Spatial Mapping and Optimization of Ion Intensity for Nanoelectrospray in the Plume-Inlet Region

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Introduction

Spatio-chemical distributions exist within the electrospray plume, such as changes in pH1. Previous work described the development and application of a digital spatial mapping system with XYZ control for the construction of a spatial map of the volumetric space in the front of the mass spectrometer inlet2. In this study, the digital spatial mapping system was used to characterize the distribution of both singly and multiply charged molecular ions within the electrospray plume at nanoelectrospray flow rates (ca. 250 nL/min. to 8 µL/min.) The precise control of the XYZ stage enables the optimization of ion current and reproducible positioning of the emitter to within a fraction of a millimeter, even within the mass spectrometer inlet, enabling facile operation at ultra-low flow rates3,4.

PV Acquire™ (v. 1.51) software for the Digital PicoView® nanospray source was modified and combined with an additional scanning module to generate a raster scan pattern of the nanospray emitter with respect to the mass spectrometer inlet. Each movement to a given nanospray emitter XYZ position triggered an MS acquisition and resulting data file. After conversion of these RAW-formatted files to mzXML data format, a parsing and data visualization program was used to reconstruct the data into a mass-filtered ion current image. Images were 2 x 2 mm with a pixel step size of 200 µm. The acquisition of a 100-point (10 x 10) data set took approximately 30 minutes (20 sec./pixel). Ion current maps resulting from a sample mixture of a singly-charged low molecular weight drug, a multiply charged peptide, and a small protein in aqueous-organic mobile phase were acquired. Target flow rate: 300 ± 20 nL/min.

Methods

Samples:
- Continuous infusion experiments:
  - Human angiotensin I, (Sigma-Aldrich®), buspirone (Sigma-Aldrich), and ubiquitin (Sigma-Aldrich) were prepared to a final concentration of 1 µM, 1.4 µM, and 1 µM respectively (30% ACN, 0.1% formic acid)
- Continuous infusion pump & flow rate monitor:
  - Syringe pump (Harvard Apparatus, PHD Model) with 250 µL Gastight Syringe (Hamilton™)
  - In-line digital flow rate monitor (Upchurch®);
  - Target flow rate: 300 ± 20 nL/min.

Mass Spectrometer:
- LCQ Deca™ (Thermo Scientific)
  - 3 Microscans/spectra, 300-1400 m/z full scan
  - Emitter-to-inlet distance: 0.25, 0.5, 1.0, or 2.0 mm (as stated)
  - ESI voltage: 1.4, 2.2 or 3.2 kV (as stated in figures)
  - Each image data point is from a 12 sec. RAW file acquisition
- Digital PicoView® DPV-150 nanospray source (New Objective) modified for scanned spray
  - PicoClear™ conductive union (New Objective) used for application of high-voltage
  - PicoView® Acquire™ 1.51 software with scanning module
  - Raster scanning step size: 200 µm
  - Image size: 10 x 10 pixels; 2 x 2 mm typical
  - Self-pack PicoFrit® Emitter, PF360-20-10 (New Objective)

Data Analysis:
- ReAdW software program (Institute for Systems Biology; http://tools.proteomecenter.org/ReAdW.php)
  - Conversion of Thermo-generated RAW files to mzXML format
  - ViewImage (LabVIEW™ 8.2, National Instruments)
  - Parses mzXML file and extracts mass spectrum
  - Generates an image from multiple files and compiles ratio of user selected m/z values
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FIGURE 1

Schematic of the mapping experiment setup. The nanospray emitter mounted on the XYZ stage of the source is scanned in front of the MS inlet in a raster pattern. The source generates a contact closure after each stage movement, triggering the acquisition of a RAW file for that point. After a 100 point scan (10 x 10 image) the ReAdW program is used to convert files to the mzXML format. The ViewImage program loads the mzXML files for parsing and allows for the selection of the m/z range for image reconstruction. The orange circle in each map represents the size of the spectrometer inlet (~ 0.5 mm).

