Characterization of a spleen sulphotransferase responsible for the 6-O-sulphation of the galactose residue in sialyl-N-acetyl-lactosamine sequences

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An enzyme which catalyses the transfer of sulphate from 3'-phosphoadenosine 5'-phosphosulphate (PAPS) to C-6 of galactose in the NeuAc2-3Gal/β1-4GlcNAc (3'SLN) sequence has been found in rat spleen microsomes and its specificity indicates that it is well suited to participate in the assembly of 3'-sialyl-6-sulpho-LacNAc [NeuAc2-3Gal(6-SO3)β1-4GlcNAc] and 3'-sialyl-6-sulpho-LewisX [NeuAc2-3Gal(6-SO3)β1-4(Fucβ1-3)GlcNAc] saccharide groups which have been implicated as selectin ligands. This sulphotransferase has a strict requirement for oligosaccharide acceptors which are capped by an α2-3-linked sialic acid residue, although GlcNAc in 3'SLN can be substituted by Glc, and Gal/β1-4GlcNAc can be replaced by Gal/β1-4GlcNAc without loss of activity. The finding that 3'-sialyl LewisX was inert as an acceptor suggested that fucosylation, in contrast with sialylation, follows the addition of the sulphate group. Since fetuin glycopeptides containing the NeuAc2-3Gal/β1-4GlcNAc sequence had a similar affinity for the enzyme as the unattached 3'SLN, it would appear that the acceptor determinants reside primarily in the peripheral trisaccharide constellation. The position of the sulphate on C-6 of galactose was elucidated by Smith periodate oxidation, hydrazine/nitrous acid/NaBH₄ treatment and elder (Sambucus nigra) bark lectin chromatography of the desialylated [35S]sulphate-labelled products of the enzyme. Assays carried out with 3'SLN as acceptor indicated that the sulphotransferase had a pH optimum between 6.5 and 7.0 and a dependence on a bivalent cation best met by Mn²⁺ (12–25 mM); Triton X-100 (0.02 to 0.35 %) brought about maximal stimulation. Tentative Km values determined for this enzyme were 4.7 µM for PAPS, and 0.72 mM and 1.16 mM for 3'SLN and fetuin glycopeptides respectively. A survey of several rat organs indicated that the PAPS:3'SLN-6-O-sulphotransferase is selectively distributed with maximal activity occurring in spleen which was substantially greater than thymus or lymph nodes. In contrast, other enzymes (i.e. PAPS:Gal-β1-3-O-glucosyltransferase, PAPS:Gal-β1-4-O-glucosyltransferase, PAPS:GalNAc-β1-3-O-glucosyltransferase) involved in the sulphation of sialyl-lactosamine and lactosamine sequences, which in the sulphated form are believed to also be selectin ligands, were more evenly distributed in lymphoid tissues. Relatively high activities for all three enzymes were found in brain.

INTRODUCTION

It has now been well established that N- and O-linked oligosaccharides of a diverse assortment of secreted as well as membrane-associated glycoproteins contain sulphate substituents which have been localized to defined positions on Gal [1–4], GalNAc [3,5], GlcNAc [1,2,4,6–11] and Man [12] residues. Studies in various cells have indicated that the addition of these anionic groups occurs as a late, trans-Golgi network-localized event [11,13]. Moreover, a number of specific sulphotransferases have now been characterized, and these include enzymes which attach sulphate to C-3 of Gal [14], C-4 of GalNAc [15] and C-6 of GlcNAc [16].

Although the physiological role of sulphate groups in glycoproteins remains to a large extent undetermined, their presence on the Gal and GlcNAc residues of ligands for selectins appears to provide an intriguing example in which they have been implicated in a major biological process represented by the attachment of leukocytes to endothelial cells (see [17–19] for reviews). A number of binding studies with various oligosaccharides have suggested that sulphate groups may be involved in the carbohydrate-dependent interaction of circulating leukocytes with vascular endothelial cells in lymphoid tissues during the homing phenomenon [20–24]. Furthermore, structural investigations have demonstrated that the carbohydrate chains of GlyCAM-1, an endothelial-derived ligand for L-selectin, contains 6'-sulphated sialyl LewisX [NeuAc2-3Gal(6-SO3)β1-4(Fucβ1-3)GlcNAc] capping groups [25], which in subsequent studies have been implicated in leukocyte-endothelial cell adhesion [23,24].

