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Molecular Cloning, Expression, and Chromosomal Mapping of Human Chondroitin 4-Sulfotransferase, Whose Expression Pattern in Human Tissues Is Different from That of Chondroitin 6-Sulfotransferase[1]

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Chondroitin 4-sulfotransferase (C4ST) catalyzes the transfer of sulfate from 3′-phosphoadenosine 5′-phosphosulfate to position 4 of the *N*-acetylgalactosamine residues of chondroitin. We previously reported the cloning of C4ST cDNA from mouse brain. We here report the cloning and expression of human C4ST cDNA. The cDNA was isolated from a human fetal brain cDNA library by hybridization with a DNA probe prepared from rat poly(A)$^+$ RNA used for the cloning of mouse C4ST cDNA. The cDNA comprises a single open reading frame that predicts a Type II transmembrane protein composed of 352 amino acids. The protein has an amino acid sequence homology of 96% with mouse C4ST. When the cDNA was introduced into a eukaryotic expression vector and transfected in COS-7 cells, the sulfotransferase activity that transfers sulfate to both chondroitin and desulfated dermatan sulfate was overexpressed. Northern blot analysis indicated that human C4ST mRNAs (6.0 and 1.9 kb) are expressed ubiquitously in various adult human tissues. Dot blot analysis has shown that human C4ST is strongly expressed in colorectal adenocarcinoma and peripheral blood leukocytes, whereas strong expression of human chondroitin 6-sulfotransferase (C6ST) is observed in aorta and testis. These observations suggest that the expression of C4ST and C6ST may be controlled differently in human tissues. The *C4ST* gene was localized to chromosome 12q23.2-q23.3 by fluorescence *in situ* hybridization.

Key words: chondroitin sulfate, chromosome mapping, molecular cloning, proteoglycan, sulfotransferase.

Chondroitin sulfate from various sources bears sulfate groups at position 4 and/or position 6 of GalNAc residues, and position 2 or 3 of GlcA residues. Chondroitin sulfate isomers with different sulfation patterns have been shown to be involved in different biological functions: chondroitin sulfate A, containing GalNAc(4SO_4) residues, is a receptor for *Plasmodium falciparum*–infected erythrocytes (*1*); chondroitin sulfate C, containing GalNAc(6SO_4) residues, has been reported to be involved in the binding of a receptor-like protein, tyrosine phosphatase (PTPζ), to pleiotrophin and midkine (*2*, *3*); chondroitin sulfate E, containing GalNAc(4,6 bisSO_4), is a component of granules in mucosal mast cells (*4*); chondroitin sulfate D, containing GlcA-(2SO_4)-GalNAc(6SO_4), is reported to stimulate neurite outgrowth (*5*); and chondroitin sulfate A, but not chondroitin sulfate C, inhibits the Cu^{2+}-catalyzed oxidation of LDL (*6*, *7*). The characterization and molecular cloning of sulfotransferases, which participate in the formation of the defined structure of chondroitin sulfate, are important to clarify the functional roles of chondroitin sulfate isomers.

We previously purified (*8*) and cloned (*9*, *10*) chondroitin 6-sulfotransferase (C6ST), which catalyzes the sulfation of position 6 of the GalNAc residues of chondroitin. We also purified rat chondrosarcoma chondroitin 4-sulfotransferase (C4ST) (*11*), which catalyzes the sulfation of position 4 of the GalNAc residues of chondroitin, and cloned mouse

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Abbreviations: The abbreviations used are: C6ST, chondroitin 6-sulfotransferase; C4ST, chondroitin 4-sulfotransferase; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; GalNAc, *N*-acetyl-D-galactosamine; GlcA, D-glucuronic acid; ΔDi-0S, 2-acetamide-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-D-galactose; ΔDi-4S, 2-acetamide-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-4-*O*-sulfo-D-galactose; ΔDi-6S, 2-acetamide-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-6-*O*-sulfo-D-galactose; EST, expressed sequence-tagged.

C4ST (*12*). C4ST also transfers sulfate to position 4 of GalNAc residues in GlcA-rich regions of partially desulfated dermatan sulfate. We here report the cloning of human C4ST from a fetal brain cDNA library, and compare the expression pattern of C4ST with that of C6ST in various human tissues. While this manuscript was being prepared, human C4ST cDNA was reported as a cDNA showing homology with HNK-1 sulfotransferase (*13*).

EXPERIMENTAL PROCEDURES

Materials—The following commercial materials were used: $H_2{}^{35}SO_4$ was from Dupont/NEN; [α-^{32}P]dCTP (110 TBq/mmol) and Hybond N$^+$ were from Amersham Japan, Tokyo; a fetal human brain cDNA library, Human Multiple Tissue Northern Blots, and Multiple Tissue Expression Array were from CLONTECH, Palo Alto, CA; unlabeled PAPS was from Sigma, St. Louis, MO; Fast Desalting Column HR 10/10 was from Amersham Pharmacia Biotech, Tokyo; chondroitinase ACII, chondroitinase ABC, chondroitin sulfate A (whale cartilage), and dermatan sulfate (pig skin) were from Seikagaku Corporation, Tokyo; Dulbecco's modified Eagle's medium and fetal bovine serum were from Life Technologies.

