Biochem. J. (1992) 288, 161–165 (Printed in Great Britain)

Substrate specificity and distribution of UDP-GalNAc:sialylparagloboside *N*-acetylgalactosaminyltransferase in the human stomach

Taeko DOHI,*†** Atsushi NISHIKAWA,‡ Ineo ISHIZUKA,§ Masayuki TOTANI,† Kenji YAMAGUCHI,‖ Ken NAKAGAWA,¶ Osamu SAITOH,¶ Sabro OHSHIBA¶ and Mieko OSHIMA*

*Division of Clinical Biochemistry, Clinical Research Institute, National Medical Center, Tokyo 162, Japan, †National Institute of Health and Nutrition, Tokyo 162, Japan, ‡Department of Biochemistry, Osaka University Medical School, Suita 565, Japan, §Department of Biochemistry, Teikyo University School of Medicine, Tokyo 173, Japan, ‖School of Home Economics, Tokyo Kasei University, Tokyo 173, Japan and ¶Second Department of Internal Medicine, Osaka Medical School, Takatsuki 569, Japan

The detailed substrate specificity of the UDP-GalNAc:sialylparagloboside *N*-acetylgalactosaminyltransferase to form the Sd(a+) blood group active carbohydrate determinant GalNAcβ1-4(NeuAcα2-3)Gal was studied using a membrane fraction prepared from human gastric fundic mucosa. Various sialosylated oligosaccharides and gangliosides were examined as acceptor substrates. Oligosaccharide substrates were fluorescence-labelled with 2-aminopyridine, and the transferase activity was quantified by h.p.l.c. using a reversed-phase column. The structures of the products were determined by glycosidase degradation and proton n.m.r. 3′-Sialyl-lactose (II^3NeuAcLac), 3′-sialyl-lactotetraose (IV^3NeuAcLc$_4$), and 3′-sialyl-lactoneotetraose (IV^3NeuAcnLc$_4$) were good substrates for the β1-4GalNAc transferase in gastric fundic mucosa, but 6′-sialyl-lactoneotetraose (IV^6NeuAcnLc$_4$) or 6′-sialyl-lactose (II^6NeuAcLac) were not. Gangliosides with a terminal NeuAcα2-3Gal residue such as G_{M3}, sialylparagloboside, G_{M1b} and G_{D1a} were also studied. The activity of β1-4GalNAc transfer to sialylparagloboside was much higher than that to G_{M2}, G_{M1b} or G_{D1a} in spite of them having the same terminal residue. Measurement of the activity of the β1-4GalNAc transferase in biopsy specimens demonstrated that the activity was localized in gastric fundic mucosa and was absent in pyloric mucosa, intestinal metaplasia and gastric cancer tissue. Thus the β1-4GalNAc transferase present specifically in fundic mucosa required a NeuAcα2-3Gal residue connected to either type-1-chain or type-2-chain oligosaccharides. In glycolipids, the acceptor specificity was restricted to NeuAcα2-3Galβ1-4GlcNAc because the NeuAcα2-3Galβ1-3GalNAc structure in ganglio-series glycolipids was not a good acceptor substrate.

INTRODUCTION

Previously we have reported the presence of a sialylpentaosylceramide, localized specifically in human gastric fundic mucosa by using the anti-G_{M2} monoclonal antibody [1]. (The nomenclature used for glycolipids and oligosaccharides is that of the system of Svennerholm [2] or the IUPAC–IUB [3]). This glycolipid, temporally named NGM-1, was commonly found in the stomach of Japanese people, and was characterized as GalNAcβ1-4(NeuAcα2-3)Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer which shares the same terminal epitope as the G_{M2} ganglioside. We also found that the activity of UDP-GalNAc:sialylparagloboside β1-4GalNAc *N*-acetylgalactosaminyltransferase (NGM-1 synthase) was high in the fundic mucosa, while the activity of UDP-GalNAc:G_{M3} β1-4GalNAc *N*-acetylgalactosaminyltransferase (G_{M2} synthase) was very low. In contrast, an increased G_{M2} synthase activity was found in gastric cancer cell lines and gastric cancer tissue, and the activity of NGM-1 synthase was absent in pyloric mucosa and gastric cancer [4].

