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Characterization of distinct Gal : 3-O-sulfotransferase activities in human tumor epithelial cell lines and of calf lymph node GlcNAc : 6-O-sulfotransferase activity

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We found earlier in human breast and colon tumors, an augmented level of Gal : 3-O-sulfotransferase activities showing, respectively, an acceptor preference to blood group T-hapten (Group A enzymes) or Galβ1,4GlcNAc (Group B enzymes) on the mucin Core 2 structure [Chandrasekaran EV, Jain RK, Vig R, and Matta KL (1997) *Glycobiology* 7: 753–68]. The present study reports these enzyme activities in human tumor cell lines and additional tumor specimens. The human colon tumor epithelial cell lines, akin to their parent tumors, express Group B enzyme activity. The acceptor specificity and kinetic properties, such as divalent metal ion activation and pH dependent activity profile, of the colon cancer line LS180 enzyme activity are identical to those of colon tissue specimens. Consistent with breast tumor specimens, the Group A enzyme activity is present in human breast tumor epithelial cell lines, with some exceptions. The Gal : 3-O-sulfotransferases show specific binding to *Aleuria aurantia* lectin, suggesting the presence of asparagine linked carbohydrate chains containing an inner core α1,6-fucosyl residue on these enzymes. Calf lymph nodes contain GlcNAc : 6-O-sulfotransferase as well as Group A Gal : 3-O-sulfotransferase activities, which differ in pH dependent profiles, pH optima (7.6 and 7.0, respectively) and the influence of Mn^{2+}.

Keywords: **cancer cells/glycan:sulfotransferases/kinetic properties/lymph nodes/specificities/tumors**

Abbreviations: **AA/CP, acrylamide copolymer; Al., allyl; AAL, Aleuria aurantia lectin; BAL., British anti-lewisite (2,3-dimercaptoethanol); Bn, benzyl; CALN, calf axial lymph node; CMLN, calf mesenteric lymph node; CEA, carcinoembryonic antigen; Me, methyl; PAPS, 3′phosphoadenosine 5′-phosphosulfate; PNA, peanut agglutinin; pNP, p-nitrophenyl; RM, reaction mixture; TLC, thin layer chromatography.**

Introduction

Chemical and enzymatic syntheses of sialyl Lewis x, sialyl Lewis a, their sulfated analogs and modified structures related to these motifs [1–12] are being pursued with the goal of developing potential drugs for the treatment of inflammatory diseases and cancer. Since fucose, sialic acid and sulfate groups have a significant role in the natural ligands for selectins, a focus on the specificities of fucosyl, sialyl and sulfotransferases can provide valuable information for the synthetic strategy toward the procurement of ligands for selectins [13]. The major capping group of the L-selectin ligand on high endothelial venules in human lymph nodes has been identified as 3′-sialyl, 6-sulfo Lewis x [14]. Our recent investigation on the specificities of sulfotransferases with a battery of well-defined acceptors [15] demonstrated two Gal : 3-O-sulfotransferase activities showing preference in utilizing mucin core 2 Galβ1,4GlcNAc or Galβ1,3GalNAc as acceptors. During these studies we also found that the branched structure Galβ1,4(Fucα1,3)GlcNAcβ1,6(Galβ1,3)GalNAcα-OBn can be converted to Galβ1,4(Fucα1,3)GlcNAcβ1,6(3-O-sulfoGalβ1,3)GalNAcα-OBn, which has been identified recently as a high affinity ligand for L- and P-selectins [12]. In our continuing interest in the study of glycan:sulfotransferases, in the present paper, we report a) the presence of distinct Gal : 3-O-sulfotransferase activities in human tumor cell lines akin to the parent tu-

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mors and a GlcNAc: 6-O-sulfotransferase activity in calf lymph nodes, b) the distinct interactions of fucose-specific *Aleuria aurantia* lectin with various glycan:sulfotransferases, and the divalent cation dependence of these enzymes.

Materials and methods

Cell culture

The colon carcinoma cell line, Colo 205, and the breast carcinoma cell lines, BT20 and MCF-7, were grown in minimal essential medium, the colon carcinoma cell line LS180 and the breast carcinoma cell line DU4475 in RPMI 1640, the colon cancer cell lines SW1116 and HT29, the ovarian carcinoma cell line SW626 and the breast carcinoma cell lines MDA-MB-231, MDA-MB-435S, MDA-435/LCC6, and MDA-435/LCC6^{MDRI}, were grown in Leibovitz's L-15 medium [all media were supplemented with 10% fetal bovine serum and the antibiotics—penicillin, streptomycin, amphotericin B] in 250 ml T-flasks under the conditions as recommended by American Type Culture Collection except for DU4475 grown as suspension. MDA-435/LCC6 and MDA-435/LCC6^{MDR1} were kindly provided by Dr. Ralph Bernacki of this Institute. The cells were homogenized with 0.1 M Tris-Maleate pH 6.3 containing 2% Triton X-100 using a Dounce all-glass hand-operated grinder. The homogenate was centrifuged at 16,000 g for 1 h at 4°C. Protein was measured on the supernatants by the BCA micro method (Pierce Chemical Co.) with BSA as the standard. The supernatants were adjusted to 5 mg of protein/ml by adding the necessary volume of the extraction buffer and then stored frozen at −20°C; 10 µl aliquots of these extracts were used in assays run in duplicate.

Calf mesenteric axial lymph nodes were made available from the animal facility through the courtesy of Dr. M.P. McGarry. Human breast metastatic tumor specimen BC 9380 was obtained from the National Disease Research Interchange, Philadelphia. Human ovarian and breast tumors and the metastatic omentum specimen MOM 9214 were obtained from the tissue procurement facility of Roswell Park Cancer Institute. All tissue specimens were kept frozen at −70°C until use.

Tissue extract

The tissue samples were homogenized at 4°C with 4 volumes of 0.1 M Tris Maleate pH 6.3, 0.1% NaN_3 using kinematica. After adjusting the concentration of Triton X-100 to 2%, these homogenates were mixed in the cold room for 1 h using Speci-Mix (Thermolyne) and then centrifuged at 20,000 g for 1 h at 4°C. The clear fat-free supernatant was stored frozen at −20°C until use.

Preparation of microsomal fractions

The buffer used in the homogenization of tissues was 50 mM Tris-HCl pH 7.4 containing 25 mM KCl, 0.25 M sucrose, 5 mM β-mercaptoethanol and 5 mM Mg acetate [16]. Tumor tissues (4.0 g in each case) were homogenized in 20 ml buffer in a Dounce all-glass hand-operated grinder. The homogenates were centrifuged at 16,000 g for −½ h at 4°C. The fat-free supernatants were centrifuged at 180,000 g in a Beckman ultracentrifuge using SW65 Ti rotor and polyallomer tubes for 1 h at 10°C. The microsomal pellets were dissolved in each case in 0.4 ml of 0.1 M Tris-Maleate pH 6.3 containing 2% Triton X-100 and 0.1% NaN_3. These solutions were dialyzed against the same buffer at 4°C for 48 h with three changes and stored frozen at −20°C until use.

Synthetic compounds

The syntheses of several compounds used in the present study have already been published [15,17]. The synthesis of Galβ1,4GlcNAcβ1,6(Galβ1,3) GalNAcα-O-Bn structures bearing a C-3 block (methyl or sulfate group) on either Gal has been reported [18]. The details on the synthesis of GlcNAcβ1,6Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn, GlcNAcβ1,3Galβ1,4Glc, 3-O-MeGalβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn, etc. will be reported elsewhere. The purity of the synthetic compounds was checked by TLC. The structural assignments were confirmed by ^{13}CNMR and fast atom bombardment mass spectroscopy.

Macromolecular and natural acceptors: Acrylamide copolymer of GlcNAcβ-O-A1 (GlcNAcβ-O-A1/AA-CP) was synthesized by following the procedure of Horejsi *et al.* [19]. About 1 µmol of GlcNAc was present in 1.0 mg copolymer (hexosamine determination after acid hydrolysis); a molecular weight of ~40,000 was assigned as judged by chromatography on a Biogel P-60 column with dextran (39,200 av. MW) as the marker.

Fetuin triantennary asialoglycopeptide and bovine IgG diantennary glycopeptide were available from earlier studies of this laboratory [20,21]. 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.).

