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Expression Cloning of a Human Sulfotransferase That Directs the Synthesis of the HNK-1 Glycan on the Neural Cell Adhesion Molecule and Glycolipids*

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Edgar Ong‡, Jiunn-Chern Yeh, Yili Ding, Ole Hindsgaul, and Minoru Fukuda§

From the Glycobiology Program, La Jolla Cancer Research Center, The Burnham Institute, La Jolla, California 92037

The HNK-1 carbohydrate is expressed on various adhesion molecules in the nervous system and is suggested to play a role in cell-cell and cell-substratum interactions. Here we describe the isolation and functional expression of a cDNA encoding a human sulfotransferase that synthesizes the HNK-1 carbohydrate epitope. A mutant Chinese hamster ovary cell line, Lec2, which stably expresses human neural cell adhesion molecule (N-CAM) (Lec2-NCAM), was first established. Lec2-NCAM was co-transfected with a human fetal brain cDNA library, a cDNA encoding the rat glucuronyltransferase that forms a precursor of the HNK-1 carbohydrate, and a vector encoding the polyoma large T antigen. The transfected Lec2-NCAM cells expressing the HNK-1 glycan were enriched by fluorescence-activated cell sorting. Sibling selection of recovered plasmids resulted in a cDNA encoding a sulfotransferase, HNK-1ST, that directs the expression of the HNK-1 carbohydrate epitope on the cell surface. The deduced amino acid sequence indicates that the enzyme is a type II membrane protein. Sequence analysis revealed that there is a short amino acid sequence in the presumed catalytic domain, which is highly homologous to the corresponding sequence in other Golgi-associated sulfotransferases so far cloned. The amount of HNK-1ST transcript is high in fetal brain compared with fetal lung, kidney, and liver. Expression of HNK-1ST resulted in the formation of the HNK-1 epitope on N-CAM and a soluble chimeric form of HNK-1ST was shown to add a sulfate group to a precursor, GlcAβ1→3Galβ1→4GlcNAcβ1→R, forming sulfo→3GlcAβ1→3Galβ1→4GlcNAcβ1→R. The results combined together indicate that the cloned HNK-1ST directs the synthesis of the HNK-1 carbohydrate epitope on both glycoproteins and glycolipids in the nervous tissues.

Neural cells express unique carbohydrates that are often shared by immune cells (1, 2). One of them is the HNK-1 carbohydrate epitope, originally discovered by a monoclonal antibody raised against human natural killer cells (3), although its role in immune cells is not known. The functional significance of the HNK-1 carbohydrate was first recognized as an auto-antigen involved in peripheral demyelinative neuropathy. The structural analysis of glycolipids reacting with these auto-antibodies led to the discovery that the HNK-1 epitope is sulfo→3GlcAβ1→3Galβ1→4GlcNAcβ1→R (4, 5).

By using HNK-1-specific antibodies and carbohydrate structural studies, the HNK-1 glycan has been found in a number of neural cell adhesion molecules including N-CAM,[1] myelin-associated glycoprotein, L1, contactin, and P0 (2, 6–9). The studies, using either monoclonal antibodies or isolated carbohydrates, demonstrated that the HNK-1 glycan is involved in cell-cell and cell-substratum interactions (10, 11). In one study, the inhibition by HNK-1 oligosaccharide was abolished by desulfation of the HNK-1 glycan indicating the critical role of the sulfate group (11). The expression of the HNK-1 epitope is spatially and developmentally regulated, and is found on migrating neural crest cells, cerebellum, and myelinating Schwann cells in motor neurons but not on those in the sensory neurons (12–14). In addition, the HNK-1 carbohydrate was shown to bind to P- and L-selectins (15), suggesting that the interactions between immune cells and the nervous system may be mediated through the binding of the HNK-1 carbohydrate in neural cells.

The HNK-1 carbohydrate is synthesized in a stepwise manner by the addition of a β-1,3-linked glucuronic acid to a precursor *N*-acetyllactosamine followed by the addition of a sulfate group to GlcAβ1→3Galβ1→4GlcNAc→R (16, 17). Recently, Terayama *et al*. (18) reported the cloning of a β-1,3-glucuronyltransferase, GlcAT-P, that forms an HNK-1 precursor carbohydrate, GlcAβ1→3Galβ1→4GlcNAcβ1→R, in glycoproteins (18). As a part of our systematic studies on neural cell glycoconjugates (19–22, 45), we describe herein the expression cloning of HNK-1 sulfotransferase, HNK-1ST. Using the cDNA isolated, the expression profile of the HNK-1ST transcripts was compared with that of GlcAT-P transcripts for various fetal and adult tissues. We also demonstrate the HNK-1ST activity *in vivo* and *in vitro* using N-CAM, synthetic oligosaccharides and glycolipids as acceptors.

