SHIMADZU

WP 254

Multidirectional Analysis of Plant Alkaloids Using MS Imaging (MSI) and Oxygen Attachment Dissociation (OAD)

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1. Introduction

Plant alkaloids, naturally occurring compounds containing nitrogen atoms, are widely distributed in plants and are known for their pharmacological activities. Because many of them exhibit toxicity, they function as defense mechanisms against herbivores and pathogens. Well-known examples include morphine from the poppy plant (used as an analgesic) and quinine from cinchona bark (used in malaria treatment). Other notable alkaloids include caffeine, nicotine, and atropine. These compounds are of critical importance in medicine, agriculture, and the economy, making alkaloid research indispensable for novel drug development and understanding plant biology. In this study, we employed Oxygen Attachment Dissociation (OAD) a novel radical-induced dissociation methodtogether with conventional collision-induced dissociation (CID) to investigate both the distribution and chemical structures of plant alkaloids.

2. Methods

OAD uses neutral radicals such as atomic oxygen and hydroxyl radicals O/OH•, generated from water vapor, to induce radical-based fragmentation of precursor ions. O/OH• radicals produced by a microwave-driven source were introduced via a quartz tube into the q2 collision cell to acquire OAD-MS/MS spectra. Commercial potato (*Solanum tuberosum L*) sprouted under sunlight were used as a model. Potato slices with sprout were coated with CHCA (0.7 µm) via an iMLayer™ (Shimadzu). MSI, CID-MS/MS, and OAD-MS/MS were performed on an iMScope™ QT with an OAD Radical Source I (Shimadzu). MS images were generated with IMAGEREVEAL™ MS (Shimadzu), and in silico CID MS/MS data were analyzed using LabSolutions Insight Explore™ (Shimadzu).

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*

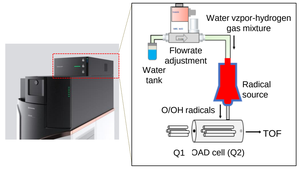


Fig. 1 Shimadzu LCMS™-9050 (Q-TOF) with OAD unit.

Table 1 Analytical settings of MS

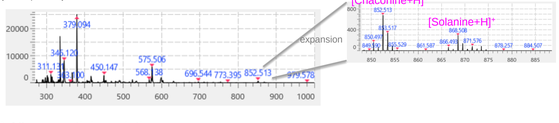
Mass Spectrometer	
System	: OAD iMScope QT system
Polarity	: Positive
DL temp	: 290 ℃
Heat block temp	: 450 ℃
MS Range	: MS *m/z* 270-1000 MS/MS *m/z* 100-1000
Spatial Resolution (Pitch)	: 10 / 25 / 50 µm
Laser Diameter Setting	: 1 / 2 / 4
Laser Intensity	: 38 / 46 / 63
Laser Repetition Frequency	: 1 kHz
Collision Energy	: 10 / 80 V
Matrix Coating	
System	: iMLayer
Matrix Used	: a-Cyano-4-hydroxycinnamic Acid (CHCA)
Coating Method	: Deposition with 0.7 µm Thickness

3. Results

Distribution Analysis of Plant Alkaloids Using MSI

The distribution of plant alkaloids in sections prepared from the sprout of the potato was analyzed by MSI. The Mass Spectra and Mass images of the entire section at 25 µm spatial resolution and the area observed with an optical microscope at 5x objective lens at 10 µm spatial resolution are shown in Fig. 2 and Fig. 3. MSI revealed that plant alkaloids such as solanine and chaconine were abundantly distributed in potato sprouts and surrounding tissues. These glycoalkaloids are known to accumulate in potato sprouts to defend against herbivores and pathogens.

