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IDENTIFICATION OF A α-NeuAc-(2→3)-β-D-GALACTOPYRANOSYL *N*-ACETYL-β-D-GALACTOSAMINYLTRANSFERASE IN HUMAN KIDNEY

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ABSTRACT

Microsomal preparations from human kidney were found to contain enzymic activity capable to transfer *N*-acetylgalactosamine from UDP-*N*-acetylgalactosamine to native bovine fetuin. The acceptor structures on the fetuin molecules were identified as *N*- as well as *O*-linked glycans with a markedly higher incorporation into the *N*-linked carbohydrate chains. Analysis of the alkali-labile transferase products by thin-layer chromatography indicated that the enzyme is able to synthesize structures having mobilities identical with those found on glycophorin from Cad erythrocytes. Mild acid treatment and enzymic hydrolysis with *N*-acetylhexosaminidase from jack beans of the *N*-linked transferase products suggested that β-D-Gal*p*NAc-(1→4)-[α-NeuAc-(2→3)]-β-D-Gal*p*-(1→ structures were formed by the enzymic reaction on both *N*- and *O*-linked acceptors. The enzyme might, therefore, be involved in the biosynthesis of Sd^a (and Cad) antigenic structures. By use of various oligosaccharides, glycopeptides, and glycolipids having well characterized carbohydrate sequences, the acceptor-substrate specificity of the *N*-acetylgalactosaminyltransferase was determined. The enzyme generally recognized α-NeuAc-(2→3)-β-D-Gal groups as acceptors, but in a certain conformation. Thus, tri- and tetra-saccharide alditols, native human glycophorin A, and GM_3 were not acceptor substrates although they carry the potential disaccharide acceptor unit. When these structures were presented as sialyl-(2→3)-lactose or as a tryptic peptide from glycophorin A, they were shown to be rather good acceptor substrates for the *N*-acetyl-β-D-galactosaminyltransferase from human kidney.

INTRODUCTION

Carbohydrate structures having a β-D-Gal*p*NAc-(1→4)-[α-NeuAc-(2→3)]-β-D-Gal*p* group at the nonreducing end have been recently discovered. They are

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associated with the Cad phenotype of human erythrocytes, where these structures are found on the *O*-glycans of the main sialoglycoproteins[1,2] and on a novel ganglioside[3] derived from sialosylparagloboside. In human urine, the same trisaccharide sequence was identified on the *N*-glycosyl chains of the major urinary glycoprotein (Tamm–Horsfall glycoprotein) and shown to be associated with the blood-group Sd^a phenotype[4]. Likewise, in human urine, a mucin carrying only *O*-glycosyl-linked oligosaccharides was found to react strongly with anti-Sd^a antiserum and, therefore, may be assumed to contain the same terminal trisaccharide group[5]. Very recently, the presence of oligosaccharide structures identical with those found on the glycophorins from Cad individuals has been reported on a murine cytotoxic T-lymphocyte cell line[6].

So far, nothing is known about the biosynthesis of blood-group Cad or Sd^a reactive structures in human tissues. It has been shown that the microsomes from guinea pig kidney contain an enzymic activity able to transfer GalNAc to native bovine fetuin and to Tamm–Horsfall glycoprotein[7]. Furthermore, an enzyme was detected in the murine cytotoxic T-cell line capable of transferring GalNAc to human glycophorin A and to bovine fetuin[8]. This enzymic activity was absent in a mutant cell that did not contain the structure β-D-Gal*p*NAc-(1→4)-[α-NeuAc-(2→3)]-D-Gal at its cell surface. On human glycophorin, the murine T-cell enzyme synthesized oligosaccharide structures identical with those found on glycophorin from Cad individuals. From the data presented in the two papers, it is clear that the enzyme from guinea pig kidney and the enzyme from the murine T-cell line are very similar.

We report herein on an *N*-acetyl-β-D-galactosaminyltransferase in microsomal preparations from human kidney, its enzymic properties, and its specificity for various acceptor substrates. The human enzyme is furthermore compared to the *N*-acetyl-β-D-galactosaminyltransferase from guinea pig kidney.

