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Isolation and characterization from porcine serum of a soluble sulfotransferase responsible for 6-O-sulfation of the galactose residue in 2′-fucosyllactose: Implications in the synthesis of the ligand for L-selectin

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A soluble sulfotransferase from porcine serum which catalyzes the transfer of sulfate from adenosine 3′-phosphate 5′-phosphosulphate (PAPS) to 2′-fucosyllactose (2′-FL) was purified 36,333-fold using a combination of conventional and affinity chromatographic steps. The purified enzyme preparation after non-denaturing discontinuous-PAGE exhibited a molecular mass of about 80 kDa by reducing SDS-PAGE. However, when a partially purified enzyme preparation was subjected to gel filtration on Sephacryl S-300, the enzyme activity eluted in the void volume, which indicated that the native enzyme existed as an oligomer. The purified enzyme showed K_m values of 9.15 μM for PAPS and 15.38 mM for 2′-FL at the optimum pH value of 7.4. The substrate specificity of the purified enzyme was evaluated with various sugars that are structurally similar to sialyl Lewis^x (sLe^x). Results indicated that 3′-sialyllactose and lactose were efficient acceptors of sulfation, whereas 6′-sialyllactose and 6′-sialyllactosamine were poor substrates for this sulfotransferase. Further, the reaction product analysis revealed that the sulfate substitution, when using 2′-FL as the substrate, was at the C-6 position of the galactose residue. Coincidentally, a similar enzyme activity was also found in porcine lymphoid tissues such as, lymph nodes (peripheral and mesenteric) and spleen. Collectively, these findings suggest that this enzyme might be involved in the synthesis of the ligand for L-selectin.

Keywords: **L-selectin, sialyl Lewis^x, sulfation, sulfotransferase and inflammation**

Abbreviations: **2′-FL, 2′-fucosyllactose; PAPS, adenosine 3′-phosphate 5′-phosphosulphate; PLP, pyridoxal 5′ phosphate; DTT, dithiothreitol; SDS, sodium dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis; HP-TLC, high performance-thin layer chromatography and Galactose-6-O sulfotransferase, Gal-6-O-Stase**

Introduction

Leukocyte adhesion to activated endothelium is a key initial event in the trafficking and recruitment of circulating leukocytes into lymphoid tissues, and at sites of inflammation [1]. The first step in the recruitment process is the interaction between circulating leukocytes and endothelial cells lining the blood vessels [2]. L-selectin is a constitutively expressed cell adhesion molecule on all classes of circulating leukocytes, which participates in leukocytes binding to their cognate ligands on the endothelial cells [3]. Recent studies indicate that a sulfated, sialylated and fucosylated oligosaccharide(s) is a component of the ligand for L-selectin and that the binding of L-selectin to its ligand can be abolished by pretreatment of high endothelial venules with neuraminidase [4–7]. The high-affinity binding of selectins to their ligands is facilitated when carbohydrate epitopes are arranged as clusters on the protein backbone of specific glycoproteins [8–10]. GlyCAM-1 and the 120 kDa glycoform of CD-34 have been identified as two natural glycoprotein ligands for L-selectin [8–10]. Whereas GlyCAM-1 is a secreted protein, the glycoform of the CD-34 is a membrane bound protein on the endothelial cell surface [11–12].

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Both GlyCAM-1 and CD-34 likely function as ligands through the presentation of highly clustered *O*-linked chains to L-selectin on the leukocyte cell surface [10]. Sugar structural analysis revealed that GlyCAM-1 contains sulfate on both C-6 of galactose (6′-sulfo sialyl Le^x) and C-6 of N-acetylglucosamine (6-sulfo sialyl Le^x) [13–15].

We have previously reported the identification of peripheral addressin (PNAd), a 120 kDa glycoform of CD-34 [16], as the membrane bound ligand for L-selectin in porcine lymph nodes (PLN) and demonstrated that the sulfation and sialylation of this ligand was critical for its binding to L-selectin [16–18]. In addition, we also showed that the binding between PNAd and L-selectin was inhibited by 6-sulfo sialyl Le^x and other highly sulfated molecules such as fucoidin, heparin and hepta-sulfated β-cyclodextrin [16–18]. Collectively, these findings suggested that the clusters of sulfated sLe^x on the PNAd may be the critical determinants for its binding to L-selectin. The complete synthesis of a sulfated derivative of sLe^X from the precursor N-acetyllactosamine requires actions of a sialyltransferase, a fucosyltransferase and a sulfotransferase. Although the isolation and characterization of sialyltransferases, fucosyltransferases [19–22], and a number of different sugar-specific sulfotransferases have been identified [23–27], little is known about sulfotransferases specific for sulfation at the C-6 position of the galactose. Chondroitin 6-sulfotransferase has been shown to catalyze the transfer of sulfate not only to the C-6 position of GalNAc residues of chondroitin but also to the C-6 position of galactose residues in keratan sulfate [22,25]. In addition, a sulfotransferase activity was identified in spleen that catalyzed the sulfation at the C-6 position of galactose residues of a number of sugars [29]. We have recently reported the purification of a membrane-bound sulfotransferase responsible for sulfation at the C-6 position of galactose residues in sugars structurally similar to sLe^X [30]. We describe here the isolation and characterization of a soluble sulfotransferase from porcine serum which specifically transfers sulfate from PAPS to the C-6 position of galactose residues.