[^{35}S]PAPS was prepared as described (*14*). Partially desulfated dermatan sulfate was prepared from pig skin dermatan sulfate according to Nagasawa *et al.* (*15*). Solvolysis with dimethyl sulfoxide was carried out at 100°C for 60 min. The degree of desulfation was calculated as 83% from the proportion of ΔDi-0S to the total unsaturated disaccharides formed after chondroitinase ABC digestion. Chondroitin (squid skin) was prepared as previously described (*16*).

Screening of a λgt 11 Library—Approximately 4 × 10^5 plaques were screened. Hybond N$^+$ nylon membrane (Amersham) replicas of the plaques from the λgt 11 cDNA library were fixed by the alkali fixation method recommended by the manufacturer, and prehybridized in a solution containing 50% formamide, 5 × SSPE, 5 × Denhardt's solution, 0.5% SDS, and 0.04 mg/ml of denatured salmon sperm DNA for 3.5 h at 42°C. Hybridization was carried out in the same buffer containing ^{32}P-labeled probe for 16 h at 42°C. The preparation of the ^{32}P-labeled probe used for screening from the rat poly(A)$^+$ RNA was described previously (*12*). The filters were washed at 55°C in 1× SSPE, 0.1% SDS, and subsequently in 0.1× SSPE, 0.1% SDS, and positive clones were detected by autoradiography.

DNA Sequence Analysis—DNA from λgt 11 positive clones were isolated and cut with *Eco*RI, which excised the cDNA insert in two fragments. The fragments were simultaneously subcloned into pBluescript II plasmid, and a clone containing the two fragments was selected. A clone that contained the whole open reading frame when compared with the sequence of the mouse C4ST cDNA was obtained. The complete nucleotide sequence of this clone was determined independently on both strands by the dideoxy chain termination method using a DNA sequencer (Applied Biosystem Model 373A).

Construction of pcDNAC4ST—To construct a plasmid containing the human C4ST cDNA, named pcDNAC4ST, the pBluescript II plasmid containing human C4ST cDNA was cut with *Hin*dIII and *Xba*I, and the 1,584 nucleotide fragment, of which 1,533 nucleotides were derived from the cDNA, was ligated into the *Hin*dIII and *Xba*I sites of pcDNA3 (Invitrogen).

Transient Expression of Human C4ST cDNA in COS-7 Cells—COS-7 cells were transfected with pcDNAC4ST or vector alone by the DEAE-dextran method as described previously (*9*, *17*). After transfection, the cells were extracted with 10 mM Tris-HCl, pH 7.2, 10 mM $MgCl_2$, 2 mM $CaCl_2$, 0.5% Triton X-100, and 20% glycerol for 30 min on a rotatory shaker. The extracts were centrifuged at 10,000 ×*g* for 20 min, and the sulfotransferase activities in the supernatant fractions were measured using chondroitin or desulfated dermatan sulfate as acceptors.

Northern Blot and Dot Blot Hybridization—Human Multiple Tissue Northern Blot Filters were prehybridized in ExpresHyb solution (CLONTECH) at 68°C. Hybridization was carried out in the same solution containing ^{32}P-labeled probe for 1 h at 68°C. The radioactive probe was prepared from the cDNA fragment excised from the pBluescript II plasmid with *Hin*dIII and *Xba*I by the random oligonucleotide-primed labeling method using [α-^{32}P]dCTP and a DNA random labeling kit (Takara Shuzo). The filters were washed at room temperature in 2× SSC, 0.05% SDS, and subsequently in 0.1× SSC, 0.1% SDS at 50°C. The membrane was exposed to X-ray film at –80°C with an intensifying screen. Human Multiple Tissue Expression Array was prehybridized in ExpresHyb solution (CLONTECH) at 68°C. Hybridization was carried out in the same solution containing ^{32}P-labeled probe for 1 h at 68°C. The radioactive probe for human C4ST and the hybridization conditions were the same as those used for the Northern blot as above. The radioactive probe for human C6ST was prepared from human C6ST cDNA as described (*10*).

Assay of Sulfotransferase Activity—The sulfotransferase activities were assayed by the method described previously (*8*). The standard reaction mixture contained 2.5 μmol of imidazole-HCl, pH 6.8, 1.25 μg of protamine chloride, 0.1 μmol dithiothreitol, 25 nmol (as glucuronic acid) chondroitin, 50 pmol [^{35}S]PAPS (about 5.0 × 10^5 cpm), and enzyme in a final volume of 50 μl. When 25 nmol (as GalNAc) partially desulfated dermatan sulfate was used as an acceptor, the amount of protamine chloride was increased to 10 μg. The reaction mixtures were incubated at 37°C for 20 min and the reaction was stopped by immersing the reaction tubes in a boiling water bath for 1 min. After the reaction was stopped, ^{35}S-labeled glycosaminoglycans were isolated by precipitation with ethanol followed by gel chromatography on a Fast Desalting Column as described previously, and the radioactivity was determined. For determining the incorporation into position 4 and position 6 of GalNAc residues, ^{35}S-labeled chondroitin and ^{35}S-labeled desulfated dermatan sulfate were digested with chondroitinase ACII and chondroitinase ABC, respectively. The resulting unsaturated disaccharides (ΔDi-4S and ΔDi-6S) were separated by paper chromatography (*8*), and their radioactivities were measured.

