THE BIOSYNTHETIC PATHWAY OF THE ASPARAGINE-LINKED OLIGOSACCHARIDES OF GLYCOPROTEINS

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I. INTRODUCTION*

The study of protein glycosylation started shortly after the discovery of the role of sugar nucleotides as glycosyl donors. The transfer of sugars to serine, threonine, and hydroxylysine residues in polypeptides was obtained in vitro, but the addition of the sugar moiety to asparagine residues proved to be more complicated. This process required a different line of attack and this was provided by the discovery of the role of polyprenols in the biosynthesis of bacterial polysaccharides by Robbins, Strominger, and co-workers. Some lipid-linked sugars were detected in animal tissues and the lipid moiety was identified as the polyprenol:dolichol. A new glycosylation pathway was uncovered. Besides the direct transfer from sugar nucleotides, it was found that sugars could be added with dolichyl phosphates as intermediates. The latter mechanism is used for the glycosylation of asparagine residues in polypeptides, and seems to be more closely related to the process of protein synthesis in the ribosomes than the other pathway which leads to the addition of saccharide residues to hydroxyaminoacids by direct transfer from sugar nucleotides.

This review is restricted to the glycosylation of proteins through lipid intermediates, a field that has become one of very active research in recent years. For previous reviews see References 1 through 8.

II. ASPARAGINE-LINKED OLIGOSACCHARIDES

In 1961-1963 Marshall, Neuberger, and co-workers showed that in ovalbumin an N-acetylglucosamine residue is joined to the amide nitrogen of asparagine.[9-11] In 1970 Tarentino et al.[12] detected di-N,N'-acetylchitobiose as part of the linkage region of ribonuclease B, and later these workers with Sukano found that α mannosidase could remove all the mannoses but one.[13] The remaining mannose turned out to link β to the N,N'di-acetylchitobiose. We now know that asparagine-linked oligosaccharides have a common pentasaccharide core, with the structure shown in Figure 1, which may carry

* Abbreviations: G-oligosaccharide is the oligosaccharide containing: glucose$_{1-3}$, mannose$_9$, and N-acetylglucosamine$_2$ which occurs combined with dolichyl diphosphate. The subscripts G_0-, G_1-, G_2-, and G_3- refer to the number of glucose residues. Dol = dolichyl; Dol-P = dolichyl monophosphate; Dol-P-P = dolichyl diphosphate.

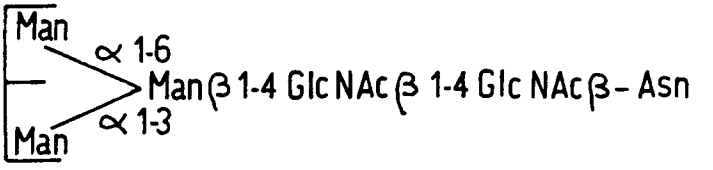


FIGURE 1. The common core of the asparagine-linked oligosaccharides. In some of the figures that follow, the core is represented by a large E, as shown.

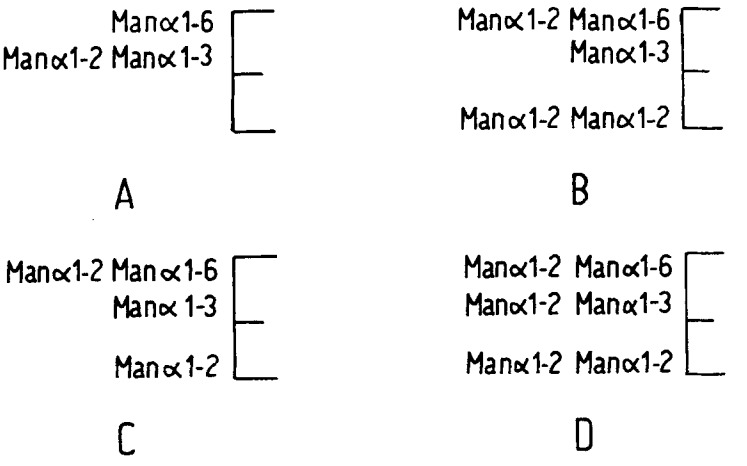


FIGURE 2. High mannose oligosaccharides. (A) and (B) Immunoglobulin;[14,15] (C) ovalbumin;[16] (D) thyroglobulin.[17] The core is represented by a large E, as shown in Figure 1. The substituents shown above or below the middle line of the E correspond to the α 1-6 and α 1-3-linked mannoses, respectively.

various substituents. However some atypical cases have been described. The number of known structures has increased considerably, and at the same time great progress has been achieved in the knowledge of the biosynthetic steps.

A. The High Mannose Type

The oligosaccharides of the high mannose type have a variable number of mannose residues joined to N,N'di-acetylchitobiose. Some representative types are shown in Figure 2. In each case the oligosaccharides may have a slightly variable number of mannose residues and this constitutes the so called microheterogeneity.

It is interesting to point out that an oligosaccharide of thyroglobulin shown in Figure 2D has nine mannoses linked exactly the same as in the oligosaccharide containing glucose$_3$, mannose$_9$, and N-acetylglucosamine$_2$ which occurs joined to dolichyl diphosphate and which we call G_3-oligosaccharide.

B. The High N-Acetylglucosamine Type

In addition to the high mannose type there are oligosaccharides which contain mannose and more than two N-acetylglucosamines. These have been found in ovalbumin and may be present in other glycoproteins.

The structure of two representatives of this type, which may be referred to as the high N-acetylglucosamine type, is shown in Figure 3. It may be observed that in Figure 3A, a β

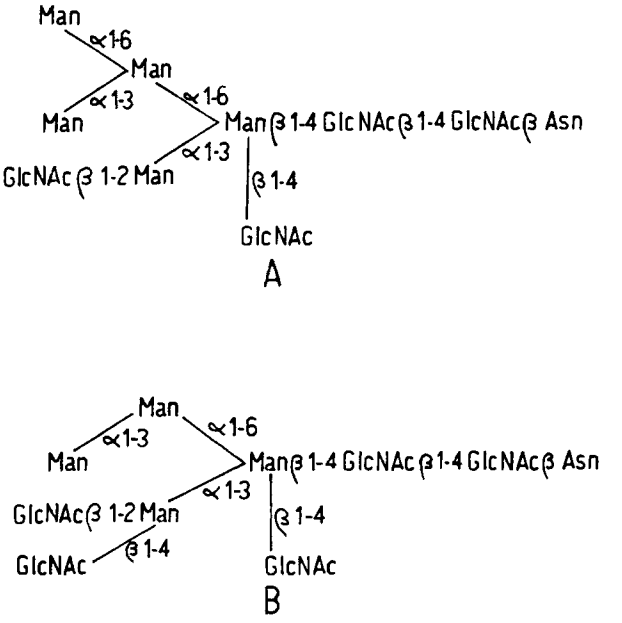


FIGURE 3. Oligosaccharides of the high N-acetylglucosamine type from ovalbumin.[16]

1-4-linked N-acetylglucosamine residue is attached to the internal mannose, and another β-1-2 residue is combined to the α 1-3 mannose of the core. Thus this oligosaccharide has 4 N-acetylglucosamines, and 5 mannoses in total. The oligosaccharide shown in Figure 3B has one mannose less, but an additional N-acetylglucosamine-linked β 1-4 (thus totaling 5 N-acetylglucosamines) to the α 1-3-linked mannose residue of the core.

C. The Complex Type

The characteristic feature of the complex type of oligosaccharides is the sequence sialic acid-galactose-N-acetylglucosamine joined to the core. The disaccharide galactosyl β 1-4 N-acetylglucosamine is called N-acetyllactosamine, and for this reason the complex saccharides are often referred to as the lactosamine type.[18] Some typical representatives of this type are shown in Figure 4. In many cases some of the sialic acid residues are missing. The lactosamine-containing chains may vary in number from 2 to 4. Some workers refer to these oligosaccharides as bi-, tri-, and tetraantenary, according to the number of substitutent chains.[19] The biantenary type (Figure 4A) is found in many glycoproteins, including transferrin, several immunoglobulins, cold insoluble globulin, and several others.[20–22] In immunoglobulin E, besides the complex biantenary, there are oligosaccharides of the high mannose type but with a different core *vide infra*. Human α_1-protease inhibitor has both bi- and triantenary oligosaccharides with structures as those shown in Figure 4 A, B, and C.[23–24] Cold-insoluble globulin has a biantenary oligosaccharide, and contains sialic acid residues linked α 2-6 or α 2-4, and some β 1-3 galactosyl residues.[22] The tetraantenary structure shown in Figure 4D corresponds to the oligosaccharide of human plasma α_1 acid glycoprotein.[19] Some of these oligosaccharides may have some fucose residues joined to α 1-6 to the innermost N-acetylglucosamine, while others have fucose residues linked α 1-3 to the antennae as shown in Figure 4D.

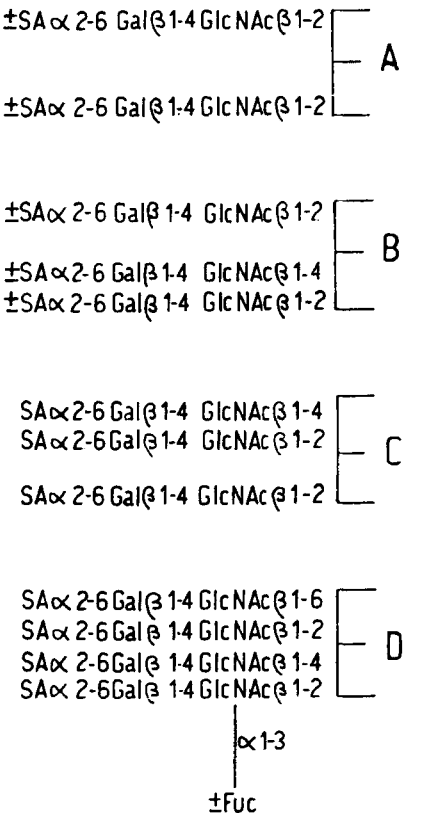


FIGURE 4. Oligosaccharides of the complex or lactosamine type. (A) Human sero transferrin,[20] IgE,[21] cold insoluble globulin,[22] human α_1 protease inhibitor;[23] (B) human α_1 protease inhibitor,[23] human plasma α_1 acid glycoprotein;[19] (C) human α_1, protease inhibitor;[24] (D) human α_1 acid glycoprotein.[19] The large E represents the core, as described in Figure 1.

D. Miscellaneous Oligosaccharides

Some oligosaccharides have the common pentasaccharide core, but are different from both the high mannose and the complex type. One of these was isolated by Krusius et al.[25] It has 5 to 14 lactosamine units joined to the common core. It was isolated from red blood cells and had A and B blood group activities. A similar oligosaccharide was detected in CHO cells.[26] It had about 12 acetyllactosamine residues joined to the pentasaccharide core (Figure 5A). Corneal keratan sulfate also has lactosamine-repeating units and is believed to be joined to protein by a mannose-N-acetylglucosamine core. Its biosynthesis seems to involve lipid intermediates since it is inhibited by tunicamycin.[27]

Studies on calf thymocytes have revealed the presence, in the asparagine-bound oligosaccharides, of the grouping: Gal β 1-3 Gal β 1-4 GlcNAc — which occurs in di-, tri-, or tetraantenary complex structures.[27a]

Another interesting compound has been isolated from bovine kidney cells.[28] The proposed structure is shown in Figure 5B. There are two galactose disaccharides substituting the mannoses of the common core. Two acetaldehyde in acetal linkages are

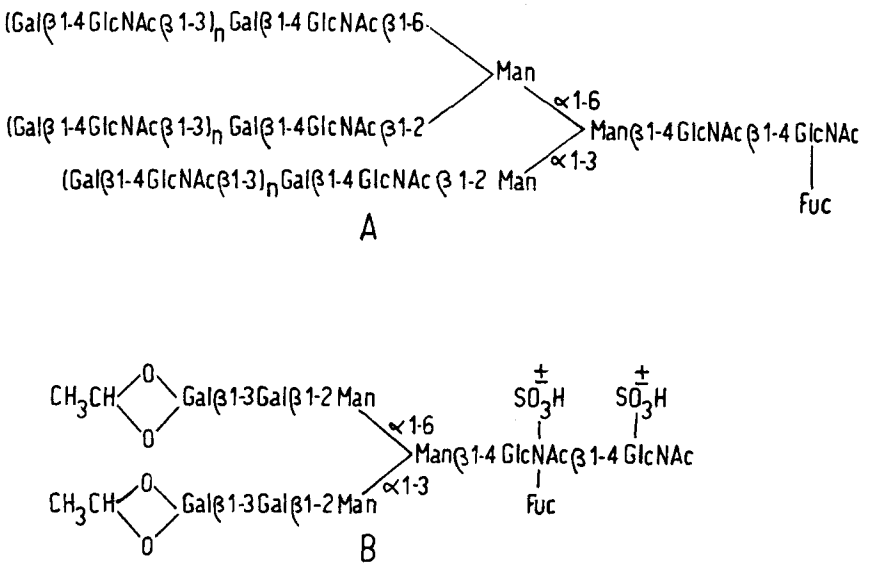


FIGURE 5. Miscelaneous oligosaccharides, (A) Chinese hamster ovary glycoprotein;[26] (B) bovine kidney cell oligosaccharide.[28]

present, probably on the terminal galactose. Furthermore, two sulfate groups and a fucose substitute for the N-acetylglucosamine residues of the core. This unorthodox compound was first detected in paramyxovirus-infected bovine kidney cells, but was also found in uninfected cells.

Sulfated oligosaccharides bound to asparagine also have been found in embryonic liver and lung. They contain terminal fucose and sialic acid residues, as well as O-sulfated N-acetylglucosamine residues.[28a]

E. Atypical Cases

Some oligosaccharides have been described which do not conform to the rule of having the common pentasaccharide core. One of these exceptions is an oligosaccharide isolated from immunoglobulin E which has the structure shown in Figure 6A. It does not have the N,N'di-acetylchitobiose residue, and has an α-linked N-acetylglucosamine.[29] Furthermore, it has no microheterogeneity.

Another atypical case was detected by Jouanneau and Bourrilon in a human pathological immunoglobulin M.[30] It contained only one N-acetylglucosamine to which six mannose were joined as shown in Figure 6B.

The biosynthesis of these compounds is a new challenge, but before embarking on this study, perhaps it would be advisable to confirm the structures.

F. Oligosaccharides of Viral Glycoproteins

The *Sindbis* virus has two envelope glycoproteins.[31] Each of these glycoproteins has two types of oligosaccharides, one complex like the one shown in Figure 4A but with α 2-3 linked sialic acid residues, and one high mannose identical to the ovalbumin oligosaccharide of Figure 2C. This oligosaccharide is shown with seven mannose residues, but it is heterogenous and it can have more or less mannose residues.[32]

From the Sindbis glycoprotein E2, three glycopeptides of the complex type have been isolated. The glycopeptide S1 has two sialic acid residues, while glycopeptides S2 and S3 have only one or no sialic acid residues, respectively.[33]

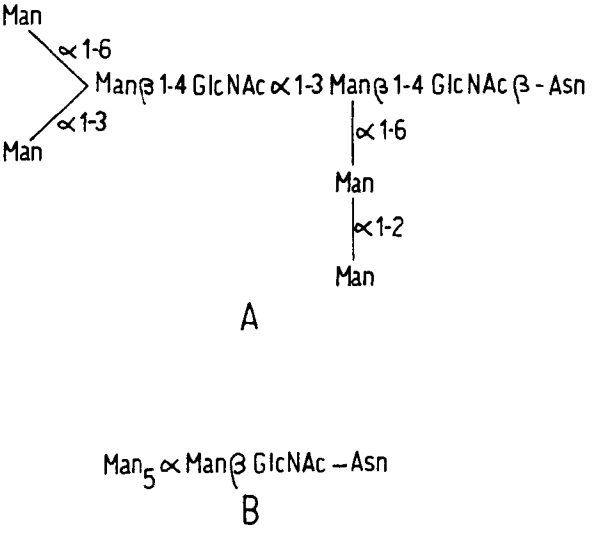


FIGURE 6. Some atypical oligosaccharides. (A) Immunoglobulin E oligosaccharide;[29] (B) Pathological immunoglobulin M oligosaccharide.[30]

The *Vesicular stomatitis* virus has five polypeptides. One of them, glycoprotein G, is inserted assymetrically in the virus membrane. Its oligosaccharides and a large part of the polypeptide chain are on the external side of the membrane. Glycoprotein G has two oligosaccharides of the complex type (Figure 4C), with three chains capped with α 2-3-linked sialic acid and a fucose-joined α 1-6 to the innermost acetylglucosamine.[34]

The *Semliki* virus has three glycoproteins (E_1, E_2, and E_3). Two of them (E_1 and E_3) are glycoproteins with oligosaccharides of the complex type similar to those of Vesicular stomatitis virus.[35]

G. Oligosaccharide of Plant Glycoproteins

Several plant glycoproteins contain oligosaccharides with an N-acetylglucosamine linked to asparagine (see Sharon and Lis for a review[36]). An oligosaccharide of the high mannose type is found in soya bean agglutinin.[37] Another oligosaccharide containing the common core plus one residue each of α 1-2 mannose, α 1-6 fucose, and β-xylose, has been detected in bromelain, the proteolytic enzyme of pineapple.[21]

III. THE RELEASE OF OLIGOSACCHARIDES FROM PROTEINS

The separation of the oligosaccharide moiety from the protein is an important step in the study of glycoproteins. Besides removal of most of the amino acids by proteolytic enzymes, several procedures are now available for isolating the oligosaccharide.[38] These are: (a) treatment with alkali, (b) hydrazinolysis, (c) the use of endo-β-N-acetylglucosaminidases.

A. Treatment with Alkali

The conditions used for the alkali treatment vary with the different types of glycoproteins. The galactosyl-hydroxylysine linkage of glycoprotein as found in collagen is very stable to alkali. Thus the glycopeptide can be isolated after heating at 90° C in 2 N NaOH for 10 hr.[39] In contrast, the linkage of sugars (xylose, N-acetylgalactosamine or mannose) to hydroxyaminoacids such as serine or threonine is alkali labile. For

instance, it can be split by treatment with 0.05 N NaOH for 12 to 30 hr at 25°. The process is referred to as β elimination. It requires that the amino and carboxyl groups of the hydroxyaminoacid be substituted, as occurs in proteins.

