Molecular Cloning and Expression of a Novel Human β-Gal-3-O-sulfotransferase That Acts Preferentially on N-Acetyllactosamine in N- and O-Glycans*

Atsushi Suzuki, Nobuyoshi Hiraoka, Masami Suzuki, Kiyohiko Angata, Anup K. Misra, Joseph McAuliffe, Ole Hindsgaul, and Minoru Fukuda†

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

A novel cDNA-encoding galactose 3-O-sulfotransferase was cloned by screening the expressed sequence tag data base using the previously cloned cDNA encoding a galactosyl ceramide 3-O-sulfotransferase, which we term Gal3ST-1. The newly isolated cDNA encodes a novel 3-O-sulfotransferase, termed Gal3ST-3, that acts exclusively on N-acetyllactosamine present in N-glycans and core2-branched O-glycans. These conclusions were confirmed by analyzing CD43 chimeric proteins in Chinese hamster ovary cells expressing core2 β1,6-N-acetylglucosaminyltransferase. The acceptor specificity of Gal3ST-3 contrasts with that of the recently cloned galactose 3-sulfotransferase, which forms a sulfatide, sulfo–Galβ1→4(Fucα1→3)GlcNAcα1→R. Gal3ST-3 can act on Galβ1→4(Fucα1→3)GlcNAcα1→R while not Galβ1→4(Fucα1→3)GlcNAcα1→R. Finally, Gal3ST-3 was shown to be expressed in ovarian cystadenoma glycoprotein and conjugated to 3′-sulfogalactose in both O- and N-glycans.

Sulfate groups in carbohydrates play important roles in conferring highly specific functions on glycoproteins, glycolipids, and proteoglycans (1–3). One of these sulfated glycans is 3′-sulfogalactose, which is present in type 1 and type 2 oligosaccharides (N-acetyllactosamine), sulfo–Galβ1→4GlcNAc→R, or attached to a type 1 oligosaccharide, sulfo–Galβ1→3GlcNAc→R. Feizi and colleagues (4) demonstrated that 3′-sulfogalactose, in a type 1 or type 2, fucosylated oligosaccharide, functions as an E-selectin ligand (4). When oligosaccharides were released from ovarian cystadenoma glycoprotein and conjugated to lipids, sulfo–Galβ1→3/4(Fucα1→3)GlcNAcβ1→3Gal was found to bind to Chinese hamster ovary (CHO) cells expressing E-selectin (4). On the other hand, sulfo–Galβ1→4(Fucα1→3)GlcNAc acted as a P-selectin ligand when a synthetic oligosaccharide with this structure was transferred to cell surface glycoproteins through a fucose residue by α1,3-fucosyltransferase III (5). These studies suggest that 3′-sulfogalactose on the cell surface plays a role in carbohydrate-protein interactions, including those involved with selectin.

Recently, a comparison of the amino acid sequences of cloned sulfotransferases demonstrated that there is a weak but discernible homologous sequence motif among Golgi-associated sulfotransferases (6–9). In particular, the amino acid sequences that are responsible for binding 5′-phosphatase and 3′-phosphatase groups of the donor substrate, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), are well conserved and are often highly homologous to each other among those that share the same acceptor specificity (10–15). Previously, galactosyl ceramide 3′-sulfotransferase, which forms a sulfatide, sulfo–Gal→ceramide, has been cloned based on the amino acid sequence of purified protein (16). Because this enzyme, which we now term Gal3ST-1, is thought not to add a sulfate to glycoproteins (17), galactose 3-O-sulfotransferase was molecularly cloned by searching for an enzyme homologous to Gal3ST-1. This reported enzyme, which we now term Gal3ST-2, has the unique property of adding a sulfate on both type 1 and type 2 oligosaccharides and core1 O-glycans, Galβ1→3GalNAc→R (18). On the other hand, the structures of O-linked oligosaccharides containing 3′-sulfogalactose reported to date show that 3′-sulfogalactose is present in N-acetyllactosamine in core2 O-glycans, sulfo–Galβ1→4GlcNAcβ1→6Galβ1→3GalNAcα1→R (19); core3 O-glycans, sulfo–Galβ1→4GlcNAcβ1→3GalNAc (20); and core1 extended structures, sulfo–Galβ1→4GlcNAcβ1→3Galβ1→3GalNAc (21). Moreover, no 3′-sulfogalactose in core1 O-glycans such as sulfo–Galβ1→3GalNAc→R has been previously reported. Similarly, galactose 3-O-sulfotransferase in human respiratory mucosa was found to act exclusively on N-acetyllactosamine in core2-branched O-glycans and not on core1 O-glycans (17). The presence of 3′-sulfogalactose in N-glycans has been extensively studied in human, bovine, and porcine thyroglobulins, and these