EXPERIMENTAL PROCEDURES

Preparation of Recipient Cells and Plasmids—A mutant cell line of Chinese hamster ovary cells, Lec2, was used as recipient cells. β-Glucuronylation of *N*-acetyllactosamines is extremely efficient in Lec2 cells (18) because sialylation is absent in this cell line (23). Lec2 cells were first transfected with pHβAPr-1-neo-*NCAM* 140 (24) and a stable cell line expressing human N-CAM, Lec2-NCAM, was selected as described before (19). For cloning of GlcAT-P, the cDNA was synthesized from poly(A)$^+$ RNA of rat brain (CLONTECH) using a reverse transcription-PCR kit (Stratagene). Using the cDNAs synthesized as templates, PCR

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF033827.

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§ To whom correspondence should be addressed: The Burnham Institute, 10901 N. Torrey Pines Rd., La Jolla, CA 92037. Tel.: 619-646-3144; Fax: 619-646-3193; E-mail: minoru@burnham-inst.org.

[1] The abbreviations used are: N-CAM, the neural cell adhesion molecule; GlcAT-P, glycoprotein-specific glucuronyltransferase; HNK-1ST, HNK-1 sulfotransferase; PCR, polymerase chain reaction; PAPS, 3′-phosphoadenosine 5′-phosphosulfate.

was performed to amplify the GlcAT-P sequence under the conditions described previously (21). The 5′- and 3′-primers correspond to nucleotides −32 to −10 and nucleotides 1047–1027, respectively, of the reported rat GlcAT-P sequence (18). The 5′- and 3′-primers also contain *Hin*dIII and *Xho*I sites, respectively. The PCR product was digested with *Hin*dIII and *Xho*I, then cloned into pcDNA3, resulting in pcDNA3-GlcAT-P.

Isolation of a Human HNK-1ST cDNA Clone—Lec2-NCAM cells were found to be negative for the HNK-1 antigen after pcDNA3-*GlcAT-P* was transiently expressed. Lec2-NCAM cells were thus co-transfected with 18 μg of a human fetal brain cDNA library in pcDNAI (19), 6 μg of pcDNA3-*GlcAT-P*, and 6 μg of pPSVE1-PyE harboring the polyoma large T cDNA (25), using LipofectAMINE™ (Life Technologies, Inc.) as described previously (19). After 62 h, the transfected cells were dissociated into monodispersed cells using the enzyme-free cell dissociation solution (Hanks' based, purchased from Cell and Molecular Technologies, Lavellette, NJ), followed by fluorescence-activated cell sorting of the HNK-1-positive cells using anti-HNK-1 monoclonal antibody (Becton Dickinson). Plasmid DNA from the sorted cells was isolated by the Hirt (26) procedure and amplified in the host bacteria *Escherichia coli* MC1061/P3 in the presence of ampicilin and tetracycline. The pcDNAI vector contains the supF suppressor tRNA, so that MC1061/P3 cells containing pcDNAI are resistant to both ampicillin and tetracycline. In contrast, MC1061/P3 cells harboring pcDNA3-GlcAT-P or pPSVE1-PyE are resistant to ampicillin but not to tetracycline. Because of this difference, only plasmids derived from pcDNAI were rescued and amplified by this procedure (19), allowing the isolation of plasmids responsible for the HNK-1 glycan expression.

Bacteria harboring plasmids, which were isolated by the Hirt procedure, were divided into 20 plates. Plasmid DNA was prepared from each plate and separately transfected into Lec2-NCAM cells together with pcDNA3-*GlcAT-P*. The transfectants were screened by immunofluorescence microscopy using anti-HNK-1 antibody to identify a plasmid pool that directed the expression of the HNK-1 glycan. By narrowing down the plasmid pools using the same procedure, it was possible to isolate a single clone containing the plasmid DNA (pcDNAI-*HNK-1ST*) encoding a human sulfotransferase that directed the expression of the HNK-1 carbohydrate epitope.

Construction of Vectors Harboring Short 5′- and 3′-Untranslated Sequences—To shorten the long 3′-untranslated sequence of pcDNAI-*HNK-1ST*, HNK-1ST cDNA was digested utilizing an internal *Eco*RI site 15 nucleotides downstream of the stop codon and cloned into pcDNA3, resulting in pcDNA3-HNK-1ST (short). A truncated cDNA containing only 9 and 6 nucleotides of 5′- and 3′-untranslated sequences in addition to the coding sequence was prepared by PCR. The 5′- and 3′-primers for the PCR were 5′-GTCAAGCTTTGTGACAAACATGCACCACCAGTGGCT-3′ and 5′-GCGCTCGAGTATGCATTAGTTTAGCAAAAAGTC-3′. *Hin*dIII and *Xho*I sites are singly underlined, while HNK-1ST-coding sequences are doubly underlined. After restriction enzyme digestion, the PCR product was cloned into pcDNA3, yielding pcDNA3-*HNK-1ST* (ORF). Nucleotide sequences were determined in both strands by an automated sequencer (Applied Biosystems 377XL).