Chromosome Preparation and In Situ Hybridization—The direct R-banding FISH method was used for the chromosomal assignment of the human *C4ST* gene. The preparation of R-banded chromosomes and FISH were performed as described by Takahashi *et al.* (*18*, *19*). Mitogen-stimulated human peripheral blood lymphocytes were cultured and synchronized by thymidine blockage. The incorporation of 5-bromodeoxyuridine during the late replication stage

was made for differential replication staining after the release from excessive thymidine. R-band staining was performed by exposing chromosome slides to UV light after staining with Hoechst 33258.

The chromosome slides were hardened at 65°C for 3 h, and then denatured at 70°C in 70% formamide in 2× SSC, and dehydrated in 70–85–100% ethanol series at 4°C. The human 1.4 kb cDNA fragment inserted in the *Eco*RI site of pBluescript II KS was labeled by nick translation with biotinylated 16-dUTP (Roche Diagnostics) following the manufacturer's protocol. The labeled cDNA fragment was ethanol-precipitated with salmon sperm DNA and *E. coli* tRNA, and then denatured at 75°C for 10 min in 100% formamide. The denatured probe was mixed with an equal volume of hybridization solution to make a final concentration of 50% formamide, 2× SSC, 10% dextran sulfate, and 2

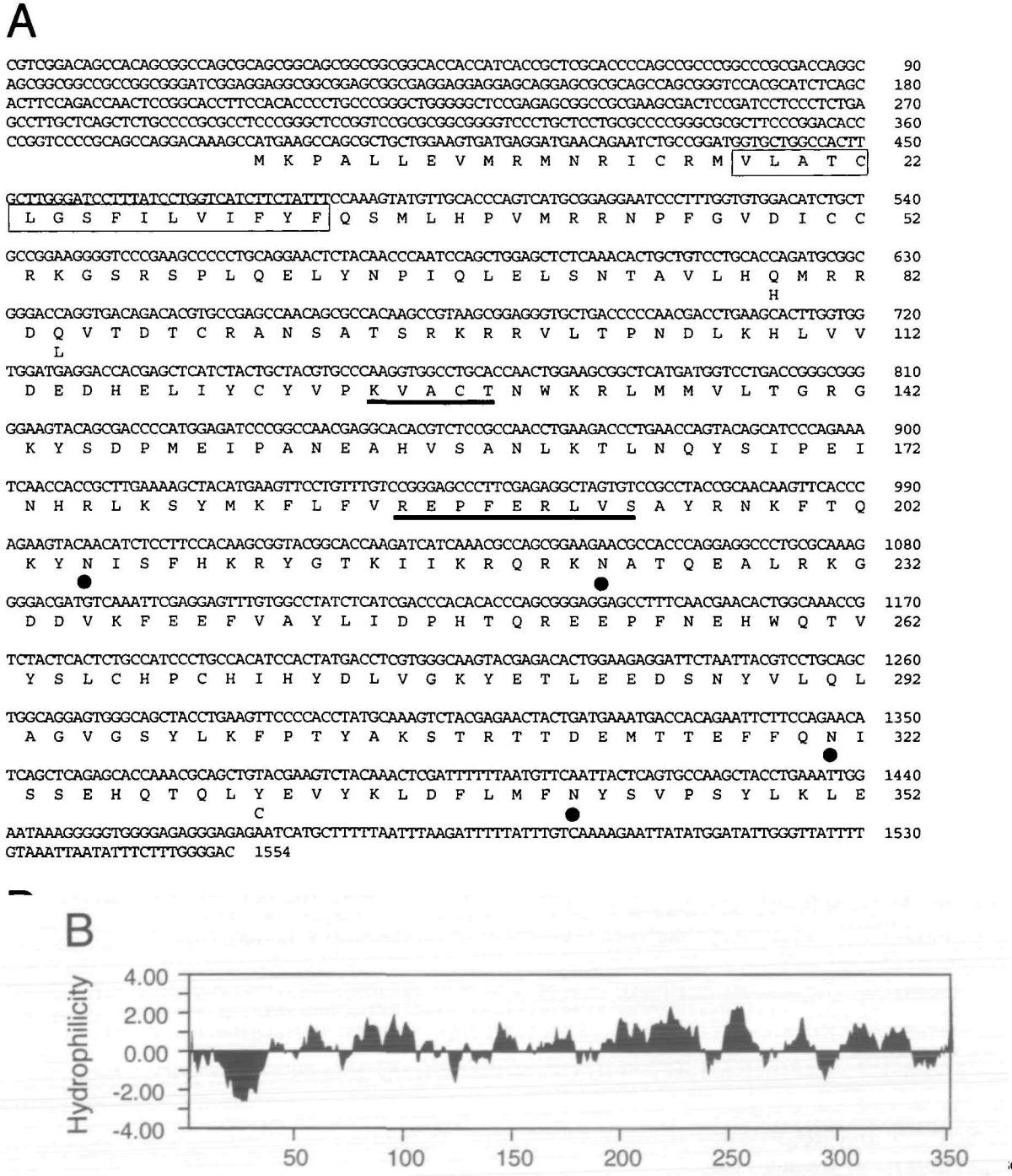

Fig. 1. **(A) predicted amino acid sequence.** The predicted amino acid sequence is shown below the nucleotide sequence. Four potential *N*-linked glycosylation sites are indicated by dots. The putative transmembrane hydrophobic domain is boxed. The amino acid residues of e-quence (*13*) are indicated under the sequence of human C4ST. **(B) Hydropathy plot of human C4ST.** The hydropathy plot was calculated by the method of Kyte and Doolittle (*31*) with a window of 11 amino acids.

μg/μl BSA (Sigma). A 20 μl mixture containing 250 ng labeled DNA was put on the denatured slide, covered with parafilm and incubated overnight at 37°C. The slides were washed for 15 min in 50% formamide in 2× SSC at 37°C, and in 2× SSC and 1× SSC for 20 min each at room temperature. After rinsing in 4× SSC, they were incubated under coverslips with goat anti-biotin antibodies (Vector Laboratories) at a 1:500 dilution in 1% BSA/4× SSC for 1 h at 37°C. They were washed with 4× SSC, 0.1% Nonidet P-40 in 4× SSC, 4× SSC for 5 