Mucin biosynthesis

Properties of a bovine tracheal mucin β -6-*N*-acetylglucosaminyltransferase

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We have characterized a bovine tracheal mucin β -6-*N*-acetylglucosaminyltransferase that catalyses the transfer of *N*-acetylglucosamine from UDP-*N*-acetylglucosamine to the C-6 of the *N*-acetylgalactosamine residue of galactosyl- β 1 $\rightarrow$ 3-*N*-acetylgalactosamine. Optimal enzyme activity was obtained between pH 7.5–8.5, at 5 mM-MnCl₂, and at 0.06–0.08% (v/v) Triton X-100 (or Nonidet P-40), or 0.5–5.0% (v/v) Tween 20. Ba²⁺, Mg²⁺ and Ca²⁺ could partially replace Mn²⁺, but Co²⁺, Fe²⁺, Cd²⁺ and Zn²⁺ could not. Sodium dodecyl sulphate, cetylpyridinium chloride, sodium deoxycholate, octyl β -D-glucoside, digitonin and alkyl alcohols were less effective in enhancing enzyme activity, and dimethyl sulphoxide was ineffective. The apparent Michaelis constants were 1.25 mM for UDP-*N*-acetylglucosamine, 0.94–3.34 mM for freezing-point-depressing glycoprotein and 0.19 mM for periodate-treated blood-group-A porcine submaxillary mucin. Asialo ovine submaxillary mucin could not serve as the glycosyl acceptor. The structure of the ¹⁴C-labelled oligosaccharide obtained by alkaline-borohydride treatment of the product was identified as Gal β 1 $\rightarrow$ 3(GlcNAc β 1 $\rightarrow$ 6)*N*-acetylglactosaminitol by β -hexosaminidase treatment, gas chromatography–mass spectrometry and ¹H-n.m.r. (270 MHz) analysis. The enzyme is important in the regulation of mucin oligosaccharide biosynthesis.

Mucous glycoprotein (mucin) is a major determinant of the rheological properties of mucus secretion (Allen, 1978; Rose *et al.*, 1979). The carbohydrate moiety of mucous glycoprotein constitutes 50–80% of the molecule by weight (Boat & Cheng, 1976). Carbohydrate can contribute to the aggregation of mucin (Hill *et al.*, 1977), which in turn could affect the rheological properties of mucus secretion. Mucin oligosaccharide chains, which are heterogeneous in nature, are synthesized by the

sequential addition of sugar from sugar nucleotide to the acceptor (Roseman, 1970). Thus the sequence of sugar addition determines the final oligosaccharide structure (Beyer *et al.*, 1981). Following the transfer of the first sugar, *N*-acetylgalactosamine to serine/threonine in the peptide, one of the three sugars galactose, *N*-acetylglucosamine (Lamblin *et al.*, 1981) and *N*-acetylneuraminic acid may be added to the *N*-acetylgalactosamine residue. If NeuAc α 2 $\rightarrow$ 6GalNAc is formed first, synthesis of the oligosaccharide will be terminated at the disaccharide level (Carlson *et al.*, 1973; Beyer *et al.*, 1979). However, if Gal β 1 $\rightarrow$ 3GalNAc or GlcNAc β 1 $\rightarrow$ 3GalNAc is formed, then, depending on whether a chain-elongation sugar, e.g. galactose or *N*-acetylglucosamine, or a chain-terminating sugar, e.g. *N*-acetylneuraminic acid or L-fucose, is added, the oligosaccharide chain synthesis could either continue or be limited (Schachter, 1978; Beyer *et al.*, 1981).

Increase in oligosaccharide chain length has been reported for tracheal (Lamblin *et al.*, 1977) as

Abbreviations used: asialo-OSM, ovine submaxillary mucin (OSM) treated with neuraminidase and β -galactosidase (Cheng & Bona, 1982); IO₄-PSM, blood-group-A porcine submaxillary mucin (PSM) treated with periodate (Wingert & Cheng, 1984); FPDG, freezing-point-depressing glycoprotein; NeuGc (in sequences), *N*-glycolylneuraminic acid; GalNAc-H₂ (in sequences), *N*-acetylglactosaminitol.

