Mucin Biosynthesis: Characterization of Rabbit Small Intestinal UDP-*N*-Acetylglucosamine:Galactose β-3-*N*-Acetylgalactosaminide (*N*-Acetylglucosamine → *N*-Acetylgalactosamine) β-6-*N*-Acetylglucosaminyltransferase†

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ABSTRACT: We have characterized a UDP-GlcNAc:Gal β-3-GalNAc (GlcNAc → GalNAc) β-6-*N*-acetylglucosaminyltransferase from rabbit small intestinal epithelium by using freezing point depression glycoprotein as the acceptor. Optimal enzyme activity was obtained at pH 7.0–7.5, at 3 mM $MnCl_2$, and at 0.08% Triton X-100. Ca^{2+}, Mg^{2+}, and Ba^{2+} also enhanced enzyme activity. The apparent Michaelis constant was 4.80 mM for freezing point depression glycoprotein, 0.59 mM for periodate-treated porcine submaxillary mucin, 0.49 mM for Galβ1→3GalNAcαPh, and 1.03 mM for UDP-GlcNAc. No enzyme activity was observed when asialo ovine submaxillary mucin was used as the acceptor. The ^{14}C-labeled oligosaccharide obtained by alkaline borohydride treatment of the product was shown to be a homogeneous trisaccharide by compositional analysis, Bio-Gel P-4 gel filtration, and high-performance liquid chromatogrophy. The structure of the trisaccharide was identified as Galβ1→3-(GlcNAcβ1→6)GalNAc-H_2 by (a) identification of 2,3,4,6-tetramethyl-1,5-diacetylgalactitol and 1,4,5-trimethyl-3,6-diacetyl-2-*N*-methylacetamidogalactitol by gas–liquid chromatography–mass spectrometry and (b) the complete cleavage of the newly formed glycosidic bond by jack bean β-hexosaminidase. The structure of the trisaccharide was confirmed by ^1H nuclear magnetic resonance (270 MHz) and also by periodate oxidation of the trisaccharide followed by $NaBH_4$ reduction, 4 N HCl hydrolysis, a second $NaBH_4$ reduction, and the identification of threosaminitol on an amino acid analyzer. By acceptor competition studies, the enzyme activity was shown to be a mucin *N*-acetylglucosaminyltransferase. We postulate that this glycosyltransferase may play a key role in the regulation of mucin oligosaccharide synthesis.

The carbohydrate moiety of mucous glycoproteins consists of *N*-acetylgalactosamine (GalNAc),[1] *N*-acetylglucosamine (GlcNAc), galactose (Gal), fucose, and neuraminic acid. These five sugars constitute at least 50% of the dry weight of the molecule (Boat & Cheng, 1976; Pigman et al., 1977). Peptide synthesis occurs at rough endoplasmic reticulum. The assembly of oligosaccharides is initiated at the rough endoplasmic reticulum and completed within the Golgi apparatus (Jentoft et al., 1976; Schachter et al., 1978). The structures of mucin oligosaccharides are determined by the sequence of sugar additions (Beyer et al., 1981). Each step is catalyzed by a glycosyltransferase that is specific not only for the nucleotide sugar donor but also for a specific carbohydrate sequence in the acceptor. A number of mucin glycosyltransferases have been well characterized (Beyer et al., 1981) including sialyltransferases (Sadler et al., 1979a,b), fucosyltransferases (Beyer et al., 1980; Prieels et al., 1981), galactosyltransferases (Cheng & Bona, 1982; Mendicino et al., 1982; Sheares et al., 1982), and *N*-acetylgalactosaminyltransferases (Schwyzer & Hill, 1977; McGuire & Roseman, 1967). Although mucin oligosaccharides containing *N*-acetylglucosaminides with β1→3 (Rovis et al., 1973; Lamblin et al., 1980), β1→4 (Newman & Kabat, 1976), β1→6 (Yurewicz et al., 1982; Lombart & Winzler, 1974), and α1→4 (Newman & Kabat, 1976; Kochetkov et al., 1976) linkages have been reported, only the enzyme responsible for the synthesis of GlcNAcβ1→6GalNAc has been studied (Williams et al., 1980; William & Schachter, 1980). The enzyme source was canine submaxillary glands. We report the characterization of a mucin *N*-acetylglucosaminyltransferase in rabbit intestinal epithelium that catalyzes the following reaction:

UDP-GlcNAc + Galβ1→3GalNAcαR →
Galβ1→3(GlcNAcβ1→6)GalNAcαR + UDP

where R is a protein or other group such as phenyl.

Experimental Procedures

Materials

Unless otherwise stated, all the reagents used in this study were reagent grade and used without further purification. The following reagents were obtained from the respective supplier: UDP-[U-^{14}C]GlcNAc (49 mCi/mmol) and Aquassure, New England Nuclear Corp.; porcine and ovine submaxillary glands, freshly frozen and shipped, Pel Freez Biological, Inc.;

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[1] Abbreviations: UDP-GlcNAc, uridine diphosphate *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; Ac, acetyl; Me, methyl; MOPS, 3-(*N*-morpholino)propanesulfonic acid; FPDG, freezing point depression glycoprotein; PPO, 2,4-diphenyloxazole; POPOP, 1,4-bis(5-phenyloxazol-2-yl)benzene; MES, 2-(*N*-morpholino)ethanesulfonic acid; NeuGc, *N*-glycolylneuraminic acid; GalNAc-H_2, *N*-acetylgalactosaminitol; AraN-H_2, arabinosaminitol; ThrN-H_2, threosaminitol; asialo-OSM, ovine submaxillary mucin of which sialic acid and galactose have been removed by neuraminidase and β-galactosidase (Cheng & Bona, 1982); PSM, porcine submaxillary mucin; IO_4^--PSM, periodate-treated A^+ porcine submaxillary mucin; GC–MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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stachyose, raffinose, galactose pentaacetate, methyl α-glucopyranoside, and methyl α-galactopyranoside, Pfanstiehl Laboratories; sodium hydride, Alfa Products, Inc.; 2,4,6-tri-2-pyridyl-*s*-triazine, Aldrich Chemical Co., Inc.; trimethylsilyl reagent, Pierce Chemical Co.; $NaBH_4$, 2,4-diphenyloxazole (PPO), 1,4 bis(5-phenyloxazol-2-yl)benzene (POPOP), acetonitrile (HPLC grade), methyl iodide, and ethyl acetate, Fisher Scientific Co.; UDP-GlcNAc, ATP, Me_2SO, 2,3-dimercaptopropanol, sodium metaperiodate, and *N*,*N*′-diacetylchitobiose, Sigma Chemical Co.; Bio-Gel P-2 (–400 mesh), Bio-Gel P-4 (–400 mesh), Econo column (30 × 1 cm), Cellex D anion exchanger (high capacity), 3-(*N*-morpholino)propanesulfonic acid (MOPS), and 2-(*N*-morpholino)ethanesulfonic acid (MES), Bio-Rad Laboratories; Triton X-100, Research Product International Corp.; Spherisorb 5-μm bonded primary amine HPLC column (25 cm × 4.6 mm) and 3% OV-225 (80–100 mesh), Supelco Co.; D_2O, 99.75%, KOR Isotopes; $NaBD_4$, Merck Sharp & Dohme. *Dissostichus mawsoni* serum and fractions 7 and 8 of freezing point depression glycoprotein (FPDG) were supplied by Dr. Arthur DeVries, Department of Physiology and Biophysics, University of Illinois, Urbana-Champaign. Jack bean β-hexosaminidase (Li et al., 1975) was a gift from Dr. Y.-T. Li at the Department of Biochemistry, Tulane University, and bovine testicular β-galactosidase (Distler & Jourdian, 1978) was provided by Dr. George W. Jourdian at the Department of Biochemistry, University of Michigan. Galβ1→3GalNAcαPh (Matta & Barlow, 1975) was supplied by Dr. K. Matta at the Roswell Memorial Institute, Buffalo, NY. Galβ1→3GalNAc-H_2 and Fucα1→2Galβ1→3GalNAc-H_2 were prepared from porcine submaxillary mucin according to the procedure of Carlson (1968).