The assembly of a NeuAc2-3Gal/β1-4GlcNAc (3'SLN) sequence with a sulphate group on C-6 of the galactose residue would appear to require a sulphotransferase distinct from those which have already been described and which, in view of the in vivo demonstration that sulphation occurs subsequent to sialylation [11], should act optimally on oligosaccharides containing a terminal neuraminic acid residue. In the present study, we undertook a search in various rat tissues for an enzyme with such a specificity employing 3'SLN as an exogenous acceptor probe. This investigation led us to the finding that 3'-phosphoadenosine 5'-phosphosulphate (PAPS):3'SLN-6-O-sulphotransferase activity is most prominently expressed in spleen. Accordingly, we utilized microsomal membranes from this lymphoid organ to characterize a unique enzyme which appears to be well suited for sulphating galactose at the C-6 position in preassembled NeuAc2-3Gal/β1-4GlcNAc sequences. The tissue distribution of this novel sulphotransferase was found to be quite distinct from those of two other enzymes which are believed to participate.

Abbreviations used: 3'SLN, 3'-sialyl-N-acetyl-lactosamine (NeuAc2-3Gal/β1-4GlcNAc); PAPS, 3'-phosphoadenosine 5'-phosphosulphate; LacNAc, N-acetyl-lactosamine; SNA, Sambucus nigra agglutinin; R₂Lact, chromatographic mobility relative to lactitol; AnManH₂; 2,5-anhydro mannitol; for brevity the saccharides 3'SL, 6'SL, LSTa, LSTc, 3'SLeα, 3'SLeβ, DSLNT and DSL are defined in the text; -O-Me, methyl glycoside.

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in the sulphation of the N-acetyl-lactosamine (LacNAc) and sialyl-LacNAc sequences.

**EXPERIMENTAL**

**Preparation of rat spleen membranes**

After an overnight fast, male rats (200–250 g; CD strain; Taconic Inc., Germantown, NY, U.S.A.) were killed by decapitation and their spleens were rapidly excised. This tissue was subsequently cut into small pieces, followed by disruption with a Polytron homogenizer (2 × 5 s at a setting of 7) in 4 vol. of ice-cold 50 mM Tris-acetate buffer, pH 7.0, containing 25 mM potassium acetate and 0.35 M sucrose. The homogenate was then centrifuged successively in Berkmann L5-65 ultracentrifuge at 2000 g for 10 min, 12000 g for 15 min and 144000 g for 45 min. The last pellet, representing the microsomal fraction, was washed with 50 mM Tris-acetate buffer, pH 7.0, containing 25 mM potassium acetate, and then suspended in the same buffer at a protein concentration of approx. 18 mg/ml with a Dounce homogenizer.

**Assay of 3′-sialyl-N-acetyl-lactosamine sulphotransferase**

In the standard assay, the microsomal membranes (90–180 µg of protein) were incubated with 0.5 µCi of 3′-phospho[35S]sulphate (PAPS; 1.8–2.4 Ci/mmol; DuPont–New England Nuclear) and 50 nmol of 3′SLN (Oxford Glycosystems, Abingdon, Oxon, U.K.) for 60–90 min at 30 °C in 65 µl of 50 mM Tris-acetate buffer, pH 7.0, containing 100 mM NaF, 2 mM ATP, 25 mM manganese acetate and 0.1% (v/v) Triton X-100. The reaction was terminated by the addition of 5 µl of a 100 µg/ml BSA solution, followed by 3 µl of ice-cold 80% (v/v) ethanol. After mixing, the samples were centrifuged at 600 g for 15 min at 2 °C and the protein pellet was then washed twice with 2 ml of ice-cold 80% (v/v) ethanol. The combined supernatants were dried under a vacuum at 35 °C, dissolved in 3 ml of 0.2 M NaCl and applied to a column of charcoal/Celite [Darco G-60/Celite 535; 1:1 (w/w)] [26] from which, after washing with 10 ml of 0.1 M NaCl and 15 ml of water, the saccharides were eluted with 12 ml of 50% (v/v) ethanol. TLC of the eluted material was carried out on cellulose-coated plates in solvent system A (see below) and after detection by fluorography, the sulphated saccharide products were quantified by liquid-scintillation counting subsequent to being scraped into vials to which 0.5 ml of water followed by 4 ml of scintillation fluid (Monofluor; National Diagnostics, Manville, N.J., U.S.A.) were added.

