Molecular Cloning and Characterization of a Human Uronyl 2-Sulfotransferase That Sulfates Iduronyl and Glucuronyl Residues in Dermatan/Chondroitin Sulfate*

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A partial-length human cDNA with a predicted amino acid sequence homologous to a previously described heparan sulfate iduronyl 2-sulfotransferase (Kobayashi, M., Habuchi, H., Yoneda, M., Habuchi, O., and Kimata, K. (1997) J. Biol. Chem. 272, 13980–13985) was obtained by searching the expressed sequence-tagged (EST) data bank. Northern blot analysis was performed using this homologous cDNA as a probe, which demonstrated ubiquitous expression of messages of 5.1 and 2.0 kilobases in a number of human tissues and in several human cancer cell lines. Since the human lymphoma Raji cell line had the highest level of expression, it was used to isolate a full-length cDNA clone. The full-length cDNA was found to contain an open reading frame that predicted a type II transmembrane protein composed of 406 amino acid residues. The cDNA in a baculovirus expression vector was expressed in Sf9 insect cells, and cell extracts were then incubated together with 3-[35S]phosphoadenosine 5'-phospho[35S]sulfate and potential glycosaminoglycan acceptors. This demonstrated substantial sulfotransferase activity with dermatan sulfate, a small degree of activity with chondroitin sulfate, but no sulfotransferase activity with desulfated N-resulfated heparin. Analysis of [35S]Sulfate-labeled disaccharide products of chondroitin ABC, chondroitin AC, and chondroitin B lyase treatment demonstrated that the enzyme only transferred sulfate to the 2-position of uronyl residues, which were preponderantly iduronyl residues in dermatan sulfate, but some lesser transfer to glucuronyl residues of chondroitin sulfate.

Dermatan sulfate is a glycosaminoglycan polysaccharide consisting of N-acetylgalactosamine (GalNAc) residues alternating with varying proportions of glucuronyl (GlcA)¹ and iduronyl (IdeA) residues that are formed from the GlcA by epimerization during polymerization and GalNAc 4-sulfation (1–4). Thus dermatan sulfate can be considered as a variant of chondroitin 4-sulfate, containing some IdeA as well as GlcA, with the IdeA only found next to 4-sulfated GalNAc residues (5). In addition the IdeA of dermatan sulfate is frequently 2-sulfated (6). Some 2-sulfation of GlcA on chondroitin sulfate has also been found but only next to GalNAc 6-sulfate rather than GalNAc 4-sulfate. Proteoglycans containing dermatan sulfate are ubiquitously present in most tissues, where the dermatan sulfate portion may be involved in various biological activities presumably relating in great part to its fine structure. Activities include interaction with heparin cofactor II (7, 8) requiring repeating 2-sulfated iduronyl-containing disaccharide units (7), hepatocyte growth factor/scatter factor (9), and promotion of fibroblast growth factor-2 during wound repair (10). Although there is little information concerning detailed biological activities based on the structural diversity of the dermatan sulfate, the 2-sulfation of IdeA would appear to be of special interest.

The only galactosaminoglycan sulfotransferase that has been cloned to date is a chondroitin 6-sulfotransferase (11). However, several glucosaminoglycan sulfolysotransferases have been cloned (12–19), including an IdeA 2-sulfotransferase for heparan sulfate (15, 20). It seemed likely that this enzyme would have similarities to IdeA 2-sulfotransferase for dermatan sulfate. Therefore, in an attempt to find such an IdeA 2-sulfotransferase, we employed the heparan sulfate IdeA 2-sulfotransferase sequence to obtain a related expressed sequence-tagged (EST) clone. This provided for the molecular cloning of a human cDNA which we found to encode a uronyl 2-sulfotransferase. We have found this enzyme to have no 2-sulfotransferase activity with heparan sulfate but to be involved in the sulfation of the IdeA residues of dermatan sulfate with some lesser activity in 2-sulfation of GlcA residues in chondroitin sulfate.