Methods

Preparation of a Microsomal Fraction from Rabbit Small Intestine. The small intestine was removed from 3-kg adult New Zealand male rabbits anesthetized by 2.5 mg of pentobarbital administered intravenously. The tissue was gently rinsed in ice-cold 0.9% NaCl. Epithelial scrapings from the proximal one-third of the intestine were homogenized (10 strokes) in 4 volumes of 0.25 M sucrose with a glass homogenizer. After centrifugation at 10000*g* for 15 min at 4 °C, the supernatant fraction was decanted and further centrifuged at 4 °C and 100000*g* for 1 h in a Beckman Model L preparative ultracentrifuge. The pellet was resuspended in 0.25 M sucrose with 0.1 the volume of the original homogenate. This microsomal preparation was stored at –70 °C. Approximately 80% of enzyme activity was retained after four cycles of freezing and thawing over a period of 2 weeks.

Preparation of Acceptors. Ovine and porcine submaxillary mucins were prepared from the respective submaxillary glands according to the procedure of de Salegui & Plonska (1969). Asialo ovine submaxillary mucin was prepared by treatment with neuraminidase and bovine testicular β-galactosidase as previously described (Cheng & Bona, 1982). Periodate-treated PSM (IO_4^--PSM) was prepared by dissolving 90 mg of A^+ PSM in 40 mL of 0.05 M sodium acetate, pH 5.2, to which 5 mL of 0.2 M $NaIO_4$ was added. The reaction was allowed to proceed in the dark at 0 °C until consumption of IO_4^- was leveled off. About 3.4 μmol of IO_4^- was consumed/mg of A^+ PSM. After dialysis against water, the PSM solution was adjusted to pH 9.1 with 0.8 M potassium borate and then treated with 0.5 M $NaBH_4$ for 6 h. Following the addition of 4 N acetic acid to destroy the residual $NaBH_4$, the sample was dialyzed against water; then, the pH was adjusted to 1.5 with 0.1 N H_2SO_4 and the sample incubated at 80 °C for 1 h. The sample was then dialyzed against water and centrifuged at 500*g* for 5 min to remove the particulate matter. The IO_4^--PSM was further purified on Bio-Gel P-4 (200–400 mesh). The IO_4^--PSM prepared by this procedure does not contain a detectable amount of fucose and neuraminic acid as analyzed by GC. Removal of the oligosaccharides from IO_4^--PSM by alkaline (0.05 M)–NaB^3H_4 (0.5 M, 5 mCi) treatment (50 °C, 20 h) (Carlson, 1968) followed by Dowex 50 H^+ treatment and then Bio-Gel P-4 (200–400 mesh) column (2.5 × 110 cm) chromatography gave mainly ^3H-labeled GalNAc-H_2 and Galβ1→3GalNAc-H_2. On the basis of the radioactivity under each peak, the ratio of GalNAc-H_2 to Galβ1→3GalNAc-H_2 was estimated to be approximately 3 to 2. Freezing point depression glycoprotein (FPDG) was isolated from the serum of the antarctic fish *Dissostichus mawsoni* by DEAE-cellulose chromatography as described by Lin & DeVries (1976). The first peak from the DEAE-cellulose column was used for this study unless otherwise indicated.

Enzyme Assays. (*A*) *Column Method.* This method was employed when macromolecules were utilized as the acceptors. The assay mixture had a total volume of 50 μL and contained the following: MOPS, pH 7.5, 2.5 μmol; $MnCl_2$, 0.15 μmol; Triton X-100, 0.08%; ATP, 0.25 μmol; UDP-[^{14}C]GlcNAc (2700 dpm/nmol), 0.1 μmol; bovine serum albumin (BSA), 50 μg; FPDG, 0.5 μmol (expressed as available Gal-GalNAc sites), and 80–150 μg of enzyme protein. The reaction mixture was incubated at 37 °C for 60 min and the reaction stopped by the addition of 15 μL of 0.25 M GTP followed by 0.4 mL of ice-cold Hank's balanced salt solution containing 1 M NaCl. A 10-μL aliquot was removed, mixed with 10 mL of Aquassure scintillation fluid, and counted. A 350-μL aliquot was applied to a Bio-Gel P-4 (200–400 mesh) column (29 × 1 cm) to separate the radiolabeled product from unreacted UDP-[^{14}C]GlcNAc and degraded glycosyl donor. The column was eluted with 0.1 M Tris-HCl, pH 7.0, and a multichannel proportioning pump (Technicon Instruments) to process up to 15 samples simultaneously as previously described (Cheng & Bona, 1982). The void volume was collected as one fraction, and a 2-mL aliquot was counted in 10 mL of Aquassure. The percent transfer of [^{14}C]GlcNAc to the macromolecular acceptor was calculated after subtraction of the percentage transfer of the control (without exogenous acceptor) from that of the test sample. Mean values of duplicate samples were expressed as nanomoles of GlcNAc transferred per hour per milligram of protein. All enzyme assays were performed with conditions under which product formation was proportional to incubation time and enzyme amount. Linearity was maintained for at least 60 min and up to 20% transfer of [^{14}C]GlcNAc from UDP-[^{14}C]GlcNAc to the acceptor. The addition of 5 mM ATP in the reaction mixture minimizes the degradation of the glycosyl donor and maintains the linearity mentioned above.

(*B*) *Paper Chromatography Method.* When Galβ1→3GalNAcαPh (2 mM) was used as the acceptor, the product was isolated by a paper chromatography method described below. The enzyme reaction was carried out as described above and the reaction stopped by the addition of 15 μL of 0.25 M GTP. A 5-μL aliquot was applied to a 1.5 × 1.5 in. Whatman 3MM paper and counted in 10 mL of toluene scintillator (19 g of PPO and 1.14 g of POPOP in 3.8 L of toluene). A 40-μL aliquot was applied separately to Whatman 3MM paper and subjected to descending paper chromatography for 16 h with the eluting solvent, ethyl acetate/pyri-

dine/H_2O, 10/4/3 (v/v/v). UDP-[^{14}C]GlcNAc moved approximately 5 cm, and its degraded products, [^{14}C]GlcNAc-1-PO_4 and [^{14}C]GlcNAc, moved 3 and 25 cm, respectively. The [^{14}C]GlcNAc-labeled product was located approximately 20 cm from the origin by a Packard radiochromatogram scanner, cut out, and counted in 10 mL of toluene scintillator. The data were calculated as described above.

