THE STRUCTURE OF THE TETRASIALOGANGLIOSIDE FROM HUMAN BRAIN

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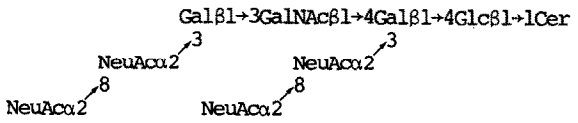
1. Introduction

The development of a new procedure for isolating gangliosides from tissue and separating them on an anion exchange resin, Spherosil–DEAE-dextran, according to their number of sialic acids has made it possible to isolate gangliosides with 3 or more sialic acids with a homogenous carbohydrate moiety in a high yield [1–3]. We have applied the new procedure in the isolation of tetra- and pentasialogangliosides from human infant brain. By partial sialidase and mild acid hydrolysis, component and methylation analysis and mass spectrometry of the permethylated and the permethylated-reduced-silylated derivatives of the intact ganglioside, the structure of the major tetrasialoganglioside from human brain was shown to be:



Abbreviations and nomenclature: GC-MS, gas-liquid chromatography–mass spectrometry; GalNAc, *N*-acetylgalactosamine; NeuAc, *N*-acetylneuraminic acid; cer, ceramide; PTLC, high performance liquid chromatography

Abbreviations for gangliosides follow the nomenclature system of Svennerholm, IUPAC–IUB Commission on Biochemical Nomenclature CBN, The Nomenclature of Lipids [14]

GM1, II^3NeuAc-$GgOse_4$Cer; GD1a, IV^3NeuAc, II^3NeuAc-$GgOse_4$Cer; GD1b, $II^3(NeuAc)_2$-$GgOse_4$Cer; GT1a, $IV^3(NeuAc)_2$, II^3NeuAc-$GgOse_4$Cer; GT1b, IV^3NeuAc, $II^3(NeuAc)_2$-$GgOse_4$Cer; GQ1b, $IV^3(NeuAc)_2$, $II^3(NeuAc)_2$-$GgOse_4$Cer

2. Materials and methods

2.1. *Chemicals*

The materials and methods for isolating a crude ganglioside extract and the assay with (TLC) were described in [1]. Spherosil–DEAE-dextran, consisting of porous glass beads covered with cross-linked DEAE-dextran, was prepared as in [2]. References sugars were puchased from Pfanstiehl Labs., Waukegan, IL. *Vibrio cholerae* sialidase (EC 3.2.18) was from Behring-Werke AG, Marburg-Lahn.

2.2. *Isolation of tetrasialoganglioside*

Crude gangliosides were isolated from 500 g of a 3-month-old human infant forebrain with chloroform/methanol/water (4:8:3, by vol.) and after purification [1] separated on two identical Spherosil–DEAE-dextran columns [3]. The gangliosides were eluted with a discontinuous gradient of potassium acetate in methanol. Tetrasialogangliosides appeared in the 0.5 M KAc in methanol effluent. After dialysis against water the fraction was redissolved in 10 ml chloroform/methanol/water (30:60:20, by vol.), and undissolved material was removed by centrifugation. The solution was then passed through a column of 2 g silica gel. The gangliosides were eluted with 40 ml of the same solvent. A major tetrasialoganglioside band was isolated by chromatography on precoated TLC plates developed in 1-propanol/0.25% aqueous KCl (7:3, v/v) for 4 h. The plates were sprayed with bromthymol blue, the tetrasialoganglioside band was scraped off and eluted from the gel, placed in small columns with sintered discs, with chloroform/methanol/water (30:60:20, by vol.). The purity of the tetrasialoganglioside fraction was assayed by HPTLC in several solvent systems [3].

2.3. *Determination of components*

Sialic acid was assyed with the resorcinol procedure [4] and the other components with methods described in [5].

2.4. *Partial acid and enzymic degradation*

Partial acid hydrolysis was performed with either 0.01 M formic acid at 80°C for 30 min or with 1.0 M formic acid for 30 min in a boiling water bath. Gangliosides and neutral glycolipids formed were analysed by TLC with solvents and spray reagents described in [5].