Although kinetic studies were carried out under conditions in which less than 1% of the substrate was utilized, there was a fall-off from linearity with time which made it possible to obtain only approximate Km values. The values for fetuin glycopeptides and 3′SLN were calculated with the Michaelis–Menten equation, while the Km for PAPS was determined with the Hill equation. The software for these calculations [27] was kindly made available by Dr. P.J. Brooks (Carleton University, Ottawa, Canada).

For specificity studies, this assay system was used with the following saccharides: 6′-sialyl-N-acetyl-lactosamine (6′SLN), 3′-sialyl-lactose (3′SL), 6′-sialyl-lactose (6′SL), NeuAcc2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc (LSTA), NeuAcc2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc (LSTC), 3′-sialyl LewisX (3′SLX), 3′-sialyl-3-fucosyl-lactose (3′SL3F) and disialyl lacto-N-tetraose (DSLNT) from Oxford GlycoSystems; N-acetyl-lactosamine (LacNAc) and disialyl-lactose (DSL) from Sigma; and 3′-sialyl LewisX methyl glycoside from Toronto Research Chemicals (North York, Ontario, Canada). Fetuin glycopeptides containing either the native or the desialylated N-linked carbohydrate units were prepared from Pronase digests as previously described [14].

When glycopeptides were used as acceptors, their radiolabelled sulphated products were recovered at the origin after TLC in solvent system A.

**Determination of tissue distribution of several sulphotransferases**

Several tissues from freshly killed overnight-fasted male rats (200–250 g) as well as frozen rat lymph nodes (Pel-Freeze Biologicals, Rogers, AR, U.S.A.) were disrupted with a Polytron homogenizer in 5 vol. of buffer in a manner similar to that described for spleen. Postnuclear supernatants (600 g, 10 min) of the homogenates were then centrifuged for 60 min at 100000 g to obtain membrane pellets which, after two washes with 100 mM Tris-acetate, pH 7.0, containing 25 mM potassium acetate, were suspended in this buffer at a protein concentration of about 20 mg/ml. These postnuclear membranes were then assayed for 3′SLN-6-O-sulphotransferase activity in a manner described above, as well for PAPS:Gal-3-O- and GlcNAc-6-O-sulphotransferase activities, as previously reported [14,16]. These three comparative assays were carried out with 0.5 µCi of [35S]PAPS and 65 nmol of 3′SLN, LacNAc, or GlcNAcβ1-6Manα1-0-Me acceptors respectively for 90 min at 30 °C in a total volume of 65 µl (O-Me is methyl glycoside).

**Neuraminidase digestions**

Purified [35S]sulphate-labelled 3′SLN, LSTA and fetuin glycopeptides were incubated with *Arthrobacter ureafaciens* neuraminidase (50 munits) in 50 µl of 0.15 M sodium acetate, buffer pH 5.0, at 37 °C for 24 h in the presence of toluene. At that time, a further addition of enzyme (25 munits) was made and the incubations were continued for another 24 h. The digestions were terminated by heating, and the radiolabelled products were deproteinized and desalted by adsorption on a charcoal/Celite column from which they were eluted with 50% (v/v) ethanol as described above.

