A Remodeling System of the 3'-Sulfo-Lewis a and 3'-Sulfo-Lewis x Epitopes*

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It has been reported that the chemically synthesized 3'-sulfo-Le" and 3'-sulfo-Le° epitopes have a high potential as a ligand for selectins. To elucidate the physiological functions of 3'-sulfated Lewis epitopes, a remodeling system was developed using a combination of a βGal3-O-sulfotransferase GP3ST, hitherto known α1,3,4-fucosyltransferases (FucT-III, IV, V, VI, VII, and IX) and arylsulfatase A. The pyridylaminated (PA) lacto-N-tetraose (Galβ1–3GlcNAcβ1–3Galβ1–4Glc) was first converted to 3'-sulfalacto-N-fucopentaose II (sulfal-3Galβ1–3(Fucα1–4)GlcNAcβ1–3Galβ1–4Glc)-PA by sequential reactions with GP3ST and FucT-III. The 3'-sulfalacto-N-fucopentaose III (sulfal-3Galβ1–4(Fucα1–5)GlcNAcβ1–3Galβ1–4Glc)-PA was then synthesized from lacto-N-neotetraose (Galβ1–4GlcNAcβ1–3Galβ1–4Glc)-PA by GP3ST and FucT-III. -IV, -V, -VI, -VII, or -IX in a similar manner. The substrate specificity for the 3'-sulfated acceptor of the α1,3-fucosyltransferases was considerably different from that for the non-substituted and 3'-sialylated varieties. When the GP3ST gene was introduced into A549 and Chinese hamster ovary cells expressing FucT-III, they began to express 3'-sulfo-Le° and 3'-sulfo-Le" epitopes, respectively, suggesting that GP3ST is responsible for their biosynthesis in vivo. The expression of the 3'-sialyl-Le" epitope on Chinese hamster ovary cells was attenuated by GP3ST and FucT-III, indicating that GP3ST is responsible for their biosynthesis in vivo. The expression of the 3'-sialyl-Le" epitope on Chinese hamster ovary cells was attenuated by GP3ST and FucT-III, indicating that GP3ST is responsible for their biosynthesis in vivo. The expression of the 3'-sialyl-Le" epitope on Chinese hamster ovary cells was attenuated by GP3ST and FucT-III, indicating that GP3ST is responsible for their biosynthesis in vivo.

Sulfated glycoconjugates occur in a wide range of biological compounds, including glycoproteins, proteoglycans, glycolipids, and polysaccharides (for a review, see Ref. 1). The negative charge of the sulfate group is thought to serve as an adherent force in interactions with a variety of functional molecules, which include growth factors, cellular adhesion molecules, and extracellular matrix proteins (1). In fact, a considerable body of evidence has accumulated relative to the biological importance of sulfation of carbohydrate chains (2–6).

The sulfation group is attached to positions 3 and 6 of Gal, positions 3 and 6 of GlcNAc, and position 4 of GalNAc, in the case of N-linked or O-linked glycopolypeptides (1, 7). The 3-sulfo-βGal linkage is found in both N-glycans (8, 9) and O-glycans (10–17). Among these are the sulfo-3Galβ1–3(Fucα1–4)GlcNAc-R (3'-sulfo-Le") and sulfo-3Galβ1–4(Fucα1–3)GlcNAc-R (3'-sulfo-Le") structures (12, 14, 15, 17), which have been shown to be more potent ligands for both L- and E-selectin than the 3'-sialylated-Le" and -Le° determinants as evidenced by a binding assay using chemically synthesized oligosaccharides (14, 18, 19). The expression of the 3'-sulfo-Le" epitope decreases with increasing depth of invasion of human colon carcinomas (20), and human colon carcinoma cells expressing the 3'-sulfo-Le" epitope show a lower tumorigenicity in nude mice (21). On the other hand, the 3'-sulfo-Le" and/or -Le° determinants have been detected in cancer cells as well as in surrounding nonmalignant epithelia in human colon cancer tissues (22) and the 3'-sulfo-Le" epitope has been found to be a major carbohydrate motif in a human colon carcinoma cell line with a high metastatic tendency (15). These findings indicate that 3'-sulfated Lewis epitopes may serve as a relevant ligand for selectins in vivo and that their expression modulates tumor progression, in the case of human colon cancer. However, the lack of genetic tools for the remodeling of such epitopes has hampered the complete characterization of their biological functions.