Preparation and Purification of the ^{14}C-Labeled Product and the Trisaccharide. A large-scale preparation of the ^{14}C-labeled product was carried out by incubating a mixture containing the following components in a final volume of 1 mL: MOPS buffer, pH 7.5, 50 μmol; Triton X-100 (1.25%, v/v), 100 μL; BSA, 1 mg; FPDG, 30 mg; UDP-[^{14}C]GlcNAc (900 dpm/nmol), 6 μmol; ATP, 5 μmol; 2,3-dimercaptopropanol (Faltynek et al., 1981), 2.5 μmol; 6.8 mg of enzyme protein; 2 drops of toluene. After incubation at 37 °C for 16 h, the sample was applied to a Bio-Gel P-4 (200–400 mesh) column (29 × 1 cm) to isolate the ^{14}C-labeled product. Fractions containing the product were pooled, dialyzed extensively against water with a M_r 2000 cut-off dialysis tubing, and dried under reduced pressure. Oligosaccharides were removed from the ^{14}C-labeled product by the alkaline borohydride procedure of Carlson (1968), applied to a Dowex 50 H^+ column (25 × 1 cm), and eluted with water. Borate was removed by coevaporation with methanol. A total of 20 mg of BSA, 0.3 g of NaCl, and 2 μmol of galactose were added to the ^{14}C-labeled oligosaccharide prior to chromatography on a Bio-Gel P-2 column (−400 mesh) (2.5 × 180 cm), which had previously been calibrated with galactose, lactose, GlcNAc, Galβ1→3GalNAc-H_2, Fucα1→2Galβ1→3GalNAc-H_2, raffinose, and stachyose. The column was eluted with distilled water, and fractions containing the ^{14}C-labeled oligosaccharide were combined.

Gas Chromatography and Gas Chromatography (GC)–Mass Spectrometry (MS) Analysis of the Partially Methylated Alditol Acetates. Permethylation of the trisaccharide (360 μmol) was carried out under dry N_2 according to the method of Hakomori (1964). Following the removal of Me_2SO by H_2O extraction, the permethylated oligosaccharide was extracted with $CHCl_3$, hydrolyzed in acid, reduced by $NaBD_4$, and then acetylated as described by Stellner et al. (1973). The partially methylated alditol acetates along with standards were chromatographed isothermally at 180 °C on a 3% OV-225 glass column (6 ft × 2 mm) with a Hewlett-Packard gas chromatograph Model 5800A equipped with a flame ionization detector. The standards include 2,3,4,6-tetramethyl-1,5-diacetylglucitol prepared from methyl α-glucopyranoside, and 2,3,4,6-tetramethyl-1,5-diacetylgalactitol and 2,3,6-trimethyl-1,4,5-triacetylglucitol prepared from lactose, 1,4,5,6-tetramethyl-3-acetyl-2-(*N*-methylacetamido)galactitol prepared from Galβ1→3GalNAc-H_2 by Hakomori's procedure (1964), methyl 2,3,4,6-tetraacetylgalactopyranoside prepared by acetylation of methyl α-galactopyranoside in acetic anhydride at 100 °C for 4 h (Stellner et al., 1973), and galactose pentaacetate. Mass spectra were taken at 70 eV on a Hewlett Packard 5985A GC–MS at the MS facility, Department of Biochemistry, Michigan State University. The analysis was performed on a glass column (6 ft × 2 mm) packed with 3% OV-225 with a temperature program of 170–210 °C at 2 °C/min.

Periodate Oxidation of the Trisaccharide. The trisaccharide (100 nmol), dried in a screw-cap test tube (125 × 14 mm), was treated with 0.15 mL of 30 mM $NaIO_4$ in 50 mM sodium acetate buffer, pH 5.1, at 4 °C for 16 h in the dark. Excess IO_4^- was destroyed by adding 5 μL of ethylene glycol followed by a 4-h incubation at room temperature. Reduction was carried out by adding 0.1 mL of 1 M $NaBH_4$ in 1 M NaOH followed by a 16-h incubation. Hydrolysis was performed at 100 °C for 4 h after 2 mL of 4 N HCl was added. HCl was removed under reduced pressure. The sample was evaporated twice after the addition of 1 mL of water. A second reduction employing the same conditions was carried out. Finally, excess BH_4^- was destroyed with several drops of glacial acetic acid and the borate removed by evaporation 3 times with 1 mL of MeOH. The amino alcohols were separated on a Beckman amino acid analyzer Model 119 CL with a pH 5.28 citrate–borate buffer containing 15% 1-propanol as previously described (Cheng & Boat, 1978).

Glycosidase Treatment of the Trisaccharide. For β-hexosaminidase treatment, a reaction mixture containing 20 nmol of ^{14}C-labeled trisaccharide, 50 μg of BSA, 2 μmol of citrate–phosphate buffer, pH 4.2, 5.2 units of jack bean β-hexosaminidase, and 1 drop of toluene in a total volume of 25 μL was incubated for 16 h at 37 °C. Five milligrams of BSA and 2 μmol each of galactose, raffinose, stachyose, *N*-acetylglucosamine, and *N*,*N*′-diacetylchitobiitol were added to the reaction mixture to a final volume of 1 mL prior to Bio-Gel P-4 (−400 mesh) column (215 × 1.6 cm) chromatography. The column was eluted with 0.1 M pyridinium acetate, pH 5.0. BSA was monitored by absorbance at 280 nm, ^{14}C-labeled sugar was monitored by scintillation counting, neutral sugars were monitored by the anthrone procedure (Morris, 1948), and sugar alcohols were monitored by the chromotropic acid method (O'Dea & Gibbons, 1953).

For β-galactosidase treatment, a reaction mixture (40 μL) that contained 15 nmol of the ^{14}C-labeled trisaccharide, 2 μmol of citrate–phosphate buffer, pH 4.3, 50 μg of BSA, 0.32 unit of bovine testicular β-galactosidase, and 1 drop of toluene was incubated at 37 °C for 16 h. The rest of the procedure was the same as that described above. Hydrolysis of the disaccharide, Galβ1→3GalNAc-H_2(44 nmol), was carried out under identical conditions. Gas chromatography using trimethylsilyl derivatives was employed as previously described (Cheng & Bona, 1982) for detection of the disaccharide and the liberated sugars.

Proton Nuclear Magnetic Resonance Studies. ^{1}H NMR spectra of the ^{14}C-labeled trisaccharide (0.4 μmol) and Galβ→3GalNAc-H_2 (2.0 μmol) were obtained on a Bruker 270/180 Fourier-transform NMR spectrometer. Exchangeable hydrogens were removed by three cycles of dissolving the sample in 1.0 mL of 99.75% D_2O and removing the solvent under reduced pressure. The oligosaccharides were then dissolved in 0.3 mL of 99.75% D_2O and 270-MHz spectra obtained in a 5-mm NMR tube at ambient temperature. A total of 56 and 316 scans were accumulated for the di- and trisaccharide, respectively. Residual D_2O was suppressed by saturating the resonance for 2 s prior to a 90° (6-μs) observation pulse (Redfield & Gupta, 1971). A spectral width of 4000 Hz was used with an acquisition time of 2 s. Acetone, which has a chemical shift of 2.225 ppm from tetramethylsilane, was included as a secondary internal standard. Final spectra contained 8K real data points giving 0.244 Hz/point resolution.

