We have previously cloned chondroitin 6-sulfotransferase (C6ST) cDNA from chick embryo chondrocytes. C6ST catalyzes sulfation of chondroitin, keratan sulfate, and sialyl N-acetyllactosamine oligosaccharides. In this study, we report the cloning and characterization of a novel sulfotransferase that catalyzes sulfation of keratan sulfate. This new sulfotransferase cDNA clone was obtained from a human fetal brain library by cross-hybridization with chick C6ST cDNA. The cDNA clone obtained contains a single open reading frame that predicts a type II transmembrane protein composed of 411 amino acid residues. When the cDNA was introduced into a eukaryotic expression vector and transfected in COS-7 cells, keratan sulfate sulfotransferase activity was overexpressed, but C6ST activity was not increased over that of the control. Structural analysis of 35S-labeled glycosaminoglycan, which was formed from keratan sulfate by the reaction with 35S-labeled 3-phosphoadenosine 5'-phosphosulfate and the recombinant sulfotransferase, showed that keratan sulfate was sulfated at position 6 of Gal residues. On the basis of the acceptor substrate specificity, we propose keratan sulfate sulfotransferase (KSST)1 has so far been obtained.

We have previously purified and cloned chondroitin 6-sulfotransferase (C6ST) from the culture medium of chick embryo chondrocytes (11, 12). We found that C6ST catalyzes sulfation of chondroitin, keratan sulfate, and sialyl N-acetyllactosamine oligosaccharides (11, 13, 14). This enzyme may, therefore, participate in the biosynthesis of both chondroitin sulfate and keratan sulfate in tissues such as cartilage, in which both chondroitin 6-sulfate and keratan sulfate are actively synthesized. On the other hand, in the developing cornea, keratan sulfate is actively synthesized, but synthetic activity of chondroitin 6-sulfate seems to be minimal (7, 8). In addition, the expression of C6ST mRNA was found to be much weaker in the chick cornea compared with that in cartilage (13). These observations suggest the possible existence of a different sulfotransferase in the cornea, which catalyzes mainly the sulfation of keratan sulfate. In this study we report cloning of a novel sulfotransferase cDNA that encodes a protein with sulfotransferase activity toward keratan sulfate. This sulfotransferase transferred sulfated to position 6 of the Gal residue of keratan sulfate but showed no activity toward chondroitin.

**EXPERIMENTAL PROCEDURES**

**Materials**—The following commercial materials were used: H32SO4 was from DuPont NEN; [3H]NaBH4 (16.3 GBq/mmol) [35S]dCTP (110 TBq/mmol) and Hybond N were from Amersham Japan, Tokyo; the fetal human brain cDNA library and human multiple tissue Northern blots were from CLONTECH, Palo Alto, CA; unlabeled PAPS, N-acetylgalcosamine 6-sulfate, and galactose 6-sulfate were from Sigma; Hiload heparin; CDSNS-heparin (shark cartilage), dermatan sulfate, and completely desulfated N-sulfated heparin (CDSNS-heparin) were from Seikagaku Corporation, To-
Cloning of Keratan Sulfate Gal-6-Sulfotransferase

kyo, Partisil SAX-10 was from Whatman. Keratan sulfate from bovine cornea was a product of Seikagaku Corporation and generously donated by that company. 

[3H]PAPS was prepared as described previously (15). [3H]GlcNAc6S and [3H]Gal(6S) were prepared from GlcNAc(6S) and Gal(6S), respectively, by using the Dounce homogenizer in 1.5 ml/dish of 0.25M sucrose, 10 mM Tris-HCl, pH 7.2, and 0.5% Triton X-100. The homogenates were centrifuged for 10,000 g for 20 min, and the activities of C6ST, C4ST, and KSST in the supernatants were measured as described above.

