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N-ACETYL-β-D-GLUCOSAMINYLTRANSFERASES RELATED TO THE SYNTHESIS OF MUCIN-TYPE GLYCOPROTEINS IN HUMAN OVARIAN TISSUE*

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ABSTRACT

The presence of *N*-acetyl-β-D-glucosaminyltransferases in microsome preparations from human ovarian tissues was investigated with UDP-GlcNAc and several synthetic oligosaccharides as acceptors. The products were identified by paper chromatography and the linkage of the 2-acetamido-2-deoxy-β-D-glucopyranosyl group incorporated into oligosaccharides was determined by exoglycosidase digestions, ^{1}H-n.m.r. spectroscopy, and methylation analysis. These results showed that ovarian microsome preparations contain both β-(1→3)- and β-(1→6)-*N*-acetyl-D-glucosaminyltransferase activities which might be involved in the synthesis of mucin-type glycoproteins. Substrate competition tests suggested that both UDP-GlcNAc:-Bn glycoside of β-D-Glc*p*NAc-(1→6)-α-D-Gal*p*NAc [GlcNAc to GalNAc] and -Bn glycoside of β-D-Gal*p*-(1→3)-[β-D-GlcNAc-(1→6)]-α-D-Gal*p*NAc [GlcNAc to Gal] β-(1→3)-*N*-acetyl-D-glucosaminyltransferase activities reside in a single enzyme species.

INTRODUCTION

The oligosaccharide chains of mucin glycoproteins are attached to the peptide backbone by an *O*-glycosyl linkage from a 2-acetamido-2-deoxy-α-D-galactopyranosyl residue to a serine or threonine residue. These oligosaccharides vary in size and complexity, and contain three main regions, *i.e.*, the core, the backbone, and the peripherial regions[1]. The linkages[2] of the 2-acetamido-2-deoxy-β-D-glucopyranosyl residues found in these regions are β-D-Glc*p*NAc-(1→3)-D-GalNAc, β-D-Glc*p*NAc-(1→6)-D-GalNAc, β-D-Glc*p*NAc(1→3)-D-Gal, and β-D-Glc*p*NAc-(1→6)-D-Gal. The last two structures are also important for the formation of the branched structure, β-D-Glc*p*NAc-(1→3)-[β-D-Glc*p*NAc-(1→6)]-D-Gal associated with the I antigenic determinant[3]. *N*-Acetyl-β-D-glucosaminyltransferases respons-

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ible for these structures have been identified in canine submaxillary glands[4–7]; Novikoff ascites tumor cells[8]; hog[6,7,9,10], rat, monkey, and sheep[7] gastric mucosa; rat[6,7], hog, human, and monkey[7] colon; rabbit small intestine[11]; human serum[12–15]; mouse lymphoma[16]; and bovine trachea[17].

In the present investigation, an attempt was made to establish whether the same enzyme or different β-(1→3)- and β-(1→6)-*N*-acetylglucosaminyltransferases catalyze the synthesis of these structures. Oligosaccharides prepared by chemical synthesis in our laboratory were used as acceptors for these enzymes[4–7,9,11]. The use of such compounds has advantages for both detection and characterization of these enzymes. With the aid of these synthetic substrates, we present evidence herein for the existence of a β-(1→3)- and β-(1→6)-*N*-acetylglucosaminyltransferase in human overian microsomes. Substrate competition tests also suggested that two different β-(1→3)-*N*-acetylglucosaminyltransferase activities reside in a single enzyme species.