* This work was supported by Grants R01 CA48737 and P01 CA71932 awarded by the NCI, National Institutes of Health. 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 abbreviations used are: CHO, Chinese hamster ovary; PAPS, 3′-phosphoadenosine 5′-phosphate; Gal3ST, galactose 3-O-sulfotransferase; EST, expressed sequence tag; PCR, polymerase chain reaction; MES, 2-(N-morpholino)ethanesulfonic acid; C2GnT-1, core2 β1,6-N-acetylglucosaminyltransferase-1; HPLC, high performance liquid chromatography; pNP, p-nitrophenol; NCAM, the neural adhesion molecule.

‡ The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF026481.
studies showed that 3'-sulfo galactose is present in N-acetylated
tosamine in complex N-glycans (22–24). Notably, galactose 3-
sulfotransferase present in the thyroid was found to act only on
N-acetylatedtosamine but not on type 1 oligosaccharides, which
differs from the properties of Gal3ST-2 (25). Although no glyco-
protein acceptor was tested for Gal3ST-2, these results suggested
that there is another galactose 3-O-sulfotransferase (Gal3ST-3) yet
to be identified.

In the present study, we first identified a novel cDNA by
screening the EST data base for cDNAs related to human
Gal3ST-1 (16). The expression of a full-length cDNA revealed
that this cDNA encodes a novel galactose 3-O-sulfotransferase,
termed Gal3ST-3, that adds a sulfate exclusively in the 3'-position
of galactose in N-acetylatedtosamine in both N- and
O-glycans but not on a type 1 Galβ1–3GlcNAc or core1
Galβ1–3GalNAc structure. When CD43 (leukosialin) was tested as an acceptor, Gal3ST-3 preferentially acted on
N-acetyllactosamine present in core2-branched
contrast to the previously reported ubiquitous expression of
whereas Gal3ST-2 acted on both core1- and core2-branched
O-glycans. Moreover, we show that this novel enzyme is ex-
pressed almost exclusively in the thyroid, kidney, and brain, in
contrast to the previously reported ubiquitous expression of
Gal3ST-2 (18).

EXPERIMENTAL PROCEDURES

Isolation of cDNAs Encoding Galactose 3-O-Sulfotransferase—
Galactosyl ceramide 3'-sulfotransferase (Gal3ST-1) shares homologous se-
quences to the binding sites for 3'-phosphate and 5'-phosphosulfate
with other Golgi-associated sulfotransferases. The amino acid se-
quences of residues 76–100 and 156–179, which include the above
motifs, were used as probes to search the dbEST data base using the
TBlastN program. Initially two ESTs, AI160920 and AA100976, were
identified that exhibited a significant homology to the 3'-phosphate
binding site and 5'-phosphosulfate binding site of Gal3ST-1. After se-
quence analysis of both cDNAs obtained from Incyte Genomics (St.
Louis, MO), a BLAST search of the working draft of the human genome
identified that exhibited a significant homology to the 3
9primers for the PCR were

3'-CGGAGAGAGGAGCTGGTGT-3

and

5'-AAGATCCAGGACCT-3'. (BamHI site is underlined and nucleo-
tides 217–236 are doubly underlined) and 5'-AAATCTAGG-
GAGGAAAGCTGCTGTG-3' (XbaI site is underlined and nucleotides
1362–1380 are doubly underlined). PCR products were digested with
BamHI and XbaI and cloned into the same sites of the pcDNA3/HSH-Gal3ST-2 (encoding amino acid resi-
dues 29–398 of Gal3-ST-2).

