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# STRUCTURE OF A NEW DISIALOGANGLIOSIDE GD1c FROM SPONTANEOUS MURINE THYMOMA*

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ABSTRACT

A major mono- and a di-sialoganglioside were isolated and purified to homogeneity from a spontaneous thymoma that occurs in AKR mice. Compositional and methylation analyses and the use of exoglycosidases established the monosialoganglioside to be αNeu(2→3)βGal(1→3)βGalNAc(1→4)βGal(1→4)-Glc(1→1)Cer and the disialoganglioside to be αNeuAc(2→8)αNeuAc(2→3)βGal-(1→3)βGalNAc(1→4)βGal(1→4)Glc(1→1)Cer (GD1c). A possible pathway for the biosynthesis of this disialoganglioside is presented.

## INTRODUCTION

Gangliosides, sialic acid-containing glycosphingolipids, are ubiquitous components of the plasma membrane of the animal cell. The structure and functions of glycosphingolipids have been extensively revieved[1–3], with special emphasis on changes in their composition associated with ontogenesis and oncogenesis as well as possible involvement in regulation of cell proliferation[4,5].

We have recently compared the ganglioside content and composition of murine thymoma occurring spontaneously in AKR mice with those of normal thymus[6]. This work is a continuation of an earlier research on glycoconjugates in murine thymoma initiated by Prof. Wojciech Rossowski at Department of Radiobiology and Health Protection, Institute of Nuclear Research, Warsaw, Poland. In this report we present structures of the major monosialoganglioside and disialoganglioside of this thymoma. A disialoganglioside having the structure of a $IV^3(NeuAc)_2GgOse_4$ceramide* has not yet been described.

*Dedicated to Roger W. Jeanloz.

*Nomenclature proposed by Svennerholm[7] and recommended by IUPAc-IUB[8]. Neu = *N*-acetyl- or *N*-glycolyl-neuraminic acid.

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EXPERIMENTAL

*Animals.* — Mice of the AKR strain were purchased from Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Wrocław, kept in our colony, and fed standard diet *ad lib.* The animals developed thymoma spontaneously at about 4–7 months. Mice were killed, thymomas or thymuses removed, washed in saline, blotted with filter paper, and stored at −20° until needed.

*Glycosphingolipid standards and other materials.* — Gangliosides and neutral glycosphingolipids used as standards were the same preparations as previously described[6]. Preparative t.l.c. plates coated with silica gel 60 (cat. No. 5745) and analytical t.l.c. plates also coated with silica gel 60 (cat. No. 5721) were purchased from Merck. Solvent mixtures are all v/v. Other reagents were obtained from following sources: Bio-Sil A 200–400 mesh, and *Vibrio cholerae* neuraminidase, Calbiochem; DEAE-Sepharose CL-6B and Sephadex G-25 Superfine, Pharmacia; jack bean β-D-galactosidase, Sigma; *Charonia lampas* β-D-galactosidase and hexosaminidase, Seikagaku Kogyo Co; green coffee bean α-D-galactoside, Boehringer; sodium taurocholate, BDH.

*Thin-layer chromatography.* — Gangliosides were separated on preparative and analytical t.l.c. plates with solvent system *A* (1 + 2) of Rösner[9]. Plates were first developed in 60:35:7.5:3 chloroform–methanol–12mM $MgCl_2$–15M $NH_4OH$, dried, and developed again in 58:40:9 chloroform–methanol–12mM $MgCl_2$. Analytical t.l.c. plates were also used with solvent systems *B*, 60:35:8 chloroform–methanol–0.25% aqueous $CaCl_2$, and *C* which was the lower layer of a 65:30:8 chloroform–methanol–water mixture. Separated gangliosides were located on chromatograms with iodine vapor or stained with resorcinol or orcinol. Neutral glycosphingolipids were stained with orcinol.

