The Role of Polyprenol-Linked Sugars in Eukaryotic Macromolecular Synthesis

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Since the Lipid Group last included polyprenol-linked sugars as a colloquium topic over four years ago there has been a great deal of research activity, which has generated several reviews (Behrens, 1974; Hemming, 1974, 1977a,b; Lucas & Waechter, 1976; Waechter & Lennarz, 1976). The object of the present paper is to discuss some of the more recent developments, most of which have concerned eukaryotic, especially vertebrate, systems.

Polyprenol-linked sugars feature in schemes to explain the addition of core sugar residues to some N-glycosidic glycoproteins. Fig. 1 (after Behrens, 1974) was proposed primarily on the basis of experiments with cell-free membrane preparations of rat liver. Using slices of several tissues Spiro et al. (1976, 1976a,b) have elegantly confirmed and elaborated this scheme. The Dol-P₂-oligos* resulting from reaction 7 usually has \( n = 11 \) and one or two glucose residues. These workers report the absence of glucose from the product from pancreas, but Herscovics et al. (1977a,b) have shown this tissue to make a glucosylated Dol-P₂-oligos also. The process occurs most efficiently in thyroid and oviduct, and Spiro's group have concentrated on the former.

Sugar analysis, periodate treatment, acetylation and \( \alpha \)-mannosidase treatment lead to the oligosaccharide structure (Fig. 2), which contains glucose attached to the proximal core. This arrangement protects the core from attack by \( \alpha \)-mannosidase. Thyroid slices incorporate \( ^{14} \text{C} \)-labelled sugars, \( ^{32} \text{P} \)P₁ and \( ^{3} \text{H} \)mevalonate into Dol-P₂-oligos, and it is calculated that the oligosaccharide portion is synthesized at a faster rate than is the Dol-P portion, suggesting a recycling of Dol-P.

Puromycin inhibited glycosylation of both soluble and insoluble protein and stopped the turnover of Dol-P₂-oligos. However, the concentration of Dol-P₂-oligos did not build up, suggesting that the amount of Dol-P is limiting. Attempts to alter this amount by changing pools of putative precursors failed, possibly because of a large pool of an immediate precursor (dolichol?). One of the major soluble glycoproteins of thyroid is

* Abbreviations: Dol-P, dolichol monophosphate; Dol-P-Man, dolichol monophosphate mannose; Dol-P₂-oligos, dolichol diphosphate oligosaccharide; Dol-P₂-GlcNAc₂, dolichol diphosphate di-(N-acetylglucosamine), etc.
Fig. 1. Role of Dol-P in glycosyl transfer to glycoprotein in rat liver (after Behrens, 1974)

Dol-P, \( \text{H}[\text{H}_2\text{C}-(\text{CH}_3)-\text{CH}-\text{CH}_2]_n-\text{CH}_2-\text{CH}(\text{CH}_3)-\text{CH}_2-\text{CH}_2\text{O}-\text{PO}_3^-\text{H} \). In mammals \( n = 14-21 \).

Thyroglobulin, the oligosaccharide chains of which (Fig. 2) lack glucose and contain less mannose. If Dol-P_2-oligos is to be a precursor of these groups considerable glycosidase activity on the first glycoprotein formed, followed by further glycosylations in the case of group B (Fig. 2), must occur. Alternatively, the glycoprotein (especially the insoluble product) resulting in Fig. 1 may be an end product [in keeping with the increasing number of reports of glucose in membrane-bound glycoproteins (e.g. Spiro et al., 1976a; Kitamura et al., 1976)].

In slices step 8 (Fig. 1) is rate limiting, and Dol-P_2-oligos accumulates to a much greater degree than Dol-P-Man. In cell-free membrane preparations this situation is reversed, owing possibly to membrane damage. Membrane preparations of fibroblasts in fact exhibit a high affinity for UDP-glucose in the glucosylation of Dol-P_2-oligos (Robbins et al., 1977). The present report also indicated that the concentration of exogenous nucleotide diphosphate sugars is a critical factor if unnatural blockages in Fig. 1 in cell-free systems are to be avoided. Kerr (1976) has observed in liver preparations the presence of two glucosyl transferases (step 6, Fig. 1), one of which is readily inhibited by GDP-mannose (<10^-6 M) and other nucleotide diphosphate sugars. These studies also revealed that the pool of Dol-P available for mannosyltransferase (step 4) is not available for the glucosyltransferase (step 6). Probably each glycosyltransferase (steps 4+5 and 5+6) is a complex containing its own tightly bound coenzyme (Dol-P). In fact direct unequivocal evidence for the presence of dolichol in endogenous Dol-P_2-oligos is
Fig. 2. Oligosaccharide structures

Structures of oligosaccharide unit A of calf thyroglobulin (Spiro, 1973) (a), oligosaccharide unit B of pig thymoglobulin (Toyoshima et al., 1973) (b), and of the oligosaccharide unit (provisional) of Dol-P₅-oligos (Fig. 1, Spiro et al., 1976) (c). →, Uncertainty of adjoining monosaccharide.