On the other hand, the carbohydrate moiety of NGM-1 was shown to be identical to an immunodominant ganglioside of a rare blood group called Cad or Sd(a++) [5]. The incidence of Cad/Sd(a++) individuals, whose erythrocytes are rich in the NGM-1 glycolipid, is extremely low (reviewed in [6]), and estimated as 0.03% in the Japanese race [7]. In contrast, the antigen carrying GalNAcβ1-4(NeuAcα2-3)Gal as the terminal structure, probably on a glycoprotein, is widely distributed in mammalian tissues [8], and is specified as Sd(a+) to distinguish it from Cad/Sd(a++). In human tissues, this carbohydrate determinant has been reported to be present in the urine, kidney and colon in more than 90% of the Caucasian population [8,9], and the β1-4GalNAc transferase involved in the synthesis of this carbohydrate determinant has been detected in the kidney, serum and colon [7,10,12]. Studies on the β1-4GalNAc transferase in the stomach, however, have not been reported. We found the NGM-1 synthase to be a glycolipid synthase that is functionally different from the Sd^a antigen synthase which is considered to be responsible for producing soluble antigen. Besides Sd^a antigens, glycolipids with the same terminal structure, GalNAcβ1-4-(NeuAcα2-3)Gal, as NGM-1, GalNAc-G_{D1a} and GalNAc-G_{M1b}, have been detected in neural tissue [13,14]. These studies on the β1-4GalNAc transferase activity synthesizing GalNAcβ1-4(NeuAcα2-3)Gal prompted our investigation into the substrate specificity of the NGM-1 synthase in the human stomach.

In the present paper we describe a membrane fraction of the gastric fundic mucosa which was able to transfer GalNAc to an oligosaccharide acceptor, as well as sialylparagloboside. The detailed substrate specificity of the β1-4GalNAc transferase that produces GalNAcβ1-4(NeuAcα2-3)Gal in human gastric fundic mucosa was determined using a panel of oligosaccharides and

Abbreviation used: -PA, pyridylaminated (i.e. sugar-PA).

** To whom correspondence should be directed at the following address: Division of Clinical Biochemistry, Clinical Research Institute, National Medical Centre, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162, Japan.

glycolipids of well-defined structure. Furthermore, we found that the activity of β1-4GalNAc transferase towards sialosyl-oligosaccharides was localized in fundic mucosa as well as that to sialylparagloboside.

MATERIALS AND METHODS

Tissue

An intact mucosal layer from the greater curvature of the gastric body (fundic area) was obtained from a surgically resected stomach from a patient treated for gastric cancer in the National Medical Center Hospital (Tokyo, Japan). After rinsing the tissue in cold phosphate-buffered saline (0.15 M-NaCl, 8 mM-Na_2HPO_4, 1.5 mM-KH_2PO_4, 2.7 mM-KCl, pH 7.2), it was minced, homogenized with a glass micro-homogenizer (Radnoti Glass, Monrovia, CA, U.S.A.) in 3 vol. of homogenizing buffer containing 250 mM-sucrose and 10 mM-Tris/HCl, pH 7.4, and centrifuged at 900 *g* for 10 min. In preparing the membrane fraction, the 900 *g* supernatant was centrifuged at 3000 *g* for 10 min, and the resulting supernatant was centrifuged at 100000 *g* for 1 h. The pellet was suspended in a homogenizing buffer. The biopsy samples were obtained from patients who had had an endoscopic examination of the stomach (Osaka Medical School Hospital) and had been found to have lesions requiring a histological examination. Each specimen was divided into two parts, one for histological examination and the other for assay of glycosyltransferase activity. In the latter case, samples were homogenized with a glass microhomogenizer in 100 μl of homogenizing buffer, and the supernatant obtained after centrifugation with a microcentrifuge Capsule HF 120 (TOMY, Tokyo, Japan) was used for the assay. The protein concentration was determined by a Bio-Rad protein assay kit using BSA as a standard.

