Imaging Mass Microscope iMScope™
Oxygen Attachment Dissociation MS/MS Option Kit

Application News

Analysis of C=C Positions of Lipids in Mouse Brain Sections Using MALDI-OAD-MS/MS

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User Benefits

◆ It is possible to identify the C=C positions of fatty acids in sections quickly and easily without the need for extraction or other complicated processes.

◆ It can be applied to both alkali metal adducts and proton adducts.

◆ It can be applied to both positive and negative ion polarities.

■ Introduction

Tandem mass spectrometry (MS/MS) is an essential technique for the structural analysis of biomolecules, with Collision Induced Dissociation (CID)-MS/MS being widely used, which utilizes gas collisions with sample ions. However, CID preferentially dissociates at points in the molecular structure where bond energies are weak, often resulting in insufficient structural information for substances with unknown chemical structures. Therefore, we have developed a new MS/MS method (Oxygen Attachment Dissociation, OAD-MS/MS) that can be used complementarily with CID. Oxygen Attachment Dissociation (OAD)[1-3] is a fragmentation method that specifically dissociates carbon-carbon double bonds (C=C).

In this report, we conducted C=C position analysis of lipids in mouse brain sections using MALDI-OAD-MS/MS, combining an OAD-TOF system equipped with the OAD RADICAL SOURCE I option kit on the LCMS™-9050 with an imaging mass microscope, iMScope™ QT (Fig. 1).

Fig. 1 Combination of the OAD-TOF System and iMScope™ QT

■ OAD-TOF system

OAD induces radical-based fragmentation of precursor ions using atomic oxygen and hydroxyl radicals (O/OH•), which are neutral radicals generated from water vapor. The O/OH• radicals produced by a microwave-driven source are introduced into the Q2 collision cell via a quartz tube, allowing the acquisition of OAD-MS/MS spectra. Atomic oxygen selectively oxidizes and cleaves C=C bonds, and OAD-MS/MS clearly provides positional information of the C=C bonds.
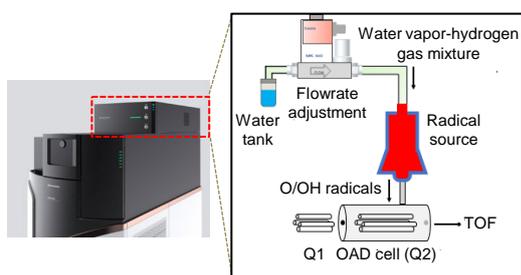

Fig. 2 LCMS-9050 equipped with OAD RADICAL SOURCE I (OAD-TOF system)

■ Pre-treatment and Analysis Conditions

The mouse brain was frozen, and sections with a thickness of 10 μm were cut using a cryomicrotome and placed on ITO-coated glass slides. To remove metal ions from the sections, The sections were washed three times with 50 mM ammonium formate solution at 4 ˚C[4]. The matrix was applied using the iMLayer™ deposition system (Fig. 3), depositing 2,5-dihydroxybenzoic acid (DHB) at a thickness of 1.2 μm. The cerebellar region was analyzed using the OAD iMScope QT system in both positive and negative ion modes (Table 1). Data analysis was performed using IMAGEREVEAL™ MS (Fig. 4) to confirm the OAD-MS/MS spectra, and the C=C positions were identified from the neutral loss values of the fragment ions.

