Kinetic Properties and Acceptor Substrate Preferences of Two Kinds of Galβ1,3GalNAc α2,3-Sialyltransferase from Mouse Brain†

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ABSTRACT: The cDNAs encoding two kinds of Galβ1,3GalNAc α2,3-sialyltransferases (ST3GalA.1 and ST3GalA.2) have been cloned from mouse brain, both of which could synthesize the NeuAcα2,3Galβ1,-3GalNAc sequence of gangliosides as well as O-glycosidically linked oligosaccharides of glycoproteins [Lee *et al.* (1993) *Eur. J. Biochem. 216,* 377–385; Lee *et al.* (1994) *J. Biol. Chem.* (in press)]. Kinetic analysis of the two sialyltransferases using Galβ1,3GalNAc, asialoGM1, or asialofetuin revealed that ST3GalA.1 exhibits the highest K_m value for asialoGM1 (K_m = 1.25 mM) and the lowest one for asialofetuin (K_m = 0.10 mM), whereas the K_m values of ST3GalA.2 for the substrates are very similar ($K_m \approx 0.5$ mM). The synthesis of GM1b from asialoGM1 by ST3GalA.1 was clearly inhibited in the presence of Galβ1,3GalNAc or asialofetuin, but that by ST3GalA.2 was not at all. On the other hand, the activity of ST3GalA.2 toward Galβ1,3GalNAc or asialofetuin was inhibited by asialoGM1 or GM1. The results of acceptor competition experiments involving asialoGM1, Galβ1,3GalNAc, and asialofetuin indicated that ST3GalA.2 exhibits noncompetitive inhibition between asialoGM1 and Galβ1,3GalNAc or between asialoGM1 and asialofetuin, whereas ST3GalA.1 exhibits competitive inhibition between all kinds of acceptors. These results strongly indicate that acceptor preference of ST3GalA.1 is different from that of ST3GalA.2, although their acceptor substrate specificities are the same; *i.e.,* gangliosides serve as predominant acceptors for the latter over O-glycosidically linked oligosaccharides of glycoproteins, which are much better acceptors for the former.

Sialic acid residues occur at the terminal positions of the carbohydrate groups of three types of glycoconjugates (N- and O-glycosidically linked oligosaccharides of glycoproteins, and glycosphingolipids) and play important roles in a variety of biological processes (Dennis *et al.*, 1982; Feizi, 1985; Paulson & Colley, 1989; Schnaar, 1991). The transfer of sialic acid from CMP-sialic acid to these glycoconjugates is catalyzed by a family of sialyltransferases (Beyer *et al.*, 1981; Kornfeld & Kornfeld, 1985). Although the same carbohydrate sequences containing sialic acid are found in the three types of glycoconjugates, it is not clear whether the sialyltransferases for one type of glycoconjugate are different from those for others. For example, the carbohydrate sequence NeuAcα2,-3Galβ1,3GalNAc was found in gangliosides as well as O-glycosidically linked oligosaccharides of glycoproteins (Thomas & Winzler, 1969; Spiro & Bhoyroo, 1974; Fishman & Brady, 1976). A β-galactoside α2,3-sialyltransferase, purified from human placenta and porcine submaxillary gland, has been shown to catalyze the α2,3-sialyltransfer to galactose residues of the Galβ1,3GalNAc sequences of some glycoproteins as well as those of some gangliosides (Rearick *et al.*, 1979; Joziasse *et al.*, 1985). On the other hand, sialyltransferase IV has been shown to synthesize GM1b,[1] GD1a, and GT1b through α2,3-sialyltransfer to the Galβ1,3GalNAc sequences of asialoGM1, GM1, and GD1b, respectively, using rat liver Golgi vesicles as the enzyme source (Pohlentz *et al.*, 1988; Iber *et al.*, 1991).