min each, and then stained with fluoresceinated donkey anti-goat IgG (Nordic Immunology) at a 1:500 dilution for 1 h at 37°C. After washing with 4× SSC, 0.1% Nonidet P-40 in 4× SSC, 4× SSC for 10 min each on a shaker, the slides were rinsed with 2× SSC and stained with 0.75 μg/ml propidium iodide. FISH images were observed under Nikon fluorescence microscope *in situ* hybridization using Nikon filter sets B-2A and UV-2A. Kodak Ektachrome ASA100 films were used for microphotography.

RESULTS

cDNA and Predicted Protein Sequence of Human C4ST—We have cloned human C4ST cDNA from a fetal brain library. The nucleotide sequence of the human C4ST cDNA and the predicted amino acid sequence are shown in Fig. 1. Although this clone, as well as another C4ST clone reported by Hiraoka *et al.* (*13*), does not contain an in-frame stop codon in the 5′ noncoding region, $A^{387}TG$ appears to be a start codon as judged from the high sequence homology with mouse C4ST (Fig. 2). A putative hydrophobic transmembrane domain, 16 residues in length, was found in the amino-terminal region extending from amino acid residue 18 to 33. Putative PAPS binding sites (*20*) were found: one is K^{125}VACT for a 5′-phosphosulfate binding site and another is R^{186}EPFERLVS for a 3′-phosphate binding site.

Comparison of the coding sequence of human C4ST with that of mouse C4ST (*12*) and human HNK-1 sulfotrans-

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mC4ST     MKPALLEVMRMNRICRMVLATCFGSFILVIFYFQSMLHPVMRRNPFGVDICCRKG-SRSP  59
hC4ST     MKPALLEVMRMNRICRMVLATCLGSFILVIFYFQSMLHPVMRRNPFGVDICCRKG-SRSP  59
hHNK-1    MHHQWLLLAACFWVIFMFMVASK--FITLTFKDPDVYSAKQEFLFLTTMPEVRKLPEEKH  58

mC4ST     LQELYNPIQLELSNT----AILHQMRRDQVTDTCRANSAMSRKRRVLTPNDLKHLVVDED  115
hC4ST     LQELYNPIQLELSNT----AVLHQMRRDQVTDTCRANSATSRKRRVLTPNDLKHLVVDED  115
hHNK-1    IPEELKPTGKELPDSQLVQPLVYMERLELIRNVCRDDALKNLSHTPVSKFVLDRIFVCDK  118

mC4ST     HELIYCYVPKVACTNWKRLMMVLSGRGKYSDPMEIPANEAHVSAN--LKTLNQYSIPEIN  173
hC4ST     HELIYCYVPKVACTNWKRLMMVLTGRGKYSDPMEIPANEAHVSAN--LKTLNQYSIPEIN  173
hHNK-1    HKILFCQTPKVGNTQWKKVLIVLNG--AFSSIEEIPENVVHDHEKNGLPRLSSFSDAEIQ  176

mC4ST     HRLKSYMKFLFVREPFERLVSAYRNKFTQ--KYNTSFHKRYGTKIIRRQRKNATQEALRK  231
hC4ST     HRLKSYMKFLFVREPFERLVSAYRNKFTQ--KYNISFHKRYGTKIIKRQRKNATQEALRK  231
hHNK-1    KRLKTYFKFFIVRDPFERLISAFKDKFVHNPRFEPWYRHEIAPGIIRKYRRNRTETRG--  234

