SHIMADZU

Application News

MALDI-TOF Mass Spectrometry No.B12

On-Tissue Direct Analysis: MALDI Mass Spectrometric Imaging for Tryptic Digest Peptides

MALDI imaging, a type of mass spectrometry using the MALDI technique, can display the distribution of biomolecules such as peptides and proteins without having to conduct such operations as the extraction and labeling of the biomolecules. Up to now, biomolecular MS imaging for a variety of tissue specimens has been reported, and recently there have been published reports showing the distribution of disease-specific protein biomarker candidates.

However, proteins that have been detected by conducting mass spectrometry directly on a tissue section are extremely difficult to identify using the mass information alone. The typical method for identifying proteins is to conduct PMF (peptide mass fingerprinting) utilizing "tryptic digest peptides" of the target protein, together with in-silico generated database searches. Since multiple proteins are present in a tissue section, PMF cannot be used for identification of these proteins. This makes it necessary to conduct the MS/MS ion search for the tryptic digested peptides.

By utilizing a spotter instrument to apply a coat of enzyme solution on the tissue section, it becomes possible to limit the analysis to micro regions of the tissue. This also allows the use of smaller amounts of expensive enzyme solution as compared to the spray method of coating. The use of spray techniques, including air brushing, can also lead to peptide diffusion on the tissue, complicating analyses.

Here we present an example of protein identification conducted at a micro region of a rat liver tissue section, and an example of MALDI imaging of a tryptic digest site on a rat brain tissue section.

First, trypsin solution (40 µg/mL, 5 mM NH_4HCO_3) was deposited on a micro region of the liver tissue section using a chemical inkjet printer (CHIP-1000). Repeat deposition of 1.0 nL of trypsin solution (10 nL/spot) was conducted at 500 µm intervals on the tissue section. After allowing the enzymatic reaction to continue for 2 hours at 37 ˚C in an external incubator, matrix solution (5 mg/mL DHB, 50 % methanol, 0.1 % trifluoroacetic acid) was dispensed onto the tissue. Fig. 1 shows an image of the liver tissue section with the deposited matrix.

Next, after drying the tissue section in a desiccator, we conducted mass spectrometry using the AXIMA-QIT. Then, MS/MS analysis was conducted for the two peaks *m/z* 1572.78 and 1817.01 which exhibited high signal intensity in the initial mass spectrum. The obtained MS/MS spectra and the respective database search results are shown in Fig. 2.