EXPERIMENTAL

Materials. — A fresh human kidney from an unmatched organ donor (blood group O) was obtained through the courtesy of Dr. D. Droz, Hôpital Necker, Paris, France, and stored at −80° until used. Guinea pigs were purchased from IFFA–CREDO, Lyon, France, and sacrificed by decapitation. Microsomes from fresh or frozen tissues were prepared as described[9] and stored at −80°. UDP-2-acetamido-2-deoxy-D-[^{14}C]galactose (1.75 GBq/mmol) was obtained from New England Nuclear. Unlabeled UDP-2-acetamido-2-deoxy-D-galactose was chemically synthesized[10,11] as described. Bovine fetuin, 2-acetamido-2-deoxy-D-glucose, ATP, Triton X-100, sucrose, lactose, α-NeuAc-(2→3 or 6)-β-D-Gal*p*-(1→4)-D-Glc (sialyllactose) from bovine colostrum, lactosylceramide, TPCK-treated trypsin (EC 3.4.21.4), and *N*-acetyl-β-hexosaminidase (EC 3.2.1.30) from jack beans were purchased from Sigma Chemical Co. *Vibrio cholerae* neuraminidase (EC 3.2.1.18) was from Behringwerke. α_1-Acid glycoprotein was obtained from the Centre

National de Transfusion Sanguine, Production, Orsay, France. Glycophorin A was prepared from human erythrocyte stroma as described[1], and the tryptic peptides were obtained according to the published procedures[12]. The peptides T_1 (amino acids 1–39) and T_2 (1–31) were separated from T_3 (40–61) by gel filtration. Reduced sialyllactose was prepared according to Takasaki and Kobata[13].

The chemically synthesized[14] β-D-Gal*p*-(1→4)-D-GlcNAc was kindly supplied by Dr. A. Veyrières, Orsay, France. The oligosaccharides[15] β-D-Gal*p*-(1→3)-GalNAc, α-NeuAc-(2→3)-β-D-Gal*p*-(1→3)-[α-NeuAc-(2→6)]-D-GalNAcol, and α-NeuAc-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-D-GlcNAc and the glycopeptide[15] α-NeuAc-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-Asn (S1) were generous gifts of Dr. J. C. Michalski and G. Strecker, Lille, France. A biantennary glycopeptide of the complex type isolated from Cohn Fraction IV was donated by Dr. V. Michel, Orléans, France. α-NeuAc-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-ceramide[16] (sialosylparagloboside), β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-ceramide (paragloboside), and α-NeuAc-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-ceramide (GM3) were donated by Dr. D. M. Marcus, Houston, Texas, U.S.A. Asialoglycophorin that had been specifically resialylated with a pure preparation of the α-D-galactoside α-(2→3)-*N*-acetylneuraminyltransferase from porcine submaxillary glands[17] was a generous gift of Dr. J. C. Paulson, Los Angeles, California, U.S.A. Oligosaccharides, glycopeptides, and glycoproteins were desialylated either by treatment with *V. cholerae* neuraminidase according to the instructions given by the supplier or by mild acid treatment[18]. *N*-Acetylneuraminic acid was quantitatively determined by following the published procedures[19].

N-*Acetyl-β-D-galactosaminyltransferase assays.* — Depending on the acceptor substrates used in the assays, different methods were employed to separate the products of the transferase reaction from the radiolabeled substrate and its degradation products.

Method (a). In a total volume of 30μL, the reaction mixture contained UDP-[^{14}C]GalNAc (3 nmol; specific activity, 10^5 c.p.m./nmol), bovine fetuin or other glycoprotein acceptors (260 μg), sodium cacodylate (pH 6.9, 1.25 μmol), $MnCl_2$ (0.75 μmol), 0.8% Triton X-100, GlcNAc (2.5 μmol), ATP (0.1 μmol), and microsomal protein (20 μg). The incubation was carried out at 37° for 3 h and was stopped by adding cold 0.15M NaCl (170 μL). The glycoproteins were separated from UDP-[^{14}C]GalNAc by gel filtration on a column (0.7 × 10 cm) of Sephadex G-50 (fine) equilibrated and developed in 0.15M NaCl. The fractions eluted in the void volume were collected in scintillation vials and the radioactivity counted. Equally good separations could be obtained by depositing the incubation mixtures on Whatman 3MM paper and chromatographing overnight in 5:2 (v/v) 95% ethanol-M ammonium acetate (pH 7.0; solvent *A*). The spots containing the proteins were cut out and the radioactivity was counted in a liquid-scintillation counter.

Method (b). When oligosaccharides or glycopeptides were used, the incubation mixture was as described in Method (*a*), but the glycoprotein acceptor was replaced by oligosaccharides (23 nmol). The incubation was stopped by addition of 5mM NaH_2PO_4 (pH 6.8; 1 mL) and the mixture applied to a column (0.5 × 3 cm) of Dowex 1-X8 (PO_4^{3-}) equilibrated and developed in the same buffer. Neutral and sialic acid-bearing oligosaccharides and glycopeptides passed unretarded through the column, whereas UDP-GalNAc and GalNAc-1-*P* remained bound. The effluent and a 0.5-mL wash were collected and either their radioactivity was counted directly or they were dried and the residues further analyzed by descending paper chromatography. Neutral oligosaccharides were chromatographed for 16 h in 5:5:1:3 (v/v; solvent *B*) ethyl acetate–pyridine–acetic acid–water, the paper was dried, and the radioactive spots were detected by a Packard Radiochromatogram Scanner. Acidic oligosaccharides and glycopeptides were submitted to paper chromatography in solvent *A*. Radioactive areas on the paper strips were cut out and quantitated by liquid-scintillation counting.