Assay of sulfotransferase [15]

The incubation mixtures run in duplicate contained 100 mM Tris-maleate pH 7.2, 5 mM Mg Acetate, 5 mM ATP, 10 mM NaF, 10 mM BAL., 7.5 mM acceptor (unless otherwise stated) and 0.5 µCi of$^{[35]}$S PAPS (2.4 Ci/mmol) and 10 µ1 of cell or tissue extract or 5 µ1 of microsomal preparation in a total volume of 30 µ1; the control incubation mixtures contained everything except the acceptor. Incubation was carried out for 2 h at 37°C. Depending on the acceptor used in the incubation, either Dowex-1-Cl fractionation or TLC was employed to measure the radioactive product (see below).

Dowex-1-Cl method [15,22,23]

The incubation mixture was diluted with 1.0 ml water and passed through Dowex-1 X-8 (200–400 mesh chloride form) (1 ml in a Pasteur pipet). The column was washed twice with 1.0 ml water. A quantitative elution of the radioactive product ([^{35}S] sulfated compound) was achieved by eluting the column with 3.0 ml of 0.2 M NaCl. This was established by subjecting the [^{35}S] sulfated compounds resulting from the acceptors 3-O-MeGalβ1,4GlcNAc β1,6(Galβ1,3) GalNAcα-O-Bn, Galβ1,4GlcNAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn, Galβ1,4GlcNAcβ-O-Al and Galβ1,3GalNAcα-O-Al (isolated by Dowex-1-Cl fractionation and then Biogel P_2 gel filtration for desalting) to Dowex-1-Cl fractionation under identical conditions. The radioactivity present in the NaCl eluate was measured using 3a70 scintillation mixture (Research Products International., Mount Prospects, IL) and a Beckman LS 9000 instrument. The 0.2 M NaCl eluate from the control reaction mixture (containing no acceptor) always had negligible amount of radioactivity (<50 CPM). The values for the duplicate runs did not vary more than 5%.

TLC method [15,22,23]

The reaction mixtures (30 μ1) were subjected to thin layer chromatography (silica gel GHLF; 250 microns, scored 20 × 20 cm; Analtech, NJ) after a quantitative transfer as 2 cm streaks. The solvent system, chloroform-methanol-water (5:4:1, v/v), was used to separate the [^{35}S] sulfated allyl or benzyl glycoside (migrated >12 cm from the origin) from the much slower migrating [^{35}S] PAPS (<3 cm) from the origin. The radioactive content of 1/2 cm width segments of silica scraped into scintillation vials and soaked in 2 ml of water was determined by liquid scintillation. Variation in the values for reaction mixtures run in duplicate were all within 5%.

PNA-agarose affinity chromatography

A column of 2 ml bed volume of PNA-agarose (Vector Lab, Burlingame, CA) was employed using 10 mM Hepes pH 7.5, containing 0.1 mM Ca^{++}, 0.01 mM Mn^{++} and 0.1% NaN_3 as the eluting buffer as recommended by the manufacturer. The fractionation was done at room temp. The [^{35}S] sample was applied to the column in 1.0 ml of the above buffer. After entry of the sample into that column bed, it was allowed to remain in contact with the gel for 20 min before starting elution with the same buffer. Fractions of 1 ml were collected and counted for radioactivity by liquid scintillation. In order to see the effect of 3-O-sulfated analogs of mucin core 2 structure, 1.5 μmol of each compound was mixed separately with [^{35}S] sulfated product before its application to the PNA-agarose column.

Effect of pH and divalent cations on glycan:sulfotransferases

Tris-maleate buffer in the pH range 5.2–8.4 (final concentration in reaction mixture 0.1 M) was used under the standard incubation conditions. For seeing the effect of divalent metal ions, the incubation mixture contained varying concentrations (1–50 mM) of Mg acetate, Mn acetate or Ca acetate under the standard incubation conditions.

Testing for competitive inhibition

For studying the competitive inhibition of calf lymph node Gal: 3-O-sulfotransferase and GlcNAc: 6-O-sulfotransferase activities, we took advantage of the fact that the radioactive product arising from the competitive inhibitor, 3-O-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn namely 3-O-sulfoGalβ1,4GlcNAcβ1,6(3-O-[^{35}S] sulfo Galβ1,3)GalNAcα-O-Bn cannot be eluted, whereas, the product from the neutral as well as monosialyl acceptors can be eluted from the Dowex-1-Cl column by 0.2 M NaC1. The concentration of the neutral as well as monosialyl acceptors was left constant (5 mM) and that of the sulfated acceptor was varied from 0 to 6 mM and incubated under the standard conditions.

Study on the affinity interaction of AAL with Gal: 3-O-sulfotransferases of different sources

a) Colo 205 glycan:sulfotransferases. Colo 205 cells (1.2×10^9 cells) were homogenized with 25 ml of 0.1 M Tris-Maleate pH 6.3 - 0.1% NaN_3 - 2% Triton X-100 using a Dounce all-glass hand-operated grinder. The 20,000 g supernatant was applied to an AAL-agarose (Vector Lab, Burlingame, CA) column (bed volume of 8 ml) which had been washed with 0.1 M Tris Maleate pH 6.3, 0.1% NaN_3 - 0.1% Triton X-100. After sample entry, the column was washed with 50 ml of the equilibration buffer. The breakthrough and washings were collected together. Elution was then done with 50 ml of the above buffer containing 0.1 M fucose followed by 50 ml buffer containing 2.0 M NaCl. The breakthrough solution and the two eluates were concentrated to ~4 ml by ultrafiltration using PM10 membrane then dialyzed against 2 liters of 0.1 M Tris-Maleate pH 6.3 - 0.1% NaN_3 - 2% Triton X-100 and stored frozen at −20°C until use.

b) Human breast metastatic tumor glycan:sulfotransferases. The large tumor specimen BC 9380 (snap-frozen and powdered; 11.7 g) was homogenized with 100 ml of 0.1 M Tris Maleate pH 6.3 - 0.1% NaN_3, at 4°C using kinematica. After adjusting the concentration of Triton X-100 to 2%, this homogenate was mixed in the coldroom for 1 h using Speci-Mix (Thermolyne) and then centrifuged at 20,000 g for 1 h at 4°C. This supernatant was subjected to AAL-agarose column (8 ml bed volume) chromatography as mentioned for Colo 205.

c) Human ovarian tumor glycan:sulfotransferases. Ovarian tumor tissues from six patients were pooled (total weight 26.5 g) and homogenized with 130 ml of 0.1 M Tris-Maleate pH 6.3 - 0.1% NaN_3. The homogenate after adjusting the concentration of Triton X-100 to 2%, was stirred on a magnet stirrer for 2 h at 4°C, and centrifuged at 20,000 g for 1 h at 4°C. The fat free supernatant was saturated to 40% with $(NH_4)_2SO_4$. The precipitate was dissolved in 20 ml of 0.1 M Tris-Maleate pH 6.3 - 0.1% NaN_3 - 2% Triton X-100 and dialyzed against 1 liter of the same buffer with three changes for 24 h at 4°C. About 70% of the glycan:sulfotransferase activity was recovered in this fraction and that fraction was subjected to AAL-agarose column (8 ml bed volume) chromatography as mentioned for Colo 205.

Results

Glycan:sulfotransferase activities in human tumor cell lines (Table 1)

Gal : 3-O-sulfotransferase activities towards the terminal Gal moieties of Mucin Core 2 analogues were measured with the acceptors, 3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3) GalNAcα-O-Bn and Galβ1,4GlcNAcβ1,6(3-O-Me Galβ1,3) GalNAcα-O-Bn. In addition, GlcNAc: 6-O-sulfotransferase was measured using GlcNAcβ1,6(3-O-Me Galβ1,3) GalNAcα-O-Bn. The colon cell lines Colo 205, LS180 and SW1116, as well as the ovarian line SW626, expressed predominantly the Gal : 3-O-sulfotransferase activity towards the β1,4 linked-Gal in the β1,6 branch of mucin Core 2, whereas, the breast tumor cell lines, except for DU4475 and MCF-7, contained only the Gal : 3-O-sulfotransferase activity acting on the T-hapten of mucin Core 2. Among the colon cell lines, LS180 showed the highest enzyme activity (216.0) followed by SW1116 (92.5) and Colo 205 (6.4). The T-hapten Gal : 3-O-sulfotransferase activities in breast cell lines were as follows: MDA-435/LCC6 (62.6), MDA-435/LCC6^{MDRI} (28.1), MDA-MB-435S (7.2) and MDA-MB-231 (1.2). The colon cancer line HT29 and the breast cancer line BT20 showed negligible Gal : 3-O-sulfotransferase activity. Sulfotransferase activity on the terminal GlcNAc moiety was either absent or at a very low level in all the cell lines examined (see the last column in Table 1).