Oligosaccharide Acceptors—Galβ1–3GlcNAcβ1–octyl, Galβ1–
4GlcNAcβ1–octyl, Galβ1–4GlcNAcβ1–6Galβ1–3GalNAcβ1–octyl,
y and GlcNAcβ1–6Galβ1–3GalNAcβ1–octyl were synthesized as described previously (28, 29). NeuNac2–3Galβ1–3GalNAcβ1–octyl
2 was alkylated using mGLP 2-sulfotransferase (Calbiochem, San
Diego, CA) and Galβ1–3GalNAcβ1–octyl as a precursor. Galβ1–4Fucα1–3Galβ1–octyl, Galβ1–4(sulfo)–6GlcNAcβ1–octyl, NeuNac-
2α1–3Galβ1–4GlcNAcβ1–octyl, and NeuNac2–3Galβ1–3GalNAcβ1–octyl
were chemically synthesized as described previously (30, 31). Galβ1–4GlcNAcβ1–2Manα1–6Manβ1–octyl, Galβ1–
4GlcNAcβ1–6Manα1–6Manβ1–octyl, Galβ1–4GlcNAcβ1–3Ga-
1–4GlcNAcβ1–6Manα1–6Manβ1–octyl, and (Galβ1–
4GlcNAcβ1–3) Galβ1–4GlcNAcβ1–6Manα1–6Manβ1–octyl were
synthesized as described previously (32, 33). Galβ1–3GlcNAcβ1–p-
nitrophenol and Galβ1–3Fucα1–3Galβ1–3GalNAcβ1–4Glc-
β1–2aminopyridine were purchased from Tokyo Research
Chemicals and Takara Biochemicals, respectively.

CHO cells were transfected with pcDNA3.1/HSH-Gal3ST-1, pcDNA3.1/HSH-Gal3ST-2, pcDNA3.1/HSH-
Gal3ST-3, or mock pcDNA3.1/HSH using LipofectAMINE Plus (Life
Technologies) as described previously (34). 24 h after transfection, the
medium was changed to serum-free OptiMEM medium (Life
Technologies). After an additional 48 h of culture, the supernatant was filtered and concentrated with Microcon 30 (Millipore) and used as the enzyme source as described previously (35).

Sulfotransferase activities of Gal3ST-3 were assayed as described previously with modification (18). Briefly, the reaction mixture (20 μl)
contained 50 mM MES (2-N-morpholinoethanesulfonic acid) buffer
(pH 7.0), 10 mM MgCl2, 0.1% Triton X-100, 10 mM NaF, 2 mM ATP, 500
μM acceptor, 0.08 nmol of [35S]PAPS, and 10 μl of enzyme solution.
After incubation at 37 °C for 2 h, the reaction was terminated by boiling
for 2 min. The reaction products were then adjusted to 0.25 M ammo-
nium formate, pH 4.0, and applied to C18 reverse phase columns
(Alltech) as described previously (6). After washing the column with
the same product, the solution was eluted with 30% acetonitrile.
Radioac-
tivity was measured by scintillation counting. Gal3ST-2 activity was
assayed according to the previously described reaction mixture (16).

Construction of Vectors Encoding Soluble Forms of the Enzymes
To create a soluble form of the enzymes, cDNAs encoding the transmembrane portions of the enzymes were
removed and the resultant cDNA fragments were cloned into a
pcDNA3.1/HSH vector. This pcDNA3.1/HSH vector harbors cDNAs en-
coding a signal peptide for the pcDNA3.1-A vector (26) and 6'His
peptide, which had been constructed as described previously (27).
pcDNA3-Gal3ST-3 was digested with BglII and EcoRI, blunt-ended, and
cloned into the blunt BglII site of pcDNA3.1/HSH, resulting in
pcDNA3.1/HSH-Gal3ST-3 (encoding amino acid residues 41–431 of
Gal3ST-3). pcDNA3-Gal3ST-1 cDNA was similarly digested with BglII
and BamHI, blunt-ended, and cloned into the blunt BamHI site of
pcDNA3.1/HSH vector resulting in pcDNA3.1/HSH-Gal3ST-1 (encoding
amino acid residues 40–423 of Gal3ST-1). cDNA encoding a soluble
form of Gal3ST-2 was prepared by PCR using 5'-AAGATCCAGGACCT-3'
A Novel Galactose 3'-O-Sulfotransferase Acting on N-Acetyllactosamine

FIG. 1. Nucleotide and translated amino acid sequences of Gal3ST-3. The signal membrane anchoring domain is denoted by a bold underline, and N-glycosylation sites are marked by closed circles.