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well as intestinal (Wesley *et al.*, 1983) mucins from patients with cystic fibrosis. Studies of the key enzymes involved in the regulation of tracheal mucin oligosaccharide synthesis may yield some important insight into the possible causes for this alteration. The present paper describes a bovine tracheal mucin *N*-acetylglucosaminyltransferase activity that catalyses the following reaction:

Experimental

Preparation of microsomal fraction from bovine tracheal epithelium

Epithelial scrapings from bovine tracheas obtained fresh from a local slaughterhouse were homogenized (12 strokes) in 4 vol. of 0.25 M-sucrose with a glass homogenizer. The microsomal fraction (Wingert & Cheng, 1984) was resuspended in 0.25 M-sucrose, approx. one-tenth the volume of the original homogenate, and then stored at -20°C . About half of the enzyme activity remained after 1 year.

Preparation of acceptors

OSM and PSM were prepared from sheep and pig submaxillary glands (Pel Freez Biological, Rogers, AR, U.S.A.) respectively, by the procedure of de Saegui & Plonska (1969). Asialo-OSM was prepared by the treatment of OSM with neuraminidase and bovine testicular β -galactosidase as previously described (Cheng & Bona, 1982). IO_4^- -PSM was prepared by treating PSM (2 mg/ml) with 20 mM- NaIO_4 as described previously (Wingert & Cheng, 1984). The IO_4^- -PSM contained no detectable amounts of fucose and sialic acid, and the ratio of *N*-acetylgalactosaminitol to $\text{Gal}\beta 1 \rightarrow 3\text{GalNAc-H}_2$ was found to be approx. 3:2. FPDG was isolated from the serum of an antarctic fish, *Dissostichus mawsoni*, by DEAE-cellulose column chromatography by using the procedure of Lin & DeVries (1974). The fraction eluted with 2.5 mM-Tris/HCl, which contains FPDG fractions 1–5, was used as the acceptor to carry out the studies except for the acceptor competition study, where FPDG fractions 7 and 8 (DeVries *et al.*, 1970) were used.

Enzyme assays

The assay mixture in a total volume of 50 μl contained 5 μl of 0.5 M-Mops adjusted to pH 7.5 with 1 M-NaOH, 5 μl of 0.8% (v/v) Triton X-100, 5 μl of 50 mM-MnCl₂, 5 μl of 50 mM-ATP adjusted to pH 7.5 with 1 M-NaOH, 5 μl of 20 mM-UDP-[^{14}C]-*N*-acetylglucosamine (0.7 mCi/mmol), 5 μl of bovine serum albumin solution (10 mg/ml), 4.5 μmol

(expressed as available Gal-GalNAc sites) of FPDG and 50–200 μg of enzyme protein. The UDP-[^{14}C]-*N*-acetylglucosamine was prepared by diluting the UDP-[U- ^{14}C]-*N*-acetylglucosamine (49 mCi/mmol) obtained from the New England Nuclear Corp. (Boston, MA, U.S.A.) with unlabelled UDP-*N*-acetylglucosamine (Sigma Chemical Co., St. Louis, MO, U.S.A.). The reaction

mixture was incubated at 37°C for 60 min, and the reaction was stopped by the addition of 0.4 ml of ice-cold 1 M-NaCl solution containing Methyl Red to monitor the column performance. The ^{14}C -labelled product was isolated by chromatography on Bio-Gel P-4 columns (29 cm $\times$ 1 cm) as previously described (Cheng & Bona, 1982). Average values of the results of duplicate samples after subtracting those of the controls, which contained no acceptor, are expressed as nmol of *N*-acetylglucosamine transferred/h per mg of protein. Protein was assayed by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard. All enzyme assays were performed under conditions where product formation was linear with respect to incubation times and enzyme amounts.