Materials and methods

Materials

Porcine serum was obtained from Pel-Freeze Biologicals. Dowex AG 1 × 8 resin and Poly Prep columns for enzyme assay were from Bio-Rad Laboratories. ADP-agarose was purchased from Sigma Chemicals. Acceptor sugars (lactose, 2′-fucosyllactose, 2,3′-sialyllactose, lactodifucotetraose, 2′,6′-sialyllactosamine, 2′,6′-sialyllactose) were purchased from Sigma Chemicals or Vector Labs. 2′-Fucosyllactose, lactose and 2′,3′-sialyllactose were also isolated directly from human milk as described by Kobata [29] and Scudder *et al.* [17]. Purity of the isolated oligosaccharides was determined by FAB mass spectrometry. Pyridoxal 5′-phosphate agarose columns were prepared following the method as described by Churchich [31]. DEAE-Sepharose, Sephacryl S-300, heparin-agarose, WGA-agarose and PD-10 columns were from Pharmacia LKB Biotechnology. Centriprep-10 concentrators with a molecular weight cutoff of 10 kDa were from Amicon. [^{35}S] PAPS (1.6 Ci/mmol) Adenosine 3′-phosphate 5′-phosphosulphate was from New England Nuclear. The other reagents used were of the highest grade commercially available. Unless otherwise indicated, all buffers used were 20 mM Hepes buffer, pH 7.4 containing 1 mM DTT (Buffer A).

Enzyme assay

Enzyme activity was measured using an ion-exchange methodology. The reaction mixture contained 20 mM 2′-FL, 40 μM [^{35}S] PAPS (about 1×10^6 cpm), 80 μM PAPS, 2 mM ATP, 10 mM NaF, 10 mM $MgCl_2$, 1 mM DTT, 100 mM Hepes buffer, pH. 7.4 and enzyme solution (1–5 μl) in a final volume of 20 μl. The reaction mixture was incubated at 25 °C for 1 hr and the reaction was stopped by addition of 0.98 ml of 10 mM ammonium bicarbonate buffer, pH 8.0. The reaction mixture was applied to a small Dowex AG 1 × 8 column (0.75 ml, Poly Prep column) previously equilibrated with the above buffer. The sulfated product was eluted from the column with 10 ml of 100 mM ammonium bicarbonate buffer, pH 8.0, mixed with an equal volume of Ultima Gold XR scintillation cocktail and its radioactivity was counted. One unit of enzyme activity was defined as that amount required to catalyze the transfer of 1 pmole of sulfate per min. Specific activity was expressed as units per mg of protein.

Purification procedures

All purification steps described below were performed at 4 °C unless otherwise noted.

DEAE-sepharose

Porcine serum (2 liters) was filtered through Whatman #1 paper and incubated for 1 hr with 1 liter of DEAE-sepharose which was equilibrated with Buffer A. The DEAE-sepharose was then washed with 4 liters of the Buffer A and with 2 liters of Buffer A containing 150 mM KCl followed by 1 liter of Buffer A containing 300 mM KCl. Sulfotransferase activity was found in the last wash.

Sephacryl S-300

Solid ammonium sulfate was added to the active fraction from the DEAE-sepharose step to 25% saturation. After stirring for 1 hr, the suspension was centrifuged and solid ammonium sulfate was then added to the supernatant to 60% saturation. After stirring overnight, the precipitate was collected by centrifugation of the suspension for 1 hr at 30,000 × g, dissolved in 20 ml of Buffer A and applied to a Sephacryl S-300 column (5 × 100 cm) equilibrated with

Buffer A containing 50 mM KCl and eluted with the same buffer.

Heparin-agarose

Active fractions were collected and incubated with 100 ml of Heparin-agarose in Buffer A for 2 hrs with constant stirring. The Heparin-agarose was washed with 2 liters of Buffer A and with 500 ml of Buffer A containing 150 mM KCl. The sulfotransferase was released from the Heparin-agarose by adding 500 ml of Buffer A containing 1 M KCl and stirring the mixture for 2 hrs followed by centrifugation. The supernatant, which contained sulfotransferase, was concentrated to 25 ml by Centriprep-10 concentrators and desalted using PD-10 columns.

