 Participation of a Trisaccharide-Lipid in Glycosylation of Oviduct Membrane Glycoproteins*

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Preincubation of a hen oviduct membrane preparation with UDP-\(\beta\)-acetyl\(^{14}\)C-glucosamine and bacitracin, followed by incubation with GDP-mannose, leads to formation of a chloroform/methanol (2/1)-extractable glycolipid. Treatment of the lipid with mild acid results in release of a trisaccharide shown to have the structure \(\beta\)-mannosyl-N-acetylgalactosaminyl-N-acetylgalactosamine. Incubation of purified trisaccharide-lipid with oviduct membranes in the presence of sodium deoxycholate, Mn\(^{2+}\), and GDP-mannose leads to formation of a labeled glycoprotein with an apparent molecular weight of 25,000. Under these conditions no oligosaccharide-lipid is formed from the trisaccharide-lipid. Structural studies revealed that the labeled glycoprotein contained a trisaccharide side chain. When incubation conditions were altered by omission of sodium deoxycholate and Mn\(^{2+}\), trisaccharide-lipid was converted to an oligosaccharide-lipid containing 7 to 9 glucose units. Under these conditions both the trisaccharide-lipid, and the oligosaccharide-lipid formed from it, served as a donor of their carbohydrate chains to form labeled glycoprotein(s). These results provide direct evidence for the enzymatic formation of a lipid-linked trisaccharide containing a \(\beta\)-mannosyl unit, and its subsequent participation in the glycosylation of membrane glycoprotein(s).

Although the participation of polylsoprenol-linked sugars in synthesis of the oligosaccharide chains of certain glycoproteins is now well established (for reviews see Refs. 1 and 2), a number of key questions about this process remain unanswered. One of these questions relates to the intermediates involved in the assembly of the oligosaccharide chain that is ultimately transferred to protein. The enzymatic synthesis of a disaccharide-lipid, shown by Herscovics et al. to be identical with synthetic \(\alpha\)-lygogalic, has been demonstrated in a number of systems (4-8). Moreover it has been shown that this disaccharide-lipid is converted to an oligosaccharide-lipid containing multiple \(\alpha\)-mannosyl units (5, 7, 9). However, little is known about possible intermediates between the disaccharide-lipid and the oligosaccharide-lipid. Levy et al. (7) have presented preliminary evidence suggesting the formation of a trisaccharide-lipid. Recently Heifitz et al. (8) reported the synthesis of a family of oligosaccharide-lipids of differing carbohydrate chain lengths, one of which has been shown to contain a trisaccharide unit. Smith degradation of this trisaccharide yielded a trisaccharide with the structure \(\beta\)-mannosyl-N-acetylgalactosaminyl-N-acetylgalactosamine (Hamman-GlcNAC-GlcNAc). Similar findings have been reported in a mouse myeloma system by Hsu et al. (12). In this study we have investigated the possible involvement of a trisaccharide-lipid in synthesis of this oligosaccharide-lipid and glycoprotein (Fig. 1). These studies have established that indeed, such a trisaccharide-lipid, containing a \(\beta\)-mannosyl residue linked to the disaccharide, \(N\),\(N\)-diacetyglactosamn, is formed by oviduct membranes. Moreover, it has been found that, under appropriate conditions, this lipid-linked trisaccharide can either be elongated to the oligosaccharide-lipid, or can serve as a direct donor of its trisaccharide unit to an endogenous protein acceptor. Some aspects of this work have been reported in preliminary form (13).

**Experimental Procedures**

*Materials and Methods* — GDP-(\(\beta\)-acetyl\(^{14}\)C)mannose (210 mCi/mmol) was purchased from New England Nuclear Corp. UDP-\(\beta\)-acyltyll\(^{14}\)Cglucosamine (300 mCi/mmol) was obtained from Amersham/Searle. Purified protease type V was purchased from Sigma Chemical Co. Bacitracin was purchased from Nutritional Biochemicals Corp. Highly purified \(\alpha\)-mannosidase (Jack Bean) and \(\beta\)-mannosidase (Polyporus sulfurus) was generously supplied by Dr. Y. T. Li, Tulane University. All other chemicals were purchased from commercial sources unless otherwise noted. Radioactivity was measured on a Packard Tri-Carb liquid scintillation counter.