Method (c). When glycolipids were used as acceptors, the incubation mixtures were as described in Method (*a*), but the glycoprotein acceptors were replaced by glycolipids (30 nmol). The incubations were stopped by addition of 2:1 (v/v) chloroform–methanol (100 μL), and deposited on Whatman 3MM paper. Sugar nucleotides and sugars were removed from the glycolipids by descending paper chromatography in water (for neutral glycolipids) or 1% $Na_2B_4O_7$ (for ganglioside acceptor)[20]. The glycolipids remained in both solvents at the origin and could be quantitatively eluted by five washings with 2:1 (v/v) chloroform–methanol (3 mL). The eluents were dried in scintillation vials and the radioactivity was counted directly, or the samples were dried and the residues deposited on precoated silica gel plates (Merck, Kieselgel 60) and developed in 10:9:2 (v/v) chloroform–methanol–water containing 0.02% of $CaCl_2$. The radioactive material was detected by fluorography with En^{3}Hance (New England Nuclear) and Fuji X-ray film. The carbohydrates were detected with the orcinol–H_2SO_4 reagent.

Larger-scale preparation and analysis of biosynthetically labeled fetuin. — Fetuin (120 mg) was incubated in a scaled up experiment with microsomal protein (6.5 mg) and UDP-[^{14}C]GalNAc (1.5 μmol; specific activity 10^4 c.p.m./nmol) in a total volume of 10 mL. The other components needed for the enzymic activity were added in the appropriate amounts as described for Assay Method (*a*). After 18 h of incubation, the proteins were separated from the substrate and monosaccharides by gel filtration on a column (2.5 × 50 cm) of Sephadex G-50 (fine) equilibrated and developed in PBS, pH 7.4. The radioactive material eluting in the void volume was extensively dialyzed against de-ionized water and freeze-dried.

Oligosaccharides *O*-glycosyl-linked to serine or threonine were liberated by β-elimination as described[2], but unlabeled $NaBH_4$ was used. Hydrazinolysis was performed according to the published procedure[21]. The liberated *N*-glycans were *N*-acetylated, reduced, and desalted on a column of TSK G-2000 with LKB HPLC equipment. Liberated and desalted *O*- as well as *N*-linked oligosaccharide alditols

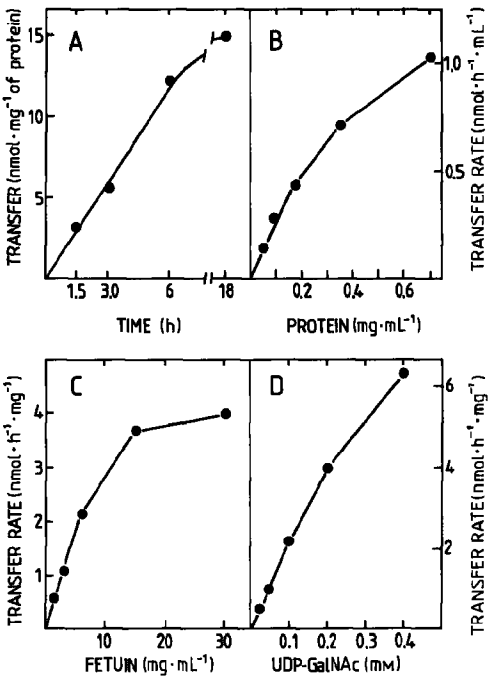

Fig. 1. Dependence of the *N*-acetyl-β-D-galactosaminyltransferase reaction on time (A), microsomal protein concentration (B), and on the concentrations of acceptor (C) and donor (D) substrates. The incubation conditions were those described under Assay Method (*a*); only the parameters indicated on the x-axis were changed accordingly.

were analyzed by t.l.c. on precoated silica gel plates in 30:10:10:3:1 (v/v) ethanol–water–butanol–pyridine–acetic acid (solvent *C*). Fluorography of the radioactive material was done as described in Assay Method (*c*). Enzymic hydrolysis of labeled 2-acetamido-2-deoxy-D-galactose incorporated into the *N*-linked chains of fetuin was carried out as described[8].

RESULTS

Transfer of [^{14}C]*GalNAc from UDP-*[^{14}C]*GalNAc to bovine fetuin.* — When microsomal preparations from fresh human kidney samples were incubated in the presence of manganese ions and Triton X-100 with UDP-[^{14}C]GalNAc and bovine fetuin, transfer of radioactivity to the exogenous acceptor as well as some transfer to endogenous acceptors were observed. The rate of the transfer was dependent on the incubation time, on the amount of microsomal protein added, and on the concentrations of exogenous acceptor and donor substrates (Fig. 1).

