Analytical Biochemistry 524 (2017) 45–55

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Advances and unresolved challenges in the structural characterization of isomeric lipids

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ARTICLE INFO

Article history:
Received 22 August 2016
Received in revised form
11 September 2016
Accepted 16 September 2016
Available online 17 September 2016

Keywords:
Lipidomics
Mass spectrometry
Ion mobility
Chromatography
Lipid isomers

ABSTRACT

As the field of lipidomics grows and its application becomes wide and varied it is important that we don't forget its foundation, i.e. the identification and measurement of molecular lipids.

Advances in liquid chromatography and the emergence of ion mobility as a useful tool in lipid analysis are allowing greater separation of lipid isomers than ever before. At the same time, novel ion activation techniques, such as ozone-induced dissociation, are pushing lipid structural characterization by mass spectrometry to new levels. Nevertheless, the quantitative capacity of these techniques is yet to be proven and further refinements are required to unravel the high level of lipid complexity found in biological samples.

At present there is no one technique capable of providing full structural characterization of lipids from a biological sample. There are however, numerous techniques now available (as discussed in this review) that could be deployed in a targeted approach. Moving forward, the combination of advanced separation and ion activation techniques is likely to provide mass spectrometry-based lipidomics with its best opportunity to achieve complete molecular-level lipid characterization and measurement from complex mixtures.

1. Introduction

Several definitions of lipidomics have been published, yet the most comprehensive was provided back in the nascent era of the field: “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” [1]. In the same editorial the authors listed three “first tasks” that the field of lipidomics needed to achieve in order to meet this definition: (i) new analytical approaches for mapping the lipidome, (ii) the application of biophysical methods to understand lipid-protein interactions, and (iii) identifying the lipid network, including lipid mediators, for metabolic and gene regulation and its integration with non-lipid signaling. Thirteen years later, lipidomics researchers are still searching for the best approaches to achieve these “first tasks”. All three are still relevant and necessary if lipidomics is to provide the biological and physiological insight it promises; however, the ability to fully characterize the lipidome is required before lipid-protein interactions or pathway mapping can be fully realized.

We have previously discussed the importance of lipid structural characterization [2–4] and most recently reviewed the area in 2012 [5]. Since that time there has been significant growth in the number of researchers working on new techniques to determine lipid structure and several new and innovative approaches have been published. While our increasing armory of analytical techniques aides our endeavor to resolve and measure molecular lipid isomers, i.e. sn-position isomers (Fig. 1a vs 1b), double bond position isomers (Fig. 1a vs 1c), double bond stereochemical isomers (Fig. 1a vs 1d), and enantiomers (Fig. 1a vs 1e), much work still remains. This review will describe and evaluate the analytical techniques being developed to resolve and measure these lipid isomers with a particular focus on developments that have occurred since 2012.

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http://dx.doi.org/10.1016/j.ab.2016.09.014
0003-2697/

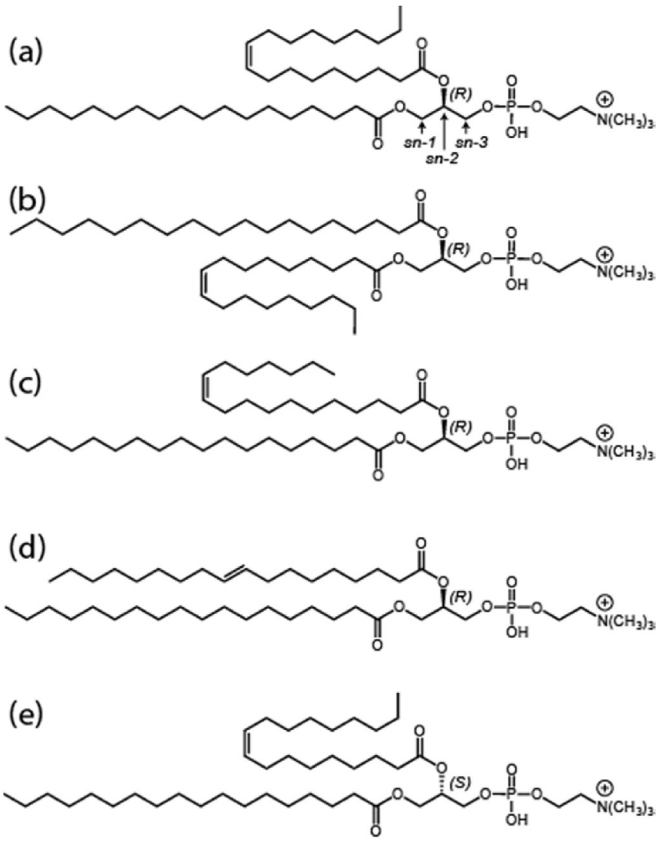

Fig. 1. Examples of some of the possible isomeric forms of PC 36:1 arising from sn-positional isomerism, double bond positional isomerism, *cis/trans* isomerism about the double bond and possible R/S chiral variation for (a) PC 18:0/18:1 (9Z), (b) PC 18:1 (9Z)/18:0, (c) PC 18:0/18:1 (7Z), (d) PC18:0/18:1 (9E) (e) PC 18:0/18:1 (9Z).