Recently we successfully cloned two kinds of cDNA encoding mouse brain Galβ1,3GalNAc α2,3-sialyltransferases (ST3GalA.1 and ST3GalA.2) (Lee *et al.*, 1993, 1994), by a PCR method directed to a highly conserved region (sialylmotif) of the sialyltransfease gene family (Livingston & Paulson, 1993; Drickamer, 1993; Kurosawa *et al.*, 1994). They exhibit the same acceptor substrate specificities and are able to only synthesize the NeuAcα2,3Galβ1,3GalNAc sequence in gangliosides and glycoproteins as well as oligosaccharides (Lee *et al.*, 1993, 1994). The deduced amino acid sequence of ST3GalA.1 exhibits 80% identity to that of Galβ3GalNAc α2,3-sialyltransferase cloned from porcine submaxillary gland (Galβ3GalNAc-α3STPSM) (Lee *et al.*, 1993), whereas no identity (20% or less) was observed between ST3GalA.1 and other types of sialyltransferases so far cloned (Weinstein *et al.*, 1987; Wen *et al.*, 1992; Bast *et al.*, 1992; Gillespie *et al.*, 1992; Lee *et al.*, 1993; Sasaki *et al.*, 1993; Kurosawa *et al.*, 1994; Kitagawa & Paulson, 1994), indicating that mouse brain ST3GalA.1 is the same type of sialyltransferase as Galβ3GalNAc-α3STPSM. On the other hand, comparison of the deduced amino acid sequences of ST3GalA.2 and other sialyltransferases, including ST3GalA.1, revealed that ST3GalA.2 was a new type of sialyltransferase; it shows 20% or less identity to all sialyltransferases except ST3GalA.1, exhibiting 46% identity to ST3GalA.1 (Lee *et al.*, 1994).

The existence of two different kinds of sialyltransferase with the same acceptor substrate specificity in the same tissue

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[1] Abbreviations: The nomenclature for gangliosides and glycolipids follows the system of Svennerholm (1964). BSM, bovine submaxillary gland mucin; Galβ1,3GalNAc-α3STPSM, Galβ1,3GalNAc α2,3-sialyltransferase cloned from porcine submaxillary gland; HPTLC, high-performance thin-layer chromatography; PCR, polymerase chain reaction.

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(mouse brain) leads us to the assumption that these enzymes are responsible for the biosynthesis of the NeuAcα2,3Galβ1,-3GalNAc sequences in gangliosides and O-glycosidically linked oligosaccharides of glycoproteins, respectively. In this study, we compared their kinetic properties using three kinds of glycoconjugates as acceptors, *i.e.*, oligosaccharides, gangliosides, and glycoproteins.

MATERIALS AND METHODS

Materials. CMP-[^{14}C]NeuAc (11 GBq/mmol) was purchased from Amersham Corp. (U.K.) and used after dilution with nonradiolabeled nucleotide sugar obtained from Sigma (St. Louis, MO). The glycosphingolipids used here, Galβ1,-3GalNAc, asialofetuin, and Triton CF-54 were from Sigma. Other materials were the same as previously reported (Lee *et al.*, 1993). Glycosphingolipids were dissolved in 1% Triton CF-54 at the concentration of 10 mM.

Preparation of Soluble Forms of Sialyltransferases. To express the soluble form of ST3GalA.1 or ST3GalA.2, a cDNA of the truncated form of the enzyme, *i.e.*, lacking the putative cytoplasmic and transmembrane domains (55 and 57 amino acids from the N-termini for ST3GalA.1 and ST3GalA.2, respectively) of the open reading frame, was prepared by PCR amplification, and each fragment (956 and 930 bp for ST3GalA.1 and ST3GalA.2, respectively) was inserted into the pcD-SRα vector plasmid containing the signal peptide of mouse immunogloblin M, as described in previous papers (Lee *et al.*, 1993, 1994). COS-7 cells were then transiently transfected with 3 μg of each constructed plasmid containing enzyme sequences, using the DEAE-dextran procedure (Sambrook *et al.*, 1989). The medium in each case was harvested at 48 h post-transfection, concentrated 10-fold, and then used for the enzyme assay. The expressed enzymes only exhibited activities toward the disaccharide Galβ1,3GalNAc. The medium from the cells transfected with pcD-SRα alone did not show any sialyltransferase activity.

