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Dolichol Phosphate, a Coenzyme in the Glycosylation of Animal Membrane-Bound Glycoproteins

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The last few years have seen a tremendous increase in interest in, and understanding of, the biochemistry of membranes. One membrane-associated activity is the catalysis of the glycosylation of complex polysaccharides and glycoproteins. The formation of bacterial wall polysaccharides by preparations of plasma membrane has been studied extensively, and the role of undecaprenol phosphate as a lipid-soluble coenzyme in the enzyme complexes involved is now well established (Lennarz & Scher, 1972; Hemming, 1974).

It is clear that dolichol phosphate plays an analogous role in the glycosylation of several membrane-bound glycoproteins of eukaryotes, although the details and especially the nature of the glycoproteins concerned are still being worked out. The present short review summarizes the current position in animal systems. The reader could consult with advantage other more-detailed reviews (see, for example, Waechter & Lennarz, 1976; Behrens, 1974).

Membrane-bound glycoproteins

Functions. Glycoproteins are important components of membranes, both internally and at the periphery of living cells. They are currently considered to be cell-surface receptors to several biological effectors, including some hormones, viruses and mitogens, and also to asialoglycoproteins in their hepatic elimination from the serum. Roles in immunoprotection and in cell-cell interactions of both developing embryonic tissues and adult tissues have been proposed. In addition it has been shown that several glycoproteins have enzymic activity, and for others a structural role is a possibility.

The significance of the carbohydrate moiety in all of these molecules is not yet clear. In some it provides a part, or possibly all, of the functional site. In others its presence is probably more directly related to the localization of the protein portion and possibly to its rate of turnover.

Structures. The major group of glycoproteins concerned have an N-glycosidic linkage between N-acetylglucosamine and the amide N of an asparagine residue of the protein portion. The N-acetylglucosamine residue may be at the reducing terminus of a simple oligosaccharide containing N-acetylglucosamine and mannose residues (Fig. 1) and possibly in some cases also glucose (Fig. 2). Alternatively, a complex oligosaccharide containing a similar proximal core of N-acetylglucosamine and mannose residues with side chains of N-acetylglucosamine, galactose and sialic acid (or fucose) may be present (Fig. 1).

Sites of biosynthesis

It is generally accepted that the protein moiety of these glycoproteins is synthesized in the ribosomes of the rough endoplasmic reticulum. The ribosomes are on the cytoplasmic face of the double membrane and in some way as yet not understood the nascent protein, or that part that is to be glycosylated, crosses the membrane to become
Simple: \( \text{Man}_x\text{GlcNAc}_y\text{N-C-Asp} \)

Complex: \( [(\text{SA or Fuc})\text{-Gal-GlcNAc}]_x\cdot\text{Man}_y\text{GlcNAc}_z\text{N-C-Asp} \)

**Fig. 1. Generalized structures of oligosaccharide moieties of \( N \)-glycosidically linked glycoproteins**

\( x = 1-5, y = 3-16, z = 1 \text{ or } 2 \). Abbreviation: SA, sialic acid.

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**Fig. 2. Role of dolichol monophosphate in glycosyl transfer to glycoprotein in rat liver (Behrens, 1974)**

Abbreviation: Dol, dolichol.

Located on the luminal face. Cytological and radioautographic evidence and analogy with current ideas on the production of secreted glycoproteins leads to the view that 'membrane flow' occurs from rough to smooth endoplasmic reticulum and thence via the Golgi apparatus and vesicles to the plasma membrane or to intracellular structures such as lysosomes, and also that during this movement of membrane, progressive glycosylation of its component glycoproteins occurs (Fig. 3).
Addition of peripheral sugars

The most fully understood of these glycosylation steps are those resulting in the addition of side-chain sugars of the complex type of oligosaccharide moiety (Fig. 1). The enzymes responsible, glycosyltransferases, occur in the membranes of the Golgi and vesicles, and can be solubilized and purified relatively easily. They exhibit a high degree of specificity for sugar donor and for the oligosaccharide structure of the glycoprotein acceptor. The preferred donor forms are the nucleoside diphosphate sugars listed in Table 1(a). The specificity leads to an ordered sequence of glycosylations appropriate for producing structures of the pattern generalized in Fig. 1. It appears that inversion of enantiomeric configuration usually occurs at each glycosylation such that the configuration of the newly attached sugar is opposite to that in the donor.