The transfer of 2-acetamido-2-deoxy-D-galactose increased with the time of incubation at a linear rate for up to 6 h at 37°, and the transfer reaction was found to be linear for up to 20 μg of microsomal protein per incubation mixture. The

transfer rate increased with the concentration of acceptor and donor substrates up to 15 mg/mL and 0.4mM concentration, respectively, and was dependent on the pH of the incubation mixture with a broad optimum between 6.5 and 8.0. The transferase was found to have an absolute requirement for Mn^{2+} ions which could not be replaced in the incubation medium by Mg^{2+} or Ca^{2+} (not shown).

Microsomes from most tissues contain enzymic activities that are able to hydrolyze rapidly the labeled UDP-GalNAc substrate. Therefore, inhibitors of these hydrolytic enzymes were included in the assay mixtures, noteably 80mM 2-acetamido-2-deoxy-D-glucose, which was found to have no effect on the transferase reaction, and 3mM ATP, which slightly inhibited the GalNAc-transferase. Under the conditions employed, about 15% of the total UDP-[^{14}C]GalNAc were degraded during the incubation, and 4–5% were specifically incorporated into fetuin. Very little transfer to endogenous acceptors was observed; in general it did not exceed 10% of the incorporation into fetuin. Controls without exogenous acceptor were performed for all assays.

When fetuin was submitted to mild acid hydrolysis or to hydrolysis by *V. cholerae* neuraminidase, the desialylated product was found to be a very poor acceptor for the GalNAc-transferase. The specific transfer rate for asialofetuin decreased to less than 5% as compared to native fetuin (see later).

Identification of the product of the GalNAc-transferase. — In a scaled-up experiment, fetuin (120 mg) was incubated with microsomal protein (6.5 mg) and UDP-[^{14}C]GalNAc (1.5 μmol), as described in the Experimental section, and ~250 nmol of [^{14}C]GalNAc could be incorporated into fetuin within 18 h. However, only 70% of the label were covalently linked to the acceptor glycoprotein as judged by $NaDodSO_4$–PAGE analysis of the product. Although a clear baseline separation of UDP-GalNAc and fetuin could be obtained by gel filtration on Sephadex G-50 (fine), some of the UDP-GalNAc remained nonspecifically bound to fetuin. After this observation, blanks without enzyme were included in all assays. However, the nonspecific absorption of UDP-GalNAc was found only with one batch of bovine fetuin. An aliquot of the labeled fetuin was further analyzed by β-elimination and the elution profile of the liberated oligosaccharides on Biogel P-6 is shown in Fig. 2A. Gel filtration in 0.5% acetic acid resolved two major peaks of radioactivity. The first peak was close to the void volume of the column, and the second peak very near to the included volume; between the two pronounced peaks was a broad zone of low, but detectable radioactivity. The column effluents were pooled into three fractions (F1, F2, and F3), freeze-dried, and further analyzed by t.l.c. in solvent *C*. The chromatograms were exposed to X-ray film for 5 days at −80° and then stained for sugars with the orcinol reagent (Fig. 2B).

The autoradiography showed radioactive spots in all three fractions. F1 contained only material that remained at the origin, thus indicating uncleaved glycopeptides or high-mol.wt. oligosaccharides. In this fraction, the radioactive spot coincided with the total sugar as shown by the orcinol spray. F2 contained the oligosaccharides liberated by the elimination reaction. The products that stained