Glycolipids and oligosaccharides

G_{M3} and sialylparagloboside were purified from human erythrocytes, G_{M2} and G_{D1a} from calf brain, and NGM-1 from human gastric mucosa, and they were used as substrates or reference standards. G_{M1b} was a gift from Dr. Hirabayashi (The Riken Institute of Physical and Chemical Research, Wako, Japan). 3′-Sialyl-lactose (II^3NeuAcLac), 3′-sialyl-lactoneotetraose (IV^3NeuAcnLc$_4$), GalNAc-sialyl-lactose (II^4GalNAc-II^3NeuAcLac), and GalNAc-3′-sialyl-lactoneotetraose (IV^4GalNAcIV^3NeuAcnLc$_4$) were prepared from G_{M3}, sialylparagloboside, G_{M2} and NGM-1 respectively by treatment with endoglycoceramidase provided by Dr. M. Ito (Mitsubishi Kasei Institute of Life Sciences, Machida, Japan). 6′-Sialyl-lactose (IV^6-NeuAcLac), 6′-sialyl-lactoneotetraose (IV^6NeuAcnLc$_4$), and 3′-sialyl-lactotetraose (IV^3NeuAcLc$_4$) were purchased from Biocarb (Lund, Sweden). Lactoneotetraose (nLc$_4$) and lactotetraose (Lc$_4$) were prepared by incubation of 3′-sialyl-lactoneotetraose and 3′-sialyl-lactotetraose respectively in 1% acetic acid at 100 °C for 1 h.

Measurement of the glycosyltransferase activity using fluorescence-labelled oligosaccharide as substrate

The assay was done following the modified method described by Taniguchi *et al.* [15]. Oligosaccharides were fluorescence-labelled with 2-aminopyridine according to the method described by Hase *et al.* [16]. The assay solution had the following final concentration in a total volume of 0.05 ml: 0.1 M-cacodylate buffer (pH 7.5), 0.01 M-$MnCl_2$, 0.45% Triton X-100, 0.01 M-UDP-GalNAc, 0.08 mM-pyridylaminated oligosaccharide (oligosaccharide-PA) as the acceptor substrate, and 50 μg of membrane protein or 20 μl of crude homogenate. After incubation for 2 h, the reaction was stopped by boiling the samples for 2 min, and a 3 μl aliquot of the reaction mixture was analysed with an h.p.l.c. system equipped with a fluorescence monitor (Shimadzu RF-535T) using the reversed-phase column (TOSO TSK-Gel ODS-80TM, 0.45 cm × 25 cm) kept at 50 °C. Ammonium acetate buffer (20 mM; pH 4.0) was used at a flow rate of 1 ml/min for a mobile isocratic phase. The reaction product was identified with the reference of the assay solution incubated in the absence of UDP-GalNAc. The amount of product was quantified by the fluorescence intensity calculated with a Shimadzu CR4-A Chromatopac using pyridylaminated lactose as a standard.

Structural identification of the oligosaccharide product by enzymic degradation

The reaction product separated from the substrate by h.p.l.c. was first incubated with 0.3 unit of *N*-acetylneuraminidase/ml from *Anthrobacter* (Nakarai, Kyoto, Japan) in 10 mM-ammonium acetate buffer (pH 4.6) for 24 h at 37 °C. Then *N*-acetylhexosaminidase from Jack bean (Sigma) was added to the reaction mixture to give a final concentration of 1.25 units/ml, and subsequently incubated for 24 h at 37 °C. Aliquots of the reaction mixture, after treatment with each enzyme, were analysed by h.p.l.c. as described above.

Structural identification of the oligosaccharide product by ^{1}H n.m.r.

