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STUDIES ON UDP-*N*-ACETYLGLUCOSAMINE : α-MANNOSIDE β-*N*-ACETYLGLUCOSAMINYLTRANSFERASE OF RAT LIVER AND HEPATOMAS

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When homogenates of rat liver and hepatomas were centrifuged at 78 000 × *g*, over 90% of liver *N*-acetylglucosaminyltransferase assayed with β-galactosidase- and β-*N*-acetylhexosaminidase-treated asialofetuin as acceptor was recovered in the particulate fraction, while as much as 24% of hepatoma transferase was in the supernatant fraction. The particulate transferase solubilized by 0.2% sodium deoxycholate emerged from a DEAE-cellulose column at 0.04 M NaCl (transferase A). The supernatant fractions from all the hepatomas tested contained a second *N*-acetylglucosaminyltransferase eluted from the column at 0.02 M NaCl (transferase B). Transferase B was absent from liver supernatant fraction. The activities of these transferases toward various acceptors and the effect of β-*N*-acetylhexosaminidase on their products suggest that both transferases are UDP-*N*-acetylglucosamine : α-mannoside β-*N*-acetylglucosaminyltransferase. Although ovalbumin and glycopeptide V, which was isolated from pronase digest of ovalbumin, were good acceptors, transferase A utilized ovalbumin and glycopeptide V with apparent K_m values of 0.44 and 0.33 mM, respectively, whereas the corresponding values for transferase B were 4.5 and 0.050 mM.

Introduction

While neoplastic transformation is known to be associated with alterations in the carbohydrate moieties of glycoproteins (for review, see Ref. 1), the nature of the alterations has not as yet been fully understood. Earlier studies in this laboratory [2,3] demonstrated that glucosamine-6-phosphate synthase (EC 5.3.1.19) in rat liver specifically increases upon hepatocarcinogenesis with concomitant replacement of the original liver-type by a fetal-type isozyme. Since the enzyme is the rate-limiting enzyme of UDP-*N*-acetylglucosamine synthesis [4,5], these observations may offer a key for the elucidation of the neoplastic alterations in the carbohydrate moieties of glycoproteins. UDP-*N*-Acetylglucosamine donates *N*-acetylglucosamine to nascent glycoproteins, and this donation is catalyzed by *N*-acetylglucosaminyltransferase.

The present report describes the characterization of *N*-acetylglucosaminyltransferase of rat hepatomas in comparison with the enzyme of rat liver using β-galactosidase- and β-*N*-acetylhexosaminidase-treated asialofetuin and ovalbumin as the major acceptors. A few studies were reported in the past concerning *N*-acetylglucosaminyltransferase of rat liver [6–8] and hepatomas [7], but they dealt simply with the activity of the enzyme in crude tissue fractions.

Materials and Methods

Materials. Fetuin was purchased from Sigma, St. Louis, MO, U.S.A., and purified by Sephadex G-200 chromatography before use. Ovalbumin (crystalline), transferrin and dolichol phosphate were also the products of Sigma. Disialyl-oroso-*N*-octaose from the urine of a mucolipidosis patient [9] has a structure of

NeuAcα2-6Galβ1-4GlcNAcβ1-2Manα1
NeuAcα2-6Galβ1-4GlcNAcβ1-2Manα1
$\}$ 6,3 Manβ1-4GlcNAc ,

and was kindly provided by Dr. Koichi Tsurumi, Fukushima Medical College, Fukushima, Japan. Bovine submaxillary mucin was prepared as described by Tsuiki et al. [10]. β-Galactosidase, β-*N*-acetylhexosaminidase and α-mannosidase were purified from Jack bean by the method of Li and Li [11]. Pronase, *Arthrobacter* neuraminidase and calf intestine alkaline phosphatase were the products of Kaken Chemicals, Tokyo, Japan, Nakarai Chemicals, Kyoto, Japan, and Boehringer, Mannheim, F.R.G., respectively. UDP-*N*-[^{14}C]acetylglucosamine was obtained from New England Nuclear, Boston, MA, U.S.A., and used after dilution with UDP-*N*-acetylglucosamine to give a final specific radioactivity of 1 mCi/mmol. UDP-*N*-Acetylglucosamine and UDP-galactose were obtained from Boehringer.

Liver. Male Donryu rats weighing 150–200 g and fed ad libitum were killed by decapitation. The livers were immediately excised and rinsed in cold physiological saline.