mC4ST     GDDVKFEEFVAYLIDP-HTQREEPFNE---HWQTVYSLCHPCHIHYDLVGKYETLEEDSN  287
hC4ST     GDDVKFEEFVAYLIDP-HTQREEPFNE---HWQTVYSLCHPCHIHYDLVGKYETLEEDSN  287
hHNK-1    ---IQFEDFVRYLGDPNHRWLDLQFGDHIIHWVTYVELCAPCEIMYSVIGHHETLEDDAP  291

mC4ST     YVLQLAGVSGYLKFPTYAK-STRTTDEMTTEFFQNISAEHQTQLYEVYKLDFLMFNYSVP  346
hC4ST     YVLQLAGVGSYLKFPTYAK-STRTTDEMTTEFFQNISSEHQTQLYEVYKLDFLMFNYSVP  346
hHNK-1    YILKEAGIDHLVSYPTIPPGITVYNRTKVEHYFLGISKRDIRRLYARFEGDFKLFGYQKP  351

mC4ST     NYLKLD  352
hC4ST     SYLKLE  352
hHNK-1    DFLLN   356
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Fig. 2. **Sequence comparison of human C4ST, mouse C4ST, and human HNK-1 sulfotransferase.** mC4ST, hC4ST, and hHNK-1 represent mouse C4ST, human C4ST, and human HNK-1 sulfotransferase, respectively. Asterisks indicate that the predicted amino acid in the alignment is identical between the two sequences.

TABLE I. **Overexpression of human C4ST in COS-7 cells.** COS-7 cells were transfected with a plasmid containing the C4ST cDNA (pcDNAC4ST) or vector alone (pcDNA3). Sulfotransferase activity was determined using chondroitin or desulfated dermatan sulfate as acceptor. The incorporation of $^{35}SO_4$ into ΔDi-4S and ΔDi-6S after digestion with chondroitinase ACII (for chondroitin) or chondroitinase ABC (for desulfated dermatan sulfate) was determined. Values represent averages ± SD of triplicate cultures.

Plasmid	Acceptor			
	Chondroitin		Desulfated dermatan sulfate	
	ΔDi-4S	ΔDi-6S	ΔDi-4S	ΔDi-6S
	(pmol/min/mg protein)			
pcDNAC4ST	38.1 ± 1.6	3.6 ± 0.7	7.5 ± 2.3	1.6 ± 0.4
pcDNA3	0.3 ± 0.1	5.1 ± 0.9	0.1 ± 0.1	1.1 ± 0.4
None	0.3 ± 0.1	4.8 ± 0.7	0.1 ± 0.1	1.1 ± 0.3

ferase (*21*) revealed that 96 and 29% identity, respectively, at the amino acid level (Fig. 2). Four potential *N*-linked glycosylation sites are completely conserved between mouse and human C4ST. The three amino acid residues indicated under the sequence of human C4ST in Fig. 2 are those of human C4ST reported in another paper, in which human C4ST cDNA was isolated from an EST clone from human germinal center B cells (*13*).

Expression of Human C4ST cDNA in COS-7 Cells—To confirm that the cDNA encodes a protein with C4ST activity, we constructed an expression vector containing the sequence for the whole open reading frame and transfected it into COS-7 cells. As shown in Table I, when the vector containing the isolated cDNA was used, the sulfotransferase activity, which transfers sulfate to position 4 of GalNAc residues of chondroitin, and the sulfotransferase activity, which transfers sulfate to position 4 of GalNAc residues of partially desulfated dermatan sulfate, increased more than 100- and 7-fold, respectively, above control levels. In contrast, the sulfotransferase activity, which transfers sulfate to position 6 of GalNAc residues, was not increased.

Northern Blot and Dot Blot Analysis—Northern blot analysis was performed using Human Multiple Tissue Northern Blots (CLONTECH), on which poly(A)$^+$ RNAs derived from various adult human tissues were applied (Fig. 3). A C4ST message of 6.0 kb was expressed strongly in heart, brain, placenta, and lung, and weakly in other tissues examined. The expression pattern of the 1.9 kb message is almost the same as that of the 6.0 kb message, except that the expression of the 1.9 kb message in the brain was much weaker than in heart, placenta and lung. The 1.3 kb message was expressed only in the heart. A 5.0 kb transcript was observed in liver, skeletal muscle, kidney and pancreas. The expression pattern of C4ST was clearly distinct from that of human C6ST; C6ST is expressed mainly in heart, placenta, skeletal muscle and pancreas (*10*). Dot blot analysis using Human Multiple Tissue Expression Array (CLONTECH) showed that C4ST is expressed widely in various tissues; among these tissues, the strongest expression was observed in colorectal adenocarcinoma, SW480 (10G) and peripheral blood leukocytes (7E). In contrast, C6ST was expressed in the order of aorta (4B), testis (8F), ovary (8G), bone marrow (7G), adrenal gland (9C), corpus callosum (2C), spinal cord (3E), heart (4A), and fetal kidney (11C). C6ST expression was hardly detected in peripheral blood leukocytes and colorectal adenocarcinoma, SW480, in which the expression of C4ST was