Preparation of ^{14}C -labelled product and isolation of the trisaccharide

A large-scale preparation of the ^{14}C -labelled product was carried out by incubating the following mixture in a final volume of 1 ml: Mops buffer, pH 7.5, 50 μmol ; Triton X-100 (0.8%, v/v), 100 μl ; bovine serum albumin, 1 mg; MnCl₂, 3 μmol ; FPDG, 20 mg; UDP-[^{14}C]-*N*-acetylglucosamine (900 d.p.m./nmol), 6 μmol ; ATP, 5 μmol ; 2,3-dimercaptopropanol (Faltynek *et al.*, 1981), 5 μmol ; 10.4 mg of enzyme protein; 2 drops of toluene. After incubation at 37°C for 14 h, the ^{14}C -labelled product was isolated by chromatography on Bio-Gel P-4. Oligosaccharides were removed from the ^{14}C -labelled product under alkaline borohydride conditions (Carlson, 1968). The ^{14}C -labelled oligosaccharide was subsequently purified by chromatography on a Bio-Gel P-2 (–400 mesh) column (2.5 cm $\times$ 180 cm) that had previously been calibrated with galactose, lactose, *N*-acetylglucosamine, $\text{Gal}\beta 1 \rightarrow 3\text{GalNAc-H}_2$ (Cheng & Bona, 1982), $\text{Fuc}\alpha 1 \rightarrow 2\text{Gal}\beta 1 \rightarrow 3\text{GalNAc-H}_2$ (Carlson, 1968), raffinose and stachyose (Pfanstiehl Laboratories, Wankegan, MI, U.S.A.).

Preparation of partially methylated alditol acetates from the trisaccharide

Permethylation of the trisaccharide was carried out by the method of Hakomori (1964). Approx.

480 nmol of the trisaccharide was treated with anhydrous dimethyl sulphoxide anion for 2 h and then methylated with methyl iodide overnight. After the addition of 10 ml of chloroform, dimethyl sulphoxide was removed by extraction with an equal volume of water. The chloroform layer was extracted three more times with water and then dried under N_2 . The samples were then subjected to acetolysis, reduced with $NaBH_4$ (Merck, Sharp and Dohme, Rahway, NJ, U.S.A.) and acetylated with acetic anhydride as previously described (Stellner *et al.*, 1973).

Gas-chromatography and gas-chromatography-mass-spectrometry analysis of the partially methylated alditol acetates

The partially methylated alditol acetates were separated isothermally at 180°C on a 3% OV-225 glass column (1.8 m $\times$ 2 mm) using a Hewlett-Packard model 5800A gas chromatograph equipped with a flame ionization detector. The column was standardized with the following alditol acetates: 1,5-diacetyl-2,3,4,6-tetramethylglucitol prepared from α -methyl glucopyranoside, 1,5-diacetyl-2,3,4,6-tetramethylgalactitol and 1,4,5-triacetyl-2,3,6-trimethylglucitol prepared from lactose, and 3-acetyl-1,4,5,6-tetramethyl-2-*N*-methylacetamidogalactitol prepared from Gal β -1 $\rightarrow$ 3GalNAc- H_2 by Hakomori's (1964) procedure. Mass spectra were then taken on a Hewlett-Packard 5958A gas chromatograph-mass spectrometer at the mass-spectrometry facility, Department of Biochemistry, Michigan State University, East Lansing, MI, U.S.A.) (Wingert & Cheng, 1984).

β -Hexosaminidase treatment of the trisaccharide

Treatment of the trisaccharide with the jack-bean β -hexosaminidase (Li *et al.*, 1975) was carried out with a 25 μ l reaction mixture containing 48 nmol of ^{14}C -labelled trisaccharide, 50 μ g of bovine serum albumin, 30 mM-citric acid/80 mM- Na_2HPO_4 , pH 4.3, 5.2 units of jack-bean β -hexosaminidase and 1 drop of toluene. The reaction mixture was incubated for 16 h at 37°C and then applied to a Bio-Gel P-2 (-400 mesh) column (2.5 cm $\times$ 180 cm) to separate the reactant from the products.

1H -n.m.r. studies

The 1H -n.m.r. spectrum of the trisaccharide (0.7 μ mol) was obtained on a Bruker 270/180 Fourier-transform n.m.r. spectrometer operating at 270 MHz. After the repeated exchange of hydrogen with 99.8% 2H_2O (KOR Isotopes, Cambridge, MA, U.S.A.), the trisaccharide was

dissolved in 0.1 ml of 99.8% 2H_2O and transferred to a 5 mm probe. Acetone, which has a chemical shift of 2.225 p.p.m. from tetramethylsilane, was included as a secondary internal standard. The data were acquired as described previously (Wingert & Cheng, 1984).