Primary hepatomas. Male Donryu rats weighing 100–120 g were fed a commercial stock diet containing 0.06% (w/v) 3′-methyl-4-dimethylaminoazobenzene for 10 weeks, then fed the same diet, free of carcinogen, until death (5–10 weeks). When diethylnitrosamine was the carcinogen employed, it was administered to the rats with drinking water at a level of 0.01% (w/v) for 3 weeks while they were fed normal diet; the rats were killed 20–25 weeks after the withdrawal of carcinogen. After decapitation, livers were quickly excised and tumors formed were dissected from the surrounding and necrotic tissues. Small sections were taken for histological examination and hepatocellular carcinomas diagnosed were rinsed in cold physiological saline.

Transplantable hepatomas. A strain of transplantable rat hepatoma, AH-109A [12], was inoculated into male Donryu rats (150–200 g) either intraperitoneally or subcutaneously. The tumor was harvested 5 (for ascitic form) or 12 (for solid form) days after inoculation. Ascitic tumor cells were collected and packed by centrifugation. Solid tumors were dissected as described for the primary hepatomas. Morris hepatoma 5123D, which was maintained by subcutaneous inoculation in male Buffalo rats, was a gift of Dr. Minro Watanabe, Cancer Chemotherapy Department of this Institute.

Preparation and fractionation of homogenates. All subsequent procedures were carried out at 0–4°C unless otherwise specified. Solid tissues were homogenized in 4 vol. 0.25 M sucrose/25 mM KCl/1 mM EDTA/20 mM Tris-HCl, pH 7.4 (buffer A) by the use of a glass-Teflon homogenizer with six strokes. The low speed supernatant (600 × *g*, 10 min) was recentrifuged at 78 000 × *g* for 70 min and the resultant particulate and supernatant fractions were saved. Ascitic hepatoma cells were packed in buffer A and homogenized in an equal volume of water. A glass-Teflon homogenizer was used with six strokes. The homogenate was then made isotonic by addition of 0.5 M sucrose/50 mM KCl/2 mM EDTA/40 mM Tris-HCl, pH 7.4, and centrifuged at 78 000 × *g* for 70 min.

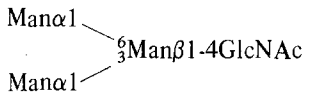
Preparation of N-acetylglucosaminyltransferase A. The particulate fraction from livers (approx. 50 g) or hepatomas (approx. 20 g) was suspended in 50 (for livers) or 20 (for hepatomas) ml buffer A and made 0.2% (w/v) in sodium deoxycholate. The suspension was then homogenized by a glass-Teflon homogenizer with three strokes, centrifuged at 78 000 × *g* for 70 min and the resulting supernatant was fractionated by addition of $(NH_4)_2SO_4$. The precipitate formed at 35% saturation was collected by centrifugation, dissolved in a minimum volume of 20 mM Tris-HCl, pH 7.4/0.1% (w/v) Triton X-100/5% (w/v) glycerol (buffer B), passed through a desalting column of Sephadex G-25 and applied to a DEAE-cellulose (DE-52) column (1.5 × 10 cm) previously equilibrated with buffer B. The column was washed with 100 ml buffer B and eluted by a linear 400 ml NaCl gradient from 0 to 0.1 M. Fractions collected in 10 ml were assayed for *N*-acetylglucosaminyltransferase under the standard conditions; and those containing the transferase were pooled and the volume reduced to a few millilitres by ultrafiltration using an Amicon PM-10 membrane.

Preparation of N-acetylglucosaminyltransferase B. The 78 000 × *g* supernatant fraction from hepatoma homogenates was fractionated by addition of $(NH_4)_2SO_4$. The precipitate formed at 35% saturation was collected by centrifugation, dissolved in buffer B, desalted by Sephadex G-25 and chromatographed on DEAE-cellulose as described for transferase A. The fractions containing transferase B were pooled and the volume reduced to a few millilitres using an Amicon PM-10 membrane.

Analytical methods. Total sialic acid was determined by the method of Jourdian et al. [13] and mannose by a phenol-H_2SO_4 method [14]. Sialic acid [15], galactose [16] and *N*-acetylglucosamine [17] made free from glycoproteins were assayed as described in the references. Protein content was estimated by the method of Lowry et al. as modified by Dulley and Grieve [18].