Abbreviations: AcNeu, N-acetylneuraminic acid; GclNeu, glycolloylneuraminic acid. The type of linkage is indicated above the bonds (arrows); when in parentheses the linkage is uncertain. In (a) the subscripts indicate different mannose chain lengths.
still lacking, although the evidence based primarily on chromatographic and hydrolytic data and precursor studies has been strengthened by results of ozonolytic studies (Kerr, 1976).

The enzymes catalysing Fig. 1 are concentrated in the endoplasmic reticulum (e.g. Spiro et al., 1976a). However, Heifetz & Elbein (1977) have reported solubilization of various mannosyltransferase activities from aorta with Nonidet P.40. In particular the solubilized enzyme catalysing step 3 has been isolated and detailed studies confirm a β-mannosyl residue in the product, in keeping with the view that an inversion of enantiomeric configuration occurs each time a glycosyl 1-phosphate bond is broken in Fig. 1. Solubilized enzymes should allow a more detailed enzymology of Fig. 1.

Effects of different steps in Fig. 1 have been reported. Bacitracin surprisingly causes the accumulation of Dol-P_2-GlcNAc_2-Man in oviduct preparations, possibly by limiting the supply of Dol-P to step 4 (Chen & Lennarz, 1976). On the other hand, Godelaine et al. (1977) report a strong stimulation of step 1 by GTP, specifically, by using pyrophosphate-washed rough endoplasmic reticulum of rat liver. It is suggested that the pyrophosphate may remove ribosomes from the membrane exposing the transferase to GTP.

A powerful negative effector of step 1 that is proving very useful is the antibiotic tunicamycin (Takatsuki et al., 1975; Tkacz & Lampen, 1975). The relevance of this inhibition to a block in the biosynthesis of viral-envelope glycoprotein and hence to the antiviral activity of tunicamycin is discussed elsewhere (Hemming, 1977a). Robbins et al. (1977) also demonstrated the probable involvement of reactions depicted in Fig. 1 in the biosynthesis of Sindbis-virus glycoprotein, suggesting the possible subsequent modification of the oligosaccharide chain to provide the final form of oligosaccharides of virus glycoproteins (cf. Fig. 2b). The reactions in Fig. 1 have been demonstrated in preparations of hen oviduct leading to the formation of membrane-bound glycoproteins, but not of ovalbumin (Lucas et al., 1975). However, Struck & Lennarz (1977) reported the inhibition of N-glycosylation of ovalbumin by tunicamycin and a block in mannosylation at steps 3 and 4 (Fig. 1) (presumably owing to the lack of acceptor), which suggests a role for Fig. 1 in ovalbumin glycosylation.

The inhibitory effect of 2-deoxyglucose on the infectivity of enveloped viruses may well involve interference with mannosyl transfer (Schmidt et al., 1976) through Dol-P, especially in view of the formation of dolichol phosphate 2-deoxyglucose by yeast preparations (Lehle & Schwarz, 1976). This could also explain the effect of 2-deoxyglucose on glycosylation of immunoglobulins (see, e.g., Eagon et al., 1975) and on receptor-mediated phagocytosis by macrophages (Mickl et al., 1976).

Although most reports indicate that the major involvement of Dol-P in glycosylation occurs in intracellular membranes, several convincing observations of cell-surface-catalysed glycosylation of Dol-P have appeared (e.g. Patt & Grimes, 1976; Struck & Lennarz, 1976; Arnold et al., 1976). The last of these concerned isolated liver cells of embryonic chicks.

The embryonic system of fertilized sea-urchin eggs has also been studied by Schneider & Lennarz (1976). Mannosyl transfer activity to dolichol phosphate is reported to be present. Another invertebrate, the fruit fly Ceratitis capitata has been shown (Quesada-Allue et al., 1976) to form all three monosaccharide derivatives of Dol-P shown in Fig. 1.

