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MALDI-TOF Mass Spectrometry Analysis MALDI-8020/MALDI-8030

Application News

Protein and Peptide Mass Spectrometry Imaging on the MALDI-8020 Benchtop MALDI-TOF Mass Spectrometer

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User Benefits

- Simple imaging analysis of peptides and proteins in the rat brain on an affordable, easy-to-use benchtop MALDI-TOF system
- High quality spectra with class-leading mass resolution and sensitivity producing detailed MALDI images
- Workflow applicable to many tissue types and with different methods of matrix application

■ Introduction

MALDI mass spectrometry imaging (MSI) is a powerful technique that utilizes the capabilities of the spectrometer to collect thousands of individual spectra at various positions on a sample. Subsequently, using dedicated software, the resulting detected ions can be spatially represented as pseudo-colour images to visualize key molecules relative to their position in the sample / tissue.

The MALDI-8020 linear time-of-flight (TOF) mass spectrometer (Fig. 1) is capable of generating quality MS images, such as lipids in rat brains. The ability to rapidly exchange sample plates in the instrument (<3 mins) and the short 'instrument-ready' time mean that acquisitions can be quickly started, which is advantageous during optimisation of imaging methods. We have previously demonstrated MALDI imaging for a variety of target analytes on the MALDI-TOF benchtop system with the analysis of fingerprints, soybeans and PET films (Shimadzu Application News; 01-00389-EN and 01-00392-EN). Here, we demonstrate the capability of our imaging platform to easily achieve quality mass spectra and MS images for intact proteins from tissue sections and for peptides following on-tissue digestion, with linear mode analysis of rat brain tissue.

Fig. 1 MALDI-8020 Benchtop linear TOF mass spectrometer

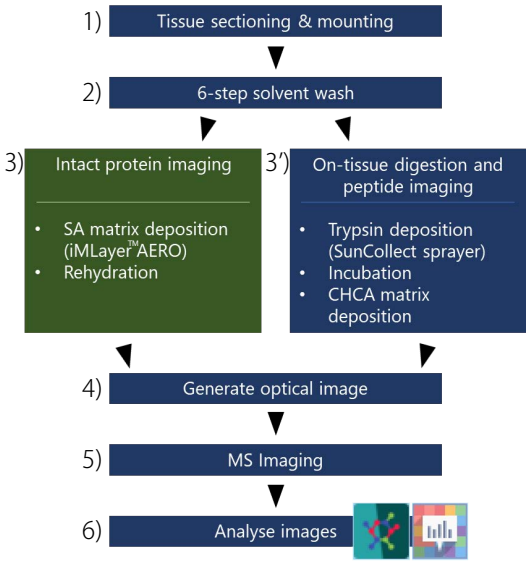

Fig. 2 Workflow for protein and peptide MS imaging with Sinapinic acid (SA) and α-cyano-4-hydroxycinnamic acid (CHCA) matrices, respectively

■ Measurement Conditions and Samples

Rat brain samples embedded in CMC media were prepared on FlexiVision-mini ITO Slides by AMSBIO (Oxford, UK). The slide was stored at -80 °C and vacuum desiccated to room temperature, prior to sample processing.

To prepare the tissue for peptide and protein analysis (Fig. 2-2), the lipids and salts need to be removed as they will dominate the mass spectrum and suppress the signal of the proteins. For delipidation, the slides were washed using a 6-step solvent wash protocol [1] : 1) 30 seconds in 70% ethanol, 2) 30 seconds in 100% ethanol, 3) 2 minutes in Carnoy's solution (60:30:10 of ethanol:chloroform:acetic acid), 4) 30 seconds in 100% ethanol, 5) 30 seconds in H2O, and 6) 30 seconds in 100% ethanol. After washing, the slides were vacuum desiccated before matrix deposition.

For on-tissue digestion, porcine trypsin (67 ng/µL in 90:10 of 20 mM ammonium bicarbonate:acetonitrile (ACN)) was deposited on tissue using a SunCollect sprayer (SunChrom, Germany). The slide was then placed in a rehydration chamber with 50:50 ACN:H2O at 37 °C for 18 hours. After the incubation, α-Cyano-4-hydroxycinnamic acid (CHCA, 10 mg/mL in 50:50 ACN:0.1 % trifluoroacetic acid (TFA)) was applied, again using the SunCollect sprayer, followed by rehydration for 2 minutes with no solvent and then 3 minutes with 5% acetic acid at 85 °C . Rehydration extracts analytes to the surface.