**Structural and analytical procedures**

[35S]Sulphate-labelled fetuin glycopeptides were submitted to hydrazine/HNO3/NaBH4 treatment as previously described [1,14], and the radiolabelled products were examined in solvent system B (see below). [3H]labelled sulphated disaccharide standards were prepared as already reported [14]. Neuraminidase-treated [35S]3′SLN was allowed to react with sodium metaperiodate as described previously [1]. After NaBH4 reduction of the product, the sample was desalted on a charcoal/Celite column and submitted to Smith degradation (0.05 M HCl, 50 °C, 1 h) followed by TLC. For the preparation of radiolabelled α-glycerol sulphate standard, the [35S]GlcNAc(6- SO3)β1-6Manα1-0-Me product of rat liver Golgi PAPS:GlcNAc-6-O-sulphotransferase [16] was submitted to a Smith periodate degradation. The α-glycerol [35S]sulphate, which is the only radiolabelled product of this reaction, was then isolated by preparative TLC. Protein was quantified by the Bio-Rad binding assay [28], using BSA as a standard.

**Elder (Sambucus nigra) bark lectin (SNA) chromatography**

A column (0.7 cm × 9.0 cm) of agarose-linked SNA (Vector Laboratories, Burlingame, CA, U.S.A.) was equilibrated at 4 °C with 0.15 M NH4HCO3 containing 0.1 mM calcium acetate prior to the application of [35S]sulphate-labelled 3′SLN before and after its desialylation with neuraminidase. After application of the sample, the column was eluted with the equilibration buffer, 1 ml fractions were collected, and their radioactivity was...
monitored by liquid-scintillation counting. Other sulphated disaccharides submitted to the lectin chromatography were prepared by sulphotransferase ([35S]PAPS to 3’SLN) or galactosyltransferase ([35S]PAPS to 3’SLN) action as previously described [14,16].

TLC
Chromatography was performed on plastic sheets precoated with cellulose (0.1 mm thick; Merck) in pyridine/ethyl acetate/water/acetic acid, 6:3:3:1 (by vol.) (solvent system A) or pyridine/ethyl acetate/water/acetic acid, 5:5:3:1 (by vol.) (solvent system B). A wick of Whatman no. 3 paper was clamped to the top of the plate during chromatography; components were revealed by fluorography and quantified by scintillation counting after elution with water. For preparative separations the radioactive saccharides were solubilized with water and the eluates were extracted with peroxide-free diethyl ether to remove scintillants.

Radioactive measurements
Liquid scintillation counting was carried out in Monofluor with a Beckman LS 7500 instrument. Components on thin-layer plates were located by fluorography at −80 °C on X-Omatic AR film (Eastman Kodak) after being sprayed with a scintillation mixture containing 2-methyl-naphthalene [29].

RESULTS
Assay of spleen sulphotransferase activity towards sialylated oligosaccharides
Rat spleen microsomes were observed to effectively catalyse the transfer of sulphate from [35S]PAPS to 3’SLN, although no sulphation of the α2-6-linked isomer (6’SLN) took place (Figure 1). In a similar fashion, incorporation of sulphate into 3’-sialyl-lactose (3’SL) and the α2-3-sialylated pentasaccharide, LSTa, was evident, in contrast with the absence of transfer activity when the sialyl α2-6 structural analogues (6’SL and LSTc) were tested as acceptors (Figure 1). Since a comprehensive survey of rat organs indicated that spleen had, to a substantial extent, the highest PAPS:3’SLN-sulphotransferase activity (see section below), we carried out our detailed investigations of this enzyme with this lymphoid tissue.

Properties 3’SLN-sulphotransferase
Enzyme activity towards 3’SLN increased with time for at least 2 h at 30 °C, although there was a departure from linearity by 60 min; no detectable activity was observed in the absence of the exogenous acceptor (results not shown). Furthermore, this sulphotransferase activity increased in a linear manner with the amount of microsomal membranes when assay in the 40–240 µg protein range under standard conditions for 60 min (results not shown).