The pooled product (approx. 10 nmol) was purified by h.p.l.c. as mentioned above together with gel filtration with Toyopearl HW40 FINE (1 cm × 55 cm) in 10 mM-ammonium acetate buffer (pH 6.0), the eluate being monitored for fluorescence intensity. The product was lyophilized and dissolved in 99.96% ^2H_2O (MSD Isotopes, Montreal, Canada) three times. ^{1}H n.m.r. analysis at 400 MHz was performed at 30 °C in ^2H_2O with a JNM-GX400 FT n.m.r. spectrometer in the Fourier-transform mode.

Measurement of the glycosyltransferase activity using glycolipid as substrate

The assay was performed using the optimized conditions described previously [4], with the exception that radiolabelled sugar nucleotide was used following the method described by Kawano *et al.* [17]. The standard assay, in a total volume of 0.05 ml, consisted of the following components (final concentrations): 0.1 M-cacodylate buffer (pH 7.5), 0.01 M-$MnCl_2$, 0.18% Triton X-100, 84 μM-UDP-[^{14}C]GalNAc (Amersham, 60 mCi/mmol), 0.08 mM substrate glycolipid and 50 μg of membrane protein. After incubating the assay for 2 h at 37 °C, the reaction was stopped by adding 1 ml of chloroform/methanol/water (30:60:8 by vol.) and the mixture was applied on 1 ml of DEAE Sephadex A-25 column equilibrated with the same solvent. After eluting the column with 2 ml of chloroform/methanol/water (30:60:8 by vol.) followed by 3 ml of methanol, the ganglioside fraction was eluted in 6 ml of 40 mM-ammonium acetate in methanol. The radioactivity was determined in 10 ml of scintillation fluid using a liquid scintillation spectrometer (Packard 4530).

RESULTS

Activity of GalNAc transferase towards oligosaccharide-PA

Fig. 1 shows the h.p.l.c. elution pattern of the assay mixture when 3′-sialyl-lactose-PA or 3′-sialyl-lactoneotetraose-PA were used as the substrates. Fig. 2 demonstrates the results of the enzyme degradation of the products formed from 3′-sialyl-lactose-PA, 3′-sialyl-lactoneotetraose-PA and 3′-sialyl-lactotetraose. Treatment with neuraminidase and *N*-acetylhexosaminidase resulted in the appearance of sugar-PA eluting with an identical retention time to lactose-PA, lactoneotetraose-

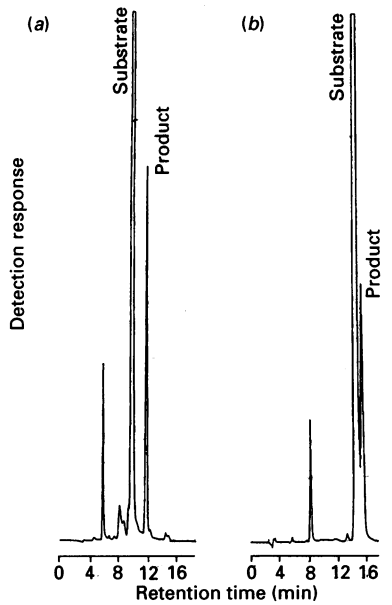

Fig. 1. Elution pattern of h.p.l.c. for the separation of substrate sugar-PA and product

Substrate as sugar-PA was incubated with the membrane fraction of fundic mucosa in the assay solution, and an aliquot (3 μl) was subjected to h.p.l.c. as described in the Materials and methods section. The substrate in (*a*) is 3′-sialyl-lactose-PA and that in (*b*) is 3′-sialyl-lactoneotetraose-PA.

