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

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INTRODUCTION

Gangliosides are essential components made up of a polar headgroup containing a sialic acid and a carbohydrate and a hydrophobic fatty acid chain. It is the polar headgroup that is responsible for the unique interaction properties of gangliosides, which are organized in multiple lipid rafts in the cell membrane. Ganglioside composition is tissue specific and unique to specific cell types. Abnormalities in ganglioside metabolism lead to defects in cell-cell interactions, and adhesion and tissue differentiation. The transmembrane gangliosides are believed to play an important role in cell-matrix interactions, cell-cell interactions, and cell adhesion. An understanding of ganglioside composition in disease is of great importance. For example, gangliosides are known to play a role in the pathogenesis and progression of several diseases. In order to monitor ganglioside changes in biological samples, one needs to develop high resolution tandem mass spectrometry methods with high sensitivity and high specificity.

METHOD

Sample Preparation

Bovine brain gangliosides GM1, GD1a and GD1b were purchased from Sigma-Aldrich. Ganglioside samples were reconstituted in 2-3% methanol, and then diluted to ~50 pmol/L 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 (Cyclotron, Los Alamos National Laboratory). The magnet was operated at 15.13 T and 1.14 K. Blank ESI-FT-ICR experiments were performed using 200 µL of methanol/water, 3% double distilled acetic acid. The ESI source was a Nanomate (Advion Biosciences) ceramic nanospray tip that aspirated a 5 µL sample and was set at 2000 V. Nanospray was used to eliminate the ionization of polar compounds. The ESI source was operated at an ion current of 10 µA. The FT-ICR mass spectrometer was operated in a 90° mode with a 180° collision angle. The collision energy was 20 eV. The spectra were acquired using a standard lock mass measurement of d7-Valine.

RESULTS

Figure 1. Chemical structure of GM1a.

Figure 2. Schematic structures of the GM1 and GD1 gangliosides. GM1a contains no sialic acids whereas GM1b has one and GM1a has two sialic acids. Previous work at the NMRB confirmed that GM1a is primarily composed of the GM1a isoform.

REFERENCES


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 samples. The high resolution, high mass accuracy, high sensitivity and multiple ion manipulations (SWIFT, MS²) 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 tissues will provide pre-experiment of ganglioside species. High resolution MS/MS verified the isoforms of the gangliosides based on the unique IRMPD fragmentation patterns and mass accuracy of FT-ICR MS.