Removal of the asparagine-linked oligosaccharides also can be carried out by alkaline treatment. In order to avoid degradation, borohydride may be added so that the reducing group is reduced immediately after it is liberated. The procedure consists in heating at 100° for 4 to 6 hr in 1 *M* NaOH plus 1 *M* $NaBH_4$. The monosaccharide residues are not degraded by this treatment, but the most internal N-acetylglucosamine is reduced, and the acetyl groups are removed. The oligosaccharide therefore acquires positive charges and can be characterized by electrophoresis.[40] Another analytical procedure consists of treating the deacetylated oligosaccharide with nitrous acid followed by paper chromatography.[41]

B. Hydrazinolysis

Removal of the oligosaccharide has been effected by heating with anhydrous hydrazine.[42] The procedure has been used successfully by several workers, apparently without undue decomposition of the oligosaccharides.[22,43]

Usually the glycopeptide or glycoprotein is heated in anhydrous hydrazine for 30 hr at 100°.[44] The hydrazine is then distilled under vacuum. The acetyl groups are removed by this treatment, and the amino groups can then either be reacetylated or the oligosaccharide can be treated with nitrous acid, thus splitting the molecule at the points where hexosamine residues are present.

C. Endo-β-N-Acetylglucosaminidases

The discovery of endo-β-N-acetylglucosaminidases provided a method for removing the oligosaccharide from the polypeptide without submitting it to a drastic chemical treatment. These enzymes hydrolyze the bond between the two β 1-4-linked N-acetylglucosamine residues of the oligosaccharide core.

The first of these enzymes to be discovered was found in *Streptomyces griseus* extracts by Tarentino and Maley.[45] The enzyme was called endo-β-N-acetylglucosaminidase H because it acts on the high mannose type oligosaccharides, while another enzyme, endo-β-N-acetylglucosaminidase L, acts on the low molecular weight oligosaccharide such as Man $(GlcNAc)_2$ Asn.[46] The last enzyme also hydrolyzes β 1-4-linked N-acetylhexosamine oligosaccharides obtained from chitin. The N-acetylglucosamine tri- and tetrasaccharides are split at twice the rate observed from Man$(GlcNAc)_2$ Asn.

Another endo-β-N-acetylglucosaminidase was detected in *Diplococcus pneumonia.* At first, it was thought that this enzyme could hydrolyze complex oligosaccharides, but then it was found that the purified enzyme only acted after removal of the peripheral sugars (sialic acid, galactose, and N-acetylglucosamine).[47] Two endo-β-N-acetylglucosaminidases, C_I and C_{II}, have been isolated from the bacteria *Clostridium perfringens.*[48]

Considerable work has been carried out on the specificity of these enzymes. Table 1 summarizes the activity of the endo-β-N-acetylglucosaminidases on different substrates. The meaning of M_{α_3}, M_{β_4}, and M_{α_6} is explained in the legend. Endo-β-N-acetylglucosaminidase H acts on oligosaccharides containing 3 to 50 mannose residues.[49] Its specificity can be summarized as follows: it requires an α 1-3 mannose or an α 1-6 mannose on M_{α_6} (a Man α 1-2 residue on the above mentioned α 1-3-linked mannose does not abolish activity). (See Table 1, Numbers 6 and 7). The M_{α_3} residues may be absent, unsubstituted, or substituted (Numbers 1 through 8). An α 1-6 fucose residue on the internal N-acetylglucosamine suppressed the activity.[49] Endo-β-N-acetylglucosaminidase H acts on oligosaccharides joined to protein or to an asparagine residue, as well as on the free oligosaccharide or one having the first N-acetylglucosamine reduced.[50]

Trimble et al.[54] reported that the relative rate of hydrolysis of the different

Table 1
THE SPECIFICITY OF ENDO-β-N-ACETYLGLUCOSAMINIDASES

	Substituents on the mannoses of the core			Endo-β-N-acetylglucosaminidases			
				Streptomyces griseus	*Diplococus pneumoniae*	*Clostridium perfrigens*	
	$M_{\alpha 6}$	$M_{\alpha 3}$	$M_{\beta 4}$	Endo H	Endo D	Endo C_{II}	Endo C_I
1	Man α 1-6 Man α 1-3	Galβ 1-4GlcNAcβ 1-4 GlcNAcβ 1-2	GlcNAcβ 1-4	$+^{53}$		$-^{53}$	
2	Man α 1-6 Man α 1-3	GlcNAcβ 1-4 GlcNAcβ 1-2	GlcNAcβ 1-4	$+^{53}$		$-^{53}$	
3	Man α 1-6 Man α 1-3	GlcNAcβ 1-2	GlcNAcβ 1-4	$+^{53}$		$+^{53}$	
4	Man α 1-6 Man α 1-3	Man α 1-2	None	$+^{51,52}$	$-^{51,52}$	$+^{48}$	$-^{48}$
5	Man α 1-6 Man α 1-3	None	None	$+^{51}$	$+^{51,52}$	$+^{48}$	
6	Man α 1-6	None	None	$+^{51}$			
7	Man α 1-3	None	None	$+^{53}$		$+^{53}$	
8	Man α 1-3	$M_{\alpha 3}$ absent	None	$+^{51}$			
9	None	None	None	$-^{51}$	$+^{51}$		
10	None	$M_{\alpha 3}$ absent	None	$-^{51}$			
11	$M_{\alpha 6}$ absent	$M_{\alpha 3}$ absent	None	$-^{51}$		$-^{48}$	$-^{48}$

Note: The mannose residues of the core oligosaccharide shown in Figure 1 and linked α 1-3, β 1-4 and α 1-6 are referred to as $M_{\alpha 3}$, $M_{\beta 4}$ and $M_{\alpha 6}$, respectively. The substituents on each of these mannoses are shown in the rows numbered 1 to 11. For instance, No. 3 corresponds to the oligosaccharide shown in Figure 3A, and Number 9, to the naked core of Figure 1. The + sign means that the enzyme is active on the corresponding substrate, and the − sign that it is inactive. The numbers represent the literature references.

oligosaccharides with endo-β-N-acetylglucosaminidase H is as follows (numbers in parenthesis refer to those of Table I): core with a Man α 1-6 substituent on M_{α_6} (6) > core with a Man α 1-3 substituent on M_{α_6} and with M_{α_3} absent (8) > core with Man α 1-6 and Man α 1-3 substituents on M_{α_6} (5) >>> naked core (9) > core with M_{α_3} absent (10) >> core with M_{α_6} and M_{α_3} absent (11).

Endo-β-N-acetylglucosaminidase C_{II} requires the M_{α_3} residue (which may have an α 1-2 mannose or GlcNAc β 1-2 — see Table I, Numbers 3, 4) and a Man α 1-3 joined to M_{α_6} (the α 1-3 Man may carry an α 1-2 mannose). An α 1-6 fucose on the internal N-acetylglucosamine suppresses the activity. There are differences between the specificities of endo-β-N-acetylglucosaminidase H and C_{II}. The latter only acts on oligosaccharides of the high mannose type, while endo-β-N-acetylglucosaminidase H can also hydrolyze oligosaccharides substituted with N-acetylglucosamine or lactosamine in position 4 of M_{α_3}.

The specificities of endo-β-N-acetylglucosaminidases D and C_I are equal. They require a free M_{α_3} mannose, and may have no substituent, several mannoses, or a lactosamine chain on M_{α_6}. The internal N-acetylglucosamine may be joined to various residues, or carry an α 1-6 fucose residue. The substitution of the M_{α_3} mannose produces a decrease of 1000-fold in the reactivity of these glycopeptides towards endo-β-N-acetylglucosaminidase D.[52]

An endo-β-N-acetylglucosaminidase extracted from a *Basidiomycete* has been found to act on asialo biantenary glycopeptide, and more slowly on the monosialo derivative. The presence of fucose on the innermost acetylglucosamine decreased the rate of hydrolysis. Tri- and tetraantenary glycopeptides were not acted upon.[55]

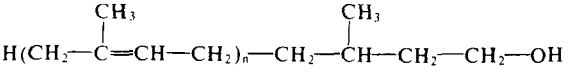
Endo-β-N-acetylglucosaminidase activity has also been detected in animal tissues, such as hen oviduct and pig and rat liver.[56,57] They presumably are involved in the breakdown of glycoproteins. This would explain why oligosaccharides that lack one N-acetylglucosamine, like the products of endo-β-N-acetylglucosaminidase, are found in the urine of patients with mannosidosis. All the oligosaccharides excreted by patients with mannosidosis have the basic structure of the G-oligosaccharide, but without glucose and with fewer mannoses.[58] The most abundant is a Man α 1-3 Man β 1-4 GlcNAc, followed by Man α 1-2 Man α 1-3 Man β 1-4 GlcNAc.

IV. LIPID INTERMEDIATES

The early work on lipid intermediates has been reviewed previously.[1-3] The compounds involved in the glycosylation of asparagine residues in proteins are derivatives of dolichol. The derivatives of retinol have received considerable attention but their role is not quite clear as yet.[59] Two types of dolichol compounds are found in cells: some with one phosphate residue, and others with two.

A. Dolichol

Dolichol was first detected in chemical studies on the unsaponifiable fraction of biological material.[60] Its structure is as follows:



The number of isoprene residues in dolichol isolated from animals varies from 17 to 21, and from 14 to 24 for yeast, fungi, and plants. However, a polyprenol with 11 isoprene residues has been isolated from pig liver. Like dolichol, it is α-saturated.[61] Undecaprenol,

a polyprenol which acts as a cofactor in the formation of certain polysaccharides in bacteria, has 11 isoprene residues, all unsaturated. Ficaprenol isolated from plant material, also has 11 isoprene residues, but differs in that it has three *trans* double bonds instead of the two found in undecaprenol.

Besides being present in most animal tissues, dolichol probably is present in plants. It has been found that a phosphorylated form, apparently containing 17 or 18 isoprenes units and α-saturated, occurs in plant material as the phosphate.[62,63] The synthesis of dolichyl phosphate from mevalonic acid or isopentyl pyrophosphate has been obtained in cell-free extracts from peas or from the liver of chicken or rabbits. Of the subcellular fraction, the most active was the mitochondrial outer membrane.[64,65]

Biosynthesis with a hen oviduct extract resulted in the isolation of 2-3 dehydrodolichol monophosphate as if phosphorylation occurred before saturation of the double bond.[66]

B. The Phosphorylation and Dephosphorylation of Dolichol

There is evidence that dolichol phosphate arises from dolichol by the addition of phosphate, but another possibility is that the carbon chain grows with the phosphate already present.

Phosphorylation of dolichol has been detected in eucaryotic cell extracts according to the following reaction:[67,68]

$$\text{CTP} + \text{Dol} \rightarrow \text{Dol-P} + \text{CDP} \qquad (1)$$

Phosphorylation of dolichol has also been detected by incubation of the pupae of the Mediterranean fly *(Ceratitis capitata)* with radioactive ATP.[69] In this case the phosphate donor might also have been CTP, but this point was not established. A dephosphorylation of dolichyl diphosphate was first observed to be catalyzed by rat liver microsomes.[70] A phosphatase active on dolichyl phosphate and diphosphate has been detected in human lymphocytes[71] and in neuronal perikaria.[72] It does not require divalent metals for activity.

C. Dolichyl Derivatives with One Phosphate

Compounds of dolichyl monophosphate with glucose, mannose, xylose, and galactose have been described.[7] In every case the sugar is present as the β-anomer. All these compounds lose the sugar residue on treatment with dilute acid.

1. Dolichyl Phosphate Glucose

The early work on Dol-P-Glc has been reviewed previously.[7] The effect of added dolichyl phosphate was first tested on the formation of Dol-P-Glc catalyzed by the microsome fraction of liver or other tissues[73] as follows:

$$\text{UDPGlc} + \text{Dol-P} \rightarrow \text{Dol-P-Glc} + \text{UDP} \qquad (2)$$

The reaction requires Mg^{++} ions and a neutral detergent such as Triton X-100. The amount of Dol-P-Glc formed is proportional within limits to the amount of Dol-P added, so that it can be used as a method for measuring Dol-P. It was with this procedure that Dol-P was first detected as a cofactor in oligosaccharide biosynthesis. It was later found that the method is not quite specific because other polyprenol phosphates may be used as substrates for Dol-P. For instance, chemically phosphorylated ficaprenol has been found to be active, although much less than Dol-P. The glucose residue is labile to acid, i.e., when heated at pH2 for 5 to 10 min it is liberated completely. Since very small amounts of buffering substances affect the pH, it is convenient to adjust the latter with an

internal indicator such as thymol blue. In 0.1 N acid at 18° C in chlorofom-methanol 2:1, and half-life of Dol-P-Glc is 5 to 6 min.[74] Treatment with alkali (45 min at 100° in 1 N NaOH in aqueous propanol) leads to the decomposition with formation of 1-6 anhydro glucosan.[73] This was taken as an indication of the β configuration of the glucosyl residue. This anomeric structure, as well as that of the rest of the molecule, was made certain by the experiments of Herscovics et al.[75] They synthesized Dol-P α Glc and Dol-P β Glc and found that only the latter produced 1-6 anhydroglucosan when heated 30 min at 60 to 90° in 0.1 N NaOH.

2. Dolichyl Phosphate Mannose

An enzyme present in many, or perhaps all, mammalian tissues and in some microorganisms catalyzes the following reaction:

$$\text{GDP Man} + \text{Dol-P} \rightarrow \text{Dol-P-Man} + \text{GDP} \qquad (3)$$

The reaction product, Dol-P Man, was studied by several workers,[7] and was synthesized by Warren and Jeanloz.[76-78] They also prepared Dol-P-α-Man and Dol-P-P-α-Man. The product formed according to Equation 3 with endogenous Dol-P and calf pancreas microsomes was clearly different from Dol-P-P-α-Man as judged by its mobility in thin layer chromatography (TLC).

Comparison of synthetic Dol-P-α-Man with the compound formed by the enzyme showed that although they have very similar mobilities in TLC in several solvents they could be slightly separated with others. On the other hand, Dol-P-β-Man obtained by synthesis ran like the enzymatically synthesized compound in all solvents.[79,80] Both the synthetic and natural compounds when treated with alkali yielded mainly mannose-2-phosphate, while Dol-P-α-Man gave only degradation products of mannose.[80,81]

Studies by Mankowski et al.[82,83] in which other polyprenols were substituted for Dol-P in enzymatic tests showed that the most important structural requirement was the saturation of the α-isoprene residue. The chain length of the polyprenol and the *cis/trans* configuration of the double bond were less important. This was true for the formation of the mannose, glucose, and N-acetylglucosamine containing lipids from the corresponding sugar nucleotides.

3. Dolichyl Phosphate Derivatives of Other Sugars

By incubation of UDP-xylose with hen oviduct microsomes, Waechter et al.[84] obtained Dol-P-xylose. It has not been determined if a specific enzyme is involved or if it is produced by the enzyme that normally makes Dol-P-glucose. This seems likely, due to the fact that D-xylose differs only from glucose in having an H residue instead of CH_2OH.

The xylosyl transferase involved in joining xylose to serine in the linkage region of chondroitin sulfate seems to use directly UDP-xylose as a substrate, and not a lipid intermediate.

Another derivative which has been prepared is Dol-P-galactose. It has been obtained by incubation of UDP-galactose, Dol-P, and an enzyme from *Acetobacter xylinum*.[85] Its formation with enzymes from eukaryotes is doubtful, but it functions as a galactose donor to an endogenous acceptor of liver, resulting in the formation of a lipid-linked galactose containing oligosaccharide which has a chromatographic mobility similar to G-oligosaccharide.[86]

D. Dolichyl Derivatives with Two Phosphates

The series of compounds containing two phosphates involves some with only one

sugar residue up to the largest known, which has 14 residues (dolichyl diphosphate G_3-oligosaccharide).

1. Dolichyl Diphosphate N-acetylglucosamine

The first compound of the series is dolichol diphosphate N-acetylglucosamine. It was first detected as a product of incubation of radioactive UDP-N-acetylglucosamine and liver microsomes.[7] In the process a glycosyl phosphate is transferred and UMP is formed, in contrast to the previously mentioned reactions in which only the sugar residue is transferred and UDP is formed. The reaction is

$$\text{UDP-GlcNAc} + \text{Dol-P} \rightarrow \text{Dol-P-P-GlcNAc} + \text{UMP} \qquad (4)$$

It requires a divalent cation (Mg^{++} or Mn^{++}) and is activated by neutral detergents. Chemical synthesis of Dol-P-P-GlcNAc has been carried out by Warren et al.,[87] and the product has been compared with the compound obtained by enzymatic synthesis. Both substances behaved the same when run in DEAE cellulose and silicic acid columns or in TLC with different solvents and gave N-acetylglucosamine by mild acid hydrolysis and GlcNAc-1-P by treatment with phosphodiesterase.

2. Dolichyl Diphosphate N,N'di-acetylchitobiose

Paper chromatography of the reaction products of Reaction 4 after mild acid hydrolysis shows the presence of N-acetylglucosamine, and sometimes also of a slower moving substance which was identified as N-acetylglucosaminyl β 1-4-N-acetylglucosamine, or N,N'di-acetylchitobiose.[88] *A priori* it could be thought that the added N-acetylglucosaminyl residue could originate from UDP-GlcNAc or from Dol-P-P-GlcNAc. Studies in which the labeled donor was one or the other compound led to the conclusion that the reaction occurred as follows:[88]

$$\text{UDP-GlcNAc} + \text{Dol-P-P-GlcNAc} \rightarrow \text{Dol-P-P-GlcNAc}\ \beta\text{-1-4 GlcNAc} + \text{UDP} \qquad (5)$$

That is, the donor is the sugar nucleotide. The reaction product has been chemically synthesized and found to be the same as the one formed enzymatically.[89]

3. Dolichyl Diphosphate Oligosaccharide Containing N-acetylglucosamine and Mannose Residues

The early experiments showed that incubation of microsomes with labeled GDP-Man or Dol-P-Man led to the incorporation of label in dolichyl diphosphate oligosaccharides.[7] The evidence that has been gathered since then shows that the first five mannoses are added directly from GDP-Mannose, and the rest probably from dolichyl diphosphate mannose.