High-Performance Liquid Chromatography (HPLC) Analysis. The oligosaccharides (5–20 nmol) were chromatographed on a Varian 5000 liquid chromatograph equipped with a bonded primary amine column (25 cm × 4.6 mm), at a linear gradient of 85% (v/v) acetonitrile in water to 60% (v/v) acetonitrile in water for a 90-min program and a flow rate of 1 mL/min (Lamblin et al., 1981). Oligosaccharides

Table I: Rabbit Intestinal FPDG *N*-Acetylglucosaminyltransferase. Effect of Divalent Cations[a]

divalent cations (3 mM)	relative activity	divalent cations (3 mM)	relative activity
	100[b]	$Co(NO_3)_2$	67
$MnCl_2$	140	$FeSO_4$	24
$CaCl_2$	146	$CdCl_2$	13
$MgCl_2$	150	$ZnCl_2$	9
$BaCl_2$	134		

[a] The incubation mixture in a total volume of 50 μL contained the following components: UDP-[^{14}C]GlcNAc (sp act. 1.23 mCi/mmol), 0.1 μmol; MOPS buffer, pH 7.5, 2.5 μmol; Triton X-100, 1.25% (v/v); $MnCl_2$ (or other divalent cation), 0.15 μmol; BSA, 50 μg; FPDG acceptor, 0.5 μmol; enzyme protein, 85 μg; ATP, 0.25 μmol. After incubation at 37 °C for 60 min, the enzyme reaction was stopped by addition of 15 μL of 0.25 M GTP and 0.40 mL of ice-cold 1 M NaCl. The ^{14}C-labeled product was then isolated by the Bio-Gel P-4 column method (Cheng & Bona, 1982). [b] The specific activity without added divalent cations was 31.1 nmol h^{-1} (mg of protein)$^{-1}$.

were monitored by the absorbance at 206 nm with a Varian UV-50 detector.

Other Methods. Protein was estimated by the procedure of Lowry et al. (1951) with BSA as the standard. Quantitation of hexosamines and hexosaminitol was performed by the method of Cheng & Boat (1978) with ξ-aminocaproic acid as the internal standard. Galactose was analyzed by gas–liquid chromatography following methanolysis in 0.5 N HCl in anhydrous methanol (Boat et al., 1976) as modified from the procedure of Clamp et al. (1971). The standard amino alcohols, serinol (SerN-H_2), threosaminitol (ThrN-H_2), arabinosaminitol (AraN-H_2), and galactosaminitol (GalN-H_2), were prepared by limited periodate oxidation of *N*-acetylgalactosaminitol as previously described (Cheng & Bona, 1982).

Results

Requirements for Enzyme Activity. Optimal FPDG *N*-acetylglucosaminyltransferase activity was obtained in the presence of 0.08% (v/v) Triton X-100. At this concentration, the enzyme activity was enhanced approximately 5-fold. At Triton X-100 concentrations between 0.5 and 5%, the enhancement of enzyme activity was dropped to 3-fold. Enzyme activity was slightly enhanced by 3 mM $MnCl_2$. However, inhibition occurred at Mn^{2+} concentrations exceeding 10 mM. In the absence of added divalent cations, EDTA at concentrations ranging from 1 to 20 mM did not significantly affect enzyme activity. Ca^{2+}, Mg^{2+}, and Ba^{2+} all increased the enzyme activity, while Co^{2+}, Fe^{2+}, Cd^{2+}, and Zn^{2+} were inhibitory (Table I). FPDG *N*-acetylglucosaminyltransferase exhibited maximal enzyme activity at pH 7.0–7.5. Addition of GTP (35 mM) inhibited at least 90% of the enzyme activity.

Kinetic Parameters and Acceptor Specificity. The apparent Michaelis constants for UDP-GlcNAc, freezing point depression glycoprotein, Galβ1→3GalNAcαPh, and IO_4^--PSM were 1.03, 4.80, 0.49, and 0.59 mM, respectively. No transfer of [^{14}C]GlcNAc to the acceptor was observed when asialo ovine submaxillary mucin (0.25–1.00 mg) was used as the acceptor.

Purification and Characterization of the ^{14}C-Labeled Trisaccharide from the Product of FPDG N-Acetylglucosaminyltransferase. The ^{14}C-labeled product of FPDG *N*-acetylglucosaminyltransferase from a large-scale preparation was isolated on Bio-Gel P-4 (Figure 1). About 40% of the [^{14}C]GlcNAc was transferred from UDP-[^{14}C]GlcNAc to the acceptor. Following alkaline borohydride treatment of the

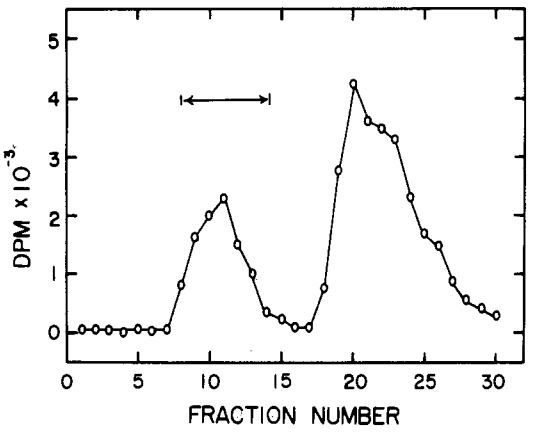

FIGURE 1: Bio-Gel P-4 (200–400 mesh, 29 × 1 cm) isolation of ^{14}C-labeled product of FPDG *N*-acetylglucosaminyltransferase. 2 mL of the large-batch reaction mixture containing 1 M NaCl was applied. Fraction size was 0.9 mL. 40% of the total radioactivity was recovered in the first peak, which contained the product.

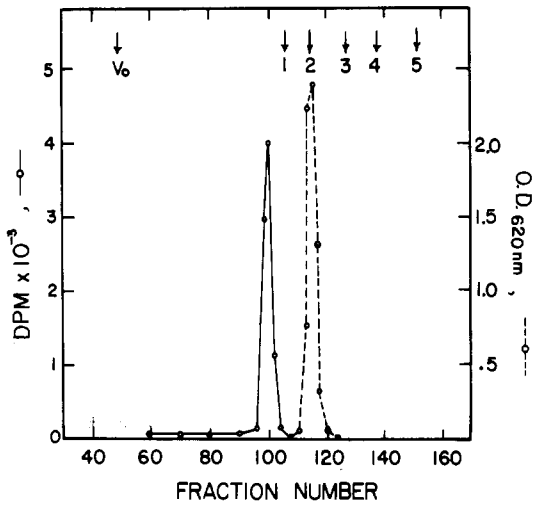

FIGURE 2: Bio-Gel P-2 (–400 mesh, 2.5 × 180 cm) purification of the ^{14}C-labeled oligosaccharide obtained by alkaline–borohydride (Carlson, 1968) treatment of the ^{14}C-labeled product (Figure 1). The column was standardized with the following oligosaccharides: (1) Fucα1→2Galβ1→3GalNAc-H_2; (2) Galβ1→3GaNAc-H_2; (3) GalNAc-H_2; (4) GlcNAc and lactose; (5) Gal. The column was eluted with glass-distilled water at the rate of 27 mL/h, and the volume of each fraction collected was 4.6 mL. Aliquots were analyzed by both radioactivity (—) and the anthrone (– –) assay (Morris, 1948).

^{14}C-labeled product, only one radioactive peak, which corresponded to a trisaccharide, was recovered by Bio-Gel P-2 column chromatography (Figure 2). Since GlcNAc behaves like dihexose and GalNAc-H_2 like trihexose (Cheng & Bona, 1982), the trisaccharide eluted like a hexahexose and Galβ1→3GalNAc-H_2 like a tetrahexose on Bio-Gel P-2 chromatography. This elution profile suggests that only one GlcNAc was added to the FPDG dissacharide side chains. Forty percent of the [^{14}C]GlcNAc label in peak 1 of the Bio-Gel P-4 column (Figure 1) was recovered in the trisaccharide. The rest of the radioactivity was either lost during the dialysis step (20%) or not completely eliminated and got trapped on the Dowex 50 H^+ column (30%). The trisaccharide exhibited a symmetrical peak on Bio-Gel P-4 chromatography (Figure 3A), gave only one major peak at a retention time of 53.72 min by HPLC on a bonded primary amine column (Figure 4), and contained equimolar amounts of GlcNAc, Gal, and GalNAc-H_2 by compositional analysis.

