Enzymatic Excision of Glucosyl Units Linked to the Oligosaccharide Chains of Glycoproteins*

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Studies in the accompanying paper (Chen, W. W., and Lennarz, W. J. (1978) J. Biol. Chem. 253, 5774-5779) showed that hen oviduct membranes catalyze synthesis of a Glc-containing oligosaccharide-lipid and that the oligosaccharide moiety of this compound is transferred en bloc to an endogenous protein as well as to an exogenous, soluble protein. In this study we have established that the endogenous proteins in oviduct membranes are devoid of Glc. In view of these findings, the possibility that Glc was excised from the oligosaccharide chain after it had been transferred to protein was examined. Kinetic studies involving the use of endogenous membrane proteins as acceptors of the oligosaccharide chain suggested that the Glc residues were released without significant degradation of the core oligosaccharide. Direct evidence for the presence of a membrane-bound glucosidase was obtained using either free, Glc-containing oligosaccharide or Glc-containing oligosaccharide linked to S-carboxymethylated α-lactalbumin as substrates. Experiments utilizing [3H]Glc- and [14C]GlcNAc-labeled, glycosylated S-carboxymethylated α-lactalbumin established that, under conditions leading to extensive removal of [3H]Glc, there was essentially no degradation of the [14C]-GlcNAc-labeled oligosaccharide core.

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EXPERIMENTAL PROCEDURES

Materials and Methods—UDP-[3H]mannose (210 mCi/mmol) and UDP-N-acetyl-[14C]glucosamine (6.6 Ci/mmol) were purchased from New England Nuclear Corp., Boston, Mass. UDP-[3H]glucose (310 mCi/mmol), UDP-[14C]glucose (3.7 Ci/mmol), and UDP-N-acetyl-[14C]glucosamine (300 mCi/mmol) were purchased from Amer sham Corp., Arlington Heights, Ill. The preparation of membranes from hen oviduct has been previously reported (5). The labeled (α-Man)₆β-Man-(1→4)-β-GlcNAC-(1→4)-β-GlcNAC-peptide and β-Man-(1→4)-β-GlcNAC-(1→4)-β-GlcNAC-peptide were prepared as previously described (6, 7). The preparations of [14C]oligosaccharide-lipid, [GlcNAC-14C, Glc-3H]oligosaccharide-lipid, S-carboxymethylated α-[Glc-14C]lactalbumin and α-[GlcNAC-14C, Glc-3H]lactalbumin were performed as reported in the preceding paper (1). Descending paper chromatography was performed on Whatman No. 3MM paper in Solvent System A, which consisted of nitromethane:acetic acid:ethanol:H₂O saturated with boric acid (8:1:1:1). Radioactivity on paper chromatograms was detected by scanning with a Packard model 7801 radiochromatogram scanner.

Analysis of 3H-Glucose in Oviduct Membranes—Oviduct membranes (430 mg of protein) were subjected to a multiple extraction procedure (9). The protein content of the CHCl₃:CH₃OH (2:1) extract, the CHCl₃:CH₃OH:H₂O (10:10:3) extract, and the residual fractions were determined by the Lowry method (10). A sample of each fraction was hydrolyzed with 1.0 ml of 2 N trifluoroacetic acid at 121°C for 60 min. The hydrolysates were neutralized and eluted through 2-ml columns containing equal amounts of Dowex 1 and Dowex 50 with 1 M H₂O to remove amino acids and other charged by-products. The effluent fractions were evaporated to dryness and analyzed for total hexose (11) and for Glc (12).

Kinetics of Incorporation of [3H]Glc from UDP-[3H]Glc into Oligosaccharide-Lipid and Glycoprotein—Oviduct membranes (0.4 mg of protein) were incubated with 3.9 µM UDP-[3H]Glc (310 mCi/mmol), 10 mM MgCl₂ in 200 µl of 50 mM Tris-HCl, pH 7.2, containing 5% sucrose, 0.9% NaCl, 1 mM dithiothreitol, and 1 mM EDTA (hereafter termed Tris/sucrose buffer). After incubation at 37°C for the times indicated the reaction was terminated by addition of 4 ml of CHCl₃:CH₃OH (2:1). Labeled glycosyl lipids, oligosaccharide-lipid, and glycoprotein were measured after separation by the multiple extraction procedure (9).

