Polyprenol-linked sugars and glycoprotein synthesis in plants

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Introduction

Protein glycosylation is a universal feature of all eukaryotic organisms and has been the subject of intensive studies for several years. Lipid-linked saccharides, or lipid-intermediates, of a type similar to those involved in the biosynthesis of cell wall polysaccharides in bacteria (see, for example, Hemming, 1974) have also been shown to participate in the formation of glycoproteins. The present short review tries to summarize the current state in plant systems. Since much of the initial work has been carried out in animal and yeast, reference will be made to those studies. For a more detailed bibliography the reader is referred to recent reviews (Parodi & Leloir, 1979; Elbein, 1979; Hubbard & Ivatt, 1981; Lehle, 1981a).

Structure of glycoproteins

As is the case with glycoproteins of animal origin, plant glycoproteins are a diverse group with regard to their structure and include compounds such as structural proteins, enzymes, storage proteins, lectins, toxins and glycoproteins for which no biological role has emerged yet (Brown & Kimmis, 1977; Sharon & Lis, 1979; Selvendran & O'Neill, 1982). Whereas the structures of many animal glycoproteins have been characterized (Kornfeld & Kornfeld, 1976), considerably less information is available in plants.

N-Glycosidically linked chains. One major group of glycoproteins have an N-glycosidic linkage between the anomeric carbon of N-acetylglucosamine and the amide N of asparagine. In spite of the enormous variety and heterogeneity of these oligosaccharide chains in terms of their size and structure, they can be classified into three groups as outlined in Fig. 1. In particular it should be noted that they have the pentasaccharide structure GlcNAc$_2$Man$_3$ in common, which led to the concept, later proven, that there is a common route for the biosynthesis of these oligosaccharides (see below). The best example of a well-characterized high-mannose type structure in plants is that of soya bean lectin (Fig. 2; Lis & Sharon, 1978; Dorland et al., 1981). Lima-bean agglutinin (Misaki & Goldstein, 1977), bromelain (Fukuda et al., 1976; Ishihara et al., 1979), a-amylase (Minobe et al., 1979), legumin (Browder & Beevers, 1978; Basha & Beevers, 1976), vicilin (Ericson & Chrispeels, 1973; Chrispeels et al., 1982) and horseradish peroxidase (Clarke & Shannon, 1976) are other examples for which structural information is available. No typical complex mammalian type oligosaccharide structures have been found in plants. But as one may realize in the case of bromelain, plants seem to modify the

![Pentasaccharide structure](image_url)

**Fig. 1. Generalized structures of N-glycosidically linked oligosaccharides**

The broken arrows indicate possible linkages with various sugar residues. In high-mannose type chains these are exclusively mannose residues, whereas in complex-type chains they consist of GlcNAc, Gal and sialic acid.

Abbreviations used: Dol, dolichol; Ret, retinol.

*Professor Tanner was to have presented this talk at the Colloquium but regrettably was unable to attend due to illness. However, Dr. Lehle and he kindly provided this review manuscript on this topic and the organizers wish to express their appreciation.
ooligomannosidic structure in a different way to give what we might consider as a 'pseudo-complex type' structure. Moreover, various reports in the literature about the sugar composition of plant glycoproteins, and the glycosylation reactions which occur (Davies & Delmer, 1981) lead one to suspect further structures, which cannot be assigned to any of the groups described above, nor to the types discussed below.

O-Glycosidically linked chains. A major group among the O-glycosidic plant glycoproteins are hydroxyproline-rich cell wall glycoproteins found in green plants and green algae. The so called 'extensin' is characterized by small arabinose side chains linked to hydroxyproline (Hyp-O-Ara,1) and the occurrence of galactosyl-O-serine (Lampert, 1967; Lampert et al., 1973; Lampert & Catt, 1982). The same type of linkages has been described for the well-characterized potato lectin (A. K. Allen et al., 1978). Other hydroxyproline glycosides not found in extensin are Hyp-O-galactose (Lampert & Miller, 1971) in algae cell walls and in arabinogalactan proteins (Fincher et al., 1974). Recently, preliminary evidence was also obtained for the existence of Hyp-O-glucose (Mani et al., 1979). In maize roots an O-glycosidic linkage between xylose and threonine has been found. A polysaccharide moiety is assembled on a protein primer and subsequently secreted by the cap cells (Green & Northcote, 1978).