EXPERIMENTAL

Materials. — They were obtained as follows. UDP-*N*-acetyl-D-[U-^{14}C]glucosamine (11 GBq/mmol) and UDP-D-[1-^{3}H]galactose (380 GBq/mmol) from New England Nuclear; UDP-GlcNAc, Triton X-100, HEPES, *N*-acetyl-β-D-glucosaminidase (jack bean), and BSA from Sigma Chemical Co.; MES from Calbiochem Behring Corp.; Bio-Gel P-2, Dowex AG 1-X8 (100–200 mesh), and Dowex AG 50W-X8 (100–200 mesh) from Bio-Rad Lab., and TLC aluminum sheet Silica gel 60 F_{254} from Merck Chemical Div. The following compounds were synthesized by methods described earlier: β-D-Glc*p*NAc-(1→3)-α-D-Gal*p*NAcOBn and β-D-Glc*p*NAc-(1→6)-α-D-Gal*p*NAcOBn[18]; β-D-Glc*p*NAc-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn[19]; β-D-Glc*p*NAc-(1→6)-β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn and β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn[20]; β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→3)-α-D-Gal*p*NAcOBn[21]; β-D-Gal*p*-(1→3)-α-D-Gal*p*NAcO(2-NP)*, β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcO(2-NP), β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAc, α-D-Gal*p*NAcOBn, and β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn[22].

Enzyme preparations. — Ovarian tissues were obtained during surgical procedures from patients with ovarian cancer. Control tissues corresponding to these tumors were obtained from patients with non-ovarian cancer disease as well as healthy females. Tissues were homogenized with a Polytron in ice-cold 0.25M sucrose containing 0.2M NaCl (10 g of tissue/20 mL of homogenizing fluid). All subsequent steps were done at 4°. The homogenate was centrifuged at 10 000*g* for 20 min, and the supernatant at 100 000*g* for 60 min in a Beckman Model L3-50 ultracentrifuge. The precipitated microsome preparation was resuspended in minimal amounts of homogenizing fluid and stored at −70°.

*Abbreviation: 2-NP, 2-nitrophenyl.

Enzyme assay. — The reaction mixtures used to assay for *N*-acetyl-β-D-glucosaminyltransferase activity contained the following components in a total volume of 70 μL: UDP-D-[^{14}C]GlcNAc (1.85 kBq), UDP-D-GlcNAc, (1 μmol); $MnCl_2$ (0.5 μmol), ATP (0.5 μmol), HEPES (pH 7.5) (5 μmol), Triton X-100 (0.3%), acceptor (0.2 μmol), and microsome preparation (~0.4 mg of protein). After incubation at 37° for 3 h, an equal volume of ethanol was added and the mixture was centrifuged at 4000*g* for 20 min. The supernatant was deposited onto Whatman 3 MM paper and chromatographed in 12:5:4 (v/v) ethyl acetate–pyridine–water (solvent *A*), or in 6:4:3 (v/v) butanol–pyridine–water (solvent *B*). The radioactive-labeled oligosaccharides formed were estimated by reference to compounds of known structure run on the same chromatogram. The radioactivity was counted with a Beckman LS 9000 liquid-scintillation spectrometer.

Preparation of the ^{14}C-labeled products. — A large-scale synthesis of the labeled products was carried out with the reaction mixture containing 3mM UDP-[^{14}C]GlcNAc (37 kBq), 10mM $MnCl_2$, 10mM ATP, 100mM HEPES (pH 7.5), Triton X-100 (2.5 μL), 3mM acceptor, and enzyme preparation (0.4 mg of protein) in a total volume of 1 mL. After incubation at 37° for 3 h, ethanol (1 mL) was added and the mixture was centrifuged as described above. The supernatant was applied to a column (0.8 × 5 cm) of Dowex AG 1-X8 (Cl^-). The product was eluted with water and then lyophilized. The sample was applied onto a Whatman paper and developed as described earlier. The radioactive product was eluted off the paper with water and lyophilized, then applied to a column (1 × 50 cm) of Bio-Gel P-2, equilibrated with water. The radioactive compounds were eluted with water and subjected to structural analysis.

Preparation of ^{3}H-labeled N*-acetyllactosamine and 2-acetamido-2-deoxy-glucopyranosyl-*N*-acetyl[^{3}H]lactosamine.* — *N*-Acetyllactosamine labeled in the D-galactopyranosyl group was synthesized from UDP-[^{3}H]Gal, *N*-acetyl-D-glucosamine, and human serum according to the method of Fujita-Yamaguchi and Yoshida[23]. The composition of the reaction mixture in a total volume of 300 μL was $MnCl_2$ (5.0 μmol), ATP (1.0 μmol), NaN_3 (1.0 μmol), sodium cacodylate (pH 7.5; 20 μmol), and human O-type serum, (200 μL). After incubation at 37° for 24 h, ethanol (300 μL) was added and the mixture centrifuged. The supernatant was applied to a mixed-bed column of Dowex AG 1-X8 (Cl^-) and AG 50W-X8 (H^+) ion-exchange resins and the eluate was lyophilized. The ^{3}H-labeled *N*-acetyllactosamine was purified by paper chromatography and Bio-Gel P-2 column chromatography as described earlier.

