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Pentasialogangliosides of human brain

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A pentasialoganglioside fraction of the ganglio series of glycosphingolipids has been isolated from human cerebellar cortex with a yield of 8.8 nmol NeuAc per g fresh tissue. The structural analysis showed that the material was a mixture of GP1b and GP1c gangliosides.

Complex ganglioside; Pentasialoganglioside; Complex glycosphingolipid; Human brain; Human cerebellum

1. INTRODUCTION

Gangliosides constitute a heterogenous group of sialylated glycosphingolipids differing in both the oligosaccharide and ceramide composition. The majority of brain gangliosides belong to a ganglio series of glycolipids and contain invariable tetrasaccharide backbones with variable numbers of sialic acid residues [1,2]. The most abundant human brain gangliosides are GD1a, GD1b and GT1b, the most complex one described so far is GQ1b. Gangliosides with more than 4 sialic acid residues per mol commonly occur in brains of some phylogenetically lower animals, e.g. fish [3], but not in the human brain. The presumable biological function of highly sialylated glycosphingolipids includes the participation in developmental processes [4–6] and in conductance of signals in nervous cells [7,8]. Findings concerning cholinergic-specific oligosialogangliosides Chol-1 [8,9] are of great interest.

In a next paper (H. Miller-Podraza et al., in preparation) we will report on chromatographic evidence for the presence in the human brain of low quantities of highly sialylated ganglioside fractions migrating on thin-layer plates below ganglioside GQ1b. We also showed that the cerebellar cortex was a good source of pentasialogangliosides. In this paper we report data concerning structures of these new human brain gangliosides.

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Abbreviations: NeuAc, *N*-acetylneuraminic acid; Gal, galactose; Glc, glucose; HexNAc, *N*-acetylhexosamine; GalNAc, *N*-acetylgalactosamine. The ganglioside nomenclature is according to Svennerholm (Eur. J. Biochem. (1977) 79, 11–21). GP1, pentasialoganglioside; GP1b, $IV^3(NeuAc)_3II^3(NeuAc)_2GgOse_4Cer$; BP1c, $IV^3(NeuAc)_2II^3$-$(NeuA)_3GgOse_4Cer$.

2. EXPERIMENTAL

2.1. *Materials*

Human cerebellar tissue was obtained from the Department of Forensic Medicine, University of Göteborg. The cerebellar cortex was dissected from organs in our laboratory. Silica gel 60, 230–400 mesh and glass-backed HPTLC plates of silica gel 60 were from Merck, Darmstadt (Germany). Sephadex G-25 (fine) was from Pharmacia, Uppsala (Sweden). Standard human brain (GM3-GQ1b) and fish brain (GP1) gangliosides were isolated and characterized in our laboratory. All organic solvents used were of HPLC quality. All other chemicals used were of analytical quality and used without further purification.

2.2. *Isolation of pentasialogangliosides*

The cerebellar cortex (400 g) was homogenized in 1 vol. of water in a scissors homogenizer. Methanol and chloroform were added to make the final proportions of chloroform/methanol/water, 8:4:3 (v/v), and the tissue was extracted during stirring for 2 h. The mixture was centrifuged and the pellet was re-extracted once in 4 l of the same solvent system under the same conditions. The combined supernatants were evaporated and the material was divided into 2 parts, which were processed separately under the same conditions. Complex gangliosides were isolated from the crude lipids according to the method described in a forthcoming paper (H. Miller-Podraza et al., in preparation). Briefly: the material (corresponding to 200 g of the fresh tissue) was dissolved in 300 ml of chloroform/methanol/water, 65:25:4 (v/v), and applied to a column of 150 g of silica gel (silica gel packed in chloroform/methanol, 4:1, v/v). The column was eluted with: (1) chloroform/methanol/water, 65:25:4 (v/v), 600 ml; (2) chloroform/methanol/0.25% KCl in 2.5 M ammonia, 50:40:10 (v/v), 300 ml; (3) propanol/water, 7:3 (v/v). A fraction collector was used to monitor the separation of gangliosides in the last eluate (propanol/water). The fractions containing complex gangliosides were combined, evaporated, dissolved in a small volume of water and lyophilized. The dry material was suspended in 150 ml of chloroform/methanol/water, 65:25:4 (v/v), and applied to another silica gel column (150 g silica gel). The insoluble part of the material, which remained attached to the walls of the flask was dissolved in the smallest possible volume of water, followed by the addition of chloroform and methanol to make final proportions of chloroform, methanol and water, 65:25:4 (v/v). The washing was applied to the