Addition of core sugars

The transfer of proximal N-acetylglucosaminyl and mannosyl residues to glycoproteins is less completely understood. Radioautographic studies on tissues, and biochemical studies on subcellular fractions, show that these sugars are added to secreted glycoproteins mainly in the rough and smooth endoplasmic reticulum. Studies with subcellular fractions support a similar localization for proximal glycosylations of membrane-bound glycoproteins. Clarification of the biochemical details of this addition of sugar residues is complicated by the current failure to solubilize and purify the transferase activity. The fact that the transferases present in microsomal preparations will not function with exogenous putative acceptor proteins but only with the small
Table 1. *Sugar donors involved in glycosylation of glycoproteins*

**Abbreviations:** Dol, dolichol; SA, sialic acid. $x = 1-2$, $y = 0-16$, $z = 0-2$.

(a) UDP-Glc, UDP-Gal, GDP-Man, UDP-GlcNAc, CMP-SA
(b) Dol-P-Man, Dol-P-Glc, Dol-P-Gal, Dol-P-Xyl
(c) Dol-P$_2$-GlcNAc$_x$-Man$_y$-Glc$_z$

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**Dolichol monophosphate**

\[
\begin{array}{c}
\text{CH}_3 \\
H \hspace{0.5cm} \text{H}_2\text{C} \cdots \text{CH} \cdots \text{CH}_2 \\
\end{array}
\begin{array}{c}
\text{CH}_3 \\
\text{CH}_2 \cdots \text{CH}_2 \cdots \text{CH}_2 \text{O} \cdots \text{P} \cdots \text{OH}
\end{array}
\]

**Retinol monophosphate**

\[
\begin{array}{c}
\text{CH}_3 \\
\beta \text{-ionone} \cdots \text{C} \cdots \text{CH} \cdots \text{CH} \cdots \text{CH}_2 \text{O} \cdots \text{P} \cdots \text{OH} \\
\end{array}
\]

**Fig. 4. Structures of dolichol and retinol phosphates**

In mammalian cells, $n = 14-21$ (mainly).

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amounts of endogenous acceptor protein in the membrane preparation, also limits the studies in this area. However steady progress has been made and, whereas it is not possible to demonstrate direct addition of proximal N-acetylglucosamine and mannose residues from appropriate nucleotide donors, transfer from lipid-soluble donors has been reported in membrane preparations of many animal tissues and of plants. With two exceptions, discussed below, the endogenous proteins accepting these sugars have not yet been characterized, but it is likely that they include various membrane-bound proteins, and it is possible that some are subsequently further glycosylated as described above without the intermediacy of lipid-soluble donors to form glycoproteins with complex oligosaccharide portions (Fig. 1).