GlcNAc-*N*-acetyl[^{3}H]lactosamine was synthesized enzymically as described earlier for "Enzyme assay", except that UDP-[^{14}C]GlcNAc and the acceptor were replaced by UDP-GlcNAc (1 μmol) and *N*-acetyl[^{3}H]lactosamine (1.26×10^6 c.p.m.), respectively, and in addition NaN_3 (1.0 μmol) and enzyme preparation (4.0 mg of protein) were also added. The incubation time was prolonged to 24 h. The products were also purified by paper chromatography as described earlier and used for methylation analysis.

Methylation of oligosaccharides. — Methylation of the products and reference compounds was performed with dimethylsulfinyl carbanion, dimethylsulfoxide, and methyl iodide by the method of Hakomori[24]. The methylated oligosaccharides were hydrolyzed with 0.25M H_2SO_4 in 95% acetic acid for 18 h at 80°, and the solution was de-ionized by passing it through a column of Dowex AG 50-X8 (H^+) surmounted with Dowex AG 1-X8 (AcO^-) ion-exchange resin. The hydrolyzates were deposited on a t.l.c. Silica gel 60 F_{254} aluminum sheet, and the chromatogram was developed with 500:9 (v/v) acetone–4.5M NH_4OH. The radioactive compounds on the chromatogram were detected with a Packard radiochromatogram scanner, model 7220/21 and the nonradioactive compounds with H_2SO_4.

Treatment with glycosidases. — [^{14}C]GlcNAc-labeled oligosaccharides were treated with *N*-acetyl-β-D-glucosaminidase from jack bean and with β-D-galactosidase from bovine testes[25] as described later.

Protein assay. — Protein was estimated by the method of Lowry *et al.*[26] with bovine serum albumin as the standard.

^{1}H-N.m.r. spectroscopy. — The samples were prepared and the spectroscopy was performed as previously described[7].

RESULTS AND DISCUSSION

Transfer of [^{14}C]GlcNAc from UDP-[^{14}C]GlcNAc to β-D-GlcpNAc-(1→3)-α-D-GalpNAcOBn and β-D-GlcpNAc-(1→6)-α-D-GalpNAcOBn. — Human ovarian microsome preparations contained enzyme(s) that catalyze the transfer of GlcNAc from UDP-GlcNAc to β-D-Glc*p*NAc-(1→3)-α-D-Gal*p*NAcOBn and β-D-Glc*p*NAc-(1→6)-α-D-Gal*p*NAcOBn. The rates of GlcNAc incorporation into these acceptors were found to be 9.62 ±3.53 and 3.39 ±2.65 nmol · mg^{-1} of protein · h^{-1}, respectively. No significant difference was observed in both enzyme activities in ovarian tissues of cancer patients as compared to that of normal ones. The enzymes that catalyze the aforementioned reactions have been shown to be β-(1→6)- and β-(1→3)-*N*-acetylglucosaminyltransferases, respectively, in hog gastric mucosa[6], and rat[6,7] and hog[7] colon mucosa.

The enzyme products of both substrates showed four radioactive peaks, respectively, on the paper chromatogram. One of the peaks that had an R_{GlcNAc} value of 0.81 in solvent (*B*) yielded [^{14}C]GlcNAc on hydrolysis with *N*-acetyl-β-D-glucosaminidase from jack bean, and this peak was detected in all the reactions. The mobility of this peak also corresponded with that of authentic β-D-Glc*p*NAc-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn. This result confirmed the presence of two enzymes which catalyze the transfer of [^{14}C]GlcNAc to O-6 and -3 of β-D-Gal*p*NAcOBn, *i.e.*, β-(1→6)- and β-(1→3)-*N*-acetylglucosaminyltransferase. The activity of β-(1→3)-*N*-acetylglucosaminyltransferase was lower than that of β-(1→6)-*N*-acetylglucosaminyltransferase in all enzyme preparations tested.