Glucose-containing glycoproteins. Plant glycoproteins with glucose as a permanent constituent (which is not the case for glycoproteins from other eukaryotic tissues) have recently gained considerable attention as primers for plant polysaccharide synthesis such as starch (Tandecarz et al., 1975; Tandecarz & Cardini, 1978), paramylon (Tomas & Northcote, 1978) and cellulose (Hopp et al., 1978a,b). The nature of the protein–carbohydrate linkage is unknown and unambiguous data concerning the structure of the carbohydrate portion do not exist.

Participation and nature of lipid carriers

Almost simultaneously in the early 1970’s the existence of sugar-lipid derivatives with the possible function as intermediates in the synthesis of glycoproteins was reported for animal systems (Caccam et al., 1969; Behrens & Leloir, 1970), yeast (Tanner, 1969) as well as for plants (Kauss, 1969). Since then it has become clear that with some exceptions the lipid portions are phosphorylated polyprenols of variable chain length containing a saturated a-isoprene unit (Fig. 3). These polyprenols of the so-called dolichol type differ from those found in bacteria, in which all the isoprene residues are unsaturated (Hemming, 1974) and are, as already mentioned, intermediates in the wall formation of these organisms. As several such unsaturated aliphatic polyprenols occur also in plants (Fig. 3; Wellburn & Hemming, 1966) any of them could be the endogenous lipid acceptor.

Nature of the lipid acceptor. The first strong evidence for the dolichol nature of the lipid acceptor in glycoprotein transfer in plants was obtained by Pont Lezica et al. (1975) in soya bean, wheat germ and pea. Lehle et al. (1976) and Brett & Leloir (1977) reported that the lipid attached to mannose and N-acetylglucosamine is also of the dolichol type. The structure of the mannolipid from kidney bean has in the meantime been characterized unambiguously as phosphoryl dolichol, using mass spectrometry, treatment with phenol and catalytic hydrogenation (Delmer et al., 1978). It appears that the lipid acceptor in all eukaryotic cells is of the dolichol type. This has been questioned, however, by the observation that with a particular enzyme preparation from mung beans GDP-Man does not compete with UDP-GlcNAc for the lipid acceptor (Ericson et al., 1978a). It has been suggested that the use of different lipids could be a regulatory mechanism in glycoprotein synthesis. On the other hand, the above effect could merely reflect different pools of polyprenols, which in fact is substantiated by the experiments using liver microsomes (Oliver & Hemming, 1975). Concerning the different chain lengths of dolichols, it was speculated that they may serve different functions, in a way that dolichol of a certain size carries N-acetylglucosamine, while others function as acceptor for mannose or glucose. However, Reuvens et al. (1978) examined the lipid moiety of Dol-PP-GlcNAc from yeast by h.p.l.c. and concluded that no particular homologue was preferred in the synthesis of this compound. Investigations carried out in yeast using solubilized glycosyltransferases and polyprenylphosphates of different chain length gave the following results. The mannosyl-dolichyl-phosphate transferase (DGP-Man + Dol-P→Dol-P-Man +
GDP did reveal a critical lower chain length of about C35 but otherwise no dramatic effects both with respect to length and saturation could be observed, i.e. unsaturated polyprenols can also function in vitro as acceptors (Palamarczyk et al., 1980). The minimum chain length requirement, however, was no longer seen when the purified transferase was used (Lehle et al., 1983). In contrast to the formation of Dol-P-Man the UDP-GlcNAc:dolichyl phosphate:glucosamine phosphate transferase showed an absolute dependence on polyprenols of the dolichol type. So far, however, no clear picture has yet emerged from these studies as to the rational explanations for the possible reaction of different chain lengths and saturation. Also obscure is the function of retinyl phosphate (Fig. 3) for which an involvement in the transfer of mannose and perhaps galactose to protein was demonstrated (De Luca et al., 1970; De Luca, 1977). This polyprenol differs from dolichol in that it contains an additional double bond per isoprene unit, has an allylic phosphate and a β-ionone ring structure at the opposed terminal end. Retinol has been proposed to play a role in the glycosylation of glycoproteins, which, though not further characterized, were distinct from the ones synthesized via Dol-P (Sasaki & De Luca, 1980). Very recently (Lehle et al., 1983) it was demonstrated that yeast membranes catalyse synthesis of Ret-P-Man and that Ret-P-Man can replace Dol-P-Man in terminal mannosyl-transfer reactions in the assembly of Dol-PP-GlcNAc-Manα (see below).

