Isolation and characterization of the trisialogangliosides from bovine adrenal medulla

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Abstract Trisialogangliosides were isolated from bovine adrenal medulla by DEAE-Sephadex A-25 and Iatrobeads column chromatography. Their structures were elucidated by sugar analysis, neuraminidase digestion, and permethylation studies. The complete structures of trisialogangliosides, A to D, were identified as follows. A: G_{T1b}, IV^{3}NeuAc, II^3 (NeuAc)$_2$-GgOse$_4$Cer. B: G_{T1b} (NeuAc/NeuAc-NeuGc-); IV^{3}NeuAc, II^3 (NeuAcα2-8NeuGc-)GgOse$_4$Cer. C: G_{T1b} (NeuGc/NeuAc-NeuAc-); IV^{3}NeuGc, II^3 (NeuAcα2-8NeuAc-)GgOse$_4$Cer. D: G_{T1b} (NeuAc/NeuGc-NeuGc-); IV^{3}NeuAc, II^3 (NeuGcα2-8NeuGc-)GgOse$_4$Cer. Gangliosides B, C, and D, which contain N-glycolylneuraminic acid, have not previously been reported in the literature.— **Ariga, T., M. Sekine, R. K. Yu, and T. Miyatake.** Isolation and characterization of the trisialogangliosides from bovine adrenal medulla. *J. Lipid Res.* 1983. **24:** 737–745.

Supplementary key words N-glycolylneuraminic acid

Gangliosides are a family of sialic acid-containing glycosphingolipids. Several anion exchange resins have been developed that greatly facilitate the quantitative separation of gangliosides (4–7). Recently Nagai and coworkers devised a ganglioside mapping method which permits the discovery of new ganglioside species (8–12). Two-dimensional thin-layer chromatographic (TLC) technique and the development of the new solvent system for TLC have also enhanced the identification and detection of several minor gangliosides (13–18).

Bovine adrenal medulla contains predominantly N-glycolylneuraminic acid-containing gangliosides (19–22). In a previous paper (23) we have described the isolation of several disialogangliosides containing N-glycolylneuraminic acid from bovine adrenal medulla and characterized the structures of these gangliosides. They are G_{D3} (NeuAc/NeuGc); II^3 (NeuAcα2-8NeuGc-) LacCer, G_{D3} (NeuGc)$_2$; II^3 (NeuGcα2-8NeuGc-) LacCer, G_{D1a} (NeuAc/NeuGc); IV^{3}NeuAc, II^{3}NeuGc-GgOse$_4$Cer, and G_{D1a} (NeuGc)$_2$; IV^{3}NeuGc, II^{3}NeuGc-GgOse$_4$Cer.

However several minor gangliosides in adrenal medulla, particularly N-glycolylneuraminic acid-containing gangliosides, are still not characterized. In the present report, we describe the isolation of four trisialogangliosides, A to D, from bovine adrenal medulla. Ganglioside A has the same structure of brain G_{T1b} as previously characterized by Kuhn and Wiegandt (24). Other gangliosides are G_{T1b} analogues containing one or two N-glycolylneuraminic acid residues in addition to N-acetylneuraminic acid.

MATERIALS AND METHODS

Isolation of trisialogangliosides

Bovine adrenal medulla tissue, 10 kg, was homogenized in 5 vol of cold acetone. The dried acetone powder was extracted successively with chloroform–methanol 1:1 (v/v), chloroform–methanol 1:2 (v/v), and methanol. The combined extracts were evaporated and subjected to mild alkaline degradation, dialysis, and DEAE-Sephadex A-25 column chromatography as described previously (23). The trisialoganglioside fractions were combined, dialyzed against distilled water for 3 days, and lyophilized. The lyophilized materials were dissolved in a small volume of n-propanol–water 9:1 (v/v) and applied to an Iatrobeads column (45 g, 1.5 cm i.d. × 76 cm) with 1.2 liters of a linear gradient system prepared from n-propanol–water–28% ammonia 85:10:5 and 70:25:5 (v/v/v). Fractions of 7 ml of

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography. The nomenclature for gangliosides is based on the system of Svennerholm (1). The glycolipid nomenclature and symbols follow recent recommendations (2, 3).

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the effluent were collected. Final purification of each ganglioside was achieved by Iatrobeads column chromatography (15 g, 1.2 cm i.d. × 56 cm) with 400 ml of a linear gradient system prepared from n-propanol–water 80:20 and 70:30 (v/v). The purity of the isolated gangliosides was examined by thin-layer chromatography with the following solvent systems: (A) chloroform–methanol–water 55:45:10 (v/v/v) containing 0.02% $CaCl_2 \cdot 2H_2O$; (B) chloroform–methanol–5 M NH_4OH–0.4% $CaCl_2 \cdot 2H_2O$ 60:40:4:5 (v/v/v/v); and (C) n-propanol–water 80:20 (v/v) containing 0.02% $CaCl_2 \cdot 2H_2O$.

