Molecular Cloning of a Novel α2,3-Sialyltransferase (ST3Gal VI) That Sialylates Type II Lactosamine Structures on Glycoproteins and Glycolipids*

(Received for publication, November 18, 1998, and in revised form, February 4, 1999)

Tetsuya Okajima‡, Satoshi Fukumoto‡‡, Hiroshi Miyazaki‡, Hideharu Ishida‡, Makoto Kiso‡, Keiko Furukawa‡, Takeshi Urano‡, and Koichi Furukawa‡‡

From the ‡Department of Biochemistry, Nagoya University School of Medicine, Tsuromai, Nagoya 466-0065, the ¶Department of Pediatric Dentistry, Nagasaki University School of Dentistry, Sakamoto, Nagasaki 852-8501, and the §Department of Applied Bio-organic Chemistry, Faculty of Agriculture, Gifu University, Gifu 501-1193, Japan

A novel member of the human CMP-NeuAc-β-galactoside α2,3-sialyltransferase (ST) subfamily, designated ST3Gal VI, was identified based on BLAST analysis of expressed sequence tags, and a cDNA clone was isolated from a human melanoma line library. The sequence of ST3Gal VI encoded a type II membrane protein with 297 amino acids; and showed homology to previously cloned ST3Gal III, ST3Gal IV, and ST3Gal V at 34, 38, and 33%, respectively. Extracts from L cells transfected with ST3Gal VI cDNA in an expression vector and a fusion protein with protein A showed an enzyme activity of α2,3-sialyltransferase toward Galβ1,4GlcNAc structure on glycoproteins and glycolipids. In contrast to ST3Gal III and ST3Gal IV, this enzyme exhibited restricted substrate specificity, i.e. it utilized Galβ1,4GlcNAc on glycoproteins, and neolactotetraosylceramide and neolactohexaosylceramide, but not lactotraosylceramide, lactosylceramide, or asialo-GM1. Consequently, these data indicated that this enzyme is involved in the synthesis of sialyl-paragloboside, a precursor of sialyl-Lewis X determinant.

Sialyltransferases are a family of more than 14 enzymes that catalyze the transfer of sialic acid from cytidine 5'-monophospho-N-acetylneuraminic acid (CMP-NeuAc) to terminal positions on sugar chains of glycoproteins and glycolipids. Terminal NeuAc residues are key determinants of carbohydrate structures involved in a variety of biological processes and are widely distributed in many cell types (1–3). For example, sialyl-Lewis X (sialyl-Le^a^) determinants have been reported to be ligands for the three known selectins (E-, P-, and L-selectins), which are cell adhesion molecules involved in the recruitment of leukocytes to lymphoid tissues and the sites of inflammation (4–6). Furthermore, increased expression of sialyl-Le^a^ determinants was suggested to contribute to the metastatic behavior of carcinoma cells (7). Glycosyltransferases involved in the synthesis of sialyl-Le^a^ structures are β1,3-N-acetylglucosaminyltransferase (8), β1,4-galactosyltransferase (9, 10), α1,3-fucosyltransferases (11–15), and α2,3-sialyltransferases, as described below.

To date, four enzymatically distinct human α2,3-sialyltransferase genes have been cloned and exhibited distinct acceptor substrate specificities. ST3Gal I (16, 17) and II (18, 19) synthesize the sequence NeuAcα2,3Galβ1,3GlcNAc common to many O-linked oligosaccharides and glycolipids like GM1b, GD1a, and GT1b. ST3Gal III (20, 21) forms a less common sequence, NeuAcα2,3Galβ1,3GlcNAc. ST3Gal IV (22, 23) is capable of forming the terminal NeuAcα2,3Galβ1,3GlcNAc and the NeuAcα2,3Galβ1,4GlcNAc sequence found in the carbohydrate moieties of glycoproteins and glycolipids. Recently, we have cloned mouse ST3Gal V (GM3 synthase) (24), which exhibits activity almost exclusively toward LacCer. Among five α2,3-sialyltransferases so far isolated, human ST3Gal III and IV are candidates for involvement in the formation of the sialyl-Le^a^ determinant in vivo. However, human ST3Gal III has been shown to utilize Galβ1,3GlcNAc much more efficiently than Galβ1,4GlcNAc as an acceptor in vitro. The reported substrate specificities of ST3Gal IV have been contradictory for its preference between Galβ1,3GlcNAc and Galβ1,4GlcNAc structure, and for utilization of glycolipids. Therefore, to date, no human α2,3-sialyltransferase that shows high and clear acceptor specificity toward Galβ1,4GlcNAc sequence has yet been identified.

