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Human ovarian cancer, lymphoma spleen, and bovine milk GlcNAc:β1,4Gal/GalNAc transferases: two molecular species in ovarian tumor and induction of GalNAcβ1,4Glc synthesis by α-lactalbumin

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Abstract

Affinity Gel-UDP was utilized to purify GlcNAc:β1,4Gal/GalNAc transferases (Ts) from human lymphoma spleen, ovarian tumor, and ovarian cancer sera. Mn^{2+} was found to be an absolute requirement for activity. Two molecular species containing both β1,4Gal/GalNAc-T activities were discernible when the purified ovarian tumor microsomal enzyme was subjected to Sephacryl S-100 HR column chromatography as well as native polyacylamide gel-electrophoresis. Acceptor specificity studies of the affinity-purified lymphoma spleen and ovarian tumor microsomal enzymes and the conventionally purified, as well as the cloned, bovine milk GlcNAc:β1,4Gal-Ts using a number of synthetic acceptors showed that the β(1,6)-linked GlcNAc moiety to α-GalNAc was the most efficient acceptor. As compared to the purified milk enzyme, the recombinant form exhibited sixfold GlcNAc:β1,4 GalNAc-T activity and up to eightfold GlcNAc6SO_3β-:β1,4Gal-T activity. Further, the recombinant enzyme catalyzed the transfer of GalNAc to the terminal β-linked GlcNAc6SO_3 moiety. Alpha-lactalbumin (α-LA) inhibited up to 85%, the transfer of Gal to the GlcNAc moiety linked either to Man or GlcNAc. On the contrary, α-LA had no significant influence on the transfer of GalNAc to the above acceptors. α-LA had no appreciable effect on the recombinant enzyme, except for the transfer of Gal or GalNAc to Glc. Both α- and β-glucosides, as well as α-*N*-acetylglucosaminide, did not serve as acceptors.

Keywords: Bovine milk; Cancer sera; Human ovarian tumor; Lymphoma-spleen; GlcNAc:β1,4Gal/GalNAc transferases; Specificities; Kinetic properties

1. Introduction

N-Acetyllactosamine units (Galβ1,4-GlcNAcβ-) of glycoproteins serve as the backbone structures built on an N- or O-linked core structure for carrying the terminal carbohydrate epitopes such as sialyl Lewis x. The

Abbreviations: AA-CP, acrylamide copolymer; All, allyl; Bn, benzyl; Gal-T, galactosyltransferase; α-LA, alpha lactalbumin; Me, methyl; Ph, phenyl; RM, reaction mixture; TLC, thin-layer chromatography.

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single step of forming a Galβ1,4GlcNAcβ linkage is performed by a family of β1,4 Gal-Ts, and each member of this family may play a distinct role in different cells.[1] Differences in sensitivity to α-lactalbumin modulation of putative members of the β1,4Gal-T family have been observed.[1–6] A variation of the *N*-acetyllactosamine unit, namely GalNAcβ1,4GlcNAcβ-, is quite diverse in vertebrates and comprises membrane as well as secreted glycoproteins, enzymes, hormones, transport proteins and protective glycoproteins.[7–12] Bovine pituitary gland GlcNAc:β1,4GalNAc-T was shown to act with a 100–200-fold greater efficiency on glycoprotein hormones.[13,14] On the other hand, Van den Nieuwenhof et al.,[15] found that bovine mammary gland GlcNAc:β1,4GalNAc-T was non-specific for pituitary hormone and interacted with α-lactalbumin.

LacdiNAc (GalNAcβ1,4GlcNAcβ-) based chains occur relatively abundantly on invertebrate glycoconjugates. An active GlcNAcβ-R:β1,4GalNAc-T has been identified in cercariae of the schistosome, *Trichobilharzia ocellata*, in the albumen gland and the prostate gland of the snail, *Lymnaea stagnalis*, and in several insect cell lines, as well as in adult worms of *Schistosoma mansoni*.[16–19] This enzyme resembles mammalian β1,4Gal-T in having very similar acceptor properties being capable of acting on non-reducing terminal β-GlcNAc residues regardless of the underlying structure and aglycone. Furthermore, it does not share acceptor properties with other described β1,4GalNAc-T such as the enzymes involved in the synthesis of the Sd^a blood group determinant, ganglioside GM_2 and chrondroitin sulfate.

The ovarian cancer antigen, CA125, is a highly glycosylated glycoprotein containing predominantly a mucin core 2 that consists of carbohydrate chains carrying the blood group Lewis x and Lewis y determinants.[20] An elevation of GlcNAc:β1,4Gal-T activity in the sera of human ovarian cancer patients has been consistently observed by this laboratory.[21,22] A recent study of Udagawa et al. found tumor-associated galactosyltransferase to be a suitable marker for distinguishing ovarian cancer from benign gynecological disorders.[23] As this enzyme extends the carbohydrate chain from the GlcNAc moiety of mucin core 2, it may have an oncogenic role in CA125 biosynthesis. We have now studied the properties and the acceptor–substrate specificities of GlcNAc:β1,4Gal/GalNAc-T present in ovarian tumor, ovarian cancer sera and spleen tissue from a lymphoma patient and also of the purified preparation and the recombinant form of bovine milk β1,4Gal-T. The present paper reports: (a) the occurrence of two molecular species containing both β1,4Gal/GalNAc-T activities in ovarian tumor; (b) α-LA-mediated synthesis of GalNAcβ1,4Glc in ovarian tumor and bovine milk; and (c) substantial difference in acceptor utilization between the purified and the cloned preparations of bovine milk Gal-T.

2. Experimental

General.—Pooled sera from ovarian cancer patients (Roswell Park Cancer Institute) were stored frozen at −70 °C until use. Ovarian tumor tissue and the spleen from a lymphoma patient were obtained during surgical procedures at Roswell Park Cancer Institute and stored frozen, within 1 h, at −70 °C. A purified preparation of bovine milk galactosyltransferase (EC 2.4.1.22) and a recombinant form of the same enzyme (EC 2.4.1.90) were purchased from Sigma Chemical Co. (St. Louis) and Calbiochem (San Diego), respectively, and stored at −20 °C until use.

Purification of GlcNAc:β1,4Gal/GalNAc-Ts Hodgkins lymphoma spleen # 7184-4S. The tissue (140 g) was homogenized using Polytron in 560 mL of 0.1 M Tris–Maleate pH 7.2 containing 2% Triton X-100, 20 mM Mn acetate and 0.1% NaN_3; the homogenate was stirred in the cold room for 2 h, centrifuged at 10,000*g* (5 °C) for 1 h and the resulting supernatant was then subjected to $(NH_4)_2SO_4$ fractionation. The 20–60% satd $(NH_4)_2SO_4$ precipitate was dissolved in 100 mL of the above buffer and dialyzed against 2 L of the same buffer for 48 h in the cold room, with five changes. About 65% of the enzyme activity was recovered at this stage. The dialyzed preparation was applied to a column of

Affinity Gel-UDP (Calbiochem) (25-mL bed volume) in the cold room. After the application of the enzyme solution, the column was washed with 100 mL of the same buffer. The bound protein was eluted with 100 mL of 2 M NaCl in the above buffer, concentrated by Amicon PM10 membrane ultrafiltration, dialyzed against the above buffer in the cold room, and stored frozen at −20 °C. The enzyme had thus been purified ~500-fold with 35% recovery.

Human ovarian tumor microsomes. The primary ovarian tumor #10315-1P (124 g) was cut into small pieces in the cold room and homogenized with 620 mL of 0.05 M Tris–HCl pH 7.4 containing 25 mM KCl, 0.25 M sucrose, 5 mM each of Mg acetate and β-mercaptoethanol,[24,25] in a hand-operated, all-glass homogenizer. The homogenate was stirred for 1 h at 4 °C and centrifuged at 10,000*g* for 1 h at 4 °C. The supernatant was centrifuged with SW 27.7 rotor at 25,000 rpm in polyallomer tubes (15.5 mL/tube) for 2 h at 8 °C. The microsomal pellets were pooled and solubilized by stirring in the cold room for 2 h in 50 mL of 0.1 M Tris–Maleate pH 7.2 containing 2% Triton X-100, 20 mM Mn acetate and 0.1% NaN_3. The solubilized extract was continuously mixed for 2 h with 25 mL of the Affinity Gel-UDP in the cold room using the Speci-Mix. After this mixing the solution was allowed to drain off. The column was then washed with 100 mL of the buffer. The bound protein was eluted with 100 mL of 2 M NaCl in the same buffer and concentrated by ultrafiltration and stored frozen as above. A purification of ~800-fold with 60% recovery of the enzyme activity had been attained.