Analytical procedures

Compositional analysis was carried out by gas–liquid chromatography. Neutral sugar, sialic acid, fatty acids, and long chain bases were analyzed as previously described (23). The sialic acid species were determined by the method of Yu and Ledeen (25) with slight modifications. Samples containing 10 μg of sialic acid were methanolyzed at 90°C for 1 hr with 0.05 N hydrochloric acid in methanol and trimethylsilylated. Aliquots were injected into a column of 3% OV-101 maintained at 255°C. In order to determine sialosyl-sialosyl linkages in gangliosides, periodate oxidation followed by borohydride reduction was carried out according to the method of Ando and Yu (26). The reaction products were desalted by Sephadex LH-20 (fine) column chromatography (1 cm i.d. × 48 cm) by elution with methanol. The ganglioside fraction was then subjected to methanolysis and trifluoroacetylation and analyzed by GLC (27).

Neuraminidase digestion

Enzymatic treatment by neuraminidase from *Cl. perfringens* (EC 3.2.1.18, type IX, Sigma Chemical Co., St. Louis, MO) was carried out by the method of Ando and Yu (26) and the procedure described previously (23). The ganglioside samples, containing 40 μg of sialic acid, were dissolved in 150 μl of 0.1 M sodium acetate buffer (pH 5.0) and 15 μl of neuraminidase solution (1 unit in 1 ml of 0.1 M sodium acetate buffer) was added. The solution was first incubated for 150 min at 20°C. One-third of the solution was removed and the reaction was terminated by the addition of 1 ml of chloroform–methanol 1:1 (v/v). The remaining solution was further incubated by adding 15 μl of enzyme solution for 16 hr at 37°C. One-half of the solution was removed and the reaction was terminated. Then the other half of the solution was further incubated for 24 hr at 37°C in the presence of 15 μl of 1% sodium taurocholate and 100 μl of neurmainidase solution. Each sample under the different hydrolytic conditions was dried under a stream of nitrogen and salts were removed by Sephadex LH-20 column chromatography. The glycolipid products were examined by TLC using the following developing solvent systems: (A) chloroform–methanol–water 55:45:10 (v/v/v) containing 0.02% $CaCl_2 \cdot 2H_20$; (B) chloroform–methanol–5 M NH_4OH–0.4% $CaCl_2 \cdot 2H_2O$ 60:40:4:5 (v/v/v/v). In a separate experiment, ganglioside samples containing 70 μg of sialic acid were digested by neuraminidase and the glycolipid products were separated by TLC with the solvent system (A) as described above. Each gangioside was scraped off the thin-layer plates, extracted with a solvent mixture of chloroform–methanol–water 30:60:8 (v/v/v), and applied to DEAE-Sephadex A-25 column chromatography. The ganglioside fraction was eluted with 0.2 M sodium acetate in methanol (5) and salts were removed by Sephadex LH-20 column chromatography. The N-acetyl- and N-glycolylneuraminic acid species of each ganglioside were determined by GLC as their trimethylsilyloxy derivatives (25). Enzymatic treatment by neuraminidase from *A. ureafaciens* (EC 3.2.1.18, Nakarai Chemical Co., Kyoto, Japan) was carried out as follows. The ganglioside samples, containing 5 μg of sialic acid, were dissolved in 70 μl of distilled water and 100 μl of 0.1 M sodium acetate buffer (pH 5.0); then 20 μl of neuraminidase solution (1 unit in 1 ml of 0.01 M phosphate buffer, pH 6.8) and 10 μl of 1.3% sodium cholate were added and the reaction mixtures were incubated for 48 hr at 37°C. The reaction was terminated by the addition of 1 ml of chloroform–methanol 2:1 (v/v). The lower phase was dried and the glycolipid products were examined by TLC with the solvent system (A) as described above.

Permethylation study

Permethylation of gangliosides was carried out according to the method of Ando et al. (28) with slight modifications (23, 29). Purification of the permethylated gangliosides was achieved by TLC with a developing solvent system of chloroform–methanol–n-hexane 4:1:2 (v/v/v). Permethylated gangliosides were divided into two portions. One portion was hydrolyzed in 90% acetic acid containing 0.3 N sulfuric acid in the presence of nitrogen gas at 80°C for 16 hr, followed by reduction with sodium borohydride, and acetylated according to the method of Yang and Hakomori (30). Aliquots of their acetylated derivatives were analyzed by GLC and GLC–mass spectrometry using a column of 3% OV-225 at 220°C. Another portion of permethylated gangliosides was methanolyzed with 0.5 ml of 0.3 N hydrochloric acid in methanol for 18 hr at 75°C in order to analyze the substitution site of sialic acid residues (31). The methanolyzates were analyzed by GLC and GLC–mass spectrometry as their trimethylsilyloxy derivatives.