Requirements for enzyme activity. — Requirements for the activity of β-(1→6)-*N*-acetylglucosaminyltransferase to transfer [^{14}C]GlcNAc to β-D-Glc*p*NAc-

TABLE I

REQUIREMENTS FOR THE INCORPORATION OF [^{14}C]GlcNAc INTO β-D-Glc*p*NAc-(1→3)-α-D-Gal*p*NAcOBn

Incubation mixture	*Incorporation into β-D-Glc*p*NAc-(1→3)-α-D-Gal*p*NAcOBn (%)*
Complete system[a]	100
Minus acceptor	1.4
Minus Mn^{2+}	37.8
Minus Mn^{2+} plus Mg^{2+}	84.5
Minus ATP	28.4
Minus Triton X-100	30.4
Plus EDTA	12.2

[a]The complete system contained the components of the standard incubation system using β-D-Glc*p*NAc-(1→3)-α-D-Gal*p*NAcOBn as an acceptor. To the system, 0.5 μmol of various divalent cations and EDTA were added.

(1→3)-α-D-Gal*p*NAcOBn are shown in Table I. Mn^{2+} ions were required for the enzyme, but Mg^{2+} ions could be used instead of Mn^{2+} ions. In the absence of these divalent cations and of Triton X-100, or in the presence of EDTA, the enzyme activity was reduced significantly. The effect of pH on the transferase activity was studied over a pH range of 4.0 to 9.0 with acetate, MES, HEPES, and Tris buffers. The enzyme had pH optima from 7.0 to 8.5 in buffers of HEPES as well as Tris. The requirements and pH optima of β-(1→3)-*N*-acetylglucosaminyltransferase, which transfers a GlcNAc group to β-D-Glc*p*NAc-(1→6)-α-D-Gal*p*NAcOBn, were almost the same as those of the β-(1→6)-*N*-acetylglucosaminyltransferase.

Substrate specificity. — In order to examine further the substrate specificity of the β-*N*-acetylglucosaminyltransferases in ovarian microsome preparations, several chemically synthesized 2-nitrophenyl and benzyl oligosaccharides were tested as acceptors of [^{14}C]GlcNAc (Table II). The results suggest two different modes of elongation of saccharide chains through attachment of GlcNAc group, as shown in Scheme 1. Although the trisaccharide β-D-Glc*p*NAc-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn can be synthesized from α-D-Gal*p*NAcOBn either through Pathway (*1*) or Pathway (*2*), the enzyme rates suggest Pathway (*1*) to be the preferred one. Similarly, enzyme rate studies show that Pathway (*3*) is preferred for the synthesis of the tetrasaccharide to Pathway (*4*).

In addition to these reactions, the enzyme preparations can catalyze the incorporation of a GlcNAc group into *N*-acetyllactosamine, although to a lesser extent under identical conditions.