In this study, using human expressed sequence tags, we have cloned a novel α2,3-sialyltransferase, designated ST3Gal VI. ST3Gal VI is a novel Galβ1,4GlcNAc α2,3-sialyltransferase with high specificity for neolactotetraosylceramide and neolactohexaosylceramide as glycolipid substrates. Moreover, ST3Gal VI prefers oligosaccharides containing the terminal Galβ1,4GlcNAc structure much more than those containing the

*This work was supported by Grant-in-aid for Scientific Research in Priority Areas 10176105, by a Core of Excellence grant from the Ministry of Education, Science, Sports and Culture of Japan, and by a grant-in-aid from Ono Medical Research Foundation. 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‡‡ and EBI Data Bank with accession number(s) AB022918.† To whom correspondence should be addressed: Dept. of Biochemistry II, Nagoya University School of Medicine, 65 Tsurumai, Showa-ku, Nagoya 466 Japan. Tel.: 81-52-744-2070; Fax: 81-52-744-2069; E-mail: koichi@med.nagoya-u.ac.jp.

1 The abbreviations used are: CMP-NeuAc, cytidine 5'-monophospho-N-acetylneuraminic acid; mAb, monoclonal antibody; RT, reverse transcription; PCR, polymerase chain reaction; DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; PBS, phosphate-buffered saline; TLC, thin layer chromatography; PAGE, polyacrylamide gel electrophoresis; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPTLC, high performance thin layer chromatography; kb, kilobase pair(s); SPG, sialyl-paragloboside. The nomenclature of gangliosides is based on that of Svennerholm (54). The abbreviated nomenclature for cloned sialyltransferases follows Ref. 2A. The designations of glycosphingolipids are abbreviated according to the recommendations of the IUPAC-IUB Commission on Nomenclature (55). Leα, Galβ1,3GlcNAcβ1,3Galβ1,4Glcβ1-Cer; nLeα; Galβ1,4GlcNAcβ1,3Galβ1,4Glcβ1-Cer; nLeα, Galβ1,4GlcNAcβ1,3Galβ1,4Glcβ1-Cer. 2 Fukumoto, S., Miyazaki, H., Urano, T., Furukawa, K., and Furukawa, K. (1999) J. Biol. Chem. 274, in press.
Galβ1,3GlcNAc structure, suggesting it is involved in the formation of the sialyl-Le\(^{a}\) determinant on glycoproteins and glycolipids. The expression of the gene among normal human tissues and cell lines was also analyzed.

**EXPERIMENTAL PROCEDURES**

**Nomenclature of Cloned Sialyltransferase**—So far five members of human α2-3-sialyltransferase (ST3Gal) have been cloned: ST3Gal I (16), ST3Gal II (18), ST3Gal III (21), ST3Gal IV (22, 23), and ST3Gal V (GenBank\(^{TM}\) accession no. AB013302). The α2,3-sialyltransferase cloned in this study is referred to as ST3Gal VI according to Tsuji et al. (24).

**Materials**—CMP-NeuAc, LacCer, GM2, GM1, GD1a, GalCer, GTb, GQ1b, GA1, asialofetuin, and bovine submaxillary asialomucin, were purchased from Sigma. GM3 was purchased from Snow Brand Milk Products Co. (Tokyo, Japan). \[\text{α-}[\text{32P}]\text{dCTP}\] was from ICN (Costa Mesa, CA). Le\(^{a}\) ceramide (Galβ1,4(Fuc)x1,3GlcNaNcβ1,3Galβ1,4GlcP1Cer) and lactotetraosylceramide (Le\(^{a}\)) were chemically synthesized as described previously (25). Sialyl-neolactotetraosylceramide (sialyl-NeLc4) and sialyl-neolactohexaosylceramide (sialyl-NeLc6) were prepared from bovine blood cells as described previously (26). The asialo compounds were prepared by digestion with neuraminidase from Vibrio cholerae (Sigma) as described previously (26).

**Isolation of ST3Gal VI**—Mouse expressed sequence tags (GenBank\(^{TM}\) accession nos. W52470, N49607, H06247, AA883549, and H22233) with similarity to mouse ST3Gal V (GM3 synthase) were amplified by the reverse transcription polymerase chain reaction (RT-PCR) method using total RNA prepared from a human melanoma cell line SK-MEL-37 as a template. The sense primer 5'-TTGGGAGAAGGAGGATGGTACTCTATGG-3' and the antisense primer 5'-CCACGTGAGCAAAGCTCTTACTGTTCTGACTGATACTTCTGA-3' were used for PCR amplification, which was carried out as follows; 94 °C for 1 min, 25 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, and 72 °C for 1 min. The RT-PCR-amplified 630-base pair cDNA was cloned into pCR\(^{TM}\)2.1-TOPO vector (Invitrogen, San Diego, CA). The DNA insert was \[\text{α-}[\text{32P}]\text{dCTP}\]-labeled with a Megaprime\(^{TM}\) DNA labeling system (Amersham, Buckinghamshire, UK) and used to screen the SK-MEL-37 cDNA library. Approximately 4 \times 10\(^5\) recombinant MC1061P3s from a cDNA library prepared from human SK-MEL-37 cells were screened by colony hybridization. Colony lifts were prepared with GeneScreen Plus membrane (NEN Life Science Products). The nucleotide sequence was determined by the dideoxy termination method using an ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, CA).

