Purification of Rat Liver N-Heparan-sulfate Sulfotransferase*

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N-Heparan-sulfate sulfotransferase catalyzes the transfer of sulfate from 3'-'phosphoadenylyl) sulfate to the nitrogen of glucosamine in heparan sulfate. This reaction is an obligatory step for subsequent epimerization of D-glucuronic to L-iduronic acid and of O-sulfation of the sugar chains. We have purified this sulfotransferase from rat liver membranes to apparent homogeneity using a combination of conventional and affinity chromatography on DEAE-Sepharose, heparin-agarose, 3',5'-ADP-agarose, wheat germ-Sepharose, and finally a glycerol gradient. The pure enzyme is a glycoprotein with an apparent molecular weight of 97,000. It was enriched in specific activity 65,000-fold over the homogenate. The recovery of activity was 4% of that of the homogenate.

Preliminary enzymatic characterization of the purified sulfotransferase indicates a high degree of substrate specificity. Transfer of sulfate occurs to heparan sulfate, N-heparan sulfate, and N-desulfated heparin, but not to N-acetylated heparan sulfate, N-acetylated heparin, chondroitin, chondroitin sulfate, and tyrosine-containing tripeptides.

Sulfotransferases are enzymes involved in the transfer of sulfate from PAPS1 to specific substrates such as growing proteoglycans (1, 2), tyrosines of proteins (3), and sugars of glycoproteins and glycolipids (4, 5). It has been shown that sulfation occurs in the Golgi apparatus through pulse-chase studies in vivo with radioactive sulfate (2, 6, 7) and studies in vitro using subcellular fractionation (5, 8).

Among the substrates for sulfotransferases, heparan sulfate proteoglycans have been the focus of intense studies in recent years (9-11). These proteoglycans are present in a variety of basement membranes (12), extracellular matrices (13), and cell membranes (14). Different functions have been proposed for these compounds depending on the type of tissue where they reside. In the basement membrane, they may control permeability by acting as a charge barrier (15); and in the plasma membrane and basal lamina, they may function as anchorage receptors for enzymes such as lipoprotein lipase and acetylcholinesterase (16, 17). Recently, it has been shown that heparan sulfate proteoglycan present in the membrane of endothelial cells displays anticoagulant activity. This suggests that the proteoglycan may be responsible, in part, for the nonthrombogenic properties of the vascular endothelium (18). Some of these functions appear to be the result of a specific location of sulfate within the sugar chains of the proteoglycans (18, 40, 41).

The polysaccharide portion of heparan sulfate proteoglycan is composed of alternating D-glucuronic and hexuronic acids (D-glucuronic or L-iduronic). Sulfate substituents occur either as sulfamino groups at C-2 of the glucosamine or as sulfate ester groups at C-6 of the glucosamine or C-2 of the iduronic acid. Recently, the presence of a sulfate ester in C-3 of the glucosamine has been shown to occur in heparan sulfate proteoglycan (18). This appears to be a unique feature of heparin (38).

The polysaccharide chains are initially synthesized by polymerization of disaccharides containing D-glucuronic acid and N-acetylglucosamine residues in alternating sequence. The resulting polymer subsequently undergoes a series of modification reactions. These include, in sequence, N-deacetylation and N-sulfation of glucosamine, C-5 epimerization of D-glucuronic acid to L-iduronic acid, 2-O-sulfation of L-iduronic acid, and finally 6-O- and 3-O-sulfation of glucosamine. In liver, the synthesis of the polysaccharide chains and the sulfation of the proteoglycans occur exclusively in the Golgi apparatus (10).

Many glycosyltransferases as well as sulfotransferases are located in the Golgi apparatus (39). To date, several mammalian glycosyltransferases have been purified to apparent homogeneity (20-25). These enzymes have served as antigens against antibodies which have been used to study, by immunoelectronmicroscopy, the spatial localization of the glycosyltransferases within the Golgi apparatus (26-28). Until now, no Golgi sulfotransferases have been purified, and the localization of these enzymes within the Golgi apparatus remains unknown. In addition, the substrate specificity of sulfotransferases has not been studied in detail, and it is not known whether the different sulfotransferases have structural similarities. Answers to these questions require the isolation and purification of these enzymes. Toward this objective, we have purified the N-heparan-sulfate sulfotransferase to apparent homogeneity.