Kinetics of Synthesis of Glc-[14C], GlcNAC-[14C]-Labeled Protein from UDP-[14C]Glc and UDP-N-Acetyl-[14C]glucosamine—Oviduct membranes (3.4 mg of protein) were preincubated with 7.6 µM UDP-N-acetyl-[14C]glucosamine (660 mCi/mmol) and 10 mM MnCl₂ in 200 µl of Tris/sucrose buffer for 5 min at 37°C. Then, 10 µl of 500 µM unlabeled GDP-Man, and 10 µl of 248 µM UDP-[14C]Glc (10 mCi/mmol) were added. After incubation for the times indicated the reaction was terminated as described above, and the formation of [Glc-14C, GlcNAC-14C]glycoprotein was measured by the multiple extraction procedure (9).

Kinetics of Transfer of [Glc-14C]Oligosaccharide from Oligosac-
charide-Lipid to Protein—Large quantities of \([\text{Glc}^{14}\text{C}]\)oligosaccharide-lipid (approximately 500,000 cpm) were prepared and isolated from 30 incubation mixtures as described in the preceding report (1). An aliquot of \([\text{Glc}^{14}\text{C}]\)oligosaccharide-lipid (200,000 cpm) in CHCl<sub>3</sub>CH<sub>2</sub>OH·H<sub>2</sub>O (10:10:3) was transferred to a conical tube and evaporated to dryness. The residue was dispersed in 200 ~1 of Tris/sucrose buffer by means of a Vortex mixer. Aliquots (20 ~1) of the dispersed \([\text{Glc}^{14}\text{C}]\)oligosaccharide-lipid (20,000 cpm) were incubated with oviduct membranes (3.4 mg of protein) and 10 mM MnCl<sub>2</sub> in 200 ~1 of Tris/sucrose buffer at 37°C. Incubations were terminated at the indicated times and \([\text{Glc}^{14}\text{C}]\)glycoprotein formation was determined by the multiple extraction procedure (9). Triton X-100 Extraction of Oviduct Membranes—Oviduct membranes (340 mg of protein) were suspended in 20 ml of 0.1% Triton X-100, 50 mM Tris·HCl, pH 7.2, 5% sucrose, 0.9% NaCl, 1 mM dithiothreitol, and 1 mM EDTA, and homogenized in a glass vessel with 20 strokes of a motor-driven Teflon pestle. The suspension was centrifuged at 30,000 × g for 15 min. The supernatant fraction was collected and concentrated by filtration through a Amicon apparatus equipped with a Diaflo PM 30 ultrafiltration membrane to a protein concentration of 30 to 40 mg/ml. The pellet was suspended to a protein concentration of 30 to 40 mg/ml with buffer. The Triton X-100 containing buffer and then repeating the same homogenization and centrifugation process described above. This process was repeated four times. The final pellet was suspended to a protein concentration of 30 to 40 mg/ml with buffer. The Triton X-100 extract and the extracted membranes were tested for their ability to catalyze enzymatic release of Glc from the Glc-labeled oligosaccharide moiety as described below. Assay for the Enzymatic Release of Glc from \([\text{Glc}^{14}\text{C}]\)oligosaccharide—\([\text{Glc}^{14}\text{C}]\)oligosaccharide (250,000 cpm) prepared from \([\text{Glc}^{14}\text{C}]\)oligosaccharide-lipid as described in the preceding report (1) was purified by gel filtration on a Bio-Gel P-4 column (2.0 × 98 cm) eluted with H<sub>2</sub>O. The fractions containing a single peak of \([\text{Glc}^{14}\text{C}]\)oligosaccharide were combined, lyophilized, and subsequently dissolved in 1 ml of Tris/sucrose buffer. This preparation of \([\text{Glc}^{14}\text{C}]\)oligosaccharide was used as substrate in preliminary experiments to detect enzymatic release of Glc. The assay mixture contained 2.8 mg of protein from either oviduct membranes or Triton X-100 extracted oviduct membranes, 5,000 cpm of \([\text{Glc}^{14}\text{C}]\)oligosaccharide, and Tris/sucrose buffer in 200 ~1. Incubations were carried out at 37°C for the indicated times and then terminated by addition of 100 ~1 of 0.1 N HCl. The reaction mixture was centrifuged for 15 min at 39,000 × g and the supernatant fraction analyzed by gel filtration on a Bio-Gel P-2 column (1.0 × 17 cm). Unreacted substrate ([\text{Glc}^{14}\text{C}] oligosaccharide) eluted in the void volume, whereas the hydrolytic product was retained and co-eluted with Glc at V<sub>e</sub>. Analysis of the radioactive product by paper chromatography in Solvent System A established that it was \([\text{Glc}^{14}\text{C}]\)Glc. Hydrolysis of α-[\text{Glc}^{14}\text{C}]Lactalbumin and α-[\text{GlcNAc}^{14}\text{C}] Lactalbumin with Membrane-bound Glucosidase—S-Carboxymethylated α-[\text{Glc}^{14}\text{C}]Lactalbumin and α-[\text{GlcNAc}^{14}\text{C}] Lactalbumin were prepared and purified by gel filtration on a Sephadex G-75 column as described in the preceding report (1). Aliquots of Glc-labeled S-carboxymethylated α-lactalbumin (2,000 cpm) were incubated at 37°C with oviduct membranes (5.6 mg of protein) in the presence of 10 mM MnCl<sub>2</sub> in 200 ~1 of Tris/sucrose buffer. After 3 h the reaction was terminated by addition of 100 ~1 of 0.1 N NaOH and the mixture was then centrifuged at 39,000 × g for 15 min. The resulting supernatant fraction was analyzed by gel filtration on a Sephadex G-75 column (0.7 × 48 cm). Intact, Glc-labeled α-lactalbumin was eluted at V<sub>e</sub>/V<sub>0</sub> = 1.38, whereas the labeled product of enzymatic hydrolysis eluted at V<sub>e</sub>. Paper chromatography of this product in Solvent System A demonstrated that the product was labeled Glc.

