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Multistage mass spectrometry of phospholipids using collision-induced dissociation (CID) and metastable atom-activated dissociation (MAD)

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ABSTRACT

We herein demonstrate an approach to gas phase ion manipulation that provides MS^3-level CID spectra of phospholipid radical cations that are almost independent of the original charging adduct ions. In the MS^2 He-MAD spectra of the protonated, sodiated and potassiated adducts of POPC, the different adducts induce different primary fragmentation pathways and provide significantly different spectra, as is commonly observed by other activation methods. In separate experiments, the even-electron adduct ions ($[M+H]^+$, $[M+Na]^+$, $[M+K]^+$) of 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) were first converted to radical cations $[POPC]^{+\bullet}$ by using helium metastable atom-activated dissociation (He-MAD) to eject the charging adduct ions, then exposed to low-energy collision induced dissociation (CID) to induce extensive fragmentation along the acyl chains. Such charge-remote fragmentation is generally inaccessible through low-energy CID of the even-electron precursor ions. The combination of He-MAD and CID provides radical-induced spectra that show very major similarities and only minor differences, and therefore overcomes major differences in chemistry that are otherwise observed by the original adducting species. Collisional activation of even-electron $[POPC+H]^+$ required higher CID amplitudes than odd-electron $[POPC]^{+\bullet}$ to effect fragmentation—as expected—and the latter provided fragments within the acyl chains that were influenced by the double bond position.

1. Introduction

Lipids are the major building blocks of cellular membranes and possess crucial relevance in signal transduction and the storage of energy in biological systems [1]. Changes in lipid profiles and their distribution are found to be closely related to many pathological conditions such as Alzheimer's disease, Down syndrome, and diabetes [2]. Because of the biological relevance of lipids in organisms, substantial efforts have been devoted to the study of lipids, or lipidomics [3,4].

Mass spectrometry (MS) is a powerful method that has become an indispensable tool in the study of biomolecules. Mass spectrometric applications in lipid analysis started in the early 1970s with electron ionization mass spectrometry (EI-MS) [5,6] and fast atom bombardment mass spectrometry (FAB-MS) [7]. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) and electrospray ionization mass spectrometry (ESI-MS) became available in the 1990s [2,8]. Being soft ionization techniques, MALDI and ESI exhibit a sensitivity that is 2–3 orders of magnitude greater than that achieved by FAB-MS [8].

The aforementioned soft ionization techniques excel in preserving intact molecular ions, but at the expense of useful structural information, that is, they provide molecular ions but no fragment ions. To enhance the structural information, tandem mass spectrometry (MS^n) experiments are often performed, and the most common of which is collision-induced dissociation (CID) [8–12]. CID-based mass spectra of phospholipids are typically dependent on the adduct form of precursor ion. For example, the CID spectrum of protonated adducts of phosphatidylcholines are dominated by a phosphocholine ion at m/z 184, whereas CID of alkali metal-adducted ions produces several fragment ions that allow elucidation of the identities and positions of fatty acid substituents

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[10]. CID of proton-bound dimers has recently been shown to distinguish cis- and trans-isomers of double bonds in addition to their position in the acyl chains [13].

In addition to CID, other methods of tandem mass spectrometry—such as post source decay (PSD) [14] and ozone induced dissociation (OzID) [15–20]—have also been applied in lipid analysis. In OzID, mass-selected lipid cations are exposed to ozone vapor to initiate the gas-phase ion-molecule reaction. Subsequent ozonolysis results in diagnostic fragment ions that can unambiguously identify C=C double bond location(s). Alternative fragmentation methods, such as infrared multiphoton dissociation (IRMPD) [21], ultraviolet photodissociation (UVPD) [22], electron transfer dissociation (ETD) [23], and electron impact excitation of ions from organics (EIEIO) [24] have also been recently employed in the characterization of glycerolipids. These high energy and radical-based methods typically activate more pathways than even-electron low energy rearrangements, so they provide complementary fragments to conventional CID [25].

Metastable-atom activated dissociation (MAD) is another developing tandem MS fragmentation method [26–32]. To date, MAD has been used in a variety of studies concerning peptide structures, including multiply charged cations and anions, 1+ cations, phosphorylated cations, disulfide bonds, to cleave the amide ring structure of proline and to differentiate isoleucine from leucine [30–32]. In addition to fragmenting peptides, MAD has been shown to provide high energy and radical-induced fragmentation of lipid cations [33]. In contrast to CID, which exclusively proceeds through even-electron mechanisms, MAD produces both even-electron and odd-electron fragments.

To explore the unique features of MAD, we herein demonstrate the ability to acquire CID spectra of radical cations that are independent of the charging adduct ions. Isolated, even-electron adduct ions ($[M+H]^+$, $[M+Na]^+$, $[M+K]^+$) are first converted to odd-electron molecular ions $[M]^{+\bullet}$ through the use of He-MAD. Low-energy collisional activation of the isolated radical cations ions then induces extensive fragmentation along the acyl chains of the lipids through mechanisms that are not achievable from CID of even-electron precursor ions. Distinctive radical fragments are also observed and described, which illustrate the potential utility of this type of gas-phase ion manipulation.

