SHIMADZU

ThP 474

MS imaging enabling visualization of lipid C=C positional isomers in biological tissues using Oxygen Attachment Dissociation (OAD)

Kaoru Nakagawa, Hidenori Takahashi, Satoshi Kasamatsu, Kengo Takeshita, Manami Kobayashi
Shimadzu Corporation, Analytical & Measuring Instruments Division, Japan

1. Introduction

Lipids are essential biomolecules that function as signaling molecules, energy sources, and skin barriers, in addition to constituting cell membranes. These diverse physiological functions are supported by the structural diversity of lipids. In particular, the position of the double bond (C=C) in the lipid structure is an important factor that determines the physical properties of biomembranes, substrate selectivity in metabolic reactions, and physiological functions of signaling molecules. Chemical derivatization, ozone-induced dissociation (OzID), ultraviolet photodissociation (UVPD), or electron-induced dissociation (EID), and ion mobility have been reported as separation techniques for lipid isomers in MS imaging (MSI).

In this study, we implement Oxygen Attachment Dissociation (OAD)[1-3], a fragmentation method that specifically dissociates C=C, in MSI to demonstrate the utility of this approach.

1) Takahashi.H et al. Anal. Chem. 2018, 90 (12), 7230-7238.
2) Takahashi.H et al. Mass Spectrometry. 2019, S0080.
3) Uchino.H et al. Commun Chem. 5, 162 (2022).
4) Peggi M. Angel et al. Anal. Chem. 2012, 84 (3), 1557-1564

OAD MS/MS

Neutral radical-based

Charge-neutral radical-induced dissociation is available in both positive and negative ion modes!

O• **OAD (Oxygen Attachment Dissociation)-MS/MS**

$[M+H]^+ + O^\bullet \rightarrow [M + H + O]^{+\bullet} \rightarrow$ *fragments*

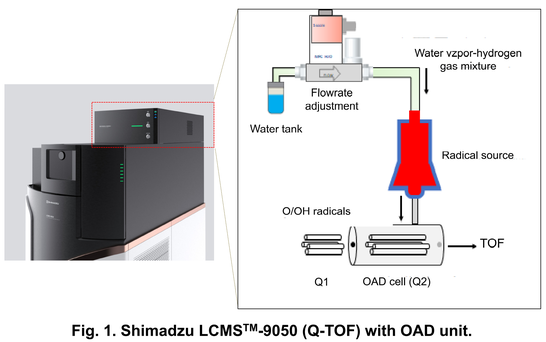


Fig. 1. Shimadzu LCMS™-9050 (Q-TOF) with OAD unit.

Lipid MS/MS: CID vs. OAD

◆ **CID-MS/MS** does not provide detailed structural information within carbon chains. Instead, CID selectively cleaves labile polar head groups (*m/z* 184).

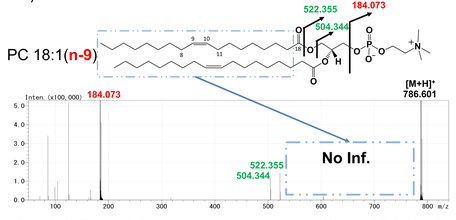


Fig. 2-1. Typical CID spectrum of a model lipid of PC (18:1).

◆ **OAD-MS/MS** clearly provides C=C positional information. Atomic oxygen selectively oxidizes and cleaves at C=C.

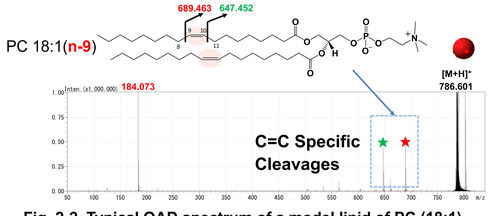


Fig. 2-2. Typical OAD spectrum of a model lipid of PC (18:1).

2. Methods

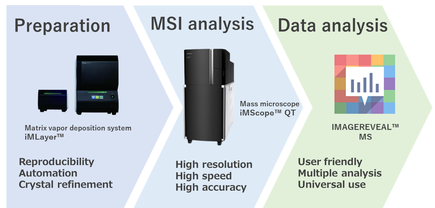


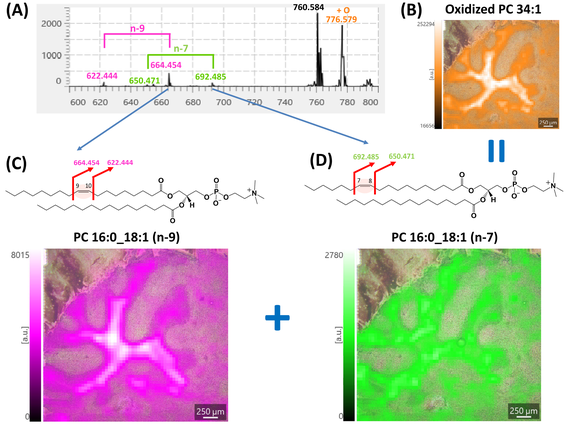
Fig. 3. MS imaging workflow.