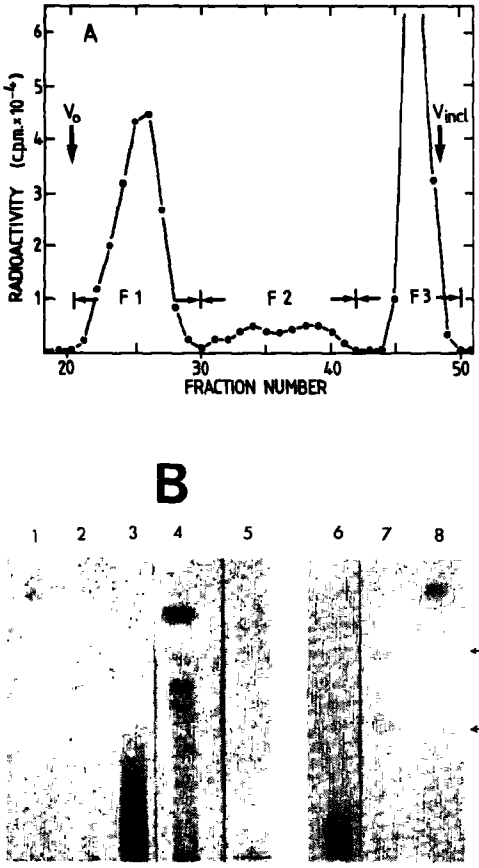

Fig. 2. (A) Elution profile, from a Bio-Gel P-6 column, of the glycopeptides and oligosaccharides obtained by β-elimination of biosynthetically labeled bovine fetuin. The column (1.5 × 100 cm) was equilibrated and developed in 0.5% acetic acid at a flow rate of 10 mL/h. Fractions (3.3 mL) were collected and aliquots taken out for liquid-scintillation counting. The column effluents were pooled into three fractions (F1, F2, and F3) corresponding to 47, 9, and 44%, respectively, of the total recovered radioactivity. (B) The pooled fractions were further analyzed by t.l.c. on Silica gel plates in solvent *C*: Lanes 1–5 were stained with the orcinol–H_2SO_4 reagent and lanes 6–8 show an autoradiography of the same plate before staining. Lanes 1 and 2 contain standards of 2-acetamido-2-deoxy-D-galactitol and NeuAcα→3Galβ→3(NeuAcα→6)GalNAcol, respectively. Lanes 3 and 6 correspond to F1, lanes 4 and 7 to F2, and lanes 5 and 8 to F3. The arrows indicate the position of: (*a*) GalNAcβ→4(NeuAcα→3)Galβ→3GalNAcol and (*b*) GalNAcβ→4(NeuAcα→3)Galβ→3(NeuAcα→6)-GalNAcol, both obtained by β-elimination of Cad-specific glycophorin A.

with the orcinol reagent migrated with a R_F of 0.43 and 0.30, indicating the presence of NeuAcα→3Galβ→3GalNAcol and NeuAcα→3Galβ→3(NeuAcα→6)GalNAcol, respectively. The radioactive products of the transferase reaction were detected by autoradiography and showed R_F values of 0.37 and 0.23, suggesting that each of the two *O*-linked oligosaccharides present in the fetuin molecule had

been elongated with a 2-acetamido-2-deoxygalactopyranosyl group. The R_F values of the radioactive products were found to be identical with those of the major oligosaccharides released from glycophorin A of a Cad individual. F3 contained mostly salts and no carbohydrate could be found with the orcinol reagent. However, autoradiography revealed one spot comigrating with 2-acetamido-2-deoxygalactitol originating mainly from the nonspecifically adsorbed UDP-GalNAc. About five times more radioactivity was incorporated into the *N*-linked glycopeptide fraction (F1) than in F2, which contained the alkali-labile oligosaccharides.

In a parallel experiment, another sample of the labeled fetuin was analyzed by hydrazinolysis. The radioactive reaction products obtained were found to migrate on t.l.c. with the other liberated *N*-glycan chains, and no separation between orcinol-positive and radioactive spot could be observed. The radioactive material liberated by hydrazinolysis closely corresponded to the amount of radioactivity found in Fraction F1 (Fig. 2) after alkali–borohydride treatment (data not shown). Together, these results show that both *O*- as well as *N*-glycans in fetuin are acceptors for the GalNAc-transferase. However, about five times more radioactivity was incorporated into *N*- than into *O*-glycans.

The oligosaccharides obtained through hydrazinolysis were further analyzed according to the method described by Conzelmann and Kornfeld[8] which indicated the structure GalNAc$\beta\rightarrow$4(NeuAc$\alpha\rightarrow$3)Gal$\beta\rightarrow$R. The radioactive label incorporated by the GalNAc-transferase could not be removed from the oligosaccharide with an *N*-acetyl-β-hexosaminidase from jack beans. Likewise, the neuraminidase-treated product resisted the action of the hexosaminidase. Only after the product had been treated with 2M acetic acid for 1 h at 100°, freeze-dried, and desalted over Bio-Gel P-2, was the *N*-acetyl-β-hexosaminidase able to remove specifically the labeled 2-acetamido-2-deoxy-β-D-galactopyranosyl group from the transferase product.

Acceptor-substrate specificity. — The specificity of the GalNAc-transferase towards its acceptors was investigated by use of various oligosaccharides and glycopeptides of well established primary structures as acceptor substrates. The results obtained with oligosaccharides and glycopeptides are listed in Table I. Only substrates containing sialic acid residues were found to be acceptors. Sialyllactose (**4**) from bovine colostrum, which contains ~85% of the neuraminic acid group α-(2$\rightarrow$3)-linked was found to be an acceptor. However, reduced oligosaccharides **5** and **6** were no longer recognized by the transferase as acceptor substrate. The two compounds containing exclusively α-(2$\rightarrow$3) linked sialic acid groups (**7** and **8**) were both very good acceptors for the GalNAc-transferase. The glycopeptide from human serum, Cohn Fraction IV (**9**), which carries preponderantly (87%) an α-(2$\rightarrow$6)-linked sialic acid group[22], was found to be a rather poor acceptor. Compounds **7, 8,** and **9** lost their acceptor ability after desialylation (data not shown).

