SHIMADZU

WP 250

High-Sensitivity MALDI MS Imaging of Lipid C=C Positional Isomers via O_2-Enhanced Oxygen Attachment Dissociation (O_2E-OAD)

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1. Introduction

Oxygen Attachment Dissociation (OAD) enables localization of carbon-carbon double bonds (C=C) in lipids without derivatization[1-3]. We developed an oxygen-enhanced OAD (O_2E-OAD) to improve radical generation and imaging sensitivity. This approach allows high-sensitivity MALDI imaging of C=C positional isomers.

2. Method

OAD (Oxygen Attachment Dissociation)-MS/MS

OAD is a new fragmentation technology that enables identification of structural isomers not achievable with CID. As shown in Fig. 1, charge-neutral atomic oxygen or hydroxyl radicals are introduced into q2, inducing gas-phase dissociation. Applicable to ions of any charge state, including negative and multiply charged ions, OAD selectively dissociates C=C in lipids, allowing positional isomer differentiation. In this study, we found that pre-irradiation of the radical source with pure oxygen O_2 enhances radical generation—a phenomenon defined as the O_2-Enhanced OAD.

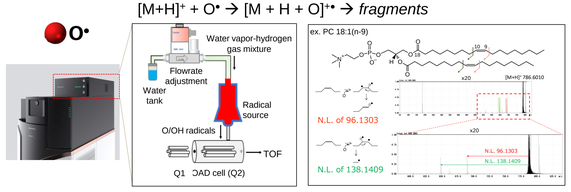


Fig. 1 Shimadzu LCMS-9050 (Q-TOF) with OAD unit.

OAD LCMS-9050 system

The LCMS-9050 offers a unique capability to easily exchange ion sources and acquire both LC-MS and MSI data within a single platform. OAD-MS/MS further enables C=C localization and isomer distinction.

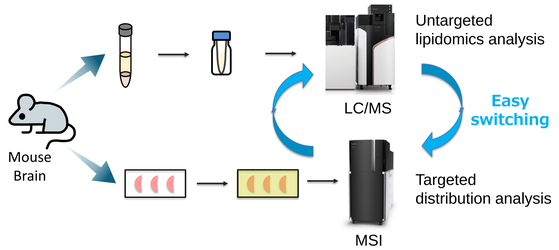


Fig. 2 Analysis workflow.

3. Results

3.1. Untargeted OAD-LCMS analysis

Table 1 LCMS Analysis Parameters

Mass Spectrometer	
System	: LCMS-9050 with OAD unit
Polarity	: Positive
MS/MS mode	: OAD
Collision energy	: 30 V
Data-Dependent MS/MS	: Top10 precursors from MS1
Event time	: 100 msec for MS1 and MS/MS
LC	
System	: Shimadzu UPLC system
RP-LC Conditions	: Same as described in Uchino *et al.*[3]

Untargeted OAD-Lipidomics Workflow by LCMS

Lipid extracts from mouse tissue sections were analyzed using OAD-LCMS. Fig. 3 shows the workflow and a representative result. Spectra were automatically assigned to lipid structures using MS-DIAL. As an example, C=C positional isomers of PC 16:0_18:1 were identified, and their relative abundances are shown in the bar graph. PC 16:0_18:1 (n-7) was found at about half the abundance of the n-9 isomer, prompting further spatial analysis by MS imaging to investigate potential differences in their distribution within mouse brain tissue.

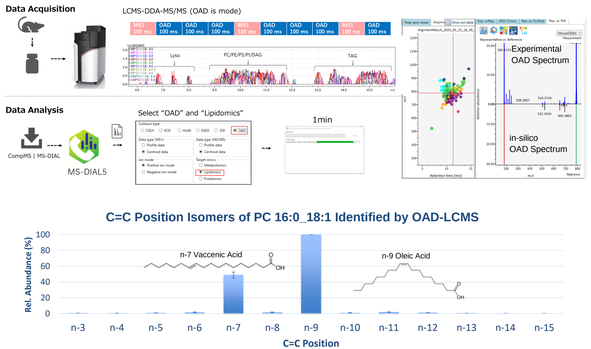


Fig. 3 Workflow of untargeted OAD-lipidomics using LC-MS.