The membrane fraction from the magnum section of freshly killed laying hens was prepared as previously described (11). [\(\beta\)-Man-\(^{14}\)C]Oligosaccharide-lipid, [GlcNAC-\(^{14}\)C]oligosaccharide-lipid, and [\(\beta\)-Man-\(^{14}\)C]glycoprotein were prepared by using this membrane preparation (5). Oligosaccharide, reduced oligosaccharide, authentic \(\beta\)-Man-(1→4)-\(\beta\)-GlcNAC-(1→4)-GlcNAC and \(\beta\)-Man-(1→4)-\(\beta\)-GlcNAc-
Proteolytic Digestion of [GlcNAc-4Cl]Glycoprotein - The labeled glycoprotein (27,000 cpm) was subjected to mild acid hydrolysis (5), and the water-soluble oligosaccharide product was analyzed by paper chromatography in Solvent System C, CHCl₃/CH₃OH/H₂O (65/25/4). Paper electrophoresis of samples at 30 V/cm for 1.5 h was performed in the following electrolyte systems: System D, 1.5 mM formic acid; and System E, 1% sodium borate. Radioactivity on paper chromatograms was detected by scintillation counting vial by rinsing the tube three times with 5 ml of Hydromix (14) or anilinediphenylamine (15). Thin layer chromatography was performed on Silica Gel 60-pretreated plates purchased from E.M. Laboratories, Inc. in Solvent System F, CHCl₃/CH₃OH/H₂O (10/35/6). Radioactivity was detected and quantitated by scraping 0.5-cm segments of the silica gel into scintillation vials for counting.

Preparation and Partial Purification of [GlcNAc-4Cl]Trisaccharide-Lipid - Forty-eight tubes, each containing oviputum membranes (3.8 mg of protein), 5.3 µM UDP-[1-4]Clglucosamine (50,000 cpm), 1 mM NaCl, 10 mM MnCl₂, and 25 mM Tris/HCl (pH 7.2) in a final volume of 200 µl were incubated for 15 min at 37°C. After 20 µl of 400 µM GDP-mannose were added, the incubation was continued for an additional 10 min. The reaction was terminated by addition of 8 ml of CHCl₃/CH₃OH (2/1) in the same manner. The combined supernatant extracts (8 ml) were washed with 1.6 ml of CHCl₃/CH₃OH (2/1). Unreacted oligosaccharide product was analyzed by paper chromatography as described under "Results." The fractions comprising the single peak of radioactivity were pooled and lyophilized.

Alkaline Degradation of [GlcNAc-4Cl]Glycopeptide - The labeled glycopeptide (8,500 cpm) was heated with 1 mM NaOH, 1 mM NaN₃, for 18 h at 100°C in a tightly capped tube. The reaction mixture was immersed in an ice bath and treated with a few drops of glacial acetic acid to destroy the excess NaN₃. The reaction mixture was then centrifuged and the supernatant was concentrated as described above. The products of α- or β-mannosidase digestion were examined by paper chromatography as described under "Results."
mild acid hydrolysis the N-acetyl[14C]glucosamine-containing carbohydrate moieties of the three radioactive peaks were identified as N-acetylglucosamine, N,N'-diacetylchitobiose, and a trisaccharide subsequently shown to be β-Man-GlcNAc-GlcNAc (see below).

Experiments undertaken to increase the level of the trisaccharide-lipid revealed that, when the membranes were preincubated with UDP-[14C]GlcNAc in the presence of 1 mM bacitracin and then incubated with GDP-mannose, the amount of trisaccharide-lipid recovered in the CHCl3/CH3OH-extractable fraction was increased more than 10-fold (Table I). This increase was dependent on the concentration of bacitracin up to 1 mM and reached a maximum in 10 min (data not shown).