Discerning the preference shown by Group A and Group B Gal : 3-O-sulfotransferases for one of the Gal moieties in the mucin core 2 structure Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn

The [^{35}S] sulfated compounds arising from Galβ1,4Glc NAcβ1,6(Galβ1,3)GalNAcα-O-Bn by incubating with the Triton X-100 extract of LS180 or MDA-435-LCC6 were isolated by chromatography on a Biogel P_2 column. When the [^{35}S] product resulting from the action of LS180 enzyme was subjected to PNA-agarose chromatography, most of the product showed distinct loose binding to this column (Fig. 1B), whereas the other [^{35}S] product did not bind at all to the column (Fig. 1A). The specific binding of the former

Table 1. Identification of specific glycan:sulfotransferase activities in human colon, ovarian and breast tumor cell lines

	Sulfotransferase Activity: Incorporation of [^{35}S] Sulfate (CPM × 10^{-3}) into the acceptor catalyzed by 1 mg of protein / 2h.		
Cell line	*3-O-MeGalβ1,4GlcNAcβ1,6 (Galβ1,3)GalNAcα-O-Bn (7.5 mM)*	*Galβ1,4GlcNAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn (7.5 mM)*	*GlcNAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn (7.5 mM)*
Colon:			
Colo205	1.3	6.4	0.1
LS180	33.4	216.0	0.2
SW1116	17.3	92.5	0.4
HT29	0.1	0.1	<0.1
Ovary:			
SW626	2.5	15.7	0
Breast:			
MDA-MB 231	1.2	0.2	0.3
MDA-435/LCC6	62.6	0.5	0.2
MDA-435/LCC6^{MDR1}	28.1	0.3	0.2
MDA-MB 435S	7.2	0.1	0.0
DU4475	51.8	361.2	0.2
MCF-7	3.4	19.4	0
BT20	<0.1	0.3	0.1

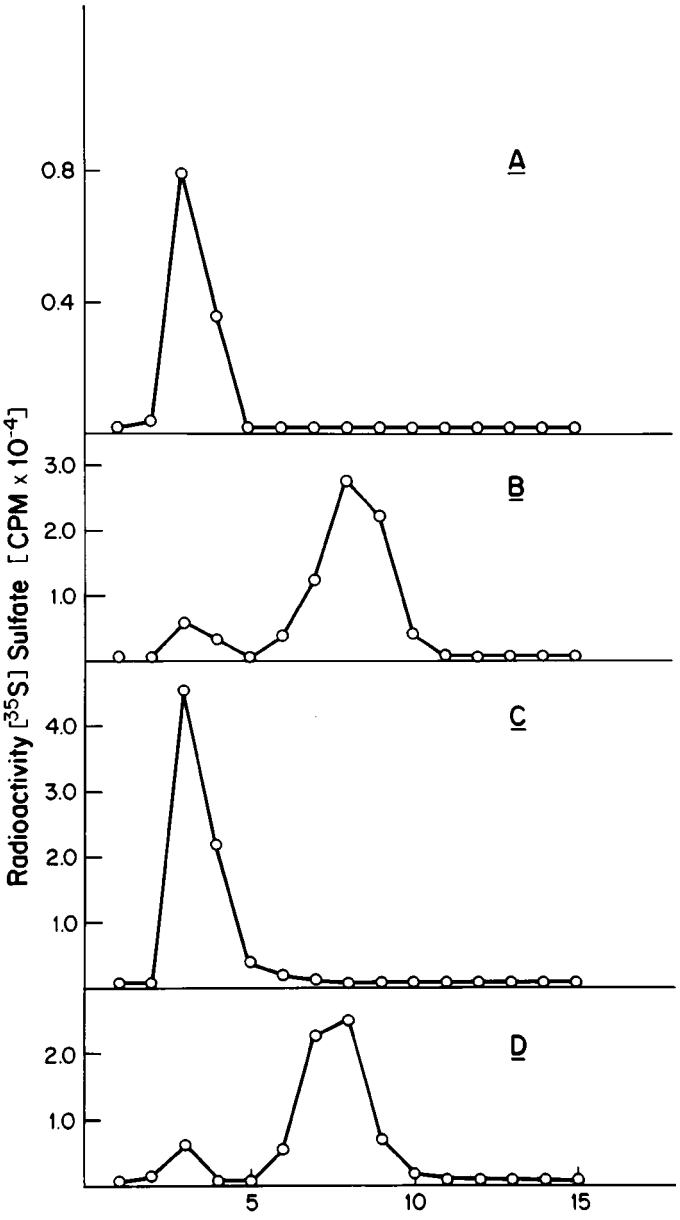

Figure 1. Localization of 3-O-sulfo Gal in mucin core 2 structure after the enzymatic [^{35}S] sulfation, by PNA-agarose chromatography. The [^{35}S] product arising from Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn by the action of A: MDA-435/LCC6 enzyme extract; B: LS180 enzyme extract; C: as in B in the presence of cold authentic 3-O-sulfo Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn (1.5 μmol); D: as in B in the presence of cold authentic Galβ1,4GlcNAcβ1,6(3-O-sulfo Galβ1,3)GalNAcα-O-Bn (1.5 μmol).

to PNA-agarose was further demonstrated by showing, respectively, the complete inhibition and non-inhibition of this binding with authentic cold compounds obtained through chemical synthesis [18], 3-O-sulfo Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn and Galβ1,4GlcNAcβ1,6(3-O-sulfo Gal β1,3)GalNAcα-O-Bn (Fig. 1C and 1D respectively).

The characterization of colon carcinoma cell line LS180 Gal : 3-O-sulfotransferase Activity employing various synthetic acceptors (Table 2). The acceptors Galβ1,4 GlcNAcβ-O-Al, Galβ1,3GlcNAcβ-O-Al, 3-O-MeGalβ1,4 GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn and Galβ1,4Glc NAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn showed 101.7%, 22.7%, 23.7% and 248.8% activities, respectively, as compared to Galβ1,3GalNAcα-O-Al. The results indicate that LacNAc type 2 (Galβ1,4GlcNAc) occurring as a mucin Core 2 structure is the best acceptor for the enzyme(s) of this source. LacNAc type 1 (Galβ1,3GlcNAcβ-O-Al) as compared to LacNAc type 2 (Galβ1,4Glc NAcβ-O-Al) was a poor acceptor (activity: 10.2 vs. 45.7). The acceptor Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn, containing two possible sites for activity, was less efficient than Galβ1,4GlcNAcβ1,6(3-O-MeGalβ1,3)Gal NAcα-O-Bn (75.1 versus 111.8). It is interesting to note that 3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn is better than Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn as an acceptor for Group B activity (activity:10.6 and 4.3 respectively). The GlcNAc sulfotransferase activity in LS180 as measured with synthetic acceptors namely, Glc NAcβ1,6Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn,3-O-Me Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn, Fucα1,2Galβ1,3 (GlcNAcβ1,6)GalNAcα-O-Bn and NeuAcα2,3Galβ1,3 (GlcNAcβ1,6)GalNAcα-O-Bn was found to be negligible.

Microsomal glycan:sulfotransferase activities of normal and tumor tissues (see Table 3)

Our earlier study [15] on glycan:sulfotransferases used Triton X-100 solubilized extracts of human tumor as well as control tissues. Since sulfotranferases are known to consist of two groups, namely cytosolic and membrane-bound enzymes [24], we have prepared in the present study crude microsomes from tissue specimens to demonstrate that glycan : sulfotransferases are membrane bound enzymes. The microsomes of lymphoma spleen displayed a higher level of the three distinct glycan : sulfotransferase activities than the normal spleen microsomes. The microsomes of lung tumor also contained a significant level of these three distinct glycan:sulfotransferase activities. The microsomes of ovarian tumors (both primary and metastatic) contained a high level of Group A Gal : 3-O-sulfotransferase activity. Some of the ovarian tumor specimens [OT (PW), OT9387, MO9420] also exhibited a high level of Gal : 3-O-sulfotransferase activity towards Galβ1,4GlcNAcβ-. However, the microsomes of all the ovarian tumor specimens contained a relatively low level of GlcNAc : sulfotransferase activity.

