Molecular Cloning and Expression of a Novel Chondroitin 6-O-Sulfotransferase

Hiroshi Kitagawa, Masaki Fujita, Nobue Ito, and Kazuyuki Sugahara†
From the Department of Biochemistry, Kobe Pharmaceutical University, Higashinada-ku, Kobe 658-8558, Japan

A novel human chondroitin 6-O-sulfotransferase, designated C6ST-2, was identified by BLAST analysis of expressed sequence tag using the sequence of a previously described human chondroitin 6-O-sulfotransferase (C6ST-1) as a probe. The new cDNA sequence revealed an open reading frame coding for a protein of 486 amino acids with a type II transmembrane protein topology. The amino acid sequence displayed 24% identity to the human C6ST-1, and the highest sequence identity was found in the COOH-terminal catalytic domain. The expression of a soluble recombinant form of the protein in COS-1 cells produced an active sulfotransferase with marked specificity for polymer chondroitin. In contrast, keratan sulfate and oligosaccharides containing the Galβ1→4GlcNAc sequence, which are good acceptor substrates for the C6ST-1, hardly served as acceptors. The identification of the reaction product indicated that the enzyme is a novel chondroitin 6-O-sulfotransferase (C6ST-2) that mainly transfers sulfate to N-acetylgalactosamine. The coding region of C6ST-2 was contained in a single exon and localized to chromosome Xp11. Northern blot analysis of human brain poly(A)+ RNA revealed a single transcript of 2.4 kilobase pairs. Reverse transcription-polymerase chain reaction analysis showed that C6ST-2 is developmentally regulated in various tissues with expression persisting through adulthood in the spleen. Thus, we demonstrated the redundancy in chondroitin 6-O-sulfotransferases capable of forming chondroitin 6-sulfate, which is important for understanding the mechanisms leading to specific changes in the sulfation profile of chondroitin sulfate chains in various tissues during development and malignant transformation.

Chondroitin sulfates are synthesized as proteoglycans that can be expressed on the surfaces of most cells and in extracellular matrices that are covalently linked to a wide range of core protein families. Chondroitin sulfate proteoglycans are increasingly implicated as important regulators of many biological processes, such as cell migration and recognition, extracellular matrix deposition, and morphogenesis (for reviews see Refs. 1 and 2). Growing evidence indicates that many of their functions are associated with the sulfated glycosaminoglycan (GAG)3 moieties (for a review see Ref. 3).

Chondroitin sulfate GAG has a linear polymer structure that possesses repetitive, sulfated disaccharide units containing glucuronic acid (GlcA) and N-acetylgalactosamine (GalNAc). These sulfated GAGs are generated by a family of sulfotransferases that transfer sulfate from its high energy donor PAPS to the C-2 or C-3 position of GlcA residues or to the C-4 or C-6 position of GalNAc residues. Sulfotransferases generate considerable structural diversity by transferring sulfate with remarkable specificity for the underlying oligosaccharide substrate (for a review see Ref. 4). Chondroitin sulfate GAGs considerably change in the position and degree of sulfation during normal embryonic development, growth, and malignant transformation (3, 5–7). The regulated expression of sulfated GAGs appears to be dependent on many factors including the availability of PAPS to the Golgi lumen; various competing sulfotransferases; and co-localization of appropriate acceptors, sulfotransferases, and PAPS transporters within a particular Golgi cisterna. However, the most important determinant of sulfated GAGs expression is probably the regulated expression observed for each member of the sulfotransferase gene family (7).

Based on known sulfated structures for chondroitin sulfate, the sulfotransferase gene family has been estimated to consist of at least four independent gene products described above, although it is possible that more sulfotransferases with different specificities exist. In terms of chondroitin 6-O-sulfotransferase (C6ST) that catalyzes the transfer of sulfate from PAPS to the C-6 position of the GalNAc residue, only one orthologous gene, designated C6ST-1, has been cloned to date from chickens, mice, and humans (8–10), despite the growing number of sulfotransferase cDNAs homologous to C6ST-1 that have been cloned (11–14). To search for additional members of the sulfotransferase gene family involved in chondroitin sulfate biosynthesis, the C6ST-1 protein sequence was used to screen the translated data base of expressed sequence tags (EST). Here, we describe the cloning of a human cDNA encoding a novel chondroitin 6-O-sulfotransferase, designated C6ST-2, with high specificity for polymer chondroitin.