EXPERIMENTAL PROCEDURES

Heparan Sulfate 2-Sulfotransferase Homologous cDNA and Generation of PCR Probe to Isolate Full-length Clone—The National Center for

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MTN Blot membranes were prehybridized in an ExpressHyb at 68 °C SSC, 0.5% SDS at 55 °C for 20 min. The human and cancer cell line host strain Y1090r were plated at 4

Northern Blot Hybridization—To obtain full-length clones, we first scanned tissue-specific and cell type-specific expression of the cDNA by Northern blot hybridization using the32P-labeled PCR probe. Tissue

Scanning of cDNA Library—The human lymphoma 5'-STRETCH PLUS cDNA Library (CLONTECH) was obtained from mRNA from Burkitt’s lymphoma Raji cell line at EcoRI-cloning sites of the αgt11 vector by the priming method with oligo(dT) and random primers. The host strain Y1090r—cells were infected with phage from the library, plated at 4 × 107 plaque-forming units/dish, and approximately 1.2 × 108 plaques were screened. Colony/Plaque screen™ (NEN Life Science Products) membrane replicas of the plaques were fixed by the rapid autodigestion of DNA by phosphodiesterase and blotted onto nitrocellulose. A single hybrization was performed in an ExpressHyb™ hybridization solution (CLONTECH) for 30 min at 68 °C, and hybridized in the same solution containing the denatured 32P-labeled probe at 68 °C for 16 h according to the manufacturer’s protocol with a 16-h modification. The filters were washed twice with 2× SSC, 0.05% sodium dodecyl sulfate (SDS) for 20 min at 22 °C, and then twice with 0.1× SSC, 0.1% SDS for 20 min at 50 °C. The positive clones were detected by autoradiography.

Characterization of cDNA Clones—Plaque solutions from the positive clones were initially characterized by LD-insert screening amplifier sets (CLONTECH) according to the manufacturer’s PCR protocol. The resultant PCR products were subjected to agarose gel electrophoresis to determine the sizes of the inserts, recovered from the gel, and sequenced. Agt11 DNA of the clones was isolated from its plate lysate using a Qiagen lambda kit (Qiagen), subcloned into pcDNA3 vector (Invitrogen) at the EcoRI sites, and sequenced again to confirm sequence data. For DNA sequencing, the 5’ and 3’ insert regions were enzymatically sequenced from flanking primer sites of the respective PCR fragments or vectors. The remaining sequences of both strands were obtained with internally priming oligonucleotides. Primers were spaced every 100 bp apart with a 400-bp overlap to minimize the 3’-end overlap. Automated fluorescence sequencing was performed with Perkin-Elmer Applied Biosystems models 373A and 477 DNA Sequencers. The DNA sequence files obtained were aligned and compiled with Sequencher (Gene Codes Corp.) and GENETYX-MAC (Software Development Corp.) computer programs. Sequence comparison searches were performed on the data bases of GenBank™, EMBL, PDB, SwissProt, SPudate, PIR, and dbEST.

Construction of Baculovirus Expression Vector—The PufII-EcoRI fragment containing the coding region from positions 193 to 1,382 shown in Fig. 1 was excised from the αgt11 cloning vector, blunted with T4 DNA polymerase, and ligated into the StuI site of the pFAStBAC™ competent cells (Life Technologies, Inc.) transformed with the recombinant pFAStBAC HTa donor plasmid according to the manufacturer’s instructions. The recombinant molecules were isolated and amplified in Sf9 insect cells (Invitrogen) seeded onto 35-mm culture dishes containing 2 ml of SF-900 II SFM (Life Technologies, Inc.) according to the manufacturer’s instructions with slight modifications. Sf9 insect cells (Invitrogen) were seeded onto 35-mm culture dishes containing 2 ml of SF-900 II SFM (Life Technologies, Inc.) were transfected with bacmid-sulfotransferase (presumptive) or bacmid-heparan sulfate 2-sulfotransferase using CELLfectIN™ reagent (Life Technologies, Inc.). The medium was replaced with Grace’s insect medium (Invitrogen), 10% fetal bovine serum (JRH Biosciences), and the culture was continued for another 3 days at 27 °C. The spent medium was centrifuged for 5 min at 500 × g to obtain the virus-containing supernatant as viral stock.