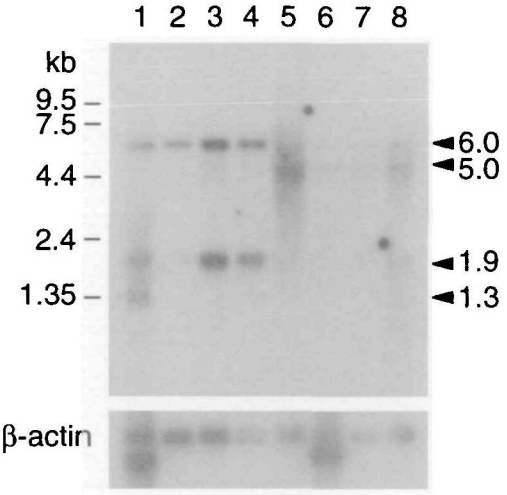


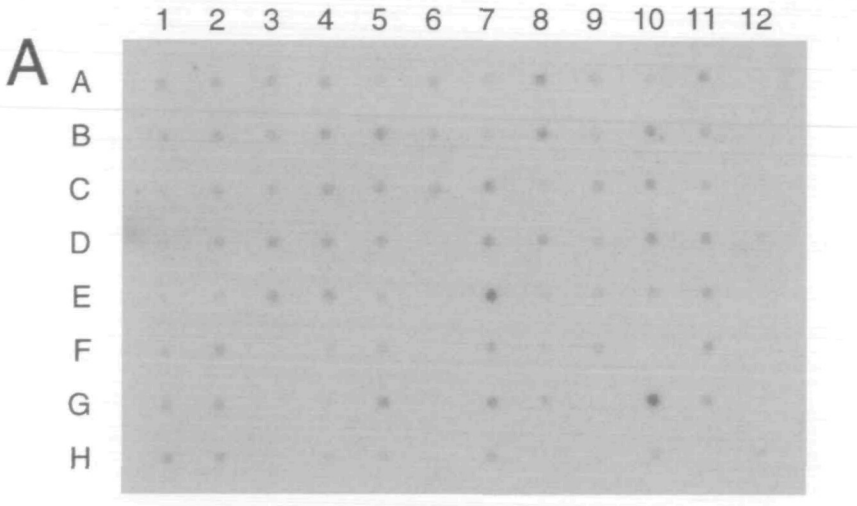
Fig. 3. **Northern blot analysis of C4ST messages in various adult human tissues.** Northern blots with poly(A)$^+$ RNA from heart (lane 1), brain (lane 2), placenta (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7), pancreas (lane 8) were hybridized with ^{32}P-labeled DNA probe for human C4ST cDNA.

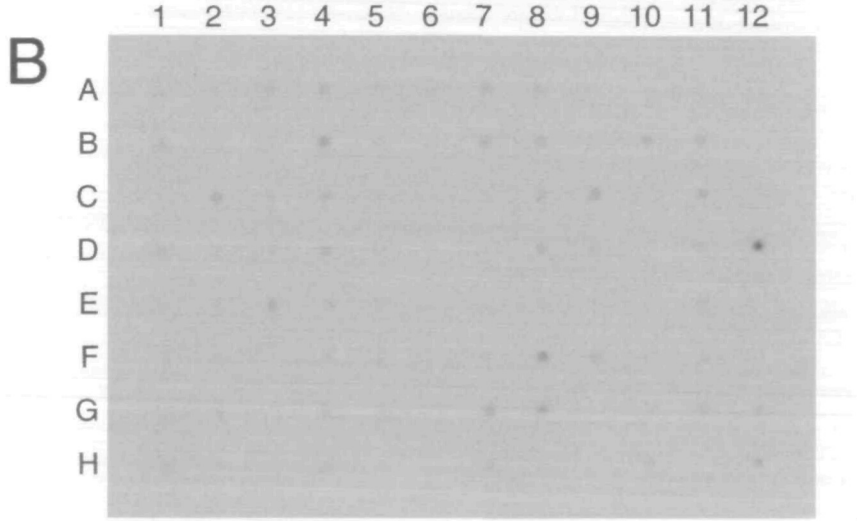
prominent (Fig. 4). *E. coli* DNA was stained with C6ST cDNA, but the reason is not clear. These observations suggest that C4ST and C6ST should play different functional roles. To obtain a semiquantitative comparison between C4ST expression and C6ST expression, we determined the density of each dot using NIH-Image software and calculated the ratio of the intensity of C4ST expression and C6ST expression in each sample. The patterns of the ratio shown in Fig. 5 clearly indicate that the relative expression of C4ST and C6ST depends on the tissue or cell. Higher ratios of C4ST/C6ST were observed in several immunologically relevant tissues and cells such as spleen (7C), thymus (7D), peripheral blood leukocytes (7E), lymph node (7F), and several kind of leukemia or lymphoma cells such as HL-60 (10A), MOLT-4 (10D), and Burkitt's lymphoma Raji (10E). Colorectal adenocarcinoma, SW480 (10G), also showed a high ratio. On the other hand, higher ratios of C6ST/C4ST were observed in aorta (4B), ventricle (4F), interventricular septum (4G), kidney (7A), skeletal muscle (7B), prostate (8E), testis (8F), ovary (8G), adrenal gland (9C), and fetal kidney (11C).