**EXPERIMENTAL PROCEDURES**

**Materials**

Frozen rat livers were purchased from Rockland, Inc. [α35S]PAPS (1.3 Ci/mmol) was purchased from Du Pont-New England Nuclear. Heparan sulfate, heparin, DEAE-Sepharose, heparin-agarose, 3',5'-ADP-agarose, and wheat germ-Sepharose were obtained from Sigma.

**Methods**

Purification of Rat Liver N-Heparan-sulfate Sulfotransferase

All operations described below were performed at 4 °C.

**Step 1: Detergent Extraction**—Up to 1 kg of frozen rat livers was...
processed at a time. Livers were thawed and rinsed with distilled water. They were then suspended in 10 mM Hepes buffer, pH 7.2, containing 10 mM MgCl₂ (w/v, 1%). The tissue was homogenized with a Polytron tissue grinder at low setting by two 20-s bursts separated by a 20-s interval. The crude homogenate was spun at 9000 rpm for 40 min in a Sorvall GS rotor. The pellets were resuspended in the above homogenization in a Dounce homogenizer with a high clearance pestle. The suspension was brought to 0.3% Triton X-100 (v/v). After stirring for 45 min, the suspension was centrifuged as described before. The supernatant solution was discarded, and the pellet was resuspended in 1.5 liters of Triton X-100 (1.4% final concentration). The mixture was stirred for 45 min and centrifuged as described before. The supernatant solution was decanted and filtered through layers of cheesecloth, yielding a Triton-containing extract which was saved. The pellet was resuspended with 1.0 liter of 1.4% Triton X-100 and extracted as described before. Both Triton extracts were pooled, and glycerol was added to the suspension to a final concentration of 20%.

Step 2: DEAE-Sephacel (NaCl Pulse) - The Triton extract was loaded onto a 1-liter DEAE-Sephacel column (15 × 50 cm) equilibrated in buffer A (10 mM Hepes buffer, pH 7.2, containing 10 mM MgCl₂, 0.1% Triton X-100, and 20% glycerol). The flow rate was 100 ml/h. The column was first eluted with 2 liters of buffer A followed by 600 ml of buffer A plus 0.6 M NaCl. The sulfotransferase activity eluted at approximately 180 mM NaCl. They were diluted with buffer A to a final concentration of 0.15 M NaCl.

Step 3: Heparin-Agarose (NaCl Gradient) - The fraction containing the sulfotransferase activity from Step 2 was applied to a heparin-agarose column (5 × 20 cm) equilibrated in buffer A containing 0.15 M NaCl. The flow rate was 70 ml/h. The column was first eluted with 1 liter of buffer A containing 0.15 M NaCl, followed with buffer A containing 0.75 M NaCl. This fraction, containing the sulfotransferase activity, was dialyzed against 30 volumes of buffer A with two changes every 12 h.

Step 4: 3',5'-ADP-Agarose (NaCl Gradient) - The sulfotransferase-containing fraction from Step 3 was applied to a 3',5'-ADP-agarose column (1 × 20 cm, 1.3 μmol of cADP/ml of gel) equilibrated in buffer A. The flow rate was 15 ml/h. The column was first washed with 150 ml of buffer A containing 50 mM NaCl. The sulfotransferase activity was then eluted with a linear gradient of 150 ml of buffer A containing 50 mM to 1 M NaCl. The fractions containing sulfotransferase activity eluted at approximately 180 mM NaCl. They were pooled and dialyzed for 18 h against 30 volumes of buffer A.

Step 5: 3',5'-ADP-Agarose (3',5'-ADP Gradient) - The sulfotransferase-containing fraction from Step 4 was applied to a 3',5'-ADP-agarose column (1 × 8 cm, 0.8 μmol of 3',5'-ADP/ml of gel) equilibrated in buffer A. The flow rate was 15 ml/h. The column was washed with 100 ml of buffer A containing 50 mM NaCl. The sulfotransferase activity was eluted with a linear gradient of 3',5'-ADP (75 ml of buffer A containing 50 mM NaCl as starting buffer and 75 ml of buffer A containing 500 mM 3',5'-ADP as final buffer). Following the gradient elution, the column was washed with 50 ml of buffer A containing 0.65 M NaCl to recover additional bound proteins.