ADP-agarose column

The enzyme solution was then applied to an ADP-agarose column (0.9 × 15 cm) equilibrated with Buffer A. Sulfotransferase was eluted from the column using a linear gradient of 0 to 0.2 mM PAPS in Buffer A. Active fractions were collected, concentrated to 4 ml and adjusted to a final concentration of 1 M KCl by the addition of 4 ml of 2 M KCl in Buffer A. After incubation for 8 hrs, the enzyme solution was applied to PD-10 columns to remove any remaining PAPS.

PLP-agarose column

The desalted enzyme solution was applied to a PLP-agarose column (0.9 × 15 cm) equilibrated with Buffer A. After washing with 100 ml with the same buffer, the sulfotransferase was eluted with the above buffer containing 1 mM PLP. Active fractions were collected, concentrated to 5 ml and desalted.

WGA-agarose column

The enzyme was then applied to a WGA-agarose column (0.9 × 15 cm) equilibrated with Buffer A. After washing with 100 ml of Buffer A, the enzyme was eluted from the column with Buffer A containing 0.5 M N-acetyl glucosamine. Active fractions were collected and desalted.

Non-denaturing discontinuous PAGE

The purified enzyme was then subjected to non-denaturing discontinuous polyacrylamide gel electrophoresis for 16 hrs at 3 mA per gel in 4 °C. The gel was then manually sliced into (1 mm) sections and the activity of sulfotransferase in each section was determined by incubating these sections with 100 µl of reaction mixture. Although the enzyme was very unstable under these conditions, about 5% of the initial enzyme activity was recovered in three gel slices, # 3–5, with the highest activity located in gel slice # 4 (Fig. 2). SDS-PAGE with silver staining of gel slice # 4 showed a protein band with a molecular weight of about 80 kDa (Fig. 2, *Inset*). However, we were unable to detect this protein band from gel slices # 3 and 5 (data not shown). The extremely low levels of the enzyme activity in these gel slices suggest that the protein content is beyond the detection limit of SDS-PAGE followed by the silver staining.

Substrate specificity

Purified enzyme was incubated with 4 mM oligosaccharides for 16 hrs at 25 °C in 20 mM Hepes buffer, pH 7.4 containing 40 µM [^{35}S] PAPS, 80 µM PAPS, 1 mM DTT, 10 mM NaF, 1mM ATP, 10% glycerol and 10 mM $MgCl_2$. A 2 µl aliquot from each sample was spotted onto a 10 × 10 cm HP-TLC plate (Merck 5633) and developed in ethanol:*n*-butanol:pyridine:acetic acid:H_2O = 100:10:10:3:30. Sulfon-

Table 1. Purification of sulfotransferase from 2 liters of porcine serum.

Step	*Volume (ml)*	*Total activity*[a] *(units)*	*Total protein*[b] *(mg)*	*Specific activity*[a] *(units/mg)*	*Fold*	*Yield (%)*
Porcine serum	2000	32280.0	117800.0	0.27	1.0	100.0
DEAE-sepharose	1600	29052.0	7536.0	3.85	14.2	90.0
Sephacryl S-300	350	23564.4	3794.0	6.21	23.0	72.9
Heparin-agarose	300	22385.8	155.4	144.0	533.5	69.3
ADP-agarose	50	5596.2	1.2	4663.5	17272.2	17.3
PLP-agarose	10	1175.2	0.14	8374.0	31014.8	3.6
WGA-agarose	7	196.2	0.02[c]	9810.0	36333.3	0.6
Electrophoresis[d]	0.4		0.001[c]			

[a]One unit of enzyme activity was defined as the amount required to catalyze the transfer of 1 pmole of sulfate per min. Specific activity was expressed as units per mg of protein.
[b]Protein concentration was determined by the Bradford method using bovine serum albumin as the standard.
[c]Protein concentration was estimated by SDS-PAGE with silver staining using bovine serum albumin as the standard.
[d]Since the enzyme activity was significantly decreased after the non-denaturing discontinuous PAGE step, its specific activity, fold of purification and yield were not included.

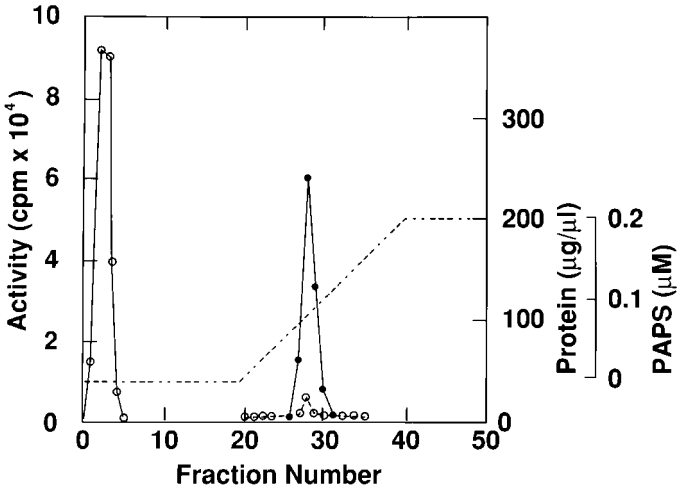

Figure 1. Purification of sulfotransferase by ADP-agarose affinity chromatography. The active fractions from the Heparin-agarose step were combined and applied to an ADP column (0.9 × 15 cm) which was previously equilibrated with Buffer A. Sulfotransferase was eluted from the column using a linear gradient of 0 to 0.2 mM PAPS in Buffer A (—). The active fractions (•) were concentrated and desalted using a Pharmacia PD-10 columns as described. Protein concentration (❍) was determined by the Bradford method [32].

ated oligosaccharides were detected by autoradiography. R_f values for the oligosaccharide acceptors were detected by orcinol:sulfuric acid staining.