*Ganglioside isolation and purification.* — Pooled thymomas or thymuses were extracted with chloroform and methanol by the procedure of Svennerholm and Fredman[10] with minor changes[6]. Lipid extracts were evaporated, dissolved in 2:1 chloroform–methanol and, after addition of water, partitioned into upper and lower phases[11]. The lower phase was extracted three times with the Folch upper phase, 3:47:48 chloroform–methanol–water. The ganglioside-rich upper phase was evaporated and treated with 0.5M sodium hydroxide in 1:3 chloroform–methanol overnight at room temperature. The samples were made neutral with acetic acid, evaporated, the residues suspended in water, and dialyzed against water for 4 days. Ganglioside-containing retentates were evaporated, the residues dissolved in a small volume of 2:1 chloroform–methanol, and applied to columns of Bio-Sil A. After washings the columns with 5 column volumes of 9:1 chloroform–methanol, gangliosides were eluted with 10 column volumes of 2:3 chloroform–methanol and 5 column volumes of 1:3 chloroform–methanol. Eluates were checked for their sialic acid content by the color reaction with resorcinol[10], and then pooled and evaporated. Gangliosides were redissolved in 3:9:1 chloroform–methanol–water and fractionated on DEAE-Sepharose columns[12,13]. Monosialo-, disialo-, and

trisialo-gangliosides were eluted with methanolic 0.02, 0.1, and 0.2M ammonium acetate, respectively, followed by a final wash with methanolic 0.5M ammonium acetate. After evaporation and dialysis for 4 days, ganglioside fractions were assayed for sialic acid content with resorcinol and their patterns studied by t.l.c. Final purification of the major monosialoganglioside $G_M$ and disialoganglioside $G_D$ from murine thymoma was achieved by repeated t.l.c. on preparative silica gel plates with solvent system *A* (1 + 2) and on analytical silica gel plates with solvent system *A* (1 + 2) and *B*.

*Gas–liquid chromatography and mass spectrometry.* — All g.l.c. analyses were performed with a Pye Unicam GCV gas chromatograph[14]. Sugar constituents of murine thymoma $G_M$ and $G_D$ gangliosides were identified and quantitated after their conversion into trimethylsilyl derivatives and separation on a column of 3% SE-30 operated between 150° to 250° with a temperature increment of 3° per min[15].

Fatty acid methyl esters obtained after hexane extraction of methanolyzed samples were separated[16] on a column of 10% Silar 10 C operated isothermally at 200°. Long-chain bases of murine thymoma $G_D$ ganglioside were isolated as described[17] and analyzed after conversion into trimethylsilyl ether derivatives, on a column of 3% SE-30 maintained at 220°. Methylation of glycosphingolipids was performed according to Stellner *et al.*[18]. Partially methylated alditol and hexosaminitol acetates were prepared under modified conditions of Zdebska *et al.*[14]. Mass spectrometry was performed with a Hewlett–Packard 9995A mass spectrometer[19] using a 12-m capillary column made of fused silica and coated with crosslinked 5% phenylmethyl silicone. The column was heated at 100° for 2 min and then programmed to 240° at 2°/min. Methylation analysis of neuraminic acids was performed as follows. Methylated ganglioside samples were purified on columns of LH-20 Sephadex and methanolyzed[20] in 0.5M HCl in methanol for 24 h at 85°. After removal of fatty acid methyl esters with hexane, the residue was dried under nitrogen and acetylated[21] in acetic 1:1 anhydride–pyridine for 30 min at 85°. The reagents were removed under nitrogen after addition of toluene. The residue was dissolved in chloroform and analyzed by g.l.c. on a column of 3% SE-30 at 230° and by g.l.c.–m.s. using the same column as for partially methylated alditol and hexosaminitol acetates, but programmed from 100 to 240° at 4°/min.

*Determination of anomeric configuration and sequences of carbohydrate residues by use of exoglycosidases.* — *Vibrio cholerae* neuraminidase was used as described by Mullin *et al.*[22]. Incubation mixtures contained in 100 μL: ganglioside 10 nmol; sodium acetate buffer, pH 5.5, 5 μmol; $CaCl_2$ 0.9 μmol; NaCl 15 μmol; and neuraminidase, 50 mU. After incubation for 20 h, samples were desalted on columns of Sephadex G-25 Superfine[23]. *Charonia lampas* β-D-galactosidase and β-hexosaminidase were used under conditions specified by Zieleński and Kościelak[24]. Incubations mixtures contained in 100 μL: glycosphingolipid, 10 nmol; sodium taurocholate, 50 μg; sodium citrate buffer, pH 4.0, 5 μmol; NaCl, 50 μmol; and enzymes, 25 mU. Reactions were terminated after 72 h by the addition of 2:1 chloroform–methanol. Glycosphingolipids were recovered in the Folch lower layer

