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SHORT COMMUNICATION

Acidic glycosphingolipids of cock testis elucidated by mass spectrometry and NMR spectroscopy

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The main acidic glycosphingolipids (GSLs) of cock testis were identified as GalCer I³-sulfate and gangliosides GM4, GM3, GD3 and GT3. They contained *N*-acetylneuraminic acid as the major sialic acid, and ceramides composed mainly of sphingosine (dl8:1) and C18–24 non-hydroxy fatty acids. Appreciable amounts of hydroxy fatty acids were detected only in the GM4 preparation.

Keywords: **ganglioside, sufatide, cock testis**

Introduction

Glycosphingolipids (GLSs) have been isolated from many tissues [1] including human [2,3] and animal [2,4–7] male germ cells. In mammalian sperm and testis GSLs constitute only a minor part of all glycolipids, while the major components are sulfogalactoglycerolipids (seminolipids) [8]. Non-mammalian vertebrates such as birds [2,9], fish [2,9], reptiles [9] or amphibians [9] contain, on the other hand, among germ cell glycolipids mainly GSLs. Information on testicular GSLs in different species is still limited. In the present investigation we report on the ganglioside and sulfatide composition of cock testis.

Materials and methods

Gangliosides were obtained by standard procedures [10,11]. Mass spectrometry analyses were performed on a Jeol SX-102 mass spectrometer. Negative ion fast atom bombardment (FAB MS) spectra were produced by Xe atoms (6kv) using triethanolamine as matrix. Electron ionization mass spectrometry (EI MS) of permethylated [12] GSLs was performed as described [13], and 500 MHz ^{1}H-NMR of GSLs was done in dimethylsulfoxide-d_6/D_2O (98:2) at 25° C.

Results and discussion

Chromatographic purity of the GSLs is shown in Fig. 1. GM4 resulted after desialylation in GalCer, while the other three gangliosides gave LacCer (D.P. Iga, unpublished data). The concentrations of gangliosides GM4, GM3, GD3, GT3 and sulfatide were 10.1, 6.8, 27.6, 12.6 and 400 nmol/g of wet tissue, respectively.

Fig. 2 shows FAB-MS spectra of the investigated GSLs. The dominating ceramide compositions are listed in Table 1. The predominant sphingosine in all samples agreed with dl8:1, however the sulfatide ion at m/z 862.6 could contain either d18:1 with 24:2 or d18:2 with 24:1. GM4 contained both hydroxylated and non-hydroxylated fatty acids as well as NeuAc and NeuGc, therefore different molecular species might overlap with each other. The same was the case for GM3, although the predominant ions agree well with

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The nomenclature is according to recommendations of IUPAC-IUB Commission on Biochemical Nomenclature (*Lipids* (1997) **12: 456–68; *J Biol Chem* (1982) **257:** 3347–51).

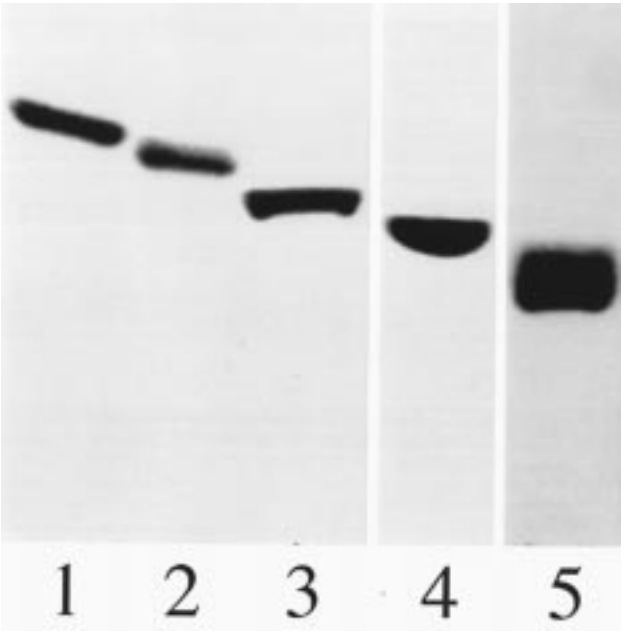

Figure 1. Purity verification of gangliosides GM4, GM3, GD3 and GT3 and sulfatide (shown in lanes 1–5, respectively). Silica gel 60 TLC plates (Merck, Germany) were developed in n-propanol-water-concentrated ammonia, 6:2:1, (v/v) (lanes 1–4) or in chloroform-methanol-acetone-acetic acid-water, 65:25:4 (v/v), lane 5), and visualised with resorcinol (lanes 1–4) or orcinol (lane 5).

common ceramides with non-hydroxylated fatty acids. The main sialic acid identified in GM3, GD3 and GT3 was NeuAc.