H.p.l.c. analysis of unmodified trisaccharide and benzoylated trisaccharide

Unmodified oligosaccharides were analysed on a primary-amine column (25 cm $\times$ 4.6 mm) with a linear gradient of 85% (v/v) acetonitrile in water to 60% (v/v) acetonitrile in water by the method of Lamblin *et al.* (1981). Oligosaccharides were monitored by the absorbance at 206 nm with a Varian UV-50 detector.

The trisaccharide isolated from bovine tracheal enzyme product and Gal-GalNAc- H_2 , *N*-acetylgalactosaminitol and NeuAc α 2 $\rightarrow$ 6GalNAc- H_2 were perbenzoylated by using the procedure of Daniel *et al.* (1981). The benzoylated derivatives were chromatographed on an Ultrasphere-Octyl column (4.6 mm $\times$ 25 cm) with a linear gradient of 70% (v/v) acetonitrile in water to 100% (v/v) acetonitrile. Oligosaccharide derivatives were monitored by the absorbance at 230 nm.

Results

Requirements for enzyme activity

A 6–7-fold enhancement of enzyme activity was observed when 0.06–0.08% (v/v) Triton X-100 or Nonidet was included in the assay mixture (Fig. 1). High concentrations ($\geq 0.5\%$) of Tween 20 and even higher concentrations ($\geq 10\%$) of Tweens 80 and 60 were needed to give approximately the same effect. At 5 mM concentration of Mn^{2+} , enzyme activity was enhanced maximally by 70%. At concentrations higher than 15 mM, Mn^{2+} was inhibitory. However, enzyme activity was not affected by the addition of 20 mM-EDTA in the absence of Mn^{2+} . Ca^{2+} , Mg^{2+} and Ba^{2+} enhanced enzyme activity by 26–55%. Co^{2+} did not affect enzyme activity, and Fe^{2+} , Cd^{2+} and Zn^{2+} inhibited enzyme activity by 40%, 75% and 100% respectively. Optimal enzyme activity was observed at pH 7.5–8.5 with Mops buffer. Spermidine at 2 mM increased the enzyme activity by 13–17%. The apparent Michaelis constants obtained under the conditions given in the Experimental section for UDP-*N*-acetylglucosamine, FPDG fractions 1–5 (Lin & DeVries, 1974), FPDG fractions 7–8 (DeVries *et al.*, 1970) and IO $_4$ -PSM were 1.25 mM, 3.34 mM, 0.95 mM and 0.19 mM respectively. No enzyme activity was detected when asialo-OSM (10 mM) was used as the acceptor.

Fig. 1. Effects of type A non-ionic detergents on bovine tracheal mucin *N*-acetylglucosaminyltransferase activity

A reaction mixture in a total volume of 50 μ l contained the following components: UDP-[14 C]-*N*-acetylglucosamine (0.7 mCi/mmol), 0.10 μ mol; Mops buffer, pH 7.5, 2.5 μ mol; $MnCl_2$, 2.5 μ mol; bovine serum albumin, 50 μ g; ATP, 0.25 μ mol; FPDG, 0.25 mg; microsomal membrane protein, 60 μ g; detergents, as indicated. After incubation at 37°C for 60 min, the enzyme reaction was stopped by the addition of 0.4 ml of ice-cold 1 M-NaCl containing Methyl Red. The [14 C]-labelled product was immediately isolated by the Bio-Gel P-4 multiple-column method (Cheng & Bona, 1982). The detergents used were Triton X-100 (○), Tween 20 (●), Tween 60 (▲) and Tween 80 (△).

Effects of ionic and type B non-ionic detergents, organic solvents and salts

In contrast with the effects of the type A non-ionic detergents (Helenius & Simons, 1975), the ionic detergents, which included sodium dodecyl sulphate, cetylpyridinium chloride and sodium deoxycholate, gave 3–5-fold increase in enzyme activity at low (<0.1%) concentrations but completely inhibited enzyme activity at higher (0.5%) concentration. The type B non-ionic detergents including octyl β -D-glucoside and digitonin, on the other hand, either gave small enhancement in enzyme activity or did not affect enzyme activity at all at low (<0.1%) detergent concentrations, but exerted a moderate (2.0–3.5-fold) increase in enzyme activity at higher (0.5–1.0%) concentrations.