Assay of Sialyltransferases. In a total volume of 10 μL, the assay mixture containing 50–1000 μM glycolipid or oligosaccharide (asialoGM1, GM1, or Galβ1,3GalNAc) or 0.5–5 mg/mL glycoprotein acceptor (asialofetuin, 34–340 μM as Galβ1,3GalNAc residues), 0.5% Triton CF-54, 100 mM sodium cacodylate buffer (pH 6.0), 10 mM $MgCl_2$, 50 μM CMP-[^{14}C]NeuAc (0.9 MBq/pmol), and 1 μL of concentrated COS cell medium was incubated at 37 °C for 1 h. For separation of the product from CMP-NeuAc, the assay mixture was applied on a silica gel 60 HPTLC plate (Merck, Germany) and then developed with $CHCl_3$/methanol/0.02% $CaCl_2$ (55:45:10) for glycolipid acceptors or with ethanol/pyridine/1-butanol/acetic acid/water (100:10:10:3:30) for disaccharide acceptors and glycoprotein acceptors. The radioactivity of the corresponding product and total radioactivity were detected and counted using the BAS2000 radioimage analyzer (Fuji Film, Japan). The rates of all reactions described here were linear with time, at least to 1 h. Product identification was performed as described previously (Lee *et al.*, 1993, 1994). For kinetic analysis, the concentration of asialofetuin was calculated based on the number of O-glycosidically linked Galβ1,3GalNAc residues (68 nmol/mg) (Rearick *et al.*, 1979). The K_m values presented are apparent K_m values determined in detergent-containing assays.

RESULTS

Kinetic Properties of the Two Kinds of Sialyltransferase. Since both ST3GalA.1 and ST3GalA.2 exhibit the same acceptor substrate specificities toward gangliosides and O-glycosidically linked oligosaccharides in glycoproteins having terminal Galβ1,3GalNAc sequences (Lee *et al.*, 1993, 1994), it was of particular interest the possibility that one of the gangliosides and O-glycosidically linked oligosaccharides in glycoproteins containing the Galβ1,3GalNAc sequence serves as a predominant acceptor over the others for ST3GalA.1 or ST3GalA.2. To clarify this possibility, their kinetic properties were compared using Galβ1,3GalNAc, asialoGM1, GM1, or asialofetuin as an acceptor. The results are summarized in Table 1. The K_m value of ST3GalA.1 for Galβ1,3GalNAc was greatly different from those for asialoGM1 and GM1 (8–10-fold), whereas it was similar to that for asialofetuin. In contrast, ST3GalA.2 showed similar K_m values for Galβ1,-3GalNAc, asialoGM1, GM1, and asialofetuin, respectively. It should be noted that the K_m value of ST3GalA.1 for oligosaccharides or glycoproteins was 3–4-fold smaller than that of ST3GalA.2, while the K_m values for glycosphingolipids (asialoGM1 and GM1) of ST3GalA.1 were, in contrast, 3–4-fold greater than those of ST3GalA.2. The V_{max} value of ST3GalA.1 for asialoGM1 was remarkably smaller than that for Galβ1,3GalNAc, but that of ST3GalA.2 for asialoGM1 was, on the contrary, greater than for the disaccharide. As shown in Table 1, the relative activities for gangliosides

Table 1: Kinetic Properties of ST3GalA.1 and ST3GalA.2

	ST3GalA.1			ST3GalA.2		
substrate	K_m (mM)	V_{max}[a]	V_{max}/K_m[c]	K_m (mM)	V_{max}	V_{max}/K_m
Galβ1,3GalNAc	0.16	100	625	0.50	100	200
asialoGM1	1.25	33.3	26.6	0.56	122	218
GM1	1.67	71.8	43.0	0.45	52.3	116
asialofetuin[b]	0.10	29.1	291	0.48	16.0	33.3

[a] Relative values. [b] The K_m values for asialofetuin shown are based on the number of O-glycosidically linked Galβ1,3GalNAc residues in asialofetuin (68 nmol/mg) (Rearick *et al.*, 1979). [c] Relative substrate activity.