and washed once with "theoretical" upper layer. The products of reaction with the other glycosidases were extracted by the same procedure. Jack bean β-D-galactosidase was incubated with glycosphingolipids according to Li *et al.*[25]. Incubation mixtures contained in 300 μL: glycosphingolipid, 10 nmol; sodium taurocholate, 150 μg; sodium citrate buffer, pH 4.0, 12 μmol; and β-D-galactosidase, 100 mU. Incubations when jack bean β-D-galactosidase was used together with *Charonia lampas* β-hexosaminidase also contained NaCl, 50 μmol and β-hexosaminidase, 50 mU. Incubations were terminated after 72 h. Green-coffee bean α-D-galactosidase was employed according to Clarke *et al.*[26]. Incubation mixtures contained in 250 μL: glycosphingolipid, 10 nmol; sodium taurocholate, 100 μg; citrate–phosphate buffer, pH 3.8, 10 μmol; and α-D-galactosidase, 500 mU. Incubations were terminated after 20 h. All samples were kept at 37° in the presence of toluene. Glycosphingolipids recovered from the incubation mixtures were examined by t.l.c. in solvent system *B* or *C*.

*Mild acid hydrolysis.* — To remove sialic acid residues, samples (10–50 nmol) of ganglioside were hydrolyzed for 90 min at 100° in 200 μL of 1% acetic acid. The samples were then concentrated to ~50 μL under a stream of nitrogen. The remaining water and acetic acid were frozen and removed in a dessicator over NaOH pellets.

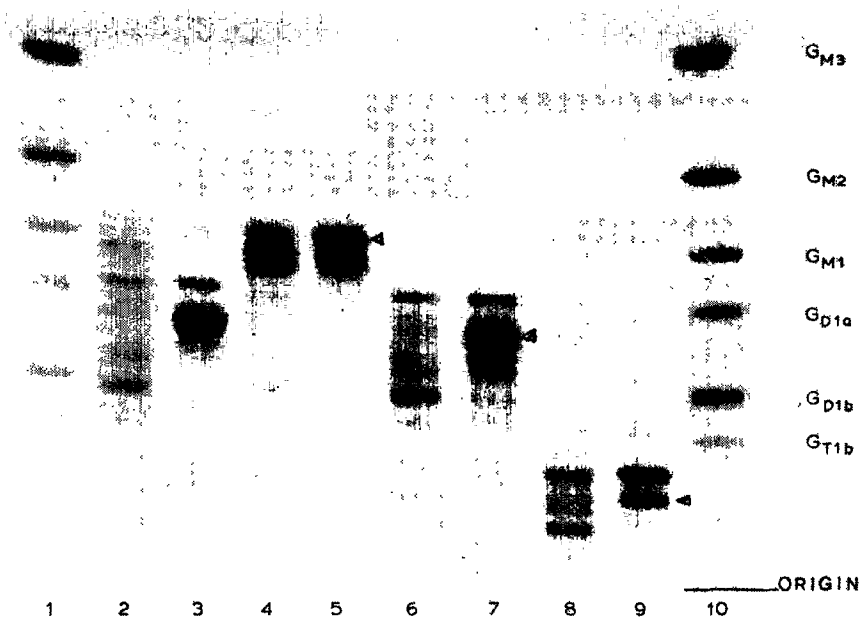


Fig. 1. Thin-layer chromatogram of murine thymus and thymoma gangliosides: 1 and 10, ganglioside standards; 2, unfractionated thymus gangliosides; 3, unfractionated thymoma gangliosides; 4, thymus monosialogangliosides; 5, thymoma monosialogangliosides; 6, thymus disialogangliosides; 7, thymoma disialogangliosides; 8, thymus trisialogangliosides; and 9, thymoma trisialogangliosides. Plate was developed with solvent system *A* (1 + 2) and stained with resorcinol–HCl. The major monosialo- and disialo-ganglioside, and also the trisialoganglioside that accumulates in thymoma are indicated by ▲.