Subcellular distribution and synthesis. Dolichols occur membrane-bound in all subcellular fractions from tissues (Dallner et al., 1972). They occur as the free form, but the largest proportion is esterified with fatty acids and only a smaller amount, e.g. in yeast 10-20% is phosphorylated (Jung & Tanner, 1973). This synthesis follows the early steps in the pathway of cholesterol biosynthesis. In plants, cell-free preparations from pea epicotyls or from the green alga Chlamydomonas demonstrated (De Luca et al., 1970; De Luca, 1977), this polyprenol differs from dolichol in that it contains an additional double bond per isoprene unit, has an allylic phosphate and a β-ionone ring structure at the opposed terminal end. Retinol has been proposed to play a role in the glycosylation of glycoproteins, which, though not further characterized, were distinct from the ones synthesized via Dol-P (Sasaki & De Luca, 1980). Very recently (Lehle et al., 1983) it was demonstrated that yeast membranes catalyse synthesis of Ret-P-Man and that Ret-P-Man can replace Dol-P-Man in terminal mannosyl-transfer reactions in the assembly of Dol-PP-GlcNAc-Manα (see below).

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Since retinol has not been proven to be a natural constituent of this organism the physiological significance of this reaction has to await further elucidation.

Lipid-linked saccharides. Several dolichol-linked monosaccharides and oligosaccharides have been found and described in the past, and a detailed presentation of this aspect in plants has been given by Elbein (1979). For reasons of completeness and for the discussion below it should be mentioned that the lipid-linked monosaccharides include Dol-P-Man, Dol-P-Glc, Dol-P-GlcNAc and in plants also Dol-PP-Glc, whereas lipid-linked oligosaccharides of importance in terms of protein glycosylation are Dol-PP-GlcNAc-Manα, Dol-PP-GlcNAc-Manβ, Dol-PP-Glcα, and Dol-PP-Glcβ. The finding of a polyisoprenyl-fucosylated and its possible role in slime polysaccharide production (Green & Northcote, 1979) should be considered preliminary and needs further elucidation. So far no other polyisoprenyl-fucosylated sugars have been found in plants. An earlier report that mild acid labile glycolipids (a characteristic of polyprenyl derivatives) have been synthesized from UDP-xylose, UDP-arabinose, UDP-galactose or UDP-glucuronic acid has not been confirmed (Villemez & Clark, 1969). Likewise fucose-containing lipid soluble products synthesized in castor bean endosperm (Roberts et al., 1980) on incubation with GDP-fucose do not seem to have a function in protein glycosylation. The product of transfer of galactose from UDP-Gal to Dol-P in Vohoux turns out to be Dol-P-galactose and not Dol-P-galactose, due to the presence of an effective epimerase in the tissue (Bause & Jaenicke, 1979). The same observation was made in Chlamydomonas (Lang, 1982a) and in a previous report with liver homologate formation of Dol-PP-N-acetylmannosamine from UDP-GlcNAc and Dol-P was catalysed besides the expected product Dol-PP-N-acetylglucosamine (Palamarczyk & Hemming, 1975). These results clearly emphasize the necessity of a thorough analyses of the reaction products, which are occasionally lacking in earlier studies in this field.