Northern Blot Hybridization—Poly(A)+ RNAs (5 μg) prepared from chick embryo tissues were denatured in 50% formamide (v/v), 5% formaldehyde (v/v), 20 mM MOPS, pH 7.0, at 65 °C for 10 min, electrophoresed in 1.2% agarose gel containing 5% formaldehyde (v/v), and transferred to a Hybond N+ nylon membrane. The membrane was washed with NaBH4 (13). Oligonucleotides prepared as previously described (16). Partially desulfurated keratan sulfate (sulfate/glucosamine = 0.62) was prepared from corneal keratan sulfate according to Nagasawa et al. (17). Solvolyis with dimethyl sulfoxide was carried out at 80 °C for 45 min. The molar ratios of Galβ1→4GlcNAc to Galβ1→4GlcNAc(6S) of the desulfated keratan sulfate were determined by the paper chromatographic separation of [3H]Galβ1→4MANβ(27), and [3H]Galβ1→4GlcNAc(6S) after the reaction sequence of hydrazinolysis, deaminative cleavage, and reduction with NaBH4(27), was 0.73. A mixture of [3H]Galβ1→4GlcNAc and [3H]Galβ1→4GlcNAc(6S) was prepared by partial acid hydrolysis (0.1 M HCl, 100 °C, 40 min) of [3H]Galβ1→4GlcNAc(6S) as described previously (14).

Screening of λgt 1 Library—Approximately 2 × 106 plaques were screened. Hybond N+ nylon membrane (Amersham Corp.) replicas of the plaques from the λgt 1 cDNA library were fixed by the alkalai fixation method recommended by the manufacturer, prehybridized in a solution containing 50% formamide, 5 × SSPE, 5 × Denhardt’s solution, 0.5% SDS, and 0.04 mg/ml denatured salmon sperm DNA for 3.5 h at 42 °C. Hybridization was carried out in the same buffer containing a 32P-labeled probe for 16 h at 42 °C. The radioactivity probe for screening the cDNA library was prepared from chik C6ST cDNA previously reported (12) by the random oligonucleotide-primed labeling method (18) using [α-32P]dCTP (Amersham Corp.) and a DNA random labeling kit (Takara Shuzo). The filters were washed at 65 °C in 1 × SSPE, 0.1% SDS, and subsequently at 1 × SSPE, 0.1% SDS, and positive clones were detected by autoradiography.

DNA Sequence Analysis—DNA from λgt 1 positive clones were isolated and cut with EcoRI, which excised the cDNA insert in a single fragment. The fragments were inserted into Bluescript plasmid, and deletion clones were prepared as described previously (19, 20) using a DNA deletion kit (Takara Shuzo). The complete nucleotide sequence was determined independently on both strands using the dyeoxy chain termination method (21) with [α-32P]dCTP and Sequenase (U. S. Biochemical Corp.). The DNA sequence was also determined using synthetic oligonucleotide primers. DNA sequences were compiled and analyzed using the Gene Works computer programs (IntelliGenetics).

Construction of pCXNKSGal6ST—For the construction of pCXNKSGal6ST, the EcoRI fragment containing the 2415-base pair cDNA was excised from the Bluescript plasmid and was ligated into the EcoRI site of pCXN2 expression vector (the pCXN2 vector was constructed by Dr. Jun-ichi Miyazaki, Department of Disease-Related Gene Regulation, Faculty of Medicine, University of Tokyo (22) and provided by Dr. Yasuhiro Hashimoto, Tokyo Metropolitan Institute of Medical Sciences). Recombinant plasmids were analyzed by restriction mapping using BamHI to correct the orientation of pCXN2. The plasmid that contained the cDNA fragment in the reverse orientation was designated as pCXNKSGal6ST2 and used for control experiments.