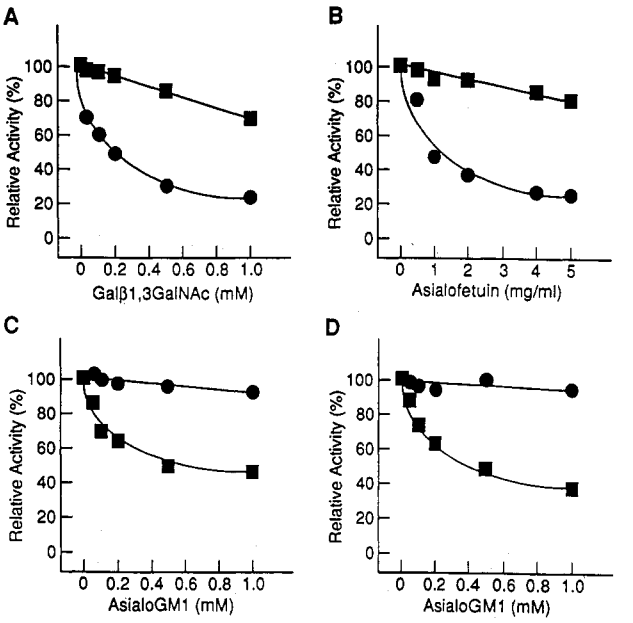

FIGURE 1: Effects of different types of acceptors on the activity toward asialoGM1, Galβ1,3GalNAc, or asialofetuin. AsialoGM1 (1 mM) was incubated in an assay mixture (see Materials and Methods) with ST3GalA.1 (circles) or ST3GalA.2 (squares) in the presence of various concentrations of Galβ1,3GalNAc (panel A) or asialofetuin (panel B). Galβ1,3GalNAc (1 mM) (panel C) or asialofetuin (2 mg) (panel D) was incubated in the presence of various concentrations of asialoGM1. Each activity was expressed as the relative value toward that without the inhibitor.

Table 2: Summary of K_m, K_i, and V_{max} Values of Sialyltransferases, Obtained in Competition Experiments, with Different Types of Glycoconjugates

		K_m (mM) for acceptors		K_i (mM) for inhibitors		V_{max} (pmol/h)	
acceptor	inhibitor[a]	ST3GalA.1	ST3GalA.2	St3GalA.1	ST3GalA.2	ST3GalA.1	ST3GalA.2
asialoGM1	none	1.25	0.56			41.3	111.1
	Galβ1,3GalNAc	5.00	0.58	0.15	>3.0	43.5	50.6
	asialofetuin	2.10	0.62	0.08	NC[c]	40.3	71.4
Galβ1,3GalNAc	none	0.16	0.50			125.0	90.9
	asialoGM1	0.71	0.50	NC	0.11	136.1	42.3
	asialofetuin	0.58	0.96	0.07	0.08	119.8	87.2
asialofetuin[b]	none	0.10	0.48			29.1	16.0
	asialoGM1	0.26	0.56	NC	0.44	29.6	7.2

[a] 1 mM asialoGM1 or GAlβ1,3GalNAc, or 2 mg/mL asialofetuin was used. [b] K_m and K_i values for asialofetuin were calculated based on Galβ1,3GalNAc in asialofetuin (Rearick *et al.*, 1979). [c] NC, could not be calculated.

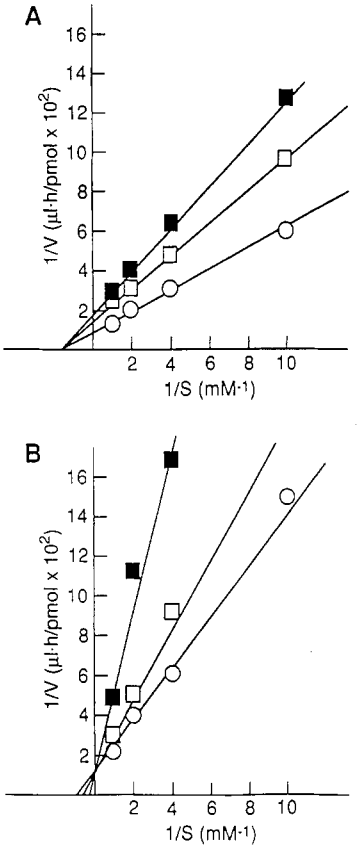

FIGURE 2: Lineweaver–Burk plots of GM1b synthesis with and without an inhibitor. Various concentrations of asialoGM1 (0.05–1.0 mM) were incubated with or without 1 mM Galβ1,3GalNAc and 2 mg/mL asialofetuin, respectively. (A) ST3GalA.2; (B) ST3GalA.1. Open circles, without any inhibitor; closed squares, with Galβ1,-3GalNAc; open squares, with asialofetuin.

