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Characterization of protonated phospholipids as fragile ions in quadrupole ion trap mass spectrometry

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ABSTRACT

Some ions exhibit "ion fragility" in quadrupole ion trap mass spectrometry (QIT-MS) during mass analysis with resonance ejection. In many cases, different ions generated from the same compound exhibit different degrees of ion fragility, with some ions (e.g., the $[M+H]^+$ ion) stable and other ions (e.g., the $[M+Na]^+$ ion) fragile. The ion fragility for quadrupole ion trap (QIT) mass spectrometry (MS) for protonated and sodiated ions of three phospholipids, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, PC (16:0/16:0), 1,2-dipalmitoyl-*sn*-glycero-3-phophoethanolamine, PE (16:0/16:0), and N-palmitoyl-D-*erythro*-sphingosylphosphorylcholine, SM (d18:1/16:0), was determined using three previously developed experiments: (1) the peak width using a slow scan speed, (2) the width of the isolation window for efficient isolation, and (3) the energy required for collision-induced dissociation. In addition, ion fragility studies were designed and performed to explore a correlation between ion fragility in QIT mass analysis and ion fragility during transport between the ion source and the ion trap. These experiments were: (1) evaluating the amount of thermal-induced dissociation as a function of heated capillary temperature, and (2) determining the extent of fragmentation occurring with increasing tube lens voltage. All phospholipid species studied exhibited greater ion fragility as protonated species in ion trap mass analysis than as sodiated species. In addition, the protonated species of both SM (d18:0/16:0) and PC (16:0/16:0) exhibited greater tendencies to fragment at higher heated capillary temperatures and high tube lens voltages, whereas the PE (16:0/16:0) ions did not appear to exhibit fragility during ion transport.

1. Introduction

Glycerophospholipids (GPLs) serve one primary function in biology, as structural components of cell membranes [1]. GPLs typically consist of a glycerol backbone with a polar head group and two non-polar fatty acid tails. GPLs with this basic structure are phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), phosphatidylinositols (PIs), and phosphatidylserines (PSs), with the classes determined by the composition of the head group.

Because of their biological abundance and importance, GPLs have been studied by mass spectrometry for many years, from electron ionization (EI) coupled to gas chromatography/mass spectrometry (GC/MS) [2] (not as intact ions, but as derivatized species where the head group and tails are removed from the glycerol backbone) to more recently matrix-assisted laser desorption/ionization (MALDI) [3–6] and electrospray ionization (ESI) [7–10]. Under appropriate experimental conditions, the latter ionization techniques produce a predominant ion ($[M+H]^+$ or $[M+cation]^+$) corresponding to the molecular weight of the intact molecule M, whereas EI causes a high degree of fragmentation, and thus requires derivatization and only analyzes the fatty acid tails. In ESI, relying solely on the molecular weight for compound identification is typically inadequate because of the wide variety of GPLs present (varying fatty acyl chains and head groups) and the possibility of isomers; thus compound identification is typically performed by tandem MS with triple quadrupole or ion trap mass spectrometers [7,9,11,12].

Many previous studies have focused on differences in fragmentation between protonated and cationized GPLs [8,11–14]. For positive ions, experiments have shown that protonated and sodiated PCs produce very different fragmentation patterns, with sodiated PCs providing a more informative fragmentation (identification of fatty acyl substituents) pattern [9]. Upon collision-induced dissociation (CID), protonated PCs produce primarily one fragment ion, m/z 184, corresponding to the polar head group, indicating that the charge is retained on the head group. In contrast, CID of

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sodiated PCs produces fragment ions that correspond to losses of the head group with retention of the charge on the glycerol backbone. The most abundant fragment ion in MS/MS shows a neutral loss of 59, which corresponds to the loss of trimethylamine, $-N(CH_3)_3$ [9].

The difference in fragmentation of protonated and cationized GPLs has been of considerable interest, particularly in the study of different metal ions for cationization. Due to the limitation of only a single stage of tandem MS (MS/MS or MS^2) provided by a triple quadrupole instrument, more informative fragment ions after MS/MS are desired. The use of lithiated adducts was shown to provide many structurally informative fragment ions for PCs [12] and PEs [13] after a single stage of tandem MS, and many other cations have been evaluated for other GPLs [11]. A structurally significant fragment ion in the analysis of lipids allows for the correct identification of the fatty acid tails and their location on the glycerol backbone. A novel adduction with trifluoroacetic acid (TFA)/K^+ for PCs was determined to provide an abundance of structurally informative fragment ions as well [15]. The resonating theme of most positive ion studies was that the cationized GPLs, or adducts with other complexes, should be preferred over protonated GPLs for structural identification of fatty acyl substituents.

This difference in fragmentation behavior under CID is also of concern in ion trap mass spectrometry for both ion production and ion fragility. In previous studies from our laboratory [16], the fragility of an ion within the ion trap was quantified and results showed that different ions formed from the same molecule can exhibit varying degrees of fragility. For example, cationized oleandomycin is a stable ion, whereas its protonated counterpart is fragile; in contrast, acylcarnitines are more stable protonated ions. A consequence of fragility is an inability to efficiently isolate a fragile ion without widening the isolation notch, as well as mass shifts and reduced mass resolution, particularly at slow scan speeds [16]. Thus far, a relationship between differences seen in fragmentation behavior between different ions of the same compound in tandem MS experiments and differences in ion fragility of those ions has not been evaluated; we explore this relationship using GPLs as a test case in this paper. In addition, we expand the concept of ion fragility in the ion trap to fragility in ion transport.