**Structural Studies on Trisaccharide Moiety of [GlcNAc-14C]Trisaccharide-Lipid** – [GlcNAc-14C]Trisaccharide-lipid purified as described under "Experimental Procedures" was radiochemically homogenous as judged by paper chromatography in Solvent System A (Rf = 0.90); on EDTA-pretreated SG-81 paper in Solvent System C (Rf = 0.14); and on thin layer chromatography in Solvent System F (Rf = 0.27). To characterize the carbohydrate moiety, reduced [GlcNAc-14C]trisaccharide was prepared from purified [GlcNAc-14C]trisaccharide-lipid by mild acid hydrolysis followed by treatment with NaBH₄ (5), and examined by paper chromatography in Solvent System B and by paper electrophoresis in Electrolyte System E. The results, shown in Fig. 3, indicate that this product is chromatographically identical with authentic β-Man-(1→4)-β-GlcNAc-(1→4)-N-acetylglucosaminotol, prepared as previously described (10). To determine the sequence of linkage and the anomeric configuration of the trisaccharide moiety in the trisaccharide-lipid, the reduced [GlcNAc-14C]trisaccharide from purified [GlcNAc-14C]trisaccharide was subjected to digestion with purified α- and β-mannosidase. As shown in Fig. 4, reduced [GlcNAc-14C]trisaccharide from [GlcNAc-14C]trisaccharide-lipid was unchanged after incubation with purified α-mannosidase. In contrast, after incubation of the reduced trisaccharide with purified β-mannosidase, a major product which was chromatographically identical with authentic N,N'-diacetylchitobiose, was formed. These findings indicate that the mannos unit is at the nonreducing terminus and that it is linked by a β-glycosidic bond to a disaccharide of N-acetylglucosamine.

[GlcNAc-14C]Trisaccharide-lipid was isolated and purified as described under "Experimental Procedures." Exogenous [GlcNAc-14C]trisaccharide-lipid was incubated with ovicidal membranes, and the transfer of radioactivity into oligosaccharide lipid and endogenous protein acceptors was measured. As shown in Fig. 5A, efficient, time-dependent transfer of labeled trisaccharide to protein occurs, but no labeled oligosaccharide lipid is detected. The dependence of glycoprotein labeling from [GlcNAc-14C]trisaccharide-lipid on the amount of membrane protein is shown in Fig. 5D. Although under optimal conditions about 25% of the radioactivity from [GlcNAc-14C]trisaccharide-lipid is transferred into protein acceptors, less than 2% is incorporated into oligosaccharide-lipid. Almost all of the remaining radioactivity can be recovered from the incubation mixture as intact trisaccharide-lipid.
Role of Trisaccharide-Lipid in Glycosylation of Proteins

FIG. 4. Paper chromatography of the products formed upon α-mannosidase or β-mannosidase digestion of reduced [GlcNAc-\(^{14}\)C]trisaccharide prepared from [GlcNAc-\(^{14}\)C]trisaccharide-lipid. Chromatography was performed in Solvent System B. Panel A, no enzyme treatment; Panel B, product after treatment with α-mannosidase; Panel C, product after treatment with β-mannosidase. As shown, the latter treatment results in formation of a small amount of N-acetylglucosaminitol, in addition to the major product, N,N'-diacetylchitobitol.

In these incorporation studies sodium deoxycholate was used to disperse the [GlcNAc-\(^{14}\)C]trisaccharide-lipid in water; with this detergent the incorporation of trisaccharide-lipid into protein acceptors remains constant in the concentration range of sodium deoxycholate from 0.01% up to 0.1%. The incorporation into glycoprotein was stimulated 2-fold by addition of 5 mM Mn\(^{2+}\), but even in the absence of Mn\(^{2+}\) significant incorporation was observed (see below).