Expression of cDNA—cDNA was expressed using a BAC-TO-BAC™ HT baculovirus expression system (Life Technologies, Inc.) according to the manufacturer’s instructions with slight modifications. Sf9 insect cells (Invitrogen) seeded onto 35-mm culture dishes containing 2 ml of SF-900 II SFM (Life Technologies, Inc.) were transfected with bacmid-sulfotransferase (presumptive) or bacmid-heparan sulfate 2-sulfotransferase using CELLfectIN™ reagent (Life Technologies, Inc.). The medium was replaced with Grace’s insect medium (Invitrogen), 10% fetal bovine serum (JRH Biosciences), and the culture was continued for another 3 days at 27 °C. The spent medium was centrifuged for 5 min at 500 × g to obtain the virus-containing supernatant as viral stock. 150-mm Petri dishes of Sf9 cells were then infected by each recombinant viral stock and incubated at 27 °C for 3 days. After collecting the spent medium, the 150-mm Petri dishes of infected Sf9 cells were washed with phosphate-buffered saline, scraped, and homogenized in a 3-ml solution of 10 mM Tris-HCl, pH 7.4, 0.5% (w/v) Triton X-100, 0.15 M NaCl, 10 mM MgCl2, 2 mM CaCl2, 20% (v/v) glycerol, and a mixture of protease inhibitors (5 µg Na-p-tosyl-l-lysine chloromethyl ketone, 3 µg N-tosyl-l-phenylalanine chloromethyl ketone, 30 µg phenylmethylsulfonyl fluoride, and 3 µg pepstatin A) as described previously (15, 20). After 1 h of gentle stirring at 4 °C, the homogenate was centrifuged at 4 °C for 30 min at 10,000 × g. Sulfoaminotransferase activities in the supernatant fractions (cell extracts) were measured as described below. Protein contents of the cell extracts were estimated by a micro-BCA protein assay reagent kit (Pierce) using bovine serum albumin as a standard. Assay for Sulfoaminotransferase Activity—Completely desulfated and N-resubstituted heparan (CDNSN-heparin) and shark cartilage chondroitin sulfate B (CSB) were obtained from Seikagaku; porcine skin dermal sulfate was obtained from Sigma. Chemical desulfation (23) was used to obtain dermatan and chondroitin, which resulted in apparent complete desulfation of the chondroitin but left small amounts of 4-sulfate on the dermatan. A standard reaction mixture (25 µl) contained 1.25 µmol of imidazole HCl, pH 6.8, 1.88 µg of proteomine chloride, 12.5 µg of glycosaminoglycan, 0.5 mmol (2.5 µC/ 15 µCi) 35S-labeled 35S-labeled 3-phosphoadenosine 5-phosphate ([35S]PAP) (Seikagaku Biobest, Inc.), and 5 µl of the cell extract. After incubation at 37 °C for 30 min, the reaction mixtures were directly spotted on Whatman No. 1 paper and chromatographed in ethan, 1 ml ammonium acetate (5.2 (v/v)) overnight. The origins, which contained the sulfated products, were assayed for radioactivity as described previously (24).

Structural Analysis of 35S-Labeled Products—In order to obtain sufficient labeled products for detailed analyses, higher specific activity 5’-phosphothreo[35S]sulfate (~150 µCi/nmol) prepared as described previously (25) was used. After phenol:chloroform:isoamyl alcohol (25:24:1) treatment and subsequent ethanol precipitation, the 35S-labeled glycosaminoglycans were digested with protease-free chondroitin ABC lyase (20 million units/µg substrate), chondroitin AC I lyase (100 million units/µg substrate), or chondroitin B lyase (100 million units/µg substrate) (Seikagaku Corp.) for 16 h at 37 °C (30 °C for B lyase) and boiled at 100 °C for 1 min to terminate the reaction. Products (~9,000 pmol) were applied on Bio-Gel P-2 (Bio-Rad) columns (0.75 × 200 cm) that were equilibrated and eluted with 0.1 M ammonium bicarbonate at a flow rate of 4 ml/h and assayed for radioactivity. Aliquots of the boiled ABC lyase degradation mixture were then incubated for an additional 16 h at 37 °C with the dermatan sulfate substrate (100 µg) provided by K. Yoshida, Seikagaku Corp.). (b) chondroitinase A (100 units/µg substrate) or (c) chondroitinase B (100 units/µg substrate) (Seikagaku Corp.). ~35S-Labeled digests (~3,000 cpmp) after and sulfatase treatment were then analyzed by high performance liquid chromatography (HPLC) on a column of YMC-Pack Polyimine II (0.46 × 25 cm) (YMC), injected together with unsaturated saccharide

Dermatan/Sulfate 2-Sulfotransferase
standards (Seikagaku Corp.) and eluted with a linear gradient from 16 to 530 mM NaH₂PO₄ over a 60-min period at a flow rate of 1.0 ml/min (27). Fractions of 0.5 ml were collected and mixed with 6 ml of Eco-Lume™ (ICN Biomedicals), and the radioactivity was determined.

