

Metabolic Profiling of a Single Live Cells by Laser Ablation Electrospray Ionization Mass Spectrometry

Bindesh Shrestha; Akos Vertes

George Washington University, Washington ,

Novel Aspect:

Single cell ablation and LAESI-MS enabled metabolic profiling of live cells of different age and derived from different species.

Introduction:

Biochemical processes within a cell are linked to the cell cycle, disease states, and ecological effects. Thus even cells of the same type exhibit diverse metabolic makeup depending on their age and interactions with the environment. In vivo analysis of metabolites in a single cell is challenging because of the limited size and complexity of the sample. Mass spectrometric techniques, such as matrix-assisted laser desorption ionization (MALDI) and secondary ion mass spectrometry (SIMS), in a vacuum environment have demonstrated the analysis of single cells. Here we show that metabolic profiles of single epidermal cells obtained by laser ablation electrospray ionization (LAESI) mass spectrometry (MS) vary for different plant species and change with the age of the cells.

Methods:

Subcellular mid-infrared laser ablation (~ 25 μm in diameter) for LAESI-MS was achieved by an etched GeO₂ optical fiber tip as a focusing device. A long-distance video microscope was utilized to maintain a constant distance between the fiber tip and the sample surface to avoid breaking the tip or puncturing the cell. A second video microscope, under right angle to the sample surface, was utilized to visualize and select a single cell for the LAESI-MS analysis. The ablation plume produced by the Nd:YAG OPO laser pulse (2.94 μm wavelength, 100 Hz) was postionized by a nano-electrospray source, and analyzed by an orthogonal acceleration time-of-flight mass spectrometer.

Preliminary Results:

Epidermal cells of *Allium cepa* bulb, a model system due to the large homogeneous cells that form a monolayer, were used to study metabolic profiles of single cells and cell populations in intact tissues. The LAESI mass spectra of a single epidermal cell of *A. cepa* showed more than 46 ions that were assigned to 20 endogenous metabolites. Most of the smaller ions were protonated and assigned to smaller metabolites, such as alliin, 2-aminoacrylate, thioacrolein found in the allin degradation cycle and the larger ions corresponded to sodiated or potassiated polysaccharide ions, possibly fructans found in fructan biosynthesis cycle. Tandem MS, based on collision activated dissociation, aided in the assignment of metabolites. The neighbouring epidermal cells of *A. cepa* from the same membrane showed a homogeneous metabolic profile. The epidermal cells from different scale leaves in the same bulb of *A. cepa* showed metabolic differences according to their age. The metabolic profile of epidermal cells in the *A. cepa* bulb was compared with cells of the same type from a different species, *Narcissus pseudonarcissus*. Single cell LAESI-MS spectra of the *A. cepa* were dominated by carbohydrates, whereas for the *N. pseudonarcissus* various alkaloids were the most abundant. Further developments in single cell LAESI-MS analysis focus on reducing the ablation spot size while obtaining information rich mass spectra. Proper selection of laser parameters depends on the cell types and the nature of the extracellular matrix. Avoiding damage to neighboring cells, while performing single-cell analysis, is the prerequisite to studying intact multilayered cell populations. An important extension of the LAESI-MS method is the analysis of biological tissues cell-by-cell, with the ultimate goal of molecular imaging based on cells as the natural voxels.