Galactosylceramide containing ω-amino-fatty acids: preparation, characterization, and sulfotransferase acceptor

Kouichi Kamio, Shinsei Gasa,[1] and Akira Makita

Biochemistry Laboratory, Cancer Institute, Hokkaido University School of Medicine, Sapporo 060, Japan

Abstract For preparation of an affinity ligand, an N-fatty acyl moiety of galactosylceramide (GalCer) was chemically replaced with ω-amino-fatty acid including amino-*n*-hexanoic acid or amino-*n*-dodecanoic acid to obtain ω-aminoGalCer. For the synthesis of the compound, galactosylsphingosine (GalSph) was coupled with N-trifluoroacetyl ω-amino-fatty acid which was prepared by a reaction with S-ethyltrifluorothioacetate. After removal of the N-trifluoroacetyl group in a mild alkaline solution, in which an N-fatty acyl group was retained, aminoGalCer composed of an N-hexanoyl or an N-dodecanoyl group was obtained with an overall yield of 90%. Their chemical structures were confirmed by proton nuclear magnetic resonance and fast atom bombardment–mass spectrometries. These aminoGalCers and GalSph as well as immobilized aminoGalCer were sulfated by a glycolipid sulfotransferase from rat kidney. Furthermore, immobilized aminoGalCer on gel matrix was used for affinity chromatography of the sulfotransferase, resulting in an excellent increase in the purification (14,000-fold) with a recovery rate of 40%.—**Kamio, K., S. Gasa, and A. Makita.** Galactosylceramide containing ω-amino-fatty acids: preparation, characterization, and sulfotransferase acceptor. *J. Lipid Res.* 1992. **33:** 1227–1232.

Supplementary key words ω-aminogalactosylceramide • galactosylsphingosine • lysogalactosylceramide • sulfotransferase

Galactosylceramide (GalCer) is the simplest glycosphingolipid, and is widely distributed in mammalian tissues such as brain, intestine, and kidney. GalCer is metabolitically converted into sulfatide (SO_4-3GalCer), ganglioside GM_4 (sialyl α2-3GalCer), and galabiosylceramide (Gal α1-4Gal β1-1Cer) (for a review, see ref. 1). GalCer at the cell surface was recently demonstrated to specifically bind human immunodeficiency virus type 1 and to internalize the virus, suggesting that this lipid is an essential component in the receptor for the virus in CD4-negative cells (2, 3).

For immobilization of glycolipids for affinity chromatography, the glycolipid acid was first used as a ligand, which was obtainable by oxidative cleavage at the double bond on sphingosine (4–6). Using the acid, three neutral glycolipid synthases were effectively purified (7–9). Furthermore, the glycolipid aldehyde, also an oxidized derivative at the sphingosine moiety, worked as an affinity ligand for purification of sialyltransferase (10) after immobilization. As an alternate glycolipid derivative for immobilization at the lipid moiety, a deacylated glycolipid (lysoglycolipid) was usable, because it possessed an amino residue in the molecule. The immobilized lysolipid, however, was not effective for purification of glycolipid synthase (11). On the other hand, with regard to chemical modification of the lipid moiety in glycolipids, a fatty acyl residue at the ceramide moiety was replaced by the appropriate acid through the coupling reaction of lysoglycolipid with the acid chloride (12) or with the acid by carbodiimide (13). In fact, Erickson and Radin (14) synthesized several N-alkyl glucosylsphingosines having alkyl carbon chains with different lengths, and demonstrated an inhibitory effect of the N-hexyl derivative on β-glucosidase. Furthermore, the ω position at the fatty acyl moiety in gangliosides has been labeled by pyrene to investigate the formation of the labeled ganglioside micelles and their diffusion and distribution in phospholipid membranes (15). Hence, the glycolipid for immobilization was derived as naturally as possible, by application of the above fatty acyl replacement after introduction of a blocked amino residue. In addition, we aimed to improve the effect of lysolipid-affinity chromatography in the

Abbreviations: GalCer, galactosylceramide; GalSph, galactosylsphingosine; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; NMR, nuclear magnetic resonance spectrometry; FAB-MS, fast atom bombardment-mass spectrometry; SETTA, S-ethyltrifluorothioacetate; TLC, thin-layer chromatography; TFAc, trifluoroacetyl; DMSO, dimethyl sulfoxide; EDTA, ethylene diamine tetraacetate; HFA, α-hydroxy fatty acid; NFA, nonhydroxy fatty acid.