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column. The column was run under identical conditions as the first one. Fractions containing gangliosides GQ1b and GP were collected, evaporated, subjected to alkaline hydrolysis (0.2 M NaOH, 37°C, 4 h, methanol/water, 2:1, v/v) and dialysed against distilled water for two days. The gangliosides were evaporated, dissolved in water and separated preparatively on HPTLC plates. After application of the material the plates were dreid carefully in a stream of warm air. The plates were developed in chrloroform/methanol/0.25% KCl in 2.5 M ammonia, 50:40:10 (v/v), dried in a stream of warm air, and developed once again in propanol/0.25% KCl in water, 7:3 (v/v). The gangliosides were visualized with a Bromophenol blue indicator. The band containing ganglioside GP was scraped from plates and extracted with chloroform/methanol/water, 30:60:20 (v/v) two times, and with propanol/water, 7:3 (v/v) three times. The combined extracts were evaporated and the material was dissolved in 1 ml of water. 6.25 ml of methanol and 16.2 ml of chloroform were added to the solution and the material was applied to a silica gel column (10 g of silica gel suspended in chloroform/methanol, 4:1 (v/v)). The column was eluted with 20 ml of chloroform/methanol/0.25% KCl in 2.5 M ammonia, 50:40:10 (v/v), and next with propanol/water 7:3 (v/v). The last eluate was monitored for the presence of gangliosides. Ganglioside GP was collected, evaporated, dissolved in water (buffered with 25 μM potassium carbonate buffer, pH 8.8) and stored frozen.

2.3. *Analytical methods*

Sialic acid was determined according to the method of Svennerholm [10], and sphingosine according to the modified procedure of Lauter and Trams [11]. The sensitivity of GP to sialidase was tested twofold. In the first test, the ganglioside (15 nmol NeuAc) was hydrolysed in 30 μl of 0.01 M Tris-maleate buffer (pH 6.5) containing 2 mM $CaCl_2$, 0.25% Triton X-100 and 0.5 U/ml of *Vibrio cholerae* neuraminidase (E.C. 3.2.1.18, Behringwerke, Marburg Lahn, Germany) for 20 h at 37°C. Samples of 5 μl were put directly on HPTLC plates, which were developed in chloroform/methanol/0.25% KCl in water, 50:40:10 (v/v). The plates were stained with orcinol or resorcinol and evaluated visually. In the second test, the ganglioside (1–6 pmol) was applied to the plate, which was then developed in chloroform/methanol/0.25% KCl in 2.5 M ammonia and treated with *Clostridium perfringens* neuraminidase (Sigma, USA) for 5 h at 37°C. The released ganglioside GM1 was quantified with cholera toxin B subunit and anti-B monoclonal antibody according to the TLC ELISA method previously described [12]. Fast-atom-bombardment mass spectrometry (FAB-MS) of the permethylated ganglioside was performed on a VG-ZAB mass spectrometer as previously described [13,14] with the exception that the material was acetylated prior to methylation. For acetylation the ganglioside was dissolved in formamide-pyridine-acetic anhydride, 10:5:4 (v/v) [15] and kept at room temperature overnight. The solution was next diluted 10 times with cold distilled water and dialysed against distilled water for two days. The acetylated ganglioside was evaporated, dissolved in chloroform/methanol, 2:1 (v/v) and applied to a 10×20 cm HPTLC plate, which was developed in chloroform/methanol/0.25% KCl, 50:40:10 (v/v) for approx. 10 min (solvent migration = 2.5 cm). The gel corresponding to ganglioside GP (1.3 cm wide zone adjacent to the solvent front) was scraped from the plate and extracted three times with chloroform/methanol/water, 60:30:4.5 (v/v). After evaporation of solvents, the ganglioside was dissolved in 2 ml of chloroform/methanol/water, 60:30:4.5 (v/v), and applied to 0.5 g of Sephadex G-25 (fine) suspended in the same solvent. The column was eluted with 2.5 ml of chloroform/methanol/water, 60:30:4.5 (v/v) and 2.5 ml of chloroform/methanol, 2:1 (v/v). The combined eluates were evaporated. The material was dried in vacuum and used for methylation [14].

3. RESULTS AND DISCUSSION

The pentasialoganglioside fraction was obtained from human cerebellar cortex with a yield of 8.8 nmol NeuAc per g of fresh tissue. The fraction migrated on HPTLC plates as a homogenous band (Fig. 1) and had a chromatographic mobility similar to that of standard fish brain GP1. The relative migrations of the isolated GP compared to human brain GD1b (R_{GD1b}), GT1b (R_{GT1b}) and GQ1b (R_{GQ1b}) were 0.42, 0.60 and 0.66, respectively. The isolated gangliosides contained sialic acid and sphingosine in a molar ratio of 1:4.53. The fraction was susceptible to sialidase from *Clostridium perfringens* and *Vibrio cholerae*, and was converted to ganglioside GM1. The formation of ganglioside GM1 from GP was demonstrated in a test tube reaction and on a plate treated with sialidase (see Section 2). The yield of the reaction after 5 h of incubation with the excess of *Clostridium perfringens* sialidase was 95%, as determined using the quantitative TLC ELISA method.

