Mass Spectrometry Imaging with Isomeric Resolution Enabled by Ozone-Induced Dissociation

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Abstract:

Mass spectrometry imaging (MSI) enables the spatial distributions of molecules possessing different mass-to-charge ratios to be mapped from biological tissues. For the first time, we demonstrate both enabling the differential imaging of isomeric lipid molecules directly from tissues. Here, this chemical ambiguity is addressed through an innovative combination of ozone-induced dissociation reactions with MSI, and identifying the association with disease-driven chemical alterations.

Nowhere is the need for isomer-resolved MSI more apparent than in lipid biology where the cumulative variation results in more than 100,000 possible lipid structures in the cellular lipidome. In part, this complexity arises because of structural isomers varying only in fatty acyl chain composition, sites of unsaturation (double bond-positional isomers, db), and esterification position of fatty acyl chains in glycerolipids (sn-positional isomers). While the structural differences between these isomeric variants are subtle, they demarcate distinct biosynthetic origins and biological functions. Isomeric resolution is therefore essential to understand the origin of de novo synthesized lipids from enzymatic processes, such as the action of specific elongase and desaturase enzymes in creating double bond-positional isomers. Resolving structural isomers is also vital for better understanding disease progression. For instance, the dysregulation of pathways involving the phospholipase A2 (PLA2) family of enzymes – responsible for selective hydrolysis of fatty acids localized to the sn-2 position on the glycerol backbone – has been implicated in a variety of cancers.

Conventional tandem mass spectrometry provides distinct spectra for different fatty acyl chain combinations but is largely blind to the presence of regiosomers arising from db- or sn-positional variation. By necessity, alternative ion activation strategies have been developed to resolve signals arising from isomeric lipids. These include photochemically activated Paternó-Büchi reactions, ultraviolet photodissociation, electron-based activation and ozone-induced dissociation (OzID). OzID has proven to be particularly versatile due to the production of easy to interpret spectra and ability to resolve both db- and sn-positional isomers. Ion mobility coupled with mass spectrometry has also emerged as an alternative method to resolve isomeric lipids. Application of ion mobility to complex biological extracts however, does not always yield separation of isomers and, in instances where the presence of multiple isomeric contributors is demonstrated, the structural differences between them remain to be elucidated. It is perhaps most remarkable that a mixture of isomeric lipids is observed in almost every case where isomeric resolution is achieved for biological extracts. For example, using photochemically activated Paternó-Büchi reactions, lipids with double bonds located at the n-7 position (with respect to the methyl terminus) were observed to be elevated relative to the corresponding n-9 isomers in cancerous breast tissue. The relative abundance of db- and sn-positional isomers has also been observed to alter dramatically across different tissue types providing a strong motivation to visualize selective and conserved alterations in lipid isomer populations by MSI.