Among the glycoproteins tested as acceptor substrates for the GalNAc-transferase, bovine fetuin was found to be the best acceptor (Table II). α_1-Acid glycoprotein from human serum was only slightly less active as acceptor. When sialic

TABLE I

OLIGOSACCHARIDE AND GLYCOPEPTIDE ACCEPTOR SPECIFICITY OF THE *N*-ACETYL-β-D-GALACTOSAMINYL-TRANSFERASE FROM HUMAN KIDNEY

Acceptor (0.75mM)	*Transferase activity*		
	C.p.m.	*nMol · h^{-1} · mg^{-1} of protein*	*Relative rate*
Galβ→4Glc (**1**)	<50		
Galβ→4GlcNAc (**2**)	<50		
Galβ→3GalNAc (**3**)	<50		
NeuAcα→3 or 6Galβ→4Glc (**4**)	4260	0.71	0.3
NeuAcα→3 or 6Galβ→4Glcol (**5**)	<50		
NeuAcα→3Galβ→3(NeuAcα→6)GalNAcol (**6**)	<50		
NeuAcα→3Galβ→4GlcNacβ→2Manα→3Manβ→4GlcNAc (**7**)	9970	1.66	0.7
NeuAcα→3Galβ→4GlcNAcβ→3Galβ→4GlcNAcβ→Asn (S1) (**8**)	13 400	2.34	1.0
NeuAcα→6 or 3Galβ→4GlcNAcβ→2Manα→6(NeuAcα→6 or 3Galβ→4GlcNAcβ→2Manα→3)Manβ→4GlcNAcβ →4GlcNAcβ→Asn (**9**)[a]	1870	0.31	0.14

[a]0.37mM.

acid groups were removed from the two glycoproteins, either by mild acid treatment or by the action of *V. cholerae* neuraminidase, both glycoproteins were no longer recognized as acceptors by the GalNAc-transferase. Surprisingly, native glycophorin A from human erythrocytes was not an acceptor at all. The glycophorin preparation was not inhibitory since mixing glycophorin A with fetuin in the incuba-

TABLE II

GLYCOPROTEIN AND GLYCOLIPID ACCEPTOR SPECIFICITY OF THE *N*-ACETYL-β-D-GALACTOSAMINYL-TRANSFERASE FROM HUMAN KIDNEY

Acceptor (0.75mM)[a]	*Transferase activity*		
	C.p.m.	*nMol · h^{-1} · mg^{-1} of protein*	*Relative rate*
Fetuin (260 μg)	12 400	2.07	1.00
Asialofetuin (260 μg)	500	0.08	0.04
α_1-Acid glycoprotein (600 μg)	9300	1.55	0.75
Asialo-α_1-acid glycoprotein (600 μg)	400	0.07	0.03
Glycophorin A (66 μg)	120	0.02	0.01
Asialoglycophorin A (66 μg)	<50		
Glycophorin A (66 μg) and fetuin (260 μg)	11 000	1.84	0.89
Sialosylparagloboside (50 μg)	800	0.13	0.06
Lactosylceramide (50 μg)	<50		
Paraglobise (50 μg)	<50		
GM_3 (50 μg)	<50		

[a]As NeuAcα→3Gal or equivalent.

TABLE III

COMPARISON OF ACCEPTOR SPECIFICITY OF *N*-ACETYL-β-D-GALACTOSAMINYLTRANSFERASES FROM HUMAN AND FROM GUINEA PIG KIDNEYS

Acceptor (0.75mM)[a]	*Transferase activity*			
	Human		*Guinea pig*	
	C.p.m.	*nMol · h^{-1} · mg^{-1} of protein*	*C.p.m.*	*nMol · h^{-1} · mg^{-1} of protein*
Fetuin	12 900	2.15	34 400	5.73
Glycophorin A	125	0.02	33 200	5.53
α-(2→3)-Resialylated asialoglycophorin	<50	<50	18 900	3.15
Glycopeptides $T_1 + T_2$ from glycophorin A	3200	0.53	43 800	7.30
Glycopeptide T_3 from glycophorin A	800	0.13	36 200	6.03
NeuAcα→3Galβ→3(NeuAcα→6)GalNAcol	<50	<50	20 700	3.45
NeuAcα→3Galβ→4GlcNAcβ→3Galβ→ 4GlcNAcβ→Asn (S1)	13 400	2.23	44 600	7.43

[a] As NeuAcα→3Gal.

tion mixture did not reduce significantly the transfer to fetuin. Several glycolipids were also tested as acceptor substrates. The neutral glycolipids were not acceptors and, among the gangliosides, only sialosylparagloboside was found to incorporate a 2-acetamido-2-deoxygalactosyl group. GM_3, on the other hand, which also contains the NeuAcα→3Galβ→ group at its nonreducing end was not recognized as an acceptor substrate by the GalNAc-transferase.

