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A comprehensive profiling of sulfatides in myelin from mouse brain using liquid chromatography coupled to high-resolution accurate tandem mass spectrometry

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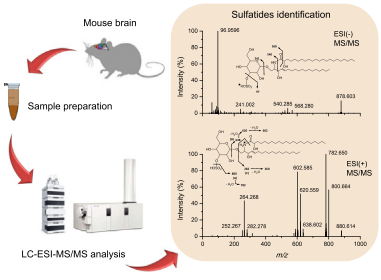
HIGHLIGHTS

- Thirty seven sulfatides have been simultaneously determined in myelin by LC-ESI-MS/MS.
- Wrong-way-round ionization effect allowed to obtain very good sensitivity in ESI(+).
- MS/MS fragmentation pattern allowed differentiating structural isomers.
- High mass accuracy was necessary to get a reliable identification of sulfatides.
- Sulfatides with hydroxylated and/or odd numbered fatty acid chains increase with age.

GRAPHICAL ABSTRACT



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ABSTRACT

Sulfatides are sulfoglycolipids found in the myelin sheath. The composition ratio of sulfatide molecular species changes with age, and it has also been associated with the pathogenesis of various human central nervous system diseases. However, profiling sulfatides in biological samples is difficult, due to the great variety of molecular species. In this work, a new, easy and reliable liquid chromatography-electrospray tandem mass spectrometry (LC-ESI(+)-MS/MS) method has been developed to profile sulfatide content in biological samples of myelin. The 'wrong-way-round' ionization effect has been described for this type of molecules for the first time, making it possible to correctly identify as many as 37 different sulfatides in mouse brain myelin samples, including molecules with different fatty acid chain lengths and varying degrees of unsaturation and hydroxylation. A chemometric analysis of their relative abundances showed that the main difference among individuals of different ages was the content of sulfatides with odd-numbered fatty acid chains, in addition to hydroxylated species.

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1. Introduction

Myelin is the insulating sheath around axons, which is essential for high-speed transmission of electrical impulses [1] and also provides trophic support to axons [2]. While myelin in the central nervous system (CNS) is formed by the cell membrane of oligodendrocytes, the myelin-forming cells in the peripheral nervous system are Schwann cells [3]. The myelin sheath is particularly rich in lipids [4]; more than 70% of its dry weight is composed of lipids, mainly cholesterol, galactosylceramides and sulfatides. In particular, the glycolipid sulfatides are amphiphilic molecules containing a polar head: namely, a galactose residue with a sulfate group at position 3, bound to a ceramide moiety via a glycosidic linkage (Fig. 1). The ceramides are composed of a fatty acid residue linked to the amine group of sphingosine. Sulfatide species vary in length, as well as degree of unsaturation and/or hydroxylation of their fatty acid chain [5]. In the CNS the most common species found in myelin are composed of an acyl chain of 24 carbons with or without an unsaturated bond [5].

Sulfatides are found on the extracellular leaflet of myelin [4]. The composition ratio of sulfatide molecular species in human and mouse brains changes with age [5–8], although the most pronounced variations occur during myelination. Thus, an increase of hydroxyl fatty acid derivatives is observed during myelination in the entire neonatal mouse brain [6]. This increase correlates with upregulation of the FA2H gene expression, which encodes the enzyme fatty acid 2-hydroxylase. A mutation in the FA2H gene has been identified in patients with leukodystrophy or complex spastic paraparesis [9], highlighting the importance of hydroxylation for myelin maturation. Furthermore, the acyl chain length varies during tissue development. In mice, sulfatide species with a short acyl chain (16:0 and 18:0) are more abundant in membranes of an immature brain, while those with a longer chain (24:1 and 24:0) are enriched in membranes of an adult mouse cerebellum [10].

Changes in sulfatide levels have been associated with the pathogenesis of various human CNS diseases [11], including multiple sclerosis [12], Parkinson's disease [13], leukodystrophy [9] and Alzheimer's disease [14,15]. In particular, the most typical and frequent primary demyelinating disease is multiple sclerosis, which is caused by the loss of myelin-forming oligodendrocytes [16]. The aforementioned studies have pointed out that sulfatides can potentially be used as biomarkers in neurological diseases.