These findings strongly suggested the possibility of direct transfer of the trisaccharide moiety from trisaccharide-lipid into endogenous protein acceptors, without intermediate formation of oligosaccharide-lipid. This possibility was supported by the following observations (data not shown): (a) the labeling of protein from trisaccharide-lipid does not require the presence of GDP-mannose; (b) the addition of excess unlabeled GDP-mannose (up to 500 \(\mu\)M) does not affect the level of incorporation of radioactivity from trisaccharide-lipid into either glycoprotein or oligosaccharide-lipid; and (c) the glycosylation of protein acceptors is not affected by preincubation of oviduct membranes with 200 \(\mu\)M GDP, which might be expected to deplete any endogenous pool of mannosylphosphoryl dolichol (16).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of [GlcNAc-\(^{14}\)C]Glycoprotein — A sample of [GlcNAc-\(^{14}\)C]glycoprotein was prepared by using [GlcNAc-\(^{14}\)C]trisaccharide-lipid as a trisaccharide donor and then isolated as described under "Experimental Procedures." A fraction of the reaction mixture was analyzed by polyacrylamide disc gel in the presence of sodium dodecyl sulfate and urea. As shown in Fig. 6, the major labeled polypeptide migrates with an apparent molecular weight of 25,000. Only a minor fraction of the radioactivity (less than 5%) is associated with either ovalbumin or the 85,000 molecular weight polypeptide previously detected (11).

Preparation of [GlcNAc-\(^{14}\)C]Glycopeptide — [GlcNAc-\(^{14}\)C]Glycoprotein prepared from [GlcNAc-\(^{14}\)C]trisaccharide-lipid was found to resist β elimination after incubation with 0.2 N NaOH, 1 M NaBH\(_4\), at 37° for 24 h, thus indicating that the trisaccharide is not linked via a O-glycosidic bond to a serine or threonine residue (17). When the labeled glycoprotein was subjected to extensive pronase digestion more than 95% of the total radioactivity was recovered in a water-soluble glycopeptide. Analysis of the glycopeptide by gel filtration on a

FIG. 5. Transfer of radioactivity from exogenous [GlcNAc-\(^{14}\)C]trisaccharide-lipid to glycoprotein and oligosaccharide-lipid. Incubation and assay conditions were as described under "Experimental Procedures." A, dependence on time; B, dependence on the concentration of oviduct membrane protein.

FIG. 6. Electrophoretic analysis of [GlcNAc-\(^{14}\)C]glycoprotein prepared from exogenous [GlcNAc-\(^{14}\)C]trisaccharide-lipid. Incubation conditions were as described under "Experimental Procedures." A fraction of reaction mixture containing 370 \(\mu\)g of protein was directly applied on a 7.6% polyacrylamide gel in the presence of sodium dodecyl sulfate and urea. Electrophoresis was carried out for 6 h at 18°.
Sephadex G-25 column (Fig. 7A) clearly showed that the molecular weight of the glycopeptide is distinctly lower than that of the glycopeptide derived from protein labeled using oligosaccharide-lipid as substrate (10). Further evidence supporting this conclusion was obtained by paper electrophoresis of these two glycopeptides (Fig. 7B).

Comparison of Product of Reductive Alkaline Hydrolysis of \([\text{GlcNAc}^{14}\text{C}]\text{Glycopeptide with Reduced Trisaccharide from }[\text{GlcNAc}^{14}\text{C}]\text{Trisaccharide-Lipid}\) — The resistance of the labeled glycoprotein to \(\beta\) elimination (see above) is consistent with the possibility that the trisaccharide is linked to an asparaginyl residue of the polypeptide by an \(N\)-glycosidic bond. Therefore the \([\text{GlcNAc}^{14}\text{C}]\)glycopeptide was treated with \(\text{NaOH-}\text{NaBH}_4\), under the vigorous conditions necessary to cleave such a bond. Following \(N\)-acytlylation of the cleavage product, it was compared with reduced \([\text{GlcNAc}^{14}\text{C}]\)trisaccharide prepared from \([\text{GlcNAc}^{14}\text{C}]\)trisaccharide-lipid by mild acid hydrolysis followed by reduction with \(\text{NaBH}_4\) (10).