Reaction product analysis

Enzyme (100 µl) was incubated with 10 mM 2′-FL, 40 µM [^{35}S] PAPS, 80 µM PAPS, 2 mM ATP, 10 mM NaF, 10 mM $MgCl_2$, 1 mM DTT, 100 mM Hepes buffer, pH. 7.4 in a final volume of 500 µl. The reaction mixture was incubated at 25 °C for 6 hrs and the reaction was stopped by the addition of 1.95 ml of 10 mM ammonium bicarbonate buffer, pH 8.0. The reaction mixture was applied to a small Dowex AG 1 × 8 column (1.5 ml, Poly Prep column) previously equilibrated with the above buffer. The sulfated product was eluted from the column with 10 ml of 100 mM ammonium bicarbonate buffer, pH 8.0, and the eluate was lyophilized. The lyophilized powder was dissolved in 2 ml of water and lyophilized again. Two cycles of lyophilization removed most of the ammonium bicarbonate from the reaction product. The reaction product was dissolved in 100 µl of water. Purity and authenticity of reaction product was checked by HP-TLC.

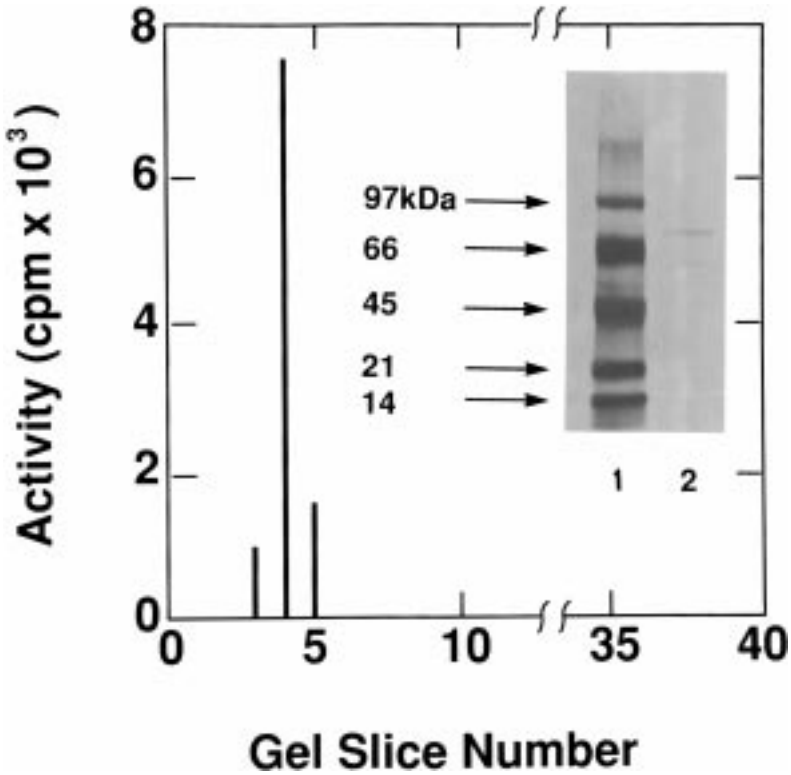

Figure 2. Activity of sulfotransferase obtained from gel slices of non-denaturing discontinuous polyacrylamide gel electrophoresis. Sulfotransferase from the WGA-agarose affinity column was applied to a gel tube (130 × 5 mm ID glass tubes). Electrophoresis was run for 16 hrs at 4 °C using a current of 3 mA per tube [32]. The gel was then manually sliced into (1 mm) sections and the enzyme activity from each section was determined by incubating it with 100 µl of reaction mixture overnight followed by performing a sulfotransferase assay. *Inset:* SDS-PAGE of gel slice #4 obtained from nondenaturing polyacrylamide gel electrophoresis: *lane 1,* molecular weight standards (from top: rabbit skeletal muscle phosphorylase b, 97 kDa; bovine serum albumin, 66 kDa; hen egg ovalbumin, 45 kDa; Carbonic anhydrase, 31 kDa; Trypsin inhibitor, 21 kDa and Lysozyme, 14 kDa) and *lane 2,* gel slice #4 obtained from gel slices derived from nondenaturing polyacrylamide gel electrophoresis.