**Expression Vector**—A cDNA fragment encoding the open reading frame of ST3Gal VI was PCR-amplified by using a 5'-primer containing a XhoI site, 5'-CTCCCTGGAGGTAGCCAGCATGGAGGG-3', and a 3' primer containing a XhoI site, 5'-TCTCTTCTAGTCAATCTTGAGGAATTCGGAATGAAACG-3' using total RNA prepared from a human melanoma cell line ST3Gal VI-protA. Five \[\mu\text{g}\] of GM3 was sialylated with GD3 synthase prepared form the L cells transfected with pMIKneo-ST3Gal I (29) using CMP-[\text{3}^{-}\text{H}]\text{NeuAc} (8, 800 \mu\text{m}) and the products were purified by C18 Sep-Pak cartridge, dried, and resolved in 25 \mu\text{l} of 50 \text{mM} sodium citrate (pH 6.0) and 100 \mu\text{M} NaCl containing 100 \mu\text{g/mL} bovine serum albumin. The resulting products were incubated for 2 h at 37 °C after the addition of 0.85 unit Salmonella typhimurium LT2 sialidase (New England Biolabs, Beverly, MA). The digestion products were separated by two volumes of chloroform/methanol (1:1), and the organic phase was collected by partition, dried, and subjected to HPTLC with a solvent system of chloroform/methanol/0.02% CaCl\(_2\) (55:45:10). The plate was exposed to an imaging plate and then analyzed by BAS 2000 image analyzer.

**TLC Immuno-staining**—Twenty \[\mu\text{g}\] of LN4c was sialylated with a soluble form of ST3Gal VI (ST3Gal VI-protA). Five \[\mu\text{g}\] of GM3 was sialylated with GD3 synthase prepared form the L cells transfected with pMIKneo-ST3Gal I (29) using CMP-[\text{3}^{-}\text{H}]\text{NeuAc} (8, 800 \mu\text{m}) and the products were purified by C18 Sep-Pak cartridge, dried, and redissolved in 25 \mu\text{l} of 50 \text{mM} sodium citrate (pH 6.0) and 100 \mu\text{M} NaCl containing 100 \mu\text{g/mL} bovine serum albumin. The resulting products were incubated for 2 h at 37 °C after the addition of 0.85 unit Salmonella typhimurium LT2 sialidase (New England Biolabs, Beverly, MA). The digestion products were separated by two volumes of chloroform/methanol (1:1), and the organic phase was collected by partition, dried, and subjected to HPTLC with a solvent system of chloroform/methanol/0.02% CaCl\(_2\) (55:45:10). The plate was exposed to an imaging plate and then analyzed by BAS 2000 image analyzer.

**Cloning of Mouse cDNA**—Total RNA was prepared from human cancer cell lines using TRIZOL\(^{\text{TM}}\) reagent (Life Technologies, Inc.) according to the manufacturer's instruction. Total RNA (10 \mug) was separated on 1.2% agarose-formaldehyde gel, then transferred onto a GeneScreen Plus\(^{\text{TM}}\) membrane. Human Multiple Tissue Northern Blot\(^{\text{TM}}\) was purchased from CLONTECH. The blots were probed with a gel-purified, \[\alpha-\text{P}\]dCTP-labeled ST3Gal VI cDNA or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as described previously (26).

**Flow Cytometry**—Adherent cells were detached in PBS containing 0.5 mM EDTA and 1 mg/ml glucose. After washing with PBS, approximately 5 \times 10\(^5\) cells were incubated with mAb 2H5 (CD16s; Pharmingen, San Diego, CA) at a dilution of 1:500 (1 \muM) for 30 min on ice. After washing twice, the cells were stained with fluorescein isothiocyanate-conjugated goat anti-mouse IgM (\mu\text{g} chain specific) (Zymed Laboratories Inc.) at a 1:200 dilution. After a 30-min incubation on ice, they were washed twice and subjected to analysis on a FACS Calibur with Cell Quest\(^{\text{TM}}\) Version 3.1 software (Becton Dickinson). Thresholds for antigen positivity were set at a fluorescence intensity level that excluded 99% of the cells that had been stained without mAb 2H5.