[1]To whom correspondence should be addressed at: Biochemistry Laboratory, Cancer Institute, Hokkaido University School of Medicine, Kita-ku, N15 W7, Sapporo 060, Japan.

purification of glycolipid synthase described above by using immobilized aminoglycolipid, which has a longer spacer toward the gel matrix. Introduction of the amino residue at the fatty acyl ω position in the glycolipid has not been investigated.

In the present report, we describe the synthesis of ω-aminoGalCer having a fatty acyl moiety with a different carbon chain length for immobilization of the lipid. The synthesized compounds were further capable of being acceptor substrates for glycolipid sulfotransferase, and useful for affinity chromatography as a ligand in the purification of the transferase.

MATERIALS AND METHODS

Chemicals

S-Ethyltrifluorothioacetate (SETTA) and anhydrous hydrazine were the products of Aldrich Chemical (Milwaukee, WI); [^{35}S]3'-phosphoadenosine 5'-phosphosulfate (PAPS) and unlabeled PAPS were from New England Nuclear (Boston, MA) and Sigma Chemical (St. Louis, MO), respectively; CNBr-activated Sepharose 4B was from Pharmacia-LKB (Uppsala, Sweden) and 6-amino-*n*-hexanoic and 12-amino-*n*-dodecanoic acids were from Wako Pure Chemicals (Tokyo). GalCer was purified from porcine brain, and GalCer containing α-hydroxy fatty acid (HFA) and nonhydroxy fatty acid (NFA) were separated by silica gel column chromatography. The fatty acids of the former were composed of 98% HFA and the latter of 96% NFA. Other reagents were of analytical grade.

N-Trifluoroacetylation of ω-amino-n-hexanoic acid and ω-amino-n-dodecanoic acid

The ratio of solvent mixture is expressed by volume. N-Trifluoroacetylation of the ω-amino-fatty acids was carried out according to the method of Barker et al. (16), with slight modifications, where the different solvents were used to solubilize the amino-fatty acids. Briefly, 10 mmol of the aminohexanoic acid in 10 ml of water or 5 mmol of aminododecanoic acid in 50 ml of $CHCl_3$-CH_3OH-H_2O 30:60:8 (solvent A) was vigorously stirred at 0°C. After the mixture was adjusted to pH 9.5 with 5 N KOH, 1 ml of SETTA was added, and the mixture was stirred in an ice bath for 1 h. Further addition of SETTA and adjustment of pH were required to increase the yield of the product. After three or four additions of SETTA, the mixture was adjusted to pH 5.0 with trifluoroacetic acid and the solvents were evaporated in vacuo below 40°C. Evaporation was repeated 3 times by the addition of 10 ml of water. The residue was dissolved in 25 ml of water or solvent A for the starting aminohexanoic acid or aminododecanoic acid, respectively, and adjusted to pH 1.5 with trifluoroacetic acid. The acidified solution was applied on a column with a 10-ml bed of Dowex 50 (H^+form) that had been equilibrated with water or solvent A. The column was washed with water or solvent A, and acidic washings were collected. The washings were evaporated to dryness. The powder obtained was recrystallized with water and methanol to give N-trifluoroacetyl (TFAc) ω-amino-fatty acid as needles.

