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Mapping of phospholipids by MALDI imaging (MALDI-MSI): realities and expectations

Louis J. Sparvero[a,c,*], Andrew A. Amoscato[a,c], C. Edward Dixon[b,e], Joseph B. Long[f], Patrick M. Kochanek[b,d], Bruce R. Pitt[c], Hülya Bayır[a,b,c,d], Valerian E. Kagan[a,c,*]

[a] Center for Free Radical and Antioxidant Health, Pittsburgh, PA 15213, USA
[b] Safar Center for Resuscitation Research, Pittsburgh, PA 15213, USA
[c] Department of Environmental and Occupational Health, Pittsburgh, PA 15213, USA
[d] Department of Critical Care Medicine, Pittsburgh, PA 15213, USA
[e] Department of Neurosurgery, University of Pittsburgh, Pittsburgh, PA 15213, USA
[f] Center for Military Psychiatry and Neuroscience, Walter Reed Army Institute of Research, Silver Spring, MD 21910, USA

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ABSTRACT

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) has emerged as a novel powerful MS methodology that has the ability to generate both molecular and spatial information within a tissue section. Application of this technology as a new type of biochemical lipid microscopy may lead to new discoveries of the lipid metabolism and biomarkers associated with area-specific alterations or damage under stress/disease conditions such as traumatic brain injury or acute lung injury, among others. However there are limitations in the range of what it can detect as compared with liquid chromatography–MS (LC–MS) of a lipid extract from a tissue section. The goal of the current work was to critically consider remarkable new opportunities along with the limitations and approaches for further improvements of MALDI-MSI. Based on our experimental data and assessments, improvements of the spectral and spatial resolution, sensitivity and specificity towards low abundance species of lipids are proposed. This is followed by a review of the current literature, including methodologies that other laboratories have used to overcome these challenges.

Abbreviations: 9-AA, 9-aminoacridine; ALI, acute lung injury; CA, cornu ammonis; CCI, controlled cortical impact; Cer, ceramide; CL, cardiolipin; CNS, central nervous system; DAG, diacylglycerol; DESI, desorption electrospray ionization; DHA, docosahexaenoic acid; DHB, 2,5-dihydroxybenzoic acid; ESI, electrospray ionization; FT-ICR, Fourier transform ion cyclotron resonance; HBSS, Hank's balanced salt solution; H&E, hematoxylin and eosin; 4-HHE, 4-hydroxyhexenal; 4-HNE, 4-hydroxynonenal; IMS, ion mobility spectrometry; ISD, in-source decay; ITO, indium tin oxide; LC, liquid chromatography; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; MS/MS, tandem mass spectrometry; MS^n, multiple-stage tandem mass spectrometry; MSI, mass spectrometry imaging; NALDI, nano-assisted laser desorption/ionization; NAO, 10-N-nonyl-acridine orange; NIMS, nanostructure initiator mass spectrometry; PA, phosphatidic acid; PBS, phosphate-buffered saline; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, phospholipid; PLC, phospholipase-C; PND, postnatal day; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid or fatty acyl; Q-TOF, quadrupole time-of-flight; SIMS, secondary ion mass spectrometry; SM, sphingomyelin; ST, sulfatide; TBI, traumatic brain injury; TFA, trifluoroacetic acid; TIC, total ion count; TLCL, 1,1′,2,2′-tetralinoleoylcardiolipin; TOCL, 1,1′,2,2′-tetraoleylcardiolipin; TOF, time-of-flight.

* Corresponding authors at: Center for Free Radical and Antioxidant Health, Department of Environmental and Occupational Health, University of Pittsburgh, Bridgeside Point, 100 Technology Drive, Suite 350, Pittsburgh, PA 15219-3130, USA. Tel.: +1 412 624 9479; fax: +1 412 624 9361.

E-mail addresses: ljs39@pitt.edu, sparverolj@upmc.edu (L.J. Sparvero), amoscatoaa@upmc.edu (A.A. Amoscato), dixoec@upmc.edu (C.E. Dixon), joseph.long@us.army.mil (J.B. Long), kochanekpm@ccm.upmc.edu (P.M. Kochanek), brucep@pitt.edu (B.R. Pitt), bayihx@ccm.upmc.edu (H. Bayır), kagan@pitt.edu (V.E. Kagan).

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1. Introduction

"It is always wise to look ahead, but difficult to look further than you can see." (Winston Churchill)

1.1. Multiple roles and molecular diversity of phospholipids and their oxidation products

Lipids are fundamental components of tissue architecture and are critical for tissue function. According to current estimates, the mammalian lipidome is comprised of 10,000–100,000 individual species of lipid molecules (Schuhmann et al., 2011) making it more diversified than the proteome or genome. This remarkable diversity of tissue-specific lipids originates from a few hundred lipid classes, including phospholipids (PLs), glycolipids, sphingolipids, and neutral lipids (Shevchenko and Simons, 2010). Phospholipids in particular consist of a headgroup, a phosphoester-connected glycerol backbone, and (usually ester-linked) two fatty acid chains. Different headgroups distinguish different phospholipid classes. These headgroups tend to be exposed to the aqueous environment while the hydrophobic fatty acid chains are usually embedded in cellular and subcellular membranes. There are over 15 common fatty acid residues that exist in varying combinations in different phospholipids. One notable exception from this general plan of architecture is cardiolipin (CL), a phospholipid that contains four fatty acid chains and is found exclusively in mitochondria. Its molecular diversity can thus be much larger. Most tissues such as lung, liver, and heart have only a few major species of CL in high abundance (Minkler and Hoppel, 2010). Brain tissue, on the other hand, is extremely diverse with respect to the number of CL species. A typical CL spectrum of murine brain exhibits approximately 10–12 major CL clusters, most of which have multiple individual CL species at several isobaric masses. This gives over eighty major molecular species of brain CL, all at relatively similar abundances (Kiebish et al., 2008; Bayir et al., 2007; Tyurin et al., 2008a; Sparvero et al., 2010; Samhan-Arias et al., 2012).