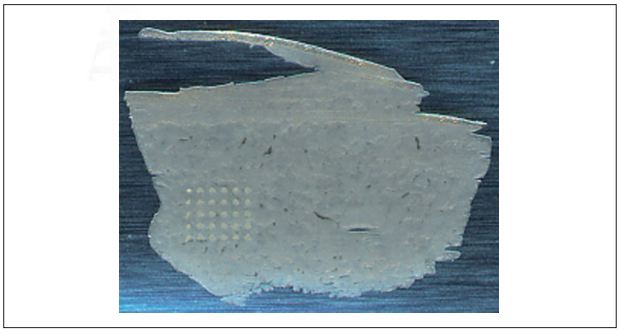

Fig. 1 Liver Tissue Section with Deposited Matrix

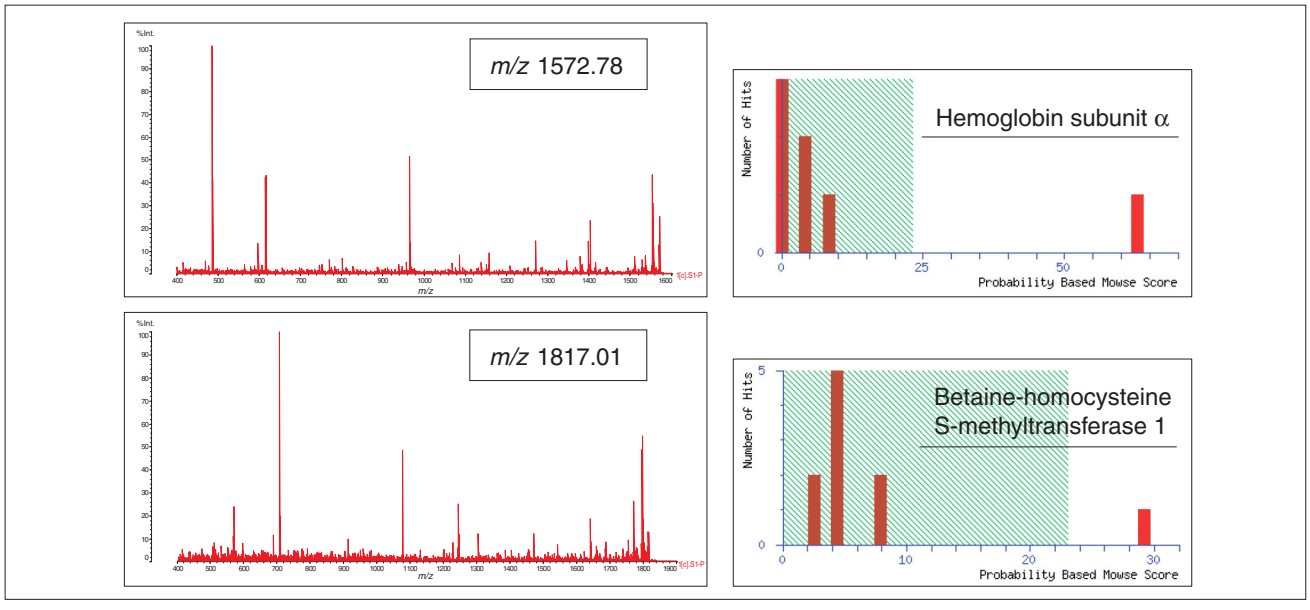

Fig. 2 MS/MS Spectra of Tryptic Digest Peptides and Database Search Results

The AXIMA-QIT, an ion trap mass spectrometer, allows MS/MS analysis with high mass accuracy, as shown in Fig. 2. When used in conjunction with the chemical inkjet printer which can dispense micro volumes of enzyme solution, direct protein identification within a micro region of a tissue section becomes possible.

Next, with the rat brain tissue section as the sample, MALDI imaging of the tryptic digest was conducted using the chemical inkjet printer and the AXIMA-Performance. Trypsin solution (40 μg/mL) was deposited at 250 μm intervals at a micro volume of 300 pL per deposition, and following completion of the enzymatic reaction, 300 pL each of 10 mg/mL CHCA (50 % acetonitrile, 0.1 % trifluoroacetic acid) was dispensed (9 nL/spot). Analysis was conducted using the AXIMA-Performance, and then MS images were generated based on the obtained mass spectrum positional information and the molecular ion intensity ratios. The BioMap software (http://www.maldi-msi.org/) was used to create the MS images. Fig. 3 shows the MS images for *m/z* 726.46 and *m/z* 1198.66, respectively. Each of the digested peptides shows a characteristic distribution, as can be seen in Fig. 3. In addition, MS/MS analysis of the molecular ions confirmed that they are derived from the Myelin basic protein (*m/z* 726.46) and Actin (*m/z* 1198.66), respectively (Fig. 4).

Regarding the distribution of Myelin basic protein, all of the citations are reported, and the results here correlate with the reported distribution of protein in brain tissue.

Thus, we have confirmed that on-tissue protein identification using the chemical inkjet printer in conjunction with the AXIMA Series, and MALDI imaging of a tryptic digest are useful for the examination of protein information obtained directly from biological tissue sections.