Transient Expression of Keratan Sulfate Gal-6-Sulfotransferase cDNA in COS-7 Cells—COS-7 cells (obtained from Riken Cell Bank, Tsukuba, Japan) were plated in 100-mm culture dishes at a density of 8 × 105 cells/dish. Volume of the medium was 10 ml. The medium used was DMEM containing penicillin (100 units/ml), streptomycin (50 μg/ml), and 10% fetal bovine serum (Life Technologies, Inc.), and cells were grown at 37°C in 5% CO2, 95% air. When the cell density reached 3 × 106 cells/dish (48 h after plating), COS-7 cells were transfected with pCXNKSGal6ST or pCXNKSGal6ST2. The transfection was performed using the DEAE-dextran method (23). 5 ml of the prewarmed DMEM containing 10% Nu serum (Collaborative Biomedical Products) were mixed with 0.2 ml of phosphate-buffered saline containing 10 mg/ml DEAE-dextran plus a 2.5 mM chloroquine solution. 15 μg of the recombinant plasmid was mixed with the solution, and the mixture was added to the cells. The cells were incubated for 4 h in a CO2 incubator. The medium was then replaced with 5 ml of 10% dimethyl sulfoxide in phosphate-buffered saline. After the cells were left at room temperature for 15 min, 5 ml of DMEM without transfected solution was added. The DMEM containing penicillin (100 units/ml), streptomycin (50 μg/ml), and 10% fetal bovine serum were added. The cells were incubated for 67 h, washed with DMEM alone, scraped, and homogenized with a Dounce homogenizer in 1.5 ml/dish of 0.25 M sucrose, 10 mM Tris-HCl, pH 7.2, and 0.5% Triton X-100. The homogenates were centrifuged at 10,000 × g for 20 min, and the activities of C6ST, C4ST, and KSST in the supernatants were measured as described above.

Fluorescence in Situ Hybridization—To determine the chromosomal localization of KSGal6ST, fluorescence in situ hybridization was performed. Metaphase chromosomes were prepared from normal male lymphocytes using the thymidine synchronization, a bromodeoxyuridine release technique for the delineation of R- and G-bands (24). Before hybridization, chromosomes were stained in Hoechst 33258 and irradiated with UV. A 2.4-kb cDNA shown in Fig. 1 was labeled with biotin-16-UTP by nick translation and hybridized to the denatured chromosomes. The hybridization signals were detected by fluorescein isothiocyanate-avidin (Boehringer Mannheim GmbH, Mannheim, Germany), and chromosomes were counterstained with propidium iodide (1 μg/ml). The fluorescent signals were examined using epifluorescent microscope and precise positions of the signals were determined according to the G-bands delineated by Hoechst 33258 through UV filter.

Assay of Sulfotransferase Activity—in the early experiments (Fig. 3), C6ST activity and KSST activity were assayed by the method described previously (11). The reaction mixture used for the early experiments contained, in a final volume of 50 μl, 2.5 μmol of imidazole-HCl, pH 6.8, 1.25 μg (for chondroitin) or 3.75 μg (for keratan sulfate) of proteamine chloride, 0.1 μmol of diethiothreitol, 0.025 μmol of glycosaminoglycans (as glucosamine or galactosamine), 50 μmol of [3H]PAPS (about 5.0 × 106 cpm), and enzyme. 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. 35S-Labeled glycosaminoglycans were isolated by precipitation with ethanol followed by gel chromatography with a fast desalting column as described previously, and radioactivity was determined. The reaction mixtures described above developed for C6ST were, however, not optimum for KSST, and the activity of KSST was underestimated. After the optimum conditions for KSST were revealed, the following modification of the reaction mixture was adopted unless otherwise stated. 2.5 μmol of imidazole-HCl, pH 6.4, and 0.5 μmol of CaCl2 were added to the reaction mixture in place of 2.5 μmol of imidazole-HCl, pH 6.8, and proteamine chloride, respectively. KSST activity and chondroitin sulfotransferase activity were determined using keratan sulfate and chondroitin, respectively, as acceptor. For assaying the activity of KSST, 35S-labeled chondroitin was digested with chondroitinase ACII, and the unsaturated deacidscharides formed were separated by paper chromatography.