Determination of the N-Acetylglucosaminide Linkage. GC analysis under isothermal (180 °C) conditions of the partially methylated alditol acetate derivatives of the trisaccharide

Table II: ^{1}H NMR Chemical Shifts of the C-3, C-4, C-5, and C-6 Protons of GalNAc-H_2 in the Oligosaccharides

GalNAc-H_2 residue	chemical shift (ppm)			
	^{14}C-labeled trisaccharide[a]	Galβ1→3(NeuGcα2→6)GalNAc-H_2[b]	Galβ1→3GalNAc-H_2 this study[c]	Galβ1→3GalNAc-H_2 literature[b]
H-3	4.06	4.06	4.06	4.06
H-4	3.50	3.53	3.50	3.51
H-5	4.28	4.15	4.20	4.20
H-6	3.9[d]	3.86	3.69	3.69
H-6′	3.7[d]	3.49	3.65	3.65

[a] 0.4 μmol of the trisaccharide was dissolved in 0.3 mL of D_2O. [b] Van Halbeek et al., 1981. [c] 2.0 μmol of the disaccharide was dissolved in 0.3 mL of D_2O. [d] Chemical shift determined from decoupling the H-5 proton of GalNAc-H_2.

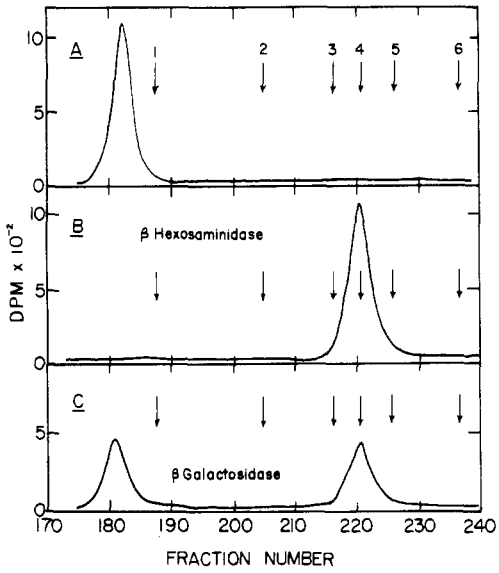

FIGURE 3: Bio-Gel P-4 (−400 mesh, 1.6 × 215 cm) separation of the ^{14}C-labeled trisaccharide before and after glycosidase treatment. The column was eluted with 0.1 M pyridine acetate, pH 5.0, at a flow rate of 20 mL/h. The fraction size was 1.8 mL. The P-4 column was standardized with the following oligosaccharides: (1) *N,N′*-diacetylchitobiitol; (2) stachyose; (3) raffinose; (4) GlcNAc, (5) lactose; (6) Gal. The column fractions were monitored by radioactivity measurement. (Panel A) ^{14}C-Labeled trisaccharide; (panel B) trisaccharide after jack bean β-hexosaminidase treatment; (panel C) trisaccharide after bovine testicular β-galactosidase treatment.

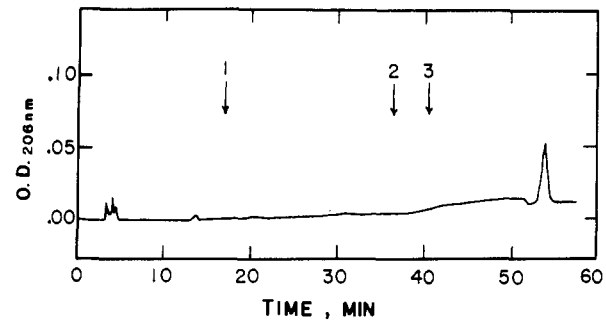

FIGURE 4: HPLC profile of the ^{14}C-labeled trisaccharide (8 nmol). The bonded primary amine (5-μm sphere particle) column (25 × 0.46 cm) was eluted with a gradient of acetonitrile in water 85–60% for 90 min at a flow rate of 1 mL/min. The column was standardized with the following oligosaccharides (8–20 nmol): (1) GalNAc-H_2 (16.91 min); (2) Galβ1→3GalNAc-H_2 (36.34 min); (3) Fucα1→2Galβ1→3GalNAc-H_2 (40.57 min). The retention time for the ^{14}C-labeled trisaccharide was 53.62 min.

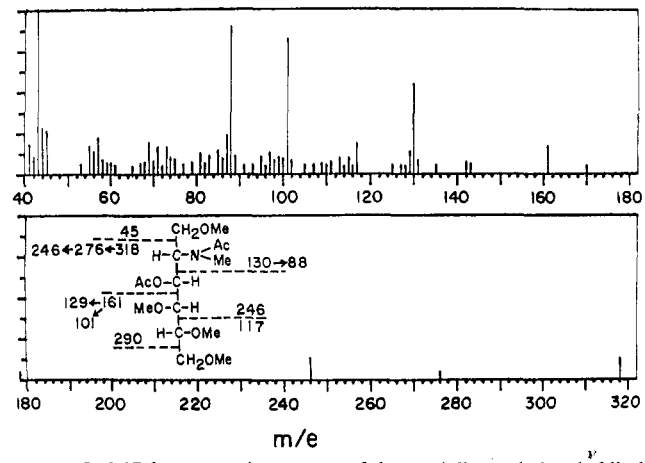

FIGURE 5: MS fragmentation pattern of the partially methylated alditol acetate of *N*-acetylgalactosaminitol obtained from the trisaccharide. The abscissa indicates ion fragments (*m/e*), and the vertical axis is the relative ion intensity. The insert shows the primary and secondary ion fragmentation schemes.

showed three major carbohydrate peaks, 1–3, with retention times of 1.18, 7.14, and 9.76 relative to that of 2,3,4,6-tetramethyl-1,5-diacetylglucitol. The relative molar ratios of peaks 1–3 obtained from the GC analysis were 1.00:0.71:0.31. Peak 1 coeluted with standard 2,3,4,6-tetramethyl-1,5-diacetylgalactitol, suggesting that the galactose residue is unsubstituted. This was confirmed by the presence of primary ion fragments at *m/e* 161, 162, and 205 in the mass spectrum of peak 1 (Bjorndahl et al., 1967). Peak 2 gave primary ion fragments of *m/e* 45, 159, 161, 203, 205, and 245, indicating its structure to be 3,4,6-trimethyl-1,5-diacetyl-2-(*N*-methylacetamido)glucitol derived from unsubstituted GlcNAc. The primary mass fragments of peak 3 had *m/e* values of 45, 117, 130, 161, 246, and 318 with significant secondary fragments at 101 and 88 (Figure 5). The presence of ion fragment 246 indicates that the GalNAc residue is substituted at C-3 and C-6. Thus, GlcNAc must be linked to C-6 rather than C-4 of GalNAc-H_2.

The ^{1}H NMR spectrum of the trisaccharide is shown in Figure 6. The resonances of the Gal and GalNAc-H_2 protons were assigned by comparison with published results (Van Halbeek, 1981) and by homodecoupling techniques. Table II shows the chemical shifts of each proton at C-3 to C-6 of GalNAc-H_2 in the [^{14}C]GlcNAc-labeled trisaccharide, Galβ1→3(NeuGcα2→6)GalNAc-H_2, and Galβ1→3GalNAc-H_2. The chemical shifts of H-6′ and the protons at C-3 and C-4 are the same in both the tri- and disaccharides. A slight shift (+0.08 ppm) of H-5, together with a larger shift for H-6 (+0.2 ppm) from the corresponding protons in the disaccharide, suggests that GlcNAc is linked to C-6 of GalNAc-H_2.