We recently reported on the cDNA cloning of a βGal3-O-sulfotransferase (GP3ST) that acts on both type 1 (Galβ1–3GlcNAc-R) and type 2 (Galβ1–4GlcNAc-R) chains and is expressed in human colonic mucosa (23), based on its similarity to

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1 The abbreviations used are: GP3ST, βGal3-O-sulfotransferase; CHO cells, Chinese hamster ovary cells; MES, 2-N-morpholinoethanesulfonic acid; mAb, monoclonal antibody; PA, 2-aminopyridine; PAPS, adenosine 3'-phosphate, 5'-phosphosulfate; LeC, lacto-N-tetraose, Galβ1–3GlcNAcβ1–3Galβ1–4Glc; nLeC, lacto-N-neotetraose, Galβ1–4GlcNAcβ1–3Galβ1–4Glc; lacto-N-fucopentaose II, Galβ1–3(Fucα1–4)GlcNAc-R (3'-sulfo-Le") and sulfo-3Galβ1–4(Fucα1–3)GlcNAc-R (3'-sulfo-Le") structures (12, 14, 15, 17), which have been shown to be more potent ligands for both L- and E-selectin than the 3'-sialylated-Le" and -Le° determinants as evidenced by a binding assay using chemically synthesized oligosaccharides (14, 18, 19). The expression of the 3'-sulfo-Le" epitope decreases with increasing depth of invasion of human colon carcinomas (20), and human colon carcinoma cells expressing the 3'-sulfo-Le" epitope show a lower tumorigenicity in nude mice (21). On the other hand, the 3'-sulfo-Le" and/or -Le° determinants have been detected in cancer cells as well as in surrounding nonmalignant epithelia in human colon cancer tissues (22) and the 3'-sulfo-Le" epitope has been found to be a major carbohydrate motif in a human colon carcinoma cell line with a high metastatic tendency (15). These findings indicate that 3'-sulfated Lewis epitopes may serve as a relevant ligand for selectins in vivo and that their expression modulates tumor progression, in the case of human colon cancer. However, the lack of genetic tools for the remodeling of such epitopes has hampered the complete characterization of their biological functions.

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glycolipid 3-O-sulfotransferase (24). Its molecular cloning enabled us to develop a remodeling system of 3′-sulfated Lewis epitopes. The enzymatic degradation of these epitopes is also discussed.

**EXPERIMENTAL PROCEDURES**

**Materials**—PAPS was purchased from Sigma; lacto-N-tetraose and lacto-N-neotetraose were purchased from Seikagaku Kogyo (Tokyo, Japan); 1-fucose and GDP-Fuc from Nacalai Tesque (Kyoto, Japan). Lc4-PA and nLc4-PA were synthesized by the pyridylation of lacto-N-tetraose and lacto-N-neotetraose using a GlycoTAG Reagent kit (Takara, Shiga, Japan) with an automated pyridylation apparatus (GlycoTAG, Takara). FucT-III was isolated from a conditioned medium of CHO cells that had been transfected with pSec-FucT-III, which was constructed by recombination of the DNA fragments encoding the open reading frame portion of human FucT-III (25) into an expression vector pSec-FucT-III, which was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes. Mouse IgG1 (Dako, Santa Barbara, CA) was used for the detection of carbohydrate epitopes.