Table 1 MSI Analysis Conditions

| Mass spectrometer	
System	: iMScope QT＋OAD-TOF System
Polarity	: Positive / Negative
DL temp	: 290 ˚C
Heat block temp	: 400 ˚C
MS/MS Range	: MS/MS m/z 500-920
Spatial Resolution (Pitch)	: 50 μm
Laser Diameter Setting	: 4
Laser Intensity	: 75
Laser Repetition Frequency	: 100 Hz
Q1 Resolution	: 5.0 Da
Collision Energy	: 10 V
Matrix Coating	
System	: iMLayer
Matrix Used	: DHB
Coating Method	: Deposition with 1.2 μm Thickness

Fig. 3 iMLayer™

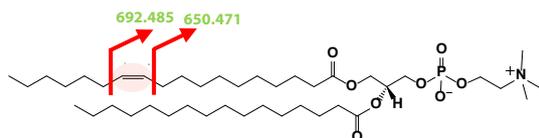Fig. 4 IMAGEREVEAL™ MS

■ MALDI-OAD-MS/MS in Positive Ion Mode

In positive ion mode, we conducted C=C position analysis of phosphatidylcholine (PC) 16:0_18:1 in the cerebellar region of mouse sections. Using DHB as the matrix, PC 16:0_18:1 was detected primarily as the ion species [M+K]$^+$ (m/z 798.543). When this [M+K]$^+$ was used as the precursor ion for MALDI-OAD-MS/MS analysis, fragment ions indicative of the C=C position isomers PC 16:0_18:1 (n-7) (Fig. 5 (B)) and PC 16:0_18:1 (n-9) (Fig. 5 (C)) were detected (Fig. 5 (A)).

Next, by washing the brain sections with ammonium formate solution to remove salts, we detected only [M+H]$^+$ (m/z 760.584). When this [M+H]$^+$ was used as the precursor ion for MALDI-OAD-MS/MS analysis, fragment ions indicative of PC 16:0_18:1 (n-7) and PC 16:0_18:1 (n-9) were detected (Fig. 5(D)). Thus, it was confirmed that MALDI-OAD-MS/MS can be applied not only to alkali metal adducts but also to proton adducts. Furthermore, in the analyzed mouse cerebellum, it was confirmed that PC 16:0_18:1 (n-7) was present in several times lower quantities compared to PC 16:0_18:1 (n-9) (Fig. 5 (A) and (D)).).
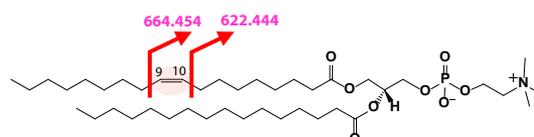
Precursor [PC 16:0_18:1 + K]$^+$ *m/z* 798.543 （Unwashed）

(A)
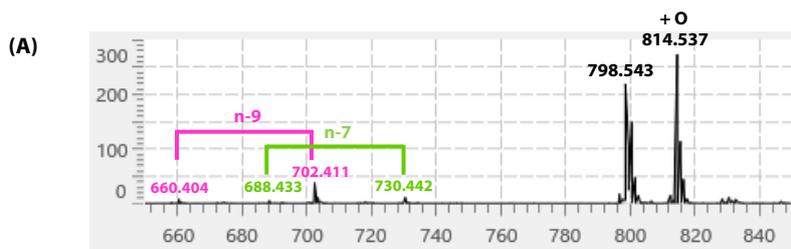
(B) PC 16:0_18:1 (n-7)

(C) PC 16:0_18:1 (n-9)

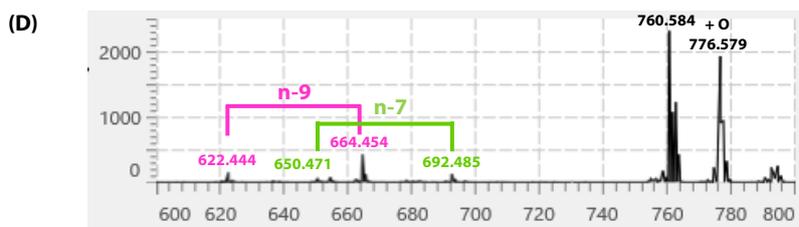Precursor [PC 16:0_18:1 + H]$^+$ *m/z* 760.584 （Washed）

(D)