Periodate oxidation of the trisaccharide destroyed all of the GalNAc-H_2 and 80–90% of the GlcNAc. Following periodate oxidation, $NaBH_4$ reduction, 4 N HCl hydrolysis, and a second $NaBH_4$ reduction, two amino alcohols were identified on an amino acid analyzer (Figure 7) as serinol (peak 4) and threosaminitol (peak 3), respectively. Serinol was produced from GlcNAc while the detection of threosaminitol indicates

Table III: Rabbit Intestinal Mucin *N*-Acetylglucosaminyltransferase Acceptor Competition Study[a]

	acceptor concn (mM)		[^{14}C]GlcNAc transferred [nmol h^{-1} (mg of protein)$^{-1}$]			
				theoretical value		
expt	FPDG[b]	IO_4^--PSM[c]	exptl value	1 E[d]	2 E[e]	conclusion
1	1.28		57.6			
2	2.56		77.8			
3	5.10		87.1			
4		0.43	32.3			
5		0.86	43.7			
6		1.29	51.8			
7	1.28	0.43	63.0	62.0	89.9	
8	2.56	0.86	78.8	75.6	121.5	1 E
9	5.10	1.29	90.1	84.0	138.9	

[a] The complete reaction mixture and the detailed experimental conditions with the exception of acceptors are given in Table I. [b] Fractions 7 + 8 of FPDG (DeVries et al., 1970). [c] A$^+$ PSM treated successively with periodate, $NaBH_4$, and mild acid. [d] Equation for the calculation of the apparent velocity for the one-enzyme-two-activities system (Dixon & Webb, 1964) is $v = (V_1S_1/K_1 + V_2S_2/K_2)/(1 + S_1/K_1 + S_2/K_2)$, where v, V, S, and K are apparent velocity, maximal velocity, substrate concentrations, and Michaelis constant, respectively. 1 and 2 represent the acceptors, FPDG and IO_4^--PSM, respectively. V_1 = 105.3 nmol h^{-1} (mg of protein)$^{-1}$; V_2 = 75.2 nmol h^{-1} (mg of protein)$^{-1}$; K_1 = 1.06 mM; K_2 = 0.59 mM. The kinetic constants were obtained from experiments 1-6. [e] The sum of apparent velocities obtained from the assay of each acceptor separately.

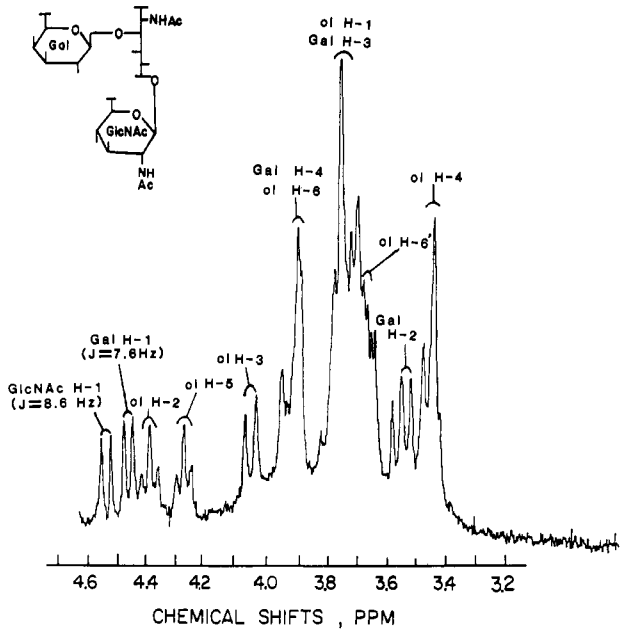

FIGURE 6: ^{1}H NMR (270-MHz) spectrum of the ^{14}C-labeled trisaccharide. 0.4 μmol of the trisaccharide was dissolved in 0.3 mL of 99.75% D_2O. The spectrum was taken after scanning for 2 h at room temperature. Acetone was included as the secondary internal standard, which appears at 2.225 ppm relative to the primary standard tetramethylsilane. The proton resonances that have been identified are given in the figure. ol represents *N*-acetylgalactosaminitol.

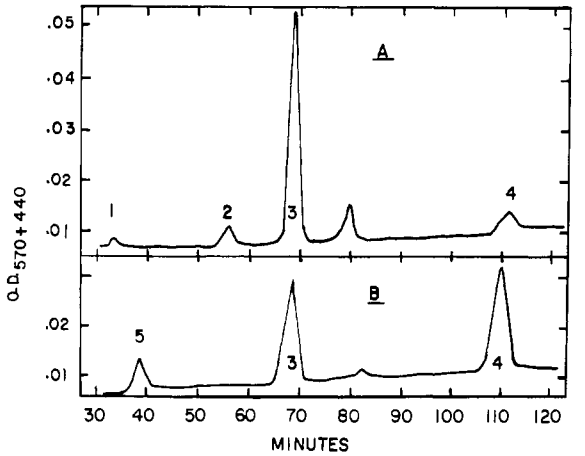

FIGURE 7: Amino acid analyzer separation of amino alcohols. (A) Separation profile of the standard amino alcohols obtained from GalNAc-H_2 by limited periodate oxidation as previously described (Cheng & Bona, 1982): (peak 1) GalN-H_2; (peak 2) AraN-H_2; (peak 3) ThrN-H_2; (peak 4) SerN-H_2. (B) Separation profile of the amino alcohols obtained from the ^{14}C-labeled trisaccharide after treatment with periodate. Peak 5 is GlcN-H_2. The amino alcohols were eluted from the column (0.6 × 30 cm), packed with sperical type W-3H resin, with a citrate–borate buffer, pH 5.28, containing 15% 1-propanol (Cheng & Boat, 1978). The column temperature was 55 °C and the buffer flow rate 44 mL/h (Cheng & Bona, 1982). The amino alcohols were detected with ninhydrin reagent.

that the linkage of GlcNAc to GalNAc-H_2 is 1→6.

Determination of the Anomeric Configuration of the N-Acetylglucosaminide. As shown in Figure 3B, jack bean β-hexosaminidase completely cleaved the GlcNAc residue from the ^{14}C-labeled trisaccharide, suggesting that the newly formed *N*-acetylglucosaminide was a β-anomer. However, bovine testicular β-galactosidase, which readily cleaved the galactose residue from Galβ1→3GalNAc-H_2, failed to cleave the galactose residue from the trisaccharide under identical experimental conditions (Figure 3C). Partial cleavage of [^{14}C]-GlcNAc was due to trace contamination of β-hexosaminidase in the testicular β-galactosidase preparation.

Proton NMR (270 MHz) identified the GlcNAc linkage as a β-anomer by the coupling constant ($J_{1,2}$ = 8.6 ± 0.2 Hz) of the C-1 proton at the chemical shift of 4.54 ppm. The coupling constant of the C-1 proton of Gal residue was 7.6 ± 0.2 Hz at the chemical shift of 4.47 ppm, indicating a β-anomer (Van Halbeek, 1981). The ratio of the peak areas of the corresponding anomeric hydrogens of GlcNAc and Gal was 1.00:1.02 (Figure 6).

