complex is formed. A model for this complex is proposed in which the metal

[65] H. Laatsch, *Liebigs Ann. Chem.*, 1982, 28.
[66] I. Oberbaumer, E. Grell, F. Raschdorf, and W. J. Richter, *Helv. Chim. Acta*, 1982, **65**, 2280.
[67] L. K. Steinrauf, J. A. Hamilton, and M. N. Sabesan, *J. Am. Chem. Soc.*, 1982, **104**, 4085.

ion binds to the ester carbonyls of the D-valine residues, the remaining ligands probably being solvent acetonitrile molecules.[68] A hexapeptide containing a 12-membered thiolactone ring (40) has been synthesized (Scheme 9). Macrocycle thiolactones are common to the metastable binding site of three serum proteins and in principle could exist as either 12- or 15-membered rings. Hydrolysis of (40) proceeded 900 times faster than hydrolysis of a 15-membered thiolactone ring peptide prepared earlier.[69]



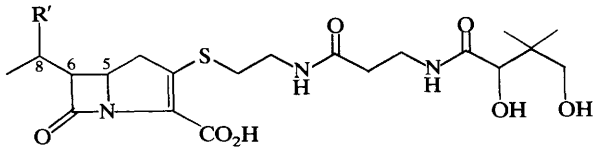
(40)

Reagents: i, liquid HF; ii, *NN'*-dicyclohexylcarbodi-imide, 1-hydroxybenzotriazole; iii, NEt_3.

Scheme 9

4 Highly Modified Cyclic Peptides

Bicyclic β-Lactams.—New carbapenems continue to be isolated. *Streptomyces* sp. OA-6129A has yielded four compounds designated as OA-6129A, B_1, B_2, and C (41), which are potent anti-microbials against both Gram-positive and



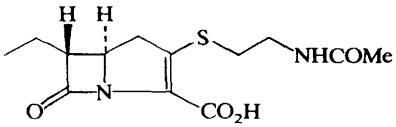
OA-6129	R'	C_5	C_6	C_8	*Pantoyl group*
A	H	R	R	—	R
B_1	OH	R	R	*	R
B_2	OH	R	S	S	R
C	OSO_3H	R	R	*	R

* Not determined.

(41)

-negative bacteria. The structure of OA-6129A was established by its semisynthesis from PS-5 (42).[70] Three relatively acidic β-lactams, the pluracidomycins (43), have been obtained from *Streptomyces*

[68] M. B. Sankaram and K. R. K. Easwaran, *Biopolymers*, 1982, **21**, 1557.
[69] S. A. Khan and B. W. Erickson, *J. Am. Chem. Soc.*, 1982, **104**, 4283.
[70] T. Yoshioka, I. Kojima, K. Isshiki, A. Watanabe, Y. Shimauchi, M. Okabe, Y. Fukagawa, and T. Ishikura, *Tetrahedron Lett.*, 1982, **23**, 5177.



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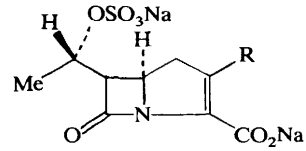
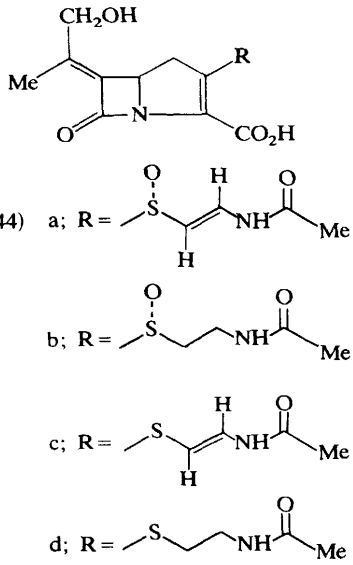


(43) Pluracidomycin
A; R = —SO_3Na
B; R = —S—CH_2CO_2Na
C; R = —S(=O)—CHO

pluracidomyceticus,[71] and a new strain of *Streptomyces* sp. KC-6643 that no longer produces carpetimycins A and B generates instead a compound identified as dihydroasparenomycin C (44d).[72] Asparenomycins A, B, and C (44a, b, and c) are novel carbapenems recently isolated from both *Streptomyces tokunonsis* and *Streptomyces argenteolus*, which bear a hydroxyisopropylidene group on the β-lactam ring. Also isolated was a lactone (45) assumed to arise



(44) a; R = —S(=O)—CH=CH—NH—C(=O)—Me

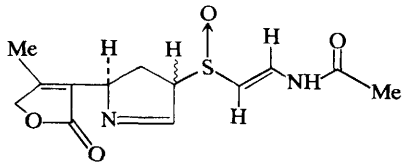
b; R = —S(=O)—CH_2CH_2—NH—C(=O)—Me

c; R = —S—CH=CH—NH—C(=O)—Me

d; R = —S—CH_2CH_2—NH—C(=O)—Me

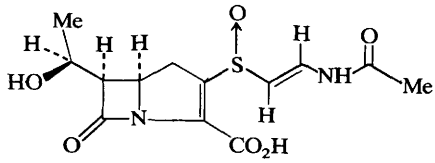
[71] N. Tsuji, K. Nagashima, M. Kobayashi, Y. Terui, K. Matsumoto, and E. Kondo, *J. Antibiot.*, 1982, **35**, 536.

[72] S. Tanabe, M. Okuchi, M. Nakayama, S. Kimura, A. Iwasaki, T. Mizoguchi, A. Murakami, H. Itob, and T. Mori, *J. Antibiot.*, 1982, **35**, 1237.



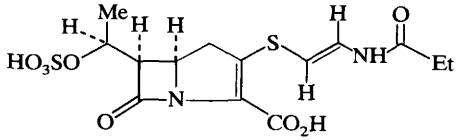
(45)

from the *Z*-isomer of asparenomycin A.[73] The structure (46) of a minor component of the culture filtrate of *Streptomyces griseus* subsp. cryophilus C-19393 has been confirmed by partial synthesis from epithienomycin B; it has broad antibiotic activity and is a strong β-lactamase inhibitor.[74] Two other

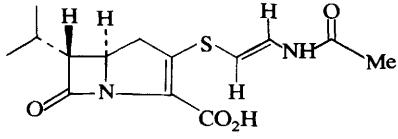


(46)

minor components newly identified are an olivanic acid derivative (47) from *Streptomyces olivaceus* BCS 349.80[75] and PS-8 (48) from *Streptomyces cremeus* subsp. auratilis A 271.[76]



(47)



(48)

Two genera of bacteria, *Serratia* sp. and *Erwinia* sp., have been found to produce a simple carbapenem (49) that is highly unstable. It was isolated as its *p*-nitrobenzyl ester, which is cleaved by NEt_3/MeOH to (50).[77] Deacetoxycephalosporin C (51) has been unexpectedly isolated from *Flavobacterium*

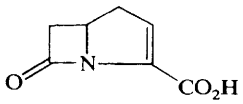
[73] N. Tsuji, K. Nagashima, M. Kobayashi, J. Shoji, T. Kato, Y. Terui, H. Nakai, and M. Shiro, *J. Antibiot.*, 1982, **35**, 24.

[74] S. Harada, Y. Nozaki, S. Shinagawa, and K. Kitano, *J. Antibiot.*, 1982, **35**, 957.

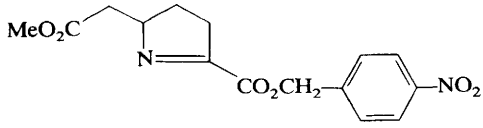
[75] S. J. Box, D. F. Corbett, K. G. Robins, S. R. Spear, and M. S. Verrall, *J. Antibiot.*, 1982, **35**, 1394.

[76] N. Shibamoto, M. Nishino, K. Okamura, Y. Fukugawa, and T. Ishikura, *J. Antibiot.*, 1982, **35**, 763.

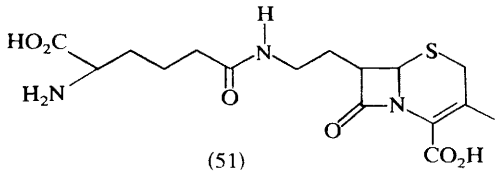
[77] W. L. Parker, M. L. Rathnum, J. S. Wells, jun., W. M. Trejo, P. A. Principe, and R. B. Sykes, *J. Antibiot.*, 1982, **35**, 653.



(49)



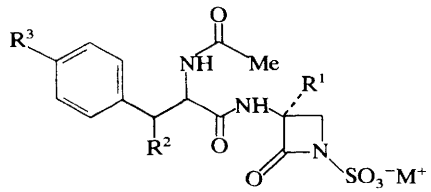
(50)



(51)

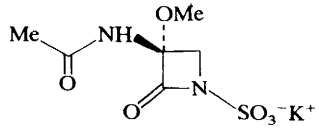
and *Xanthomonos* sp. It was previously thought that the 'lowly' bacteria would produce only simple monocyclic β-lactam antibiotics.[78]

Monocyclic β-Lactams.—A series of novel monocyclic β-lactams (52) have been isolated from a strain of *Agrobacterium radiobacter* and are of weak anti-bacterial activity but highly stable to hydrolysis by β-lactamases.[79] The simpler compound (53) has been obtained from *Chromobacterium violaceum*.



SQ	R^1	R^2	R^3	M
26/823	OMe	H	H	Na
26/875	OMe	H	OH	K
26/700	H	H	OH	K
26/970	OMe	OH	$OSO_3^-Na^+$	Na
26/812	OMe	$OSO_3^-Na^+$	$OSO_3^-Na^+$	Na

(52)

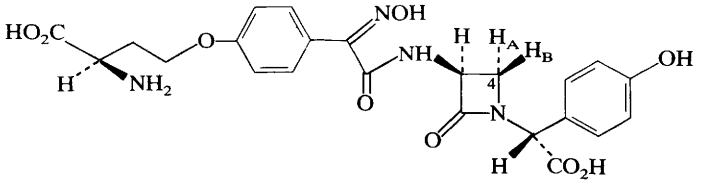


(53)

Confirmation of its structure and assignment of its absolute configuration was made by synthesis from 6-aminopenicillanic acid. Sulfazecin was also produced

[78] P. D. Singh, P. C. Ward, J. S. Wells, C. M. Ricca, W. H. Trejo, P. A. Principe, and R. B. Sykes, *J. Antibiot.*, 1982, **35**, 1397.

[79] J. S. Wells, W. H. Trejo, P. A. Principe, K. Bush, N. Georgopapadakou, D. P. Bonner, and R. P. Sykes, *J. Antibiot.*, 1982, **35**, 295, 300.



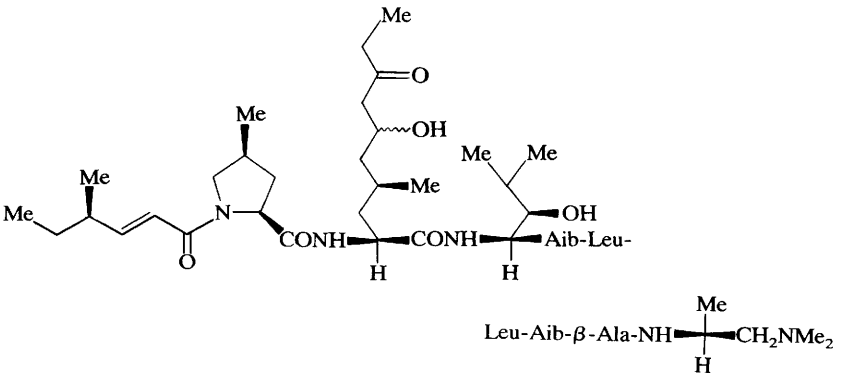
(54)

by this bacterium.[80] In the biosynthesis of nocardicin A (54), L-serine bearing a label at the (3*R*)-locus gives rise to enhanced ^2H content at position 4A while (3*S*)-label specifically enriches position 4B. The stereochemical course of β-lactam formation *in vivo* is therefore substantially, if not exclusively, inversion.[81]

Other Modified Cyclic Peptides.—Full details of the use of the catalytic hydrogenation of carbobenzoxy peptide pentafluorophenyl esters in the synthesis of peptide alkaloids (see last year's report) have now been published.[82]

5 Linear Peptides

Peptides Containing α-Aminoisobutyric Acid.—A new antibiotic leucinostatin A (55) has been found in *Paecilomyces lilacinus*. It contains the unusual



(55)

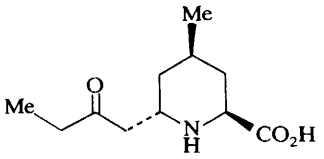
amino-acids *cis*-4-methyl-L-proline, L-*threo*-β-hydroxyleucine, and 2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid; on acid hydrolysis the latter cyclizes to 4-methyl-6-(2-oxobutyl)-2-piperidinecarboxylic acid (56). This antibiotic has anti-tumour, anti-bacterial, and anti-fungal activities.[83] The 'hexapep-

[80] W. L. Parker, W. H. Koster, C. M. Cimarusti, D. M. Floyd, W.-C. Liu, and M. L. Rathnum, *J. Antibiot.*, 1982, **35**, 189.

[81] C. A. Townsend and A. M. Brown, *J. Am. Chem. Soc.*, 1982, **104**, 1748.

[82] U. Schmidt, A. Lieberknecht, H. Griesser, and J. Talbiersky, *J. Org. Chem.*, 1982, **47**, 3261; U. Schmidt, A. Lieberknecht, H. Griesser, and J. Hausler, *Liebigs Ann. Chem.*, 1982, 2153.

[83] Y. Mori, M. Tsubio, M. Suzuki, K. Fukushima, and T. Arai, *J. Chem. Soc., Chem. Commun.*, 1982, 94.



(56)

tide' H-Pro-Leu-Aib-Aib-Glu-valinol has been synthesized and found identical with a *C*-terminal fragment obtained from natural trichotoxin-A40. A similar peptide in which glutamine replaced glutamic acid was clearly different from the natural material. This work,[84] together with a mass-spectrometric study,[85] has led to a revised sequence (57) for trichotoxin A in which the amino-acids at positions 6 and 17 have been interchanged. Although the main component contains isovaline at position 16, the minor component [amino-acids in brackets replace those preceding in (57)] *C*-terminus was synthesized in preference as α-aminoisobutyric acid is more readily available.[84]

MeCO-Aib-Gly(Ala)-Aib-Leu-Aib-Gln-Aib-Aib-Aib(Ala)-Ala-
-Aib-Aib-Pro-Leu-Aib-D-Iva(Aib)-Glu-Valinol
(6) (17)

(57)

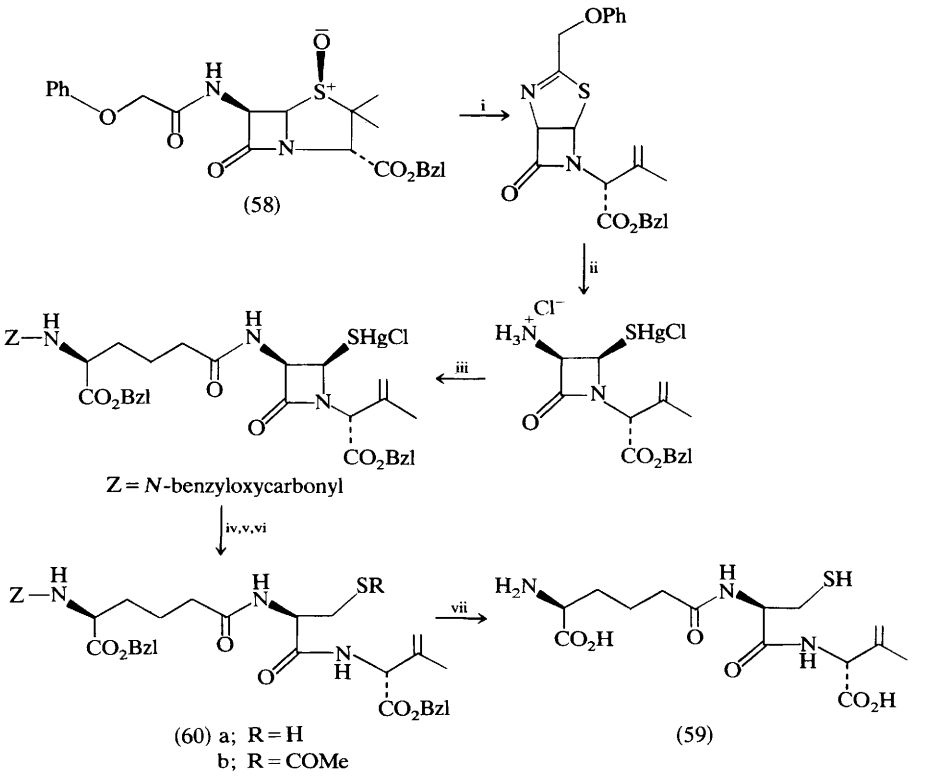
X-Ray and i.r. studies show that in the solid state the oligomer series Z-(Aib)$_n$-OH ($n = 3$, 4, or 5) forms incipient 3_{10}-helices, the observations being incompatible with α-helical structures. In solvents of low polarity, i.r. spectra favour the occurrence of the same intramolecularly H-bonded forms, but high peptide concentration leads to aggregation.[86] Similarly, in the series acetyl-(Aib)$_n$-NHMe ($n = 1$, 2, or 3) n.m.r. and i.r. studies indicate that in solution a 3_{10}-helical-type conformation is adopted, supporting the hypothesis that the Aib residue has asymmetric geometry at the C^{α} atom in solution as well as in the crystalline state.[87] Whereas Pro-Aib segments of some tetrapeptides have been found to form type III β-turns,[88] *X*-ray and spectroscopic examination of pivaloyl-Pro-Aib-Aib-phenylalaninol, an analogue of the *C*-terminal tetrapeptide of the antibiotic antiamoebin I, shows the presence of a single turn of a 3_{10}-helix. Each peptide molecule also donates and accepts a pair of intermolecular H-bonds.[89]

X-Ray analysis of an *N*-protected amino-terminal pentapeptide fragment of suzukacillin, Boc-Aib-Pro-Val-Aib-Val-OMe, shows a four-fold helix with a pitch of 5.58 Å stabilized by two $5 \rightarrow 1$ type H-bonds (α-helix) and one $4 \rightarrow 1$ type (3_{10}-helix). However, two of these H-bonds involve the carbonyl oxygen

[84] H. Bruckner and G. Jung, *Liebigs Ann. Chem.*, 1982, 1677.
[85] M. Aydin, D. H. Bloss, W. A. Konig, H. Bruckner, and G. Jung, *Biomed. Mass Spectrom.*, 1982, **9**, 39.
[86] E. Benedetti, A. Bavoso, B. Diblasio, V. Pavone, C. Pedone, M. Crisma, G. M. Bonara, and C. Toniolo, *J. Am. Chem. Soc.*, 1982, **104**, 2437.
[87] Y. Paterson, E. R. Stimson, D. J. Evans, S. J. Leach, and H. A. Scheraga, *Int. J. Pept. Protein Res.*, 1982, **20**, 468.
[88] B. V. Venkataram Prasad, H. Balaram, and P. Balaram, *Biopolymers*, 1982, **21**, 1261.
[89] P. van Roey, G. D. Smith, T. M. Balasubramanian, A. S. Redlinski, and G. R. Marshall, *Int. J. Pept. Protein Res.*, 1982, **19**, 499.

of the t-butyloxycarbonyl group.[90] Two other pentapeptide derivatives, Boc-Ala-Aib-Ala-Aib-Aib-OMe and Boc-Leu-Aib-Pro-Val-Aib-OMe, which correspond to sequences 6—10 and 16—20, respectively, of suzukacillin, adopt 3_{10}-helical structures. These results support the presence of a largely helical conformation for the suzukacillin membrane channel.[91] All seven of the possible isomers of bradykinin in which the three proline residues have been replaced partially or totally by Aib residues have been prepared. Substitution for Pro^2 and/or Pro^3 appears to stabilize a type III β-turn involving the *N*-terminal residues; biological potencies are low in these analogues. The relatively high activity of $[Aib]^7$-bradykinin suggests that the complexed form of the peptide is characterized by a *cis*-Pro^7 conformation.[92]

Peptides Containing Dehydroamino-acids.—The benzyl ester sulphoxide of penicillin N (58) has been converted to L-α-aminoadipyl-L-cysteinyl-D-3,4-di-

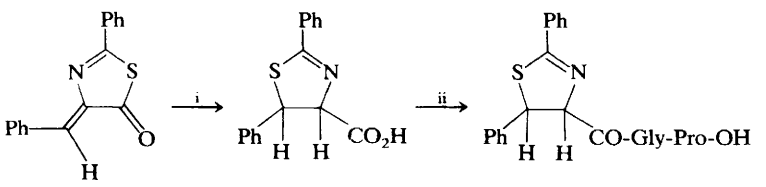


Reagents: i, $(MeO)_3P$, C_6H_6; ii, $HgCl_2$, $Me_2C(CH_2OH)_2$; iii, EEDQ, Z-NHCH(CO_2Bzl)-$(CH_2)_3CO_2H$; iv, H_2S; v, $NaBH_3CN$; vi, Ac_2O, pyridine; vii, Na, liquid NH_3.

Scheme 10

[90] A. K. Francis, M. Iqbal, P. Balaram, and M. Vijayan, *J. Chem. Soc., Perkin Trans. 2*, 1982, 1235.
[91] A. K. Francis, Ch. P. Rao, M. Iqbal, R. Nagaraj, M. Viajayan, and P. Balaram, *Biochem. Biophys. Res. Commun.*, 1982, **106**, 1240.
[92] R. E. London P. G. Schmidt, R. J. Vavrek, and J. M. Stewart, *Int. J. Pept. Protein Res.*, 1982, **19**, 334.

dehydrovaline (59) (Scheme 10) *via* the thiol (60a) isolated as its 5′-acetyl derivative (60b). On incubation of the latter with an active cell-free extract of *Cephalosporium acremonium*, no conversion to active antibiotic took place. On co-incubation with its dihydro derivative (*i.e.* the Arnstein tripeptide, with valine at the *C*-terminus), strong inhibition of conversion of the latter to isopenicillin N was observed.[93] A number of enkephalin analogues containing dehydroalanine (Δ-Ala) have been prepared. The peptide chains were assembled with serine in the positions where the unsaturated residue was required. Tosylation followed by treatment with diethylamine effected the required elimination. [D-Ala^2, Δ-Ala^3, Leu^5]-enkephalin maintained most of the receptor-binding affinity for both δ- and μ-receptors, but the [Δ-Ala^3, Leu^5] analogue showed a drastic drop in this property.[94] [Δ-Ala^2, Leu^5]-enkephalin was more active than the parent in this assay, showing, however, a preference for μ-opiate receptors. Although [Δ-Ala^2, des-Leu^5]-enkephalin was only weakly active, it had a high selectivity for μ-receptors.[95] A dehydro analogue of the angiotensin-converting enzyme inhibitor *N*-Bzl-Phe-Gly-Pro-OH (ACE) has been prepared by condensation of the oxazolinone of *N*-benzoyl-(*Z*)-dehydrophenylalanine with glycylproline. Use of the corresponding (*E*)-isomer gave the same (*Z*)-tripeptide. Attempts to prepare a thio analogue using the thio-oxazolinone (61) were unsuccessful. Base was found to open the ring of (61) to yield a thiazoline (62) that could be elaborated into a tripeptide (63) (Scheme 11). Both this tripeptide and *N*-Bzl-(*Z*)-Δ-Phe-Gly-Pro-OH were moderate inhibitors of ACE.[96]



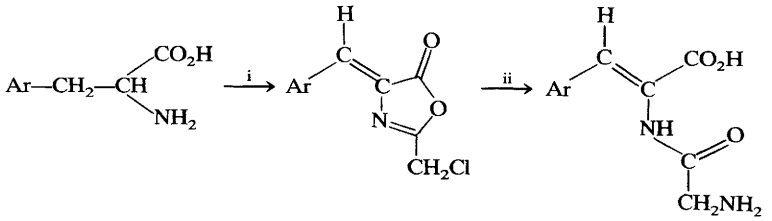
(61) (62) (63)

Reagents: i, OH^-, H_2O; ii, H-Gly-Pro-ONa, *NN*′-dicyclohexylcarbodi-imide, 1-hydroxybenzotriazole.

Scheme 11

A number of new approaches to the generation of αβ-unsaturated amino-acid residues have been developed. *N*-Protected methyl esters of serine and threonine can be conveniently dehydrated to their dehydro analogues using *NN*′-carbonyldi-imidazole. Protected dipeptides such as Z-Val-Ser-OMe that contain serine in the *C*-terminal position give dehydroalanine derivatives with this reagent, but if the β-hydroxyamino-acid is *N*-terminal, as in Z-Ser-Gly-OMe, no dehydration occurred. Likewise, Z-Ser-NH_2 was unaffected by *NN*′-

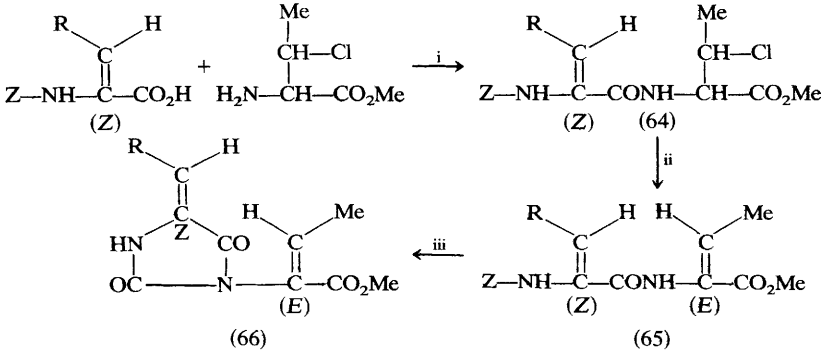
[93] J. E. Baldwin, B. Chakravarti, L. D. Field, J. A. Murphy, K. R. Whitten, Sir E. P. Abraham, and G. Jayatilake, *Tetrahedron*, 1982, **38**, 2773.
[94] Y. Shimogashi and C. H. Stammer, *Int. J. Pept. Protein Res.*, 1982, **20**, 199.
[95] Y. Shimogashi and C. H. Stammer, *Int. J. Pept. Protein Res.*, 1982, **19**, 54.
[96] T. J. Nitz, J. Lindsay, and C. H. Stammer, *J. Org. Chem.*, 1982, **47**, 4029.



Reagents: i, $(Cl_2CHCO)_2O$, EtOAc; ii, NH_3, MeOH, H_2O.

Scheme 12

carbonyldi-imidazole.[97] By a general two-step process β-arylalanines can be converted to glycyl-(β-aryl)dehydroalanines (Scheme 12). By analogy to the related Ehrlenmeyer azlactone synthesis, a (*Z*)-configuration of the product is assumed.[98] Z-(*Z*,*E*)-dehydrodipeptides have been synthesized by dehydrochlorination of Z-(*Z*)-dehydrodipeptides containing an *erythro*-3-chloro-2-aminobutanoic moiety (Scheme 13). This is the first report of a peptide



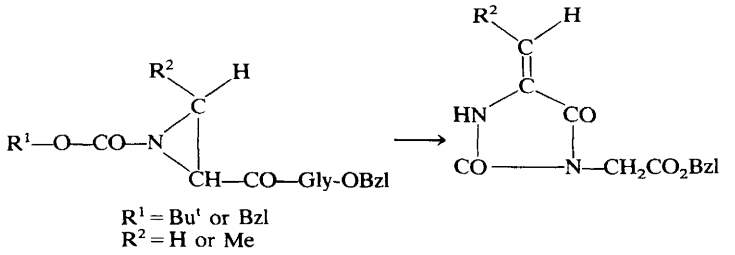
Reagents: i, *NN'*-dicyclohexylcarbodi-imide; ii, NEt_3, 20 °C, 15 h; iii, DBU.

Scheme 13

containing (*E*)-dehydrobutyrine. If the chlorine atom in (64) is replaced by hydroxyl, dehydration with $MeSO_2Cl/NEt_3$ gives the Z-(*Z*,*Z*)-dehydrodipeptide. Treatment of (65) with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gives a hydantoin derivative (66). On prolonged contact with DBU the (*E*)-dehydrobutyrine residue of the hydantoin isomerizes to the (*Z*)-isomer.[99]

Urethane-protected 2-aziridine carboxylates (Azy residues) also give hydantoins on treatment with tertiary amines (Scheme 14). If the Azy residue is not *N*-terminal, such treatment more usefully opens the heterocyclic ring to form a dehydroamino-acid residue.[100] Sodium iodide in acetone solution will effect a similar isomerization (Scheme 15). Where a dehydrobutyrine residue is generated, only the (*Z*)-isomer is formed.[101] A generally applicable synthesis of *N*-

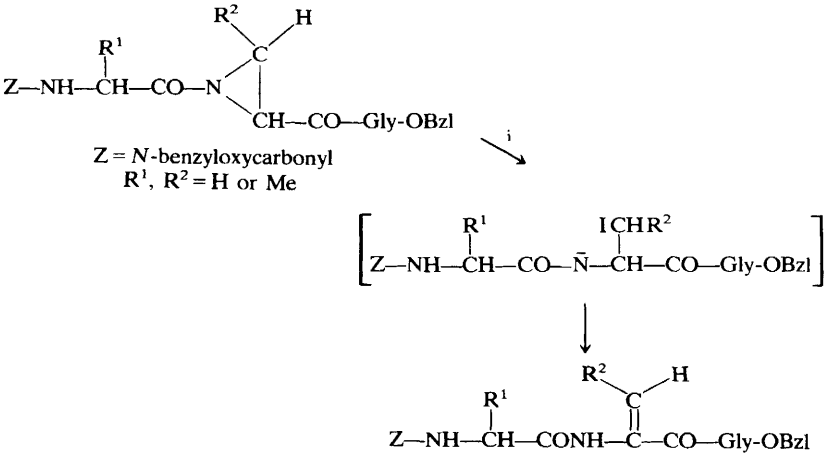
[97] R. Andruszkiewicz and A. Czerwinski, *Synthesis*, 1982, 968.
[98] D. J. Phelps and F. C. A. Gaeta, *Synthesis*, 1982, 234.
[99] C. Shin, Y. Yonezawa, T. Yamada, and J. Yoshimura, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 2147.
[100] K. Nakajima, H. Oda, and K. Okawa, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 3232.
[101] K. Okawa, K. Nakashima, T. Tanaka, and M. Neya, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 174.



R^1 = Bu^t or Bzl
R^2 = H or Me

Reagent: NEt_3 or DBU.

Scheme 14



Z = *N*-benzyloxycarbonyl
R^1, R^2 = H or Me

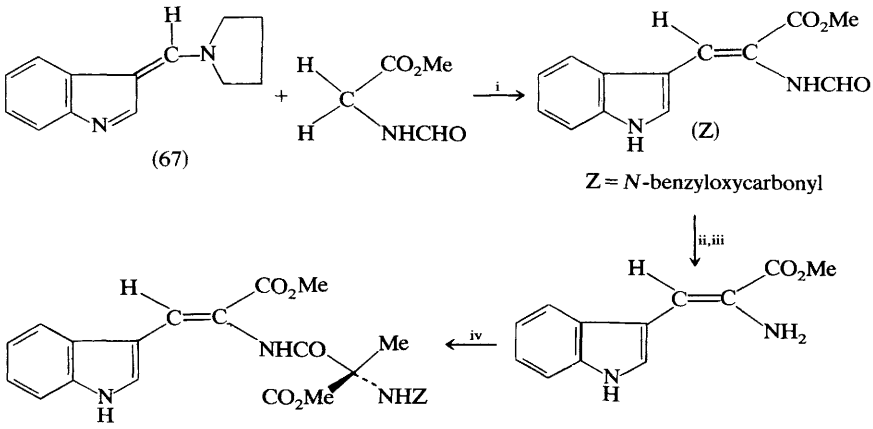
Reagent: i, NaI, acetone.