Although several analytical techniques may be used to analyse sulfatides [17], mass spectrometry is currently the method of choice. This technique had already been used for sulfatide analysis between the 1960s and the 1980s [18–20], and it has become the most popular analytical technique since the late 1990s thanks to the widespread use of electrospray (ESI) and matrix-assisted laser desorption/ionization (MALDI).

Nowadays, these compounds have been the focus of a number of qualitative and quantitative studies, most of them involving the use of mass spectrometry imaging (MSI) [10,21–25] or liquid chromatography coupled to mass spectrometry [26–34]. Recently, the leading methodological advances introduced in sulfatide analysis have involved the use of both ultra-high performance liquid chromatography (UHPLC) [30–32], in order to obtain better and faster separations, and high resolution mass analysers such as quadrupole-time of flight (Q-TOF) [29].

All the studies referred to above have contributed to establishing the content of sulfatides in biological tissues and their relationship with several diseases. However, the number of sulfatides detected is usually scarce, and the confidence that they are properly identify is sometimes limited. For example, a very recent paper [35] carries out a thorough qualitative and quantitative analysis of sulfatides using an LC-MS method, but the authors do not use accurate masses to identify the different molecular species, with the evident risk of misidentification. Taking these limitations into account, a new high-performance liquid chromatography-mass spectrometry method, capable of carrying out comprehensive and reliable sulfatide profiling in biological samples is presented in this paper. The method is based on obtaining quasimolecular wrong-way-round ions using positive electrospray ionization, then recording the precursor and product mass spectra in the high resolution accurate-mass mode. The applicability and performance of the developed method were confirmed using myelin samples isolated from adult mice at different ages (at the first step for mature myelination, i.e., 60 days, and at successively older stages, i.e., 120, 240 and 365 days), in order to reflect known dynamic changes in the plasticity of myelin over the course of a lifetime [36].

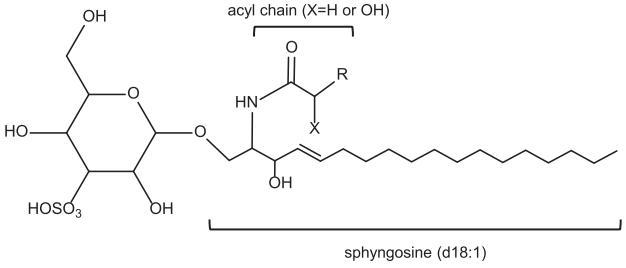

Fig. 1. Chemical structure of sulfatides.

2. Materials and methods

2.1. Reagents and standards

HPLC grade methanol with 99.9% purity (Merck) and chloroform with 99.8% purity (Sigma-Aldrich) were used. Ultrapure water was obtained from the Milli-Q system (Millipore). Ammonium acetate, acetic acid and acetonitrile used for the mobile phase were of LC-MS grade (Sigma-Aldrich). The pH of the aqueous and hydro-organic solutions was measured using an Oakton pH Tutor Benchtop Meter calibrated with aqueous buffers (pH 7.00 and 4.01). Sulfatide standard mixture, in the form of ammonium salt, was purchased from Avanti Polar Lipids (Porcine Brain, Ref. 131305). Stock solutions were prepared when needed in chloroform/methanol/water (2:1:0.1, v:v:v) and stored at −20 °C in glass vials until analysis.

2.2. Sample treatment

C57/Bl6 animals were obtained from Harlan, Spain and maintained in a temperature controlled room, with 12:12 h light/dark schedule and received food and water ad libitum. Animal care and procedures were performed according to our institutional animal use and care committee (Hospital Nacional de Parapléjicos, registered as SAPA001), following the European Parliament and Council Directive (2013/63/EU) and the Spanish regulation (RD 53/2013) on the protection of animals for experimental use. Myelin was isolated, as previously reported in Larocca and Norton [37], from animals of different ages: 60 (P60), 120 (P120), 240 (P240) and 365 (P365) postnatal days old. Animals were sacrificed by cervical dislocation.

The described protocol can be used to isolate myelin from different sources (developing and adult whole brain, spinal cord, and white matter from animals of different species) and from fresh or frozen tissue. The following describes the isolation of myelin from fresh, whole, adult mousebrain.

Brains were homogenized with a Dounce homogenizer in an ice-cold 0.3 M sucrose buffer solution containing 20 mM Tris·Cl