RESULTS

cDNA and Predicted Protein Sequence of the Presumptive Sulfotransferase—In order to screen for IdecA 2-sulfotransferase cDNA, the predicted amino acid sequence is shown below the nucleotide sequence. The putative transmembrane hydrophobic domain (see Fig. 2) is boxed, and the polyadenylation signal is underlined with a dashed line. The five possible sites for N-glycosylation are shown with black dots. A point mutation found in the isolated clone is underlined with a solid line.

Fig. 1. Nucleotide sequence and predicted amino acid sequence of the presumptive sulfotransferase cDNA. The predicted amino acid sequence is shown below the nucleotide sequence. The putative transmembrane hydrophobic domain (see Fig. 2) is boxed, and the polyadenylation signal is underlined with a dashed line. The five possible sites for N-glycosylation are shown with black dots. A point mutation found in the isolated clone is underlined with a solid line.

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ferases the NCBI Data Bank of EST cDNA clones was probed with the deduced amino acid sequence of CHO cell heparan sulfate IdecA 2-sulfotransferase cDNA (15). As described under "Experimental Procedures," a human partial-length cDNA clone, Clone ID HE9M306, was found, encoding a novel related species. The cDNA from this clone was 3,743-bp (positions 473–4215) in length as shown in Fig. 1. The 614-bp (positions 473–1086) PCR probe for library screening and Northern hybridization was generated as described under "Experimental Procedures." Approximately 1.2 × 10^6 plaques of a Agt11 human lymphoma Raji cell cDNA library were screened using this PCR fragment as a probe, resulting in 25 positive clones. Sixteen insert cDNAs of these clones were selected, amplified, and sequenced as described under "Experimental Procedures," but only one (1.4 kb) appeared to have the complete coding sequence of the presumptive sulfotransferase.

The amino-terminal sequence of this clone was found to contain three in-frame ATG codons and a TGA stop codon in frame at position 56 upstream from the first ATG codon. A single open reading frame beginning at the first ATG codon predicted a protein of 406 amino acid residues with a molecular mass of 47,672 Da with five potential N-linked glycosylation sites. Hydropathic analysis (28) of the predicted amino acid sequence of the presumptive sulfotransferase revealed that it had a prominent type II membrane protein hydrophobic segment in the amino-terminal region, 18 residues in length at positions 48–65 (Figs. 1 and 2).

Comparison of the sequence of this human presumptive sulfotransferase with CHO cell heparan sulfate IdecA 2-sulfotransferase (GenBank™ accession number D88811) (15) revealed ~30% identity and ~50% similarity at the amino acid level. In particular, extensive homology existed across 210 amino acid residues from 114 to 320 for a consensus sequence in the middle region of these enzymes, which included the 5'-phosphophosphate binding motif, its catalytic Lys, and 3'-phosphate binding motif corresponding to the reports of new algorithms using PAPS on substrates (29–31) (Fig. 3). There was no significant homology at the nucleotide level. In addition there was considerable homology and identity with a protein from Caenorhabditis elegans (GenBank™ accession number Z81479) (32) and Drosophila melanogaster segregation distorting protein (GenBank™ accession number P25722) (33) (Fig. 3). It shared little overall sequence similarity and no common sequence elements with any glycosaminoglycan sulfotransferase previously reported (11–14, 16–19) other than the heparan sulfate IdecA 2-sulfotransferase (15), indicating that it was also most likely an IdecA 2-sulfotransferase.