Northern Blot Analysis of Various Human Tissues—Human multiple tissue Northern blots of poly(A)$^+$ RNA (CLONTECH) were hybridized sequentially with gel-purified cDNA inserts of pcDNA3-*HNK-1ST* (ORF) and pcDNA3-*GlcAT-P*, after labeling with [^{32}P]dCTP by random oligonucleotide primers (Prime-IT II labeling kit, Stratagene).

Western Blot Analysis of N-CAM Expressing the HNK-1 Carbohydrate—Lec2-NCAM cells were transiently transfected with pcDNAI-*HNK-1ST* and pcDNA3-*GlcAT-P*, pcDNAI-*HNK-1ST* alone or pcDNA3-*GlcAT-P* alone. Forty-eight h after transfection, cell lysates were made from the transfected cells and incubated with a mouse anti-human N-CAM monoclonal antibody (ERIC-1, Santa Cruz Biotechnology) (27), followed by protein G-agarose (Pierce). After solubilization, the immunoprecipitates were separated by SDS-polyacrylamide (5%) gel electrophoresis and transferred onto nitrocellulose membrane. The blot was then incubated with the anti-N-CAM antibody, anti-HNK-1 antibody, or M6749 antibody (28) followed by horseradish peroxidase-conjugated sheep anti-mouse immunoglobulins and visualized by an ECL kit (Amersham Corp.).

Expression of the Protein A-HNK-1ST Fusion Protein—The cDNA fragment encoding the stem region plus catalytic domain of HNK-1ST was prepared by PCR using pcDNA3-*HNK-1ST* (short) as a template and fused with the cDNA encoding a signal peptide sequence and the IgG binding domain of *Staphylococcus aureus* protein A (20, 29). The 5′-primer for this PCR is TTAGATCTACCAGATGTGTACAGTGCC, where *Bgl*II site is underlined, and the coding sequence of HNK-1ST is doubly underlined. The 3′-primer is SP6 promoter sequence. The PCR product was digested by *Bgl*II and *Xho*I then cloned into *Bam*HI and *Xho*I sites of pcDNAI-A (20), yielding pcDNAI-A·HNK-1ST. pcDNAI-A·HNK-1ST and pcDNAI-A were separately transfected to COS-1 cells and the enzyme was adsorbed to IgG-Sepharose 6FF (Pharmacia Biotech Inc.) as described previously (20).

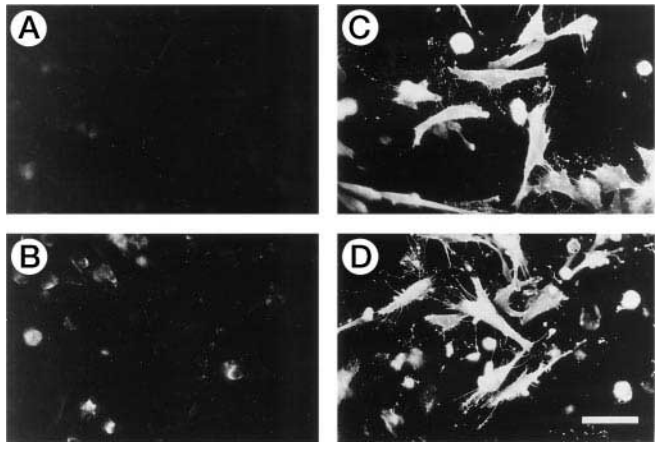

FIG. 1. **Immunofluorescence staining of transfected Lec2-NCAM cells by anti-HNK-1 antibody.** Lec2-NCAM cells, transfected with pcDNA3-*GlcAT-P* (*A*), pcDNAI-*HNK-1ST* (*B*), pcDNAI-*HNK-1ST* and pcDNA3-*GlcAT-P* (*C*), and pcDNA3-*HNK-1ST* (ORF) and pcDNA3-GlcAT-P (*D*), were incubated with mouse anti-HNK-1 antibody followed by fluorescein isothiocyanate-conjugated goat anti-mouse IgM antibodies. *Bar* = 40 μm.

Assay of in Vitro Activity of HNK-1ST—Galβ1→4GlcNAcβ1→octyl, GlcAβ1→3Galβ1→4GlcNAcβ1→octyl, and sulfo→3GlcAβ1→3Galβ1→4GlcNAcβ1→octyl were synthesized according to the reported procedures (30, 31) with a slight modification. The detailed procedure for the synthesis of these oligosaccharides will be published elsewhere.[2] Key ^{1}H NMR (600 MHz, D_2O) for GlcAβ1→3Galβ1→4GlcNAcβ1→octyl is included; δ 4.680 (*d*, *J* = 8.0 Hz, H-1 GlcA), 4.520 (secondary order, $J_{1,2}$ = 8.1 Hz) and 4.502 (*d*, *J* = 7.9 Hz) (H-1 Gal and H-1 Glc), 4.190 (*d*, *J* = 3.1, H-4 Gal), 2.040 (*s*, $NCOCH_3$). GlcAβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→ceramide was prepared by acid hydrolysis from its sulfated form (4) and kindly provided by Dr. Firoze Jungalwala.

