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Lipid intermediates in protein glycosylation

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The oligosaccharide moieties of animal glycoproteins that involve linkage between carbohydrate and asparagine are built up on dolichol pyrophosphate before incorporation into the protein. The use of dolichol pyrophosphate in place of the commoner nucleoside diphosphate as the sugar carrier may be because the former is more compatible with the hydrophobic environment of the membrane where the glycoprotein synthesis occurs.

The glycosylation of proteins occurs in some cases by direct transfer of monosaccharides from sugar nucleotides to polypeptide chains, while in other cases it occurs by a mechanism involving lipid-bound mono- and oligosaccharides as intermediates. Work on the latter mechanism is reported in this review (see also refs [1–3]).

The pathway involving lipids seems to function in the biosynthesis of those glycoproteins which contain oligosaccharides linked to asparagine residues. The glycoproteins in which the saccharide moieties are joined to hydroxylysine or to serine or threonine are usually believed to be built up by direct and sequential transfer from the sugar nucleotides but further work may uncover new facts(for reviews see refs [4,5]).

It seems important to understand the mechanism of biosynthesis of glycoproteins because they are widely distributed in nature, from bacteria to animal tissues. They can be found among cytoplasmic, secreted or membrane-bound proteins and may function as enzymes, hormones, antibodies, etc. (reviewed in refs [5–7]). They are believed to have a key role in determining the specificity of cell surface recognition phenomena.

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Polyprenols

The role of lipid intermediates in sugar transfer was first detected in studies on the formation of some bacterial polysaccharides. The lipids involved are derivatives of polyprenols that have the general structure

$$H-\left[CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH_2\right]_n OH.$$

The compound which acts as an intermediate in bacteria has $n=11$ and two trans double bonds. It was isolated as the mono- or diphosphate combined to sugars [3].

The lipid intermediates of animal tissues are derivatives of dolichol which is a very long polyprenol ($n=17$–21). It has two trans double bonds and the isoprene which carries the –OH group is saturated. The compound was found partly free and partly esterified to fatty acids [3]. At present it is known to occur also as the monophosphate and the diphosphate, free or combined with sugars.

Dolichol phosphate sugars

Several reactions in which dolichol derivatives are involved have been described (see Table I). Reaction 1.1. which leads to the formation of Dol-P-Glc was first detected on incubation of glucose labeled UDP-Glc with liver microsomes and magnesium ions [8]. A radioactive compound soluble in organic solvents was formed, and its amount could be greatly increased by the addition of a lipid extract of liver. The acceptor contained in the extract was purified and its properties found to be identical to a sample of dolichol phosphorylated by a chemical method. It differed from undecaprenol phosphate in that it was acid stable, a difference that can be accounted for by the absence of the double bond in the first isoprene. The product formed by transfer of glucose from UDP-Glc to Dol-P, namely Dol-P-Glc, was found to be acid labile and to give 1,6-anhydroglucose by alkaline treatment. This was taken as an indication that the glycosidic bond in Dol-P-Glc is β because the same anhydride is formed by alkaline treatment of phenyl β-glucosides. A transfer reaction was also detected with GDP-Man as donor (reaction 1.2) [9]. The product, Dol-P-Man, has been studied more thoroughly and compared with a synthetic specimen [10–11]. It is also the β anomer.

The transfer of xylose to Dol-P (reaction 1.3) has also been detected [12]. Since xylose is identical to glucose except for the absence of the $-CH_2OH$ residue the reaction might be attributed to loose specificity of the glucose transfer reaction. Transfer of xylose occurs in the synthesis of the linkage region of proteoglycans (serine-xylose), but the corresponding enzyme has been purified and no lipid requirement was detected [13].

Dolichol diphosphate sugars

When UDP-GlcNAc is used as donor and Dol-P as acceptor a transfer of sugar phosphate takes place so that the product is Dol-P-P-GlcNAc (reaction 2.1) [14]. A second addition of acetylglucosamine can occur so that Dol-P-P-$(GlcNAc)_2$ is formed (reaction 4.1). There is evidence that the donor is UDP-GlcNAc and not Dol-P-P-GlcNAc [14]. The next reaction appears to be a transfer from GDP-Man so that a trisaccharide residue is formed (reaction 4.2) [15]. This trisaccharide (β-Manl-4β-GlcNAc-4-GlcNAc) is of special interest because it occurs linked to asparagine residues in many glycoproteins such as immunoglobulins, ovalbumin, thyroglobulin, etc. However, there are exceptions since, for instance, immunoglobulin E contains an oligosaccharide with the sequence α-GlcNAcl-3β-Manl-4β-GlcNAc-β-asparagine [16].

The transfer of mannose residues from Dol-P-Man to the Dol-P-P linked trisaccharide leads to the formation of larger oligosaccharides (reaction 5.1) [15]. Such compounds can be detected by incubation of labeled GDP-Man or Dol-P-Man with liver or hen oviduct microsomes [17–18]. The reaction products, soluble in organic

solvents, are then submitted to mild acid hydrolysis and run on paper chromatography. Radioactive oligosaccharides can be observed which run like malto-oligosaccharides of 5–12 units. The amount of radioactive Dol-P-P-oligosaccharide is greatly increased if a lipid extract of liver known to contain the unlabeled dolichyl-pyrophosphate derivatives is added to the incubation mixture.