Coupling of GalSph with N'-TFAc ω-aminofatty acids

GalSph was prepared from GalCer by hydrazinolysis as described previously (17). N'-TFAc amino-fatty acid, 1.4 equivalent to GalSph (10–50 μmol), was refluxed in 1 ml of thionyl chloride for 30 min under anhydrous conditions. After evaporation of thionyl chloride, the residue was dried over sodium hydroxide in a desiccator. The N'-TFAc amino-fatty acyl chloride was dissolved in 3 ml of ether and supplemented with 3 ml of a solution containing GalSph in 0.2 M aqueous sodium hydrogen carbonate. The coupling reaction was performed at room temperature for 20 h with stirring. The mixture was neutralized with acetic acid followed by evaporation of the ether. The residual aqueous solution was put in a C_{18} cartridge (Waters, MA) to remove salt by washing with water (18), eluted with solvent A, and the solvent was evaporated to dryness. The coupled product, N-(N'-TFAc ω-aminohexanoyl) GalSph or N-(N'-TFAc ω-aminododecanoyl) GalSph (N'-TFAc aminoGalCers) was purified by silica gel column chromatography in a $CHCl_3$-CH_3OH-H_2O solvent system by raising the polarity. Both the N'-TFAc aminoGalCers were eluted from the column by the solvent mixture with a ration of 80:20:2.

Removal of the TFAc group from N'-TFAc aminoGalCer

To the coupled product dissolved in 2–4 ml of solvent A was added sodium methoxide (28%) in methanol to a final concentration of 1% followed by stirring at room temperature. The reaction was monitored by thin-layer chromatography (TLC) with $CHCl_3$-CH_3OH-H_2O 60:35:8 as developing solvent and by visualizing the product with an orcinol-sulfuric acid reagent. After completion of the reaction, the mixture was neutralized with acetic acid followed by evaporation of the solvent. The residue dissolved in methanol-water 1:1 was desalted with a C_{18} cartridge, concentrated, and chromatographed on a silica gel column as described above to purify the product. The N'-deTFAc product was eluted with a ratio of 60:40:4 in the above solvent mixture. N-AminohexanoylGalSph or N-aminododecanoylGalSph was quantitatively obtained from its N'-TFAc derivative.

Immobilization of aminododecanoylGalSph to Sepharose

CNBr-activated Sepharose was washed with 20 vol of 1 mM HCl per ml of the gel, and then with 10 vol of 10%

aqueous dimethyl sulfoxide (DMSO). To the gel suspension, two equivalents of aminododecanoylGalSph dissolved in a minimum volume of DMSO was added, and the mixture was gently shaken on a rotator for 48 h at room temperature. The gel was further treated with 1 M ethanolamine to block unbound residues. The resultant gel was collected, washed with 20% DMSO in water, and stored in phosphate-buffered saline containing 0.02% sodium azide until use. The ligands bound to the gel were estimated to be 2 μmol per ml of the gel.

Glycolipid sulfotransferase assay

A light membrane fraction was prepared from rat kidney, solubilized, and used as an enzyme preparation (19).

The sulfotransferase reaction was performed similarly to the previously reported method except for the added acceptor glycolipid (20). Briefly, the mixture contained 0.1 mM [^{35}S]PAPS (2×10^5 cpm), 0.3 mM aminoGalCer, 5 mM $MnCl_2$, 5 mM $MgCl_2$, 10 mM ATP, 1.25 mM dithiothreitol, 5 mM sodium fluoride, and enzyme in a final volume of 50 μl. The mixture was incubated at 37°C for 1 h, and supplemented with 0.45 ml of 5 mM EDTA, pH 7.5, to stop the reaction. The whole mixture was poured onto a reversed-phase column (RP-18, Merck, Darmstadt), and the column was washed with 20 ml of water using a washing apparatus (Adsorbex, Merck, Darmstadt) under reduced pressure (21). The radiolabeled product was eluted by a combination of 1 ml of ethanol and then by 4 ml of $CHCl_3$–CH_3OH–H_2O 60:30:4.5. After evaporation of the solvent, the radioactivity of the product was measured in 10 ml of liquid scintillant using a liquid scintillation counter. Through this method, the sulfatide was recovered with a yield of 90% (n = 5). Specific enzyme activity was calculated by subtraction of the blank value, which was obtained without the added lipid or in the presence of EDTA. Protein concentration was determined with a BCA-Protein kit (Kanto Chemicals, Tokyo) using bovine serum albumin as the standard.