Pseudomolecular ions of GD3 appeared in FAB spectra as M-H and as sodium and potassium adduct ions. Pseudomolecular ions in EI spectra (M-59) as well as ceramide ions confirmed structures listed in Table 1. The ion at *m/z* 737 showed the presence of $(NeuAc)_2$. The FAB spectrum of GT3 was complicated since sodium and potassium adduct ions appeared in different combinations. Ceramide composition of this fraction was similar to GD3. EI MS confirmed the presence of $(NeuAc)_2$ and $(NeuAc)_3$. The major species of GD3 and GT3 may overlap with minor structures. For example, the NeuAc-GD3 ions at 1576.9, 1520.9, 1536.9 and 1592.9 (Table 1) agree also with NeuGc-GD3 and ceramides t18:0, 22:0 (or d18:1, h22:1), t18:0, 18:0 (or d18:1, h18:1), t18:0, h18:0, and t18:0, h22:0, respectively. However, these ceramides were not detected in appreciable amounts.

The sulfatide NMR spectrum revealed one β signal in the anomeric region at 4.15 ppm. The other ring proton signals were established by COSY and especially H-3 was found to be downfield-shifted (3.94 ppm) compared to the corresponding proton (3.29 ppm) of GalβCer [14], indicating a sulfate group in 3-position.

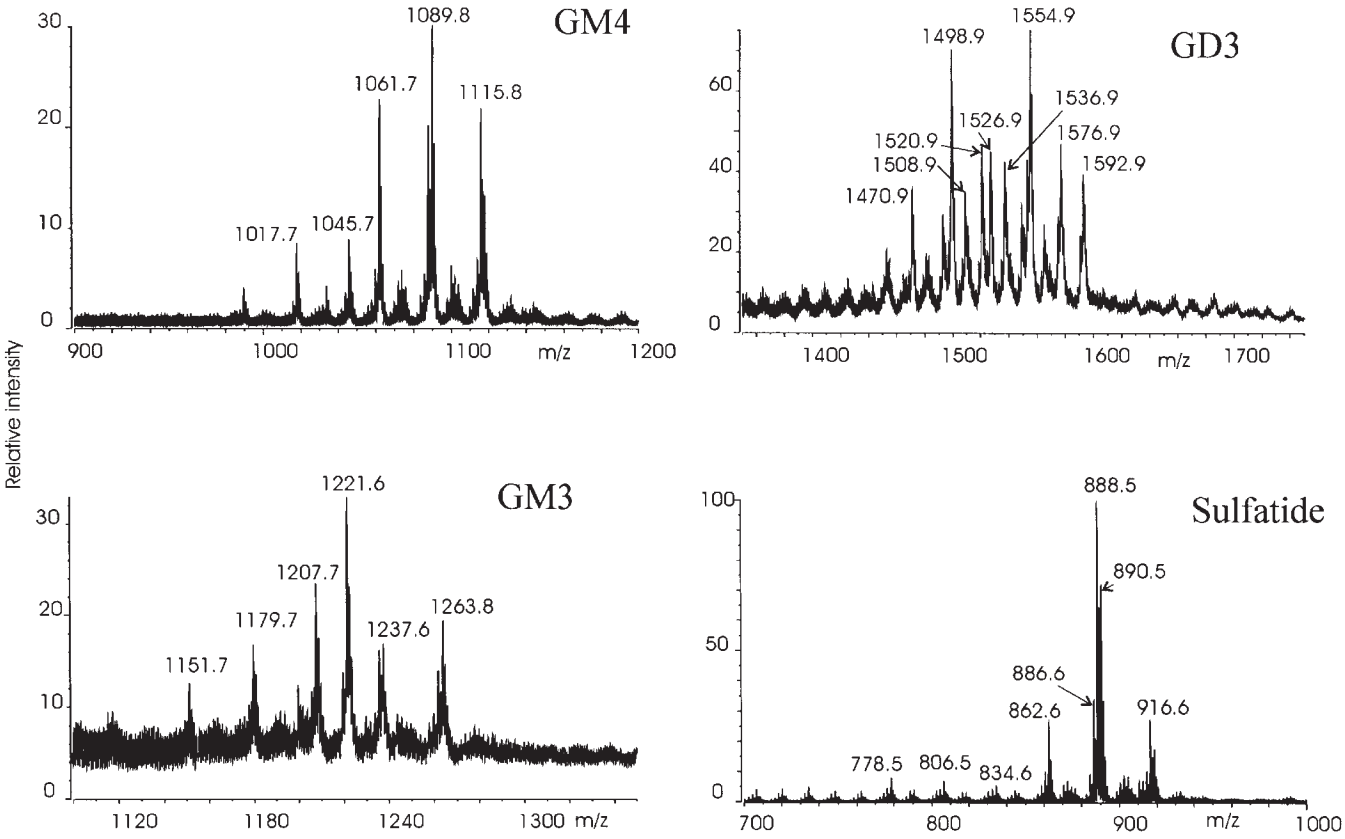

Figure 2. Negative ion fast atom bombardment (FAB MS) spectra of gangliosides GM4, GM3 and GD3 and sulfatide.

TABLE 1. Major molecular ions identified in negative ion FAB mass spectra of acidic glycosphingolipids prepared from cock testis. The ions (*m/z*) are shown in decreasing order of abundance. Data in parentheses indicate less probable or less abundant structures (based on the presence of ceramide and fatty acid ions).