Starting with dolichyl diphosphate N, N' di-acetylchitobiose as acceptor and GDP-Mannose as donor, a dolichyl diphosphate trisaccharide was found to be formed as follows:

$$\text{Dol-P-P-GlcNac}_2 + \text{GDP-Man} \rightarrow \text{Dol-P-P-GlcNac}_2\text{-Man} + \text{GDP} \qquad (6)$$

Dol-P-Man was inactive as donor, and Dol-P did not accelerate the reaction.[90] It may be pointed out that the first mannose to be added has a β configuration, whereas the others are α.

In the presence of EDTA, which inhibits dolichyl phosphate mannose formation, and GDP-Man as donor, a dolichyl diphosphate oligosaccharide containing five mannoses accumulates.[91,92] A mouse lymphoma cell mutant Thy-1$^-$E which is defective in dolichyl

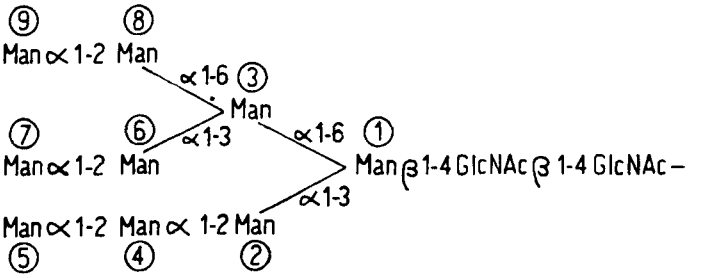


FIGURE 7. The order of addition of the mannose residues of dolichyl diphosphate G-oligosaccharide.

phosphate mannose formation also accumulates a lipid oligosaccharide containing five mannoses (numbers 1-5 of Figure 7).[93] Membrane enzymes from this mutant were found to be able to use dolichyl phosphate mannose as a donor of mannose number 6, and probably 7, 8, and 9 of Figure 7.

A mannose transferase which is involved in the process of formation of dolichyl diphosphate oligosaccharides has been solubilized and partially purified.[94] It catalyzes the transfer of mannose from GDP-Man to an exogenous acceptor yielding a five-mannose compound in which the newly added mannose joins α 1-2. This mannose seems to be that marked number 5 in Figure 7.

C. Dolichyl Diphosphate G-Oligosaccharide

1. The Structure of G-oligosaccharide

Dolichyl diphosphate G-oligosaccharide was discovered some 10 years ago as a product of the reaction of Dol-P-Glc with an endogenous acceptor present in liver microsomes. The substance was found to be insoluble in most organic solvents, but soluble in chloroform-methanol-water (1:1:0.3).[74] Mild acid treatment led to the liberation of a substance which appeared to be dolichyl phosphate, and of another compound which had the properties of an oligosaccharide.[95] If the acid treatment was carried out in a solvent containing methanol and low water content, the methyl glycoside of an oligosaccharide was formed, and when the latter was treated with alkali (2 N at 100°), two positive charges appeared due to the deacetylation of two hexosamine residues.[40] Periodate oxidation of G_3-oligosaccharide labeled in the glucose residues showed that the radioactive formic acid liberated corresponded to less than 1 molecule per molecule of glucose. Theoretically, only those residues which have unsubstituted 2, 3, and 4 hydroxyl groups should yield formic acid. The conclusion was that only half or less of the glucose residues corresponded to unsubstituted end groups, and that probably some of the glucose residues were joined to each other.[40]

Further studies on Dol-P-P-G-oligosaccharide were carried out by Spiro et al.[96,97] They incubated calf thyroid slices with labeled monosaccharides and isolated the Dol-P-P-G-oligosaccharide. They concluded that N-acetylglucosamine, mannose, and glucose were part of the same compound. After labeling with ^{3}(H)mevalonic acid, [^{32}P]phosphate, and [^{14}C]glucose, a glycolipid with the three labels was found to be formed.

The structure for the G-oligosaccharide shown in Figure 8 was proposed by Li et al.,[98] based on the studies carried out on a dolichyl diphosphate G-oligosaccharide obtained from *Vesicular stomatitis* virus-infected cells incubated with labeled monosaccharides. These studies consisted of acetolysis, Smith degradation, methylation analysis, and periodate oxidation. The linkages of the mannose and N-acetylglucosamine are the same as those of thyroglobulin oligosaccharide shown in Figure 2D.

Liu et al.[99] arrived at the same structure in studies on G-oligosaccharide synthesized by

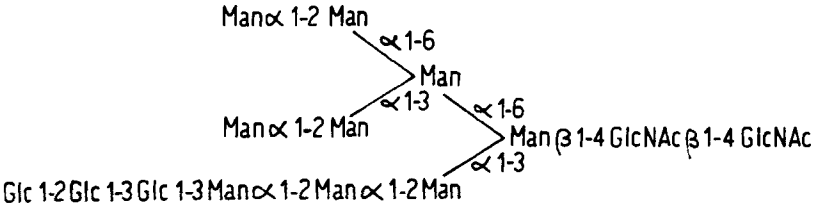


FIGURE 8. The structure of G_3-oligosaccharide.

NIL 8 fibroblasts. The anomeric configuration of the glucoses has not been established with complete certainty, but most of the evidence indicates an α configuration. The commonly available α and β glucosidases act very slowly or not at all on the G-oligosaccharide. Microsomal enzymes release the glucose rapidly, but their specificity is not certain. Studies on the inhibition by disaccharides has shown that kojibiose (glucosyl α 1-2 glucose) inhibits the glucosidase acting on the most external glucose, and that nigerose and maltose (which have α 1-3 and α 1-4 linkages, respectively) inhibit the glucosidase acting on the two internal glucoses.[100] None of the β disaccharides tested were inhibitory. This point, and some contradictory results with *p*-nitrophenylglucosides, are mentioned in the section on glucosidases.

Chromium trioxide has been used by Spiro et al.[101] for distinguishing α from β anomers. The results were in favor of an α configuration. Considering all the evidence available, it seems that the three glucoses of G-oligosaccharide are α.

2. The Biosynthesis of Dolichyl Diphosphate G-oligosaccharide

A scheme of the biosynthetic pathway of dolichyl diphosphate G-oligosaccharide is presented in Figure 9. There are still many details to fill in, but on the whole the scheme has solid foundations and is applicable to most, or perhaps to all, eukaryotic organisms.

The first enzyme in the pathway is an N-acetylglucosamine transferase which leads to the formation of dolichyl diphosphate N-acetylglucosamine and uridine monophosphate. The second reaction is another N-acetylglucosamine transferase which yields dolichyl diphosphate, N, N′ di-acetylchitobiose. The next step is the introduction of mannose residues, the donor being either GDP-mannose directly, or dolichyl phosphate mannose. The last step in the biosynthesis of the G-oligosaccharide is the addition of the three glucoses. The glucose acceptor seems to be a dolichyl diphosphate oligosaccharide with nine mannoses and two acetylglucosamine (the compound with eight mannoses has also been suggested).[102] The donor of all three glucoses is Dol-P-Glc.[103,104]

All these steps are catalyzed by enzymes present in the microsomal fraction of tissue extracts. The most available material is liver but many other tissues have been used. Microsomes usually contain acceptors so that the different reactions can be detected with only the addition of sugar nucleotides. However, in some cases addition of exogenous acceptors greatly increases the yield of the product. For instance, the yield of mannose-labeled lipids from GDP-[^{14}C]-Man was greatly increased by the addition of an exogenous acceptor.[105]

The last reaction of G-oligosaccharide biosynthesis, that is the addition of glucose from Dol-P-glucose, was not influenced by the addition of an exogenous acceptor. In all these reactions an important factor is the detergent concentration. The quantity added for optimal yields depends on the amount of microsomes and lipids added, and can only be determined by previous tests.

In some experiments more than one sugar nucleotide was incubated with the microsomes. Robbins et al.[106] observed that the size of the lipid-linked oligosaccharides was larger when UDP-Glc was added besides labeled GDP-Man. The action of UDP-Glc

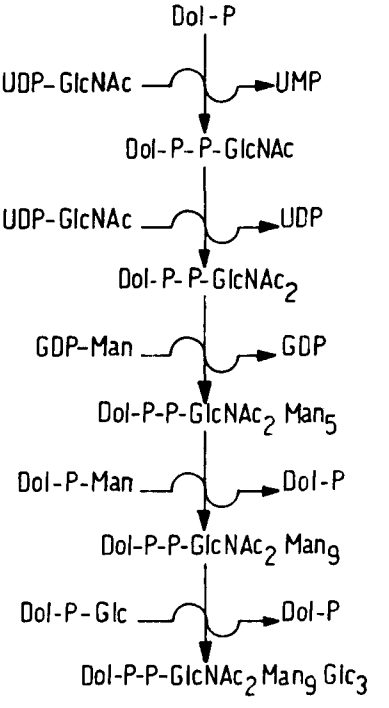


FIGURE 9. The pathway of dolichyl diphosphate G-oligosaccharide biosynthesis.

was manifest in the range of $10^{-7}M$. This concentration is three or four orders of magnitude lower than the K_m for UDP-Glc of glycogen synthetase.

Based on a study of the lipid-linked intermediates found in CHO cells incubated with [^{3}H]galactose or [^{3}H]mannose, in order to label glucose (or both glucose and mannose, respectively), Chapman et al.[107] deduced a sequence of addition of the mannoses. The intermediates were extracted and hydrolyzed to release the oligosaccharides. After separation of the latter by paper chromatography, the oligosaccharides which contained one to eight mannose residues were identified by α-mannosidase digestion methylation analysis and acetolysis. Knowing the structure of the different oligosaccharides, the order of mannose addition shown in Figure 7 was proposed.

V. PROTEIN GLYCOSYLATION

A. Transfer of the Oligosaccharides to Protein

The first experiments of transfer of oligosaccharide from the dolichyl diphosphate derivative to protein were carried out with Dol-P-P-G-oligosaccharide as donor and endogenous protein of liver microsomes as acceptor.[108] Subsequently, other Dol-P-P-saccharides were tested as donors and exogenous polypeptides were used as acceptors.

B. Different Dolichyl-P-P-Saccharides as Donors

Qualitative tests showed that many compounds can be used as saccharide donors. Mannose-labeled Dol-P-P-oligosaccharides, presumably containing five or more mannoses and probably no glucose, were found to act as donors.[105,109-111]

Dolichyl derivatives containing saccharides as small as N,N′ di-acetylchitobiose,[112-114] or a trisaccharide,[113-115,116] also served as donors. Transfer to protein has also been

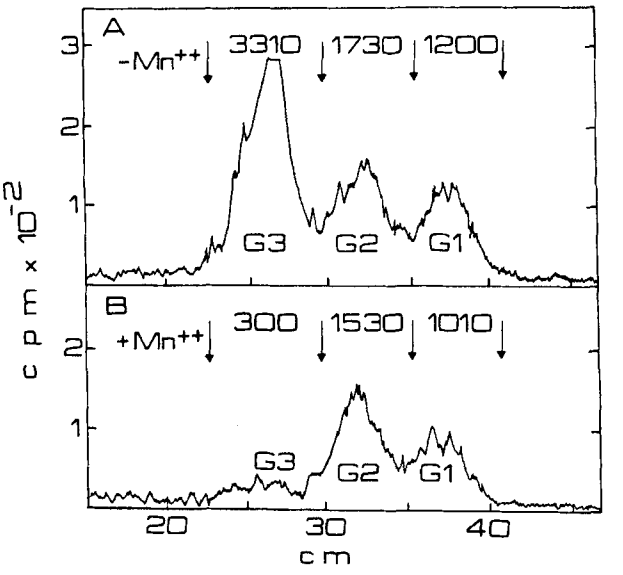


FIGURE 10. The transfer of G_3-oligosaccharide to protein. Dolichyl diphosphate G-oligosaccharide labeled in the glucose was incubated at 37° with liver microsomes with (lower frame), or without Mn^{++} (upper frame). After 10 min, the proteins were precipitated off, and the fraction soluble in chloroform-methanol-water 1:1:0.3 containing the unreacted dolichyl diphosphate oligosaccharides was hydrolyzed with 0.01 *M* acid at 100° for 10 min. and the oligosaccharides were separated by paper chromatography — G_3, G_2, and G_1, correspond to oligosaccharides with 3, 2, and 1 glucose residues. The numbers at the top represent the radioactivity in cpm of the corresponding piece of paper. (From Staneloni, R. J., Ugalde, R. A., and Leloir, L. F., *Eur. J. Biochem.*, 105, 275, 1980. With permission.)

observed with a sugar nucleotide as donor. Thus, Khalkhali and Marshall reported a transfer of N-acetylglucosamine from UDP-N-acetylglucosamine to ribonuclease A.[117,118]

However, when the rate of transfer to protein was measured some striking differences were observed. Turco et al.[119] compared Dol-P-P-G-oligosaccharides with similar compounds free from glucose. In order to measure the concentration of the substrates they submitted the substances to mild acid hydrolysis followed by reduction with tritium-labeled borohydride. When they tested the rate of transfer of the oligosaccharides from the Dol-P-P derivative to protein at equal concentrations of the donors, they found that the oligosaccharides containing glucose were transferred to endogenous protein about eight times faster.

Transfer is much faster with dolichyl diphosphate G-oligosaccharide containing three glucoses than with the corresponding compound with only one or two glucoses. Thus, Staneloni et al[103] carried out the transfer reaction to endogenous protein and then examined the unreacted products by paper chromatography. It was observed that only the compound containing three glucoses disappeared (Figure 10). In a control without Mn^{++} in which no transfer to protein occurred, there was no disappearance of the G_3-oligosaccharide.

The presence of the peripheral mannose residues of G-oligosaccharide does not seem to be necessary for transfer from the dolichyl derivative to protein. Thus treatment of

dolichyl diphosphate G-oligosaccharide with α-mannosidase did not decrease the donor activity as compared with the untreated compound. In contrast, treatment with α-glucosidase decreased activity drastically.

A dolichyl derivative detected in Thy-1⁻E mutant of mouse lymphoma cells, which is like dolichyl-G-oligosaccharide but of smaller size, containing 3 glucoses, only five mannoses, and two N-acetyl-glucosamine, was found to be transferred to endogenous protein.[120]

C. Acceptor Proteins

Tests carried out with native exogenous protein acceptors usually have been negative, but some successes have been reported. Eagon et al.,[121] found that a carbohydrate-free form of Kappa-type immunoglobulin light chain synthesized by mouse myeloma tumor MOPC 46 was glycosylated by the enzyme system of the same tissue. Several proteins could be glycosylated when added in denatured forms. Pless and Lennarz[122] tested some sulfitolysed proteins based on the finding of Tarentino et al.[123] that endo-β-N-acetylglucosaminidase H acted on the oligosaccharide of ovalbumin only after sulfitolysis. Ovalbumin, α lactalbumin, and ribonuclease A after sulfitolysis acted as oligosaccharide acceptors from a dolichyl diphosphate oligosaccharide, while other proteins which did not have the sequence Asn-X-Thr(Ser) were inactive. This confirmed the generalization of Marshall,[124] who after examining the amino acid sequence of many glycoproteins concluded that a necessary condition for glycosylation is the sequence Asn-X-Thr(Ser), but that other factors are also involved. Hart et al.[124a] tested many peptides as acceptors using hen oviduct enzymes. They found that the above mentioned tripeptide was practically inactive, but became a good acceptor when substituted in both terminals. One of the best acceptors was the tripeptide N-acetyl-Asn-Leu-Thr-N-CH_3.

Various sythetic peptides were tested by Ronin et al.[125,126] A tripeptide H-Asn-Ala-Thr-NH_2 was found to be used as acceptor in a system containing mannose-labeled dolichyl diphosphate oligosaccharides and pig thyroid rough microsomes, but it gave the lowest V_{max}/ Km ratio of the peptides tested. The best substrate was the hexapeptide H-Tyr-Gln-Ser-Asn-Ser-The-Met-NH_2, which is found in porcine ribonuclease. Another good substrate was the DNP-hexapeptide from thyroglobulin: DNP-Ala-Leu-Glu-Asn-Ala-Thr-Arg-NH_2.

The oligosaccharide-transferring enzyme from hen oviduct was solubilized and purified 2000-fold by adsorption to an α-lactalbumin Sepharose column and elution by removal of divalent cation.[126a] The purified enzyme had an absolute requirement for Mn^{++} (Mg^{++} was 30% as effective), was completely dependent on an exogenous acceptor and had a pH optimum between 7 and 7.5.

D. Studies on Protein Glycosylation in Intact Cells

Some studies on the site of subcellular glycosylation have been carried out with labeled sugars. Melchers[127] incubated plasma tumor cells (BALB mice MOPC46) with labeled monosaccharides and then separated the different organelles. The myeloma protein associated with the rough endoplasmic reticulum contained glucosamine and mannose, traces of galactose, and no fucose. The myeloma protein associated with the smooth endoplasmic reticulum contained as much mannose and glucosamine as the completed secreted form, but only half the amount of galactose and traces of fucose. The conclusion was that sugar residues are added stepwise at different subcellular sites.

Other experiments showed that glucosamine and mannose are incorporated soon after translation, but more glucosamine also is added later in the Golgi apparatus.[128] Incorporation of galactose in the Golgi has also been detected by radioautography.[129]

Experiments on the synthesis of thyroglobulin have been carried out on thyroid slices. It is known that at first, subunits of 3-8 S and 12 S are formed, and that they then

aggregate to form intermediates of 17-18 S, which are finally iodinated to yield thyroglobulin (19 S). After incubating rat thyroid slices with labeled leucine and either mannose or galactose, these were found to become incorporated at the same time in the 3-8 S and 12 S precursor, but galactose only appeared later in the 17-18 S precursor.[130] Fucose was found to behave like galactose and is incorporated in the Golgi apparatus.[131] The latter conclusion also has been reached by radioautography.[132,133]

Studies on rat thyroid with labeled N-acetylmannosamine as precursor showed that the label appeared in the 19 S precursor and that 86% was sialic acid.[134] All these results agree with the fact that galactose, fucose, and sialic acid are incorporated in the Golgi membranes.