Scheme 15

acetyldehydroamino-acid esters involving treatment of esters of 2-azidocarboxylic acid in acetic anhydride with a catalytic amount of rhenium heptasulphide has been reported. The required 2-azidocarboxylic acid esters are accessible from the corresponding α-halogen derivatives *via* azide exchange using a phase-transfer reaction.[102] *N*-Acyl-$\alpha\beta$-dehydrotryptophan esters, especially *N*-formyl derivatives, have hitherto been difficult to synthesize by conventional methods. A new route involving the reaction of 3-[(1-pyrrolidinyl)methylene]-3*H*-indole (67) with *N*-acylglycinates conveniently makes such derivatives available for incorporation into peptides (Scheme 16).[103]

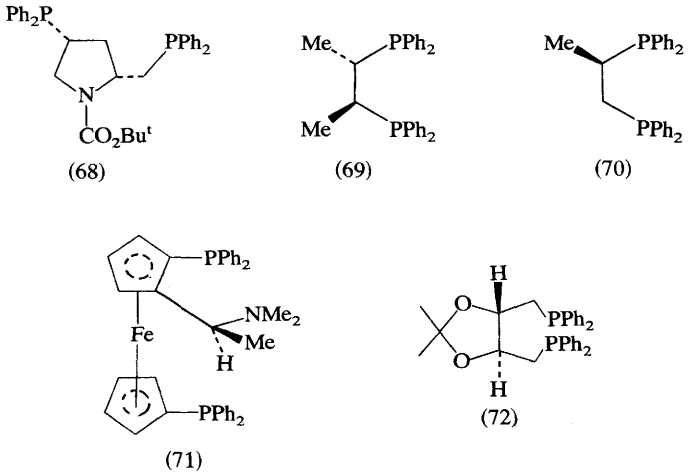
A study of the effect of varying the chiral diphosphine ligand of the rhodium complex catalyst in the homogeneous hydrogenation of various dipeptides containing a single dehydroamino-acid residue has been made. The highest stereoselectivity (up to 99.6%) was found with pyrrolidinophosphines such as

[102] F. Effenberger and T. Beisswenger, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 203.
[103] T. Mariya, N. Yoneda, M. Miyoshi, and K. Matsumoto, *J. Org. Chem.*, 1982, **47**, 94.

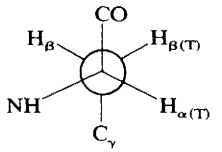


Scheme 16

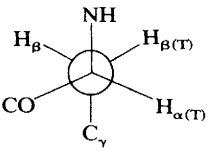
Bppm (68); chiraphos (69), prophos (70), and Bppfa (71) gave only poor results.[104] Catalytic tritiation of Ac-Δ-Phe-(*S*)-Phe-OMe in the presence of the rhodium complex of DIOP (72) gave tritiated Ac-(*S*)-Phe-(*S*)-Phe-OMe : Ac-(*R*)-Phe-(*S*)-Phe-OMe in the proportions 88 : 12, both diastereoisomers having nearly theoretical incorporations of radioactivity. Use of ^{3}H–^{3}H and ^{1}H–^{1}H coupling constants allowed the unequivocal assignment of the three staggered rotamers around C_α-C_β in the *N*-terminal Phe residue. In the (*S*)-residue (73) predominates (61%) whereas in the (*R*)-residue (74) makes the major (67%)



[104] I. Ojima, T. Kogure, N. Yoda, T. Suzuki, M. Yatabe, and T. Tanaka, *J. Org. Chem.*, 1982, **47**, 1329.

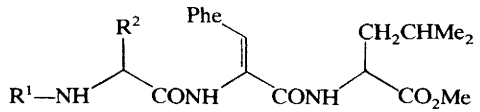


(73)



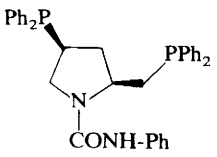
(74)

contribution.[105] In the synthesis of tripeptides by the asymmetric hydrogenation of the central dehydro residue of (75) using the rhodium complex of Ph-Capp (76) as the catalyst, although the ratio of the diastereoisomers produced varied somewhat with the *N*-terminal amino-acid, the *N*-protecting group showed more remarkable effects on stereoselectivity and reactivity. The t-butyloxycarbonyl group gave the best stereoselectivity and catalyst efficiency.[106] Acetyl-(*S*)-Phe-(*S*)-Phe-OMe has been obtained in high diastereoselectivity (98%) and enantioselectivity by the catalytic hydrogenation of acetyl-Δ-Phe-Δ-Phe-OMe using as chiral catalyst [Rh(dipamp)(cyclo-octa-1,5-diene)]$^+BF_4^-$ [see (77)].[107]

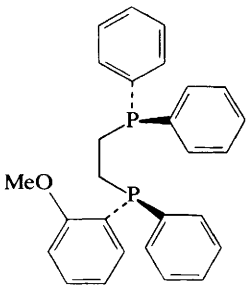


(75) R^1 = Boc, *N*-benzyloxycarbonyl, or CF_3CO
R^2 = H, Me, or CH_2Ph

(75)



(76)

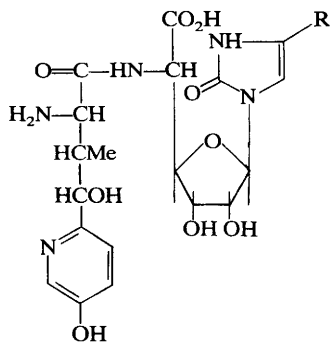


(77) dipamp

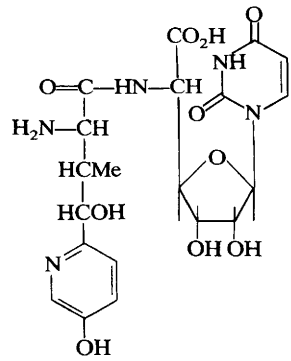
In the crystal structure of Ac-Δ-Phe-Pro-OH·$\frac{1}{4}H_2O$ one water molecule co-ordinates four dipeptide molecules in a tetrahedral arrangement. The conformation of the Δ-Phe resembles that of the central residue of (Δ-Phe)$_2$-Gly rather than that of (Δ-Phe)$_2$-Ala, indicating the influence of a saturated residue on the preceding unsaturated one.[108]

[105] H. Levine-Pinto, J. L. Morgat, P. Fromageot, D. Meyer, J. C. Poulin, and H. B. Kagan, *Tetrahedron*, 1982, **38**, 119.
[106] I. Ojima and N. Yoda, *Tetrahedron Lett.*, 1982, **23**, 3913.
[107] J.-C. Poulin and H. B. Kagan, *J. Chem. Soc., Chem. Commun.*, 1982, 1261.
[108] D. Ajo, V. Busetti, and G. Granozzi, *Tetrahedron*, 1982, **38**, 3329.

Other Dipeptide Derivatives.—Three new chitin synthetase inhibitors, neopolyoxins A, B (78), and C (79), have been isolated from *Streptomyces*

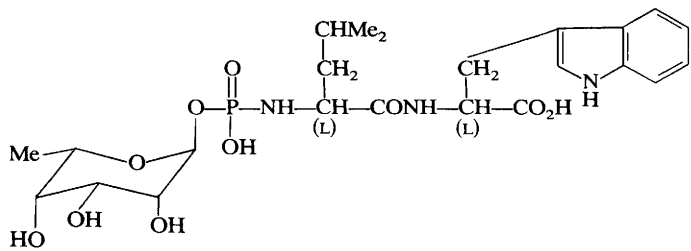


(78) a; R = CHO
b; R = CO_2H



(79)

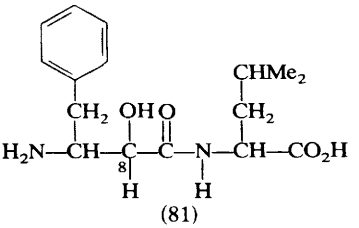
cacaoi. It is thought that neopolyoxin C is the biosynthetic precursor of neopolyoxins A and B, ring contraction of uracil to the imidazoline ring occurring at the nucleoside level. This benzilic acid rearrangement-type reaction played a key role in structure determination of the nucleosides of neopolyoxins A and B.[109] Talopeptin (MK-1), a novel metalloproteinase inhibitor produced by *Streptomyces mozunensis* MK-23, has been shown to be the 6-deoxy-α-L-talopyranosyloxyphosphadipeptide (80). The α-pyranose



(80)

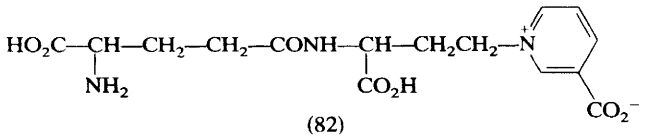
form of the sugar was deduced from the ^1H and ^{13}C n.m.r. spectra.[110] The *X*-ray crystal structure of the hog-kidney leucine aminopeptidase (LAP) inhibitor bestatin (81) has been reported. The non-polar side chains are oppositely disposed and separated by 10 Å. The peptide bond is *trans* and there is no hydrogen bonding between the OH on C-8 and the adjacent CO. It is suggested that bestatin binds more tightly to LAP than normal dipeptides because the C-8 tetrahedral atom is a closer reflection of the active site than the planar carbon. Bestatin can also ligate the activation-site Zn^{II} with some

[109] M. Uramoto, K. Kobinata, K. Isono, T. Higoshima, T. Miyozawa, E. E. Jenkins, and J. A. McCloskey, *Tetrahedron*, 1982, **83,** 1599.
[110] K.-I. Fukuhara, S. Murao, T. Nozawa, and M. Hatano, *Tetrahedron Lett.*, 1982, **23,** 2319.



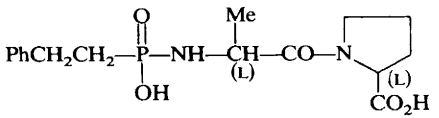
(81)

combination of the C-8—OH as well as the CO oxygen and the α-NH_2 nitrogen.[111] The γ-glutamyl derivatives of both acidic and neutral amino-acids have been found earlier in the mushroom *Lentinus edodes*; γ-Glu-His, γ-Glu-α-Orn, and γ-Glu-α-Lys have now been isolated from the same source, their first reported occurrence in any plant or mushroom. *L. edodes* also contains γ-glutamylnicotianine (82).[112]



(82)

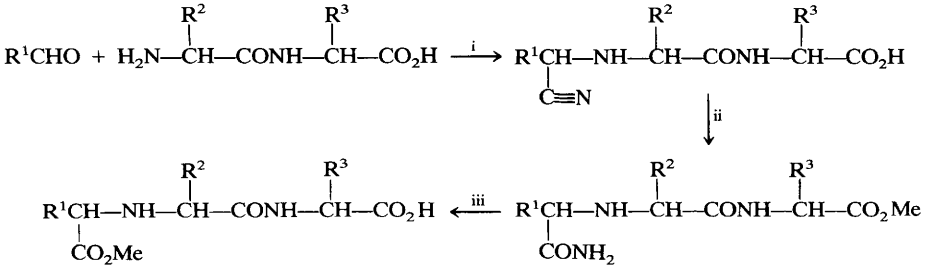
Several phosphonamides, phosphoramides, and phosphates have been prepared and tested as inhibitors of angiotensin-converting enzyme. Maximal inhibition (50% at 7 nM) was found with the *N*-(phenethylphosphoryl) dipeptide (83).[113] The readily available diphenyl-1-aminoalkanephosphonates have



(83)

been used in the synthesis of phosphonodipeptides. After dipeptide formation the phenyl groups can be removed either by transesterification (MeOH, KF, crown ether) followed by HBr–HOAc treatment or by hydrogenolysis on Adams catalyst.[114] Diastereoisomeric mixtures of phosphonodipeptides prepared from *N*-acyl L-α-amino-acids and racemic 1-aminoalkanephosphonates can be simply separated by ion-exchange chromatography on Dowex 50W (H^+).[115] The use of dipeptides as amine components in a Strecker reaction with aldehydes provides a useful route to substituted *N*-carboxymethyl dipeptides (Scheme 17). Yields range from 25% with Me_2CHCHO and Ala-Phe to 83% with PhCHO and Val-Tyr.[116]

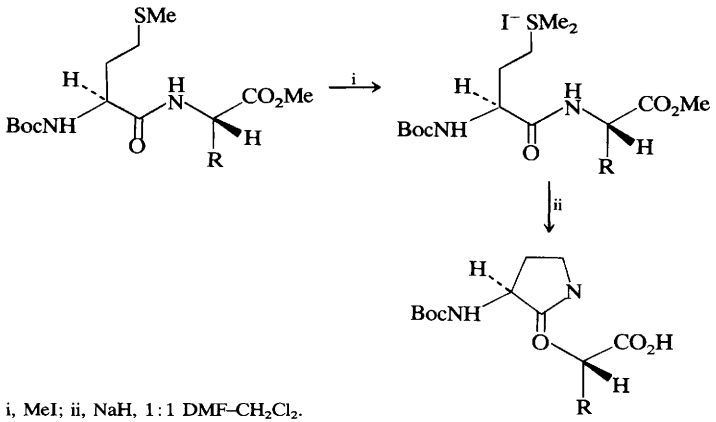
[111] J. C. Ricci, A. Bousvaros, and A. Taylor, *J. Org. Chem.*, 1982, **47**, 3063.
[112] Y. Aoyagi, T. Suguhara, T. Hasegawa, and T. Suzuki, *Agric. Biol. Chem.*, 1982, **46**, 987, 1939.
[113] E. D. Thorsett, E. E. Harris, E. R. Peterson, W. J. Greenlee, A. A. Patchett, E. M. Ulm, and T. C. Vassil, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79**, 2716.
[114] B. Lejczak, P. Kafarski, and J. Szewezyk, *Synthesis*, 1982, 412.
[115] J. Szewezyk, B. Lejczak, and P. Kafarski, *Experientia*, 1982, **38**, 983.
[116] W. J. Greenlee, *Tetrahedron Lett.*, 1982, **23**, 3995.



Reagents: i, KCN, MeOH, HOAc; ii, HCl–MeOH; iii, Amberlyst resin, MeOH, 60 °C.

Scheme 17

A design effective for generating inhibitors of angiotensin-converting enzyme has been successfully extended to inhibitors of another Zn^{II} peptidase, the metalloendopeptidase associated with porcine-kidney microsomes. These inhibitors, of the general structure (*S*)-*N*-(1-carboxy-3-phenylpropyl)-Phe-X, were most active when X = Gly, β-Ala, or γ-aminobutyric acid.[117] Z-Thioglycyl-Phe-OH has been synthesized as a thioamide analogue of Z-Gly-Phe-OH, a known substrate of carboxypeptidase A. It proved to be a weak competitive inhibitor as well as a substrate, binding with comparable affinity to Z-Gly-Phe-OH but hydrolysing only 10% as fast.[118] A number of protected lactam-bridged dipeptides have been prepared for use as conformational restraints in peptides. Five-membered rings were obtained by an intramolecular alkylation route (Scheme 18), six- and seven-membered rings by intramolecular acylation (Scheme 19). In an alternative route to a six-membered ring (Scheme 20), extensive racemization of the cysteine α-C atom occurred.[119]



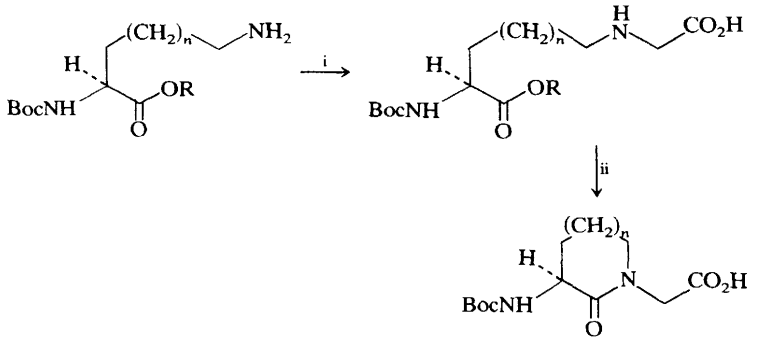
Reagents: i, MeI; ii, NaH, 1:1 DMF–CH_2Cl_2.

Scheme 18

[117] R. A. Mumford, H. Zimmerman, J. ten Broeke, D. Taub, H. Joshua, J. W. Rothrock, J. M. Hirschfield, J. P. Springer, and A. A. Patchett, *Biochem. Biophys. Res. Commun.*, 1982, **109**, 1303.

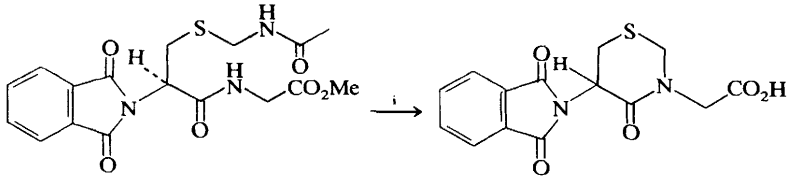
[118] P. D. Bartlett, K. L. Spear, and N. E. Jacobsen, *Biochemistry*, 1982, **21**, 1608.

[119] R. M. Friedinger, D. S. Perlow, and D. F. Veber, *J. Org. Chem.*, 1982, **47**, 104.



Reagents: i, CHO—CO_2H; ii, for $n = 1$, R = H, DMF, 55 °C, for $n = 2$, R = Me, MeCN, NEt_3.

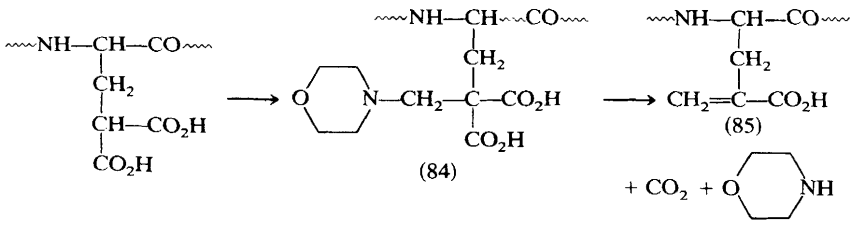
Scheme 19



Reagent: i, $(CH_2O)_x$, Tos—OH, CH_2Cl—$CHCl_2$.

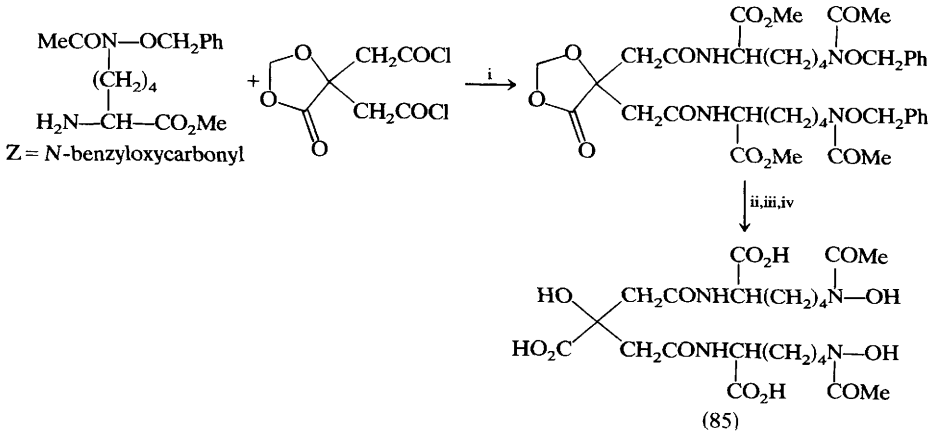
Scheme 20

A method for converting residues of γ-carboxyglutamic acid (Gla) into γ-methyleneglutamic acid (γ-MGlu) has been developed. This involves treating the peptide with a morpholine–HCHO mixture at pH 4.5 to form the Mannich base adduct (84), which fragments rapidly in 50% aqueous DMF to give the alkene derivative (85) (Scheme 21). Even Z-Gla-Gla-OMe gives a 72% yield of Z-γ-MGlu-γ-MGlu-OMe in this reaction.[120] Synthetic lysinoalanine, [N^{ε}-DL-(2-amino-2-carboxyethyl)-L-lysine], has been found to have a strong chelating ability for metals, being able to remove Zn^{II} from carboxypeptidases A and B and yeast alcohol dehydrogenase at millimolar concentration. On the



Scheme 21

[120] N. T. Boggs, H. D. Bruton, D. H. Craig, J. A. Helpern, H. C. Marsh, M. D. Pegram, D. J. Vandenbergh, K. A. Koehler, and R. G. Hiskey, *J. Org. Chem.*, 1982, **47**, 1812.



(85)

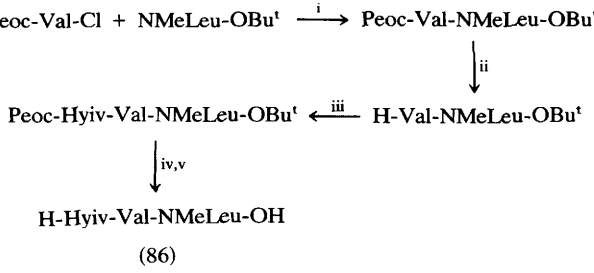
Reagents: i, NEt_3, $CHCl_3$; ii, NaOH, H_2O, THF; iii, H^+; iv, Pd–H_2.

Scheme 22

basis of models it is suggested that the LD- rather than the LL-isomer is involved.[121]

The total synthesis of aerobactin (85) has been reported (Scheme 22) in an overall yield of 26% from ε-hydroxynorleucine.[122] Although technically not a dipeptide, this linking of two amino-acid residues is of some interest.

Other Larger Linear Peptides.—2-(Triphenylphosphonio)ethoxycarbonyl (Peoc) amino-acids and hydroxy-acids are converted by treatment with oxalyl chloride into acid chlorides stable at 20 °C. These are useful activated intermediates for the generation in particular of sterically hindered amide bonds in peptides and depsipeptides. Using this method sporidesmolic acid (86), identical with that obtained from sporidesmolide, has been synthesized (Scheme 23).



(86)

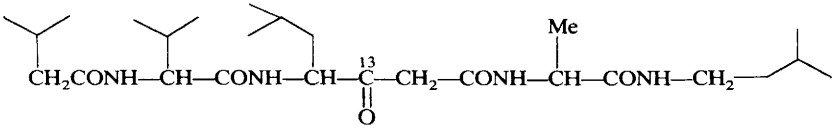
Reagents: i, pyridine, CH_2Cl_2, −20 °C; ii, $HNMe_2$, MeOH, 0 °C; iii, Peoc-Hyiv-Cl, pyridine, CH_2Cl_2, −20 °C; iv, HCl–CH_2Cl_2, 0° C; v, $HNMe_2$, MeOH, 0 °C.

Scheme 23

[121] R. Hayashi, *J. Biol. Chem.*, 1982, **257**, 13896.
[122] P. J. Maurer and M. J. Miller, *J. Am. Chem. Soc.*, 1982, **104**, 3096.

No racemization occurred during the two acid chloride couplings.[123] Homotripeptides of 3-aminobutyric acid (Abu) have been prepared. With Z-*N*-protection, 2,4,5-trichlorophenyl active esters proved the most successful of the conventional coupling methods. *N*-Benzoyl-Abu or Z-Abu_2-OH could be coupled cleanly using the oxazinones prepared from them by treatment with isobutyl chloroformate.[124] Alanine oligopeptides containing up to three amino-acid residues have been attached to the desosamine hydroxyl group of erythromycin A and the 9-amino group of 9-amino-erythromycylamine in an attempt to facilitate transport of the antibiotic into Gram-negative bacterial cells, but no activity was obtained.[125]

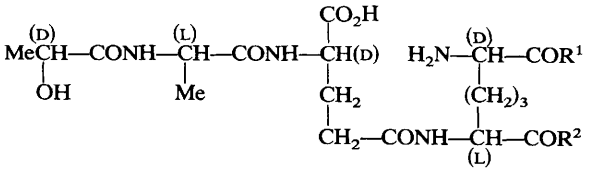
Bastadins 1-, 2-, and 3-, the novel metabolites derived from four bromotyrosine units reported last year, have been synthesized, proving that the geometry at the oxime double bonds is unambiguously *anti*.[126] Direct ^{13}C n.m.r. evidence has been obtained for a tetrahedral intermediate in the binding of the pepstatin analogue (87) to porcine pepsin; the C3 resonance



(87)

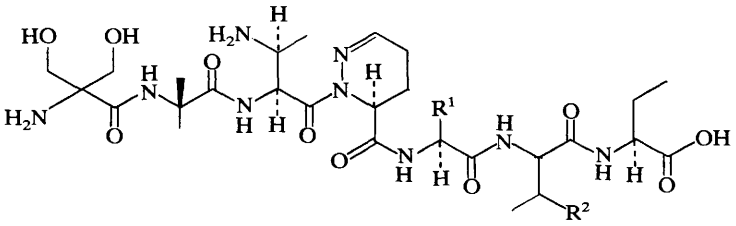
shifts 105—108 p.p.m. up-field. Oxygen is the most likely nucleophile to have attacked since only oxygen ligands or water would be close to the ketonic group of the inhibitor at the pepsin active site.[127] Biotin-containing tetrapeptides that correspond to the local sequence of biotin carboxyl carrier protein subunit of *E. coli* acetyl-CoA carboxylase have been synthesized. In biotin-containing carboxylases the prosthetic group is attached to the ε-amino moiety of a lysine residue. The synthesis therefore involved attachment of biotin to protected Met-Lys-Met-Met; the *p*-nitrophenyl ester used, however, only gave a 28—38% yield of product. ^{13}C n.m.r. spectroscopy indicated that the amide carbonyls of the model compounds interacted principally with Mg^{2+} ions rather than the sulphide side chains.[128] A second group has sequenced the new immunostimulant FK-156 produced by *Streptomyces olivaceogriseus violaceus*, which contains four amino-acids and one hydroxy acid (88a).[129] Its structure has been proved by synthesis; an isomer (88b) also prepared had a much lower biological activity than FK-156.[130]

[123] H. Kunz and H.-H. Bechtolsheimer, *Liebigs Ann. Chem.*, 1982, 2068; *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 630.
[124] C. N. C. Drey and E. Mtetwa, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1587.
[125] R. A. Lemahiev, D. Pruess, and M. Carson, *J. Antibiot.*, 1982, **85**, 1063.
[126] S. Nishiyama and S. Yamamura, *Tetrahedron Lett.*, 1982, **23**, 12381.
[127] D. H. Rich, M. S. Bernatowicz, and P. G. Schmidt, *J. Am. Chem. Soc.*, 1982, **104**, 3535.
[128] H. Kondo, F. Moriuchi, and J. Sunamoto, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 1579.
[129] Y. Kawai, K. Nakahara, T. Gotoh, I. Uchida, H. Tonaka, and H. Imanaka, *J. Antibiot.*, 1982, **35**, 1293.
[130] K. Hemmi, M. Aratani, H. Takeno, S. Okada, Y. Miyazaki, O. Nakaguchi, Y. Kitawa, and M. Hoshimoto, *J. Antibiot.*, 1982, **35**, 1300; K. Hemmi, H. Takeno, S. Okada, O. Nakaguchi, Y. Kitawa, and M. Hoshimoto, *Tetrahedron Lett.*, 1982, **23**, 693.



(88) a; $R^1 = OH$, $R^2 = -NHCH_2CO_2H$
b; $R^1 = -NHCH_2CO_2H$, $R^2 = OH$

The structure of the toxic octapeptide lophyrotomin from the larva of the sawfly *Lophyrotoma interrupta* has been determined to be PhCO-D-Ala-D-Phe-Val-Ile-D-Asp-Asp-D-Glu-Gln-OH by f.a.b.–m.s. The presence of glutamine rather than isoglutamine was determined by the detection of 2,4-diaminobutyric acid on amino-acid analysis after carrying out a Hofmann degradation with [bis(trifluoroacetoxy)iodo]benzene. The absolute configuration of the constituent amino-acids was found by g.l.c. on a chiral column of their *N*-Tfa isopropyl esters. The unprecedented occurrence of four D-amino-acid residues in an animal peptide leads to the suspicion that it may originate from simpler organisms.[131] The structure of antrimycin A from *Streptomyces xanthocidicus* was reported in 1981; the structures of several minor congeners (89) have now been elucidated.[132] Cirratiomycins A and B from *Streptomyces cirratus* have proved to be identical to antrimycins D and D_v, respectively.[133]



Antrimycin	R^1	R^2
A_v	Me	Me
(*cf.* A	Me	Et)
B_v	Et	Me
B	Et	Et
C_v	Pr	Me
C	Pr	Et
D_v	Bu^i	Me
D	Bu^i	Et

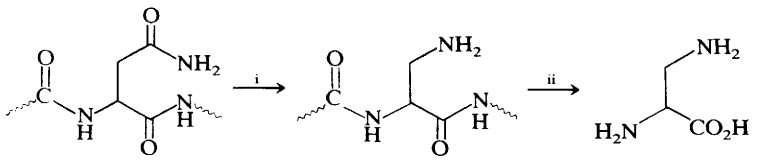
(89)

[131] D. H. Williams, S. Santicorn, P. B. Oelrichs, F. De Angelis, J. K. Macleod, and R. J. Smith, *J. Chem. Soc., Chem. Commun.*, 1982, 1394.
[132] K. Morimoto, N. Shimada, H. Naganawa, T. Takita, H. Umegawa, and H. Kambara, *J. Antibiot.*, 1982, **35**, 378.
[133] T. Shiroza, N. Ebisawa, K. Furihata, T. Endo, H. Seto, and N. Otake, *Agric. Biol. Chem.*, 1982, **46**, 865, 1891.

One approach to pinpointing the exact binding sites of the gramicidin A channel is to label individually the peptide carbonyls with ^{13}C-enriched amino-acids and then to study the cation interaction after incorporating these products into phospholipid structures. As a start on such a programme [^{13}C-D-Leu^{12,14}]-gramicidin A has been prepared by a solid-phase synthesis. The [15]-peptide was removed from the support with ethanolamine, the *N*-Boc group then being removed and replaced by a formyl group. The single channel conductance of the synthetic product in lipid bilayer membranes was indistinguishable from the natural compound, as were its c.d. and h.p.l.c. characteristics.[134]

6 Glycopeptides

Glycopeptide Antibiotics.—The structure proposed for vancomycin has been revised. Vancomycin itself has not given crystals suitable for *X*-ray, but such analysis has been made on a product (CPD-I) obtained by heating the antibiotic at pH 4—5. The difference between CDP-I and its parent was previously assumed to be the presence of an isoasparaginyl residue in the latter and an isoaspartyl residue in the former. It has now been found that a Hofmann-type oxidative degradation of *O*-methylated aglycovancomycin gives 2,3-diaminopropionic acid, which can arise from asparagine but not isoasparagine (Scheme 24). Possible routes for the rearrangement of asparagine to

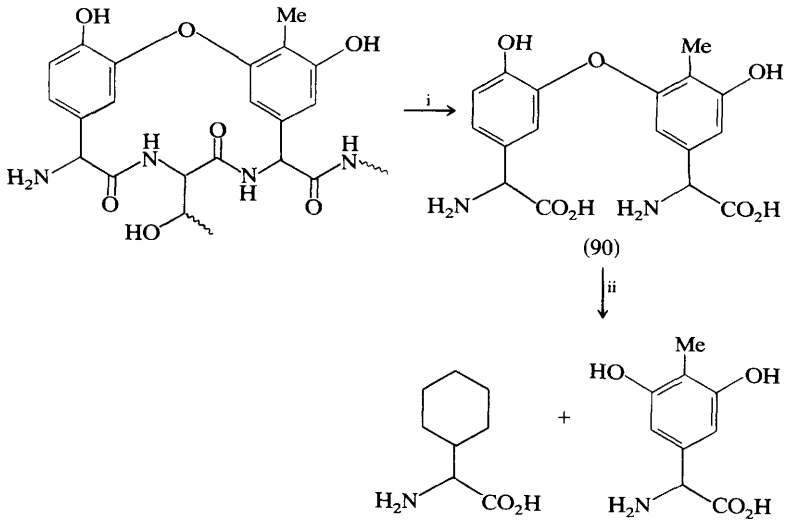


Reagents: i, $PhI(OAc)_2$; ii, H^+/H_2O.