Pooled human ovarian tumors [OT 188718 (160 g) and OT 9780 (115 g)]. The pooled tissue (275 g) was homogenized using polytron in 1100 mL of 0.1 M Tris–Maleate pH 7.2, 2% Triton X-100 and 0.1% NaN_3 and centrifuged at 10,000*g* for 1 h at 4 °C. The fat-free supernatant was adjusted to 0–60% $(NH_4)_2SO_4$ saturation and centrifuged at 10,000*g* for 1 h at 4 °C. The precipitate was dissolved in 200 mL of 20 mM Tris–HCl pH 9.0, 2% Triton X-100 and 0.1% NaN_3 and dialyzed against 4 L of the same buffer at 4 °C with three changes for 24 h. The dialyzed solution was passed through a Q-Sepharose column (150-mL bed volume), and equilibrated with the same buffer. The column was washed with 500 mL of the same buffer and then eluted with 500 mL of 0.2 M NaCl in the same buffer; ~80% of the enzyme activity present in $(NH_4)_2SO_4$ fraction was recovered in this eluate. This fraction was dialyzed against 0.1 M Tris–Maleate pH 7.2 containing 2% Triton X-100, 20 mM Mn acetate and 0.1% NaN_3 and passed through the Affinity Gel-UDP column (25-mL bed volume). The column was washed with 100 mL of the buffer, and the bound protein was eluted with 100 mL of 2.0 M NaCl in the same buffer. The enzyme solution was concentrated by ultrafiltration as above. The enzyme had been purified ~400-fold with a 30% recovery of the activity.

Pooled ovarian cancer sera. Sera (250 mL) were diluted with 250 mL of 0.1 M Tris–Maleate pH 7.2 containing 20 mM Mn acetate, 2% Triton X-100 and 0.1% NaN_3 and passed through the column of Affinity Gel-UDP (25-mL bed volume). After the entry of the sample, the column was washed with 100 mL of the buffer. The bound protein was eluted with 100 mL of 2.0 M NaCl in the same buffer. The eluate was concentrated by ultrafiltration and stored frozen at −20 °C. A purification of ~400-fold with 25% recovery of the enzyme activity had been achieved.

Assay of βGlcNAc:β1,4Gal-T and αGalNAc:β1,3Gal-T (EC 2.4.1.122) activities.—The reaction mixture in duplicate contained 0.1 M Hepes–NaOH pH 7.0, 7 mM ATP, 20 mM Mn acetate, 1 mM UDP-Gal, UDP [^{14}C]Gal (0.05 μCi; 327 mCi/mmol; Amersham), 0.5 mM acceptor (unless otherwise stated) and the enzyme in a total volume of 20 μL. It was incubated for 4 h at 37 °C. The radioactive product was quantitated by the Dowex 1 Cl-method[26,27] as follows.

The incubation mixture was diluted with 1.0 mL of water and passed through Dowex 1 × 8 (200–400 mesh Cl^-) (1 mL in a Pasteur pipet). The column was washed twice with 1.0 mL of water. The radioactivity present in the water eluate was measured by liquid scintillation counting. The variation between duplicate values was within 5%.

The radioactive products from the sulfated acceptors containing the benzyl aglycone were quantitated by the Sep-Pak C_{18} cartridge (Waters Corp., MA) method.[28]

Assay of βGlcNAc:β1,4GalNAc-T activity.—Duplicate reaction mixtures contained 0.1 M Hepes–NaOH pH 7.0, 7 mM ATP, 20 mM Mn acetate, UDP [^{3}H] GalNAc (0.20 μCi; 7.8 Ci/mmol; New England Nuclear Corp.) 7.5 mM acceptor (unless otherwise stated) and the enzyme in a total volume of 20 μL and incubated for 4 h at 37 °C. The radioactive product was quantitated by the Dowex 1 Cl-method[26,27] or the Sep-Pak cartridge method.[28] For assessing the affect of α-lactalbumin on the enzymatic transfer of Gal/GalNAc moiety, 200 μg of bovine milk α-lactalbumin (Sigma L6010) was included in the reaction mixture.

Preparation of glycopeptides.—Fetuin triantennary asialoglycopeptide and bovine IgG diantennary glycopeptide were available from earlier studies of this laboratory.[29,30] Agalacto derivatives of these glycopeptides were made by digestion with β-galactosidases of *Aspergillus niger* (Calbiochem) and bovine testes (Oxford Glycosciences) as recommended by the supplier and then isolated by chromatography on a BioGel P2 column (1.0 × 116.0 cm).[25] About 10 mg of Fetuin triantennary asialo agalacto glycopeptide was dissolved in 100 μL of water, mixed with 2 U of β-*N*-acetylhexosaminidase (jack bean) (EC 3.2.1.30) in 200 μL of 2 × buffer (Oxford Glycosciences) and incubated for 20 h at 37 °C. The resulting non-reducing terminal GlcNAc-free glycopeptide was isolated by BioGel P2 chromatography (yield 5.2 mg). Acrylamide copolymer of GlcNAcβ-*O*-All(-GlcNAcβ-*O*-Al/AA-CP) was synthesized by following the procedure of Horejsi et al.[31]

Synthetic compounds.—The chemical syntheses of acceptors used in the present study have already been published.[32–35]

Gel-permeation chromatography.—The Affinity Gel-UDP purified GlcNAc:β1,4Gal/GalNAc-T preparation (2 mL) was applied to a Sephacryl S-100 HR column (2.5 × 118.0 cm) equilibrated at 4 °C with 0.1 M Tris–Maleate pH 6.3 containing 0.1% Triton X-100 and 0.02% NaN_3. Fractions of 2 mL at a flow rate of 6 mL/h were collected, and 10 μL of alternate fractions were assayed for Gal/GalNAc-T activities using from two to four different acceptors.

Polyacrylamide gel-electrophoresis.—Ready-made polyacrylamide gels (10% resolving gel and 4% stacking gel containing no SDS) were used for native PAGE. Bio-Rad Mini-Protean II Electrophoresis Cell was used. Native PAGE was performed in the cold room with Tris–glycine pH 8.3 containing 0.1% Triton X-100. The enzyme sample (100 μL) was mixed with 100 μL of 2 × buffer containing glycerol, Triton X-100, and Bromophenol Blue, applied in equal volume (33 μL) to each of the middle six wells and run at 30 mA/gel using a Bio-Rad Power Pac 1000 at 200 constant volts. After electrophoresis each gel was removed and cut into 3 mm slices (22 slices total). Each gel slice was shaken in the cold room in 200 μL of 0.1 M Hepes–NaOH pH 7.0 containing 2% Triton X-100 and 0.1% NaN_3 for 24 h using a Speci-mix aliquot mixer (Thermolyne). The eluates were assayed for GlcNAc:β1,4 Gal-T activity.

3. Results

Properties of the affinity-purified, human spleen GlcNAc:β1,4 Gal/GalNAc-T

Influence of divalent metal ions and pH. The βGlcNAc:β1,4Gal-T activity (see Fig. 1(B)) reached maximum at 5 mM Mn^{2+}, whereas, the βGlcNAc:β1,4GalNAc-T activity reached optimum at 50 mM Mn^{2+} (acceptor: Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα - *O* - Bn). The coexisting αGalNAc:β1,3Gal-T activity in this preparation was stimulated to a maximum at 20 mM Mn^{2+} (acceptor: GlcNAc4Fβ1,6-GalNAcα-*O*-Bn). There was no stimulation of these activities by Mg^{2+} or Ca^{2+} (data not shown). The stimulation of bovine milk Gal/GalNAc-T activities by Mn^{2+} was quite similar to that of the spleen enzyme (see Fig. 1(A)). Using Hepes buffer in the pH 6.0–8.4 range, the optimum pH of human spleen GlcNAc:β1,4GalNAc-T activity was found to be 6.8 (data not shown).

Mutual inhibition of Gal/GalNAc-T activities by the sugar donors (see Fig. 2). When human spleen βGlcNAc:β1,4GalNAc-T activ-

ity was measured with the acceptor Gal3Meβ1,3 (GlcNAcβ1,6)GalNAcα-*O*-Bn, the activity gradually decreased by increasing the concentration of UDP-Gal (Fig. 2(B)). The K_i for UDP-Gal inhibition was 0.91 μM. Human spleen βGlcNAc:β1,4Gal-T activity as measured with the same acceptor, Gal3-Meβ1,3(GlcNAcβ1,6)GalNAcα-*O*-Bn, decreased with an increase of UDP-GalNAc (Fig. 2(A)) and the K_i for this inhibition was 0.59 mM. Bovine milk βGlcNAc:β1,4 Gal-NAc-T activity was inhibited by UDP-Gal (Fig. 2(C)) (K_i 2.22 μM). The bovine milk βGlcNAc:β1,4 Gal-T activity was not inhibited by UDP-GalNAc at the maximum concentration (0.5 mM) tested (data not shown).