Partial Purification of Keratan Sulfate Gal-6-Sulfotransferase—The homogenate of COS-7 cells transfected with pCXNKSGal6ST (224 mg as protein obtained from 80 10-cm dishes) was applied to a DEAE-Sephadex A-50 column (2.2 × 13 cm) equilibrated with buffer A (10 mM Tris-HCl, pH 7.2, containing 20% glycerol, 20 mM MgCl2, 2 mM CaCl2, and 10 mM 2-mercaptoethanol) containing 50 mM NaCl. After the column was washed with 500 ml of the same buffer, the absorbed materials were eluted with 0.5 M NaCl in buffer A. About 20% of KSST activity was recovered in the flow through fractions. The remaining KSST activity and all of chondroitin sulfotransferase activity were eluted in 0.5 M NaCl fractions. The flow-through fractions were pooled, dialyzed against 0.15 M NaCl in buffer B, and applied to a Heparin-Sepharose CL 6B column (1.2 × 8.0 cm) equilibrated with buffer A containing 0.15 M NaCl. The materials absorbed to the Heparin-Sepharose CL 6B column were eluted in 0.5 M NaCl in buffer A, dialyzed against buffer A containing 30 mM NaCl, and used for KSGal6ST preparation devoid of C6ST activity. As a control, the homogenate of COS-7 cells transfected with pCXNKSGal6ST was obtained without transfected solution. The flow-through fractions were also separated with DEAE-Sephadex in the same procedures as described above except that the column size, elution volume, and fraction size were reduced to one-fourth.

Keratanase II Digestion of 35S-Labeled Glycosaminoglycan Formed from Keratan Sulfate or Desulfated Keratan Sulfate—35S-Labeled glycosaminoglycan was prepared by incubating keratan sulfate or desulfated keratan sulfate with keratanase II.
fated keratan sulfate with $[^{35}S]$PAPS and the partially purified KSGal6ST (2 μg as protein) as described above for 18 h. $[^{35}S]$-Labeled glycosaminoglycans were separated from $[^{35}S]$O4 and $[^{35}S]$PAPS with the fast desalting column and desalted by lyophilization. The desalted samples from four reaction tubes were pooled and digested with keratanase II in the reaction mixture containing, in a final volume of 50 ml, 0.005 unit of keratanase II and 2.5 μmol of acetate buffer, pH 6.5 (25, 26). The reaction mixtures were incubated at 37 °C for 24 h. $[^{35}S]$-Labeled disaccharides formed after the keratanase II digestion were separated with an anion exchange HPLC. The keratanase II digests were applied to a Whatman Partisil 10-SAX column (4.5 × 25 cm) equilibrated with 5 mM KH2PO4. The column was developed with 5 mM KH2PO4 for 5 min followed by a 20-min gradient from 5 mM to 250 mM of KH2PO4. The flow rate was 1 ml/min. Under the chromatographic conditions, elution time of Gal(6S)b1–4GlcNAcR(6S) and Galb1–4GlcNAcR detected by absorption at 210 nm were 14 min and 22 min, respectively. 0.5-ml fractions were collected, and 10-μl aliquots were used for determination of radioactivity. Each radioactive peak was collected, dried with a centrifuging vacuum evaporator, and redissolved in a small volume of water. Potassium phosphate was removed by Superdex 30 column chromatography, and the eluate from the Superdex 30 column was lyophilized.