Scheme 24

isoaspartic acid are discussed.[135] A change of configuration at residue 1 in ristocetin A is also proposed. The (*S*)-configuration deduced on n.m.r. evidence is at odds with the (*R*)-configuration assigned to residue 1 of ristomycin A, which is now known to be identical with ristocetin A. After removal of the sugars from ristocetin A, the peptide was hydrolysed and ristomycinic acid (90) isolated. Degradation of the single stereoisomer obtained by hydrogenolysis gave (*R*)-cyclohexylglycine and an (*S*)-amino-acid (Scheme 25), indicating residue 1 to have an *R*-configuration.[136] In the biosynthesis of vancomycin by *Streptomyces orientalis* it has been shown that di-*m*-hydroxyphenylglycine can be derived from acetic acid, whereas *p*-hydroxyphenylglycine and *m*-chloro-β-hydroxytyrosine are derived from tyrosine.[137]

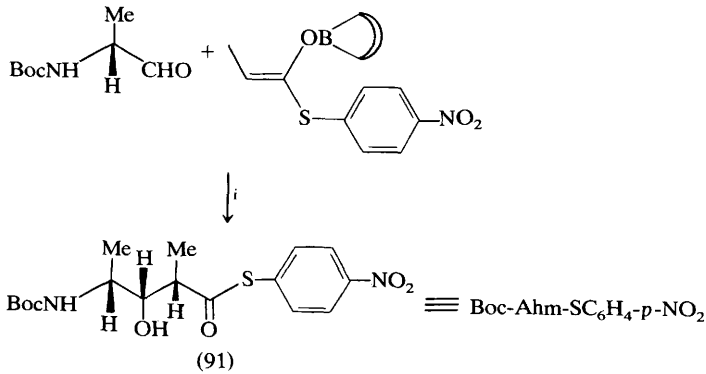
[134] K. V. Prasad, T. L. Trapane, D. Busath, G. Szabo, and D. W. Urry, *Int. J. Pept. Protein Res.*, 1982, **19**, 162.
[135] C. M. Harris and T. M. Harris, *J. Am. Chem. Soc.*, 1982, **104**, 4293.
[136] C. M. Harris and T. M. Harris, *J. Am. Chem. Soc.*, 1982, **104**, 363.
[137] S. J. Hammond, M. P. Williamson, D. H. Williams, L. D. Boeck, and G. G. Marconi, *J. Chem. Soc., Chem. Commun.*, 1982, 344.



Reagents: i, 2M HCl; ii, PtO_2, H_2.

Scheme 25

In last year's report the total synthesis of deglycobleomycin A_2 was reported. Two total syntheses of bleomycin A_2 itself have now been recorded.[138,139] Because attempts to glycosylate a protected deglycobleomycin A_2 have been unsuccessful,[138] this compound was not an intermediate in either synthesis. In one synthesis a (2*S*, 3*S*, 4*R*)-4-amino-3-hydroxypentanoic acid derivative (91) was prepared stereoselectively through an aldol condensation in 64% yield (Scheme 26).[140] This thiolester was then coupled with the *C*-terminal fragment



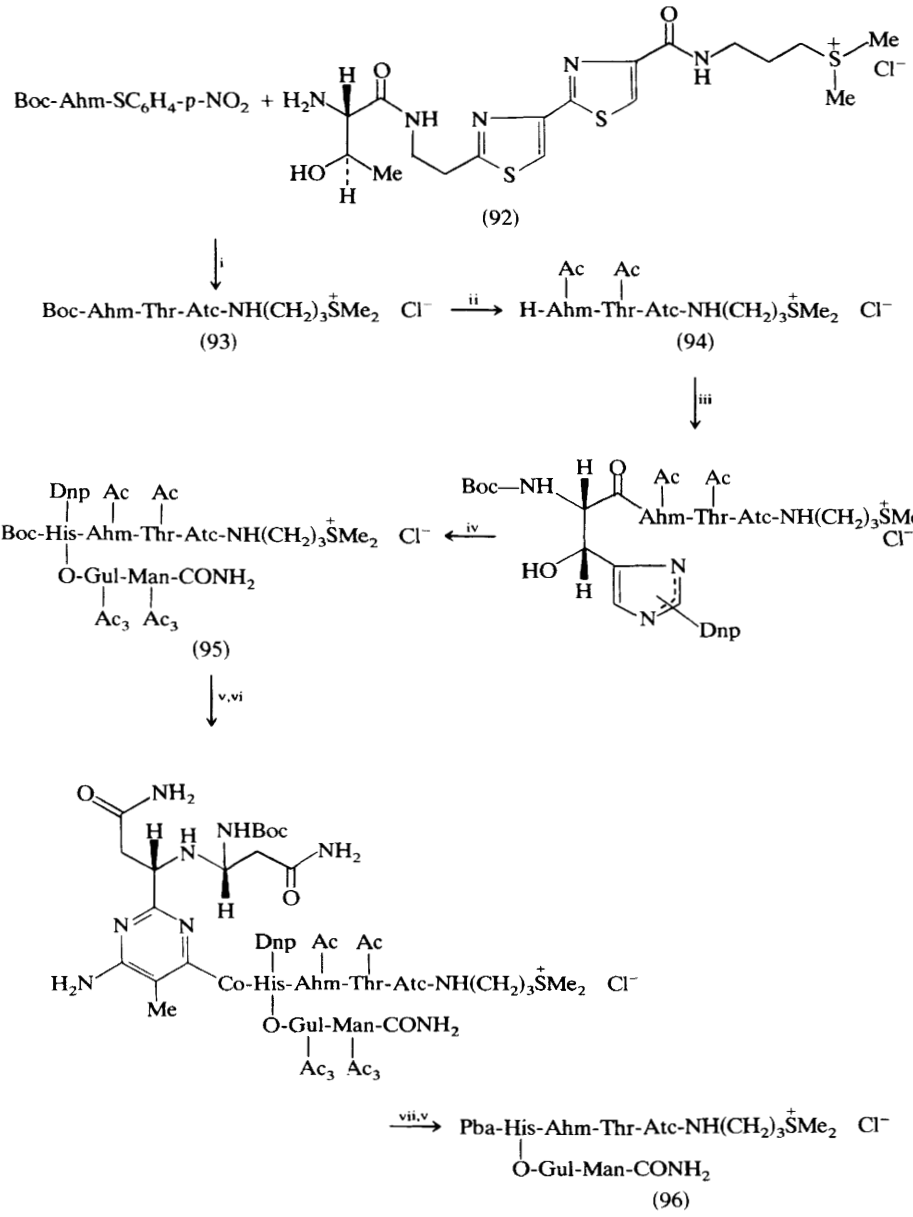
Reagent: i, DMF, 20 °C.

Scheme 26

[138] T. Takita, Y. Umezawa, S. Aito, H. Morishima, H. Naganawa, M. Umezawa, T. Tsuchiya, T. Miyake, S. Kageyama, S. Umezawa, Y. Muraoka, M. Suzuki, M. Otsuka, M. Narita, S. Kobayasi, and M. Ohno, *Tetrahedron Lett.*, 1982, **23,** 521.

[139] Y. Aoyagi, K. Katano, H. Suguna, J. Primeau, L.-H. Chang, and S. M. Hecht, *J. Am. Chem. Soc.*, 1982, **104,** 5537.

[140] M. Narita, M. Otsuka, S. Kobayoshi, M. Ohno, Y. Umezawa, H. Morishima, S. Saito, T. Takita, and H. Umezawa, *Tetrahedron Lett.*, 1982, **23,** 525.

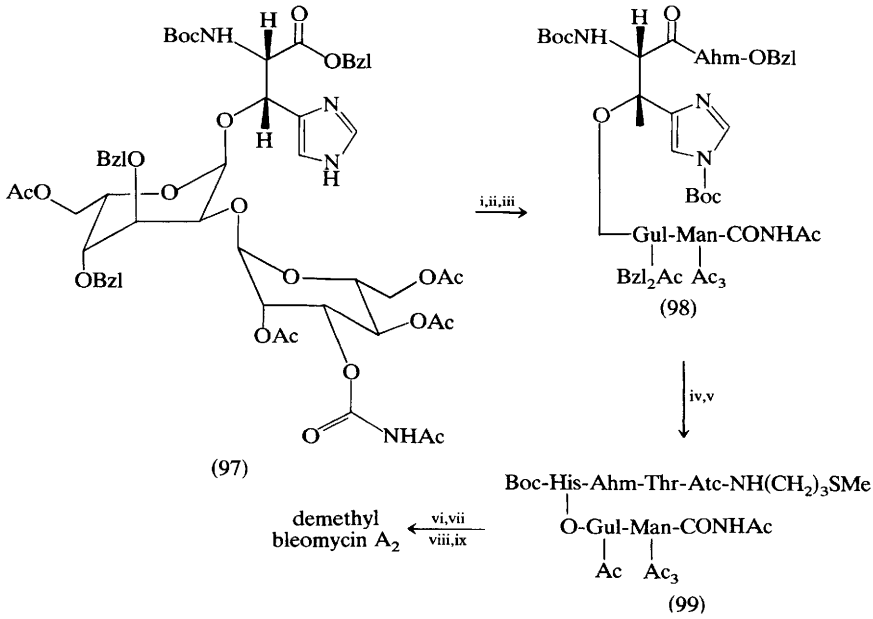


Reagents: DMF, 20 °C; ii, MeCOCl, 6M HCl–HOAc, 20 °C; iii, N^{α}-Boc-N^{im}-Dnp-L-*erythro*-β-hydroxyhistidine, *NN'*-dicyclohexylcarbodi-imide (DCC), 1-hydroxybenzotriazole (HOBt); iv, Br-Gul(Ac_3)-Man(Ac_3)-$CONH_2$, $Hg(CN)_2$, sulpholane, molecular sieve 4A, 40 °C; v, CF_3CO_2H; vi, Boc-pyridoblamic acid, DCC, HOBt; vii, 0.1M NaOH–MeOH (1:1), 20 °C.

Scheme 27

(92) (Scheme 27). The resulting tripeptide (93) was *N*-deprotected and the hydroxyl groups were protected by acetylation to give (94) before addition of the β-hydroxyhistidine derivative. The disaccharide unit was then added to give (95). After deprotection, Boc-pyridoblamic acid was coupled. Removal of all remaining protecting groups then gave bleomycin A_2 (96). Compound (95) was not purified owing to its instability. The mixture of compounds resulting was not separated until after the final deprotection, when bleomycin A_2 was the minor product.[138]

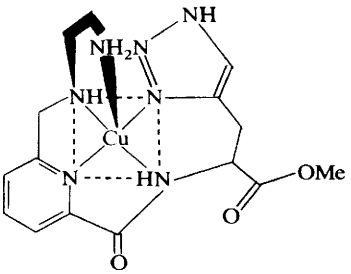
In the second synthesis a protected disaccharide unit was coupled to $N^{\alpha}N^{im}$-diBoc-L-*erythro*-β-hydroxyhistidine benzyl ester to give (97), the N^{im}-Boc being lost in this reaction. After some adjustment of protecting groups (Scheme 28), the 4-amino-3-hydroxy-2-methylvaleric acid residue was added as its benzyl ester. All the benzyl groups of the product (98) were cleaved by hydrogenolysis, a procedure that also solvolysed the N^{im}-Boc group, and the product was coupled with the dipeptide Thr-Atc-NH$(CH_2)_3$SMe to give a product (99) differing from intermediate (95) in the first synthesis only in its pattern of protecting groups and the presence of the sulphide rather than the sulphonium salt. After the final coupling to link the pyridoblamic acid residue, the Boc and acetyl groups were removed before the final alkylation of the sulphide to give bleomycin A_2.[139]



Reagents: i, $(Me_3CO)_2CO$, pyridine, 1 h, 25 °C; ii, Pd/H_2, EtOAc; iii, $H_2NCHMeCHOHCHMeCO_2Bzl$, DCC, HOBt; iv, Pd Black, H_2, 55 °C, EtOH; v. H-Thr-Atc-NH$(CH_2)_3$SMe, DCC, HOBt; vi, CF_3CO_2H, Me_2S, 0 °C, 1 h; vii, Boc-Pba-OH, diphenylphosphoryl azide, DMF; viii, 0.1 M NaOH, 0 °C, 22 h; ix, Me_2S, CF_3CO_2H, 0 °C, 1 h.

Scheme 28

The material prepared in both syntheses proved identical to natural bleomycin A_2 in all respects. The synthesis of deglycobleomycin A_2 reported last year has also been improved,[141] and an independent synthesis has also been described, whose product, like bleomycin A_2 itself, proved capable of transferring oxygen in a fashion reminiscent of cytochrome P450 and also of effecting DNA degradation *via* aerobic or anaerobic activation.[142] Both Fe^{III} and Cu^{II} complexes of bleomycin (BLM) (but not *N*-Ac-BLM–Fe^{III}) mediate the transfer of oxygen from iodosobenzene to *cis*-stilbene, forming the epoxide. In both the presence and absence of oxygen, BLM–Cu^{II}–PhIO degrades DNA. These findings provide a new assay system for the study of bleomycin analogues and extend the analogy between bleomycin and cytochrome P450.[143] Comparative ^1H n.m.r. studies of bleomycin and deglycobleomycin B_2 complexes suggest that all metal complexes of bleomycin capable of DNA degradation need not have the same geometry.[144] A simple analogue of bleomycin has been synthesized; the e.s.r. spectral parameters of its Cu^{II} complex (100) correspond with the characteristics of the Cu^{II}–bleomycin system.[145]



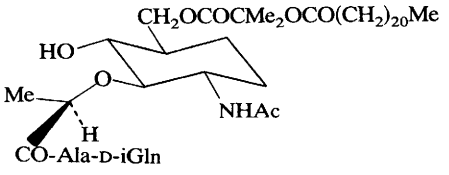
(100)

Other Glycopeptides.—Synthetic acetylmuramyl-Ala-D-iGln (muramyl dipeptide, MDP) and its lysyl derivative have been found on intraventricular administration to mimic the somnogenic effects of a natural muramyl peptide that induces slow-wave sleep in animals. Both compounds are also pyrogenic, which may modify the response. The somnogenic effect probably has been overlooked previously because animals need to be studied in an environment free from disturbance for its effects to be observed.[146] A ^1H n.m.r. examination of MDP confirms an earlier report of an anomeric equilibrium ratio of 2:1 for $\alpha:\beta$. The alanine amide group is intramolecularly H-bonded, probably to the

[141] S. Saito, Y. Umezawa, H. Morishima, T. Takita, H. Umezawa, M. Narita, M. Otsuka, S. Kobayashi, and M. Ohno, *Tetrahedron Lett.*, 1982, **23,** 529.
[142] Y. Aoyagi, H. Suguna, N. Murugesan, G. M. Ehrenfeld, L.-H. Chang, T. Ohgi, M. S. Shekhani, M. P. Kirkup, and S. M. Hecht, *J. Am. Chem. Soc.*, 1982, **104,** 5237.
[143] N. Murugesan, G. M. Ehrenfeld, and S. M. Hecht, *J. Biol. Chem.*, 1982, **257,** 8600.
[144] N. J. Oppenheimer, C. Chang, L.-H. Chang, G. M. Ehrenfeld, L. O. Rodriguez, and S. M. Hecht, *J. Biol. Chem.*, 1982, **257,** 1606.
[145] J.-P. Hénichart, R. Houssin, J.-L. Bernier, and J.-P. Catteau, *J. Chem. Soc., Chem. Commun.*, 1982, 1295.
[146] J. M. Kruegar, J. R. Pappenheimer, and M. L. Karnovsky, *Proc. Natl. Acad. Sci. U.S.A.*, 1982, **79,** 6102.

side-chain carboxyl of isoglutamine.[147] A sleep-promoting small glycopeptide, Factor S, has been isolated from human urine. It contains Glu, Ala, diaminopimelic acid, and muramic acid in molar ratios 2:2:1:1, but their mode of linkage is not yet known. Factor S induces slow-wave sleep in rabbits that resembles the deep sleep occurring when animals are allowed to sleep following prolonged sleep deprivation.[148]

Analogues of MDP containing modifications of the carbohydrate moiety or replacements for it continue to attract synthetic interest. Of the modified forms, the long-chain derivative (101) causes an antibody response greater than that of MDP;[149] two disaccharide derivatives, (102) and (103), also have high immunoadjuvant activity.[150] On the other hand galacto-MDP, allomuramyl-MDP, and norallomuramyl-MDP have been prepared but increase the amount of circulating antibodies to a smaller degree than MDP itself.[151] Of a number of long-chain fatty acylamino analogues of MDP, all as active as MDP itself, *N*-(3-hydroxytetradecyloctadecanoylamino)muramyl-Ala-D-iGln was one of four compounds that also showed strong anti-tumour activity.[152]



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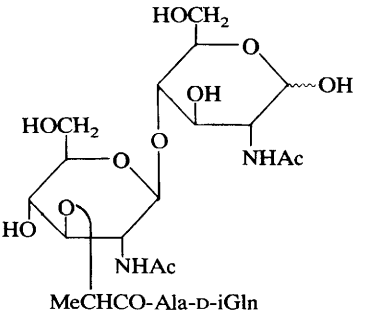

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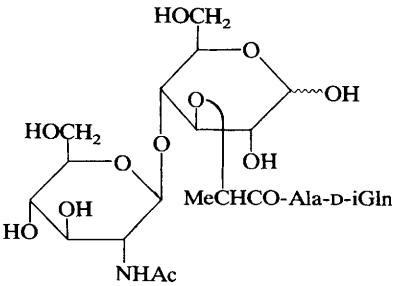

(103)

[147] B. E. Chapman, M. Batley, and J. W. Redmond, *Aust. J. Chem.*, 1982, **35,** 489.
[148] J. M. Krueger, J. R. Pappenheimer, and M. L. Karnovsky, *J. Biol. Chem.*, 1982, **257,** 1664.
[149] P. L. Durette, C. P. Dorn, T. Y. Shen, and A. Friedmann, *Carbohydrate Res.*, 1982, **108,** 139.
[150] M. Kiso, Y. Kaneda, R. Shimizu, A. Hasegawa, and I. Izuma, *Carbohydrate Res.*, 1982, **104,** 253.
[151] M. Zaoral, J. Jezek, and J. Rotta, *Collect. Czech. Chem. Commun.*, 1982, **47,** 2989.
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5

Metal Complexes of Amino-acids, Peptides, and Proteins

BY R. W. HAY AND K. B. NOLAN

1 Introduction

This chapter describes work published during the years 1981 and 1982. As before, we have attempted to satisfy the requirements of chemists with different interests in the field. Biological inorganic chemistry is a substantial growth area, and many more inorganic chemists are becoming interested in the interaction of metal ions with biologically important ligands such as amino-acids, peptides, and proteins. An annual review of inorganic biochemistry is available.[1]

Books of interest include the following: 'Biochemistry of Nonheme Iron' (transferrins, ferritins, iron–sulphur proteins, and miscellaneous non-haem iron proteins such as oxygenases),[2] 'Inorganic Chemistry in Biology and Medicine' (radiopharmaceuticals, carcinogenesis and anti-cancer activity of metal complexes, ligand design and chelation, therapy for iron, copper, and other metal-overload conditions, ionophores, and trace metals),[3] Volume 6 of 'Chemistry and Biochemistry of Amino Acids, Peptides and Proteins', which contains a chapter on peptide and protein complexes of transition metals as modulators of cell growth,[4] and Volumes 13 and 14 of Sigel's 'Metal Ions in Biological Systems', which have now been published (Volume 13 deals with copper proteins and Volume 14 with inorganic drugs in deficiency and disease).[5]

Sigel and Martin[6] have produced a useful review on the co-ordinating properties of the amide bond, dealing with the stability and structure of metal complexes of peptides and related ligands. Other reviews of interest have been made on the following: metal-tolerant plants,[7] metal clusters in biology and attempts to model the catalytic site of nitrogenase,[8] the co-ordination chemis-

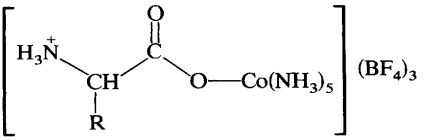
[1] 'Inorganic Biochemistry', ed. H. A. O. Hill (Specialist Periodical Reports), The Chemical Society, London, 1979, Vol. 1; The Royal Society of Chemistry, London, 1981, Vol. 2.
[2] A. Bezhorovainy, 'Biochemistry of Nonheme Iron', Plenum Press, New York, 1980.
[3] 'Inorganic Chemistry in Biology and Medicine', Am. Chem. Soc. Symp. Ser., No. 140, ed. A. E. Martell, American Chemical Society, Washington, D.C., 1980.
[4] 'Chemistry and Biochemistry of Amino Acids, Peptides and Proteins', ed. B. Weinstein, Marcel Dekker, Basel, 1982.
[5] 'Metal Ions in Biological Systems', ed. H. Sigel, Marcel Dekker, Basel, 1981, Vol. 13; 1982, Vol. 14.
[6] H. Sigel and R. B. Martin, *Chem. Rev.*, 1982, **82**, 385.
[7] M. E. Farago, *Coord. Chem. Rev.*, 1981, **36**, 155.
[8] R. H. Holm, *Chem. Soc. Rev.*, 1981, **10**, 455.

try of bleomycin,[9] and the photochemical properties of copper complexes, including amino-acid and peptide complexes.[10]

Books have appeared dealing with the following topics: iron–sulphur proteins,[11] copper proteins,[12] metals in biochemistry,[13] molybdenum chemistry of biological significance,[14] and invertebrate oxygen-binding proteins.[15]

2 Amino-acids

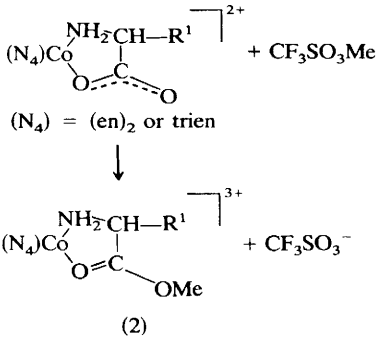
Synthetic and Spectroscopic Studies.—A number of interesting papers have appeared dealing with the use of cobalt(III) complexes in peptide synthesis. The penta-amminecobalt(III) moiety is a useful *C*-terminal protecting group for sequential peptide synthesis.[16] Complexes of the type $[Co(AA)_1]$ (1) can be readily prepared. The reaction of these complexes with Boc-amino-acid active esters or Boc-symmetric anhydrides results in the formation of $[Co(AA)_1(AA)_2Boc]$. The Boc group is removed with 95% CF_3CO_2H to give $[Co(AA)_1(AA)_2]$, which can be used for further sequential peptide synthesis. The $[(NH_3)_5Co^{III}]$ group is selectively removed by rapid reduction with $NaBH_4$ or NaSH. The syntheses of Leu_5-enkephalin, Met_5-enkephalin, and the hexapeptide His-Gly-His-Gly-His-Gly are described.



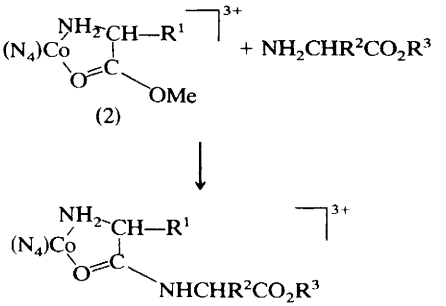
(1)

The use of cobalt(III) as both an *N*-protecting and activating group has been recognized for some time.[17] A viable peptide synthesis using the reactions shown in Schemes 1 and 2 has been developed.[18] Chelated amino-acids can be alkylated in dry trimethylphosphate with CF_3SO_3Me to give high yields of the chelated amino-acid esters (2). The coupling reaction (Scheme 2) is rapid in dry Me_2SO, acetonitrile, or methanol. The method has been applied to the synthesis of tetrapeptides and Leu_5-enkephalin.[19]

[9] J. C. Dabrowiak, *J. Inorg. Biochem.*, 1980, **13,** 317.
[10] G. Ferraudi and S. Mualidharan, *Coord. Chem. Rev.*, 1981, **36,** 45.
[11] 'Iron–Sulphur Proteins', ed. T. G. Spiro, John Wiley, Chichester, 1982.
[12] 'Copper Proteins', ed. T. G. Spiro, John Wiley, Chichester, 1981.
[13] 'Metals in Biochemistry', P. M. Harrison and R. J. Hoare, Chapman Hall, London, 1980.
[14] W. E. Newton and S. Otsuka, 'Molybdenum Chemistry of Biological Significance', Plenum, New York, 1980.
[15] 'Invertebrate Oxygen-Binding Proteins, Structure, Active Site and Function', ed. J. N. Lamy and J. Lamy, Marcel Dekker, New York, 1981.
[16] S. S. Isied, A. Vassilian, and J. M. Lyon, *J. Am. Chem. Soc.*, 1982, **104,** 3910.
[17] For a review see R. W. Hay and P. J. Morris, 'Metal Ions in Biological Systems', ed. H. Sigel, Marcel Dekker, New York, 1976, Vol. 5.
[18] C. R. Clark, R. F. Tasker, D. A. Buckingham, D. R. Knighton, D. R. I. Harding, and W. S. Hancock, *J. Am. Chem. Soc.*, 1981, **103,** 7023.
[19] D. R. Knighton, D. R. K. Harding, M. J. Friars, W. S. Hancock, G. D. Reynolds, C. R. Clark, R. F. Tasker, and D. A. Buckingham, *J. Am. Chem. Soc.*, 1981, **103,** 7025.



Scheme 1

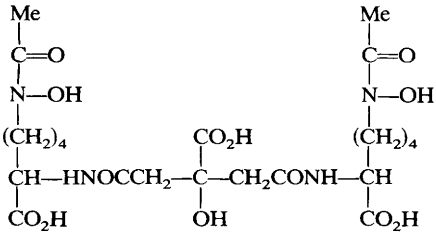


Scheme 2

Several complexes of amino-acids, $[Co(en)_2Gly]^{2+}$, $[Co(tren)(AA)]^{2+}$, *trans*-$[Pt(Gly)_2]$, and $[Mo(\eta_5\text{-}C_5H_5)_2(Gly)]^+$, have been esterified either by alkylation with dimethyl sulphate or methyl toluene-*p*-sulphonate or by acid-catalysed esterification in methanol.[20] Dipeptide complexes of the type [Co(tren)-(dipeptideOR)]$(p\text{-}MeC_6H_4SO_3)_3$ have been prepared[21] by the coupling of a chelated ester complex with a free amino-acid ester. The rate of the reaction appears to be quite sensitive to steric hindrance. With chiral amino-acids the product is a mixture of diastereoisomers, a result consistent with a rapid racemization coupled with a slight asymmetric induction. The half-life for proton exchange in the complex $[Co(en)_2(GlyOMe)]^{3+}$ under the conditions of peptide synthesis is 20 s. The hydrolysis of this ester in methanol (1 mol dm^{-3} in H_2O) is nevertheless faster than racemization.

The synthesis of the natural iron(III) ionophore (−)-aerobactin (3) and of its constituent amino-acid N^6-acetyl-N^6-hydroxylysine has been described.[22] Trinuclear oxo-bridged iron(III)–L-amino-acid perchlorates (4) derived from a variety of L-amino-acids have been prepared, and their spectral and magnetic properties have been studied.[23] These complexes provide a good model for the ferritin iron core. The preparation and properties of the nitrate salts have also

[20] H. Wautier, V. Daffe, M.-N. Smets, and J. Fastrez, *J. Chem. Soc., Dalton Trans.*, 1981, 2479.
[21] H. Wautier, D. Marchal, and J. Fastrez, *J. Chem. Soc., Dalton Trans.*, 1981, 2484.
[22] P. J. Maurer and M. J. Miller, *J. Am. Chem. Soc.*, 1982, **104**, 3096.
[23] R. N. Puri, R. O. Asplund, and S. L. Holt, *J. Coord. Chem.*, 1981, **11**, 125.



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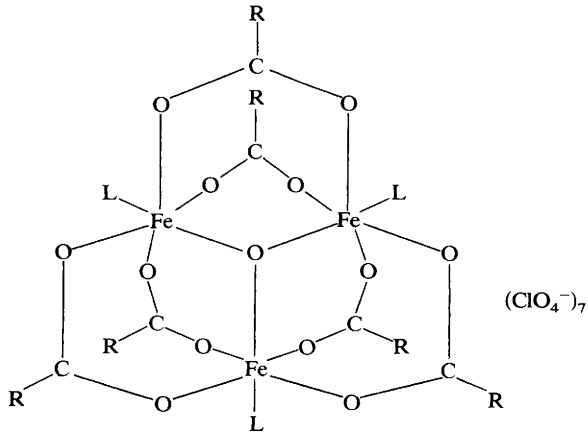
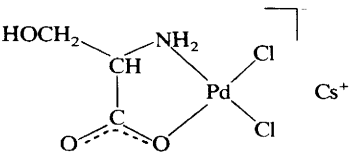


(4) L = H_2O

been described.[24] Further synthetic studies have dealt with the $[Fe_3O(\text{L-amino-acid})_3(H_2PO_3)_3(H_2O)_3](NO_3)_4$ complexes, which also contain the $[Fe_3O]^{7+}$ unit.[25] The presence of the phosphorus-containing ligand has a minimal effect on the spectral and magnetic properties of iron(III)–L-amino-acid complexes containing the $[Fe_3O]^{7+}$ unit. Similar observations have been made with ferritin. Characterization of dinuclear dialkoxo-bridged iron(III)–L-proline complexes has also been described.[26] These complexes were obtained by thermal, photolytic, and chemoreductive rearrangement of trinuclear oxo-bridged iron(III)–L-proline complexes. Circular dichroic spectra of the $[Fe_3O(\text{L-amino-acid})_6(H_2O)_6](ClO_4)_7$ complexes have been investigated in detail.[27]

The discovery of *cis*-$[Pt(NH_3)_2Cl_2]$ as an effective anti-tumour drug has led to considerable interest in the preparation of Pt^{II} and Pd^{II} complexes of amino-acids. Several platinum complexes can induce filamentous growth in *Escherichia coli*,[28–30] but similar effects appeared to be lacking with Pd^{II}. It has

[24] R. N. Puri and R. O. Asplund, *Inorg. Chim. Acta*, 1981, **54,** L187.
[25] R. N. Puri and R. O. Asplund, *Inorg. Chim. Acta*, 1982, **66,** 49.
[26] R. N. Puri and R. O. Asplund, *J. Coord. Chem.*, 1981, **11,** 73.
[27] R. N. Puri, R. O. Asplund, and W. F. Tucker, *Inorg. Chim. Acta*, 1982, **66,** 7.
[28] B. Rosenberg, L. Van Camp, and T. Krigas, *Nature (London)*, 1965, **203,** 698.
[29] B. Rosenberg, *Platinum Met. Rev.*, 1971, **15,** 42.
[30] M. J. Cleare, 'Recent Results Cancer Res.', Springer-Verlag, Berlin, 1974, Vol. 48, p. 12.



