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Review

# Analysis of unsaturated lipids by ozone-induced dissociation ☆

Simon H.J. Brown [a,b], Todd W. Mitchell [b], Stephen J. Blanksby [a,*]

[a] School of Chemistry, University of Wollongong, Wollongong, NSW, 2522, Australia
[b] School of Health Sciences, University of Wollongong, Wollongong, NSW, 2522, Australia

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ABSTRACT

Recent developments in analytical technologies have driven significant advances in lipid science. The sensitivity and selectivity of modern mass spectrometers can now provide for the detection and even quantification of many hundreds of lipids in a single analysis. In parallel, increasing evidence from structural biology suggests that a detailed knowledge of lipid molecular structure including carbon–carbon double bond position, stereochemistry and acyl chain regiochemistry is required to fully appreciate the biochemical role(s) of individual lipids. Here we review the capabilities and limitations of tandem mass spectrometry to provide this level of structural specificity in the analysis of lipids present in complex biological extracts. In particular, we focus on the capabilities of a novel technology termed ozone-induced dissociation to identify the position (s) of double bonds in unsaturated lipids and discuss its possible role in efforts to develop workflows that provide for complete structure elucidation of lipids by mass spectrometry alone: so-called top-down lipidomics. This article is part of a Special Issue entitled: Lipodomics and Imaging Mass Spectrom.



## 1. Introduction

Diversity in lipid molecular structures differs from that of other classes of biological molecules. Macromolecules such as proteins are composed of a relatively limited array of potential "building blocks" (e.g., 20 naturally occurring amino acids). The diversity of these macromolecules arises from long chains composed of permutations of the same set of building blocks bound together utilizing a single bonding motif (e.g., amide linkages). Conversely, any given lipid is composed of fewer components, but the large array of structurally diverse building blocks creates a highly varied lipid pool. In addition, lipids display a variety of bonding motifs joining individual components (e.g., ether or ester bonds can link a hydrocarbon chain to a glycerol backbone). The large array of potential components and bonding motifs presents a unique analytical challenge in the elucidation of lipid structure. Taking glycerophospholipids (GPL) as an example, Fig. 1A shows how variation in the headgroup type, acyl chain length, position of attachment, degree of unsaturation and stereochemistry, can provide a staggering array of structurally diverse molecules. Additionally, as shown in Fig. 1B, for each isobaric GPL multiple isomers sampling the array of possible acyl chain lengths (e.g., 18:1/16:0 and 20:1/14:0), glycerol backbone positional isomers (e.g., *sn*-1 and *sn*-2), double bond regioisomers (e.g., *n*-7 and *n*-9) [1,2], and stereoisomers (e.g., *Z* and *E*) may exist.

The striking diversity in lipids induces one to ask the question of why such variation might arise. Take the example of the lipid bilayer. The traditional model of a lipid bilayer consists of homogenous layers of phospholipids, intersected by membrane-bound proteins. In fact, lipids are highly organized and heterogeneous, even within a single membrane. It has been suggested there are over 1000 lipid species in membranes and all membranes differ in lipid composition and organization [3]. Additionally, membranes are asymmetric with the outer leaflet often composed of primarily phosphatidylcholine (PC) and sphingolipids, while the cytosolic leaflet is rich in phosphatidylethanolamines (PE), phosphatidylserines (PS) and phosphatidylinositols (PI) [4]. Substantial ATP activity is expended in membrane bound enzymatic pumps to maintain this asymmetry [5]. Such complicated mechanisms and expenditure of energy to segregate lipids would only have been likely to evolve if the structure of the lipids on each leaflet were necessary for membrane function. If one takes into account the diversity of lipids found in signalling, for example secondary messenger systems, hormonal pathways and enzyme targeting and localization, the true scale of lipid diversity is realized.

The variety of cellular functions in which lipids are involved highlights the importance of structural diversity in lipid species [6]. Mediation of membrane trafficking occurs through interaction of

*Abbreviations:* CL, Cardiolipin; CID, Collision induced dissociation; DAG, Diacylglycerol; ESI, Electrospray ionization; FA, Fatty acid; GC, Gas chromatography; GPL, Glycerophospholipid; LC/MS, Liquid chromatography mass spectrometry; MS, Mass spectrometry; OzESI-MS, Ozone electrospray ionization-mass spectrometry; OzID, Ozone-induced dissociation; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PG, Phosphatidylglycerol; PI, Phosphatidylinositol; PKC, Protein kinase C; PS, Phosphatidylserine; TAG, Triacylglycerol; ToF, Time-of-flight

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* Corresponding author. Tel.: +61 2 4221 5484; fax: +61 2 4221 4287.
*E-mail address:* blanksby@uow.edu.au (S.J. Blanksby).