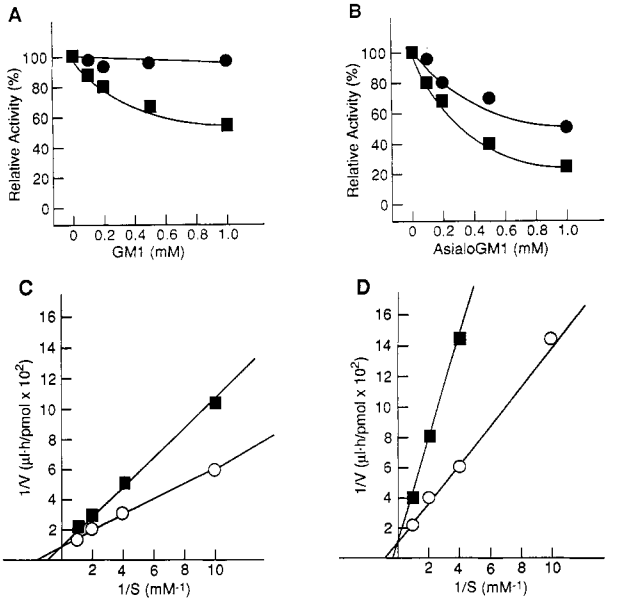

FIGURE 3: Effect of GM1 on GM1b synthesis and effect of asialoGM1 on GD1a synthesis. (Panels A and B) 1 mM asialoGM1 (panel A) or GM1 (panel B) was incubated in an assay mixture with ST3GalA.1 or ST3GalA.2 in the presence of various concentrations of GM1 and asialoGM1, respectively. The symbols are the same as in Figure 1. (Panels C and D) Various concentrations (0.05–1.0 mM) of asialoGM1 were incubated with (closed squares) or without (open circles) 1 mM GM1. Panels C and D show the activities of ST3GalA.2 and ST3GalA.1, respectively.

expressed by V_{max}/K_m of ST3GalA.2 were considerably higher than the corresponding activities of ST3GalA.1, whereas the relative activity of ST3GalA.2 for asialofetuin was clearly smaller than that of STGalA.1. These results suggest that ST3GalA.1 and ST3GalA.2 exhibit different substrate preferences; *i.e.*, gangliosides seem to serve as much better acceptors for the latter than for the former.

Competition between Different Types of Acceptors. To prove that ST3GalA.2 has a different substrate preference to ST3GalA.1, competition experiments were performed using different types of glycoconjugates as acceptors, *i.e.*, disaccharides, gangliosides, and glycoproteins. When asialoGM1 was used as the substrate at a fixed concentration and Galβ1,-3GalNAc or asialofetuin was used as the inhibitor at various concentrations, GM1b synthesis by ST3GalA.1 was clearly inhibited in the presence of each type of inhibitor (Figure 1A,B). On the other hand, the activity of ST3GalA.1 toward Galβ1,3GalNAc or asialofetuin was not inhibited at all by asialoGM1 (Figure 1C,D). The apparent K_i values of Galβ1,-3GalNAc and asialofetuin for GM1b synthesis were similar to the respective K_m value (Table 2). Thus, Galβ1,3GalNAc and asialofetuin serve as dominant acceptors for ST3GalA.1.

The inhibition of GM1b synthesis of ST3GalA.2 by Galβ1,-3GalNAc or asialofetuin was only slight, even at the inhibitor concentration of 1 mM or 5 mg/mL (Figure 1A,B), although the apparent K_m values of asialoGM1, Galβ1,3GalNAc, and asialofetuin were very similar (Table 2). Conversely, when the disaccharide, Galβ1,3GalNAc, or asialofetuin was used as the substrate and asialoGM1 was used as the inhibitor, the activity of ST3GalA.2 toward each substrate was clearly inhibited by asialoGM1 (Figure 1C,D). The apparent K_i values of asialoGM1 toward the disaccharide and the glycoprotein were smaller than the K_m value for asialoGM1 (Table 2). These results strongly indicate that ganglioside asialoGM1 serves as a predominant acceptor for ST3GalA.2 over Galβ1,-3GalNAc or asialofetuin.