† To whom correspondence should be addressed: Dept. of Biochemistry, Kobe Pharmaceutical University, 4-19-1 Motomakita-machi, Higashinada-ku, Kobe 658-8558, Japan. Tel.: 81-78-441-7570; Fax: 81-78-441-7569; E-mail: k-sugar@kobe-pharma-u.ac.jp.

* This work was supported in part by the Science Research Promotion Fund of the Japan Private School Promotion Foundation (to K. S.), Grants-in-aid for Encouragement of Young Scientists 11771460 (to H. K.) and for Scientific Research on Priority Areas #10178102 (to K. S.) from the Ministry of Education, Science, Culture, and Sports of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EBI Data Bank with accession number(s) AB037187.
Cloning of a Second Chondroitin 6-O-Sulfotransferase

EXPERIMENTAL PROCEDURES

MATERIALS—[35S]PAPS and unlabel PAPS were purchased from NEN Life Science Products and Sigma, respectively. Chondroitin (a chemically desulfated derivative of whale cartilage chondroitin sulfate A), various chondroitin sulfate isoforms, desulfated keratan sulfate from bovine cornea, completely desulfated N-sulfated heparin from porcine intestine, four unsaturated standard disaccharides derived from N-acetylated 3-sulfated, 3-sulfate, 4-sulfated, 4-sulfate, 3,6-diacetyl-2-sulfate, and 4,6-diacetyl-2,3-disulfate, were purchased from Seikagaku Corporation (Tokyo, Japan). GlcNAc3P-O-CH, was purchased from Nakarai Tesque (Kyoto, Japan). GlcNAcI-4GlcNAcI-4GlcNAcII-4GlcNAcII-4GlcNAcII (Seikagaku Corporation), Superdex 78 Peptide HH10/30 and HITRAP™ desalting columns were purchased from Amersham Pharmacia Biotech.

Cloning of the Novel Sulfotransferase cDNA—BLASTn analysis of the EST data base at the National Center for Biotechnology Information (National Institutes of Health, Bethesda, MD) with sequences from human C6ST-1 (10), revealed an EST (GenBank™ accession number AI088880). The EST clone (IMAGE™ catalog number 1686986) was obtained from Genome Systems, Inc. (St. Louis, MO). Sequencing of this clone revealed a partial open reading frame with significant sequence similarity to human C6ST-1 and a 3′-untranslated region containing a potential polyadenylation signal. An additional sequence was obtained by 5′-rapid amplification of the cDNA ends (RACE) with the human brain Marathon-Ready™ cDNA kit (CLONTECH, Palo Alto, CA) using an oligo(dT) primer 5′-CTTGGGAACCGAGGAGACTGGTTC-3′. PCR reactions were carried out with Ex Taq polymerase (Takara Shuzo Co., Kyoto) in 5% (v/v) dimethyl sulfoxide for 25 cycles at 94 °C for 10 s and 68 °C for 4 min after 5 cycles at 94 °C for 10 s and 72 °C for 4 min. The PCR products were subcloned into pGEM®-T Easy vector (Promega, Tokyo) and sequenced using a 377 DNA sequencer (Applied Biosystems, Foster City, CA). Clones were sequenced to compensate for misreading by Ex Taq polymerase. The combined cDNA sequence of the EST clone and the 5′-RACE product showed a single open reading frame. After completion, the database search of the Human Genome Project, which recently became available (November 23, 1999) showed a genome sequence (Human Genome Project accession number AL022165) identical to the cDNA sequence. Comparison between the cDNA and the genome sequence revealed the genomic organization of the novel sulfotransferase gene.