*Identified glycolipid**	*m/z** composition	*Ceramide*
sufatide	888.5	d18.1, 24:1
SO_3-3GalβCer	890.5	d18:1, 24:0
	886.6	d18:1, 24:2 or d18:2, 24:1
	862.6	d18:1, 22:0
	916.6	d18:1, 26:1
	778.5	d18:1, 16:0
	806.5	d18:1, 18:0
	834.6	d18:1, 20:0
GM4 NeuAc(NeuGc)α3Galβ Cer	1089.8	d18:1, h22:0, NeuAc (d18:1, 22:0, NeuGc) (t18:0, 22:1, NeuAc)
	1061.7	d18.1, h20:0, NeuAc (d18:1, 20:0, NeuGc) (t18:0, 20:1, NeuAc)
	1115.8	d18:1, h24:1, NeuAc (d:18:1, 24:1, NeuGc)
	1087.8	d18:1, h22:1, NeuAc (d18:1, 22:1, NeuGc)
	1045.7	d18:1, 20:0, NeuAc
	1017.7	d18:1, 18:0, NeuAc
GM3 NeuAc(NeuGc)α3Galβ 4GlcβCer	1221.6	d18:1, 20:1 NeuGc and/or d18:1, h20:1, NeuAc
	1207.7	d18:1, 20:0, NeuAc
	1263.8	d18:1, 24:0, NeuAc
	1179.7	d18:1, 18:0, NeuAc
	1237.6	d18:0, 18:0, NeuAc
	1235.8	d18:1, 22:0, NeuAc
	1151.7	d18:1, 16:0, NeuAc
GD3 NeuAc(Gc)α8NeuAc (Gc)α3Galβ4GlcβCer	1554.9	d18:1, 24:0, NeuAc
	1576.9 (M-2H +Na)	
	1592.9 (M-2H +K)	
	1498.9	d18:1, 20:0, NeuAc
	1520.9 (M-2H +Na)	
	1536.9 (M-2H+K)	
	1526.9	d18:1, 22:0, NeuAc
	1470.9	d18:1, 18:0, NeuAc

*These structures are from combined interpretation of mass and NMR spectra.
**The values correspond to M-H, unless otherwise indicated.

The GM4 spectrum was similar to earlier published data for NeuAc-GM4 [15] as exemplified by the Galβ1 anomeric signal at 4:07 ppm and H-3_{eq} of NeuAcα3 at 2.77 ppm. However, the signal at 3.93 ppm indicates a significant proportion of *N*-glycolyl sialic acid [16]. GD3 displayed two β-anomeric signals at 4.17 ppm (Glcβ1) and 4.33 ppm (Galβ4). The H-3_{eq} and H-3_{ax} of NeuAcα8 were identified at 2.84 ppm and 1.25 ppm, respectively, whereas the corresponding signals of NeuAcα3 were difficult to locate. On the other hand, the acetamido methyl resonance at 1.87 ppm corresponds to at least six protons which could indicate two sialic acids. GT3 was not analyzed in detail due to the presence of other minor GSLs, but β-anomeric signals at 4.14 ppm (Glcβ1) and 4.31 ppm (Galβ4) were clearly seen as were two signals at 2.65 ppm and 2.70 ppm, the latter having twice the intensity of the former, due H-3_{eq} of

NeuAcα3 and NeuAcα8 [17]. The presence of NeuAcα3 in GT3 indicates that also in precursor gangliosides (GM3 and GD3) sialic acid is connected with Gal in 3-position.

The combined MS and NMR analyses agree with the following structures: SO_3-3GalβCer (sulfatide), NeuAc (Gc) α3GalβCer (GM4), NeuAc(Gc)α3Galβ4GlcβCer (GM3), NeuAc(Gc)α8NeuAc(Gc)α3Galβ4GlcβCer (GD3), and NeuAc (Gc)α8NeuAc(Gc)α8NeuAc(Gc)α3Galβ4GlcβCer (GT3).

Quantitative data show, that the predominant acidic GSLs of cock testis are SO_3-3GalβCer, GD3 and GT3, which is in agreement with previous reports on high concentrations of sulfogalactolipids in spermatocytes and spermatozoa of vertebrates [8]. The results also support earlier findings on the presence of sulfatides in bird germ cells. In testis of fowl and duck sulfogalactosylceramide was described as the only detectable sulfoglycolipid [2,9]. GD3 has earlier been reported as a characteristic component of undifferentiated tissues [18] and one of the major gangliosides of human semen [3]. It is symptomatic that the majority of acidic glycolipids of the male germ cells are highly negatively charged and may potentially bind cations and mediate ion transport or contribute to cell adhesion [8]. The importance of sulfoglycolipids in the fertilization process was supported by studies on a conserved, germ cell-specific protein SLIP1 which binds specifically *in vitro* to seminolipids and sulfatides. SLIP1 has been shown by various immunological techniques to be involved in the initial event in egg-sperm interaction [19]. Surface glycoconjugates are believed to be of importance for differentiation [20] and interaction [19,21] of gametes, however the full understanding of cellular functions of these components requires further studies.

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