To examine the competition by Galβ1,3GalNAc or asialofetuin in GM1b synthesis, asialoGM1 at various concentra-

Table 3: K_m, K_i, and V_{max} Values of ST3GalA.1 and ST3GalA.2 as to Competition between AsialoGM1 and GM1

acceptor	inhibitor[a]	K_m (mM) for acceptors		K_i (mM) for inhibitors		V_{max} (pmol/h)	
		ST3GalA.1	ST3GalA.2	ST3GalA.1	ST3GalA.2	ST3GalA.1	ST3GalA.2
asialoGM1	none	1.25	0.56			41.3	111.1
	GM1	3.30	1.05	NC[b]	0.51	42.0	108.5
GM1	none	1.67	0.45			100.0	47.6
	asialoGM1	3.41	2.50	1.29	0.53	90.1	49.5

[a] 1 mM asialoGM1 or GM1 was used. [b] NC, could not be calculated.

tions was incubated with or without 1 mM Galβ1,3GalNAc or 2 mg/mL asialofetuin. The resulting Lineweaver–Burk plots of GM1b synthesis by ST3GalA.1 clearly showed competitive inhibition of GM1b synthesis by Galβ1,3GalNAc or asialofetuin (Figure 2B). As summarized in Table 2, ST3GalA.1 activity toward each oligosaccharide, glycosphingolipid, or glycoprotein was competitively inhibited by another. In contrast, Lineweaver–Burk plots of GM1b synthesis by ST3GalA.2 indicated noncompetitive inhibition by either Galβ1,3GalNAc or asialofetuin (Figure 2A). The activity toward Galβ1,3GalNAc or asialofetuin was also noncompetitively inhibited by asialoGM1 (Table 2).

Competition between AsialoGM1 and GM1 as Acceptors. The same competition experiments were performed using the same types of glycoconjugates as acceptors, *i.e.*, asialoGM1 and GM1. GM1b synthesis by ST3GalA.2 was inhibited by GM1, but that by ST3GalA.1 was inhibited only slightly (Figure 3A). On the other hand, GD1a synthesis was inhibited by asialoGM1 in the cases of both ST3GalA.1 and ST3GalA.2 (Figure 3B). In contrast with the inhibition manner between different types of substrates, GM1b synthesis by ST3GalA.2 as well as ST3GalA.1 was competitively inhibited by GM1 (Figure 3C,D). In addition, competitive inhibition by asialoGM1 was also observed in GD1a synthesis by ST3GalA.2 (Table 3). These results were coincident with the results observed in the case of sialyltransferase IV, which acts as a GM1b-, GD1a-, and GT1b-synthase (Pohlentz *et al.*, 1988; Iber *et al.*, 1991).

DISCUSSION

The carbohydrate sequence NeuAcα2,3Galβ1,3GalNAc was so far known to exist in two distinct classes of glycoconjugates, namely, O-glycosidically linked oligosaccharides of glycoproteins and gangliosides (Thomas & Winzler, 1969; Spiro & Bhoyroo, 1974; Fishman & Brady, 1976). Although this sequence in glycoproteins and gangliosides is thought to be synthesized in the Golgi apparatus by distinct classes of enzymes (Roseman, 1970; McGuire, 1976), the possibility has been shown that a single enzyme could synthesize the sequence in both types of glycoconjugates (Rearick *et al.*, 1979; Joziasse *et al.*, 1985). In addition, a single β-galactoside α2,3-sialyltransferase, so-called sialyltransferase IV, has been shown to catalyze the sialic acid transfer of GM1b, GD1a, and GT1b from asialoGM1, GM1, and GD1b, respectively (Pohlentz *et al.*, 1988; Iber *et al.*, 1991). In previous papers (Lee *et al.*, 1993, 1994), we reported the isolation of full-length cDNAs encoding two distinct mouse brain sialyltransferases which exhibit the same acceptor substrate specificity for the structure of Galβ1,3GalNAc of glycoproteins and gangliosides, and which form NeuAcα2,3Galβ1,3GalNAc sequences. One of them, ST3GalA.1, has been shown to exhibit high identity in the nucleotide sequence and the deduced amino acid sequence to the Galβ1,3GalNAc α2,3-sialyltransferase cloned from porcine submaxillary gland (Lee *et al.*, 1993), while the other, ST3GalA.2, shows very low identity to all other sialyltransferases so far known (Lee *et al.*, 1994).