**Dolichol phosphate as a coenzyme**

The type of lipid-soluble donors involved are summarized in Table 1(b). The lipophilic portions of the molecule is the polyisoprenoid alcohol dolichol (Fig. 4). This effectively anchors the lipid-linked carbohydrate donors on the membrane, presumably in a manner better suited than nucleotide-linked carbohydrate donors to act as substrate for the next transferase. Each of the dolichol monophosphate monosaccharide donors is formed directly from the usual nucleotide-linked donor with the appropriate specific transferase. The maximal transferase activities reported in any tissue studied so far are in the order mannosyl > glucosyl > galactosyl. In fact the galactosyltransferase activity is generally very low and its significance is uncertain. Transfer of xylose to dolichol monophosphate has been reported only in oviduct preparations, where the transferase has relatively low activity. These transferases are highly specific for nucleoside and sugar structure and are tightly bound to the rough and smooth endoplasmic reticulum. They are less specific for lipid phosphate acceptor, and in *vitro* several other long-chain alcohols can substitute for dolichol. It is relevant that the sugar-1-phosphate linkage and hence the transfer potential of the donor is retained. The compounds are well suited to formation of glycosides in a relatively hydrophobic environment.
In fact, most of the new glycosidic linkages formed by transfer of mannose from dolichol phosphate mannose in liver are part of an oligosaccharide linked to dolichol diphosphate (Table 1c and Fig. 2). The oligosaccharide is then transferred to protein en bloc. In the formation of at least some glycoproteins in the liver, glucose is added as indicated (Behrens, 1974). A similar scheme has been revealed as operating in other animal tissues, notably aorta, lymphocytes, myeloma, pancreas and oviduct, although the transfer of glucose (steps 6 and 7, Fig. 2) is not yet widely established. The existence of transferases for steps 1, 4 or 6 has also been demonstrated in many other tissues. In most tissues, with the exception of myeloma, the protein that has become glycosylated was extremely difficult to solubilize, and it is generally assumed to be a naturally membrane-bound glycoprotein. Indeed, experiments designed to reveal an involvement of dolichol phosphate in the glycosylation of ovalbumin secreted by the oviduct demonstrated that the predominant product was membrane-bound and not ovalbumin, as judged by immunological criteria (Lucas et al., 1975). On the other hand there is one notable observation of the involvement of dolichol diphosphate oligosaccharides, essentially as outlined in Fig. 2, in the formation of a secreted glycoprotein. This is in the formation of a $k$-type immunoglobulin light chain by a myeloma system (Hsu et al., 1974; Eagon et al., 1975). In effect, in all of these situations, dolichol monophosphate is acting as a lipophilic coenzyme to the multienzyme complexes involved in glycosyl transfer to glycoprotein.

The transferases, operating from dolichol monophosphate monosaccharides to dolichol diphosphate oligosaccharides (steps 5 and 7, Fig. 2) and from the latter to protein (step 8) have not been studied as much as the others, but it does appear that a fairly high specificity exists for the dolichol phosphate moiety of the donor. Conversely, the specificity of the size of the oligosaccharide transferred in step 8 seems to be quite loose, in that oligosaccharide with and without glucose and, in the latter case, with the value of $n$ in Fig. 2 varying from 3 to 16 have been observed to be transferred in liver systems (Oliver et al., 1975; Behrens, 1974). Again, during glycosyl transfer by rupture of a sugar-1-phosphate bond, an inversion of configuration is believed to occur (steps 2, 3, 4, 6 and 8, Fig. 2). This has been confirmed experimentally in the case of steps 4 and 6. In this way Fig. 2 explains the configuration of glycosidic linkages found in the core region of many N-glycosidic glycoproteins.

With regard to Fig. 3 it should be appreciated that the liberation of dolichol pyrophosphate at step 8 and its dephosphorylation (step 9) are presumptive, and also that the nature of the protein acceptor is uncertain. It is clear that the glycoprotein produced is reasonably stable to alkali, and that the oligosaccharide is therefore probably not O-glycosidically linked to serine or threonine. The oligosaccharide composition, ignoring the glucose, is one expected of an N-glycosidic link via asparagine, but this linkage has not been established experimentally. Otherwise, the intermediate compounds in the Figure have been identified, and in some cases characterized unequivocally, in a satisfactory manner, and all of the enzymes required have been detected in liver and several other tissues.

Control of dolichol monophosphate concentrations

The concentration of dolichol monophosphate in mammalian tissues is very low and is probably rate-limiting for these glycosylation processes. It has been reported that in liver cells this is at its highest concentration in nuclear, Golgi and rough-endoplasmic- reticulum membranes (Dallner et al., 1972). Variation of the concentration presents a possible way of controlling the rate of glycosylation of at least some membrane-bound glycoproteins. The hypothetical scheme in Fig. 5 indicates reactions which may be particularly relevant to the formation and metabolism of dolichol monophosphate. It is noteworthy that the early steps in the dolichol biosynthetic pathway are common to sterol biosynthesis, and parameters known to affect hepatic hydroxymethylglutaryl-CoA reductase and other early steps may well affect dolichol phosphate concentrations. In bacteria, control of polyprenol phosphate concentrations has been linked with changes n the rate of reactions analogous to steps i and j in Fig. 5.
Formation of viral envelope glycoproteins