**Sialyltransferase Assay—**The sialyltransferase assay was performed in a mixture containing 10 mM MgCl\(_2\), 0.3% Triton CF-54, 100 mM sodium cacodylate buffer, pH 6.0, 0.66 mM CMP-NeuAc (Sigma), 4,400 dpm/\muL CMP-\([\text{3}^{-}\text{H}]\)NeuAc (Amersham Pharmacia Biotech), the enzyme solution (containing 25 \mug/ml of sialyltransferase activity), and 20 \muL of glycoprotein acceptors or 20 \muL of oligosaccharides and asialoglycoproteins. The reaction mixture was incubated at 37 °C for 2 h. For glycolipid acceptors, the reaction was terminated by addition of 500 \muL of water. The products were isolated by C18 Sep-Pak cartridge (Waters, Milford, MA) and analyzed by thin layer chromatography (TLC). High performance thin layer chromatography (HPTLC) plates (E. Merck, Darmstadt, Germany) were used. For oligosaccharide substrates, the reaction was terminated by the addition of 20 \muL of methanol. Oligosaccharide products were separated by TLC with a solvent system of ethanol/pyridine/n-butanol/acetic acid (100:10:10:3). For glycoprotein acceptors, the reaction was terminated by the addition of 20 \muL of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer and the mixtures were subjected to SDS-PAGE. The radioactivity on each plate and gel was visualized with a BAS 2000 image analyzer (Fuji Film, Tokyo, Japan).

**Linkage Analysis by Sialidase Digestion—**Five \mug of leoctacto- tetraosylceramide (nLc4) was sialylated with a soluble form of ST3Gal VI (ST3Gal VI-protA). Five \mug of GM3 was sialylated with GD3 synthase prepared form the L cells transfected with pMIKneo-ST3Gal I (29) using CMP-[\text{3}^{-}\text{H}]\text{NeuAc} (8, 800 \muM). The products were purified by C18 Sep-Pak cartridge, dried, and redissolved in 25 \muL of 50 \text{mM} sodium citrate (pH 6.0) and 100 \muM NaCl containing 100 \mug/mL bovine serum albumin. The resulting products were incubated for 2 h at 37 °C after the addition of 0.85 unit Salmonella typhimurium LT2 sialidase (New England Biolabs, Beverly, MA). The digestion products were separated by two volumes of chloroform/methanol (1:1), and the organic phase was collected by partition, dried, and subjected to HPTLC with a solvent system of chloroform/methanol/0.02% CaCl\(_2\) (55:45:10). The plate was exposed to an imaging plate and then analyzed by BAS 2000 image analyzer.
RESULTS

Molecular Cloning of a cDNA Encoding a Novel α2,3-Sialyltransferase—We previously cloned a cDNA encoding mouse ST3Gal V (GM3 synthase) using an expression cloning method. By searching the expressed sequence tag data base, we found sequences (GenBankTM accession nos. W52470, N40607, H06247, AA883549, and H22233) with similarity to mouse ST3Gal V, and obtained the corresponding cDNA fragment by RT-PCR (nucleotide numbers 728–1375 in Fig. 1A). Approximately 4 × 10⁵ colonies of a human melanoma cell line SK-MEL-37 cDNA library were screened using the cDNA fragment as a probe, and nine independent clones (clones 1–9) were obtained. Characterization of the positive clones revealed that clone 2 contained a 1-kilobase pair (kb) insert, clone 3 was 1.2 kb, clone 5 was 1.6 kb, and clone 9 was 1.5 kb in length. From the nucleotide sequence, clone 9 was found to contain a whole open reading frame (Fig. 1A). The nucleotide sequence revealed that the cDNA contains an open reading frame encoding a protein of 331 amino acids with a calculated molecular mass of 38,213 daltons, with six potential N-linked glycosylation sites. The position of the AUG start codon was determined according to the Kozak consensus sequence (32), and the upstream region contained an in-frame stop codon. Hydropathy analysis determined by the Kyte and Doolittle method (33) indicated one prominent hydrophobic segment of 32 residues in length in the amino-terminal region (Gly3–Val34), predicting that the protein has type II transmembrane topology characteristic of many other glycosyltransferases cloned to date (Fig. 1B). Comparison of the primary structure of ST3Gal VI protein and the 14 other cloned sialyltransferases indicated that there is significant similarity in two regions, so-called sialylmotifs (34, 35). In addition, ST3Gal VI has a TXXXXYPE sequence near the C-terminal end of L-sialylmotif, which is found conserved among the members of ST3Gal subfamily (Fig. 2). These results indicated that this protein belongs to the sialyltransferase gene family and likely the α2,3-sialyltransferase subfamily. The predicted protein shows 38%, 34%, and 33% sequence identity to human ST3Gal IV, human ST3Gal III, and mouse ST3Gal V, respectively (Fig. 2). No significant homology in amino acid sequence was observed between the predicted protein and other known sialyltransferases.