Construction of a Soluble Form of the Novel Sulfotransferase—The cDNA fragment of a truncated form of the novel sulfotransferase, lacking the first NH2-terminal 63 amino acids of the novel sulfotransferase, was amplified by PCR using a 5′ primer (5′-CGGATCCCGAGCGAGGAGTTCAAGAGTGATG-3′) containing an in-frame XhoI site and a 3′ primer (5′-CGGATCCCGAGCGAGGAGTTCAAGAGTGATG-3′) containing a partial open reading frame of 1458 bp coding for a protein of 486 amino acid residues in length in the NH2-terminal region, conformed to the Kozak consensus sequence for initiation (18), an in-frame stop codon was present upstream of the asparagine-accepting initiation codon, and an in-frame polyadenylation signal was present. An NheI fragment containing the fusion protein was inserted into the XhoI site of the expression vector pSVL (Amersham Pharmacia Biotech).

Expression of a Soluble Form of the Novel Sulfotransferase and Enzyme Assay—The expression plasmid (6 μg) was transfected into COS-1 cells on 100-mm plates using LipofectAMINE PLUS™ (Life Technologies, Inc.) according to the instructions provided by the manufacturer. Two days after transfection, 1 ml of the culture medium was collected and incubated with 10 μl of IgG-Sepharose (Amersham Pharmacia Biotech) for 1 h at 4 °C. The beads recovered by centrifugation were washed with and then resuspended in the assay buffer and tested for sulfotransferase activity using various polymer GAGs and oligosaccharide derivatives (see “Materials”) as sulfate acceptor substrates and [35S]PAPS as a sulfate donor. The reaction mixtures contained 10 mmoles of [35S]PAPS (about 1.0 × 108 dpm) in a total volume of 60 μl. The reaction mixtures were incubated at 37 °C for 1 h, and the reactions were terminated by boiling for 1 min. After centrifugation at 16,000 × g for 5 min at 4 °C, the supernatant was subjected to gel filtration on a HITRAP™ desalting column when various polymer GAGs were used as acceptors or to anion exchange HPLC on an amine-bound silica PA03 column (YMC Co., Kyoto) using a linear salt gradient from 16 to 530 mmoles of NaH2PO4, as described previously (16), when oligosaccharide derivatives were used as acceptors. The radioactivity was measured with a liquid scintillation counter.

Characterization of the Reaction Products—The reaction products were isolated by gel filtration on a HITRAP™ desalting column equilibrated with 0.25 m NaHCO3/7% 1-propanol. The radioactivity containing the product was pooled and evaporated to dryness. The isolated products were digested with 20 μl of chondroitin ABC. The amino acid composition was determined by an automated amino acid HPLC on an amine-bound silica PA03 column as described previously (16). To confirm the disaccharide structure, chondroitin-4-O-sulfate or 6-O-sulfatase digestion of the chondroitin ABC digest was conducted with the remainder of the [35S]-labeled isolated product as described previously (7).

RESULTS

Molecular Cloning of a Human cDNA Homologous to C6ST-1—We found a short sequence of 494 bp (GenBank™ accession number AI088880) through an EST approach in which we screened human EST data bases for sequences that were homologous to a human C6ST-1 (10). The EST clone from human brain was fully sequenced and found to be a sequence containing a partial open reading frame with significant sequence similarity to human C6ST-1 and a 3′-untranslated region containing a potential polyadenylation signal. An additional sequence was then obtained by 5′-RACE using the human brain cDNA as a template (see “Experimental Procedures”).

The combined cDNA sequence of the EST clone and the 5′-RACE product showed a 5′-untranslated region of 10 bp, a single open reading frame of 1458 bp coding for a protein of 486 amino acids with four potential N-glycosylation sites, and a 3′-untranslated region of 0.7 kb, including a poly(A) tail (Fig. 1). Northern blot analysis indicated that the mRNA was about 2.4 kb in length in various human brain tissues (see below), suggesting that the cDNA was approximately full length. The open reading frame had a high G + C content of 46%, a deduced amino acid sequence corresponded to a 54,294-Da polypeptide. The predicted translation initiation site corresponds to the Kozak consensus sequence for initiation (18), and an in-frame stop codon was present upstream of the assigned initiating ATG codon. A Kyte-Doolittle hydropathy analysis (19) revealed one prominent hydrophobic segment of 19 amino acid residues in length in the NH2-terminal region, predicting that the protein has a type II transmembrane topology characteristic of many Golgi-localized sulfotransferases.
Cloning of a Second Chondroitin 6-O-Sulfotransferase

and glycosyltransferases cloned to date (Fig. 1). An additional characteristic feature in the amino acid sequence of the newly cloned gene was a cluster of six consecutive arginine residues in the cytoplasmic tail, which was located three residues from the amino terminus (Fig. 1).