3.2. Targeted OAD-MSI analysis

Table 2 MS Imaging Analysis Parameters

MS Imaging	
System	: OAD iMScope QT system
Polarity	: Positive
MS/MS mode	: OAD
Collision energy	: 10 V
Spatial Resolution (Pitch)	: 50 µm
Laser Settings	: Repetition 100Hz, Diameter 4
Laser Intensity	: 72
Matrix Coating	
System	iMLayer
Matrix Used	2,5-Dihydroxyacetophenone (DHAP)
Coating Method	Sublimation

MALDI Matrix Optimization

In the analysis of phospholipids, DHB is commonly used as a matrix in positive ion mode; however, it has been reported that the optimal matrix varies depending on the lipid subclass [4]. Among them, we conducted the analysis using DHAP, which is expected to enhance signal intensity in the analysis of PC. In mouse brain sections, PC 16:0_18:1 (m/z 798.542 $[M+K]^+$) showed over 5× higher ion intensity with DHAP than with DHB.

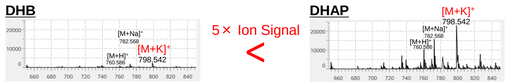


Fig. 4 Comparison of ion intensities using DHB and DHAP in MS1.

$[M+H]^+$ vs $[M+K]^+$: Optimal Precursor for OAD

The mouse brain sections were washed with 50 mM ammonium formate [5] to remove salts and concentrate the ion species to $[M+H]^+$. Washing gave >2× higher $[M+H]^+$ intensity, but $[M+K]^+$ showed ~2.5× higher OAD efficiency. Thus, OAD product ion intensities were comparable, and $[M+H]^+$ was selected as it provides more informative fragment ions for structural analysis by CID-MS/MS.

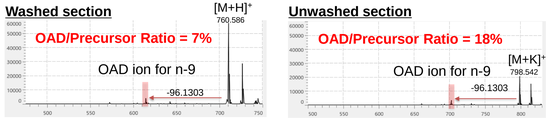
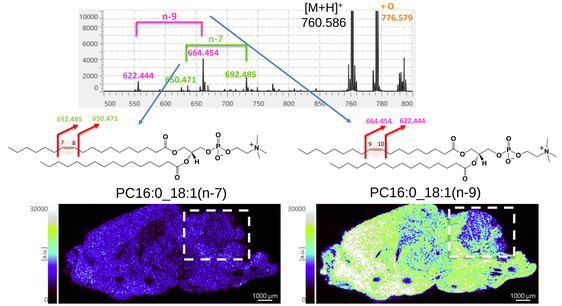


Fig. 5 OAD-MS/MS with [M+H]+ vs. [M+K]+ precursors in mouse brain.

Spatial Distribution of C=C Isomers in Mouse Brain

MS imaging based on OAD-MS/MS enabled spatial differentiation of lipid C=C positional isomers in mouse brain tissue. For PC 16:0_18:1, the n-9 isomer was broadly distributed in white matter, while the n-7 isomer was locally concentrated in gray matter. Ratio images (e.g., n-7 / (n-7 + n-9)) clearly visualized the regional differences in isomer localization.



Ratio Images of C=C Positional Isomers in the Cerebellum

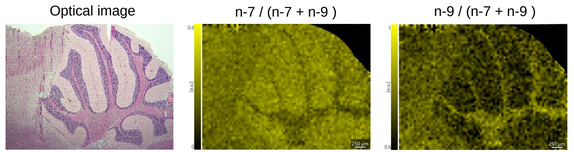


Fig. 6 C=C isomer distribution of PC 16:0_18:1 in mouse brain.

4. Conclusion

- OAD-MS/MS visualized lipid C=C isomers in mouse brain.
- OAD-LCMS and MSI were integrated for lipid identification and mapping.
- $[M+H]^+$ suited OAD-LCMS; $[M+K]^+$ gave higher OAD efficiency in MSI.
- The workflow enables confident structural and spatial analysis.

Reference

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3) Uchino.H *et al.* Commun Chem. 5, 162 (2022).
4) Perry WJ et al. J Mass Spectrum. 2020;55:e4491
5) Angel MP *et al.* Anal. Chem. 2012, 84 (3), 1557-1564.