Assignment of the Human C4ST Gene by Fluorescence In Situ Hybridization—The chromosomal location of the human *C4ST* gene was determined by fluorescence *in situ* hybridization (Fig. 6). The *C4ST* gene was localized to chromosome 12q23.2-q23.3 (*22*).

DISCUSSION

Human C4ST cDNA has been reported independently by three groups including us. A comparison of amino acid sequences among these reports reveals some inconsistencies. Three amino acid residues of the human C4ST cloned in this paper are different from those reported by Hiraoka *et al.* (*13*), and one amino acid residue is different from that reported by Xia *et al.* (accession number, AJ269537). The reason for this inconsistency is not clear. We cloned C4ST from fetal brain, whereas Hiraoka *et al.* obtained C4ST





C

	1	2	3	4	5	6	7	8	9	10	11	12
A	whole brain	cerebellum, left	substantia nigra	heart	esophagus	colon, transverse	kidney	lung	liver	leukemia, HL-60	fetal brain	yeast total RNA
B	cerebral cortex	cerebellum, right	accumbens nucleus	aorta	stomach	colon, descending	skeletal muscle	placenta	pancreas	HeLa S3	fetal heart	yeast tRNA
C	frontal lobe	corpus callosum	thalamus	atrium, left	duodenum	rectum	spleen	bladder	adrenal gland	leukemia, K-562	fetal kidney	*E. coli* rRNA
D	parietal lobe	amygdala	pituitary gland	atrium, right	jejunum		thymus	uterus	thyroid gland	leukemia, MOLT-4	fetal liver	*E. coli* DNA
E	occipital lobe	caudate nucleus	spinal cord	ventricle, left	ileum		peripheral blood leukocyte	prostate	salivary gland	Burkitt's lymphoma, Raji	fetal spleen	Poly r(A)
F	temporal lobe	hippo-campus		ventricle, right	ilocecum		lymph node	testis	mammary gland	Burkitt's lymphoma, Daudi	fetal thymus	human C_0t -1 DNA
G	p. g.* of cerebral cortex	medulla oblongata		inter-ventricular septum	appendix		bone marrow	ovary		colorectal adeno-carcinoma, SW480	fetal lung	human DNA 100 ng
H	pons	putamen		apex of the heart	colon, ascending		trachea			lung carcinoma, A549		human DNA 500 ng

* paracentral gyrus

Fig. 4. **Dot blot analysis of C4ST (A) and C6ST (B) messages in various human tissues.** The sources of the poly(A)$^+$ RNA are indicated in C.

from human germinal center B cells, suggesting that tissue specific variants in C4ST might exist. Since the C4ST cloned from fetal brain and C4ST cloned from germinal center B cells were both active, amino acid substitutions (Gln^{79} to His, Gln^{84} to Leu, Tyr^{331} to Cys) do not affect the sulfotransferase activity.

Both mouse and human C4ST contain a Cys^{128} residue in the 5′-phosphosulfate binding domain. On the other hand,

HNK-1 sulfotransferase does not have Cys, although the sequence of the 5'-phosphosulfate binding domain is well conserved. C4ST was stimulated by dithiothreitol but HNK-1 sulfotransferase showed activity in the absence of the sulfhydryl compound, suggesting that the Cys residue contained in the 5'-phosphosulfate binding domain may be relevant to the requirement of C4ST for sulfhydryl compounds.

As shown in Fig. 3, various sized messages of C4ST were expressed in a tissue-specific manner, suggesting the presence of tissue-specific regulation of C4ST gene expression. Mouse C4ST was expressed mainly in the brain and kidney (*12*), whereas the expression of human C4ST in the kidney was weak. It is not clear why the expression pattern of the mouse C4ST gene is different from that of human C4ST. Since 4-sulfated glycosaminoglycans such as chondroitin 4-sulfate and dermatan sulfate are synthesized in most tissues, isozymes of C4ST with different expression patterns and different specificities may be present. Hiraoka *et al.* reported the cloning of C4ST-2, whose expression pattern in human tissues differs from that of C4ST (*13*).

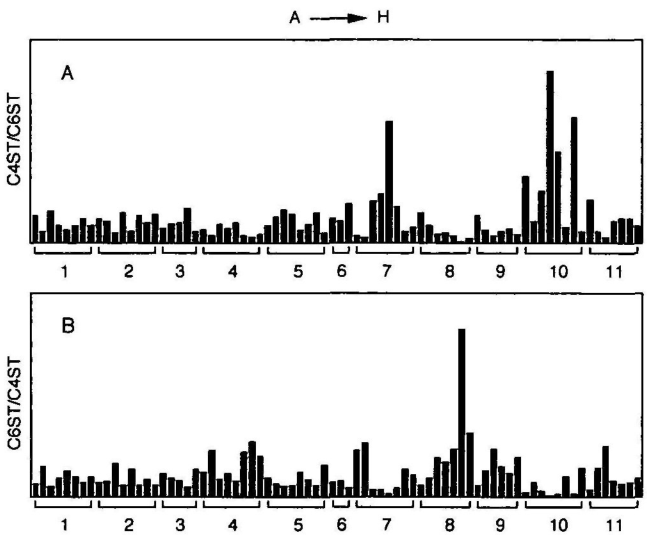


Fig. 5. **Comparison between C4ST expression and C6ST expression.** The density of each dot shown in Fig. 4 was determined by NIH-Image. The ratios of (intensity of each dot of C4ST)/(intensity of corresponding dot of C6ST) (A) and (intensity of each dot of C6ST)/(intensity of corresponding dot of C4ST) (B) were calculated. Data are shown in arbitrary units.