Affinity chromatography of glycolipid sulfotransferase

The affinity chromatography was performed at 4°C. Membrane (5 mg/ml) from rat kidney solubilized by 1% Lubrol PX containing 10 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$ (buffer A) and 20% glycerol, 20 ml, was diluted 5 times by buffer A, and applied on an ω-aminododecanoylGalSph-Sepharose column (1 × 5 cm) previously equilibrated in buffer A containing 0.2% of the detergent and 5% glycerol (buffer B) with a flow rate of 5 ml/h. The flow-through was fractionated to 4.5 ml per tube. After the column was washed thoroughly with buffer B, elutions were carried out stepwise with buffer B containing 1 M and 3 M NaCl. Twenty-eight μl of each fraction was assayed for sulfotransferase activity as described above.

Proton NMR measurement

The proton NMR spectra of synthetic intermediates and aminoGalCer were obtained in DMSO-d_6 containing 2% deuterium oxide at 90°C on a Varian JNM GX-500 spectrometer in a Fourier-transform mode, at the High Resolution NMR Laboratory of Hokkaido University, as described previously (22). The frequency and the sweep width were 500 MHz and 5 kHz, respectively. Chemical shifts were indicated by distance (ppm) from tetramethylsilane as an internal standard.

Fast atom bombardment–mass spectrometry (FAB–MS)

The negative FAB–MS of synthetic intermediates and aminoGalCer was measured in a glycerol matrix on a JEOL JMS-DX-300 mass spectrometer equipped with a JMA-DA-5000 Mass Data System at the GC–MS and NMR Laboratory of the Faculty of Agriculture of Hokkaido University. The sample was bombarded by Xe gas at 6 kV (20 mA), and the fragments were accelerated at 2 kV.

RESULTS AND DISCUSSION

Synthesis of aminoGalCer

The yields were 95% for N-TFAc aminohexanoic acid and 40% for N-TFAc aminododecanoic acid through the above reactions. The poor yield of the latter compound may be ascribed to weak solubility in the solvent; this requires further improvement. Through the present procedures, the overall yield starting from GalSph and N-TFAc

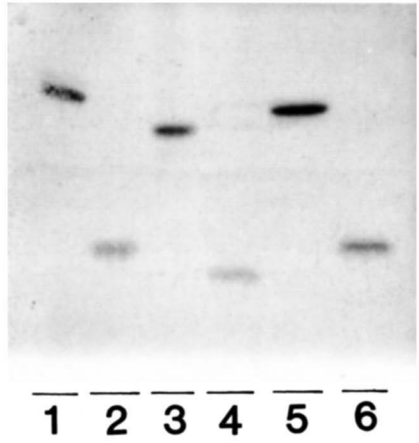

Fig. 1. TLC pattern of synthetic intermediates for aminoGalCer. Lane 1 shows GalCer containing NFA; lane 2, GalSph; lane 3, N-(N'-TFAc ω-aminohexanoyl)GalSph; lane 4, N-(ω-aminohexanoyl) GalSph; lane 5, N-(N'-TFAc ω-aminododecanoyl)GalSph; lane 6, N-(ω-aminododecanoyl)GalSph. The plate was developed in chloroform–methanol–water 60:35:8, and stained by an orcinol-sulfuric acid reagent. GalSph and aminoGalCer were ninhydrin-positive.