**Flow Cytometry Analysis of CHO Cells**—A549 Cells Transfected with or without GP3ST and FucT-IIIG CHO and A549 cells were transfected with linearized pCXN2-GP3ST and/or pDNA-FucT-II genes using the Effectene Transfection Reagent (Qiagen, Hilden, Germany) according to the standard protocol for stable transfection and selected for clones stably expressing these genes, based on their resistance to G418 (Sigma) and/or Zeocin (Invitrogen) followed by measurement of the enzyme activities, as described above. The cloned cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 0.1 mg/ml streptomycin, 400 μg/ml G418, and 150 μg/ml Zeocin and harvested with PBS containing 1 mM EDTA. Fifty μl of each suspensions (5 × 10^6 cells) were incubated with a primary antibody (SU59 diluted 1:5; P12 and KM93 diluted 1:25; MAB2108, ZY-C09, 91.9H, and control immunoglobulin G at a dilution of 1:50) for 30 min on ice. Cells were then washed with 1 ml of PBS, suspended in 100 μl of fluorescein isothiocyanate-conjugated F(ab’)_2 fragment of goat anti-mouse immunoglobulins (Dako) diluted 1:25 and incubated for 30 min on ice. Flow cytometry analyses were performed using a FACScan instrument (Becton Dickinson, Franklin Lakes, NJ) operating with CELLQuest software.

**Enzymatic Synthesis of 3′-Sulfated Lewis Epitopes**—Parental CHO cells and CHO cells transfected with the GP3ST and/or FucT-III genes were suspended in 4 volumes of 10 mM Tris-HCl buffer (pH 7.4) containing 1% Triton X-100, 1 mM EDTA, and 0.1% protease inhibitor mixture for mammalian cell and tissue extracts (Wako, Osaka, Japan). After incubation on ice for 1 h, the solution was centrifuged at 15,000 rpm for 30 min and the supernatants were used as cell lysates. Protein concentration was assayed by means of the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). The cell lysates were then washed and incubated with fluorescein isothiocyanate-conjugated F(ab’)2 fragment of goat anti-mouse immunoglobulins diluted 1:25 for 30 min on ice. Flow cytometry analyses was performed as described above.

**RESULTS**

**Enzymatic Synthesis of 3′-Sulfated Lea and 3′-Sulfated Leb Structure in Vitro**—In a previous study (23), GP3ST was found to act on both lacto-N-tetraose and lacto-N-neotetraose, but not on lacto-N-fucopentaose II or lacto-N-fucopentaose III, suggesting that 3′-sulfation of the terminal Gal occurs prior to the 3′-sulfosylation of the penultimate GlcNAc in the biosynthetic pathway of the 3′-sulfated Lea and Leb structures. Our knowledge is incomplete on the use of crude enzyme sources on lacto-N-fucosyltransferase. Therefore, we investigated biosynthesis of 3′-sulfated Lea and Leb structures extensively using recombinant GP3ST and a3,1,4-sulfotransferases. Since 3′-sialylation also occurs prior to the 3′-sulfosylation in the synthetic path-
Recombinant FucT-III, which is the sole way of 3'-sialyl-Le^a and -Le^x (34, 35), the effects of α1,3/4-fucosyltransferases on non-substituted 3'-sialylated and 3'-sulfated Galβ1-3/4GlcNAc-R oligosaccharides were compared.

The α1,4-fucosylation of the 3'-sulfated type 1 chain (Galβ1-3GlcNAc-R) was examined first. 3'-Sulfo-Le^a-PA was synthesized from Le^a-PA via catalysis by GP3ST (25). The resulting 3'-sulfo-Le^a-PA was then subjected to fucosylation by recombinant FucT-III, which is the sole α1,4-fucosyltransferase (25). A strong product peak appeared, as shown by an arrow, in Fig. 1b in the presence of GDP-Fuc (Fig. 1b), whereas no peak was detected in the absence of the donor substrate (Fig. 1a). The m/z value of the material in the product peak was 1010.4, corresponding to that of 3'-sulfolacto-N-fucopentaose II-PA (Fig. 1c). These results indicate that FucT-III has the capability to act on the 3'-sulfated type 1 chain and to synthesize the 3'-sulfo-Le^a structure. The efficiency of FucT-III for non-substituted 3'-sialylated and 3'-sulfated acceptors was also compared (Table I). The result indicates that FucT-III prefers 3'-sulfo-Le^a-PA to the non-substituted Le^a-PA or 3'-sialyl-Le^a-PA.