Step 6: Wheat Germ-Sepharose - Fractions containing sulfotransferase activity from Step 5 were pooled and loaded onto a wheat germ-Sepharose column (0.65 cm diameter). The flow rate was 15 ml/h. The column was washed with 10 ml of buffer A containing 0.1 M GcNac. The sulfotransferase activity eluted in this fraction. Finally, the column was washed with 10 ml of buffer A containing 1 M NaCl to recover additional bound proteins.

Step 7: Glycerol Gradient - The sulfotransferase activity obtained from Step 6 was concentrated and washed with buffer A in Ca³⁺-free filters (Amicon) to reduce the final glycerol concentration to 8%. This sample was loaded on top of a 8–30% glycerol gradient containing 10 mM Hepes, pH 7.2, 10 mM MgCl₂, 0.08 mM NaCl, and 0.1% Triton X-100. The gradient was centrifuged in a SW 50 rotor at 46,000 rpm for 17 h at 4°C. Fractions of 0.20 ml were collected.

**Sulfotransferase Assay**

The incubation mixture contained (except where stated otherwise) enzyme (200 μg of protein for crude fractions and 20–100 μg of protein for the purest fractions), heparan sulfate (50 μg), [³²P]PAPS (550,000 cpm; 2.5 nmol), MgCl₂ (1 μmol), NaF (1 μmol), 2,3-dimercaptopropanol (30 mM), Triton X-100 (250 μg), and Hepes buffer, pH 7.2 (1 μmol), in a final volume of 0.05 ml. Incubations were done for 10 min at 37°C. The amount of sulfate transferred to heparan sulfate was determined as described by Rapraeger and Bernfield (30) with small modifications. The reaction mixtures were spotted on polyethyleneimine chloride-impregnated Whatman No. 3MM filter discs (prepared by soaking the filters in 2.5% polyethyleneimine chloride, followed by drying). The filters with the radioactive products were washed with water for 20 min and soaked for 30 min in 25 ml of 1 M sodium sulfate, followed by a 30-min rinse with water. The discs were then soaked in 20% trichloroacetic acid for 1 h, followed by a 15-min wash with water. Finally, the filters were soaked in 95% ethanol and air-dried before liquid scintillation spectrometry.

**Acceptor Substrates for Sulfotransferase**

Different glycosaminoglycan acceptors for the sulfotransferase assays were prepared as previously described: N-desulfoheparan sulfate and N-desulfoheparin (31), N-desulf-N-acetylatedheparan sulfate and N-desulf-N-acetylatedheparin (32), and chondroitin (33).

Degradation of [³²S]-labeled glycosaminoglycans with nitrous acid was performed using protocol A as described by Lindahl et al. (34). The reaction was stopped by adding 2 volumes of 2 M ammonium sulfamate. The reaction mixture was then chromatographed on a Sephadex G-50 column (6.0 × 100 cm), and the column was eluted with 0.2 M ammonium acetate, pH 6.0. The flow rate was 10 ml/h. The radioactive free sulfate represents the sulfate bound to the nitrogen of the glucosamine of heparan sulfate.

**RESULTS**

**Heparan-sulfate Sulfotransferase Activity of Rat Liver Is Localized in Membranes of the Golgi Apparatus**

Rat liver homogenate can catalyze the transfer of sulfate from [³²P]PAPS to exogenous glycosaminoglycan acceptors. When heparan sulfate chains are used as acceptor substrate, the sulfotransferase is found to be highly concentrated in membranes of the Golgi apparatus compared to those of the rough and smooth endoplasmic reticulum (Table I). This activity is only expressed if the vesicles are permeabilized with detergents, suggesting, as a first approximation, that the active site of the enzyme faces the lumen of the Golgi apparatus. The heparan-sulfate sulfotransferase activity has an apparent Kₘ for PAPS of 5.0 μM. Maximal incorporation of sulfate is found when heparan sulfate is present at a concentration of 0.5 mg/ml. The sulfotransferase has a pH optimum of 7.2, whereas incubations at pH 5.7, 6.1, 6.4, 6.9, 7.4, 7.7, and 8.1 result in incorporation of sulfate of 33, 35, 54, 76, 90, 71, and 54%, respectively, compared to that at pH 7.2. The sulfotransferase requires metals for optimal activity. Maximal activation is found with 5 mM Mg²⁺, 5 mM Mn²⁺, or 5 mM Ca²⁺ stimulated the sulfotransferase activity 62 and 22%, respectively.