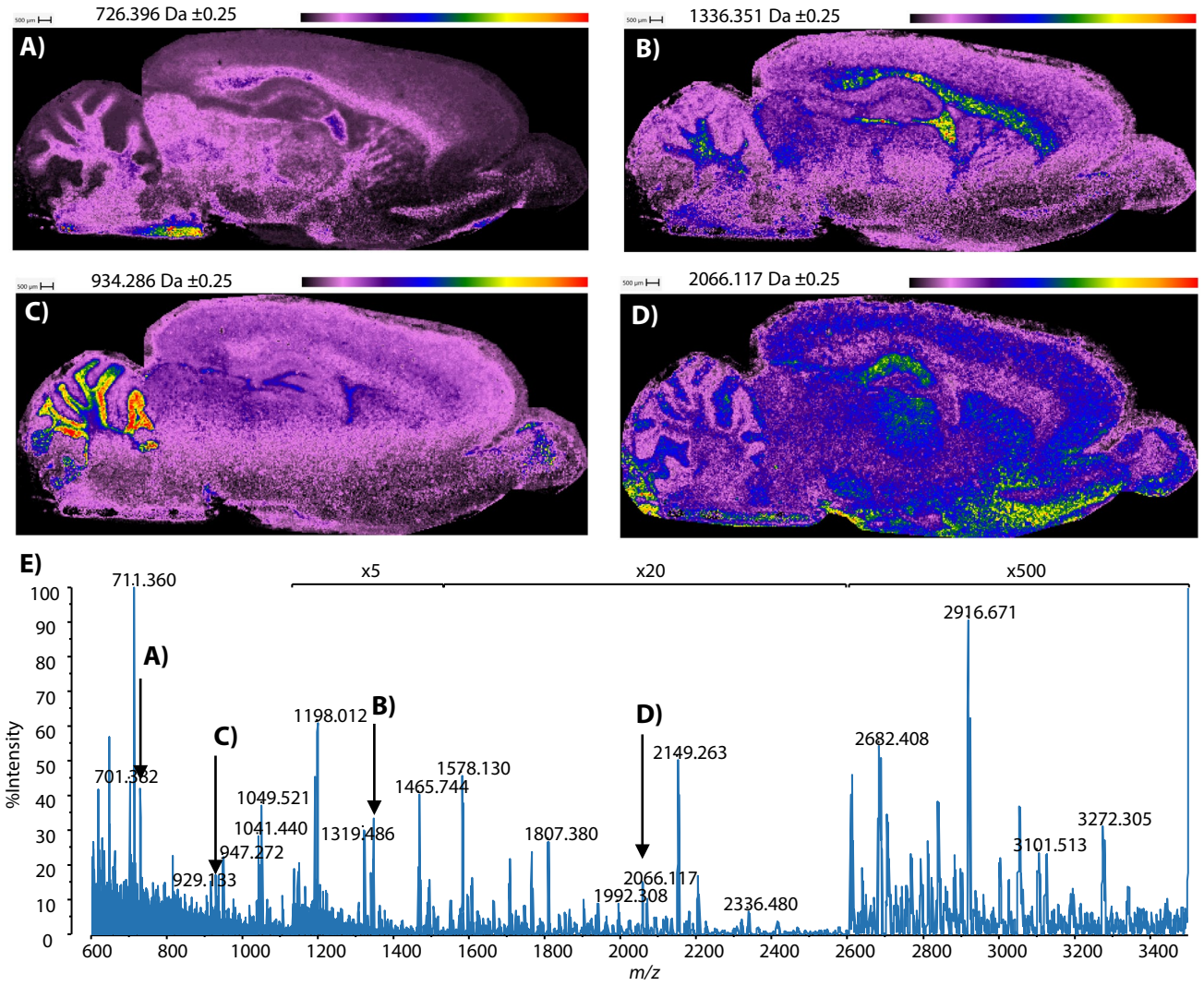

Fig. 3 MS Images of digested peptides: Tentative assignments to A) MBP peptide: HGFLPR, T160-165 (m/z 726.394), B) MBP peptide: YLATASTMDHAR, T148-159 (m/z 1336.633) localized in the white matter [4], C) & D) unknown peptides localized in the cerebellum and grey matter respectively, E) TIC spectrum of rat brain peptides