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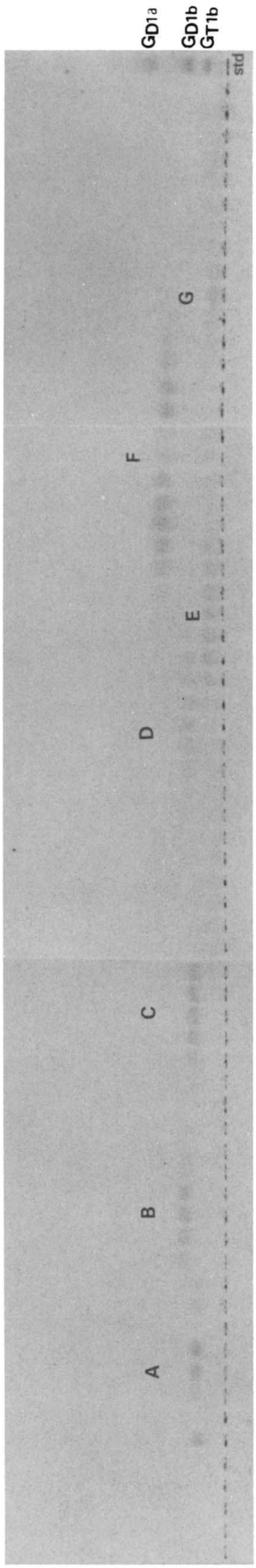

Fig. 1. Gangliosides elution profiles from Iatrobeads column chromatography. The trisialoganglioside fraction was separated into seven different components. Four gangliosides, A to D, were isolated and their structures characterized in this study. (std, gangliosides from human grey matter). The plate was developed with n-propanol–water 80:20 (v/v) containing 0.02% $CaCl_2 \cdot 2H_2O$. The bands were visualized by heating at 95°C with the resorcinol-HCl reagent.

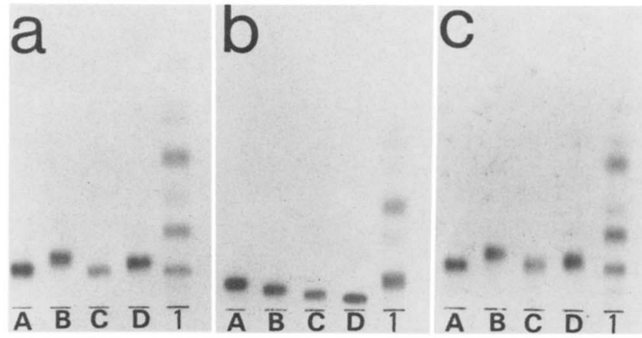

Fig. 2. Thin-layer chromatogram of bovine adrenal medulla trisialogangliosides. A to D, purified trisialogangliosides; 1, gangliosides from human grey matter. Plate (a) was developed with chloroform–methanol–water 55:45:10 (v/v/v) containing 0.02% $CaCl_2 \cdot 2H_2O$; plate (b) with chloroform–methanol–5 M NH_4OH–0.4% $CaCl_2 \cdot 2H_2O$ 60:40:4:5 (v/v/v/v); and plate (c) with n-propanol–water 80:20 (v/v) containing 0.02% $CaCl_2 \cdot 2H_2O$. The bands were visualized by heating at 95°C with the resorcinol-HCl reagent.

RESULTS

The content of lipid-bound sialic acid in the trisialoganglioside fraction was 3.0 μg/g fresh tissue, which accounted for about 1.4% of total lipid-bound sialic acid. The trisialoganglioside fraction was separated into at least seven different components by Iatrobeads column chromtography (**Fig. 1**). Four gangliosides, A to D, were isolated and purified to homogeneity as revealed by TLC with three different solvent systems as shown in **Fig. 2.**

Compositional analysis

The sugar composition of these gangliosides is summarized in **Table 1.** These gangliosides contained glucose, galactose, N-acetylgalactosamine, and sialic acid in a molar ratio of 1:2:1:3. Periodate oxidation– borohydride reduction experiments showed these gangliosides yielded 2 mol of the C7 derivative of sialic acid and 1 mol of intact sialic acid (**Table 2**). The composition of the sialic acid species of these gangliosides is presented in **Table 3.** All of the sialic acids of ganglioside A were identified as N-acetylneuraminic acid. Gangliosides B and C were found to contain N-acetylneuraminic acid and N-glycolylneuraminic acid in a molar

TABLE 1. Carbohydrate analysis of purified gangliosides from bovine adrenal medulla

	A	B	C	D
Molar ratio of carbohydrate				
Glucose	1.00	1.00	1.00	1.00
Galactose	2.28	2.07	2.14	1.87
N-acetylgalactosamine	0.97	0.92	0.97	1.19
Sialic acid	3.26	3.05	2.89	3.07

TABLE 2. Periodate oxidation–borohydride reduction analysis of purified gangliosides from bovine adrenal medulla

Gangliosides	Sialic Acid	
	C7	C9
A	2.16	1.00
B	2.15	1.00
C	2.23	1.00
D	2.09	1.00
G_{T1b}[a]	2.03	1.00
G_{D1b}[a]	0.97	1.00

[a] Authentic gangliosides, G_{T1b} and G_{D1b}, were obtained from bovine brain.