| Table I | Distribution of heparan-sulfate sulfotransferase in rat liver organelles |
|---------|------------------------------------------------------------------------|
| Assays for heparan-sulfate sulfotransferase were carried out in 10 mM Hepes, pH 7.2, 10 mM MgCl₂, 5 mM NaF, 0.5% Triton X-100 for 10 min at 37°C with 3.5 X 10⁶ cpm of [³²P]PAPS in the presence of membranes of rat liver organelles (10–100 μg of protein) using bovine kidney heparan sulfate (2.0 mg/ml) as substrate. |
| Specific activity | nmol/mg/min |
|-------------------|-------------|
| Golgi apparatus   | 2.34        |
| Fleischer and Kervina (45) | 2.55 |
| Leelavathi et al. (46) | 0.02 |
| Rough endoplasmic reticulum (45) | 0.25 |
| Smooth endoplasmic reticulum (45) | 0.15 |

**SDS Gel Electrophoresis**

Sodium dodecyl sulfate-polyacrylamide (10%) gel electrophoresis was performed as described by Laemmli (29). Samples were precipitated by adding 2 volumes of cold ethanol and allowed to stand at −20°C for 1 h. Precipitates were collected by centrifugation and resuspended in loading buffer (29).
TABLE II
Purification of N-Heparan-sulfate sulfotransferase

| Step       | Total protein | Specific activity | Activity | Purification |
|------------|---------------|------------------|----------|--------------|
|            | mg            | units/mg protein | units    | -fold        |
| Homogenate | 500,000       | 0.00003          | 15.0     | 1            |
| 1 Triton extract | 130,000    | 0.00008          | 10.4     | 2.7          |
| 2 DEAE-Sepharose | 52,000     | 0.00016          | 8.3      | 5.3          |
| 3 Heparin-agarose | 11,333      | 0.00066          | 6.8      | 20           |
| 4 3',5'-ADP-agarose | 191         | 0.012            | 2.3      | 400          |
| (NaCl pulse) |               |                  |          |              |
| 5 3',5'-ADP-agarose | 3.64        | 0.33             | 1.9      | 11,000       |
| (3',5'-ADP gradient) |            |                  |          |              |
| 6 Wheat germ-Sepharose | 0.89        | 0.93             | 0.83     | 31,000       |
| 7 Glycerol gradient | 0.27        | 1.95             | 0.53     | 65,000       |

*1 unit of activity is defined as 1 μmol/min sulfate transferred to kidney heparan sulfate used as substrate under standard assay conditions as described under “Experimental Procedures.”

![Fig. 1. NaCl gradient elution of heparan-sulfate sulfotransferase from a 3',5'-ADP-agarose column.](image1)

**Step Drotein**

**Method**

The sulfotransferase was purified to apparent homogeneity about 65,000-fold over the specific activity of the total homogenate. A summary of the purification from 3.0 kg of rat liver is shown in Table II.

**Step 1: Triton X-100 Extraction**—Rat liver membranes were obtained from homogenates by low speed centrifugation in the presence of 10 mM MgCl₂. Triton X-100 was used to extract the membrane-bound sulfotransferase. Whereas other detergents (Triton CF-54, Nonidet P-40, Lubrol PX) gave satisfactory extraction, the heparan-sulfate sulfotransferase was more stable in Triton X-100 than in other detergents. Approximately 70% of the total activity from the crude extract could be solubilized with Triton X-100.

**Step 2: DEAE-Sepharose Chromatography**—The Triton X-100 extract, containing the sulfotransferase activity, was loaded on a DEAE-Sepharose column. Virtually all the enzyme activity bound to the column. The bulk of the enzyme activity (98%) was eluted with 0.65 M NaCl. Pooled enzyme activity was diluted with buffer to reduce the NaCl concentration to 0.15 M and was used for the next purification step.