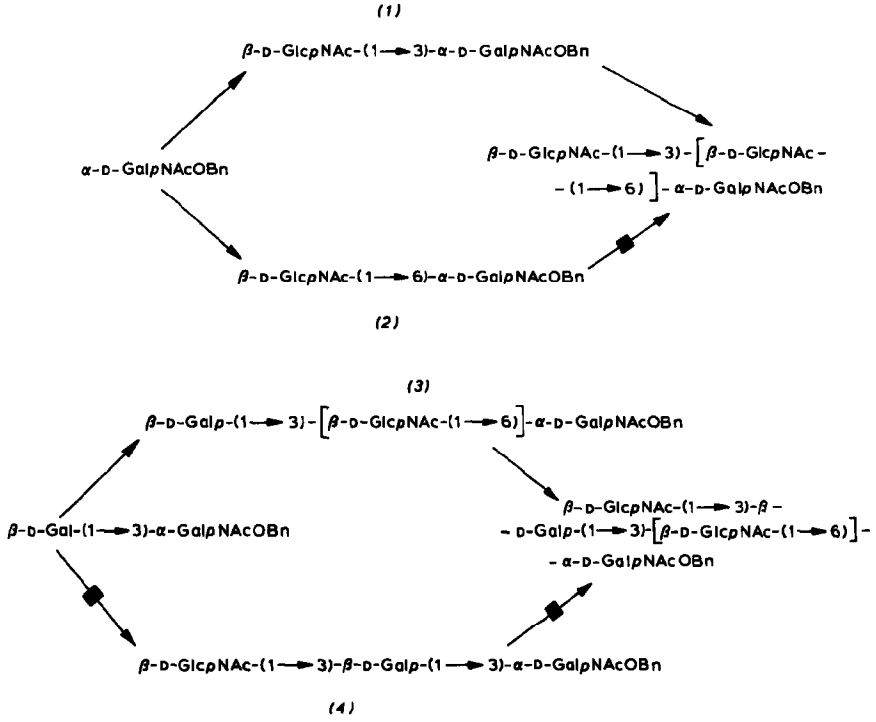
*Determination of the 2-acetamido-2-deoxy-*D*-glucopyranosyl group linkage.* — In order to study the linkage of the GlcNAc group incorporated into some of the acceptors, the radioactive product, β-D-Gal-(1→3)-{β-D-[^{14}C]Glc*p*NAc-(1→6)}-α-D-Gal*p*NAcOBn (Fig. 1a) was digested with either *N*-acetyl-β-D-glucosaminidase or β-D-galactosidase, and the hydrolyzates were subjected to paper chromatography. Radiolabeled GlcNAc was completely released with *N*-acetyl-β-D-glucos-

TABLE II

SUBSTRATE SPECIFICITY OF β-(1→6)- AND β-(1→3)-*N*-ACETYLGLUCOSAMINYLTRANSFERASES IN OVARIAN MICROSOME PREPARATION

Acceptor	*Mobility of product* (R_{GlcNAc})[a]	*Probable structure*	*Enzyme rate* (*nmol* · h^{-1} · mg^{-1})
βGal→3αGalNAcO(2-NP)	0.84	βGal→3(βGlcNAc→6)αGalNAcO(2-NP)	13.0
	0.45	βGlcNAc→3βGal→3(βGlcNAc→6)αGalNAcO(2-NP)	3.1
βGal→3αGalNAcOBn	1.10	βGlcNAc→3βGal→3αGalNAcOBn	2.4
	0.78	βGal→3(βGlcNAc→6)αGalNAcOBn	17.2
	0.57	βGlcNAc→3βGal→3(βGlcNAc→6)αGalNAcOBn	2.9
βGlcNAc→3βGal→3αGalNAcOBn	0.57	βGlcNAc→3βGal→3(βGlcNAc→6)αGalNAcOBn	1.6
βGal→3(βGlcNAc→6)αGalNAcO(2-NP)	0.45	βGlcNAc→3βGal→3(βGlcNAc→6)αGalNAcO(2-NP)	3.0
βGal→3(βGlcNAc→6)αGalNAcOBn	0.57	βGlcNAc→3βGal→3(βGlcNAc→6)αGalNAcOBn	3.3
αGalNAcOBn	1.23	βGlcNAc→6αGalNAcOBn	2.5
	1.18	βGlcNAc→3αGalNAcOBn	1.8
	0.81	βGlcNAc→3(βGlcNAc→6)αGalNAcOBn	2.8
βGlcNAc→3αGalNAcOBn	0.81	βGlcNAc→3(βGlcNAc→6)αGalNAcOBn	8.1
βGlcNAc→6αGalNAcOBn	0.81	βGlcNAc→6(βGlcNAc→3)αGalNAcOBn	2.3

[a]Solvent systems: 2-ONP glycosides, solvent (*A*); Bn glycosides, solvent (*B*).



Scheme 1.

aminidase (Fig. 1b). After treatment of the labeled trisaccharide with β-D-galactosidase, almost all of the radioactivity was detected as a compound having the same mobility as β-D-Glc*p*NAc-(1→6)-α-D-Gal*p*NAcOBn (Fig. 1c). On the other hand, [^{14}C]GlcNAc was released from β-D-[^{14}C]Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn by digestion with *N*-acetyl-β-D-glucosaminidase, but no [^{14}C]GlcNAc was liberated by hydrolysis with β-D-galactosidase (data not shown).