RESULTS

Gangliosides isolated from murine thymomas or thymuses migrated on t.l.c. as at least 17 separate bands, some visible only after fractionation on columns of DEAE-Sepharose (Fig. 1). These patterns, though more complex than already described for extracts of murine thymus[15], are still simplified, as the alkaline hydrolysis used in our purification procedure removes *O*-acetyl groups known to occur in the sialic acid of murine thymus gangliosides[15]. After sequential t.l.c. in solvent systems *A* (1 + 2) and *B*, the major murine thymoma monosialo- $G_M$ and disialo-ganglioside $G_D$ migrated on chromatograms as single bands (Fig. 2). From ~140 g of wet thymoma tissues we recovered 190 nmol of $G_M$ ganglioside and 390 nmol of $G_D$ ganglioside. These preparations were analyzed in their native and asialo forms (obtained after removal of sialic acid residues by acid hydrolysis). Slightly less-pure fractions (not purified by t.l.c. in solvent system *B*) were examined for their susceptibility to *Vibrio cholerae* neuraminidase (Fig. 3).

*Carbohydrate composition.* — GM and $G_D$ gangliosides consisted of glucose, galactose, and *N*-acetylgalactosamine in a ratio close to 1:2:1. Moreover, $G_M$

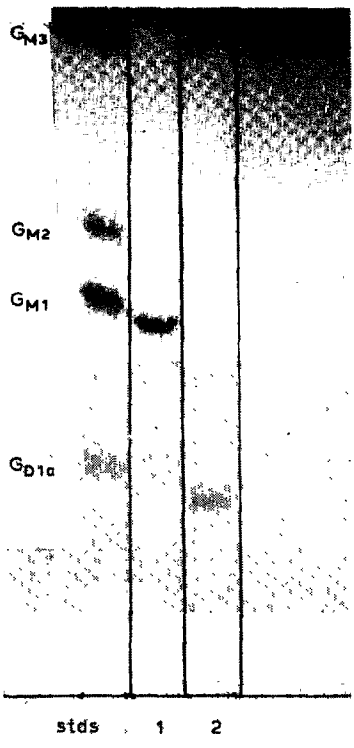


Fig. 2. Thin-layer chromatogram of purified murine thymoma gangliosides: 1, monosialoganglioside, $G_M$; 2, disialoganglioside $G_D$; and Stds., ganglioside standards. The plate was developed with solvent system *B* and stained with resorcinol–HCl.

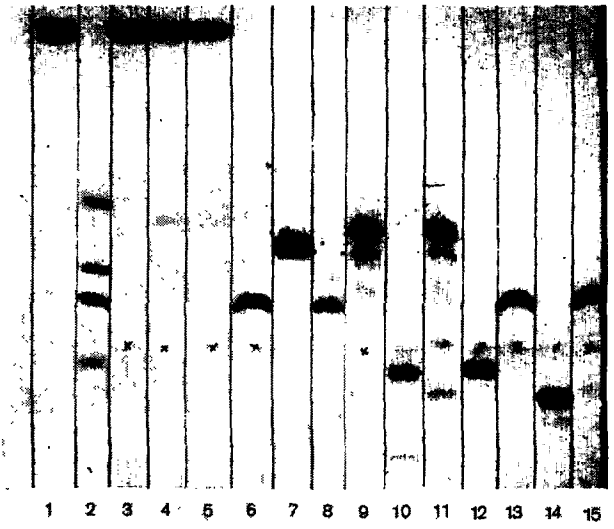


Fig. 3. Thin-layer chromatogram of gangliosides treated with *Vibrio cholerae* neuraminidase: 1, lactosylceramide standard; 2, standards of $G_{M3}$, $G_{M2}$, $G_{M1}$, and $G_{D1a}$ gangliosides (top to bottom); 3, $G_{M3}$ containing *N*-acetylneuraminic acid, treated with neuraminidase; 4 and 5, $G_{M3}$ containing *N*-glycolylneuraminic acid, treated with neuraminidase; 6, $G_{M1}$ treated with neuraminidase; 7, $GgOse_4Cer$ standard; 8, murine thymoma $G_M$; 9, murine thymoma $G_M$ treated with neuraminidase; 10, murine thymoma $G_D$; 11, murine thymoma $G_D$ treated with neuraminidase; 12, $G_{D1a}$; 13, $G_{D1a}$ treated with neuraminidase; 14, $G_{D1b}$; and 15, $G_{D1b}$ treated with neuraminidase. The plate was developed with solvent system *B*.

ganglioside contained one and $G_D$ ganglioside two molecules of neuraminic acid (Table I).