Other methods

Protein concentrations were determined by the method of Bradford [32] using bovine serum albumin as the standard. Measures of purity and a molecular weight determination of the isolated sulfotransferase were made by SDS-PAGE with silver staining [33]. Non-denaturing discontinuous polyacrylamide gel electrophoresis was performed essentially as described [34].

Results and discussion

A soluble sulfotransferase from porcine serum which catalyzes the transfer of sulfate from PAPS to C-6 position of galactose was purified 36,333-fold (Table 1) to an apparent homogeneity. The purification procedure consisted of a combination of column chromatographies on DEAE-sepharose, Sephacryl S-300, Heparin-sepharose, ADP-agarose (Fig. 1), PLP-agarose, WGA-agarose and non-denaturing discontinuous PAGE (Fig. 2). The purified enzyme showed a molecular mass of about 80 kDa on SDS-PAGE with silver staining (Fig. 2, *Inset*). However, when the partially purified enzyme was subjected to gel filtration on Sephacryl S-300 column, the enzyme activity eluted in the void volume (data not shown), suggesting that the enzyme might exist as an oligomer in its native state. The pH dependence of the purified enzyme was investigated at pH from 4.0 to 9.0. Similar to other sulfotransferases [23–24], the purified enzyme exhibited a pH optimum of 7.4. The purified enzyme was stable at −70 °C for at least six months. At 4 °C, the enzyme was stable for two weeks, however, over 90% of the enzyme activity was lost after incubation for three days at room temperature (data not shown). K_m values for the substrates 2′-FL and PAPS were calculated to be about 15.4 mM and 9.2 μM, respectively (Fig. 3).

A number of sugars that are structurally similar to sLe^X were used to define the substrate specificity of the enzyme. These sugars were incubated with the enzyme preparation after the ADP-agarose step and the reaction products were analyzed by HP-TLC (Fig. 4). While 2′,3′-sialyllactose (*lanes 1, 5 and 6*) and lactose (*lanes 7 and 8*) were found to be good substrates, the enzyme was completely inactive towards 2′,6′-sialyllactosamine (*lane 3*) and 2′,6′-sialyllactose (*lane 4*). These results suggest that the enzyme is unable to transfer the sulfate group if the C-6 position of the galactose residue is already occupied. To further confirm this observation, the reaction product, from a scaled-up reaction with the purified enzyme and 2′-FL as substrate, was isolated and purified (see Materials and Methods). As shown in Figure 5, the R_f value of the reaction product (*lanes 2 & 3*) was identical to that of an authentic sulfated derivative of [Fuc(α1,2)Gal-6-0-$S0_3$-(β1,4)Glc] (*lanes 4 & 5*). This sulfated oligosaccharide was produced by incubating 2′-FL with PAPS in the presence of a purified porcine