The reaction mixtures (100 μl total) contained 0.02 mM [^{35}S]PAPS, 25 μl of IgG bead-bound enzyme suspension, 0.1 mM acceptor oligosaccharides or 0.0265 mM acceptor glycolipids in 100 mM Tris-HCl, pH 7.2, 0.1% Triton X-100, 10 mM $MnCl_2$, and 2.5 mM ATP (17). After incubation for 2 h at 37 °C, the reaction products were adjusted to 0.25 M ammonium formate, pH 4.0, and applied to C18 reverse phase column (Alltech). After washing the column with the same solution, the product was eluted with 70% methanol. The radioactivity was measured by scintillation counting.

The products were separated by thin layer chromatography using silica gel. After separation in chloroform, methanol, 0.25% KCl (5:4:1, v/v/v), the thin layer plate was exposed to X-ray film (Kodak BioMax) for 16 h at room temperature. Standard oligosaccharides were then detected by spraying with 0.2% orcinol in 2 M H_2SO_4.

RESULTS

Isolation of a cDNA Clone That Directs the Expression of the HNK-1 Carbohydrate Epitope—Lec2-NCAM cells were co-transfected with a human fetal brain cDNA library in pcDNAI, pcDNA3-*GlcAT-P*, and pPSVE1-PyE (25). The transfected cells were incubated with anti-HNK-1 antibody followed by fluorescein isothiocyanate-conjugated secondary antibody, then subjected to cell sorting. Plasmid DNA, recovered from anti-HNK-1 antibody-positive Lec2-NCAM cells, was immediately subjected to sibling selection with sequentially smaller, active pools, identifying a single clone containing the plasmid, pcDNAI-*HNK-1ST*, that directed the expression of the HNK-1 glycan (Fig. 1*C*). Weak staining was observed when only HNK-1ST was present (Fig. 1*B*) probably due to endogenous expres-

[2] Y. Ding and O. Hindsgaul, manuscript in preparation.