RESULTS

Isolation of cDNA Encoding Novel Gal3ST—By searching the EST data base for a novel cDNA related to Gal3ST-1, two novel cDNAs were found to have homology to Gal3ST-3. The cDNA encoding Gal3ST-3 was cloned into pcDNA3 Neo, resulting in pcDNA3-Gal3ST-3. Although we searched the new human genome data base for the Gal3ST-3 sequence, the 3'-region, including the 3'-untranslated region, was not identified. We thus still have limited knowledge on the genomic structure of Gal3ST-3 other than that it is located on chromosome 11.

The comparison of the amino acid sequences of Gal3ST-3 with those of Gal3ST-1 and Gal3ST-2 reveals the following points (Fig. 2). The sequences corresponding to the binding sites for the 5'-phosphosulfate and 3'-phosphate groups are highly homologous among these enzymes. Moreover, these binding sites are close to the transmembrane/anchoring domains in the Gal3ST gene family in that in all of the other sulfotransferases cloned to date, the transmembrane/anchoring domain and 5'-phosphosulfate binding site are separated by only 21–38 residues, whereas in the majority of Golgi-associated sulfotransferases, the distance is more than 50 residues. On the other hand, the size of the entire amino acid sequence of the Gal3ST gene family is relatively large among Golgi-associated sulfotransferases, indicating that the size of the polypeptide from the 3'-phosphate binding site to the COOH terminus is larger than that of the other Golgi-associated sulfotransferases cloned to date. Overall, the amino acid sequence of Gal3ST-3 is slightly more homologous to that of Gal3ST-2 (40.1%) than that of Gal3ST-1 (38.0%). None of the amino acid sequences in the data base showed significant homology to these three sulfotransferases.

Acceptor Specificity of Gal3ST-3—To determine the acceptor specificity of Gal3ST-3, a soluble form of Gal3ST-3 was prepared as described above. The purified product was purified by Sep-Pak cartridge column chromatography. The purified product was partially hydrolyzed in 40 ml of HCl at 100 °C for 2 h (15). The hydrolysate was then purified by Bio-Gel P-4 gel filtration and analyzed by HPLC using a Whatman Partisil SAX-10 column (4.6-× 250 mm, Whatman, Clifton, NJ) equilibrated with 10 mM KH2PO4 at room temperature. The column was eluted with an isocratic buffer, pH 6.7, as described previously (11). To remove sulfate groups, the column (4.6-× 250 mm, Whatman, Clifton, NJ) was equilibrated with 3.6 mM KH2PO4 at room temperature and eluted with a linear gradient of 0-170 mM KH2PO4 (Sigma Chemical Co.) and β-N-acetylgalactosaminidase A, and then subjected to Bio-Gel P-4 gel filtration as described previously (11). β-N-Acetylgalactosaminidase A can cleave both non-sulfated and sulfated N-acetyllactosamines.

Analysis of Sulfated Products—To determine the product sulfated by Gal3ST-3, Galβ1→4GlcNAcβ1→3GlcNAc–p-nitrophenol was incubated with 35S-PAPS and a soluble form of Gal3ST-3 as prepared above. The purified product was purified by Sep-Pak cartridge column chromatography. The purified product was partially hydrolyzed in 40 ml of HCl at 100 °C for 2 h (15). The hydrolysate was then purified by Bio-Gel P-4 gel filtration and analyzed by HPLC using a Whatman Partisil SAX-10 column (4.6-× 250 mm, Whatman, Clifton, NJ) equilibrated with 10 mM KH2PO4 at room temperature. The column was eluted with an isocratic buffer, pH 6.7, as described previously (11). To remove sulfate groups, the column (4.6-× 250 mm, Whatman, Clifton, NJ) was equilibrated with 3.6 mM KH2PO4 at room temperature and eluted with a linear gradient of 0-170 mM KH2PO4 (Sigma Chemical Co.) and β-N-acetylgalactosaminidase A, and then subjected to Bio-Gel P-4 gel filtration as described previously (11). β-N-Acetylgalactosaminidase A can cleave both non-sulfated and sulfated N-acetyllactosamines.

