Structural characterization of complex lipids by ozone-induced dissociation and ultraviolet photodissociation on high-resolution mass spectrometers

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OVERVIEW

Purpose: To investigate the utility for research of new ion activation techniques, ozone-induced dissociation (OzID) and ultraviolet photodissociation (UVPD) for comprehensive lipid structure elucidation.

Methods: Lipids from human blood plasma were analyzed in positive ion mode on two different platforms: i) a modified Thermo Scientific™ Q Exactive™ HF mass spectrometer with ozone replacing nitrogen as the HCD collision gas; ii) a Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS equipped with a 213 nm UVPD laser.

Results: Unsaturation profiles in major lipid classes of human plasma (*e.g.*, cholesterol esters (ChE), phospholipids (PL) and triacylglycerol (TG)) are successfully identified with both techniques. The predominant sites of unsaturation identified are the n-6 and n-9 double bonds of linoleic acid (*e.g.*, ChE18:2 (*n-6,n-9*)).

INTRODUCTION

Dynamic alterations of the lipidome are associated with a number of human disorders including diabetes and cancer. To understand the role of lipids in physiological and pathological conditions, detailed characterization of lipid species is required, including the determination of: (1) lipid class; (2) number of carbons and double bonds; (3) relative position of acyl chains; and (4) location and stereochemistry of double-bonds.

Current tandem mass spectrometry approaches identify lipid class, total carbons and double bonds, but not further information. In this study, we investigate the utility of two different ion activation techniques, specifically, ozone-induced dissociation (OzID) and ultraviolet photodissociation (UVPD) for more comprehensive lipid structure elucidations.

MATERIALS AND METHODS

Sample Preparation

1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (PLPC) was used as standard compound and lipids from human blood plasma were extracted using the MTBE protocol [1]. The organic phase was dried under vacuum and stored at -20 ° C prior to the analysis. Lipids were dissolved in methanol containing 20 µM sodium acetate.

Mass Spectrometry

Lipids were analyzed using a shotgun approach. ESI–MS analysis was performed in positive ion mode on a modified Q Exactive HF mass spectrometer with ozone (approximately 11% O_3 in O_2) replacing nitrogen as the HCD collision gas (Figure 1a). Full MS spectra were acquired with mass resolution of R=240.000 at *m/z* 200, the ion spray voltage was set at 3.9 kV. Photodissociation was implemented on an Orbitrap Fusion Lumos Tribrid MS equipped with a 213 nm UVPD laser (Figure 1b). Full MS spectra were acquired with mass resolution of R=120.000 at *m/z* 200, the ion spray voltage was 3.5 kV. In both cases, the scan range was set at *m/z* 650-950, and MS/MS spectra were acquired for all ions in this range in a data-independent workflow at mass resolution of R=120.000 and a fill time of the collision cell of 300ms.

Data Analysis

Identification of double bond position(s) was based on:

- reaction of selected lipid ions with ozone inside the mass spectrometer produces two characteristic product ions: an aldehyde and a Criegee ion. The expected neutral losses for OzID product ions from lipids containing monounsaturated or polyunsaturated fatty acids are shown in Table 1.[4]
- Formation of predictable product ions arising from photodissociation at 213 nm (wavelength absorbance of conjugated double bonds).

Figure 1. Schematic illustrating the online setup for ozone delivery to a modified Q Exactive HF (a). Schematic of the Orbitrap Fusion Lumos equipped with a 213 nm UVPD laser (b).

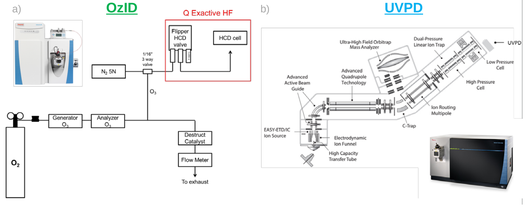


Table 1. Predicted neutral losses (or gains) for OzID (a) and UVPD (b) product ions showing the dependence on position and degree of unsaturation.