Comparison of the N-*acetyl-β-*D-*galactosaminyltransferases from human and guinea pig kidney microsomes.* — The activities of the GalNAc-transferases from human and guinea pig kidney were compared (see Table III). The specific activity towards all acceptors tested was about three times higher for the preparation from guinea pig kidney than for the microsomes from human kidney. However, in contrast to the human enzyme, the GalNAc-transferase from guinea pig was active also with glycophorin A and all its derivatives as acceptors, including the reduced tetrasaccharide obtained through β-elimination.

DISCUSSION

Human kidney was found to contain a *N*-acetyl-β-D-galactosaminyltransferase that specifically transfers 2-acetamido-2-deoxy-β-D-galactopyranosyl groups to sialylated *N*- and *O*-linked carbohydrate chains in bovine fetuin. When the product of the transferase reaction with fetuin as acceptor was submitted to β-elimination, a radioactive material was released that migrated more slowly on t.l.c. than the unlabeled, precursor oligosaccharides. The R_F values observed for the labeled oligosaccharides indicated an elongation of the precursor chains by one

additional 2-acetamido-2-deoxy-β-D-galactopyranosyl group. Furthermore, these newly synthesized products comigrated on t.l.c. plates with the oligosaccharides released by alkali–borohydride treatment of glycophorin A purified from Cad erythrocytes. The Cad-active structures are well characterized[2] and it may, therefore, be suggested that the structures formed by the *N*-acetyl-β-D-galactosaminyltransferase are β-D-Gal*p*NAc-(1→4)-[αNeuAc-(2→3)]-β-D-Gal*p*-(1→3)-D-GalNAc and β-D-Gal*p*NAc-(1→4)-[αNeuAc-(2→3)]-β-D-Gal*p*-(1→3)-[αNeuAc-(2→6)]-D-GalNAc.

Transfer was also obtained to the carbohydrate chains of the *N*-glycosyl, complex type present in the fetuin molecule. These oligosaccharides may be cleaved specifically by anhydrous hydrazine under conditions that completely eliminate the *O*-glycosyl-linked sugar molecules. Thus, after hydrazinolysis and gel filtration, pure *N*-linked oligosaccharides were obtained from the bovine fetuin that had been labeled with [^{14}C]GalNAc by the *N*-acetylgalactosaminyltransferase.

Donald *et al.*[4] and Conzelmann and Kornfeld[8] have shown that carbohydrate structures carrying the GalNAcβ→4(NeuAcα→3)Gal group are resistant to both neuraminidase from *V. cholerae* and *N*-acetylhexosaminidase from jack beans. Only after the sialic acid group has been removed by acid treatment could the *N*-acetyl-β-hexosaminidase cleave the labeled 2-acetamido-2-deoxygalactopyranosyl group. By use of this technique, it was shown that the transfer to the *N*-glycans had resulted in the same terminal trisaccharide β-D-GalNAc-(1→?)-[α-NeuAc-(2→3)]-D-Gal group as the transfer to the *O*-linked oligosaccharides. Furthermore, these sequential treatments were also applied to a pure and homogenous product that had been obtained by the transfer of 2-acetamido-2-deoxy-D-galactose to the glycopeptide NeuAcα→3Galβ→4GlcNAcβ→3Galβ→4GlcNAcβ→Asn (S_1), and, indeed, this product too proved to satisfy the criteria established for the carbohydrate structures containing the GalNAcβ→4(NeuAcα→3)Gal group.

The aforementioned results suggest that human kidney microsomes contain a β-D-(1→4)-*N*-acetylgalactosaminyltransferase capable to transfer a 2-acetamido-2-deoxygalactopyranosyl group to a galactose residue already substituted at O-3 by an *N*-acetylneuraminic acid. The transfer to the *N*-linked chains on the fetuin molecule was about five times greater than that to the *O*-linked oligosaccharides. However, it is well established[23,24] that about the same number of NeuAcα→3Gal groups are present on *O*- and *N*-linked chains of native bovine fetuin. Galactose residues carrying an NeuAc group in α-(2→6) linkage were not acceptors for the GalNAc-transferase. By use of oligosaccharides and glycopeptides having well characterized structures, it was found that the best acceptors were those carrying only *N*-acetylneuraminic acid groups in α-(2→3) linkage. The transfer rate to molecules that contained mostly α-(2→6)-linked sialic acid groups, such as the biantennary glycopeptide isolated from Cohn Fraction IV from human serum, was much lower. The observed, relative transfer rate (14%) corresponded clearly to the proportion (13%) of α-(2→3)-linked sialic acid groups present in the preparation. Among the glycoprotein acceptors, α_1-acid glycoprotein was found to be a

poor acceptor only when the proportion of 2-acetamido-2-deoxygalactose incorporated per mol sialic acid was compared to the proportion obtained for bovine fetuin. However, in contrast to bovine fetuin which contains 70% of *N*-acetylneuraminic acid groups α-(2→3)-linked, human α_1-acid glycoprotein contains only about 15% of *N*-acetylneuraminic acid groups α-(2→3)-linked to galactose, the rest being α-(2→6)-linked.