TABLE 4. Fatty acid compositions of isolated trisialogangliosides

	A	B	C	D
		%		
C16:0	tr[a]	0.8	0.5	0.6
C18:0	19.4	19.5	5.5	9.2
C18:1	tr	0.3	0.9	2.3
C19:0	0.4	0.5	tr	0.1
C19:1	tr	tr	tr	0.2
C20:0	10.4	11.7	5.6	8.8
C21:0	tr	0.5	tr	0.4
C22:0	23.5	25.2	24.7	26.5
C23:0	9.3	9.6	13.6	10.8
C23:1	tr	0.5	tr	tr
C24:0	22.3	17.6	36.0	25.3
C24:1	14.7	14.2	13.3	15.4

[a] tr, trace amounts less than 0.1%.

ratio of 2:1. Ganglioside D also contained N-acetylneuraminic acid and N-glycolylneuraminic acid in a molar ratio of 1:2. All of the trisialogangliosides contained predominantly C18 long-chain base, which was composed of sphingenine (91 ~ 93%) and sphinganine (1.4 ~ 2.8%). Lesser amounts of C16 homologues were also detected. No C20 homologues could be detected. Fatty acid compositions of these gangliosides are shown in **Table 4.** The major fatty acids were stearic, arachidic, behenic, tricosanoic, lignoceric, and nervonic acids.

Neuraminidase digestion

After neuraminidase treatment by *Cl. perfringens*, the degradation products were anayzed by TLC with different solvent system (**Fig. 3**). Under mild conditions (23, 26), the degradation product from gangliosides A and C cochromatographed on TLC with authentic G_{D1b}, while gangliosides B and D were converted to an unknown ganglioside, which migrated near G_{D1b} on TLC with neutral solvent systems (Fig. 3-I). After hydrolysis with neuraminidase in the presence of sodium taurocholate, gangliosides A and C were converted to N-acetylneuraminic acid-containing G_{M1} ganglioside, and gangliosides B and D were converted to N-glycolylneuraminic acid-containing G_{M1} ganglioside (Fig. 3-II), which was identical with the glycolipid product from bovine adrenal medulla G_{D1a} (NeuAc/NeuGc) by neuraminidase treatment (23). In a separate experiment, the neuraminidase-treated glycolipid products were isolated by preparative TLC and DEAE-Sephadex A-25 column chromatography (**Fig. 4**). These glycolipid products were subjected to methanolysis by the method of Yu and Ledeen (25) and analyzed by GLC in order to determine the identity of the sialic acid species (Table 3). The sialic acid of the mono- and disialoganglioside fractions from gangliosides A and C was identified as N-acetylneuraminic acid. The sialic acid of monosialoganglioside fraction from gangliosides B and D and the disialoganglioside fraction from ganglioside D was identified as N-glycolylneuraminic acid. The disialoganglioside fraction from ganglioside B was found to contain N-acetylneuraminic acid and N-glycolylneuraminic acid in a molar ratio of 1:1. After exhaustive hydrolysis with neuraminidase from *A. ureafaciens* in the presence of sodium cholate, these gangliosides were converted to the same asialo-ganglio-N-tetraosyl ceramide (G_{A1}), which was identical with that derived from bovine brain G_{M1}, G_{D1a}, and G_{T1b} (**Fig. 5**).

TABLE 3. Sialic acid species in glycolipid products of neuraminidase-treated trisialogangliosides (%)

	A	B	C	D
Trisialogangliosides				
N-acetyl type	100.0 (3.00)[a]	63.6 (1.75)	64.5 (1.82)	35.2 (1.00)
N-glycolyl type	0	36.4 (1.00)	35.5 (1.00)	64.8 (1.84)
Disialo-fraction				
N-acetyl type	100.0 (2.00)	42.2 (0.73)	100.0 (2.00)	10.2 (0.23)
N-glycolyl type	0	57.8 (1.00)	0	89.8 (2.00)
Monosialo-fraction				
N-acetyl type	100.0 (1.00)	1.3 (0.01)	95.2 (1.00)	0.8 (0.01)
N-glycolyl type	0	98.7 (1.00)	4.8 (0.05)	99.2 (1.00)

[a] Parentheses express the molar ratio of sialic acid species.