a) OzID

Monounsaturate			Polyunsaturate			
n-	Aldehyde loss	Criegee loss	First Double bond	*n-*	Aldehyde loss	Criegee loss
3	-26.0520	-10.0571	*n-3*	3	-26.0520	-10.0571
4	-40.0677	-24.0728		6	-66.0833	-50.0884
5	-54.0833	-38.0884		9	-106.1146	-90.1197
6	-68.0990	-52.1041		12	-146.1459	-130.1510
7	-82.1146	-66.1197		15	-186.1772	-170.1823
8	-96.1303	-80.1354		18	-226.2085	-210.2136
9	-110.1459	-94.1510	*n-6*	6	-68.0990	-52.1041
10	-124.1616	-108.1667		9	-108.1303	-92.1354
11	-138.1772	-122.1823		12	-148.1616	-132.1667
12	-152.1929	-136.1980		15	-188.1929	-172.1980
13	-166.2085	-150.2136		18	-228.2242	-212.2293
14	-180.2242	-164.2293	*n-9*	9	-110.1459	-94.1510
15	-194.2398	-178.2449		12	-150.1772	-134.1823
				15	-190.2085	-174.2136

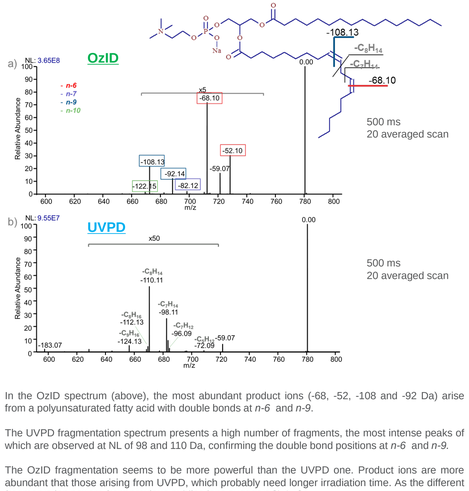
b) UVPD 213 nm

First Double bond	*n-*	Polyunsaturate	
n-3	3	-56.0626	-68.0626
	6	-96.0939	-108.0939
	9	-136.1252	-148.1252
	12	-176.1565	-188.1565
	15	-216.1878	-228.1878
	18	-256.2191	-268.2191
n-6	6	-98.1096	-110.1096
	9	-138.1409	-150.1409
	12	-178.1722	-190.1722
	15	-218.2035	-230.2035
	18	-228.2242	-212.2293
n-9	9	-140.1565	-152.1565
	12	-180.1878	-192.1878
	15	-220.2191	-232.2191

RESULTS

To benchmark the two methods we used a standard lipid, PLPC (PC 16:0/18:2(n-6,n-9)).

Figure 2. Isolation and activation of [M+Na]$^+$ ions of PLPC with OzID (a) and UVPD (b). Product ions are denoted relative to the precursor ion mass (*m/z* 782.55).



In the OzID spectrum (above), the most abundant product ions (-68, -52, -108 and -92 Da) arise from a polyunsaturated fatty acid with double bonds at *n-6* and *n-9*.

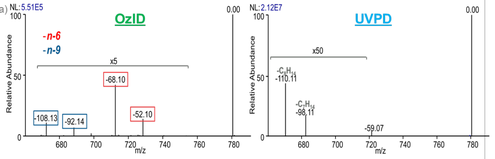
The UVPD fragmentation spectrum presents a high number of fragments, the most intense peaks of which are observed at NL of 98 and 110 Da, confirming the double bond positions at *n-6* and *n-9*.

The OzID fragmentation seems to be more powerful than the UVPD one. Product ions are more abundant that those arising from UVPD, which probably need longer irradiation time. As the different fragmentation pattern from previous publications suggests [2,3,5].

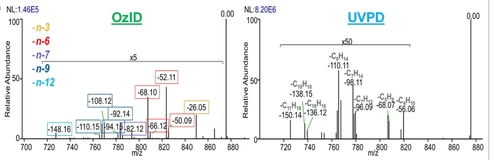
PLASMA ANALYSIS

The two techniques were applied to the analysis of plasma lipidome. It was possible to identify the double bond position in phospholipids (PLPC, Figure 2a), tryacylglycerols (TG 52:5, Figure 2b) and cholesterol esters (ChE 22:6, Figure 2c).

Figure 3. Tandem mass spectra of PLPC (a), TG 52:5 (b) and ChE 22:6 (c) obtained with OzID and UVPD.

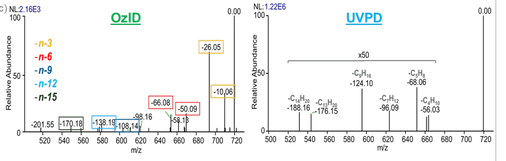


As the phospholipid example we used PLPC, in order to also compare with the standard. It is clear that PLPC in plasma is present only as PC (16:0/18:2 *n-6,n-9*). In the two spectra are present only the diagnostic ions to that relative position of the double bonds.