*Methylation analysis.* — The results obtained for native and asialo derivatives of $G_M$ and $G_D$ gangliosides are presented in Table II. Removal of neuraminic acid from $G_M$ and from $G_D$ ganglioside results in the replacement of 2,4,6-tri-*O*-methylgalactitol by 2,3,4,6-tetra-*O*-methylgalactitol. This result indicates that the penultimate galactose residue in $G_M$ ganglioside is substituted at O-3 by one molecule of neuraminic acid, and in $G_D$ ganglioside by a neuraminylneuraminyl group. The remaining, partially methylated alditol acetates (identical for both gangliosides) should result from a common ganglio-*N*-tetraose backbone.

*Characterization of the NeuAc-NeuAc linkage.* — After methylation followed by methanolysis and reacetylation, the sialic acid residues of $G_D$ ganglioside gave rise to the permethylated methyl glycoside methyl ester and 8-*O*-acetyl-4,7,9-tri-*O*-methyl derivative of the methyl glycoside methyl ester of *N*-acetylneuraminic acid (Fig. 4). This result identifies the NeuAc(2→8)NeuAc sequence in the $G_D$ ganglioside.

*Anomeric configuration and sequence of sugar units.* — Neuraminic acid residues of $G_M$ and $G_D$ gangliosides could be readily split off by *Vibrio cholerae* neuraminidase, indicating that they are α-linked to the IV galactopyranosyl residue

TABLE I

MOLAR RATIOS OF SUGAR CONSTITUENTS OF MURINE THYMOMA $G_M$ AND $G_D$ GANGLIOSIDES

| *Molar ratios of carbohydrates (Glc = 1.0)* | $G_M$ | $G_D$ |
|---|---|---|
| Glucose | 1.0 | 1.0 |
| Galactose | 1.9 | 2.1 |
| *N*-Acetylgalactosamine | 1.0 | 1.0 |
| *N*-Acetylneuraminic acid | 1.0 | 2.0 |

TABLE II

PARTIALLY METHYLATED ALDITOL ACETATES IDENTIFIED[a] IN HYDROLYZATES OF PERMETHYLATED NATIVE AND ASIALO DERIVATIVES OF MURINE THYMOMA $G_M$ AND $G_D$ GANGLIOSIDES

| *Alditol acetate* | $G_M$ | | $G_D$ | |
|---|---|---|---|---|
| | *native* | *asialo* | *native* | *asialo* |
| 2,3,4,6-Tetra-*O*-methyl-Gal-ol | – | + | – | + |
| 2,4,6-Tri-*O*-methyl-Gal-ol | + | – | + | – |
| 2,3,6-Tri-*O*-methyl-Gal-ol | + | + | + | + |
| 4,6-Di-*O*-methyl-2-deoxy-2-*N*-methylacetamido-Gal-ol | + | + | + | + |
| 2,3,6-Tri-*O*-methyl-Glc-ol | + | + | + | + |

[a]Partially methylated alditol acetates were identified by g.l.c.–m.s. as described under Experimental. The presence of a particular partially methylated alditol acetate is indicated by + and its absence by –.

of $GgOse_4Cer$ in the mono- and di-sialo sequence, respectively. The asialo ($G_M$) and asialo ($G_D$) ganglioside products from reaction with neuraminidase had identical t.l.c. mobility and migrated in solvent system *B* slightly ahead of a standard of bovine brain $GgOse_4Cer$ (Fig. 3). The sequence of the remaining sugar residues and type of linkages were determined with jack bean β-D-galactosidase and *Charonia lampas* *N*-acetyl-β-hexosaminidase (Table III). The terminal non-reducing galactopyranosyl residues of asialo $G_M$ and asialo $G_D$ gangliosides were susceptible to hydrolysis by jack bean β-D-galactosidase and gave products migrating on t.l.c. in the trihexosylceramide region (not shown). When jack bean β-D-galactosidase and *Charonia lampas* *N*-acetyl-β-hexosaminidase were used in the same incubation mixture, reaction products having t.l.c. mobility similar to GlcCer were detected (not shown). Asialo $G_M$ and asialo $G_D$ gangliosides showed susceptibility to various exoglycosidases similar to that of a $GgOse_4Cer$ standard, being resistant — under our experimental conditions — to *Charonia lampas* β-D-galactosidase.

