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Review

Surface analysis of lipids by mass spectrometry: More than just imaging

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ABSTRACT

Mass spectrometry is now an indispensable tool for lipid analysis and is arguably the driving force in the renaissance of lipid research. In its various forms, mass spectrometry is uniquely capable of resolving the extensive compositional and structural diversity of lipids in biological systems. Furthermore, it provides the ability to accurately quantify molecular-level changes in lipid populations associated with changes in metabolism and environment; bringing lipid science to the "omics" age. The recent explosion of mass spectrometry-based surface analysis techniques is fuelling further expansion of the lipidomics field. This is evidenced by the numerous papers published on the subject of mass spectrometric imaging of lipids in recent years. While imaging mass spectrometry provides new and exciting possibilities, it is but one of the many opportunities direct surface analysis offers the lipid researcher. In this review we describe the current state-of-the-art in the direct surface analysis of lipids with a focus on tissue sections, intact cells and thin-layer chromatography substrates. The suitability of these different approaches towards analysis of the major lipid classes along with their current and potential applications in the field of lipid analysis are evaluated.

Contents

Abbreviations: 2-AEP, 2-aminophosphonolipid; 9-AA, 9-aminoacridine; APCI, atmospheric pressure chemical ionization; AP-MALDI, atmospheric pressure matrix-assisted laser desorption ionization; ASAP, atmospheric pressure solids analysis probe; Cer, ceramide; Cer1P, ceramide-1-phosphate; CHCA, α-cyano-4-hydroxycinnamic acid; CID, collision-induced dissociation; DAG, diacylglyceride; DAN, 1,5-diaminonapthalene; DAPPI, desorption atmospheric pressure photoionization; DART, direct analysis in real time; DESI, desorption electrospray ionization; DHA, 2,6-dihydroxyacetphenone; DHB, 2,5-dihydroxybenzoic acid; DIOS, desorption/ionisation from porous silicon; DMAN, 1,8-bis(dimethylamino)naphthalene; EASI, easy ambient sonic spray ionization; ELDI, electrospray laser desorption ionization; ESI, electrospray ionization; FAEE, fatty acid ethyl ester; FAME, fatty acid methyl ester; FFA, free fatty acid; GAL, Cergalactosylceramide; GALDI, graphite-assisted laser desorption ionization; GL, ganglioside; GSL, glycosphingolipid; IR, infrared; IR-MALDI, infrared matrix-assisted laser desorption ionization; LacCer, lactosylceramide; LAESI, laser ablation electrospray ionization; LDI, laser desorption ionization; LESA, liquid extraction surface analysis; LIAD, laser-induced acoustic desorption; LPE, lyso phosphatidylethanolamine; LPS, lyso phosphatidylserine; LTP, low temperature plasma; MALDESI, matrix-assisted laser desorption electrospray ionization; MALDI, matrix-assisted laser desorption ionization; MBT, 2-mercaptobenzothiazole; ME-SIMS, matrix-enhanced secondary ion mass spectrometry; Met-SIMS, metal-assisted secondary ion mass spectrometry; MS/MS, tandem mass spectrometry; MTPFPP, meso-tetrakis (pentaflurophenyl) porphyrin; NALDI, nanowire-assisted laser desorption ionization; NIMS, nanostructure-initiator mass spectrometry; PA, phosphatidic acid; PC, phosphatidylcholine; PCA, principal component analysis; PE, phosphatidylethanolamine; PESI, probe electrospray ionization; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, phospholipid; PMMA, poly(methyl methacrylate); PNA, p-nitroaniline; PS, phosphatidylserine; PSI, paper spray ionization; PTFE, polytetrafluoroethylene; PVDF, polyvinylidene difluoride; REIMS, rapid evaporative ionization mass spectrometry; SIMS, secondary ion mass spectrometry; SM, sphingomyelin; SSSP, sealing surface sampling probe; ST, sulfatide; TAG, triacylglycerides; TCNQ, 7,7,8,8-tetracyanoquinodimethane; THAP, 2,4,6-trihydroxyacetophenone; TLC, thin-layer chromatography; V-EASI, venturi-easy ambient sonic spray ionization.

