

Atmospheric Pressure Infrared MALDI Mass Spectrometry: Imaging and In Vivo Studies of Metabolites in Plants

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Novel Aspect: AP IR-MALDI MS was demonstrated to be applicable for in vitro and in vivo studies in plant metabolomics.

Introduction

Many experimental techniques (including MS, NMR, IR and Raman spectroscopy, GC, LC and CE, etc.) have been developed to offer both high sensitivity and accuracy for the analysis of the ~200,000 metabolites estimated to exist in the plant kingdom. In this contribution, we report on the utility of atmospheric pressure (AP) infrared (IR) matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) for the identification and imaging of metabolites in plant tissue. We also present an example of in vivo imaging with AP IR-MALDI MS.

Methods

A Q-TOF Premier mass spectrometer (Waters Co., Milford, MA) was modified by replacing the electrospray source with a custom-made AP laser desorption ionization interface. A tunable OPO laser at 2940 nm was used to produce the ions directly from the tissue samples. Spectra were collected in both positive and negative ion modes. For selected ions MS/MS experiments provided structural information. Under the normal experimental conditions, the sensitivity of the instrument was about 1 fmol/pixel. In the imaging experiments, a stepper motor-driven 3-axis precision flexure stage was computer controlled to scan the sample surface. A LabVIEW program, which rendered the times to the corresponding X-Y coordinates, converted the ion chromatograms to two-dimensional spatial distributions.

Preliminary results

Thin sections from various plant tissues (from e.g., garlic, onion, tomato, potato, cilantro, white lily, strawberry and banana) were studied with AP IR-MALDI-MS. Analysis of high mass accuracy and collision induced dissociation data enabled the tentative identification of over 30 important metabolites including sugars, GABA, malic acid, citric acid, etc. In the positive mode, most of the ions observed in the mass spectra were produced by protonation or potassium (or occasionally sodium) adduct formation, with sugars as the dominant ions. In the negative mode, almost all the ions observed were formed through deprotonation of organic acids. Thus, the positive and negative modes provided complementary information. AP IR-MALDI MS was used to image the distributions of several metabolites in the petals of white lily flowers. In a transpiration experiment, we also demonstrated the transport of small molecules in plant vasculature. As there is no need for sample pre-treatment and with the ability to use native water as the matrix, AP IR-MALDI MS is especially suitable for in vivo studies of plants. In vivo studies were conducted on *Arabidopsis thaliana* and white lily plants. Although IR laser ablation at the applied fluences effectively removes the top few micrometers of the exposed plant tissue, the plants continue to function after the experiment. AP IR-MALDI MS imaging is expected to find a wider application in biological and medical research.