### RESULTS

Absence of D-Glucose in Oviduct Membrane Glycoproteins—In view of our findings in the preceding report (1) on the synthesis of Glc-containing glycoproteins from Glc-containing oligosaccharide-lipid, it was of interest to investigate the distribution of glucose residues in various subfractions of the oviduct membranes (5). Therefore, membranes were subjected to a multiple extraction procedure (9), and the CHCl<sub>3</sub>CH<sub>2</sub>OH (2:1) extract, CHCl<sub>3</sub>CH<sub>2</sub>OH·H<sub>2</sub>O (10:10:3) extract and residual fraction were analyzed for total hexose and for Glc. The results shown in Table I reveal that although substantial amounts of Glc are present in components extracted by CHCl<sub>3</sub>CH<sub>2</sub>OH·H<sub>2</sub>O (2:1) or by CHCl<sub>3</sub>CH<sub>2</sub>OH·H<sub>2</sub>O (10:10:3), no detectable amount of Glc is found in the residual fraction, which consists of the majority of the membrane proteins and glycoproteins. The presence of Glc in the oligosaccharide-lipid and its absence in the membrane proteins is consistent with the possibility that enzymatic removal of Glc occurs after the Glc-containing oligosaccharide moiety has been transferred from oligosaccharide-lipid to protein acceptors.