Data base searches indicated that the amino acid sequence displayed 24, 27, 40, 27, and 27% identity to human C6ST-1 (10), human keratan sulfate Gal-6-O-sulfotransferase (KSGal6ST) (11), human N-acetylglucosamine 6-O-sulfotransferase (Gn6ST) (12), human high endothelial cell N-acetylglucosamine 6-O-sulfotransferase (HEC-Gn6ST) (13), and human intestinal N-acetylglucosamine 6-O-sulfotransferase (I-Gn6ST) (14), respectively. The highest sequence identity was found in the COOH-terminal catalytic domain, in which three highly conserved motifs (I–III), reported in previous studies (13, 14), were observed (Fig. 2). Motifs I and II contained elements that conformed to the recently described consensus binding motifs for the high energy sulfate donor, PAPS, which were predicted from the x-ray crystallographic analyses of estrogen sulfotransferase (20) and N-sulfotransferase (21). These elements are found in all sulfotransferases cloned to date. Notably, six invariant cysteines were observed in the catalytic domain. Comparison of the primary structure of the newly cloned C6ST-2 displayed 24, 27, 27, and 27% identity to human C6ST-1 (10), human KSGal6ST (11), human Gn6ST (12), human HEC-Gn6ST (13), and human I-Gn6ST (14), respectively. There are two regions in which more than four consecutive amino acid clusters are identical among the six sequences. Gaps introduced for maximal alignment are indicated by dashes. Three highly conserved regions named motifs I–III, including putative PAPS binding sites (motifs I and II), are indicated by arrows.

![Fig. 1. Nucleotide and deduced amino acid sequences of the novel sulfotransferase cDNA. The putative membrane spanning domain is underlined, and four potential N-glycosylation sites are marked by asterisks. The presumptive polyadenylation signal ATTAA is boxed. The sequences are numbered relative to the translation initiation site, which begins at the first in-frame ATG codon. The location of an intron is indicated by an arrowhead.](image1)

![Fig. 2. Comparison of the predicted amino acid sequence of the human novel sulfotransferase (C6ST-2), C6ST-1, KSGal6ST, HEC-Gn6ST, and I-Gn6ST. The predicted amino acid sequences were aligned using the GENETYX-MAC (version 10) computer program. Black and shaded boxes indicate that the predicted amino acid in the alignment is identical among all six and more than any four sequences, respectively. The amino acid sequence of the newly cloned C6ST-2 displayed 24, 27, 27, and 27% identity to human C6ST-1 (10), human KSGal6ST (11), human Gn6ST (12), human HEC-Gn6ST (13), and human I-Gn6ST (14), respectively. There are two regions in which more than four consecutive amino acid clusters are identical among the six sequences. Gaps introduced for maximal alignment are indicated by dashes. Three highly conserved regions named motifs I–III, including putative PAPS binding sites (motifs I and II), are indicated by arrows.](image2)
sequences (data not shown). This gene is located on human chromosome Xp11.

Expression of a Soluble Form of the Novel Sulfotransferase and Characterization as C6ST-2—To facilitate the functional analysis of the putative sulfotransferase, a soluble form of the protein was generated by replacing the first 63 amino acids of the putative sulfotransferase with a cleavable insulin signal sequence and a protein A IgG-binding domain as described under “Experimental Procedures,” and then the soluble putative sulfotransferase was expressed in COS-1 cells as a recombinant enzyme fused with the protein A IgG-binding domain. The fused enzyme expressed in the medium was absorbed on IgG-Sepharose beads to eliminate endogenous sulfotransferases, and then the enzyme-bound beads were used as an enzyme source. The bound fusion protein was assayed for sulfotransferase activity using a variety of GAG acceptor substrates. As shown in Table I, activity was detected with polymer chondroitin and desulfated keratan sulfate, although the latter showed 20-fold less sulfate incorporation. In contrast, no activity was detected with various chondroitin sulfate isoforms or completely desulfated N-sulfated heparin. No detectable sulfotransferase activity was recovered by the affinity purification from a control pSVL transfection sample. These findings clearly indicate that the expressed protein is a sulfotransferase with marked specificity for polymer chondroitin.