*Characterization of ceramide moiety.* — The fatty acid composition of $G_M$ and $G_D$ gangliosides is presented in Table IV. The most abundant fatty acids in these gangliosides are $C_{20:0}$ and $C_{22:1}$. The major long-chain bases of $G_D$ ganglioside

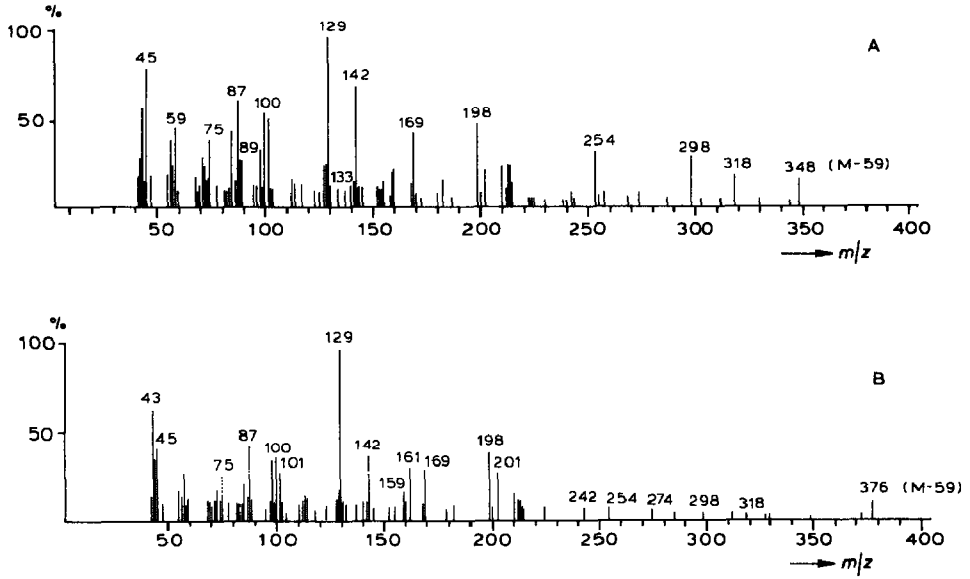


Fig. 4. A. Mass spectrum of the permethylated methyl glycoside methyl ester of *N*-methylacetamidoneuraminic acid. The ions at *m/z* 45, 89, and 133 indicate that positions 9, 8, and 7, respectively, are methylated. Methylation at position 7 is supported by the presence of the intense ion at *m/z* 318. No ion at *m/z* 161 is present. B. Mass spectrum of the 8-*O*-acetyl-4,7,9-tri-*O*-methyl derivative of the glycoside methyl ester of *N*-methylacetamidoneuraminic acid. The presence of ions at *m/z* 45 and 318 indicate methylation at positions 9 and 7, respectively. The ion at *m/z* 161 gives evidence for the presence of an acetoxyl group in the side chain at position 8, and is supported by the absence of ions at *m/z* 89 and 133. Interpretation was according to Rauvala and Kärkkäinen[21] who have not reported, however, the diagnostic value of the ion at *m/z* 133 derived from the permethylated C-7–9 fragment. M.s. conditions were: ionization potential 70 eV, temperature of ion source 148°; and temperature of the transfer line, 240°. G.l.c. conditions: a 12-m capillary column made of fused silica and coated with 5% crosslinked phenylmethyl silicone. The column was heated at 100° for 5 min and then programmed to 220° at 4°/min.

are sphingosine and phytosphingosine. The long-chain base composition of $G_M$ ganglioside was not determined.

In conclusion, the structure of $G_M$ ganglioside from murine thymoma may be described as αNeu(2→3)βGal(1→3)βGalNAc(1→4)βGal(1→4)Glc(1→1)Cer and that of $G_D$ ganglioside as αNeuAc(2→8)αNeuAc(2→3)βGal(1→3)βGalNAc-(1→4)βGal(1→4)Glc(1→1)Cer.