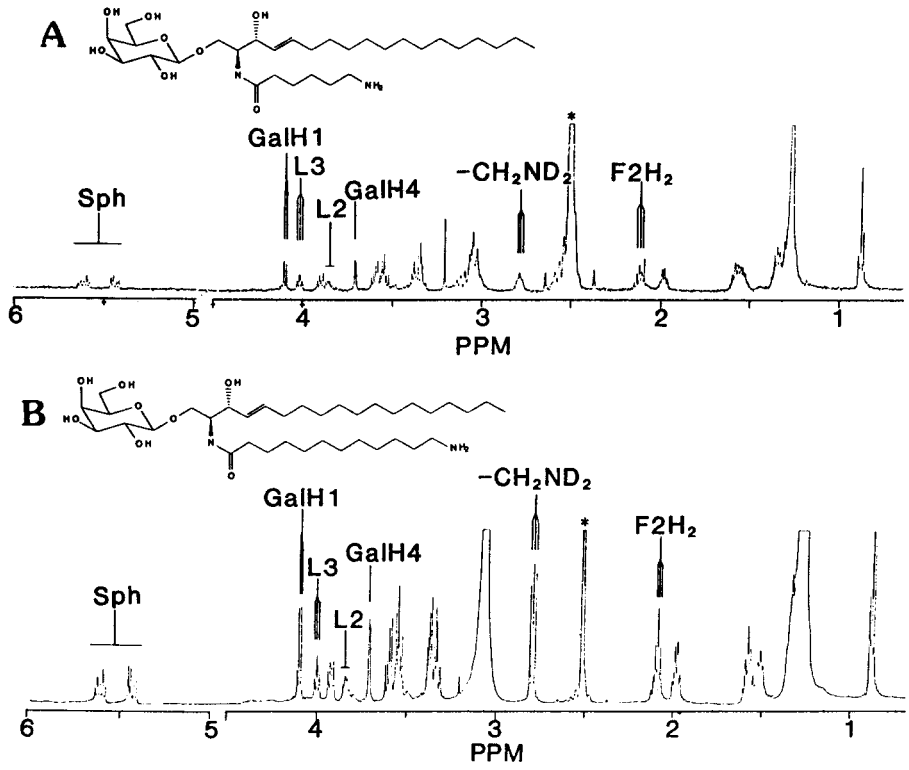

Fig. 2. Proton NMR spectra of aminoGalCers. Panel A shows the spectrum of N-(ω-aminohexanoyl) GalSph; panel B, N-(ω-aminododecanoyl) GalSph. In the spectra, Sph indicates olefinic H-4 and H-5 on sphingosine; GalH1 and GalH4, anomeric proton and C-4 proton on β-galactoside; $F2H_2$, α-methylene on the ω-amino-fatty acid; L2 and L3, C-2 and C-3 protons on sphingosine; $-CH_2ND_2$, amino methylene. All the spectra were measured at 90°C and 500 MHz in DMSO-d_6-D_2O 98:2. The asterisk indicates the peak of DMSO.

acids was more than 90% for synthesis of two GalCers containing ω-amino-fatty acids. If amino-fatty acids with different carbon chain lengths were available, aminoGalCers including these acids could be synthesized by the present procedures using suitable solvents.

The results of TLC of the synthetic intermediates and aminoGalCer are presented in **Fig. 1**. The coupling reaction of N-TFAc fatty acyl chloride and GalSph was performed in a biphasic system composed of ether and water containing a weak base giving a good yield of the coupling product, whereas the reaction in a single-phase afforded a poor yield. Alternatively, direct condensation of N-TFAc amino-fatty acid to GalSph using carbodiimide gave the same product but with a lower yield than that in the acid chloride-mediated reaction. When the carbodiimide-condensation of the amino-fatty acid was performed without blocking the amino group, the yield was much lower. It was also possible to use (9-fluorenylmethyl)-chloroformate (Fmoc) instead of TFAc residue as a blocking group of the amino residue. These blocking groups

TABLE 1. Chemical shift (ppm) of synthetic N-(ω-amino-fatty acyl) GalSphs and the intermediates[a]

	β-Gal	Sphingosine			Fatty Acid	
Derivative	H-1	H-3	H-4	H-5	H-2	Amide
GalCer HFA	4.107	4.025	5.406	5.588	2.261	7.188
NFA	4.072	4.025	5.406	5.588	2.049	7.163
GalSph	4.176	4.294	5.482	5.753		
GalSph-$COC_5H_{10}NHTFAc$	4.082	3.982	5.410	5.585	2.096	7.208
GalSph-$COC_5H_{10}NH_2$	4.095	4.016	5.440	5.612	2.122	[b]
GalSph-$COC_{11}H_{22}NHTFAc$	4.092	3.989	5.424	5.599	2.076	7.156
GalSph-$COC_{11}H_{22}NH_2$	4.091	3.996	5.425	5.601	2.081	7.178

[a]Measured at 90°C in DMSO-d_6-D_2O 98:2.
[b]Should be detected, but not observed.

bound to the primary amino residue were rapidly removed by mild saponification, while the N-acyl group remained unchanged, giving a good yield of aminoGalCer. The present procedures could be applied to synthesis of other glycolipids containing ω-amino-fatty acids starting lysoglycolipids.