Gel filtration revealed that these two reduced trisaccharides, as well as authentic \(\beta\)-Man-(1\(\rightarrow\)4)-\(\beta\)-GlcNAc-(1\(\rightarrow\)4)-\(N\)-acetylglucosaminitol (10), had identical elution profiles (data not shown). In addition, analysis by paper chromatography in Solvent System B (Fig. 8A), and paper electrophoresis in Electrolyte System E (Fig. 8B), indicated that these two reduced trisaccharides and authentic \(\beta\)-Man-(1\(\rightarrow\)4)-\(\beta\)-GlcNAc-(1\(\rightarrow\)4)-\(N\)-acetylglucosaminitol are chromatographically identical. To corroborate the conclusion that the structure of the trisaccharide in the glycoprotein is \(\beta\)-Man-GlcNAc-GlcNAc, the reduced \([\text{GlcNAc}^{14}\text{C}]\)trisaccharide isolated from \([\text{GlcNAc}^{14}\text{C}]\)trisaccharide-protein was treated with purified \(\beta\)-mannosidase; as shown in Fig. 9, the product of enzymatic digestion is chromatographically identical with \(N, N'\)-diacetyltirucallitol. These findings provide strong evidence that indeed trisaccharide-lipid does serve as a direct donor to glycosylate an endogenous protein acceptor.

Incorporation of \([\text{GlcNAc}^{14}\text{C}]\)Trisaccharide from \([\text{GlcNAc}^{14}\text{C}]\)Trisaccharide-Lipid into \([\text{GlcNAc}^{14}\text{C}]\)Oligosaccharide-Lipid and \([\text{GlcNAc}^{14}\text{C}]\)Glycoprotein — Since earlier studies (5) showed that prelabeled, endogenous \(N, N'\)-diacetyltirucallitol lipid was converted to oligosaccharide lipid, it seemed likely that under appropriate incubation conditions exogenous trisaccharide-lipid might also participate as a precursor in assembly of the oligosaccharide-lipid. To investigate this pos-
sibility incubation conditions were varied to optimize the formation of \( \text{GlcNAc-}^{14}\text{C} \text{oligosaccharide-lipid from exogenous GlcNAc-}^{14}\text{C} \text{trisaccharide-lipid.} \) As described above, the presence of Mn\(^{2+}\) ion and sodium deoxycholate was necessary for optimal direct incorporation of the trisaccharide unit of exogenous \( \text{GlcNAc-}^{14}\text{C} \text{trisaccharide-lipid into GlcNAc-}^{14}\text{C} \text{glycoprotein.} \) To facilitate possible elongation of the trisaccharide-lipid to oligosaccharide-lipid, attempts were made to depress the direct glycosylation of protein. As shown in Fig. 10A, in the absence of Mn\(^{2+}\) ion the direct transfer of the trisaccharide unit from \( \text{GlcNAc-}^{14}\text{C} \text{trisaccharide-lipid to glycoprotein is decreased, but formation of oligosaccharide-lipid is not significantly increased. However, when both Mn\(^{2+}\) and deoxycholate are omitted from the incubation mixture it is clear that not only is formation of labeled protein depressed, but \( \text{GlcNAc-}^{14}\text{C} \text{oligosaccharide-lipid is formed (Fig. 10B).} \) From these observations we conclude that the Mn\(^{2+}\) ion and sodium deoxycholate inhibit elongation of the exogenous trisaccharide-lipid to an oligosaccharide-lipid.

The kinetics of incorporation of trisaccharide from \( \text{GlcNAc-}^{14}\text{C} \text{trisaccharide-lipid into GlcNAc-}^{14}\text{C} \text{oligosaccharide-lipid and into GlcNAc-}^{14}\text{C} \text{glycoprotein are shown in Fig. 11. After 45 min, 10% of the \( \text{GlcNAc-}^{14}\text{C} \text{trisaccharide-lipid is elongated to form a \( \text{GlcNAc-}^{14}\text{C} \text{oligosaccharide-lipid.} \) Although formation of \( \text{GlcNAc-}^{14}\text{C} \text{glycoprotein is observed, its level is depressed (compare with Fig. 5A).} \) To obtain more structural information on the oligosaccharide chain, the \( \text{GlcNAc-}^{14}\text{C} \text{trisaccharide-lipid was prepared from \( \text{GlcNAc-}^{14}\text{C} \text{trisaccharide-lipid and then subjected to mild acid hydrolysis (9).} \) Analysis of the resulting oligosaccharide by paper chromatography in Solvent System A revealed that the major labeled peak \( (R_\text{f} = 0.15), \) accounting for 80% of the radioactivity, was identical with authentic \( \alpha\)-Man-(1→4)-\( \beta\)-Man-(1→4)-\( \beta\)-GlcNAc-(1→4)-GlcNAc (5). A minor product \( (R_\text{f} = 0.49) \) had the same mobility as authentic \( \beta\)-Man-(1→4)-\( \beta\)-GlcNAc-(1→4)-GlcNAc. In earlier studies (11) we showed the exogenous oligosaccharide-lipid can serve as a donor of its oligosaccharide chain to glycoprotein. It was of interest, therefore, to examine the product(s) formed when both trisaccharide lipid, and oligosaccharide-lipid formed from it, were present as potential substrates. Accordingly, \( \text{GlcNAc-}^{14}\text{C} \text{glycoprotein was prepared using \( \text{GlcNAc-}^{14}\text{C} \text{trisaccharide-lipid as substrate under conditions that favor formation of \( \text{GlcNAc-}^{14}\text{C} \text{oligosaccharide-lipid.} \) The labeled glycoprotein fraction was isolated and a \( \text{GlcNAc-}^{14}\text{C} \text{glycopeptide fraction was prepared from it by pronase digestion.} \) As shown in Fig. 12, analysis of the glycopeptide fraction by gel filtration revealed the presence of two distinct \( \text{GlcNAc-}^{14}\text{C} \text{glycopeptide peaks that correspond to authentic reference trisaccharide-peptide and oligosaccharide-peptide. It}

