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Re-print of "Radical Delivery and Fragmentation for Structural Analysis of Glycerophospholipids"

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ABSTRACT

Conventional tandem mass spectrometry relies on even-electron fragmentation that provides limited structural information for glycerophospholipids (GP), which are key constituents of all cell membranes. Different GP classes are chemically very similar and subtle variations in carbon-carbon bonding features and linkages can lead to numerous isomeric structures that are challenging to distinguish with traditional mass spectrometry. In this study, we demonstrate that the primary amine groups in many GP classes can be modified with either noncovalent attachment of crown ether derivatives containing an iodobenzoyl moiety, or by direct covalent attachment of the iodobenzoyl moiety. Radical lipids can be generated using these modifications via photoactivation of labile carbon-iodine bonds, providing rich information about headgroup and fatty acid chain structure. The method is demonstrated for lipid standards containing various carbon chain motifs and linkages, as well as phospholipids extracted from a soybean mixture. Numerous lipids were examined, including plasmalogen-, lyso-, diacyl- types of phospholipids containing mono-/poly- unsaturated fatty acid (FA) substituents, and branched-/nonbranched-FA chains. Interestingly, the presence of double bond and/or vinyl ether linkage leads to the formation of a signature fragment ion that facilitates rapid structural identification.

1. Introduction

Glycerophospholipids (GP) are key constituents of all cell membranes and important targets for characterization by lipidomics [1–3]. Unfortunately, traditional tandem mass spectrometry utilizing collisional activation of even electron ions provides limited structural information about the carbon-carbon bonding features in the various GP classes, which are chemically very similar and often isomeric [4,5]. Tandem mass spectrometry can typically reveal information about headgroup class and general fatty acid composition via collision induced dissociation (CID) experiments [6,7]. However, subtle variations in carbon-carbon bonding features and linkages to the glycerol backbone are not easily distinguished by CID. Nevertheless, the separation and analysis of such isomeric species by mass spectrometry remains an important goal and is an active area of research [8–13].

Radical-based dissociation techniques have been used to provide unique information in experiments targeting peptides and proteins, [14–20] and these radical-based methods have recently started to attract attention in the investigation of lipids as well [21–25]. One route for generating radical species is to use electrons as in electron capture dissociation (ECD) or electron-transfer dissociation (ETD). Applications of ECD/ETD in lipidomics are hampered by the fact that multiply positively charged species must initially be produced so that the product ions will be detectable, yet lipids are not typically multiply charged species. This problem can be ameliorated to some extent by the addition of divalent metal adducts or multiple metal adducts (to form doubly positively charged lipid ions) [21,22]. In many cases, characterization of lipids by ECD/ETD under these conditions yielded in similar information to that obtainable by conventional CID [21,22]. The most abundant dissociation channels provide information about headgroup class, carbon number, and degree of unsaturation on each acyl chain constituent. Very little information on other structural features, such as double bond or sn-positions of the fatty acyl chains on the glycerol backbone is typically provided.

Interestingly, photochemistry can also be used to generate radial species. In these experiments, absorption of 266 nm photons leads to population of dissociative excited states, resulting in homolytic cleavage of specific bonds [23]. The radical species generated by PD can be collisionally activated to stimulate migration of the radical that eventually leads to radical directed dissociation (RDD). This method differs from other photoactivation experiments where

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photons are the primary initiators of the observed fragmentation, as is the case for ultraviolet photodissociation (UVPD) [26–29] and infrared multi-photon dissociation (IRMPD) [30,31] based experiments.

In an RDD experiment, photons are used to generate the radical, which is then the primary initiator of fragmentation. Native lipids do not contain bonds that undergo excited state dissociation upon photoactivation, requiring that such bonds be added to the lipid via covalent or noncovalent modifications. The noncovalent strategy relies on bifunctional molecules that contain a photo-caged radical initiator and a sticky lipid-adducting group. For example, 4-iodobenzoic acid is well suited to attach to the choline headgroup thanks to the ionic attraction between the fixed positive charge and the negatively charged carboxylic acid. Generation of lipid radicals in this fashion was used to successfully discriminate between branched and non-branched phosphatidylcholine (PC) lipid isomers [23]. Alternatively, 4-iodobenzoyl 18-crown-6 (IB-18C6) interacts very specifically with primary amine containing lipids such as those with phosphoethanolamine (PE) and phosphoserine (PS) headgroups [25]. In this case, noncovalent attachment of 18C6 is so efficient that it can quantitatively mass-separate PE lipids from their PC isomers in biological samples. A novel product ion from RDD in experiments with IB-18C6 was used to determine the location of the double bond for lipids with an unsaturated fatty acid chain [25].

In this study, we further investigate methods for generating lipid radicals and the utility of radical fragmentation for structural elucidation of lipids. We demonstrate that the primary amine groups in various GP classes with different linkages can be modified with either noncovalent attachment of IB-18C6 or by covalent attachment of an iodobenzoyl functional group for radical delivery. Subsequent collisional activation and RDD is shown to be useful for characterization of plasmalogen-, lyso-, diacyl- types of glycerophospholipids, which contain mono-/poly- unsaturated as well as branched-/nonbranched- fatty acid (FA) substituents. The results further support the hypothesis that radical chemistry is useful for both headgroup and FA chain structural elucidation.