In this study, the properties of these two distinct Galβ1,-3GalNAc α2,3-sialyltransferases (ST3GalA.1 and ST3GalA.2) were compared using different types of glycoconjugates as acceptors. Although both sialyltransferases used in the experiments were soluble forms which lack the hydrophobic transmembrane regions, the kinetic analysis and competition experiments revealed that, as compared with ST3GalA.1, ST3GalA.2 exhibits some different characteristics in the following two points: (i) acceptor substrate preference and (ii) inhibition mode with different types of acceptors, *i.e.*, between gangliosides and glycopoteins or oligosaccharides. The activity of ST3GalA.2 toward a ganglioside is noncompetitively inhibited by Galβ1,3GalNAc and asialofetuin, respectively, whereas the activity of ST3GalA.1 shows competitive inhibition with each different type of acceptor. On the other hand, ST3GalA.2 as well as ST3GalA.1 showed competitive inhibition between asialoGM1 and GM1. These results suggest that ST3GalA.2 recognizes gangliosides and glycoproteins as individual acceptors at their individual binding sites on the enzyme, or it may recognize not only the Galβ1,-3GalNAc sequence but also the whole structure of acceptors including lipid moieties; there may be a recognition site for the ceramide moiety in addition to one for the Galβ1,3GalNAc moiety. In contrast, ST3GalA.1 recognizes them without any distinction and probably only recognizes the Galβ1,3GalNAc sequence of an acceptor at a single acceptor binding site. Furthermore, the apparent K_m values of ST3GalA.2 for asialoGM1 and GM1 were smaller than those of ST3GalA.1. Although the apparent K_m values of ST3GalA.2 for Galβ1,-3GalNAc, asialoGM1, GM1, and asialofetuin were very similar, the competition experiments revealed that gangliosides asialoGM1 and GM1 serve as predominant acceptors for ST3GalA.2 over Galβ1,3GalNAc and asialofetuin, which serve as much better substrate for ST3GalA.1. Therefore, we concluded that ST3GalA.2 was most probably the enzyme involved in the biosynthesis of gangliosides, particularly of GM1b, GD1a, and GT1b from asialoGM1, GM1, and GD1b, respectively. In contrast, ST3GalA.1 is involved in the biosynthesis of O-glycosidically linked oligosaccharide of glycoproteins rather than ganglioside biosynthesis.

The sialyltransferases cloned so far have been shown to exhibit remarkable tissue-specific expression (Weinstein *et al.*, 1987; Paulson & Colley, 1989; Gillespie *et al.*, 1992), which is correlated with the existence of cell type-specific carbohydrate structures. If ST3GalA.1 and ST3GalA.2 are involved in the biosynthesis of O-glycosidically linked oligosaccharides of glycoproteins and in ganglioside biosynthesis, respectively, the expression pattern of their mRNAs should be clearly different. The expression of ST3GalA.2 mRNA is prominent in brain (Lee *et al.*, 1994), which is known to contain a particularly high amount of ganglioside (Weigandt, 1967; Svennerholm, 1980), but not in submaxillary gland, which expresses high amounts of mucin-type glycoproteins, whereas that of ST3GalA.1 mRNA is abundant in submaxillary gland but is at a particularly much lower level in brain (Lee *et al.*, 1993).

It is not clear at this time whether or not ST3GalA.2 or ST3GalA.1 is different from sialyltransferase IV. Using rat liver Golgi vesicles as the enzyme source, the reported K_m values of sialyltransferase IV for asialoGM1 and GM1 were remarkably smaller than those of ST3GalA.2 and ST3GalA.1 (Pohlentz *et al.*, 1988). In addition, kinetic analysis revealed that sialyltransferase IV was able to catalyze sialic acid transfer to lactosylceramide as well as asialoGM1, GM1, and GD1b (Iber *et al.*, 1991). Since the ST3GalA.2 as well as ST3GalA.1 used in this experiment lacks the hydrophobic transmembrane region, it is possible that such a form of sialyltransferase shows larger K_m values for gangliosides and does not exhibit intrinsic activity for GM3 synthesis. On the other hand, association of the sialyltransferases with Golgi membranes may be important for the activity, particularly for ganglioside synthesis. It is also possible that some activating factors existing in the Golgi apparatus may enhance the activity of sialyltransferase IV. An alternative possibility is the existence of sialyltransferase IV "subtypes". The *in vivo* expression of ST3GalA.2 cDNA as well as ST3GalA.1 cDNA will provide further information for elucidation of the biological functions of these enzymes. Experiments along these lines are currently in progress.

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