![Image](96x296 to 250x730)

**Fig. 1. Enzymatic synthesis of 3'-sulfo-Le^a and 3'-sulfo-Le^x structures in vitro.** Le^a-PA (panels a and b) or nLc4-PA (panels d and e) were incubated with recombinant human FucT-III in the absence (panels a and d) or presence (panels b and e) of GDP-fucose. Reaction products were isolated by reverse-phase HPLC, with fluorescence monitoring as described under “Experimental Procedures.” The arrows indicate the elution position of the products. The fractions containing the products were pooled, dried, and characterized by mass spectrometry. The determined m/z of the products corresponded with 3'-sulfo-lacto-N-fucopentaose II-PA (panel c) and 3'-sulfo-lacto-N-fucopentaose III-PA (panel f), respectively.

Thus far, six α1,3-fucosyltransferase isozymes, FucT-III, -IV, -V, -VI, -VII, and -IX, are known (26, 36, 37). Since the substrate specificity of α1,3-fucosyltransferases for 3'-sulfated acceptors has not been investigated, this was examined, compared with that for non-substituted and 3'-sialylated acceptors. When 3'-sulfo-nLc4-PA was incubated with FucT-III in the presence of GDP-Fuc, 3'-sulfolacto-N-fucopentaose III was produced (Fig. 1, c and f). As shown in Table I, FucT-III preferred the 3'-sulfated nLc4-PA to nLc4-PA or 3'-sialyl-nLc4-PA, and preferred the type 1 chain to the type 2 chain, as described previously (25). The α3-fucosylation of 3'-sulfo-nLc4-PA was then examined with respect to the other α1,3-fucosyltransferases. Since the sources and specific activities of the fucosyltransferases used were different, the activities toward individual acceptors are expressed relative to those toward nLc4-PA (FucT-III, -IV, -V, -VI, and -IX) or 3'-sialyl-nLc4-PA (FucT-IV) in Table II. The preference for the sulfated acceptor among the α1,3-fucosyltransferases was considerably different from that for the sialylated or non-substituted acceptors. All the α1,3-fucosyltransferases acted on the sialated acceptor unlike the sialylated one, although the extent of relative reaction efficiency varied, depending on the specific enzyme. It was noted that the 3'-sulfated oligosaccharide was a better substrate than the non-substituted or 3'-sialylated oligosaccharide for FucT-III, -V, and -VI.

**Recollection of 3'-Sulfo-Le^a and 3'-Sulfo-Le^x Epitopes on Living Cells**—To analyze biological roles of the 3'-sulfated Lewis epitopes, information on the expression of these epitopes on the living cell surface is required. Therefore, the GP3ST and FucT-III genes, which had been inserted into the expression vectors, were transfected into CHO cells and the expression of 3'-sulfated Lewis epitopes was examined by flow cytometry analysis using specific antibodies against the 3'-sulfated Lewis epitopes. The mAb SU59 recognizes both 3'-sulfo-Le^a and 3'-sulfo-Le^x epitopes (29), but mAb 91.9H recognizes only the 3'-sulfo-Le^a epitope (30, 31).

The parent CHO cells (Fig. 2, panels a, e, and i) and CHO cells transfected with the GP3ST gene alone (Fig. 2, panel b, f, and j) expressed neither Le^a (recognized by mAb P12), 3'-sialyl-Le^a (recognized by mAb KM93), nor 3'-sulfo-Le^a (recognized by mAb SU59). The CHO cells that had been transfected with only the FucT-III gene expressed Le^a and 3'-sialyl-Le^a (Fig. 2, panel c and g), but did not express 3'-sulfo-Le^a (Fig. 2, panel h), indicating that CHO cells do not express the βGal-3-O-sulfotransferase. In addition, FucT-III-transfected CHO cells expressed neither Le^a nor 3'-sialyl-Le^a (data not shown), consistent with the previously reported observation that CHO cells express only the type 2 chain (38).