Butan-1-ol did not affect enzyme activity at 0.1% concentration, but increased enzyme activity 1.4–3.0-fold at concentrations in the 1–5% range. Identical results were obtained for hexan-3-ol, pentan-2-ol and cyclohexanol. However, in the presence of 2% Tween 20, these alkyl alcohols became inhibitory. Under the same conditions, glycerol did not seem to affect the enzyme activity appreciably. Dimethyl sulphoxide did not affect

Fig. 2. Purification by Bio-Gel P-2 (–400 mesh) column (2.5 cm $\times$ 180 cm) chromatography of the [14 C]-labelled oligosaccharide obtained by alkaline-borohydride treatment of the [14 C]-labelled product

The following oligosaccharides were used as standards: 1, Fuc α 1 $\rightarrow$ 2Gal β 1 $\rightarrow$ 3GalNAc-H $_2$; 2, stachyose; 3, raffinose; 4, lactose. The column was eluted with distilled water at a rate of 27 ml/h, and the volume of each fraction was 4.6 ml. Samples were analysed by liquid-scintillation counting (○) and the anthrone assay (●). Gal β 1 $\rightarrow$ 3GalNAc-H $_2$ was co-eluted with stachyose.

the enzyme activity over a wide range (1–10%) of concentrations when detergent was not present. In addition, enzyme activity was inhibited gradually by NaCl and KCl at increasing concentrations (0.1–0.8 M), which was fully recoverable once the salt had been removed. In addition, (NH $_4$) $_2$ SO $_4$ at 0.8 M concentration inhibited enzyme activity to the same extent (76%) as NaCl and KCl did.

Isolation and purification of the [14 C]-labelled trisaccharide from the product of *N*-acetylglucosaminyltransferase

The [14 C]-labelled product of *N*-acetylglucosaminyltransferase with FPDG as the acceptor was prepared on a large scale and isolated by chromatography on Bio-Gel P-4. About 70% of the [14 C]-*N*-acetylglucosamine was transferred from UDP-[14 C]-*N*-acetylglucosamine to the acceptor. Treatment of the [14 C]-labelled product with alkaline borohydride followed by purification by chromatography on Bio-Gel P-2 yielded only one radioactive peak (Fig. 2). This elution profile suggests that only one *N*-acetylglucosamine residue was added to the original disaccharide side chain of the FPDG acceptor. The trisaccharide gave only one peak, with a retention time of 43.55 min, by h.p.l.c. on a bonded primary-amine column (Fig. 3a). This trisaccharide co-chromatographed with Gal β 1 $\rightarrow$ 3(GlcNAc β 1 $\rightarrow$ 6)GalNAc-H $_2$ (Wingert & Cheng, 1984). Furthermore, the benzoylated derivative of the trisaccharide and that of

Gal β 1 $\rightarrow$ 3(GlcNAc β 1 $\rightarrow$ 6)GalNAc-H₂ had the same retention time on a C-8 reverse-phase column (Fig. 3b).

Determination of the *N*-acetylglucosaminide linkage

Gas-chromatographic analysis of the partially methylated alditol acetates prepared from the trisaccharides gave three major carbohydrate peaks under isothermal (180°C) conditions. Peak 1 was co-eluted at 5.4 min with standard 1,5-diacetyl-2,3,4,6-tetramethylgalactitol, indicating that the galactose residue is unsubstituted. Peaks 2 and 3 had relative retention times of 5.96 and 8.19 with respect to peak 1. Since *N*-acetylglucosamine could be added to either C-4 or C-6 of the *N*-acetylglucosamine residue, confirmation of the trisaccharide structure as Gal β 1 $\rightarrow$ 3(GlcNAc β 1 $\rightarrow$ 6)GalNAc-H₂ was obtained by mass-spectrometric analysis of each partially methylated alditol acetate. Peak 1 gave the expected primary m/z ion fragments of 161, 162 and 205. The ion-fragment pattern agreed with that of 1,5-diacetyl-2,3,4,6-tetramethylgalactitol (Bjorndahl *et al.*, 1967). Peak 2 had primary m/z ion fragments of 45, 117, 159, 161, 203 and 205, and thus was derived from unsubstituted *N*-acetylglucosamine (Wrann & Tappy, 1978). Peak 3 gave primary m/z ion fragments of 45, 117, 130, 161, 246 and 318. The fragmentation pattern, which is essentially identical with that of 3,6-diacetyl-1,4,5-trimethyl-2-*N*-methylacetamidogalactitol (Wingert & Cheng, 1984), indicates that both C-3 and C-6 are substi-

