Journal of Chromatography B, 947–948 (2014) 1–7

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Rapid and sensitive LC-ESI-MS of gangliosides

Aldo D. Garcia, Jorge L. Chavez, Yehia Mechref*

Department of Chemistry and Biochemistry, Texas Tech University, Lubbock 79409, TX, USA

ARTICLE INFO

Article history:
Received 25 May 2013
Received in revised form
12 November 2013
Accepted 17 November 2013
Available online 25 November 2013

Keywords:
Glycolipids
Gangliosides
LC-ESI-MS
Hydrophilic interaction chromatography

ABSTRACT

Gangliosides are a class of sphingolipids characterized by a ceramide lipid chain attached to an anionic oligosaccharide moiety that varies in complexity based on the level of sialylation. Heterogeneity in the oligosaccharide chain of gangliosides is a direct result of the monosaccharide structure, content, sequence, and connections. Gangliosides are highly concentrated in the central nervous system, and are cell type-specific as well as development-dependent and their quantities and species can undergo drastic changes during cell differentiation. Specific localization of gangliosides also allows for interaction with a variety of bioeffectors, including glycoproteins, antibodies, peptide hormones, and growth factors. There are currently no rapid analytical assays capable of identifying and quantifying gangliosides. The aim of this study is to establish a reliable chromatographic mass spectrometry based assay capable of profiling ganglioside levels in complex biological samples at high sensitivity. We describe here a chromatographic method using an amino column on which the separation is based on hydrophilic interaction with the sugar moiety of gangliosides. Several gangliosides, including GM1–3, $GD1_{a,b}$, GD2–3, and $GT1_{a,b}$, were efficiently separated in less than 10 min at a limit of detection ranging between 10–50 pg on column with a concentration dynamic range extending over 4 orders of magnitude. The developed method allowed the sensitive quantitation of gangliosides derived from the blood serum of patients with different esophagus diseases, including, adenocarcinoma, high-grade dysplasia, and Barrett's.

1. Introduction

Gangliosides are a class of sphingolipids characterized by a ceramide lipid chain attached to an anionic oligosaccharide moiety that varies in complexity based on the level of sialylation (N-acetylneuraminic acid or Neu5Ac). Heterogeneity in the oligosaccharide chain of gangliosides is a direct result of the monosaccharide structure, content, sequence, and connections [1]. When taking the variability of the lipid moiety into account, this makes gangliosides a very large family of compounds. Gangliosides are integral constituents of cells and are especially abundant in neuronal membranes. Most importantly, gangliosides are known to serve a wide array of biological functions, including cellular recognition, adhesion, and cellular signaling [2]. They have even been reported to play critical structural roles as well. Recently, theoretical considerations and experimental data suggest that gangliosides actively participate in the organization and maintenance of membrane lipid domains/zones [3].

Moreover, ganglioside expression is developmentally regulated and is not only cell type specific, but also goes hand in hand with the differentiation state of the cell [4,5]. Multiple investigations have also postulated that changes of ganglioside profiles during cellular differentiation is closely related to their metabolism, and in particular their biosynthesis [6]. Interestingly, gangliosides may even have neuroprotective effects to the cell [7–9]. Many studies have also highlighted the neuroprotective effects of GM1 in diseases such as Alzheimer's disease [10], Parkinson's disease [11], stroke, and Guillain–Barré syndrome [12].

Recently, gangliosides have also become an attractive target of biomarker discovery because of their biological significance. Even though gangliosides have neuroprotective effects, unusual ganglioside proliferation can also signify diseases, such as Tay-Sachs, Sandhoff disease, and the AB variant of GM2 gangliosidosis [13–17]. Glycoconjugates have also been associated with tumor development. In the mid 1980s, it was discovered that glycoconjugates are often expressed as onco-developmental antigens [18]. Today, there is growing evidence to suggest that glycosphingolipids (GSLs) play an important role in tumor biology. For instance, multiple studies have discovered increased levels of GSL-bound sialic acid found in the serum of patients with mammary carcinoma [19], as well as colorectal cancer [20] and melanoma [21]. Abnormally high levels of GM3 and GD3 in the presence of melanoma and elevated levels of GD2 in retinoblastoma patients have also been found through targeted monitoring studies [22]. Establishment of reliable cancer biomarkers is still severely lacking as there is yet no streamlined, facile, rapid, and sensitive assay capable of being applied in a clinical setting.

* Corresponding author at: Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409-1061, 806-834-8246806 742 1289.
E-mail address: yehia.mechref@ttu.edu (Y. Mechref).

1570-0232/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jchromb.2013.11.025

Accordingly, significant research efforts have been invested for the development of more robust and reliable ganglioside analytical assays. A conventional method for ganglioside analysis has been thin-layer chromatography (TLC) [23] but has been recently outpaced by other technologies due to its low resolutions that are simply inadequate for detecting subtleties in the heterogeneity of the gangliosides. The coupling of TLC with densitometric or immunochemical detection [12,23], HPLC, supercritical fluid chromatography, and enzyme-linked immunosorbent assay (ELISA) techniques have also been explored [24]. Most of these techniques require extensive sample preparations with multiple steps that only offer limited resolution and sensitivity. Recently, mass spectrometry based techniques have been explored due to its high resolution, sensitivity, and accuracy. For instance, Vukelic et al. developed multiple MS methods using nano-electrospray ionization (ESI), Fourier-transform ion cyclotron resonance (FT-ICR), and chip nano-ESI quadrupole time-of-flight (QTOF) for successful ganglioside profiling of human gliosarcoma tissue in which 73 distinct ganglioside species were observed [25]. Matrix-assisted laser desorption/ionization (MALDI) has also offered promising results for ganglioside analysis. Specifically, numerous MALDI-TOF studies have been performed with limitations being observed in resolving structures with similar mass [26–29]. As a result, mass analyzers with high resolution are becoming a necessity for reliable structural elucidation of complex polar lipids.