On dot blot analysis, C4ST was found to be expressed strongly in leukocytes and various leukemia cells. The ratio of C4ST/C6ST was also higher in these cells. Chondroitin 4-sulfate proteoglycans constitute the predominant proteoglycans produced by activated human monocyte/macrophages (*23*). Regardless of their different maturation stages, B-lymphocyte cell lines secrete proteoglycans possessing chondroitin sulfate chains having chondroitin 4-sulfate as the prevalent disaccharide unit (*24*). Surface-expressed neutrophil glycosaminoglycans, which inhibit the binding of platelet factor 4 to neutrophils, are chondroitin sulfate composed of approximately 85–90% chondroitin 4-sulfate units and 10–15% chondroitin 4,6-bissulfate units (*25*). Serglycin secreted by the mouse T cell line CTLL2 binds to the lymphocyte adhesion molecule CD44 through the glycosaminoglycan chains. The glycosaminoglycan of serglycin is composed mainly of chondroitin 4-sulfate (*26*). These observations coincide with the strong expression of C4ST in leukocytes, and suggest that the expression of C4ST may be important for the cellular interaction of leukocytes. C4ST was also expressed strongly in colorectal adenocarcinoma, SW480. In the development of colon cancer, the proportion of the synthesis of chondroitin sulfate, 32% 4-sulfate and 68% 6-sulfate, has been reported to increase in contrast to a concomitant decrease in dermatan sulfate and heparan sulfate (*27*). Chemical analysis of purified glycosaminoglycans from colonic tumors revealed a 12-fold increase in the concentration of chondroitin 4- and 6-sulfate as compared with controls (*28*). These observations suggest that an increase in the concentration of chondroitin 4/6-sulfate and an increase in the rate of synthesis of chondroitin 4/6-sulfate may be closely related to the development of colon cancer, and that the strong expression of C4ST in colorectal adenocarcinoma may reflect the increase in chondroitin sulfate; however, it is not clear why the expression of C6ST in colorectal adenocarcinoma is hardly observed.

Chondroitin sulfate obtained from human aorta is reported to be mainly 6-sulfated isomers (*29*, *30*). The relatively strong expression of C6ST in aorta seems to reflect the composition of aortic chondroitin sulfate. The relative expression of C6ST is higher in reproductive organs such as prostate, testis and ovary. Chondroitin 6-sulfate may function in these tissues.

C4ST transfers sulfate to desulfated dermatan sulfate; however, it remains to be determined whether C4ST is

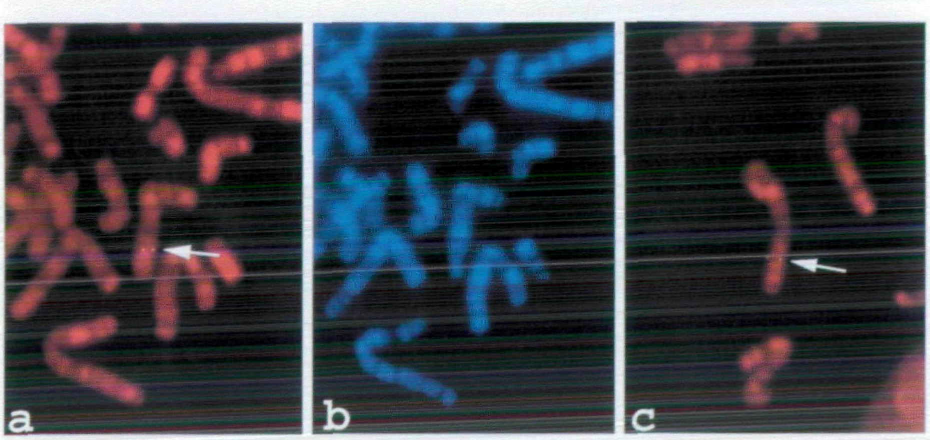


Fig. 6. **Chromosomal localization of the human *C4ST* gene.** A 1.0 kb cDNA fragment was used as a biotinylated probe. Arrows indicate the hybridization signals. The *C4ST* gene was localized to chromosome 12q23.2-q23.3. The metaphase spreads were photographed with Nikon B-2A (a, c) and UV-2A (b) filters. R- and G-banded patterns are demonstrated in (a, c) and (b).

involved *in vivo* in the synthesis of dermatan sulfate as well, or whether another isoform of C4ST, which might participate mainly in the synthesis of dermatan sulfate, is present.

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