Preparation of β-galatosidase- and β-N-acetylhexosaminidase-treated asialofetuin. The purified fetuin containing 58.4 μmol sialic acid was subjected to mild acid hydrolysis as described by Spiro [19]: 56.5 μmol or 95% of the sialic acid were removed (asialofetuin). After dialysis against water, asialofetuin was incubated with β-galactosidase (20 U) in 10 ml 50 mM citrate buffer, pH 3.5, at 37°C for 48 h under toluene. This treatment removed 20.5 μmol galactose. The solution was then adjusted to pH 5.0 and further incubated with β-*N*-acetylhexosaminidase (40 U) at 37°C for 48 h under toluene: 18.5 μmol *N*-acetylglucosamine were released and this quantity was equivalent to almost 80% of the removable *N*-acetylglucosamine of fetuin inferred from its oligosaccharide structure and its sensitivity to Jack bean exoglycosidases [20]. The final solution was heated at 70°C for 30 min to inactivate the exoglycosidases and dialyzed against water. The concentration of β-galactosidase- and β-*N*-acetylhexosaminidase-treated asialofetuin was expressed in terms of acceptor sites for *N*-acetylglucosamine based on the quantity of *N*-acetylglucosamine removed from β-galactosidase-treated asialofetuin. In one experiment, β-galactosidase- and β-*N*-acetylhexosaminidase-treated asialofetuin was further treated with α-mannosidase (U not determined) in 50 mM citrate buffer, pH 5.0, at 37°C for 48 h under toluene.

Preparation of other acceptors. β-Galactosidase- and β-*N*-acetylhexosaminidase-treated asialotransferrin was prepared from transferrin in the same manner as described above. The same procedures were also applied to disialyl-oroso-*N*-octaose: although the quantity of the starting material was too small to carry out the entire procedures quantitatively, the extent of the release of *N*-acetylglucosamine suggested that the bulk of the products (β-galactosidase- and β-*N*-acetylhexosaminidase-treated oroso-*N*-octaose) had the following structure.



Asialo-bovine submaxillary mucin was prepared from bovine submaxillary mucin by the same procedure as described for asialofetuin. The asialomucin, 15 μmol in acceptor sites based on the quantity of sialic acid removed, was incubated with galactosyltransferase partially purified from rat liver using a α-lactalbumin affinity column [21]. The incubation mixture also contained 50 μmol UDP-galactose, 100 μmol $MnCl_2$ and 500 μmol 2-(*N*-morpholino)ethanesulfonate, pH 6.2, in a final volume of 10 ml. After 24 h at 37°C, the mixture was heated at 70°C for 30 min, passed through a Sephadex G-25 column and lyophilized. The mucin incorporated 4.5 μmol galactose under these conditions.

Preparation of glycopeptides from ovalbumin. 2 g crystalline ovalbumin were digested with pronase (40 mg) in 15 ml 0.1 M Tris-HCl, pH 8.0/10 mM $CaCl_2$ at 37°C for 48 h, and the digest was passed through a Sephadex G-25 column (1.5 × 90 cm). The glycopeptide fraction was then fractionated on a Dowex 50W-X2 column (1.5 × 90 cm) in sodium acetate, pH 2.6/1 mM Na^+ into subfractions I to V as described by Tai et al. [22]. Glycopeptide VI, a minor subfraction reported later by the same authors [23], was scarcely detectable. The subfractions were assayed for mannose and the results were used to quantify each glycopeptide.

Assay of N-acetylglucosaminyltransferase. The standard assay mixture contained 30 nmol UDP-*N*-[^{14}C]acetylglucosamine, 100 nmol β-galactosidase- and β-*N*-acetylhexosaminidase-treated asialofetuin, 0.5 μmol $MnCl_2$, 5 μmol *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate, pH 7.5, 0.1 mg Triton X-100 and enzyme in 0.1 ml. After 1 h at 37°C, the reaction was terminated by addition of 1 ml of 1% (w/v) phosphotungstic acid in 0.5 M HCl. The precipitate formed was collected, washed twice with 5% (w/v) trichloroacetic acid in 1% phosphotungstic acid, placed on a glass filter (Whatman GF/A) and washed with ethanol/ether (1 : 1, v/v). The filter was dried and counted in a liquid scintillation spectrometer with the use of toluene scintillator containing 0.6% (w/v) 2,5-diphenyloxazole and 0.01% (w/v) 1,4-bis(5-phenyloxazole-2-yl)benzene.

1 unit of the enzyme was defined as the amount which catalyzed the transfer of 1 nmol of *N*-acetylglucosamine in 1 h.

When ovalbumin-derived glycopeptides were the acceptor, the reaction was terminated by freezing the mixture at −20°C and the glycopeptide was separated from UDP-[^{14}C]*N*-acetylglucosamine by high voltage paper electrophoresis in 1% (w/v) sodium tetraborate (70 V/cm, 40 min) using Whatman 3 MM paper. The glycopeptide remained at the origin. To remove any free radioactive *N*-acetylglucosamine, the electrophoretogram was further developed by descending paper chromatography with 80% (v/v) ethanol overnight. The paper was then cut into strips and those containing the glycopeptide were counted in a liquid scintillation spectrometer as described above.