Hydrolysis with *Vibrio cholerae* sialidase was performed in 0.01 M Tris–maleate buffer (pH 6.5) containing 4 mM $CaCl_2$. Incubation was done in a water bath at 37°C and samples were taken out after 15, 30 and 60 min. After removal of salts and liberated sialic acids on mini-columns with Sephadex G-25 [6], the glycolipid products were assayed by HPTLC [5].

2.5. *Periodate oxidation–borohydride reduction*

Periodate oxidation and borohydride reduction of the tetrasialoganglioside was performed according to [7]. The ganglioside was thereafter subjected to methanolysis and with GLC [8] the sialic acids were analyzed as their TMS derivatives.

2.6. *Permethylation studies*

The ganglioside was permethylated by the method in [9], hydrolysed, reduced, acetylated and analysed by GC–MS, as in [5].

2.7. *Mass spectrometric analyses*

The gangliosides were permethylated according to [9], reduced with $LiAlH_4$ and silylated [10]. The mass spectrometer used was a MS 902 instrument (AEI Ltd, Manchester). The handling of the samples has been discussed in [10]. To obtain fragments of higher weight the temperature of the ion source was adjusted to the evaporation temperature of the sample, and the sample cuvette was placed just in the electron beam [11]. The mass numbers were obtained by counting by hand and are therefore nominal masses. Owing to the high content of hydrogen atoms (mass 1.008) the exact masses in the upper regions are one unit higher than those reproduced.

3. Results and discussion

The concentration of the tetrasialoganglioside of human infant forebrain was found to be 16 μmol ganglioside/kg wet wt or 4% of the total lipid-NeuAc. The recovery of the tetrasialoganglioside was ~80%. The molar ratio of the components confirmed that it was a tetrasialosylgangliotetraosylceramide (table 1). Hydrolysis of the tetrasialoganglioside in 0.01 M formic acid led to the formation of roughly equal amounts of gangliosides, which at TLC migrated like GT1a, GT1b, GD1a, GD1b and GM1. When 1.0 M formic acid was used, the predominant product was a glycolipid, which migrated like gangliotetraosylceramide. Minor portions of gangliotetraosylceramide and lactosylceramide were also formed. On TLC the primary product of hydrolysis after sialidase treatment of the tetrasialoganglioside migrated like authentic GT1b. Continued hydrolysis led to the formation of ganglioside GD1b and minor amounts of GD1a (the ratio was 10–20:1 in different experiments). The final product was ganglioside GM1, which was identified by comparing its capacity to bind cholera toxin with authentic GM1 [12].

Table 1
Molar ratio of components of tetrasialoganglioside

Sphingosine	1.00
N-Acetylneuraminic acid	3.93
Glucose	1.03
Galactose	1.84
Galactosamine	0.73

Mass spectra of derivatives of the intact ganglioside provide conclusive evidence for the GQ1b sequence. The cluster of ions seen at *m/e* 2747 in fig.1 and produced from the permethylated-reduced derivative is characteristic [10,13] of the complete saccharide and the fatty acid (see top formula). This supports the data in table 1 giving all together 8 sugars including 4 sialic acids. Specific primary octasaccharide ions are indicated at *m/e* 2422, 2438 and 2452, and these may lose a varying number of mass units including part of a sialic acid (e.g., 391 mass units, which is 406–15). This is shown at *m/e* 1235, 1640, 2031, 2350, 2364 and 2378.

The sequence of the 8 sugars is inferred from a number of peaks in both spectra. A terminal saccharide of two sialic acids is shown in fig.1 by peaks at *m/e* 360, 374, 390, 392, 406 and 797, and in fig.2 by peaks at *m/e* 330, 344, 362, 376, 677 and 737. There is no evidence of a trisaccharide of sialic acids (expected at *m/e* 1188 in fig.1 or 1098 in fig.2). Two

