

Imaging at Atmospheric Pressure by MALDI Mass Spectrometry

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Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry is becoming a powerful tool to map the spatial distributions of biomolecules in tissue sections. In its current form, however, this molecular imaging technique requires that the samples are transferred into vacuum environment and covered with matrix material. These requirements limit the scope of applications. For example, the need for vacuum excludes the possibility of *in vivo* studies, and the deposition of matrixes potentially obscures or distorts the studied component distributions. Here we report our first results on atmospheric pressure (AP) MALDI imaging using UV and IR lasers as ionization sources.

A Q-TOF Premier mass spectrometer (Waters Co.) was modified by replacing the electrospray source with a custom-made AP interface featuring a 130 μm ID capillary inlet. The output of a nitrogen laser (337 nm) or a Nd:YAG laser with tunable optical parametric oscillator (OPO) (~ 2940 nm) was projected onto the target plate to produce ions. In order to improve the sensitivity of the instrument, pulsed dynamic focusing was implemented. In the imaging experiments, a 3-axis precision flexure stage (NanoMax TS, Thorlabs Inc.) with stepper motor drives and computer control scanned the sample surface by moving the target plate perpendicular to the MS inlet.

UV-AP-MALDI mass spectra and imaging. Figure 1a shows an averaged AP-MALDI mass spectrum of a mock peptide mixture containing bradykinin, angiotensin I and substance P from 2,5-dihydroxybenzoic acid (DHB) matrix. With a pipette tip as a pen and this mixture as the ink, the letters "T", "O" and "F" were scribed on the target plate. The three peptides were used separately to produce the three strokes in the letter "F". The results presented in Figure 1b indicate that the total ion current intensity distribution (represented by false colors) clearly reflects the original pattern. Figure 1c shows the mass selected images for the molecular ions of the three peptides and for m/z 1046 in the letter "F". The distribution of the three molecular ions corresponds to the original arrangement for the three strokes in the letter. Figure 1c also shows that the distribution of the m/z 1046 ion closely follows that of the angiotensin I. Thus, this ion is very likely a contamination, a degradation product or a fragment of angiotensin I. Such spatial correlations can be particularly helpful for the identification of unknown ions, e.g., in the analysis of complex mixtures.

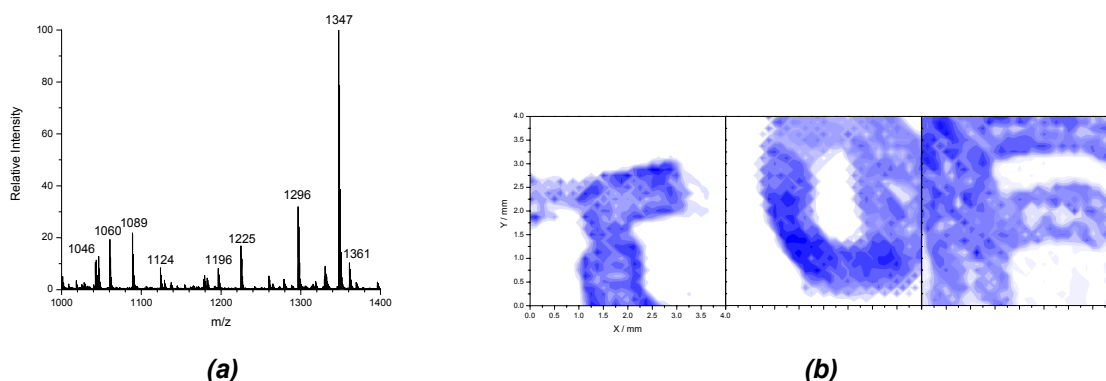


Figure 1: (a) UV-AP-MALDI mass spectrum of a bradykinin, angiotensin I and substance P mixture; (b) AP-MALDI image of three letters, "T", "O" and "F".