Product characterization. For this purpose, the standard assay mixture was scaled up 10-fold and the incubation lasted 10 h. The mixture was then passed through a Sephadex G-25 column (1.5 × 20 cm), and the resultant high molecular weight fraction was treated with β-*N*-acetylhexosaminidase (3 U) in 50 mM citrate buffer, pH 5.0, for 48 h at 37°C under toluene; the final volume was 1 ml. After lyophilization, the digest was subjected to high voltage paper electrophoresis in 1% sodium tetraborate (70 V/cm, 50 min, Whatmann 3 MM paper) and the electrophoretogram was further chromatographed in a descending manner with 1-butanol/pyridine/water (6 : 4 : 3, v/v) for 8 h. The paper was cut into 1-cm strips and counted in a liquid scintillation spectrometer.

Results

Level and distribution of N-acetylglucosaminyltransferase

Tissue homogenates were centrifuged at 78 000 × *g* and the resulting particulate and supernatant fractions were assayed for *N*-acetylglucosaminyltransferase using β-galactosidase- and β-*N*-acetylhexosaminidase-treated asialofetuin as acceptor. Under the conditions employed, the rate of transferase reaction was proportional to the amount of enzyme and the duration of incubation. As shown in Table I, the particulate fractions from three rat hepatomas were somewhat less active than that from rat liver. More significant is the difference in the subcellular distribution of the enzyme: while over 90% of liver transferase was associated with the particulate fraction, as much as 24% of hepatoma transferase was recovered in the supernatant fraction. Actually, hepatoma induced by 3'-methyl-4-dimethylaminoazobenzene had significantly more transferase in the supernatant fraction compared to liver. The transferase of 40 h-regenerating liver, however, exhibited a a subcellular distribution very similar to that of control rat liver.

TABLE I

LEVEL AND DISTRIBUTION OF *N*-ACETYLGLUCOSAMINYLTRANSFERASE IN RAT LIVER AND HEPATOMAS

The particulate and supernatant fractions were assayed for *N*-acetylglucosaminyltransferase under the standard conditions except that 0.1 μmol NAD^+ was added to suppress pyrophosphatase activity [24]. The values are means ± S.E. of the means of three to five experiments. 5–10 rats were killed for each experiment. The figures in parenthesis are percentage of the total activity.

Tissue	*N*-Acetylglucosaminyl transferase activity (units/g tissue)	
	Particulate	Supernatant
Liver:		
normal adult	25.74 ± 2.96	1.76 ± 0.71 (6.4)
regenerating [a]	12.00	0.46 (3.7)
Hepatomas:		
3'-Methyl-4-dimethylaminoazobenzene-induced	15.30 ± 2.70	4.80 ± 0.60 (23.9)
Diethylnitrosamine-induced	12.67 ± 1.16	2.69 ± 0.49 (17.5)
AH-109A (solid)	8.30	1.80 (17.0)

[a] 40 h regenerating liver was prepared as described previously [25].

Characterization of particulate-associated N-acetylglucosaminyltransferase

Although Bosman [26] extracted *N*-acetyl-

glucosaminyltransferase from rabbit liver with 0.1% Triton X-100, this condition was far from satisfactory for the solubilization of rat liver particulate transferase. Increasing the amount of Triton X-100 resulted in the solubilization of up to 20% of the particulate activity at a Triton X-100 concentration of 0.5% followed by gradual decrease at higher concentrations. Nearly quantitative solubilization of rat liver particulate transferase, however, was achieved by using 0.2% sodium deoxycholate. The same results were obtained with the particulate fractions from various hepatomas.

N-Acetylglucosaminyltransferase solubilized by sodium deoxycholate was precipitated with $(NH_4)_2SO_4$ and chromatographed on a DEAE-cellulose column. Fig. 1 shows the results obtained for liver and dimethylaminoazobenzene-induced hepatoma. In either case, transferase activity emerged from the column as a single symmetrical peak at an NaCl concentration of 0.04 M. This transferase was designated *N*-acetylglucosaminyltransferase A. The particulate fractions from diethylnitrosamine-induced hepatoma and AH-109A (solid and ascitic) all exhibited a single *N*-acetylglucosaminyltransferase peak corresponding to transferase A (data not shown).