For intact protein analysis, sinapinic acid (10 mg/mL in 70:30 ACN: 0.1% TFA) was sprayed onto the tissue using an iMLayer™ AERO automated spraying device (Shimadzu, Japan). The tissue was coated with 8 layers at 50 mm/s and 4 layers at 30 mm/s. Following coating, the slide was placed in a rehydration chamber with 5% acetic acid for 3.5 minutes at 85 °C .

Samples were analysed at 50 µm spatial resolution (50 µm stage step size) in linear mode on a benchtop MALDI-TOF instrument (MALDI-8020, Shimadzu Corporation).

The acquisition method used for the peptides was 30 shots/profile, 200 Hz repetition rate, mass range 300-3000 m/z, and pulsed extraction at 2700 Da. There was a total of 67,482 profiles and the acquisition period was 2 hrs 50 mins.

The acquisition method used for the proteins was 20 shots/profile, 100 Hz repetition rate, mass range 3000-30000 m/z, and pulsed extraction at 22000 Da. There was a total of 68,836 profiles and the acquisition period was 3 hrs 47 mins.

Data was analyzed in IonView™ software (Shimadzu Corporation).

■ Results of peptide and protein imaging

In an earlier work (not shown), on-tissue digestion and protein imaging protocols were successfully validated on a MALDI-TOF-TOF (MALDI-7090, (Shimadzu Corporation)) instrument. In this work, we have transferred these methods to a benchtop linear MALDI-TOF MS instrument (MALDI-8020) .

MSI of the trypsin digestion of the rat brain showed that on the MALDI linear TOF system it was still possible to tentatively assign peptides based on mass (in the absence of MS/MS), to commonly observed rat brain proteins, e.g. myelin basic protein (MBP) is characteristically localized in the white matter, and this was consistent with the MALDI images obtained (Fig. 3) [2].

Intact protein MSI similarly revealed analytes corresponding to common proteins in the rat brain (e.g. MBP) (Fig. 4). The tentative protein identifications in Fig. 4 are based on observed mass of the detected species [3,4]. It was possible to obtain data at much higher masses compared to routine lipid MSI analysis showing suitability of this workflow for protein imaging. Signals beyond 16 kDa were low, but this was due to the embedding media used to improve the sectioning of the rat brain specimen [5].

The quick acquisition times of 2 hrs 50 mins and 3 hrs 47 mins respectively for the peptide and intact protein imaging of the full rat brains facilitated optimisation of the method.