Identification of GlcNAc: sulfotransferase activity in human breast tumor

Eight breast tumor specimens were examined for Groups A and B Gal : 3-O-sulfotransferase activities using the

Table 2. Specificities of colon carcinoma cell line LS180 glycan:sulfotransferase

Acceptor (7.5 mM)	*Sulfotransferase Activity Incorporation of [^{35}S] sulfate (CPMx10^{-3})into the acceptor catalyzed by 1 mg of protein / 2h.*
Galβ1,3GalNAcα-O-Al	45.0
Galβ1,4GlcNAcβ-O-Al	45.7
Galβ1,3GlcNAcβ-O-Al	10.2
3-O-MeGalβ1,4GlcNAβ1,6(Galβ1,3)GalNAcα-O-Bn	10.6
Galβ1,4GlcNAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn	111.8
Galβ1,4GlcNAβ1,6(Galβ1,3)GalNAcα-O-Bn	75.1
Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	4.3
GlcNAcβ1,6Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	0.2
3-O-MeGalβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	0.2
Fucα1,2Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	0.1
NeuAcα2,3Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	0.2

acceptors Galβ1,3GalNAcα-O-A1 and Galβ1,4Glc NAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn, and for GlcNAc : 6-O-sulfotransferase activity using the acceptors GlcNAcβ1,6Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn and GlcNAcβ-O-A1/AA-CP. It is evident from the results reported in Table 4 that all these tissue specimens are active towards GlcNAcβ1,6Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn indicating a significant expression of GlcNAc: sulfotransferase activity. These specimens, however, showed very low activity towards GlcNAcβ-O-A1/AA-CP. In order to further substantiate the presence of GlcNAc : sulfotransferase activity in breast tumor, we have arbitrarily chosen two breast tumor specimens for testing with various GlcNAc terminating compounds as the acceptors (see Table 5). We found all these compounds except for 3-O-MeGalβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn to be significantly active with the enzymes present in the two breast specimens.

Characterization of glycan:sulfotransferase activities of calf lymph node

The specificities of glycan:sulfotransferase activities of calf mesenteric lymph node were discerned by using synthetic acceptors as well as glycopeptides (see Table 6). Both Galβ1,3GalNAcα-O-A1 and 3-O-MeGalβ1,4GlcNAcβ1,6 (Galβ1,3)GalNAcα-O-Bn served equally well as efficient acceptors (activity: 54.3 versus 58.3), whereas, Galβ1,4 GlcNAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn showed low activity (4.4). The following acceptors with the terminal β1,6 linked GlcNAc moiety, namely, Galβ1,3(GlcNAcβ1,6) GalNAcα-O-Bn, 3-O-MeGalβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn, GlcNAcβ1,6Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn, NeuAcα2,3Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn, and Fucα1,2Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn acted as acceptors (activities: 20.0, 18.7, 54.5, 11.5 and 37.4, respectively). The acrylamide copolymer GlcNAcβ-O-A1/

Table 3. Microsomes as the source of various glycan:sulfotransferase activities in human spleen and tumor tissues

	Glycan:sulfotransferase activity[a]								
Acceptor (7.5 mM)	*NS*[b] *7665*	*LS 7643*	*LT 9287*	*OT (PW)*	*OT 9287*	*OT 9303*	*MO 9307*	*OT 9387*	*MO 9420*
3-O-MeGalβ1,4GlcNAcβ1,6 (Galβ1,3)GalNAcα-O-Bn	0.1	0.7	0.8	10.3	39.3	14.9	6.7	199.6	41.4
Galβ1,4GlcNAcβ1,6 (3-O-MeGalβ1,3)GalNAcα-O-Bn	0	0.6	0.8	7.4	2.5	0.7	2.0	159.3	41.5
GlcNAcβ1,6 (3-O-MeGalβ1,3)GalNAcα-O-Bn	<0.1	1.2	1.3	2.2	4.7	1.1	1.5	5.4	2.4

[a]incorporation of [^{35}S] sulfate (CPM $\times$ 10^{-3}) catalyzed by 1 mg of microsomal protein / 2h.
[b]NS, normal spleen; LS, lymphoma spleen; LT, lung tumor; OT, ovarian tumor; MO, metastatic ovary.

Table 4. Identification of GlcNAc: sulfotransferase activity in human breast tumor

	Sulfotransferase Activity Incorporation of [^{35}S] sulfate (CPM $\times$ 10^{-3}) into the acceptor catalyzed by 1 mg of protein of tissue extract / 2h.			
	Gal: 3-O-Sulfotransferase		*GlcNAc: sulfotransferase*	
Breast tumor specimen	*Galβ1,3GalNacα-O-A1 (7.5mM)*	*Galβ1,4GlcNAcβ1,6(3-OMeGalβ1,3)GalNAcα -O-Bn (7.5mM)*	*GlcNAcβ1,6Galβ 1,3(GlcNAcβ1,6) GalNAcβ-O-Bn (7.5mM)*	*GlcNAcβ-O-A1/AA-CP (50; μg)*
BT 7764	181.9	17.6	13.5	0.2
BT 7803	46.3	18.9	5.6	0.7
BT 8565	38.6	2.8	4.6	1.8
BT 8640	104.4	12.3	9.2	0.4
BT 8600	47.7	18.8	3.7	1.0
BT 7549	173.4	3.6	17.7	1.2
BT 9060	144.4	7.4	14.7	0.9
BT 9380	67.8	44.6	8.4	2.0

AA-CP at a low concentration (50 μg:62.5 μM) showed significant activity (activity: 3.6). Fetuin triantennary asialo glycopeptide and bovine IgG glycopeptide became slightly better acceptors after exposing the GlcNAc moiety by de-galactosylation (activity: 2.1→3.8; 0.4→0.8).

Identification of the enzymatic sulfation as the C-6 hydroxyl of terminal GlcNAc moiety

The compound GlcNAcβ1,3Galβ-O-Me was subjected to [^{35}S] sulfation using calf lymph node microsomal extract in a ten-fold reaction mixture under the standard incubation conditions. The radioactive product was isolated by chromatography of the reaction mixture on a Biogel P2 column (1.0 $\times$ 116.0 cm) and then subjected to thin layer chromatography along with authentic compounds, namely, GlcNAcβ1,3Galβ-O-Me, 3-O-sulfo-GlcNAcβ1,3Galβ-O-Me and 6-O-sulfoGlcNAcβ1,3Galβ-O-Me (see Fig. 2A). It was found that the radioactive material moved identical to the standard 6-O-sulfoGlcNAcβ1,3Galβ-O-Me, indicating that the enzymatic sulfation of the terminal GlcNAc occurs on the C-6 position of this sugar moiety. The [^{35}S] sulfated product from another acceptor GlcNAcβ1,3Galβ1,4Glc by the action of calf lymph node microsomal enzyme was also isolated as above. On TLC this radioactive product migated slower than the acceptor compound (Fig. 2B). These two [^{35}S] sulfated compounds when subjected to TLC exhibited single spots of much lower mobilities than the [^{14}C] fucosylated compound used as a marker in autoradiography.