Proton NMR and FAB-MS of ω-aminoGalCer

The chemical structures of synthetic intermediates and aminoGalCer were confirmed by proton NMR and FAB-MS. The partial NMR spectra of these final lipids are shown in **Fig. 2**, together with that of GalCer for comparison, and the chemical shifts are summarized in **Table 1**. Comparing the spectra of GalCer and GalSph, *cisoid* olefinic protons at 5.321 ppm and α-methylenic protons at 2.261 ppm on fatty acid in GalCer disappeared in GalSph. In the spectrum of GalSph, H-3 at 4.294 ppm as well as *transoid* olefinic protons H-4 at 5.482 ppm and H-5 at 5.753 ppm on sphingosine were characteristically shifted downfield as compared to those of GalCer or aminoGalCer or the N-TFAc derivatives (Table 1). α-Methylenic protons on fatty acid appeared in the spectra of N'-TFAc derivatives of N-aminohexanoylGalSph and N-aminododecanoylGalSph, indicating insertion of the N'-TFAc amino-fatty acid into the amino residue on sphingosine in the coupling reaction. The methylenic protons were retained after removal of the N'-TFAc group. Molecular masses of the intermediates and the aminoGalCers were confirmed by means of negative FAB-MS analysis (data not shown). A pseudomolecular ion, *m/z* 460, was observed in GalSph (M_r = 461) though its intensity was very low. This low intensity may be ascribed to an unsuitable matrix (glycerol). *M/z* 298 due to $[M\text{-}Gal]^-$ was not detected in the spectrum. However, *m/z* 669 and 507 due to $[M\text{-}H]^-$ and $[M\text{-}Gal]^-$, respectively, were clearly observed in N-(N'-TFAc aminohexanoyl) GalCer (M_r = 670). Similarly, *m/z* 753 and 591 due to $[M\text{-}H]^-$ and $[M\text{-}Gal]^-$ fragment ions were detected in N-(N'-TFAc aminododecanoyl)-GalCer (M_r = 754). Removal of the N'-TFAc group from the aminoGalCers gave *m/z* 573 and 411 due to $[M\text{-}H]^-$ and $[M\text{-}Gal]^-$ for the N-aminohexanoyl derivative (M_r = 574), and *m/z* 657 and 495 due to the fragments for N-aminododecanoyl derivative (M_r = 658). Interestingly, *m/z* 609 and 693 due to $[M\text{-}H + 2H_2O]^-$ were both observed in the aminoGalCer with high intensities.

TABLE 2. Glycolipid sulfotransferase activity toward aminoGalCer

Substrate[a]	Activity	
	pmol/mg protein/h	%
GalCer containing		
HFA	313	100
NFA	248	79
GalSph	196	63
GalSph-$CO(CH_2)_5NH_2$	275	82
GalSph-$CO(CH_2)_{11}NH_2$	246	79

[a]Substrate concentration was 0.3 mM of the lipids.

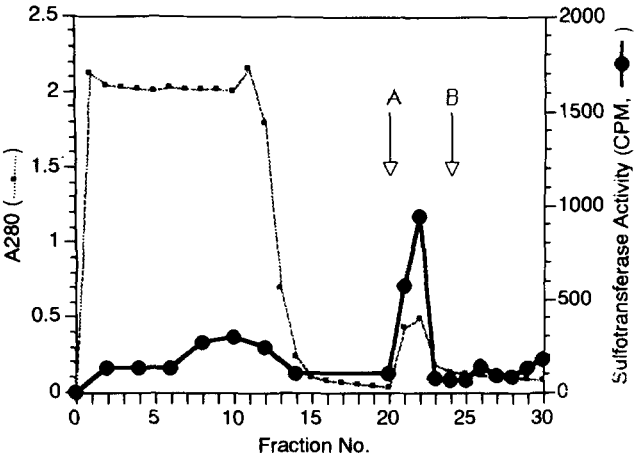

Fig. 3. Affinity chromatography of glycolipid sulfotransferase. The solubilized membrane from rat kidney was chromatographed on aminododecanoylGalSph-Sepharose. The fractions were assayed for sulfotransferase as described under Methods. Arrows A and B indicate elution buffer containing 1 M and 3 M of NaCl, respectively.