Carried out in 10 mM pyridine-acetate buffer, pH 5.5, and stepwise eluted with increasing concentrations of NaCl (11). By using standard oligosaccharides, we found that monosialosyl, disialosyl, and trisialosyl oligosaccharides elute with 70 mM, 120 mM, and 140 mM NaCl, respectively, and 6-sulfo Gal and 6-sulfo GlcNH2 elutes with 70 mM NaCl. Bio-Gel P-4 gel filtration was carried out in 0.1 M ammonium acetate buffer, pH 6.7, as described previously (11). To remove sulfate groups, samples were treated with solvolysis in 90% dimethyl sulfoxide/10% methanol at 80 °C for 5 h as described previously (11). To identify the core2 oligosaccharide Galβ1→4GlcNAcβ1→3GlcNAcOH, HPLC was carried out using a column (4.6-× 300 mm) of NH2-bonded silica (Varian Micropack AX-5) equipped in Gilson 306 as described previously with slight modification (41). The AX-5 column was equilibrated with 3.6 mM KH2PO4 at room temperature and eluted with a solution linearly increased to 4.2 mM KH2PO4 in the first 5 min, then again linearly increased to 9 mM KH2PO4 in the next 60 min at a flow rate of 0.8 ml/min. Oligosaccharides were sequentially digested with α,3-specific sialidase (NANaseI, Glyko) and jack bean β-galactosidase (Sigma Chemical Co.) and β-N-acetylgalactosaminidase A, and then subjected to Bio-Gel P-4 gel filtration as described previously (11). β-N-Acetylgalactosaminidase A can cleave both non-sulfated and sulfated N-acetyllactosamines.
hand, the enzyme exhibited relatively weak activity toward Galβ1→4GlcNAcβ1→2Manα1→6Manβ1→octyl and no activity toward core1 O-glycan, Galβ1→3GlcNAcα1→octyl, or type 1 oligosaccharide, Galβ1→3GlcNAcβ1→octyl. This acceptor specificity contrasts with that of Gal3ST-2, which can act on GlcNAcβ1→6Galβ1→3GalNAcα1→octyl and type 1 oligosaccharide but not on Galβ1→4(sulfo)→6GlcNAcβ1→octyl (Fig. 3). Moreover, Gal3ST-3 prefers a side chain derived from the 6-position of α-mannose over that derived from the 2-position of α-mannose, which is opposite to the preference by Gal3ST-2. Both enzymes did not act on Lewis x structures, Galβ1→4(Fucα1→3)GlcNAcβ1→octyl. These results demonstrate that Gal3ST-2 and Gal3ST-3 differ significantly in acceptor specificity.

Incorporation of $[^{35}S]$Sulfate to NCAM and CD43 (Leukosialin) Chimeric Proteins by Gal3ST-3—The substrate specificity of Gal3ST-3 was examined using NCAM-IgG and CD43-IgG chimeric proteins in the absence and presence of core2 β1,6-N-acetylgalcosaminyltransferase (C2GnT-1). NCAM contains almost exclusively N-glycans, whereas CD43 contains one N-glycan and ~70 O-glycans (37, 42). As the first of a series of experiments, CHO cells were transfected with vectors encoding NCAM-IgG or CD43-IgG chimeric protein with or without expression of full-length cDNA encoding Gal3ST-3. Because CHO cells lack C2GnT activity (41, 42), core2 O-glycans were absent when C2GnT was not transfected.

Fig. 4A illustrates that the incorporation of $[^{35}S]$sulfate into CD43 chimeric protein was significantly increased by the expression of Gal3ST-3. The incorporated $[^{35}S]$sulfate was mostly removed by N-glycanase treatment. $[^{35}S]$sulfate incorporation into NCAM was moderately increased by Gal3ST-3, and the majority of the incorporated radioactivity was removed by N-glycanase treatment. These results indicate that $[^{35}S]$sulfate was incorporated by Gal3ST-3 mainly into N-glycans in the absence of C2GnT. In contrast, there was only a slight increase in $[^{35}S]$sulfate incorporation into NCAM by Gal3ST-2. Moreover, $[^{35}S]$sulfate incorporated by Gal3ST-2 into CD43 was less susceptible to N-glycanase treatment (Fig. 4A).