Other mannose transfer reactions, not shown in Table I, were described by Adamany and Spiro [19]. They observed that Dol-P-Man can act as donor to methyl α-mannoside or to a dinitrophenyl glycopeptide.

When microsomes are incubated with labeled UDP-Glc or Dol-P-Glc, only one lipid oligosaccharide becomes labeled (reaction 5.2). This compound is insoluble in chloroform methanol (2:1) but is soluble in chloroform-methanol-water (1:1:0.3). The oligosaccharide moiety obtained by mild acid hydrolysis chromatographs on paper like a malto-oligosaccharide of about 17 glucose units [20]. It contains two hexosamine residues, and combines with concanavalin A [21]. Its molecular weight as measured with Sephadex G-50 is about 3400. This glucose containing compound has been prepared starting from several kg of liver. Although only minute amounts were obtained, the oligosaccharide could be analyzed by gas chromatography. It contained GlcNAc, Man and Glc in the proportion 2:12:4.

Reaction 3.1 in which Dol-P-P-GlcNAc is transformed into Dol-P-P-N-acetylmannosamine has been detected upon incubation of liver microsomes with UDP-GlcNAc and Dol-P. Evidence was presented indicating that epimerization occurred on the dolichol derivative [22].

Transfer to protein

The transfer of the oligosaccharide moieties to protein has been detected both with the mannose- and the glucose-labeled Dol-P-P oligosaccharides (reaction 6.1). The same enzyme preparations used for the other reactions in Table I that is, microsomal fractions from various different animal tissues, have been used in this case, with the addition of Mn^{2+} ions and detergents [17,23]. The glycoproteins synthesized seem to be of the asparagine-linked type. The basis of this belief is that in all cases studied the mannose-GlcNAc-GlcNAc containing oligosaccharides have been found joined to an asparagine residue. The proteins glycosylated by the pathway involving dolichol intermediates have not been clearly identified. Thus, Lucas et al. [18] used microsomes from hen oviduct, a tissue in which about 50% of the soluble protein synthesized is ovalbumin, but only less than 10% of the protein labeled from Dol-P-P-oligosaccharide was precipitated with ovalbumin antiserum. Similarly Hsu et al. [24] used a mouse myeloma which synthesizes immunoglobulin light chain and obtained only 10–20% of the label in the expected glycoprotein. It seems that part of the glycoproteins formed by this pathway are membrane constituents. One of the great difficulties in these studies on transfer to protein is the lack of adequate methods for preparing the non-glycosylated protein for use as acceptor.

Glycoproteins of the asparagine type may have various peripheral sugars besides the internal mannose and GlcNAc residues. These external sugars are commonly galactose, GlcNAc and sialic acid. The transfer of the peripheral residues probably occurs after that of the core oligosaccharide to the protein. It has been shown that membranes of the Golgi reticulum catalyze the sequential addition of such residues to glycoproteins from which the corresponding monosaccharides had been previously removed [4]. As no dolichol derivatives of galactose and sialic acid have been detected in animal tissues these sequential transfer reactions presumably occur directly from the sugar nucleotides.

The reason why dolichol intermediates are involved in some cases and not in others is difficult to understand. It has been suggested that the dolichol moiety may embed itself in the membrane lipids thus permitting the saccharide residue to go through or position itself on the membrane at some stage in the synthetic process.

TABLE I
Dolichol phosphates in glycoprotein synthesis

1. Synthesis of dolichol monophosphate sugars
   - 1.1 UDP-Glc + Dol-P = Dol-P-Glc + UDP
   - 1.2 GDP-Man + Dol-P = Dol-P-Man + GDP
   - 1.3 UDP-xylose + Dol-P = Dol-P-xylose + UDP
2. Synthesis of dolichol pyrophosphate sugars
   - 2.1 UDP-GlcNAc + Dol-P = Dol-P-P-GlcNAc + UMP
3. Transformation of the saccharide moiety
   - 3.1 Dol-P-P-NAc = Dol-P-P-N-acetylmannosamine
4. Direct transfer from sugar nucleotides to dolichol pyrophosphate sugars
   - 4.1 UDP-GlcNAc + Dol-P-P-GlcNAc = Dol-P-P-$(GlcNAc)_2$ + UDP
   - 4.2 GDP-Man + Dol-P-P-$(GlcNAc)_2$ = Dol-P-P-$(GlcNAc)_2$ + GDP
5. Dolichol monophosphate monosaccharides as donors
   - 5.1 Dol-P-Man + Dol-P-P-Oligosac = Dol-P-P-Oligosac-Man + Dol-P
   - 5.2 Dol-P-Glc + Dol-P-P-Oligosac = Dol-P-P-Oligosac-Glc + Dol-P
6. Dolichol pyrophosphate oligosaccharides as donors to protein
   - 6.1 Dol-P-P-Oligosac + Protein = Dol-P-P + glycoprotein

Note: Dol: dolichol; Glc: glucose; Man: mannose; GlcNAc: N-acetylglucosamine

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