In addition to their structural role in membranes, polyunsaturated lipids are precursors of important signaling molecules that are formed by multistage oxygenation of polyunsaturated fatty acid residues (PUFAs) – linoleic (C18:2), arachidonic (C20:4), eicosapentaenoic (C20:5), docosapentaenoic (C22:5) and docosahexaenoic (C22:6) acids (Guichardant et al., 2011). Usually, phospholipase A2-catalyzed hydrolysis precedes the enzymatic oxygenation stages and represents a rate-limiting stage in the overall biosynthetic process. A huge number of different oxygenated fatty acids formed via this general pathway act as potent intra- and extracellular regulators of multiple biological functions (Marcheselli et al., 2003). Alternatively, esterified fatty acid residues of phospholipids that contain multiple double bonds are prone to oxidative modifications yielding predominantly hydroperoxy-, hydroxy-, epoxy- and oxo-functionalities to phospholipids (Domingues et al., 2008; Fuchs et al., 2011; Kim et al., 2011). Phospholipids with oxidized fatty acid residues can undergo subsequent hydrolysis by specialized phospholipases – e.g., lipoprotein lipase A2 – to yield oxygenated fatty acids and lyso-phospholipids (Vaishnav et al., 2010). The oxidatively modified phospholipids can also generate a number of secondary degradation products with truncated fatty acid chains (Tyurin et al., 2009; Yin et al., 2011; Liu et al., 2011; Hall et al., 2010). Peroxidation of polyunsaturated phospholipids may occur as a random – likely non-enzymatic – free radical oxidation commonly associated with oxidative stress and injury (Girotti, 1998; Hall et al., 2004; Singh et al., 2006). This type of oxidative damage can result from a variety of insults, including overload with catalytic transition metals (Valko et al., 2005), chemical poisoning with carbon-tetrachloride (Weber et al., 2003), and ischemia/reperfusion (Zhang et al., 1996). In many cases, phospholipid peroxidation and tissue damage and cell death can be selective whereby different phospholipids undergo oxidative modification at different rates (Tyurin et al., 2008b). Two anionic phospholipids – a mitochondria-specific CL and extramitochondrial phosphatidylserine (PS) – are the most reactive to oxidative stress during apoptotic cell death (Tyurin et al., 2008a; Korytowski et al., 2011). Of the many polyunsaturated fatty acid chains that these phospholipids exhibit, only a select few are prone to biological oxidative modification during apoptosis (Sparvero et al., 2010) due to the involvement of a specific catalyst, cytochrome c, in the oxidation process (Kagan et al., 2005; Tyurin et al., 2010). While these features of random (non-enzymatic) and selective (enzymatic) peroxidation reactions have been known for a long time, their spatial confinements to specific regions and to specific types of cells and subcellular compartments have not been definitively established. This is mostly due to the insufficiency of methodological approaches for the detailed and accurate mapping of lipids and lipid oxidation products in tissues.

1.2. Mass spectrometry and matrix-assisted laser desorption/ionization (MALDI) imaging in lipidomics

Mass spectrometry (MS) has emerged as the analytical technology with sufficient sensitivity and specificity to quantitatively analyze the complexities of biological materials at the molecular level (Gross, 2011; Murphy et al., 2011b). The advent of soft ionization technologies (laser desorption or electrospray ionization) enabled mass spectra of biological molecules (proteins, lipids, etc.) to be obtained. Coupling MS to separation techniques (such as liquid chromatography (LC)) and selective fragmentation by tandem mass spectrometry (MS/MS) markedly improves the analysis of complex mixtures. MALDI and electrospray ionization (ESI) are two of the most prominent soft-ionization MS methods for the direct, label-free analysis of intact lipids and each has its own strengths and limitations (Fuchs et al., 2010; Han et al., 2012). MALDI analysis can be performed on intact tissue sections (MALDI imaging or MALDI-MSI[1]) to provide spatial localization for various lipid species, while ESI–LC–MS of lipid extracts allows for a global assessment of lipid species (Delvolve et al., 2011; Goto-Inoue et al., 2011; Zemski-Berry et al., 2011; Fernández et al., 2011; Amstalden van Hove et al., 2010). Although lipid extracts are devoid of spatial information, microdissected regions of interest within a tissue section and their subsequent extraction can determine localization of lipid species (Burnum et al., 2009; Hankin and Murphy, 2010). Once an array of MALDI mass spectra is obtained using a pulsed laser across a tissue section, it is possible to create a lipid-ion image that is associated with a particular mass-to-charge (m/z) ratio. This image represents the spatial distribution and relative abundance of that particular lipid-ion, and can be correlated with histological features (Burnum et al., 2009; Delvolve et al., 2011; Carter et al., 2011). By adding internal standards, absolute abundances of several lipid classes can be measured with high precision using MALDI-MSI (Landgraf et al., 2011). MALDI-MSI does not require specific antibodies such as those used in immunohistochemical methods. This allows for direct analysis and discovery of molecular species. In contrast to radioactive or fluorescent labeling-based techniques, one is not limited to a small number of detectable species. Breakdown products (including lysophospholipids and oxidatively modified species) are also detectable as distinct molecular species (Koizumi et al., 2010; Wang et al., 2010).

[1] We have chosen to use "MALDI-MSI" although both it and "MALDI-IMS" are used by many authors in the field.