No apparent inhibition of GlcNAc : 6-O-sulfotransferase

Table 5. Further evidence for GlcNAc: sulfotransferase activity in human breast tumor using specific acceptors

	Sulfotransferase Activity Incorporation of [^{35}S] sulfate (CPM X 10^{-3}) into the acceptor catalyzed by 1 mg of protein of the tissue extract / 2h.	
Acceptor (7.5 mM)	*BT 7549*	*BT 9060*
Galβ1,3GalNAcα-O-A1	95.5	81.5
3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn	121.7	111.2
Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	7.8	6.3
GlcNAcβ1,3Galβ1,4Glc	9.3	24.1
3-O-MeGalβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	0.2	1.3
Fucαl,2Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	7.2	6.0
NeuAcα2,3Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	6.2	4.9

Table 6. Identification of both Gal: and GlcNAc: sulfotransferase activities in calf mesenteric lymph nodes

Accept or (7.5 mM)	*Sulfotransferase Activity Incorporation of [^{35}S] sulfate (CPM × 10^{-3}) into the acceptor catalyzed by 1 mg of microsomal protein / 2h.*	*(%)*
Galβ1,3GalNAcα-O-Al	54.3	(100.0)
3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn	58.3	(107.4)
Galβ1,4GlcNAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn	4.4	(8.1)
Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	20.0	(36.7)
3-O-MeGalβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	18.7	(34.5)
GlcNAcβ1,6Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	54.5	(100.4)
NeuAcα2,3Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	11.5	(21.1)
Fucαl,2Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	37.4	(68.8)
GlcNAcβ1,3Galβ1,4Glc	52.6	(96.9)
GlcNAcβ1,3Galβ-O-Me	5.8	(10.7)
GlcNAcβ1,6Manα-O-Al	0.1	(0.2)
GlcNAcβ-O-Al/AA-CP (50μg)	3.6	
Bovine IgG gp (100μg)	0.4	
Bovine IgG agalacto gp (100μg)	0.8	
Fetuin triantennary asialo gp (100μg)	2.1	
Fetuin triantennary aisalo agalacto gp (100μg)	3.8	

activity by the competitive inhibitor of Gal : 3-O-sulfotransferase activity (see Fig. 3). The sulfated compound 3-O-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn is an acceptor for Group A Gal : 3-O-sulfotransferase acting on the Gal moiety of T-hapten. When Galβ1,3GalNAcα-O-Al, 3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn, GlcNAcβ1,6Galβ1,3(GlcNAcβ1,6)GalNAcβ-O-Bn, Fucα1,2Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn, 3-O-MeGalβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn and NeuAcα2,3Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn were tested as acceptors for calf lymph node sulfotransferases in the presence of 3-O-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn (0→6.0 mM) [see Fig. 3], it was found that the transfer of [^{35}S] sulfate to the acceptors Galβ1,3GalNAcα-O-Al and 3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn decreased steadily with an increase in the concentration of the sulfated acceptor. On the other hand, the sulfation of the other acceptors mentioned above was not impaired. The results would suggest the presence of two enzyme activities in calf lymph nodes, one specific for 3-O-sulfation of Gal and the other for 6-O-sulfation of GlcNAc of mucin core 2.

GlcNAcβ-O-Allyl/Acrylamide copolymer is a high affinity acceptor for calf lymph node GlcNAc : 6-O-sulfotransferase

The activity of calf lymph node GlcNAc : 6-O-sulfotransferase was measured using this copolymer, at various concentrations, as the acceptor (0.025 mM→1.25 mM) [data not shown]. The K_m as determined by Lineweaver-Burk plot was 2.0 mM indicating the usefulness of this copolymer as a high affinity acceptor for this enzyme.

Aleuria aurantia lectin (AAL) interacts with Gal : 3-O-sulfotransferases

Enzymes from three different sources (Colo 205, human breast cancer metastases, and human ovarian tumors) were examined in order to ascertain this phenomenon.

a) Colo 205 Gal : 3-O-sulfotransferase activity. Triton X-100 solubilized extract of Colo 205 was subjected to AAL-agarose column chromatography (see Table 7). Only a small amount of enzyme activity, as determined by the acceptors, 3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3) GalNAcα-O-Bn and Galβ1,4GlcNAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn, did not bind to the column under the experimental conditions. The major activity found in the NaCl eluate (26.1) was towards the acceptor, Galβ1,4GlcNAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn. The enzyme activity towards Galβ1,3GalNAcα-O-Al appears to be the predominant activity in the fucose eluate (149.3). Interestingly, the mucin core 2 acceptor, 3-O-MeGalβ1,4GlcNAcβ1,6 (Galβ1,3)GalNAcα-O-Bn displayed very low activity (1.5).

b) Gal : 3-O-sulfotransferases of human breast cancer metastasis (see Table 7). The AAL non-binding fraction contained enzyme activity which was mostly active towards Galβ1,3GalNAcα-O-Al and towards the same structural unit in mucin Core 2 [3-O-MeGalβ1,4GlcNAcβ1,6 (Galβ1,3)GalNAcα-O-Bn] (activity; 33.9 versus 37.8). On the other hand, an enrichment of enzyme activity towards Galβ1,4GlcNAcβ1,6(3-O-MeGalβ1,3)

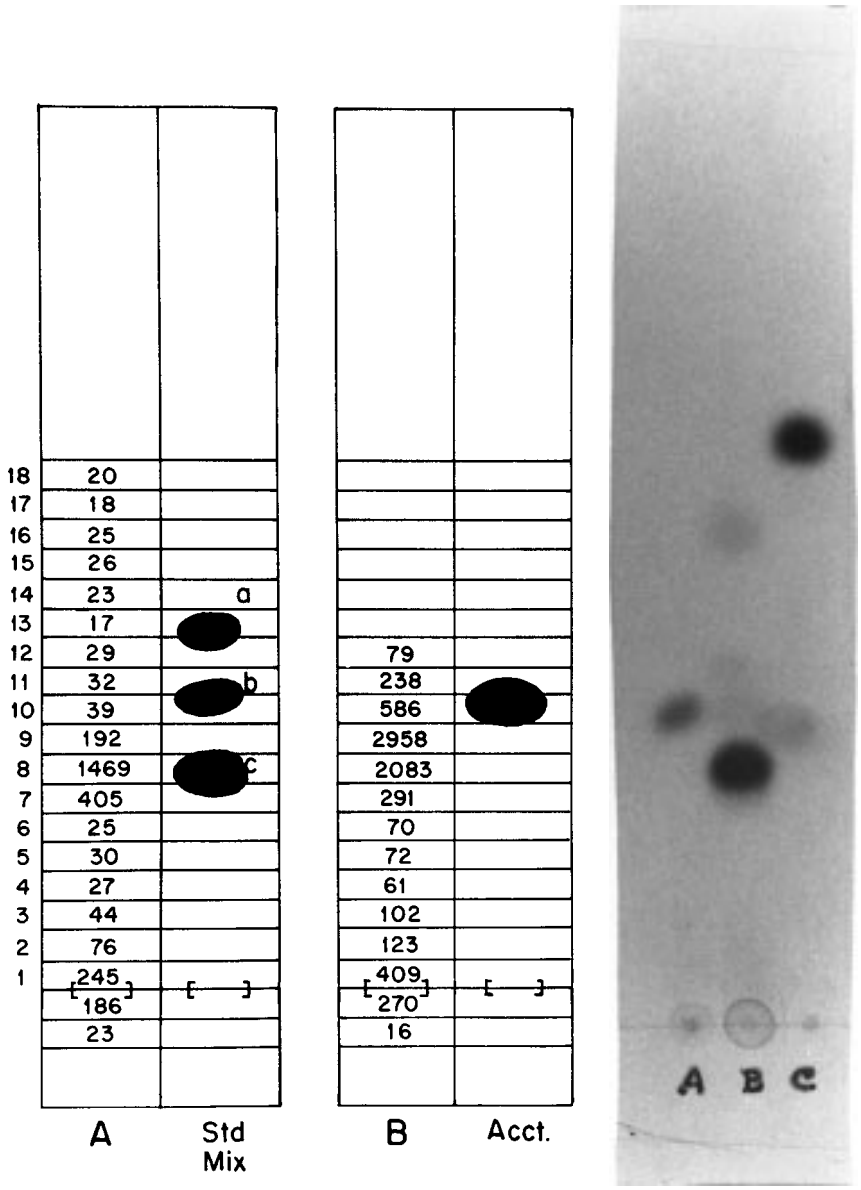

Figure 2. Thin layer chromatography of the [^{35}S] sulfated compounds arising from the acceptors GlcNAcβ1,3Galβ-O-Me and GlcNAcβ1,3Galβ1,4Glc by the action of calf lymph node microsomal preparation. The TLC plate was developed twice in the solvent system n-butanol:acetic acid:water (3/2/1). A. [^{35}S] sulfated compound resulting from GlcNAcβ1,3Galβ-O-Me Std. Mix: a - GlcNAcβ1,3Galβ-O-Me; b - 3-O-sulfoGlcNAcβ1,3Galβ-O-Me; c - 6-O-sulfoGlcNAcβ1,3Galβ-O-Me. B. [^{35}S] sulfated compound resulting from GlcNAcβ1,3Galβ1,4Glc Acct(acceptor):GlcNAcβ1,3Galβ1,4Glc. C. [^{14}C] fucosylated compound resulting from GlcNAcβ1,4GlcNAcβ-O-Bn by the action of LS180 cell extract. Note: Since the TLC of A, B and ABC together (autoradiogram) were carried out on three separate occasions, they are not comparable with each other.

