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Liquid chromatography with dual parallel mass spectrometry and ^{31}P nuclear magnetic resonance spectroscopy for analysis of sphingomyelin and dihydrosphingomyelin I. Bovine brain and chicken egg yolk

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Abstract

Liquid chromatography coupled to atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) mass spectrometry (MS), in parallel, was used for detection of bovine brain and chicken egg sphingolipids (SLs). APCI–MS mass spectra exhibited mostly ceramide-like fragment ions, [Cer-H_2O + H]$^+$ and [Cer-2H_2O + H]$^+$, whereas ESI–MS produced mostly intact protonated molecules, [M + H]$^+$. APCI–MS/MS and MS^3 were used to differentiate between isobaric SLs. APCI–MS/MS mass spectra exhibited long-chain base related fragments, [LCB]$^+$ and [LCB-H_2O]$^+$, that allowed the sphinganine backbone to be differentiated from the sphingenine backbone. Fragments formed from the fatty amide chain, [FA(long)]$^+$ and [FA(short)]$^+$, allowed an overall fatty acid composition to be determined. The presence of both dihydrosphingomyelin (DSM) and sphingomyelin (SM) sphingolipid classes was confirmed using ^{31}P NMR spectroscopy.

Keywords: Sphingolipids; Sphingomyelin; Dihydrosphingomyelin; APCI–MS; ESI–MS

1. Introduction

Since Hannun and Bell [1] first described the stimulus-regulated metabolism of sphingomyelin in 1989, known as the Sphingomyelin Cycle, the interest in sphingolipids (SLs) has greatly increased. SLs play two crucial roles in cellular systems: (1) they participate in cellular signaling pathways and (2) they are principal components of the structural matrices of cellular membranes (both the plasma membrane bilayer and intracellular membranes). Sphingomyelin and its metabolites have been implicated as important components of signaling cascades associated with apoptosis, cell cycle arrest, cell senescence, differentiation, T-cell receptor signaling, Ca^{2+} mobilization, lipid transport, and many more. The roles of sphingolipids in a wide variety of cell signaling pathways have been the subject of extensive review [2–11].

In their structural role, SLs have a higher gel to liquid crystalline phase transition temperature than glycerophospholipids [12–14], which means that they maintain their highly ordered state at higher temperatures (i.e. biological temperature) than glycerophospholipids [15]. SLs also have the unique characteristic that they self-associate into extended 'membrane rafts', which exhibit special characteristics, discussion of which is beyond the scope of this report, but which have been extensively reviewed [16–19]. The phase transition temperature and the degree of order of a lipid membrane is determined in great part by the composition of the fatty chains that make up the lipids [20,21]. The identities of the molecular species also have an important impact on the rate of *sphingomyelinase* turnover of SLs [14]. Thus, knowledge of the identities of the molecular species that make up a mixture of SLs is an important prerequisite for understanding their roles in cellular processes.

Phosphosphingolipids are large (∼700–900 Da) zwitterionic molecules that are not amenable to direct gas-phase analysis

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