Synthetic neutral compounds as acceptors (Table 1). The enzyme exhibits higher GalNAc-T activity towards the GlcNAc moiety, which is linked to αGalNAc, whereas GlcNAc residues linked β(1,3) to Gal, β(1,2) to Man or

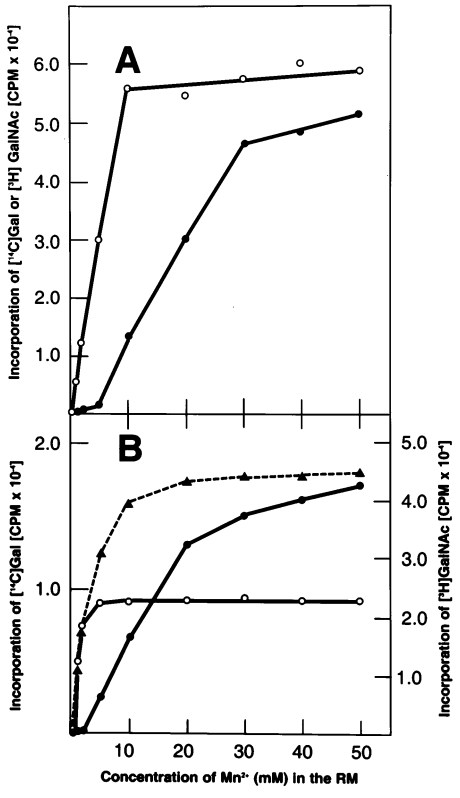

Fig. 1. Effect of Mn^{2+} on βGal/βGalNAc-T activities. (A) Bovine milk enzyme: acceptor: Gal3Meβ1,3(GlcNAcβ1,6)-GalNAcα-*O*-Bn. GlcNAc:β1,4Gal-T activity: (○–○–○). GlcNAc:β1,4GalNAc-T activity: (●–●–●). (B) Human spleen enzyme: GlcNAc:β1,4Gal-T activity: (○–○–○). GlcNAc:β1,4GalNAc-T activity: (●–●–●). α-GalNAc: β1,3Gal-T activity: (▲···▲···▲). (acceptor: GlcNAc-4Fβ1,6GalNAcα-*O*-Bn.)

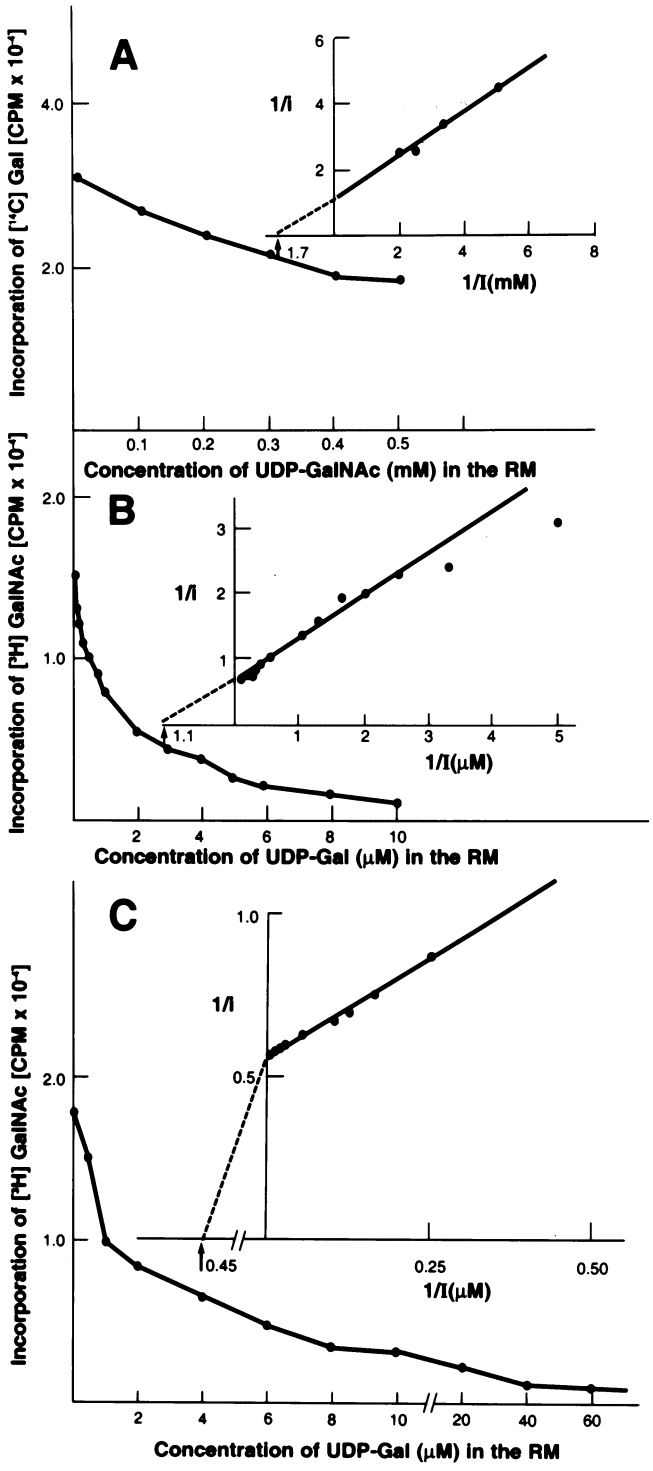

Fig. 2. Inhibition of GlcNAc:β1,4GalNAc and GlcNAc:β1α1,4 Gal-T activities by UDP-Gal and UDP-GalNAc, respectively. Human spleen enzyme: (A) Inhibition of Gal transfer by UDP-GalNAc. (B) Inhibition of GalNAc transfer by UDP-Gal. Bovine Milk enzyme: (C) Inhibition of GalNAc transfer by UDP-Gal.

β(1,4) to GlcNAc are only one third as efficient in accepting GalNAc. The influence of the aglycone on the monosaccharide acceptor

Table 1
Discerning the nature of β-GalNAc and β-Gal-T activities present in a purified enzyme preparation from human lymphoma spleen using various synthetic acceptors

Acceptor	βGalNAc-T activity [a] (CPM × 10^{-3})	βGal-T activity [b] (CPM × 10^{-3})	
	7.5 mM	0.2 mM	3.0 mM
Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα-*O*-Bn	36.7	10.8	2.2
GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-All	33.8	10.7	3.0
GlcNAcβ1,6GalNAcα-*O*-Bn	37.5	17.4	32.0
GlcNAc1,3GalNAcα-*O*-Bn	29.8	6.4	5.3
GlcNAcβ-*O*-Bn	29.6	8.7	4.8
GlcNAcβ1,6Manα-*O*-All	24.0	9.2	5.1
GlcNAcβ1,6Manα-*O*-Me	ND [c]	6.9	8.9
GlcNAcβ1,3Galβ-*O*-Me	13.4	4.6	11.1
GlcNAcβ1,3Galβ1,4Glc	13.2	4.2	11.2
GlcNAcβ1,2Manα1,6Glcβ-*O*-All	13.0	5.2	8.7
GlcNAcβ1,4GlcNAcβ-*O*-Bn	12.9	6.1	9.2
GlcNAcβ-*O*-All	9.6	6.5	9.7
GlcNAcβ-OAll/AA-CP	2.5 [d]	11.0 [e]	
GlcNAc4Fβ1,6GalNAcα-*O*-Bn	0.4 [f]	9.0	20.8 [f]

[a] Incorporation of [^{3}H]GalNAc catalyzed by 2 μg protein.
[b] Incorporation of [^{14}C]Gal catalyzed by 2 μg protein.
[c] ND, not determined.
[d] 100 μg acceptor.
[e] 40 μg acceptor.
[f] 0.5 mM acceptor since higher concentrations could not be used due to the poor solubility of this acceptor.

was also quite evident (activities: GlcNAcβ-*O*-Bn, 29.6; GlcNAcβ-*O*-All, 9.6). The acrylamide copolymer (100 μg: 0.125 mM in RM) was found to be significantly active in accepting GalNAc. As anticipated, 4-fluoro GlcNAcβ1,6GalNAcα-*O*-Bn did not serve as an acceptor for GalNAc transfer.

An examination of Gal-T activity revealed that at 0.2 mM concentration, the GlcNAc moiety β(1,6)-linked to GalNAc was the most efficient acceptor. At a higher acceptor concentration (3.0 mM), the acceptor with a mucin core 2 structure showed considerably less activity, indicating the phenomenon of substrate inhibition. The differential influence of aglycone on the monosaccharide acceptor was also evident (GlcNAcβ-*O*-Bn: activities 8.7 (0.2 mM) → 4.8 (3.0 mM); GlcNAcβ-*O*-All: 6.5 (0.2 mM) → 9.7 (3.0 mM)). The acrylamide copolymer GlcNAcβ-*O*-Al AA-CP was identified as an efficient acceptor as it was highly active at 40 μg (0.05 mM in RM).