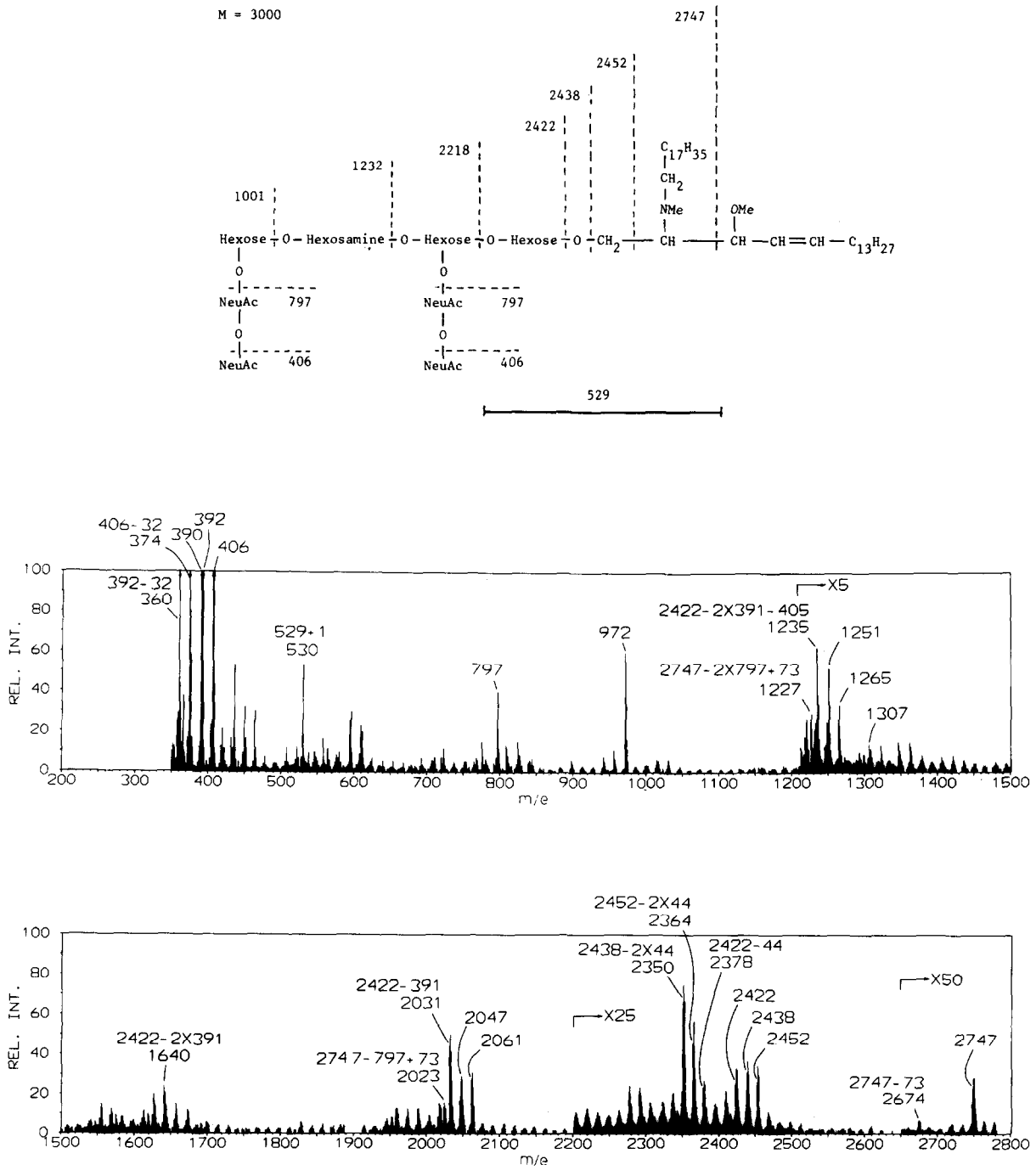


Fig.1. Mass spectrum of the permethylated, reduced and trimethylsilylated ganglioside. The formula above the spectrum gives the major molecular species found. Ions were recorded in the molecular peak region (*m/e* 3000), but their nominal masses were uncertain and therefore not included in the figure. The peaks at *m/e* 360, 374, 390, 392, 406 and 407 exceeded the range of the oscillographic recording but are given as 100% in the figure. Ions below *m/e* 350 were not recorded. The sample cuvette was introduced just at the electron beam (in-beam technique) at an ion source temperature of 280°C. Further conditions of analysis were: electron energy 38 eV; trap current, 500 μA; acceleration voltage, 3 kV.