To identify the sulfotransferase reaction products, polymer chondroitin was labeled with [35S]sulfate by incubation with [35S]PAPS and its degradation products by gel filtration chromatography on a HITRAP™ desalting column and were quantified by liquid scintillation counting. Relative rates for each enzyme are calculated as percentages of the incorporation obtained with polymer chondroitin. The incorporation was 7,342 dpm for C6ST-2 and 58,402 dpm for C6ST-1. The values represent the averages of two independent experiments, where the two series of experiments gave essentially identical results.

| Acceptor Substrate                      | C6ST-2 Relative Rate (%) | C6ST-1 Relative Rate (%) |
|-----------------------------------------|--------------------------|--------------------------|
| Chondroitin                             | 100                      | 100                      |
| Chondroitin sulfate A                   | 0                        | 29                       |
| Chondroitin sulfate B                   | 0                        | 23                       |
| Chondroitin sulfate C                   | 0                        | 26                       |
| Chondroitin sulfate D                   | 0                        | 40                       |
| Chondroitin sulfate E                   | 0                        | 1                        |
| Desulfated keratan sulfate              | 5                        | 38                       |
| CDSNS-heparin*                         | 0                        | 0                        |

*CDSNS-heparin, completely desulfated N-sulfated heparin.*

To distinguish the substrate specificity of C6ST-2 from that of previously cloned C6ST-1, the recombinant soluble C6ST-2 was compared with recombinant human C6ST-1 (10) for their utilization of a variety of GAG acceptor substrates as well as various compounds structurally related to sulfated GAGs including GlcNAcβ1→3Galβ1→4GlcNAc, GlcNAcβ1→3Galβ1→4GlcNAc, GlcAβ1→3Galβ1→4GlcNAc. As shown in Tables I and II, C6ST-2 mainly utilized polymer chondroitin consisting of a nonsulfated disaccharide unit, GlcAβ1→3GalNAc, whereas C6ST-1 utilized various substrates including polymer chondroitin, various chondroitin sulfate isoforms, desulfated keratan sulfate, and

FIG. 4. Identification of the novel sulfotransferase reaction products. Enzymatic reactions were carried out using polymer chondroitin as an acceptor substrate under the incubation conditions described under “Experimental Procedures,” and the reaction products were separated from [35S]PAPS and its degradation products by gel filtration chromatography on a HITRAP™ desalting column and were quantified by liquid scintillation counting. Relative rates for each enzyme are calculated as percentages of the incorporation obtained with polymer chondroitin. The incorporation was 7,342 dpm for C6ST-2 and 58,402 dpm for C6ST-1. The values represent the averages of two independent experiments, where the two series of experiments gave essentially identical results.

FIG. 3. Genomic organization of the human novel sulfotransferase (C6ST-2) gene. Not(N), PvuI(P), and BamHI(B) restriction sites are shown as vertical lines. Exon regions are denoted by boxes. A closed box represents the coding sequence, and open boxes denote the 5' and 3' untranslated sequences. The translation initiation codon (ATG) and the termination codon (TAG) are also shown. The black horizontal bar denotes the intron.
both enzymes utilized the GlcAβ1–3GalNAc sequence as their best substrates, C6ST-2 exhibited a more strict specificity, exhibiting negligible sulfate incorporation into galactosides that are good acceptor substrates for C6ST-1. These findings indicate that the two chondroitin 6-O-sulfotransferases differ in their substrate specificity and that C6ST-2 mainly transfers sulfate to the C-6 position of GalNAc in a nonsulfated disaccharide unit, GlcAβ1–3GalNAc.

Expression Pattern of C6ST-2—Northern blot analysis of mRNA demonstrated a single band of 2.4 kb for all human brain tissues examined (Fig. 5). The expression of the C6ST-2 gene was next determined in various human tissues using PCR-based methods with normalized cDNA pools. A single amplified DNA of the expected size (1328 bp) was obtained from most cDNA preparations of the 18 adult and 8 fetal human tissues examined, although the amounts of the amplified cDNAs varied considerably (Fig. 6). Notably, expression was not detected in adult skin and appears to be developmentally regulated in various tissues with expression persisting through adulthood in the spleen.