GalNAcα-O-Bn could be found in the fucose and NaCl eluates (64.7 and 59.0). Further, these eluates also showed considerable activities (31.7 and 35.8) towards 3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn. These results indicate that the Gal : 3-O-sulfotransferase activities present in this breast cancer tissue specimen are capable of acting on the two terminal Gal moieties of mucin Core 2 and that both can bind to *Aleuria aurantia* lectin.

c) Human ovarian tumor glycan:sulfotransferases (see Table 7). The AAL non-binding fraction contained very low glycan: sulfotransferase activities. The AAL-agarose binding fractions (the fucose and NaCl eluates) showed predominant activity towards Galβ1,4GlcNAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn (107.6 and 63.2 respectively). The other activity acting on T-hapten (either as such or as part of mucin Core 2) also constitutes a significant portion in these binding materials. In addition, the sulfotransferase acting on terminal GlcNAc moiety was present to a small

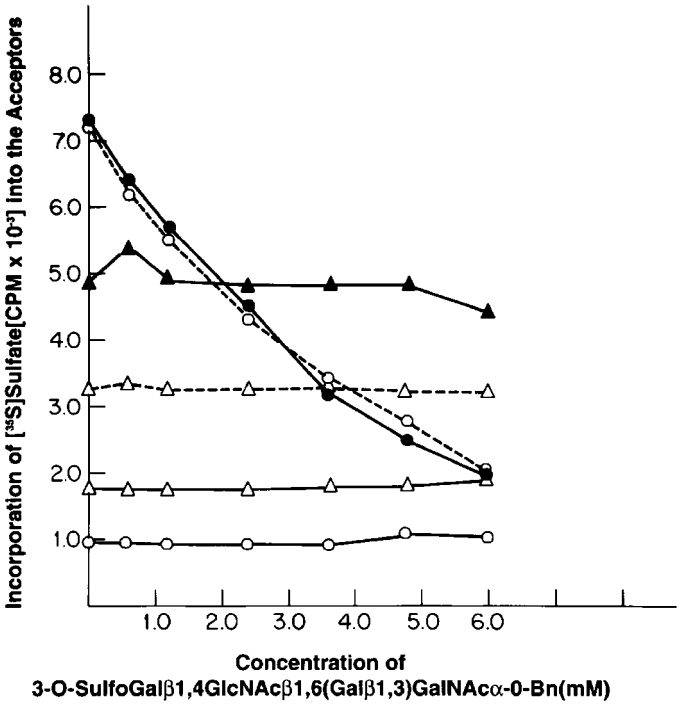

Figure 3. Effect of 3-O-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn on calf lymph node. Gal: and GlcNAc: sulfotransferase activities. Gal: 3-O-sulfotransferase activity: Acceptors: ●—●—● Galβ1,3GalNAcα-O-Al ○---○---○ 3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn GlcNAc: Sulfotransferase activity: Acceptors: ▲—▲—▲ GlcNAcβ1,6 Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn; △---△---△ Fucα1,2Galβ1,3 (GlcNAcβ1,6)GalNAcα-O-Bn; △—△—△ 3-O-MeGalβ1,3(GlcNAcβ1,6) GalNAcα-O-Bn; ○—○—○ NeuAcα2,3Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn.

extent in the fucose as well as in the NaCl eluates, as judged with the acceptors, GlcNAcβ1,6Galβ1,3(GlcNAcβ1,6) GalNAcα-O-Bn and GlcNAcβ-O-A1/AA-CP.

pH optimum of Glycan : sulfotransferase activities.

a) LS180 Gal : 3-O-Sulfotransferase: The enzyme activity was measured over a pH range of 5.2–8.4 (data not shown). The activity gradually increased from zero at pH 5.2 to an optimum at pH 7.2 and then dropped steadily when measured with the acceptor, Galβ1,4GlcNAcβ1,6(3-O-Me Galβ1,3)GalNAcα-O-Bn.

b) A substantial difference in the influence of pH on Glycan:Sulfotransferase activities in lymph nodes. Gal : 3-O-sulfotransferase and GlcNAc : 6-O-sulfotransferase activities were measured in calf mesenteric and axial lymph nodes using Galβ1,3GalNAcα-O-Al and GlcNAcβ1,6 Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn, respectively (Fig. 4A and 4B). The former activity in both lymph nodes increased sharply after pH 5.6 reaching an optimal level around pH 7.0. On the other hand, the other activity in both lymph nodes increased very slowly with an increase in pH attaining a maximum at pH 7.6. At pH 8.4 Gal : 3-O-sulfotransferase activity amounted to only 15% of the optimal activity, whereas, GlcNAc : 6-O-sulfotransferase activity remained at ~65% level.

Influence of divalent metal ions on Glycan : sulfotransferase activities

a) LS180 Gal : 3-O-sulfotransferase: When the LS180 Gal : 3-O-sulfotransferase activity was measured in the presence of varying concentrations of Mn^{2+}, Mg^{2+} or Ca^{2+}, the activity steadily increased (50%–90%) up to a 10 mM level of any of these divalent cations (data not shown). These metal ions exhibited no apparent large difference in the stimulation of activity. The enzyme activity decreased at higher concentrations of these metal ions although Mg^{2+} and Ca^{2+} showed considerable stimulation of activity, even at 50 mM.

b) Effect of Mn^{2+} on Gal : 3-O-sulfotransferases activities of calf lymph nodes and human metastatic omentum. The Gal : 3-O-sulfotransferase activities of both calf mesenteric and axial lymph nodes as measured with the acceptor 3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn showed very little stimulation of activity up to 5 mM Mn^{2+} and then a steady decline of activity reaching ~40% at 50 mM (data not shown). A measurement of the activity in metastatic omentum using the same acceptor indicated a small stimulation of activity reaching optimal at 10 mM Mn^{2+} and then a decline (~45% at 50 mM). The omentum also contained Gal : 3-O-sulfotransferase activity reactive towards the LacNAc type 2 structure. A significant stimulation of this activity as measured with Galβ1,4GlcNAcβ1,6 (3-O-MeGalβ1,3)GalNAcα-O-Bn acceptor was seen at 10 mM Mn^{2+}. The activity then dropped slightly reaching its initial level at 50 mM Mn^{2+}. The above results indicate that Mn^{2+}, indeed, has a distinct effect on Group A and Group B Gal: 3-O-sulfotransferase activities.

c) Differential Effect of Mn^{2+} on Gal : 3-O-sulfotransferase and GlcNAc : 6-O-sulfotransferase activities present in calf lymph nodes. The above sulfotransferase activities were measured using the acceptors Galβ1,3GalNAcα-O-A1 and GlcNAcβ1,6Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn, respectively, in the presence of varying concentrations of Mn^{2+}, Mg^{2+} or Ca^{2+} (Fig. 5A and 5B). Mg^{2+} stimulated both activities to a small extent, whereas, Ca^{2+} inhibited both activities causing >65% inhibition of Gal : 3-O-sulfotransferase activity at 50 mM and ~50% inhibition of GlcNAc: 6-O-sulfotransferase activity at 20 mM. There was no further inhibition of this activity at higher concentrations of Ca^{2+}. A distinct differential effect on these two sulfotransferase activities was found only with Mn^{2+}. There was about 60% stimulation of GlcNAc : 6-O-sulfotransferase activity at 5 mM Mn^{2+} and then a decline occurred reaching 50% at 50 mM Mn^{2+}. On the contrary, Gal : 3-O-sulfotransferase activity did not show any stimulation but

Table 7. Distinct interaction of *Aleuria aurantia* lectin with Glycan:sulfotransferases of Colo 205, human breast cancer metastases and ovarian tumors.