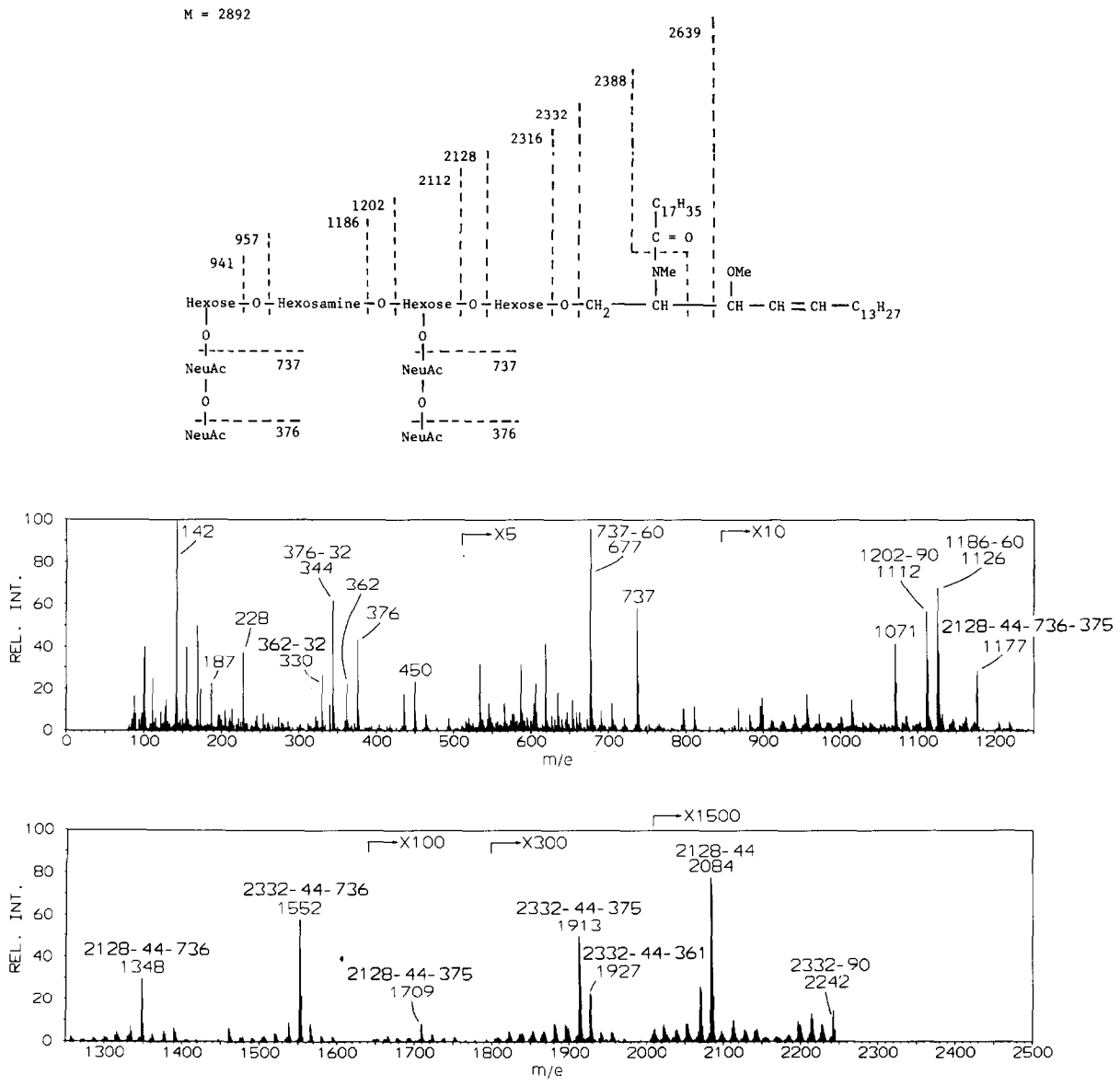
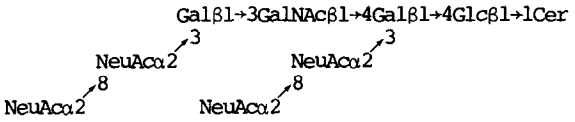