DISCUSSION

We cloned the second chondroitin 6-O-sulfotransferase, C6ST-2, which is homologous to but distinct from previously cloned C6ST-1. The two chondroitin 6-O-sulfotransferases exhibit distinct but overlapping acceptor substrate specificities as shown in Tables I and II. Polymer chondroitin is the best substrate for both enzymes, yet C6ST-2 exhibits a more strict specificity, exhibiting negligible sulfate incorporation into other substrates. Although no chondroitin 6-O-sulfotransferase with the specificity demonstrated for C6ST-2 has been reported so far, it has been suggested that at least one such sulfotransferase might exist, based on the observation that C6ST-1 knockout mice synthesize a small amount of chondroitin 6-sulfate and the mice are apparently normal and viable through adulthood (24). This hypothesis has now been confirmed by the cDNA cloning of C6ST-2. It is likely that additional distinct chondroitin 6-O-sulfotransferases exist. For example, because neither C6ST-1 nor -2 transferred sulfate to the C-6 position of a GalNAc residue in a monosulfated disaccharide unit, GlcAβ1–3GalNAc(4-O-sulfate), another chondroitin 6-O-sulfotransferase responsible for the synthesis of a disulfated disaccharide unit, GlcAβ1–3GalNAc(4, 6-O-disulfate) probably exists. Likewise, a chondroitin 6-O-sulfotransferase that transfers sulfate to a monosulfated disaccharide unit, GlcA(2-O-sulfate)β1–3GalNAc to form GlcA(2/O-sulfate)β1–3GalNAc(6-O-sulfate) may also exist. The presence of multiple sulfotransferase isoforms has been reported for heparan sulfate D-glucosaminyl 3-O-sulfotransferases, where four homologous enzymes with distinct but overlapping acceptor specificities have been identified (25). Furthermore, three heparan sulfate D-glucosaminyl 6-O-sulfotransferases have recently been reported (26).

The recombinant C6ST-2 showed high specificity toward polymer chondroitin (Table I). No other chondroitin sulfate isoforms were utilized by the enzyme despite these chondroitin sulfate isoforms containing a small but significant amount of a nonsulfated disaccharide unit, GlcAβ1–3GalNAc. In strong contrast, the recombinant C6ST-1 utilized the nonsulfated disaccharide unit, GlcAβ1–3GalNAc in these isoforms (Ref. 10 and Table I). These findings suggest that the prior sulfation of the acceptor substrates strongly influences subsequent sulfations especially by C6ST-2. Thus, it will be interesting to determine how a preceding sulfation of saccharide residues on the reducing and/or the nonreducing side(s) of saccharide sequences influences the 6-O-sulfation of the penultimate GalNAc using sulfated hexasaccharides such as GlcAβ1–3GalNAc(4-O- or 6-O-sulfate)β1–4GlcAβ1–3GalNAcβ1–4GlcAβ1–3GalNAc(4-O- or 6-O-sulfate) when such hexasaccharides become available.

C6ST-2 may provide functional redundancy with C6ST-1, as implied by the C6ST-1 knockout experiment that showed no apparent anomalies (24). Alternatively or in addition, C6ST-2 may play a unique role in the expression of the sulfation pattern of chondroitin sulfate in some tissues in view of the different specificity (Tables I and II) and distinct tissue expres-
Cloning of a Second Chondroitin 6-O-Sulfotransferase

The human C6ST-1 gene is relatively simple, its protein-coding sequences (30). In contrast, the genomic organization of the C6ST-2 gene in the adult spleen, with modest expression in the heart, lung, skeletal muscle, and spleen. Particularly striking is the abundant expression of the C6ST-2 gene in the adult spleen, with modest expression in the lung, pancreas, ovary, peripheral blood leukocytes, and small intestine (Fig. 6). In contrast, human C6ST-1 exhibited abundant expression in the adult heart, placenta, skeletal muscle, and thymus and very little in adult lung and peripheral blood leukocytes (23). Furthermore, a deficiency in chondroitin 6-O-sulfotransferase activity is reportedly associated with a heritable form of spondyloepiphyseal dysplasia (27, 28), suggesting that the chondroitin 6-O-sulfotransferase appears to be essential for normal skeletal development. Therefore, the products of the genes may not provide functional back-up in all cells. More detailed characterization of the C6ST-1 knockout mice will be required. In addition, generation and analysis of C6ST-2 knockout mice will provide further insights into the possible distinct functions of these genes.