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1. Introduction

Technological developments, particularly those in the field of mass spectrometry, have been central to the rapid growth in the field of lipidomics [1,2]. The ultimate goal of lipidomics is to quantitatively describe all lipids within a cell and their associated functions [3]. However, the extensive structural diversity observed in lipid populations poses a significant challenge to this endeavor. For example, variations in common structural motifs observed in mammalian lipids (i.e., headgroup structure, chain length and degree of unsaturation in fatty acyl chains and the degree of sphingolipid glycosylation) have led to estimates of over 180,000 possible lipid structures in nature [4]. Moreover, isomeric variants arising due to alterations in double bond position, backbone substitution of fatty acyls, and stereochemistry, further increase the number of possible lipid structures. Thus comprehensive analysis of biological lipids requires analytical techniques possessing: (i) a high level of molecular specificity that allows one to differentiate the many molecular lipid structures invariably present in any lipid extract; and (ii) the ability to provide detailed structural information on the individual lipids present in a given system.

The excellent sensitivity and molecular specificity offered by modern mass spectrometry has made it arguably the method of choice for lipid analysis. No other analytical method allows for both the simultaneous and differential detection of individual lipid variants and acquisition of the detailed structural information that is required by the lipid researcher. Mass spectrometric analysis of lipids is traditionally achieved following lipid extraction from a sample (typically tissue or cells) and analysis by infusion-based electrospray ionization (ESI), with or without prior chromatographic separation. During ESI the sample solution is infused through a small capillary that has a high voltage applied (3–5 kV) [5]. This results in a charged spray being emitted from the capillary, which, following solvent evaporation assisted by a nebulizing gas, produces intact gas-phase lipid ions that then enter the mass spectrometer for analysis. Once transferred into the mass spectrometer, lipid ions can be subjected to tandem mass spectrometry (MS/MS), primarily through collision-induced dissociation (CID). CID analysis readily allows the identification of a range of structural motifs, such as headgroup structure and the length and degree of unsaturation of fatty acid chains [6,7]. These approaches have been invaluable for the structural, qualitative and quantitative analysis of lipids and form the foundation of most lipidomic workflows. For an in-depth discussion on the field of lipidomics and the role of mass spectrometry in lipid research please see references [1,8].

In recent years there has been a plethora of new mass spectrometry approaches developed that provide direct surface analysis capabilities. These approaches allow direct detection of lipids from surfaces that are typically encountered in lipid analysis, such as tissue sections and intact cells, without prior extraction. Furthermore, many of these approaches can also be coupled with additional analytical techniques such as thin-layer chromatography (TLC), a popular method used for the separation of complex lipid extracts on a silica gel surface [9]. Perhaps the most powerful capability of these methods in regards to lipid analysis however, is their ability to acquire position-correlated spectra that allow the spatial distribution of lipids within a sample to be visualized (so-called mass spectrometry imaging or MSI). Thus surface analysis can provide complementary information to that typically obtained by infusion-ESI of biologically derived lipid extracts. Most notably, surface analysis can elucidate the spatial distribution(s) of lipids within a sample, complementing quantitative analysis on lipid extracts from the same source.

Regardless of the approach, there are three requirements for successful and efficient mass spectrometric surface analysis, namely: (i) desorption of desired analytes from the surface by the interaction of a sampling probe with the surface (e.g., spray, laser, or plasma); (ii) ionization of desorbed analytes (note that some lipids, such as certain classes of phospholipids, are already charged and do not require post-desorption ionization); and (iii) analysis of the gas-phase analyte ions in the mass spectrometer. Crucially, the energy deposited during the desorption and ionization events determines the structure of the detected ions (i.e., fragments or intact molecules). Fortunately, for most (but not all) surface analysis methods, ionization is generally "soft". This means intact ions (e.g., $[M+H]^+$, $[M+alkali]^+$, $[M-H]^-$) are the dominant ionic products. This is advantageous as the lack of fragmentation occurring during "soft" ionization produces easy-to-interpret spectra. For further structural information (e.g., headgroup and fatty acid composition) these ions can then be subjected to collision-induced dissociation (CID) [6].

Traditional mass spectrometry surface analysis methods include matrix-assisted laser desorption ionization (MALDI) [10,11] and secondary ion mass spectrometry (SIMS) [12]. Both methods have been used extensively for lipid analysis with great success, albeit with different capabilities. Importantly, in their conventional forms, MALDI and SIMS require desorption and ionization be performed in vacuo. This can complicate sample introduction and requires that the sample be resistant to modification in the vacuum environment (e.g., dehydration of untreated tissue samples can lead to deformation, which may introduce artifacts). As a result,

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