N-Glycosylation: lipid-oligosaccharide assembly, processing reactions, inhibitors. Studies in the last 5 years have made clear that the pathway of formation of asparagine-linked carbohydrate chains in animals and yeast reflect a common glycosyltransferase concept. Recent reports reveal that this is also valid for plants, emphasizing the universality of this mechanism, which has been conserved during evolution. The sequence of reactions (most of which have been demonstrated in plants) is summarized in Fig. 4. The glycosylation process starts while a nascent polypeptide chain is synthesized on endoplasmic reticulum-bound ribosomes. An oligosaccharide with the composition Glc,Man,GlcNAc linked to dolichyl diphosphate is the immediate precursor, and is subsequently transferred en bloc to the protein. In animal and yeast cells the transfer of sugar residues proceeds stepwise as indicated either direct from the corresponding sugar nucleotides (Forsee et al., 1977) or from additional lipid intermediates like Dol-P-Man and Dol-P-Glc. The latter is concluded from the observations that when no sugar residues proceeds stepwise as indicated either direct from the corresponding sugar nucleotides (Forsee et al., 1977) or from additional lipid intermediates like Dol-P-Man and Dol-P-Glc. The latter is concluded from the observations that when no for Glc, Man,GlcNAc (Lehle, 1980). Although in plants such a detailed analysis has not been made, the formation of lipid-oligosaccharides from GDP-Man and UDP-GlcNAc have been well established in membrane preparations from a variety of sources, i.e. from cotton fibres, mung, kidney, soya and castor beans, peas and algae (Forsee & Elbein, 1975; Forsee et al., 1976; Alam & Hemming, 1973; Lehle et al., 1976; Ericson & Delmer, 1977, 1978; Brett & Leloir, 1977; Beever & Mense, 1977; Mellor & Lord, 1979; Baily et al., 1979; Romero et al., 1979). Recently the occurrence of the glucose-containing oligosaccharides, Dol-P-GlcNAc-Manα, Dol-P-GlcNAc-Manβ, Dol-PP-Glcα, and Dol-PP-Glcβ has been demonstrated in plants (Stanek, 1980; Lehle, 1981b). Moreover, the lipid-bound oligosaccharides was shown to be transferred to endogenous protein and could be released subsequently by endo-N-acetylgalactosaminidase treatment, as one expects for an asparagine-bound high-mannose oligosaccharide (Stanek, 1981). The function of the glucose residues, which are not constituents of the asparagine-linked
A proposed scheme for the reactions leading to high-mannose type and plant 'pseudo-complex type' glycoproteins. The latter expression is introduced to emphasize that these oligosaccharide chains differ from the mammalian ones. So far, no occurrence of sialic acid has been described in plants.

The dolichyl phosphate-linked saccharide pathway of N-glycosylation

A lipid-oligosaccharide in plants has appeared for the mannose-containing lipid-oligosaccharide Man,GlcNAc, which was synthesized in vitro by Phaseolus aureus seedlings (Hori & Elbein, 1982). Since the mannose residues were probably not uniformly labelled the data are not unambiguous. Nevertheless, the structure for Man,GlcNAc, is consistent with the oligosaccharide for soya bean lectin (Fig. 2) and with the lipid-oligosaccharide structure elucidated in animals (Li et al., 1978).
In mammalian cells, after the oligosaccharide is transferred to the protein, the glucose and some of the mannose residues are removed (processing or trimming reactions) to give rise to either high-mannose type or complex type oligosaccharides (if additional glycosylation occurs subsequently). In plants no processing reactions have been described so far. As is evident from Fig. 2, if the synthesis follows this biosynthetic route, then such reactions have to be postulated to attain the oligosaccharide chains, e.g. α-amylase or bromelain. A fucosyl transferase described in castor bean endosperm (Roberts et al., 1980) and an N-acetylgalactosamine transferase from Phaseolus aureus (Davies & Delmer, 1982) could be involved in reactions following chain elongation. Thus in the latter report, part of the GlcNAc transferred to protein was incorporated into terminal linkages in a reaction insensitive to tunicamycin but most probably attached to an asparagine-linked oligosaccharide.