Separation and Identification of Monosulfated Disaccharide Fraction Formed from $[^{35}S]$Gal(6S)b1–4GlcNAcR(6S) by the Partial Acid Hydrolysis—To determine which sulfate of Gal(6S)b1–4GlcNAcR(6S) contained $^{35}$S radioactivity, monosulfated disaccharide fraction was prepared from $[^{35}S]$Gal(6S)b1–4GlcNAcR(6S) with partial acid hydrolysis. $[^{35}S]$Gal(6S)b1–4GlcNAcR(6S) obtained by Partisil-10 SAX HPLC and Superdex 30 chromatography was reduced with NaBH4 as described elsewhere (14), and hydrolyzed with 50 μl of 0.1 M HCl at 100 °C for 40 min. After re-N-acetylation with acetic anhydride, the hydrolysate was spotted on a strip of Whatman No. 3 and developed with a solvent described below for 48 h. The second radioactive peak, which potentially contains Galb1–4GlcNAcR(6S), Gal(6S)b1–4GlcNAcR, Gal(6S), and SO4, was eluted and subjected to paper electrophoresis. The faster migrating peak in the paper electrophoresis was assigned as $[^{35}S]$O4. The slower migrating peak, which potentially contains Galb1–4GlcNAcR(6S), Gal(6S)b1–4GlcNAcR, and Gal(6S) was reduced with NaBH4 as described previously (14) and analyzed with Partisil-10 SAX HPLC as described below.
rate was 1 ml/min, and the column temperature was 40 °C. 0.5-ml fractions were collected.

RESULTS

cDNA and Predicted Protein Sequence of the Keratan Sulfate Gal-6-Sulfotransferase—When approximately 2 \( \times \) 10^6 plaques of a human fetal brain cDNA library were screened using chick C6ST cDNA as a probe, two cDNA clones (1.2 and 2.4 kb) other than human C6ST cDNA clones were obtained. These clones were clearly distinguished from the C6ST clones on the autoradiogram due to their weaker signals. We will report the human C6ST cDNA elsewhere. From the nucleotide sequence, the longer cDNA clone was found to contain a whole open reading frame. The nucleotide sequence of the KSGal6ST cDNA and the predicted amino acid sequence are shown in Fig. 1A. A single open reading frame predicts a protein of 411 amino acid residues with five potential N-linked glycosylation sites.

To determine the location of any transmembrane domain, a hydropathy plot was generated from the translated sequence. Analysis of the plot revealed one prominent hydrophobic segment in the amino-terminal region, 14 residues in length, that extends from amino acid residues 7–20 (Fig. 1B).

Comparison of the coding sequence of human KSGal6ST with that of chick C6ST has revealed that there is 37% identity on the amino acid level (Fig. 2). There are 5 regions in which more than 6 consecutive amino acid residues are identical. Homology of N-terminal region was lower than that of the C-terminal region. No significant homology in amino acid sequence was observed between human KSGal6ST and any other sulfotransferases previously reported involving heparan sulfate N-sulfotransferase (29), heparan sulfate 2-sulfotransferase (30), and galactosylceramide 3'9-sulfotransferase (31).

Expression of Keratan Sulfate Gal-6-Sulfotransferase cDNA in COS-7 Cells—COS-7 cells were transfected with the pCXNKSGal6ST, a recombinant plasmid containing the isolated cDNA in the mammalian expression vector pCXN2. The transfected cells were scraped at 67 h after transfection, homogenized with a buffer containing 0.5% Triton X-100, and centrifuged. Activities of C6ST, C4ST, and KSST contained in the supernatant fractions were determined. Control experiments without vector and with vector containing the cDNA in the reversed orientation (pCXNKSGal6ST2) were also done. As shown in Table I, when the cells were transfected with pCXNKSGal6ST2, KSST activity in the transfected cells was unchanged compared with nontransfected cells, whereas about 10-fold increase in KSST activity was observed in the cells transfected with pCXNKSGal6ST. In contrast, both C6ST and C4ST activities were not increased above the control, indicating that the isolated cDNA encodes a protein with KSST activity alone.