2. Experimental methods

2.1. Chemicals

Methanol and chloroform (HPLC grade) were purchased from Thermo-Fisher Scientific (Waltham, MA), other organic solvents were purchased from Sigma–Aldrich or Acros Organics and used without purification unless otherwise noted. Water was purified to 18.2 MΩ resistivity using a Millipore Direct-Q water purification system.

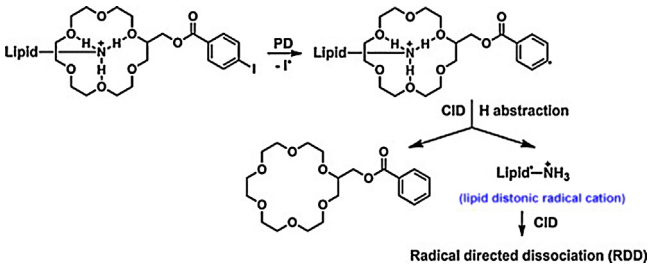
2-hydroxymethyl-18-crown-6 ether was obtained from TCI America (Waltham, MA), *N*-hydroxysuccinimide was purchased from Sigma–Aldrich (St. Louis, MO), 4-iodobenzoic acid was purchased from Acros Organics (Geel, Belgium). The procedure for synthesis of 4-iodobenzoyl 18-crown-6 (IB-18C6) and 4-iodobenzoyl *N*-hydroxysuccinimide ester (IB-NHS ester) were previously reported [32].

Phospholipid standards were purchased from Avanti Polar Lipids (Alabaster, Alabama, USA), including phosphatidylethanolamine: non-branched PE 16:0/16:0, branched PE br-20:0/br-20:0 or specifically PE 16:0(3Me,7Me,11Me,15Me)/16:0(3Me,7Me,11Me,15Me), unsaturated PE 18:1(9Z)/18:1(9Z), poly-unsaturated PE 16:0/18:2(9Z,12Z), deuterium labeled standard PE 16:0-d31/18:1(9Z), plasmalogen PE or plasPE 18:0p/18:1(9Z), lyso-phosphatidylethanolamine LPE 18:1(9Z), phosphatidylserine PS 16:0/18:1(9Z) and lyso-phosphatidylserine LPS 18:1(9Z). Asolectin from soybean mixture of lipids was obtained from Sigma–Aldrich (St. Louis, MO). Lipid nomenclature and shorthand notation is employed from Liebisch et al [33].

2.2. Sample preparation

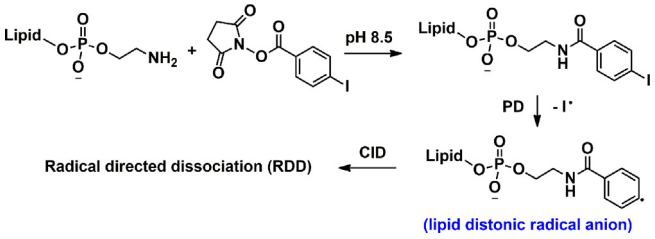
2.2.1. Noncovalent modification

Fresh lipid samples were prepared by diluting chloroform stock solutions or lipid extracts in methanol to a final concentration of 1–5 μM. When desired, IB-18C6 (with a ratio lipid:crown 1:4) was added to solution immediately prior to positive ion ESI–MS experiments [25].



2.2.2. Covalent modification

Lipids (~20 nmoL) were derivatized with IB-NHS ester by reacting 1:2 ratio (lipid: IB-NHS ester) in 100 μL 1:1 borate buffer (pH 8.5):organic solvent (DMSO) for 30 min at 40 °C in a water bath. After cooling, water (0.5 mL) and chloroform (0.5 mL) were sequentially added to the solution. The chloroform phase was then collected and diluted in methanol to a final lipid concentration of 5 μM prior to negative ion ESI–MS experiments. Reaction yields were estimated to be ~90–100% for PE lipids and ~30–50% for PS lipids (see Fig. S-5).



2.3. Instrumentation

Experiments were performed using a modified linear ion-trap mass spectrometer, Thermo Scientific LTQ (San Jose, CA). Typical source parameters: spray voltage 4–5 kV; capillary voltage 25 V; tube lens voltage 125 V; capillary temperature 215 °C; sheath gas flow 8 (arbitrary units); and sweep and auxiliary gas flow set at between 0 and 5 (arbitrary units).

The linear ion trap has been modified as previously described to enable photodissociation (PD) experiments [19]. Laser pulses (266 nm) were produced by a flashlamp-pumped Nd:YAG laser (Continuum, Santa Clara, CA), and were synchronized to the beginning of the activation step of a typical MS^n experiment via a digital delay generator (Berkeley Nucleonics, San Rafael, CA). The MS stage that is synchronized with the laser can be chosen using the diagnostics menu of the default instrument X-Calibur software. Ions were