DESI-IMS coupled with triple quadrupole mass spectrometry demonstrated the capability of NMN imaging in Shelfordella lateralis tissue

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論文題目

DESI-IMS coupled with triple quadrupole mass spectrometry demonstrated the capability of NMN imaging in *Shelfordella lateralis* tissue

（DESI-IMS とトリプル四重極質量分析計により可能となった *Shelfordella lateralis* 組織における NMN イメージング）

論文の内容の要旨

[Introduction]

Imaging mass spectrometry (IMS) has been used to visualize biomolecules due to its high sensitivity and label-free nature. Now a days, desorption electrospray ionization (DESI) combined with triple quadrupoles (TQ) mass spectrometry has emerged and offers advantages over DESI coupled with quadrupole-time-of-flight (Q-TOF). Despite the significant advantage of IMS, detecting and imaging of many biologically important molecules in tissue is still challenging.

Nicotinamide mononucleotide **(**NMN), a vital precursor of nicotinamide adenine dinucleotide (NAD^+), has been difficult to detect by IMS. NMN is a nucleotide, found in all living cells as a co-enzyme. Increasing NAD^+ levels improve insulin sensitivity, reverses mitochondrial dysfunction, and enhances lifespan. Thus, NMN has important applications in medicine and healthcare, but the chemical synthesis of NMN is complex. Therefore, it is worth exploring a rich source of natural NMN and its imaging in medical research. Unlike other natural products, insects have received comparatively little attention in imaging studies. In this study, we first screened NMN content in six insect species applying ultra-performance liquid-chromatography–tandem mass spectrometry (UPLC-MS/MS). Finally, we employed both DESI-Q-TOF and DESI-TQ to observe the distribution of NMN in *S. lateralis,* which showed the highest NMN content among the other species under this study.

[Materials and Methods]

The insect samples were freeze-dried, ground into powder, vacuum-sealed, and stored at −20 ℃ freezer until use for analyses. UPLC-MS/MS was used to screen the insect extracts. Chromatographic separation was carried out on an Intrada Amino Acid column. The electrospray ionization (ESI) source was used on the mass spectrometer in positive ion mode. The elution flow rate was 0.6 mL/min, and total run time was 7 minutes. The selected reaction monitoring (SRM) transition at *m/z* 335.1 to *m/z* 123.0 using a collision energy of 10.0 eV was used for NMN quantification.

For imaging study, first we employed DESI-IMS using DESI source attached to a Q-TOF mass analyzer at a scan rate of 200 μm/sec, and the pixel size was 100 μm ×

100 µm. Finally, we performed A Xevo TQ-XS equipped with a DESI source in SRM mode at a scan rate of 10 scan/sec. The pixel size was set as 50 µm × 50 µm for tissue and 100 µm × 100 µm for standard. A collision energy of 20 eV, and the SRM dwell time was 0.0016 sec/pixel used for NMN transition. Finally, the tissue sections were stained with hematoxylin and eosin and DESI-TQ data was analyzed using MassLynx (version 4.2) and HDI Imaging (version 1.6) software.

[Results]

We quantified NMN in six different insect species by LC-MS methods. A linear calibration curve (Y= 9.3056X-154.914, R= 0.99) of NMN standards was observed in the range of (20–500 ng/mL). A Chromatogram of NMN (SRM transition at *m/z* 335.1 to *m/z* 123.0) was observed with high intensities in five insect species. *S. lateralis* showed significantly higher NMN content 15.5 mg/100g (dry weight) than all other species (p <0.0001) in this study. *G. bimaculatus* showed the second highest content of NMN, which was significantly higher than *H. illucens, R. speratus*, and *Z. atratus* (p <0.005).

For the imaging study, we first performed DESI-Q-TOF analysis in the whole body of *S. lateralis* and could not detect NMN. Next, we performed DESI-TQ in the SRM mode to observe the distributions of NMN in the sagittal sections of *S. lateralis*. NMN (SRM transition at *m/z* 335.1 to *m/z* 123.0) was successfully detected in almost entire regions of the *S. lateralis* section. To observe the region-specific distribution of NMN, the histological images were overlaid on the ion images.

[Discussion]

For the first time, we demonstrated NMN imaging in biological tissue employing DESI-TQ in SRM mode that showed higher sensitivity and excellent selectivity than DESI-Q-TOF. NMN detection is challenging by IMS since this molecule is very labile and readily undergoes in-source fragmentation under standard ESI settings. DESI-Q-TOF is typically used for untargeted analysis where the target ion is mixed with myriads of other ions and could not be detected. Recently, DESI-TQ offered about ten to a thousand times higher sensitivity than the DESI-Q-TOF. In this study, DESI-TQ successfully detected NMN in *S. lateralis,* which could be attributed to its improved sensitivity.

Another significant finding was that cockroaches showed NMN content 3 to 13 times higher than edamame, which previously had the highest NMN content among natural foods. Cockroach extracts have been traditionally used for various infections, and their gut microbial metabolites show therapeutic potential. Cockroaches also carry pathogens and allergens; therefore, safety and sanitation are must require.

[Conclusion]

This study demonstrated the NMN imaging in tissue by DESI-TQ and revealed *S. lateralis* as a rich source of NMN, specifically in the abdomen. In future, NMN imaging could aid in understanding age-related changes in NAD^+ metabolism and its potential role in human health.