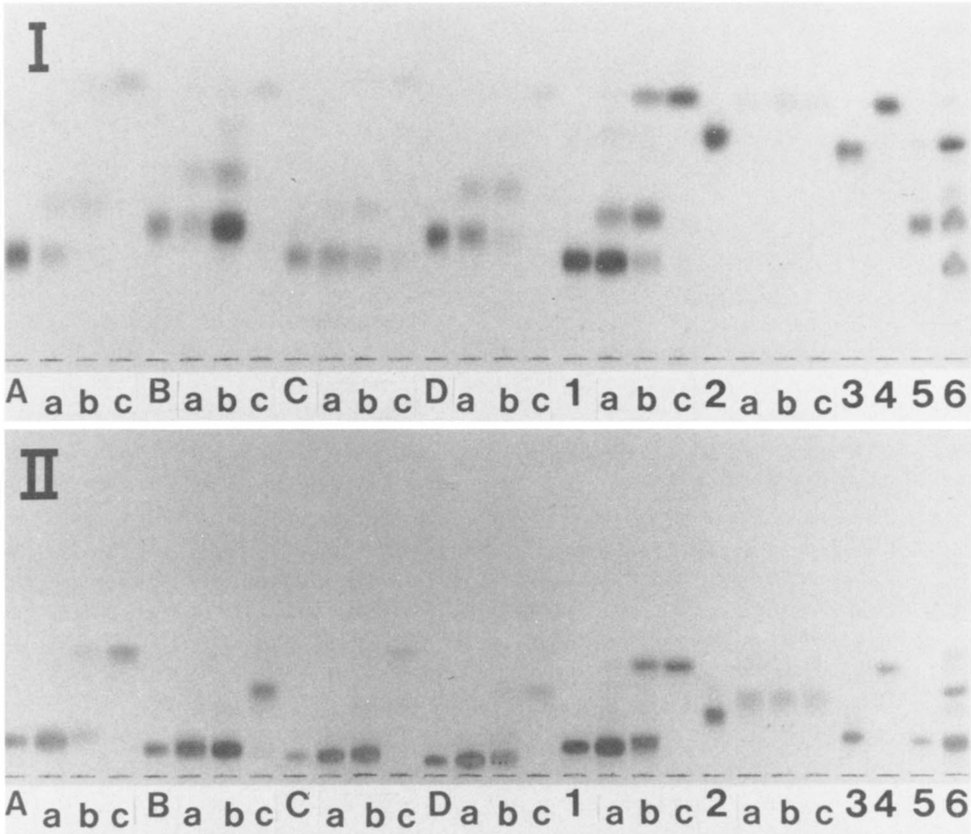

Fig. 3. Thin-layer chromatogram of glycolipid products of gangliosides, A to D, after neuraminidase (*Cl. perfringens*) treatment. A to D, purified trisialogangliosides; 1, authentic G_{T1b} from bovine brain; 2, G_{D1a} (NeuAc/NeuGc) from bovine adrenal medulla (23); 3, G_{D1a} $(NeuGc)_2$ from bovine adrenal medulla (23); 4, authentic G_{M1} from bovine brain; 5, authentic G_{D1b} from bovine brain; 6, gangliosides from human grey matter. Trisialogangliosides, A to D, G_{T1b}, and G_{D1a} (NeuAc/NeuGc) were digested as follows: a, glycolipid products after hydrolysis for 150 min at 20°C; b, glycolipid products after hydrolysis for 16 hr at 37°C; c, glycolipid products after hydrolysis for 24 hr at 37°C in the presence of sodium taurocholate (23, 26). The plates were developed with (I) chloroform–methanol–water 55:45:10 (v/v/v) containing 0.02% $CaCl_2 \cdot 2H_2O$, and (II) chloroform–methanol–5 M NH:i4OH–0.4% $CaCl_2 \cdot 2H_2O$ 60:40:4:5 (v/v/v/v). The bands were visualized by heating at 95°C with the resorcinol-HCl reagent.

Permethylation study

Analyses by GLC and GLC–mass spectrometry revealed that these gangliosides produced 2,3,6-tri-O-methyl-1,4,5-tri-O-acetylglucitol; 2,4,6-tri-O-methyl-1,3,5-tri-O-acetylgalactitol; 2,6-di-O-methyl-1,3,4,5-tetra-O-acetylgalactitol; and 4,6-di-O-methyl-1,3,5-tri-O-acetyl-2-deoxy-2-N-methylacetamidogalactitol, suggesting the presence of ganglio-N-tetraosyl ceramide backbone. The sialic acid linkage sites were also analyzed by permethylation studies (**Fig. 6** and **Fig. 7**). The gangliosides, A to D, produced the same terminal sialic acid that was identified as 2,4,7,8,9-penta-O-methyl-N,N-acetyl,methyl-neuraminic acid methyl ester by the presence of the molecular ion m/z 407 and the fragment ions m/z 129, 254, 298, 318, 348, and 392 (Fig. 6a).The gangliosides C and D yielded a different type of the terminal sialic acid which was identified as 2,4,-7,8,9 - penta - O - methyl - N,N - glycolylmethyl, methyl-neuraminic acid methyl ester by the detection of the molecular ion m/z 437 and the fragment ions m/z 159, 284, 328, 348, 378, and 422 (Fig. 6b). Gangliosides A and C yielded the same inner sialic acid that was identified as 2,4,7,9-tetra-O-methyl-8-O-trimethylsilyloxy-N,N-acetyl,methyl-neuraminic acid methyl ester by virtue of the molecular ion m/z 465 and the fragment ions m/z 147, 254, 318, 356, 406, and 450 (Fig. 6c). The inner sialic acid in gangliosides B and D was characterized as 2,4,7,9-tetra-O-methyl-8-O-trimethylsilyloxy-N,N-glycolylmethyl,methyl-neuraminic acid methyl ester by the detection of the molecular ion m/z 495 and the fragment ions m/z 147, 159, 284, 348, 436, and 480 (Fig. 6d).