Sialyltransferase Activity of the Newly Cloned Enzyme—To analyze the sialyltransferase activity of ST3Gal VI, the expression vector of the cDNA, pMIKneo-ST3Gal VI, was transfected

Fig. 1. Nucleotide and deduced amino acid sequences of human ST3Gal VI and hydropathy plot of the protein. A, the deduced amino acid sequence is shown below the nucleotide sequence. The putative transmembrane hydrophobic domain is underlined, and six potential N-linked glycosylation sites are boxed. B, the hydropathy plot was calculated by the method of Kyte and Doolittle (33) with a window of 11 amino acids.
into L cells, and the extracts of the transfected cells were assayed for sialyltransferase activity using CMP-[14C]NeuAc as a donor and glycolipid mixture from bovine blood cells as acceptors. As shown in Fig. 3, the enzyme sialylated asialoglycolipids containing LacCer, nLc4, and nLc6 prepared from acidically glycosphingolipids of bovine red blood cells, and the products co-migrated with sialyl-nLc4 and sialyl-nLc6. In contrast, purified LacCer did not serve as an acceptor for ST3Gal VI. No activity was detected in the extracts from mock-transfected cells. Similar results were obtained using a soluble fusion enzyme ST3Gal VI-protA (data not shown).

**Fig. 2.** Comparison of sialylmotif L and sialylmotif S of ST3Gal VI with that of 14 previously cloned sialyltransferases. The previously cloned sialyltransferases are the human ST3Gal I (17), human ST3Gal II (19), human ST3Gal III (20), human ST3Gal IV (22, 23), mouse ST3Gal V (GenBank™ accession no. AB013302), human ST3Gal I (43, 44), the chick ST6GalNAc I (45), the mouse ST6GalNAc II (46), the rat ST6GalNAc III (47), the human ST8Sia I (29, 48, 49), human ST8Sia II (50), human ST8Sia III (GenBank™ accession no. AF004668), human ST8Sia IV (51), and human ST8Sia V (GenBank™ accession no. U91641). The sialyltransferase motifs are grouped by the linkages that they form. Shaded letters represent highly conserved amino acids in many sialyltransferases. Single and double shaded letters indicate conserved amino acid residues among the members of ST3Gal subfamily.
Various acceptor substrates were incubated in the standard assay mixture using ST3Gal VI-protA as an enzyme source. Each substrate was used at a concentration of 0.1 mM for glycolipids and oligosaccharides and 0.2 mg/ml for glycoproteins. Relative rates are calculated as a percentage of the incorporation obtained with nLc6.

| Acceptor | Structure(s) | Relative rate |
|----------|--------------|---------------|
| Glycoproteins | Galβ1,3GalNAc Ser/Thr and Galβ1,4GalNAcβ1-R | 22 |
| Asialofetuin | Galβ1,3GalNAc Ser/Thr and Galβ1,4GalNAcβ1-R | 0 |
| Glycolipids | - | - |
| LacCer | Galβ1,4Glcpβ1-Cer | 0 |
| GM3 | NeuAcβ2,3Galβ1,4Glcpβ1-Cer | 0 |
| GM2 | Galβ1,3GalNAcβ1,4 NeuAcα2,3Galβ1,4Glcpβ1-Cer | 0 |
| GM1 | Galβ1,3GalNAcβ1,4 NeuAcα2,3Galβ1,4Glcpβ1-Cer | 0 |
| GD1a | NeuAcα2,3Galβ1,3GalNAcβ1,4 NeuAcα2,3Galβ1,4Glcpβ1-Cer | 0 |
| GD1b | Galβ1,3GalNAcβ1,4 NeuAcα2,3Galβ1,4Glcpβ1-Cer | 0 |
| GT1b | NeuAcα2,3Galβ1,3GalNAcβ1,4 NeuAcα2,3Galβ1,4Glcpβ1-Cer | 0 |
| GQ1b | NeuAcα2,8 NeuAcα2,3Galβ1,3GalNAcβ1,4 NeuAcα2,8 NeuAcα2,3Galβ1,4Glcpβ1-Cer | 0 |
| GalCer | Galβ1-Cer | 0 |
| GA1 | Galβ1,3GalNAcβ1,4 Galβ1,4 Glcpβ1-Cer | 0 |
| Le4 | Galβ1,3 GlcpNAcβ1,4 Galβ1,4 Glcpβ1-Cer | 0 |
| nLc4 | Galβ1,4 GlcpNAcβ1,3 Galβ1,4 Glcpβ1-Cer | 49 |
| nLc6 | Galβ1,4 GlcpNAcβ1,3 Galβ1,4 Glcpβ1-Cer | 100 |
| Lea | Galβ1,4 Fucα1,3 GlcpNAcβ1,3 Galβ1,4 Glcpβ1-Cer | 0 |
| Oligosaccharides | Type I | 8 |
| Type II | Galβ1,4 GlcpNAc | 47 |
| Type III | Galβ1,3 GlcpNAc | 0 |

* Data from Ref. 52 for fetuin and Ref. 53 for BSM (bovine submaxillary mucin).
+ R represents the remainder of the N-linked oligosaccharide chain.