^{1}H-N.m.r. spectroscopy at 360 MHz was also used to identify the anomeric configuration of the products formed with β-D-Gal-(1→3)-α-D-Gal*p*NAcOBn as an acceptor. Fig. 2 showed the spectrum for the product corresponding to β-D-Gal-(1→3)-{β-D-[^{14}C]-Glc*p*NAc-(1→6)}-α-D-Gal*p*NAcOBn. This spectrum was identical to the spectrum reported previously[9] for β-D-Gal-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn. Therefore, it was concluded that a β-D-Glc*p*NAc group was incorporated at O-6 of α-D-Gal*p*NAcOBn.

The products formed by transfer of GlcNAc to ^{3}H-labeled *N*-acetyllactosamine were subjected to methylation and hydrolysis, yielding at least three radioactive spots on t.l.c. plate (Fig. 3). The main radioactive spots could be identified as those corresponding to 2,4,6- and 2,3,4-tri-*O*-methylgalactose, and other weak spots corresponded to 2,3,4,6-tetra-*O*-methylgalactose, which might indicate contamination of the products with a ^{3}H-acceptor, and di-*O*-methylgalactose which

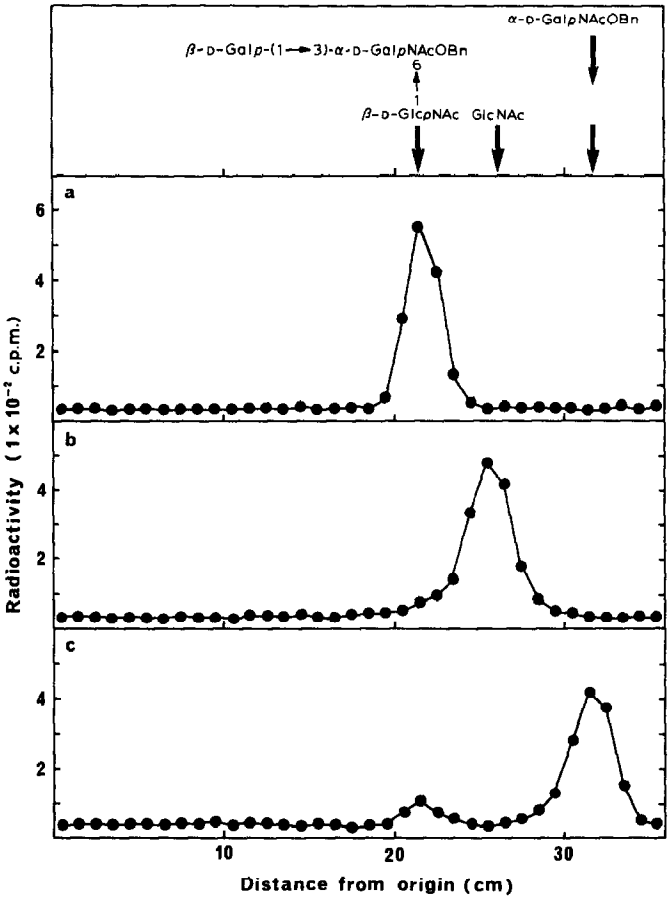


Fig. 1. Paper chromatography of the ^{14}C-labeled trisaccharide formed by the action of human ovarian microsome preparation on β-D-Gal*p*-(1→3)-α-D-Gal*p*NAcOBn: (a) ^{14}C-labeled trisaccharide, (b) trisaccharide after *N*-acetyl-β-D-glucosaminidase treatment, and (c) trisaccharide after β-D-galactosidase treatment.

migrated between the origin and 3,4,6-tri-*O*-methylgalactose. All [^{14}C]GlcNAc incorporated into *N*-acetyllactosamine was completely released by the action of *N*-acetyl-β-D-glucosaminidase (data not shown). These results showed that the reaction products with *N*-acetyllactosamine as an acceptor are two trisaccharides having 2-acetamido-2-deoxy-β-D-glucopyranosyl groups linked to O-3 and -6 of the penultimate D-galactosyl residue.