FIGURE 2

Screen shot of the prototype scan module within Digital PicoView® (right, DPV-550 shown). The starting stage position, step size (µm), point dwell time, and number of points for the raster scan are all under user control.

FIGURE 3

Representative full scan mass spectra for the sample mixture consisting of 1.4 µM buspirone (B-), 1 µM human angiotensin I peptide (A-), 1 µM bovine ubiquitin (U-); 30% ACN, 0.1% formic acid. Multiply charged molecular ions indicated by Xn+, are molecular ions of composition (X +nH)n+.

FIGURE 4

Inlet gas flow perturbates the spray plume Active droplet/cluster sampling

The ion mapping experiment reveals spatial inhomogeneity of relative ion distribution for singly charged vs. multiply charged ions. The radial (X,Y) distribution of multiply charged ions appears to be larger than the distribution of singly charged species. This is possibly due to ion mobility and space charge effects within the plume. Viscous mass transfer of neutral and charged species into the heated capillary of the mass spectrometer convolutes the distribution.
Ion map images (mass-to-charge ratio and spray voltage noted) for four different emitter-to-inlet distances (0.25, 0.5, 1.0 and 2.0 mm). The scan range is 2 mm, with a 0.2 mm step per pixel. Each pixel represents the extracted ion current from a 12 sec. RAW file. All images are plotted on the same absolute peak ion intensity. The orange circle represents the size of the MS inlet (0.5 mm).
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Ion map images and the image of numerical ratio for the $U^{12+}$ ion to $A^{3+}$ (left) and $B^{1+}$ (right) ions. ESI voltage = 1.4 kV, inlet distance was 0.5 mm. The $U^{12+}$ and $A^{3+}$ ions show even, nearly identical distributions. The $A^{3+}$ and $U^{12+}$ ions show a nearly identical distributions. The $U^{12+}$ and $B^{1+}$ ion show markedly different distributions, with the relative intensity of the $B^{1+}$ reduced significantly at the edges of the distribution. Note the symmetrical ring shaped distribution—a strong indication of spatial inhomogeneity. Full scan mass spectra from points 1, 2, 3, and 4 are shown in Figure 6.

Full scan mass spectra (average of 10 scans each) for points 1, 2, 3, and 4 shown in Figure 5. ESI voltage = 1.4 kV, inlet distance = 0.5 mm. Molecular ions are indicated as in Figure 3. Note the significant decrease in intensity of $B^{1+}$ relative to $A^{3+}$ and $U^{12+}$ at points 1 and 4, which are at the edge of the plume/inlet capture zone.
The digital stage control enables precise, repeatable positioning within the MS inlet. Photos of a 10 µm ID fritted emitter precisely positioned on-axis, and slightly recessed into the mass spectrometer inlet (0.5 mm diameter) of the LCQ Deca ion trap. Mobile phase (30% ACN, 0.1% formic acid) flow rate and ESI applied voltage are as shown in each figure. The efficient capture of plume droplets across flow rates from 250 to 8,500 nL/min. are shown. Ramified-jet spray modes are visible at the higher flow rates (5,300 and 8,500 nL/min).

Total ion current, selected ion current, and full scan mass spectra acquired at (A) 250 nL/min. and (B) 8,500 nL/min. for the emitter position shown in Figure 7. Molecular ions are indicated as in Figure 3.

Signal vs. Flow Rate

Conclusions

- The distribution of singly and multiply charged ions is inhomogeneous
- Maximum changes in relative ion intensity are observed at the “edges” of the plume/inlet sampling region
- Multiply charged ions exhibit a broader spatial distribution than singly charged species
- Precise and reproducible positioning of the spray emitter within the inlet capillary is enabled by the digital stage control
- Signal intensity remained relatively constant with changes in flow rate (200 to 8,500 nL/min.)

References