The existence of the dolichol cycle in plants is also supported by experiments in vivo originally described for pea cotyledons using metabolic labelling with glucosamine and mannose (Lehle & Elbein, 1981). The observation that tunicamycin inhibits formation of lipid-linked oligosaccharides and protein glycosylation in soya bean cells (Hori & Elbein, 1981). Tunicamycin has become a very valuable tool to examine the role of glycosylation, for it blocks the first enzyme of the dolichol cycle, the GlcNAc1–P transferase, i.e. the formation of Dol-PP-GlcNAc (Tkacz & Lampen, 1975; Takatsuki et al., 1975; Lehle & Tanner, 1976). This mode of inhibition, originally elucidated in yeast and animals, has been shown to occur also in cell free extracts from higher plants and algae (Eriscon et al., 1977; Müller et al., 1981). Other inhibitors of glycosylation effective in plants, which are not so specific and effective at low concentrations in their action, are bacitracin (inhibition of GlcNAc and Man transferase) and phytokinase (inhibition of Dol-P-Man formation; Eriscon, 1978c), showedmycin (inhibition of Dol-PP-GlcNAc and Dol-P-Glc formation; Müller et al., 1981).

Characterization of the glycoprotein products. With regard to the characterization of the glycoprotein products synthesized in vitro or in vivo our knowledge is very poor and this will be one of the main areas of attention in the future. Promising results come from experiments dealing with the glycosylation with recombinant glycoprotein. Davies & Delmer (1979) demonstrated a transfer of GlcNAc from UDP-GlcNAc to vicillin (=phaseolin) and phytohaemagglutinin. The product was identified on the basis of its mobility on polyacrylamide-gel electrophoresis and, in the case of vicillin, with antiserum raised against this protein. Since there was no simultaneous incorporation from GDP-Man into the product, the GlcNAc transfer probably reflects a terminal glycosylation reaction. In this connection two reports should be mentioned which emphasize the endoplasmic reticulum as the locus for glycosylation of vicillin (see also below). However, glycosylation per se did not seem to be a prerequisite for transport of this glycoprotein from the endoplasmic reticulum to protein bodies and assembly into oligomers (Chrispeels & al., 1982; Budnik & Jones, 1982). Horsecad radiolysis treated with a mixture of glycosidases was shown to act as an acceptor for N-acetylgalactosamine transfer from UDP-GlcNAc catalysed by a membrane fraction from the same tissue (Sever & Shannon, 1977). A lipid–oligosaccharide-mediated glycosylation of chemically unfolded ribonuclease A was demonstrated with endoplasmic reticulum membranes from castor bean (Mellor et al., 1979). It has been postulated (Neuberger & Marshall, 1968) that a marker sequence of the type Asn-Xaa-Ser or Asn-Xaa-Thr is a necessary condition for N-glycosylation to occur. This hypothesis has been proven now by using synthetic peptides of defined and systematically altered amino acid sequences as glycosyl acceptors in several tissues such as liver, ovotid and yeast (Ronin et al., 1978; Hart et al., 1979; Bause, 1979; Bause & Lehle, 1979). In plants such studies have not been carried out. The few examples of plant glycoproteins with known amino acid sequence next to the Asn–carbohydrate linkage (Sharon & Lis, 1979) allow the conclusion that the "sequon hypothesis" is also valid in these organisms.