Substrate competition tests. — Competition experiments were done (Table III) to determine whether the transferases discussed herein are different from those that transfer a GlcNAc group to benzyl oligosaccharides discussed earlier, and also whether the same enzyme or different β-(1→3)- and β-(1→6)-*N*-acetylglucosaminyltransferase catalyze the synthesis of the various structures mentioned above. The acceptors used for β-(1→3)-*N*-acetylglucosaminyltransferase were β-D-

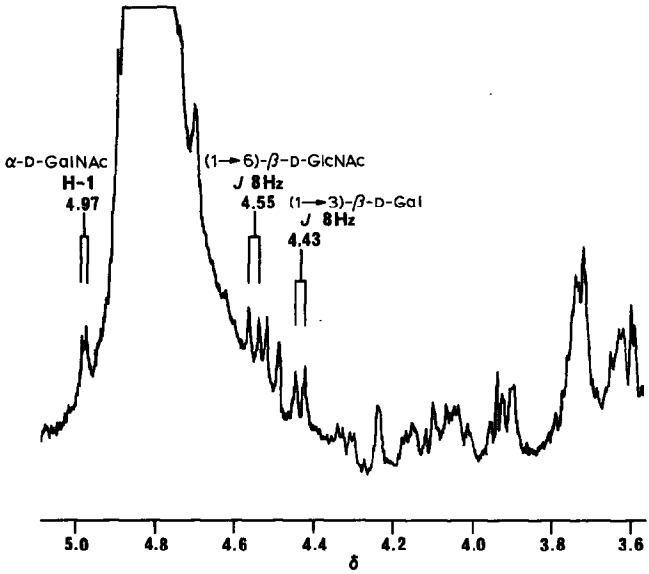


Fig. 2. ^{1}H-N.m.r. spectra of room temperature between δ 3.6 and 5.0 of β-D-Gal*p*-(1→3)-{β-D-[^{14}C]Glc*p*NAc-(1→6)}-α-D-Gal*p*NAcOBn formed by the action of human ovarian microsome preparation on β-D-Gal*p*-(1→3)-α-D-Gal*p*NAcOBn.

Glc*p*NAc-(1→6)-α-D-Gal*p*NAcOBn and β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn. The results showed that the two substrates competed with each other for a common enzyme site and, thus, a single enzyme catalyzed the incorporation of a GlcNAc group into the penultimate GalNAc residue and terminal Gal group, respectively. The substrate competition tests for β-(1→6)-*N*-acetylglucosaminyltransferase were carried out with β-D-Gal*p*-(1→3)-α-D-Gal*p*-NAcOBn and β-D-Glc*p*NAc-(1→3)-α-D-Gal*p*NAcOBn, and the results showed that the two activities were also due to a single enzyme.

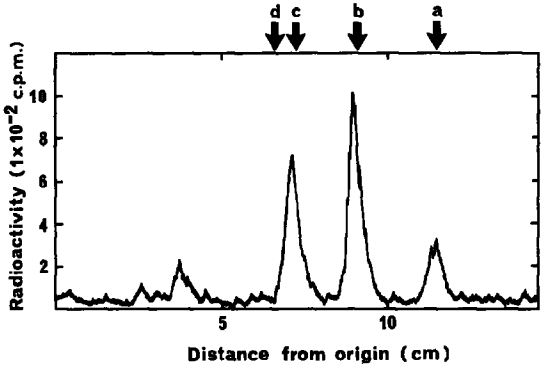


Fig. 3. Thin-layer chromatography of ^{3}H-labeled methyl galactosides from *N*-acetylglucosaminylated *N*-acetyl[^{3}H]lactosamine. The migration of the following references is indicated: (a) 2,3,4,6-tetra-*O*-methylgalactose, (b) 2,4,6-tri-*O*-methylgalactose, (c) 2,3,4-tri-*O*-methylgalactose, and (d) 3,4,6-tri-*O*-methylgalactose.