E. Subcellular Localization

All the evidence available indicates that transfer to protein occurs on nascent polypeptides. Melchers[135] studied the kinetics of sugar incorporation in immunoglobulin G1 secreted by mouse plasma cell tumor MOPC21 and found that it occurs while the polypeptide chain is still attached to the ribosomes. Similar results have been obtained by following the attachment of N-acetylglucosamine and mannose containing oligosaccharide to ovalbumin.[136] The sugars were incorporated to the nascent chain of ovalbumin when it was still attached to the rough endoplasmic reticulum. Experiments on the in vitro synthesis of virus proteins in a system containing crude wheat germ showed that when the G protein of vesicular stomatitis virus was translated from the corresponding messenger RNA, it was not glycosylated, and still had the signal sequence at the amino terminal.[137] If pancreatic microsomal membranes were added before starting the translation, the resulting G protein had oligosaccharides and lacked the signal sequence. The membranes had to be added before the polypeptide was 70 amino acids long in order to obtain the correct insertion in the membrane and glycosylation. Detergent (Triton X-100®) was found to inhibit the glycosylation. If it was added before the polypeptide was 150 aminoacids long, protein G was not glycosylated. If the polypeptide had more than 150 and less than 400 amino acids, the resulting protein G had only one oligosaccharide. If the peptide was larger than 400 when detergent was added, the protein G had two oligosaccharides.[137] It seems, therefore, that the two oligosaccharides are added sequentially, while the polypeptide transverses the membranes. The glycosylation of placental peptide hormones has been found to be catalyzed by both rough and smooth microsomes.[138] The activity of some of the enzymes of the dolichol pathway has been determined in the rough and in the smooth microsomes. The enzyme that catalyzes transfer to protein from dolichyl diphosphate G-oligosaccharide,[139] or from lower homologues without glucose,[140] was found to be present both in rough and smooth microsomes, and also in cell membranes of reticulocytes.[141] On the other hand, the transferring activity with GDP-[^{14}C]mannose as donor and endogenous or exogenous acceptor (S carboxymethylated α lactalbumin) showed a higher activity in the rough membranes.[142] The formation of some of the lipid intermediates was also higher in the rough fraction.

VI. THE PROCESSING OF PROTEIN-BOUND OLIGOSACCHARIDES

The Dol-P-P-G-oligosaccharide was first considered to be a curiosity since a glucose-containing oligosaccharide of that type had never been detected in glycoproteins. It was later found that G-oligosaccharide could be transferred to protein, and soon after evidence was presented showing that Dol-P-P-G-oligosaccharide was a donor for the glycosylation of some or perhaps all the N-glycosylated glycoproteins. Once transferred to protein, the oligosaccharide loses its glucoses and may be processed to yield saccharides of the high mannose or complex type.

The liberation of glucose during the transfer of G-oligosaccharide to protein was observed in the initial in vitro experiments, but its physiological role was not appreciated.[108] Several groups of workers[143-145] studied the changes of the oligosaccharide which follow transfer from lipid to protein.

A. Experiments with Virus-Infected Cells

In some experiments, use has been made of virus-infected cells which synthesize only the virus glycoprotein but use the host's saccharide-synthesizing machinery. Sefton[146] compared the size of the lipid- and protein-bound oligosaccharides at different times after administration of [^{3}H]mannose to Sindbis virus-infected cells. After short incubation times (50 sec), the labeled glycopeptides were as large or larger than the high mannose glycopeptide (S4) of the Sindbis virus — that is, no smaller intermediates were detectable as expected if synthesis occurred by successive addition of monosaccharides. On the other hand, the oligosaccharides linked to lipid appeared as several compounds of different sizes, and the largest increased with time at the expense of the smallest as if synthesis occurred on the lipid-linked oligosaccharide. These experiments were consistent with the idea that oligosaccharides are transferred to protein after assembly on a lipid intermediate. However, evidence on the trimming required direct comparison of the protein and lipid-linked saccharides.

It may be mentioned that Sefton[146] also observed that the viral glycoproteins synthesized during glucose starvation lacked oligosaccharides or had some abnormal ones. This observation was similar to that of Kaluza,[147] who found that the electrophoretic mobility of the glycoproteins synthesized by cells infected with Semliki Forest virus was greater when growth took place in glucose-free medium.

Robbins et al.[144] gave pulses [^{3}H]mannose and [^{35}S] methionine to embryonic cells infected with vesicular stomatitis virus and examined the proteins by polyacrilamide gel electrophoresis, with or without previous treatment with endo-β-N-acetylglucosaminidase H. All the protein-linked oligosaccharides formed initially were released by endo-β-N-acetylglucosaminidase H, but during a chase period the glycoproteins became increasingly resistant to the enzyme. This is as if the oligosaccharide formed initially belonged to the high mannose type, and progressively became of the complex type. In other experiments the G-oligosaccharide was released from the dolichyl diphosphate by mild acid hydrolysis and treated with endo-β-N-acetyl glucosaminidase H; the product was then compared by gel filtration with the oligosaccharide released from the glycoproteins. After a short pulse (2.5 min) of [^{3}H]mannose, the oligosaccharide released from the glycoproteins was nearly the same size as the G-oligosaccharide, but as time passed the size decreased progressively. Similar results were obtained with Sindbis virus-infected chick embryo fibroblasts. From these results Robbins et al.[144] proposed that glycosylation of asparagine residues occurs by transfer of a common oligosaccharide from the dolichyl diphosphate G-oligosaccharide to proteins. Following the transfer the oligosaccharide would be processed to give the high mannose or complex oligosaccharides.

Careful investigation of the processing of the protein-bound glucose containing oligosaccharide was carried out by Tabas et al.[143] Their results and conclusions greatly clarified the events leading to the formation of the N-bound oligosaccharide of proteins. The general technique used in these experiments consisted in measuring by gel filtration the size of the oligosaccharides from vesicular stomatitis virus-infected cells labeled with [^{3}H]mannose for a short time or after pulse-chase for different periods of time. The oligosaccharides were released by endo-β-acetylglucosamindase C_{II} from *Clostridum perfringens* (Table 1). It was found that the processing of the oligosaccharide begins immediately after the transfer of the oligosaccharide to protein. After 30 min the

oligosaccharide had been transformed into a protein-bound pentasaccharide. The outer sugar residues (GlcNAc, Gal, sialic acid) were then added.[143]

Similar results were reported by Hunt et al.[145] They gave pulses of [^{3}H]mannose to HeLa cells infected with vesicular stomatitis virus and observed that the protein-bound oligosaccharides which were labeled initially had seven to nine mannoses. After some time only three mannoses were found, and complex type oligosaccharides appeared.

Experiments were carried out by Kornfeld and co-workers[148] with vesicular stomatitis virus-infected CHO cells labeled with radioactive mannose or galactose. The glycopeptides were obtained by treatment with pronase and isolated by gel filtration. The oligosaccharides were released from the glycopeptides with the endo-β-N-acetyl-glucosaminidase C_{II}. They were then reduced with $NaBH_4$ and subjected to paper chromatography in n-butanol pyridine water (4:3:4). Each oligosaccharide was then subjected to acetolysis, methylation, and α mannosidase treatment to determine its structure. After 20 to 30 min of labeling, $Glc_1\ Man_9\ GlcNac_2$ and $Man_9\ GlcNac_2$ were the major processing intermediates. There were also small amounts of $Man_8\ GlcNac_2$ and $Man_7\ GlcNac_2$ and trace amounts of $Glc_2\ Man_9\ GlcNac_2$ and $Man_6\ GlcNac_2$. These results show that a few minutes after transfer, the processing of the oligosaccharide begins with the removal of two to three glucoses residues. At 20 to 30 min, all the glucose is removed and the high mannose intermediate is then rapidly processed to give the $Man_3\ GlcNAc_2$ core.[148]

The scheme shown in Figure 11 has been proposed by Kornfeld et al.[148] for the changes which occur in the formation of complex oligosaccharides. The G-oligosaccharide A loses glucose to give B, which has nine mannoses. An α 1-2 manosidase would then remove four mannoses, leaving oligosaccharide C which has an α 1-3 and an α 1-6 mannose residue. This is the compound which accumulates in clone 15 B cells which lack N-acetylglucosaminyl transferase I (see Section VI. C.) The next step is the addition of an N-acetylglucosamine residue to give oligosaccharide D, after which two mannoses are removed to give E. The following steps are the addition of more N-acetyl-glucosamine, fucose, galactose, and sialic acid to give the complex oligosaccharide.

B. Experiments With Noninfected Cells

Tabas et al.[143] examined the changes in the oligosaccharides in LPCl mouse plasmocitoma cells that synthesize the oligosaccharide joined to the heavy chain. After a 12 min incubation with [^{3}H]mannose, the intracellular immunoglobulin G was immunoprecipitated and the glycopeptides were prepared with pronase. The product obtained was of the high mannose type, as judged by the fact that it was hydrolyzed by endo-β-N-acetylglucosaminidase C_{II}, whereas the glycopeptide obtained from the immunoglobulin G secreted into the medium was not acted upon — that is, it appeared to be of the complex type. Its behavior when treated with β-galactosidase, endo-β-N-acetylglucosaminidase and α mannosidase confirmed this assumption. Therefore it seems that processing also occurs in the formation of immunoglobulin G, which is a soluble protein, in contrast to those of the viruses, which are membrane-bound.

Staneloni and Leloir[149] incubated thyroid slices with [^{14}C]-glucose, and liberated the oligosaccharides from protein by treatment with alkali and borohydride. The oligosaccharides found were about four hexose units smaller than the G-oligosaccharide and had traces of glucose. These results are consistent with the existence of a trimming process.

C. Mutant Cells

The use of mutant cells has already yielded interesting results and will surely yield many more in the future. Some mutants have been produced by treating cells with a mutagenic agent and then selecting them for resistance to lectins.

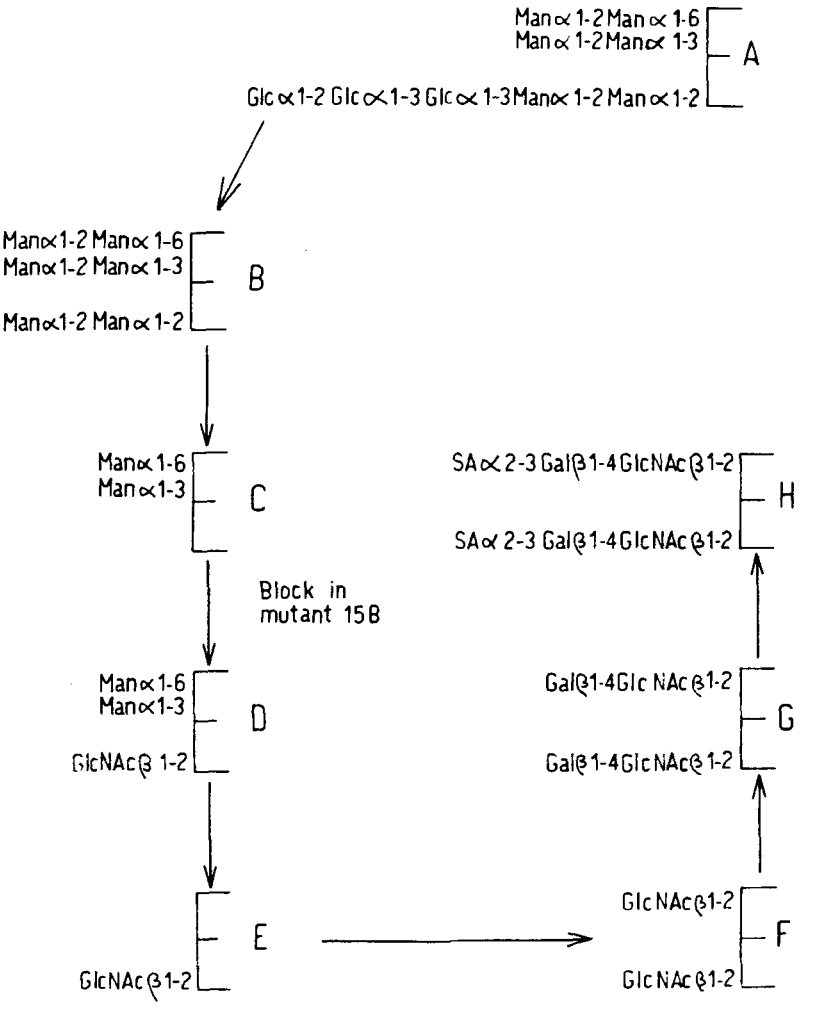


FIGURE 11. The pathway of formation of a protein-bound complex oligosaccharide from G-oligosaccharide. The large E represents the core, as described in Figure 1.

Trowbridge and Hyman[150] found that certain cells (Class E, Thy⁻1 negative mouse lymphoma) obtained by immunoselection are unable to synthesize Dol-P-P-G-oligosaccharide, and have a smaller lipid-linked oligosaccharide than the parent lymphoma cells.

The structure of this oligosaccharide was determined by Chapman et al.[151] and found to be the same as that of the G-oligosaccharide, but lacking the four mannoses joined to the α 1-6 mannose of the core. The lymphoma mutant has been found to lack the enzyme responsible for the formation of Dol-P-Man, which seems to be the donor for the addition of the sixth and probably the rest of the mannoses.[93]

The five-mannose-containing compound formed by transfer from GDP-Man can then accept three glucoses, as does G_0-oligosaccharide. It is believed that in normal cells the pathway going through Dol-P-P-Glc_3 Man_5 $GlcNAc_2$ oligosaccharide is a minor one in relation to that which goes through the G-oligosaccharide derivative. Normal cells have been found to contain lipid-linked $Glc_3Man_5GlcNAc_2$ and, furthermore, its oligosaccharide moiety can be transferred to protein by normal cell enzymes.[151]

This is additional evidence showing that the glucoses are necessary for transfer to

protein. Once bound to protein the $Glc_3Man_5GlcNAc_2$-oligosaccharide is processed and gives rise to oligosaccharides of the complex type. This process was studied by Kornfeld et al.[120] by performing pulse-chase experiments on vesicular stomatitis virus-infected lymphoma mutant cells using tritium-labeled mannose. The protein-bound oligosaccharides were then released with endo-β-N-acetylglucosaminidase D or with alkaliborohydride, and the structure of some of them was determined. The major protein-bound intermediates were: Glc $Man_5GlcNAc_2$, $Man_5GlcNAc_2$, $Man_4GlcNAc_2$, and $Man_3GlcNAc_2$. They all had the structure expected for degradation products of $Glc_3Man_5GlcNAc_2$ oligosaccharide.

The processing which is shown in Figure 12 therefore is similar to that of the G-oligosaccharide in that the glucoses are removed first, but is different in the mechanism of addition of N-acetylglucosamine. In the $Glc_3Man_5GlcNAc_2$ oligosaccharide pathway, N-acetylglucosamine is added to the three mannose compound, whereas as mentioned before in the G-oligosaccharide scheme, N-acetylglucosamine is added to a compound with five mannoses (Figure 11), and then two mannoses are removed to form the acceptor for the next N-acetylglucosamine. The removal of the two mannoses by α mannosidase before N-acetylglucosamine transfer, in the G-oligosaccharide pathway, is not necessary in the $Glc_3Man_5GlcNAc_2$. The steps which follow, addition of galactose, sialic acid, and fucose, would be the same in the two schemes.

N-acetylglucosaminyl transferase I can use as substrate both the $mannose_3$ and the $mannose_5$ peptides.[152] Thus the enzymes of normal and mutant cells are all the same, except that the mutant does not form Dol-P-Man and cannot add the four mannoses to the α 1-6 linked mannose of the core.

A comparison of the protein-bound oligosaccharides of purified vesicular stomatitis virus grown in parent and in mutant cells did not reveal any difference.[120] After treatment with neuraminidase, the glycopeptides behaved the same when submitted to Gel filtration, Concanavalin A-Sepharose separation, and methylation analysis. It was concluded that the vesicular stomatitis virus glycoprotein grown in the mutant cells contained complex type oligosaccharides that were very similar or identical with those found in the parent lymphoma cells. However, the glycopeptides of several cell surface proteins of the mutant had smaller high mannose oligosaccharides than those of the normal counterpart.[153] It seems, therefore, that whereas no changes can be detected when the cells make complex type oligosaccharides only, there is a difference under conditions in which the high mannose are also synthesized.

A mutant (clone 15 of CHO cells) selected for resistance to ricin has been found to lack N-acetylglucosamine transferase I (see Section VI.E.3.). Its membrane oligosaccharides are deficient in sialic acid, galactose, and N-acetylglucosamine.

D. The Pathway to the High Mannose Oligosaccharides

There is evidence indicating that the oligosaccharides of the high mannose type arise by transfer of G-oligosaccharide from dolichyl diphosphate derivative to protein. A high mannose oligosaccharide with a structure identical to the G-oligosaccharide but without the glucoses has been isolated from calf thyroglobulin.[17] Its structure is shown in Figure 2D. It seems obvious that in this case the precursor should be the G-oligosaccharide. The G-oligosaccharide can be transformed, at least on paper, into any of the other high mannose saccharides shown in Figure 2. It has been mentioned previously that a lymphoma mutant which does not form dolichyl G-oligosaccharide, forms instead a compound having five mannoses, and that when these lymphoma mutants were infected with Sindbis virus the glycoproteins formed contained smaller high mannose oligosaccharide than the Sindbis-infected nonmutant lymphoma.[150] This is another indication that dolichyl diphosphate oligosaccharide is the precursor of the high mannose oligosaccharides.