tuted. Since C-3 of *N*-acetylglucosaminitol had already been occupied by galactose, *N*-acetylglucosamine had to be linked to C-6 of *N*-acetylglucosaminitol. The ¹H-n.m.r. analysis of the trisaccharide yielded essentially the same resonance spectrum as that of the standard trisaccharide Gal β 1 $\rightarrow$ 3(GlcNAc β 1 $\rightarrow$ 6)GalNAc-H₂ (Wingert & Cheng, 1984). The resonance of galactose and *N*-acetylglucosaminitol protons were located by the homodecoupling technique and by comparing them with the published values for Gal β 1 $\rightarrow$ 3GalNAc-H₂ and Gal β 1 $\rightarrow$ 3(NeuGc α 2 $\rightarrow$ 6)GalNAc-H₂ (Van Halbeek *et al.*, 1981). A significant downfield shift (0.2 p.p.m.) was observed for one of the C-6 protons in the *N*-acetylglucosaminitol residue of the trisaccharide as compared with that of the disaccharide, suggesting that *N*-acetylglucosamine is linked to C-6 of *N*-acetylglucosaminitol.

Assignment of the anomeric configuration of the *N*-acetylglucosaminide

¹H-n.m.r. (270 MHz) analysis of the trisaccharide showed that the coupling constant of the GlcNAc C-1 proton, which was located at 4.54 p.p.m., was 8.5 ± 0.2 Hz, indicating a β -anomer, since an α -anomer would have a coupling constant of approx. 3.5 Hz (Van Halbeek *et al.*, 1981). The β -configuration of the galactose C-1 proton was also confirmed by its coupling constant ($J_{1,2} = 7.8 \pm 0.2$ Hz) at 4.46 p.p.m. (Van Halbeek *et al.*, 1981). Furthermore, the ratio of the peak areas of the corresponding anomeric hydrogen atoms of

Fig. 3. H.p.l.c. elution profile of (a) the ¹⁴C-labelled trisaccharide (10 nmol) obtained from alkaline-borohydride treatment of the product of bovine tracheal FPDG β -6-*N*-acetylglucosaminyltransferase and (b) of the benzoylated derivative of the trisaccharide (200 pmol)

The bonded primary-amine (5 μ m particle size) column (25 cm $\times$ 4.6 mm) was eluted with an acetonitrile gradient of 85–60% in water for 60 min at a flow rate of 1 ml/min. The oligosaccharides were monitored by the absorbance at 206 nm. *N*-Acetylglucosaminitol was eluted at where the arrow is indicated. (b) The C-8 (5 μ m particle size) reversed-phase column (25 cm $\times$ 4.6 mm) was eluted with a gradient of 70–100% acetonitrile in water for 40 min at a flow rate of 1 ml/min. Benzoyl derivatives were monitored by the absorbance at 230 nm. The column was standardized with the perbenzoylated derivatives of the following oligosaccharides (100–200 pmol): 1, *N*-acetylglucosaminitol (11.29 min); 2, NeuAc α 2 $\rightarrow$ 6GalNAc-H₂ (13.00 min); 3, Gal β 1 $\rightarrow$ 3GalNAc-H₂ (23.34 min); 4, Gal β 1 $\rightarrow$ 3(GlcNAc β 1 $\rightarrow$ 6)GalNAc-H₂ (31.30 min). The retention time for the benzoylated derivative of the ¹⁴C-labelled trisaccharide was 31.36 min.