**Fig. 3. Glycerol gradient sedimentation profile of heparan-sulfate sulfotransferase.** A, heparan-sulfate sulfotransferase eluted from a wheat germ-Sepharose column was loaded onto an 8-30% glycerol gradient and centrifuged as described under “Methods.” Fractions of 0.2 ml were collected. The arrows indicate the sedimentation position of β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), and bovine serum albumin (66 kDa). B, aliquots of different fractions from the glycerol gradient were pooled, as indicated, and analyzed by SDS-PAGE. Proteins were visualized with silver nitrate stain. Molecular size standards were the following: myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), and egg albumin (45 kDa). Bands in the M₀ 50,000-65,000 range are an artifact of silver staining when 2-mercaptoethanol was present in the loading buffer (25).
Step 3: Heparin-Agarose Chromatography—One of the substrates of heparan-sulfate sulfotransferase is heparan sulfate. It therefore appeared logical to use a heparin matrix as a possible affinity ligand for the above sulfotransferase. This column was loaded to a heparin-agarose column. The column was then eluted with 1.0 M NaCl. This resulted in a 28-fold purification of the sulfotransferase activity. This column resulted in a 2.0 M NaCl gradient from a 3'5'-ADP-agarose column (low sulfotransferase activity); lane 9, 3 µg of protein eluted with buffer A containing 3',5'-ADP from a 3',5'-ADP-agarose column (no sulfotransferase activity); lane 10, 3 µg of protein eluted with buffer A containing 0.65 M NaCl after 3',5'-ADP gradient elution of a 3',5'-ADP-agarose column (low sulfotransferase activity); lane 11, 3 µg of protein eluted with buffer A from a wheat germ-Sepharose column (low sulfotransferase activity); lane 12, 3 µg of protein eluted with buffer A containing GlcNac from a wheat germ-Sepharose column (high sulfotransferase activity). Molecular size standards are the same as used in Fig. 3B.

Step 4: 3',5'-ADP-Agarose Chromatography I: Elution with NaCl—Heparan-sulfate sulfotransferase transfers sulfate from PAPS to heparan sulfate. 3',5'-ADP, in addition to being one of the products of the reaction, is also a strong inhibitor, suggesting a specific interaction between the nucleotide diphosphate and the sulfotransferase. As shown in Fig. 1, when the enzyme is loaded onto a 3',5'-ADP-agarose column, 85% of the activity bound to the resin, whereas 90% of the protein did not. Additional proteins were eluted with buffer A containing 0.05 M NaCl. The bound enzyme activity was eluted with a gradient of buffer A containing NaCl. This step resulted in a 20-fold purification.

Step 5: 3',5'-ADP-Agarose Chromatography II: Elution with 3',5'-ADP—The dialyzed sulfotransferase activity from Step 4 was applied to another 3',5'-ADP-agarose column. This column was washed with buffer A containing 0.05 M NaCl, which removed some additional protein but no enzyme activity. The column was then eluted with a 3',5'-ADP gradient (0-1.0 mM in buffer A). As shown in Fig. 2, the sulfotransferase activity eluted as a sharp peak with very little protein. The column was then eluted with 1.0 M NaCl. This resulted in elution of some protein, but only 6% of sulfotransferase activity. This column resulted in a 28-fold purification of the sulfotransferase.

Step 6: Wheat Germ-Sepharose Chromatography—The enzyme from Step 5 was loaded onto a wheat germ-Sepharose column. 93% of the sulfotransferase activity was retained on this column. Approximately 90% of the activity was eluted specifically with 0.5 M N-acetylglucosamine. This suggests that the sulfotransferase may be a glycoprotein.

Step 7: Glycerol Gradient—As a final step in the purification of the sulfotransferase, the active enzyme was concentrated by ultrafiltration, loaded on top of a 8-30% glycerol gradient, and centrifuged for 17 h. Fig. 3A shows the profile of sulfotransferase activity throughout the gradient. The enzyme activity migrated as a symmetric peak, with the peak corresponding to a M, of 94,000. At this stage, the sulfotransferase was purified about 65,000-fold over the homogenate (Table II), with an overall yield of 4%. The enzyme activity was stable for at least 6 months at -70 °C in the presence of 20% glycerol.