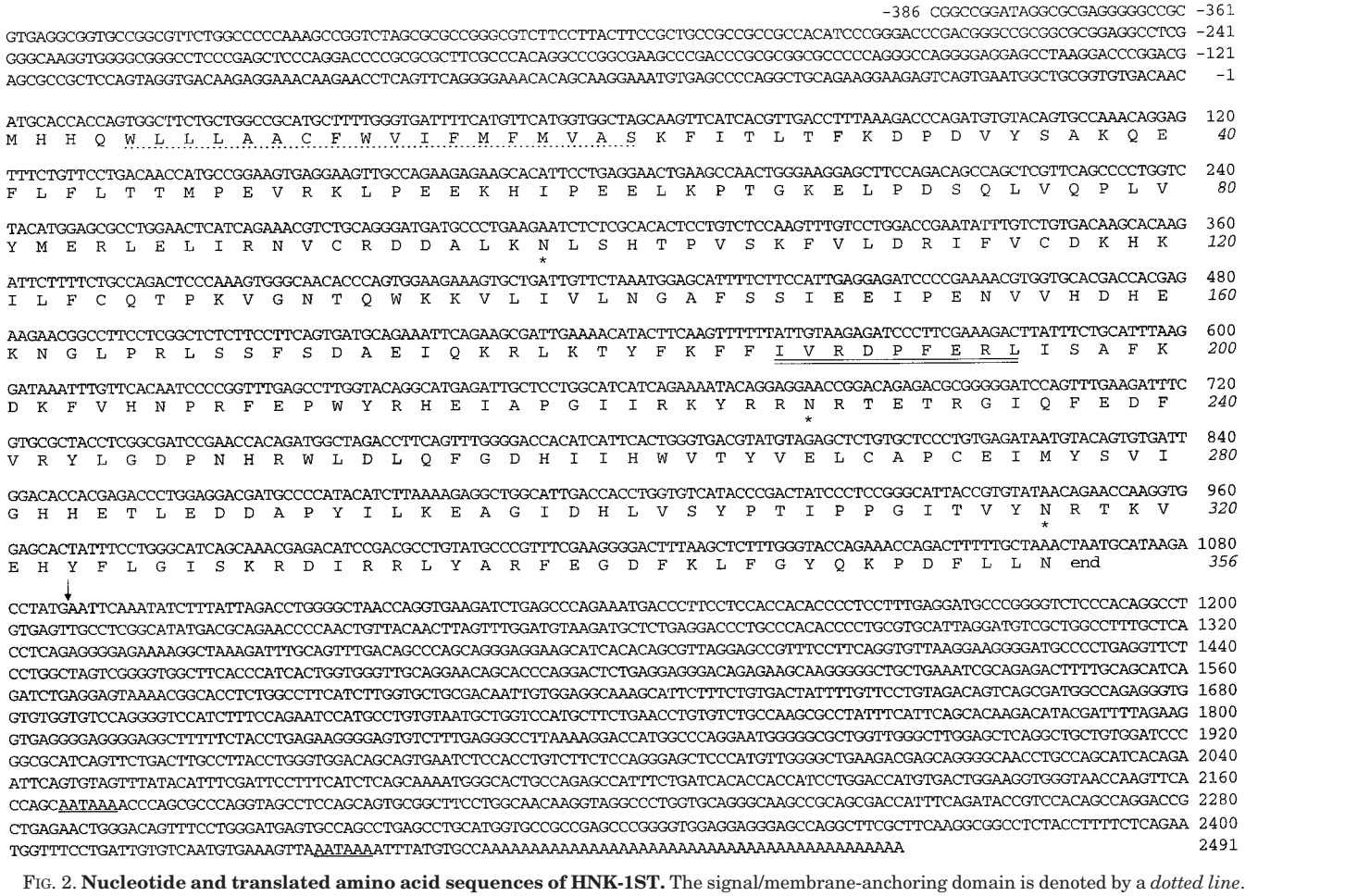

FIG. 2. **Nucleotide and translated amino acid sequences of HNK-1ST.** The signal/membrane-anchoring domain is denoted by a *dotted line*. A sequence homologous to that found in other Golgi-associated sulfotransferases is *doubly underlined*, and the polyadenylation consensus sequences are *singly underlined*. Potential *N*-glycosylation sites are marked with *asterisks*. The *Eco*RI site used for construction of a shorter cDNA HNK-1ST (short) is indicated by an *arrow*.

sion of a small amount of GlcAT-P. No staining was observed when HNK-1ST cDNA was not expressed (Fig. 1*A*).

Predicted Amino Acid Sequence of HNK-1ST—The cDNA insert encoding HNK-1ST contains an open reading frame predicting a protein of 356 amino acid residues (42,206 Da) (Fig. 2). A hydropathy plot predicts that this protein has a type II membrane topology, and the transmembrane domain (residues 5–22) is flanked by basic amino acids. This topology has been found in almost all mammalian glycosyltransferases so far cloned (32). Although there is no significant similarity between the cloned HNK-1ST and sequences for other proteins deposited in GenBank™, the amino acid sequence of residues 187–195 (see the doubly underlined sequence in Fig. 2 and Fig. 3) has homology with the sequences found in other Golgi-associated sulfotransferases such as chick chondroitin sulfate 6-*O*-sulfotransferase, human galactosylceramide sulfotransferase, hamster heparan sulfate 2-*O*-sulfotransferase, and human heparan sulfate 3-*O*-sulfotransferase (33–36). In particular, the RDP sequence (residue 189–191) is completely conserved, and hydrophobic amino acids are shared among these amino acid sequences (Fig. 3). Moreover, the amino acid sequence of the rat HNK-1ST reported recently (37) has the identical sequence as the human HNK-1ST in residues 187–195.

To determine if the translation starts at the presumed methionine, a truncated cDNA starting from 9 base pairs upstream of the initiation codon was constructed in pcDNA3, yielding pcDNA3-*HNK-1ST* (ORF). This plasmid directed the expression of the HNK-1 carbohydrate (Fig. 1*D*), confirming

huHNK-1ST	187-IV**RDP**FERL-195
raHNK-1ST	187-IV**RDP**FERL-195
haHep2ST	162-VI**RDP**IERL-170
chCho6ST	279-LV**RDP**RAVL-287
huGalCerST	162-VL**RDP**ARLF-170
hu3-*O*ST	145-IL**RDP**SERV-153
hu3-*O*ST	220-LI**RDP**FPEI-228
consensus	xx**RDP**zzzx

FIG. 3. **Comparison of amino acid sequences of different sulfotransferases.** The amino acid sequences of human HNK-1ST (*huHNK-1ST*) is compared with that of rat HNK-1ST (*raHNK-1ST*), hamster heparan sulfate 2-*O*-sulfotransferase (*haHep2ST*), chick chondroitin sulfate 6-*O*-sulfotransferase (*chCho6ST*), human galactosylceramide sulfotransferase (*huGalCer-ST*), and human heparan sulfate 3-*O*-sulfotransferase (*hu3-OST*). In the consensus sequence, *x* and *z* denote hydrophobic amino acids and any amino acids, respectively. The number of the amino acid residue is shown at both ends. The compared sequences were reported in Refs. 33–37.

that nucleotides 1–1068 encode the coding region of HNK-1ST (Fig. 2).