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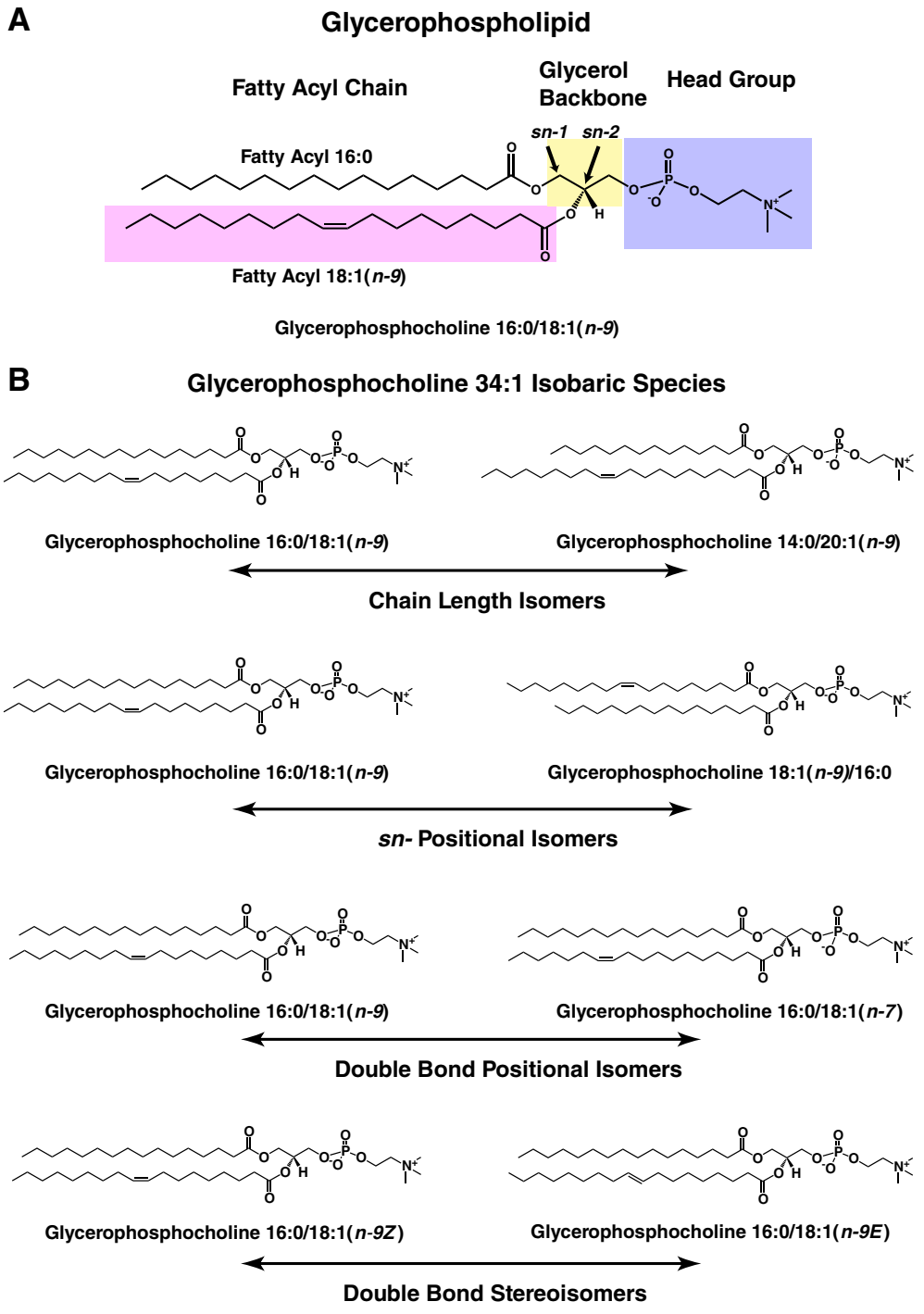


**Fig. 1.** (A) Representative structure of a glycerophospholipid. The glycerol backbone (yellow), head group (purple), and a fatty acyl chain (pink) are highlighted. (B) Examples of possible isomers of PC 34:1, arising from differences in acyl chain lengths (18:1/16:0 and 20:1/14:0), relative glycerol backbone position (*sn*-1 and *sn*-2), double bond position (*n*-9 and *n*-7), and stereochemistry about the double bond (*Z* and *E*).

lipids with protein complexes on membranes [7]. Recent exploration into lipid rafts has expanded our knowledge of membrane diversity and emphasized the importance of protein–lipid interactions. Two common functions of protein-lipid interactions are for enzyme targeting and messenger signalling. A protein domain can recognize a membrane-tethered lipid and be targeted to a specific location in the cell (i.e., a membrane sub-compartment) localizing the protein function often in partnership with other enzyme molecules. Conversely, a lipid may act as a signalling molecule, and the production of a localized pool of lipid allows dynamic modulation of enzyme function. Diacylglycerol (DAG) was identified as a lipid second messenger that could modulate the function of protein kinase C (PKC) more than 25 years ago [8], and has served as a long-standing model of lipid signalling [9,10]. In the years since this discovery, the importance of lipids as both signalling and structural molecules has been steadily realised.

Multiple X-ray protein crystal structures have demonstrated the structural basis for regulation of the nuclear receptor SF-1 by lipid second messengers [11–13]. These protein structures share a common protein fold and hydrophobic lipid-binding pocket. In two