A $\beta 1,3$-galactosyltransferase that transfers galactose from UDP-galactose to $\beta 1,3$-$N$-acetyl$\beta 1,3$-$N$-acetylgalactosaminide mucin was purified from swine trachea membrane homogenates. The enzyme which was present in the microsome fraction was solubilized and purified to homogeneity by procedures which included affinity chromatography on Sepharose 4B containing covalently bound asialo Cowper's gland mucin. The enzyme showed a high specificity for $N$-acetyl$\beta 1,3$-$N$-acetylgalactosaminide residues linked to serine and threonine, and the most active glycosyl acceptors were macromolecular mucin glycoproteins containing free $N$-acetyl$\beta 1,3$-$N$-acetylgalactosaminyl residues linked to a polypeptide chain. The purified enzyme did not transfer galactose to the terminal $N$-acetyl$\beta 1,3$-$N$-acetylgalactosaminyl residues of type A blood group glycoprotein. Only a single band with molecular weight of 84,000 was observed upon gel electrophoresis in the presence of dodecyl sulfate, and a molecular weight of 90,000 was obtained by exclusion chromatography on Sephadex G-100 in the presence of 0.1% Triton X-100. The solubilized enzyme was unstable in the absence of Triton X-100 or Nonidet P-40. The $K_m$ for asialo Cowper's gland mucin was 0.35 $\mu$M, and the $K_m$ for UDP-galactose was 20 $\mu$M. The presence of sialic acid-linked $\alpha 2,6$ to the terminal $N$-acetyl$\beta 1,3$-$N$-acetylgalactosaminyl residues of Cowper's gland mucin completely inhibited the transfer of galactose. The product formed in a large scale incubation of asialo Cowper's gland mucin with UDP[$^14$C]galactose and the purified transferase was isolated. A disaccharide, [$^14$C]galactose $\beta 1,3$-$N$-acetylgalactosaminitol, containing more than 90% of the incorporated radioactivity was released by treatment with alkaline borohydride and characterized by enzymatic and methylation analysis.

The structures of oligosaccharide chains in acidic mucin glycoproteins may play an important role in regulating the rheological properties of respiratory mucus. Alterations in the structures of glycoproteins present in mucus secretions are accompanied by changes in the viscoelastic properties of mucus and in the cilary clearance of mucus which is observed in many chronic obstructive lung diseases (1, 2). The altered physical properties of the mucus brought about by changes in the structures of acidic mucin glycoproteins may in turn result from changes in the activities of glycosyltransferases which synthesize these glycoproteins. In order to understand the regulation of glycoprotein biosynthesis in respiratory tissues it is necessary to carefully characterize the individual glycosyltransferases and then examine their interactions with glycoprotein substrates in reconstructed multienzyme systems.

A number of glycosyltransferases with specificities for mucin glycoproteins have been detected in canine tracheal extracts (3, 4). Glycosyltransferases in these preparations catalyzed the transfer of galactose to terminal GalNAc in asialoovine submaxillary mucin (3). A sialyltransferase, fucosyltransferase, and N-acetyl$\beta 1,3$-$N$-acetylgalactosaminyl transferase were also present in these particulate preparations (3). Modified samples of ovine submaxillary mucins, bovine submaxillary mucins, fetuin, $\alpha_1$-acid glycoprotein, and ovalbumin were used as glycosyl acceptors in these studies. A $N$-acetyl$\beta 1,3$-$N$-acetylgalactosaminyltransferase which transfers GalNAc to serine residues in a modified mucin polypeptide chain was also found (4, 5). A second $N$-acetyl$\beta 1,3$-$N$-acetylgalactosaminyltransferase which catalyzes the transfer of GalNAc to blood group H substance to form a product with blood group A activity was shown to be present in microsomal preparations isolated from canine trachea (5). These studies further showed that the activity of this enzyme coincided with the blood group activity present in canine respiratory secretions, and only trachea from dogs secretting A-active substance contained the enzyme. Glycosyltransferases which synthesize the terminal residues of $\beta 1,3$-$N$-acyl$\beta 1,3$-$N$-acylgalactosaminyltransferases have been purified from bovine colostrum and other tissues (6). However, glycosyltransferases catalyzing the synthesis of the $\beta 1,3$-linked galactosyl residue in the inner chain of $\beta 1,3$-$N$-acyl$\beta 1,3$-$N$-acylgalactosaminyltransferases have been demonstrated in various tissues but have not yet been purified.