Purity of the N-Heparan-sulfate Sulfotransferase

Assessment of the purity of the N-heparan-sulfate sulfotransferase during different purification steps was measured by SDS-PAGE of different purified fractions of heparan-sulfate sulfotransferase. Lane 1, 20 µg of protein of a total homogenate Triton X-100 extract; lane 2, 20 µg of protein eluted with buffer A from a DEAE-Sephacel column (no sulfotransferase activity); lane 3, 20 µg of protein eluted with buffer A containing 0.6 M NaCl from a DEAE-Sephacel column (high sulfotransferase activity); lane 4, 20 µg of protein eluted with buffer A containing 0.15 M NaCl from a heparin-agarose column (no sulfotransferase activity); lane 5, 20 µg of protein eluted with buffer A containing 0.75 M NaCl from a heparin-agarose column (high sulfotransferase activity); lane 6, 20 µg of protein eluted with buffer A containing 50 mM NaCl from a 3',5'-ADP-agarose column (no sulfotransferase activity); lane 7, 20 µg of protein eluted with a NaCl gradient from a 3',5'-ADP-agarose column (low sulfotransferase activity); lane 8, 3 µg of protein eluted with buffer A containing 50 mM NaCl from a 3',5'-ADP-agarose column (low sulfotransferase activity); lane 9, 3 µg of protein eluted with buffer A containing 3',5'-ADP from a 3',5'-ADP-agarose column (high sulfotransferase activity); lane 10, 3 µg of protein eluted with buffer A containing 0.65 M NaCl after 3',5'-ADP gradient elution of a 3',5'-ADP-agarose column (low sulfotransferase activity); lane 11, 3 µg of protein eluted with buffer A from a wheat germ-Sepharose column (low sulfotransferase activity); lane 12, 3 µg of protein eluted with buffer A containing GlcNac from a wheat germ-Sepharose column (high sulfotransferase activity). Molecular size standards are the same as used in Fig. 3B.

TABLE III

| Substrate | [³⁵S]Sulfate incorporation | Golgi membrane | Purified sulfotransferase |
|-----------|---------------------------|----------------|-------------------------|
| Heparan sulfate | 42,510 | 43,300 |
| N-Desulfated heparan sulfate | 43,640 | 46,300 |
| N-Acetylated heparan sulfate | 1,680 | 1,180 |
| Heparin | 1,510 | 2,260 |
| N-Desulfated heparin | 44,040 | 50,530 |
| N-Acetylated heparin | 980 | 300 |
| Chondroitin | 2,700 | 0 |
| Chondroitin sulfate | 2,200 | 0 |
| Glc-Ala-Tyr-OH (3) | 11,150 | 180 |
by SDS-PAGE as shown in Fig. 4. It can be seen that a protein band of $M_r$ 97,000 was predominantly stained with silver nitrate after elution from the wheat germ-Sepharose column with N-acetylgalcosamine (Fig. 4, lane 12). This protein band appears almost exclusively when the peak fractions containing sulfotransferase activity from the glycerol gradient were subjected to SDS-PAGE (Fig. 3B). The molecular weight determined by SDS-PAGE is consistent with that obtained by sedimentation in the glycerol gradient ($M_r$, 94,000). This result suggests that the active form of the sulfotransferase may be a monomer.

**Acceptor Substrates for N-Heparan-sulfate Sulfotransferase**

The purified N-heparan-sulfate sulfotransferase was incubated with different acceptors in order to study the specificity of the enzyme. Table III shows that the apparently pure sulfotransferase was able to transfer sulfate to heparan sulfate, N-desulfated heparan sulfate, and N-desulfated heparin. The enzyme was not active toward N-acetylated heparan sulfate, N-acetylated heparin, chondroitin, chondroitin sulfate, and a tyrosine-containing tripeptide. However, chondroitin, chondroitin sulfate, and a tyrosine-containing tripeptide were acceptors for other sulfotransferases of the Golgi membranes, suggesting the presence of specific sulfotransferases in this organelle (Table III). The above results suggest that the purified heparan-sulfate sulfotransferase transfers sulfate to the nitrogen of the glucosamine in heparan-like molecules.

In order to obtain direct evidence for an N-heparan-sulfate sulfotransferase, heparan sulfate was labeled with $[^{35}S]PAPS$ in the presence of the purified sulfotransferase. The reaction product was then treated with nitrous acid and chromatographed on a Sephadex G-50 column. More than 94% of $[^{35}S]$ sulfate migrated as free sulfate, indicating that the purified sulfotransferase had catalyzed the transfer of sulfate to the nitrogen of the glucosamine in the heparan sulfate chains.