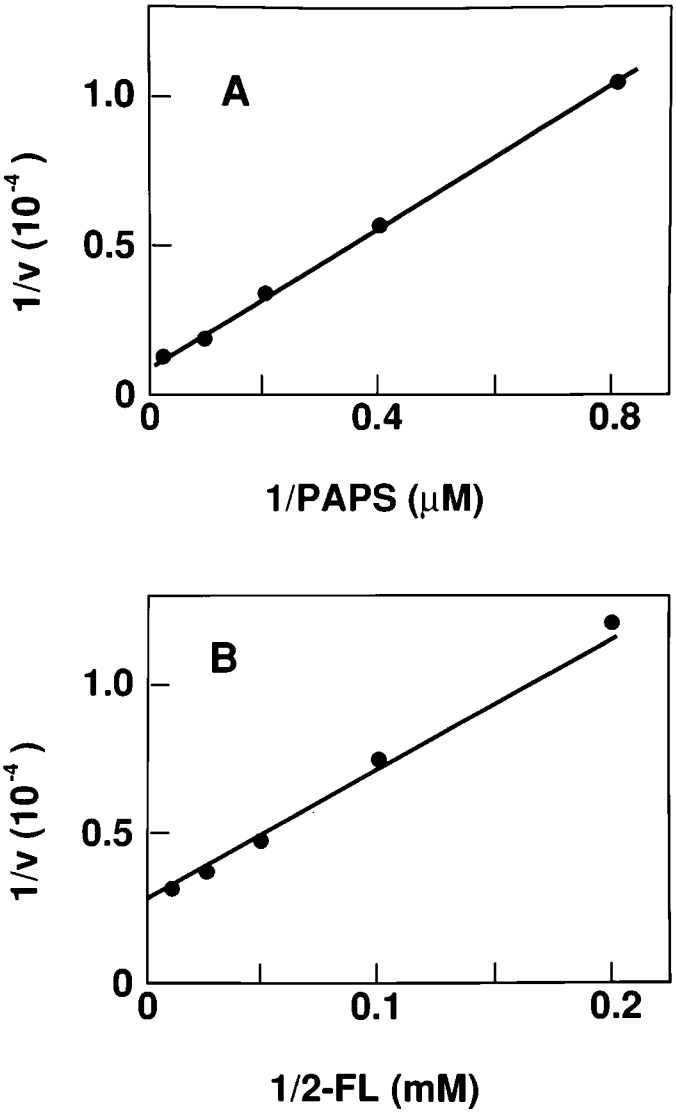

Figure 3. Effect of various concentrations of PAPS (A) and 2′-FL (B) on sulfotransferase activity. The sulfotransferase activity was measured with increasing concentrations of (A) PAPS and (B) 2″-FL by using the procedure as described in "Materials and Methods."

lymph nodes Gal-6-O-Stase [30]. The structure analysis of this sulfated-oligosaccharide by the FAB mass spectrophotometry indicated that the sulphate substitution was at the C-6 position of the galactose residue [Detail of enzyme assay and structure analysis of sulfated oligosaccharides by FAB mass spectrometry will be published elsewhere (K. O. Broschat, C. C. Gorka, A. Dell, G. S. Jacob *et al.,* Manuscript in preparation).] Furthermore, when the reaction product was mixed with the authentic Fuc(α1,2) Gal-6-0-$S0_3$-(β1,4)Glc, the mixture showed only one radioactive band (*lane 1*), confirming that the reaction product is identical to Fuc(α1,2)Gal-6-O-sulphate(β1,4) Glc. These findings suggest that the purified serum sulfotransferase is

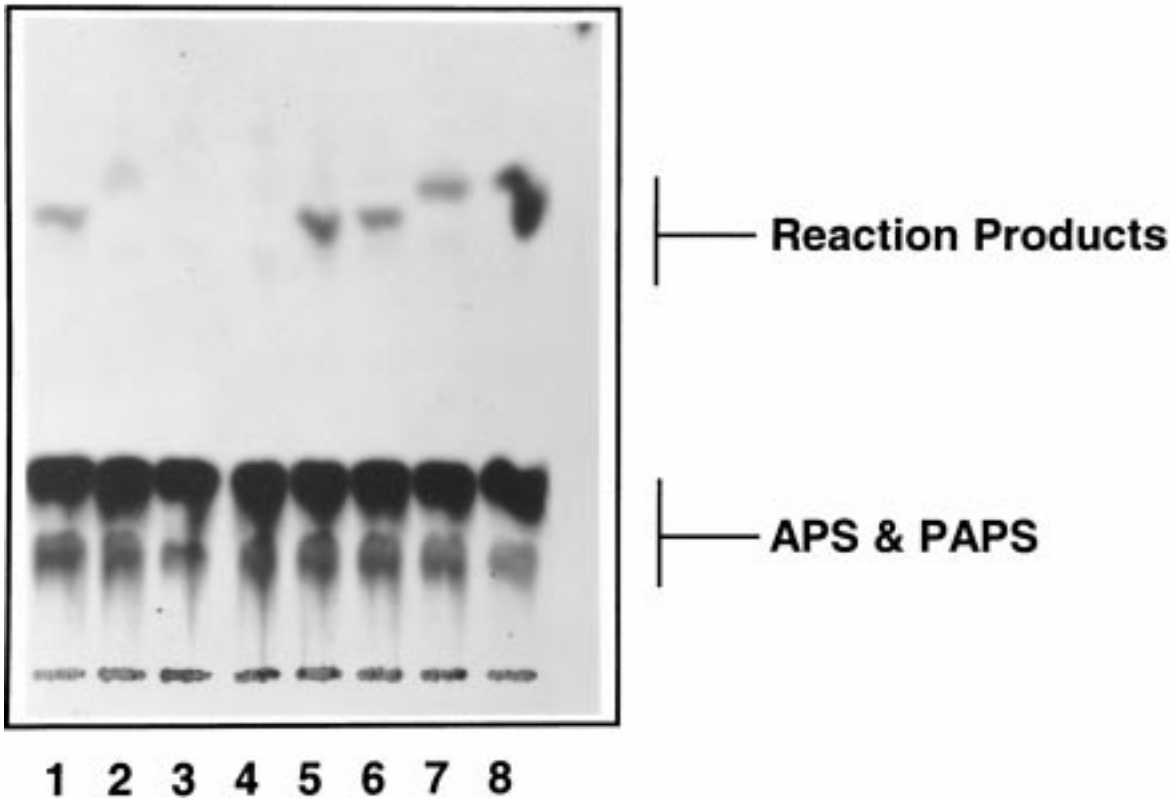

Figure 4. Substrate specificity of the purified sulfotransferase from porcine serum. Purified enzyme was incubated with 4 mM oligosaccharides for 16 hrs at 25 °C in the standard reaction mixture. Two μl aliquot from each sample was spotted onto a HP-TLC plate and developed. *Lane 1,* 2′,3′-sialyllactose; *lane 2,* Lactodifucotetraose; *lane 3,* 2′,6′-sialyllactosamine, *lane 4,* 2′,6′-sialyllactose, *lane 5,* 2′,3′-sialyllactose (purified from human milk); *lane 6,* 2,3′-sialyllactose (from Sigma Chemicals), *lane 7,* 20 mM lactose (purified from human milk) and *lane 8,* 100 mM lactose. Sulfonated reaction products, residual [^{35}S] PAPS and its degradation product [^{35}S] APS (Adenosine-5′-phosphosulfate) are indicated on the figure.