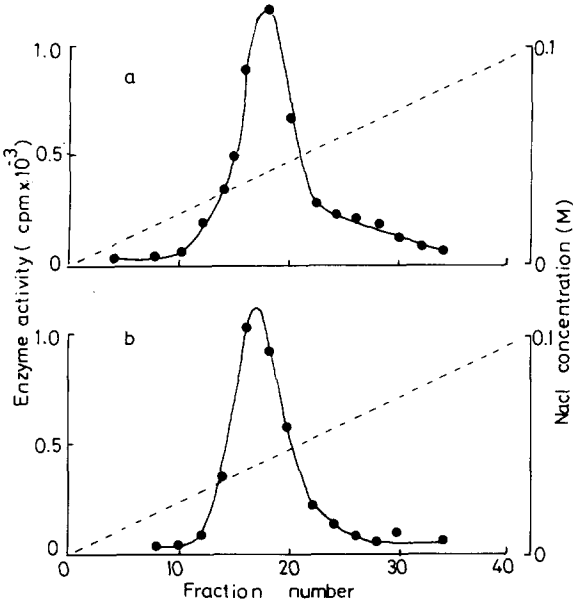


Fig. 1. Chromatography of solubilized particulate *N*-acetylglucosaminyltransferase on DEAE-cellulose. a, liver; and b, 3'-methyl-4-dimethylaminoazobenzene-induced hepatoma. ●———●, enzyme activity and - - - - - -, NaCl concentration.

Characterization of supernatant N-acetylglucosaminyltransferase

When the 78 000 × *g* supernatant fraction from liver homogenate was fractionated with $(NH_4)_2SO_4$ and chromatographed on DEAE-cellulose, *N*-acetylglucosaminyltransferase activity emerged as a single peak at an NaCl concentration of 0.04 M (Fig. 2c).

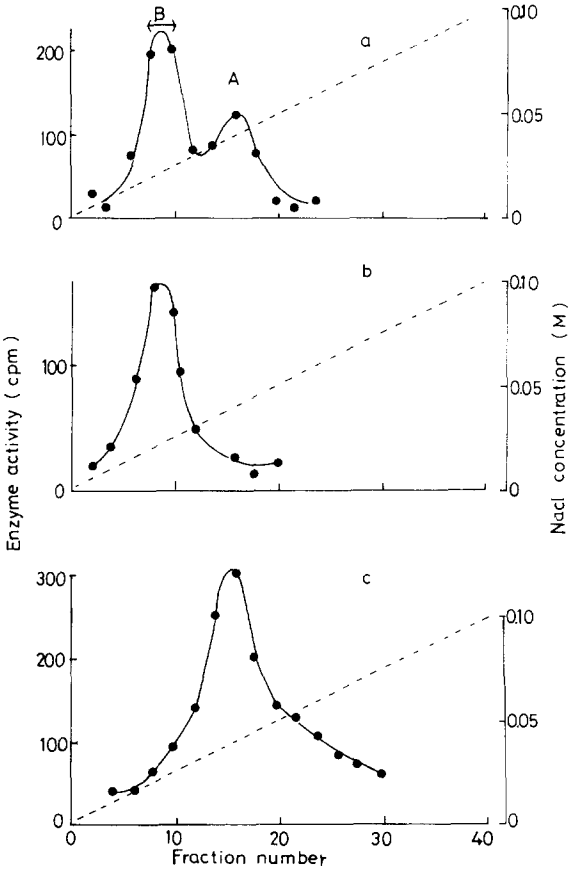


Fig. 2. Chromatography of supernatant *N*-acetylglucosaminyltransferase on DEAE-cellulose. a, The supernatant fraction from diethylnitrosamine-induced hepatoma was chromatographed. b, Peak B in (a) was pooled, concentrated by ultrafiltration and rechromatographed under the same conditions. c, The supernatant fraction from liver was chromatographed under the same conditions as for (a) except that 4-times as much protein was applied to the column. ●———●, enzyme activity, and - - - - - -, NaCl concentration.

The transferase of liver supernatant thus appears to be transferase A rendered soluble during tissue homogenization, although the possibility cannot be ruled out that it is by nature a cytosolic enzyme.

The supernatant fraction from diethylnitrosamine-induced hepatoma, on the other hand, exhibited a second peak of transferase, *N*-acetylglucosaminyl-transferase B, resolvable from transferase A by DEAE-cellulose column chromatography (Fig. 2a). Transferase B was eluted from the column at an NaCl concentration of 0.02 M (Fig. 2a) and this elution characteristic was reproducible on rechromatography (Fig. 2b). When the study was extended to the supernatant fractions from four other hepatomas, namely dimethylaminoazobenzene-induced hepatoma, Morris hepatoma 5123D and the solid and ascitic forms of AH-109A, all were found to contain transferase B besides transferase A (data not shown). In the sections to follow, the properties of transferase B prepared from the supernatant fraction of dimethylaminoazobenzene-induced hepatoma were compared with those of transferase A which was from the particulate fraction of rat liver. Typical preparations of transferases A and B had specific activities of 39 and 0.9 units/mg protein, respectively, under the standard assay conditions. Although not pure, each preparation was free of the other enzyme activity.