Fig. 5 MALDI-OAD-MS/MS of PC 16:0_18:1 in Positive Ion Mode
(A) MALDI-OAD-MS/MS spectrum of [PC 16:0_18:1+K]$^+$
(B) Structural formula of PC 16:0_18:1 (n-9) and cleavage position by OAD
(C) Structural formula of PC 16:0_18:1 (n-7) and cleavage position by OAD
(D) MALDI-OAD-MS/MS spectrum of [PC 16:0_18:1+H]$^+$

■ MALDI-OAD-MS/MS in Negative Ion Mode

In negative ion mode, we conducted C=C position analysis of sulfatide (SHexCer) d18:1/24:1 in the cerebellar region of mouse sections. To remove salts from the brain sections, they were washed with ammonium formate solution. From the MALDI-OAD-MS/MS spectrum of SHexCer d18:1/24:1 [M-H]⁻ (*m/z* 888.623), fragment ions indicating the presence of two carbon double bond positions were detected, corresponding to SHexCer d18:1 (Δ4) / 24:1 (n-9) (Fig. 6 (A)). It was confirmed that MALDI-OAD-MS/MS can also be applied in negative ion mode.

■ Conclusion

In this report, we performed C=C position analysis of lipids in the cerebellar region of mouse brain sections using MALDI-OAD-MS/MS, combining the iMScope QT and OAD-TOF systems. As a result, it was confirmed that MALDI-OAD-MS/MS can be applied in positive ion mode to precursor ions not only with alkali metal adducts but also with proton adducts. Additionally, it was confirmed that the method is applicable to both positive and negative ions. This is because OAD uses neutral charge radicals for fragmentation. Thus, MALDI-OAD-MS/MS allows for simple and rapid C=C position analysis of lipids without the need to extract lipids from sections, making it a promising tool.

<References>

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

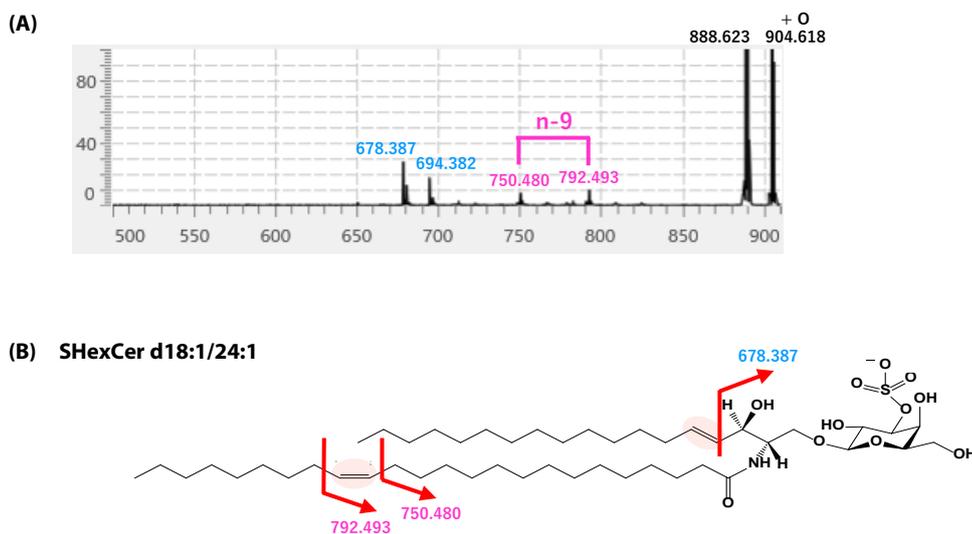Precursor   [SHexCer d18:1/24:1-H]⁻ *m/z* 888.623 （Washed）

Fig. 6 MALDI-OAD-MS/MS of SHexCer d18:1/24:1 in Negative Ion Mode
(A) MALDI-OAD-MS/MS spectrum of [SHexCer d18:1/24:1-H]⁻
(B) Structural formula of SHexCer d18:1/24:1 and cleavage position

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