Fig. 5. Incorporation of sialic acid into asialofetuin by ST3Gal VI. Sialyltransferase activity of ST3Gal VI toward glycoproteins was measured. Forty μg of glycoproteins acceptors was incubated with 8 μg of the cell extracts in the standard assay condition. The samples were boiled in Laemmli sample buffer and subjected to 12.5% SDS-PAGE. The gel was dried and exposed to an imaging plate and then analyzed with a BAS 2000 radioimage analyzer. Additional details were provided under “Experimental Procedures.” For Fig. 4, [14C]NeuAc was incorporated into nLc4 and nLc6 containing Galβ1,4GlcpNAc sequence at the non-reducing end. ST3Gal VI-protA did not exhibit activity toward Le4, GA1, and lactosylceramide. Then, we determined the acceptor specificity of the enzyme toward oligosaccharides. As summarized in Table I, ST3Gal VI-protA utilized Galβ1,4GlcpNAc as the best substrate. Galβ1,3GlcpNAc showed much less incorporation of [14C]NeuAc. The kinetic analysis using the L. cell extracts transfected with pMIKneo-ST3Gal VI showed that the K_m value for nLc4 was 0.22 mM.

Next, we examined the sialyltransferase activity toward various glycoproteins. As shown in Fig. 5 and summarized in Table I, asialofetuin served as an acceptor. However, mucin from bovine submaxillary gland did not. No activity was detected in the extracts from mock-transfected cells.

**Expression of the ST3Gal VI Gene**—To determine the size of

**Linkage Analysis by Sialidase Digestion**—To determine the incorporated sialic acid linkage, nLc4 was labeled with CMP-[14C]NeuAc using ST3Gal VI-protA and GD3 were produced from nLc4 and GM3 using ST3Gal VI-protA and GD3 synthase, respectively. The labeled products were then subjected to the treatment with α2,3 sialidase as described under “Experimental Procedures.” The resulting glycolipids were separated on a TLC plate with a solvent system of chloroform/methanol/0.2% CaCl2 (55:45:10) and detected with a BAS 2000 radioimage analyzer. SA-nLc4, sialyl-neolactotetraosylceramide.
ST3Gal VI mRNA and its expression, Northern blot analysis was conducted using the full-length fragment of cDNA as a probe. As shown in Fig. 8, 1.8- and 3.0-kb mRNAs of ST3Gal VI were detected. In adult tissues, the gene expression is abundant in heart, placenta, and liver (Fig. 8A). In human cancer cell lines, strong signals were observed in melanoma lines SK-MEL-37 and SK-MEL-23, while signals were hardly detected in hematopoietic cell lines and an astrocytoma AS or a neuroblastoma line IMR32 (Fig. 8B).

**Correlation of the Expression Levels of ST3Gal VI Gene with Sialyl-Le^a Expression**—In order to investigate whether ST3Gal VI was involved in sialyl-Le^a expression, we examined the expression of ST3Gal VI gene along with sialyl-Le^a expression levels in several human cancer cell lines. As shown in Fig. 9A, a flow cytometric analysis indicated that these cancer cell lines strongly expressed sialyl-Le^a except for MOLT-3. Subsequently, expression of ST3Gal VI in these cells was analyzed by semiquantitative RT-PCR analysis. As shown in Fig. 9B, melanoma cell lines, especially SK-MEL-37 showed a high level of the STGal VI mRNA expression. Among colon cancer cell lines, Lovo and DLD-1 expressed sialyl-Le^a on the cell surface, but the expression of ST3Gal VI gene was not observed. Thus, the expression levels of ST3Gal VI did not correlate well with sialyl-Le^a expression among cell lines.

**DISCUSSION**

Since Weinstein et al. isolated a cDNA clone of β-galactoside α2,3-sialyltransferase in 1987 (37), a number of sialyltransferase genes have been cloned. These cloned sialyltransferases can be classified into four subfamilies based on the linkages they form, i.e. the ST3Gal-, ST6Gal-, ST6GalNAc-, and ST8Sia-subfamilies. In a subfamily, some enzymes utilize certain acceptors with high efficiency, but use other acceptors with less efficiency. Therefore, substrate specificities of these enzymes frequently overlap, at least in *in vitro* analysis. It seems reasonable to think that an acceptor showing the strongest substrate activity *in vitro* is also the best acceptor *in vivo*. However, an acceptor that exhibits very weak activity for an enzyme *in vitro* can be a major acceptor *in vivo* depending on the expression levels of itself and another enzymes that share the same substrate (38).