Data base searches indicated that the amino acid sequence of C6ST-2 displayed 24, 27, 29, 30, and 27% identity to the human C6ST-1 (10), KSGal6ST (11), Gn6ST (12), high endothelial cell Gn6ST (HEC-Gn6ST) (13), and intestinal Gn6ST (I-Gn6ST) (14), respectively, showing that they constitute a family of highly conserved enzymes. Although C6ST-2 showed the highest sequence homology among the other five family members to Gn6ST that transfers sulfate exclusively to the B-glucosaminyl (1-3)galactosylacyllactosamine sequence (12), the recombinant C6ST-2 did not show the enzyme activity toward oligosaccharides such as GlcNAcβ1-O-CH₃ and GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc (Table II). Conversely, both C6ST-1 and -2 utilized polymer chondroitin as the best substrates despite the lowest sequence homology between C6ST-1 and -2 among the six cloned sulfotransferases. Thus, it is difficult to predict the substrate specificity of a sulfotransferase simply from its primary sequence analysis. Specificity studies using appropriate oligosaccharide substrates are inevitable.

The genomic organization has been elucidated for several sulfotransferases involved in GAG formation. The protein-coding sequences of the human heparan sulfate N-deacetylase/N-sulfotransferase-1 and -2 genes are distributed over 14 and 13 exons that span approximately 35- and 6.5-kb lengths (29, 30), respectively. Comparison of the genomic organization of these genes shows a similar genetic exon-intron organization within the coding sequences (30). In contrast, the genomic organization of the human C6ST-1 gene is relatively simple, its protein-coding sequence being divided into only two exons that span approximately 8 kb of the genomic sequence (10). In addition, the gene structure of the human KSGal6ST (31), Gn6ST(2), high endothelial cell Gn6ST (HEC-Gn6ST) (13) and intestinal Gn6ST (I-Gn6ST) (14) exhibits an intron-less coding region. Human C6ST-2 falls into the same pattern. Interestingly, chromosomal assignments of the three sulfotransferases, human KSGal6ST, Gn6ST, and C6ST-2, indicate that these genes are localized on different human chromosomes, 11p11.1-11p12.2, 7q31, and Xp11 (Refs. 11 and 12 and this study), respectively, despite the significant homology in nucleotide and amino acid sequences observed among the three genes. These findings strongly suggest that the three sulfotransferases diverged from an ancestor gene early in evolution. It remains to be determined whether the three other sulfotransferase genes, C6ST-1, HEC-Gn6ST, and I-Gn6ST, are likewise dispersed in the human genome.

Acknowledgment—We thank Dr. K. Yoshida for the donation of the enzyme substrate.