In Vitro Studies on the Kinetics of Incorporation of Glc into Oligosaccharide-Lipid and Glycoproteins—To investigate this possibility, oviduct membranes were incubated with UDP-[\text{Glc}^{14}\text{C}] and the formation of \([\text{Glc}^{14}\text{C}]\)oligosaccharide-lipid and \([\text{Glc}^{14}\text{C}]\)glycoprotein (from endogenous precursors) as a function of time were measured. As shown in Fig. 1, synthesis of \([\text{Glc}^{14}\text{C}]\)oligosaccharide-lipid precedes synthesis of \([\text{Glc}^{14}\text{C}]\)glycoprotein, as expected if the former serves as a precursor of the latter. However, the most striking finding is that the amount of \([\text{Glc}^{14}\text{C}]\)Glc in the glycoprotein decreases after reaching a maximum at 30 min. This finding is in marked contrast to earlier studies (9) showing that under identical incubation conditions \([\text{Glc}^{14}\text{C}]\) Man incorporation into glycoproteins continues for 2 h. To confirm this apparent selective loss

### Table I

<table>
<thead>
<tr>
<th>Fractiona</th>
<th>Total hexose</th>
<th>Glucoseb</th>
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</thead>
<tbody>
<tr>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;OH (2:1) extract</td>
<td>19.4</td>
<td>5.1</td>
</tr>
<tr>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;OH·H&lt;sub&gt;2&lt;/sub&gt;O (10:10:3) extract</td>
<td>12.5</td>
<td>4.8</td>
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<tr>
<td>Lipid-free macromolecular fraction</td>
<td>23.9</td>
<td>0</td>
</tr>
</tbody>
</table>

a Preparation and analysis of these fractions is described under "Experimental Procedures." The three fractions contained 3.9, 13.6, and 226 mg of protein, respectively.
b The lower limit of detection in this analysis was 2 µg of glucose.
of Glc from newly glycosylated proteins, the incorporation of \([^{14}C]\text{Glc}\) and \([^{3}H]\text{GlcNAc}\) into endogenous proteins was studied. Oviduct membranes were preincubated with UDP-\([^{14}C]\text{Glc}\) and then UDP-\([^{14}C]\text{Glc}\) and unlabeled GDP-Man were added. The incorporation of the two labeled sugars into proteins as a function of time is shown in Fig. 2. The results clearly show that \([^{3}H]\text{GlcNAc}\) incorporation into glycoproteins continues for over 3 h; in contrast, \([^{14}C]\text{Glc}\) incorporation reaches a maximum at 30 min and subsequently decreases.

**In Vitro Study of the Transfer of Oligosaccharide Moiety from \([^{14}C]\text{Oligosaccharide-Lipid}\) to Glycoprotein**—To simplify studies on the kinetics of incorporation of the \([^{14}C]\text{oligosaccharide}\) into protein, \([^{14}C]\text{oligosaccharide-lipid}\), prepared and characterized as described in the preceding paper (1), was used as a \([^{14}C]\text{oligosaccharide donor. Oviduct membranes were incubated with \([^{14}C]\text{oligosaccharide-lipid}\) and the level of \([^{14}C]\text{oligosaccharide}\) incorporated into protein was measured as a function of time. As shown in Fig. 3, initially there is relatively rapid incorporation of \([^{14}C]\text{oligosaccharide}\) into protein. However, after maximal incorporation at 90 min there is a loss of \([^{14}C]\text{Glc}\) from the glycoprotein fraction. It should be noted that the incubation time required for maximal incorporation of \([^{14}C]\text{oligosaccharide}\) in protein is longer using the oligosaccharide-lipid than when the sugar nucleotides are used to generate the oligosaccharide-lipid (cf. Figs. 1 and 2). This difference is most likely related to a lower efficiency of transferring a \([^{14}C]\text{oligosaccharide}\) than when the sugar nucleotides are used to generate the oligosaccharide-lipid.