Glycopeptide acceptors (Table 2). Complete removal of Gal residues from fetuin trianten-

Table 2
Glycopeptides as the acceptors for GlcNAc:β1,4GalNAc-T purified from human spleen

Glycopeptide 100 μg	Incorporation of [^{3}H]GalNAc (%) [a]
(i) Fetuin triantennary asialoglycopeptide	2.3
(ii) (i) treated with β-galactosidase (*A. niger*)	29.4
(iii) (i) treated with β-galactosidase (*A. niger* and bovine testes)	44.1
(iv) (iii) treated with β-*N*-acetylhexosaminidase	0
(v) bovine IgG glycopeptide	4.9
(vi) (v) treated with α-L-fucosidase (bovine epididymis)	4.8
(vii) (v) treated with β-galactosidase (*A. niger*)	15.3
(viii) (vi) treated with β-galactosidase (*A. niger*)	17.8

[a] The activity is expressed as percent of the activity towards the synthetic acceptor Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα-*O*-Bn (2.5 mM) tested at the same time along with the glycopeptides.

Table 3
Differential regulation of human ovarian tumor microsomal GlcNAc:β1,4Gal and GlcNAc:β1,4GalNAc-T activities by α-lactalbumin (α-LA)

Acceptor	Gal-T activity			GalNAc-T activity		
	Transfer of [^{14}C]Gal (CPM × 10^{-3})			Transfer of [^{3}H]GalNAc (CPM × 10^{-3})		
	+α-LA	−α-LA	a/b (%)	+α-LA	−α-LA	c/d (%)
	a	b		c	d	
Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα-*O*-Bn	4.7	5.8	80.9	10.2	8.8	115.8
Galβ1,3(GalNAcβ1,6)GalNAcα-*O*-All	4.7	8.3	56.3	8.7	8.4	102.9
GlcNAc4Fβ1,6GalNAcα-*O*-Bn	8.4	7.0	119.4	0	0	
GlcNAcβ1,6GalNAcα-*O*-Bn	9.5	11.3	84.0	7.1	9.7	73.7
GlcNAcβ1,6Manα-*O*-All	3.9	13.3	29.5	4.3	5.6	77.2
GlcNAcβ1,6Manα-*O*-Me	1.6	10.6	14.8	5.8	5.0	116.8
GlcNAcβ1,2Manα1,6Glcβ-*O*-All	1.2	6.7	17.9	2.1	2.7	76.7
GlcNAcβ1,4GlcNAcβ-*O*-Bn	1.3	6.8	19.1	2.4	3.1	78.8
GlcNAcβ-*O*-Bn	4.6	12.6	36.7	7.8	7.9	99.0
GlcNAc	0.3	1.9	14.9	10.9	2.3	473.6
Glc	1.8	0		2.2	0	

nary asialo glycopeptide using *A. niger* and bovine testes β-galactosidases increased the transfer of GalNAc to this glycopeptide from 29.4 to 44.1%. A complete removal of GlcNAc residues from the agalacto glycopeptide abolished the acceptor activity. Bovine Ig glycopeptide, which is known to contain some terminal GlcNAc resides, served as an acceptor to a small extent. Removal of the internal α(1,6)-linked Fuc using α-L-fucosidase (bovine epididymis) did not increase the acceptor activity, but removal of the Gal moieties with *A. niger* β-galactosidase converted this glycopeptide into a moderate acceptor for GalNAc transfer.

Effect of α-lactalbumin on the acceptor specificities of GlcNAc:β1,4Gal- and GlcNAc:β1,4GalNAc-T activities.—The affinity-purified enzyme preparation from human ovarian tumor microsomes, as well as the purified enzyme and recombinant form from bovine milk, were studied using a battery of well-defined synthetic acceptors. The data are reported in Tables 3–5.

Ovarian tumor microsomal enzyme (see Table 3). α-Lactalbumin inhibited to a great extent (up to 85% inhibition) the transfer of Gal to GlcNAc linked either to Man or GlcNAc, as well as to the monosaccharide acceptors. It did not have much influence on the transfer of Gal to the GlcNAc moiety linked to αGalNAc. The transfer of Gal to Glc took place only in presence of α-LA. On the other hand, α-LA had no significant influence on the transfer of GalNAc to the various acceptors. But the transfer of GalNAc to the monosaccharide GlcNAc was enhanced ~fivefold by α-LA. As in the case of Gal transfer, GalNAc transfer to Glc took place only in presence of α-LA.

Purified bovine milk β1,4Gal-T (see Table 4). The enzymatic transfer of Gal was inhibited more by α-LA when the terminal GlcNAc residue was linked either to Man or GlcNAc rather than to α-GalNAc. The transfer of Gal to Glc occurred only in presence of α-LA. Both α- and β-glucosides were not active in accepting Gal or GalNAc either in the presence or absence of α-LA. The transfer of GalNAc to the terminal GlcNAc moiety of the various acceptors examined was not much influenced by α-LA. But the transfer of GalNAc to the monosaccharide GlcNAc was stimulated >threefold by α-LA. As in the case of Gal transfer, GalNAc transfer to Glc took place only in the presence of α-LA.

Cloned bovine milk β1,4Gal-T (Table 5). Contrary to the purified bovine milk enzyme, α-LA did not have any significant influence on the levels of cloned GlcNAc:β1,4Gal/GalNAc-T activities, but it was a requirement for the

Table 4
Discerning the effect of α-lactalbumin on purified bovine milk GlcNAc:β1,4Gal-T and GlcNAc:β1,4GalNAc-T activities using various synthetic acceptors

Acceptor	Gal-T activity			GalNAc-T activity		
	Transfer of [^{14}C]Gal (CPM × 10^3)			Transfer of [^{3}H]GalNAc (CPM × 10^3)		
	+α-LA	−α-LA	a/b (%)	+α-LA	−α-LA	c/d (%)
	a	b		c	d	
Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα-*O*-Bn	32.1	58.1	55.2	15.6	18.0	86.9
Galβ1,3(GalNAcβ1,6)GalNAcα-*O*-All	26.4	57.0	46.3	14.5	18.9	77.1
GlcNAc4Fβ1,6GalNAcα-*O*-Bn	0.3	0.5	NA	0.1	0.4	NA
GlcNAcβ1,6GalNAcα-*O*-Bn	32.7	61.6	53.1	16.8	21.2	79.3
GlcNAcβ1,6Manα-*O*-All	13.3	45.0	29.6	9.0	14.1	64.0
GlcNAcβ1,6Manα-*O*-Me	5.8	31.9	18.3	11.6	6.7	172.0
GlcNAcβ1,2Manβ1,6Glcβ-*O*-All	2.3	19.0	12.3	4.5	5.9	75.2
GlcNAcβ1,4GlcNAcβ-*O*-Bn	2.8	20.5	13.6	5.7	7.8	73.2
GlcNAcβ-*O*-Bn	13.2	43.3	30.4	13.3	16.9	78.7
GlcNAc	9.8	6.5	151.2	24.6	7.6	322.8
Glcβ-*O*-All	0	0	NA [a]	0	0	NA
Glc	14.7	0	NA [a]	7.8	0	NA

[a] NA, not applicable. As anticipated, milk GlcNAc:β1,4Gal/GalNAc-T did not utilize GlcNAc4Fβ1,6GalNAcα-*O*-Bn, which is a specific acceptor for α-GalNAc:β1,3Gal-T. We also found that this enzyme did not transfer GalNAc to Glcα-*O*-Me, Glcβ-*O*-Me and GlcNAcα-*O*-Ph both in the presence and absence of α-LA.

Table 5
The effect of α-lactalbumin on the activities of the cloned form of bovine milk β1,4Galactosyltransferase

Acceptor	Gal-T activity			GalNAc-T activity		
	Transfer of [^{14}C]Gal (CPMx10^3)			Transfer of [^{3}H]GalNAc (CPMx10^3)		
	+α-LA	−α-LA	a/b (%)	+α-LA	−α-LA	c/d (%)
	a	b		c	d	
Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα-*O*-Bn	52.7	51.7	101.5	109.1	116.8	93.4
Galβ1,3(GalNAcβ1,6)GalNAcα-*O*-All	52.9	54.9	96.4	110.9	121.8	91.1
GlcNAc4Fβ1,6GalNAcα-*O*-Bn	0.6	0.5	NA	6.7	9.6	NA
GlcNAcβ1,6GalNAcα-*O*-Bn	56.4	56.5	99.8	111.2	120.2	92.5
GlcNAcβ1,6Manα-*O*-All	53.1	51.1	103.9	98.3	111.3	88.3
GlcNAcβ1,6Manα-*O*-Me	43.0	47.2	91.1	110.2	115.7	95.2
GlcNAcβ1,2Manα1,6Glcβ-*O*-All	30.6	53.2	57.5	78.9	96.8	81.5
GlcNAcβ1,4GlcNAcβ-*O*-Bn	36.2	56.8	63.7	85.0	101.8	83.5
GlcNAcβ-*O*-Bn	48.6	44.8	108.5	100.5	106.8	94.1
GlcNAcβ-*O*-All	44.0	50.8	86.6	86.4	98.7	87.5
GlcNAc	23.7	45.6	52.0	112.5	93.0	121.0
Glcβ-*O*-Al	0.3	0.9	NA [a]	0	0.5	NA
Glc	57.3	0.5	NA [a]	92.7	0.8	NA

[a] NA, not applicable. As anticipated, the cloned bovine milk β1,4Gal-T did not utilize GlcNAc4Fβ1,6GalNAcα-*O*-Bn, which is a specific acceptor for α-GalNAc:β1,3Gal-T. We also found that this enzyme did not transfer either Gal or GalNAc to Glcβ-*O*-Me, Glcα-*O*-Me and GlcNAcα-*O*-Ph both in presence and absence of α-LA.

