Structural Characterization of the Brain Gangliosides GM1 and GD1 by High Resolution FT-ICR Tandem Mass Spectrometry

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INTRODUCTION

Gangliosides are amphiphilic molecules made up of a polar carbohydrate head that contains sialic acid linked to a ceramide (**Figs. 1 and 2**). Ceramides are N-acyl sphingoid bases. The fatty acids are designated by Cx:y to reflect the chain length (x) and number of double bonds (y). Because gangliosides are poised in the outer leaf of cell membranes, they are important mediators of cell-cell recognition, cell adhesion and tissue differentiation. The ganglioside composition of cell membranes is complex and can change in response to internal and external stimuli. The ability to monitor changes in gangliosides from low-level samples would benefit our understanding of the impact of these changes in brain disease. In order to monitor ganglioside patterns in biological samples, new analytical methods have been developed which focus on small sample volumes, high sensitivity and high analytical specificity.

METHOD

Sample Preparation.
Bovine brain gangliosides GM1, GD1a and GD1b were purchased from Sigma-Aldrich. Ganglioside samples were reconstituted in 100% methanol (1000 pmol/μL), and then diluted to ~50 pmol/μL with 50:50 methanol/water, 3% double distilled acetic acid for ESI analysis.

FT-ICR Mass Spectrometry.
FT-ICR mass spectrometry was performed with a homebuilt instrument equipped with a 7 Tesla, unshielded superconducting magnet (Oxford Instruments, Oxford, UK).[1] The electrospray (ESI) robot (Nanomate, Advion Biosciences) aspirated a 5 μL sample volume from a 10 μL total sample volume, and initiated the electrospray infusion of the sample through a 400-nozzle ESI chip to the FT-ICR MS. The ions were transported into the mass spectrometer through a Chait-style atmosphere-to-vacuum interface and externally accumulated in a short (15 cm) octopole. After accumulation, the collected ions were transferred through a hexapole ion guide and captured by gated trapping in an open cell with rectangular electrodes. Ions of interest were isolated by stored-waveform inverse Fourier transform (SWIFT) ejection[2]. Isolated ions were fragmented by IRMPD with a 40 W, CO_2 laser (Synrad, Mukilteo, WA). Photon irradiation was performed for 150 ms at 12-18 % laser power. Control of the instrument and data acquisition was achieved with a modular ICR data acquisition system (MIDAS) data station.

LC-MS.
Separation of sphingoid bases by C18 reversed-phase LC-MS has been previously described[3]. However, we were unable to separate ganglioside isomers by the reverse phase method. Separation of 10 μL GM1 was performed in a 150 X 0.3 mm polystyrene-divinylbenzene column (Polymer Laboratories) packed with 100 Å particles and a nano-LC system (Eksigent). The flow rate was 1000 nL/min, isocratic (97% methanol and 3% acetic acid), for 15 minutes. The eluent flowed through a fused silica emitter and was ionized by microelectrospray.

CONCLUSIONS

Analysis of small volumes of ganglioside samples is possible with electrospray FT-ICR MS.

Low-flow electrospray is necessary to deal with the small sample volumes and low concentrations of the biological isolates.

The high resolution, high mass accuracy, high sensitivity and multiple ion manipulations (SWIFT, MS^n) of FT-ICR MS greatly enhance the ability to perform structural analysis of single components in highly complex biological samples.

Further developments in LC-MS of gangliosides in biological isolates will provide pre-separation of ganglioside isomers.

High resolution MS/MS verified the isoforms of the gangliosides based on the unique IRMPD fragmentation patterns and mass accuracy of FT-ICR MS.

Figure 1

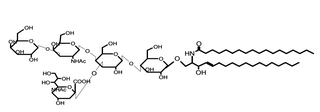

Figure 1. Chemical structure of GM1a.

Figure 2

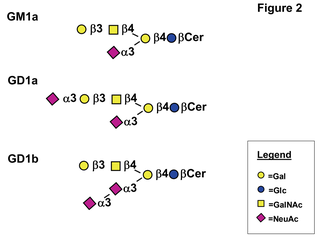

Figure 2. *Schematic structures of the GM1 and GD1 gangliosides.* GD1 contains two sialic acid residues whereas GM1 only has one sialic acid residue. Previous work done at the NHMFL confirmed that GM1 is predominantly composed of the GM1a isoform[4].

REFERENCES

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2. Guan, S.; Marshall, A. G. *Int. J. Mass Spectrom. Ion Processes* **1996**, *157/158*, 5-37.

3. Merrill, A. H.; Sullards, M. C.; Allegood, J. C.;Kelly, S.; Wang, E. *Methods* **2005**, *36*, 207-224.

4. McFarland, M.A., Marshall, A.G., Hendrickson, C.L., Nilsson. C.L., Fredman, P., Månsson, Jan-Eric, J. *Am. Soc. Mass Spectrom.* **2005**, *16*, 752-762.

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RESULTS

Figure 3

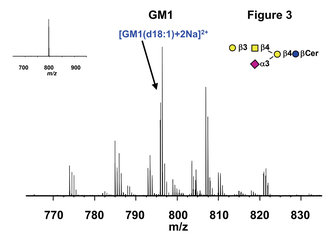

Figure 3. *Broadband (zoom) FT-ICR MS Spectrum of Bovine GM1 Ganglioside.* The peaks were assigned to protonated and metal cation-adducted GM1 (**Table 1**). The most abundant species was disodiated GM1 (d18:1/C18:0). The top left inset shows the SWIFT isolation of the *m/z* 795.920 $^{2+}$ ion prior to IRMPD fragmentation. The top right is a schematic representation of the known structure of GM1a.