To determine the size of the oligosaccharide chain remaining linked to glycoprotein after the Glc residues have been removed, the \([^{14}C]\text{GlcNAc-14C, Glc-3H}\)glycoproteins synthesized in the above experiment were subject to protelytic digestion (7). The resulting double-labeled glycopeptide fraction was compared to authentic \((\alpha-Mann)_{4-6}(1+4)-\beta\text{GlcNAc-peptide}\) and authentic \((\alpha-Mann)_{4-6}(1+4)-\beta\text{GlcNAc-peptide}\) (7) by gel filtration (Fig. 5). The \([^{14}C]\text{oligosaccharide}\)-peptide described in the preceding report (1) is shown for reference in Fig. 5A. The profiles of the \([^{14}C]\text{GlcNAc-14C, Glc-3H}\)glycopeptides obtained from the glycoprotein after incubation for 60 and 240 min are shown in Fig. 5, B and C. It is clear that as \([^{14}C]\text{Glc}\) is progressively lost from the glycoprotein there is production of a smaller glycopeptide that lacks \([^{3}H]\text{Glc}\). The mobility of this single-labeled \([^{14}C]\text{GlcNAc}\)-glycopeptide indicates that its apparent molecular weight is similar to that of \((\alpha-Mann)_{4-6}(1+4)-\beta\text{GlcNAc-14C-}\text{peptide}\) (7). All of these findings indicate the intact \([^{14}C]\text{GlcNAc-14C, Glc-3H}\)oligosaccharide moiety of oligosaccharide-lipid is transferred en bloc into endogenous protein acceptors. Subsequent action of a hydrolytic
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by mild acid hydrolysis (1) was used as a model substrate. Oviduct membranes were subjected to extraction with 0.1% Triton X-100 solution as described under “Experimental Procedures” and the pellet and supernatant fraction were assayed for their activity in catalyzing release of $^{14}$C-Glc from $[^{14}$C]-oligosaccharide. It was found that about 70% of the total activity is retained in the pellet; after the first extraction with Triton X-100 only about 20% of the total activity is detected in the supernatant fraction. Even after repeating the Triton X-100 extraction four times, the pellet still retains one-fifth of the initial hydrolytic activity. Therefore, it seems likely that the hydrolyase is tightly associated with the membrane. The product formed upon incubation of $[^{14}$C]-oligosaccharide with oviduct membranes was shown to be free Glc. As shown in Fig. 6, the mobility of the product on paper chromatography in Solvent System A is identical with that of authentic Glc.

The presence of α-methylmannoside (100 mM) in the incubation mixture has no inhibitory effect on release of free Glc. This finding suggests that the production of free Glc is not the result of the combined action of an α-mannosidase and the glucosidase.

Fig. 5. Analysis of $[^{14}$C, Glc-$^3$H]glycopeptides prepared from $[^{14}$C, Glc-$^3$H]glycoprotein. Gel filtration was performed on a Bio-Gel P-4 column (2.0 x 98 cm). The positions of blue dextran (V₀), Man (V₁), authentic (α-Man), α-Man-(1 → 4)-β-GlcNAc-(1 → 4)-GlcNAc-peptide and β-Man-(1 → 4)-β-GlcNAc-(1 → 4)-GlcNAc-peptide are indicated. A, reference peptide. [Glc-$^3$H]glycopeptide; B, $[^{14}$C, Glc-$^3$H]glycopeptide fraction prepared from glycoprotein after incubation with membranes for 60 min; C, $[^{14}$C, Glc-$^3$H]glycopeptide fraction prepared from glycoprotein after incubation with oviduct membranes for 240 min. Incubation conditions and the preparation of glycopeptides are described under “Experimental Procedures.”

Fig. 6. Analysis of the product formed upon incubation of $[^{14}$C]-oligosaccharide with oviduct membranes. The product was separated from unreacted substrate by gel filtration of the reaction mixture. The product, which eluted at Vᵢ, was lyophilized to dryness and analyzed by paper chromatography in Solvent System A. Details of the incubation conditions and the assay method are described under “Experimental Procedures.”