On the basis of these results, the chemical structures of these purified trisialogangliosides, A to D, from bovine adrenal medulla are shown as follows.

A: IV^{3}NeuAc, II^3(NeuAc)$_2$-GgOse$_4$Cer;

```
Gal(β1 → 3)GalNAc(β1 → 4)Gal(β1 → 4)Glc(β1 → 1′)ceramide
  3                     3
  ↑                     ↑
  2                     2
  α                     α
NeuAc                 NeuAc
                        8
                        ↑
                        2
                        α
                      NeuAc
```

B: IV^{3}NeuAc, II^3(NeuAcα2-8NeuGc-)GgOse$_4$Cer;

```
Gal(β1 → 3)GalNAc(β1 → 4)Gal(β1 → 4)Glc(β1 → 1′)ceramide
  3                     3
  ↑                     ↑
  2                     2
  α                     α
NeuAc                 NeuGc
                        8
                        ↑
                        2
                        α
                      NeuAc
```

C: IV^{3}NeuGc, II^3(NeuAcα2-8NeuAc-)GgOse$_4$Cer;

```
Gal(β1 → 3)GalNAc(β1 → 4)Gal(β1 → 4)Glc(β1 → 1′)ceramide
  3                     3
  ↑                     ↑
  2                     2
  α                     α
NeuGc                 NeuAc
                        8
                        ↑
                        2
                        α
                      NeuAc
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D: IV^{3}NeuAc, II^3(NeuGcα2-8NeuGc-)GgOse$_4$Cer;

```
Gal(β1 → 3)GalNAc(β1 → 4)Gal(β1 → 4)Glc(β1 → 1′)ceramide
  3                     3
  ↑                     ↑
  2                     2
  α                     α
NeuAc                 NeuGc
                        8
                        ↑
                        2
                        α
                      NeuGc
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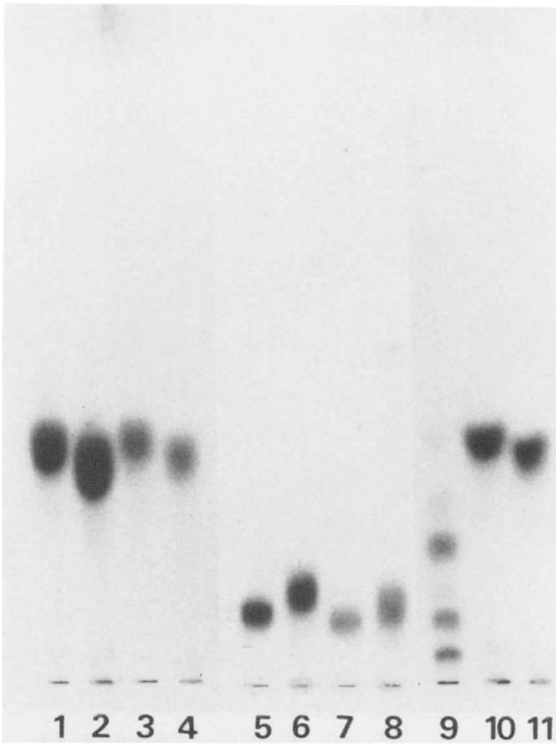

Fig. 4. Glycolipid products from trisialogangliosides, A to D, after-neuraminidase (*Cl. perfringens*) digestion. 1–4, monosialo fraction; 5–8, disialo fraction; 1 and 5, glycolipid products from ganglioside A; 2 and 6, glycolipid products from ganglioside B; 3 and 7, glycolipid products from ganglioside C; 4 and 8, glycolipid products from ganglioside D; 9, gangliosides from human grey matter; 10, authentic G_{M1} (NeuAc) from bovine brain; 11, authentic G_{M1} (NeuGc) from bovine spinal cord. The plate was developed with chloroform–methanol–water 55:45:10 (v/v/v) containing 0.02% $CaCl_2 \cdot 2H_2O$. The bands were visualized by heating at 95°C with the resorcinol-HCl reagent.