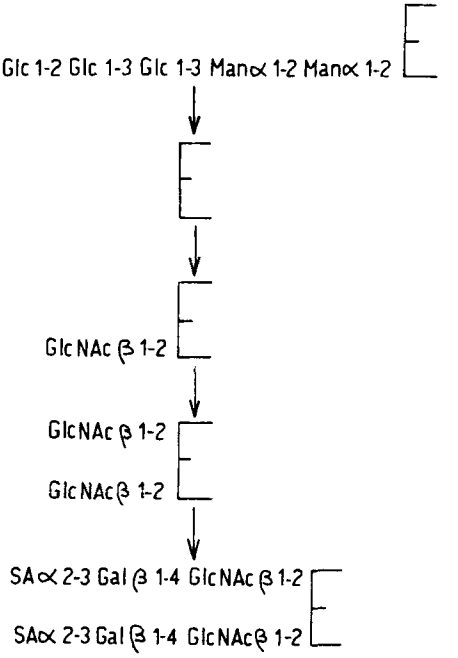


FIGURE 12. The formation of a complex oligosaccharide from protein-bound Glc_3 Man_5 $GlcNAc_2$ in lymphoma mutant cells. The large E represents the core, as described in Figure 1.

E. Studies on Isolated Enzymes

The study of the enzymes involved in the processing of the oligosaccharides has only started. One of the difficulties is that most of these enzymes are membrane-bound, and that methods for the purification of this type of proteins are not adequately developed.

1. Glucosidases

In the first experiments on the transfer of G-oligosaccharide from the dolichyl diphosphate derivative to protein, it was observed that glucose was liberated, and that the amount was higher under conditions which led to maximal transfer.[108] This was an indication of the presence of glucosidases acting on protein-bound G-oligosaccharide. Since the latter is not an easily available substrate for glycosidase estimation, either free or peptide-bound labeled G-oligosaccharide have been used. The radioactive glucose liberated has been measured after separation by paper chromatography or after phosphorylation with hexokinase and ATP followed by passing through an anion exchange resin. In liver extracts most of the activity was found in the microsomal fraction and could be detected using oligosaccharide, either free or combined with dolichyl diphosphate or with a peptide.[154] Active preparations also have been obtained from hen oviduct[155] and from thyroid glands.[101]

Measurements on the glucosidase activity on the separated G_1-, G_2-, and G_3-oligosaccharides have shown that at least two different enzymes are involved — one that acts on G_3-, and another acting on G_2- and G_1-oligosaccharides.[156,157,100]

Ugalde et al.[158] separated two liver microsomal enzymes by differential extraction. With a nonionic detergent (Nonidet P-40®) the glucosidase active on G_1- and G_2-oligosaccharide was extracted and on increasing the ionic strength (160 m*M* phosphate buffer) the enzyme acting on G_3-oligosaccharide could be solubilized. The properties of the two enzymes were clearly different. The glucosidases seem to be

localized on the cisternal side of the microsomal vesicles.[156] This was deduced from experiments in which the microsomes were treated with proteases.

Oligosaccharides treated with α mannosidase so as to remove the peripheral mannoses were found to be poorer substrates for the glucosidases.[101,157] The action of several disaccharides have been tested on the different glucosidases.[100] It was found that 1 m*M* kojibiose (glucosyl α 1-2 glucose) inhibits 91% the hydrolysis of the most external glucose which is joined by a 1-2 linkage. Nigerose (glucosyl α 1-3 glucose) and maltose (glucosyl α 1-4 glucose) inhibit the action of the glucosidase which hydrolyses the two internal glucoses which are linked 1-3.

Other disaccharides such as sophorose (glucosyl β 1-2 glucose) and cellobiose (glucosyl β 1-4 glucose) were found to be inactive. Glucose at 8 m*M* inhibited 40, 30, and 0% the glucosidase activity on G_1-, G_2-, and G_3-oligosaccharides, respectively.

The action of kojibiose also was tested on protein-linked G_3-oligosaccharide as substrate, and the result was an inhibiton of glucosidase activity.[100] This is an indication that the same enzyme acts on the free and on the protein-bound oligosaccharide.

Contradictory evidence has been obtained with p-nitrophenylglycosides. Some workers found no inhibition,[159] others reported that the α isomer, but not the β, inhibited the hydrolysis of the internal glucose.[157] Scher and Waechter[160] reported that p-nitrophenyl-β-glucoside inhibited the hydrolysis of G-oligosaccharide more than the α anomer. Ugalde[161] measured the action of the above mentioned compounds on the hydrolysis of glucose from G_2- and G_3-oligosaccharide and confirmed that the β glucoside inhibits G_3-oligosaccharide hydrolysis more than the α. This can be taken as evidence in favor of the β configuration of the glucose of G_3-oligosaccharide, but all the other facts are more in favor of an α configuration. The effect of p-nitrophenylglucoside on the enzyme acting on G_2-oligosaccharide is different since only the α anomer produces inhibition. This agrees with the results of the inhibition by the α-linked disaccharide.

2. Mannosidase

After the G-oligosaccharide joined to protein loses its three glucose residues, it may be trimmed further by specific α mannosidases (Figure 11).

Touster et al.[162,163] purified an α mannosidase obtained from rat liver Golgi membranes. The purification was followed by measuring the activity with p-nitrophenyl α D-mannoside, but the enzyme was also active on oligosaccharides containing α 1-2-linked mannose. It may be pointed out that G_0-oligosaccharide has four external α 1-2 mannose residues.

A liver Golgi mannosidase was also purified by Tabas and Kornfeld,[164] and they measured its activity on oligosaccharides containing 9, 8, 7, 6, and 5 mannoses. These oligosaccharides were obtained by treatment of the glycopeptides with endo- β-N-acetylglucusaminidase C_{II}. All those that contained α 1-2 mannoses were good substrates but the five mannose compound (which did not contain α 1-2 linkages) was not hydrolyzed. Analysis of the intermediate products obtained by the action of the enzyme on G_0-oligosaccharide led to the conclusion that the order of removal of the mannoses was as shown in Figure 13.

The first residue removed could be that marked 1 or 2, but the sequence of removal of 3 and 4 was specific and different from that found in the processing of the CHO cells infected with vesicular stomatitis virus.[148] Golgi mannosidases active on the α 1-3 and α 1-6-linked mannoses have also been detected.[165]

3. N-acetylglucosaminyl Transferases

Two different transferases with different specificities have been described which are referred to as I and II.[152] Transferase I from bovine colostrum was purified by Harpaz

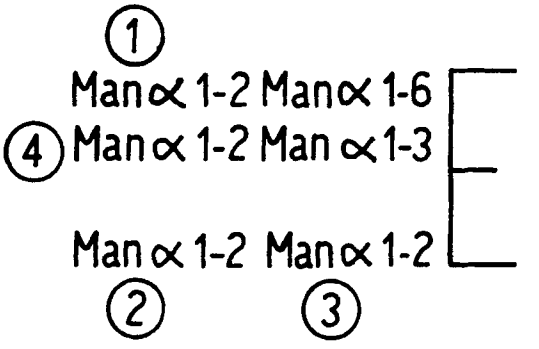


FIGURE 13. Order of removal of the mannose residues of G-oligosaccharide bound to protein. The large E represents the core as described in Figure 1.

and Schachter[166] and its action on different substrates was studied. They found that the enzyme can transfer N-acetylglucosamine to several glycopeptides obtained from ovalbumin and immunoglobulin G. The best substrate (Km = 0.12 m*M*) was the five-mannose-containing compound shown in Figure 14A. If the α 1-3 mannose is substituted with a β 1-2 N-acetylglucosamine (Figure 14C), the enzyme can transfer another N-acetylglucosamine on the α 1-6 mannose. However, the affinity for this substitute is much lower (Km = 10 m*M*). The presence of fucose on the internal N-acetylglucosamine does not affect the activity.

In a variant line of CHO cells and in clone 15 selected for resistance to phytohemaglutinin and to ricin, respectively, transferase I is lacking and protein-bound oligosaccharide A of Figure 14 is not processed further.

The addition of N-acetylglucosamine can be considered to be the signal for α mannosidase to remove the two mannoses from the α 1-6-linked mannose residue of the core.[165] Direct evidence for this was obtained by incubation of CHO cell extracts with labeled $Man_5GlcNAc_2$ peptide and UDP-GlcNAc.[165] It was found that the release of mannose occurred only when UDP-GlcNAc was present.

Transferase II catalyzes the addition of N-acetylglucosamine to oligosaccharide C of Figure 14 with Km = 0.1 m*M*. As mentioned before, transferase I can also catalyze this reaction, but with a Km 100 times higher.

Another transferase (III) has been postulated to catalyze the transfer of N-acetylglucosamine forming a β 1-4 linkage on the β 1-4-linked mannose of the core.[167] This structure is found in some ovalbumin glycoproteins (see Figure 3).

Information is lacking on the transferases responsible for the introduction of the additional N-acetylglucosamines in tri- and tetraantenary compounds.

4. Fucosyl Transferases

Several enzymes which catalyze the transfer of D-fucose from GDP-fucose have been detected. These are of two types: one for which the acceptor is a galactosyl residue, and another for which it is N-acetylglucosamine. An α 1-2 fucosyltransferase of the first type, which transfers to galactosyl residues, has been found in porcine submaxillary glands and is partially purified.[168]

An enzyme present in human milk catalyzes the incorporation of fucose in α 1-3 linkage to acceptors that have following sequence Gal β 1-4 GlcNAc-R to produce Gal β 1-4 (Fuc α 1-3)GlcNAc-R.[169]

Another fucosyl transferase found in liver microsomes catalyzes transfer to the most

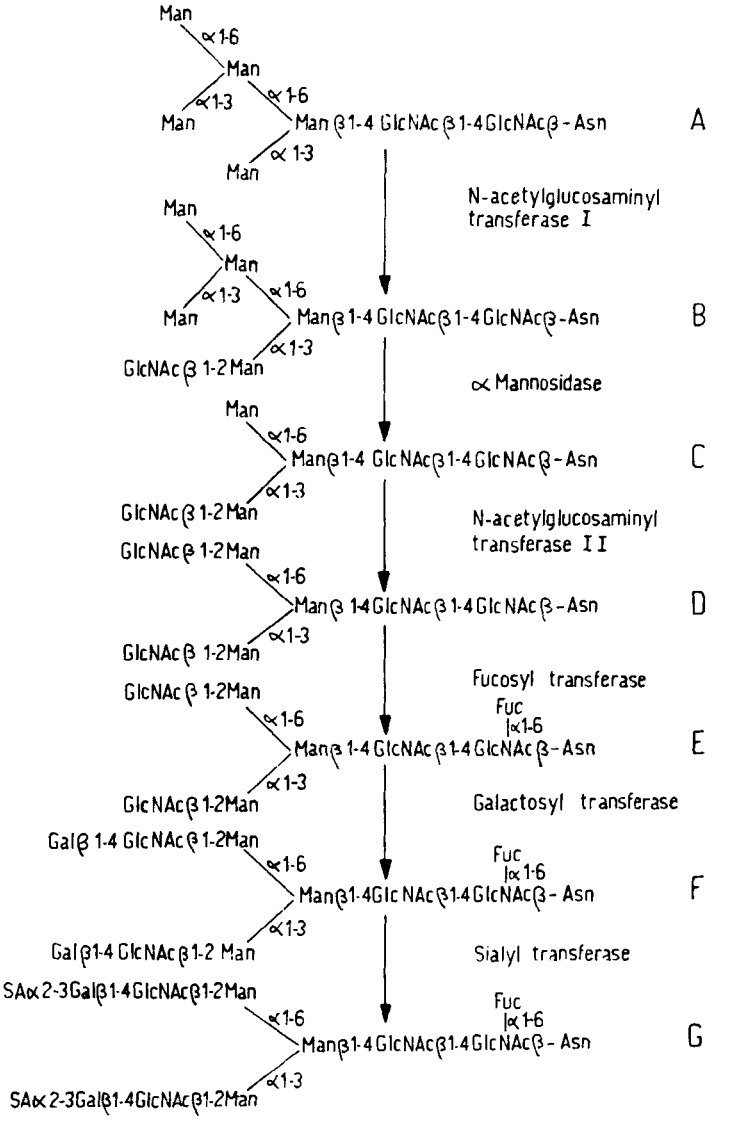


FIGURE 14. The enzymes involved in the formation of complex oligosaccharides.

internal N-acetylglucosamine of human plasma α_1 acid glycoprotein.[170] The corresponding glycopeptide, freed from sialic acid and galactose (Figure 14D), was a good substrate. Removal of an N-acetylglucosamine (Figure 14C) also produced a good substrate, but at least one β 1-2 linked N-acetylglucosamine is required for fucose transfer. This agrees with the fact that a CHO cell mutant lacking N-acetylglucosamine transferase I does not transfer fucose.[171]

5. Galactosyl Transferases

The transfer of galactose from UDP-galactose to position 4 of an N-acetylglucosamine residue is catalyzed by the β 1-4 galactosyl-transferase, which was purified from human and bovine milk.[172,173] It is well known that the enzyme will use glucose as acceptor, in the presence of α lactalbumin, and N-acetylglucosamine in its absence. In the first case the product is lactose and the second acetyllactosamine (galactosyl β 1-4 N-acetylglucosamine).

Combined N-acetylglucosamine residues are also used as substrates (Figure 14E). It also is known that this occurs not only with the mammary gland enzyme but with those of liver and other tissues.[174]

What is not so well known is how many transferases are involved in the different catalytic processes and their specificity. Besides the β 1-4-linked galactose, some β 1-3 linkages have been detected in oligosaccharides linked to asparagine.[22,43]

6. Sialyl Transferases

The transfer of sialic acid from CMP sialic acid is a process which has received considerable attention. Many sialyl transferases acting on different acceptors have been described.[175-178]

A sialyl transferase from bovine colostrum has been purified 400,000 times.[176] It catalyzes the incorporation of sialic acid in the sequence SA α 2-6 Gal β 1-4 GlcNac-R (Figure 14G). As mentioned before this sequence is found in many complex type oligosaccharides (Figure 4). Beyer et al.[179] have studied the addition of different monosaccharides, which purified enzymes, to different acceptors prepared from human asialotransferrin and antifreeze protein. The enzymes used were three sialyl transferases, two fucosyl transferases, and an N-acetyl-glucosaminyl transferase. It was concluded that the final product formed depended not only on the type of enzymes present, but also on the order of addition.

VII. DISTRIBUTION OF THE DOLICHYL PHOSPHATE PATHWAY

The protein glycosylating mechanism involving dolichyl derivatives seems to be present in most or perhaps all eukaryotic cells. It has been detected in animals, insects, plants and fungi.

A. Insects

Some of the intermediates of the dolichyl phosphate pathway have been found in insects and it seems likely that the whole scheme is applicable. The insects investigated were the Mediterranean fruit fly *(Ceratitis capitata)* and the Chagas disease vector *Triatoma infestans.* A compound having the properties of dolichyl phosphate was detected as well as the in vitro formation of dolichyl phosphate glucose, dolichyl phosphate mannose, and of lipid-linked N-acetylglucosamine and N,N'di-acetyl-chitobiose.[180,181] A substance similar or identical to dolichol also was isolated and found to behave as if it had 17 to 18 isoprene residues.

B. Plants

Progress in the study of glycoprotein biosynthesis in plants has been slower than in mammalian tissues.[36,182] The formation of several of the dolichyl derivatives present in animal tissues has been detected using enzymes from cotton fibers,[183] *Phaseolus aureus* seedlings, or hypocotyls[184,185] and pea seedlings.[63]

Lipid-bound oligosaccharides containing N-acetylglucosamine and four to eight mannoses have also been detected.[186] As with animal enzymes, tunicamycin has been found to inhibit the formation of dolichyl diphosphate N-acetylglucosamine.[187] The formation of dolichyl diphosphate G-oligosaccharide by plant material with the procedure used for animal tissues has given negative results. This procedure consisted of incubating microsomes with labeled UDP-Glc or Dol-P-Glc in order to obtain the transfer of radioactive glucose to an endogenous acceptor. However, experiments with alfalfa root segments incubated with radioactive glucose allowed the detection of a compound having the properties of dolichyl diphosphate G-oligosaccharide.[188] Paper and column chromatography, alkaline deacylation, specific glucosidases, and total

hydrolysis gave the same results for the compounds from plant and animal origin. The yield of dolichyl diphosphate G-oligosaccharide from plants was very low, and this may explain the difficulties in its detection.

Some polyprenyl intermediates have been detected in algae. The formation of dolichyl-P-glucose, dolichyl diphosphate glucose, and some related compounds by *Prototheca zoopffi* has been studied, as well as their role in cellulose biosynthesis.[189] In extracts of another algae, *Volvox carteri F. nagariensis,* several lipid intermediates were detected. The formation of Dol-P-Glc was particularly rapid.[190]

C. Yeast

The glycoproteins of yeast have similarities with those of animal tissues but there are, however, some important differences. Yeast does not contain sialic acids and therefore it has no oligosaccharides of the complex type. Furthermore, it has saccharides of the high mannose type but with a much higher mannose content.

Studies on the glycosylation mechanism have progressed considerably. A transfer of mannose to serine/threonine residues of yeast protein was found years ago to be mediated by dolichyl phosphate mannose. Only one mannose residue is transferred by this mechanism, but the first residue may serve as acceptor for more residues transferred from GDP-Man, resulting in the formation of di-, tri-, and tetra-mannosyl residues linked α 1-2 and α 1-3.[191-103] Besides this process of serine glycosylation, there is another one which leads to the transfer of oligosaccharide to asparagine residues.

There are many polymannose-containing proteins in yeast which apparently have a core very similar or identical to the G-oligosaccharide. The saccharide moieties of these mannoproteins are of different sizes. Carboxypeptidase Y of yeast contains 4 oligosaccharide chains, each composed of 2 N-acetylglucosamines and about 13 mannose residues.[194,195] The biosynthesis of carboxypetidase Y is perturbed by tunicamycin, which is known to inhibit dolichyl diphosphate N-acetylglucosamine synthesis and consequently glycosylation through the dolichyl diphosphate G-oligosaccharide mechanism. Another mannoprotein is yeast invertase which contains about 50% of carbohydrate, mainly mannose. Endo-β-N-acetylglucosaminidase H releases polysaccharide chains of two classes, one of about 26, and another of 52 mannose residues.[194] Yeast alkaline phosphatase is also a mannoprotein[196] and its carbohydrate moiety is released by endo-β-N-acetylglucosaminidase H. The synthesis of the enzyme is not depressed by tunicamycin, but the result is the formation of a carbohydrate-free form.