CHO cells transfected with both the GP3ST and FucT-III genes were SU59-positive (Fig. 2, panel l) but 91.9H-negative (data not shown), indicating that the cells express the 3'-sulfo-Le^a determinant but not the 3'-sulfo-Le^x, which is in good agreement with the conclusion that CHO cells expresses only the type 2 chain. Furthermore, the expression of the 3'-sialyl-Le^a epitope on both gene-transfected cells was remarkably reduced, compared with that on only the FucT-III gene-transfected cells (Fig. 2, panels g and h). This finding indicates that the expression of GP3ST interferes with the biosynthesis of 3'-sialyl-Le^a epitope in vivo.

To analyze the specific molecules on which 3'-sulfo-Le^a epitope is carried, glycoproteins were extracted from CHO cells.
transfected with the GP3ST and FucT-III genes and examined by Western blotting. As shown in Fig. 3a, several protein bands with a relatively high molecular weight were specifically stained with mAb SU59 (lanes 4 and 5), indicating that the 3'-sulfo-Lea epitope was contained by several different proteins. These SU59-positive bands were nearly identical to the bands stained with anti-3'-sialyl-Lea antibody KM93 in CHO cells transfected with only the FucT-III gene (Fig. 3b, lane 3). In addition, the reactivity with anti-3'-sialyl-Lea antibody was reduced in CHO cells that had been transfected with both the GP3ST and FucT-III genes (Fig. 3b, lanes 4 and 5), consistent with the flow cytometry results. These observations suggest that 3'-sulfation and 3'-sialylation occur on common glycoproteins. In addition, most SU59-positive bands in Fig. 3a, lanes 4 and 5, disappeared after treatment with N-glycanase (Fig. 3c, lanes 1 and 2). When glycolipids were extracted from the doubly transfected CHO cells with this enzyme and analyzed by thin-layer chromatography immunostaining, no SU59-positive band could be detected (data not shown). These findings indicate that 3'-sulfo-Lea epitope is mainly carried on N-linked glycoproteins in the CHO cells.

To examine the ability of GP3ST to synthesize 3'-sulfo-Lea epitope in living cells, its gene was transfected into a human lung carcinoma cell line A549, which expresses Lea antigen but does not react with mAb 91.9H (Fig. 4a). After the introduction of the GP3ST gene, the cells became 91.9H positive (Fig. 4b, several). Mouse IgG1 was used as a negative control (dotted line). Note that only the CHO cells transfected with both GP3ST and FucT-III genes are SU59-positive (panel l), while the expression of 3'-sialyl-Lea is remarkably reduced, compared with those transfected only with the FucT-III gene (panels g and h).

**TABLE I**

| Acceptor | Activity (pmol/min/ml) |
|----------|------------------------|
| Galβ1→4GlcNAcβ1→3Galβ1→4Glc→PA (Le4-PA) | 217.3 |
| NeuAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→PA (3’-sialyl-Le4-PA) | 60.7 |
| SO3−→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→PA (3’-sulfo-Le4-PA) | 320.1 |
| Galβ1→4GlcNAcβ1→3Galβ1→4Glc→PA (nLe4-PA) | 2.6 |
| NeuAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→PA (3’-sialyl-nLe4-PA) | 3.3 |
| SO3−→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→PA (3’-sulfo-nLe4-PA) | 20.7 |

* The concentration of acceptors was 25 μM, except for 3'-sialyl-Le4-PA, which was used at a concentration of 12.5 μM.

**TABLE II**

| Acceptor | Relative activity (%) |
|----------|-----------------------|
| Galβ1→4GlcNAcβ1→3Galβ1→4Glc→PA (Le4-PA) | 100 |
| NeuAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→PA (3’-sialyl-Le4-PA) | 128 |
| SO3−→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→PA (3’-sulfo-Le4-PA) | 803 |

* The concentration of acceptors was 25 μM.