DISCUSSION

Permethylation studies suggest that the trisialogangliosides isolated from bovine adrenal medulla have the same basic ganglio-N-tetraosyl ceramide structure that is found in the major mammalian brain gangliosides. These gangliosides have their sialic acid residues linked to both the internal and the external galactose molecules in a 2 → 3 linkage. Periodate oxidation experiments and permethylation studies of the sialic acid residues indicate the presence of a sialosyl (2 → 8) sialosyl residue attached to a ganglio-N-tetraosyl ceramide (asialo-G_{M1}) backbone (26, 31). Further structural analyses were carried out on these gangliosides that included carbohydrate analysis and neuraminidase digestion. Our results indicate that each ganglioside contains a disialosyl residue attached to the inner galactose molecule and the remaining sialosyl residue linked to the terminal galactose molecule of the ganglio-N-tetraosyl ceramide backbone (32). Hence all these gangliosides can be considered as structural analogues of brain G_{T1b}. The only difference among them is the type of sialic acid species they contain. Gangliosides B and C contain 1 mol of N-glycolylneuraminic acid each; and ganglioside D contains 2 mol of N-glycolylneuraminic acid. Ganglioside A contains only N-acetylneuraminic acid, therefore, its structure is identical to that of brain G_{T1b}. Determination of the sialic acid residues in the glycolipid products produced by neuraminidase and permethylation studies of the sialic acid residue suggest that gangliosides A and C have a N-acetylneuraminosyl (2 → 8) N-acetylneuraminosyl residue and that ganglioside D has only the N-glycolyl type. However, ganglioside B contains a N-acetylneuraminosyl (2 → 8) N-glycolylneuraminosyl residue. The glycosidic linkage of the sialic acid residue is of α-D configuration on the basis of neuraminidase study (33). Therefore, the chemical structures of these purified gangliosides are proposed

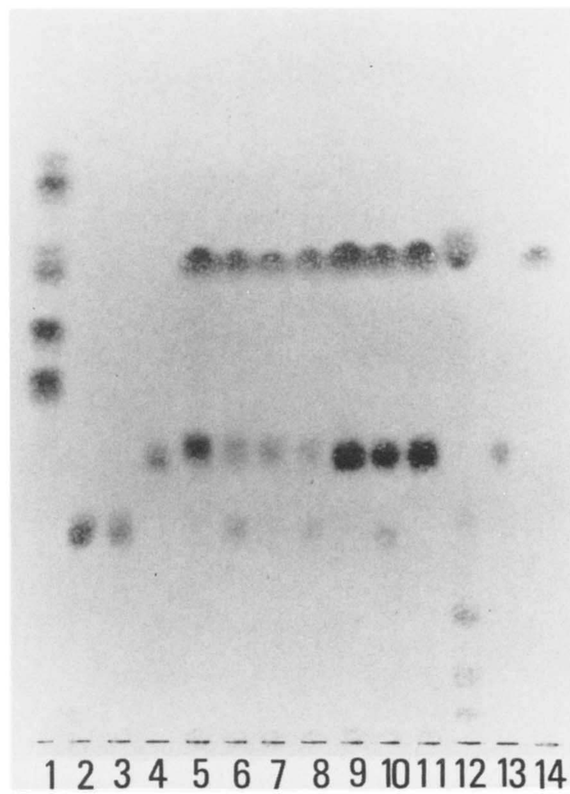

Fig. 5. Thin-layer chromatogram of glycolipid products of trisialogangliosides, A to D, after neuraminidase (*A. ureafaciens*) treatment. 1, authentic GalCer from bovine brain and LacCer, $GbOse_3Cer$, and $GbOse_4Cer$ from pig erythrocyte membranes (from top to bottom); 2, G_{M1} (NeuAc) from bovine brain; 3, G_{M1} (NeuGc) from bovine spinal cord; 4 and 13, asialo-G_{M1} (G_{A1}) from bovine brain; 5–8, glycolipid products of trisialogangliosides, A to D, from bovine adrenal medulla after neuraminidase digestion; 9, 10, and 11, glycolipid products of bovine brain gangliosides, G_{T1b}, G_{D1a}, and G_{M1}, after neuraminidase digestion, respectively; 12, gangliosides from human grey matter; 14, authentic sodium cholate. The plate was developed with chloroform–methanol–water 55:45:10 (v/v/v) containing 0.02% $CaCl_2 \cdot 2H_2O$. The bands were visualized by heating at 110°C with the α-naphthol-H_2SO_4 reagent.