Studies of this area in higher plants has progressed less rapidly than in animals, but evidence for a scheme of mannosyl and N-acetylglucosaminyl transfer to protein approximating to Fig. 1 has been obtained by using cell-free preparations of Phaseolus aureus (Forsee et al., 1976; Lehle et al., 1976; Roberts & Pollard, 1975), of cotton-boll fibres (Forsee et al., 1976), of Phaseolus vulgaris (Ericson & Delmer, 1977) and of Pisum sativum (Brett & Leloir, 1977). The glucosylation of polypremol phosphate occurs in the Golgi apparatus of Phaseolus aureus (Bowles et al., 1977) and in Pisum sativum (Pont-Lezica et al., 1975, 1976), although in both it is a quantitatively minor process compared with sterol glucosylation. The latter two reports and Brett & Leloir (1977) indicate a dolichol type of polypremol, but it is not clear if the process is involved in a reaction scheme such as Fig. 1, or solely in the formation of glucans, as suggested by Brett & Northcote (1975).
The elegant work demonstrating the role of Dol-P-Man in O-glycosylation of membrane proteins of Saccharomyces cerevisiae (Sharma et al., 1974) has been extended (Lehle & Tanner, 1975) to show the formation of a Dol-P, -oligos containing mannose and N-acetylglucosamine during the N-glycosylation of membrane proteins. Reuvers et al. (1977) have studied in detail the formation of Dol-P,2-GlcNAc, in this organism, and report in cell-free preparations the transfer of the disaccharide to endogenous protein. The same membrane preparation will also catalyse the N-acetylglucosaminyla-
tion of bovine pancreatic ribonuclease A (Khalkhali et al., 1976). Inhibition by tunicamycin suggests involvement of step 1 of Fig. 1 in the process. Nakayama et al. (1976) (see also Palamarczyk, 1976) report that yeast preparations mannosylate Dol-P,2-
GlcNAc, by reaction 3 of Fig. 1 and that the resulting trisaccharide can be transferred to protein. Parodi (1976) reports further that incubation with Dol-P,Glcn gives rise to Dol-P,2-oligos containing approximately 16 sugar residues and that this oligosaccharide is then transferred to protein. Mannosylation of yeast mannoprotein by Dol-P-Man appears to be concentrated in polyribosomes (Larriba et al., 1976), although the formation of Dol-P,2-GlcNAc has been observed in endoplasmic reticulum and in mitochondrial membranes (Palamarczyk, 1976).

In Aspergillus niger (Letoublon & Got, 1974) and Neurospora crassa (Gold & Hahn, 1976) good evidence for a role of Dol-P-Man in the O-mannosylation of protein has been reported. An involvement in N-glycosylation of proteins of these fungi has not yet been demonstrated, although it is relevant that such glycoproteins appear to exist in A. niger (Rudick & Elbein, 1975) and that a specific inhibition of synthesis of cell-surface components occurs when this organism is exposed to tunicamycin (Katoh et al., 1976).

In summary there is now evidence for a scheme similar to that in Fig. 1 operating in most eukaryotic organisms. In filamentous fungi and invertebrates the evidence is little more than hints, whereas in yeast and higher plants the evidence is becoming quite convincing. In many tissues of the vertebrates studied strong evidence for this scheme is accumulating. Although results with cell-free systems inspired the scheme, only in whole cells is the formation of the oligosaccharide chain completed. Cell-free studies are complicated by the mixture of oligosaccharide derivatives formed and by the low degree of specificity of step 8 to oligosaccharide size. In vertebrates glucose is linked to a proximal part of the oligosaccharide chain that eventually forms an N-glycosidic link with protein. Whether or not this glycoprotein is an end product or is further modified to form the previously described types of N-glycosidically linked oligosaccharide chains (Fig. 2) is still uncertain. Equally uncertain in most tissues is the nature of the protein glycosylated although it now appears that in some cases both soluble and membrane-bound products are formed. A further involvement of Dol-P in O-mannosylation of glycoproteins in yeasts and fungi has been established. It may also be involved in the formation of glucans in higher plants. Interference with steps in these schemes provides a method of controlling the synthesis of these macromolecules.


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Mycobacterial Glycolipids as Bacterial Antigens

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The role of mycobacteria as immunological adjuvants was established indirectly from the observation (Lewis & Loomis, 1924) that a higher titre of haemolysin was produced in tuberculous than in normal guinea pigs. Later Freund (1956) reported that the addition of paraffin oil to killed tubercle bacilli stimulated antibody production against non-mycobacterial protein antigens. Whole mycobacteria as the adjuvant can be replaced by a number of bacterial fractions. For instance, pure mycobacterial cell walls were equally as effective as whole cells in Freund's adjuvant (Kotani et al., 1960).