Acceptor Competition Studies. To determine whether the FPDG *N*-acetylglucosaminyltransferase is a mucin *N*-acetylglucosaminyltransferase, a mixed acceptor experiment was carried out. IO_4^--PSM was employed as the mucin acceptor. The experimental conditions and the results are shown in Table III. The enzyme activities obtained from the mixed acceptor experiment, as shown in experiments 7–9, were compared with the theoretical values calculated from the experimental data from experiments 1–6. The experimental data agree with those calculated for one enzyme system, indicating that probably the mucin *N*-acetylglucosaminyltransferase catalyzes the reaction for both acceptors.

Discussion

We have characterized a rabbit small intestinal epithelial enzyme that transfers GlcNAc from UDP-GlcNAc to FPDG

as UDP-GlcNAc:Gal β-3-GalNAc (GlcNAc → GalNAc) β-6-*N*-acetylglucosaminyltransferase. The Galβ1→3-(GlcNAcβ1→6)GalNAc-Ser(Thr) linkage has been identified previously in several mucous glycoproteins including human tracheobronchial (Lamblin et al., 1981), gastric (Oates et al., 1974), ovarian cyst (Rovis et al., 1973), and cervical (Yurewicz et al., 1982) mucins, and equine (Newman & Kabat, 1976) and hog (Kochetkov et al., 1976) gastric and canine submaxillary (Lombart & Winzler, 1974) mucins. However, the enzyme responsible for this structure was only recently identified and described in the canine submaxillary glands by Williams et al. (1980). In addition, we have found FPDG *N*-acetylglucosaminyltransferase activity in bovine, human, rabbit, and cat tracheal epithelia and human intestinal epithelium (data not included). The enzyme from rabbit intestine shares many properties with the canine submaxillary enzyme. Enzyme activities from both sources have an optimal pH near neutral, are slightly stimulated in the presence of a low Mn^{2+} concentration (3 mM), are not affected significantly by EDTA, require approximately 0.1% Triton X-100 for maximal activity, and have apparent Michaelis constant of approximately 1 mM for UDP-GlcNAc and 0.5–1.0 mM for the phenyl derivatives of Galβ1→3GalNAc. However, the rabbit intestinal enzyme has a lower apparent K_m for FPDG (4.80 mM). The rabbit intestinal enzyme has lower K_m (0.59 mM) for PSM than does canine enzyme (5.2 mM) (Williams & Schachter, 1980; Williams et al., 1980). The possible explanation for this discrepancy may well be that the IO_4^--PSM we prepared for this study not only is devoid of the peripheral sugars but also retains a more intact mucin peptide than does the acid- (in HCl, 70 °C, 2 h) treated PSM used for the previous studies. Furthermore, the canine submaxillary enzyme is unstable at 37 °C, but rabbit intestinal enzyme is stable at this temperature for at least 60 min. In our study, only one product, a trisaccharide, was formed, whereas the canine enzyme produced not only a trisaccharide but also a disaccharide, GlcNAcβ1→6GalNAc-*O*-*p*-NO_2Ph.

The product of rabbit intestinal FPDG *N*-acetylglucosaminyltransferase was identified as Galβ1→3(GlcNAcβ1→-6)GalNAc-H_2 following alkaline borohydride treatment of the ^{14}C-labeled product. GC–MS analysis of the partially methylated alditol acetates prepared from the trisaccharide showed that galactose was unsubstituted and GalNAc-H_2 was substituted at both C-3 (by galactose) and C-6 (by GlcNAc) (Figure 5). Periodate oxidation of the trisaccharide followed by $NaBH_4$ reduction, 4 N HCl hydrolysis, and a second $NaBH_4$ reduction resulted in two amino alcohols, serinol (peak 4) and threosaminitol (peak 3) (Figure 7). Serinol was formed from GlcNAc. Depending upon the linkage of GlcNAc to GalNAc-H_2, different amino alcohols can be formed from GalNAc-H_2 following periodate oxidation as described above. For example, if the linkage is 1→4, arabinosaminitol will be obtained, and if it is a 1→6 linkage, threosaminitol will be formed. The absence of AraN-H_2 and the presence of ThrN-H_2 (Figure 7) support the conclusion that GlcNAc is linked 1→6 to GalNAc-H_2. ^1H NMR studies of the trisaccharide also confirmed the assignment of the 1→6 linkage of GlcNAc to GalNAc-H_2 (Figure 6 and Table II). The β-anomeric configuration of the *N*-acetylglucosaminide was identified by its complete cleavage with β-hexosaminidase (Figure 3B). The H-1 in GlcNAc should give a coupling constant of 3.5 Hz for the α-anomer and 8.0 Hz for the β-anomer (Kamerling et al., 1975). The coupling constant (8.6 Hz) obtained for the GlcNAc C-1 proton confirms the β-configuration assignment obtained from the β-hexosaminidase experiment. The failure of bovine testicular β-galactosidase to hydrolyze galactose from the trisaccharide was unexpected (Figure 3C) because the same enzyme can readily hydrolyze Galβ1→3GalNAc-H_2 under identical conditions (Cheng & Bona, 1982). A similar observation was also made recently by Yurewicz et al. (1982), who reported that *Aspergillus niger* β-galactosidase could not hydrolyze the galactose from the same trisaccharide until GlcNAc had been removed.

Microheterogeneity of mucin oligosaccharides is a general phenomenon. Carbohydrate chains with lengths ranging from a single sugar up to 20 sugars with branching structures have been reported. The presence of GlcNAc in the oligosaccharide chain may determine the potential for formation of long carbohydrate chains. For example, the longest carbohydrate chain that does not contain GlcNAc is the A^+ PSM pentasaccharide GalNAcα1→3(Fucα1→2)Galβ1→3(NeuGcα2→-6)GalNAc (Carlson, 1968). All mucin oligosaccharides can thus be divided into two groups, the simple chains that do not contain GlcNAc and the complex oligosaccharide chains that do. The structures of *N*-acetylglucosaminides found in complex oligosaccharide chains are GlcNacα1→4Gal (Kochetkov, 1976), GlcNAcα1→4GlcNAc (Newman & Kabat, 1976), GlcNAcβ1→4Gal (Rovis et al., 1973), GlcNAcβ1→6Gal (Rovis et al., 1973), GlcNAcβ1→6GalNAc (Yurewicz et al., 1982a; Lombart & Winzler, 1974), GlcNAcβ1→3Gal, and GlcNAcβ1→3GalNAc (Lamblin et al., 1980). When Galβ1→3GalNAcαR is used as the acceptor, five potentially known structures (Rovis et al., 1973; Lamblin et al., 1980; Kochetkov et al., 1976) can be formed. However, only one product, i.e., Galβ1→3(GlcNAcβ1→6)GalNAcαR, was formed. The acceptor competition study indicated that the enzyme activity that catalyzed the transfer of GlcNAc from UDP-GlcNAc to FPDG was probably the same enzyme as that utilizing IO_4^--PSM as the acceptor (Table III). No enzyme activity was detected when asialo ovine submaxillary mucin was employed as the acceptor although two potential structures, 1→3 and 1→6, are possible. It appears that the Gal residue is essential for the β-6- (to GalNAc) *N*-acetylglucosaminyltransferase activity. A similar observation also was reported for canine submaxillary enzyme (Williams & Schachter, 1980; Williams et al., 1980). The reason why only Galβ3(GlcNAcβ6)GalNAcαR was synthesized by intestinal and submaxillary tissue is not clear. We postulated that C-6 of GalNAc is an important regulation point for mucin carbohydrate chain synthesis (Cheng & Bona, 1982). Either neuraminic acid, a chain-terminating sugar, or GlcNAc, a sugar that permits additional chain growth, can be attached at this site. The addition of GlcNAc to C-6 of GalNAc may be a prerequisite for the synthesis of other *N*-acetylglucosaminides, such as α1→4Gal, β1→4Gal, β1→3Gal, and β1→-6Gal. Although this sugar addition prevents the formation of oligosaccharides containing neuraminic acid linked 2→6 to GalNAc, it does not preclude the formation of other sialylated oligosaccharides. The GlcNAc-containing sialylated oligosaccharides have been found in human cervical mucin (Yurewicz et al., 1982b). Characterization of the mucin *N*-acetylglucosaminyltransferase is an important step in understanding the regulation of the synthesis of complex oligosaccharide chains in mucins.