Table 1. *Acceptor competition study of bovine tracheal mucin β -6-N-acetylglucosaminyltransferase*
 The reaction mixture (50 μ l) contained the following components: MnCl₂, 0.25 μ mol; Mops buffer, pH 7.5, 2.5 μ mol; Triton X-100, 0.08% (v/v); ATP, 0.25 μ mol; bovine serum albumin, 50 μ g; UDP-[¹⁴C]*N*-acetylglucosamine (0.7 mCi/mmol), 0.1; enzyme protein, 75 μ g; acceptors as indicated in the Table. The reaction was allowed to proceed at 37°C for 60 min, and then the product was isolated by chromatography on a Bio-Gel P-4 column and quantified as described in the text.

			[¹⁴ C]GlcNAc transferred (nmol/h per mg of protein)			
Acceptor concentration (mM)			Experimental value	Theoretical value		Conclusion
Expt. no.	FPDG*	IO ₄ ⁻ -PSM†		One enzyme‡	Two enzymes§	
1	1.28		22.7			One enzyme
2	2.55		25.9			
3	5.10		31.4			
4		0.29	8.7			
5		0.43	10.4			
6		0.86	11.8			
7	1.28	0.29	22.4	18.8	31.4	
8	2.55	0.43	22.4	22.4	36.3	
9	5.10	0.86	26.2	24.4	43.2	

* Fractions 7 and 8 of FPDG (DeVries *et al.*, 1970).

† PSM treated successively with periodate, NaBH₄ and mild acid (Wingert & Cheng, 1984).

‡ Equation for the calculation of the apparent velocity for the one-enzyme-two-activities system (Dixon & Webb, 1964) is shown below:

$$v = \frac{V_1([S_1]/K_1) + V_2([S_2]/K_2)}{1 + ([S_1]/K_1) + ([S_2]/K_2)}$$

where v , V , $[S]$ and K are apparent velocity, maximal velocity, substrate concentration and Michaelis constant respectively. 1 and 2 represent the acceptors FPDG and IO₄⁻-PSM respectively. $V_1 = 37.3$ nmol/h per mg of protein; $V_2 = 14.5$ nmol/h per mg of protein; $K_1 = 0.94$ mM; $K_2 = 0.19$ mM. The kinetic constants were obtained from Expts. 1–6.

§ The sum of apparent velocities obtained from the results of assaying the enzyme activity in the presence of each acceptor separately.

N-acetylglucosamine and galactose was 1.00:1.14, indicating that only one *N*-acetylglucosamine residue was transferred to the disaccharide. The newly formed *N*-acetylglucosaminide was completely cleaved by jack-bean β -hexosaminidase, further confirming its β -configuration.

Acceptor competition study

A mixed-acceptor experiment was carried out to determine whether the *N*-acetylglucosaminyltransferase is a mucin *N*-acetylglucosaminyltransferase. The IO₄⁻-PSM was utilized as the mucin acceptor. The experimental conditions and results are shown in Table 1. The apparent velocities obtained from the mixed-acceptor experiment, as shown in Expts. 7–9, were compared with the theoretical values calculated from the experimentally obtained data from single-acceptor Expts. 1–6. The experimentally obtained data agreed with those calculated for one enzyme system, indicating that the enzyme activity catalysing the reaction for both ac-

ceptors is a mucin *N*-acetylglucosaminyltransferase.

Discussion

Previous reports on the respiratory (Boat *et al.*, 1976; Lamblin *et al.*, 1977) and intestinal (Wesley *et al.*, 1983) mucins from patients with cystic fibrosis have described alteration in their chemical properties, namely an increase in more-acidic mucous glycoproteins that contain longer carbohydrate chains. This alteration may be explained by elevation of the relative activities between carbohydrate chain elongation versus chain-terminating enzymes in these two mucin-secreting tissues in patients with cystic fibrosis. Whether these changes are caused by the primary or secondary gene defects remains to be established. To date, there have been only limited studies on tracheal mucin glycosyltransferases (Baker & Munro, 1971; Baker *et al.*, 1972; Baker & Sawyer, 1975;

Mendicino *et al.*, 1982; Cheng & Bona, 1982; Sheares & Carlson, 1983) but none on tracheal mucin *N*-acetylglucosaminyltransferases.