It is likely that the most rapid evaluation of the significance of the involvement of dolichol phosphate in glycoprotein biosynthesis will be made with suitable model biological systems in which a defined, recognizable and assayable glycoprotein is being formed predominantly. The myeloma system used by Heath and co-workers (Hsu et al., 1974) to study the production of a secretory glycoprotein (an immunoglobulin κ-type light chain) has already yielded valuable results. For membrane-bound glycoproteins the formation of the envelope glycoprotein of some viruses of mammalian cells is a promising system. On invasion of the host cell, transcription of the viral genome results in the formation of the protein portion of the glycoprotein, which becomes a major component of the viral envelope and which is a key factor in maintaining the infectivity of the viral particles. The formation of the host proteins for glycosylation is switched off and the host glycosylating machinery is used for the formation of the viral envelope glycoprotein, which in fact is of the complex N-glycosidic type (Fig. 1).

It has been observed that tunicamycin, a glucosamine-containing antibiotic, has antiviral activity by virtue of inhibiting formation of envelope glycoprotein. This has been shown with Newcastle disease virus (Takatsuki & Tamura, 1971), and with Semliki-Forest virus, Rous sarcoma virus, and fowl plague virus (Schwarz et al., 1976). Very low concentrations of antibiotic (<1 μg/ml) have also been shown to inhibit step 1 in Fig. 2 in microsomal preparations of chick-embryo fibroblasts, the host cells for these viral studies, and of ox liver (Takatsuki et al., 1975; Tkacz & Lampen, 1975). It appears that tunicamycin inhibits glycosylation of the protein by blocking the glycosylation process, involving dolichol phosphate specifically at the first step. (It has been demonstrated that it does not, for example, block step 4 of Fig. 2). It may well be that the inhibition of infectivity of enveloped viruses observed in the presence of 2-deoxyglucose and some fluorosugars is also due to a similar block in protein glycosylation (Schmidt et al., 1976).
In chick-embryo fibroblasts infected with Semliki-Forest virus and treated with tunicamycin, it was possible to detect the formation of the protein moiety but not the whole envelope glycoprotein. With fowl-plague-virus- and Rous-sarcoma-virus-infected cells treated with tunicamycin, the formation of neither envelope glycoprotein nor its protein moiety could be detected. However, if in addition the fowl-plague-virus system also contained a proteinase inhibitor, the formation of the protein moiety could be detected (Schwarz et al., 1976). It is possible, therefore, that the oligosaccharide portion of the envelope glycoprotein of fowl-plague virus protects the molecule from attack by proteinases present in chick-embryo fibroblasts. In these viral systems the absence of an oligosaccharide portion on the protein resulted either in a loss of infectivity of the viral particles and a decrease in their haemaglutinin and neuraminidase activity, or a failure to release viral particles at all.

Control of glycosyltransferase activity

The mechanism by which tunicamycin inhibits the N-acetylgalactosaminyltransferase is not understood, but, in view of the antibiotic's glucosamine content, it may well be due to a substrate-analogue-inhibition phenomenon. The transferases in Fig. 2 may also be sensitive to allosteric control and, for example, the powerful and specific inhibition of step 6 by physiological concentrations of GDP-mannose (Kerr, 1976) may have biological significance in the normal cell. The loose specificity of step 4 (Fig. 2) for dolichol phosphate but the apparently tighter specificity for the dolichol phosphate moiety in subsequent steps also opens the door for the manipulation of the rate of protein glycosylation by administration of substrate analogues of dolichol phosphate. Analogues which act in vitro as good acceptors for mannose, but which in the mannosylated form are relatively poor donors of mannose, have been described (Richards & Hemming, 1972).

Involvement of retinol

Retinol phosphate (Fig. 4) has been reported to substitute adequately for dolichol phosphate as an acceptor for mannose and galactose, and evidence suggests the further transfer of sugar to protein (Rosso et al., 1975). Whether or not both function together or as alternative coenzymes and whether or not the oligosaccharides and glycoproteins formed are the same for each has yet to be determined. The fact that retinoic acid, which appears to be incapable of biological reduction to retinol, will relieve most symptoms of vitamin A deficiency suggests that this role in glycosylation cannot explain the complete mode of action of retinol.