Optimal transfer activity occurred between pH 6.5 and 7.0, with a sharp decline in both the acidic and alkaline range (Figure 2). Sulphation of 3’SLN was stimulated by manganese with an optimum between 12 and 25 mM, while Mg²⁺, Ca²⁺ and Zn²⁺ had no perceptible effect. In the presence of Co²⁺, however, a stimulation equivalent to 32 % of that brought about by Mn²⁺ was observed. The inclusion of Triton X-100 in the incubation resulted in a pronounced stimulation of sulphotransferase action that was already evident at 0.02 % (v/v) and levelled off after a concentration of 0.035 % (v/v) had been reached (results not shown).

Kinetic measurements with various PAPS concentrations (at 90 min) yielded a sigmoidal curve for which a Kₘ value of 4.7 µM was calculated; an examination of the effect of increasing concentrations of 3’SLN on enzyme activity (at 60 min) gave a Kₘ value of 0.72 mM. For the reasons stated in the Experimental section, these Kₘ values must be considered tentative.

Substrate specificity of sulphotransferase
When fetuin glycopeptides with an N-linked carbohydrate unit in which the oligosaccharide branches contain the NeuAc2-
Table 1  Specificity of spleen PAPS:3’SLN-6-O-sulphotransferase towards oligosaccharide acceptors

Incubations were carried out with rat spleen microsomes (138 µg of protein) and [35S]PAPS (0.5 µCi) for 90 min at 30 °C with the indicated acceptor (50 nmol; 0 ± 77 mM) under the conditions described in the Experimental section. The products were quantified by liquid-scintillation counting after column-chromatographic and TLC purifications. Acceptor abbreviations are defined in the text.

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>Relative activity</th>
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<tbody>
<tr>
<td>3’SLN</td>
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</tr>
<tr>
<td>6’SLN</td>
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</tr>
<tr>
<td>3’SLeα</td>
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<tr>
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<tr>
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<td>DSLNT</td>
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<tr>
<td>DSL</td>
<td>0</td>
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<tr>
<td>LacNAc</td>
<td>0†</td>
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</table>

* Values are expressed relative to 3’SLN, into which 2450 d.p.m. was incorporated during the period of assay.
† TLC indicated that sulphate was incorporated exclusively into Gal(3-SO4)β1-4GlcNAc.

3Galβ1-4GlcNAc sequence [30] were tested as acceptors, effective transfer of sulphate took place and an approximate K_m value of 1.16 mM was determined.

An examination of a number of acceptors indicated that the sulphotransferase acted only on oligosaccharides terminating in sialic acid linked to galactose by an α2-3 bond. Although the GlcNAc in the sialyl-lactosamine sequence could be exchanged for a Glc residue and a Galβ1-3GlcNAc linkage could be substituted for Galβ1-4GlcNAc without much change in activity, the linkage of fucose to the GlcNAc, as in 3’SLeX, or NeuAc, as in DSLNT, resulted in totally inert acceptors (Table I). Although desialylated fetuin glycopeptides and LacNAc incorporated radioactivity, the [35S]sulphate was found to be exclusively located on C-3 of the terminal galactose residue, and this was attributed to the action of the previously described [14] PAPS:galactose 3-O-sulphotransferase that is also present in spleen microsomes (see section below).

Figure 3  Effect of neuraminidase (‘Neurase’) treatment on the TLC migration of 35S-labelled sulphated 3’SLN and LSTa

After purification by preparative TLC, [35S]3’SLN and [35S]LSTa produced by the spleen enzyme were incubated with (+) or without (−) with Arthrobacter ureafaciens neuraminidase as described in the Experimental section and subsequently chromatographed on a cellulose-coated plate in solvent system B for 46 h (left side) or 90 h (right side). The components were detected by fluorography.

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Characterization of the sulphotransferase product

Treatment of the chromatographically purified [35S]sulphated sulphated 3’SLN and LSTa with Arthrobacter ureafaciens neuraminidase resulted in an increase in the TLC migration (solvent system B) of these two components from R_LacH≈0.40 to 1.20 and from R_LacH≈0.13 to 0.30 respectively (Figure 3). As no radioactivity was lost by this
The disaccharide resulting from neuraminidase treatment of $[^{35}S]3'SLN$ (see Figure 3) was examined by TLC with (+) or without (−) undergoing prior Smith periodate degradation (NaIO$_4$). The standard (STD) x-glycerol $[^{35}S]$sulphate (Gly(S)) was prepared in the manner described in the Experimental section. Chromatography was carried out on a cellulose-coated plate in solvent system B for 24 h. Components were revealed by fluorography; the arrow indicates the migration of standard galactitol.

