SHIMADZU

Identification of Double Bond Positions in Ceramides from the Stratum Corneum Using OAD-TOF System

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Overview

Oxygen Attachment Dissociation (OAD) is a novel method for fragmentation, providing additional fragmentation to CID on the LCMS-9050. To enhance identification of ceramide which is one of the lipid group, OAD-MS/MS has been applied to provide double bond specific dissociation, enabling C=C position assignments.

1. Introduction

Ceramides has an important role in the skin by contributing to moisture retention and acting as a barrier against the entry of pathogens, allergens, and other external factors, and it is estimated that there are over 1,000 different species. The different structures of ceramides influence their functions and interactions, so accurately analyzing the types and molecular structures of ceramides is crucial for comprehending their roles in skin health.

LC-MS/MS is the one of major options to analyze these compounds simultaneously, but it is difficult to identify each isomer that has different double bond positions. A brand-new oxygen attachment dissociation (OAD) provides diagnostic fragment ions enabling the assignment of double-bond positions. In this study, it was confirmed that OAD-TOF system, a novel technique for identifying the position of carbon double bond position was effective in this challenge.

2. Materials and Methods

All experiments were performed using an ESI Q-TOF LCMS-9050 (Shimadzu, Kyoto, Japan). OH and O radicals generated by a compact microwave-driven radical source was introduced into the collision cell (Q2) through the quartz tube to obtain OAD-MS/MS spectrum [1][2]. Shimadzu Nexera™ LC system was used for the separation of the ceramides extracted from the stratum corneum.

Extracts were subjected to analysis with LCMS-9050, and the positive ion mode was used to generate OAD-MS/MS based spectra to determine the positions of the double-bonds.

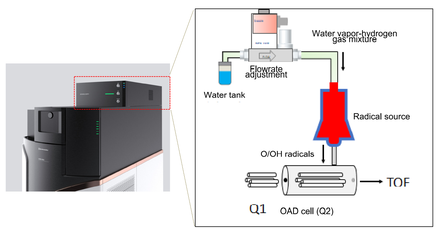

Fig. 1 Shimadzu LCMS-9050 and OAD system

Human SC samples were collected from healthy volunteers by tape stripping. To collect the SC samples, the inner forearm was cleaned with water and dried up before tape stripping was performed. A 20 x 50 mm piece of film masking tape No.644(Teraoka Seisakusho, Tokyo, Japan) was stuck to the inner forearm and the pressed and removed. The procedure was repeated three times using a new piece of tape. The collected tape samples were transferred into a glass tube containing 4 mL methanol. After vortex for 3 min, the tapes were removed. The samples were transferred to 1.5 mL micro-tube and centrifuged at room temperature(15,000g, 10min). The supernatant was transferred into glass vial and subjected to LC-OAD-TOF analysis.

Table 1 Analytical Condition

HPLC condition	MS condition
System: Shimadzu Nexera X3 Flow rate: 0.3 mL/min Gradient Elution Mobile phase A: 20mM Ammonium formate Mobile phase B ACN/IPA = 1/1 (v/v) Column: Shim-pack Scepter Claris C18-120 (1.9 μm, 2.1 mmI.D. x 100 mmL.) Column Oven Temp. 50 ˚C	System: Shimadzu LCMS-9050 + OAD RADICAL SOURCE I Mode: CID/OAD Polarity: Positive CE: 15V NEBU Gas: 3 mL/min Heating Gas: 10 mL/min Drying Gas: 10 mL/min

3. How to Identify C=C double bond positions

OAD-MS/MS clearly provides C=C positional information. The neutral loss(NL) corresponding to C=C double bond position is detected by OAD-MS/MS.

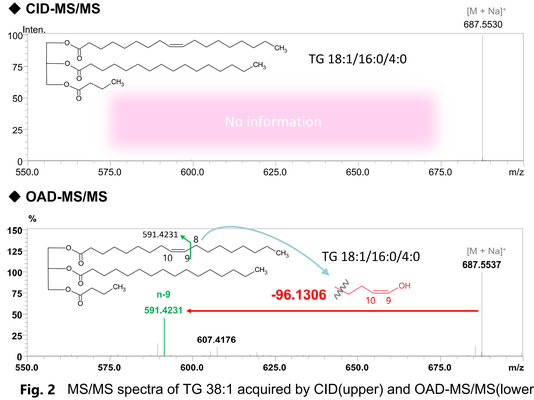

Fig. 2 MS/MS spectra of TG 38:1 acquired by CID(upper) and OAD-MS/MS(lower)