This report describes our studies on the purification and properties of this $\beta 1,3$-galactosyltransferase from tracheal membrane. We have used a unique well characterized glycoprotein substrate, asialo Cowper's gland mucin, to assay, characterize, and purify the enzyme because preliminary studies from this laboratory showed that the enzyme had a high affinity for this substrate. The transferase participates in the synthesis of the core portion of $\beta 1,3$-$N$-acyl$\beta 1,3$-$N$-acylgalactosaminyltransferase secreted by this tissue.

**EXPERIMENTAL PROCEDURES**

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**Experimental Procedures** are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 81M2654, cite authors, and include a check for $2.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
UDP-Gal:GalNAc-mucin:β1,3-galactosyltransferase

UDP-Gal:GalNAc-mucin:β1,3-galactosyltransferase was cloned into pBluescript II SK minus and expressed in Escherichia coli strain BL21 (DE3). The protein was purified from the cell culture supernatant by affinity chromatography on a GalNAc-Sepharose column. The purified protein was used for the production of polyclonal antibodies in rabbits. The antibodies were used to detect the protein in Western blots and immunohistochemistry.

The enzyme activity was measured by the formation of β1,3-galactosyltransferase acceptor. The reaction mixture contained UDP-Gal, GalNAc, and the donor sugar. The reaction was initiated by adding the enzyme and monitored by measuring the decrease in the concentration of UDP-Gal.

The enzyme showed high specificity for GalNAc as the acceptor and Gal as the donor sugar. The optimal pH for the enzyme activity was 7.5, and the optimal temperature was 37°C. The enzyme was stable at pH 7.0-8.0 and was active for at least 6 months at 4°C.

RESULTS

Subcellular Distribution of GalNAc-mucin:β1,3-galactosyltransferase in Swine Trachea Mucosa—The relative distribution of transferase activity in subcellular fractions isolated from homogenates of trachea mucosa is shown in Table I. The activity of the enzyme was measured with an excess of asialo GM2 and UDP-Gal (0.5 μmol), and 1 μmol of NAD+ was added to the reaction mixture to provide an alternate substrate for hydroxylases present in the membrane preparations. Under these conditions linear initial rates were obtained with all of the fractions. Triton X-100, 0.1%, was added to the reaction mixture since preliminary data showed that the addition of a detergent stimulated the activity about 3-fold with particulate preparations. Disruption of the membrane preparations by sonication also increased the observed transferase activity. These treatments presumably make the vesicular inner membrane surfaces more accessible to the macromolecular glycosyl acceptor (7).

The Golgi and microsome membrane fractions contain most of the β1,3-galactosyltransferase activity. At least 70% of the total activity present in trachea mucosa homogenates was consistently recovered in the microsome fraction. Some activity was found in the supernatant fraction obtained by centrifugation.
The subcellular fractions were isolated from a 20% homogenate prepared from 50 g of trachea as described in the text. Each of the particulate fractions was washed twice with 10 volumes of 0.02 M Tris, pH 7.0, 0.25 m sucrose, 2 mM MnCl2. The final pellet was suspended in this buffer and assayed for galactosyltransferase activity by the standard procedure. Values are taken from the average of five preparations.

| Fraction            | Total activity | Specific activity |
|---------------------|----------------|------------------|
|                     | nmol/min/g tissue | nmol/min/mg protein |
| Homogenate          | 43.5           | 0.8              |
| Supernatant fraction| 3.5            | 0.1              |
| Plasma membranes    | 1.8            | 0.7              |
| Golgi membranes     | 3.2            | 19.4             |
| Microsomes          | 28.0           | 4.5              |
| Rough microsomes    | 4.2            | 2.4              |
| Smooth microsomes   | 5.1            | 6.3              |

Table I

Subcellular distribution of GalNAc-Mucin:β1,3-galactosyltransferase in swine trachea mucosa

The subcellular fractions were isolated from a 20% homogenate prepared from 50 g of trachea as described in the text. Each of the particulate fractions was washed twice with 10 volumes of 0.02 M Tris, pH 7.0, 0.25 m sucrose, 2 mM MnCl2. The final pellet was suspended in this buffer and assayed for galactosyltransferase activity by the standard procedure. Values are taken from the average of five preparations.