Many studies have been carried out on yeast mannan which is a mannoprotein. The polysaccharide residue consists of a core portion and an outer chain built up on a mannose α 1-6 chain and carrying mannosyl α 1-2 and α 1-3 substituents and totaling 100 to 150 residues.

Many of the enzymes and intermediates present in animal tissues have been detected in yeast,[7] and the scheme of biosynthesis of Dol-P-P-G-oligosaccharide seems to be wholly applicable to it. Parodi[104,197] isolated Dol-P-P-G-oligosaccharide from yeast and compared its properties with the animal counterpart. The oligosaccharide had the same mobility in paper chromatography, gave a compound with two positive charges on alkaline treatment, and contained mannose and glucose. Transfer of the oligosaccharide to protein was observed to occur only, or at least much faster, with Dol-P-P-G_3-oligosaccharide than with compounds with one or two glucoses.[198] In some experiments yeast was given a pulse of glucose and the protein-bound oligosaccharides were examined after different times.[199] Liberation of the oligosaccharides from the glycoproteins was carried out by treatment with alkali-borohydride, and the products were chromatographed on paper. The oligosaccharides first decreased in size, and then increased as if first the glucoses, and at the most one mannose, were removed. Afterwards mannoses

were added to the trimmed oligosaccharide. Besides experiments with wild type cells, some mutants which synthesize an incomplete mannan were examined. Mutants carrying the mnn_1 mutation, which are known to be devoid of terminal α 1-3 mannose residues in the outer chain and inner core of mannan, were found not to add α 1-3-linked mannoses to the oligosaccharide after it became protein bound and lost the glucoses.[198,199]

D. Other Fungi

The formation of lipid intermediates has been detected in various fungi — for instance, extracts of *Neurospora crassa*[200] *Aspergillus niger,*[201] and *Dictyostelium discoideum*[202] have been found to catalyze the transfer of mannose to lipid in the presence of divalent cations.

E. Protozoa

Cell-free extracts from *Tetrahymena pyriformis* catalyze the transfer of glucose from UDP-glucose to an endogenous acceptor yielding a compound similar or identical to dolichyl phosphate glucose. The latter compound can then serve as donor to an oligosaccharide lipid and to glycoproteins.[203]

VII. FINAL COMMENTS

The knowledge that has accumulated on the metabolism of glycoproteins will allow the clarification of problems which have remained obscure up to now. One of these is the function of the "glyco" part of glycoproteins. More information on this problem will be obtained by the use of inhibitors which can interrupt oligosaccharide formation, by experiments with cell mutants that have blocks in glycoprotein synthesis, and with further studies on the role of sugars in the uptake of proteins by cells.

Some inhibitors that act specifically on protein glycosylation have been found. The most well known is tunicamycin, which inhibits the formation of dolichyl diphosphate N-acetylglucosamine, and consequently of the lipid-linked oligosaccharide which is transferred to protein. Tunicamycin, as well as some other antibiotics, has been tested on many different cells and under various circumstances in order to find out which processes require glycoprotein biosynthesis.

Experiments with cell mutants provide a powerful method for unraveling the biosynthetic pathways and for discovering the role of the oligosaccharide moiety of glycoproteins. Mutants have been selected for resistance to lectins and found to have alterations in their surface carbohydrates. Some of these mutants have been studied carefully, but the great potentialities of the method have only started to be exploited.

Another line of work which will yield more information on the role of oligosaccharides consists in studies on the uptake of glycoproteins by cells. The work started with observations on the disappearance from the blood of proteins containing unsubstituted galactosyl residues and has become a very active field of research. Several different carbohydrate moieties have been identified as responsible for specifically directing the uptake of glycoproteins by certain cells. It seems that with all this information we will soon have a clear picture of the function of the sugar moieties of glycoproteins.

Another aspect of glycoproteins which deserves comment is the distribution of the biosynthetic pathway in various living organisms. The dolichyl phosphate derivatives, as well as the corresponding enzymes, have been found in many different organisms such as yeasts, fungi plants, and animals (including insects). They appear to be present in all eukaryotes. Such a wide distribution of cell constituents and reactions suggests that the dolichyl phosphate sugar mechanism appeared in the first eukaryotes — i.e., about 1500 million years ago — and cannot be altered without producing profound disturbances in cell function.

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REFERENCES

1. **Hemming, F. W.,** Lipids in glycan biosynthesis, in *Biochemistry of lipids,* Vol. 4, Goodwin, T. W., Ed., Butterworths and University Park Press, London and Baltimore, 1974, 33.
2. **Behrens, N. H.,** Polyprenol sugars and glycoprotein synthesis, in *Biology and Chemistry of Eucaryotic Cell Surfaces,* Lee, E. Y. C. and Smith, Eds. Academic Press, New York, 1974, 159.
3. **Beytia, E. D. and Porter, J. W.,** Biochemistry of polyisoprenoid biosynthesis, *Annu. Rev. Biochem.,* 45, 113, 1976.
4. **Lucas, J. J. and Waechter, C. J.,** Polyisoprenoid glycolipids involved in glycoprotein biosynthesis, *Mol. Cell. Biochem.,* 11, 67, 1976.
5. **Waechter, C. J. and Lennarz, W. J.,** The role of polyprenol-linked sugars in glycoprotein synthesis, *Annu. Rev. Biochem.* 45, 95, 1976.
6. **Schachter, H.,** Glycoprotein biosynthesis, in *The Glycoconjugates,* Vol. 2, Horowitz, M. I. and Pigman, W., Eds., Academic Press, New York, 1978, 87.
7. **Parodi, A. J. and Leloir, L. F.,** The role of lipid intermediates in the glycosylation of protein in the eucaryotic cell, *Biochim. Biophys. Acta.,* 559, 1, 1979.
8. **Staneloni, R. J. and Leloir, L. F.,** The biosynthetic pathway of the asparagine-linked oligosaccharides of glycoproteins, *Trends Biochem. Sci.,* 4, 65, 1979.
9. **Johansen, P. G., Marshall, R. D., and Neuberger, A.,** Carbohydrates in proteins. The preparation and some of the properties of a glycopeptide from hen's egg albumin., *Biochem. J.,* 78, 518, 1961.
10. **Fletcher, A. P., Marks, G. S., Marshall, R. D., and Neuberger, A.,** Carbohydrates in proteins. Procedures for the isolation of glycopeptides from hen's egg albumin and their oxidation by periodate, *Biochem. J.,* 87, 265, 1963.
11. **Marks, G. S., Marshall, R. D., and Neuberger, A.,** Carbohydrates in proteins. Studies on the carbohydrate-peptide bond in hen's egg albumin. *Biochem. J.,* 87, 274, 1963.
12. **Tarentino, A., Plummer, T. H., and Maley, F.,** Studies on the oligosaccharide sequence of ribonuclease B, *J. Biol. Chem.,* 245, 4150, 1970.
13. **Sukeno, T., Tarentino, A. L., Plummer, T. H., Jr., and Maley, F.,** Purification and properties of α-D- and β-D-mannosidases from hen oviduct, *Biochemistry,* 11, 1493, 1972.
14. **Chapman, A. and Kornfeld, R.,** Structure of the high mannose oligosaccharides of human IgM myeloma protein. I. The major oligosaccharides of the two high mannose glycopeptides, *J. Biol. Chem.,* 254, 816, 1979.
15. **Chapman, A. and Kornfeld, R.,** Structure of the high mannose oligosaccharides of a human IgM myeloma protein. II. The minor oligosaccharides of high mannose glycopeptide I., *J. Biol. Chem.,* 254, 824, 1979.
16. **Tai, T., Yamashita, K., Ito, S., and Kobata, A.,** Structures of the carbohydrate moiety of ovalbumin glycopeptide III and the difference in specificity of Endo-β -N-acetylglucosaminidases C_{II} and H, *J. Biol. Chem.,* 252, 6687, 1977.
17. **Ito, S., Yamashita, K., Spiro, R. G., and Kobata, A.,** Structure of a carbohydrate moiety of a unit A glycopeptide of calf thyroglobulin, *J. Biochem.,* 81, 1621, 1977.
18. **Strecker, G. and Montreuil, J.,** Glycoproteines et glycoproteinoses, *Biochimie,* 61, 1199, 1979.
19. **Fournet, B., Montreuil, J., Strecker, G., Dorland, L., Haverkamp, S., Vliegenthart, F. G., Binette, J. P., and Schmid, K.,** Determination of the primary structures of 16 asialo-carbohydrate units derived from human plasma α_1-acid glycoprotein by 360-MH_z ^1H NMR spectroscopy and permethylation analysis, *Biochemistry,* 17, 5206, 1978.
20. **Spik, G., Bayard, B., Fournet, B., Strecter, G., Bouquelet, S., and Montreuil, J.,** Studies on glycoconjugates. LXIV. Complete structure of two carbohydrate units of human serotransferrin, *FEBS Lett.,* 50, 296, 1975.
21. **Kornfeld, R. and Kornfeld, S.,** Comparative aspects of glycoprotein structure, *Annu. Rev. Biochem.,* 45, 217, 1976.

22. **Takasaki, S., Yamashita, K., Suzuki, K., Iwanaga, S., and Kobata, A.,** The sugar chains of cold-insoluble globulin. A protein related to fibronectin, *J. Biol. Chem.,* 254, 8548, 1979.
23. **Hodges, L., Laine, R., and Chan, S. K.,** Structure of the oligosaccharide chains in human α_1 protease inhibitor, *J. Biol. Chem.,* 254, 8208, 1979.
24. **Mega, T., Lujan, E., and Yoshida, A.,** Studies, on the oligosaccharide chains of human α_1-protease Inhibitor II. Structure of oligosaccharides, *J. Biol. Chem.,* 255, 4057, 1980.
25. **Krusius, T., Finne, J., and Rauvala, H.,** The poly (glycosyl) chains of glycoproteins. Characterisation of a novel type of glycoprotein saccharides from human erythrocyte membrane, *Eur. J. Biochem.,* 92, 289, 1978.
26. **Li, E., Gibson, R., and Kornfeld, S.,** Structure of an unusual complex-type oligosaccharide isolated from chinese hamster ovary cells., *Arch. Biochem. Biophys.,* 199, 393, 1980.
27. **Hart, G. W. and Lennarz, W. J.,** Effects of tunicamycin on the biosynthesis of glycosaminoglycans by embryonic chick cornea, *J. Biol. Chem.,* 253, 5795, 1978.

27a. **Yoshima, H., Takasaki, S., and Kobata, A.,** The asparagine-linked sugar chains of the glycoproteins in calf thymocyte plasma membrane, *J. Biochem.,* 88, 819, 1980.

28. **Prehm, P., Scheid, A., and Choppin, P. W.,** The carbohydrate structure of the glycoproteins of the paramyxovirus SV5 grown in bovine kidney cells, *J. Biol. Chem.,* 254, 9669, 1979.

28a. **Heifetz, A., Kinsey, W. H., and Lennarz, W. J.,** Synthesis of a novel class of sulfated glycoproteins in embryonic liver and lung, *J. Biol. Chem.,* 255, 4528, 1980.

29. **Baenziger, J. and Kornfeld, S.,** Structure of the carbohydrate units of IgE immunoglobulin I overall composition glycopeptide isolation and structure of the high mannose oligosaccharide unit, *J. Biol. Chem.,* 249, 1889, 1974.
30. **Jouanneau, J. and Bourrillon, R.,** Characterization of a glycopeptide from pathological human IgM with an unusual oligosaccharide core, *Biochem. Biophys. Res. Commun.,* 91, 1057, 1979.
31. **Schlesinger, M. J., Schlesinger, S., and Burge, B. W.,** Identification of a second glycoprotein in Sindbis virus, *Virology,* 47, 539, 1972.
32. **Burke, D. J. and Keegstra, K.,** Purification and composition of the proteins from Sindbis virus grown in chick and BHK cells, *J. Virol.,* 20, 676, 1976.
33. **Burke, D. J. and Keegstra, K.,** Carbohydrate structure of Sindbis virus glycoprotein E2 from virus grown in hamster and chicken cells, *J. Virol.,* 29, 546, 1979.
34. **Reading, C. L., Penhoet, E. E., and Ballou, C. F.,** Carbohydrate structure of Vesicular stomatitis virus glycoprotein, *J. Biol. Chem.,* 253, 5600, 1978.
35. **Pesonen, M. and Renkonen, O.,** Serum glycoprotein type sequence of monosaccharides in membrane glycoproteins of Semliki Forest virus, *Biochim. Biophys. Acta,* 455, 510, 1976.
36. **Sharon, N. and Lis, H.,** Comparative biochemistry of plant glycoproteins, *Biochem. Soc. Trans.,* 7, 783, 1979.
37. **Lis, H. and Sharon, N.,** Soybean aglutinin. A plant glycoprotein. Structure of the carbohydrate unit, *J. Biol. Chem.,* 253, 3468, 1978.
38. **Spiro, R.,** The study of the carbohydrates of proteins, in *Methods in Enzymology,* Vol. 28, Ginsburg, V., Ed., Academic Press, New York, 1972, 3.
39. **Butler, W. T. and Cunningham, L. W.,** Evidence for linkage of a disaccharide to hydroxylysine in tropocollagen, *J. Biol. Chem.,* 241, 3882, 1966.
40. **Parodi, A. J., Staneloni, R. J., Cantarella, A. I., Leloir, L. F., Behrens, N. H., Carminatti, H., and Levy, J. A.,** Further studies on a glycolipid formed from dolichyl-D-glucosyl monophosphate, *Carbohydr. Res.,* 26, 393, 1973.
41. **Montreuil, J.,** Recent data on the structure of the carbohydrate moiety of glycoproteins. Metabolic and biological implication, *Pure Appl. Chem.,* 42, 431, 1975.
42. **Yosizawa, Z., Sato, T., and Schmid, K.,** Hydrazinolysis of α_1-acid glycoprotein, *Biochim. Biophys. Acta,* 121, 417, 1966.
43. **Mizuochi, T., Yamashita, K., Fujikawa, K., Kisiel, W., and Kobata, A.,** The carbohydrate of bovine prothrombin. Occurrence of Gal-β-1-3 GlcNAc grouping in asparagine-linked sugar chains, *J. Biol. Chem.,* 254, 6419, 1979.
44. **Bayard, B. and Roux, D.,** Hydrazinolysis and nitrous deamination of glycoproteins. Evidence for a common inner core in carbohydrate moiety, *FEBS Lett.,* 55, 206, 1975.
45. **Tarentino, A. L. and Maley, F.,** Purification and properties of an Endo-β-N-acetylglucosaminidase from *Streptomyces griseus, J. Biol. Chem.,* 249, 811, 1974.
46. **Trimble, R. B., Tarentino, A. L., Evans, G. and Maley, F.,** Purification and properties of Endo-β-N-acetylglucosaminidase L from *Streptomyces plicatus, J. Biol. Chem.,* 254, 9708, 1979.
47. **Muramatsu, T., Atkinson, P. H., and Nathenson, S.,** Cell-surface glycopeptides: growth-dependent changes in the carbohydrate-peptide linkage region, *J. Mol. Biol.,* 80, 781, 1973.