In order to overcome some of the aforementioned limitations, we developed a facile extraction and purification protocol for reliable ganglioside profiling using LC-ESI-MS. Since blood serum, or plasma are noninvasively acquired and are slowly becoming the media of choice for disease biomarker screen testing, the newly developed methodology was applied for ganglioside profiling of human blood serum (HBS) collected from patients suffering from cancer, high grad dysplasia (HGD), Barrett's syndrome, and their matching control. Herein is described a previously unachieved rapid chromatographic method that allows for ganglioside analysis of HBS at microliter amounts with high sensitivity.

2. Materials and methods

2.1. Materials

A standard mixture of bovine brain gangliosides was attained from Santa Cruz Biotechnology (Dallas,TX). HPLC grade methanol was purchased from JT Baker (Phillipsburg, NJ). HPLC grade water was purchased from Mallinckrodt Chemicals (Phillipsburg, NJ). The Imtakt Unison UK amino column was purchased from Imtakt USA (Philadelphia, PA). The Isoelute C8 end-capped SPE cartridges (25 mg bedweight) were obtained from Fisher Scientific (Pittsburgh, PA). MS grade formic acid and ammonium hydroxide were purchased from Fisher Scientific (Pittsburg, PA).

2.2. Liquid/liquid extraction

First, liquid/liquid extraction was optimized using triplicates of 10 ng of the mixture of bovine brain gangliosides. The ganglioside standard was spiked into 1.3 ml of a mixture of chloroform/methanol/water (30/60/8, v/v/v), vortexed briefly, and sonicated for 15 min. The solution was then centrifuged at high speed for 15 min. The aqueous liquid phase containing the enriched gangliosides was collected and set aside while the organic phase was then subjected to the same extraction procedure again by adding another 1.3 ml of mixture of chloroform/methanol/water (30/60/8, v/v/v). The aqueous supernatants from the first and second extractions were combined and dried under vacuum. Once dry, the lipid extracts were re-suspended in 10 μL of the starting buffer composition of the LC system at 10%A and 90%B (buffer composition is discussed in depth in the next section) and were later analyzed by LC-MS. Standards that were subjected to several freeze-thaw cycles ($N = 3$) were also extracted as described above. This was performed to assess the effect of such cycles on quantitation. The establishment of a successful extraction protocol allowed for the subsequent analysis of biological samples, such as 10 μl of HBS that was pooled ($N = 10$ in each case) from patients suffering from adenocarcinoma, high-grade dysplasia (HGD), Barrett's disease (BS), and their matching pooled disease free counterpart. All biological samples were subjected to the same numbers of freeze-thaw cycles, thus eliminating any bias associated with these biological samples. Triplicates of the four samples were dried using a Labconco concentrator (Kansas City, MO). Next, each sample was reconstituted in 1.3 ml of chloroform/methanol/water (30/60/8, v/v/v), vortexed briefly, and sonicated for 15 min following the aforementioned protocol for the ganglioside standards. After sonication and centrifugation, the supernatant was collected and set aside. The pellet was then subjected to the same extraction procedure again. The supernatants from the first and second extractions were combined and dried under vacuum. Once dry, the lipid extracts were re-suspended in 10 μL of the starting buffer composition of the LC system at 10%A and 90%B.

2.3. C8 SPE purification

The lipid extracts were then purified using Isoelucte C8 SPE columns. The C8 SPE cartridges were first washed with 3 ml of methanol, and conditioned with a 2 ml of 60% aqueous methanol solution. The lipid extract was then loaded onto the column and allowed to pass through using gravitational forces. The pass through was then re-applied to the same column in order to ensure appropriate binding of the analyte to the media. The bound analyte was then de-salted with 3 ml of 60% aqueous methanol solution and was eluted with neat methanol, 1.5 ml (twice), for a total elution volume of 3.0 ml. The eluents were then dried under vacuum and prepared for LC-MS analysis by reconstituting the samples in 10 μL of the starting buffer composition of the LC system at 10%A and 90%B.

2.4. LC-MS analysis

Gangliosides from bovine brain (GM1, GM2, GM3, $GD1_{a,b}$, GD2, GD3, and $GT1_{a,b}$) were separated utilizing a Unison UK-Amino column (2.0 mm × 200 mm). In order to enhance separation, several parameters were optimized including the mobile phase "A" composition (consisting of water with 0.75% ammonium hydroxide and 0.2% formic acid) and the organic phase "B" additives (consisting of 95% acetonitrile with 0.07% ammonium hydroxide and 0.1% formic acid). Optimum separation conditions were attained at a flow rate of 0.5 ml/min and a separation temperature of 60 °C. Separation was achieved using the Ultimate 3000 rapid separation liquid chromatography instrument (RSLC) from Dionex; the effluent of which is interfaced to an Exactive mass spectrometer equipped with a heated electrospray ionization (HESI-II) probe from Thermo Scientific. The spray voltage was set to −4.5 kV, while the vaporizer and capillary temperatures were set to 250 and 300 °C, respectively. The sheath and auxiliary gas pressures were set to 40 and 5 psi, respectively. In order to quantify the different ganglioside species, 3 ganglioside standards of GM1, $GD1_a$, and $GT1_b$ were selected to represent each different degree of sialylation (monsialylated, disialylated, and trisialylated) and were used to create a calibration curve. A series of dilutions were created for each standard for the following ganglioside amounts loaded onto the LC-MS system, 50, 100, 300, 500, 700 pg, and 1 ng.