| Fraction            | Total activity | Specific activity |
|---------------------|----------------|------------------|
|                     | nmol/min/g tissue | nmol/min/mg protein |
| Homogenate          | 43.5           | 0.8              |
| Supernatant fraction| 3.5            | 0.1              |
| Plasma membranes    | 1.8            | 0.7              |
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| Microsomes          | 28.0           | 4.5              |
| Rough microsomes    | 4.2            | 2.4              |
| Smooth microsomes   | 5.1            | 6.3              |

Table I

Subcellular distribution of GalNAc-Mucin:β1,3-galactosyltransferase in swine trachea mucosa

The subcellular fractions were isolated from a 20% homogenate prepared from 50 g of trachea as described in the text. Each of the particulate fractions was washed twice with 10 volumes of 0.02 M Tris, pH 7.0, 0.25 m sucrose, 2 mM MnCl2. The final pellet was suspended in this buffer and assayed for galactosyltransferase activity by the standard procedure. Values are taken from the average of five preparations.
satisfaction with ammonium sulfate (47.2 g per 100 ml). The suspension was centrifuged at 34,000 × g for 10 min, and the precipitate which collected at the top of the centrifuge tube was collected carefully discarding the clear solution. The precipitate was dissolved in 40 ml of 0.05 M Tris-HCl, pH 7.5, 0.25 M sucrose, 0.1% Triton X-100, and it was dialyzed twice against 1 liter of this buffer (fraction 4).

The solution was adjusted to 10 mM MgCl₂, 1 mM UMP, and 0.1 M NaCl, and it was passed through Sepharose 4B column (2.2 × 10 cm) containing covalently bound p-aminophenyl-GlcNAc and then through a Sepharose 4B column (2.2 × 10 cm) containing covalently bound p-aminophenyl-thio-β-D-galactoside. The column was washed with three bed volumes of 0.05 M Tris-HCl, pH 7.5, containing 0.1% Triton X-100, 0.25 M sucrose, 10 mM MgCl₂, 1 mM UMP. These columns removed β1,4-galactosyltransferase and GlcNAc transferases. The filtrate and washes were combined and concentrated by ultrafiltration on a PM-10 Amicon membrane and dialyzed against 0.05 M Tris-HCl, pH 7.5, 0.25 M sucrose, 0.1% Triton X-100.

The solution was adjusted to 10 mM MgCl₂, 1 mM UMP, and 0.1 M NaCl, and it was passed into a DEAE-cellulose column (2.2 × 15 cm) containing bound asialo CGM. The column was previously equilibrated against a solution containing 10 mM MgCl₂, 1 mM UMP, 0.05 M Tris-HCl, pH 7.5, 0.25 M sucrose, 0.1% Triton X-100. The column was washed with 100 ml of the same buffer containing 0.5 mg per ml of bovine albumin. β1,3-Galactosyltransferase was then eluted with 60 ml of 0.05 M Tris-HCl, pH 7.5, containing 50 mM EDTA, 0.1% Triton X-100, 0.5 mg/ml of albumin, and 0.25 M sucrose. The fractions containing transferase activity were collected and dialyzed against 0.05 M Tris-HCl, pH 7.5, 0.25 M sucrose, 0.1% Triton X-100. The solution was concentrated to 10 ml by ultrafiltration with a PM-10 Amicon membrane, and it was dialyzed against 0.05 M Tris-HCl, pH 7.5, 0.25 M sucrose, 0.1% Triton X-100 (fraction 5).

The procedure outlined in Table II for a typical preparation of β1,3-galactosyltransferase yields a homogenous enzyme with a yield of 20%. A final specific activity of 0.810 μmol min⁻¹ mg⁻¹ was obtained with saturating concentrations of UDP-Gal and asialo Cowper's gland mucin.

Studies on the Solubilization and Purification of β1,3-Galactosyltransferase by Affinity Chromatography on Immobilized Asialo Cowper's Gland Mucin—The ability of different detergents, including Triton X-100, Nonidet P-40, sodium dodecyl sulfate, cholate, deoxycholate, and Tween 80, to solubilize galactosyltransferase activity was examined. The detergent was added to the microsomes at a final concentration of 1%, and the resulting suspension was stirred at 3 °C for 15 min. The samples were then centrifuged at 100,000 × g for 90 min, and the supernatant solutions were dialyzed against 0.05 M Tris-HCl, pH 7.5, 0.25 M sucrose and assayed for galactosyltransferase activity. At this concentration, Triton X-100 and Nonidet P-40 released more than 75% of the activity into the high speed supernatant fraction. The other detergents were less effective, and some inactivated the enzyme. The most effective method for separating the solubilized enzyme from the disrupted membrane was to pass the suspension into DEAE-columns in the presence of 0.1 M NaCl and Triton X-100. Under these conditions the membranes bound to the DEAE-cellulose column and the solubilized enzymes could be eluted from the column under conditions favoring dissociation. More than 90% of the membrane-associated glycosyltransferases were solubilized by this method.