2. Experimental

2.1. Instrumentation

All experiments were performed on a modified Bruker amaZon ETD mass spectrometer (Bruker Daltonics, Bremen, Germany). The modification method, the connection between electronic components and the working principles are described elsewhere [32,33].

2.2. Materials

The lipid used in this study was 1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine PC(16:0/18:1(9Z)), which is abbreviated as POPC. POPC was diluted to a final concentration of 60 μM using a 9:1 (v/v) mixture of methanol to water containing 1% (v/v) acetic acid to obtain the protonated ions and 0.05 M NaCl or KCl to obtain metal-adducted ions. Ultra high purity helium (Airgas, Parkersburg, WV) was used in the FAB gun and was further purified using a noble gas purifier (HP2, VICI, Houston, TX) to remove impurity gases that can rapidly quench metastable atoms.

2.3. Methods

Singly charged lipid precursor ions were generated through electrospray ionization (ESI) using an electronic syringe pump

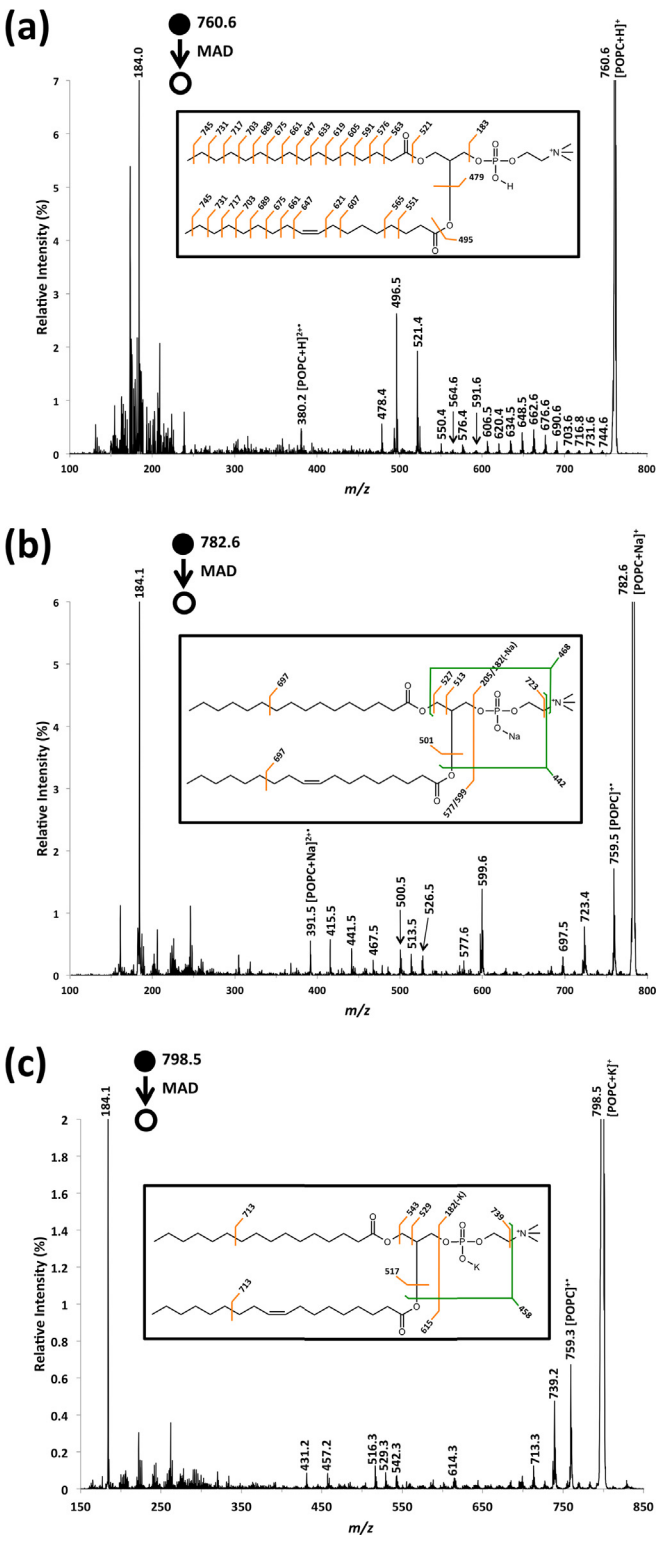

Fig. 1. He-MAD spectra of (a) protonated form of POPC, (b) sodiated form of POPC, (c) potassiated form of POPC. Insets show possible cleavages and theoretical masses for fragmentations without hydrogen rearrangements.

(#1725, Hamilton Company Reno, Nevada, NV) with a flow rate of 160 μL/h. The ion of interest was usually isolated using an isolation window of 4 Da before exposure to the helium metastable beam. Occasionally, isolation windows of 1 Da were used to obtain the mono-isotopic form of the precursor ion and help differentiate ^{13}C isotope peaks from H-atom transfer peaks. The low mass