48. **Ito, S., Muramatsu, T., and Kobata, A.,** Endo-β-N-acetylglucosaminidase active on carbohydrate moieties of glycoproteins: purification and properties of the two enzymes with different specificities from *Clostridium perfringens, Arch. Biochem. Biophys.,* 171, 78, 1975.
49. **Tarentino, A. L. and Maley, F.,** A comparison of the substrate specificity of Endo-β-N-acetylglucosaminidase from *Streptomyces griseus* and *Diplococcus pneumoniae, Biochem. Biophys. Res. Commun.,* 67, 455, 1975.
50. **Arakawa, M. and Muramatsu, T.,** Endo-β-N-acetylglucosaminidase acting on the carbohydrate moieties of glycoproteins. The differential specificities of the enzymes from *Streptomyces griseus* and *Diplococcus pneumoniae, J. Biochem.,* 76, 307, 1974.
51. **Maley, F., Tarentino, A. L., and Trimble, R. B.,** Endo-β-N-acetylglucosaminidase. Their metabolic role in disease processes and their use in the study of glycoprotein structure and function, in *Glycoproteins and Glycolipids in Disease Processes,* Walborg, E. F., Jr., Ed., ACS Symposium Series 80, American Chemical Society, Washington, D.C., 1978, chap. 4.
52. **Tai, T., Yamashita, K., Ogata-Arakawa, M., Koide, N., Muramatsu, T., Iwashita, S., Inoue, Y., and Kobata, A.,** Structural studies of two ovalbumin glycopeptides in relation to the Endo-β-N-glucosaminidase specificity, *J. Biol. Chem.,* 250, 8569, 1975.
53. **Kobata, A.,** Use of endo and exoglucosidases for structural studies of glycoconjugates, *Anal. Biochem.,* 100, 1, 1979.
54. **Trimble, R. B., Tarentino, A. L., Plummer, T. H., and Maley, F.,** Asparaginyl glycopeptides with a low mannose content are hydrolyzed by endo-β-N-acetylglucosaminidase H, *J. Biol. Chem.,* 253, 4508, 1978.
55. **Bouquelet, S., Strecker, G., Montreuil, J., and Spik, G.,** Characterization of a novel endo-N-acetyl-β-D-glucosaminidase from the culture filtrate of a Basidiomycete *Sporotricum dimor phosporum* active on biantennary mono- and asialo glycoasparagines of the N-acetyllactosamine type, *Biochimie,* 62, 43, 1980.
56. **Tarentino, A. L. and Maley, F.,** Endo-β-N-acetylglucosaminidase from hen oviduct, in *Methods in Enzymology,* Vol L, Ginsburg, V., Ed., Academic Press, New York, 1978, 580.
57. **Nishigaki, M., Muramatsu, T., and Kobata, A.,** Endoglycosidases acting on carbohydrate moieties of glycoproteins: demonstration in mammalian tissue, *Biochem. Biophys. Res. Commun.,* 59, 638, 1974.
58. **Yamashita, K., Tachibana, Y., Mihara, K., Okada, S., Yabuuchi, H., and Kobata, A.,** Urinary oligosaccharides of mannosidosis, *J. Biol. Chem.,* 255, 5126, 1980.
59. **De Luca, L. M.,** The direct involvement of vitamin A in glycosyl transfer reactions of mammalian membranes, in *Vitamins and Hormones,* Vol. 35, Munson, P. L., Diczfalusy, E., Glover, J., and Olson, R. E., Eds., Academic Press, New York, 1977, 1.
60. **Burgos, J., Hemming, F. W., Pennock, J. F., and Morton, R. A.,** Dolichol: a naturally-occurring C_{100} isoprenoid alcohol, *Biochem. J.,* 88, 470, 1963.
61. **Mankowski, T., Jankowski, W., Chojnacki, T., and Franke, P.,** C_{55}-Dolichol: occurrence in pig liver and preparation by hydrogenation of plant undecaprenol, *Biochemistry,* 15, 2125, 1976.
62. **Pont Lezica, R., Brett, C. T., Romero, P., and Dankert, M.,** A glucose acceptor in plants with the properties of an α-saturated polyprenyl-monophosphate, *Biochem. Biophys. Res. Commun.,* 66, 980, 1975.
63. **Brett, C. T. and Leloir, L. F.,** Dolichyl monophosphate and its sugar derivatives in plants, *Biochem. J.,* 161, 93, 1977.
64. **Daleo, G. R. and Pont Lezica, R.,** Synthesis of dolichol phosphate by a cell-free extract from pea, *FEBS Lett.,* 74, 247, 1977.
65. **Daleo, G. R., Hopp, H. E., Romero, P. A., and Pont Lezica, R.,** Biosynthesis of dolichol phosphate by subcellular fractions from liver, *FEBS Lett.,* 81, 411, 1977.
66. **Grange, D. K. and Adair, W. L., Jr.,** Studies on the biosynthesis of dolichyl phosphate: evidence for the *in vitro* formation of 2,3-dehydro dolichyl phosphate, *Biochem. Biophys. Res. Commun.,* 79, 734, 1977.
67. **Allen, C. M., Kalin, J. R., Sack, J., and Verizzo, D.,** CTP-Dependent dolichol phosphorylation by mammalian cell homogenates, *Biochemistry,* 17, 5020, 1978.
68. **Burton, W. A., Scher, M. G., and Wechter, C. J.,** Enzymatic phosphorylation of dolichol in central nervous tissue, *J. Biol. Chem.,* 254, 7129, 1979.
69. **Quesada-Allue, L.,** Phosphorylation of dolichol by insect enzymes. The incorporation of phosphate from ATP into dolichyl phosphate mannose, *FEBS Lett.,* 97, 225, 1979.
70. **Tabora, E.,** Thesis doctoral, Estructura de un lipido intermediario en la biosintesis de glicoproteinas. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1976.
71. **Wedgwood, J. F. and Stominger, J. L.,** Enzymatic activities in cultured human lymphocytes that dephosphorylate dolichyl pyrophosphate and dolichyl phosphate, *J. Biol. Chem.,* 255, 1120, 1980.

72. **Idoyaga Vargas, V., Belocopitow, E., Mentaberry, A., and Carminatti, H.,** A phosphatase acting on dolichyl phosphate in membranes from neuronal perikaria, *FEBS Lett.*, 112, 63, 1980.
73. **Behrens, N. H. and Leloir, L. F.,** Dolichol monophophate glucose: an intermediate in glucose transfer in liver, *Proc. Natl. Acad. Sci. U.S.A.*, 66, 153, 1970.
74. **Behrens, N. H., Parodi, A. J., and Leloir, L. F.,** Glucose transfer from dolichol monophosphate glucose: the product formed with endogenous microsomal acceptor, *Proc. Natl. Acad. Sci. U.S.A.*, 68, 2857, 1971.
75. **Herscovics, A., Bugge, B., and Jeanloz, R. W.,** Glucosyltransferase activity in calf pancreas microsomes: formation of dolichyl D-[^{14}C]-glucosyl phosphate and [^{14}C]-labeled lipid-linked oligosaccharides from UDP-D-[^{14}C]-glucose, *J. Biol. Chem.*, 252, 2271, 1977.
76. **Warren, C. D. and Jeanloz, R. W.,** Chemical synthesis of dolichyl α-D-mannopyranosyl phosphate, and citronellyl α-D-mannopyranosyl phosphate, *Biochemistry*, 12, 5038, 1973.
77. **Warren, C. D., and Jeanloz, R. W.,** The characterization of glycolipids derived from long-chain polyprenols: chemical synthesis of α-D-mannopyranosyl dolichyl phosphate, *FEBS Lett.*, 31, 332, 1973.
78. **Warren, C. D. and Jeanloz, R. W.,** Synthesis of P^1-dolichyl P^2-α-D-manopyranosyl pyrophosphate. The acid and alkaline hydrolysis of polyisoprenyl α-D-mannopyranosyl mono- and pyrophosphate diesters, *Biochemistry*, 14, 412, 1975.
79. **Tkacz, J. S., Herscovics, A., Warren, C. D., and Jeanloz, R. W.,** Mannosyltransferase activity in calf pancreas microsomes. Formation from guanosine diphosphate-D-[^{14}C]-mannose of a [^{14}C]-labeled mannolipid with properties of dolichyl mannopyranosyl phosphate, *J. Biol. Chem.*, 249, 6372, 1974.
80. **Herscovics, A., Warren, C. D., Jeanloz, R. W., Wedgwood, J. F., Liu, I. Y., and Strominger, J. L.,** Occurrence of a β-D-mannopyranosyl phosphate residue in the polyprenyl mannosyl phosphate formed in calf pancreas microsomes and in human lymphocytes, *FEBS Lett.*, 45, 312, 1974.
81. **Herscovics, A., Warren, C. D., and Jeanloz, R. W.,** Anomeric configuration of the dolichyl D-mannosyl phosphate formed in calf pancreas microsomes, *J. Biol. Chem.*, 250, 8079, 1975.
82. **Mankowski, T., Sasak, W., and Chojnacki, T.,** Hydrogenated polyprenol phosphates exogenous lipid acceptors of glucose from UDP-glucose in rat liver microsomes, *Biochem. Biophys. Res. Commun.*, 65, 1292, 1975.
83. **Mankowski, T., Sasak, W., Janczura, E., and Chojnacki, T.,** Specificity of polyprenyl phosphates in the *in vitro* formation of lipid-linked sugars, *Arch. Biochem. Biophys.*, 181, 393, 1977.
84. **Waechter, C. J., Lucas, J. J., and Lennarz, W. J.,** Evidence for xylosyl lipids as intermediates in xylosyl transfer in hen oviduct membranes, *Biochem. Biophys. Res. Commun.*, 56, 343, 1974.
85. **Romero, P., Garcia, R. C., and Dankert, M.,** Synthesis of polyprenol monophosphate β galactose by *Acetobacter xylinum*, *Mol. Cell. Biochem.*, 16, 205, 1977.
86. **Iannino, N. I. de, Staneloni, R. J., Behrens, N. H., and Dankert, M.,** Galactose transfer from dolichyl monophosphate galactose in animal tissues, *FEBS Lett.*, 99, 73, 1979.
87. **Warren, C. D. and Jeanloz, R. W.,** Chemical synthesis of P^1-2-acetamido-2-deoxy-α-D-glucopyranosyl-P^2-dolichyl pyrophosphate, *Carbohydr. Res.*, 37, 252, 1974.
88. **Leloir, L. F., Staneloni, R. J., Carminatti, H., and Behrens, N. H.,** The biosynthesis of a N,N'-diacetylchitobiose containing lipid by liver microsomes. A probable dolichol pyrophosphate derivative, *Biochem. Biophys. Res. Commun*, 52, 1285, 1973.
89. **Herscovics, A., Warren, C. D., Bugge, B., and Jeanloz, R. W.,** Biosynthesis of P^1-di-N-acetyl α-chitobiosyl P^2-dolichyl pyrophosphate in calf pancreas microsomes, *J. Biol. Chem.*, 253, 160, 1978.
90. **Levy, J. A., Carminatti, H., Cantarella, A. I., Behrens, N. H., Leloir, L. F., and Tabora, E.,** Mannose transfer to lipid-linked di-N-acetylchitobiose, *Biochem. Biophys. Res. Commun.*, 60, 118, 1974.
91. **Chambers, J., Forsee, W. T., and Elbein, A. D.,** Enzymatic transfer of mannose from mannosyl-phosphoryl-polyprenol to lipid-linked oligosaccharides by pig aorta, *J. Biol. Chem.*, 252, 2498, 1977.
92. **Spencer, J. P. and Elbein, A. D.,** Transfer of mannose from GDP-mannose to lipid-linked oligosaccharide by soluble mannosyl transferase, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 2524, 1980.
93. **Chapman, A., Fujimoto, K., and Kornfeld, S.,** The primary glycosylation defect in Class E Thy-1-negative mutant mouse lymphoma cells is an inability to synthesize dolichol-P-mannose, *J. Biol. Chem.*, 255, 4441, 1980.
94. **Schutzback, J. S., Springfield, J. D., and Jensen, J. W.,** The Biosynthesis of oligosaccharide-lipids. Formation of an α 1-2 mannosyl mannose linkage, *J. Biol. Chem.*, 255, 4170, 1980.
95. **Parodi, A. J., Behrens, N. H., Leloir, L. F., and Dankert, M.,** Glucose transfer from dolichol monophosphate glucose: the lipid moiety of the endogenous acceptor, *Biochim. Biophys. Acta*, 270, 529, 1972.
96. **Spiro, M. J., Spiro, R. G., and Bhoyroo, V. D.,** Lipid-saccharide intermediates in glycoprotein biosynthesis. I. Formation of an oligosaccharide lipid by thyroid slices and evaluation of its role in protein glycosylation, *J. Biol. Chem.*, 251, 6400, 1976.

97. **Spiro, R. G., Spiro, M. H., and Bhoyroo, V. D.,** Lipid-saccharide intermediates in glycoprotein biosynthesis, II. Studies on the structure of an oligosaccharide-lipid from thyroid, *J. Biol. Chem.*, 251, 6409, 1976.
98. **Li, E., Tabas, I., Kornfeld, S.,** The synthesis of complex-type oligosaccharides. I. Structure of the lipid-linked oligosaccharide precursor of the complex type oligosaccharides of the vesicular stomatitis virus G protein, *J. Biol. Chem.*, 253, 7762, 1978.
99. **Liu, T., Stetson, B., Turco, S. J., Hubbard, S. C., and Robbins, P. W.,** Arrangement of glucose residues in the lipid-linked oligosaccharide precursor of asparaginyl oligosaccharides, *J. Biol. Chem.*, 254, 4554, 1979.
100. **Ugalde, R. A., Staneloni, R. J., and Leloir, L. F.,** Rat liver microsomal glucosidases. Partial purification and inhibition by disaccharides, *Eur. J. Biochem.*, 113, 97, 1980.
101. **Spiro, R. G., Spiro, M. J., and Bhoyroo, V. D.,** Processing of carbohydrate units of glycoproteins. Characterization of a thyroid glucosidase, *J. Biol. Chem.*, 254, 7659, 1979.
102. **Hubbard, S. C. and Robbins, P. W.,** Synthesis and processing of protein linked oligosaccharides *in vivo*, *J. Biol. Chem.*, 254, 4568, 1979.
103. **Staneloni, R. J., Ugalde, R. A., and Leloir, L. F.,** Addition of glucose to dolichyl diphosphate oligosaccharide and transfer to protein, *Eur. J. Biochem.*, 105, 275, 1980.
104. **Parodi, A. J.,** Biosynthesis of yeast mannoproteins. Synthesis of mannan outer chain and of dolichol derivatives, *J. Biol. Chem.*, 254, 8343, 1979.
105. **Behrens, N. H., Carminatti, H., Staneloni, R. J., Leloir, L. F., and Cantarella, A. I.,** Formation of lipid-bound oligosaccharides containing mannose. Their role in glycoprotein synthesis, *Proc. Natl. Acad. Sci. U.S.A.*, 70, 3390, 1973.
106. **Robbins, P. W., Krag, S. S., and Liu, T.,** Effects of UDP-glucose addition on the synthesis of mannosyl lipid-linked oligosaccharides by cell-free fibroblast preparations, *J. Biol. Chem.*, 252, 1780, 1977.
107. **Chapman, A., Li, E., and Kornfeld, S.,** The biosynthesis of the major lipid-linked oligosaccharide of chinese hamster ovary cells occurs by the ordered addition of mannose residues, *J. Biol. Chem.*, 254, 10243, 1979.
108. **Parodi, A. J., Behrens, N. H., Leloir, L. F., and Carminatti, H.,** The role of polyprenol-bound saccharides as intermediates in glycoprotein synthesis in liver, *Proc. Natl. Acad. Sci. U.S.A.*, 69, 3268, 1972.
109. **Lucas, J. J., Waechter, C. J., and Lennarz, W. J.,** The participation of lipid-linked oligosaccharide in synthesis of membrane glycoproteins, *J. Biol. Chem.*, 250, 1992, 1975.
110. **Oliver, G. J. A., Harrison, J., and Hemming, F. W.,** The mannosylation of dolichol-diphosphate oligosaccharides in relation to the formation of oligosaccharides and glycoproteins in pig liver endoplasmic reticulum, *Eur. J. Biochem.*, 58, 223, 1975.
111. **Pless, D. D. and Lennarz, W. J.,** A lipid-linked oligosaccharide intermediate in glycoprotein synthesis. Characterization of Man-[^{14}C]-glycoproteins labeled from Man-[^{14}C]-oligosaccharide-lipid and GDP-[^{14}C]-Man, *J. Biol. Chem.*, 250, 7014, 1975.
112. **Reuvers, F., Habets-Willems, C., Reinking, A., and Boer, P.,** Glycolipid intermediates involved in the transfer of N-acetylglucosamine to endogenous proteins in a yeast membrane preparation, *Biochim. Biophys. Acta*, 486, 541, 1977.
113. **Nakajama, K., Araki, Y., and Ito, E.,** The formation of a mannose-containing trisaccharide on a lipid and its transfer to proteins in yeast, *FEBS Lett.*, 72, 287, 1976.
114. **Bause, E. and Lehle, L.,** Enzyme N-glucosylation and O-glycosylation of synthetic peptide acceptors by dolichol-linked sugar derivatives of yeast, *Eur. J. Biochem.*, 101, 531, 1979.
115. **Chen, W. W. and Lennarz, W. J.,** Participation of a trisaccharide lipid in glycosylation of oviduct membrane glycoprotein, *J. Biol. Chem.*, 251, 7802, 1976.
116. **Lehle, L. and Tanner, W.,** Glycosyl transfer from dolichyl phosphate sugars to endogenous and exogenous glycoprotein acceptors in yeast, *Eur. J. Biochem.*, 83, 563, 1978.
117. **Khalkhali, Z. and Marshall, R. D.,** Glycosylation of ribonuclease A catalysed by rabbit liver extracts, *Biochem. J.*, 146, 299, 1975.
118. **Khalkhali, Z. and Marshall, R. D.,** UDP-N-acetyl-D-glucosamine-asparagine sequon N-acetyl-β-D-glucosaminyl-transferase-activity in human serum, *Carbohydr. Res.*, 49, 455, 1976.
119. **Turco, S. J., Stetson, B., and Robbins, P. W.,** Comparative rates of transfer of lipid-linked oligosaccharides to endogenous glycoprotein acceptors *in vitro*, *Proc. Natl. Acad. Sci., U.S.A.*, 74, 4411, 1977.
120. **Kornfeld, S., Gregory, W., and Chapman, A.,** Class E Thy-1 negative mouse lymphoma cells utilize and alternate pathway of oligosaccharide processing to synthesize complex-type oligosaccharides, *J. Biol. Chem.*, 254, 11649, 1979.
121. **Eagon, P. K., Hsu, A. F., and Heath, E. C.,** Role of dolichol intermediates in glycosylation of endogenous and exogenous proteins, *Fed. Proc.* 34, 678, 1975.