There are three potential *N*-glycosylation sites (see *asterisks* in Fig. 2) in the human HNK-1ST sequence. A consensus sequence for polyadenylation signal is present at nucleotides 2432–2437 followed by a poly(A) tail. Judging from the size of

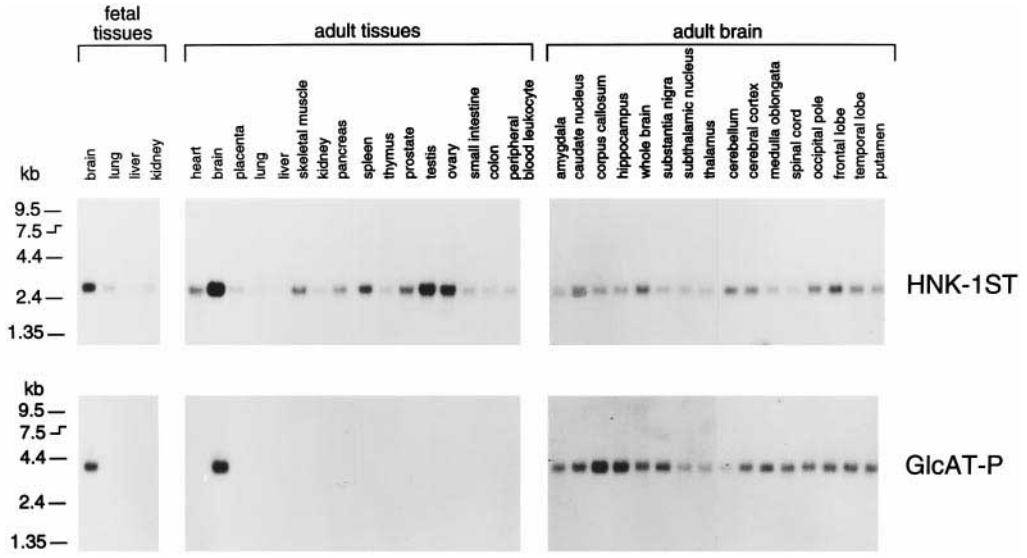

FIG. 4. **Northern blot analysis of HNK-1ST and GlcAT-P.** Each lane contained 2 μg of poly(A)$^+$ RNA. The blots were hybridized with ^{32}P-labeled HNK-1ST cDNA followed by GlcAT-P cDNA.

the mRNA (see below), the whole cDNA (2877 base pairs) nearly covers the whole transcript.

Expression of HNK-1ST mRNA in Human Tissues—Northern blots of poly(A)$^+$ RNA derived from various human tissues were examined (Fig. 4). An *HNK-1ST* transcript of ~3 kilobases was prominently detected in fetal brain, moderately in lung, and kidney but barely in liver. The same transcript was strongly detected in adult brain, testis, ovary, and moderately in heart, skeletal muscle, pancreas, spleen, and thymus, but weakly in other tissues. Among various parts of the brain, *HNK-1ST* transcript is expressed more in the frontal lobe than the other parts.

The transcript of *GlcAT-P* is almost exclusively expressed in both fetal and adult brains, as shown previously for rat tissues (18). Moreover, the *GlcAT-P* transcript is ubiquitously expressed in various parts of the brain and it is most abundant in the corpus callosum and hippocampus (Fig. 4). Since GlcAT-P is necessary to form the HNK-1 glycan in glycoproteins, expression of the HNK-1 carbohydrate in glycoproteins of nervous tissue may be determined by the regulation of GlcAT-P expression. In other tissues, it is possible that HNK-1ST may utilize acceptors other than those synthesized by GlcAT-P.

Expression of the HNK-1 Epitope on N-CAM—To determine if HNK-1ST is capable of adding the HNK-1 epitope on N-CAM, Lec2-NCAM cells were transiently transfected with pcDNAI-*HNK-1ST* and pcDNA3-*GlcAT-P*, pcDNAI-*HNK-1ST* alone or pcDNA3-*GlcAT-P* alone. Western blot analysis of N-CAM derived from those transfected cells demonstrated that the HNK-1 glycan was formed on N-CAM when both HNK-1ST and GlcAT-P were expressed while the HNK-1 glycan was not expressed in the absence of either enzyme (Fig. 5*B*, *lanes 1–3*).

The expression of GlcAT-P alone resulted in the binding of the M6749 antibody (Fig. 5*C*, *lane 3*), which was shown to react with both sulfated and non-sulfated forms of the HNK-1 carbohydrate (18, 28). These results clearly establish that HNK-1ST forms the HNK-1 carbohydrate epitope on N-CAM when a glucuronyl precursor structure is present.