(5)

now been shown[31] that caesium *cis*-dichloro-L-serinato-palladate(II) (5) induces filamentous growth in *E. coli*. The syntheses of *cis*-[M(thioproline)$_2$X$_2$] complexes (M = Pt^{II} or Pd^{II}, X = Cl, Br, or I) and some thioproline salts of Pt^{IV}, Rh^{III}, and Au^{III} have been described and their cytostatic activity has been studied.[32] Six platinum complexes of the type [Pt(bpy)(amino-acid)]Cl (amino-acid = Gly, Ala, Leu, Ser, Cys, and Met) have been prepared.[33] All of them, with the exception of the leucine derivative, display anti-viral activity against tobacco mosaic virus.

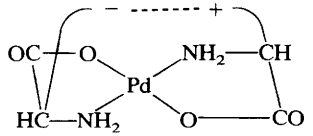
The Pt^{II} and Pd^{II} complexes of *S*-2-aminoethyl-L-cysteine and *S*-2-aminoethyl-DL-penicillamine have been characterized.[34] The amino-acids behave as bidentate ligands, *via* the sulphur atom and the amino group lying closest to the carboxyl. The complexes are being tested for anti-tumour activity. The reaction of cisplatin, *cis*-[Pt(NH$_3$)$_2$Cl$_2$], with cysteine and glutathione has been studied[35] as a possible model for protein binding. Solid derivatives were characterized.

The reaction of L-glutamine with K_2PtCl_4 has been shown[36] to give a blue paramagnetic complex and a greenish-white diamagnetic species. Such 'blue' platinum complexes often have enhanced anti-tumour activity. The activity of cisplatin is believed to be due to specific interactions with the purine and pyrimidine bases of DNA. Pyrimidines react with cisplatin to give the so-called Pyrimidine Blues.

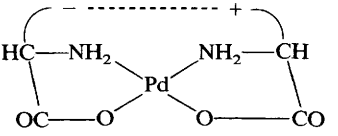
The synthesis and characterization of mixed-ligand complexes of Pt^{II} with amino-acids as primary ligands and purine and pyrimidine bases as secondary lignads have been reported.[37] In these complexes adenine and guanine are co-ordinated to the metal ion *via* N-7 whereas hypoxanthine acts as a bridging ligand *via* N-3 and N-9. The pyrimidines are co-ordinated to the metal *via* N-3. The amino-acids are monodentate *via* the α-amino group.

Synthetic and spectroscopic studies have been carried out on electrostatic ligand–ligand interactions in ternary amino-acid–palladium(II) complexes containing an acidic amino-acid (A) and a basic amino-acid (B).[38] The ternary complexes Pd(A)(L-B)(H) [A = L- or D-aspartate, L- or D-glutamate, or L-cysteinate, B = argininate with a proton (H) attached to the basic side group]

[31] A. J. Charlson, N. T. McArdle, and E. C. Watton, *Inorg. Chim. Acta*, 1981, **56**, L35.
[32] D. G. Craciunescu, A. Doadrio, A. Furlani, and V. Scarcia, *Inorg. Chim. Acta*, 1982, **67**, L11.
[33] L. Kumar, N. R. Kandasamy, and T. S. Srivastava, *Inorg. Chim. Acta*, 1982, **67**, 139.
[34] S. Mylonas, A. Valavanidis, V. Voukouvalidis, and M. Polyssiou, *Inorg. Chim. Acta*, 1982, **66**, 25.
[35] B. Odenheimer and W. Wolf, *Inorg. Chim. Acta*, 1982, **66**, L41.
[36] P. Arrizabalaga, P. Castan, J.-P. Laurent, and A. J. Charlson, *Inorg. Chim. Acta*, 1982, **66**, L9.
[37] B. T. Khan, S. V. Kumari, and G. N. Goud, *J. Coord. Chem.*, 1982, **12**, 19.
[38] O. Yamauchi and A. Odani, *J. Am. Chem. Soc.*, 1981, **103**, 391.



(6) Pd(L-A)(L-B)(H)



(7) Pd(D-A)(L-B)(H)

have been characterized and are illustrated in (6) and (7). Preferential incorporation of an enantiomer of DL-aspartic acid and DL-arginine is observed in ternary complex formation with optically pure arginine and aspartic acid, respectively. The stereoselectivity arises owing to ligand–ligand interaction. Side-chain motions in such ternary complexes have been measured by the use of ^{13}C spin–lattice relaxation times.[39] Ternary amino-acid–palladium(II) complexes that involve ligand–ligand hydrogen bonding have also been studied,[40] and the complexes $[Pd(L\text{-His})(D\text{-His})]\cdot H_2O$, $[Pd(L\text{-Asn})(L\text{-His})]\cdot 2H_2O$, and $[Pd(L\text{-Gln})(L\text{-His})]\cdot 2H_2O$ have been characterized. A further interesting paper in this area has dealt with the proximity of metal ions and hydrocarbon side chains of chelated α-amino-acids and peptides in Pd^{II} complexes.[41]

The Pt^{II} and Pd^{II} complexes of DL-phenylglycine, DL-phenylglycine methyl ester, and DL-phenylglycine benzyl ester have been prepared.[42] The amino-acid derivatives behave as unidentate ligands co-ordinating *via* the amino group. The formation of a metal–thioether sulphur bond results in a new chiral centre on sulphur. Studies have now been reported on the c.d. spectra of palladium(II)–methionine complexes and methionine-containing peptides.[43]

A dimethylplatinum(IV) complex containing deprotonated glycinate as an amido-bridging ligand, $[PtMe_2(OH)(\mu\text{-}NHCH_2CO_2)]_2^{2-}$, has been characterized.[44] Platinum(IV) complexes of EDTA have also been studied in detail, and three isomers of $[Pt(EDTAH_2)Cl_2]$, with the ligand tetradentate, have been isolated.[45]

Metal thiolate cluster compounds of composition $[M^I{}_8M^{II}{}_6L_{12}Cl]^{5-}$ have been prepared $\{M^I = Cu^I$ or Ag^I, $M^{II} = Cu^{II}$, Ni^{II}, or Pd^{II}, L = deprotonated penicillamine $[SCMe_2CH(NH_2)COO]^{2-}\}$.[46] The *X*-ray crystal structure of $[Co(NH_3)_6]_5[Ag_8Ni_6L_{12}Cl]_3\cdot\sim 197H_2O$ has been solved. Crystalline derivatives of mixed-valence Cu^I–Cu^{II} cluster complexes of composition $[Cu^{II}{}_6Cu^I{}_8L_{12}Cl]^{5-}$ (L = deprotonated α-mercaptoisobutyric acid or D-penicillamine) have also been obtained with $[Co(NH_3)_6]^{3+}$ as counter-ion.[47]

Reduction of vanadate(V) to oxovanadium(IV) by cysteine has been studied and an oxovanadium(IV)–cysteine complex characterized.[48] In nitrogenase,

[39] A. Odani and O. Yamauchi, *Inorg. Chim. Acta*, 1982, **66**, 163.
[40] A. Odani and O. Yamauchi, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3773.
[41] P. I. Vestues and R. B. Martin, *J. Am. Chem. Soc.*, 1980, **102**, 7906.
[42] S. Mylonas, A. Valavanidis, V. Voukovalidis, and M. Polyssiou, *Inorg. Chim. Acta*, 1981, **55**, 125.
[43] T. Kowalik, H. Kozlowski, and B. Decock-le-Reverend, *Inorg. Chim. Acta*, 1982, **67**, L39.
[44] N. H. Agnew, T. G. Appleton, and J. R. Hall, *Inorg. Chim. Acta*, 1981, **50**, 137.
[45] T. G. Appleton, J. R. Hall, and M. A. Williams, *Inorg. Chim. Acta*, 1982, **61**, 51.
[46] P. J. M. W. L. Birker, J. Reedijk, and G. C. Verschoor, *Inorg. Chem.*, 1981, **20**, 2877.
[47] H. van Kempen, J. A. A. J. Perenboom, and P. J. M. W. L. Birker, *Inorg. Chem.*, 1981, **20**, 917.
[48] H. Sakurai, S. Shimomura, and K. Ishizu, *Inorg. Chim. Acta*, 1981, **55**, L67.

molybdenum is bound to a cysteine residue of the protein, and there has been considerable interest in molybdenum–cysteine complexes. The dinuclear dioxo-bridged Mo^{V}–cysteine complex $[Mo^{V}_2O_4(Cys)_2]^{2-}$ has been widely studied[49—51] and its *X*-ray structure determined.[52] Spectroscopic evidence for a new binuclear Mo^{III}–cysteine complex has been presented.[53]

Technetium-99m-labelled complexes have been a major contributing factor to the rapid growth of nuclear medicine,[54] but relatively little is known about the chemistry of technetium and its complexes. The complex 1-oxo-2,3,6-(D-penicillaminato-*NSO*)-4,5-(D-penicillaminato-*NS*)technetium(V), $TcO(C_5H_9NO_2S)(C_5H_{10}NO_2S)$, has now been characterized and its crystal structure determined.[55] The technetium is six-co-ordinate, being bonded to oxygen [Tc—O = 1.657(4) Å], to the S, N, and O atoms of a D-penicillamine dianion deprotonated at S and O, and to the S and N atoms of a D-penicillamine anion deprotonated at S.

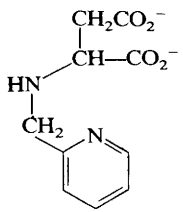
The synthesis and *X*-ray structure of the tetranuclear heterometallic anion $Na_2\{Au^{I}_2Ni^{II}_2[SCMe_2CH(NH_2)COO]_4\}\cdot x$(solvent) containing the deprotonated penicillamine ligand have also been reported.[56]

An improved preparation of iron(II) complexes of cysteine and methyl cysteinate has been described, and Mössbauer parameters have been determined.[57] Variable-temperature tin-119m Mössbauer studies of chlorodimethyltin(IV) derivatives of L-cysteine and DL-penicillamine have also been described.[58]

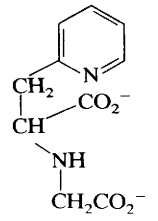
Copper(II) complexes of *N*-acetyl and *N*-benzoyl amino-acids have received considerable attention. The blue $Cu(Ac\text{-}\beta\text{-}Ala)_2\cdot 2H_2O$ and the green $[Cu(Ac\text{-}\beta\text{-}Ala)_2\cdot H_2O]_2\cdot 2H_2O$ (Ac-β-Ala = *N*-acetyl-β-alaninato ion) have been characterized by *X*-ray analysis.[59] The green $Cu(Bz\text{-}Leu)_2\cdot 2H_2O$ and mixed complexes with amines of the type $Cu(Bz\text{-}Leu)_2\cdot B_n\cdot mH_2O$ ($n = 2$, $m = 0$, B = pyridine, 3- or 4-picoline, or morpholine; $n = 1$, $m = 2$, B = 1,10-phenanthroline, 2,2-bipyridyl, or piperazine) have been prepared.[60] The amino-acid derivative co-ordinates only *via* the carboxylate group. Magnetic studies of some copper(II) complexes of *N*-acetyl- and *N*-benzoyl-alanine have also been reported.[61] Iron(II) hippurate, $Fe(PhCO\text{-}Gly)_2(H_2O)_3\cdot 2H_2O$, has been prepared and characterized by *X*-ray diffraction.[62]

[49] A. Kay and P. C. H. Mitchell, *Nature (London)*, 1968, **219**, 267.
[50] L. R. Melby, *Inorg. Chem.*, 1969, **8**, 349.
[51] A. Kay and P. C. H. Mitchell, *J. Chem. Soc. (A)*, 1970, 2421.
[52] J. R. Knox and C. K. Prout, *Acta Crystallogr., Sect. B*, 1969, **25**, 1857.
[53] M. Lamache-Duhameaun, *J. Inorg. Nucl. Chem.*, 1981, **43**, 208.
[54] R. L. Hayes in 'The Chemistry of Radiopharmaceuticals', ed. N. D. Heindel, H. D. Burns, T. Honda, and L. W. Brady, Masson et Cie., Paris, 1978, p. 155.
[55] K. J. Franklin, H. E. Howard-Lock, and C. J. L. Lock, *Inorg. Chem.*, 1982, **21**, 1941.
[56] P. J. M. W. L. Birker and G. C. Verschoor, *Inorg. Chem.*, 1982, **21**, 990.
[57] G. Terzian, R. Panossian, D. Benlian, C. More, and Y. Richard, *Inorg. Chim. Acta*, 1981, **54**, L153.
[58] K. C. Molloy, J. J. Zuckerman, G. Domazetis, and B. D. James, *Inorg. Chim. Acta*, 1981, **54**, L217.
[59] L. P. Battaglia, A. B. Corradi, G. Mareotrigiano, L. Menabue, and G. C. Pellacani, *Inorg. Chem.*, 1981, **20**, 1075.
[60] L. Antolini, L. Menabue, P. Prampolini, and M. Saladini, *Inorg. Chim. Acta*, 1982, **66**, 19.
[61] F. Cariati, L. Erre, G. Micera, L. Menabue, M. Saladini, and P. Prampolini, *Inorg. Chim. Acta*, 1982, **63**, 85.
[62] M. M. Morelock, M. L. Good, L. M. Trefonas, R. Majeste, and D. G. Karraker, *Inorg. Chem.*, 1982, **21**, 3044.

Nickel(II) and copper(II) complexes of *N*-(2-pyridylmethyl)-L-aspartate, plasp^{2-} (8), stereoselectively co-ordinate optically active amino-acidates (AA^-) to give [M(plasp)(AA)]$^-$ complexes.[63] A series of mixed-ligand cobalt(III) complexes [Co(plasp)(AA)] have now been characterized (AA^- = Gly, α-aminoisobutyrate, L-Ala, L-Thr, L-Pro, D- and L-Asp, D- and L-Phe, or D-, L-, or DL-Val).[64] Only the *fac*-CoN_3O_3 isomer in which the β-CO_2^- group of plasp^{2-} is co-ordinated *trans* to the pyridyl group of plasp^{2-} was isolated. Potentiometric studies have shown that β-(2-pyridyl)-α-alanine, $H_3\overset{+}{N}CH(CH_2C_5H_4N)CO_2^-$(pyAlaH), binds to transition-metal ions to give M(pyAla)$_2$ with a significant degree of enantioselectivity.[65] The complexes Cu(D-pyAla)$_2$·2H$_2$O and Zn(D-pyAla)$_2$ have now been characterized.[66] Spectral studies (i.r., ^{1}H n.m.r., vis., and c.d.) of Cu(D-pyAla)$_2$·2H$_2$O in aqueous solution support a structure that contains *cis*-amino and *cis*-carboxylate groups with one pyridine nitrogen occupying an apical position and the other pyridine nitrogen un-co-ordinated. This structure has been confirmed in the solid by *X*-ray diffraction. In Zn(D-pyAla)$_2$ both ligands are tridentate.



(8) plasp^{2-}



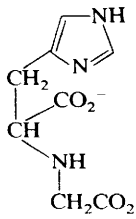
(9) *N*-Cm-L-pyAla^{2-}

Oxidation of Co^{II} to Co^{III} in the presence of *N*-(carboxymethyl)-L-β-(2-pyridyl)-α-alaninate (*N*-Cm-L-pyAla^{2-}) (9) and D-threoninate (D-Thr-) produces two isomers of the complex [Co(*N*-Cm-LL-pyAla)(D-Thr)] that are easily separated on acidic alumina.[67] On the basis of their visible spectra, one isomer is assigned a structure in which the nitrogens are arranged meridionally on cobalt, while the other is assigned to the only possible facial isomer. The crystal structure of *mer*-[Co(*N*-Cm-L-pyAla)(D-Thr)]·0·5H$_2$O confirms that the amino nitrogen of the D-Thr ligand is co-ordinated *trans* to the pyridyl nitrogen of the *N*-Cm-L-pyAla^{2-} ligand.

The ligand *N*-(carboxymethyl)-L-histidinate (*N*-Cm-L-His^{2-}) (10) has been prepared[68] by $NaBH_4$ reduction of the Schiff base formed from L-histidinate and glyoxylate. Several mixed-ligand complexes of the type [Co(*N*-Cm-L-His)(AA)] (AA^- = amino-acid anion) have been characterized.

A series of mixed-ligand complexes of the type [Co(*N*-Cm-L-pyAla)(AA)] has also been prepared (AA^- = glycinate, α-aminoisobutyrate, DL-alaninate, D-threoninate, D-, L-, or DL-valinate, or D-asparaginate).[69] The *fac*-CoN_3O_3

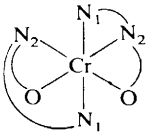
[63] R. Nakon, P. R. Rechani, and R. J. Angelici, *Inorg. Chem.*, 1973, **12**, 2431.
[64] L. A. Meiske and R. J. Angelici, *Inorg. Chem.*, 1980, **19**, 3783.
[65] P. R. Rechani, R. Nakon, and R. J. Angelici, *Bioinorg. Chem.*, 1976, **5**, 329.
[66] S. R. Ebner and R. J. Angelici, *Inorg. Chem.*, 1981, **20**, 2971.
[67] L. A. Meiske, R. A. Jacobson, and R. J. Angelici, *Inorg. Chem.*, 1981, **20**, 1794.
[68] L. A. Meiske and R. J. Angelici, *Inorg. Chem.*, 1982, **21**, 738.
[69] L. A. Meiske and R. J. Angelici, *Inorg. Chem.*, 1982, **21**, 731.



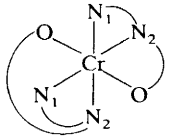
(10) *N*-Cm-L-His^{2-}

isomers in which the *N*-carboxymethyl group is co-ordinated *trans* to the pyridyl group and the *mer*-CoN_3O_3 isomers in which the amino nitrogen of the amino-acidate is co-ordinated *trans* to the pyridyl group were characterized.

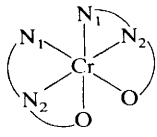
Chromium(III) complexes of amino-acids are beginning to attract attention, including chromium(III) complexes with DL-methionine and L-cysteine.[70] Mixed-ligand chromium(III) complexes [Cr(L- or D-Asp)(L-His)] and $[Cr(L\text{-}Asp)_2]^-$ have been prepared.[71] Two isomers of bis-(L-histidinato)chromium(III), in which histidine functions as a tridentate, have been characterized;[72] there are the *trans* (imidazole) (11) and *trans* (carboxylate) (12) isomers. The *trans* (amine) isomer (13) has yet to be characterized. All three isomers with Co^{III} are known,[73–75] the *trans* (imidazole) complex being the most predominant. A useful review article on transition-metal complexes with tridentate amino-acids appeared in 1975.[76]



(11) *trans* (imidazole)

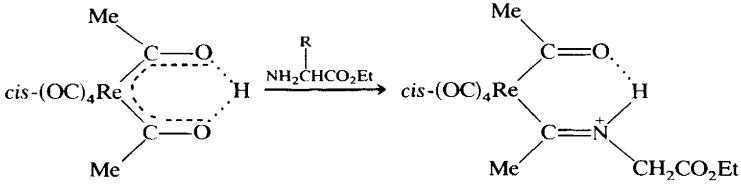


(12) *trans* (carboxylate)



(13) *trans* (amine)

The rhena-acetylacetone molecule *cis*-$(OC)_4Re(MeCO)_2H$ (14) condenses with ethyl glycinate and ethyl L-alaninate to give the corresponding rhena-β-ketoimine derivatives of the amino-acid esters (Scheme 3).[77] The rhena moiety



(14)

Scheme 3

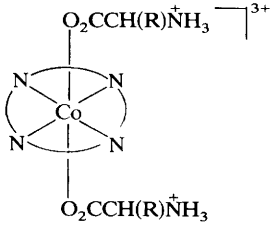
[70] J. Maslowska and L. Chrusanski, *J. Inorg. Nucl. Chem.*, 1981, **43**, 3398.
[71] M. Watabe, H. Yano, Y. Odaka, and H. Kobayashi, *Inorg. Chem.*, 1981, **20**, 3623.
[72] P. E. Hoggard, *Inorg. Chem.*, 1981, **20**, 415.
[73] L. J. Zompa, *J. Chem. Soc., Chem. Commun.*, 1969, 783.
[74] H.-H. Schmidtke, *Chem. Phys. Lett.*, 1969, **4**, 451.
[75] S. Bagger, K. Gibson, and C. S. Sørensen, *Acta Chem. Scand.*, 1972, **26**, 2503.
[76] S. T. Chow and C. A. McAuliffe, *Prog. Inorg. Chem.*, 1975, **19**, 51.
[77] A. J. Baskar, C. M. Lukehart, and K. Srinivasan, *J. Am. Chem. Soc.*, 1981, **103**, 1467.

is covalently bonded to the amino group of the amino-acid residue and can function as a heavy-atom label for *X*-ray structural studies. Base hydrolysis of the rhena-amino-acid esters gives the rhena derivatives of the free amino-acids. When the rhenaglycine complex is treated with DCC and ethyl glycinate the ethyl rhenaglycylglycinate complex is formed, thus establishing the value of the rhena moiety as an *N*-terminal protecting group during peptide synthesis. Cleavage of the amino-acid derivatives from the rhena moiety using acidic solvolysis and oxidation by iodosobenzene is also described. The technique is complementary to the use of the penta-ammine cobalt(III) moiety[16] as a C-protecting group in peptide synthesis.

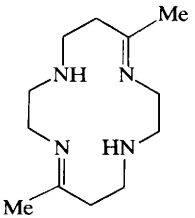
A new synthesis of *R*- and *S*-2-deuterioamino-acids using stereochemically inert cobalt(III) complexes has been described.[78] The reaction of penta-cyanonitrosylferrate(II) with α,δ- and α,ε-diamino-acids gives heterocyclic amino-acids;[79] thus ornithine gives proline and lysine gives pipecolic acid.

The protonation equilibrium $[Co(en)_2(GlyO)]^{2+} + D^+ \rightleftharpoons [Co(en)_2(GlyOD)]^{3+}$ in strongly acidic solution has been studied by ^1H n.m.r. spectroscopy.[80] Similar pK_{BD^+} values (*ca.* −1.2) are observed in D_2SO_4 and DCl solution. Complexes of the general types $\{Co(en)_2[(S)\text{-}AA]\}^{2+}$ and $\{Co(en)[(S)\text{-}AA_2]\}^{+}$ (AA = asparagine or glutamine) have been prepared.[81] Stereoselective formation of the Λ-isomers of $\{Co(en)[(S)\text{-}AA_2]\}^{+}$ depends primarily on the geometry of the co-ordination sphere, which makes possible the formation of different numbers of internal hydrogen bonds through $CONH_2$ and ethylenediamine or asparagine(glutamine)NH_2 groups.

Relatively few examples[82] are known of macrocyclic cobalt(III) complexes in which the *trans* sites are occupied by amino-acid or amine ligands. The preparations of a number of carboxylato-bonded amino-acid complexes of type (15) with Gly, L-α-Ala, β-Ala, L-Leu, L-Val, and L-His, where the macrocyclic ligand is $Me_2[14]dieneN_4$ (16), have now been described.[83]



(15)



(16)

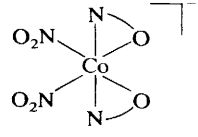
The preparation of *N*-aryl-substituted glycine complexes $[Co(en)_2(GlyN\text{-}aryl)]^{2+}$ (aryl = phenyl, 2-methylphenyl, or naphthyl) has been described.[84] The

[78] Y. N. Belokon, A. S. Melikyan, and T. F. Salel'eva, *Tetrahedron*, 1980, **36,** 2327.
[79] M. T. Beck, A. Katho, and L. Dozsa, *Inorg. Chim. Acta*, 1981, **55,** L55.
[80] J. Fastrez and V. Daffe, *J. Chem. Soc., Dalton Trans.*, 1981, 317.
[81] F. Jursik, B. Hajek, S. A. Moez, and R. D. Archer, *Inorg. Chim. Acta*, 1982, **57,** 51.
[82] J. Cragel and B. E. Douglas, *Inorg. Chim. Acta*, 1974, **10,** 33.
[83] R. W. Hay and R. Bembi, *Inorg. Chim. Acta*, 1982, **65,** L207.
[84] B. T. Golding, P. V. Ioannou, and P. J. Sellars, *Inorg. Chim. Acta*, 1981, **56,** 95.

stereoselectivity of the exchange of the methylene hydrogens H_a and H_b of the co-ordinated glycinato ligand at pD 9.7 has also been investigated and has been shown to proceed stereoselectively when aryl = phenyl.

The ^{13}C n.m.r. spectra of a series of *trans*-(*O*)-$[Co(en)_2(OOCR)_2]^{n+}$ complexes (RCOO denotes an aliphatic acid or amino-acid) have been studied, and a linear correlation has been observed between $\bar{\nu}_{max}$ (^1E_g component of the first ligand-field band) and the chemical shifts of the carboxyl and α-carbons.[85]

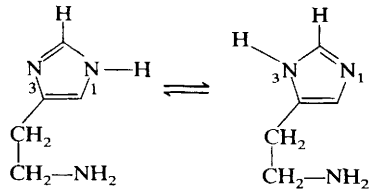
The $[Co(CN)_2(AA)]^-$ ($AA^- = Gly^-$ or β-Ala^-) complexes, which have a *cis,cis,cis* geometry with respect to the donor atoms C, N, and O, have been prepared and their chiroptical properties studied.[86] The ^{13}C downfield shifts of α-carbons in *N*-alkylglycines chelated to cobalt(III) are larger than those in *C*-alkylglycines.[87] The ^{15}N chemical shifts have been determined at natural-abundance level for complexes of the type $[Co^{III}(NO_2)_2(AA)]^{n+}$ with the stereochemistry shown in (17).[88] Co-ordination to cobalt(III) leads to displacement of the amino-acid ^{15}N resonance by 24—42 p.p.m. to higher field



(17)

compared with the protonated α-amino-acids. The ability to observe resonances at the natural-abundance level of ^{15}N (0.4%) in low-spin d^6 complexes may prove to be a very useful technique for identifying binding sites.

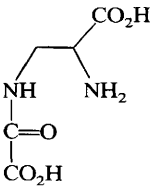
The pH dependence of Raman spectra for Cu^{II}–histamine (1:2) and Ni^{II}–histamine (1:2) aqueous solutions indicates[89] two types of co-ordination of the imidazole groups *via* N-3 and N-1, presumably as a result of the equilibrium (18) ⇌ (19).



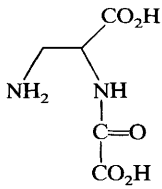
(18) (19)

The non-protein amino-acid β-*N*-oxalyl-L-α,β-diaminopropionic acid (20) has been isolated from, or detected in, the seed of many species of *Lathyrus*, *Crotalaria*, and *Acacia*. The compound undergoes spontaneous acyl transfer in aqueous solution to the α-isomer (21). The copper(II) complexes (with a 1:1

[85] T. Ama, H. Kawaguchi, and T. Yasui, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 448.
[86] S. Fujinami and M. Shibata, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 2939.
[87] T. Ama, H. Kawaguchi, and T. Yasui, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3597.
[88] N. Juranic, R. L. Lichter, M. B. Celap, M. J. Malinar, and P. N. Radivojsa, *Inorg. Chim. Acta*, 1982, **62**, 131.
[89] M. Itabashi, K. Shoji, and K. Hoh, *Inorg. Chem.*, 1982, **21**, 3484.



(20)



(21)

stoicheiometry) have now been characterized for both isomers, and their structures have been discussed in relation to the aspartate and glutamate complexes of copper(II).[90]

Purple and green copper(II) complexes of D-cycloserine have been prepared and characterized.[91] In the purple complex two cycloserine molecules are bound to copper(II), and the presence of two nitrogen donors was confirmed by e.s.r. detection of the ligand hyperfine splitting. E.s.r. spectra of copper(II)–amino-acid complexes $[Cu(AA)]^{n+}$ and $[Cu(AA)_2]^{m+}$ have been reported for twelve amino acids, and evidence has been obtained for *cis* and *trans* isomers.[92] The structure of copper(II)–histidinate complexes in aqueous solution is also discussed. Mixed 1:1:1 complexes of proline or aspartic acid with Cu^{II} and 9,10-phenanthroline or 2,2′-bipyridine show induced Cotton effects in the aromatic region.[93] The effect is ascribed to interactions between the charge-transfer transitions from the amino-acid and the longitudinal π–π^* transitions of the aromatic ligand. Further work in this area has also been reported.[94]

Transferrins, the iron-transport proteins of biological fluids, have two metal-binding sites that can accommodate a variety of multi-valent metal ions. In each site at least one tyrosyl group is co-ordinated to the metal. Copper(II) interacts with D-*o*-tyrosine to form two complexes.[95] The first complex formed at pH 6 has a c.d. spectrum characteristic of co-ordination *via* amino nitrogens and carboxylate oxygens. The second complex begins to form at pH 8 and is complete at pH 10.5. Its c.d. spectrum below 400 nm is very similar to that of iron(III) and copper(II) transferrins with well defined bands at *ca.* 400 and 330 nm. These bands are now shown to be due to phenolate oxygen to metal charge-transfer transitions.

X-Ray K-absorption spectra of some amino-acid and carbonato complexes of copper(II) have been studied.[96] Average radii of the co-ordination sphere in the amino-acid chelates are reported.

The syntheses and oxidation of cysteine- and penicillamine-containing iron(III) and cobalt(III) complexes as analogues for the active metal sites of

[90] P. O'Brien and P. B. Nunn, *Inorg. Chim. Acta*, 1982, **66**, 185.
[91] H. Sakurai, C. Shibata, H. Matsuura, and T. Yoshimura, *Inorg. Chim. Acta*, 1981, **56**, L25.
[92] B. A. Goodman, D. B. McPhail, and H. K. J. Powell, *J. Chem. Soc., Dalton Trans.*, 1981, 822.
[93] S. Bunel, C. Ibarra, M. Rodriguez, A. Urbina, and C. A. Bunton, *J. Inorg. Nucl. Chem.*, 1981, **43**, 967.
[94] S. Bunel, C. Ibara, M. Rodriguez, A. Urbina, and C. A. Bunton, *J. Inorg. Nucl. Chem.*, 1981, **43**, 190.
[95] A. Garnier-Suillerot, J.-P. Albertini, A. Collet, L. Faury, J.-M. Pastor, and L. Tosi, *J. Chem. Soc., Dalton Trans.*, 1981, 2544.
[96] C. B. Bhargava, A. N. Vishnoi, and U. C. Srivastava, *Transition Met. Chem.*, 1981, **6**, 114.

cysteine and cysteamine dioxygenases have been investigated in a recent thesis.[97] Cobalt(II) and iron(II) complexes of cystine have been studied,[98] and the syntheses of *S*-trityl-L-cysteine complexes of Pd^{II}, Cr^{III}, Mn^{II}, and Co^{II} have been described.[99]

The syntheses of $[RuCl(amino\text{-}acido)(PPh_3)_2]$ complexes have been reported;[100] these complexes act as catalysts for the oxidative dehydrogenation of glycerin.

^{199}Hg n.m.r. spectroscopy has been used to study the interaction of the toxic organomercurial ethylmercury phosphate with various amino-acids.[101] It is suggested that the ethylmercury–ribonuclease adduct involves modification of the *N*-terminal lysine residue. ^{207}Pb n.m.r. has been used to study the use of EDTA-chelation therapy in cases of lead poisoning.[102] Currently $[Na_2CaEDTA]$ available as the proprietary drug Ledclair is used to avoid the possibility of hypocalcaemia, but little is known about the precise chemistry of the interaction of the drug with lead in aqueous media.