To further examine the dependence of C2GnT on Gal3ST-3 and Gal3ST-2, Lec2 cells, which are defective in Golgi sialylation, were used as recipient cells. As shown in Fig. 4B, $[^{35}S]$sulfate incorporated into CD43 in the presence of C2GnT-T1 was only slightly removed by N-glycanase, whereas the radioactivity incorporated in the absence of C2GnT-T1 was significantly removed by the same treatment. These results indicate that $[^{35}S]$sulfate incorporation shifted from N-glycan sulfation to O-glycan sulfation with core2 branch formation.

To further examine the dependence of C2GnT on Gal3ST-3 and Gal3ST-2, Lec2 cells, which are defective in Golgi sialylation, were used as recipient cells. As shown in Fig. 4B, $[^{35}S]$sulfate incorporated into CD43 in the presence of C2GnT-T1 was only slightly removed by N-glycanase, whereas the radioactivity incorporated in the absence of C2GnT-T1 was significantly removed by the same treatment. These results indicate that $[^{35}S]$sulfate incorporation shifted from N-glycan sulfation to O-glycan sulfation with core2 branch formation.
β1→3)GalNAc structure (Fig. 3).

To further corroborate these findings, CD43 chimeric protein together with Gal3ST-3 and Gal3ST-2 were expressed in Lec1 and Lec8 cells, respectively. The results shown in Fig. 5 indicate that 35S incorporation by Gal3ST-3 is entirely dependent on the presence of core2-branched O-glycans when the recipient cells lack complex N-glycans as do Lec1 cells. Furthermore, sulfation is entirely dependent on galactose residues because no incorporation by Gal3ST-2 was observed in Lec8 cells, which lack Golgi galactosylation (Fig. 5).

Structural Analysis of O-Glycans Sulfated by Gal3ST-3—To determine the structure of sulfated O-glycans synthesized by Gal3ST-3, CD43 chimeric protein was produced in CHO cells that express Gal3ST-3 and C2GnT-1. These transfected cells were metabolically labeled with [3H]glucosamine or [3H]galactose and [35S]sulfate, and the CD43 chimeric protein released into the medium was collected. Sephadex G-50 gel filtration (Fig. 6A) revealed that the majority of the isolated O-glycans eluted as those containing two anionic charges (Fig. 6D), which were converted to a monosulfated form after desialylation (Fig. 6E). Upon Bio-Gel P-4 gel filtration, this oligosaccharide eluted at the elution position for...
core2-branched glycans, Gal\(\beta_1\)\(\rightarrow\)4GlcNAc\(\beta_1\)\(\rightarrow\)6(Gal\(\beta_1\)\(\rightarrow\)3)-GalNAcOH (Fig. 6F). Previous studies demonstrated that a monosulfated form of core2-branched O-glycans elutes at almost the same position as non-sulfated O-glycans (11). Almost identical results were obtained for O-glycans derived from Gal3ST-2-labeled CD43 in the presence of C2GnT-1 (data not shown).

After desulfation by solvolysis, the obtained O-glycans eluted at the same position as non-sulfated O-glycans (11). Almost identical results were obtained for O-glycans derived from Gal3ST-2-labeled CD43 in the presence of C2GnT-1 (data not shown). After desulfation by solvolysis, the obtained O-glycans eluted at the same position as non-sulfated O-glycans (11). Almost identical results were obtained for O-glycans derived from Gal3ST-2-labeled CD43 in the presence of C2GnT-1 (data not shown). After desulfation by solvolysis, the obtained O-glycans eluted at the same position as non-sulfated O-glycans (11). Almost identical results were obtained for O-glycans derived from Gal3ST-2-labeled CD43 in the presence of C2GnT-1 (data not shown). After desulfation by solvolysis, the obtained O-glycans eluted at the same position as non-sulfated O-glycans (11). Almost identical results were obtained for O-glycans derived from Gal3ST-2-labeled CD43 in the presence of C2GnT-1 (data not shown).

Expression of Gal3ST-3 Is Highly Restricted to Brain, Kid-
ney, and Thyroid—Northern blot analysis showed a highly specific Gal3ST-3 transcript of 2.4 kilobases expressed in fetal brain and kidney, and adult brain, kidney, and thyroid (Fig. 8). Multiple tissue expression array analysis showed almost identical results and further demonstrated that expression is highly pronounced in the putamen, caudate nucleus, and pituitary gland of the brain (Fig. 9). These results contrast with the reported expression pattern of Gal3ST-2 showing ubiquitous expression in almost all tissues tested (18).