## DISCUSSION

$^3$IVNeuGgOse$_4$ceramide, namely, the $G_{M1b}$ ganglioside identified in this report as the major monosialoganglioside in murine thymoma, has been described first as a reaction product of rat-brain sialyltransferase[27]. Later, a $G_{M1b}$ ganglioside containing *N*-acetylneuraminic acid[28–31] and *N*-glycolylneuraminic acid[32] has been isolated from several sources. In murine thymus, a monosialoganglioside having

TABLE III

THE SUSCEPTIBILITY OF ASIALO $G_M$, ASIALO $G_D$ DERIVATIVES OF MURINE THYMOMA GANGLIOSIDES AND GLYCOSPHINGOLIPID STANDARDS TO VARIOUS EXOGLYCOSIDASES[a]

| *Exoglycosidase* | *Glycosphingolipid used as substrate* | | | | |
|---|---|---|---|---|---|
| | *asialo $G_M$* | *asialo $G_D$* | *$GgOse_4Cer$* | *$nLcOse_4Cer$* | *$GbOse_3Cer$* |
| α-D-Galactosidase, coffee bean | − | − | − | − | + |
| β-D-Galactosidase, *Charonia lampas* | − | − | − | + | nt |
| *N*-Acetyl-β-hexosaminidase, *Charonia lampas* | − | − | − | −[b] | nt |
| β-D-Galactosidase, jack bean | + | + | − | + | nt |
| β-D-Galactosidase, jack bean and *N*-Acetyl-β-hexosaminidase *Charonia lampas* | ++ | ++ | ++ | ++ | nt |

[a]Glycosphingolipids were incubated with exoglycosidase preparations as described under Experimental. Removal of one sugar residue as judged by increase in t.l.c. mobility is indicated by +. Appearance of reaction product displaying t.l.c. mobility similar to GlcCer is indicated by ++. No change is marked by −; nt, not tested. [b]The *Charonia lampas* *N*-acetylhexosaminidase preparation was contaminated with β-D-galactosidase[10], resulting in some hydrolysis of $nLcOse_4Cer$.

TABLE IV

FATTY ACIDS IN MURINE THYMOMA GANGLIOSIDES (% OF TOTAL)

| *Fatty acids* | *Ganglioside* | |
|---|---|---|
| | $G_M$ | $G_D$ |
| $C_{16:0}$ | 6.9 | 4.9 |
| $C_{16:1}$ | 6.0 | 5.4 |
| $C_{18:0}$ | 6.9 | 4.3 |
| $C_{20:0}$ | 19.3 | 27.8 |
| $C_{20:1}$ | 14.5 | 5.1 |
| $C_{22:0}$ | 16.8 | 17.4 |
| $C_{22:1}$ | 18.8 | 30.6 |
| Unidentified | 10.8 | 4.5 |

neuraminidase-sensitive sialic acid and most probably a $GgOse_4$ ceramide backbone has been detected[33], but its structure has not been confirmed by chemical analysis.

The structure of $IV^3(NeuAc)_2GgOse_4$ ceramide, the major disialoganglioside in murine thymoma, has not yet been described. Nevertheless, a disialoganglioside