Expression of the Presumptive 2-Sulfotransferase in a Baculovirus System—Extracts from cells infected with recombinant viral stocks containing the bacmid-presumptive 2-sulfotransferase showed (Table I) incorporation of [35S]sulfate from 5'-phospho[35S]sulfate into dermatan sulfate, with lesser incorporation into dermatan and chondroitin sulfate, and no incorporation into chondroitin. There was also no net incorporation into CDSNS-heparin by this cell extract over that incorporated by control cell extract which contained significant endogenous heparan sulfate sulfotransferase activity. Cells infected with recombinant viral stocks containing the bacmid-heparan sulfate IdecA 2-sulfotransferase showed net incorporation of sulfate only into CDSNS-heparin, with none into dermatan sulfate, deramatan, or chondroitin sulfate. These results indicated that the isolated cDNA encoded a protein that presumably had dermatan IdecA 2-sulfotransferase activity with lesser amounts of chondroitin GlcA 2-sulfotransferase activity.

Northern Blot Analysis—Human RNA Master Blot analysis demonstrated ubiquitous expression of the gene across a wide range of human tissues (data not shown). Human MTN Blot

![Fig. 2. Hydrophobicity analysis of the predicted protein.](image)

![Fig. 3. Sequence comparison of the presumptive sulfotransferase with heparan sulfate IdecA 2-sulfotransferase and sequences from C. elegans and D. melanogaster.](image)
A 32P-labeled presumptive 2-sulfotransferase-specific probe for autoradiography as described under "Experimental Procedures." The positions of the molecular size standards are indicated at the left.

Characterization of Dermatan [35S]Sulfate and Chondroitin [35S]Sulfate—[35S]Labeled dermatan/chondroitin sulfate glycosaminoglycans were digested with chondroitin ABC lyase alone and with chondroitin ABC lyase immediately followed by disaccharide 2-sulfatase, 4-sulfatase, or 6-sulfatase. The digests were then analyzed by HPLC on a YMC-Pack Polymamine II column as described under "Experimental Procedures." The major [35S]-labeled disaccharide from dermatan sulfate was found to chromatograph with standard ΔDi-2,4S (ΔHexA-2S-GalNAc-4S) (Fig. 5A). Following 2-sulfatase digestion, the radioactivity was shifted to the position of free sulfate (Fig. 5B); following 4-sulfatase it was shifted to the position of ΔDi-2S (ΔHexA-2S-GalNAc) (Fig. 5C); but following 6-sulfatase it did not shift (Fig. 5D). These results established that the enzyme was a uronyl 2-sulfotransferase. Chondroitin B lyase, which degrades between GalNAc-4S and IdeA or IdecA-2S but not if there is GlcA or GlcA-2S, provided disaccharides as the only [35S]-labeled product (not shown). This confirmed that the enzyme was an IdecA 2-sulfotransferase.

Desulfated dermatan sulfate and chondroitin sulfate C used similarly as potential [35S]sulfate acceptors were degraded, and disaccharide products were characterized in the same fashion. Comparison with the disaccharide products from the dermatan sulfate 2-sulfation are shown (Table II). The predominant disaccharides from [35S]-labeled desulfated dermatan sulfate were shown to be ΔDi-2S and ΔDi-2,4S, indicating that 2-sulfation could take place on IdecA residues adjacent to non-

| Glycosaminoglycan acceptor | Recombinant sulfotransferases | No cDNA | DS/CS | HS |
|----------------------------|-------------------------------|---------|------|-----|
| None                       |                               | 60      | 50   | 50  |
| Dermatan sulfate           |                               | 100     | 3,400| 70  |
| Dermatan                    |                               | 90      | 1,500| 45  |
| Chondroitin                 |                               | 50      | 250  | 50  |
| Chondroitin sulfate         |                               | 70      | 45   | 65  |
| CDSNS-heparin               |                               | 2,380   | 1,250| 11,600 |

a Dermatan/chondroitin uronyl 2-sulfotransferase.
b Chondroitin sulfate 4-sulfatase.
c Chemically desulfated chondroitin sulfate with small amount of residual 4-sulfate.
d Seikagaku chondroitin sulfate C with 90% 6-sulfate, 10% 4-sulfate.
e Chemically desulfated chondroitin sulfate with no residual sulfate.
f Completely desulfated N-resultated heparin.