In preliminary studies it was observed that β1,3-galactosyltransferase required a very high concentration of low molecular weight glycosyl acceptors, >50 mM, for activity, and it did not bind with high affinity to columns containing immobilized low molecular weight GalNAc derivatives. However, the enzyme did show a high affinity for asialo Cowper's gland mucin. This property of the transferase was examined with Sepharose 4B columns containing covalently bound mucin glycoprotein.

The partially purified dialyzed enzyme (Table II, fraction 4) was adjusted to 10 mM MgCl₂ and 1 mM UMP, and it was passed through a Sepharose 4B column (2.2 × 10 cm) to remove glycosyltransferase with an affinity for this column. As seen in Fig. 3 (curve A) β1,3-galactosyltransferase does not bind to Sepharose 4B. The effluent was then passed into Sepharose 4B columns (2.2 × 10 cm) containing covalently bound asialo CGM. After washing with buffer the transferase was eluted with a solution containing EDTA. Fractions were dialyzed against 0.05 M Tris-HCl, pH 7.5, 0.25 M sucrose, 0.1% Triton X-100 to remove EDTA, and they were assayed. The data summarized in Fig. 3 show that β1,3-galactosyltransferase thus binds to asialo CGM-Sepharose 4B columns and that

Table II

| Fraction | Volume | Protein | Total activity | Specific activity | Yield |
|----------|--------|---------|----------------|------------------|-------|
|          | ml     | mg      | μmol/min       | μmol/min/mg      | %     |
| 1. Crude extract | 900    | 8100    | 3.5            | 0.00043          | 100   |
| 2. Microsome | 30     | 471     | 2.1            | 0.0045           | 60    |
| 3. Chromatography on DEAE-cellulose | 270    | 63      | 1.98           | 0.00134          | 57    |
| 4. Precipitation with ammonium sulfate | 42     | 31.5    | 1.18           | 0.0375           | 34    |
| 5. Affinity chromatography with asialo CGM | 20     | 1.2     | 0.7            | 0.810            | 20    |
increasing the amount of the transferase in the application solution results in a proportional increase in the amount of enzyme bound to the column (curve B, 2.5 ml compared to curve C, 5.0 ml). The enzyme in the combined active fractions was homogeneous, and it was completely free of other glycosyltransferases. Although the asialo CGM-Sepharose 4B column had a high specificity for the binding of β1,3-galactosyltransferase, it had a low capacity.

In order to increase the amount of asialo mucin glycoprotein in the affinity column, DEAE-cellulose was used as the supporting matrix. Mucin glycoproteins have a very high affinity for DEAE-cellulose, and 2 mM NaCl or 0.3 M Na2CO3 is required to elute from this ion exchange column. β1,3-Galactosyltransferase, on the other hand, has a very low affinity for DEAE-cellulose and does not bind to this column in the presence of 0.05 M NaCl. Therefore, it was possible that the tightly bound asialo mucin glycoprotein could be used for the purification of this transferase. Furthermore, it was found that the acidic sialic acid residues were not required for tight binding of the mucin glycoproteins to DEAE-cellulose. The effect of desialylation and disaggregation by reduction and carboxymethylation on the binding of CGM to DEAE-cellulose was examined, and the results are summarized in Table III. Surprisingly, these treatments actually increased the affinity of CGM for DEAE-cellulose. More of the intact mucin was eluted with 0.1 M NaCl than the treated samples (1,312,000 cpm compared to 774,000 cpm and 591,000 cpm for the treated samples). Mucin glycoprotein which was not removed with 100 ml of 0.3 M Na2CO3, 0.1 M NaOH was then eluted with 0.5 N KOH, 6 M urea. The amount of mucin glycoprotein removed with this solution was greater in the treated samples (970,000 and 1,200,000 compared to 340,000 in the untreated sample). These results suggest that the high affinity of CGM for DEAE-cellulose is not due to the presence of large amounts of sialic acid, 30% in CGM, nor to the high molecular weight of this glycoprotein. Instead, the tight binding appears to be a property of the polypeptide chain, since desialylation and disaggregation which increase the affinity of the mucin glycoprotein for DEAE-cellulose would also tend to make side chains of amino acids in the protein backbone more accessible to diethylaminoethanol and dodecyl sulfate as described in the legend of Fig. 4. Only one protein band was observed after this treatment, which indicates that the enzyme contains a single polypeptide chain (gels B and C). The presence of Triton X-100 caused a spreading of the band (gel B). An apparent molecular weight of 82,000 for the purified enzyme was obtained by gel electrophoresis in the presence of dodecyl sulfate, as seen in Fig. 5.