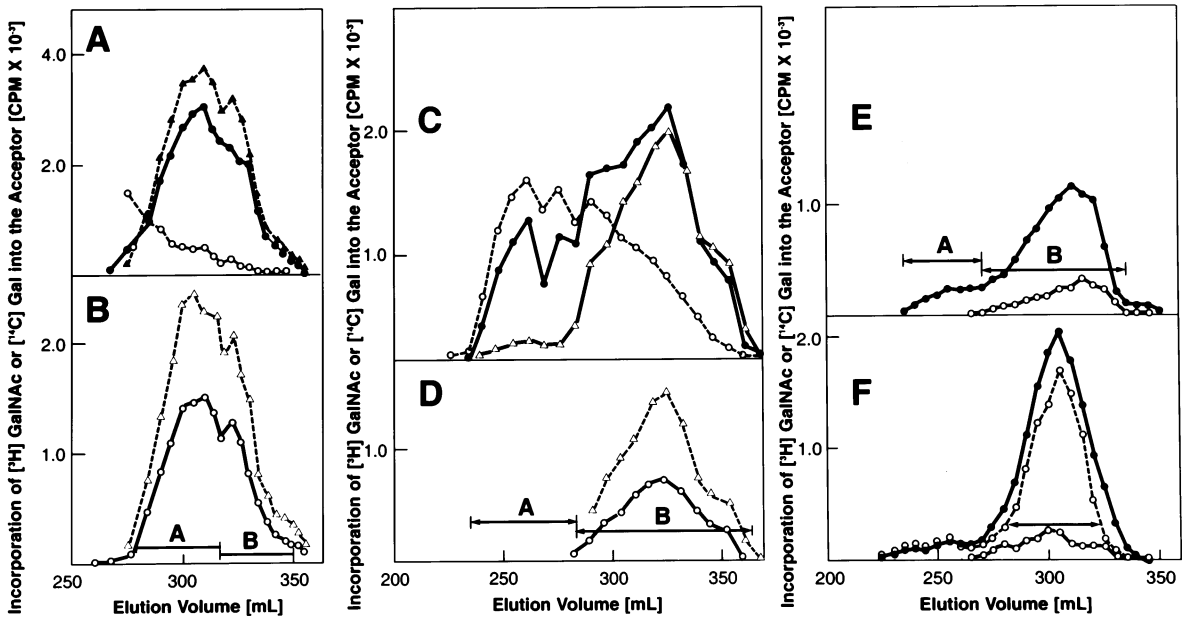

Fig. 3. Chromatography of the affinity-purified enzyme preparations from ovarian tumor microsomes, ovarian tumor extract, ovarian cancer sera and lymphoma spleen on a Sephacryl S-100 HR column. Ovarian tumor microsomal enzyme: (A) [^{14}C] Gal transfer: Acceptors: GlcNAcβ1,6Manα-*O*-All (▲···▲···▲). GlcNAcβ1,6GalNAcα-*O*-Bn (●–●–●). GlcNAc4Fβ1,6GalNAcα-*O*-Bn (○···○···○). (B) Acceptor: GlcNAcβ1,6 (Galβ1,3)GalNAcβ-*O*-All. [^{3}H]GalNAc transfer: (○–○–○). [^{14}C]Gal transfer: (▽···▽···▽). Ovarian tumor extract enzyme: (C) [^{14}C]Gal transfer: Acceptors: GlcNAcβ1,6Manα-*O*-Al: (▽–▽–▽). GlcNAcβ1,6GalNAcα-*O*-Bn: (●–●–●). GlcNAc4Fβ1,6GalNAcα-*O*-Bn: (○···○···○). (D) Acceptor: GlcNAcβ1,6(Galβ1,3)-GalNAcα-*O*-All: [^{3}H]GalNAc transfer (○–○–○). [^{14}C]Gal transfer: (▽···▽···▽). (E) Ovarian cancer sera enzyme: Acceptor: GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn. [^{3}H]GalNAc transfer: (○–○–○). Acceptor: GlcNAcβ1,6GalNAcα-*O*-Bn: [^{14}C]Gal transfer: (●–●–●). (F) Lymphoma spleen enzyme: [^{14}C]Gal transfer: Acceptors: GlcNAcβ1,6GalNAcα-*O*-Bn: (●–●–●). GlcNAc4Fβ1,6GalNAcα-*O*-Bn: (○···○···○). [^{3}H]GalNAc transfer: Acceptor: GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-All: (○–○–○).

synthesis of Galβ1,4Glc and GalNAcβ1,4Glc. GlcNAc:β1,4GalNAc-T activity was found to be present at a higher level in the cloned enzyme when compared to the purified form, illustrated as follows: For the purified bovine milk enzyme (Table 4) GalNAc-T and Gal-T activities in absence of α-LA were, respectively, 18.0 and 58.1 (acceptor: Gal3Meβ1,3 (GlcNAcβ1,6)GalNAcα-*O*-Bn), 18.9 and 57.0 (Galβ1,3(GlcNAcβ1,6)GalNAcα-*O*-All) and 21.2 and 61.6 (GlcNAcβ1,6GalNAcα-*O*-Bn). For the cloned enzyme (Table 5), these activities were 116.8 and 51.7, 121.8 and 54.9, and, 120.2 and 56.5, respectively. Calculated GalNAc-T to Gal-T ratios were 31.0, 33.2, and 34.4% for the purified enzyme and 225.9, 221.9, and 212.7% for the cloned enzyme. The above data indicate that the cloned enzyme is at least sixfold more efficient than the purified bovine milk enzyme in GalNAc-T activity.

Further examination of the affinity-purified βGal/βGalNAc-T preparations on Sephacryl S100 HR column (see Fig. 3)

Ovarian tumor microsomal enzyme preparation. The elution patterns of the ovarian tumor microsomal βGlcNAc:β1,4Gal-T activities as measured with three different acceptors, namely, GlcNAcβ1,6GalNAcα-*O*-Bn, GlcNAcβ1,6 Manα-*O*-All (Fig. 3(A)) and GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-All (Fig. 3(B)) were found to be quite identical and also indicated the presence of two molecular species. Further the elution pattern of βGlcNAc:β1,4GalNAc-T activity, as measured with GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-All (Fig. 3(B)), was also identical to the above elution patterns. But, αGalNAc:β1,3 Gal-T activity, as measured with GlcNAc4Fβ1,6 GalNAcα-*O*-β (Fig. 3(A)), appears to emerge from the column much earlier than β1,4Gal/GalNAc-T activities.

Enzyme preparation from the Triton X-100-solubilized extract of ovarian tumor. The elution patterns of GlcNAc:β1,4Gal-T activities, as measured with GlcNAcβ1,6 Manα-*O*-All (Fig. 4(C)) and GlcNAcβ1,6(Gal β1,3)GalNAcα-*O*-All (Fig. 3(D)), and that of Glc-

NAc:β1,4GalNAc-T activity, as measured with GlcNAcβ1,6(Gal β1,3)GalNAcα-*O*-All, were quite identical and appeared to coincide with the smaller species of the microsomal β1,4Gal-T activity in size. The elution pattern of βGal-T activity, as measured with GlcNAcβ1,6GalNAcα-*O*-Bn, indicated two molecular species, the smaller coinciding with β1,4Gal-T activity and the larger coinciding with α-GalNAc:β1,3 Gal-T activity, as measured with GlcNAc4Fβ1,6GalNAcα-*O*-Bn (Fig. 3(C)).

The affinity-purified enzyme preparation from ovarian cancer sera (see Fig. 3(E)). The Gal-T activity, as measured with GlcNAcβ1,6GalNAcα-*O*-Bn, indicated two molecular species, the larger being αGalNAc:β1,3Gal-T and the other βGlcNAc:β1,4Gal-T. The βGlcNAc:β1,4GalNAc-T activity coincided with the β1,4Gal-T activity.

The purified human spleen enzyme (see Fig. 3(F)). The elution patterns of βGal-T activities, as measured with GlcNAcβ1,6GalNAcα-*O*-Bn and GlcNAc4Fβ1,6GalNAcα-*O*-Bn, and that of GlcNAc:β1,4GalNAc-T activities were quite identical, indicating that human spleen αGalNAc:β1,3Gal-T and βGlcNAc:β1,4Gal/GalNAc-T have similar molecular sizes.