Further characterization of transferase B

Galactosyltransferase from human serum was recently shown to be a glycoprotein [27,28]. To determine if transferase B is simply a desialylated form of transferase A, the latter enzyme was incubated with *Arthrobacter* neuraminidase under conditions such that rat liver sialyltransferase was readily desialylated (unpublished data). The incubation, however, resulted in no alteration in the elution characteristic of transferase A. Likewise, transferase B could not be a dephosphorylated form of transferase A, since previous incubation with calf intestine alkaline phosphatase did not affect the elution pattern of transferase A. When chromatographed on Sephadex G-200, both transferases A and B behaved similarly, emerging from the column somewhat after urease (M_r 480 000) and before catalase (240 000) (Fig. 3). Although the result seems to support the view that transferase B is neither an aggregate nor disaggregate of transferase A, it should be kept in mind

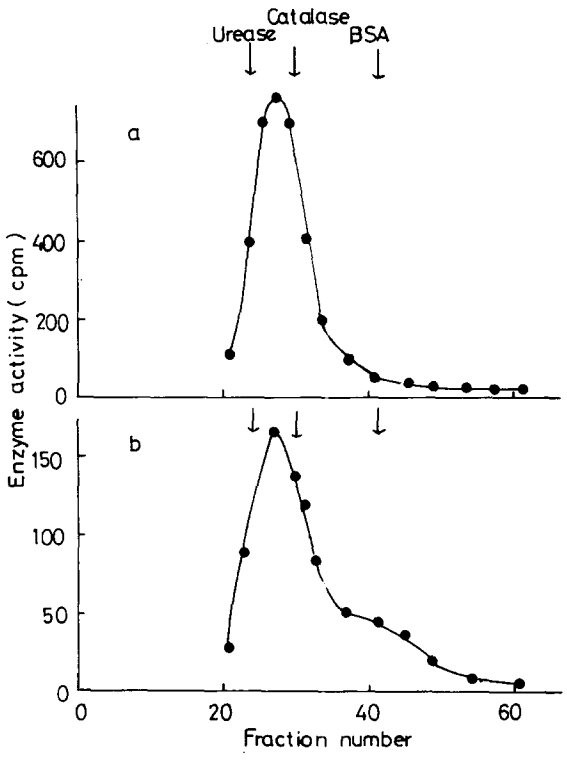


Fig. 3. Chromatography of *N*-acetylglucosaminyltransferases A and B on Sephadex G-200. Partially purified transferase A (1.5 mg in protein) (a) or B (9 mg in protein) (b) was applied to a Sephadex G-200 column (1.5 × 44 cm). The column was equilibrated and eluted with 20 mM Tris-HCl, pH 7.4/50 mM NaCl/0.1% (w/v) Triton X-100/2% (w/v) glycerol. Fractions of 1-ml were collected and assayed for *N*-acetylglucosaminyl-transferase. Soybean urease (M_r 480 000), bovine liver catalase (240 000) and bovine serum albumin (65 000) were used as markers.

that the resolving power of the gel is too poor to resolve proteins of M_r over 200 000 distinctly.

General properties of transferases A and B

Both transferases A and B obligatorily require Mn^{2+}; maximum activity was attained at 2 mM. The two transferases were stimulated equally (up to 80%) by Triton X-100 (0.1%, w/v). For either enzyme, maximum activity was detected in pH range between 7.0 and 7.3.

Acceptor specificity of transferases A and B

Transferase A transferred *N*-acetylglucosamine to β-galactosidase- and β-*N*-acetylhexosaminidase-treated

asialofetuin efficiently but to asialofetuin, β-galactosidase-treated asialofetuin and β-galactosidase-, β-*N*-acetylhexosaminidase- and α-mannosidase-treated asialofetuin only insignificantly. Using β-galactosidase- and β-*N*-acetylhexosaminidase-treated asialofetuin as acceptor, the characterization of ^{14}C-labeled product was performed: electrophoresis of the digest with β-*N*-acetylhexosaminidase gave a poor separation of free *N*-acetylglucosamine from the high molecular weight fraction and, therefore, the electrophoretogram was further developed by paper chromatography. All the radioactivity was recovered as free *N*-acetylglucosamine. When transferase B replaced transferase A, the results of acceptor and product analysis were the same. These results altogether suggest that both transferases A and B are UDP-*N*-acetylglucosamine: α-mannoside β-*N*-acetylglucosaminyltransferase. That *N*-acetylglucosamine is transferred to the terminal α-mannosine residues of the modified fetuin was also suggested from the finding that β-galactosidase- and β-*N*-acetylglucosaminidase-treated oroso-*N*-octaose exhibited a weak but significant acceptor activity (Table II). It should also be noted that dolichol phosphate was inactive as acceptor when examined according to the procedure of Waechter and Harford [29].