	Sulfotransferase Activity Incorporation of [^{35}S] sulfate (CPM $\times$ 10^{-3}) into the acceptor catalized by 1 mg of protein / 2h.		
		Subfractions	
	AAL Non-binding	*AAL Fucose Eluate*	*AAL NaCl Eluate*
Colo 205:			
Galβ1,3GalNAcα-O-A1	10.3	149.3	13.3
Galβ1,4GlcNAcβ-O-A1	1.0	11.4	11.1
3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn	0.2	1.5	5.5
Galβ1,4GlcNAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn	0.5	8.4	26.1
Human breast cancer metastases:			
Galβ1,3GalNAcα-O-A1	33.9	4.2	13.5
Galβ1,4GlcNAcβ-O-A1	0.9	5.0	6.2
3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn	37.8	31.7	35.8
Galβ1,4GlcNAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn	6.5	64.7	59.0
Human ovarian tumors:			
Galβ1,3GalNAcα-O-A1	4.1	67.7	36.1
3-O-MeGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-O-Bn	4.5	54.5	35.7
Galβ1,4GlcNAcβ1,6(3-O-MeGalβ1,3)GalNAcα-O-Bn	5.8	107.6	63.2
GlcNAcβ1,6Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn	1.0	7.1	3.5
GlcNAcβ-O-A1/AA-CP (100μg)	0	3.8	1.2

decreased steadily reaching 35% of the initial level at 50 mM Mn^{2+}.

Discussion

Several noteworthy findings on glycan:sulfotransferases have emerged from the present study: i) The colon cancer cell lines LS180 and SW1116, which synthesize copious amounts of carcinoembryonic antigen, which is known to contain several complex type LacNAc type 2 chains [25], show a very high level of Gal : 3-O-sulfotransferase activity towards the Galβ1,4 GlcNAc β-structure; ii) The specificity of Gal : 3-O-sulfotransferase activity in LS180 is directed towards LacNAc type 2 structures, as illustrated in Fig. 1, and the efficiency of this activity is increased by a C-3 block in the Gal residue of the T-hapten of mucin core 2 (see Table 2; activities:75.1 versus 111.8). iii) When a C-3 block is on the Gal moiety of LacNAc of mucin core 2, there is some extent of activity on the other Gal moiety (Table 2; activity 10.6); iv) A stimulation of the minor activity of LS180 on the Gal of T-hapten depends on the substitution of the β1,6 linked GlcNAc moiety with β1,4 Gal (Table 2; activities: 10.6 versus 4.3); v) Consistent with the pH profile of LS180, we observed earlier [15] that the Gal : 3-O-sulfotransferase of colon tissues exhibited an optimal activity at pH 7.2 and an almost identical pH-dependent activity profile; vi) LS 180, as well as the other tumor cell lines of this study contain very little GlcNAc : sulfotransferase activity (see the last column of Table 1, negligible activity towards the last four acceptors in Table 2); vii) The highly tumorigenic breast cell line MDA-435/LCC6 and its parent non-tumorigenic line MDA-MB-435S express respectively high and low levels of Gal : 3-O-sulfotransferase activity specific for T-hapten (see Table 1; activities 62.6 versus 7.2). The drug resistant tumorigenic line MDA-435/LCC6^{MDR1} possessed an intermediate level of this activity (see Table 1: activity 28.1); viii) There are two independent glycan:sulfotransferase activities in calf lymph nodes, one being directed towards the β1,3-linked Gal and the other towards the β1,6-linked GlcNAc of mucin core 2. Interestingly, they differ widely in pH-dependence and Mn^{2+} - influence.

Soluble mucins secreted by colon cancer cells gain access to the blood stream in the setting of invasive and/or metastatic epithelial tumors [26–29]. The determinant structures of GlyCAM-1,3′-sialyl, 6′-sulfo Lewis x and 3′-sialyl, 6-sulfo Lewis x are located in the β1,6 branch of mucin core 2 structure [30–32]. Soluble mucins secreted by human colon carcinoma cell line LS180 were found to bind to human L-selectin [33]. It is evident from the present investigation that LS180 has a high level of Gal : 3.0 - sulfotransferase activity towards the Gal moiety in Galβ1,4GlcNAc of mucin Core 2. But, we could not detect in LS180 6-O-sulfotransferase activities acting on Gal and GlcNAc moieties. It has been reported that C-6 sulfation of terminal Gal

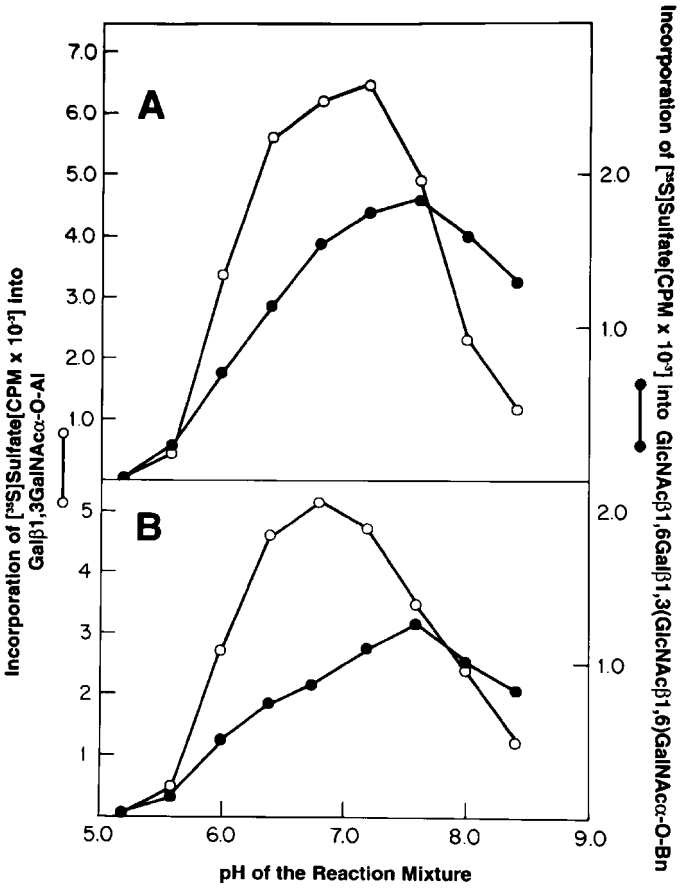

Figure 4. Influence of pH on calf lymph node Gal: and GlcNAc: sulfotransferase activities. A. Calf axial lymph node enzyme activities. ○—○—○ Galβ1,3GalNAcα-O-Al as acceptor; ●—●—● GlcNAcβ1,6 Galβ1,3 (GlcNAcβ1,6)GalNAcα-O-Bn as acceptor B. Calf mesenteric lymph node enzyme activities; [Symbols and descriptions as in A].

prevents α1,3/4-L-fucosylation of GlcNAc and α2,3 sialylation of Gal moieties in Galβ1,3/4GlcNAc [22,34]. As the sequence of events in the assembly of 3′-sialyl, 6′-sulfo Lewis x determinant appears to be in the following order - sialylation, fucosylation and sulfation, the existence of a specific 6-O-sulfotransferase acting on the structure 3′-sialyl Lewis x in LS 180 needs to be explored. Further, the structure of the L-selectin binding ligand of LS180 mucin may be more complex than the anticipated known structure of GlyCAM-1.

Brockhausen *et al.* [35] found Group A Gal: 3-O-sulfotransferase activity in the breast cell lines BT20, MCF-7 and T47D at a low level. The breast cell lines MDA-MB-231, MDA-MB-435S, MDA-435/LCC6 and MDA-435/LCC6MDR$_1$, also express Group A Gal: 3-O-sulfotransferase activity. But a vast difference has been noticed in the levels of this sulfotransferase activity between the highly tumorigenic MDA-435/LCC6 and its parent non-tumorigenic MDA-MD-435S. Our earlier observations on several breast and colon specimens [15] is consistent with our present identification of Group A Gal : 3-O-sulfotransferase activity in breast cell lines (MDA-MB-231, MDA-MB-435S, MDA-435/LCC6 and MDA-435/LCC6 MDRI) and Group B in all colon tumor cell lines. Apart from these, we also observed in the present study that all the breast tumor specimens also contained some GlcNAc : 6-O-sulfotransferase activity.

The present study has demonstrated a specific binding of the Gal : 3-O-sulfotransferase activity to AAL-agarose column by eluting the activity with fucose, using Colo 205, breast metastatic tumor and ovarian tumor as the enzyme sources. AAL binds carbohydrate structures with a fucose residue α1,6-linked to the chitobiose core [36,37]. Yazawa *et al.* [37] demonstrated the ability of immobilized AAL in their isolation of tumor-associated antigens. Mir-Shekari *et al.* [38] used agarose bound AAL., which is commercially available, in the isolation of fucosylated carbohydrate chains from influenza A virus. During the course of our investigation on the binding specificities of AAL and *Lens culinaris* lectin with various modified [^{14}C] fucosyl glycopeptides, we established that a tight binding of glycopeptide to AAL-agarose column is dependent on the presence of an α1,6-L-fucosyl residue in the chitobiose core [39]. Hence, it is likely that human Gal : 3-O-sulfotransferases bear N-linked carbohydrate chains containing the inner core α1,6-L-fucosyl chitobiose. In this context, it is noteworthy that Srikrishna *et al.* [40] have recently demonstrated that core fucosylation of N-linked oligosaccharides [GlcNAcβ1,4(Fucα1,6)GlcNAcβ1-Asn] is a common modification in animal glycans.