Native polyacrylamide gel electrophoresis (see Fig. 4).—The ovarian tumor microsomal enzyme preparations, namely Sephacryl S-100 HR Fractions A and B (refer to Fig. 4(A and B)), exhibited two peaks of βGal-T activities, as measured with GlcNAcβ1,6GalNAcα-*O*-Bn (Fig. 4(A and B)). The GlcNAc:β1,4GalNAc-T activity, as measured with GlcNAcβ1,6 GalNAcα-*O*-Bn, showed an identical pattern (see Fig. 4(A)). Significant difference in electrophoretic mobilities between Sephacryl fractions A and B was also evident (compare Fig. 4(A and B)). The Sephacryl-purified enzyme preparation (see Fig. 4(F)) from human spleen exhibited a broad range of βGal-T activity, as measured with GlcNAcβ1,6GalNAcα-*O*-Bn (Fig. 4(C)). The affinity gel-purified enzyme from ovarian cancer sera exhibited a sharp peak of βGal-T activity, as measured with GlcNAcβ1,6GalNAcα-*O*-Bn, and showed faster mobility than the other preparations (Fig. 4(D)).

Determination of the linkage of GalNAc to the GlcNAc moiety as β.—The [H^3]GalNAc-containing products from Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα-*O*-Bn, GlcNAcβ1,6 GalNAcα-*O*-Bn, GlcNAcβ1,4GlcNAcβ-*O*-Bn and GlcNAcβ-*O*-Bn when using the Affinity Gel-UDP fraction from spleen and the Sephacryl S-100 HR frac-tion A from ovarian tumor microsomes were isolated from fivefold reaction mixtures by the Sep-Pak cartridge method.[27] The methanol eluates were flash evaporated to dryness, dissolved in water, and subjected to TLC. The radioactive products in each case moved as single components on TLC [12:2:5 1-propanol–NH_4OH–water], as evident from the location of radioactivity as

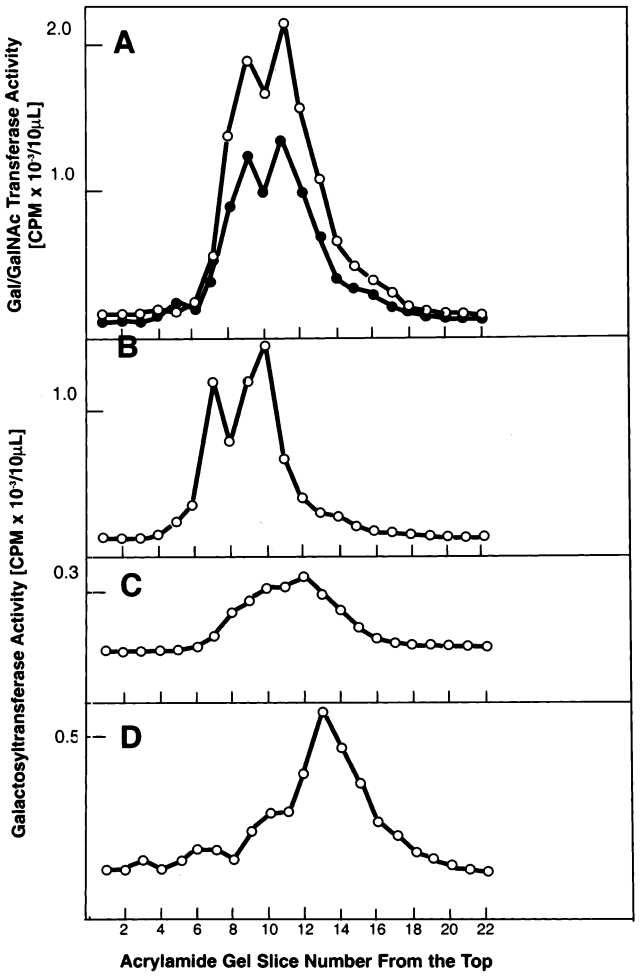

Fig. 4. Native polyacrylamide gel electrophoresis of the purified enzyme preparations. Acceptor: GlcNAcβ1,6 GalNAcα-*O*-Bn: [^{14}C]Gal transfer: (○–○–○). [^{3}H]GalNAc transfer: (●–●–●). (A) Ovarian tumor microsomal Sephacryl S-100 HR Fraction A. (B) Ovarian tumor microsomal Sephacryl S-100 HR Fraction B. (C) Lymphoma Spleen Sephacryl S-100 HR Fraction. (D) Ovarian Cancer sera enzyme purified by Affinity Gel-UDP.

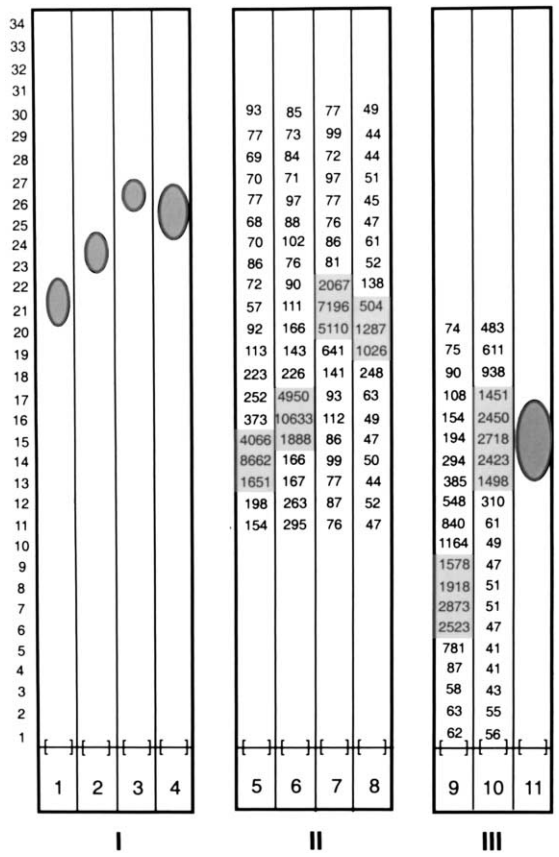

Fig. 5. Thin-layer chromatographic identification of β-GalNAc linkage. (I) TLC (12:2:5 1-PrOH–NH_4OH–water) of the acceptors: (1) Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα-*O*-Bn; (2) GlcNAcβ1,6GalNAcα-*O*-Bn; (3) GlcNAcβ-*O*-Bn; (4) GlcNAcβ1,4GlcNAcβ-*O*-Bn. (Visualized by H_2SO_4–ethanol spray and heating.) (II) TLC (12:2:5 1-PrOH–NH_4OH–water) of [^{3}H]GalNAc-products 5–8 from 1, 2, 3 and 4 respectively. (III) TLC (3:2:1 BuOH–HOAc–water) identification of the release of ^{3}[H]GalNAc. Products 9 and 10: [^{3}H]GalNAc-product from Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα-*O*-Bn before and after treatment with β-*N*-acetyl-hexosaminidase (jack bean). Product 11: Standard GalNAc.

determined by scraping 0.5 cm wide segments of silica gel from the developed plates, mixing with 2.0 mL water, and then subjecting to liquid scintillation counting. Treatment of the products with β-*N*-acetyl-hexosaminidase (jack bean) resulted in a complete release of [^{3}H]GalNAc, as identified by TLC (see Fig. 5).

Identification of the galactosyl linkage to the GlcNAc moiety at β1,4.—The [^{14}C]Gal-containing products from Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα-*O*-Bn by using the Affinity Gel-UDP fractions from spleen and ovarian cancer sera and also the Sephacryl S-100 HR fraction A from ovarian tumor microsomes were isolated as above by the Sep-Pak method. A complete release of [^{14}C]Gal from these products was observed after treatment with β1,4-galactosidase (EC 3.2.1.23) (recombinant enzyme, Calbiochem) followed by TLC (data not shown).

Differentiation of the substrate specificities of GlcNAc:β1,4Gal/GalNAc-T with sulfated and corresponding non-sulfated acceptors (see Table 6).—The Affinity Gel-UDP fraction from spleen, Sephacryl S-100 HR fraction A from ovarian tumor microsomes (see Fig. 3(B) Fraction A), and the purified and recombinant bovine milk enzyme preparations were examined for this purpose. The sulfated mucin core 2 acceptor, 3-*O*-sulfoGalβ1,3(GlcNAcβ1,6)-GalNAcα-*O*-Bn was 46.8–66.7% and 76.0–102.4% efficient as an acceptor for the transfer of Gal and GalNAc, respectively, as compared to the non-sulfated acceptor, Gal3-Meβ1,3(GlcNAcβ1,6)GalNAcα-*O*-Bn. On the other hand, the other sulfated mucin core 2 acceptor, Galβ1,3(GlcNAc6SO_3β1,6)Gal-NAcα-*O*-Bn, exhibited a considerable difference in Gal-T acceptor activity (50.0, 11.0, 7.9 and 24.0%, respectively) towards the above enzymes. Only the cloned enzyme showed a significant level of GalNAc-T activity (10.7%) towards this acceptor. Further, only the cloned enzyme was able to utilize both GlcNAcβ1,3Galβ-*O*-Me and GlcNAc6SO_3β1,3-Galβ-*O*-Me quite efficiently as acceptors for Gal-T (109.6 and 52.4%) and GalNAc-T (92.3 and 17.3%) activities, respectively.