Demonstration of In Vitro Activity of HNK-1ST—To formally prove that the cloned cDNA encodes HNK-1ST, a soluble chimeric HNK-1ST was expressed in COS-1 cells. The enzyme adsorbed to IgG-Sepharose was then incubated with acceptor oligosaccharides or glycolipid and [^{35}S]PAPS. As shown in Fig. 6, a substantial amount of ^{35}S-sulfate was incorporated to GlcAβ1→3Galβ1→4GlcNAcβ1→octyl (*lane 4*), while no incorporation was detected using the medium from mock-transfected COS-1 cells (*lane 3*), or using Galβ1→4GlcNAcβ→octyl as an acceptor (*lane 2*). Similarly, ^{35}S-sulfate was incorporated into the glycolipid acceptor (*lane 6*). Considering that the con-

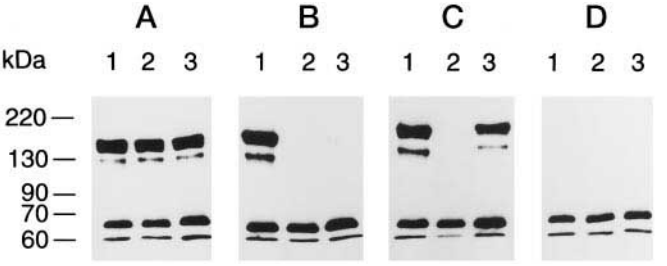

FIG. 5. **Western blot analysis of Lec2-NCAM cells transfected with pcDNAI-HNK-1ST and pcDNA3-GlcAT-P.** Lec2-NCAM cells were transiently transfected with pcDNAI-*HNK-1ST* and pcDNA3-*GlcAT-P* (*lanes 1*), pcDNAI-HNK-1ST alone (*lanes 2*), or pcDNA3-*GlcAT-P* alone (*lanes 3*). Western blot of N-CAM separated after 5% SDS-polyacrylamide gel electrophoresis was incubated with anti-N-CAM antibody (*A*), anti-HNK-1 antibody (*B*), M6749 antibody (*C*), or the secondary antibody alone (*D*).

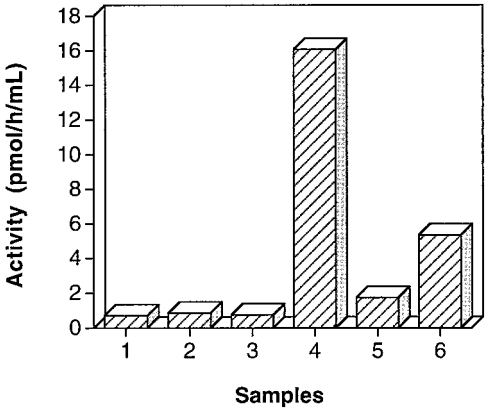

FIG. 6. **Incorporation of ^{35}S-sulfate to acceptors by HNK-1ST.** The enzyme adsorbed to IgG-Sepharose from COS-1 cells transfected with pcDNAI-A (*lanes 1*, *3*, and *5*) and pcDNAI-A-*HNK-1ST* (*lanes 2*, *4*, and *6*) was incubated with Galβ1→4GlcNAcβ1→octyl (*lanes 1* and *2*) or with GlcAβ1→3Galβ1→4GlcNAcβ1→octyl (*lanes 3* and *4*) or with GlcAβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→ceramide (*lanes 5* and *6*). The activity is expressed as picomoles of sulfate transferred from PAPS/h/ml of the culture medium.

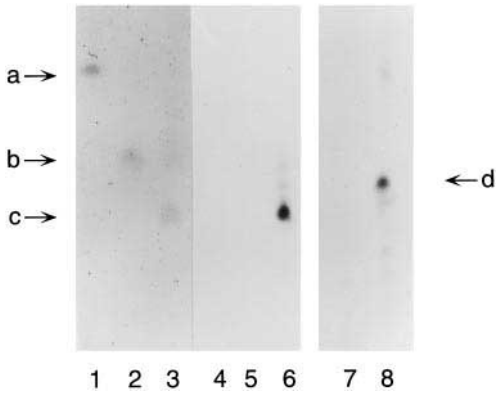

FIG. 7. **Thin layer chromatography of the reaction products after incubation with HNK-1ST.** Thin layer chromatography was carried out on the products obtained by the enzyme derived from pcDNAI-A (*lanes 5* and *7*) and pcDNAI-A-*HNK-1ST* (*lanes 4*, *6*, and *8*) and subjected to autoradiography. *a*, *b*, *c*, and *d* denote the migration position of Galβ1→4GlcNAcβ1→octyl, GlcAβ1→3Galβ1→4GlcNAcβ1→octyl, sulfo→3GlcAβ1→3Galβ1→4GlcNAcβ1→octyl, and sulfo→3GlcA-β1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→ceramide, respectively. *Lanes 1–3* represent standard oligosaccharides detected by the orcinol-H_2SO_4 reagent. *Lanes 1–6* and *lanes 7* and *8* were run separately.

centration of the oligosaccharide acceptors is 3.8 times higher than that of the glycolipid acceptor, HNK-1ST added sulfate to the oligosaccharide as efficiently as to the glycolipid. The reaction products shown in *lanes 2–6* were subjected to thin layer chromatography followed by autoradiography. The sulfated product migrated at the same position of a standard synthetic oligosaccharide, sulfo→3GlcAβ1→3Galβ1→4GlcNAcβ1→octyl (Fig. 7). These results establish that the cloned HNK-1ST transfers a sulfate group to C-3 of GlcA attached to *N*-acetyllactosamine, forming sulfo→3GlcAβ1→3Galβ1→4GlcNAcβ1→R.