The interaction of L-His, imidazole, and *N*-methylimidazole with uranyl nitrate and acetate has been studied both in D_2O and CD_3OD by ^1H and ^{13}C n.m.r.[103] The results suggest the formation of complexes with imidazole and *N*-methylimidazole *via* a nitrogen atom in CD_3OD solutions. In aqueous solution the major co-ordination site of L-His is the carboxylate group. The ^1H and ^{13}C n.m.r. spectra of $[Co(NH_3)_5ImH]^{3+}$ and the ^1H n.m.r. spectra of *cis*-α-$[Co(trien)(ImH)_2]^{3+}$ and *cis*-β-$[Co(trien)(ImH)_2]^{3+}$ indicate pK_a values (determined from the pH dependence of the chemical shift) of 10.0, 9.6, and 10.1, respectively, for the imidazole ligand.[104]

The products obtained on reaction of $SnCl_2$ with sulphur-containing amino-acids have been shown to depend on the reaction conditions.[105] Eleven new tin(II) or tin(IV) derivatives of sulphur-containing amino-acids and their esters are reported. Infrared spectra suggest co-ordination to Sn by the amino-nitrogen atoms. Other studies have dealt with lanthanide complexes of glycine and polyglycines,[106] copper(II) and palladium(II) complexes with histidine derivatives,[107] and nickel(II) and cobalt(II) complexes of aspartic and glutamic acid hydrazides.[108]

Equilibrium Studies.—Solution equilibrium studies between metal ions and amino-acid ligands continue to attract considerable attention. There have been a number of attempts to model physiological conditions. Formation constants have been determined (37 °C, $I = 0.15$ M) for the ternary systems Cu^{II}–

[97] K. J. Miller, *Diss. Abstr. Int. B*, 1981, **41,** 4113.
[98] A. Ungureanu and M. Marc, *Rev. Roum. Chim.*, 1980, **25,** 845.
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[101] D. A. Vidusek, M. F. Roberts, and G. Bodenhausen, *J. Am. Chem. Soc.*, 1982, **104,** 5452.
[102] P. G. Harrison, M. A. Healy, and A. T. Steel, *Inorg. Chim. Acta*, 1982, **67,** L15.
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[104] N. S. Rowan, C. B. Storm, and R. Rowan, *J. Inorg. Biochem.*, 1981, **14,** 59.
[105] P. A. Cusack, P. J. Smith, and J. D. Donaldson, *J. Chem. Soc., Dalton Trans.*, 1982, 439.
[106] H. G. Brittain and Z. Konteatis, *J. Inorg. Nucl. Chem.*, 1981, **43,** 1719.
[107] A. A. Danopoulos and S. M. Paraskewas, *Inorg. Chim. Acta*, 1981, **55,** 141.
[108] M. Boneva, P. Bontchev, M. Miteva, and S. Zakhariev, *Dokl. Bolg. Akad. Nauk*, 1980, **33,** 1091.

histamine-histidine, Cu^{II}–histamine-glutamine, and Cu^{II}–histamine-threonine as models for histamine as a ligand in blood plasma.[109] Metal-ion equilibria in the zinc(II)–citrate-histidinate, -glutaminate, and -threoninate systems have been studied[110] to assess the ability of citrate to mobilize the lower-molecular-weight fraction of zinc in blood plasma.

Formation constants for metal complexes of thiol-histidine have been determined by potentiometric techniques.[111] Co-ordination with Zn, Cd, Hg, and Pb occurs *via* the thiolate and amino-nitrogen donors of the ligand. Stereoselectivity in the mixed zinc(II)–histidine-threonine complex has been studied.[112]

Many investigations of ternary complexes are now being published; they include ternary complexes of copper(II) with L-histidine and a range of amino-acids,[113] binary and ternary complexes of zinc(II) involving imidazole, histamine, and L-histidine,[114] ternary complexes of zinc(II) with lysinate and some amino-acids (His, Cys, Thr),[115] ternary complexes of copper(II) with histamine and some amino-acids,[116] and the formation of binary and ternary complexes between 2,3-diaminopropionic acid, histidine, and Cu^{II}, Ni^{II}, and Zn^{II}.[117] Other similar studies include potentiometric investigations of the copper(II)–cysteinate, copper(II)–lysinate, copper(II)–histamine-cysteinate, and copper(II)–histamine-lysinate systems[118] and ternary complexes of copper(II)–glycylphenylalanine with various amino-acids.[119]

The interaction of L-histidine, imidazole, and *N*-methylimidazole with uranyl nitrate and acetate has been investigated in both D_2O and CD_3OD by ^1H and ^{13}C n.m.r. measurements.[120] The results suggest the formation of complexes of uranyl(VI) with imidazole and methyl imidazole *via* a nitrogen atom only in CD_3OD solutions.

Formation constants for complexes of uranyl(VI) with a series of amino-acids have been determined,[121] and n.m.r. and spectroscopic studies on the interaction of UO_2^{2+} with glutamic acid and glutamine have been reported.[122] The structure of the uranyl(VI) complex of EDTA has been discussed.[123]

As lead is of current environmental interest, a number of potentiometric studies of lead(II) complexes have appeared. These studies cover complexes of

[109] A. Kayali and G. Berthon, *Polyhedron*, 1982, **1**, 371.
[110] M.-J. Blais and G. Berthon, *Inorg. Chim. Acta*, 1982, **67**, 109.
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[112] A. Gergely, *Inorg. Chim. Acta*, 1981, **56**, L75.
[113] M. S. Nair, K. Venkatachalapathi, M. Santappa, and P. K. Murugan, *J. Chem. Soc., Dalton Trans.*, 1982, 55.
[114] M. S. Nair, K. Venkatachalapathi, and M. Santappa, *J. Chem. Soc., Dalton Trans.*, 1982, 555.
[115] T. Alemdaroglu and G. Berthon, *Inorg. Chim. Acta*, 1981, **56**, 51.
[116] M. S. Nair, K. Venkatachalapathi, M. Santappa, and P. K. Murugan, *Inorg. Chem.*, 1982, **21**, 2418.
[117] G. E. Jackson, P. M. May, and D. R. Williams, *J. Inorg. Nucl. Chem.*, 1981, **43**, 825.
[118] M.-J. Blais, A. Kayali, and G. Berthon, *Inorg. Chim. Acta*, 1981, **56**, 5.
[119] D. N. Shelte, *J. Coord. Chem.*, 1982, **12**, 35.
[120] A. Marzotto and H. Kozlowski, *Inorg. Chim. Acta*, 1982, **67**, 87.
[121] M. Nourmand and N. Meissami, *Polyhedron*, 1982, **1**, 537.
[122] H. Kozlowski, H. Wieczorek, B. Jezowska-Trzebiatowska, and A. Marzotto, *Inorg. Chim. Acta*, 1981, **54**, L107.
[123] M. Lurdes, S. S. Concalves, A. M. A. Mota, and J. J. R. Frausto da Silva, *Talanta*, 1983, **30**, 69.

Pb^{II} with 2-amino-2-methylpropionic acid and DL-2-aminobutyric acid[124] and with L-α-alanine, DL-α-aminoadipic acid, and L-cysteine.[125]

Cadmium(II) forms complexes with a variety of α-amino-acids, and formation constants have been determined.[126] Ternary complexes cadmium(II)–oxalate-glycinate have been studied polarographically.[127]

C.d. spectra of high-pH solutions of $[Cu(L\text{-}AlaO)_2]$ indicate a further equilibrium with a pK_a of 12.17 assigned to formation of a monomeric $[Cu(L\text{-}AlaO)_2OH]^-$ complex.[128] Similar equilibria have been observed with other amino-acids (Val, Pro, Glu, Lys, and Arg).

A novel approach based on metal-ion buffers has been used to determine metal–ligand formation constants for a variety of sparingly soluble systems.[129] It is suggested that the technique may be particularly useful in the study of metal binding by pharmaceuticals.

Mercury(II) binds strongly to soft sulphur donors; thus methylmercury(II) binds preferentially to the sulphydryl group of amino-acids and small peptides.

The toxicity of mercury results from the formation of such complexes. Complexation of $MeHg^{II}$ by mercaptoacetic acid, mercaptoethanol, mercaptosuccinic acid, cysteine, penicillamine, homocysteine, and *N*-acetylpenicillamine has been studied by ^1H n.m.r.[130] The conditional formation constants are strongly pH dependent owing to competitive reactions involving hydrogen and hydroxide ions. The results are discussed with reference to *N*-acetylpenicillamine and penicillamine as antidotes for methylmercury poisoning.

Heterobinuclear complexes of the types $[CuM(His)_2]^{2+}$ and $[CuM(Hm)_2]^{4+}$ (Hm = histamine, M = Ni^{II}, Zn^{II}, or Cd^{II}) have been characterized by potentiometric measurements,[131] and thermodynamic parameters have been obtained by direct calorimetry.

Owing to their growing use as spectroscopic probes for calcium-binding sites in proteins, lanthanide ions attract attention. Formation constants of Gd^{III} with glycine and polyglycines have been obtained.[132] Ternary complexes of praseodymium(III) with cysteine and a variety of diols have been prepared, and formation constants have been determined by potentiometric titration.[133] Lanthanide–EDTA complexes have been investigated as aqueous shift reagents.[134]

Three ligands (α-mercaptopropionylglycine, glutathione, and *N*-acetylcysteine) that contain both amide and sulphydryl groups have been studied to

[124] M. Maeda, Y. Tanaka, and G. Nakagawa, *J. Inorg. Nucl. Chem.*, 1981, **43**, 1049.
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[130] R. S. Reid and D. L. Rabenstein, *Can. J. Chem.*, 1981, **59**, 1505.
[131] P. Amico, G. Arena, P. G. Daniele, G. Ostacoli, E. Rizzarelli, and S. Sammartano, *Inorg. Chem.*, 1981, **20**, 772.
[132] Z. Konteatis and H. G. Brittain, *J. Inorg. Nucl. Chem.*, 1981, **43**, 1675.
[133] A. Kothari, R. K. Jain, A. Ahmed, and S. N. Misra, *J. Inorg. Nucl. Chem.*, 1981, **43**, 2905.
[134] A. D. Sherry, C. A. Stark, J. R. Ascenso, and C. F. G. C. Geraldes, *J. Chem. Soc., Dalton Trans.*, 1981, 2078.

establish the capability of the SH groups to serve as an anchor for Ni^{II}- and Pd^{II}-induced deprotonation of amide hydrogens.[135] The interaction of $[Pd(en)(OH_2)_2]^{2+}$ (en = ethylendiamine) with ethanolamine, L-Ser, L-Thr, L-homoserine, and L-hydroxyproline has been studied potentiometrically.[136] Mixed-ligand complexes are formed and their structures considered; co-ordination *via* deprotonated OH groups is observed in some cases.

Potentiometric and ^1H n.m.r. studies on the interaction of silver(I) with *S*-methyl-L-cysteine, L-methionine, and L-ethionine have been published.[137] Silver(I) has a high affinity for the soft sulphur donor, but the involvement of other potential donor centres NH_2 and CO_2^- requires clarification and is considered in the paper.

Complexes of the type $[CuHL]^+$ containing the protonated ligand have been characterized for L-glutamate and L-aspartate, and formation constants were obtained.[138] Visible spectra and e.p.r. parameters establish that the protonated complexes have the α-aminocarboxylate group co-ordinated to copper(II). The ternary copper(II)–malonate-glycinate system has been studied,[139] and the thermodynamic stability and structure of binary and ternary copper(II) complexes formed with L-alaninamide ($AlaNH_2$) and diethylenetriamine have been investigated.[140] For $[Cu(dien)(AlaNH_2)]^{2+}$ there is evidence that four nitrogens are equatorially co-ordinated and that the carbonyl oxygen of $AlaNH_2$ interacts to a significant extent with an apical position of Cu^{II}. It is concluded that in a mixed-ligand complex an ionized amide group may be formed and co-ordinated only if *two equatorial* co-ordination positions on Cu^{II} are accessible.

Potentiometric studies indicate that stereoselectivity occurs in the formation of ternary complexes of copper(II) with *N*-benzenesulphonyl-L-phenylalanine and D- or L-amino-acids.[141] Mixed complexes with L-Val and L-Phe are more thermodynamically stable than those with the D-enantiomers. Copper(II) complexes of serine and threonine have been studied over a wide pH range.[142] A variety of techniques indicate that the ligands can act as tridentates, owing to deprotonation of the OH groups in basic solution leading to anionic complexes.

A potentiometric study of complex formation between D-penicillamine disulphide [L = (22)] and copper(II) indicates the presence of a range of binuclear species.[143] The major species at neutral pH is $Cu_2(L)_2$, and it is

(22)

[135] I. Sovago and R. B. Martin, *J. Inorg. Nucl. Chem.*, 1981, **43,** 425.
[136] M.-C. Lim, *Inorg. Chem.*, 1981, **20,** 1377.
[137] L. D. Pettit, K. F. Siddiqui, H. Kozlowski, and T. Kowalik, *Inorg. Chim. Acta*, 1981, **55,** 87.
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[143] S. H. Laurie, E. S. Mohammed, and D. M. Prime, *Inorg. Chim. Acta*, 1981, **56,** 135.

concluded that L (the oxidized metabolite of the drug D-penicillamine) is unlikely to have therapeutic value as a chelating agent in the treatment of Wilson's disease and rheumatoid arthritis.

Formation constants of epimeric threonines (Thr, *allo*-Thr) and isoleucines (Ile, *allo*-Ile) with Cu^{II} and Co^{II} establish that the natural epimers form more stable complexes than the *allo* forms.[144] The complexation of molybdenum(VI) by L-aspartate in dilute aqueous solutions at pH 6 has been studied by visible spectra, ^1H, and ^{13}C n.m.r.[145] A single complex anion $[MoO_3(C_4H_5NO_4)]^{2-}$, where the aspartate dianion is terdentate, is observed. Formation constants of complexes of L-tyrosine with Co^{II}, Ni^{II}, Cu^{II}, and Zn^{II} have been reported.[146] The phenolic oxygen does not participate in co-ordination.

Other formation-constant studies include those on the following: DL-4,4'-dithiobis-(2-aminobutyric) acid with Mn^{II}, Co^{II}, and Ni^{II} in aqueous solution;[147] *SS'*-methylenebis-(L-cysteine) with Co^{II}, Ni^{II}, and Zn^{II};[148] cysteine and cystine with Zn^{II};[149] a series of binary and ternary complexes of Zn^{II} with phenylalanine, arginine, cysteine, and histidine;[150] glycine with Ni^{II}, Cu^{II}, and Zn^{II} in water–alcohol mixtures;[151] D-cycloserine with Co^{II}, Ni^{II}, Cu^{II}, and Zn^{II};[152] and binary and ternary complexes of Cr^{III} involving iminodiacetic acid, L-aspartic acid, L-glutamic acid, or L-cysteine as ligands.[153]

Polarographic meaurements show that nickel(II) and benzyloxycarbonyl-L-tryptophan form complexes with 1:2 and 1:4 metal-to-ligand ratios.[154] The 1:2 complex $[NiL_2]\cdot 2H_2O$ and some amine adducts have been isolated in the solid state. The basicity and metal-ion bonding capability of amine-carboxyboranes, $R_3N\cdot BH_2CO_2H$, the boron analogues of glycine, and *N*-methylated glycines have been studied.[155] Equilibria and proton-exchange processes in the VO^{2+}–glycine system in aqueous solution have been investigated by n.m.r. relaxation techniques and pH titration.[156]

Diffraction Studies.—Mugineic acid (23), an amino-acid isolated as an exudate from the roots of water-cultured barley, is the first compound to be shown to play a role in the uptake and transport of iron in higher plants. It is capable of effectively solubilizing $Fe(OH)_3$ in the pH range 4—9. The ion-solubilizing

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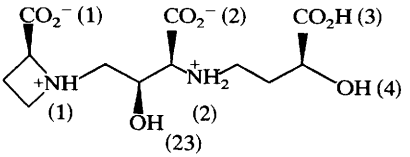
[156] I. Fabian and I. Nagypal, *Inorg. Chim. Acta*, 1982, **62**, 193.



(23)

action is strongly inhibited by divalent metal ions, $Cu^{II} > Co^{II} \geq Zn^{II} > Mn^{II}$, probably because of preferential complexation with these ions. The *X*-ray structure[157,158] of the copper(II) complex ($C_{12}H_{18}CuN_2O_8$) shows that the ligand acts as a hexadentate giving a distorted octahedral geometry on copper(II) with an N_2O_4 donor set. The co-ordination of Cu^{II} involves the azetidine nitrogen N-1 and the secondary-amine nitrogen N-2 and both terminal carboxylate oxygens O-1 and O-3 in an approximately planar co-ordination, while the hydroxyl oxgen O-4 and intermediate carboxylate oxygen O-2 are bonded axially.

The complexes $Hg[MeSCH_2CH(NH_2)COO]_2$ and $Hg[MeCH_2SCH_2CH_2CH(NH_3)COO]_2(ClO_4)_2$ obtained by reaction of yellow HgO with *S*-methyl-L-cysteine and ethionine, respectively, have been characterized by *X*-ray diffraction.[159] The former complex is *NO*-bonded, while in the ethionine derivative the Hg^{II} is bonded to two sulphur and two oxygen atoms in a distorted tetrahedron. Reactions of L-tryptophan and DL-homocysteine with mercury(II) halides in acidic ethanol–water give bis-(L-tryptophanium) trichloromercurate(II) and bis-(DL-homocysteinethiolactonium) tetrabromomercurate(II). The crystal structures of the two complexes have been determined.[160]

The structure[161] of the 1:1 complex of histamine with copper(II) chloride $\{[Cu(C_5H_9N_3)Cl_2]\}$ establishes octahedral co-ordination of the copper, with two histamine N atoms and two Cl atoms in a plane. Two symmetrically transformed Cl atoms complete the co-ordination sphere. The complex *cis*-$[Co(en)_2(Im)Cl]Cl_2$ has the expected *cis* geometry with a Co–imidazole distance of 194.5 pm.[162] The imidazole ring is planar. Dichloro(L-histidine)copper(II) consists of chains of molecules with the carboxylate oxygen, the amino nitrogen, and the two chlorine atoms forming an approximate square about the copper.[163] Two bridging chlorine atoms from two adjacent molecules complete a distorted octahedron.

A number of complexes of aspartic acid have been studied by *X*-ray diffraction: [Ni(L-Asp)(imidazole)$_3$],[164] $[Ni(L\text{-}Asp)(H_2O)_2]\cdot H_2O$,[165] and [Cu(L-

[157] K. Nomoto, Y. Mino, T. Ishida, H. Yoshioka, N. Ota, M. Inoue, S. Takagi, and T. Takemoto, *J. Chem. Soc., Chem. Commun.*, 1981, 338.
[158] Y. Mino, T. Ishida, N. Ota, M. Inoue, K. Nomoto, H. Yoshioka, T. Takemoto, Y. Sugiura, and H. Tanaka, *Inorg. Chem.*, 1981, **20,** 3440.
[159] L. Book, A. J. Carty, and C. Chieh, *Can. J. Chem.*, 1981, **59,** 144.
[160] L. Book, A. J. Carty, and C. Chieh, *Can. J. Chem.*, 1981, **59,** 138.
[161] M. L. Glowka, Z. Galdecki, W. Kazimierczak, and C. Maslinski, *Acta Crystallogr., Sect. B*, 1980, **36,** 2148.
[162] B. Zimmer-Gasser and K. C. Dash, *Inorg. Chim. Acta*, 1980, **55,** 43.
[163] K. Colyvas, H. R. Tietze, and S. K. J. Egri, *Aust. J. Chem.*, 1982, **35,** 1581.
[164] L. P. Battaglia, A. B. Corradi, L. Antolini, G. Marcotrigiano, L. Menabue, and G. C. Pellacani, *J. Am. Chem. Soc.*, 1982, **104,** 2407.
[165] L. Antolini, L. Menabue, G. C. Pellacani, and G. Marcotrigiano, *J. Chem. Soc., Dalton Trans.*, 1982, 2541.

Asp)(imidazole)]·$2H_2O$.[166] The crystal structure of bis-(*N*-benzoylglycinato)triaquozinc(II) dihydrate indicates that the zinc atom is co-ordinated by an oxygen atom of the carboxyl group of each *N*-benzoylglycinate anion and by three water molecules, resulting in a five-co-ordinate, slightly distorted, trigonal-bipyramidal geometry with two water molecules occupying the apical positions.[167] Bis-(*N*-acetyl-α-alaninato)bis-(*N*-methylimidazole)copper(II) dihydrate is planar with the copper lying on the centre of symmetry surrounded by two carboxylic oxygen and two *N*-methylimidazole nitrogen atoms.[168]

Binuclear copper sites are generally accepted as occurring as the 'e.p.r. silent' type III copper in proteins such as haemocyanin, tyrosinase, and laccase. The *X*-ray structure of the complex [*NNN'N'*-tetra-(2'-benzimidazolylmethyl)-1,2-ethanediamine]dicopper(I) perchlorate, $[Cu_2(EDTB)](ClO_4)_2$ [EDTB = $(C_7H_5N_2—CH_2)_2NCH_2CH_2N(CH_2—N_2C_7H_5)_2$], has been published;[169] this complex provides a unique example of copper(I) linearly co-ordinated by imidazole ligands.

The *X*-ray structure of dichloro-*SS'*-bis-(*O*-methylcysteinato)tin(IV), $Cl_2Sn[SCH_2CH(NH_2)COOMe]_2$, has been determined.[170] The tin is co-ordinated to two *cis*-nitrogen and two *trans*-sulphur atoms from the *O*-methylcysteinato ligands and to two *cis*-chlorine atoms in an approximately octahedral arrangement. The lack of any anti-tumour activity with the compound may be associated with the large (99.4°) *cis*-ClSnCl bond angle. The crystal structure and absolute configuration of one of the isomers of (+)K[Co(L-valinato)$_2$(CO_3)] have been determined[171] and shown to be Λ(+)-*cis*(*N*)-*cis*(*O*)-K[Co(L-valinato)$_2$δδ(CO_3)]·$2H_2O$.

Other structures of interest include $Na_2[Ni(NH_2CH_2COO)_3]ClO_4·D_2O$, in which the three nitrogens are mutually *cis*,[172] and the addition compound $(NH_2CH_2CO_2H)_3·CaI_2·H_2O$, in which the calcium is co-ordinated to seven oxygen atoms (five from glycine and two from water molecules), forming a distorted pentagonal bipyramid.[173] Dibromobis(iminodiacetamide)palladium(II) has a centrosymmetric square-planar co-ordination with two imino nitrogen and two bromine atoms in *trans* positions.[174] It should be possible to prepare other Pd^{II} complexes by deprotonation of the amide groups.

In the complex *cis*-(*S*-ethylcysteine)platinum(II) dichloride the metal is bonded *via* the sulphur and nitrogen atoms of the ligand; two diastereoisomers occur, differing in their absolute configuration on sulphur.[175]

[166] L. Antolini, G. Marcotrigiano, L. Menabue, G. C. Pellacani, and M. Saladini, *Inorg. Chem.*, 1982, **21**, 2263.
[167] H. Grewe, M. R. Udupa, and B. Krebs, *Inorg. Chim. Acta*, 1982, **63**, 119.
[168] L. P. Battaglia, A. B. Corradi, L. Menabue, G. C. Pellacani, P. Prampolini, and M. Saladini, *J. Chem. Soc., Dalton Trans.*, 1982, 781.
[169] J. J. M. W. L. Birker, H. M. J. Hendriks, and J. Reedijk, *Inorg. Chim. Acta*, 1981, **55**, L17.
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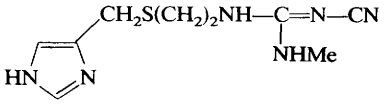
[174] M. Sekizaki, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3861.
[175] V. Theodorou, I. Photaki, N. Hadjiliadis, R. W. Gellert, and R. Bau, *Inorg. Chim. Acta*, 1982, **60**, 1.

In $[Mn_3(\beta\text{-Ala})_8](ClO_4)_6 \cdot 2H_2O$ the manganese atoms form infinite, almost linear chains linked by carboxyl bridges.[176]

One of the most potent histamine H_2 receptor antagonists is cimetidine (24), marketed under the trade name Tagamet and used for the treatment of peptic ulcer. The drug gives blue and green complexes with copper(II). The green complex contains the cimetidine ligand, but in the blue complex copper-catalysed solvolysis (by water or methanol) of the nitrile function has occurred. Such metal-ion-promoted reactions of nitriles are well established.[177] The crystal structure of the green copper(II) complex has been published,[178] as has that of the blue complex of the carboxamido derivative (25) produced on hydrolysis.



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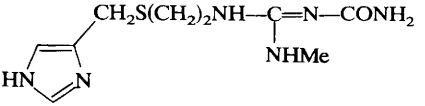

(25)

Stereochemistry and Stereoselectivity.—Aldol reactions at the α-CH_2 functions of [Cu(L-AA)(GlyO)] (L-AA^- = the anion of L-Val, L-Pro, L-Lys, or L-Glu) with benzaldehyde or butanal give β-phenylserine and β-hydroxynorleucine, respectively. An excess of *threo* over *erythro* diastereoisomers (1.6—3.1 : 1) is obtained, the ratio depending both on the reacting aldehyde and the L-amino-acid. The observed stereoselectivity is discussed in terms of a mechanism involving a bicyclic oxazolidine complex.[179] Work in this area has been recently reviewed.[180]

The rate of the α-hydrogen-exchange reaction of glycinato ligands in *mer*-tris(glycinato)cobalt(III) has been found to vary for the three stereochemically different glycinato ligands.[181] The carboxylate oxygen ligand is more effective than the amino nitrogen in labilizing the *trans* α-hydrogen. X-Ray data suggest that the kinetic *trans* influence results in part from a structural *trans* effect.

The compounds Δ,Λ-$[Ru(phen)_2(L\text{-}Trp)]ClO_4 \cdot 3H_2O$ and Δ,Λ-$[Ru(phen)_2(L\text{-}TrpO)]ClO_4 \cdot 3H_2O$ have been resolved. C.d. studies show that the Δ-diastereoisomers invert into the Λ-forms in the presence of light.[182] Light-catalysed diastereoisomeric equilibration in aqueous solutions of *cis*-$[Ru(phen)_2(L\text{-serine})]^+$ and its 2,2′-bipyridyl analogue has also been observed.[183] Models of such diastereoisomers suggest that the Λ-isomer (26) should be less sterically strained for any amino-acid of L-configuration. The Δ-diastereoisomer inverts

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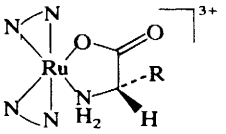
[177] See for example C. R. Clark and R. W. Hay, *J. Chem. Soc., Dalton Trans.*, 1974, 2148.
[178] F. T. Greenaway, L. M. Brown, J. C. Dabrowiak, M. R. Thompson, and V. M. Day, *J. Am. Chem. Soc.*, 1980, **102**, 7782.
[179] M. Girnth-Weller and W. Beck, *Inorg. Chim. Acta*, 1982, **57**, 107.
[180] D. A. Phipps, *J. Mol. Catal.*, 1979, **5**, 81.
[181] A. Miyanaga, U. Sakaguchi, Y. Morimoto, Y. Kushi, and H. Yoneda, *Inorg. Chem.*, 1982, **21**, 1387.
[182] R. S. Vagg and P. A. Williams, *Inorg. Chim. Acta*, 1981, **51**, 61.
[183] R. S. Vagg and P. A. Williams, *Inorg. Chim. Acta*, 1981, **52**, 69.

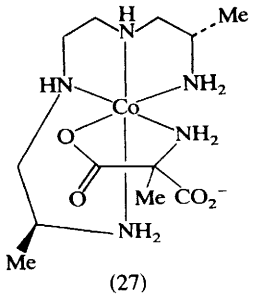


(26)

its configuration at the metal centre on exposure to light to give a thermodynamically stable equilibrium mixture in which the Λ-diastereoisomer predominates. Further studies using L-glutamate[184] and L-aspartate[185] derivatives have been published.

Complexes of the general types $[Co(en)_2(L\text{-}AA)]^{2+}$ and $[Co(en)(L\text{-}AA)_2]^{+}$ (L-AA = asparagine or glutamine) have been prepared and their detailed stereochemistry has been studied.[186] The Λ-diastereoisomers with equatorially disposed ligand side chains predominate. Stereoselective co-ordination of optically active amino-acids and their derivatives to cobalt(III) has been the subject of a recent thesis.[187] The origins of the enantioselection in asymmetric catalytic hydrogenation (using Rh^{I} complexes of chelating chiral diphosphines) of amino-acid precursors have been discussed in detail.[188]

Several years ago[189] it was shown that decarboxylation of the Λ-β_2-complex (27) in 1 mol dm^{-3} HCl, producing bound alanine, gives a predominance of the *S*-enantiomer. The use of residual circular dichroism has now been employed[190] to study such inter-ligand steric effects and to predict the outcome of asymmetric synthesis carried out on substrates bound to dissymmetric transition-metal moieties. Asymmetric induction on the decarboxylation of α-amino-α-alkylmalonic acids chelated to chiral cobalt(III) complexes has also been investigated.[191] In some cases considerable asymmetric induction was observed in the formation of the amino-acidato complexes.



(27)

[184] R. S. Vagg and P. A. Williams, *Inorg. Chim. Acta*, 1982, **58**, 101.
[185] T. J. Goodwin, P. A. Williams, and R. S. Vagg, *Inorg. Chim. Acta*, 1982, **63**, 133.
[186] F. Jursik, B. Hajek, S. A. Moez, and R. D. Archer, *Inorg. Chim. Acta*, 1982, **57**, 51.
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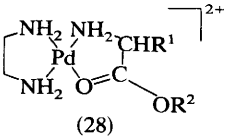
[191] M. Yamaguchi, S. Yamamatsu, H. Oikawa, M. Saburi, and S. Yoshikawa, *Inorg. Chem.*, 1981, **20**, 3179.

A number of papers have appeared dealing with stereoselectivity between Λ- and Δ-β_2-diastereoisomers of cobalt(III) complexes with the general formula [Co(Schiff base)(L-AA)] (Schiff base = sal_2en^{2-}, α-Me-sal_2en^{2-}, or 5,6-benzo-sal_2en^{2-}, L-AA^- = amino-acid anion).[192—194] The spontaneous resolution of $[Co(NH_3)_4(Val)]X_2$ and $[Co(NH_3)(Leu)]X_2$ (X = Cl^- or Br^-) has been studied,[195] as has the spontaneous resolution of $[Co(en)_2(Gly)]^{2+}$ salts.[196]

Although both *trans* (*N*, olefin) and *cis* (*N*, olefin) isomers of [Pt(amino-acid)(olefin)Cl] have previously been reported, the relative stability of the isomers has not been determined. N.m.r. evidence has now been presented for the thermodynamic preference for the *cis* (*N*, olefin) isomer.[197] Stereoselective ion-pair formation between $[Co(en)_3]^{3+}$ and $[Co(ox)_2(Gly)]^{2-}$ has been studied.[198]

Kinetics and Reactivity.—Amino-acid esters react with $[Pd(en)(OH_2)_2]$ to give complexes of the type (28). The chelate ester complexes are susceptible to nucleophilic attack by water and hydroxide ion. Substantial rate accelerations (factors of 4×10^4 for GlyOEt to 1.4×10^7 for ethyl picolinate) were observed in comparisons with base hydrolysis of the free esters.[199] Similar kinetic studies have been reported using 2,2'-bipyridyl as the inert ligand on palladium.[200]



(28)

Correlations of λ_{max} values for tridentate copper(II) complexes have been noted both with the log of the rate constant for the copper(II)-complex-catalysed hydrolysis of methyl glycinate and with the log of the formation constant of the hydroxo complex of the copper chelate.[201]

Amino-acid esters react with $[Cu(imda)]^0$ (imda = iminodiacetate) to give mixed-ligand complexes (29).[202] The complexed α-amino-acid esters undergo base hydrolysis *ca.* 10^4 times faster than the free esters $NH_2CHR^1CO_2R^2$, suggesting a weak axial interaction between the alkoxycarbonyl and copper(II).