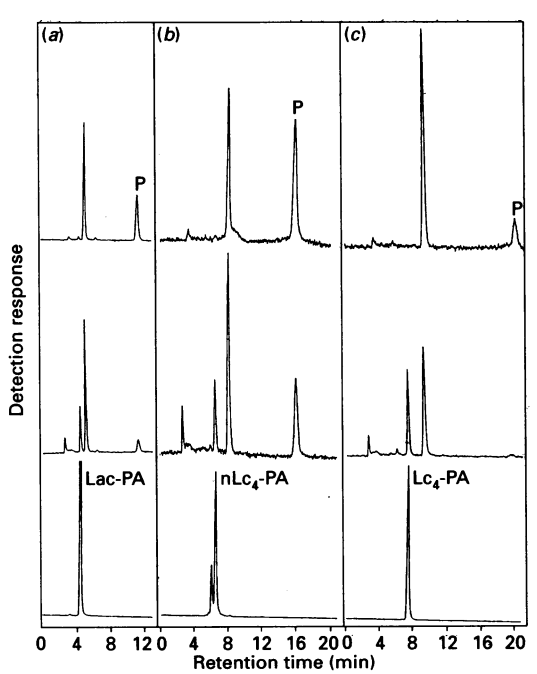

Fig. 2. Degradation of the product as sugar-PA with *N*-acetylneuraminidase and *N*-acetylhexosaminidase

An aliquot of incubation mixture was subjected to h.p.l.c. as described in the Materials and methods section. Panel (*a*), product formed from 3′-sialyl-lactose-PA; panel (*b*), product formed from 3′-sialyl-lactoneotetraose-PA; and panel (*c*), product formed from 3′-sialyl-lactotetraose-PA. The peaks marked 'P' indicate the product oligosaccharide-PA. Upper traces, after treatment with *N*-acetylneuraminidase; middle traces, after treatment with *N*-acetylneuraminidase and *N*-acetylhexosaminidase; bottom traces, standard oligosaccharide-PA indicated in the figure.

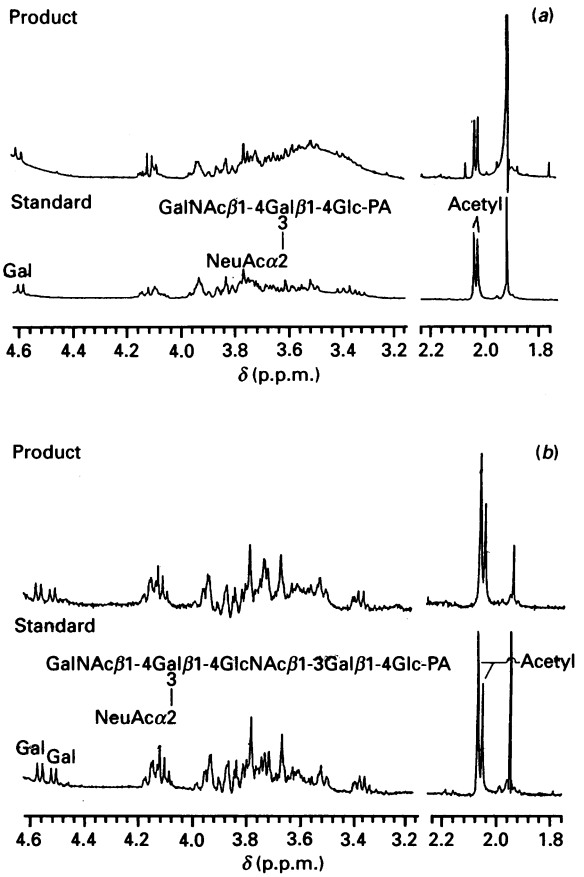

Fig. 3. ^{1}H n.m.r. spectra of products as oligosaccharides-PA formed by β1-4GalNAc transferase in gastric mucosa

(*a*) Product formed from 3′-sialyl-lactose-PA; (*b*) product formed from 3′-sialyl-lactoneotetraose-PA. The bottom of each panel shows the spectra obtained from standard oligosaccharide-PAs prepared from G_{M2} and NGM-1 in (*a*) and (*b*) respectively. The method is described in detail in the text.

Table 1. Substrate specificity of β1-4GalNAc transferase in the membrane fraction obtained from human fundic mucosa

Measurement of activity was done by quantification of fluorescence-labelled oligosaccharide or incorporation of radiolabelled GalNAc into glycolipid. Each value is the mean of the duplicated assay. The deviation of raw data from the mean was less than 11%. Methods are described in detail in the Materials and methods section. – indicates activity was below sensitivity of the assay system.