### Table III

*Influence of desialylation and reduction-carboxymethylation on the binding of Couper’s gland mucin to DEAE-cellulose*

The mucin glycoprotein was labeled with 14C-CH2COOH as described in the text. An aliquot was heated at 80°C for 1 h in 0.1 N H2SO4 to remove sialic acid. Another sample was reduced and carboxymethylated. The samples were dialyzed against 0.02 M potassium phosphate, pH 7.5, and urea was added to 6 M. About 3 mg (2,000,000 cpm of each sample) were applied to DEAE-cellulose columns (2.2 × 3.5 cm), and the columns were eluted with 100 ml of the solutions shown below.

| Eluting solution | Intact mucin | Asialo mucin | Reduced mucin |
|------------------|--------------|--------------|---------------|
| KH2PO4, 0.02 M   | 137.6        | 22           | 29            |
| KH2PO4, 0.2 M    | 221          | 25           | 26            |
| Na2CO3, 0.3 M, NaOH, 0.1 N | 1312 | 774          | 591          |
| KOH, 0.5 N, urea, 6 M | 340 | 970.2        | 1200         |

**Fig. 4. Polyacrylamide gel electrophoresis of purified β1,3-galactosyltransferase.** Albumin was removed from the purified enzyme by passing a sample through a small DEAE-cellulose column (2.2 × 4 cm). Gel A, 25 μg of protein run at pH 8.9 in Tris-glycine buffer in 7.5% acrylamide gel. Gel B, the enzyme was treated with 1% dodecyl sulfate and 1% 2-mercaptoethanol for 5 min at 100°C. Then, 50 μg was applied to the top of a gel containing 0.01 M sodium phosphate, pH 7.0, and 0.1% dodecyl sulfate. This sample contained Triton X-100. Gel C, a lyophilized sample of the purified enzyme was extracted with acetone to remove Triton X-100, and it was then treated with dodecyl sulfate and 2-mercaptoethanol. About 25 μg was layered on the top of a 7.5% gel. Electrophoresis for 4 h at 8 mA in the presence of 0.1% dodecyl sulfate yielded a single band. Gel D, standard proteins treated in the same manner as described for gel C were separated by electrophoresis under identical conditions.
A similar value was obtained when the elution volume of the purified transferase on a Sephadex G-100 column (1.8 × 100 cm) was compared with those of reference protein as described in a previous report (29). The column, the purified transferase, and the reference proteins were all equilibrated against 0.05 M Tris-Cl, pH 7.5, containing 0.25 M sucrose and 0.1% Triton X-100. The column was calibrated with [methyl-14C]phosphorylase b, bovine serum albumin, and ovalbumin, and elution volumes were monitored by radioactivity measurements since Triton X-100 interfered with protein estimations. Calculations based on plots of the log of the apparent molecular weights against elution volume yielded a molecular weight of 90,000 by this method. The somewhat higher apparent molecular weight obtained by gel filtration may be due to binding of the enzyme to Triton X-100 present in the buffer. In the absence of Triton X-100 all of the transferase activity is lost upon gel filtration.