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Registry No. UDP-GlcNAc, 528-04-1; Galβ1→3GalNAcαPh,

74211-30-6; Galβ1→3(GlcNAcβ1→6)GalNAc-H_2, 60174-22-3; UDP-acetylglucosamine–mucin acetylglucosaminyltransferase, 75718-18-2.

References

Beyer, T. A., Sadler, J. E., & Hill, R. L. (1980) *J. Biol. Chem. 225*, 5364–5372.

Beyer, T. A., Sadler, J. E., Rearick, J. I., Paulson, J. C., & Hill, R. L. (1981) *Adv. Enzymol. Relat. Areas Mol. Biol. 52*, 22–175.

Bjorndahl, H., Lindberg, B., & Svensson, S. (1967) *Acta Chem. Scand. 21*, 1808–1814.

Boat, T. F., & Cheng, P.-W. (1976) in *Cystic Fibrosis: Projections into the Future* (Mangos, J. A., & Talamo, R. C., Eds.) pp 165–172, Stratton International Medical Co., New York.

Boat, T. F., Cheng, P.-W., Iyer, R., Carlson, D. M., & Polony, I. (1976) *Arch. Biochem. Biophys. 177*, 95–104.

Carlson, D. M. (1968) *J. Biol. Chem. 243*, 616–626.

Cheng, P.-W., & Boat, T. F. (1978) *Anal. Biochem. 85*, 276–282.

Cheng, P.-W., & Bona, S. J. (1982) *J. Biol. Chem. 257*, 6251–6258.

Clamp, J. R., Bhatti, T., & Chambers, R. E. (1971) *Methods Biol. Anal. 19*, 229–344.

De Salegui, M., & Plonska, H. (1969) *Arch. Biochem. Biophys. 129*, 49–56.

DeVries, A. L., Komatsu, S. K., & Feeney, R. E. (1970) *J. Biol. Chem. 245*, 2901–2908.

Distler, J. J., & Jourdian, G. W. (1978) *Methods Enzymol. 1*, 514–520.

Dixon, M., & Webb, E. C. (1964) in *The Enzymes*, pp 84–86, Longmans, London.

Faltynek, C. R., Silbert, J. E., & Hof, L. (1981) *J. Biol. Chem. 256*, 7139–7141.

Hakomori, S. (1964) *J. Biochem. (Tokyo) 55*, 205–208.

Jentoft, N., Cheng, P.-W., & Carlson, D. M. (1976) in *The Enzymes of Biological Membranes* (Martonosi, A., Ed.) Vol. 2, pp 343–383, Plenum, New York.

Kamerling, J. P., Gerwig, G. J., & Vliegenthart, J. F. G. (1975) *Biochem. J. 151*, 491–495.

Kochetkov, N. K., Derevitskaya, V. A., & Arbatsky, N. P. (1976) *Eur. J. Biochem. 67*, 129–136.

Lamblin, G., Lhermitte, M., Boersma, A., Roussel, P., & Rheihold, V. (1980) *J. Biol. Chem. 255*, 4595–4598.

Lamblin, G., Lhermitte, M., Boersma, A., Roussel, P., Van Halbeek, H., Dorland, L., & Vliegenhart, J. F. G. (1981) *Monogr. Paediatr. 14*, 46–49.

Li, S.-C., Mazzotta, M. Y., Chien, S. F., & Li, Y.-T. (1975) *J. Biol. Chem. 250*, 6786–6791.

Lin, Y., & DeVries, A. (1976) *Biochem. Biophys. Res. Commun. 59*, 1192–1196.

Lombart, C. G., & Winzler, R. J. (1974) *Eur. J. Biochem. 49*, 77–86.

Lowry, O. D., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Matta, K. L., & Barlow, J. J. (1975) *Carbohydr. Res. 43*, 299–304.

McGuire, E. J., & Roseman, S. (1967) *J. Biol. Chem. 242*, 3745–3747.

Mendicino, J., Sivikami, S., Davila, M., & Chandrasekaran, E. V. (1982) *J. Biol. Chem. 257*, 3987–3994.

Morris, D. L. (1948) *Science (Washington, D.C.) 107*, 254–255.

Newman, W., & Kabat, E. A. (1976) *Arch. Biochem. Biophys. 172*, 535–550.

Oates, M. D. G., Rosbottom, A. C., & Schrager, J. (1974) *Carbohydr. Res. 34*, 115–137.

O'Dea, J. F., & Gibbons, R. A. (1953) *Biochem. J. 55*, 580–586.

Pigman, W., Mandel, I. D., Horowitz, M. I., Holden, K. G., Griggs, L. J., & Doehr, S. A. (1977) in *The Glycoconjugates* (Horowitz, M. I., & Pigman, W., Eds.) Vol. 1, pp 131–256, Academic Press, New York.

Prieels, J.-P., Monnom, D., Dolmans, M., Beyer, T. A., & Hill, R. L. (1981) *J. Biol. Chem. 256*, 10456–10463.

Redfield, A. G., & Gupta, R. K. (1971) *J. Chem. Phys. 54*, 1418–1419.

Rovis, L., Anderson, B., Kabat, E. A., Gruezo, F., & Liao, J. (1973) *Biochemistry 12*, 5340–5354.

Sadler, J. E., Rearick, J. I., & Hill, R. L. (1979a) *J. Biol. Chem. 254*, 5934–5941.

Sadler, J. E., Rearick, J. I., Paulson, J. C., & Hill, R. L. (1979b) *J. Biol. Chem. 254*, 4434–4443.

Schachter, H., Narasimhan, S., & Wilson, J. R. (1978) *ACS Symp. Ser. No. 80*, 108–121.

Schwyzer, M., & Hill, R. L. (1977) *J. Biol. Chem. 252*, 2338–2345.

Sheares, B. T., Lau, J. T. Y., & Carlson, D. M. (1982) *J. Biol. Chem. 257*, 599–602.

Stellner, K., Saito, H., & Hakomori, S. (1973) *Arch. Biochem. Biophys. 155*, 464–472.

Van Halbeek, H., Dorland, L., Haverkamp, J., Veldink, G., Vliegenthart, J. F. G., Fournet, B., Ricart, G., Montreuil, J., Gathmann, W. D., & Aminoff, D. (1981) *Eur. J. Biochem. 118*, 487–495.

Williams, D., & Schachter, H. (1980) *J. Biol. Chem. 255*, 11247–11252.

Williams, D., Longmore, G., Matta, K. L., & Schachter, H. (1980) *J. Biol. Chem. 255*, 11253–11261.

Yurewicz, E. C., Matsura, F., & Moghissi, K. S. (1982a) *J. Biol. Chem. 257*, 2314–2322.

Yurewicz, E. C., Matsura, F., & Moghissi, K. S. (1982b) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 41* (4), 887.