In the present paper we describe a tracheal epithelial mucin *N*-acetylglucosaminyltransferase that catalyses the transfer of *N*-acetylglucosamine from UDP-*N*-acetylglucosamine to the C-6 of the *N*-acetylgalactosamine residue in the Gal β 1 $\rightarrow$ 3GalNAc-R acceptor. In general, the enzymic properties of bovine tracheal mucin *N*-acetylglucosaminyltransferase are similar to those of the rabbit (Wingert & Cheng, 1984) and dog (Williams & Schachter, 1980; Williams *et al.*, 1980) enzymes. To search for conditions that may be suitable for the purification of the membrane-bound *N*-acetylglucosaminyltransferase, we subjected the microsomal membrane preparation to treatment with a variety of membrane-solubilizing agents. *N*-Acetylglucosaminyltransferase was found to respond differently to various detergents. Triton X-100, Nonidet P-40 and Tween detergents (Fig. 1), grouped as type A non-ionic detergents (Helenius & Simons, 1975), ranked the best among the detergents tested. However, the ability of the detergents to enhance activities did not correlate with the enzyme-solubilizing properties. For example, essentially no *N*-acetylglucosaminyltransferase activity was extracted with Tween 20 (or Tweens 80 and 60), as was judged by assaying the enzyme activity in the 100000*g* supernatant fraction and that in the pellet after detergent treatment (M. R. Little & P. W. Cheng, unpublished work). Triton X-100 at 2% concentration, on the other hand, extracted approx. 50% of the enzyme activity from the microsomal membrane fraction. However, once the enzyme was solubilized it became extremely labile.

The structures of the mucin *N*-acetylglucosaminides that have been elucidated to date include at least the following: GlcNAc β 1 $\rightarrow$ 3GalNAc (Lamblin *et al.*, 1981), GlcNAc β 1 $\rightarrow$ 6GalNAc (Rovis *et al.*, 1973; Newman & Kabat, 1976; Kochetkov *et al.*, 1976; Lamblin *et al.*, 1981), GlcNAc β 1 $\rightarrow$ 3Gal (Rovis *et al.*, 1973; Newman & Kabat, 1976; Kochetkov *et al.*, 1976), GlcNAc β 1 $\rightarrow$ 6Gal (Rovis *et al.*, 1973), GlcNAc β 1 $\rightarrow$ 4Gal (Newman & Kabat, 1976; Kochetkov *et al.*, 1976), GlcNAc α 1 $\rightarrow$ 4Gal (Kochetkov *et al.*, 1976) and GlcNAc α 1 $\rightarrow$ 4GlcNAc (Newman & Kabat, 1976). Only comparatively recently have the enzyme activities responsible for the synthesis of the *N*-acetylglucosaminide linkages of β 1 $\rightarrow$ 6 to *N*-acetylgalactosamine (Williams & Schachter, 1980; Wingert & Cheng, 1984), β 1 $\rightarrow$ 3 to *N*-acetylgalactosamine (Brockhausen *et al.*, 1983*b*) and β 1 $\rightarrow$ 3 to galactose (Brockhausen *et al.*, 1983*a*) been reported in non-respiratory epithelial tissues. The β 1 $\rightarrow$ 6 enzyme is the most active among the three *N*-acetylglucosaminyltransferases identified. The

β 1 $\rightarrow$ 3-linked galactose residue in the acceptor is required for the enzyme activity, since no enzyme activity was detected when asialo-OSM was used as the acceptor. The addition of an *N*-acetylglucosamine or galactose residue (Slomiany *et al.*, 1984) will allow the chain to grow from galactose and/or *N*-acetylglucosamine ends. The formation of α 2 $\rightarrow$ 6 sialic acid linkage to the *N*-acetylgalactosamine residue will shut off the synthesis from the *N*-acetylglucosamine end, although it will not stop the elongation from the galactose end (Beyer *et al.*, 1980). It is apparent that purification of the mucin β -6-*N*-acetylglucosaminyltransferase is necessary in order to study the acceptor specificity and to understand the regulation of mucous glycoprotein oligosaccharide synthesis.

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