Influence of the Concentration of UDP-galactose and Asialo Couper’s Gland Mucin on the Activity of β1,3-Galactosyltransferase—The effect of increasing concentration of UDP-Gal on the initial rates of the purified transferases as a function of the concentrations of asialo CGM was measured under the standard assay conditions. Lineeweaver-Burk plots of the data obtained with UDP-Gal concentrations from 0.1 mM to 2 mM were linear and intersected on the 1/[S] axis. An apparent Kₚ of 20 μM for UDP-Gal at saturating concentrations of asialo CGM (0.1 mg/0.3 ml) was calculated from the data. Double reciprocal replots of the data obtained with CGM concentrations from 0.05 μM to 50 μM as a function of the concentration of UDP-Gal yielded a series of lines which intersected on the 1/[S] axis. An apparent Kₚ of 0.35 μM for asialo CGM was calculated from this data at saturating concentrations of UDP-Gal (0.2 mM), based on a molecular weight of 200,000. The apparent Kₚ of CGM, based on the number of terminal GalNAc residues present in the glycoprotein, was 90 μM. A similar value, 80 μM, was obtained with ovine asialo submaxillary mucin, based on the number of terminal GalNAc residues present. The enzyme transferred galactose to low molecular weight glycopolypeptide acceptors such as GalNAc, GalNAcO, p-nitrophenyl-β-N-acetylgalactosamide and glycopolypeptides with less than 5 amino acids containing terminal GalNAc residues. However, the Kₚ values for these substrates, 100 mM for GalNAc, were much higher than those of asialo mucin glycoproteins. The purified enzyme did not transfer galactose to the terminal GalNAc residue of type A blood group glycoprotein or to the terminal galactosyl residue of type O blood group glycoprotein. Ovomucoid, ovalbumin, and fetuin devoid of sialic acid and galactose did not act as glycosyl acceptors for the purified β1,3-galactosyltransferase. Native CGM did not serve as a glycosyl acceptor for the purified enzyme. The presence of sialic acid-linked α2,6 to terminal GalNAc residues in the glycoprotein completely prevented the transfer of galactose.

Characterization of the Anomeric Configuration and Linkage of the Product of the Reaction—The specificity of the purified transferase was determined by characterizing the product formed in large scale reaction mixtures. Standard incubation conditions with reactants increased 50-fold and the time extended to 12 h were used to prepare the radioactive product. About 60% (6 μmol) of the galactose in the UDP [U-14C]galactose added to the reaction mixture was transferred to asialo CGM. The solution was dialyzed against distilled water, and the labeled asialo CGM was treated with alkaline borohydride as described under “Experimental Procedures” to release O-serine- and O-threonine-linked oligosaccharide chains. More than 90% of the radioactivity was released by this treatment, and nearly all of this radioactivity was recovered in a reduced disaccharide peak which eluted in the same position as the asialo A peak in Fig. 2 after chromatography on a Bio-Gel P-6 column (2.2 × 200 cm). The isolated reduced disaccharide contained only a single component which migrated the same distance as authentic Gal β1,3-GalNAc in four different solvent systems (8, 25). Hydrolysis with 2 N HCl at 100 °C for 4 h yielded equal amounts of [14C]galactose and GalNAc. These products were identified by paper chromatography in three different solvent systems (8, 24). No hydrolysis was observed when the reduced disaccharide was treated with α-galactosidase for 48 h in 0.05 mM sodium citrate, pH 4.0. This enzyme completely hydrolyzed methyl-α-D-galactoside and meibiose under these conditions. Extensive hydrolysis with high concentrations of Aspergillus niger β-galactosidase, 30 units, or Jack bean β-galactosidase, 20 units, for 48 h at 37 °C in 0.05 M potassium acetate, pH 4.0, released [14C]galactose and GalNAc which were separated and identified by paper chromatography. Chromatography was carried out with butanol-pyridine-H₂O (3:2:1) for 16 h. The paper was cut into 0.5-cm sections and counted. The positions of Galβ1,3GalNAc, galactose, and GalNAc were determined by partial hydrolysis of authentic [3H]Galβ1,3[3H]GalNAc with the same β-galactosidases.

The position of attachment of galactose to GalNAc was examined in methylation studies. Permethylation of the reduced disaccharide and analysis by gas chromatography showed the presence of only two peaks corresponding to 2,3,4,6-tetramethylgalactitol acetate and 1,4,5,6-tetramethyl-N-methylacetylgalactosaminitol acetate. The same peaks were observed when authentic Galβ1,3-GalNAc was examined by methylation analysis.

When GalNAc was used as the glycosyl acceptor in a large scale 50-fold increased reaction mixture, a radioactive disaccharide was isolated by paper chromatography in butanol-ethanol-water (10:1:2). The product had the same mobility as Gal β1,3-GalNAc in several different solvent systems (8, 24). Hydrolysis with 2 N HCl at 100 °C for 4 h yielded [14C]galactose and GalNAc. Extensive hydrolysis with β-galactosidase released [14C]galactose and GalNAc. The reducing disaccharide was examined by permethylation analysis and gas chromatography. Peaks corresponding to 2,3,4,6-tetramethylgalactitol acetate and 1,4,5,6-tetramethyl-N-methylacyetylgalactosaminitol acetate were also observed with this product. Taken collectively these results indicate that the purified transferase catalyzes the formation of a β1,3-linkage between
the transferred galactose and terminal GalNAc residues in mucin glycoproteins.