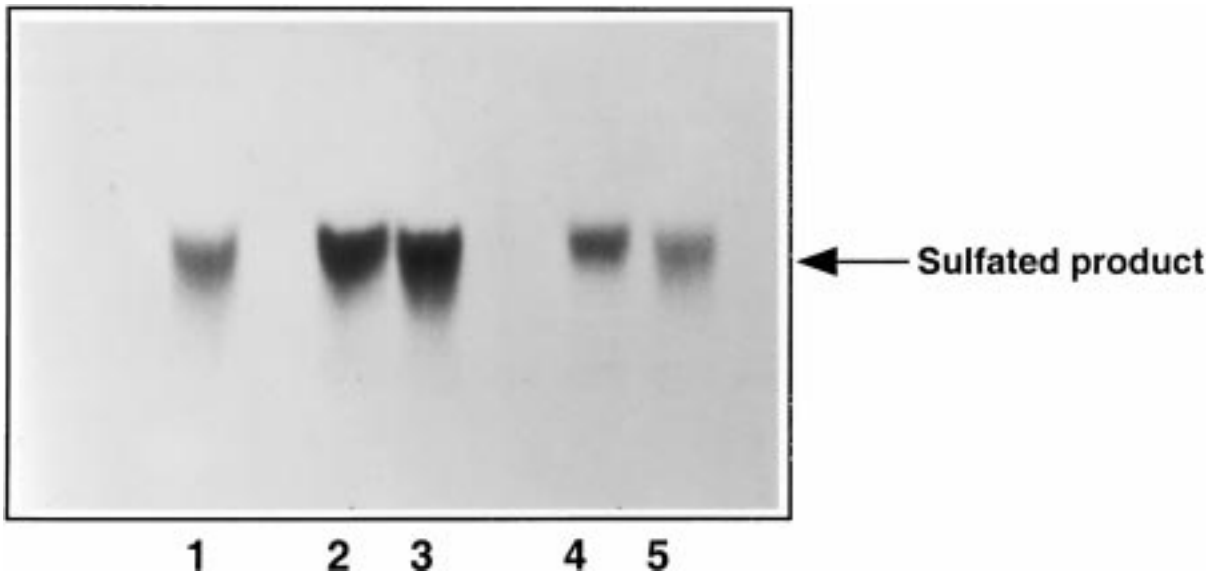

Figure 5. Validation of the reaction product as the Fuc(α1,2)Gal-6-0-sulfate(β1,4)Glc. Purified sulfotransferase from serum was incubated with 10 mM 2′-FL and 80 μM [^{35}S] PAPS in a scaled-up assay mixture and the reaction product was purified (see Materials and Methods). The purified product and Fuc(α1,2)Gal-6-0-sulfate(β1,4)Glc were compared for their migration patterns by HP-TLC. *Lane 1,* mixture of the reaction product (500 cpm) and the oligosaccharide Fuc(α1,2)Gal-6-0-sulfate(β1,4)Glc (500 cpm); *lanes 2 and 3,* 2 μl of the reaction product from serum sulfotransferase; *lanes 4 and 5,* 2,000 and 1,000 cpm of the standard oligosaccharide Fuc(α1,2)Gal-6-0-sulfate(β1,4)Glc.

specific for the sulfation at the C-6 position of the galactose residue.

The serum sulfotransferase exhibited many properties that were similar to what we found with the membrane-bound Gal-6-O-Stase from porcine lymph nodes [30]. For example, both enzymes showed similar K_m values for PAPS and 2′-FL. Like the membrane-bound Gal-6-O-Stase, the serum enzyme was also markedly activated by the treatment with 1% Triton X-100. These observations raise the possibility that Gal-6-O-Stase might exist as a soluble form in the serum and a membrane-bound form in the Golgi apparatus. Several glycosyltransferases have been purified as the soluble form from serum despite their localization in the Golgi [39–40]. For example, chondroitin 6-sulfotransferase is a membrane protein containing a type II transmembrane domain, yet this protein is also secreted in the culture medium of chondrocytes [41]. For now, it remains to be investigated whether these two enzymes are closely related proteins or are two distinct enzymes.