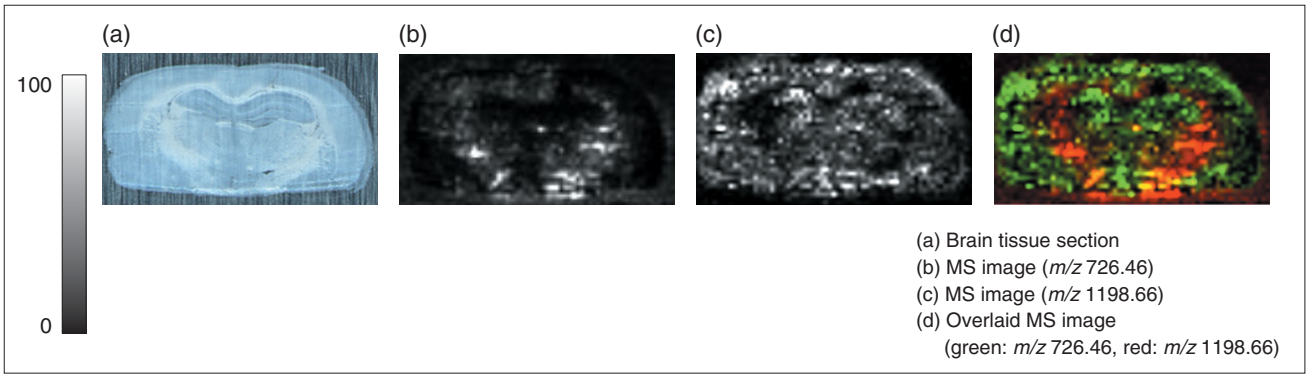

Fig. 3 MS Images of Rat Brain Tissue

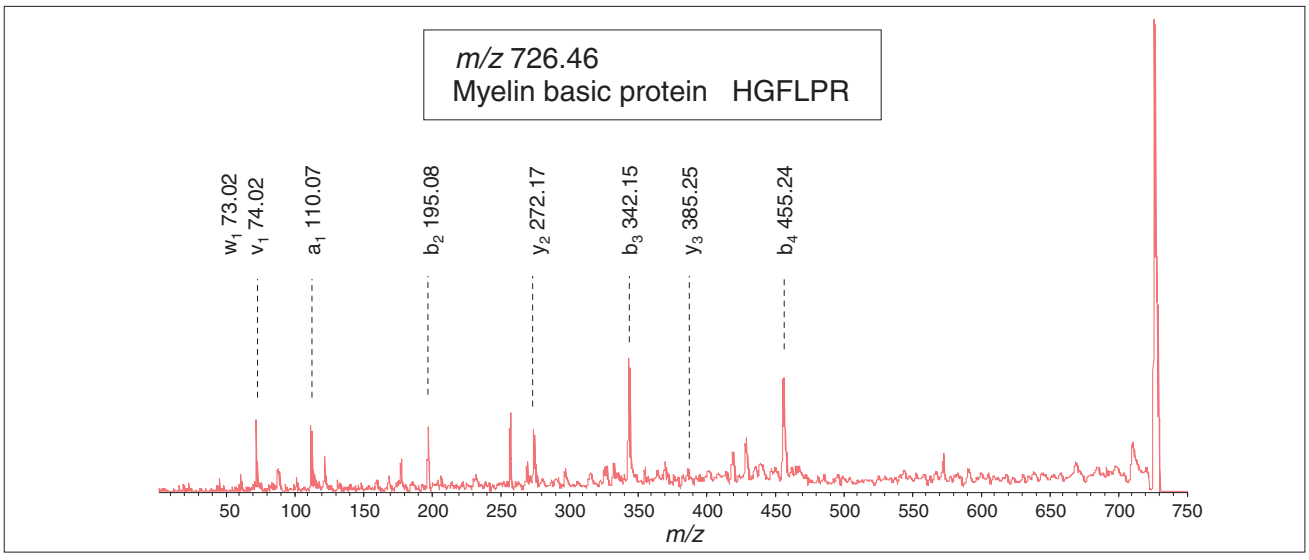

Fig. 4 MS/MS Spectrum of Tryptic Digest Peptides (Myelin basic protein)

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NOTES:
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