It has already been shown that cationized and protonated species of the same molecule can exhibit varying degrees of ion fragility in ion trap analysis, but the effect of ion fragility on transport from atmosphere to vacuum and the accompanying desolvation process has not be evaluated. The temperature of the heated capillary, and thus the effective temperature of an ion, have been shown to affect the onset of source fragmentation [17]. In MALDI mass spectrometry, source fragmentation of GPLs has been demonstrated to arise from gas-phase reactions rather than from laser-induced photodissociation [3]. This is an important factor, since gas-phase reactions occur in electrospray as well.

Fragmentation during ion transport has been studied previously. Early studies in electrospray showed the possibility of performing thermally induced dissociation (TID) of highly charged protein ions [18]. A heated capillary was used for those studies, and the results indicated that the higher charged ions (+6, +5, and +4) were more susceptible to TID due to increased coulombic repulsions. Source collision-induced dissociation (SCID) has been used for many years for controllable dissociation of complexes and to provide fragment ions that can be further fragmented in a tandem mass spectrometer.

In this paper, the concept of ion fragility in ion trap mass analysis is examined for GPLs (and sphingolipids) and the effects on fragmentation during ion transport are explored by comparing two different GPLs and one sphingolipid as protonated and sodiated species, namely PC (16:0/16:0), PE (16:0/16:0), and sphingomyelin, SM (d18:0/16:0) (a sphingolipid, but still containing a phosphate group).

2. Experimental

All experiments were performed in the positive ion mode on the Finnigan LCQ instrument (San Jose, CA), an electrospray ionization quadrupole ion trap (ESI-QIT) mass spectrometer. Analyte solutions were directly infused at a flow rate of 1 μL/min using a syringe pump, 4.5 kV applied to the electrospray needle, and nitrogen sheath gas set to 30 arbitrary units. For evaluating differences in ion fragility of protonated and cationized molecules, three related phospholipids were chosen: PC (16:0/16:0), SM (d18:1/16:0), and PE (16:0/16:0). The PC (16:0/16:0) and SM (d18:1/16:0) were chosen because they share the same head group and exhibit the same differences in fragmentation of the protonated and sodiated species upon collision-induced dissociation (CID). The main difference is in their fatty acid tail arrangement: SM (d18:1/16) has one amide-linked fatty acyl chain, palmitoyl, as it is derived from the sphingosine base, whereas PC (16:0/16:0) has two ester-linked fatty acyl chains as it is derived from glycerol. PE (16:0/16:0) was chosen because it has the same fatty acyl chain arrangement as PC (16:0/16:0), but a slightly different head group; it also exhibits a different fragmentation pathway between protonated and cationized species. The difference in the head group is the replacement of trimethylamine ($-N(CH_3)_3$) with amine ($-NH_3$) (Fig. 1). For this discussion, these three species will be referred to as phospholipids because of the presence of phosphate in the head group.

Standards of PE and PC with the desired fixed fatty acid substituents of palmitoyl (16:0) were obtained from Avanti Polar Lipids (Birmingham, AL); SM was purchased from Avanti as a chicken egg extract, but with palmitoyl (16:0) as the predominant acyl chain (80%). All phospholipids were obtained as powders and prepared to the desired concentrations. PC (16:0/16:0) and SM (d18:1/16:0) were made as stock solutions of 1000 ppm (~1.4 μM) in 50:50 isopropanol:methanol and PE was made as a stock solution of 500 ppm (~0.7 μM) in 75:25 choloroform:methanol. For ESI QIT-MS analysis, the stock solutions were diluted to 10 ppm (~14 nM) in methanol.

Due to the presence of unwanted source fragmentation and to uncover the origin of those fragment ions, solutions were prepared to ensure that either the $[M+H]^+$ ion or the $[M+Na]^+$ ion was the most abundant ion formed during ESI. Even with careful cleaning of all fittings and connectors, it was difficult to remove the presence of the $[M+Na]^+$ ions in all experiments, so we settled on ensuring that the protonated species was the most abundant for these studies. For production of protonated species, formic acid was added to a final concentration of 0.1%; for the production of sodiated species, sodium acetate was added to a concentration of 100 μM. All solvents were of HPLC grade and were obtained from Fisher Scientific (Pittsburgh, PA).

Ion fragility in the ion trap was determined using three experiments described previously [16]. These experiments were (1) measuring the peak width of the parent ion at 10% peak height ($PW_{10\%}$) using a slow scan speed (called zoom scan), (2) finding the isolation width required for isolating the desired ion, and (3) determining the amount of energy required for CID. Typically, a fragile ion will exhibit a wider peak width at a slower scan rate, will need a wider isolation window, and will require less energy to fragment under CID. These experiments were set up using Xcalibur software. Data for the zoom scan were averaged over 2 min. The isolation width was determined by changing the width from 1 to 4 amu wide in 1 amu increments, and collecting data for 1 min at each interval. In studying the amount of energy required for CID, all experiments were conducted at a heated capillary temperature of 250 °C and a tube lens offset of 30 V, while changing the %CID