The formation of $[Co(en)_2(GlyO)]^{2+}$ from the monodentate complexes derived from (30) has been studied in detail.[203] Stereospecific co-micelle-promoted hydrolysis of *N*-acylphenylalanine *p*-nitrophenyl esters in the presence and absence of cobalt(II) has been investigated.[204]

[192] Y. Fujii, K. Shiono, K. Ezuka, and T. Isago, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 3537.
[193] Y. Fujii, M. Matsufuru, A. Saityo, and S. Tsuchiya, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 2029.
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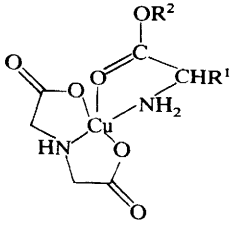
[202] R. W. Hay and P. K. Banerjee, *J. Inorg. Biochem.*, 1981, **14**, 147.
[203] C. J. Boreham and D. A. Buckingham, *Inorg. Chem.*, 1981, **20**, 3112.
[204] K. Ohkubo, K. Kawazoe, and M. Toyoda, *J. Mol. Catal.*, 1980, **9**, 219.



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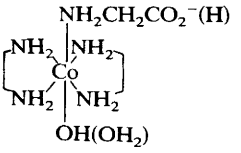
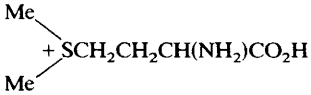


(30)

The photochemistry of copper(II)–amino-acid complexes (Glu, β-Ala, or Gly) has been studied by continuous and flash photolysis.[205] Charge-transfer irradiation causes oxidation of the ligand and the reduction of copper(II) to copper(I). Photochemical decarboxylation of $[Co(phen)(GlyO)]^{2+}$ has also been investigated.[206]

The kinetics of oxygen transfer from $[MoO_2(L\text{-}CysOEt)_2]$ to PPh_3 and the reaction between $[Mo_2O_3(L\text{-}CysOEt)_4]$ and O_2 in benzene solvent have been studied[207] and activation parameters determined. Silver(II) oxidation of amino-acids leads to decarboxylation, and the kinetics have been investigated in detail.[208]

The formation and dissociation of pentacyanoferrate(II) complexes of cysteine, glutathione, penicillamine, and 2-mercaptoethylamine have been studied.[209] A correlation between the formation rate constants and the ligand charge type was observed. A further investigation has dealt with the kinetics of the reaction of cysteine with pentacyanonitrosylferrate(2−).[210] A reinvestigation of the thermal decomposition of the copper(II) complex of L-methylsulphonium methionine (31) has been reported.[211] Dimethyl sulphide is rapidly lost at 145 °C.



(31)

N.m.r. relaxation studies have been carried out on the copper(II)–glycine system[212] and the copper(II)–bipyridylglycine system.[213] The formation of the Co^{II} and Ni^{II} complexes of *O*-phospho-DL-serine has been studied kinetically.[214] ^{13}C relaxation data have been used to study the interaction of Mn^{II} with L-proline.[215] Other kinetic studies have dealt with the anation of

[205] P. Natarajan and G. Ferraudi, *Inorg. Chem.*, 1981, **20,** 3708.
[206] A. L. Poznyak and V. I. Pavlovskii, *Zh. Neorg. Khim.*, 1981, **26,** 539.
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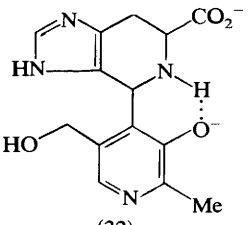
[209] D. H. Macartney and A. McAuley, *J. Chem. Soc., Dalton Trans.*, 1981, 1780.
[210] P. J. Morando, E. B. Borghi, L. M. de Schteingart, and M. A. Blesa, *J. Chem. Soc., Dalton Trans.*, 1981, 435.
[211] P. O'Brien, *Inorg. Chim. Acta*, 1981, **55,** L27.
[212] I. Nagypal, F. Debreczeni, and R. E. Connick, *Inorg. Chim. Acta*, 1981, **48,** 225.
[213] F. Debreczeni and I. Nagypal, *Inorg. Chim. Acta*, 1982, **57,** 135.
[214] T. M. Che and K. Kustin, *J. Inorg. Biochem.*, 1980, **13,** 267.
[215] B. Henry, J.-C. Boubel, and J.-J. Delpuech, *Polyhedron*, 1982, **1,** 113.

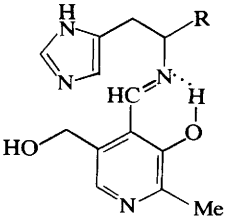
$[Cr(H_2O)_6]^{3+}$ by glycine,[216] the aquation of [Co(biguanide)(GlyO)]ClO_4,[217] the reaction of amino-acid esters with tetra-ammineruthenium-(II) and -(III),[218] temperature-jump studies of complex formation between nickel(II) and L-glutamate in aqueous solution,[219] and n.m.r. studies of intramolecular and intermolecular exchange processes in the Zn^{II}–iminodiacetate system.[220]

Schiff Bases.—As noted in the last review there seems to be a resurgence of interest in the area of Schiff bases. Structures have been reported for (pyridoxylidene-DL-valinato)copper(II) and (3-methoxysalicylidene-DL-valinato)copper(II).[221] The former compound is polymeric with square-pyramidal co-ordination to copper; the latter is monomeric with a planar co-ordination geometry that includes a donor oxygen from water. The *X*-ray structure of the 1:1 complex between copper(II) and pyridoxylidene-L-histidine establishes a trimeric molecule where each copper is co-ordinated in a plane by the phenolic oxygen, imine nitrogen, and carboxylic oxygen of one Schiff base and the histidine nitrogen of another ligand with water in the fifth apical site.[222]

Pyridoxal reacts with L-histidine, L-histidine methyl ester, histamine, and *N'*-methylhistamine to give 4,5,6,7-tetrahydropyrido[3,4-d]imidazole compounds (32) *via* the formation of Schiff-base intermediates. Zn^{II} ions can function as a trap for Schiff-base intermediates, and a number of zinc(II) complexes of *N*-pyridoxylidene-L-amino-acids and L-salicylidene-L-amino-acids have been obtained.[223] Copper(II) complexes of Schiff bases derived from pyridoxal, salicylaldehyde, or pyruvic acid and histidine, histidine methyl ester, and representative amino-acids with non-polar side chains have been prepared by metal-ion template synthesis, and their stereochemistry has been studied.[224] Japanese work[225] has also shown that cyclization (an intramolecular Mannich reaction) of the pyridoxal Schiff base of histidine or histamine (33) is inhibited by metal ions such as Cu^{II} and Zn^{II}.



(32)



(33) R = H or CO_2H

[216] I. A. Khan and K.-U. Din, *J. Inorg. Nucl. Chem.*, 1981, **43**, 1082.
[217] B. Chakravarty, S. Modak, P. K. Das, and A. K. Sil, *J. Inorg. Nucl. Chem.*, 1981, **43**, 3253.
[218] N. S. Scott and H. Taube, *Inorg. Chem.*, 1981, **20**, 3135.
[219] K. Tamura, S. Harada, Y. Funaki, and T. Yasunaga, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 813.
[220] M. C. Gennaro and P. Mirti, *J. Inorg. Nucl. Chem.*, 1981, **43**, 1711.
[221] H. M. Dawes, J. M. Waters, and T. N. Waters, *Inorg. Chim. Acta*, 1982, **66**, 29.
[222] H. M. Dawes and T. N. Waters, *J. Chem. Soc., Chem. Commun.*, 1982, 1390.
[223] L. Casella and M. Gullotti, *J. Am. Chem. Soc.*, 1981, **103**, 6338.
[224] L. Casella, M. Gullotti, and G. Pacchioni, *J. Am. Chem. Soc.*, 1982, **104**, 2386.
[225] H. Kondo, H. Yoshinaga, K. Morita, and J. Sunamoto, *Chem. Lett.*, 1982, 31.

Threonine is converted to glycine and acetaldehyde and serine is converted to glycine and formaldehyde, respectively, in pyridoxal-activated enzyme systems. The reaction has also been shown to take place with pyridoxal and metal ions at lower rates.[226] Detailed kinetic studies of the pyridoxal and the pyridoxal-metal-ion-catalysed dealdolization of threonine, β-hydroxyleucine, and β-hydroxyvaline have now been published,[227] and a possible mechanism has been suggested. Under comparable conditions the rates of Al^{III} and Ga^{III} chelate catalysis were found to be 2—6 times higher than those of the metal-free systems. Pyridoxal-catalysed β-elimination and dealdolization reactions of β-hydroxyglutamic acid have been found to occur simultaneously.[228] Metal-ion catalysis was found to be less effective than proton catalysis in the promotion of γ-decarboxylation, and the catalytic effects of metal ions were found to decrease with increasing charge on the metal ion.

The kinetics of β-elimination of *O*-phosphoserine, β-chloroalanine, *S*-ethylcysteine, and β-chloro-α-aminobutyric acid have been investigated by n.m.r. in D_2O in the presence of pyridoxal and in the presence and the absence of Ga^{III}, Al^{III}, and Zn^{II} ions.[229] Rate constants for pyridoxal catalysis in the presence of metal ions are some ten times greater than those observed in the metal-free systems.

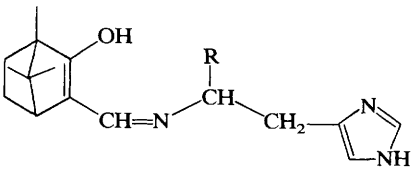
The kinetics and stereochemistry of deuterium exchange of the α-hydrogen in metal complexes of amino-acid Schiff bases with *o*-hydroxyacetophenone have been studied.[230] In such complexes steric interactions may provide a sufficiently long lifetime for the non-planar chiral carbanion, such that retention of configuration is observed.

Copper(II) complexes of Schiff bases derived from either salicylaldehyde or 2-hydroxy-1-napthaldehyde with L-serine, L-histidine, histamine, and L-tryptophan have been prepared and their spectroscopic properties examined.[231]

The Co^{III}, Cr^{III}, Mn^{III}, and Cu^{II} complexes of ethylenebis-(*o*-hydroxyphenyl)glycine have been prepared as models for the metal-binding site in the iron-transport protein transferrin.[232] E.s.r. spectra of copper(II) complexes of Schiff bases derived from salicylaldehyde and various amino-acids have been studied.[233] The complexes have a distorted planar structure with two oxygen and two nitrogen donors.

The reaction of pyridoxal and pyridoxamine with Fe^{III} has been studied kinetically.[234] C.d. spectra have been measured for copper(II) complexes of the

[226] See for example J. A. Marcello, A. E. Martell, and E. H. Abbott, *J. Chem. Soc., Chem. Commun.*, 1975, 16.
[227] J. A. Marcello and A. E. Martell, *J. Am. Chem. Soc.*, 1982, **104**, 1087.
[228] K. Tatsumoto and A. E. Martell, *J. Am. Chem. Soc.*, 1981, **103**, 6203.
[229] K. Tatsumoto, A. E. Martell, and R. J. Motekartis, *J. Am. Chem. Soc.*, 1981, **103**, 6197.
[230] Y. N. Belokon, A. S. Melikyan, V. I. Bakhmutov, S. V. Vitt, and V. M. Belikov, *Inorg. Chim. Acta*, 1981, **55**, 117.
[231] L. G. MacDonald, D. H. Brown, J. H. Morris, and W. E. Smith, *Inorg. Chim. Acta*, 1982, **67**, 7.
[232] M. G. Patch, K. P. Simolo, and C. J. Carrano, *Inorg. Chem.*, 1982, **21**, 2972.
[233] L. G. MacDonald, D. H. Brown, and W. E. Smith, *Inorg. Chim. Acta*, 1982, **63**, 213.
[234] M. S. El-Ezaby and A.-S. I. Abu-Shady, *Inorg. Chim. Acta*, 1981, **55**, 29.



(34) R = H, CO_2H, or CO_2Me

Schiff bases (34) derived from (1*R*)-3-(hydroxymethylene)camphor and histidine derivatives.[235]

General.—The regulation of ionic-nickel uptake and cytotoxicity by specific amino-acids and serum components has been studied.[236] Twenty-seven chelating agents have been screened as possible antidotes for acute nickel(II) poisoning.[237] The observation that *N*-acetyl-DL-penicillamine has none of the antidotal activity of D-penicillamine establishes the essential role of the nitrogen donor in the latter ligand.

Approximately 5% of the copper in blood plasma is bound to albumin protein, which is in equilibrium with copper bound to amino-acids:

$$Cu^{II} + \text{albumin} \rightleftharpoons [Cu^{II}(\text{albumin})]$$

$$Cu^{II}(\text{albumin}) + AA \rightleftharpoons [Cu^{II}(AA)] + \text{albumin}$$

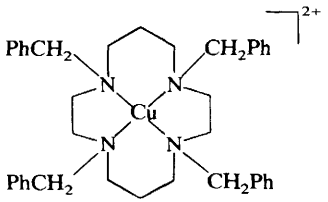
The amino-acid complexes are thought to mediate the transport of copper through biological membranes (because of their small molecular size). Histidine is believed to be the major ligand for copper in the amino-acid complexes. The influence of L-cysteine on the exchangeable copper in blood plasma has now been studied.[238]

Plants that grow on soils having normally toxic metal concentrations are described as metal tolerant. High proline levels have been observed in the roots of copper-tolerant *Armeria maritima* (Sea pink).[239] The high proline levels are believed to be associated with copper tolerance and are not a response to copper stress.

Polyamino-polycarboxylates administered as their calcium or zinc complexes are the most effective antidotes for acute cadmium poisoning.[240] Studies of cadmium poisoning are complicated by the synthesis (after a short time lag) of the cadmium-binding protein metallothionein, which immobilizes the cadmium in the liver and kidney.

Active transport of amino-acid anions by metal complexes has been studied.[241,242] Thus the copper(II) complex of the macrocyclic ligand (35) is

[235] L. Casella and M. Gullotti, *Inorg. Chem.*, 1981, **20**, 1306.
[236] M. P. Abbracchio, R. M. Evans, J. D. Heck, O. Cantoni, and M. Costa, *Biol. Trace Element Res.*, 1982, **4**, 289.
[237] M. M. Jones, M. A. Basinger, and A. D. Weaver, *J. Inorg. Nucl. Chem.*, 1981, **43**, 1705.
[238] S. H. Laurie and E. S. Mohammed, *Inorg. Chim. Acta*, 1981, **55**, L63.
[239] M. E. Farago and W. A. Mullen, *Inorg. Nucl. Chem. Lett.*, 1981, **17**, 275.
[240] M. A. Basinger, M. M. Jones, and L. A. Shinobu, *J. Inorg. Nucl. Chem.*, 1981, **43**, 3039.
[241] H. Tsukube, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 304.
[242] K. Maruyama, H. Tsukube, and T. Araki, *J. Am. Chem. Soc.*, 1982, **104**, 5197.



(35)

active in transporting amino-acid anions across synthetic membranes.[241] Presumably the amino-acid anion is carboxylate bonded to one of the axial sites in the lipophilic metal complex, thus allowing transport across the membrane.

Ligand-exchange chromatography is a useful technique for the resolution of α-amino-acids. The packing is prepared by grafting an inorganic substrate, or an organic gel, with a chiral ligand (generally an L-α-amino-acid) complexed by a transition-metal ion. Soluble models of this packing have been developed by reaction of isobutyramide and polyacrylamide with L-proline.[243] Ternary metal complexes of these ligands have been studied by potentiometry and ^{1}H n.m.r. to define the structures required for stereoselection.

Gold(0) dissolves in a wide range of aqueous amino-acid solutions exposed to dioxygen.[244] The thiol-containing molecules cysteine, penicillamine, and glutathione give solutions of gold(I) complexes that can be identified by circular dichroism. The possible significance of these results in terms of erosion of jewelry and skin-irritant reactions is also discussed.

The absorption of L-asparagine and L-alanine on the inorganic ion exchanger α-zirconium phosphate has been studied in detail.[245] Further studies with other amino-acids have been reported.[246] Iron(III) phenoxide complexes have been investigated as models for iron(III)–tyrosine co-ordination.[247] Complexes formed by naturally occurring epimers of L-threonine with divalent metal ions are more thermodynamically stable than the analogous complexes of L-*allo*-threonine. A conformational study of these complexes has been carried out, the difference in average Gibbs energies of the epimeric chelates being in rough accordance with the thermodynamic results.[248]

3 Peptides

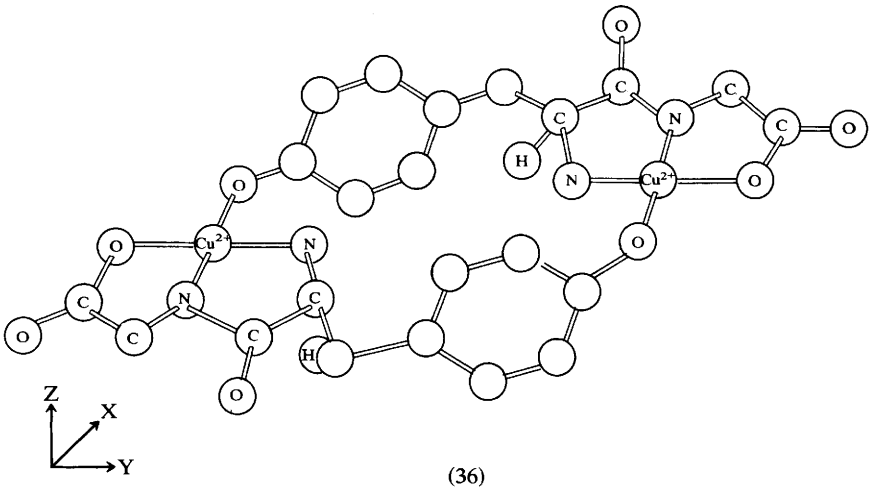
Complexes with Divalent Metal Ions.—Reports in this area are dominated by complexes of copper(II), and the majority of these deal with equilibrium studies in aqueous solution.

Formation constants are reported for the copper(II) complexes of the dipeptides (HA) β-alanyl-L-histidine (L-carnosine), L-histidylglycine, and glycyl-L-histidine, and their structures in solution are discussed.[249] In addition to

[243] F. Lafuma, J. Boue, R. Audebert, and C. Quivoron, *Inorg. Chim. Acta*, 1982, **66**, 167.
[244] D. H. Brown, W. E. Smith, P. Fox, and R. D. Sturrock, *Inorg. Chim. Acta*, 1982, **67**, 27.
[245] T. Kijima, Y. Sekikawa, and S. Ueno, *J. Inorg. Nucl. Chem.*, 1981, **43**, 849.
[246] T. Kijima, S. Ueno, and M. Goto, *J. Chem. Soc., Dalton Trans.*, 1982, 2499.
[247] S. A. Koch and M. Millar, *J. Am. Chem. Soc.*, 1982, **104**, 5255.
[248] N. Raos, S. R. Niketic, and V. Simeon, *J. Inorg. Biochem.*, 1982, **16**, 1.
[249] I. Sóvágó, E. Farkas, and A. Gergely, *J. Chem. Soc., Dalton Trans.*, 1982, 2159.

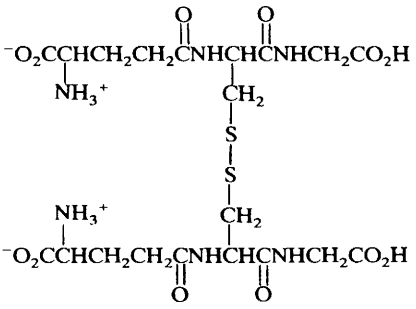
forming complexes $[CuA]^+$ where the ligand is attached to the metal through its amino, peptide oxygen, and carboxylate groups, the first two peptides both form dimers $(CuAH_{-1})_2$ in which the peptide linkages are deprotonated and the imidazole groups provide the bridges between the metal ions. In contrast, glycyl-L-histidine can function as a three-nitrogen donor (amino, deprotonated peptide, and imidazole nitrogen atoms) to give a very stable monomer $CuAH_{-1}$. At a ligand-to-metal ratio of 2:1 this binds a second peptide ligand *via* its imidazole nitrogen to give the species $Cu(AH_{-1})(AH)$ and $[Cu(AH_{-1})A]^-$. Heterobinuclear complexes of copper(II)-L-carnosine with zinc(II) and cadmium(II) have been investigated, and evidence has been presented for the existence of $[CuM(carH_{-1})]^{2+}$ and $[CuM(carH_{-2})]^+$ as the major species in solution.[250]

Formation constants have been determined potentiometrically for the 1:1 and 2:1 complexes of copper(II) with 13 aliphatic dipeptides containing various combinations of the amino-acids Gly, DL-Ala, DL-Leu, and DL-Pro.[251] Possible structures in solution as well as the influence of pH, ligand-to-metal rations, and nature of side chains on complex distribution are considered. The interaction between copper(II) and glycylglycyl-L-histidine, a ligand that simulates the binding characteristics of serum albumin, has been critically examined by combined spectrophotometric and potentiometric methods.[252] Formation constants of five 1:1 and five 2:1 complexes have been determined, and the visible spectra of the main species in solution have been computed. Formation constants are also reported for copper(II) complexes with six dipeptides involving L-tyrosine (HA).[253] Below pH 9 the major species in solution are $[CuA]^+$ and $CuAH_{-1}$. However, in the pH range 8—10.5 tyrosyl peptide complexes form green-coloured dimers $\{[Cu(AH_{-2})]_2\}^{2-}$ in which the phenolate groups provide double bridging between the metal ions (36).



(36)

[250] P. G. Daniele, P. Amico, and G. Ostacoli, *Inorg. Chim. Acta*, 1982, **66**, 65.
[251] W. S. Kittl and B. M. Rode, *Inorg. Chim. Acta*, 1981, **55**, 21.
[252] S. Lau and B. Sarkar, *J. Chem. Soc., Dalton Trans.*, 1981, 491.
[253] R. J. W. Hefford and L. D. Petit, *J. Chem. Soc., Dalton Trans.*, 1981, 1331.



(37)

Protonation constants and copper(II)-complex formation constants have been reinvestigated for oxidized glutathione, LH_4 (37).[254] This disulphide is formed in erythrocytes as the naturally occurring tripeptide glutathione and is oxidized by hydroperoxides. The complex distribution in the pH range 2—4 differs somewhat from that previously reported[255] in that an additional species $[CuLH_3]^+$ was characterized and the concentration of the neutral complex $CuLH_2$ was significantly lower. The effect of copper(II) on glutathione metabolism in leucocytes was shown to be due to inhibition of glutathione reductase.[256]

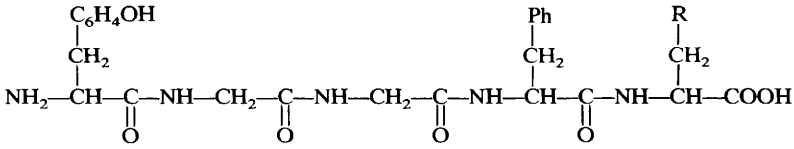
The copper(II) complexes of glycylglycine (HA) have been the subject of numerous investigations in the past. However, a recent paper[257] examines the structure in solution of the 2:1 complex $[CuA_2H_{-1}]^-$. By comparing the formation constants of glycylglycine complexes with those of glycinamide and ternary complexes involving glycylglycine and either ethylamine or 2-aminoethanol ligands, the predominant structure is suggested to be that which contains one tridentate, AH_{-1}^{-2}, and one monodentate, A^-, peptide ligand. Formation constants have been reported for the copper(II) complexes of glycinamide, β-alaninamide, and four dipeptides containing glycine and/or β-alanine.[258] The mixed-ligand complexes with 2,2′-bipyridine (bipy), $[Cu(bipy)A]^+$, and $Cu(bipy)AH_{-1}$ were also investigated. Peptide deprotonation in the binary systems was observed only in the glycine-containing ligands and in the glycinamide and glycylpeptide ternary systems.

A number of ternary copper(II) complexes with glycylglycine or glycinamide (A) and various amino-acid ligands (B) have been studied potentiometrically at 37 °C.[259] Formation constants and species distribution curves have been reported and structures for the various complexes in solution have been proposed. A previous but similar study by the same group[260] dealt with ternary complexes of copper(II) in the presence of histamine or L-histidine and a

[254] M. J. Blais and G. Berthon, *J. Chem. Soc., Dalton Trans.*, 1982, 1803.
[255] M. Micheloni, P. M. May, and D. R. Williams, *J. Inorg. Nucl. Chem.*, 1978, **40**, 1209.
[256] G. W. Rafter, *Biol. Trace Element Res.*, 1982, **4**, 191.
[257] N. Nakasuka, K. Makimura, and H. Kajiura, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3749.
[258] H. Sigel, B. Prijs, and R. B. Martin, *Inorg. Chim. Acta*, 1981, **56**, 45.
[259] M. S. Nair, *J. Chem. Soc., Dalton Trans.*, 1982, 561.
[260] M. S. Nair, M. Santappa, and P. Natarajan, *J. Chem. Soc., Dalton Trans.*, 1980, 2138.

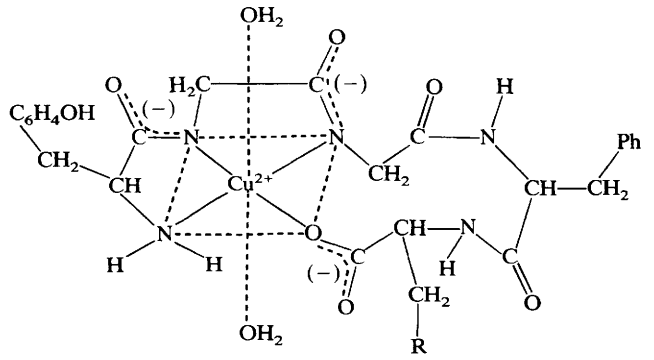
number of simple dipeptide ligands Mixed-ligand complexes of copper(II) with amino-acids and peptides, containing amide, hydroxy, or carboxylate groups in their side chains, have been investigated by potentiometric and calorimetric methods, and formation constants, species distribution, and structures in solution are discussed.[261]

A recent report describes a detailed study of the interaction between copper(II) and the Leu- and Met-enkephalins.[262] These pentapeptides (38)



(38)

were discovered in brain tissues in 1975 and later shown to be potent analgesics operating at the same receptor sites as natural opiates and their antagonists. The copper(II) complexes were investigated as a function of pH by paramagnetic n.m.r. perturbations and by electronic and e.s.r. spectroscopy. At physiological pH the major complex species contain tetradentate enkephalin ligands complexed through the amino, carboxylate, and two deprotonated peptide groups (39). The biological activity of the ligand is only slightly altered



(39)

by the metal.[263] Melanostatin, L-prolyl-L-leucylglycinamide, a hypothalamic hormone and a therapeutic agent for Parkinson's disease, forms complexes with copper(II), the structures of which are pH dependent.[264] At low pH the ligand is bonded *via* the deprotonated proline nitrogen but, with increasing pH, deprotonation and complexation of the peptide and amide groups occur to give finally at pH 8 a pink-coloured complex with four nitrogen donor atoms

[261] A. Gergely and E. Farkas, *J. Chem. Soc., Dalton Trans.*, 1982, 381.
[262] J. E. Gairin, H. Mazarguil, P. Sharrock, and R. Haran, *Inorg. Chem.*, 1982, **21**, 1846.
[263] P. Sharrock, R. Day, S. Lemaire, S. St.-Pierre, H. Mazarguil, J. E. Gairin, and R. Haran, *Inorg. Chim. Acta*, 1982, **66**, L91.
[264] G. Formicka-Kozlowska, H. Kozlowski, M. Bezer, L. D. Petit, G. Kupryszewski, and J. Przybylski, *Inorg. Chim. Acta*, 1981, **56**, 79.

around the metal. Formation constants of the copper(II) complexes and of a nickel(II) complex with this ligand are also reported. Investigations of copper(II) interactions with three tetrapeptides all containing one proline residue showed that proline co-ordination occurs only when it occupies the *N*-terminal position.[265]

The interaction of ferricyanide with the copper(II)–glycylglycine complex leads to the loss of the e.s.r. signal at 77 K and a lowering of magnetic susceptibility in solution.[266] This result has been explained in terms of antiferromagnetic coupling between copper(II) and iron(III) through cyano bridges and as such serves as a model for the interaction between ferricyanide and galactose oxidase and also for the low-spin cyano complex of oxidized cytochrome a_3.

In view of the fact that the enzyme superoxide dismutase contains imidazolate-bridged copper(II) and zinc(II) at its active site, the investigation of similarly bridged binuclear complexes has been of some interest. The crystal structure of $Na[Cu_2(AH_{-1})_2(imidazolate)]\cdot 6H_2O$ (HA = glycylglycine), in which antiferromagnetic exchange occurs between the metal ions, shows that each copper is in a square-planar environment of three nitrogens (amino, deprotonated peptido, and bridging imidazolate) and a carboxylate oxygen.[267] The mixed-ligand complexes of copper(II) with imidazole and glycylglycine or glycyl-β-alanine were found to be monomers in neutral solution but imidazolate-bridged dimers in base.[268]

A number of investigations by the Margerum group deal with reactions of metal–peptide complexes in solution. The tetraglycine (HL) complexes $[Cu(LH_{-3})]^{2-}$, $[Cu(LH_{-2})]^{-}$, and $CuLH_{-1}$ all show general acid catalysis in their reactions with acids, implying rate-determining protonation of the peptide nitrogen.[269] Nucleophilic attack by triethylenetetramine and its conjugate acids on $[Cu(LH_{-3})]^{2-}$ has also been studied, and reaction rates are compared with those previously reported for tripeptide complexes.[270] Similar investigations on nickel(II) complexes of glycylglycyl-L-histidine[271] and triglycine[272] have also been carried out. The electrochemical reduction of binary and ternary (with 2,9-dimethyl-1,10-phenanthroline, dmp) copper(II)–glycylglycine complexes has been studied by cyclic voltammetry, and in one case evidence has been obtained for the transient copper(I) complex $[Cu(AH_{-1})(A)(dmp)]^{-2}$ (HA = glycylglycine).[273] The rates of oxidation of twelve copper(II)–peptide complexes by $[IrCl_6]^{2-}$ to the corresponding copper(III) complexes have been measured,

[265] G. Formicka-Kozlowska, H. Kozlowski, I. Z. Siemion, K. Sobczyk, and E. Nawrocka, *J. Inorg. Biochem.*, 1981, **15**, 201.
[266] T. S. Srivastava and S. V. Deshpande, *Inorg. Chim. Acta*, 1981, **55**, L39.
[267] K. Matsumoto, S. Ooi, Y. Nakao, W. Mori, and A. Nakahara, *J. Chem. Soc., Dalton Trans.*, 1981, 2045.
[268] Y. Nakao, W. Mori, T. Sakurai, and A. Nakahara, *Inorg. Chim. Acta*, 1981, **55**, 103.
[269] M. P. Youngblood, K. L. Chellappa, C. E. Bannister, and D. W. Margerum, *Inorg. Chem.*, 1981, **20**, 1742.
[270] M. P. Youngblood and D. W. Margerum, *Inorg. Chem.*, 1980, **19**, 3072.
[271] C. E. Bannister, J. M. T. Raycheba, and D. W. Margerum, *Inorg. Chem.*, 1982, **21**, 1106.
[272] C. E. Bannister and D. W. Margerum, *Inorg. Chem.*, 1981, **20**, 3149.
[273] M. P. Youngblood and D. W. Margerum, *J. Coord. Chem.*, 1981, **11**, 103.

and an inner-sphere mechanism involving an axial chloride bridge is proposed for these reactions.[274]

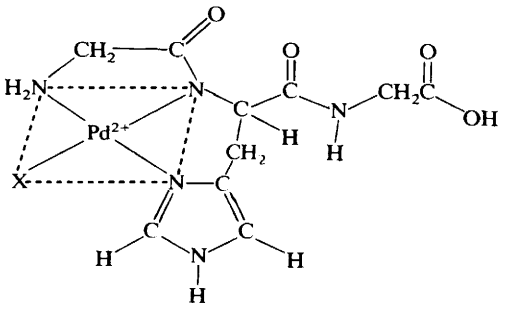
The cyclic hexapeptide *cyclo*-(Gly-His)$_3$ has been synthesized, and its 1:1 complexes with copper(II) and zinc(II) in solution have been observed.[275]

A number of papers deal with stabilities of complexes containing a series of metal ions. Formation constants and thermodynamic parameters have been determined for complexes of cobalt(II), nickel(II), copper(II), zinc(II), cadmium(II), and lead(II) with *N*-benzoylglycylglycine[276] and *N*-benzoylglycyl-L-leucine[277] in 50% water–dioxane solutions. Formation constants are also reported for complexes of cobalt(II), nickel(II), copper(II), and zinc(II) with *cyclo*-(L-histidyl-L-histidyl)[278] and for complexes of copper(II), manganese(II), nickel(II), and zinc(II) with the sulphur donor *cyclo*-(L-methionylglycyl).[279] The interaction of manganese(II), zinc(II),[280] nickel(II),[281] and cadmium(II) and mercury(II)[282] with thirteen dipeptides (HA) containing the α-amino-acid residues Gly, Ala, Leu, and Pro has been investigated potentiometrically. Formation constants, species distribution curves, and structures in solution of $[MA]^+$ and MA_2 species for all the metals and $[MA_3]^-$ for nickel(II) are discussed.