Assignment	m/z (measured)	Mass (measured)	Mass (calculated)	Error (ppm)
GM1 (d18:1) + 2H$^+$	773.9438	1547.888	1547.891	0.2
GM1 (d18:1) + H$^+$+Na$^+$	784.9366	1569.873	1569.873	0
GM1 (d20:1) + 2H$^+$	787.9609	1575.922	1575.923	0.05
GM1 (d18:1) + H$^+$+K$^+$	792.9386	1585.877	1585.982	6
GM1 (d18:1) + 2Na$^+$	795.9201	1591.840	1591.855	1
GM1 (d20:1) + H$^+$+Na$^+$	788.9440	1597.888	1597.906	1
GM1 (d20:1) + H$^+$+K$^+$	806.9265	1613.853	1614.013	9
GM1 (d20:1) + 2Na$^+$	809.9382	1619.876	1619.887	0.6

Table 1. Assignment of the major peaks detected by nano-ESI-FT-ICR MS of GM1. LC-MS of this sample separated the d18:1 from the d20:1 isomers (data not shown).

Figure 4

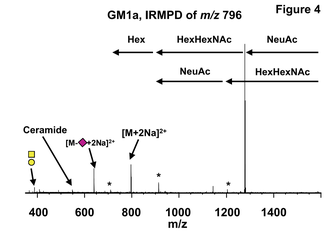

Figure 4. *IRMPD of the SWIFT isolated GM1(d18:1/C18:0), m/z 795.920 $^{2+}$ ion.* Spectrum shows the major fragmentation pathway of the bovine GM1, loss of NeuAc. Arrows point to assigned fragments (marked with asterisks). The IRMPD spectrum confirms that the biological GM1 is predominantly in the GM1a isoform as previously reported [4].

Figure 5

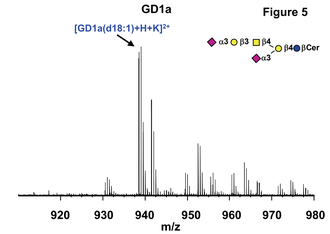

Figure 5. *Broadband (zoom) FT-ICR MS Spectrum of Bovine GD1a Ganglioside.* The most abundant species was *m/z* 938.478 $^{2+}$, corresponding to [GD1a (d18:1/C18:0)+H+K]$^{2+}$. The other peak assignments are listed in **Table 2**. The top right is a schematic representation of GD1a.

Assignment	m/z (measured)	Mass (measured)	Mass (calculated)	Error (ppm)
GD1a (d18:1) + H$^+$+Na$^+$	930.5055	1861.011	1860.969	2
GD1a (d18:1) + H$^+$+K$^+$	938.4778	1876.956	1877.077	6
GD1a (d18:1) + 2Na$^+$	941.4853	1882.971	1882.951	1
GD1a (d20:1) + H$^+$+K$^+$	952.5095	1905.019	1905.109	5
GD1a (d20:1) + 2Na$^+$	955.5218	1911.044	1910.982	3

Table 2. Assignment of the major peaks detected by nano-ESI-FT-ICR MS of GD1a.

Figure 6

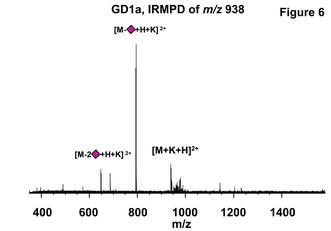

Figure 6. *IRMPD of the SWIFT isolated GD1a, m/z 938.478 $^{2+}$ ion.* The spectrum shows the two major fragments of bovine GD1a, with losses of one and two NeuAc residues. The IRMPD spectrum confirms the presence of the GD1a isoform.

Figure 7

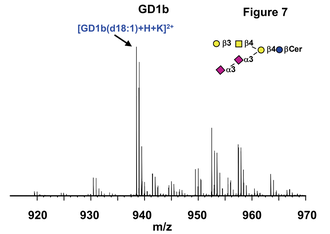

Figure 7. *Broadband (zoom) FT-ICR MS Spectrum of Bovine GD1b Ganglioside.* The most abundant species was *m/z* 938.487 $^{2+}$, corresponding to [GD1b (d18:1/C18:0)+H+K]$^{2+}$. The other peak assignments are listed in **Table 3**. The top right is a schematic representation of GD1b.

Assignment	m/z (measured)	Mass (measured)	Mass (calculated)	Error (ppm)
GD1b (d18:1) + 2H$^+$	919.5155	1839.031	1838.987	2
GD1b (d18:1) + H$^+$+Na$^+$	930.4890	1860.978	1860.969	0.5
GD1b (d18:1) + H$^+$+K$^+$	938.4866	1876.973	1877.077	6
GD1b (d18:1) + 2Na$^+$	941.4880	1882.976	1882.951	1
GD1b (d20:1) + H$^+$+K$^+$	952.5089	1905.018	1905.109	5
GD1b (d20:1) + 2Na$^+$	955.5019	1911.004	1910.982	1

Table 3. Assignment of the major peaks detected by nano-ESI-FT-ICR MS of GD1b.

Figure 8

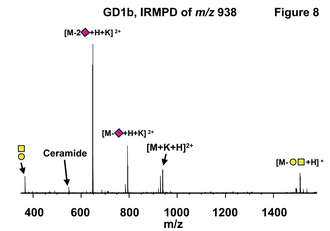

Figure 8. *IRMPD of the SWIFT isolated GD1b, m/z 938.487 $^{2+}$ ion.* The potassiated GD1b 930.470 $^{2+}$ ion was SWIFT isolated for IRMPD fragmentation for direct comparison to the potassiated GD1a ion fragmentation (**Figure 6**). The spectrum shows the loss of a terminal HexHexNAc and the loss of one and two NeuAc residues. The IRMPD spectrum confirms the presence of GD1b isoform.