Fig. 4. Northern blot analysis of the presumptive 2-sulfotransferase messages. A, tissue type-specific analysis with a human MTN Blot membrane was performed as described under "Experimental Procedures" on 2 μg of poly(A)⁺ RNA from human heart (lane 1), brain (lane 2), placenta (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7), and pancreas (lane 8). B, cell type-specific analysis with a human cancer cell line MTN Blot membrane was performed on 2 μg of poly(A)⁺ RNA from promyelocytic leukemia HL-60 (lane 1), HeLa cell S3 (lane 2), chronic myelogenous leukemia K-562 (lane 3), lymphoblastic leukemia MOLT-4 (lane 4), Burkitt’s lymphoma Raji (lane 5), colorectal adenocarcinoma SW480 (lane 6), lung carcinoma A549 (lane 7), and melanoma (lane 8). Membranes were hybridized with a [32P]-labeled presumptive 2-sulfotransferase-specific probe for autoradiography as described under "Experimental Procedures." The positions of the molecular size standards are indicated at the left.

membrane analysis (Fig. 4A) demonstrated a major band of 5.1 kb and a minor band of 2.0 kb for human tissues. Analysis with the human cancer cell line MTN Blot (Fig. 4B) showed the same two bands except with promyelocytic leukemia HL-60 and chronic myelogenous leukemia K-562. Burkitt’s lymphoma Raji cell line showed the greatest expression. For this reason the cDNA library of this lymphoma cell line was chosen as the cDNA source to isolate the present gene.
sulfated GalNAc as well as next to the small amount of GalNAc-4S that apparently had remained following chemical desulfation to prepare the dermatan. B lyase had no action on the chondroitin sulfate C, but there were 35S-sulfated disaccharides equally produced by ABC lyase or AC lyase that were found to consist almost entirely of ΔDi-2,6S (ΔHexA-2S-GalNAc-6S). This indicated that some sulfation of GlcA residues had taken place but essentially only if there were an adjacent GalNAc-6S. This was confirmed by showing that there was no incorporation of sulfate into GlcA residues of the desulfated chondroitin. It was of interest to note that the need for GalNAc-6S adjacent to a GlcA was in contrast to the sulfation of IdceA of dermatan sulfate that occurred mainly where there was an adjacent GalNAc-4S. We also used chondroitin sulfate A (Seikagaku Corp.) as an acceptor. However, chondroitin AC lyase did not degrade it completely, showing that it contained considerable dermatan residues as well as chondroitin 4-sulfate and chondroitin 6-sulfate. Analysis of the small amount of 2-sulfated AC lyase products indicated that there was mainly ΔDi-2,6S, a minor amount of ΔDi-2,4S, and no ΔDi-2S (data not shown).

### DISCUSSION

Comparison of the amino acid sequences of the dermatan/chondroitin sulfate 2-sulfotransferase with the CHO cell heparan sulfate IdceA 2-sulfotransferase (15) as well as the C. elegans (32) and D. melanogaster proteins (33) showed ~50% similarity, which was concentrated on the middle region across 210 amino acid residues. This was where the 5'-phosphosulfate binding motif, its catalytic Lys, and 3'-phosphate binding motif were found, corresponding to the previous reports concerning the heparan sulfate IdceA 2-sulfotransferase (29, 30) (Fig. 3). The homologous regions of these two uronyl 2-sulfotransferases can be partially characterized as containing four invariant cysteines (Fig. 3, consensus sequence 247, 255, 268, 274), whereas the sulfotransferase domain of heparan sulfate GlcNS 3-sulfotransferase isoforms (16, 18), 6-sulfotransferase (17), and N-sulfotransferase isoforms (12–14) contained no more than two invariant cysteines. In addition, neither the sequence of the so-called “P-loop,” GXXGXXKR, often observed in sulfotransferases and thought to be a possible ATP- or GTP-binding site (34), nor LERGCR, the putative PAPS-binding site found in arylsulfotransferase IV (35), was found in either of the two IdceA 2-sulfotransferases.

The amino-terminal sequence of the dermatan/chondroitin sulfate 2-sulfotransferase cDNA contains three in-frame ATG codons (Fig. 1). When the sequence surrounding the first ATG codon is compared with the eukaryotic consensus translation sequence (36, 37), the purine G at position −3 is conserved, whereas G at position +4 is not. The sequence surrounding the second and third ATG codons (Met-21 and Met-52 in Fig. 1) also partially fit the consensus sequence; the nucleotide at position −3 of these ATG codons is not a purine, whereas the nucleotide at position +4 is G. The third ATG codon (Met-52), however, is unlikely to be an initiation site because of its location in the amino-terminal transmembrane domain (Fig. 1). It remains to be determined which ATG codons could function as the initiation codon.