To date, four human α2,3-sialyltransferase genes have been
ities of glycosyltransferases are sometimes quite different be-
those of the other ST3Gals. This is because substrate specific-
sources to human when we discuss about the substrate speci-
available (40), it seems important to restrict the enzyme
indicating that this gene encodes a novel sialyltransferase be-
cloned, the cloned enzyme exhibit at most 40% homology, in-
being involved in the synthesis of sialyl-Le^a in some tissues.
Results of Northern blotting of ST3Gal VI gene shows predom-
inant expression in placenta, liver, heart, and skeletal
Muscle. Among human cell lines, melanoma lines exhibited
relatively high levels of the gene expression in accord with high
expression of sialyl-Le^a, indicating that ST3Gal VI might be
involved in the synthesis of sialyl-Le^a in melanomas, and prob-
ably in the synthesis of SPG in placenta (42). However, it
seemed unclear whether this gene contributes in the up-regu-
lation of sialyl-Le^a synthesis in colon cancer and hematopoietic
malignant cell lines. Actual involvement of this ST3Gal VI in
the synthesis of sialyl type II structures in vivo remains to be
analyzed.

Northern blots showed 1.8-kb major band and 3.0-kb minor
band with almost parallel intensities. These bands were rather
broad, suggesting the presence of heterogeneity in the size of
mRNAs. The fact that cloned cDNAs exhibit various patterns of
partial defects or insertions of sequences in the coding region is
probably due to alternatively spliced exons (data not shown),
explaining the observed heterogeneous mRNAs. Many of those
aberrant clones seem non-functional and may have roles in the
regulation of the enzyme activity in certain situations.

Acknowledgment—We thank Dr. S. Tsuji for providing an expression vector pCDNA for a protein A fusion enzyme.