The overall acceptor–substrate specificities of β1,4-galactosyltransferases, as evident from the present study, can be summarized as illustrated in Fig. 6.

4. Discussion

The acceptor–substrate specificities and kinetic properties of GlcNAc:β1,4GalNAc-T were studied using purified enzyme preparations from human lymphoma spleen, ovarian tumor and ovarian cancer sera along with the conventionally purified and cloned bovine milk enzyme preparations. Further, several well-defined synthetic compounds containing a terminal β-GlcNAc moiety were employed as acceptors in the present study. The identical

elution profiles of GlcNAc:β1,4Gal-T and GlcNAc:β1,4GalNAc-T activities from the Sephacryl S-100 HR column, as well as the identical migration profiles of these activities from native polyacrylamide gel electrophoresis, would suggest that these two activities are most likely associated with the same enzyme. Further, it was identified that GlcNAc:β1,4Gal/GalNAc-T of ovarian tumor microsomes consisted of two molecular species, as demonstrated by Sephacryl S-100 HR column chromatography and native polyacrylamide gel electrophoresis. We have also shown that the GlcNAc moiety β(1,6)-linked to α-GalNAc was the most efficient acceptor for GlcNAc:β1,4Gal/GalNAc-Ts, as demonstrated with the spleen, ovarian tumor microsomal and bovine milk enzymes. Asparagine-linked asialo-agalacto carbohydrate chains served as acceptors for the enzymatic transfer of GalNAc, as demonstrated with the spleen enzyme preparation. We found that the transfer of Gal, but not GalNAc, to the terminal 6-*O*-sulfo GlcNAc moiety occurred when the enzymes from spleen and ovarian tumor microsomes, and the purified bovine milk en-

Table 6
Differentiation of the substrate specificities of β1,4Gal/GalNAc-T from human lymphoma spleen, ovarian tumor microsomes and bovine milk with sulfated and corresponding non-sulfated acceptors [a]

Acceptor	Incorporation of [^{14}C]Gal into the acceptor (%)				Incorporation of [^{3}H]GalNAc into the acceptor (%)			
	A	B	C	D	A	B	C	D
Gal3Meβ1,3(GlcNAcβ1,6) GalNAcα-*O*-Bn	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Gal3SO_3β1,3(GlcNAcβ1,6) GalNAcα-*O*-Bn	66.7	48.8	46.8	48.0	76.0	87.4	89.7	102.4
Galβ1,3(GlcNAc6SO_3β1,6) GalNAcα-*O*-Bn	50.0	11.0	7.9	24.0	1.2	1.0	1.9	10.7
GlcNAcβ1,6GalNAcα-*O*-Bn	303.7	131.9	107.9	108.5	108.2	115.4	85.3	97.9
GlcNAcβ1,3Galβ-*O*-Me	138.4	60.8	46.4	109.6	29.6	37.8	35.8	92.3
GlcNAc6SO_3β1,3Galβ-*O*-Me	13.3	3.1	6.6	52.4	0	1.6	2.3	17.3

[a] A, Affinity Gel-UDP fraction from spleen; B, Ovarian tumor microsomal Sephacryl fraction A; C, Purified enzyme from bovine milk; D, the recombinant form of bovine milk enzyme. The enzyme activities [CPM $\times 10^{-3}$] towards the acceptor, Gal3Meβ1,3(GlcNAcβ1,6)GalNAcα-*O*-Bn, were 5.4, 36.0, 45.5 and 47.1 for [^{14}C]Gal transfer and 17.1, 29.3, 52.5 and 134.4 for [^{3}H]GalNAc transfer for A, B, C and D, respectively.

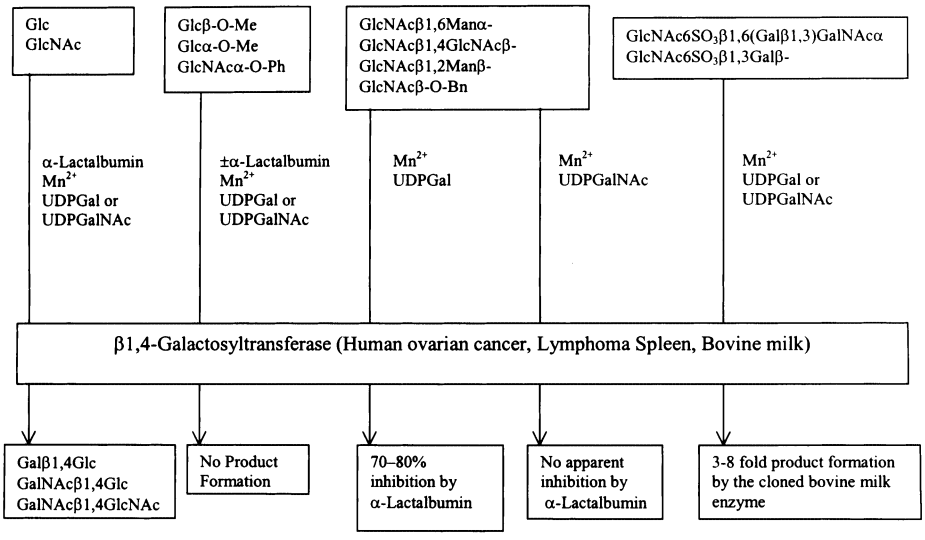

Fig. 6. Specificities of β1,4-galactosyltransferases.

zyme were examined with the GlcNAc6SO_3-β1,6(Galβ1,3)GalNAcα-O-Bn acceptor. But, the cloned bovine milk enzyme exhibited some GalNAc-T activity towards the acceptors, Galβ1,3(GlcNAc6SO_3β1,6)GalNAcα-*O*-βn and GlcNAc6SO_3β1,3Galβ-*O*-Me (activities of 10.7 and 17.3%, respectively). In this context it is interesting to note the following observations: Although GalNAcβ1,4GlcNAcβ-*O*-Bn was an efficient acceptor for α1,3-fucosyltransferase, GalNAcβ1,4GlcNAc-6SO_3β-*O*-Me was inactive (Chandrasekaran et al., unpublished results); Tandai-Hiruma et al.[36] reported a novel structure, namely, GalNAcβ1,4Gal3SO_3β- as a part of the epitope of Tamm-Horsfall glycoprotein necessary for its binding to the Interleukin-1β molecule.

We have shown that the cloned, as well as the purified, bovine milk enzymes in addition to spleen and ovarian tumor microsomal enzymes are capable of transferring Gal to the β-linked GlcNAc6SO_3. This would suggest that the transfer of Gal to the GlcNAc and the GlcNAc6SO_3 moieties may be catalyzed by the same Gal-T enzyme present in the spleen and ovarian tumor microsomal enzyme preparations. The cloned enzyme utilizes the acceptors containing the 6-*O*-sulfoGlcNAc moiety much better than the purified bovine milk enzyme. Further, the former was 6-fold more efficient at transferring GalNAc as compared to the latter. These results would suggest some structural difference between these enzymes either in the catalytic or binding sites.

We examined the effect of α-LA on GlcNAc:β1,4Gal-T and GlcNAc:β1,4GalNAc-T activities present in the purified enzyme preparation from human ovarian tumor microsomes, as well as the purified and cloned enzymes from bovine milk, using various synthetic acceptors. In all cases α-LA had no significant influence on the transfer of GalNAc to any of these acceptors. With the exception of the cloned enzyme, GalNAc transfer to the monosaccharide, GlcNAc, was enhanced, and the transfer of Gal to a GlcNAc moiety linked either to Man or GlcNAc was inhibited up to 85% by α-LA; however, it had less effect on the transfer of Gal to GlcNAc, which is β(1,6)-linked to α-GalNAc. Contrary to the finding of Do et al.[37] that bovine milk β1,4Gal-T was unable to transfer GalNAc to Glc, with or without α-LA, we have shown the transfer GalNAc to Glc in presence of α-LA using both bovine milk enzyme preparations as well as the ovarian tumor microsomal enzyme.