Northern analysis showed two transcripts of 5.1 and 2.0 kb (Fig. 4), similar to heparan sulfate IdceA 2-sulfotransferase (5.0 and 3.0 kb) (15). Such multiple transcripts of different sizes are also observed in other glycosaminoglycan sulfotransferases (11–13, 18, 38) and are likely to be due to the difference in size and sequence of the untranslated regions. The possible existence of largely different untranslated regions may be important in the function and distribution of the transcripts (38).

The expressed dermatan/chondroitin sulfate uronyl 2-sulfotransferase catalyzed some 2-sulfation of the IdceA residues of IdceA-GalNAc and better sulfation of IdceA-GalNAc-4S. No 2-sulfation of dermatan-6S or 4,6S residues was found. The enzyme also had some activity in 2-sulfation of GlcA residues of GlcA-GalNAc-6S of chondroitin sulfate, but essentially no 2-sulfation of unsulfated disaccharide residues and little or no sulfation of 4S disaccharide residues (Table II). The activity on chondroitin sulfate raises the possibility that this enzyme functions in vivo for 2-sulfation of chondroitin 6-sulfate, but alternatively it is possible that this is due to a certain degree of nonspecificity. The results conform with the 2-sulfation of IdceA and GlcA found in connective tissue of many species, where IdceA-2S has not been described in the absence of GalNAc-4S, and GlcA-2S has not been described in the absence of GalNAc-6S. The differences in GalNAc sulfation specificities are apparently due to differences in the conformation of IdceA and GlcA with their positioning relative to the GalNAc-4S and GalNAc-6S, respectively. The results show that 2-sulfation of IdceA preferentially occurs next to GalNAc-4S rather than non-sulfated GalNAc, and 2-sulfation of GlcA requires GalNAc-6S with no 2-sulfation next to non-sulfated GalNAc. Thus the biosynthetic order apparently proceeds by prior GalNAc 4-sulfation for 2-sulfation of IdceA and prior GalNAc 6-sulfation for 2-sulfation of GlcA.

Tissue-specific patterns of epimerization and 4- and 6-sulfation in dermatan sulfate have not been reported in detail, and no preferred chain location or distribution of 2-sulfated IdceA has been reported. Comparison between decorin dermatan sulfate and biglycan dermatan sulfate was reported to show a greater correlation to tissue source than to the core protein (39). It is likely, however, that contiguous disaccharide sequences containing IdceA or IdceA-2S residues might account for the weak interaction reported with many heparin/heparan sulfate-binding proteins such as basic fibroblast growth factor (40), histidine-rich glycoprotein, platelet factor 4 (41), fibromectin (42), interleukin-7 (43), and protein C inhibitor (44). In contrast to these weak interactions, a comparable high affinity interaction of dermatan sulfate has been demonstrated with heparin cofactor II (7, 8) and with hepatocyte growth factor/scatter factor (9). Furthermore, the affinity for heparin cofactor II was shown to be dependent upon 2-sulfation of the IdceA residues (45). Sulfation profiles of chondroitin sulfate have been shown to change with concomitant specific spatiotemporal patterns in various tissues, suggesting that differences in sulfation position and degree might have distinct functions in development (46).

The GlcA 2-sulfated chondroitin has been shown to be expressed by immature glial cells of the central nervous system to promote neurite outgrowth (47) and to be expressed in the cerebellum and the telencephalon of adult mouse (48). This would suggest that our finding of IdceA/GlcA 2-sulfotransferase expression in brain and especially cerebellum (data not shown) could account for the 2-sulfation of chondroitin sulfate in brain. Disaccharide residues of GlcA-2S-GalNAc-6S have also been identified in mouse mast cells derived from immune...
lymph nodes and function as an important phenotypic marker distinguishing different mast cell subsets (49) and mouse tooth germ basement membrane (46). Characteristic oligosaccharide sequences including 2-sulfated IdeA or GlcA residues in DS and CS may serve as functional domain structures recognized by some protein ligands as in heparin/heparan sulfate.

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