In addition to the modified fetuins, other native and modified glycoproteins were tested as acceptors for the two transferases. In Table II, comparisons are made relative to the activity observed with β-galactosidase- and β-*N*-acetylhexosaminidase-treated asialofetuin. In harmony with the results obtained with the modified fetuins, β-galactosidase- and β-*N*-acetylhexasaminidase-treated asialotransferrin but not asialo-bovine submaxillary mucin were good acceptors for transferases A and B. The finding that galactosylated asialomucin does not function as acceptor is significant since *O*-glycosidically linked oligosaccharide chains are believed to be present in asialofetuin [30].

Ovalbumin was found to be a good acceptor for both transferases A and B (Table II). In view of the heterogenous nature of the oligosaccharide moiety of ovalbumin [31], ovalbumin was digested with pronase and the resultant glycopeptides were subfractionated into I to V according to the method of Tai et al. [22]. When these subfractions separately served as acceptor, the two transferases were active

TABLE II

ACCEPTOR SPECIFICITY OF *N*-ACETYLGLUCOSAMINYLTRANSFERASES A AND B

Transferases A (5 μg in protein) and B (20 μg) were assayed with indicated glycoproteins as acceptors. The amount of each acceptor was 50 nmol as acceptor site except ovalbumin, whose amount was 50 nmol as ovalbumin. The amounts of the acceptor sites of asialobovine submaxillary mucin and its galactosylated product were based on the amounts of sialic acid removed and galactose incorporated, respectively, n.d. means not determined.

Glycoprotein	*N*-Acetylglucosaminyltransferase activity (%)	
	A	B
Ovalbumin	124	110
β-Galactosidase-, β-*N*-acetylhexosaminidase-treated asialofetuin (I)	100	100
Pronase-digested I [a]	18	135
β-Galactosidase-, β-*N*-acetylhexosaminidase-treated asialotransferrin	70	43
Asialo-bovine submaxillary mucin (II)	2	7
Galactosylated II	3	5
β-Galactosidase-, β-*N*-acetylhexosaminidase-treated oroso-*N*-octaose [b]	17	n.d.

[a] Digestion was carried out as described for ovalbumin.

[b] The assay was made as described for ovalbumin glycopeptides except that the paper chromatographic step was omitted.

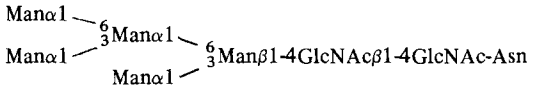
TABLE III

EFFICIENCIES OF OVALBUMIN GLYCOPEPTIDES I TO V AS ACCEPTOR FOR *N*-ACETYLGLUCOSAMINYLTRANSFERASES A AND B

Each glycopeptide (100 nmol) was incubated with *N*-acetylglucosaminyltransferases A (2 μg in protein) or B (40 μg) under the standard conditions except that incubation lasted 4 h.

Glycopeptide	*N*-Acetylglucosamine incorporated (cpm)	
	Transferase A	Transferase B
I	28	0
II	5	30
III	13	7
IV	50	21
V	656	586

only with glycopeptide V (Table III). This suggests that the *N*-acetylglucosamine-accepting site of ovalbumin is in the following structure [22,32].



Affinities of transferases A and B for ovalbumin acceptors

Table II shows that when β-galactosidase and β-*N*-acetylhexosaminidase-treated asialofetuin is digested with pronase, its acceptor efficiency toward transferase A is markedly decreased, whereas the efficiency toward transferase B is slightly increased. This prompted us to investigate the affinities of the two transferases for ovalbumin and ovalbumin-derived glycopeptide V. Fig. 4 shows the activities of transferases A and B as a function of the concentration of the two acceptors. When these data were plotted according to Lineweaver and Burk [33], transferase A was found to have apparent K_m of 0.44 and 0.33 mM for ovalbumin and glycopeptide V, respectively; the values for transferase B were 4.5 mM for ovalbumin and 0.050 mM for glycopeptide V.