**DISCUSSION**

We have purified rat liver N-heparan-sulfate sulfotransferase to apparent homogeneity. At important step in the purification of the sulfotransferase was the use of 3',5'-ADP-agarose as affinity adsorbent. 3',5'-ADP is one of the products of the sulfotransferase reaction and binds with high affinity to the enzyme. The sulfotransferase was eluted from a first 3',5'-ADP-agarose column with NaCl and from a second one with 3',5'-ADP. Both steps gave an overall purification of close to 600-fold.

Because heparan sulfate is one of the substrates for the N-heparan-sulfate sulfotransferase, a heparin-agarose column was used as an early step in the purification. It was reasoned that the structural similarities between heparan sulfate and heparin would result in specific interaction of the above sulfotransferase with heparin-agarose. We were able to elute the enzyme from a heparin-agarose column with NaCl and also with heparin (not shown). However, it was found that heparin bound to the enzyme so tightly that it interfered in subsequent purification steps. For this reason, heparin-agarose affinity chromatography was only used as a purification step when NaCl was used to elute the enzyme activity.

A similar type of experimental approach, using reaction substrates or products as affinity adsorbent ligands, has been previously used with success for the purification of several glycosyltransferases of the Golgi apparatus (20–25).

Following the 65,000-fold purification of the N-heparan-sulfate sulfotransferase, the final specific activity of the enzyme was approximately 2.0 units/mg of protein. This is in the range of values for specific activities reported for Golgi glycosyltransferases such as rat liver $\alpha 2,6$-sialyltransferase (22) and $\alpha 1,3$-mannoside $\beta 1,2$-N-acetylgalcosaminyltransferase (45).

The heparan-sulfate sulfotransferase appears to be glycoprotein because it binds to a wheat germ-Sepharose column and can be eluted specifically with N-acetylgalcosaminic acid concentrations similar to those used for elution of other glycoproteins of these columns. The enzyme appears to be a monomer based on the similarity between its molecular weight on SDS-PAGE and a glycerol gradient.

The substrate specificity of the purified N-heparan-sulfate sulfotransferase deserves some attention. At least 94% of the sulfates transferred to heparan sulfate was to the nitrogen of glucosamine. This observation and the fact that the enzyme appeared to be inactive toward N-acetylated heparan sulfate and N-acetylated heparin but was active toward N-deacetylated heparan sulfate and N-deacetylated heparin strongly suggest that the sulfotransferase has very low, if any, O-sulfotransferase activity. The enzyme also appeared to be inactive toward other glycosaminoglycan-containing structures such as chondroitin and chondroitin sulfate and toward tyrosine-containing tripeptides.

In a previous study, Göhler et al. (35, 44) reported that a sulfotransferase from calf arterial tissue microsomes exhibited both N- and O-sulfotransferase activity. One cannot rule out the presence of more than one sulfotransferase in the preparation because this enzyme was purified only 400-fold over homogenate.

The above described high degree of specificity of the N-heparan-sulfate sulfotransferase suggests that other sulfotransferases with different specificities such as for O-groups, present in C-3 and C-6 of glucosamine and C-2 of iduronic acid, will be found. The previous observation and the fact that N-sulfation of the entire polysaccharide must be terminated before O-sulfation of heparan occurs (35, 44) suggest a high degree of regulation of the synthesis of heparan sulfate proteoglycan. It is possible that this may be the result of specific spatial organization of the different sulfotransferases in the Golgi apparatus.

Recent studies (26–28) using immuno-electron microscopy have localized several glycosyltransferases to specific regions in the hepatic Golgi apparatus. No information on this is available for sulfotransferases; previous biochemical studies (19) suggest that the different reactions involved in the addition of glycosaminoglycans to proteoglycans occur in the Golgi apparatus. Kinetic studies on the synthesis of cartilage suggest that sulfation of glycosaminoglycans chains occurs late (36). The sulfation reactions may also be a late event in the biogenesis of proteoglycans of heparan sulfate. Then, one may hypothesize that the sulfotransferases are localized, in general, toward the trans Golgi or trans tubular network region of the Golgi apparatus rather than the cis side. The observation of antibodies against the purified N-heparan-sulfate sulfotransferase should enable us to explore further this particular question. These antibodies should also be useful in determining whether there is a structural relationship among the Golgi sulfotransferases, human or animal serum sulfotransferases (37), and those secreted in the medium of cells grown in tissue culture.

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