REFERENCES

1. Kimata, K., Okayama, M., Oshira, A., and Suzuki, S. (1973) Mol. Cell. Biochem. 1, 211–228
2. Kjellén, L., and Lindahl, U. (1991) Annu. Rev. Biochem. 60, 443–475
3. Poso, A. R. (1986) Biochem. J. 236, 1–14
4. Bowman, K. G., and Bertozzi, C. R. (1999) Chem. Biol. 6, 9–22
5. Roughley, P. J., and White, R. J. (1980) J. Biol. Chem. 255, 217–224
6. Inerot, S., and Heinegard, D. (1983) Collagen Relat. Res. 2, 245–262
7. Kitagawa, H., Tsutsumi, K., Tone, Y., and Sugahara, K. (1997) J. Biol. Chem. 272, 31377–31381
8. Fukuta, M., Uchimura, K., Nakashima, K., Kato, M., Kimata, K., Shimamura, T., and Habuchi, O. (1995) J. Biol. Chem. 270, 18575–18580
9. Uchimura, K., Kadomatsu, K., Fan, Q.-W., Muramatsu, H., Kurosawa, N., Kaname, T., Yamamura, K., Fukuta, M., Habuchi, O., and Muramatsu, T. (1998) Glycobiology 8, 489–496
10. Tsutsumi, K., Shimakawa, H., Kitagawa, H., and Sugahara, K. (1998) FEBS Lett. 441, 235–241
11. Fukuta, M., Inazawa, J., Torii, T., Tsvuzki, K., Shimada, E., and Habuchi, O. (1997) J. Biol. Chem. 272, 32321–32328
12. Uchimura, K., Muramatsu, H., Kadomatsu, K., Fan, Q.-W., Kurosawa, N., Matsunaka, C., Kannagi, R., Habuchi, O., and Muramatsu, T. (1998) J. Biol. Chem. 273, 22577–22583
13. Bistrup, A., Bhakta, S., Lee, J. K., Belov, Y. Y., Gunn, M. D., Zuo, F.-R., Huang, C.-C., Kannagi, R., Rosen, S. D., and Hemmerich, S. (1999) J. Cell Biol. 145, 899–910
14. Lee, J. K., Bhakta, S., Rosen, S. D., and Hemmerich, S. (1999) Biochem. Biophys. Res. Commun. 263, 543–549
15. Kitagawa, H., and Paulson, J. C. (1994) J. Biol. Chem. 269, 1394–1401
16. Sugahara, K., Okumura, Y., and Yamashina, I. (1989) Biochem. Biophys. Res. Commun. 162, 189–197
17. Kitagawa, H., Shimakawa, H., and Sugahara, K. (1999) J. Biol. Chem. 274, 13983–13997
18. Kozak, M. (1984) Nucleic Acids Res. 12, 857–872
19. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
20. Kakuta, Y., Pedersen, L. G., Carter, C. W., Negishi, M., and Pedersen, L. C. (1997) J. Biol. Chem. 272, 904–908
21. Sueyoshi, T., Kakuta, Y., Pedersen, L. C., Wall, F. E., Pedersen, L. G., and Negishi, M. (1998) FEBS Lett. 433, 211–214
22. Breathnach, R., and Chambon, P. (1981) Annu. Rev. Biochem. 50, 349–383
23. Fukuta, M., Kobayashi, Y., Uchimura, K., Kimata, K., and Habuchi, O. (1998) Biochim. Biophys. Acta 1399, 57–61
24. Uchimura, K., Kadomatsu, K., Muramatsu, H., Ishihama, H., Nakamura, E., Kurosawa, N., Habuchi, O., and Muramatsu, T. (1999) Glycoconjug. J. 16, S13–S14
25. Liu, J., Shwakar, N. W., SinaP, S., Schwartz, J. J., Zhang, L., Fritz, L. M. S., and Rosenberg, R. D. (1999) J. Biol. Chem. 274, 5185–5192
26. Habuchi, H., Tanaka, M., Habuchi, O., Yoshida, K., Suzuki, H., Ban, K., and Kimata, K. (2000) J. Biol. Chem. 275, 2859–2868
27. Toledo, S. P., Mourão, P. A., Lamego, C. A., Alves, C. A., Dietrich, C. P., Assis, L. M., and Mattar, E. (1978) Am. J. Med. Genet. 2, 385–395
28. Mourão, P. A., Kato, S., and Donnelly, P. V. (1981) Biochem. Biophys. Res. Commun. 98, 388–396
29. Gladwin, A. J., Dixon, J., Loftus, S. K., Wasmuth, J. J., and Dixon, M. J. (1996) Genomics 32, 471–473
30. Humphries, D. E., Lanciotti, J., and Karlinsky, J. B. (1996) Biochem. J. 322, 303–307
31. Mazany, K. D., Peng, T., Watson, C. E., Tabas, I., and Williams, K. J. (1998) Biochim. Biophys. Acta 1407, 92–97

2 H. Sakaguchi, H. Kitagawa, and K. Sugahara, submitted for publication.
Molecular Cloning and Expression of a Novel Chondroitin 6-O-Sulfotransferase
Hiroshi Kitagawa, Masaki Fujita, Nobue Ito and Kazuyuki Sugahara

J. Biol. Chem. 2000, 275:21075-21080.
doi: 10.1074/jbc.M002101200 originally published online April 25, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M002101200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 31 references, 12 of which can be accessed free at
http://www.jbc.org/content/275/28/21075.full.html#ref-list-1