Substrate	β1-4GalNAc transferase activity (pmol/h per mg of protein)
Oligosaccharide-PA	
3′-Sialyl-lactose-PA	5160
6′-Sialyl-lactose-PA	250
3′-Sialyl-lactotetraose-PA	3330
3′-Sialyl-lactoneotetraose-PA	3410
6′-Sialyl-lactoneotetraose-PA	–
Glycolipid	
G_{M3}	1.1
Sialylparagloboside	33.0
G_{M1b}	2.6
G_{D1a}	5.1

Table 2. Activity of β1-4GalNAc transferase (pmol/h per mg of protein) to sugar-PA in a biopsy sample of human stomach

Cases 6–10 were patients with advanced gastric cancer. – indicates activity was below sensitivity. Abbreviation: ND, not determined.

Case	Sex	Age (years)	Histological findings	Substrate... 3′-Sialyl-lactose	3′-Sialyl-lactoneotetraose
1	F	35	Fundic gland with mild inflammation	5146	9325
2	M	53	Fundic gland	9243	11509
3	M	58	Fundic gland with mild inflammation	2296	3166
1	F	35	Pyloric gland with severe inflammation	51	–
4	F	20	Pyloric gland	–	ND
5	F	43	Pyloric gland with atrophic change	–	ND
2	M	53	Intestinal metaplasia	–	–
3	M	58	Intestinal metaplasia	–	–
6	M	80	Papillary adenocarcinoma	–	–
7	M	71	Tubular adenocarcinoma	–	ND
8	M	75	Adenocarcinoma	–	ND
9	F	38	Signet ring cell adenocarcinoma	–	ND
10	F	38	Signet ring cell adenocarcinoma	–	–

PA, and lactotetraose-PA respectively. These results support the suggestion that GalNAcβ1-4(NeuAcα2-3)Gal was formed by the reaction with the membrane fraction from gastric fundic mucosa. The structure of the product was also confirmed by ^{1}H n.m.r. of pooled material compared with sugar-PA prepared from authentic G_{M2} or NGM-1 by treatment with endoglycoceramidase. Fig. 3 shows that the spectra of the product oligosaccharides were identical to those of sugar-PA derived from standard glycolipids. It was demonstrated therefore that the membrane fraction of gastric mucosa transferred GalNAc from UDP-GalNAc to the galactose residue of 3′-sialyl-lactose and 3′-sialyl-lactoneotetraose in a β1-4 linkage, constructing the same carbohydrate moiety as G_{M2} and NGM-1 respectively.

Substrate specificity of the β1-4GalNAc transferase in fundic mucosa

Table 1 shows that the membrane fraction of the gastric fundic mucosa could transfer GalNAc to 3′-sialyl-lactose-PA, 3′-sialyl-lactotetraose-PA, and 3′-sialyl-lactoneotetraose-PA, but it could not transfer to 6′-sialyl-lactoneotetraose-PA or 6′-sialyl-lactose-PA. It was also capable of transferring GalNAc to sialylparagloboside but not to G_{M3}, G_{M1b} and G_{D1a}.

Distribution of β1-4GalNAc transferase in the human stomach

Table 2 shows the β1-4GalNAc transferase activity in biopsy samples from gastric mucosa. The activity of the β1-4GalNAc transfer to sialyl-lactoneotetraose-PA and sialyl-lactose-PA was very high in gastric fundic mucosa, but the activity in most specimens from pyloric mucosa, intestinal metaplasia and cancer tissue was below detectable levels, irrespective of histopathological findings.