Separation of the Overexpressed Keratan Sulfate Gal-6-Sulfotransferase from Endogenous C6ST—When COS-7 cells were transfected with pCXNKSGal6ST, KSST activity in the transfected cells was recovered in the flow-through fraction, whereas chondroitin sulfotransferase activity was recovered only in 0.5 M NaCl fraction (Fig. 3A). When cell extracts prepared from COS-7 cells cultured without the plasmid were applied to the DEAE-Sephadex column, no KSST activity was detected in the flow-through fraction (Fig. 3B). These observations indicate that the KSST activity recovered in the flow-through fraction is due to the overexpressed enzyme, which is encoded by KSGal6ST cDNA. About 80% of KSST activity from the transfected COS-7 cells was eluted in the 0.5 M NaCl fraction (Fig. 3A). When cell extracts prepared from COS-7 cells cultured without the plasmid were applied to the DEAE-Sephadex column, no KSST activity was detected in the flow-through fraction (Fig. 3B). These observations indicate that the KSST activity recovered in the flow-through fraction is due to the overexpressed enzyme, which is encoded by KSGal6ST cDNA. About 80% of KSST activity from the transfected COS-7 cells was eluted in the 0.5 M NaCl fraction, and this activity was much higher than the activity found in 0.5 M NaCl fraction from the control COS-7 cells, suggesting that a part of overexpressed KSST activity was also eluted in 0.5 M NaCl fraction. At present, it is not clear why the overexpressed KSST activity was separated into the two fractions. The flow-through fraction was further purified with heparin-Sepharose CL-6B and used as the partially purified KSGal6ST preparation for the following experiments.

Acceptor Substrate Specificity of the Partially Purified Keratan Sulfate Gal-6-Sulfotransferase—The partially purified KSGal6ST was found to transfer sulfate exclusively to keratan sulfate and desulfated keratan sulfate. Sulfotransferase activi-
TABLE I

| Plasmid          | Sulfotransferase activity |
|------------------|--------------------------|
|                  | C6ST | C4ST | KSST |
| None             | 1.2 ± 0.2 | 0.2 ± 0.1 | 1.9 ± 0.1 |
| pCXNKSGal6ST     | 1.3 ± 0.2 | 0.2 ± 0.1 | 19.9 ± 0.3 |
| pCXNKSGal6ST2    | 1.3 ± 0.3 | 0.3 ± 0.1 | 2.1 ± 0.2 |

TABLE II

| Glycosaminoglycans | Sulfotransferase activity (pmol/min/mg protein) |
|---------------------|-----------------------------------------------|
| None                | 0.01                                         |
| Keratan sulfate     | 2.51                                         |
| Desulfated keratan sulfate | 1.25                     |
| Chondroitin         | 0.03                                         |
| Chondroitin sulfate A | 0.03                                |
| Chondroitin sulfate C | 0.02                                |
| Dermatan sulfate    | 0.02                                         |
| CDSNS-heparin       | 0.05                                         |

Fig. 3. Separation of keratan sulfate Gal-6-sulfotransferase from chondroitin sulfotransferase with DEAE-Sephadex chromatography. Crude extracts from COS-7 cells transfected with pCXNKSGal6ST (A) or control COS-7 cells (B) were applied to a DEAE-Sephadex column as described under "Experimental Procedures." After washing with buffer A containing 0.05 M NaCl, the absorbed materials were eluted with buffer A containing 0.5 M NaCl (indicated by arrows). In this experiment, KSST activity (closed circles) and chondroitin sulfotransferase activity (open circles) were assayed by the method previously described (11).

Fig. 4. Separation of keratanase II digests with Partisil 10-SAX HPLC. Conditions for keratanase digestion and HPLC were as described under "Experimental Procedures." A, keratanase II digest of 35S-labeled keratan sulfate. B, keratanase II digest of 35S-labeled desulfated keratan sulfate. Peaks 1 and 4 corresponded to Gal(6S)β1–4GlcNAc(6S). Peak 2 appeared slightly faster than the retention time of Gal(1–4GlcNAc(6S) which is indicated by an arrow in panel A. The broken line in panel A shows the profile of the eluting salt gradient.