\begin{figure}
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\includegraphics[width=\textwidth]{fig10.png}
\caption{A, dependence on Mn\(^{2+}\) ion concentration of transfer of \( \text{GlcNAc-}^{14}\text{C} \text{trisaccharide from exogenous \( \text{GlcNAc-}^{14}\text{C} \text{trisaccharide-lipid to oligosaccharide-lipid and glycoprotein.} \) Reaction mixtures contained 4.5 mg of membrane protein, 500 \( \mu \)M unlabeled GDP-mannose, 0.05% sodium deoxycholate, and MnCl\(_2\) as indicated in a final volume of 200 \( \mu \)L. B, dependence on sodium deoxycholate concentration of transfer of \( \text{GlcNAc-}^{14}\text{C} \text{trisaccharide from exogenous \( \text{GlcNAc-}^{14}\text{C} \text{trisaccharide-lipid to oligosaccharide-lipid and glycoprotein.} \) Conditions were as described in A except MnCl\(_2\) was eliminated and sodium deoxycholate was varied as indicated. In both A and B incubations were for 1 h at 0\(^{\circ}\).}
\end{figure}

\begin{figure}
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\includegraphics[width=\textwidth]{fig12.png}
\caption{Analysis of the \( \text{GlcNAc-}^{14}\text{C} \text{glycopeptide fraction prepared from \( \text{GlcNAc-}^{14}\text{C} \text{glycoprotein by extensive pronase digestion (Panel A).} \) Panel B shows the reference glycopeptides, \( \text{GlcNAc-}^{14}\text{C} \text{trisaccharide-peptide, and \( \text{Man-}^{13}\text{C} \text{oligosaccharide-peptide prepared as described in Fig. 7.} \) Gel filtration was performed in 0.1 M acetic acid on a Bio-Gel P-4 column (2.0 x 75 cm); 2-ml fractions were collected. The positions of elution of blue dextran \( (V_b) \) and mannose \( (V_m) \) are indicated.}
\end{figure}
is clear, therefore that oligosaccharide-lipid formed from trisaccharide-lipid, as well as the trisaccharide-lipid itself, can serve as donors for glycosylation of protein under appropriate incubation conditions.

**DISCUSSION**

This study has established that a hen oviduct membrane preparation catalyzes incorporation of GlcNAc from UDP-GlcNAc into three CHCl/CH₃OH-extractable lipids. Two of these are mono- and disaccharides of GlcNAc, presumably linked to dolichol via a pyrophosphate bridge. The third lipid, which contains a trisaccharide unit with the structure β-Mann-GlcNAc-GlcNAc, is present at an extremely low level. It represents only 2% of the CHCl/CH₃OH-extractable labeled products and less than 0.9% of the total labeled GlcNAc incorporated into the CHCl/CH₃OH, CHCl₃/CH₃OH/H₂O, and protein fractions. When 1 mM bacitracin is present during the incubation, incorporation of [¹⁴C]GlcNAc into the CHCl₃/CH₃OH-extractable fraction is increased 1.5-fold, and formation of labeled trisaccharide-lipid is increased 12.5-fold.