Despite the fact that a number of metal ions are known to promote the deprotonation of peptide groups, the only divalent ions of the first transition series reported to do so are cobalt(II), nickel(II), and copper(II). However, recently the related zinc(II)-promoted ionization of an amide group was claimed.[283] The ligand *N*-2-acetamidoiminodiacetate (ADA), $N(CH_2CO_2^-)_2(CH_2CONH_2)$, forms 1:1, M(ADA), and 2:1, $[M(ADA)_2]^{2-}$, complexes with cobalt(II) and zinc(II). In basic solution the 2:1 cobalt(II) complex undergoes stepwise amide-group deprotonations (pK_a values 10.03 and 11.34) with a characteristic pink to blue colour change. The 2:1 zinc(II) complex also ionizes in base (pK_a values 9.49 and 10.56), and the ^1H n.m.r. spectrum as a function of pD was claimed to imply amide-group deprotonation. However, this requires that the carboxymethyl and deprotonated amidomethyl resonances occur at exactly the same frequency. A change from an octahedral to tetrahedral zinc(II) complex is also claimed to occur with ionization.

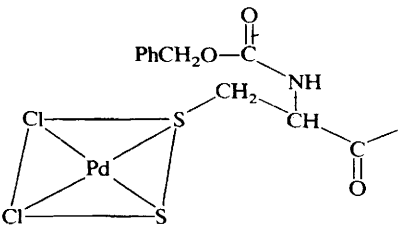
A number of reports have appeared on peptide complexes of palladium(II) and platinum(II). Complex formation between palladium(II) and glycyl-L-histidine in aqueous solution has been studied by ^1H n.m.r. spectroscopy, and the 1:1 complex was shown to contain the ligand attached to the metal ion through its

[274] G. D. Owens and D. W. Margerum, *Inorg. Chem.*, 1981, **20,** 1446.
[275] S. S. Isied, C. G. Kuehn, J. M. Lyon, and R. B. Merrifield, *J. Am. Chem. Soc.*, 1982, **104,** 2632.
[276] R. S. Sandhu and R. Kumar, *Thermochim. Acta*, 1981, **43,** 103.
[277] R. S. Sandhu and R. Kumar, *J. Indian Chem. Soc.*, 1981, **58,** 659.
[278] Y. Kojima, *Chem. Lett.*, 1981, **1,** 61.
[279] M. Bressan, M. L. Bon, and F. Marchiori, *Inorg. Chim. Acta*, 1982, **67,** L47.
[280] W. S. Kittl and B. M. Rode, *Inorg. Chim. Acta*, 1982, **63,** 47.
[281] W. S. Kittl and B. M. Rode, *Inorg. Chim. Acta*, 1982, **66,** 105.
[282] M. J. A. Rainer and B. M. Rode, *Inorg. Chim. Acta*, 1982, **58,** 59.
[283] E. A. Lance and R. Nakon, *Inorg. Chim. Acta*, 1981, **55,** L1.



(40)

amino, deprotonated peptide and imidazole nitrogen atoms (40).[284] Glycyl-L-histidylglycine was shown to co-ordinate similarly. The ligand carbobenzyloxycysteinylalanylalanylcysteine methyl ester (Z-Cys-Ala-Ala-Cys-OMe) forms the complex *cis*-$PdCl_2$(Z-Cys-Ala-Ala-Cys-OMe), in which it is chelated to the metal through its sulphur atoms (41).[285]



(41)

Although a number of studies suggest that DNA is the primary target for interaction of the anti-cancer drug *cis*-$PtCl_2(NH_3)_2$, the formation of a DNA–protein crosslink is also a possible mechanism for activity, with the protein very likely being metallated at a methionyl or cysteinyl residue.[286] As a model for such crosslinking, the *S*-bonded complex *cis*-$PtCl_2$-*cyclo*-(glycyl-L-methionyl)$_2$ has been prepared and its interaction with DNA investigated.[287] Several tetra-μ-carboxylatodirhodium(II) complexes also show significant anti-tumour activity. Since double-stranded DNA is inert towards these dimers, a mechanism involving protein binding has been proposed to account for their biological activity.[288] To understand the factors influencing the interaction of these complexes with protein functional groups, formation constants have been measured for the 1:1 and 2:1 adducts between tetra-μ-acetatodirhodium(II) and a number of nitrogen bases, including some dipeptides, in aqueous

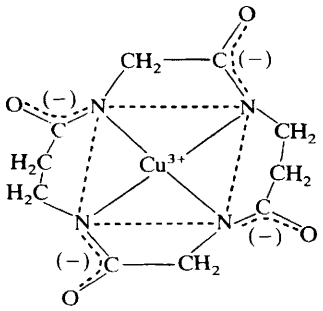
[284] D. L. Rabenstein, A. A. Isab, and M. M. Shoukry, *Inorg. Chem.*, 1982, **21**, 3234.
[285] N. Ueyama, M. Nakata, and A. Nakamura, *Inorg. Chim. Acta*, 1981, **55**, L61.
[286] L. A. Zwelling, K. W. Kohn, W. E. Ross, R. A. G. Ewig, and T. Anderson, *Cancer Res.*, 1978, **38**, 1762.
[287] V. Guantieri, L. Pandolfo, and A. M. Tamburro, *Inorg. Chim. Acta*, 1982, **67**, L51.
[288] A. M. Dennis, R. A. Howard, and J. L. Bear, *Inorg. Chim. Acta*, 1982, **66**, L341.

solution at 22 °C.[289] Adducts with imidazole bases are stabilized by π-back-bonding and have the largest formation constants of those investigated.

A number of ternary palladium(II) complexes with tridentate dipeptide and monodentate purine nucleotide ligands have been investigated by ^{1}H n.m.r. spectroscopy, and information has been deduced from the chemical shifts regarding the relative orientation of the ligands in the complexes.[290,291] Some cysteine-containing peptide (amino-protected) complexes of the type *trans*-$[PdCl_2(peptide)_2]^{2-}$, in which the peptides are thiolate-bonded to the metal, have been prepared, and their isomerization reactions as well as the activity of the metal–sulphur bonds in the *cis* products have been investigated.[292] Some *S*-methyl-L-cysteine-containing peptide complexes of palladium(II) have also been prepared and investigated by n.m.r. and c.d. spectroscopy.[293]

Complexes with Trivalent Metal Ions.—A number of papers by the Margerum group deal with peptide complexes of nickel(III) and copper(III). The kinetics of reduction of a series of copper(III) peptide complexes by $[Fe(CN)_6]^{4-}$,[294] SO_3^{2-},[295] and I^-[296] have been studied. Reduction by $[Fe(CN)_6]^{4-}$ proceeds by an inner-sphere mechanism involving a cyano bridge, provided the peptide ligand does not contain bulky groups. The rates are retarded by alkali-metal ions (M) due to formation of ion pairs $[MFe(CN)_6]^{3-}$. Reduction of the copper(III)–tetraglycine (HL) complex $[Cu(LH_{-3})]^-$ by sulphite occurs in two one-electron steps with a sulphite radical-anion intermediate. The electron-transfer self-exchange rate constant between the +III and +II oxidation states of the copper complex of tri-α-aminoisobutyric acid has been obtained by ^{1}H n.m.r. line broadening and found to be similar to rates of cross-reactions of copper(III)– and copper(II)–peptide complexes.[297] Entropy differences for several copper(III, II) and nickel(III, II) peptide couples were determined from cyclic voltammetric data and found to be of similar magnitude but opposite sign. This is consistent with the absence of axially co-ordinated H_2O in the nickel(II) and copper(III) peptide complexes but with its presence in the nickel(III) and copper(II) cases.[298] The copper(III) complex of the cyclic peptide *cyclo*-β-alanylglycyl-β-alanylglycyl (L), $[Cu(LH_{-4})]^-$ (42), is thermodynamically more stable than complexes of copper(III) with open-chain peptides, owing to the tightness of the metal cavity favouring the higher oxidation state.[299] The redox decomposition of the complex is also relatively slow because the macrocycle makes ligand substitution at equatorial positions

[289] P. N. Rao, M. L. Smith, S. Pathak, R. A. Howard, and J. L. Bear, *J. Natl. Cancer Inst.*, 1980, **64**, 905.
[290] P. I. Vestues and R. B. Martin, *Inorg. Chim. Acta*, 1981, **55**, 99.
[291] V. Scheller-Krattiger, K. H. Scheller, and R. B. Martin, *Inorg. Chim. Acta*, 1982, **59**, 281.
[292] N. Ueyama, K. Sasaki, M. Nakata, and A. Nakamura, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 2364.
[293] B. D. Reverend, C. Loncheux, T. Kowalik, and H. Kozlowski, *Inorg. Chim. Acta*, 1982, **66**, 205.
[294] J. M. Anast and D. W. Margerum, *Inorg. Chem.*, 1982, **21**, 3494.
[295] J. M. Anast and D. W. Margerum, *Inorg. Chem.*, 1981, **20**, 2319.
[296] J. M. T. Raycheba and D. W. Margerum, *Inorg. Chem.*, 1981, **20**, 45.
[297] C. A. Koval and D. W. Margerum, *Inorg. Chem.*, 1981, **20**, 2311.
[298] M. P. Youngblood and D. W. Margerum, *Inorg. Chem.*, 1980, **19**, 3068.
[299] J. S. Rybka and D. W. Margerum, *Inorg. Chem.*, 1981, **20**, 1453.



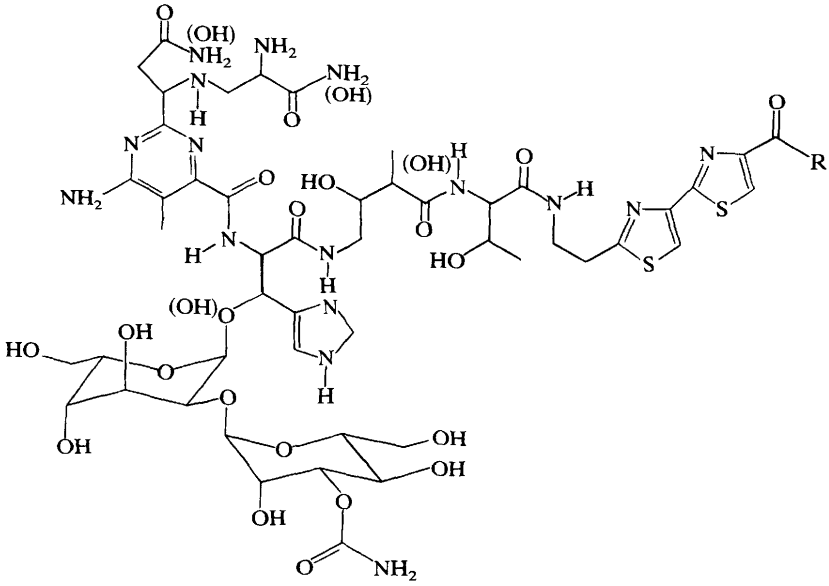
(42)

more difficult. Formation constants are reported for the axial co-ordination of monodentate nitrogen ligands to a number of nickel(III)–peptide complexes and follow the order imidazole > $NH_3 \simeq N_3^-$ > pyridine.[300]

Three cobalt(III) complexes of tetraglycine (HL), $[Co(NH_3)_2(LH_{-3})]^-$, $Co(NH_3)_3(LH_{-2})$, and $[Co(NH_3)_3(HLH_{-1})]^+$, have been prepared and fully characterized.[301] In the first of these, which contains the peptide as a tetradentate ligand, protonation of the terminal peptide nitrogen is observed in acid solution. From the reaction of the glycylglycine (HA) complex $[Co(AH_{-1})(NO_2)en]$ (en = 1,2-diaminoethane) with acetaldehyde in aqueous solution at pH 11, four different condensation products have been isolated.[302] A number of dipeptide (HA) complexes $[Co(AH_{-1})_2]^-$ have been prepared, and each was separated into two diastereoisomers by column chromatography.[303]

Interest in the co-ordination chemistry of the anti-tumour antibiotic bleomycin (43) continues. Although it has been known for some time that the antibiotic is capable of degrading DNA, recent evidence shows that an iron(II) complex of the drug reacts with molecular oxygen *in vivo* to produce radical species near the susceptible bonds of DNA. Two isomeric cobalt(III) complexes of bleomycin have been prepared, and the major product of acid hydrolysis has been studied by electronic, n.m.r., and c.d. spectroscopy.[304,305] This product is a CoN_5O-type complex having the primary amino group and water axially ligated, with a secondary amino group, a pyrimidine-ring nitrogen, a deprotonated-peptide nitrogen, and an imidazole nitrogen in the equatorial plane. A number of cobalt(III) complexes of bleomycin A_2 and B_2 have been prepared and separated by h.p.l.c.[306] Whereas the activity of bleomycin with respect to DNA degradation is reversibly inhibited by carbon monoxide and isocyanides, it is enhanced by cyanide. Indeed the interaction of

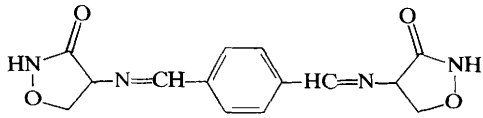
[300] C. K. Murray and D. W. Margerum, *Inorg. Chem.*, 1982, **21**, 3501.
[301] C. J. Hawkins and M. T. Kelso, *Inorg. Chem.*, 1982, **21**, 3681.
[302] Lj. R. Solujić and M. B. Ćelap, *Inorg. Chim. Acta*, 1982, **67**, 103.
[303] T. Yasui, H. Kawaguchi, and T. Ama, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 2918.
[304] M. Tsukayama, C. Randall, F. S. Santillo, and J. C. Dabrowiak, *J. Am. Chem. Soc.*, 1981, **103**, 458.
[305] J. C. Dabrowiak and M. Tsukayama, *J. Am. Chem. Soc.*, 1981, **103**, 7543.
[306] C. M. Vos and G. Westera, *J. Inorg. Biochem.*, 1981, **15**, 253.



(43) BLM A_2
$R = NH(CH_2)_3\overset{+}{S}Me_2\bar{X}$

cyanide with bleomycin–iron(II) and –iron(III) complexes was found to be unusual in that high-spin complexes are formed[307] [the adducts of iron(II)–bleomycin with carbon monoxide and isocyanides are low spin, as are the adducts of iron(III)–bleomycin with several nitrogen bases]. The total synthesis of bleomycin A_2 has recently been described.[308]

The complexes of the cycloserine derivative (44) with chromium(III), ruthenium(III), and rhodium(III) have been synthesized and structures suggested.[309]



(44)

In a recent study on the competition of enterobactin and thiols for iron it was found that glutathione can compete effectively with the siderophore at

[307] Y. Sugiura, S. Ogawa, and I. Morishima, *J. Am. Chem. Soc.*, 1980, **102,** 7944.
[308] Y. Aoyagi, K. Katano, H. Suguna, J. Primeau, L. H. Chang, and S. M. Hecht, *J. Am. Chem. Soc.*, 1982, **104,** 5537.
[309] C. Preti, L. Tassi, G. Tosi, P. Zannini, and A. F. Zanoli, *Aust. J. Chem.*, 1982, **35,** 1829.

pH <6.[310] A mechanism for iron extraction from iron(III)–enterobactin by glutathione involving initial reduction to the +II oxidation state has been postulated.

The temperature dependences of magnetic susceptibilities of aquo and hydro ferric haem *c* octapeptide in water and in water–ethyleneglycol solutions have been examined as model systems for spin equilibria in iron(III) haemoproteins.[311] Spin-state equilibrium constants and thermodynamic parameters have been calculated and compared with haemoproteins. The paramagnetic n.m.r. spectra of various ligated forms of this octapeptide complex are compared with those of corresponding cytochrome *c* complexes, and interactions responsible for the anisotropic distribution of spin density in cytochrome *c* have been suggested.[312] The electron-transfer reactivity of this haem octapeptide has also been measured.[313] Kinetics of reaction of two low-spin forms of ferric haem *c* undecapeptide with hydrogen peroxide in aqueous solution (pH 7 and 10.4) have been investigated.[314]

The complex formed between iron(III) and the protected (amino and carboxyl) tetrapeptide Z-Cys-Ala-Ala-Cys-OMe has electronic, e.s.r., and c.d. spectra similar to those of native oxidized rubredoxin.[315] Interactions between the octapeptide human angiotensin II and a number of lanthanide(III) ions in aqueous solution have been investigated by ^{1}H n.m.r. spectroscopy.[316]

Other Metal Complexes.—Attempts to synthesize active-site analogues of nitrogenase from tetrathiomolybdate and iron(II) salts in the presence of cysteine-containing peptides are reported.[317] An Mo—Fe—S unit with phenoxide ligands has also been synthesized.[318] Air oxidation of triphenylphosphine, catalysed by molybdenum(VI) complexes with cysteine-containing peptide ligands, was studied as a model system for molybdo-oxidase.[319]

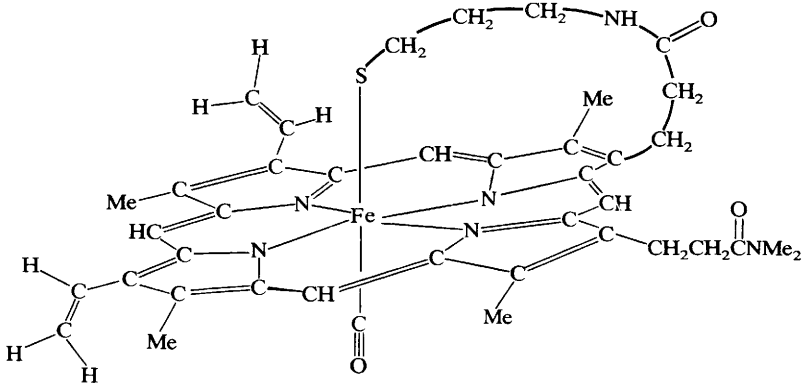
A number of diorganotin(IV) complexes with glycylglycine (HA), $R_2Sn(AH_{-1})$, have been prepared and found (in small doses) to be active against leukaemia.[320] The reaction between $BrRe(CO)_5$ and glycylglycine in boiling dioxane gives a monodentate (amino-bonded) peptide complex in the absence of base but a bidentate (amino- and carboxylate-bonded) peptide complex under basic conditions.[321]

[310] M. Y. Hamed, R. C. Hider, and J. Silver, *Inorg. Chim. Acta*, 1982, **66**, 13.
[311] Y.-P. Huang and R. J. Kassner, *J. Am. Chem. Soc.*, 1981, **103**, 4927.
[312] M. Smith and G. McLendon, *J. Am. Chem. Soc.*, 1981, **103**, 4912.
[313] G. McLendon and M. Smith, *Inorg. Chem.*, 1982, **21**, 847.
[314] G. M. Clore, M. R. Holloway, C. Orengo, J. Peterson, and M. T. Wilson, *Inorg. Chim. Acta*, 1981, **56**, 143.
[315] N. Ueyama, M. Nakata, and A. Nakamura, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 1727.
[316] R. E. Lenkinski and R. L. Stephens, *J. Inorg. Biochem.*, 1981, **15**, 95.
[317] A. Balasubramaniam and D. Coucouvanis, *Inorg. Chim. Acta*, 1982, **66**, L65.
[318] H. C. Silvis and B. A. Averill, *Inorg. Chim. Acta*, 1981, **54**, L57.
[319] N. Ueyama, E. Kamada, and A. Nakamura, *Chem. Lett.*, 1982, 947.
[320] R. Barbieri, L. Pellerito, G. Ruisi, M. T. Logiudice, F. Huber, and G. Atassi, *Inorg. Chim. Acta*, 1982, **66**, L39.
[321] A. A. Johansson, Y. G. Kovalyov, and E. D. Korniyets, *Inorg. Chim. Acta*, 1982, **65**, L31.

The binding of the oxovanadium(IV) cation to bleomycin has been investigated by potentiometric titrations and by electronic, e.s.r., and c.d. spectroscopy.[322] A ^{1}H n.m.r. study of the complexing of glutathione with trimethyllead in aqueous solution and in human erythrocytes shows the deprotonated sulphydryl group to be the strongest binding site for the metal.[323]

4 Proteins

Haem-containing Proteins.—A number of model systems for the cytochrome P450 enzymes have been investigated. Oxidation of chloro-5,10,15,20-tetramesitylporphinatoiron(III) with *m*-chloroperbenzoic acid gave a product for which an oxoiron(IV)–porphyrin cation radical structure, similar to that found in peroxidase compounds I, seems likely on the basis of spectroscopic evidence.[324] This product shows cytochrome P450-type behaviour in its ability to oxyfunctionalize hydrocarbons. Some very elegant model complexes for the active site of cytochrome P450 have been synthesized. These include a mercaptide-chelated protohaem, the iron(II) complex of which gives an adduct (45), with carbon monoxide, which has an electronic spectrum similar to that of



(45)

the cytochrome P450 adduct.[325] A series of tetra-arylporphyrins with alkyl- or aryl-mercaptan substituents, designed to serve as axial ligands, have also been synthesized.[326] The iron(II) complexes of these ligands in the presence of carbon monoxide and base give six-co-ordinate mercaptide–iron(II)–carbon monoxide complexes that reproduce the electronic and m.c.d. spectra of cytochrome P450. Electronic and m.c.d. spectra of mercaptide haem model compounds have provided evidence in favour of thiolate co-ordination in

[322] L. Banci, A. Dei, and D. Gatteschi, *Inorg. Chim. Acta*, 1982, **67**, L53.
[323] D. L. Rabenstein, S. J. Backs, and A. A. Isab, *J. Am. Chem. Soc.*, 1981, **103**, 2386.
[324] J. T. Groves, R. C. Haushalter, M. Nakamura, T. E. Nemo, and B. J. Evans, *J. Am. Chem. Soc.*, 1981, **103**, 2884.
[325] T. G. Traylor, T. C. Mincey, and A. P. Berzinis, *J. Am. Chem. Soc.*, 1981, **103**, 7084.
[326] J. P. Collman and S. E. Groh, *J. Am. Chem. Soc.*, 1982, **104**, 1391.

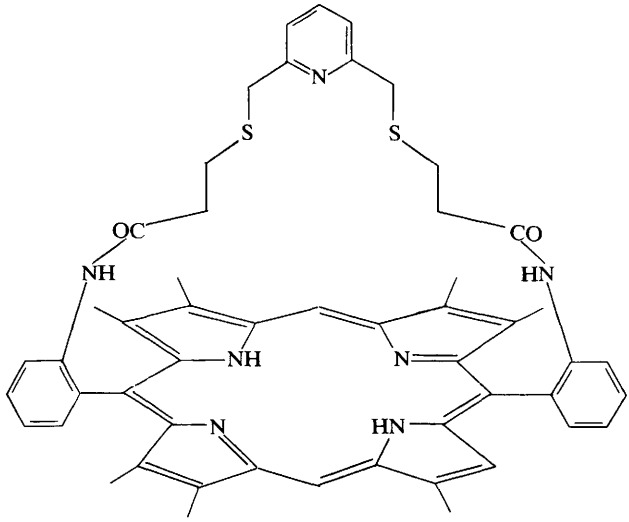
reduced high-spin cytochrome P450 and its oxygenated form.[327] The electronic and e.s.r. spectra of a number of iron(III)–protoporphyrin IX dimethyl ester complexes in the presence of various thiolate and oxygen donors as fifth and sixth ligands have been compared with those of cytochrome P450.[328] The results imply the presence of an oxygen donor ligand (rather than imidazole) at the sixth co-ordination site of the metal in the enzyme. The thiolate complex $[Fe(TPpivP)(SC_6HF_4)]^-$ (TPpivP = tetraphenylpivaloylporphyrin) forms a relatively stable dioxygen adduct that mimics the properties of the diamagnetic ferrous-oxy state in the reaction cycle of cytochrome P450.[329] The electronic spectra of derivatives of haemin incorporated into liposomes containing lipophilic imidazole have been compared with the spectra of cytochrome P450 and myoglobin derivatives.[330]

A number of papers deal with redox reactions between cytochrome *c* and simple inorganic complexes. Analysis of the kinetic data for the oxidation of ferrocytochrome *c* by $[Ru(NH_3)_5py]^{3+}$ (py = pyridine) over the pH range 1.25—6.8 points to the existence of an acid–base equilibrium (protonation constant $1.3 \times 10^3\ M^{-1}$), with the conjugate acid six times more susceptible to oxidation.[331] This is attributed to a conformational change on protonation, which allows easier access of the oxidant. The rates of oxidation of native ferrocytochrome *c* and four of its singly modified (4-carboxy-2,6-dinitrophenyl)-lysine derivatives by cationic and anionic oxidants have been measured.[332] Modification of the exposed lysine groups, Lys-13 and -72, enhances reaction rates with cationic oxidants but retards rates with anionic ones, suggesting that electron transfer occurs at the exposed haem edge. A number of six-co-ordinate bisthioether and thioether, imidazole iron–tetraphenylporphyrin complexes have been structurally investigated as models for cytochrome *c*.[333] The results imply that the iron–sulphur bond length in the protein is about 233 pm and is insensitive to the metal oxidation state. Frank–Condon effects are therefore not expected to have a major influence on rates of electron transfer. In addition the unexpectedly high affinity observed for thioether ligation to iron(III) obviates the need to invoke special protein enforcement of methionine binding in ferricytochrome *c*. Binding of aqueous copper(II) to ferricytochrome *c* over the pH range 2—9 has been investigated by e.s.r., n.m.r., and electronic spectroscopy, and the results point to histidine-33 as the main copper-binding site between pH 4 and 7.[334]

The active-site structure of cytochrome *c* oxidase still remains a challenging problem, and a number of iron–copper-containing model complexes have been

[327] S. Okubo, T. Nozawa, and M. Hatano, *Chem. Lett.*, 1981, 1625.
[328] H. Sakurai and T. Yoshimura, *Inorg. Chim. Acta*, 1982, **66**, L25.
[329] M. Schappacher, L. Ricard, R. Weiss, R. Montiel-Montoya, E. Bill, U. Gonser, and A. Trautwein, *J. Am. Chem. Soc.*, 1981, **103**, 7646.
[330] H. Sakurai, K. Iwawaki, and K. Fukuzawa, *Inorg. Chim. Acta*, 1981, **55**, L19.
[331] D. Cummins and H. B. Gray, *Inorg. Chem.*, 1981, **20**, 3712.
[332] J. Butler, D. M. Davies, A. G. Sykes, W. H. Koppenol, W. Osheroff, and E. Margoliash, *J. Am. Chem. Soc.* 1981, **103**, 469.
[333] T. Mashiko, C. A. Reed, K. J. Haller, M. E. Kastner, and W. R. Scheidt, *J. Am. Chem. Soc.*, 1981, **103**, 5758.
[334] M. A. Augustin and J. K. Yandell, *Aust. J. Chem.*, 1981, **34**, 91.

synthesized in an effort to elucidate at a molecular level the structural features responsible for the observed properties of the enzyme. Complexes of copper(II) with iron(III)–protoporphyrin IX containing 1:1 and 2:1 stoicheiometric ratios of copper to iron have been prepared.[335] In these the iron is high spin, and spectroscopic evidence suggests that the copper is bound to the propionate groups of the porphyrin and to a hydroxy bridging ligand, but antiferromagnetic coupling between the metal ions seems to be weak. Spectroscopic and magnetic evidence shows that in the trimeric complex $[Fe^{III}TPP]_2Cu^{II}MNT_2$ (H_2TPP = tetraphenylporphyrin, H_2MNT = *cis*-1,2,-dicyano-1,2-ethylenedithiolate) the iron atoms are of intermediate spin and are not strongly antiferromagnetically coupled to the copper, although the complex is almost e.s.r. silent.[336] This has been explained in terms of a combination of dipolar coupling and a small exchange coupling between the copper and the rapidly relaxing iron atoms, which results in the copper spin being relaxed and its e.s.r. signal being broadened beyond a point where it can be experimentally observed. A number of heterobinuclear iron(III)–copper(II) complexes of the strapped porphyrin (46) have been prepared, but exchange-coupling effects at least in the



(46)

solid state seem to be minimal.[337] A comparison of the electronic and resonance Raman spectra of low- and high-spin haem *a* in aprotic solvents and in water with those of reduced cytochrome a_3 show that the iron in the latter is high spin and that the dioxygen reducing site in cytochrome oxidase is

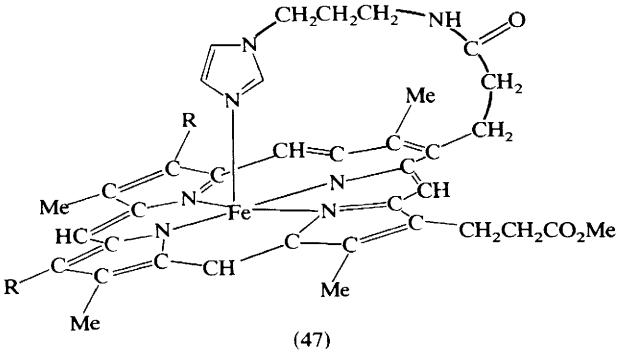
[335] B. Lukas, J. R. Miller, J. Silver, M. T. Wilson, and I. E. G. Morrison, *J. Chem. Soc., Dalton Trans.*, 1982, 1035.
[336] C. M. Elliott and K. Akabori, *J. Am. Chem. Soc.*, 1982, **104**, 2671.
[337] M. J. Gunter, L. N. Mander, K. S. Murray, and P. E. Clark, *J. Am. Chem. Soc.*, 1981, **103**, 6784.

hydrophobic.[338] Spectroscopic evidence for a superoxide bridge between copper(B) and the iron atom of cytochrome a_3 has also been reported with spin–spin coupling between the bridging ligand and copper(II) accounting for the absence of an e.s.r. signal.[339]