**DISCUSSION**

The present study describes the isolation of a novel cDNA encoding galactose 3'-O-sulfotransferase by searching the EST database for cDNAs homologous to the human galactosyl ceramide 3-O-sulfotransferase, Gal3ST-1 (16). Gal3ST-1 adds a sulfate to a β-galactose residue linked to ceramide, whereas Gal3ST-3 adds a sulfate to a β-galactose linked to N-acetylgalactosamine through a 1,4-linkage. Previously, Gal3ST-2 was also cloned based on its similarity to Gal3ST-1, but the acceptor specificities of Gal3ST-2 and Gal3ST-3 differ substantially. Although Gal3ST-3 acts exclusively on N-acetylgalactosamine, Gal3ST-2 can act also on type 1 oligosaccharide, Galβ1→3GlcNAc, and core1 oligosaccharide, Galβ1→3GlcNAcβ1→6GalNAc. On the other hand, Gal3ST-3 can act on Galβ1→4(sulfo)→6GlcNAc, whereas the best acceptor for Gal3ST-2 is likely that GalST-3 plays a major role in the brain and kidney, whereas the other (B) acts on N-acetylgalactosamine attached to an N-glycan mannose core, such as Galβ1→4GlcNAcβ1→6Manα1→6Manβ1→octyl, than on N-acetylgalactosamine itself, whereas Gal3ST-2 does not possess this preference (Fig. 3). These results indicate that Gal3ST-2 and Gal3ST-3 act differentially on various acceptor glycoproteins.

In the present study, the transcripts of Gal3ST-3 were found to be expressed selectively in the brain, kidney, and thyroid. Previously, it was reported that human thyroglobulin contains a sulfo→3Galβ1→4GlcNAcβ1→R structure in the majority of N-glycans (23). This 3'-O-sulfated N-acetylgalactosamine side chain was shown to exist in both bi-antennary and highly branched tri- and tetra-antennary N-glycans. In porcine thyroglobulin, the majority of sulfo→3Galβ1→4GlcNAcβ1→R are present in side chains derived from the 6-position of α-mannose. Moreover, the same glycoprotein contains a Galβ1→4(sulfo)→6GlcNAc side chain in a portion of the N-glycans (24). As shown previously, sulfo→6GlcNAc→R is first formed from a GlcNAc→R structure and then converted to Galβ1→4(sulfo)→6GlcNAc→R (11, 12). Galβ1→4(sulfo)→6GlcNAc→R can then be converted by Gal3ST-3 to sulfo→3Galβ1→4(sulfo)→6GlcNAc→R, considering that Gal3ST-3 can act on Galβ1→4(sulfo)→6GlcNAc→R. In the structural studies described above, no multisulfated N-glycans were analyzed (24). It is possible that sulfo→3Galβ1→4(sulfo)→6GlcNAc→R may be found with further analysis of highly sulfated N-glycans in thyroglobulin.

It has been reported that a major glycoprotein in calf thyroid contains core2-branched O-glycans (44) and that core2 branches in the thyroid are likely synthesized by C2GnT-1 and C2GnT-2 (45, 46). Core2-branched oligosaccharides from calf thyroid apparently lack 3'-sulfate galactose. In contrast to human thyroglobulin, the presence of 3'-sulfate galactose in calf thyroglobulin is minimal, presumably due to the competition with the strong α1,3-galactosyltransferase activity in calf thyroid (44). It has also been reported that galactose 3'-O-sulfotransferase in the thyroid acts only on N-acetylgalactosamine as does Gal3ST-3 (25). These results indicate that Gal3ST-3 is most likely responsible for the formation of sulfo→3Galβ1→4GlcNAc attached to both N-glycans and core2-branched O-glycans synthesized in the thyroid.