TABLE III

SUBSTRATE COMPETITION TEST FOR β-(1→3)- AND β-(1→6)-*N*-ACETYLGLUCOSAMINYLTRANSFERASES IN OVARIAN MICROSOME PREPARATION

Substrate conc. (mM)		*Enzyme activity (nmol · h^{-1} · mg^{-1})*			*Conclusion*
βGlcNAc→6αGalNAcOBn	*βGal→3(βGlcNAc→6)αGalNAcOBn*	*Experimental*	*Calc.for*		
			1E[a]	*2E*[b]	
1.0		2.2			
2.0		4.1			
3.0		5.8			
	1.0	2.4			
	2.0	4.2			
	3.0	5.3			
1.0	1.0	3.5	4.0	4.6	1E
2.0	2.0	6.4	6.4	8.3	1E
3.0	3.0	8.5	8.1	11.1	1E
βGal→3αGalNAcOBn[c]	*βGlcNAc→3αGalNAcOBn*				
1.0		17.5			
2.0		25.1			
3.0		29.5			
	1.0	5.5			
	2.0	7.7			
	5.0	10.0			
1.0	1.0	14.9	15.9	18.3	1E
2.0	2.0	21.6	20.1	32.8	1E
3.0	5.0	28.5	28.0	31.0	1E

[a]The following equation was used to calculate the amount of GlcNAc incorporated into the acceptors assuming competition for a common enzyme active site[27]: $v = V1(S1 + K1) + V2(S2 + K2)/1 + S1/K1 + S2/K2$. v, V, S, and K are apparent velocity, maximal velocity, substrate concentration, and Michaelis constant, respectively. V_{max} values for βGlcNAc→6αGalNAcOBn, βGal→3(βGlcNAc→6)αGalNAcOBn, βGal→3αGalNAcOBn[c], and βGlcNAc→3αGalNAcOBn are 29.41, 13.33, 44.44, and 12.74 nmol · mg^{-1} · h^{-1}, respectively, and the respective K_m values are 13.33, 4.72, 1.55, and 1.31mM. [b]The sum of apparent velocities obtained from the assay of each acceptor separately. [c]For this compound, the amount of GlcNAc incorporated as βGal→3(βGlcNAc→6)αGalNAcOBn.

The preliminary substrate-competition tests with *N*-acetyllactosamine and benzyl oligosaccharides showed that the addition of *N*-acetyllactosamine (20mM) to the incubation mixtures containing either β-D-Glc*p*NAc-(1→6)-α-D-Gal*p*NAcOBn or β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn inhibited the incorporation of a GlcNAc group at O-3 of the GalNAc or Gal residues of these oligosaccharides (63 and 67.5%, respectively), but the amount of GlcNAc incorporated either into β-D-Gal*p*-(1→3)-α-D-Gal*p*NAcOBn or β-D-Glc*p*NAc-(1→3)-α-D-Gal*p*NAcOBn at O-6 of the GalNAc residue was not affected by the addition of *N*-acetyllactosamine to the incubation mixtures. These results suggest that the β-(1→3)-*N*-acetylglucosaminyltransferase activities that are responsible for the synthesis of β-D-Glc*p*NAc-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn, β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn, and β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-GlcNAc might reside in a single enzyme. In contrast, the β-(1→6)-*N*-acetylglucosaminyltransferase that catalyzes the synthesis of β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn, β-D-Glc*p*NAc-(1→6)-[β-D-Glc*p*NAc-(1→3)]-α-D-Gal*p*NAcOBn, and β-D-Glc*p*NAc-(1→6)-β-D-Gal*p*-(1→4)-D-GlcNAc might be due to two different enzymes, one of which transferred a GlcNAc group to a GalNAc residue and the other to a terminal Gal group, respectively. The enzymes that transfers a GlcNAc group to the galactosyl group of *N*-acetyllactosamine have been found in Novikoff ascites tumor cells[8], human serum[12–15], and hog gastric mucosa[6,9], and they have been identified as a β-(1→6)- and β-(1→3)-*N*-acetylglucosaminyltransferase. It is also known that these two *N*-acetyl-β-D-glucosaminyltransferases synthesize the branching point in branched lactosaminoglycans, and are responsible for the synthesis of the blood group substance I and the precursor of the ABH and Lewis blood group substances[28]. Thus the enzymes described herein may be involved in the biosynthesis of the core and backbone regions of mucin-type glycoproteins, and of the branch points in branched lactosaminoglycans.

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