Although the *N*-acetyl-β-D-galactosaminyltransferase was shown to be able to use *O*-glycosyl-linked chains as acceptors in fetuin, no incorporation into glycophorin from normal human erythrocytes was observed. The presence of inhibitors in the glycophorin preparation was ruled out by mixing it with fetuin in an incubation mixture containing the transferase, no significant reduction in the transfer to fetuin being detected. Likewise, an oligosaccharide obtained through β-elimination and having a structure identical with that of the main carbohydrate chain present on human glycophorin was not an acceptor. However, this observation may be explained by the loss of the reducing terminal residue in the tetrasaccharide. Sialyllactose, which is an acceptor for the GalNAc-transferase, was no longer active after reduction with sodium borohydride. Thus, the conformation of the acceptor structure may play an important role in the transferase reaction. This could also be observed when glycolipids were used as acceptors. Both sialosylparagloboside and GM_3 carry at their nonreducing end the potential acceptor NeuAcα→3Gal group, but only sialosylparagloboside was recognized as acceptor by the GalNAc-transferase. Most probably, the two gangliosides are presented differently to the enzyme at the surface of liposomes or micelles. Similar phenomena have been observed in the study of the interaction of lectins and antibodies with glycolipids[25,26].

N-Acetyl-β-D-galactosaminyltransferases, which transfer a 2-acetamido-2-deoxy-β-D-galactopyranosyl group to sialylated glycoproteins, have been described for guinea pig kidney[7] and for a murine, cytotoxic T-lymphocyte cell line[8]. From the data presented, it is evident that these two enzymes are very closely related. When the *N*-acetyl-β-D-galactosaminyltransferase from human kidney described herein was compared with that from the kidney of guinea pigs, the specific activity of the latter enzyme in microsomes was about two- to three-fold higher towards fetuin and the glycopeptide S1 than that found in the former enzyme prepared in exactly the same way. Furthermore, the enzyme from guinea pig did not distinguish between *O*- and *N*-linked glycans and transferred 2-acetamido-2-deoxygalactose as well to fetuin as to human glycophorin A. The human enzyme was able to recognize *O*-glycan chains, but the transfer rate was much reduced. This was shown by the analysis of the fetuin product and was also evident from the transfer to a glycopeptide from human glycophorin A. At the same concentration of NeuAcα→3Gal groups, glycopeptides T_1 and T_2 incorporated only ~20% of the radioactivity that was transferred to the glycopeptide S1. The guinea pig enzyme, on the other hand, did not make any distinction; it incorporated the same amount of 2-acetamido-2-deoxygalactose into both acceptor substrates. It recognized even the reduced tetrasaccharide NeuAcα→3Galβ→3(NeuAcα→6)GalNAcol as an acceptor, however,

at about half the transfer rate. Although the N-terminal glycopeptides T_1 and T_2 are acceptors for the human kidney GalNAc-transferase at the same rate as that observed for the *O*-glycans of bovine fetuin, the glycopeptide T_3 was a very poor acceptor at the same concentration of NeuAcα→3Gal groups. Peptide T_3 is closer to the hydrophobic region of the glycophorin molecule and carries less densely packed oligosaccharide clusters than peptides T_1 and T_2. Probably the three-dimensional structure of T_3 is less extended and, therefore, the acceptor carbohydrate chains are less accessible. However, the enzyme from guinea pig kidney again seemed to be able to transfer to T_3 at about the same rate as to the glycopeptides S1, T_1, and T_2.

Together, the results presented herein indicate that human kidney microsomes contain an *N*-acetyl-β-D-galactosaminyltransferase having a marked preference for acceptor structures carrying a NeuAcα→3Galβ→4GlcNAc group at the nonreducing end. The enzyme probably transfers a 2-acetamido-2-deoxy-β-D-galactopyranosyl group from UDP-GalNAc to O-4 of the D-galactose residue and, therefore, may be involved in the synthesis of Sd^a blood-group antigens. In human kidney, this antigen is found in the *N*-glycans of the Tamm–Horsfall glycoprotein[4] or on long, endo-β-D-galactosidase-sensible structures of mucin-type, oligosaccharide chains[27]. Further studies on this enzyme will be needed to establish clearly its relationship with the blood-group Sd^a and Cad phenotypes.

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