The values represent the percentage of the activity, compared with that for nLe4-PA, except for FucT-VII in which relative activities to that for 3'-sialyl-nLe4-PA are shown. α,1,3-Fucosyltransferase activities for nLe4-PA of FucT-III, -IV, -V, -VI, and -IX were 3.6, 64.9, 75.1, 6.0, and 144.9 pmol/min/ml, respectively. α,1,3-Fucosyltransferase activity for 3’-sialyl nLe4-PA of FucT-VII was 593.9 pmol/min/ml.
then stained with mAb SU59. After blocking, the membrane was incubated with mAb SU59. Separated by SDS-PAGE and transferred to a nitrocellulose membrane.

...c, with mAb KM93. In these studies, 3-sulfated type 2 chain is of interest. Prior to this study, we anticipated a result similar to that for the 3'-sialylated acceptors. Unexpectedly, all the 1,3-fucosyltransferases act on the 3'-sialyl-Lea structure. As expected, FucT-III is largely active on type 1 but also on type 2 chains, whether they are sialylated or not. Concerning FucT-IV, the neutral type 2 chain is a good substrate while the 3'-sialylated oligosaccharide is a poor one. FucT-V and FucT-VI are active on both neutral and 3'-sialylated substrates. FucT-VII acts on only the 3'-sialylated type 2 chain whereas FucT-IX is active only on the neutral one. Therefore, the issue of which fucosyltransferases act on the 3'-sulfated type 2 chain is of interest. Prior to this study, we anticipated a result similar to that for the 3'-sialylated acceptors. Unexpectedly, all the 1,3-fucosyltransferases acted on the 3'-sulfated acceptor and no...
correlation was found for the relative activity for the sulfated substrate of individual fucosyltransferases with that for the non-substituted, sialylated, and sulfated acceptors of FucT-I, II, III, -V, and -VI may reflect the homology in their primary structures (37). The similarity in substrate specificity pending on the isozymes. The similarity in substrate specificity of 3′-sialyltransferases recognize the sulfate group or 1,3-fucosyltransferases recognize the sulfate group or for non-substituted, sialylated, and sulfated acceptors of FucT-I, II, III, -V, and -VI may reflect the homology in their primary structures (37).

The fact that GP3ST and FucT-III were collaboratively able to desulfate the 3′-sulfated Lex structure after digestion of the peptide portion attached to position 3 of the nonreducing terminal Gal in glycoproteins prompted us to examine the issue of whether it is able to desulfate the 3′-sulfated Le$^a$ and -Le$^b$ structures. As a result, arylsulfatase A was found to hydrolyze the sulfate ester bond on 3′-sulfo-Le$^a$ but not on 3′-sulfo-Le$^b$ in vitro. This suggests that arylsulfatase A can be used as a tool for the dissection of functions between the 3′-sulfated-Le$^a$ and -Le$^b$ epitopes. On the other hand, arylsulfatase A acted only weakly on the 3′-sulfogalactosyl-containing glycolipids (39).

During the preparation of this article, the molecular cloning of another βGal 3-O-sulfotransferase (Gal3ST-3, Gal3ST2), which acts on only the type 2 chain and is expressed in confined tissues such as thyroid and brain, was independently reported by two groups (42, 43). This sulfotransferase may synthesize the 3′-sulfo-Le$^a$ epitope in the thyroid, although only the sulfogalactosyl-3Galβ1-4GlcNAc-R structure without α3-fucose was found on human thyroglobulin (44). Gal3ST-3 may also be involved in the biosynthesis of 3′-sulfo-Le$^a$ epitope on the N-glycans of human thyrotropin in the anterior pituitary gland (45), where the sulfotransferase gene is expressed (44). In contrast, GP3ST is expressed in various tissues, including colon epithelia (23), and may be responsible for the biosynthesis of both 3′-sulfo-Le$^a$ and 3′-sulfo-Le$^b$ epitopes in these tissues.