Capon *et al.* [41] have reported that the oligosaccharide chains of the mucin synthesized by LS174T-HM7, a highly metastatic subline of colon carcinoma LS174T, constitute predominantly the sulfated Lewis x determinant; some chains contained up to three such determinants. We find in this study that LS180 from which LS174T was derived contains a very high level of Gal : 3-O-sulfotransferase activity towards Galβ1,4GlcNAcβ-. CEA is produced at high level by LS180 and SW1116; the tetra- and triantennary chains of this tumor glycoprotein may carry 3′-sulfo Lewis x determinants. We have also shown earlier several fold elevation of Group B Gal : 3-O-sulfotransferase activity in cancerous colon as compared to the normal colon tissue [15]. Thus, the study of glycan:sulfotransferases is emerging as an interesting and important issue in disease processes.

Though we could not detect GlcNAc : 6-O-sulfotransferase activity in LS180 which produces the mucin ligand for L-selectin [33], we found this activity in calf lymph nodes at a significant level. The identification of such activity in lymph nodes is quite consistent with the occurrence of the 6-O-sulfoGlcNAc moiety in GlyCAM-1 [30,42], which is the L-selectin ligand present in lymph nodes. Consistent with our present observation, Bowman et al. [43] found a restricted expression of GlcNAc-6-O-sulfotransferase activity in porcine lymph node high endothelial venules. Very re-

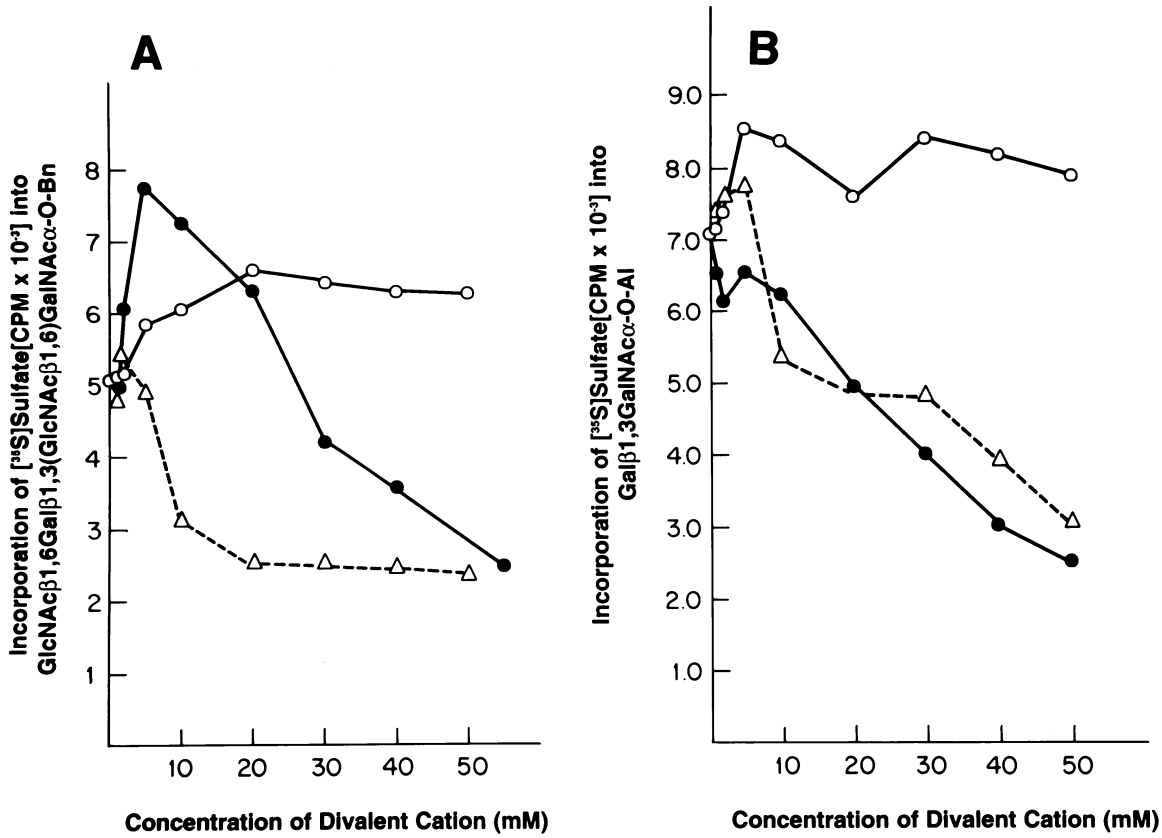

Figure 5. Effect of divalent metal ions on the sulfotransferase activities of calf mesenteric lymph nodes. A. Calf lymph node GlcNAc: sulfotransferase activity. Acceptor: GlcNAcβ1,6Galβ1,3(GlcNAcβ1,6)GalNAcα-O-Bn. ○—○—○ Mg^{2+}; ●—●—● Mn^{2+}; △---△---△ Ca^{2+}. B. Calf lymph node Gal: 3-O-sulfotransferase activity. Acceptor: Galβ1,3GalNAcα-O-Al. [Symbols and description as in A]

cently Hiraoka et al.[44] reported the molecular cloning and characterization of a murine cDNA encoding a sulfotransferase that directs the formation of 3′-sialyl, 6-sulfo Lewis^x on mucin-type O-glycans. Unlike LS180, calf lymph nodes also contained Group A instead of Group B Gal: 3-O-sulfotransferase activity. Recently, Habuchi et al. [45] reported that chondroitin-6-sulfotransferase catalyzed C-6 sulfation of the Gal moiety in NeuAcα2,3Galβ1,4 GlcNAc, but failed to act on NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAc. Spiro and Bhoyroo [46] also reported that 3′-sialyl Lewis x was inert as an acceptor for rat spleen Gal: 6-O-sulfotransferase. Habuchi *et al.,* [45] suggested that their enzyme may be involved in the biosynthesis of 3′-sialyl, 6′-sulfo Lewis x. Based on the failure of their enzyme to utilize 3′-sialyl Lewis x as an acceptor and the probable sequence in the assembly of 3′-sialyl, 6′-sulfo Lewis x appearing to be sialylation, fucosylation and sulfation [22,34], then the involvement of chondroitin-6-sulfotransferase in this biosynthesis is questionable.

The GlcNAc : 6-O-sulfotransferase activity of bronchial mucosa was reported to have an optimal pH (6.7) higher than that of Gal : 3-O-sulfotransferase activity (pH 6.1) from the same source [47]. Cystic fibrosis (CF) mucins were shown to contain predominantly 6-O-sulfoGlcNAc in their carbohydrate chains, whereas, non-CF mucins were more 3-O-sulfated on Gal [48,49]. In CF cells, the Golgi pH was found to be higher than in normal cells [50,51]. This could result in the hyperactivity of GlcNAc : 6-O-sulfotransferase leading to the predominant 6-O-sulfation of the GlcNAc moiety in CF mucins [47]. Interestingly, we do also find in calf lymph nodes that the pH optimum as 7.0 for Group A Gal : 3-O-sulfotransferase activity and 7.6 for GlcNAc : 6-O-sulfotransferase activity. At pH 8.4, the former displayed only 15% activity while the latter retained ~65% of its activity. The biological implication of such pH dependent differences between these enzymes remains unclear at present.

In contrast to the rat liver GlcNAc : 6-O-sulfotransferase reported by Spiro *et al.* [52], GlcNAcβ1,6Manα-O-Al did not serve as an acceptor for the calf lymph node enzyme. However, the macromolecular compound GlcNAcβ-O-Al/AA-CP acted as a good acceptor for the calf lymph node enzyme but we found it to be a very poor acceptor for GlcNAc: 6-O-sulfotransferase activity of the breast tumor. The biological significance of these findings remains to be seen.

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