Treatment, it was evident that the sulphate group was located internally to the sialic acid on either the Gal or GlcNAc residues. Since it has been reported that SNA binds 6’SLN but not the 3’SLN trisaccharide [31] and, moreover, that LacNAc with a sulphate substitution on C-6 of the galactose [i.e. Gal(6-SO$_4$)$_2$/1-4GlcNAc] also reacts with this lectin [32], we employed immobilized SNA to explore the position of the sulphate on our $[^{35}S]3'SLN$ product. Chromatography on SNA–agarose of the $[^{35}S]$sulphate-labelled 3’SLN both before and after neuraminidase treatment indicated that, although the native product did not bind to the column, removal of the α,3-linked sialic acid residue yielded a disaccharide which was bound to the lectin (Figure 4).

This stood in contrast with the finding that a number of other sulphated disaccharides, including Gal(3-SO$_4$)$_2$/1-4GlcNAc, did not interact with the lectin. These observations, together with the knowledge that the OH group at C-3 and C-4 of galactose are a requirement for SNA interaction [33], suggested that our radio-labelled 3’SLN was sulphated at the C-6 position of Gal.

Consistent with this structural assignment of the sulphate group was our observation that the neuraminidase-treated $[^{35}S]$sulphate-labelled 3’SLN yielded upon Smith periodate degradation a radiolabelled component which migrated on TLC to the position of x-glycerol $[^{35}S]$sulphate (Figure 5). This finding excluded the possibility that the sulphate group was situated on the GlcNAc residue or the C-3 position of the galactose component, as such locations would have imparted resistance to the periodate oxidation [34]. Moreover, C-4 sulphation of galactose would have yielded radiolabelled threitol sulphate [34]. The possibility that the sulphate is located on C-2 of galactose cannot be excluded, as the β-glycerol sulphate resulting from that situation would unlikely separate from the x-glycerol sulphate on TLC; however, in view of the SNA chromatographic data, this alternative possibility to a C-6 substitution seems unlikely.

Treatment of the $[^{35}S]$sulphated fetuin glycopeptides by the hydrazine/HNO$_2$/NaBH$_4$ procedure provided further information which was in accord with a NeuAcα2-3Gal(6-SO$_4$)$_2$/1-4GlcNAc structural formulation (Figure 6). Degradation of the native sulphated glycopeptide by this approach, subsequent to its desialylation with neuraminidase, yielded a radioactive component, most likely Gal(6-SO$_4$)$_2$/1-4AnManH$_2$(AnManH$_2$ is 2,5-anhydromannitol), which migrated to a position ($R_{GalH}$ = 1.19) that was distinguishable from Gal(3-SO$_4$)$_2$/1-4AnManH$_2$ ($R_{GalH}$ = 1.70) and Gal(1-4AnManH$_2$(6-SO$_4$) ($R_{GalH}$ = 1.27), which are the disaccharides obtained as the fragmentation products of Gal(3-SO$_4$)$_2$/1-4GlcNAc and Gal(1-4GlcNAc(6-SO$_4$) sequences respectively [1]. Indeed, when desialylated fetuin glycopeptides were used as substrate with spleen microsomes, hydrazine/HNO$_2$/NaBH$_4$ treatment of the $[^{35}S]$sulphated product yielded the Gal(3-SO$_4$)$_2$/1-4AnManH$_2$ disaccharide (Figure 6) as would be anticipated if the PAPS:galactose-3-O-sulphotransferase [14] was also present in these membranes (see section below).