Cell-surface glycosylations

It has been suggested that changes in plasma-membrane glycoproteins have an important influence on animal cell-cell interactions and on receptor properties. Some mechanisms proposed to explain changes in these parameters require the presence of glycosyltransferases on the surface of the cell. Although most glycosyltransferase activity is found associated with internal membranes, there have been several reports of this activity in cell membranes, and, in one of these (Struck & Lennarz, 1975), the involvement of dolichol phosphate-carried mono- and oligo-saccharides is suggested.

Dolichol phosphate and glycoprotein formation in non-animal systems

Some very elegant work has demonstrated a role for dolichol phosphate in yeast systems responsible for the formation of plasma-membrane N-glycosidic and O-glycosidic glycoproteins (Jung & Tanner, 1973; Lehle & Tanner, 1975). The latter is the only example of an involvement in the formation of other than N-glycosidic glycoproteins. The product is a mannoprotein, and the formation of a deoxyglucoprotein via dolichol
phosphate deoxyglucose is one aspect of the inhibition of glycoprotein formation by 2-deoxyglucose (Lehle & Schwarz, 1976).

There have been a number of reports of early reactions in Fig. 2 also taking place in fungal and higher-plant cells. Probably the best evidence for a complete scheme of this sort in higher plants was obtained by using cotton-boll preparations (Forsee & Elbein, 1975).

Conclusions

The demonstration of a role for dolichol phosphate in the glycosylation of some glycoproteins probably raises more questions than it answers. It is puzzling why, in mammalian cells, lipid-soluble donors are required for the transfer of N-acetylglucosamine and mannose to core regions of N-glycosidic glycoproteins but not for transfer of peripheral sugars or for glycosylation of O-glycosidic glycoproteins or glycolipids. Possibly the subcellular distribution of these activities provides a clue, for the latter group of glycosylations are believed to occur mainly in the Golgi and secretory-vesicle membranes, whereas the former occurs in rough and smooth endoplasmic reticulum.

The hydrophobicity of the membrane surrounding the glycosyltransferase complexes is probably less in the Golgi than in rough endoplasmic reticulum, because the former has components more heavily glycosylated than the latter. This may render the provision of a lipophilic donor in the Golgi superfluous. It may also ease the problem of movement of sugars from nucleotide donors on one side of the membrane to glycoprotein acceptors on the other side. It should be pointed out that in fact there is still no good evidence relating to vectorial aspects of the role of dolichol phosphate in sugar transfer in the rough and smooth endoplasmic reticulum.

In considering the relative constancy of the structure of the core of N-glycosidically linked oligosaccharides, Kornfeld & Kornfeld (1976) have suggested that the synthetic mechanisms may have originated early in evolution. That prokaryotic glycosylation of membrane-associated complex polysaccharides requires a polyprenol phosphate coenzyme is consistent with a general view that glycosylation via lipid-linked intermediates developed at an early stage in evolution. It is conceivable that glycosylation of membrane-linked molecules without lipid coenzymes is a relatively recent innovation. In this respect it is noteworthy that N-glycosidic glycoproteins with just the oligosaccharide cores, formed via dolichol phosphate derivatives, have been postulated as precursors, by alternative routes, of glycoproteins with both 'normal-size' and 'large' oligosaccharides. The balance between these two may be influenced by malignant transformation in favour of the 'large' group (Warren, 1976).

To explore these speculations there is a particular need for more firm data on the tissue and subcellular distribution of dolichol phosphate and its derivatives and of the relevant glycosyltransferases. Some detailed aspects of the chemistry and extent of formation of oligosaccharide derivatives in different sites will be essential, requiring an extension of the excellent chemical-synthetic work (Warren & Jeanloz, 1975) already carried out. Perhaps above all we can look forward to the characterization of the glycoproteins being achieved, a task that is dependent not only on the development of better methods for handling these compounds but also on the recognition of useful model systems.


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