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Radical-induced fragmentation of phospholipid cations using metastable atom-activated dissociation mass spectrometry (MAD-MS)

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ABSTRACT

The fragmentation pattern of several protonated 1+ phosphatidylcholines (PCs) was studied using low energy collision induced dissociation (CID) and helium metastable atom-activated dissociation (He-MAD). He-MAD of the protonated compounds produced a dominant phosphocholine head group at *m/z* 184 as well as typical sn-1 and sn-2 glycerol fragments such as $[M+H-R_{x-1}CHC{=}O]^+$ and $[M+H-R_{x-1}CO_2H]^+$. Within the aliphatic chain, He-MAD showed fragments consistent with high-energy collision induced dissociation (HE-CID) and products/pathways consistent with Penning ionization of the 1+ precursor ions to their respective radical dications. These Penning ionization products included both singly and doubly charged radical fragments, and the fragment ions are related to the number and position of double bonds in the acyl chains. Fragments created through HE-CID-like fragmentation followed classic charge remote fragmentation pathways including ladder-like fragmentation along the acyl chain, except for additional or missing peaks due to predictable rearrangement reactions. He-MAD therefore shows utility in being able to effectively fragment singly charged lipids into a variety of useful product ions using both radical and high-energy processes in the confines of a 3D ion trap.

1. Introduction

Recent advances in mass spectrometry have allowed scientists to probe different biological systems that were unattainable in the past. This increase in investigative power has contributed to the development of different fields of research including proteomics [1], genomics [2], and lipidomics [3,4]. Lipidomics is defined as the full characterization of lipid molecular species and of their biological roles with respect to expression of proteins involved in lipid metabolism and function, including gene regulation [5]. This field is expansive and covers everything from identifying which lipids are localized in cells to the role those particular lipids play in a metabolic cycle [4]. Mass spectrometry is an attractive technique for analyzing lipids due to its high selectivity and sensitivity, ability to quantify, abundance of structural information and ability to perform a variety of experiments [5,6].

Mass spectrometric characterization of lipids began with electron ionization (EI) [7–9] via GC-interfaces, but most current research now relies on low-energy collisional induced dissociation (CID) [10–13] using matrix-assisted laser desorption ionization (MALDI) or atmospheric ion sources such as electrospray ionization (ESI), APCI and atmospheric pressure (AP)-MALDI. The fragmentation products of glycerolipids are normally restricted to $[M+Y-R_{x-1}CO_2H]^+$, $[M+Y-R_{x-1}CHC{=}O]^+$, where R corresponds to the sn-1 or sn-2 fatty acid chain from which the fragment originated, and Y is the charging adduct, such as Na^+ or H^+. The phosphocholine head group is another major fragment [12,14,15]. CID can provide useful information about a lipid by identifying its class, lipid chain lengths and the degree of unsaturation [16] but this does not represent all of the pertinent information about the

Abbreviations: CID, collision induced dissociation; CRF, charge remote fragmentation; DAPC, 1,2-di-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphocholine; 9E-DOPC, 1,2-di-(9E-octadecenoyl)-sn-glycero-3-phosphocholine; 9Z-DOPC, 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine; EI, electron ionization; ESI, electrospray ionization; FT-ICR, Fourier transform ion cyclotron resonance; HE-CID, high-energy collision induced dissociation; LMCO, low mass cut-off; MAD-MS, metastable atom-activated dissociation; PC, phosphatidylcholine; PI, Penning ionization; POPC, 1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine; PSPC, 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine; SM, sphingomyelin; TOF, time of flight.

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structure/function of lipids. The position and isomeric form of double bonds are also important.

Researchers have developed a variety of tandem mass spectrometry approaches to interrogate the gas-phase structure of lipids in mass spectrometry. Some recently developed approaches include multistage mass spectrometry [16,17], post-source decay (PSD) matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF) [18], chemical IRMPD [19–21], and ion-molecule chemistry like Paternò-Büchi reactions [22], and ion-ion chemistry [23]. Blanksby's group has provided a relatively straightforward and reliable method to determine the lipid class and double bond positions of unsaturated lipids using ozone-induced dissociation (OzID) of gas-phase ions [16,24–27]. Although most lipids seem to fragment through even electron pathways using OzID, there is evidence that heavily conjugated lipids (e.g. fatty acid methyl esters, FAMES) can produce odd-electron fragments [28].

Although radical-induced dissociation of multiply-charged peptides and proteins is readily achievable via a variety of photon- or electron-based activation methods [29–34], radical ion chemistry is generally harder to drive when starting with a 1+ or 1− even-electron precursor ion, like most lipids. Recent examples of radical-induced fragmentation methods of lipids include ETD [35], UVPD [20], and electron-impact ionization excitation of ions from organics (EIEIO) [36]. These new methods have pros and cons and are still in their developing stages. They are likely to become more useful as research in these areas progresses.

An alternate activation method known as metastable atom-activated dissociation (MAD) [37–44] has been used to fragment peptides and small proteins via radical cation chemistry, and we believe this is the first report applying MAD to the fragmentation of gas-phase lipids. In contrast to CID, which almost exclusively involves even electron rearrangements, MAD causes fragmentation through radical-induced rearrangements that can induce fragmentation pathways unavailable through even electron mechanisms.