From the studies on the kinetic mechanisms of β1,4Gal-T and the influence of α-LA on enzyme activity and specificity, it was proposed that β1,4Gal-T operates by a partially ordered and sequential reaction mechanism in which the enzyme binds first to Mn^{2+} and UDP-Gal to form an E-Mn^{2+}–UDP-Gal complex. This complex then interacts with either a monosaccharide acceptor GlcNAc or α-LA. *N*-Acetyllactosamine synthesis is inhibited or stimulated depending on the relative concentration of these latter two components.[38,39] Site-directed mutagenesis studies revealed that α-LA has two aromatic clusters, and that cluster I is involved in binding to β1,4Gal-T.[40] A model was proposed whereby α-LA binding to β1,4Gal-T creates a 'monosaccharide bridge' for the binding of glucose. We found in the present study that β1,4Gal-T can transfer GalNAc to Glc, only in presence of α-LA by using the enzyme purified from human ovarian tumor microsomes, as well as the purified and cloned bovine milk enzymes. The transfer of GalNAc to Glc suggests that the conformation of the complex E-Mn^{2+}–UDP-GalNAc may be quite identical to the conformation of the complex *E*-Mn^{2+}–UDP-Gal in that α-LA can expose the binding pocket for glucose. UDP-Gal and UDP-GalNAc must occupy the same catalytic site due to the fact that β1,4Gal-T has a single binding site for UDP. Since we find in the present study that α-LA stimulates the three- to fivefold transfer of GalNAc to GlcNAc, it appears that the conformation of the complex *E*-Mn^{2+}–UDP-GalNAc may be unique since it is able to expose a binding pocket for either Glc or GlcNAc.

The present results further suggest the possibility that the aromatic aglycone moiety such as benzyl of the acceptor might substitute for the aromatic cluster of α-LA and thereby stimulate β1,4Gal-T to transfer GalNAc or Gal to GlcNAc in the absence of α-LA. In this context it is important to note that this is not the case with respect to the utilization of

glucose for Galβ1,4Glc or GalNAcβ1,4Glc formation, which takes place only in the presence of α-LA, and the aglycones (linked either α or β) cannot substitute for the role of α-LA. It also indicates that an intact anomeric hydroxyl group of glucose is essential for stimulation by α-LA.

α-LA is highly expressed in the lactating mammary gland where its expression is associated with lactose production. The UDP-GalNAc utilization by β1,4Gal-T from a different system like ovarian tumor microsomes is induced by α-LA similar to lactose production. This raises the question whether there are other modifier proteins expressed by non-mammary gland derived cells capable of mimicking α-LA and altering the sugar nucleotide specificity of β1,4Gal-T.

Acknowledgements

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References

1. Almeida, R.; Amado, M.; David, L.; Levery, S. B.; Holmes, E. H.; Merkx, G.; van Kessel, A. G.; Rygaard, E.; Hassan, H.; Bennett, E.; Clausen, H. *J. Biol. Chem.* **1997**, *272*, 31979–31991.
2. Brodbeck, U.; Denton, W. L.; Tanahashi, N.; Ebner, K. E. *J. Biol. Chem.* **1967**, *242*, 1391–1397.
3. Brew, K.; Vanaman, T. C.; Hill, R. L. *Proc. Natl. Acad. Sci. USA* **1968**, *59*, 491–497.
4. Sheares, B. T.; Carlson, D. M. *J. Biol. Chem.* **1984**, *259*, 8045–8047.
5. Neeleman, A. P.; van den Eijnden, D. H. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 10111–10116.
6. Bakker, H.; Schoenmakers, P. S.; Koeleman, C. A. M.; Joziasse, D. H.; van Die, I.; van den Eijnden, D. H. *Glycobiology* **1997**, *7*, 539–548.
7. Green, E. D.; Baenziger, J. U. *J. Biol. Chem.* **1988**, *263*, 25–35.
8. Coddeville, B.; Strecker, G.; Wieruszeski, J. M.; Vliegenthart, J. F. G.; van Halbeek, H.; Peter-Katalinic, J.; Egge, H.; Spik, G. *Carbohydr. Res.* **1992**, *236*, 145–164.
9. Sato, T.; Furukawa, K.; Greenwalt, D. E.; Kobata, A. *J. Biochem.* **1993**, *114*, 890–900.
10. Bergwerff, A. A.; Van Oostrum, J.; Kamerling, J. P.; Vliegenhart, J. F. G. *Eur. J. Biochem.* **1995**, *228*, 1009–1019.
11. Hooper, L. V.; Beranek, M. C.; Manzella, S. M.; Baenziger, J. U. *J. Biol. Chem.* **1995**, *270*, 5985–5993.
12. Taguchi, T.; Kitajima, K.; Muto, Y.; Inoue, S.; Khoo, K. H.; Morris, H. R.; Dell, A.; Wallace, R. A.; Selman, K.; Inoue, Y. *Glycobiology* **1995**, *5*, 611–624.
13. Mengeling, B. J.; Manzella, S. M.; Baenziger, J. U. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 502–506.
14. Manzella, S. M.; Hooper, L. V.; Baenziger, J. U. *J. Biol. Chem.* **1996**, *271*, 12117–12120.
15. van den Nieuwenhof, I.; Schiphorst, W. E. C. M.; van Die, I.; van den Eijnden, D. H. *Glycobiology* **1999**, *9*, 115–123.
16. Neeleman, A. P.; van der Knaap, W. P. W.; van den Eijnden, D. H. *Glycobiology* **1994**, *4*, 641–651.
17. Mulder, H.; Spronk, B. A.; Schachter, H.; Neeleman, A. P.; van den Eijnden, D. H.; De Jong-Brink, M.; Kamerling, J. P.; Vliegenthart, J. F. G. *Eur. J. Biochem.* **1995**, *227*, 175–185.
18. van Die, I.; van Tetering, A.; Bakker, H.; van den Eijnden, D. H.; Joziasse, D. H. *Glycobiology* **1995**, *6*, 157–164.
19. Srivatsan, J.; Smith, D. F.; Cummings, R. D. *J. Parasitol.* **1994**, *80*, 884–890.
20. Lloyd, K. O.; Yin, B. W.; Kudryashov, V. *Int. J. Cancer* **1997**, *71*, 842–850.
21. Madiyalakan, R.; Piskorz, C. F.; Piver, M. S.; Matta, K. L. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 901–906.
22. Odunsi, K.; Chandrasekaran, E. V.; Ghamande, S.; Ta, S.; O'Donnel, A.; Driscoll, D.; Seago, P.; Lele, S.; Matta, K. L.; *Abs. Soc. Gynecologic Oncologists Meeting*; Nashville, TN, USA, March 2001.
23. Udagawa, Y.; Aoki, D.; Ito, K.; Uejima, T.; Uemura, M.; Nozawa, S. *Eur. J. Cancer* **1998**, *34*, 489–495.
24. Lo-Guidice, J.-M.; Perini, J.-M.; Lafitte, J.-J.; Docourouble, M.-P.; Roussel, P.; Lamblin, G. *J. Biol. Chem.* **1995**, *270*, 27544–27550.
25. Chandrasekaran, E. V.; Jain, R. K.; Rhodes, J. M.; Chawda, R.; Piskorz, C.; Matta, K. L. *Glycoconjugate J.* **1999**, *16*, 523–536.
26. Chandrasekaran, E. V.; Jain, R. K.; Larsen, R. D.; Wlasichuk, K.; Matta, K. L. *Biochemistry* **1996**, *35*, 8914–8924.
27. Chandrasekaran, E. V.; Jain, R. K.; Larsen, R. D.; Wlasichuk, K.; DiCioccio, R. A.; Matta, K. L. *Biochemistry* **1996**, *35*, 8925–8933.
28. Palcic, M. M.; Heerze, L. D.; Pierce, M.; Hindsgaul, O. *Glycoconjugate J.* **1988**, *5*, 49–63.
29. Chandrasekaran, E. V.; Rhodes, J. M.; Jain, R. K.; Bernacki, R. J.; Matta, K. L. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 78–89.
30. Chandrasekaran, E. V.; Rhodes, J. M.; Jain, R. K.; Matta, K. L. *Biochem. Biophys. Res. Commun.* **1994**, *198*, 350–358.
31. Horejsi, V.; Smolek, P.; Kocurek, J. *Biochim. Biophys. Acta* **1978**, *538*, 293–298.
32. Jain, R. K.; Piskorz, C. F.; Matta, K. L. *Carbohydr. Res.* **1995**, *243*, 385–391.
33. Chandrasekaran, E. V.; Jain, R. K.; Larsen, R. D.; Wlasichuk, K.; Matta, K. L. *Biochemistry* **1995**, *34*, 2925–2936.
34. Chandrasekaran, E. V.; Jain, R. K.; Vig, R.; Matta, K. L. *Glycobiology* **1997**, *7*, 753–768.
35. Jain, R. K.; Piskorz, C. F.; Chandrasekaran, E. V.; Matta, K. L. *Glycoconjugate J.* **1998**, *15*, 951–959.
36. Tandai-Hiruma, M.; Endo, T.; Kobata, A. *J. Biol. Chem.* **1999**, *274*, 4459–4466.
37. Do, K.-Y.; Do, S.-I.; Cummings, R. D. *J. Biol. Chem.* **1995**, *270*, 18447–18451.
38. Khatra, B. S.; Herries, D. G.; Brew, K. *Eur. J. Biochem.* **1974**, *44*, 537–560.
39. Powell, J. T.; Brew, K. *J. Biol. Chem.* **1976**, *251*, 3653–3663.
40. Grobler, J. A.; Wang, M.; Pike, A. C. W.; Brew, K. *J. Biol. Chem.* **1994**, *269*, 5106–5114.