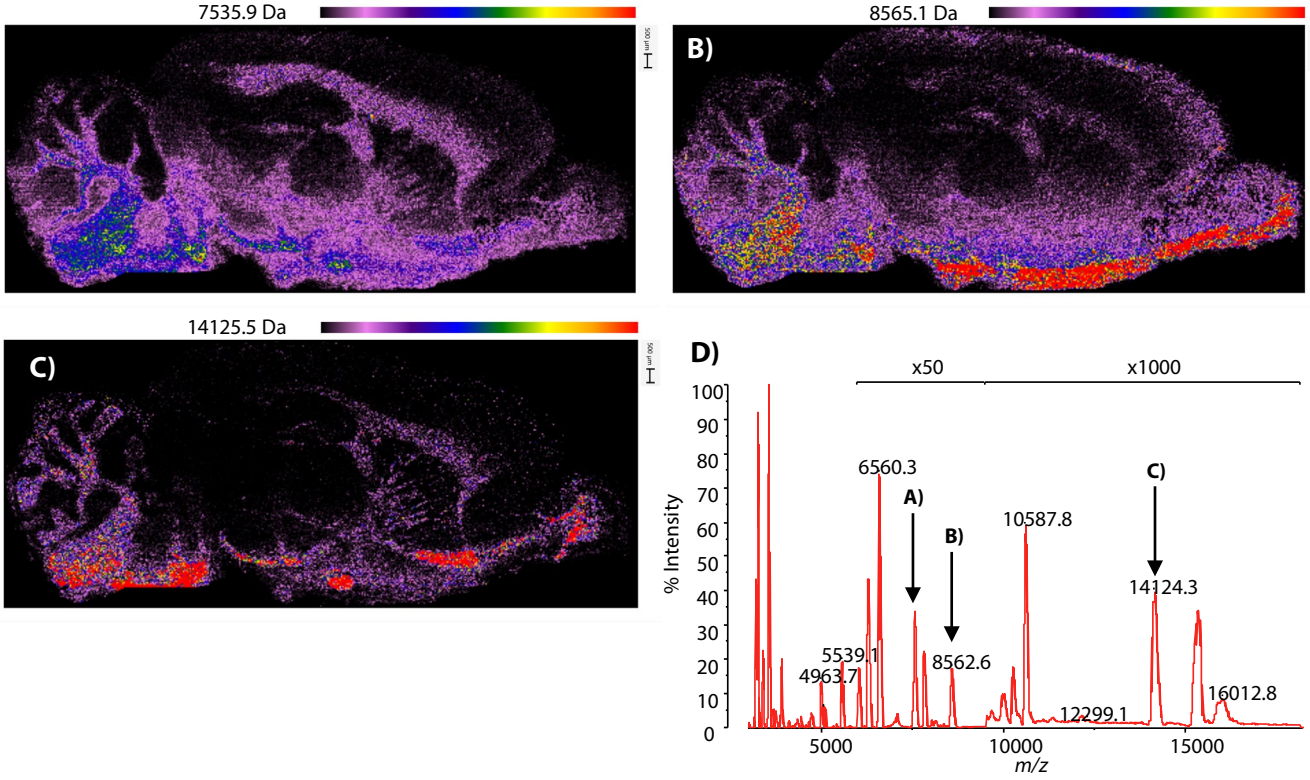

Fig. 4 MS Images of intact proteins: Tentative assignments to A) Neurogranin (m/z 7537), B) Ubiquitin (m/z 8565), C) Myelin Basic Protein (m/z 14124), D) TIC spectrum of rat brain proteins

■ Conclusion

We have demonstrated the capability of a low-cost benchtop MALDI-TOF instrument for MALDI mass spectrometric imaging, for the *in situ* analysis of peptides and proteins.

The quick sample plate exchange in the benchtop MALDI instrument (<3 mins) and fast acquisition times (2 hrs 50 mins at 200 Hz laser speed for a full rat brain spanning 67,482 profiles) facilitated development of the imaging method.

Proteins up to 16 kDa were easily detected using a simple workflow showing suitability for protein imaging.

MSI of peptides detected from the on-tissue digestion were shown on MALDI images to be consistent with characteristic localization of the corresponding proteins, showing suitability for peptide imaging and applications targeting the distribution of similar high mass species in tissue.

MS/MS identification of the peptides would be required for confirmation, but this application note suitably demonstrates protein and peptide MSI on the MALDI-8020 would be useful for screening applications.

This economical, compact, and robust instrument is ideal for those new to MALDI imaging and would represent a great resource for universities and teaching laboratories.

■ References

[1] Yang et al (2011) Matrix sublimation/recrystallization for imaging proteins by mass spectrometry at high resolution. Anal. Chem. 83, 5728-5734.

[2] Heijs et al (2015) Brain region-specific dynamics of on-tissue protein digestion using MALDI Mass Spectrometry Imaging. J Prot. Research 14, 5348-5354.

[3] Schmitt et al (2019) Genetically Encoded Fluorescent Proteins Enable High-Throughput Assignment of Cell Cohorts Directly from MALDI-MS Images. Anal. Chem., 91, 6, 3810–3817

[4] Groseclose et al (2007) Identification of proteins directly from tissue: in situ tryptic digestions coupled with imaging mass spectrometry. J. Mass Spectrom. 42, 254–262

[5] Franck & Rawlins et al (2010) MALDI mass spectrometry imaging of proteins exceeding 30000 Da. Med. Sci. Monit., 16(9): BR293 299.

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