Glycolipid sulfotransferase activity toward aminoGalCer

The sulfotransferase activity was assayed using solubilized rat kidney enzyme and synthesized aminoGalCer. The sulfotransferase from rat kidney (23) and human serum (20) utilized GalCer containing HFA better than GalCer containing NFA. This was true with the synthetic acceptor substrates as demonstrated in **Table 2.** Two aminoGalCers exhibited the same extent of acceptor capacity as GalCer containing NFA, whose chemical structure was similar to that of aminoGalCer. As for the solid acceptor substrate, the immobilized *N*-aminododecanoyl derivative was also sulfated (342 pmol of sulfate incorporated 50 μl-gel/mg-protein per h).

Affinity chromatography of glycolipid sulfotransferase

The similarity between GalCer (NFA) and aminoGalCer enables us to use the aminoGalCers as ligands for affinity chromatography for the sulfotransferase. In fact, affinity chromatography of the enzyme from rat kidney was very effective for the purification using immobilized aminododecanoylGalSph as demonstrated in **Fig. 3**. The purification of the bound fraction was elevated 14,000-fold by only the column procedure from the starting solubilized fraction, with 40% recovery. The unbound activity was slightly retarded on the column, indicating that the enzyme had affinity to the ligand and that the column had a low capacity for binding. Specific elution such as with GalCer or with GalSph was tried; however, lower recovery (10%) and lower purification (5,000-fold) than with NaCl

were obtained, because of insufficient solubility of these lipids in the low concentration (2%) of detergent used for the elution. AminoGalCer may be applicable as a probe to detect other metabolic proteins of GalCer after adequate modifications. These new lipids whose synthesized is described should contribute to methodological development in the glycolipid field. With respect to the assay method of sulfotransferase with GalCer, we previously reported chromatographic separation of the enzymatic product using an anion-exchange column (24). However, the method was unsuitable for separation of the sulfated aminoGalCer, since the sulfated product was partially bound to the ion-exchanger in spite of the neutral charge. Thus, reversed phase chromatography was used for the separation of the sulfated aminoGalCer produced by the enzymatic reaction.

We are deeply indebted to Mr. Kim Barrymore for help in the preparation of the manuscript. This work was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture, Japan, Snow Brand Milk Products Co., and Seikagakukogyo Co.

Manuscript received 19 December 1991 and in revised form 19 March 1992.