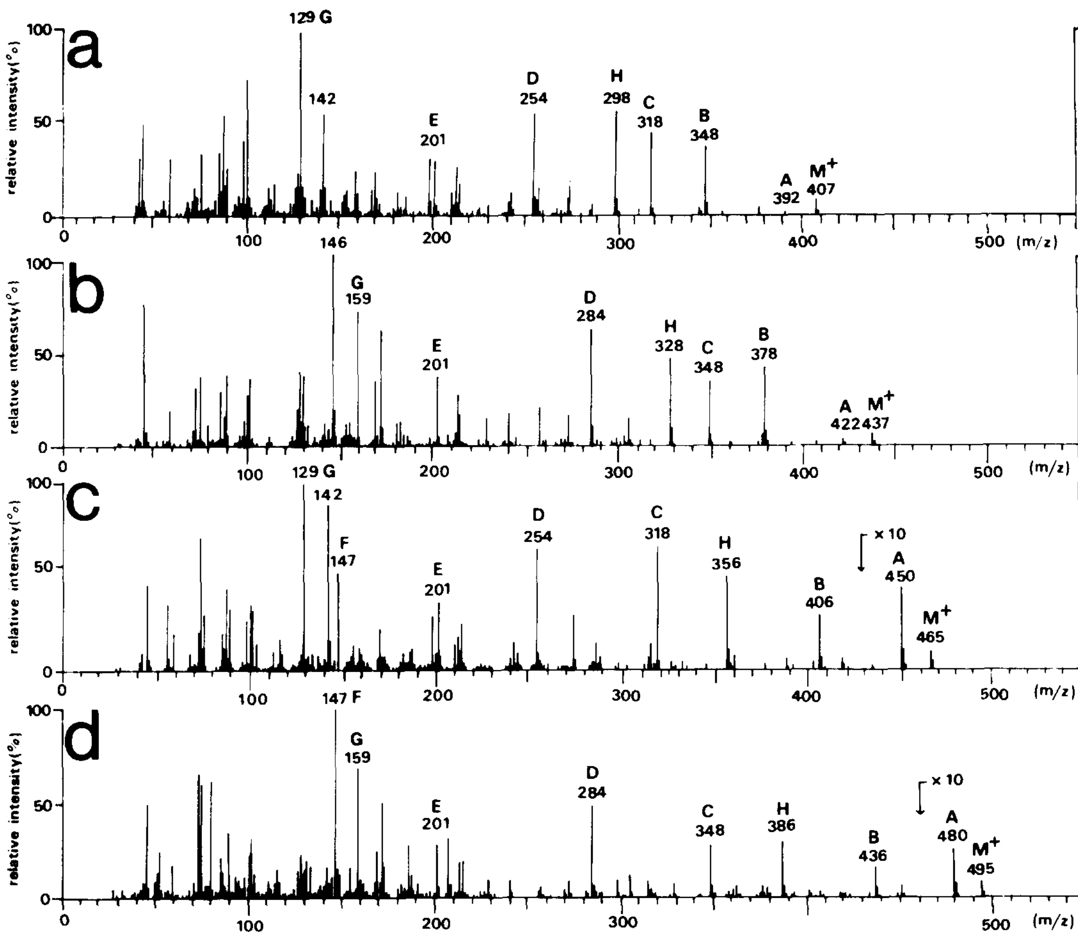

Fig. 6. Mass spectra of sialic acid derivatives from permethylated gangliosides. a, 2,4,7,8,9-penta-O-methyl-N,N-acetyl, methyl-neuraminic acid methyl ester from gangliosides, A to D; b, 2,4,7,8,9-penta-O-methyl-N,N-glycolylmethyl,methyl-neuraminic acid methyl ester from gangliosides C and D; c, 2,4,7,9-tetra-O-methyl-8-O-trimethylsilyloxy-N,N-acetyl, methyl-neuraminic acid methyl ester from gangliosides A and C; d, 2,4,7,9-tetra-O-methyl-8-O-trimethylsilyloxy-N,N-glycolylmethyl,methyl-neuraminic acid methyl ester from gangliosides B and D. Mass spectra were obtained at an electron energy of 70 eV and emission current of 60 μA.

to be as described in abstract. Gangliosides B, C, and D represent new trisialoganglioside species by virtue of their unusual sialic acid composition.

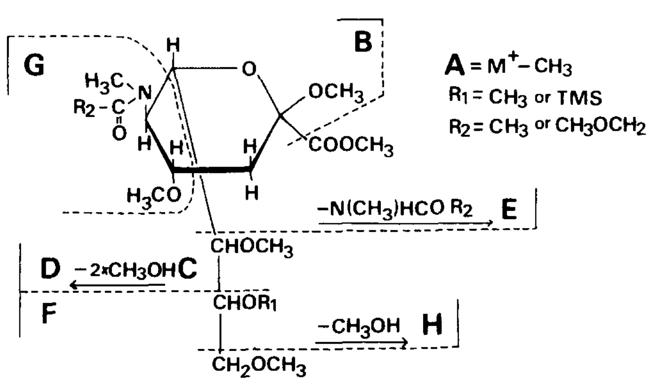

Fig. 7. Fragmentation diagram of sialic acid derivatives.

It is interesting to note that the adrenal medulla trisialogangliosides reported here and the mono- and disialogangliosides reported earlier (20, 23) all contain a significant portion of long-chain fatty acids (C > 20) in addition to stearic acid. Furthermore the long-chain base composition of these gangliosides is characterized by a preponderance of C-18 sphingenine. These features are in sharp contrast to most adult mammalian brain gangliosides which contain predominantly stearic acid and both C18- and C20-sphingenine (34, 35). It would be interesting to relate these differences in hydrophobic portions of these molecules to specific membrane functions of various tissues.

Finally, we have also isolated several other trisialogangliosides (E, F, and G). Structural analyses of these gangliosides are now in progress.

Manuscript received 25 October 1982 and in revised form 25 January 1983.

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