**Figure 5** Effect of periodate oxidation on the sulphated disaccharide obtained after neuraminidase digestion of $[^{35}S]$-labelled 3’SLN

**Figure 6** TLC examination of the $[^{35}S]$-labelled product obtained by hydrazine HNO$_2$/NaBH$_4$ treatment of sulphated fetuin glycopeptides

The purified $[^{35}S]$-labelled products resulting from the action of spleen microsomal sulphotransferase on native sialylated (Nat) and desialylated (Neu-free) fetuin glycopeptides were submitted to the hydrazine/HNO$_2$/NaBH$_4$ procedure described in the Experimental section. The native $[^{35}S]$-labelled glycopeptides were treated with neuraminidase prior to being submitted to the degradation reaction. Chromatography of the product was carried out on a cellulose-coated plate in solvent system B for 24 h. For comparison, $[^{35}S]$-labelled disaccharides (STDs) representing Galβ1→4AnManH$_2$(6-SO$_4$) and Gal(3-SO$_4$)$_2$/β1→4AnManH$_2$ were chromatographed in lanes 1 and 2 respectively; the preparation of these saccharides is described in the Experimental section. Components were revealed by fluorography.
transferrase activities that were, however, again noticeably exceeded by brain (Table 2).

**DISCUSSION**

It is apparent from the present investigation that spleen microsomes contain an enzyme which serves to transfer sulphate from PAPS to the C-6 position of the galactose residue in oligosaccharides containing the 3'SLN sequence. The specificity of this PAPS:3'SLN-6-O-sulphotransferase indicates that it is well suited to participate in the assembly of carbohydrate units in which either 3'-sialyl-6'-sulpho-LacNAc [NeuAc2-3Gal(6-SO3)β1-4GlcNAc] or 3'-sialyl-6'-sulpho-Lewis^a^ [NeuAc2-3Gal(6-SO3)β1-4(Fucα1-3)GlcNAc] saccharide constellations occur. Indeed our observation that this sulphotransferase acts exclusively on oligosaccharides which are capped by an α-2,3-linked neuraminic acid residue is consistent with the in vitro studies that indicate that sialylation precedes sulphate addition on C-6 of galactose [11] and differs from the synthetic order for the assembly of NeuAc2-3(6)Galβ1-4GlcNAc(6-SO3) sequences in which both galactosylation and sialic acid addition follow the sulphation step [16]. The inactivity of the spleen enzyme towards a 3'-sialyl Lewis^a^ acceptor suggests that fucosylation is the last step in the formation 3'-sialyl-6-sulpho Lewis^a^ and is consistent with a scheme proposed by Chandrasekaran et al. [35]. Our finding that C-6 sulphation of 3'SLN was almost as effective as that of 3'SLN has an intriguing parallel in binding studies that have indicated that substitution of glucose for GlcNAc does not impair the interaction of 3'-sialyl Lewis^a^ with L-selectin [24]. Furthermore, the comparable activity which the spleen enzyme exhibited toward NeuAc2-3Galβ1-3GlcNAc and NeuAc2-3Galβ1-4GlcNAc receptors seems to mirror the finding that both 3'-sialyl Lewis^a^ and 3'-sialyl Lewis^a^ can serve as ligands for the selectins [20,36]. Since the tentative K_a values for 3'SLN and glycoproteins containing N-linked carbohydrate units with NeuAc2-3Galβ1-4GlcNAc branches were quite similar (0.72 mM and 1.16 mM respectively), it would appear that the determinants for the newly described sulphotransferase are primarily located in the peripheral tri saccharide sequence of the acceptors. In accord with this contention is our observation that 3'SLN manifests about the same acceptor activity as the LSTα pentasaccharide.