(a) 25 µm spatial resolution



(b) 10 µm spatial resolution

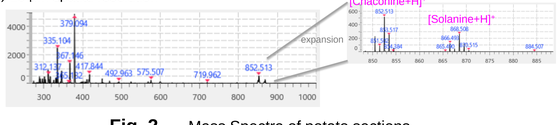
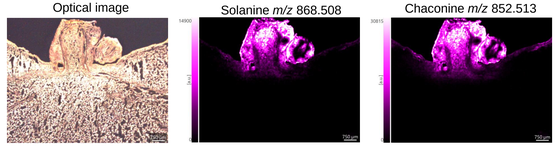


Fig. 2 Mass Spectra of potato sections

(a) Entire section (CCD camera) 25 µm spatial resolution



(b) Optical microscope at 5x objective lens 10 µm spatial resolution

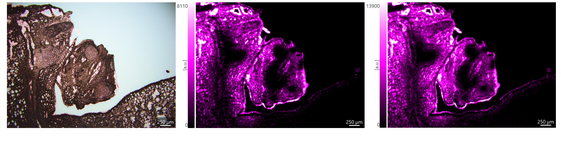


Fig. 3 Mass images of plant alkaloids in the sprout of the potato

Stractural Analysis of Plant Alkaloids Using OAD and CID

In the case of such known reference sections and compounds, identification from the precursor *m/z* and corresponding CID spectra is straightforward. However, structural elucidation of alkaloid analogs is often difficult based solely on CID spectra. Therefore, in this study, we demonstrated a proof-of-concept analytical workflow employing both CID and OAD (Fig. 4 (chaconine data not shown)). Specifically, we treated solanine and chaconine as unknowns, matched their CID spectra against in silico CID data generated by LabSolutions Insight Explore software, and derived possible structural candidates (Fig.4 (c) and Fig.5). We searched compounds with the same chemical formula registered in ChemSpider. Unsurprisingly, solanine and chaconine were among the candidates, but other structurally different compounds were also suggested (Fig. 5 (chaconine data not shown)). Thus, additional information was required to refine these candidates, and we used OAD spectra for that purpose. In OAD, a fragment ion at *m/z* 150.128, not observed under CID at the lower collision energy, appeared specifically (Fig.4 (a) and (b)). Given that OAD cleaves preferentially near heteroatoms, and exact mass analysis indicated its formula to be $C_{10}H_{16}N$, we could leverage this complementary information to narrow down the structural candidates derived from the CID data.

Disclaimer: iMLayer, iMscope, IMAGEREVEAL, LCMS and LabSolutions Insight Exploreare a trademark of Shimadzu Corporation or its affiliated companies in Japan and/or other countries.

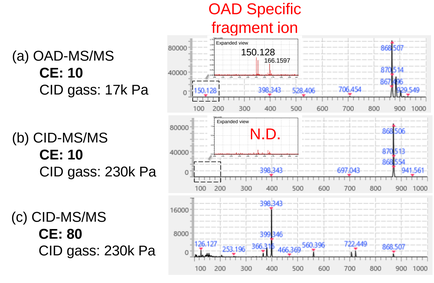


Fig. 4 OAD or CID MS/MS mass spectra of $[solanine+H]^+$

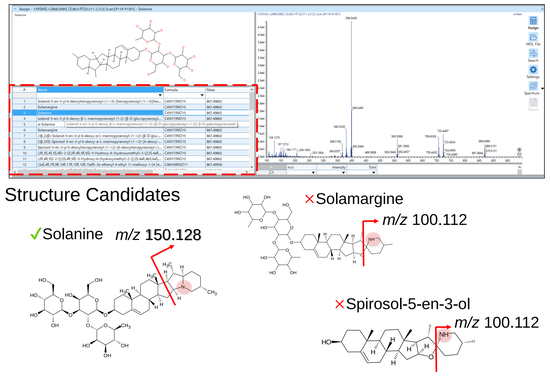


Fig. 5 Structural analysis results by LabSolutions Insight Explore

4. Conclusion

Unique cleavage patterns under OAD produce diagnostic fragments that can clarify isomeric structures or confirm functional groups, surpassing the capabilities of CID alone. By integrating OAD data within silico fragment prediction, unknown alkaloids can be characterized more confidently. Our workflow, which analyzes the distribution of compounds using MS imaging and combines OAD with CID for structural analysis of compounds, enables the verification of known compounds such as solanine and chaconine, as well as the discovery and identification of new alkaloids that may have biological or pharmacological relevance.