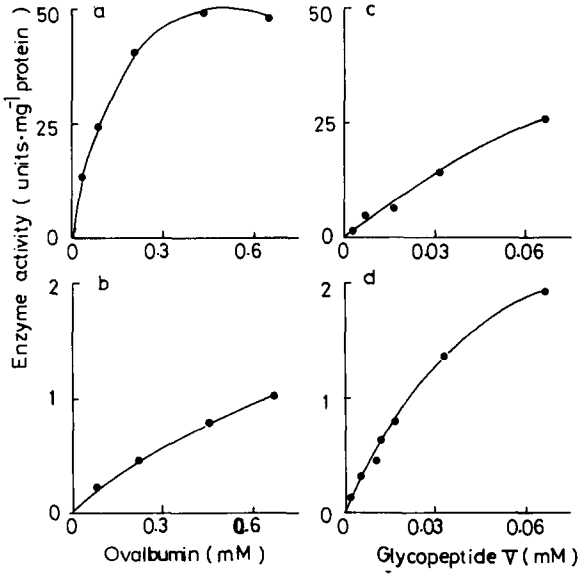


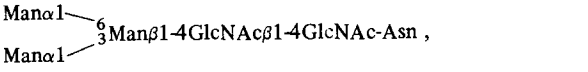
Fig. 4. Activities of *N*-acetylglucosaminyltransferases A (a and c) and B (b and d) as a function of the concentration of ovalbumin (a and b) or glycopeptide V (c and d). Partially purified transferases A and B were assayed under the standard conditions except the concentration of acceptor.

The yield of glycopeptide V more or less varied from lot to lot of ovalbumin, but in the experiment reported here, it was approx. 10% of the starting ovalbumin on molar basis. If one assumes that ovalbumin contains only one asparagine-linked oligosaccharide chain per molecule [34], the 10% yield of glycopeptide V means that only 1 out of 10 ovalbumin molecules possesses glycopeptide V, thus is capable of acting as acceptor on transferases A and B (active ovalbumin). By dividing the K_m values for ovalbumin by 10, those for active ovalbumin were estimated to be 0.044 and 0.45 mM for transferases A and B, respectively. The significance of these estimations is the disclosure that the K_m of transferase A is one order of magnitude greater for glycopeptide V than for active ovalbumin (ovalbumin-linked glycopeptide V) while the reverse is true of transferase B. Reduction in the size of ovalbumin thus affects the activities of the two transferases in the opposite directions.

Discussion

The present studies demonstrate that although rat liver and hepatomas possess *N*-acetylglucosaminyltransferase A in common, only the latter tissues contain transferase B. The two enzymes differ from each other by subcellular distribution, chromatographic behavior and some of the kinetic properties studied. In addition, attempts to convert transferase A into transferase B in vitro have been unsuccessful. It therefore appears that the two transferases represent discrete entities.

According to Harpaz and Schachter [35,36], bovine colostrum contains 2 different UDP-*N*-acetylglucosamine:α-mannoside β2-*N*-acetylglucosaminyltransferases, namely I and II, both of which are required for the synthesis of the outer chains of asparagine-linked oligosaccharides. Only transferase I, however, can utilize ovalbumin-derived glycopeptide V as acceptor and also transfers *N*-acetylglucosamine to



which is presumably identical to the *N*-acetylglucosamine-accepting sites of β-galactosidase- and β-*N*-acetylhexosaminidase-treated asialofetuin

[20,37]. Transferases A and B therefore resemble transferase I of bovine colostrum in acceptor specificity. Although transferase I was shown to attach *N*-acetylglucosamine to the position 2 of the mannose α1–3 terminus [35,36], the specificity of transferases A and B regarding the nature of the linkage and mannose terminus has not as yet been ascertained.

In addition to the asparagine-linked oligosaccharide chains, fetuin contains *O*-glycosidically linked oligosaccharide chains [30], but they must be ineffective as acceptor for transferases A and B, since asialobovine submaxillary mucin and its galactosylated product are not good acceptors for these transferases.

Although so far indistinguishable by acceptor specificity, transferases A and B differ in affinities for ovalbumin acceptors. For instance, the K_m of transferase B for ovalbumin is one order of magnitude greater than the K_m of transferase A. Much more significant, however, is the finding that the affinities of the two transferases toward ovalbumin change in the opposite directions along with reduction in the size of ovalbumin molecule. This is probably true of β-galactosidase- and β-*N*-acetylhexosaminidase-treated asialofetuin (see Table II). It therefore appears that although the two transferases may utilize the same terminal structure, transferase B has a preference for low molecular weight glycopeptides while transferase A prefers to act on high molecular weight glycoproteins.

Although the present studies have disclosed that a notable neoplatic alteration does occur at UDP-*N*-acetylglucosamine: α-mannoside β-*N*-acetylglucosaminyltransferase, the relation of this alteration to the neoplastic alterations of glycoproteins has as yet been little understood. To clarify this relation, further studies of the role played by transferase B in glycoprotein synthesis are needed and are now under way.

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