A survey of various rat tissues indicates that the distribution of the PAPS:3'SLN-6-O-sulphotransferase is quite selective, with spleen, brain and testes demonstrating high activity, while the enzyme was essentially absent from liver and kidney as well as muscle and heart. Tissues primarily devoted to the production of mucins, like lung and stomach, had low to minimal activity. Particularly revealing was the finding that, of the three lymphoid tissues analysed, spleen had substantially greater activity than either thymus or lymph nodes. It is reasonable to assume that the 3'SLN-6-O-sulphotransferase observed in the latter tissue would be involved in the assembly of the 6'-sulpho Lewis^a^ groups which have been shown to cap many of the oligosaccharide chains of GlyCAM-1 [25]. This glycoprotein, which is present in mouse lymph nodes, is known to be a ligand for L-selectin [11] and in that capacity its sulphated carbohydrate units are believed to mediate the initial adhesive interaction of lymphocytes with high endothelial venules during the homing process [24]. The particularly high 3'SLN-6-O-sulphotransferase activity of spleen suggests that comparable selectin ligands may be present in that tissue. Although spleen is the predominant organ involved in lymphocyte recirculation, surpassing the total lymphode node mass, the molecular mechanism involved in its interaction with these cells has as yet not been defined [37]. While the presence of C-6-situated sulphate groups on galactose in 3'SLN or 3'-sialyl Lewis^a^ sequences is of considerable biological interest in view of their role as selectin ligands, such anionic substituents may also be present in a variety of other as-yet-uncharacterized glycoproteins. Indeed it has been reported that the NeuAc2-3Gal(6-SO3)β1-4GlcNAc sequence occurs in the N-linked carbohydrate units of a recombinant human tissue plasminogen activator expressed in mouse epithelial cells [38].

Enzymes involved in the assembly of N- or O-linked oligosaccharides are usually not limited in the distribution to one cell type nor involved exclusively in the biosynthesis of a specific glycoprotein [39]. This generalization is supported by our survey of the tissue distribution of two other, previously described [14,16], enzymes which play a role in sulphating LacNAc and sialyl LacNAc sequences (Table 2). The high activity in lymphoid tissues of PAPS:Gal-3-O-sulphotransferase, an enzyme which was first detected in calf thyroids [14], could reflect to a certain extent the formation of capping Gal(3-SO3) groups that have been implicated as ligands in selectin interactions [17,20]. Likewise, the PAPS:GlcNAc-6-O-sulphotransferase, which was found to be present in substantial amounts in spleen, thymus and lymph nodes, may participate in the assembly of NeuAc2-3Galβ1-4GlcNAc(6-SO3) and NeuAc2-3Galβ1-4(Fucα1-3)GlcNAc(6-SO3) saccharide constellations which, on the basis of binding studies, are believed to be involved in interactions with selectins [24] and have indeed been shown to be present as L-selectin ligands in GlyCAM-1 [11]. It would appear likely that the three sulphotransferases surveyed (Table 2) acting in concert with a number of glycosyltransferases are responsible for the assembly of the sulphated selectin ligands of lymphoid tissues as well as the carbohydrate units of a variety of other glycoproteins. The ultimate biological activity imparted to glycoproteins by the anionic oligosaccharides is most likely dependent not only on the

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**Table 2** Tissue distribution of sulphotransferases which can participate in the formation of sulphated LacNAc and sialyl LacNAc sequences

<table>
<thead>
<tr>
<th>Tissue</th>
<th>3'SLN-6-O-sulphate†</th>
<th>Gal-3-O-sulphate</th>
<th>GlcNAc-6-O-sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Thymus</td>
<td>0</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td>28</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>1</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>16</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>45</td>
<td>198</td>
<td></td>
</tr>
<tr>
<td>Testis</td>
<td>51</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>1</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

* Analysis of rat stomach for 3'SLN-6-O-sulphotransferase gave a relative activity of 3.
† The three enzymes assayed are respectively PAPS:3'SLN-6-O,Gal-3-O- and GlcNAc-6-O-sulphotransferases. Subsequent to the action of Gal-3-O-sulphotransferase, sialylation does not occur, while the product of GlcNAc-6-O-sulphotransferase is a substrate for the sequential action of galactosyl- and sialyl-transferases.
‡ The activities, determined as d.p.m./mg of protein, are expressed relative to those of spleen microsomes which incorporated 14206, 108614 and 1983 d.p.m./mg of protein into 3'SLN, LacNAc and GlcNAcβ1-6Manα1-0-Me respectively during the period of the assays.
§ Activity was not detectable.
detailed structure of the saccharide groups, but also on their disposition on the polypeptide chain [19,39].

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REFERENCES


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