Properties of Glucosidase in Oviduct Membranes—To obtain some initial information on the hydrolytic activity responsible for the release of Glc residues from glycoproteins, $[^{14}$C]-oligosaccharide prepared from oligosaccharide-lipid enzyme(s) on $[^{14}$C, Glc-$^3$H]glycoprotein results in removal of the $[^{3}$H]Glc residues, with concomitant production of a smaller oligosaccharide chain containing Man and GlcNAc linked to the protein.

Fig. 7. Analysis of products formed upon enzymatic release of Glc from glycosylated α-lactalbumin by the membrane-bound glucosidase. Gel filtration was performed in 0.1 M NH₄HCO₃ on a Sephadex G-75 column (0.7 x 48 cm). The positions of blue dextran (V₀) and Man (V₁) are indicated. A, elution profile of untreated α-$[^{14}$C]-lactalbumin; B, products formed from α-$[^{14}$C]-lactalbumin after incubation with oviduct membranes; C, untreated α-$[^{14}$C, Glc-$^3$H]-lactalbumin; D, products formed from α-$[^{14}$C, Glc-$^3$H]-lactalbumin after incubation with oviduct membranes. Incubation conditions were as described under “Experimental Procedures.”
The glucosidase does not require divalent metal ions. Moreover, levels of Mn⁺⁺ or Mg⁺⁺ up to 15 mM are not inhibitory. The pH dependence of the glucosidase is very broad; approximately the same activity was detected between pH 6 to 8. The release of Glc is dependent on protein concentration up to at least 3.0 mg and on time of incubation up to at least 3 h (data not shown).

Hydrolysis of α-[Glc-¹⁴C]Lactalbumin and α-[GlcNAc-¹⁴C, Glc-³H]Lactalbumin with Oviduct Membrane-bound Glucosidase—Having established some of the basic properties of the membrane-bound glucosidase, the action of the glucosidase on a soluble Glc-containing glycoprotein was investigated. α-[Glc-¹⁴C]Lactalbumin and α-[GlcNAc-¹⁴C, Glc-³H]-lactalbumin were prepared by the enzymatic glycosylation of S-carboxymethylated α-lactalbumin as described in the preceding report (1). α-[Glc-¹⁴C]lactalbumin was incubated with oviduct membranes. After incubation the product and the remaining substrate were separated by gel filtration as shown in Fig. 7. The results show that upon incubation of α-[Glc-¹⁴C]lactalbumin (Fig. 7A) with oviduct membranes about 72% of the [¹⁴C]Glc is released; the remaining radioactivity is found in α-lactalbumin (Fig. 7B). The product found in V₁ is chromatographically identical with authentic glucose as judged by paper chromatography in Solvent System A (see Fig. 6). To demonstrate that the oligosaccharide core was still linked to the α-lactalbumin after removal of Glc, α-[GlcNAc-¹⁴C, Glc-³H]-lactalbumin (Fig 7C) was incubated with oviduct membranes and the resulting supernatant fraction was analyzed by gel filtration. It is apparent from the results shown in Fig. 7D that about 75% of the total [³H]Glc was released from the double-labeled α-lactalbumin. In contrast, no [³H]GlcNAc is released and, as expected, the ratio of [³H]Glc to [¹⁴C]GlcNAc in the doubly labeled α-lactalbumin is radically decreased.

**DISCUSSION**

In the preceding report (1) we have established that oviduct membranes catalyze the synthesis of a lipid-linked oligosaccharide containing Glc, Man, and GlcNAc. In addition, it was shown that this Glc-containing oligosaccharide chain is transferred en bloc from the lipid to both an endogenous membrane protein and exogenous S-carboxymethylated α-lactalbumin. These findings, as well as an earlier report demonstrating the occurrence of a Glc-containing oligosaccharide-lipid in oviduct tissue (17), prompted us to analyze the oviduct membrane proteins for Glc. This analysis revealed that, although the endogenous lipid fractions extracted from the membrane were rich in Glc, the membrane proteins were devoid of this hexose.