Fig. 5.结构分析 - 35S-标记的糖胺聚糖

Cloning of Keratan Sulfate Gal-6-Sulfotransferase

Sulfotransferase activities were assayed using various glycosaminoglycans as acceptors. Sulfotransferase fraction was prepared from COS-7 cells transfected with pCXNKSGal6ST as described under "Experimental Procedures." Keratan sulfate contained in the standard reaction mixture was replaced by 25 nmol (as galactosamine for chondroitin sulfate A, chondroitin sulfate C and dermatan sulfate, or as glucosamine for keratan sulfate, partially desulfated keratan sulfate and CDSNS-heparin) of glycosaminoglycans.

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KSGal6ST transfers sulfate to position 6 of Gal residues which are adjacent to GlcNAc(6S) or GlcNAc. From the observed specificity of the expressed enzyme, we proposed keratan sulfate Gal-6-sulfotransferase (KSGal6ST) for the name of this enzyme.

Northern Analysis—A Northern blot of poly(A)$^+$ RNA from adult human tissues was hybridized with a 32P-labeled probe prepared from the KSGal6ST cDNA by the random oligonucleotide-primed labeling method. As can be seen in Fig. 6, a clear mRNA band of 2.8 kb was observed in the brain, and a weaker band of slightly larger size was detected in skeletal muscle. Since keratan sulfate is known to be a major constituent of the cornea, it is important to examine the expression of KSGal6ST mRNA in the cornea. We prepared poly (A)$^+$ RNA from 12-day-old chick embryo tissues and used this for cross-hybridization with the human cDNA (Fig. 7). A band of about 2.8 kb that cross-hybridized with the human cDNA was detected in the chick embryo cornea and brain as well. No obvious bands were detected in chondrocytes in which C6ST was strongly expressed (13).

Assignment of Human KSGal6ST by Fluorescence in Situ Hybridization—Among 72 metaphase chromosome spreads analyzed, 17 showed twin-spot signals either on both or one of homologous chromosomes 11p near the centromere (Fig. 8, left). Such a specific accumulation of signals could not be detected on any other chromosomes. To determine the precise position of the signals, we detected fluorescein isothiocyanate signals on propidium iodide-stained metaphase chromosomes, and then the sublocalization was confirmed by delineation of the G-bands discernible on the same chromosomes (Fig. 8, right). This system allowed us to determine the precise locus of KSGal6ST at 11p11.1–11.2.

DISCUSSION

We report a new sulfotransferase (KSGal6ST) which catalyzes sulfation of the Gal residue of keratan sulfate. Since an amino acid sequence of KSGal6ST deduced from the nucleotide sequence of the cDNA showed 37% homology with chick C6ST, KSGal6ST and C6ST are thought to comprise a common family. Substrate specificity of KSGal6ST, however, is quite different from that of C6ST; KSGal6ST could not utilize chondroitin as an acceptor. KSGal6ST was found to catalyze the transfer of sulfate from PAPS to position 6 of the Gal residue of the disaccharide unit, Galβ1-4GlcNAc, contained in keratan sulfate. A disaccharide unit, Galβ1-4GlcNAc, which is present in the partially desulfated keratan sulfate, was also active as an acceptor. However, incorporation of 35SO$_4$ into the nonsulfated disaccharide unit was much lower than the incorporation into the monosulfated disaccharide unit. These observations suggest that KSGal6ST may prefer a Gal unit adjacent to the sulfated GlcNAc residue. The observed acceptor substrate specificity of KSGal6ST may indicate that sulfation of GlcNAc residues precedes sulfation of Gal residues during the biosynthesis of keratan sulfate. Such a mechanism was proposed previously from the structural investigation of keratan sulfate (27).