DISCUSSION

In this study, we describe the isolation of a cDNA clone encoding a human sulfotransferase, HNK-1ST, the enzyme responsible for the formation of a sulfate group attached to the C-3 of glucuronic acid residue in GlcAβ1→3Galβ1→4GlcNAc-β1→R structure. For this cloning, Lec2 cells were first stably transfected to express human N-CAM. Those Lec2 cells expressing N-CAM were then transiently co-transfected with a human fetal brain cDNA library in pcDNAI, pcDNA3-*GlcAT-P*, and pPSVE1-PyE. pcDNAI has the *supF* gene while both pcDNA3-*GlcAT-P* and pPSVE1-PyE contain only the ampicillin-resistant gene. Only plasmids derived from pcDNAI can be thus rescued and amplified in bacteria containing P3 episome such as MC1061/P3 cells in the presence of ampicilin and tetracycline (38). In theory, N-CAM cDNA could be also co-transfected transiently since its vector contains only an ampicillin resistant marker. This procedure represents an improved method over previous expression cloning strategy where a vector encoding the polyoma large T antigen was stably expressed (25, 39). By avoiding the preparation of stable transfectants, which are necessary for the expression of the acceptor carbohydrates and polyoma large T antigen, the time necessary for cloning has been shortened dramatically. The cloning of the HNK-1ST cDNA started right after the publication of GlcAT-P sequence (18) and took approximately 2.5 months to obtain a single clone.

After the submission of our paper, Bakker *et al*. (37) reported the cloning of a rat HNK-1ST. Their cloning strategy differs slightly from ours because CHOP2 cells, which are Lec2 cells stably expressing the polyoma large T antigen (40), were used as recipient cells, and the N-CAM cDNA was not co-transfected. Since we were interested in cloning the cDNA that adds the HNK-1 glycan on neural cell adhesion molecules, Lec2 cells stably expressing N-CAM were used as recipient cells in our studies. In the end, however, the cDNAs cloned by us and their group probably encode the same enzyme, since 90.2% of amino acid residues are identical between the human HNK-1ST and the cloned rat enzyme.

As shown in Fig. 3, there appears to be a consensus sequence among various Golgi-associated sulfotransferases. It is possible that the amino acid sequence may be involved in the binding of PAPS or in catalysis. Notably, this sequence is not shared by *N*-deacetylase/*N*-sulfotransferase, which has dual functions and is involved in heparan sulfate synthesis (41, 42). It is tempting to speculate that *N*-deacetylase/*N*-sulfotransferase is different in evolutionary origin than the other sulfotransferases. Moreover, the consensus sequence shown in Fig. 3 is different from the presumed PAPS binding sequences found among different soluble cytosolic sulfotransferases (43). Further studies are necessary to determine if the consensus sequence found among different Golgi-associated sulfotransferases plays a role in the enzymatic function.

Our studies revealed that the cloned human HNK-1ST adds the HNK-1 carbohydrate epitope on N-CAM. Bakker *et al*. (37) on the other hand showed that the rat GlcAT-P and rat HNK-1ST add the β-glucuronic acid and sulfate into a wide variety of glycoproteins in CHOP2 cells, although none of them was identified. These results strongly suggest that GlcAT-P can add glucuronic acid to *N*-acetyllactosamine present in a wide variety of glycoproteins and HNK-1ST can use those carbohydrates as acceptor molecules.

Our studies also demonstrate that the transcript of HNK-1ST is more widely distributed in different tissues compared with that of GlcAT-P (Fig. 4). In the brain, both the HNK-1ST and GlcAT-P transcripts are highly expressed, suggesting that the cloned HNK-1ST and GlcAT-P are most likely responsible for the formation of the HNK-1 glycan in the nervous tissues. It has been shown that there are two glucuronyltransferases specific for forming the HNK-1 precursor structure in glycoproteins and glycolipids, respectively (44). In contrast, HNK-1ST cloned in the present study was shown to add a sulfate group to both glycoproteins and glycolipids. The HNK-1ST present in tissues other than the brain may act on glycolipid acceptors. Alternatively, there is another GlcAT-P acting on glycoprotein acceptors, which may differ in tissue distribution than the one that has been cloned (18). Future studies are important to address this problem.

As described in the Introduction, the HNK-1 carbohydrate is associated with a number of cell adhesion molecules in the nervous tissues. The addition of the HNK-1 glycan to these various adhesion molecules are thought to modulate cell-cell and cell-substratum interactions. The HNK-1 cDNA obtained in the present study will be a powerful molecular tool to manipulate the expression level of the HNK-1 glycan in specific cell types, allowing us to dissect the intricate and complex cell processes of cell-cell interactions during development.

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