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Supporting information for this article is given via a link at the end of the document.
Here we present the first report of MSI offering unambiguous isomeric resolution when applied to multiple families of lipid isomers. This was accomplished by the unique coupling of matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) with gas-phase ozonolysis of mass-isolated lipid ions (OzID) in a linear ion trap mass spectrometer. This approach enabled us to visualize highly specific enrichment of multiple isomeric lipids in distinct spatial regions in the rat brain. OzID spectra were obtained at each pixel of a MALDI-MSI experiment to reveal spatial distributions of distinct lipid isomers in tissues on a modified MALDI-LTQ-Orbitrap Elite mass spectrometer (Figure S1, Supporting Information). Ozonolysis reaction schemes are provided in Supporting Information (Scheme S1 and S2). Prior to analysis tissues were coated with norharmane matrix and sodium acetate to preferentially drive formation of [M+Na]+ ions beneficial for OzID. First, we investigated the distributions of db-positional isomers corresponding to two monounsaturated lipids in rat brain; PC(34:1) and PC(36:1) with acyl chains comprising 34 and 36 carbons, and having relative abundances of ~100% and 30%, respectively, in the full-scan FTMS spectrum. The MALDI-OzID spectrum of [PC(34:1)+Na]+ is shown in Figure 1(a) and reveals two sets of product ions consistent with ozonolysis of distinct db-isomers with unsaturation at the n-9 (m/z 672.4 and 688.3) and n-7 (m/z 700.4 and 716.4) positions. In all cases the assignment of product ions is supported by high accuracy mass measurements obtained using the Orbitrap mass analyzer (Table S1, Supporting Information) and are consistent with the predicted OzID transitions (Scheme S1). We note that due to the required 10 second reaction time, only a small area of the sample could practically be measured. Figure 1(b) shows the on-tissue distribution of the [PC(34:1)+Na]+ precursor ion from Orbitrap MSI data acquired from a consecutive tissue section to that used for OzID experiments providing only the summed distribution of all contributing isomers based on exact elemental composition and reveals a relatively homogenous distribution of [PC(34:1)+Na]+ signal throughout the rat brain, with only a minor elevation of signal observed in the white matter. However, activating isomeric resolution via OzID enables the distribution of distinct db-positional isomer populations to be observed. Figure 1(c) shows the fractional distribution image (FDI) representing the intensity of n-7-related ions as a fraction of all n-9 and n-7-related ion signals (i.e., a higher intensity corresponds to a larger population of the n-7 isomer relative to the n-9 isomer) and reveals an enrichment of the n-7 isomer in the gray matter of the brain. Conversely, enrichment in signals characteristic of the PC34:1(n-9) isomer are observed in the white matter. These data reveal for the first time a differential, and cell type-specific spatial distribution of lipid db-isomers in biological tissues with a relative ~2-fold increase of PC34:1(n-7) observed in the gray matter relative to isomeric PC34:1(n-9) (Figure 1g). Importantly, given the near-identical chemical nature of these isomeric species, and thus equivalent ionization efficiencies, the ratio images are unaffected by possible differences in desorption/ionization efficiencies or changes in precursor ion abundance at different tissue regions. We note that these data reflect relative changes in product ion abundance rather than absolute isomer concentrations, however changes in FDI are a clear reflection of changing isomer populations across the tissue. An analogous assessment of PC36:1 db-positional isomers was also performed (Figure 1d-f). The full-scan MSI distribution...
PC(20:1/16:0) isomeric pair (characteristic fragment ions at CID/OzID product ions are indicative of a PC(16:0/20:1) and ratios between white and gray matter (Figure 2j). The additional very slight (~1%), although still significant, alterations in isomer corresponding FDI (Fig. 2g) of these two isomers reveals only originating from [PC(18:1/18:0)+Na] + (summed distributions to be enriched in the white matter (Figure 20). The full-scan Orbitrap MSI revealing the consistent with four unique isomeric structures, spectrum (Figure 2e) reveals a complex fragmentation profile further highlighted in the distributions of [PC(36:1)+Na] + (acquired from a consecutive tissue) reveals the summed image of all contributing isomers for the [PC(36:1)+Na] + ions and exhibits a strong enrichment throughout the white matter. OzID analysis at each pixel reveals two distinct isomer populations and distributions corresponding to n-9 (m/z 700.4 and 716.4) and n-7 (m/z 728.4 and 744.4) isomers. The corresponding FDI reveals a significant relative enrichment of the n-7 isomer in the gray matter and the n-9 isomer in the white matter (Figure 1f, h). These data suggest that individual lipid structural isomers play distinct roles in local cellular organization and metabolic processes. It is likely our MSI results reflect the underlying local activity of certain desaturase and elongase enzymes that are involved in de-novo lipid synthesis.[5] We also investigated db-isomers for polyunsaturated PC lipids (PC(36:2), PC(36:4) and PC(38:6)) but found significant less isomeric diversity (Figure S2, Supporting Information).

Using sequential CID/OzID processes to further probe isomers of PC(34:1) and PC(36:1), sn-positional variants can also be resolved and visualized throughout the rat brain. In the case of [PC(34:1)+Na] + (Figure 2a), product ions confirming the presence of PC(16:0/18:1) (m/z 379.3 and 395.3) and PC(18:1/16:0) (m/z 405.3 and 421.3) isomeric populations are observed. Rationalization of these product ions is provided in Scheme S2 (Supporting Information) and support the assignment of the presence of both possible sn-positional isomers within the tissue[10b]. The full-scan MSI distribution of the precursor ion at m/z 782.5680 reveals a relatively homogeneous distribution throughout the brain (Figure 2b) and represents a composite distribution of isomers. However, the corresponding FDI (Figure 2c) reveals a subtle, yet significant (Figure 2i) difference in the distribution of isomers. In the white matter, PC(18:1/16:0) represents ~31±3% of the lipid isomer population and this increases to 43±2% in gray matter. Importantly, for such monounsaturated sn-positional isomers relative CID/OzID product ion abundances have been demonstrated to be reflective of absolute isomer populations, thus these results are expected to reflect absolute molar fractions.[11c] Moreover, we observed an increase of LPC(18:1) relative to LPC(16:0) in the white matter (Figure S3, Supporting Information). Assuming PC(16:0/18:1) to be the largest source of these lyso lipids, these results allude to the possibility of increased phospholipase A1 activity in the white matter. These data suggest that individual lipid structural isomers play distinct roles in local cellular organization and metabolic processes. It is likely our MSI results reflect the underlying local activity of certain desaturase and elongase enzymes that are involved in de-novo lipid synthesis.[5] We also investigated db-isomers for polyunsaturated PC lipids (PC(36:2), PC(36:4) and PC(38:6)) but found significant less isomeric diversity (Figure S2, Supporting Information).