Arylsulfatase A is a lysosomal hydrolyase that catalyzes the desulfation of 3-O-sulfogalactosyl-containing glycolipids (39). The fact that arylsulfatase A hydrolyses the sulfate ester attached to position 3 of the nonreducing terminal βGal in glycolipids prompted us to examine the issue of whether it is able to desulfate the 3′-sulfated Le$^a$ and -Le$^b$ structures. As a result, arylsulfatase A was found to hydrolyze the sulfate ester bond on 3′-sulfo-Le$^a$ but not on 3′-sulfo-Le$^b$ in vitro. This suggests that arylsulfatase A can be used as a tool for the dissection of functions between the 3′-sulfated-Le$^a$ and -Le$^b$ epitopes. On the other hand, arylsulfatase A acted only weakly on the 3′-sulfogalactosyl-containing glycolipids (39).

In conclusion, the present study demonstrates that: 1) GP3ST and FucT-III catalyze the synthesis of the 3′-sulfo-Le$^a$ epitope in a collective manner; 2) GP3ST and FucT-III, IV, V, VI, VII, and IX are involved in the biosynthesis of the 3′-sulfo-Le$^a$ epitope; 3) GP3ST and α2,3-sialyltransferase compete for the common Galβ1-4GlcNAc-R oligosaccharides in CHO cells.

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FIG. 5. Enzymatic degradation of 3′-sulfo-Le$^a$ and 3′-sulfo-Le$^b$ structure in vitro. 3′-Sulfo-Le$^a$-PA (panel a), 3′-sulfo-Lc4-PA (panel b), 3′-sulfo-nLc4-PA (panel c), and 3′-sulfo-Lc4-PA (panel d) were incubated in the absence (−) or presence (+) of arylsulfatase A (ASA) and separated on a reverse-phase HPLC system as described under "Experimental Procedures." Arrows indicate the positions of desulfated products. Note that only type 2 chain carbohydrates (panels c and d) are desulfated.

FIG. 6. Effect of treatment with arylsulfatase A on the expression of 3′-sulfo-Le$^a$ epitope on GP3ST and FucT-III gene-transfected CHO cells. Both GP3ST and FucT-III gene-transfected CHO cells were incubated in 20 mM acetate-NaOH buffer (pH 5.0) and 150 mM NaCl in the presence (bold line) or absence (solid line) of arylsulfatase A (ASA), and then subjected to flow cytometry analysis with mAb SU59. Flow cytometry analysis also revealed that the robust expression of both GP3ST and FucT-III genes reacted with mAb SU59. Flow cytometry analysis, where only CHO cells transfected with both GP3ST and FucT-III genes reacted with mAb SU59. Flow cytometry analysis also revealed that the robust expression of 3′-sialyl-Le$^a$ epitope on FucT-III-transfected CHO cells was inhibited by the introduction of the GP3ST gene. This result suggests that GP3ST and α2,3-sialyltransferase are located in the same compartment of the Golgi apparatus and compete for the Galβ1–4GlcNAc-R oligosaccharide on the common oligosaccharides in CHO cells. This finding was verified by Western blotting analysis, where the protein bands with 3′-sialyl-Le$^a$ were found to be nearly identical to those with 3′-sulfated Le$^a$ and their signals were attenuated in the cells expressing GP3ST. Since the GP3ST gene is expressed in various human tissues (23), GP3ST may regulate the expression of Le$^a$ and 3′-sialyl-Le$^a$ epitopes through a similar regulation may occur in the expression of Le$^a$ and 3′-sialyl-Le$^a$ epitopes. Mutual interference by glycosyltransferases and carbohydrate-modifying enzymes in the biosynthesis of carbohydrate chains occurs under various situations (40, 41).
...sulfatase A hydrolyzes the sulfate ester bond on 3'-sulfo-Le
but not on the 3'-sulfo-Le*. In the future, the present remodeling of the 3'-sulfated Lewis epitopes may provide a useful tool for the study on their biological roles including their interaction with selectins.

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