FAB-MS of the permethylated ganglioside revealed major molecular ions at *m/z* 3270 and 3298 corresponding to the composition $NeuAc_3$*$HexNAcHex_3$*Cer with C18:0 fatty acid and 4-sphingenine or 4-eicosasphingenine as the long chain base. Fragment ions at *m/z* 376 (344), 737 (705) and 1098 showed the presence of 3 sialic acids linked together. Intense ions found at *m/z* 1186 and 1547 showed the occurrence of the terminal carbohydrate sequences NeuAc-NeuAc-Hex-HexNAc- and NeuAc-NeuAc-NeuAc-Hex-HexNAc-. Ions of equal intensity found at *m/z* 576 and 604 corresponded to the ceramide composition C18:0 fatty acid with 4-sphingenine and 4-eicosasphingenine (Fig. 2a and 2b).

The combined structural analyses of the isolated pentasialoganglioside fraction suggest the following structures: NeuAcα2-8NeuAcα2-3Galβ1-3GalNAcβ1-

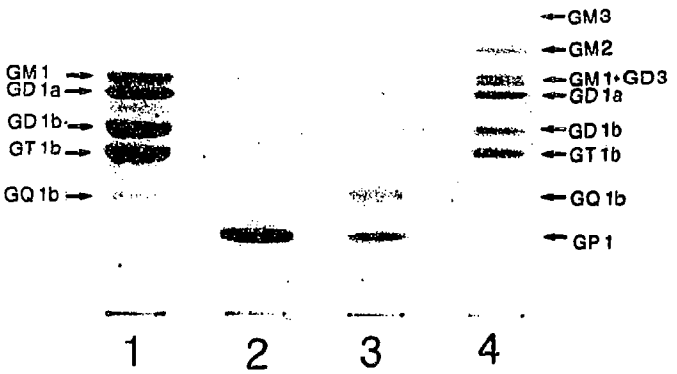

Fig. 1. Thin-layer chromatography of the pentasialoganglioside fraction isolated from human cerebellum. The plate was developed successively in two solvents: (a) chloroform/methanol/0.25% KCl in 2.5 M ammonia, 50:40:10 (v/v); (b) propanol/0.25% KCl in water, 7:3 (v/v), and stained with resorcinol. 1. Mixture of human brain gangliosides (total); 2. pentasialoganglioside fraction obtained from human cerebellar cortex; 3. standard mixture of fish brain GP1 and human brain GQ1b; 4. standard mixture of brain gangliosides.

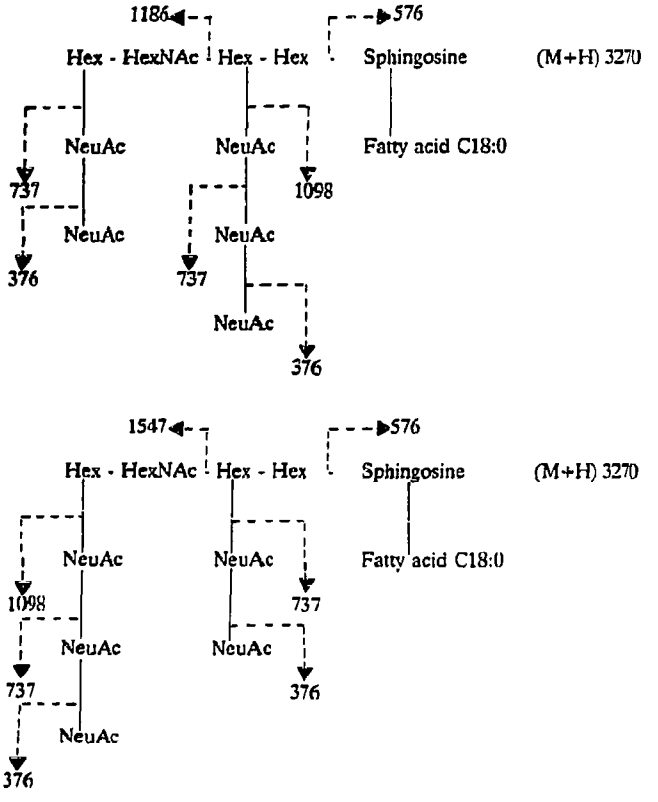

Fig. 2 (a). Fragmentation pattern obtained at positive FAB-MS of the permethylated pentasialoganglioside GP1c. (b) Fragmentation pattern obtained at positive FAB-MS of the permethylated pentasialoganglioside GP1b.

4[NeuAcα2 - 8NeuAcα2 - 8NeuAcα2 - 3]Galβ1 - 4Glcβ1-1Cer (ganglioside GP1c), and NeuAcα2-8NeuAcα2-8NeuAcα2-3Galβ1-3GalNAcβ1-4[NeuAcα2-8NeuAcα2-3] Galβ1-4Glcβ1-1Cer (ganglioside GP1b).

Gangliosides containing trisialosequences in molecules have not previously been isolated from human brain. Some indications of the presence in human nervous tissues of the gangliosides of the c-series come from immunochemical studies. The developmentally regulated antigen A2B5, which was reported to be structurally related to some oligosialogangliosides including GT3 and GQ1c [6,16], was found in human Alzheimer's neurofibrillary tangles, human fetal neurons [17], and human astrocytes [18]. The pentasialogangliosides isolated from human cerebellar cortex are probable candidates for immuno-reactive membrane components.

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