DISCUSSION

The current results demonstrated that the gastric fundic mucosa possessed the GalNAc transferase activity necessary to produce the GalNAcβ1-4(NeuAcα2-3)Gal terminal structure on both an oligosaccharide and a glycolipid. Previously, we reported that the GalNAc transferase in the gastric fundic mucosa transfers GalNAc to sialylparagloboside, but not to G_{M3}. This property of the β1-4GalNAc transferase was reminiscent of the β1-4GalNAc transferase related to the Sd^a antigen. Takeya *et al.* detected the UDP-GalNAc:NeuAcα2-3Galβ1-4GlcNAc β1-4GalNAc transferase in human blood plasma [7]. They showed that sialyl-lactose and sialylparagloboside were good acceptors, but G_{M3} was not. Piller *et al.* reported that the microsomal preparations from human kidney were capable of transferring GalNAc to *N*-linked carbohydrates, *O*-linked carbohydrates and sialylparagloboside, but not to G_{M3} [10]. Malagolini *et al.* studied the UDP-GalNAc:NeuAcα2-3Gal→R β1-4GalNAc transferase in the human colon (where R is the carbohydrate structure) and showed that the enzyme prefers NeuAcα2-3Gal→R to NeuAcα2-6Gal→R as the acceptor in the experiment using human transferrin that contains only NeuAcα2-6Galβ→R units [12]. In this paper, gastric fundic mucosa was found to be able to transfer GalNAc to oligosaccharides, and the results using sugar-PAs as substrates clearly demonstrated that the transferase worked on both type-1 chain and type-2 chains, but the NeuAc should be linked in an α2-3 linkage to terminal galactose. Thus, the substrate specificity of human gastric mucosal enzyme in this study was similar to these β1-4GalNAc transferases reported as Sd^a antigen synthases. So, is the β1-4GalNAc transferase in the human stomach identical to the Sd(a+) antigen synthase in the human colon and kidney? Although we do not have results to show the difference between enzyme molecules in these organs, the functional difference is clear; the enzyme in the human stomach is responsible for the expression of glycolipid, whereas the Sd^a synthase in the human kidney or colon is not involved in the glycolipid synthesis, apparently because the NGM-1 glycolipid is not expressed in these organs [4]. To elucidate the mechanism of differential expression of a carbohydrate epitope on the glycolipid or the glycoprotein, further studies about the enzyme molecule and the factors modulating the activity of glycosyltransferase are needed.

We have previously reported the critical difference in NGM-1 synthase and G_{M2} synthase for the glycolipid substrates. Here we report additional information about the specificity of the glycolipid acceptor. Gastric fundic mucosa could transfer GalNAc to sialylparagloboside, but its activity to ganglio-series glycolipids, G_{M1b} or G_{D1a}, was much lower than that to sialylparagloboside,

in spite of the same terminal residue NeuAcα2-3Gal. The presence of GalNAc-G_{M1b} and GalNAc-G_{D1a} in neural tissue has been reported [16,17]. As they have the same terminal structure as NGM-1 and G_{M2}, they are detected by cross-reactivity with most anti-(G_{M2}) monoclonal antibodies [4,18–20]. The β1-4GalNAc transferase in the stomach, however, recognized the difference between NeuAcα2-3Galβ1-4(or 3)GlcNAc and NeuAcα2-3Galβ1-3GalNAc, probably because of the differences in the three-dimensional structure. The β1-4GalNAc transferase involved in the synthesis of GalNAc-G_{M1b} (and GalNAc-G_{D1a}) and that involved in the synthesis of NGM-1 are apparently different, although they produced the same linkage of three sugars in the non-reduced terminal structure. It is still not clear whether G_{M2} synthase, which is believed to be the same enzyme as G_{D2} synthase [21], and GalNAc-G_{M1b} synthase can be similarly categorized. Current results make it evident that the β1-4GalNAc transferase in the stomach is distinct from these β1-4GalNAc transferases involved in the synthesis of ganglio-series glycolipid found in neural tissue. In addition, the result that high transfer activity to 3′-sialyl-lactose-PA and low activity to G_{M3} suggested the strong inhibitory effect of the ceramide moiety in G_{M3} on the activity of β1-4GalNAc transferase in gastric fundic mucosa.

The β1-4GalNAc transferase activity reported here exhibited strict localization in fundic mucosa. No activity was found in non-fundic gastric mucosa and gastric cancer tissue. This location of the enzyme suggested that the sugar moiety produced by this enzyme is possibly related to the function of the fundic mucosa in secreting HCl and pepsinogen.