Although it was reported that the transcripts of Gal3ST-2 are expressed in various tissues, the amount of transcripts was relatively low in brain and kidney when estimated by reverse transcription PCR (18). Because the transcripts of Gal3ST-3, on the other hand, are highly expressed in brain and kidney, it is likely that Gal3ST-3 plays a major role in the brain and kidney in addition to the thyroid. Previously, the existence of two different galactosyl 3'-O-sulfotransferases has been reported: one (A) acts on core1 O-glycans, whereas the other (B) acts...
on N-acetyllactosamine (47, 48). Although it is not straightforward to correlate these findings with the acceptor specificities of the cloned enzymes, it appears that A and B correspond to Gal3ST-2 and Gal3ST-3, respectively. On the other hand, the acceptor specificity of Gal3ST-3 appears to be identical to the enzyme described in human airways (17), whereas Gal3ST-2, which acts on type 1 oligosaccharides, is most likely responsible for the formation of sulfo\(^3\text{Gal}β1\)-3\(\text{GlcNAc}\)β1\(\rightarrow\)R (4, 49).

It has been reported that the sulfo\(^3\text{Gal}β1\)-4\(\text{Fuc}α1\)-3\(\text{GlcNAc}\)β1\(\rightarrow\)R structure serves as an E-selectin ligand (4, 50). The same oligosaccharide was, on the other hand, shown to be a ligand for P-selectin but not for E-selectin when a synthetic oligosaccharide attached to GDP-fucose was transferred to the cell surface (5). Recently, it has been shown that 6-sulfo sialyl Lewis x, NeuNAcα2\(\rightarrow\)3\(\text{Gal}β1\)-3\(\text{Fuc}β1\)-3\(\text{GlcNAc}\)β1\(\rightarrow\)R, is a potent physiological ligand for L-selectin (11, 12). It will be of significance to determine if all of these different oligosaccharides containing 3-sulfo galactose can be synthesized in cells with the necessary cDNAs, including those encoding Gal3ST-3.

In this context, it is noteworthy that Gal3ST-3 has a relatively narrow acceptor specificity compared with Gal3ST-2. Due to this distinct acceptor specificity, it is likely that the expression of Gal3ST-3 results in the formation of sulfo\(^3\text{Gal}β1\)-4\(\text{GlcNAc}\)β1\(\rightarrow\)R and related structures in a well-defined set of carbohydrates attached to glycoproteins. It is thus expected that the cDNA encoding Gal3ST-3, cloned in the present study, will be a powerful tool to determine the structure/function of sulfo\(^3\text{Gal}β1\)-4\(\text{GlcNAc}\)β1\(\rightarrow\)R and related structures.

Acknowledgments—We thank Dr. Yili Ding for useful discussion and Joseph P. Henig and Shizuka Mitoma for organizing the manuscript.

REFERENCES

1. Fiete, D., Sriravastava, V., Hindsgaul, O., and Baenziger, J. U. (1991) Cell 67, 1103–1110
2. Shukla, D., Liu, J., Blacklock, P., Shworak, N. W., Bai, X., Esko, J. D., Cohen, G. H., Eisenberg, R. J., Rosenberg, R. D., and Spear, P. G. (1991) Cell 99, 15–22
3. Akama, T. O., Nishida, K., Nakayama, J., Watanabe, H., Oaki, K., Nakamura, T., Dota, A., Kawasaki, S., Inoue, Y., Maeda, N., Yamamoto, S., Fujimura, T., Honar, J. J., Shimomura, Y., Kinoshita, S., Tanigami, A., and Fukuda, M. N. (2000) Nat. Genet. 25, 257–261
4. Yuen, C.-T., Lawson, A. M., Chai, W., Larkin, M., Stoll, M. S., Stuart, A. C., Sullivan, F. X., Ahern, T. J., and Feizi, T. (1992) Biochemistry 31, 9126–9131
5. Tsuoi, S., Sriravastava, O. P., Palcic, M., Hindsgaul, O., and Fukuda, M. (2000) Arch. Biochem. Biophys. 374, 100–106
6. Ong, E., Yeh, J. C., Ding, Y., Hindsgaul, O., and Fukuda, M. (1998) J. Biol. Chem. 273, 5103–5106
7. Kakuta, Y., Pedersen, L. G., Carter, C. W., Negishi, M., and Pedersen, L. C. (1997) Nat. Struct. Biol. 4, 964–968
8. Kakuta, Y., Pedersen, L. G., Pedersen, L. C., and Negishi, M. (1998) Trends Biochem. Sci. 23, 129–130
9. Ong, E., Yeh, J. C., Ding, Y., Hindsgaul, O., Pedersen, L. C., Negishi, M., and Fukuda, M. (1999) J. Biol. Chem. 274, 25608–25612