A 100 μM ceramide standard sample C18:1 Ceramide(d18:1/18:1(9Z)) (Avanti, Birmingham, AL) was also subjected to OAD-TOF system to confirm MS/MS spectrum pattern derived from OAD. As shown in Fig. 3, only OAD-MS/MS data detected the ions corresponding to C=C double bond position, but NL were different from other lipids behavior such as shown in Fig.2. In the MS/MS spectra, dehydrated ion was detected with high intensity and NL was also observed as dehydrated.

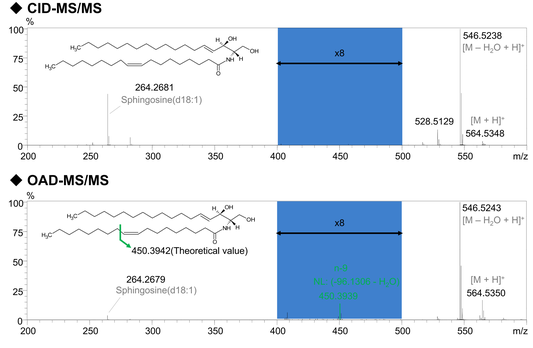

Fig. 3 Structural characterization of Cer 36:2 Ceramide using OAD-MS/MS. MS/MS spectra of C18:1 Ceramide acquired using CID(upper) and OAD(lower).

4. Results

4.1. Automated data processing and ceramide(lipid) identification using MS-DIAL with OAD-database

Data from the untargeted DDA-MS/MS analysis of SC samples were processed in MS-DIAL. Some ceramides detected in SC samples which include unsaturated fatty acid were processed in detail. The results was shown in Fig. 5

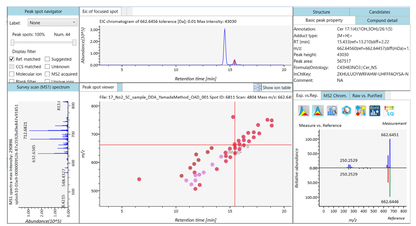

Fig. 4 Data processing and ceramide identification using MS-DIAL

4.2. Identification of double bond positions

Table 2 showed the ceramide precursor ions that we focused on and the structural candidates contained in SC. Several C=C bond positional isomers detected by OAD-MS/MS, but separations in ceramides were insufficient. To certainly identify each isomer, ceramide separation needs to be improved.

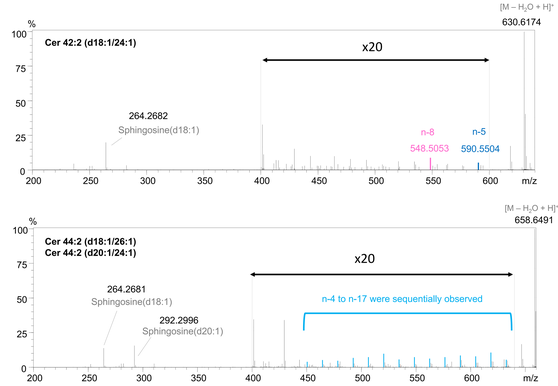

Fig. 5 Structural characterization of Cer 42:2(upper) and Cer 44:2(lower)

Table 2 Ceramide list identified C=C double bond position

m/z	Predicted compound	Detected in SC sample
648.6289	Cer 42:2 (d18:1/24:1)	Cer d18:1/24:1(n-5) Cer d18:1/24:1(n-8)
676.6602	Cer 44:2 (d18:1/26:1) Cer 44:2 (d20:1/24:1)	Cer d18:1/26:1(n-4) –d18:1/26:1(n-17)* Cer d20:1/24:1(n-4) – d20:1/24:1(n-17)**

*, ** Not clearly identified, but candidates of C=C bond positional isomers

5. Conclusion

- ✓ It found that the NL of ceramide generated by OAD-MS/MS was observed as a dehydrated form.
- ✓ It was suggested that unknown structural ceramides exist in human SC can be identified by using OAD-MS/MS.
- ✓ For more detailed analysis, it would be effective to combine OAD-MS/MS with other separation methods such as SFC.