2. Methods and instrumentation

2.1. Instrumentation

All experiments were performed on a modified Esquire-LC or amaZon QIT mass spectrometer (Bruker Daltronics, Bremen, Germany), the former of which has been described in a previous work [41]. Metastable atoms were generated with an Ion Tech FAB gun (P50, PSU, Teddington UK) and deflection electrodes were used to remove electrons and ions from the beam. The FAB gun was pulsed using custom electronics (described previously) to coincide with the fragmentation period in the scan function normally reserved for CID. The CID amplitude was set to 0 V during MAD so the ions are effectively just stored at a specified q_z while the metastable atom source is pulsed on for ~300 ms. A visual schematic and description of the connections used in this process have been provided elsewhere [41].

2.2. Reagents

All the lipids used in this experiment were purchased from Avanti Polar Lipids (Alabaster, AL). Lipids used in this experiment included 1-hexadecanoyl-2-octadecanoyl-*sn*-glycero-3-phosphocholine PSPC (PSPC, 16:0/18:0), 1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine (POPC, 16:0/18:1(9Z)), 1,2-di-(9E-octadecenoyl)-sn-glycero-3-phosphocholine (9E-DOPC, 18:1(9E)/18:1(9E)), 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine (9Z-DOPC, 18:1(9Z)/18:1(9Z)), 1,2-di-(5Z,8Z, 11Z, 14Z-eicosatetraenoyl)-sn-glycero-3-phosphocholine (DAPC, 20:4/20:4), and sphingomyelin (SM, d18:1/18:0). HPLC-grade methanol and glacial acetic acid were purchased from Sigma–Aldrich (St. Louis, MO). All lipids were reconstituted in a 9:1 mixture of methanol:water (with 1% acetic acid) to provide lipid solutions of approximately 60 μM for analysis. Ultra high purity helium (Airgas, Parkersburg, WV) was used with the FAB gun and further purified using a noble gas purifier (HP2, VICI, Houston, TX) to remove impurities that could otherwise prevent the formation of, or quench, metastable atoms.

2.3. Method

Singly charged lipid ions were generated through electrospray ionization (ESI) using an electronic syringe pump (74900, Cole-Parmer Instrument Company, Vernon Hills, IL) at a rate of 250 μL/h. After injection, precursor ions were isolated using a width of 1–4 Da before exposing them to the helium metastable atom beam at a low mass cut off (LMCO) of m/z 100. The metastable atom beam was typically pulsed on for 299 ms at an anode voltage of 6 kV. The vacuum chamber base pressure outside of the ion trap measured 1.68×10^{-5} mbar and was populated mostly by helium bath gas leaking out of the trap. After the addition of helium gas to the FAB gun, the vacuum pressure increased to 3.0×10^{-5} mbar. The holes in the ring electrode and the difference in bath gas pressure in the trap did not have any measurable effect on trap performance, but the instrument was re-tuned and re-calibrated prior to use anyway.

Collection of MAD data for each lipid took a approximately 10 min and consisted of: (1) 2 min of the full scan acquisition of the ESI spectrum of the sample; (2) 2 min of the isolated precursor ion; (3) 2 min of He MAD of the precursor with the deflection electrode on; (4) 2 min of He MAD with the deflection electrode off; and (5) 2 min of He MAD background signal (ESI off). These relatively long acquisition times enabled averaging many spectra together to improve the signal-to-noise ratio of low-abundance peaks. The MAD background is defined as the ion signal that is collected with the FAB gun on and the ESI source off, and consists of Penning ionization products of residual gases and pump oil. We expect a mixture of ion/ion and metastable atom chemistry with the deflection electrodes off, but a higher relative proportion of metastable atom-induced chemistry with the deflection electrodes on. MAD spectra shown in the figures have been background corrected using the average of the 2-min MAD background signal. The magnitude of the background signal is roughly two orders of magnitude larger than the low-abundance fragment ions of the lipids. Because the absolute magnitude of the background varies by a few percent, depending on the averaging, the variance is on the same order of magnitude as the low abundance fragment ions. It is for this reason that background subtraction in the low mass region does not result in a flat baseline.

Collisional activation (CID) analysis was then performed following MAD acquisition and included: (1) 2 min of the full-scan ESI spectrum; (2) 2 min of the isolated precursor (no CID); (3) 2 min of CID of the precursor. Ion generation, accumulation, manipulation, and detection were optimized for each lipid to provide consistent precursor ion signals of approximately 1×10^6 AU. All fragments were identified manually based on the predicted masses and were found to be within m/z 0.3 of the observed masses.

2.4. CID

All lipids were fragmented with the "SmartFrag" option in the Bruker Esquire NT 4.5 software. SmartFrag exposes the precursor ions to a linear-amplitude-modulated-waveform from 30 to 200 percent of the selected CID amplitude. The fragmentation time was set to 25 ms in all the CID experiments. The 100% amplitude setting of the CID ranged from 0.85 to 1.40 V, depending on the isolated mass. A typical acquisition spanning about 2 min contained