We are grateful to Dr. H. Morita, the National Medical Centre Hospital, for providing samples, Dr. Y. Hirabayashi, the Riken Institute of Physical and Chemical Research, Dr. Ito and Dr. Yamagata, the Mitsubishi Kasei Institute of Life Science, for providing reagents. This work was supported in part by the Japanese Health Sciences Foundation and a grant-in-aid from the Japanese Ministry of Health and Welfare for the Comprehensive 10-year Strategy for Cancer Control and the Special Coordination Funds for promoting Science and Technology from the Science and Technology Agency.

Received 13 January 1992/21 April 1992; accepted 6 May 1992

REFERENCES

1. Dohi, T., Ohta, S., Hanai, N., Yamaguchi, K. & Oshima, M. (1990) J. Biol. Chem. **265**, 7880–7885
2. Svennerholm, L. (1963) J. Neurochem. **10**, 613–623
3. IUPAC–IUB (1977) Eur. J. Biochem. **79**, 11–21
4. Dohi, T., Hanai, N., Yamaguchi, K. & Oshima, M. (1991) J. Biol. Chem. **266**, 24038–24043
5. Gillard, B. K., Blanchard, D., Bouhours, J.-F., Cartron, J.-P., van Kuik, J. A., Kamerling, J. P., Vliegenthart, J. F. G. & Marcus, D. M. (1988) Biochemistry **27**, 4601–4606
6. Donald, A. S. R., Soh, S. P. C., Yates, A. D., Feeney, J., Morgan, W. T. J. & Watkins, S. M. (1987) Biochem. Soc. Trans. **15**, 606–608
7. Takeya, A., Hosomi, O. & Kogure, T. (1987) J. Biochem. (Tokyo) **101**, 251–259
8. Morton, J. A., Pickels, M. M. & Terry, A. M. (1970) Vox Sang. **19**, 472–482
9. Morton, J. A., Pickels, M. M. & Vanhegan, R. I. (1988) Immunol. Invest. **17**, 217–224
10. Piller, F., Blanchard, D., Huet, M. & Carton, J.-P. (1986) Carbohydr. Res. **149**, 171–184
11. Reference deleted
12. Malagolini, N., Dall'Olio, F., Di Stefano, G., Minni, F., Marrano, D. & Sarafini-Cessi, F. (1989) Cancer Res. **49**, 6466–6470
13. Itoh, T., Li, Y.-T., Li, S.-C. & Yu, R. K. (1981) J. Biol. Chem. **256**, 165–169
14. Svennerholm, L., Månsson, J.-E. & Li, Y.-T. (1973) J. Biol. Chem. **248**, 740–742
15. Taniguchi, N., Nishikawa, A., Fujii, S. & Gu, J. (1989) Methods Enzymol. **179**, 397–408
16. Hase, S., Ibuki, T. & Ikenaka, T. (1984) J. Biochem. (Tokyo) **95**, 197–203
17. Kawano, M., Honke, K., Tachi, M., Gasa, S. & Makita, A. (1989) Anal. Biochem. **182**, 9–15
18. Vrionis, F. D., Wikstrand, C. J., Fredman, P., Månsson, J.-E., Svennerholm, L. & Bigner, D. D. (1989) Cancer Res. **49**, 6645–6651
19. Hiraiwa, N., Tuyuoka, K., Li, Y.-T., Tanaka, M., Seno, T., Okubo, Y., Fukuda, Y., Imura, H. & Kannagi, R. (1990) Cancer Res. **50**, 5497–5503
20. Ilyas, A. A., Li, S.-C., Chou, D. K. H., Li, Y.-T., Jungalwala, F. B., Dalakas, M. C. & Quarles, R. H. (1988) J. Biol. Chem. **263**, 4369–4373
21. Pohlentz, G., Klein, D., Schwarzmann, G., Schmitz, D. & Sandhoff, K. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 7044–7048