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References

1 Lasky LA (1992) *Science* **258:** 964–69.
2 Bevilacqua MP, Nelson RM (1993) *J Clin Invest* **91:** 379–87.
3 Gallatin WM, Weissman IL, Butcher EC (1983) *Nature* **303:** 30–34.
4 Varki A (1994) *Proc Natl Acad Sci USA* **91:** 7390–97.
5 Rosen SD, Singer MS, Yednock TA, Stoolman LM (1985) *Science* **228:** 1005–07.
6 McEver RP, Moore KL, Cummings RD (1995) *J Biol Chem* **270:** 11025–28.
7 Imai Y, Lasky LA, Rosen SD (1993) *Nature* **361:** 555–57.
8 Baumhueter S, Singer MS, Henzel W, Hemmerich S, Renz M, Rosen SD, Lasky LA (1993) *Science* **262:** 436–38.
9 Briskin MJ, McEvoy LM, Butcher EC (1993) *Nature* **363:** 461–64.
10 Brustein M, Kraal G, Mebius RE, Stults NL (1992) *J Exp Med* **176:** 1415–19.
11 Brown J, Greaves M, Molgaard H (1991) *Int Immunol* **3:** 175–84.
12 Suda J, Sudo T, Ito M, Ohno N, Yamaguchi Y, Suda T (1992) *Blood* **79:** 2288–95.
13 Hemmerich S, Rosen SD (1994) *Biochemistry* **33:** 4830–35.
14 Hemmerich S, Leffler H, Rosen SD (1995) *J Biol Chem* **270:** 12035–47.
15 Hemmerich S, Bertozzi CR, Leffler H, Rosen SD (1994) *Biochemistry* **33:** 4820–29.
16 Shailubhai K, Streeter PR, Smith CE, Jacob GS (1997) *Glycobiology* **7:** 305–14.
17 Scudder PR, Shailubhai KK, Duffin KL, Streeter PR, Jacob GS (1994) *Glycobiology* **4:** 929–33.
18 Shailubhai K, Abbas ZS, Jacob GS (1996) *Biochem Biophys Res Commun* **229:** 488–93.
19 Kitagawa H, Paulson JC (1994) *J Biol Chem* **269:** 1394–401.
20 Sasaki K, Kurata K, Funayama K, Nagata M, Watanabe R, Ohta S, Hanai N, Nishi T (1994) *J Biol Chem* **269:** 14730–37.
21 Natsuka S, Gersten KM, Zenira K, Kannagi R, Lowe JB (1994) *J Biol Chem* **269:** 16789–94.
22 Habuchi H, Habuchi O, Kimata K (1995) *J Biol Chem* **270:** 4172–79.
23 Hooper VL, Hindsgau O, Baenziger JU (1995) *J Biol Chem* **270:** 16327–32.
24 Sakakibara Y, Takami Y, Zwieb C, Nakayama T, Suiko M, Nakajima H, Liu MC (1995) *J Biol Chem* **270:** 30470–78.
25 Habuchi O, Matsui Y, Kotoya Y, Aoyama Y, Yasuda Y, Noda M (1993) *J Biol Chem* **268:** 21968–74.
26 Mitsuoka C, Sawada-Kasugai M, Ando-Furui K, Izawa M, Nakanishi H, Nakamura S, Ishida H, Kiso M, Kannagi R (1998) *J Biol Chem* **273:** 11225–33.
27 Kimura N, Mitsuoka C, Kanamori A, Hiraiwa N, Uchimura K, Muramatsu T, Tamatani T, Kansas G, Kanagi R (1999) *Proc Natl Acad Sci USA* **96:** 4530–35.
28 Spiro RG, Bhoyroo VD (1998) *Biochem J* **331:** 265–71.
29 Kobata A (1972) *Methods Enzymol* **28:** 262–71.
30 Shailubhai K, Huynh QK, Boddupalli H, Yu HH, Jacob GS (1999) *Biochem Biophys Res Commun* **256:** 170–76.
31 Churchich JE (1984) *Eur J Biochem* **38:** 327–32.
32 Bradford MM (1986) *Anal Biochem* **72:** 248–54.
33 Laemmli UK (1970) *Nature* **227:** 680–85.
34 Davis BJ (1964) *Ann NY Acad Sci* **121:** 404–27.
35 Inoue H, Otsu K, Yoneda M, Kimata K, Suzuki S, Nakashiki Y (1986) *J Biol Chem* **261:** 4460–69.
36 Mourao PAS, Kato S, Donnelly PV (1981) *Biochem Biophys Res Commun* **98:** 388–96.
37 Sugahara H, Shibamoto S, Yamashita I (1985) *FEBS Lett* **188:** 43–46.
38 Hart GW (1978) *J Biol Chem* **253:** 347–53.
39 Palace GE (1975) *Science* **189:** 347–58.
40 Horwitz AL, Dorfman A (1968) *J Cell Biol* **38:** 358–68.
41 Young RW (1973) *J Cell Biol* **57:** 175–89.

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