The fragmentation spectra of TAG 52:5 suggest that in plasma are present more isomers of this lipid, containing different acyl chains (16:0, 16:1, 18:1, 18:2, 18:3) with different relative position of the double bonds:

- One double bond (*n-3, n-6* or *n-9*);
- Two double bonds (*n-3/ n-6* or *n-6/ n-9*);
- Three double bonds (*n-3/ n-6/ n-9* or *n-6/ n-9/ n-12*).



The fragmentation spectra of ChE 22:6 present the diagnostic fragments for double bonds in position *n-3*, *n-6*, *n-9*, *n-12* and *n-15* for both techniques, so omega-3 FA can be unambiguously assigned.

Figure 4. Ion map and reconstructed 'Neutral Loss' scans with OzID (a) and UVPD (b)

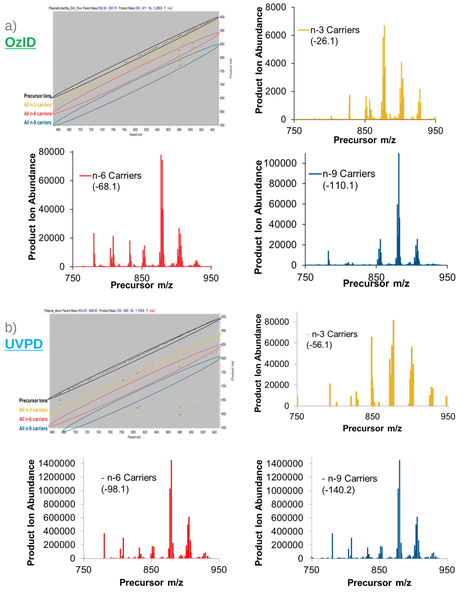
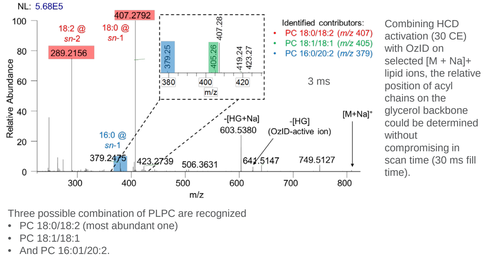


Figure 5. OzID Acyl chain relative position characterization



Combining HCD activation (30 CE) with OzID on selected [M + Na]+ lipid ions, the relative position of acyl chains on the glycerol backbone could be determined without compromising in scan time (30 ms fill time).

Three possible combination of PLPC are recognized

- PC 18:0/18:2 (most abundant one)
- PC 18:1/18:1
- And PC 16:01/20:2.

Table 2. Comparison of the possible application, the instrument used and the novelty of the two techniques

		Ozonolysis	Ultraviolet Photodissociation
Application	**Double bond position**	Monounsaturated and Polyunsaturated Acyl Chains	Monounsaturated [5] Polyunsaturated Acyl Chains
	Acyl Chain Position	Phospholipids, triacylglycerols	Phospholipids [2]
Instrument		Modified Q Exactive HF mass spectrometer using with ozone replacing nitrogen as the HCD collision gas .	Commercially available Orbitrap Fusion Lumos Tribrid MS equipped with a 213 nm UVPD laser.
Novelty of technique		-10 years ago [6]	1 year ago

CONCLUSIONS

In our study we compared ozonolysis and ultraviolet photo-dissociation for comprehensive lipid structure elucidations. Methods were set up using a polyunsaturated lipid standard and then applied to the study of the plasma lipidome. Unsaturation in cholesterol esters, phospholipids and triacylglycerols could be identified with both techniques (Figure 2) due to the generation of structural diagnostic product ions: an aldehyde and a Criegee ion for ozonolysis and a pair of ions formed by cleavage of specific conjugated double bonds upon photon uptake. Linoleic acid (18:2 n-6, n-9) was observed as the major polyunsaturated fatty acid in the plasma lipidome with both techniques.

Ozonolysis is an established technique, first being published in 2008 by Brown et al. but was implemented for the first time on a Q Exactive HF in this study. Ozonolysis is able to determine double bond position and relative acyl chain position.

Ultraviolet photodissociation was introduced last year [2,3], and the major studies were carried with 193 nm laser. While its potential is to be further investigated, it is promising detailed characterization of lipid species.

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TRADEMARKS/LICENSING

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