**DISCUSSION**

One of the principal difficulties in isolating glycosyltransferases directly from homogenates of trachea mucous membrane is that a heterogeneous population of cells is present in variable proportions (1), and the micromson fraction is, therefore, derived from several different cell types which synthesize different glycoproteins. The separation of large amounts of the major classes of individual cell types from the membrane before homogenization might help to overcome this problem. However, even if a homogeneous population of mucus-secreting cells were isolated this problem might still be present, because these cells synthesize a number of constitutive glycoproteins besides acidic mucin glycoproteins. The microsomal fraction of a single cell type which synthesizes several glycoproteins is a heterogeneous collection of vesicular membranes containing several multiglycosyltransferase systems which may be derived from the endoplasmic reticulum, Golgi apparatus, and plasma membrane. The synthesis of acidic mucin glycoprotein in trachea also requires the differentiation of basal cells to mature mucus-secreting cells (1), and the presence of intermediate cell types involved in this transformation would further add to the heterogeneity of the cells present in this tissue.

In order to circumvent these problems a highly specific purification procedure which takes advantage of all the unique properties of the β1,3-galactosyltransferase was developed. In preliminary studies it was observed that this transferase requires a macromolecular glycosyl acceptor which contains terminal GalNAc residues attached to seryl or threonyl residues in a polypeptide chain. Therefore, an affinity chromatographic procedure using Cowper's gland mucin could specifically remove this transferase from a mixture of glycosyltransferases. Evidence obtained in the present study shows that this procedure was able to separate this transferase from a number of other glycosyltransferases. The success of this step was dependent on the availability of a well characterized macromolecular substrate that satisfied only the requirements of this galactosyltransferase.

Membrane preparations from liver (30), salivary gland (31), pancreas (30), serum (32), and gastric epithelium (33) catalyze the transfer of galactose from UDP-galactose to asialo submaxillary mucins. The β-galactosyltransferase present in these particular preparations transferred galactose to free GalNAc and to nonreducing terminal GalNAc residues in asialo mucin glycoproteins. However, the enzyme has not yet been purified and characterized. Furthermore, the structure of the glycosyl acceptor and product formed in the reaction were not precisely elucidated.

The data obtained in the present studies show that a β1,3-galactosyltransferase is present in trachea membrane, and this report represents one of the first communications on the purification and characterization of a β1,3-galactosyltransferase which utilizes the O-serine-linked GalNAc residues in mucin glycoproteins. Acceptor specificity studies with the purified enzyme show that the best substrates are asialo mucin glycoproteins containing nonreducing GalNAc residues linked to a polypeptide chain. The enzyme did not transfer galactose to the terminal GalNAc residue in the oligosaccharide chain of blood group A substance. When the purified enzyme was incubated with UDP [14C]galactose and asialo Cowper's gland mucin, a disaccharide product was isolated from the glycoprotein in almost quantitative yield. Permethylation analysis and treatment with specific galactosidases showed that the disaccharide had the structure Gal β1,3-GalNAc.

In agreement with earlier studies (30, 31), the purified enzyme did not transfer galactose to sialylated GalNAc residues in CGM. These results suggest that a α2,6-sialyltransferase may compete with the β1,3-galactosyltransferase for terminal GalNAc residues in the mucin glycoprotein. The addition of sialic acid to the 6 position of the GalNAc residue would also directly block the transfer of GlcNAc by a Gal β1,3-GalNAc:β1,6 N-acetylgalcosaminyltransferase (9) which leads to an early branching of the oligosaccharide chains in mucin glycoproteins. These studies further suggest that the product of the β1,3-galactosyltransferase reaction, Gal β1,3-GalNAc-mucin, is a better glycosyl acceptor for β1,6-N-acetylgalcosaminyltransferase than the terminal GalNAc residues in asialo mucin glycoproteins. The regulation of the relative activities of β1,3-galactosyltransferase and α2,6-sialyltransferase could determine the possible number of short highly sialylated chains and long branched chains with blood group activity in a mucin glycoprotein.

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