Fig.2. Mass spectrum of the permethylated ganglioside. The formula above the spectrum gives the major molecular species found. The sample cuvette was introduced just in the electron beam (in-beam technique) at an ion source temperature of 280°C. Further conditions of analysis were: electron energy 28 eV; trap current 500 μA; acceleration voltage, 4 kV.

rather intense peaks at *m/e* 1112 and 1126 in fig.2 are produced by the terminal tetrasaccharide rest, sialyl–sialyl–hexosyl–hexosaminyl. Addition of the inner galactose with two sialic acids to this terminal tetrasaccharide gives the peak at *m/e* 2084 in fig.2. The 4 sialic acids should therefore be located as shown in the formula. That hexose is bound to ceramide is shown by the peak at *m/e* 530 in fig.1 which also contains stearic acid.

Analysis of the permethylated sugars from the tetrasialoganglioside by GLC and GC–MS showed that it contained 2,3,6-tri-*O*-methylglucitol, 2,4,6-tri-*O*-methylgalactitol, 2,6-di-*O*-methylgalactitol and 4,6-di-*O*-methyl-2-deoxy-2-*N*-methylacetamidogalac-

titol. The permethylation studies showed that sialic acid is linked to both the internal and the external galactose molecules in a 2→3 linkage. The periodate oxidation and borohydride reduction gave rise to approximately equal amounts of intact sialic acid and the 7-carbon derivative of sialic acid, indicating that 2 of the 4 sialic acids were substituted at the C8 position. The combined results of the mass spectrometric analysis, the component analysis and the studies of the partially methylated alditol acetates suggest the following structural formula for the tetrasialoganglioside isolated from human brain:



Acknowledgement

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References

[1] Svennerholm, L. and Fredman, P. (1980) Biochim. Biophys. Acta in press.
[2] Tayot, J.-L., Tardy, M., Gaffel, P., Plan, R. and Roumantzeff, M. (1978) in: Chromatography of Synthetic and Biological Polymers (Epton, R. ed) vol. 2, pp. 95–110, Ellis Horwood, Chicester.
[3] Fredman, P., Nilsson, O., Tayot, J.-L. and Svennerholm, L. (1980) Biochim. Biophys. Acta in press.
[4] Svennerholm, L. (1957) Biochim. Biophys. Acta 24, 604–611.
[5] Svennerholm, L., Vanier, M.-T. and Månsson, J.-E. (1980) J. Lipid Res. 21, 53–64.
[6] Wells, M. A. and Dittmer, J. C. (1963) Biochemistry 2, 1259–1263.
[7] Ando, S. and Yu, R. K. (1977) J. Biol. Chem. 252, 6247–6250.
[8] Yu, R. K. and Ledeen, R. W. (1970) J. Lipid Res. 11, 506–516.
[9] Hakomori, S.-I. (1964) J. Biochem. 55, 205–208.
[10] Karlsson, K.-A. (1974) Biochemistry 13, 3643–3647.
[11] Dell, A., Williams, D. H., Morris, H. R., Smith, G. A., Feeney, J. and Robert, G. C. K. (1975) J. Am. Chem. Soc. 97, 2497–2502.
[12] Holmgren, J., Lönnroth, I., Månsson, J.-E. and Svennerholm, L. (1975) Proc. Natl. Acad. Sci. USA 72, 2520–2524.
[13] Karlsson, K.-A. (1977) Progr. Chem. Fats Other Lipids 16, 207–230.
[14] IUPAC–IUB Commission on Biochemical Nomenclature CBN, The Nomenclature of Lipids (1977) Eur. J. Biochem. 79, 11–21.