The pH-rate profile for the reduction of cytochrome b_5 by $[Fe(EDTA)]^{2-}$ is explained in terms of reduction of a protonated and an unprotonated (pK_a 5.85) form of the protein. From the dependence of rate on ionic strength the charge on the enzyme has been calculated and used to obtain its self-exchange rate constant.[340]

The ^1H n.m.r. spectrum of reduced horseradish peroxidase shows a single exchangeable resonance with hyperfine shift similar to that of proximal imidazole —NH in deoxymyoglobin, haemoglobin, and model compounds, indicating that the axial imidazole in ferro-horseradish peroxidase, contrary to other spectroscopic evidence, is not deprotonated.[341] Analysis of the electron–nuclear double-resonance spectrum of horseradish peroxidase compound I shows that the oxyferryl iron of this intermediate is coupled to a porphyrin π-cation radical.[342] Charge-iterative extended-Hückel molecular-orbital calculations are presented for compounds 1 and 2 of horseradish peroxidase and compound 1 of catalase, in which these intermediates are described in terms of ferryl (compound 2) and ferrylporphyrin π-cation radical (compound 1) configurations.[343]

Electron-withdrawing effects on the binding of dioxygen and carbon monoxide to haems have been investigated using the chelated protohaem (R = —CH=CH_2), mesohaem (R = —CHMe), and diacetyldeuterohaem (R = —COMe) series (47).[344] Equilibrium constants for carbon monoxide binding



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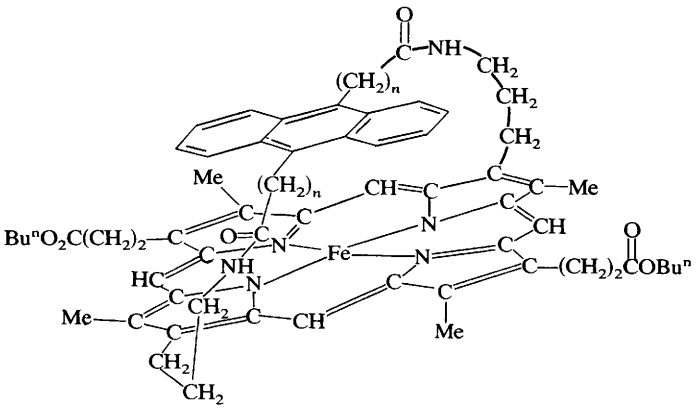
[338] J. Van Steelandt-Frentrup, I. Salmeen, and G. T. Babcock, *J. Am. Chem. Soc.*, 1981, **103**, 5981.
[339] G. M. Clore and M. Denis, *Inorg. Chim. Acta*, 1981, **55**, L47.
[340] L. S. Reid and A. G. Mauk, *J. Am. Chem. Soc.*, 1982, **104**, 841.
[341] G. N. La Mar and J. S. de Ropp, *J. Am. Chem. Soc.*, 1982, **104**, 5203.
[342] J. E. Roberts, B. M. Hoffman, R. Rutter, and L. P. Hager, *J. Biol. Chem.*, 1981, **256**, 2118.
[343] L. K. Hanson, C. K. Chang, M. S. Davis, and J. Fajer, *J. Am. Chem. Soc.*, 1981, **103**, 663.
[344] T. G. Traylor, D. K. White, D. H. Campbell, and A. P. Berzinis, *J. Am. Chem. Soc.*, 1981, **103**, 4932.

do not alter along the series, but an increase in the dioxygen dissociation rate is consistent with a structure such as $Fe^{\delta+}$—O—$O^{\delta-}$ with considerable charge separation in the iron–dioxygen group. A kinetic analysis of the reactions between carbon monoxide and dioxygen with cyclophane haems (48), providing different amounts of steric hindrance to ligand binding, has led to the conclusion that these molecules are not differentiated by distal side steric effects and therefore that such effects do not contribute significantly to the differentiation shown by haemoproteins.[345] These effects have also been investigated in iron–copper cofacial diporphyrins and in other strapped haems.[346]



(48)

The energetics of co-operativity in haemoglobin have been subjected to quantitative calculations, and it has been shown that, although the strain energy contributes a large fraction to the haem–haem interaction energy, there is also a considerable electrostatic contribution.[347] The iron–imidazole stretching modes in the resonance Raman spectra of deoxyhaemoglobin models in different solvents have been investigated and, for the complexes containing 2-methylimidazole spectral shifts, have been attributed to hydrogen bonding between the imidazole —NH group and the solvent.[348] Implications for haemoglobin co-operativity are discussed. The picosecond photodissociations of the carbon monoxide adducts of chelated protohaem and haemoglobin have been compared and the differences in the initial stages of reaction correlated with the geometry of the haem pocket.[349]

The reduction of iron(III) in bis(histidine)haemin as a model for the proton-coupled reduction of haemoproteins has been studied by cyclic voltammetry.[350]

[345] T. G. Traylor, M. J. Mitchell, S. Tsuchiya, D. H. Campbell, D. V. Stynes, and N. Koga, *J. Am. Chem. Soc.*, 1981, **103,** 5234.
[346] B. Ward, C. B. Wang, and C. K. Chang, *J. Am. Chem. Soc.*, 1981, **103,** 5236.
[347] A. Warshel and R. M. Weiss, *J. Am. Chem. Soc.*, 1981, **103,** 446.
[348] P. Stein, M. Mitchell, and T. G. Spiro, *J. Am. Chem. Soc.*, 1980, **102,** 7795.
[349] J. A. Hutchinson, T. G. Traylor, and L. J. Noe, *J. Am. Chem. Soc.*, 1982, **104,** 3221.
[350] D. A. Baldwin, V. M. Campbell, L. A. Carleo, H. M. Marques, and J. M. Pratt, *J. Am. Chem. Soc.*, 1981, **103,** 186.

Non-haem Iron-containing Proteins.—*X*-Ray absorption spectroscopy has been used to investigate the active-site structures of derivatives of haemerythrin, the naturally occurring oxygen carrier.[351] The binuclear iron cores in oxy- and met-haemerythrins are similar, with μ-oxo bridges similar to those in Fe_2O-containing standard compounds. Extended *X*-ray-absorption fine structure shows that in metazidohaemerythrin the iron is surrounded by a total of five nitrogen and oxygen ligands in addition to the bridging oxygen. The EXAFS (extended *X*-ray absorption fine structure) data for deoxyhaemerythrin indicate loss of the μ-oxo bridge upon reduction. Adduct formation between the two observed forms of semi-methaemerythrin and various anions and the kinetics and mechanism of disproportionation of these semi-met forms have been investigated.[352]

The visible spectra of transferrin and some of its metal-substituted derivatives have been analysed, and the intense absorption bands observed between 400 and 470 nm have been attributed to ligand-to-metal charge-transfer transitions originating on a phenolate oxygen.[353] The reaction between copper(II) species and apotransferrin has been the subject of a kinetic study.[354] A large number of complexes of the types FeO_6 and $FeO_2N_2O'_2$[355] and CuN_2O_2, $CuNOO'_2$, and $CuN_2OO'_2$[356] (O = phenolate oxygen, N = aliphatic or aromatic nitrogen, O' = carboxylate or methanolic oxygen) have been synthesized and examined spectroscopically in an effort to model the metal-binding sites of lactoferrin and dicopper(II)–lactoferrin. The reactions of purple uteroferrin with ferrous ion and pink uteroferrin with ferric ion have been investigated by e.s.r. and electronic spectroscopy.[357] Some complexes of general formula $[Fe_3O(\text{L-amino-acid})_3(H_2PO_3)(H_2O)_3](NO_3)_4$, containing triply bridging oxo and doubly bridging amino-acid and dihydrogen phosphite ligands, have been synthesized as models for the ferritin core.[358]

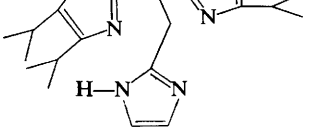
Combined EXAFS and Raman spectroscopy shows that the ferric ion at the active site of protocatechuate 3,4-dioxygenase is co-ordinated by one histidyl and two tyrosyl residues.[359,360] Ferric nitrilotriacetate has been investigated as an active-centre analogue of another non-haem iron oxygenase pyrocatechase.[361]

Some synthetic analogues of the iron–sulphur protein rubredoxin have been reported. These include a number of mono- to tetra-nuclear iron(II) tetra-

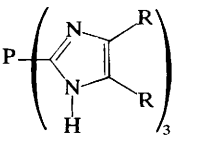
[351] W. T. Elam, E. A. Stern, J. D. McCallum, and J. Sanders-Loehr, *J. Am. Chem. Soc.*, 1982, **104**, 6369.
[352] P. C. Harrington and R. G. Wilkins, *J. Am. Chem. Soc.*, 1981, **103**, 1550.
[353] M. G. Patch and C. J. Carrano, *Inorg. Chim. Acta*, 1981, **56**, L71.
[354] T. S. Shoupe and C. D. Hubbard, *Polyhedron*, 1982, **1**, 361.
[355] E. W. Ainscough, A. M. Brodie, J. E. Plowman, K. L. Brown, A. W. Addison, and A. R. Gainsford, *Inorg. Chem.*, 1980, **19**, 3655.
[356] E. W. Ainscough, A. G. Bingham, A. M. Brodie, J. M. Husbands, and J. E. Plowman, *J. Chem. Soc., Dalton Trans.*, 1981, 1701.
[357] B. C. Antanaitis and P. Aisen, *J. Biol. Chem.*, 1982, **257**, 1855.
[358] R. N. Puri and R. O. Asplund, *Inorg. Chim. Acta*, 1982, **66**, 49.
[359] L. Que and R. M. Epstein, *Biochemistry*, 1981, **20**, 2545.
[360] R. H. Felton, W. L. Barrow, S. W. May, A. L. Sowell, S. Goel, G. Bunker, and E. A. Stern, *J. Am. Chem. Soc.*, 1982, **104**, 6132.
[361] M. G. Weller and U. Weser, *J. Am. Chem. Soc.*, 1982, **104**, 3752.

alkylthiolates[362] and tetraethylammonium tetra-(2,3,5,6-tetramethylbenzenethiolate)iron(III), the first example of a stable iron(III) tetrathiolate complex containing only monodentate ligands.[363] The use of *J*-modulated spin-echo pulse sequences for the determination of spin multiplicities in ^{13}C n.m.r. spectra has been applied to ferridoxin.[364] The complexes $[Fe_4S_4(SPh_4)]^{n-}$ ($n = 3$ or 4), $[Mo_2Fe_6S_9(SPh_8)]^{m-}$ ($m = 4$ or 5),[365] $[MoFe_4S_4(SEt)_3]^{3-}$,[366] and the double cubane cluster $[Mo_2Fe_6S_8(SEt)_6(Pr_2cat)_2]^{2-}$ (Pr_2cat = 3,6-di-n-propylcatecholate), which contains two $MoFe_3S_4(SEt)_2$ subclusters, two $Mo(Pr_2cat)$ chelates, and two unprecedented $Mo(\mu_2\text{-}SEt)Fe$ bridges,[367] have been synthesized as models for the catalytic site of the nitrogenase iron–molybdenum protein. The ^{57}Fe e.n.d.o.r. spectrum of this protein has been recorded, and six distinct iron sites were detected.[368] The solvent dependence of the electrochemical behaviour of $[Fe_6M_2S_8(SEt)_9]^{3-}$ has led to some observations concerning the participation of molybdenum but not tungsten in functional nitrogenases.[369] A simple molecular-orbital model for the delocalized *d*-electron system of polynuclear iron–sulphur clusters has been reported.[370]

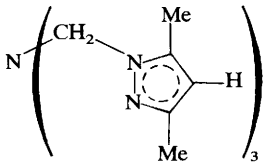
Zinc-containing Proteins.—The metalloenzyme carbonic anhydrase continues to attract much interest. The ligands (49) and (50) approximate the metal-binding sites of carbonic anhydrase, and the zinc(II) complexes of both show catalytic activity, albeit modest, towards the hydration of carbon dioxide.[371] The cobalt(II) complexes of the pyrazolyl ligand (51), L, $[Co(H_2O)L](ClO_4)_2$



(49)



(50) R = —$CHMe_2$



(51)

[362] K. S. Hagen and R. H. Holm, *J. Am. Chem. Soc.*, 1982, **104,** 5496.
[363] M. Millar, J. F. Lee, S. A. Koch, and R. Fikar, *Inorg. Chem.*, 1982, **21,** 4105.
[364] T. M. Chan, W. M. Westler, R. E. Santini, and J. L. Markley, *J. Am. Chem. Soc.*, 1982, **104,** 4008.
[365] K. Tanaka, M. Tanaka, and T. Tanaka, *Chem. Lett.*, 1981, 895.
[366] T. E. Wolff, J. M. Berg, and R. H. Holm, *Inorg. Chem.*, 1981, **20,** 174.
[367] W. H. Armstrong and R. H. Holm, *J. Am. Chem. Soc.*, 1981, **103,** 6246.
[368] B. M. Hoffman, R. A. Venters, J. E. Roberts, M. Nelson, and W. H. Orme-Johnson, *J. Am. Chem. Soc.*, 1982, **104,** 4711.
[369] C. D. Garner and R. M. Miller, *J. Chem. Soc., Dalton Trans.*, 1981, 1664.
[370] A. J. Thomson, *J. Chem. Soc., Dalton Trans.*, 1981, 1180.
[371] J. Huguet and R. S. Brown, *J. Am. Chem. Soc.*, 1980, **102,** 7572.

and $[Co(N_3)L]ClO_4$, have been synthesized, and the pH dependence of their electronic spectra is similar to that of cobalt(II)-substituted carbonic anhydrase.[372] The ^1H n.m.r. spectra of cobalt(II)-substituted carbonic anhydrase and some of its inhibitor derivatives show that all the histidine protons are present at pH 5.5—9, thus ruling out their involvement in an acid–base equilibrium in this pH range.[373] The e.s.r. spectra of inhibitor derivatives of cobalt(II)–carbonic anhydrase have been analysed, and their use in the interpretation of the geometry of the active site in cobalt enzymes is discussed.[374] Proton longitudinal relaxation rates of aqueous solutions of manganese(II), cobalt(II), and copper(II) carbonic anhydrase have been measured at frequencies between 4 and 60 MHz and used to obtain structural information on the water in the enzymatic cavity.[375] Water ^{17}O relaxation rates are reported for aqueous solutions of copper(II)–carbonic anhydrase, some of its inhibitor derivatives, and the native enzyme.[376] Spectra, formation constants, and proton longitudinal relaxation rates for ternary complexes, between various bidentate ligands and cobalt(II)–carbonic anhydrase, are discussed.[377] The reduction of cobalt(III)–carbonic anhydrase by L-ascorbic acid has been investigated.[378] The electronic and c.d. spectra of nickel(II)–carbonic anhydrase and its *p*-toluenesulphonamide adduct show low-symmetry components and are considered as five- rather than six-co-ordinate chromophores.[379] The dissociation of zinc(II) from carbonic anhydrase occurs by mechanisms involving either ternary complex formation (in the presence of 1,10-phenanthroline and 2,6-pyridinedicarboxylic acid) or spontaneous dissociation (in the presence of EDTA).[380] Electrostatic potential maps have been constructed and analysed for two isozymes of carbonic anhydrase, and the influence of solvent has been discussed.[381]

Water ^1H n.m.r. relaxation rates have been measured for solutions containing cobalt(II)-substituted carboxypeptidase A and its inhibitor derivative with β-phenylpropionate and provide evidence for co-ordinated water molecules in both cases.[382]

The catalytic zinc site of alcohol dehydrogenase has been studied by perturbed angular correlation spectroscopy on the ^{111}cadmium-substituted enzyme.[383] The spectra show no evidence for aquo-ligand ionization below pH 9 but are affected by competitive inhibitors such as pyrazole or trifluoroethanol that seemingly enter the metal co-ordination sphere as fifth ligands rather than by displacing co-ordinated water. The binding of imidazole

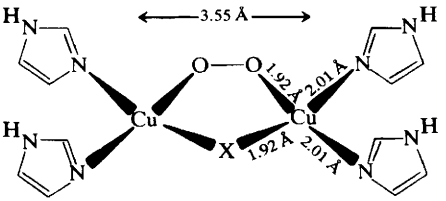
[372] I. Bertini, G. Canti, C. Luchinat, and F. Mani, *Inorg. Chem.*, 1981, **20**, 1670.
[373] I. Bertini, G. Canti, C. Luchinat, and F. Mani, *J. Am. Chem. Soc.*, 1981, **103**, 7784.
[374] A. Bencini, I. Bertini, G. Canti, D. Gatteschi, and C. Luchinat, *J. Inorg. Biochem.*, 1981, **14**, 81.
[375] I. Bertini, G. Canti, and C. Luchinat, *Inorg. Chim. Acta*, 1981, **56**, 99.
[376] I. Bertini, G. Canti, and C. Luchinat, *Inorg. Chim. Acta*, 1981, **56**, 1.
[377] J. Hirose and Y. Kidani, *J. Inorg. Biochem.*, 1981, **14**, 313.
[378] J. Hirose and Y. Kidani, *Chem. Pharm. Bull.*, 1980, **28**, 3189.
[379] I. Bertini, E. Borghi, C. Luchinat, and R. Monnanni, *Inorg. Chim. Acta*, 1982, **67**, 99.
[380] P. Hendry, L. F. Lindloy, and D. Yellowlees, *Inorg. Chim. Acta*, 1982, **65**, L237.
[381] R. P. Sheridan and L. C. Allen, *J. Am. Chem. Soc.*, 1981, **103**, 1544.
[382] I. Bertini, G. Canti, and C. Luchinat, *J. Am. Chem. Soc.*, 1982, **104**, 4943.
[383] I. Andersson, R. Bauer, and I. Demeter, *Inorg. Chim. Acta*, 1982, **67**, 53.

and bromoimidazolyl propionic acid to the native enzyme and its cobalt(II)- and cadmium(II)-substituted derivatives has been examined by visible spectroscopy.[384]

Copper-containing Proteins.—Bovine erythrocyte superoxide dismutase (Cu_2Zn_2SOD), a dimeric metalloenzyme containing imidazolate-bridged copper(II) and zinc(II) in each of its subunits, has been the subject of a number of reports. Spectroscopic evidence suggests that in the enzyme and its four-copper-substituted derivative, Cu_2Cu_2SOD, the metal ion bound to the native zinc site is reversibly released in the pH range 3—4.5.[385] Electronic and e.s.r. spectroscopies have been used to characterize the ligand environments of copper(II) in the native enzyme and in the metal-substituted derivatives Ag_2Cu_2SOD and Cu_2Cu_2SOD, which contain copper(II) bound to the native zinc site.[386] The binding of NCS^-,[387] NCO^-, N_3^-, CN^-, and F^-[388] to superoxide dismutase has been investigated by electronic, e.s.r., and multi-nuclear n.m.r. spectroscopy. The results imply that these anions bind to the copper by substituting an equatorial histidine nitrogen to give five-co-ordinate complexes in the case of NCS^-, NCO^-, and F^- but square-planar complexes (through loss of apical water) in the case of CN^- and possibly N_3^-. The structure and location of the metal-binding sites and the roles and mutual influences of the metal centres in superoxide dismutase have been discussed.[389] The reconstitution of superoxide dismutase from the copper-free enzyme offers the possibility of measuring very low copper concentrations.[390] An assay based on an oxygen electrode has been used to determine the superoxide dismutase type activity of several metalloporphyrins.[391] The manganese form of superoxide dismutase, which is found in both protearyotes and enkaryotes, was shown to be inhibited by cacodylate,[392] and its active site has been the subject of an e.s.r. and electronic spectroscopic study.[393]

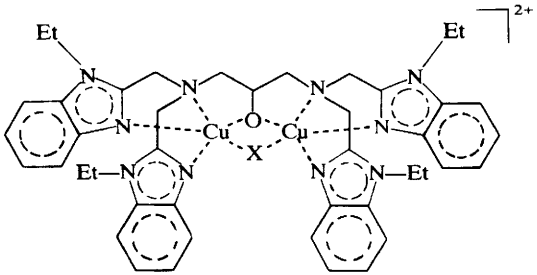
Tyrosinase and haemocyanin are members of a series of metalloproteins that contain strongly coupled, binuclear, copper-active sites (type 3). These sites in deoxyhaemocyanin[394,395] and oxyhaemocyanin[396] have been investigated by *X*-ray absorption spectroscopy. Deoxyhaemocyanin contains two copper(I) atoms both co-ordinated to two histidines with the facial sides open for attachment of dioxygen. Oxyhaemocyanin contains two copper(II) atoms, and the model emerging from EXAFS and other spectroscopic analysis suggests

[384] K. H. Dahl and J. S. McKinley-McKee, *J. Inorg. Biochem.*, 1981, **15,** 79.
[385] M. W. Pantoliano, J. S. Valentine, R. J. Mammone, and D. M. Scholler, *J. Am. Chem. Soc.*, 1982, **104,** 1717.
[386] M. W. Pantoliano, J. S. Valentine, and L. A. Nafie, *J. Am. Chem. Soc.*, 1982, **104,** 6310.
[387] I. Bertini, C. Luchinat, and A. Scozzafava, *J. Am. Chem. Soc.*, 1980, **102,** 7349.
[388] I. Bertini, E. Borghi, C. Luchinat, and A. Scozzafava, *J. Am. Chem. Soc.*, 1981, **103,** 7779.
[389] A. Scozzafava, *Inorg. Chim. Acta*, 1982, **62,** 15.
[390] R. Stevanato, P. Viglino, A. Rigo, and D. Cocco, *Inorg. Chim. Acta*, 1981, **56,** L39.
[391] R. F. Pasternak, A. Banth, J. M. Pasternak, and C. S. Johnson, *J. Inorg. Biochem.*, 1981, **15,** 261.
[392] P. J. Thornalley, A. W. Sarchet, H. A. O. Hill, J. V. Bannister, and W. H. Bannister, *Inorg. Chim. Acta*, 1982, **67,** 75.
[393] V. M. Fernandez, F. Sevilla, J. Lopez-Gorge, and L. A. del Rio, *J. Inorg. Biochem.*, 1982, **16,** 79.
[394] M. S. Co and K. O. Hodgson, *J. Am. Chem. Soc.*, 1981, **103,** 3200.
[395] M. S. Co, R. A. Scott, and K. O. Hodgson, *J. Am. Chem. Soc.*, 1981, **103,** 986.
[396] M. S. Co, K. O. Hodgson, T. K. Eccles, and R. Lontie, *J. Am. Chem. Soc.*, 1981, **103,** 984.



(52)

that they are in approximately square-planar environments, both co-ordinated to two histidines and two oxygen atoms, one of which is from a peroxo-bridging ligand and the other, X, from the protein (52). A three-co-ordinate binuclear copper(I) complex as a model for the active site of deoxyhaemocyanin[397] and the copper(II) complex (53), which mimics the structural, magnetic, and spectroscopic features of oxy- and met-haemocyanin,[398] have been synthesized. The absence of a charge-transfer band

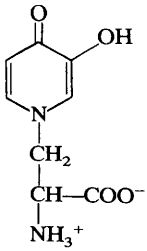


(53)

at 400—500 nm in the electronic and c.d. spectra of thioureamethaemocyanin argues against the presence of a phenolate oxygen of tyrosine as a bridging ligand at the binuclear copper site.[399] Cobalt(II)-substituted squid haemocyanin has been examined by electronic, c.d., and m.c.d. spectroscopy, and the resulting information was used to speculate on the nature of the copper environment in the native protein.[400] The reconstitution of haemocyanin from the apoprotein and copper(I) stabilized with Triton X-100 micelles at physiological pH has been the subject of a kinetic study.[401] The results of a chemical and spectroscopic study of some tyrosinase derivatives demonstrate that the geometric and electronic structure of the active site is very similar to that of oxyhaemocyanin.[402]

Mimosine (54), an inhibitor competitive with organic substrates, has been shown by electronic and e.s.r. spectroscopy to compete with azide and peroxide for direct binding to the binuclear copper site in both met and semi-met

[397] K. D. Karlin, Y. Gultneh, J. P. Hutchinson, and J. Zubieta, *J. Am. Chem. Soc.*, 1982, **104,** 5240.
[398] V. McKee, J. V. Dagdigian, R. Ban, and C. A. Reed, *J. Am. Chem. Soc.*, 1981, **103,** 7000.
[399] J. Kino, S. Suzuki, W. Mori, and A. Nakahara, *Inorg. Chim. Acta*, 1981, **56,** L33.
[400] S. Suzuki, J. Kino, M. Kimura, W. Mori, and A. Nakahara, *Inorg. Chim. Acta*, 1982, **66,** 41.
[401] P. Zatta and B. Salvato, *J. Inorg. Biochem.*, 1981, **15,** 269.
[402] R. S. Himmelwright, N. C. Eichman, C. D. LuBien, K. Lerch, and E. I. Solomon, *J. Am. Chem. Soc.*, 1980, **102,** 7339.



(54)

derivatives of tyrosinase.[403] Three binuclear copper(II) complexes with Schiff-base ligands derived from 2,6-diformyl-4-methylphenol have been synthesized and shown to model tyrosinase structurally and in their ability to act as two-electron oxidases.[404]

The so-called 'blue' (type 1) copper proteins are characterized by an intense absorption band near 600 nm, a small hyperfine splitting in the $g_{\|}$ region of the e.s.r. spectrum, and unusually high reduction potentials. The crystal structures of two of these, plastocyanin (from poplar leaves) and azurin (from *Pseudomonas aeruginosa*),[405] show that in the oxidized copper(II) state the metal is co-ordinated to two histidine nitrogen atoms and to sulphur atoms from cysteine and a more distant methionine. Single-crystal electronic absorption and e.s.r. spectra have been used to correlate the electronic structure of the copper site in plastocyanin with its geometry, and it has been shown that the methionine sulphur contributes little to ligand-field effects about the metal.[406] Polarized *X*-ray absorption spectra for oriented crystals of plastocyanin show that the contribution of methionine sulphur to the EXAFS is weak or absent.[407] The idea that the sulphur atoms are responsible for the high redox potentials of the proteins has been challenged on the basis of redox potentials and electron-transfer rates obtained for some model copper–thioether complexes.[408] Models for the copper cores and protein folding schemes in plastocyanin, azurin,[409] and stellacyanin,[410] a protein with a lower reduction potential than the first two, are discussed in detail. Quenching of the luminescent excited state of tris-(2,2′-bipyridine)ruthenium(II) by electron transfer from these three blue copper proteins has been the subject of a kinetic and mechanistic study.[411] A mechanism involving a rapid pre-equilibrium protonation followed by rate-limiting ligand rearrangement has been proposed

[403] M. E. Winkler, K. Lerch, and E. I. Solomon, *J. Am. Chem. Soc.*, 1981, **103,** 7001.
[404] K. Moore and G. S. Vigee, *Inorg. Chim. Acta*, 1982, **66,** 125.
[405] E. T. Adman and L. H. Jensen, *Isr. J. Chem.* 1981, **21,** 8.
[406] K. W. Penfield, R. R. Gay, R. S. Himmelwright, N. C. Eichman, V. A. Norris, H. C. Freeman, and E. I. Solomon, *J. Am. Chem. Soc.*, 1981, **103,** 4382.
[407] R. A. Scott, J. E. Hahn, S. Doniach, H. C. Freeman, and K. O. Hodgson, *J. Am. Chem. Soc.*, 1982, **104,** 5364.
[408] M. A. Augustin, J. K. Yandell, A. W. Addison, and K. D. Karlin, *Inorg. Chim. Acta*, 1981, **55,** L35.
[409] M. Lundeen, *J. Coord. Chem.*, 1982, **12,** 1.
[410] M. Lundeen, *Inorg. Chim. Acta*, 1981, **56,** 149.
[411] A. M. English, V. R. Lum, P. J. DeLaive, and H. B. Gray, *J. Am. Chem. Soc.*, 1982, **104,** 870.

for the acid-induced decolorization of azurin.[412] Similarities between the near-i.r. spectra of nickel(II)-substituted azurin and nickel(II) complexes with bulky Schiff-base ligands containing thiolate groups suggest that internal interactions in the polypeptide structure impose a pseudo-tetrahedral geometry on the metal centre in the protein.[413]

Galactose oxidase, which contains a single type 2 (normal) copper site, catalyses the oxidation of primary alcohols to the corresponding aldehydes. The effect of added cyanide on the ^{19}F n.m.r. relaxation rates of fluoride-bound galactose oxidase is consistent with the availability of a high-affinity equatorial site and a low-affinity axial site on the metal for exogenous ligands.[414] Strong π-bonding equatorial ligands can modify ligand binding to the axial site. The galactose oxidase-catalysed oxidation of galactose by O_2, $[IrCl_6]^{2-}$, $[Fe(CN)_6]^{3-}$, and porphyrexide has been studied kinetically, and the nature of substrate binding to the enzyme has been discussed in the light of the results.[415] Alcohol substrate binding to copper in galactose oxidase, studied by kinetic competition against cyanide and fluoride and by magnetic resonance methods, seems to be either outer sphere or possibly at an axial site.[416]

The metalloprotein laccase, which contains three types of copper site, has been investigated by electronic, *X*-ray absorption, and Raman spectroscopy.[417] Both the type 3 (coupled binuclear) and type 2 (normal) sites together are involved in binding small molecules to the native protein, and oxidation-state changes in the type 3 site lead to structural changes at the type 1 site. Several copper(II) complexes with tripod ligands containing benzimidazole, thioether, and phenolic groups reproduce the electronic and e.s.r. spectra of a number of the copper proteins discussed in this section.[418,419]

Other Metal–Protein Interactions.—A number of reports relating to complexes of serum albumin have been published. The e.s.r. spectrum of the copper(II) complex of human serum albumin at neutral pH is consistent with a ligand field containing four primary amino groups, two peptide groups, and one imidazole group.[420] Spectra of the copper(II) complexes with dog serum albumin and a number of peptides are also discussed. The affinities of bovine, dog, and rat serum albumins for copper(II) and zinc(II) have been examined by ultrafiltration methods, and the lower affinity of dog serum albumin for copper(II) has been attributed to the absence of a primary amino-terminus

[412] R. Timkovich, *J. Inorg. Biochem.*, 1981, **14,** 245.
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The co-ordination environment of molybdenum in the enzymes sulphite oxidase and xanthine dehydrogenase has been studied by fluorescence-detected EXAFS.[425] Molybdenum(V) e.s.r. signals of a number of octahedral and square-pyramidal complexes may be categorized into two types with different $g_3 - g_1$ values, thus providing a potential probe for the active sites of molybdenum-dependent enzymes.[426]

The resonance Raman spectra of tryptophan-modified manganese(III)-containing acid phosphatase and the native enzyme provide the first evidence for manganese–sulphur binding in metalloproteins.[427]

Other reports on metal–protein interactions deal with the following: the binding of terbium(III)[428] and copper(II)[429] to yeast enolase, investigations of the calcium-binding sites of calmodulin[430] and parvalbumin[431] by fluorescence spectroscopy of the substituted terbium(III) or europium(III) proteins and by ^{43}Ca n.m.r. spectroscopy,[432] the relationship between synthetic cobalt(III)–isobacteriochlorins and the prosthetic groups of some cobalt-containing proteins,[433] an *X*-ray scattering titration of α_2-macroglobin with trypsin,[434] a study of the cadmium-binding sites of metallothionein by perturbed angular correlation spectra of the partly and fully saturated ^{111}Cd protein,[435] the ^{113}Cd n.m.r. spectrum of bis(bipyridyl)cadmium(II) nitrate and its implications for the use of ^{113}Cd n.m.r. in biological systems,[436] the interaction of palladium(II) and platinum(II) albumin complexes with ATPase of sarcoplasmic reticulum,[437] the

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