KSGal6ST was clearly separated from endogenous chondroitin sulfotransferase and heparan sulfate sulfotransferase contained in COS-7. Rütter and Kresse (10) previously reported that KSST activity extracted from the bovine corneal cells was separated from chondroitin sulfotransferase activity. They showed that KSST activity was not absorbed to DEAE-tri-
sacryl, but chondroitin sulfotransferase activity was completely absorbed to this resin. We found that KSGal6ST devoid of chondroitin sulfotransferase activity could be obtained from the flow-through fraction in DEAE-Sephadex chromatography. This chromatographic behavior of KSGal6ST is similar to that of the corneal KSST. However, a large part of the expressed KSST activity was also found in the DEAE-Sephadex-absorbed fraction. It is not clear at present why KSGal6ST separated into the two fractions. Post-translational modification such as glycosylation and proteolytic cleavage may produce a heterogeneous nature in the expressed product.

In the developing chicken cornea, keratan sulfate is actively synthesized. Nakazawa et al. (8) analyzed 35S-labeled keratan sulfate synthesized by the corneal explants from various stages of developing chick embryos. They found that, when 35S-labeled keratan sulfate was digested with keratanase II, a proportion of Gal(6S)β1-4GlcNAc(6S) increased as development proceeded. Their observation seemed to indicate that sulfation of position 6 of Gal residue actually occurs during the development of the cornea. Since KSGal6ST mRNA was expressed in 12-day-old chick cornea, KSGal6ST is possibly involved in the biosynthesis of keratan sulfate in the developing cornea.

Macular corneal dystrophy is an inherited disorder characterized by corneal opacity (32). It is proposed that this disease is possibly caused by an error in the synthesis of keratan sulfate, because defective sulfation of keratan sulfate was observed in the isolated cornea of patients with macular dystrophy (9, 33). However, as far as we know, no definitive evidence concerning the specificity of the affected sulfotransferase has been reported. It is of interest to determine the expression of KSGal6ST in macular corneal dystrophy.

Since KSGal6ST mRNA was expressed most strongly in the brain among human adult tissues so far examined, KSGal6ST is expected to be involved in the synthesis of brain-specific keratan sulfate proteoglycans. Specific localization of keratan sulfate proteoglycans to the brain has been reported. Rauch et al. (6) reported a proteoglycan containing both chondroitin sulfate and keratan sulfate in rat brain. A synaptic vesicle glycoprotein, SV2, which is thought to be a transport protein (34, 35), was reported to be a proteoglycan containing keratan sulfate (4). In the neurtic plaques of Alzheimer’s disease, specific localization of keratan sulfate to the periphery of the plaques was observed (36). Lindahl et al. (37) reported that highly sulfated keratan sulfate was selectively decreased in the cerebral cortex in Alzheimer’s disease. Since the GlcNAc residue in corneal keratan sulfate was always sulfated, but about half of the Gal residue was not sulfated (27), the degree of sulfation of keratan sulfate may be increased through the sulfation of the Gal residue. Human KSGal6ST, which preferentially transfers sulfate to Gal residue of Galβ1-4GlcNAc(6S) unit, may be involved in the synthesis of highly sulfated keratan sulfate which was selectively decreased in Alzheimer’s disease.

The presence of sulfated sialyl N-acetyllactosamine was reported in the corneal keratan sulfate as a capping structure of the nonreducing end (38). We found that KSGal6ST catalyzed sulfation at position 6 of Gal residue in sialyl N-acetyllactosamine; therefore, it may be possible that KSGal6ST also catalyzes efficient sulfation of the capping structure. Chiba et al. (39) reported that sialyl N-acetyllactosamine is contained in the major sugar chain in α-dystroglycan and may be involved in the interaction of α-dystroglycan with laminin. Since KSGal6ST is expressed strongly in the brain, it is important to examine whether KSGal6ST could transfer sulfate to the oligosaccharide attached to α-dystroglycan and modify its binding activity.

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