REFERENCES

1. Makita, A., and N. Taniguchi. 1985. Glycosphingolipids. *In* New Comprehensive Biochemistry. Vol. 10 H. Wiegandt, editor. Elsevier, Amsterdam. 1–99.
2. Harouse, J. M., S. Bhat, S. L. Spitalnik, M. Laughlin, K. Stefano, D. H. Silberberg, and F. G. Scarano. 1991. Inhibition of entry of HIV-1 in neural cell lines by antibodies against galactosyl ceramide. *Science.* **253:** 320–323.
3. Bhat, S., S. L. Spitalnik, F. G. Scarano, and D. H. Silberberg. 1991. Galactosyl ceramide or a derivative is an essential component of the neural receptor for human immunodeficiency virus type 1 envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. USA.* **88:** 7131–7134.
4. Young, W. W., R. A. Laine, and S. Hakomori. 1979. An improved method for the covalent attachment of glycolipids to solid supports and macromolecules. *J. Lipid Res.* **20:** 275–278.
5. Kundu, S. K., and S. K. Roy. 1979. Aminopropyl silica gel as a solid support for preparation of glycolipid immunoadsorbent and purification of antibodies. *J. Lipid Res.* **20:** 825–833.
6. Uchida, T., and Y. Nagai. 1980. Affinity chromatographic purification of anti-glycolipid antibodies and their application to the membrane studies. *J. Biochem.* **87:** 1829–1841.
7. Taniguchi, N., N. Yokosawa, S. Gasa, and A. Makita. 1982. UDP-N-acetylgalactosamine:globoside α-3-N-acetylgalactosaminyltransferase. Purification, characterization and some properties. *J. Biol. Chem.* **257:** 10631–10637.
8. Taniguchi, N., and A. Makita. 1984. Purification and characterization of UDP-N-acetylgalactosamine:globotriaosylceramide α-3-N-acetylgalactosaminyltransferase, a synthase of human blood group P antigen, from canine spleen. *J. Biol. Chem.* **259:** 5637–5642.
9. Taniguchi, N., K. Yanagisawa, A. Makita, and M. Naiki. 1985. Purification and properties of rat liver globotriaosylceramide synthase, UDP-galactose: lactosylceramide α-1-4galactosyltransferase. *J. Biol. Chem.* **260:** 4908–4913.
10. Melkerson-Watson, L. J., and C. C. Sweeley. 1991. Purification to apparent homogeneity by immunoaffinity chromatography and partial characterization of the GM_3 ganglioside-forming enzyme, CMP-sialic acid:lactosylceramide α2,3-sialyltransferase (SAT-1), from rat liver Golgi. *J. Biol. Chem.* **266:** 4448–4457.
11. Tennekoon, G. I., J. Frangia, S. Aitchison, and D. L. Price. 1981. Cerebroside sulfotransferase: preparation of antibody and localization of antigen in kidney. *J. Cell Biol.* **91:** 332–339.
12. Kopaczyk, K. C., and N. S. Radin. 1965. In vivo conversions of cerebroside and ceramide in rat brain. *J. Lipid Res.* **6:** 140–145.
13. Selinger, Z., and Y. Lapidot. 1966. Synthesis of fatty acid anhydrides by reaction with dicyclohexylcarbodiimide. *J. Lipid Res.* **7:** 174–175.
14. Erickson, J. S., and N. S. Radin. 1973. N-Hexyl-O-glucosyl sphingosine, an inhibitor of glucosyl ceramide β-glucosidase. *J. Lipid Res.* **14:** 133–137.
15. Ollmann, M., G. Schwarzmann, K. Sandhoff, and H. J. Galla. 1987. Pyrene-labeled gangliosides: micelle formation in aqueous solution, lateral diffusion, and thermotropic behavior in phosphatidylcholine bilayers. *Biochemistry.* **26:** 5943–5952.
16. Barker, R., K. W. Olsen, J. H. Shaper, and R. L. Hill. 1972. Agarose derivatives of uridine diphosphate and N-acetylglucosamine for the purification of a galactosyltransferase. *J. Biol. Chem.* **247:** 7135–7147.
17. Kato, N., S. Gasa, A. Makita, and H. Oguchi. 1991. Improved separation of lysoglycolipids from solvolysates by reversed-phase high-performance liquid chromatography. *J. Chromatogr.* **549:** 133–139.
18. Williams, M. A., and R. H. McCluer. 1980. The use of Sep-Pak™ C_{18} cartridges during the isolation of gangliosides. *J. Neurochem.* **35:** 266–269.
19. Varki, A., and S. Kornfeld. 1980. Identification of a rat liver α-N-acetylglucosaminyl phosphodiesterase capable of removing "blocking" α-N-acetylglucosamine residues from phosphorylated high mannose oligosaccharides of lysosomal enzymes. *J. Biol. Chem.* **255:** 8398–8401.
20. Gasa, S., M. T. Casl, A. Makita, N. Sakakibara, T. Koyanagi, and T. Atsuta. 1990. Presence and characterization of glycolipid sulfotransferase in human cancer serum. *Eur. J. Biochem.* **189:** 301–306.
21. Figlewicz, D. A., C. E. Nolan, I. N. Singh, and F. B. Jungalwala. 1985. Pre-packed reverse phase columns for isolation of complex lipids synthesized from radioactive precursors. *J. Lipid Res.* **26:** 140–144.
22. Sako, F., S. Gasa, A. Makita, A. Hayashi, and S. Nozawa. 1990. Human blood group glycosphingolipids of porcine erythrocytes. *Arch. Biochem. Biophys.* **278:** 228–237.
23. Fleischer, B., and F. Zambrano. 1974. Golgi apparatus of rat kidney. Preparation and role in sulfatide formation. *J. Biol. Chem.* **249:** 5995–6003.
24. Kawano, M., K. Honke, M. Tachi, S. Gasa, and A. Makita. 1989. An assay method for ganglioside synthase using anion-exchange chromatography. *Anal. Biochem.* **182:** 9–15.