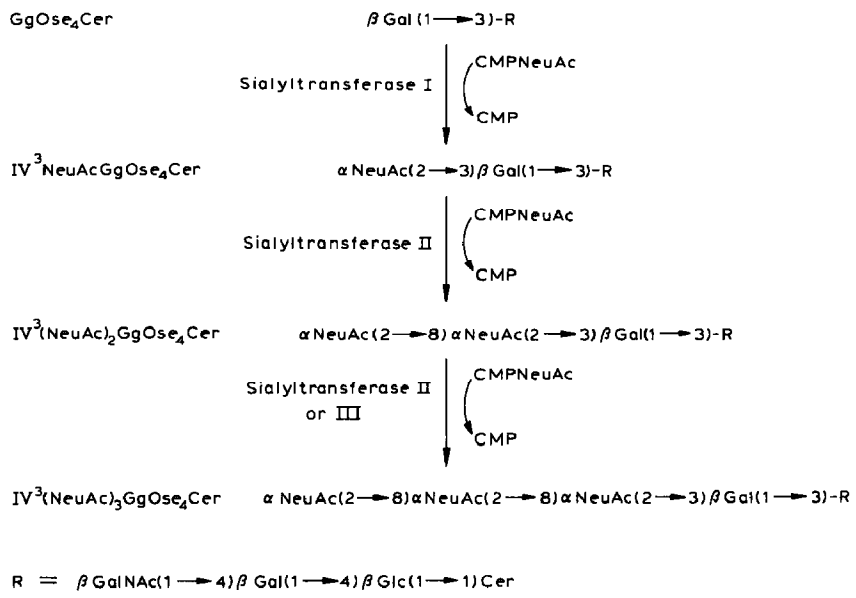


Fig. 5. Proposed biosynthetic pathway of the disialo and trisialo sequence attached to O-3 of the terminal galactose residue of $GgOse_4Cer$.

having the same sugar composition as $G_{D1a}$ ganglioside but susceptible to *Clostridium perfringens* neuraminidase has been found in murine fat-globule membrane and mammary tumor virus[34]. More recently, a neuraminidase-sensitive disialoganglioside consisting of *N*-acetylneuraminic and *N*-glycolylneuraminic acids and most probably a $GgOse_4$ceramide backbone has been isolated from murine thymus[15]. Murine fat-globule and murine thymus gangliosides may have a $IV^3(NeuAc)_2GgOse_4$ceramide structure, but the linkages of sialic acid have not been determined. The terminal galactose group of $GgOse_4Cer$ may be sialylated[35] at both O-3 and O-6 or substituted at either O-3 or O-6 by two sialyl residues.

The biosynthetic pathway leading to $IV^3(NeuAc)_2GgOse_4$ceramide should consist of two consecutive reactions catalyzed by specific sialyltransferases (Fig. 5). The first of these enzymes, synthesiszing $G_{M1b}$ ganglioside, has been detected in a variety of cells and tissues[27,30,36]. The $G_{M1b}$ ganglioside could be a substrate of the second sialyltransferase catalyzing the formation of the sialyl$\alpha$(2→8)sialyl linkage. The biosynthesis of neuraminidase-sensitive disialyl-$GgOse_4$ceramide has been reported by Kaufman *et al.*[37] and later by Stoffyn and Stoffyn[36]. The latter authors have shown that the sialyltransferase reaction-product, namely the disialyl residue, was linked at O-3 to the terminal nonreducing galactopyranosyl residue of $GgOse_4$ceramide used as substrate. This was the first example for the biosynthesis of $IV^3(NeuAc)_2GgOse_4$ceramide. Surprisingly, the intersialic linkage of the reaction product was not of the $\alpha$-(2→8) type and therefore it is probably not an intermediate in the biosynthesis of trisialogangliosides[36]. In contrast, $\alpha$NeuAc-

(2→8)αNeuAc(2→3)$GgOse_4$Cer as characterized in this report may be a substrate in reactions leading to the formation of more-complex gangliosides. Recently we have treated trisialoganglioside fractions isolated from both murine thymoma and thymus with *Vibrio cholerae* neuraminidase and have observed formation of a glycosphingolipid migrating on t.l.c. as a $GgOse_4$Cer standard[38], thus suggesting the structure $(NeuAc)_3GgOse_4$ceramide for the native compound. The biological role of $G_{M1b}$ ganglioside and its presently described disialyl derivative remains unknown as yet; because of their neuraminidase susceptibility they could be substrates in a sialidase–sialyltransferase(s) cycle. Such a cycle could provide the mechanism for fine regulation of the distribution of sialic acid, an important constituent of glycoconjugates at the surface of the cell[39].

ACKNOWLEDGMENTS

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NOTE ADDED IN PROOF

After the typesetting of this paper it came to our attention that the internal residue of sialic acid in the disialosyl-sequence is substantially de-*N*-acylated during methanolysis of methylated glycoconjugates in 0.5M methanolic HCl (S. Inoue *et al., Anal. Biochem.*, 125 (1982) 118–142). Nevertheless, this residue in GD1c should carry *N*-acetyl rather than *N*-glycolyl substituents since not even a trace of the 8-*O*-acetyl-4,7,9-tri-*O*-methyl derivative of the methyl glycoside methyl ester of *N*-glycolylneuraminic acid was found among acetylated methanolysis products of the methylated glycolipid.

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