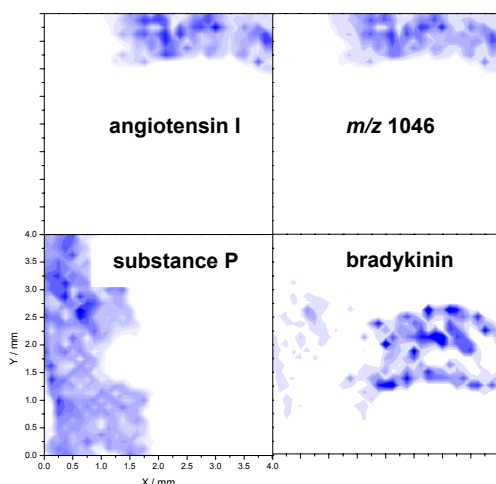


Figure 1: (c) Letter “F” imaged by UV-AP-MALDI. Three strokes in the letter were produced by substance P (vertical), angiotensin I (long horizontal) and bradykinin (short horizontal) solutions. Ion with m/z 1046 is co-located with angiotensin I, thus indicates a contamination or a fragment.

IR-AP-MALDI mass spectra and imaging. At $2.94 \mu\text{m}$ laser excitation, water was found to be a good matrix for peptide analysis; but due to its fast evaporation at room temperature, it typically gave a signal lasting only for 1 to 2 minutes. Using glycerol as a co-matrix or cooling the target plate with liquid nitrogen reduced the rate of the water evaporation and a stable signal could be obtained for over 30 minutes. Figure 2a shows a mass spectrum of strawberry skin obtained using IR-AP-MALDI with no matrix added. The dominant ions are m/z 381, identified as the potassiated sucrose ion, and the potassiated sucrose clusters at m/z 723, 1065 and 1407. The results indicate that in these experiments the native water in the strawberry serves as an efficient matrix. The potassiated sucrose ion distribution from the IR-AP-MALDI imaging experiment along with an optical image of the strawberry skin are shown in Figure 2b. The color intensity in the molecular image represents the abundance of m/z 381 ions. Interestingly, the location of the two seeds in the optical image corresponds to the low sugar concentration (white) regions in the molecular image. On test targets using step sizes smaller than the laser spot size (oversampling), a spatial resolution of $40 \mu\text{m}$ was achieved.

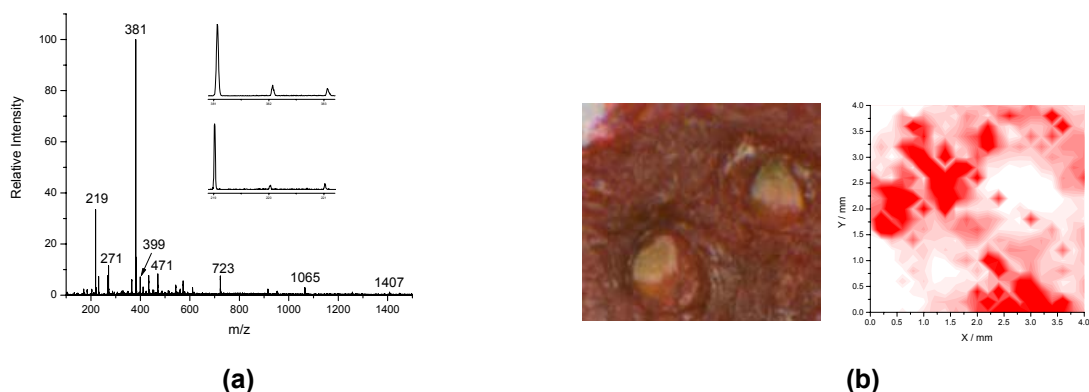


Figure 2: (a) IR-AP-MALDI mass spectrum and (b) optical and false color molecular images of strawberry skin.

AP-MALDI molecular imaging opens new avenues for the *in vivo* studies of complex biological systems in their natural environment and under controlled conditions. It has the potential to dramatically broaden the field of applications for mass spectrometry in biological and medical research.