Subcellular localization and topography. The path of biogenesis of secretory or membrane glycoproteins starts at the rough endoplasmic reticulum and is a vectorial process. According to the signal hypothesis (Blobel & Dobberstein, 1975), an N-terminal peptide causes binding of the ribosomes to a receptor site on the rough endoplasmic reticulum and subsequent discharge of the protein into the cotranslational space, with proteolytic processing and a cotranslational core glycosylation. This stringent temporal sequence has recently been shown to occur in plants for α-amylase production in rice seeds (Miya contained and fibroblasts (Snider & Robbins, 1982) showed that the synthesized lipid–oligosaccharides (the mechanism of subsequent release is not known yet).
and the core glycosylation face the luminal side of the rough endoplasmic reticulum.

**O-Glycosylation.** The participation of lipid intermediates in the formation of O-glycosidic linkages has been found so far only for the synthesis of fungal manno-oligosaccharide chains linked to serine and threonine (protein-(Ser/Thr)-Man-Man-...). The pathway is unique in that only the first mannose residue directly linked to the amino acid uses Dol-P-Man as donor, whereas further mannoses come directly from GDP-Man (Sharma et al., 1974; Brethauer & Wu, 1975). Introduction of arabinose from UDP-Ara to hydroxyproline was obtained in *vitro* with a crude membrane fraction from sycamore suspension culture (Karr, 1972). Alkaline hydrolysis of the product gave a mixture of hydroxyproline arabinosides identical in size to the P-arabinofuranose forms are attached to Hyp, although the activated form from arabinose is UDP-beta-arabinopyranose, the assembly seems to become rather complex. No lipid intermediate has been implicated in the study by Karr (1972). Recent work concerned with the biosynthesis of glycoproteins in *Chlamydomonas* demonstrated incorporation of galactose from UDP-Gal in *vitro* to form an O-glycosidic bond with peptide hydroxyproline (Lang, 1982b). No evidence was obtained for lipid intermediates in protein galactosylation (Lang, 1982a). Analysis of the labelled glycolipid fraction in this system obtained upon incubation with UDP-[14C]Gal showed that it consisted of mono- and digalactosylglycerol lipids and trace amounts of polyglucosylglycerophosphate glucose due to the presence of a 4 epimerase. No biosynthetic investigations for the Ser-O-Gal linkage have been reported so far.

**Formation of cellulose and glucoproteins**

There is good evidence that a glucoprotein as well as lipid-linked oligosaccharides are involved in the initiation of cellulose biosynthesis in green algae (Hopp et al., 1978b). Dol-PP-Glc, consisting of beta-1,4 linked glucosyl residues was synthesized partly from UDP-Glc and Dol-P-Glc in a membrane fraction from *Prototheca zopfii* and shown to be transferred to a membrane-bound protein acceptor. The latter reaction is inhibited by coumarin (Hopp et al., 1978b). A well known inhibitor of cellulose biosynthesis. When GDP glucose was added, the soluble, labelled polymer became insoluble in hot alkali, with the properties expected for cellulose. The enzymes responsible for the glucosylation of dolichol derivatives and protein were found in the endoplasmic reticulum, whereas cellulose synthetase was most active in a dictyosome-rich fraction (Hopp et al., 1978b). The results obtained in algae have not been substantiated so far with an enzyme system from higher plants.

**Concluding remarks**

As may be realized from the data summarized, considerable progress has been achieved in the past in the understanding of plant glycoproteins. In particular the biosynthetic concept of O-glycosylation turns out to be the same as in other eukaryotic cells. On the other hand a lot of old and new questions are unanswered. As yet unknown, and the subject of much speculation, is the biological function of glycoproteins, and in this context the role of the carbohydrate part itself. Besides the changes in the physical and chemical properties caused merely by the presence of carbohydrate (solubility, stability, protection against proteolytic breakdown, change in enzymic activity) more specific functions seem to be attributable to the carbohydrates, such as being signals for recognition, growth and development. It is hoped that with the advent of recent new techniques for carbohydrate and protein analysis, we might answer some of the questions raised.