One possible explanation for these findings is that the Glc residues are removed enzymatically from the oligosaccharide chain after it is transferred from the lipid to the protein. Initial kinetic experiments, following the incorporation of Glc and GlcNAc from their sugar nucleotides into protein were consistent with this possibility; incorporation of Glc into glycoproteins reached a maximum and subsequently declined, whereas incorporation of GlcNAc continued over the complete time course of the experiment. More direct evidence for the enzymatic removal of the Glc residues after transfer of the oligosaccharide chain to protein was obtained in experiments utilizing isolated, single- or double-labeled oligosaccharide-lipid as substrate. The results of these experiments indicated that substantial loss of labeled Glc from the membrane glycoproteins occurs under conditions in which there is essentially no loss of labeled GlcNAc from the oligosaccharide core.

To examine the extent of degradation of the protein-linked, Glc-containing oligosaccharide chain by the membrane preparation containing the glucosidase, samples of the Glc and GlcNAc double-labeled glycoprotein were recovered before and after extensive loss of Glc. Following pronase digestion the resulting glycopeptides were analyzed by gel filtration. The results of this analysis showed that loss of Glc from the Glc-containing glycopeptides yielded a smaller glycopeptide that was close in apparent molecular weight to (α-Man)₅,₆-β-Man-(1 → 4)-β-GlcNAc-(1 → 4)-GlcNAc-peptide.

To investigate further the loss of Glc from the oligosaccharide chain of glycoproteins we took advantage of the finding that Glc-containing saccharide-lipid serves as a donor of its oligosaccharide chain to S-alkylated derivatives of reduced α-lactalbumin (1). S-Carboxymethylated α-lactalbumin was glycosylated in vitro to produce a soluble glycoprotein containing labeled Glc residues on the oligosaccharide chain. Incubation of this isolated and purified glycoprotein with oviduct membranes resulted in a time- and membrane-dependent release of free Glc from it. Under optimal conditions more than 70% of the labeled Glc was released from the glycoprotein. Moreover, similar experiments utilizing α-[GlcNAc-¹⁴C, Glc-³H]-lactalbumin clearly established that although there was extensive excision of Glc, no loss of GlcNAc from its oligosaccharide chain occurred.

Very recently several groups have reported the results of in vitro experiments indicating that the complex oligosaccharide chain of vesicular stomatitis virus and Sindbis viral glycoproteins are derived from polymannose oligosaccharide chains (18-20), probably by selective removal of some Man units followed by addition of GlcNAc, Gal, and N-acetylneuraminic acid. In view of the fact that a Glc-containing oligosaccharide linked to lipid is found in virus-infected cells (21), and that it is probably the source of the polymannose oligosaccharide chain attached to the viral glycoproteins, it seems likely that the processing of the polymannose chain to complex chains involves not only the loss of Man residues, but Glc residues as well.

The results reported in this paper document for the first time the existence of a membrane-bound glucosidase that acts to remove Glc residues from oligosaccharide chains linked to protein. Although in the current study we have not investigated the in vitro removal of α-mannosyl residues from the Glc-containing oligosaccharide chain of proteins, it should be mentioned that a membrane-bound α-mannosidase associated with the Golgi apparatus has been reported in rat liver (22). Current efforts are directed toward a study of the subcellular distribution of the membrane bound glucosidase in oviduct, utilizing a recently reported fractionation procedure (23). In addition, we are investigating these subcellular fractions for the possible presence of an α-mannosidase. These studies, in conjunction with more detailed structural studies on Glc-containing oligosaccharide, should allow one to elucidate, at the cell-free level, the major steps involved in the processing of oligosaccharide chains of glycoproteins.

**REFERENCES**

Excision of Glucosyl Units from Glycoproteins

Enzymatic excision of glucosyl units linked to the oligosaccharide chains of glycoproteins.
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