Figure 2. (a) MALDI-CID/OzID spectrum of [PC(34:1)+Na] + ions revealing the presence of PC(16:0/18:1) and PC (18:1/16:0) structural isomers. (b) The corresponding full-scan FTMS image of the [PC(34:1)+Na] + ion and (c) fractional distribution image of PC(16:0/18:1) ions as a fraction of PC(16:0_18:1)-related ions. (d) Post-acquisition stained tissue. (e) MALDI-CID/OzID spectrum and (f) full-scan FTMS image of [PC(36:1)+Na] + revealing the presence of four distinct sn-positional isomers (PC(18:0/18:1), PC(18:1/18:0), PC(16:0/20:1) and PC(20:1/16:0). The corresponding fractional distribution images of (g) PC(18:0/18:1) as a fraction of PC(18:0_18:1); and (h) PC(16:0/20:1) as a fraction of PC(16:0_20:1)-related ions. Stacked column graphs showing relative isomer percentages for (i) the 16:0/18:1 and 18:1/16:1 isomers in PC(34:1); (j) the 18:0/18:1 and 18:1/18:0 isomers for PC(36:1); and (k) the 16:0/20:1 and 20:1/16:0 isomers for PC(36:1) within the white- and gray-matter tissue regions. Error bars represent coefficient of variation (n=5 each for white- and gray-matter regions). Statistical significance was calculated using average spectra from 5 regions corresponding to white and gray matter. Statistical significance was calculated using the area underneath a response operating characteristic curve, with p-values being related to the Mann-Whitney U statistics.
isomer-specific product ions. As with all MS/MS methods, our isomicromolecular imaging approach is applied only to targeted precursor ions. However, the deployment of multiplexed acquisition sequence enables multiple isomeric populations to be probed in a single experiment (Supporting Information). Moreover, while in this work ozonolysis reaction rates are limited by the gas requirements of the ion trap, alternative OzID implementations on instrumentation compatible with high concentrations of ozone (e.g., certain q-ToF systems[17]) would translate to faster acquisition speeds and increased sensitivity, and provides an exciting area of future research. Such approaches could even enable assignment of double bond position to individual sn-locations.[10b] Nonetheless, our data suggests that lipid isomer synthesis is highly conserved and dependent on local tissue type/environment (e.g., white and gray matter). This high-degree of structural specificity must reflect the localized activity of enzymes that synthesize and regulate lipid components (e.g., fatty acids) and assemble complex lipids. Furthermore, these distributions reflect a biological need for distinct lipid isomer ratios that could confer distinct biophysical and biochemical properties.[18] There currently exists a general lack of understanding on the biological roles of individual lipid molecules and the reasons for the breadth of molecular complexity which they encompass.[4a] In MSI, although many studies have reported altered lipid distributions throughout tissues, the biological origin of these altered lipid compositions is largely unknown. A significant contributor to this knowledge gap has been the absence of isomer-level differentiation meaning that in all MSI studies to date, lipid distributions represent an unresolved mixture of structural isomers that may not be reflective of any one particular molecule or biological process. MALDI-OzID-MSI thus provides an innovative means to unravel the biochemical origins behind the lipid distributions observed throughout biology, opening up an avenue to unambiguous lipid imaging.

Experimental Section

All mass spectrometry imaging experiments were performed using an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific GmbH, Bremen, Germany) coupled to an intermediate pressure MALDI source (Spectroglyc LLC, Kennewick, WA, USA)[19]. All brain sections were coated with norharmane matrix for MALDI-MSI analysis. Ozone-induced dissociation was conducted at each pixel by mixing high concentration ozone (~14% wt% in O2) with the helium buffer gas entering the dual pressure ion trap. Further experimental details are provided in the Supporting Information.

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Resolution and unambiguous imaging of double bond- and sn-positional lipid isomers is realized by coupling of gas-phase ozonolysis reactions on mass-selected lipid ions with mass spectrometry imaging. Distinct distributions of these isomers are observed in the rat brain, thus highlighting the biochemical conservation and selectivity of lipid isomer synthesis in biology. This approach is a key step towards the MSI of structurally-defined molecules.

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