In bacteria, bacitracin blocks the action of a phosphatase that converts undecaprenyl pyrophosphate to undecaprenyl phosphate, thus preventing it from recycling as a sugar carrier in peptidoglycan synthesis (18). Bacitracin has also been shown to inhibit bacterial and eukaryotic synthesis in cell-free preparations of rat liver and, although its mode of action in eukaryotic systems has not been studied in detail, it has been suggested that it interferes with all reactions involving polyprenol pyrophosphates (19). Possibly, its functions in a similar fashion in the oviduct membrane system and binds to polyprenol pyrophosphates, thus causing an accumulation of certain intermediates, such as the trisaccharide-lipid. However, further studies will be necessary to test this speculation.

In any case, because bacitracin causes a preferential accumulation of the trisaccharide-lipid it was practicable to isolate substrate quantities of it in radiochemically pure form. On the basis of chromatographic and enzymatic studies it has been established that the trisaccharide moiety contains a terminal β-mannosyl unit linked to N,N'-diacetylchitobiose. The origin of the mannosyl unit has not been investigated in detail, but it is clear that only trace levels of trisaccharide-lipid are formed from UDP-[¹⁴C]GlcNAc if GDP-mannose is not present in the incubation mixture. Based on the preliminary studies of Levy et al. (7) it seems likely that GDP-mannose, rather than mannosylphosphoryldolichol, serves as the donor of the β-mannosyl unit of the trisaccharide-lipid.

Addition of purified [GlcNAc-¹⁴C]trisaccharide-lipid to oviduct membranes in the presence of Mn²⁺ and sodium deoxycholate did not lead to formation of oligosaccharide-lipid, although relatively efficient transfer of the trisaccharide moiety into the protein fraction was observed. Examination of the labeled protein by sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed that virtually all of the label was associated with a protein of identical apparent molecular weight. Furthermore, when both Mn²⁺ and deoxycholate were omitted from the incubation mixture, both oligosaccharide-lipid and glycoprotein synthesis from trisaccharide-lipid was observed. Because of the relatively low level of labeling of the glycoprotein fraction under these conditions it was not possible to analyze the product by sodium dodecyl sulfate polyacrylamide gel electrophoresis. However, it was possible to establish by analysis of the pronase digestion products of the labeled glycoprotein fraction that two glycopeptides, corresponding in size to authentic trisaccharide-peptide and oligosaccharide-peptide, were present.

These findings, coupled with earlier studies of the transfer of exogenous oligosaccharide lipid to a membrane protein acceptor (5, 11), indicate that the oviduct membranes can catalyze transfer of both (α-Man)₃β-Man-GlcNAc-GlcNAc and β-Man-GlcNAc-GlcNAc from their lipid carrier to an endogenous protein of identical apparent molecular weight.

It is of interest to consider these results in relation to recently summarized information (20) on the structure of the oligosaccharide chains of glycoproteins containing an oligosaccharide linked to the polypeptide via an N-glycosidic bond between GlcNAc and an asparaginyl residue. Seventeen glycoproteins of this category are now known to contain an identical core unit with the structure β-Man-β-GlcNAc-β-GlcNAc-Asn. Excluding consideration of distal sugars (sialic acid, galactose, and N-acetylgalactosamine) the number of α-mannosyl units in these different glycoproteins varies considerably. Thus, the carbohydrate chain in human IgG, IgE, and IgA, contains only 2 α-mannosyl units whereas that in the linkage region of yeast proteo-mannan contains 11 α-mannosyl residues. Clearly, the present studies do not explain this diversity in structure of the oligosaccharide chains to different glycoproteins in terms of the enzyme(s) catalyzing transfer of carbohydrates from their lipid carriers to proteins. However, it is hoped that extension of such studies, using saccharide-lipids of known carbohydrate chain length, to a recently developed system utilizing specific exogenous protein acceptors (21) will cast light on this important question.

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Role of Trisaccharide-Lipid in Glycosylation of Proteins

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