To evaluate the performance of MSI with OAD, several unsaturated lipids of mouse cerebellum sections were subjected to positive and negative OAD-MS/MS.

Some mouse cerebellum sections were washed with 50 mM ammonium formate[4] and coated with DHB (2,5-Dihydroxybenzoic Acid) *via* vapor deposition by using the matrix sublimation apparatus iMLayer at a thickness of 1.2 μm. MSI analysis was performed using an MSI system consisting of an iMScope QT atmospheric MALDI unit connected to an OAD-TOF system. OH• and O radicals generated by a compact microwave-driven radical source were introduced into the collision cell (q2) through the quartz tube to obtain OAD-MS/MS spectrum.

3. Results

Precursor [PC 16:0_18:1 + H]$^+$ *m/z* 760.584 (Washed)



Precursor [PC 16:0_18:1 + K]$^+$ *m/z* 798.543 (Unwashed)

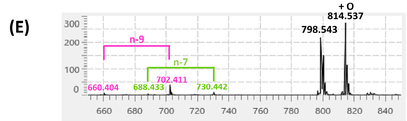


Fig. 4. OAD-MS/MS of PC 16:0_18:1 in positive ion mode.

(A) OAD-MS/MS Spectrum of [PC 16:0_18:1 + H]$^+$. (B) MS image of oxidized [PC 16:0_18:1 + H]$^+$. (C) MS image of **n-9** OAD-MS/MS fragment. (D) MS image of **n-7** OAD-MS/MS fragment. (E) OAD-MS/MS Spectrum of [PC 16:0_18:1 + Na]$^+$.

The cleavage of the C-C bond adjacent to a double bond occurs through OAD, allowing for the identification of the C=C position[1]. In positive ion mode, C=C positions of phosphatidylcholine (PC) 34:1 was successfully annotated. OAD-MS/MS spectrum of PC 16:0_18:1 ([M+H]$^+$ *m/z* 760.584 and [M+K]$^+$ *m/z* 798.543) revealed product ions indicative of two C=C positional isomers, PC 16:0_18:1(**n-7**) and PC 16:0_18:1(**n-9**) (Fig. 4 (A), (E)). In the cerebellum sections, The distribution of PC 16:0_18:1(**n-7**) and PC 16:0_18:1(**n-9**) to be different (Fig. 4 (C), (D)). PC 16:0_18:1(**n-7**) was found to have several times lower abundance compared to PC 16:0_18:1(**n-9**) (Fig. 4 (A), (E)).

Precursor [Sulfatide 24:1 - H]$^-$ *m/z* 888.623 (Washed)

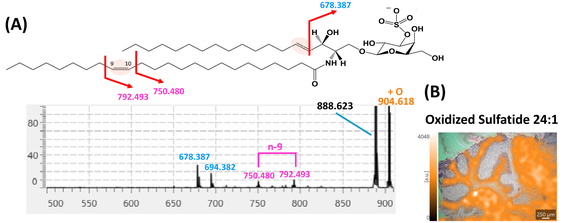


Fig. 5. OAD-MS/MS of Sulfatide 24:1 in negative ion mode.

(A) OAD-MS/MS Spectrum of [Sulfatide - H]$^-$. (B) MS image of oxidated [Sulfatide 24:1 - H]$^-$.

In negative ion mode, C=C positions of sulfatide (SHexCer was successfully annotated. OAD-MS/MS spectrum of SHexCer d18:1/24:1 ([M-H]$^-$*m/z* 888.623) revealed product ions indicative of SHexCer d18:1 (Δ**4**) / 24:1 (**n-9**) (Fig. 5 (A)).

4. Conclusion

- Since OAD utilizes charge-neutral radical species for the fragmentation, OAD can be applied to both positively charged ions and negatively charged ions.
- In addition, OAD is applicable not only alkali metal adduct ions but also protonated ions. (Lipid subclass and acyl chain length were annotated by performing CID analysis at the same laser spot where OAD was conducted.)