2. Sn regioisomers

The location of acyl chain esterification on the glycerol backbone, assigned as sn-1, sn-2 and sn-3 (Fig. 1a), is often assigned in lipidomics studies (sometimes inadvertently due to inconsistencies in nomenclature) when there is insufficient data to allow this level of structural characterization. There are some current and emerging techniques that do provide this information; however, extra analysis is often required.

2.1. Nuclear magnetic resonance (NMR) spectroscopy

NMR continues to play a significant role in lipid analysis; not only is it used for fatty acid composition and lipid class-identification, the technique has been used to provide information on the regiospecific distribution of fatty acids in triacylglycerols (TAGs) and phospholipids. Different carbon atoms produce signals in different regions of the ^{13}C NMR spectrum, i.e. carbonyl (172–174 ppm), olefinic (126–134 ppm), glycerol (74–60 ppm) and aliphatic (19–35 ppm) regions. This allows the regiospecificity (sn-distribution) of fatty acyl-chains of TAGs to be determined by ^{13}C NMR. This technique has been used to verify the authenticity and adulteration of food products, e.g. fish oils, milk and animal fat [6,7]. ^{13}C NMR is an excellent tool for regiospecific analysis of intact lipids; however, the requirement for relative large amounts of sample precludes it from many lipidomic analyses.

2.2. Mass spectrometry (MS) based analyses

Although NMR has played a significant role in understanding lipid structure, advances in MS have driven the field of lipidomics and made it the analytical tool of choice.

2.2.1. Collision-induced dissociation (CID)

Initial work studying lipids on sector-based mass spectrometers using high collision energy (keV) in the 1980s and 90s [8] contributed significantly to our current knowledge regarding the fragmentation and structural elucidation of ionized lipids [2,3]. Tandem time-of-flight (TOF/TOF) mass spectrometers provide access to similar CID energies; however, initial platforms had wide isolation windows of approximately 4 Th, hampering the ability to analyze complex lipid mixtures that require mass selection at unit resolution [9]. Recent developments in TOF/TOF technology allow higher resolution precursor ion selection, and this has been successfully utilized to determine sn-positions of TAGs in olive oil [10]. As this approach is non-targeted, i.e. almost all bonds are broken, the available charge is spread over many ions, which can limit its ability to determine sn-position in low abundance lipids.

The use of low-energy CID for structural characterization and sn-assignment in glycerophospholipids as well as the mechanisms of fragmentation processes in both polarities have been reviewed in detail [3,11]. While low energy CID allows for the identification of the most abundant sn-position isomer present, it is often difficult if not impossible to determine the presence of isomers [5] unless comparisons to calibration curves are made for each lipid of interest [12]. Multistage CID approaches can be used to generate MS^n spectra containing diagnostic ions that allow the assignment of the sn-position of triacylglycerols and glycerophospholipids [13,14]. This works well for high abundance lipids but is limited in sensitivity.

2.2.2. Ion-molecule reactions

Ozone-induced dissociation (OzID)

In combination with CID, OzID produces unique fragment ions indicative of sn-position. In such an approach, mass-selected lipids are collisionally activated before being trapped and allowed to react with ozone within the collision cell, resulting in CID/OzID spectra that can be used to determine lipid regiospecificity (reviewed by Ref. [3]). In the initial CID step either the phospholipid head group [15] or a FA from the TAG backbone [5,16] is lost. This creates a new double bond between carbons 1 and 2 of the “attacking” acyl chain that is subsequently cleaved by ozone, allowing determination of sn-position (see Refs. [15] and [16] for mechanisms). This process is fast enough to be performed on an LC timescale [17,18], and has even been used to determine the distribution of sn-positional isomers directly from sheep brain tissue [19].

Odd-electron fragmentation

Another approach to determining sn-position within lipids is generation of odd-electron products by radical-driven dissociation. Unlike low energy CID, radical-driven dissociation can break carbon-carbon bonds, which in some instances is able to produce data diagnostic of sn (and double bond) position.

Electron-induced dissociation (EID)

EID arises from the interaction of singly-charged cations at electron energies of 2–70 eV. Typically performed using Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometers, this method results in excitation and fragmentation similar to that obtained by CID. While EID is able to fragment lipids along their acyl chain it produces no unique ions diagnostic for double bond position [20]; thus providing no significant gain in sn-position information beyond that obtained from low energy CID. This was highlighted by the fact that known sn-position impurities in