122. **Pless, D. D. and Lennarz, W. J.,** Enzymatic conversion of proteins to glycoproteins, *Proc. Natl. Acad. Sci., U.S.A.*, 74, 134, 1977.
123. **Tarentino, A. L., Plummer, T. H., Jr., and Maley, F.,** The release of intact oligosaccharides from specific glycoprotein by endo-β-N-acetylglucosaminidase H, *J. Biol. Chem.*, 249, 818, 1974.
124. **Marshall, R. D.,** The nature and metabolism of the carbohydrate-peptide linkages of glycoproteins, *Biochem. Soc. Symp.*, 40, 17, 1974.
124a. **Hart, G. W., Brew, K., Grant, G. A., Bradshaw, R. A., and Lennarz, W. J.,** Primary structural requirements for the enzymatic formation of the N-glycosidic bond in glycoproteins, *J. Biol. Chem.*, 254, 9747, 1979.
125. **Ronin, C., Granier, C., Van Rietschoten, J., and Bouchilloux, S.,** Enzymatic transfer of oligosaccharide from oligosaccharide-lipids to an Asn-Ala-Thr containing heptapeptide, *Biochem. Biophys. Res. Commun.*, 81, 772, 1978.
126. **Ronin, C., Bouchilloux, S., Granier, C., and Van Rietschoten, J.,** Enzymatic N-glycosylation of synthetic Asn-X-Thr containing peptides, *FEBS Lett.*, 96, 179, 1978.
126a. **Das, R. C. and Heath , E. C.,** Dolichyl diphosphoryl oligosaccharide-protein oligosaccharyltransferase: solubilization, purification and properties, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 3811, 1980.
127. **Melchers, F.,** Biosynthesis of the carbohydrate portion of immunoglobulin. Radiochemical and chemical analysis of the carbohydrate moieties of two myeloma proteins purified from different subcellular fractions of plasma cells, *Biochemistry*, 10, 653, 1971.
128. **Schachter, H.,** The subcellular sites of glycosylation, *Biochem. Soc. Symp.*, 40, 57, 1974.
129. **Whur, P., Herscovics, A., and Leblond, C. P.,** Radioautrographic visualization of the incorporation of galactose -[^{3}H] and mannose-[^{3}H] by rat thyroids *in vitro* in relation to the stages of thyroglobulin synthesis, *J. Cell. Biol.*, 43, 289, 1969.
130. **Herscovics, A.,** Biosynthesis of thyroglobulin. Incorporation of [1-^{14}C]-galactose, [1-^{14}C]-mannose and [4,5,-$^3H_2$]-leucine into soluble proteins by rat thyroids *in vitro*, *Biochem. J.*, 112, 709, 1969.
131. **Herscovics, A.,** Biosynthesis of thyroglobulin: incorporation of [^{3}H]-fucose into proteins by rat thyroids *in vitro*, *Biochem. J.*, 117, 411, 1970.
132. **Sturgess, J. M., Minaker, E., Mitranic, M. M., and Moscarello, M. A.,** The incorporation of L-fucose into glycoproteins in the Golgi apparatus of rat liver and in serum, *Biochim. Biophys. Acta*, 320, 123, 1973.
133. **Haddad, A., Meredith, D., Herscovics, A., Nadler, N. J., and Leblond, C. P.,** Radioautographic study of *in vivo* and *in vitro* incorporation of fucose-[^{3}H] into thyroglobulin by rat thyroid follicular cells, *J. Cell. Biol.*, 49, 856, 1971.
134. **Monaco, F., and Robbins, J.,** Incorporation of N-acetylmannosamine and N-acetylglucosamine into thyrogobulin in rat thyroid *in vitro*, *J. Biol. Chem.*, 248, 2072, 1973.
135. **Melchers, F.,** Biosynthesis, intracellular transport, and secretion of immunoglobulins. Effect of 2-deoxy-D-glucose in tumor plasma cells producing and secreting immunoglobulin Gl, *Biochemistry*, 12, 1471, 1973.
136. **Kiely, M., McKnight, M., and Schimke, R. T.,** Studies on the attachment of carbohydrate to ovalbumin nascent chains in hen oviduct, *J. Biol. Chem.*, 251, 5490, 1976.
137. **Rothman, J. E. and Lodish, H. F.,** Synchronised transmembrane insertion and glycosylation of a nascent membrane protein, *Nature*, 269, 775, 1977.
138. **Bielinska, M., Rogers, G., Rucinsky, T., and Boime, I.,** Processing *in vitro* of placental peptide hormones by smooth microsomes, *Proc. Natl. Acad. Sci. U.S.A.*, 76, 6152, 1979.
139. **Idoyaga Vargas, V. and Carminatti, H.,** Glycosylation of endogenous protein(s) of the rough and smooth microsomes by a lipid sugar intermediate, *Mol. Cell. Biochem.*, 16, 171, 1977.
140. **Idoyaga Vargas, V., Perelmuter, M., Burrone, O., and Carminatti, H.,** Oligosaccharide transfer from lipid sugar intermediated to endogenous protein(s) of rat liver microsomal subfractions, *Mol. Cell. Biochem.*, 26, 123, 1979.
141. **Parodi, A. J. and Martin-Barrientos, J.,** Glycosylation of endogneous proteins through dolichol derivatives in reticulocyte plasma membranes, *Biochim. Biophys. Acta*, 500, 80, 1977.
142. **Czichi, U. and Lennarz, W. J.,** Localization of the enzyme system for glycosylation of proteins via the lipid-linked pathway in rough endoplasmic reticulum, *J. Biol. Chem.*, 252, 7901, 1977.
143. **Tabas, I., Schlesinger, S., and Kornfeld, S.,** Processing of high mannose oligosaccharides to form complex type oligosaccharides on the newly synthesized polypeptides of the vesicular stomatitis virus G protein and the IgG heavy chain, *J. Biol. Chem.*, 253, 716, 1978.
144. **Robbins, P. W., Hubbard, S. C., Turco, S. J., and Wirth, D. F.,** Proposal for a common oligosaccharide intermediate in the synthesis of membrane glycoproteins, *Cell*, 12, 893, 1977.
145. **Hunt, L. A., Etchison, J. R., and Summers, D. F.,** Oligosaccharide chains are trimmed during synthesis of the envelope glycoprotein of vesicular stomatitis virus, *Proc. Natl. Acad. Sci., U.S.A.*, 75, 754, 1978.

146. **Sefton, B. M.,** Immediate glycosylation of Sindbis virus membrane proteins, *Cell,* 10, 659, 1977.
147. **Kaluza, G.,** Effect of impaired glycosylation on the biosynthesis of Semliki Forest virus proteins, *J. Virol.,* 16, 602, 1975.
148. **Kornfeld, S., Li, E., and Tabas, I.,** The synthesis of complex-type oligosaccharides. II. Characterization of the processing intermediates in the synthesis of the complex oligosaccharide units of the Vesicular stomatitis virus G protein, *J. Biol. Chem.,* 253, 7771, 1978.
149. **Staneloni, R. J. and Leloir, L. F.,** Oligosaccharides containing glucose and mannose in glycoproteins of the thyroid gland, *Proc. Natl. Acad. Sci., U.S.A.,* 75, 1162, 1978.
150. **Trowbridge, I. S., Hyman, R.,** Abnormal lipid-linked oligosaccharides in Class E Thy-1-negative mutant lymphomas, *Cell,* 17, 503, 1979.
151. **Chapman, A., Trowbridge, I. S., Hyman, R., and Kornfeld, S.,** Structure of the lipid-linked oligosaccharide that accumulate in Class E Thy-1-negative mutant lymphomas, *Cell,* 17, 509, 1979.
152. **Narasimhan, S., Stanley, D., and Schachter, H.,** Control of glycoprotein synthesis. Lectin-resistant mutant containing only one of two distinct N-acetylglucosaminyl transferase activities present in wild-type Chinese hamster ovary cells, *J. Biol. Chem.,* 252, 3926, 1977.
153. **Trowbridge, I. S., Hyman, R., and Mazanskas, C.,** The synthesis and properties of T25 glycoprotein in Thy-1-negative mutant lymphoma cells, *Cell,* 14, 21, 1978.
154. **Ugalde, R. A., Staneloni, R. J., and Leloir, L. F.,** Action of glycosidases on the saccharide moiety of the glucose-containing dolichyl diphosphate oligosaccharide, *FEBS Lett.,* 91, 209, 1978.
155. **Chen, W. W. and Lennarz, W. J.,** Enzymatic excision of glucosyl units linked to the oligosaccharide chains of glycoproteins, *J. Biol. Chem.,* 253, 5780, 1978.
156. **Grinna, L. S. and Robbins, P. W.,** Glycoprotein biosynthesis. Rat liver microsomal glucosidases which process oligosaccharides, *J. Biol. Chem.,* 254, 8814, 1979.
157. **Michael, J. M. and Kornfeld, S.,** Partial purification and characterization of the glucosidases involved in the processing of asparagine-linked oligosaccharides., *Arch. Biochem. Biophys.,* 199, 249, 1980.
158. **Ugalde, R. A., Staneloni, R. J., and Leloir, L. F.,** Microsomal glucosidases acting on the saccharide moiety of the glucose-containing dolichyl diphosphate oligosaccharide, *Biochem. Biophys. Res. Commun.,* 91, 1174, 1979.
159. **Elting, J. J., Chen, W. W., and Lennarz, W. J.,** Characterization of a glucosidase involved in an initial step in the processing of oligosaccharide chains., *J. Biol. Chem.,* 255, 2325, 1980.
160. **Scher, M. G. and Waechter, C. J.,** A glucosylated oligosaccharide lipid intermediate in calf brain. Evidence for the transfer of the oligosaccharide into membrane glycoprotein and subsequent removal of glucosyl residues, *J. Biol. Chem.,* 254, 2630, 1979.
161. **Ugalde, R. A.,** Ph.D. thesis, 1980.
162. **Opheim, D. J. and Touster, O.,** Lysosomal α-D-mannosidase of rat liver Purification and comparison with the Golgi and cytosolic α-D-mannosidases, *J. Biol. Chem.,* 253, 1017, 1978.
163. **Tulsiani, D. R. P., Opheim, D. J., and Touster, O.,** Purification and characterization of α-D-mannosidase from rat liver Golgi membranes, *J. Biol. Chem.,* 252, 3227, 1977.
164. **Tabas, I. and Kornfeld, S.,** Purification and characterization of a rat liver Golgi α mannosidase capable of processing asparagine-linked oligosaccharides., *J. Biol. Chem.,* 254, 11655, 1979.
165. **Tabas, I. and Kornfeld, S.,** The Synthesis of complex-type oligosaccharides. III. Identification of an α mannosidase activity involved in a late stage of processing of complex type oligosaccharides., *J. Biol. Chem.,* 253, 7779, 1978.
166. **Harpaz, N. and Schachter, H.,** Control of glycoprotein synthesis. Bovine calostrum UDP-N-acetylglucosamine α-D-mannoside β 2-N-acetylglucosaminyl transferase I separation from UDP-N-acetylglucosamine α-D-mannoside β-2-N-acetylglucosaminyl transferase II partial purification and substrate specificity, *J. Biol. Chem.,* 255, 4885, 1980.
167. **Harpaz, N. and Schachter,** Control of glycoprotein synthesis, Processing of asparagine-linked oligosaccharides by one or more rat liver Golgi α-D-mannosidases dependent on prior action of UDP-N-acetylglucosamine α-D-mannoside β 2-N-acetylglucosaminyl transferase, *J. Biol. Chem.,* 255, 4894, 1980.
168. **Beyer, T. A., Sadler, J. E., and Hill, R. L.,** Purification to homogeneity of the H blood group β-galactoside α 1-2 fucosyl transferase from porcine submaxillary gland, *J. Biol. Chem.,* 255, 5364, 1980.
169. **Prieels, J. P. and Beyer, T. A.,** Purification and properties of GDP-fucose: N-acetylglucosamine α 1-3 fucosyl transferase, *Fed. Proc.,* 38(3), 631, 1979.
170. **Wilson, J. R., Williams, D., and Schachter, H.,** The control of glycoprotein synthesis: N-acetylglucosamine linkage to a mannose residue as a signal for the attachment of L-fucose to the asparagine-linked N-acetylglucosamine residue of glycopeptide from α_1-acid glycoprotein, *Biochem. Biophys. Res. Commun.,* 72, 909, 1976.

171. **Stanley, P., Narasimhans, S., Siminovitch, L., and Schachter, H.,** Chinese hamster ovary cells selected for resistance to the cytotoxicity of phytohemagglutinin are deficient in a UDP-N-acetylglucosamine-glycoprotein N-acetylglucosaminyl transferase activity, *Proc. Natl. Acad. Sci., U.S.A.*, 72, 3323, 1975.
172. **Trayer, I. P. and Hill, R. L.,** The purification and properties of the A protein of lactose synthetase, *J. Biol. Chem.*, 246, 6666, 1971.
173. **Andrews, P.,** Purification of lactose synthetase. A protein from human milk and demonstration of its interaction with α-lactalbumin, *FEBS Lett.*, 9, 297, 1970.
174. **Hudgin, R. L. and Schachter, H.,** Porcine sugar nucleotide: glycoprotein glycosyl transferase. II. Blood serum and liver galactosyl transferase, *Can. J. Biochem.*, 49, 838, 1971.
175. **Paulson, J. C., Beranek, W. E., and Hill, R. L.,** Purification of a sialyl transferase from bovine colostrum by affinity chromatography on CDP-agarose, *J. Biol. Chem.*, 252, 2356, 1977.
176. **Paulson, J. C., Rearick, J. I., and Hill, R. L.,** Enzymatic properties of β-D-galactoside α 2-6 sialyl transferase from bovine colostrum, *J. Biol. Chem.*, 252, 2363, 1977.
177. **Sadler, J. E., Rearick, J. I., Paulson, J. C., and Hill, R. L.,** Purification to homogeneity of a β-galactoside α 2-3 sialyl transferase and partial purification of an α-N-acetylgalactosaminide α 2-6 sialyl transferase from porcine submaxillary glands, *J. Biol. Chem.*, 254, 4434, 1979.
178. **Rearick, J. I., Sadler, J. E., Paulson, J. C., and Hill, R. L.,** Enzymatic characterization of β-D-galactoside α 2-3 sialyl transferase from porcine submaxillary gland, *J. Biol. Chem.*, 254, 4444, 1979.
179. **Beyer, T. A., Rearick, J. I., Paulson, J. C., Prieels, J. P., Sadler, J. E., and Hill, R. L.,** Biosynthesis of mammalian glycoproteins. Glycosylation pathways in the synthesis of the non reducing terminal sequences, *J. Biol. Chem.*, 254, 12531, 1979.
180. **Quesada-Allue, L. A., Marechal, L. R., and Belocopitow, E.,** Biosynthesis of polyprenol monophosphate sugars by *Ceratitis capitata* extracts, *FEBS Lett.*, 67, 243, 1976.
181. **Quesada-Allue, L. A. and Belocopitow, E.,** Lipid bound oligosaccharides in insects, *Eur. J. Biochem.*, 88, 529, 1978.
182. **Elbein, A. L.,** The role of lipid-linked saccharides in the biosynthesis of complex carbohydrates, *Annu. Rev. Plant Physiol.*, 30, 239, 1979.
183. **Forsee, W. T. and Elbein, A. D.,** Glycoprotein biosynthesis in plants. Demonstration of lipid linked oligosaccharides of mannose and N-acetylglucosamine, *J. Biol. Chem.*, 250, 9283, 1975.
184. **Lehle, L., Fartaczek, F., Tanner, W. and Kauss, H.,** Formation of polyprenol-linked mono- and oligosaccharides in *Phaseolus aureus, Arch. Biochem. Biophys.*, 175, 419, 1976.
185. **Forsee, W. T., Valkovich, G., and Elbein, A. D.,** Glycoprotein biosynthesis in plants. Formation of lipid-linked oligosaccharide of mannose and N-acetylglucosamine by mung bean seedlings, *Arch. Biochem. Biophys.*, 174, 469, 1976.
186. **Forsee, W. T. and Elbein, A. D.,** Biosynthesis of lipid-linked oligosaccharides in cotton fibers, *J. Biol. Chem.*, 252, 2444, 1977.
187. **Ericson, M. C., Gafford, J. T., and Elbein, A. D.,** Tunicamycin inhibits GlcNAc-lipid formation in plants, *J. Biol. Chem.*, 252, 7431, 1977.
188. **Staneloni, R. J., Tolmasky, M. E., Petriella, C., Ugalde, R. A., and Leloir, L. F.,** Presence in a plant of a compound similar to the dolichyl diphosphate oligosaccharide in animal tissue, *Biochem. J.*, 191, 257, 1980.
189. **Hopp, H. E., Romero, P. A., Daleo, G. R., and Pont Lezica, R.,** Synthesis of cellulose precursors. The involvement of lipid-linked sugars, *Eur. J. Biochem.*, 84, 561, 1978.
190. **Bause, E., Jaenicke, L.,** Formation of lipid-linked sugar compounds in *Volvox carteri F. Nagariensis Iyengar, FEBS Lett.*, 106, 321, 1979.
191. **Babczinski, P. and Tanner, W.,** Involvement of dolichol monophosphate in the formation of specific mannosyl linkages in yeast glycoproteins, *Biochem. Biophys. Res. Commun*, 54, 1119, 1973.
192. **Lehle, L. and Tanner, W.,** Membrane bound mannosyl transferase in yeast glycoprotein biosynthesis, *Biochim. Biophys. Acta*, 350, 225, 1974.
193. **Sharma, C. B., Babczinski, P., Lehle, L., and Tanner, W.,** The role of dolichol monophosphate in glycoprotein biosynthesis in *Saccharomyces cerevisiae, Eur. J. Biochem.*, 46, 35, 1974.
194. **Trimble, R. B. and Maley, F.,** The use of endo-β-N-acetylglucosaminidase H in characterizing the structure and function of glycoproteins, *Biochem. Biophys. Res. Commun.*, 78, 935, 1977.
195. **Hasilik, A. and Tanner, W.,** Carbohydrate moiety of carboxypeptidase Y and perturbation of its biosynthesis, *Eur. J. Biochem.*, 91, 567, 1978.
196. **Onishi, H. R., Thacz, J. S., and Lampen, J. O.,** Glycoprotein nature of yeast alkaline phosphatase. Formation of active enzyme in the presence of tunicamycin, *J. Biol. Chem.*, 254, 11943, 1979.
197. **Parodi, A. J.,** Synthesis of glucosyl-dolichol derivatives in baker's yeast and their role in protein glycosylation, *Eur. J. Biochem.*, 75, 171, 1977.

198. **Parodi, A. J.,** The mechanism of synthesis of the polysaccharide part of mannan in *Saccharomyces cerevisiae, Arch. Biochem. Phys.,* 210, 372, 1981.
199. **Parodi, A. J.,** Biosynthesis of yeast glycoproteins. Processing of the oligosaccharides transferred from dolichol derivatives, *J. Biol. Chem.,* 254, 10051, 1979.
200. **Gold, M. H., Hahn, H. J.,** Role of a mannosyl lipid intermediate in the synthesis of *Neurospora crassa* glycoprotein, *Biochemistry,* 15, 1808, 1976.
201. **Letoublon, R. C. P., Comte, J., and Got, R.,** Transfert du mannose, *a* partir de GDP-mannose a des accepteurs lipidiques chez *Aspergillus niger, Eur. J. Biochem.,* 40, 95, 1973.
202. **Crean, E. V. and Rossomando, E. F.,** Synthesis of a mannosyl phosphoryl polyprenol by the slime mold *Dictyostellum discoideum, Biochim. Biophys. Acta,* 498, 439, 1977.
203. **Keenan, R. W., Kruczek, M., and Fusinato, L.,** The role of glucolipid in the biosynthesis of glycoprotein in *Tetrahymena pyriformis, Arch. Biochem. Biophys.,* 167, 697, 1975.