REFERENCES
1. Hakomori, S. (1981) Annu. Rev. Biochem. 50, 733–764
2. Paulson, J. C. (1989) Trends Biochem. Sci. 14, 272–276
3. varki, A. (1992) Curr. Opin. Cell Biol. 4, 257–266
4. Valki, A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7390–7397
5. McEver, R. P. (1994) Curr. Opin. Immunol. 6, 75–84
6. Springer, T. A. (1994) Cell 76, 301–314
7. Hakomori, S. (1991) Curr. Opin. Immunol. 3, 646–653
8. Kawashima, H., Yamamoto, K., Osawa, T., and Irimura, T. (1993) J. Biol.
Chem. 268, 27118–27126
9. Narimatsu, H., Shinagawa, Y., Okumura, K., and Qasba, P. K. (1986)
Proc. Natl. Acad. Sci. U.S.A. 83, 4720–4724
10. Schwientek, T., Almeida, R., Levery, S. B., Holmes, E. H., Bennett, E., and
Clausen, H. (1998) J. Biol. Chem. 273, 29331–29340
11. Nakagawa-Latallo, J. P., Larsen, R. D., Nair, R. P., and Lowe, J. B. (1990)
Genes Dev. 4, 1288–1303
12. Weston, B. W., Smith, P. L., Kelly, R. J., and Lowe, J. B. (1992) J. Biol.
Chem. 267, 24575–24584
13. Weston, B. W., Nair, R. P., Larson, R. D., and Lowe, J. B. (1992) J. Biol.
Chem. 267, 4152–4160
14. Goed, S. E., Heston, C., Goff, D., Griffiths, B., Tizard, R., Newman, B.,
Chishty, G., and Lebb, B. (1990) Cell 63, 1348–1356
15. Sasaki, K., Kurata, K., Furumai, K., Naga, M., Watanabe, E., Ohta, S.,
Hanai, N., and Nishi, T. (1994) J. Biol. Chem. 269, 14730–14737
16. Galos, W., Kiel, S., and Paulson, J. C. (1992) J. Biol. Chem. 267, 21004–21010
17. Kitagawa, H., and Paulson, J. C. (1994) J. Biol. Chem. 269, 17872–17878
18. Lee, Y. C., Kojima, N., Wada, E., Kurosawa, N., Nakaoka, K., Hamamoto, T.,
and Tojo, Y. (1994) J. Biol. Chem. 269, 10028–10033
19. Kim, Y. J., Kim, K. S., Kim, H. C., Ko, J. H., Choe, I. S., Tsuji, S.,
and Lee, Y. C. (1996) Biochem. Biophys. Res. Commun. 229, 324–327
20. Kitagawa, H., and Paulson, J. C. (1995) J. Biol. Chem. 270, 375–382
21. Wen, D. X., Livingstone, B. D., Medzhitov, K. F., Selim, S., Buringame, A. L.,
and Paulson, J. C. (1992) J. Biol. Chem. 267, 21011–21019
22. Kitagawa, H., and Paulson, J. C. (1994) J. Biol. Chem. 269, 1394–1401
23. Sasaki, K., Watanabe, E., Kawashima, K., Sekine, S., Dohi, T., Oshima, M.,
Hanai, N., Nishi, T., and Hasegawa, M. (1993) J. Biol. Chem. 268, 21782–21787
24. Kitagawa, H., and Paulson, J. C. (1996) J. Biol. Chem. 271, 11225–11233
29. Haraguchi, M., Yamashiro, S., Yamamoto, A., Furukawa, K., Takamiya, K., Lloyd, K., Shiku, H., and Furukawa, K. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10455–10459
30. Taki, T., Handa, S., and Ishikawa, D. (1994) Anal. Biochem. 211, 312–316
31. Miyazaki, H., Fukumoto, S., Okada, M., Hasegawa, T., Furukawa, K., and Furukawa, K. (1997) J. Biol. Chem. 272, 24794–24799
32. Kosak, M. (1986) Cell 44, 283–292
33. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
34. Datta, A. K., and Paulson, J. C. (1995) J. Biol. Chem. 270, 1497–1500
35. Datta, A. K., Sinha, A., and Paulson, J. C. (1998) J. Biol. Chem. 273, 9608–9614
36. Hirabayashi, Y., Hamaoka, A., Matsumoto, M., Matsubara, T., Tagawa, M., Wakabayashi, S., and Taniguchi, M. (1985) J. Biol. Chem. 260, 13328–13333
37. Weinstein, J., de Souza-e-Silva, U., and Paulson, J. C. (1982) J. Biol. Chem. 257, 13835–13844
38. Yamashiro, S., Haraguchi, M., Furukawa, K., Takamiya, K., Yamamoto, A., Nagata, Y., Lloyd, K. O., Shiku, H., and Furukawa, K. (1995) J. Biol. Chem. 270, 6149–6155
39. Ishii, A., Ohta, M., Watanabe, Y., Matsuda, K., Ishiyama, K., Sakoe, K., Nakamura, M., Inokuchi, J., Sanai, Y., and Saito, M. (1998) J. Biol. Chem. 273, 31652–31655
40. Kono, M., Ohyama, Y., Lee, Y. C., Hamamoto, T., Kojima, N., and Tsuji, S. (1997) Glycobiology 7, 469–479
41. Miyamoto, D., Takashima, S., Suzuki, T., Nishi, T., Sasaki, K., Morishita, Y., and Suzuki, Y. (1995) Biochem. Biophys. Res. Commun. 217, 852–858
42. Taki, T., Matsuo, K., Yamamoto, K., Matsubara, T., Hayashi, A., Abe, T., and Matsumoto, M. (1988) Lipids 23, 192–198
43. Grundmann, U., Nerlich, C., Rein, T., and Zettlmeissl, G. (1990) Nucleic Acids Res. 18, 667
44. Stamenkovic, I., Asheim, H. C., Duggerdal, A., Blomhoff, H. K., Smeland, E. B., and Funderud, S. J. (1990) J. Exp. Med. 172, 641–643
45. Kurosawa, N., Yamamoto, T., Lee, Y. C., Nakaoka, T., Kojima, N., and Tsuji, S. (1994) J. Biol. Chem. 269, 1402–1409
46. Kurosawa, N., Inoue, M., Yoshida, Y., and Tsuji, S. (1996) J. Biol. Chem. 271, 15109–15116
47. Sjoberg, E. R., Kitagawa, H., Gushika, J., van Halbeek, H., and Paulson, J. C. (1996) J. Biol. Chem. 271, 7450–7459
48. Sasaki, K., Kurata, K., Kojima, N., Kurosawa, N., Ohta, S., Hanai, N., Tsuji, S., and Nishi, T. (1994) J. Biol. Chem. 269, 15950–15956
49. Nara, K., Watanabe, Y., Maruyama, K., Kasahara, K., Nagai, Y., and Sanai, Y. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7952–7956
50. Scheidegger, G. P., Sternberg, L. R., Roth, J., and Lowe, J. B. (1995) J. Biol. Chem. 270, 22685–22688
51. Nakayama, J., Fukuda, M. N., Fredette, B., Ranscht, B., and Fukuda, M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7031–7035
52. Sipro, R. G., and Bhoyroo, V. D. (1974) J. Biol. Chem. 249, 5704–5717
53. Tsuji, T., and Osawa, T. (1986) Carbohydr. Res. 151, 391–402
54. Svennerholm, L. (1963) J. Neurochem. 10, 613–623
55. IUPAC-IUB Commission on Nomenclature (1977) Lipids 12, 455–68