Published in final edited form as:

Anal Chem. 2014 August 19; 86(16): 8121–8128. doi:10.1021/ac501527y.

A New Ion Mobility–Linear Ion Trap Instrument for Complex Mixture Analysis

Gregory C. Donohoe[†], Hossein Maleki[†], James R. Arndt[†], Mahdiar Khakinejad[†], Jinghai Yi[‡], Carroll McBride[‡], Timothy R. Nurkiewicz[‡], and Stephen J. Valentine^{*†}

[†]C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, West Virginia 26506, United States

[‡]Center for Cardiovascular and Respiratory Sciences, West Virginia University School of Medicine, Morgantown, West Virginia 26506, United States

Abstract

A new instrument that couples a low-pressure drift tube with a linear ion trap mass spectrometer is demonstrated for complex mixture analysis. The combination of the low-pressure separation with the ion trapping capabilities provides several benefits for complex mixture analysis. These include high sensitivity, unique ion fragmentation capabilities, and high reproducibility. Even though the gas-phase separation and the mass measurement steps are each conducted in an ion filtering mode, detection limits for mobility-selected peptide ions are in the tens of attomole range. In addition to ion separation, the low-pressure drift tube can be used as an ion fragmentation cell yielding mobility-resolved fragment ions that can be subsequently analyzed by multistage tandem mass spectrometry (MSⁿ) methods in the ion trap. Because of the ion trap configuration, these methods can be comprised of any number (limited by ion signal) of collision-induced dissociation (CID) and electron transfer dissociation (ETD) processes. The high reproducibility of the gas-phase separation allows for comparison of two-dimensional ion mobility spectrometry (IMS)–MS data sets in a pixel-by-pixel fashion without the need for data set alignment. These advantages are presented in model analyses representing mixtures encountered in proteomics and metabolomics experiments.

Graphical abstract

^{*}Corresponding Author: stephen.valentine@mail.wvu.edu.

Supporting Information

Methods, discussion, and figures showing mass spectrum of KKDDDDDIKIIK and XIDTD. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

Notes

The authors declare no competing financial interest.

Over the last two decades, the use of ion mobility spectrometry (IMS) coupled with mass spectrometry (MS) as a means for biomolecular ion separation has grown dramatically.^{1–13} The added peak capacity afforded by the gas-phase separation (IMS) enhances the detection of lower-signal species by removing them from spectral regions containing interfering features from higher-signal ions.^{7,14–16} This advantage has driven technological development in the private sector as an increasing number of MS-based instrument platforms are adopting/developing IMS separation steps.^{17–19}

Much of the IMS-MS instrumentation demonstrated for complex mixture analysis has employed the use of time-of-flight (TOF) mass analyzers.^{1,2,20} The advantage of coupling IMS with TOFMS is that individual TOF spectra can be “nested” within the drift time (t_D) measurement.²¹ Although this instrument configuration allows the mass determination of all mobility-dispersed ions, the inability to trap ions precludes the use of multistage tandem mass spectrometry (MS^n) as a means for identifying complex mixture components. This is problematic for identifying intractable molecules for which MS/MS analysis is insufficient.^{22–25}

IMS-MS instrumentation development has also yielded instruments combining drift tubes with mass spectrometers that employ ion trapping.^{26–30} Featured designs include those utilizing high-resolution drift tubes operated at atmospheric pressure and elevated drift voltages.^{27,28} Two challenges became evident with this type of instrument design that relate to the overall measurement sensitivity. The first challenge was associated with the low duty cycle of the measurement. The second was that the addition of the required mobility-selection ion gate essentially resulted in a scanning/filtering operational mode. That is, only ions of select mobilities were transferred into the mass spectrometer. These problems may be addressed in the future using ideas presented in seminal studies demonstrating the use of Fourier Transform and Hadamard Transform IMS.^{31–33}

One problem that persists with instruments coupling high-pressure IMS and ion trap mass spectrometers is the decrease in ion transmission across the drift tube-mass spectrometer interface region. That is, ions can be lost in conductance-limiting regions as there are no means currently available to focus diffuse ion clouds at elevated pressures. The ability to compress ion packets in low-pressure drift tubes^{34,35} was one of the motivating factors for pursuing the instrumentation design described here. Additionally, the low-pressure drift tube can not only serve as the device to achieve gas-phase separation but also as an ion fragmentation cell employing collision-induced dissociation (CID).³⁶ Indeed, the higher-

pressure fragmentation process (hereafter referred to as IMS-CID) has been demonstrated to be efficient and highly tunable.^{7,36–38} The combination of the IMS-CID capabilities of the drift tube with the MSⁿ capabilities of the linear ion trap further distinguish the instrument described here.

The new analytical capabilities of the instrument are here demonstrated with the analysis of two model mixture samples. The first sample analysis represents the capability to rapidly identify phosphorylated peptides in tryptic digests such as would be encountered in post-translational modification (PTM) experiments. For these studies, IMS-CID methods show that targets for PTM analysis can rapidly be ascertained and subsequently subjected to electron transfer dissociation (ETD)³⁹ to identify phosphorylation sites. To some degree, the approach is similar to proteomics techniques that scan for neutral loss of H₃PO₄ and subject target ions to further tandem MS experiments.^{40,41} A difference is that extracted ion drift time distributions (XIDTD)⁴² help to confirm and isolate ions for subsequent tandem MS experiments. Although several instruments demonstrating fragmentation of mobility-selected ions have been described,^{7,36,37,43,44} this work presents the first combination of IMS-CID^{36,38} and ETD-MS. The second experimental example demonstrates the ability to rapidly compare IMS-MS metabolite distributions obtained on the IMS-linear ion trap (LIT) instrument across multiple samples. The advantage of the instrumental approach is that no data set alignment is required for comparisons across many data sets and mobility-selected ions can be subjected to MSⁿ in the ion trap in order to aide identification.

The described instrument is similar to that coupling IMS-LIT analysis with photodissociation capabilities;^{29,42} however, this work describes unique ion fragmentation techniques such as the combination of two different methods (IMS-CID and ETD-MS) as well as MS⁴ of mobility-selected ions. The direction of future instrumentation development is discussed while considering improvements for complex mixture analysis.

EXPERIMENTAL SECTION

Samples

Cytochrome *c* (equine, 95%) was purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Phosphorylated peptide standards (95% purity) with sequences of KRPsQRHGSKY-NH₂ (1422.5 Da) and SFVLNPTNIGMsKSSQGHVTK (2316.6 Da) were purchased from AnaSpec (Fenton CA) and used without further purification. Rat plasma samples were obtained from the West Virginia University Center for Cardiovascular and Respiratory Sciences, School of Medicine, Morgantown, WV. The ENM exposure apparatus and organism handling were described in detail previously^{45,46} and a fuller description of sample collection is provided in the Supporting Information section. Samples utilized correspond to plasma generated at specific time points after the organisms had been exposed to aerosolized TiO₂ nanoparticles. Samples from a postexposure time point of 24 h (EXP24) were utilized. Controls for the respective time point were also used (CNTRL24).

Sample Preparation

Tryptic Digest Mixture—One milligram of cytochrome *c* was diluted in 1.0 mL of deionized water. Urea was added to a final concentration of 1.5 M. TPCK-treated trypsin was added at a ratio of 1:20 (trypsin:cytochrome *c*) and the sample was incubated overnight at 37 °C. The tryptic digest (100 μ L) was diluted 10-fold in ESI solution (1:1 water:methanol and 1% formic acid) and 10 μ g (0.01 μ g· μ L⁻¹) of the phosphorylated peptide standard was added to the solution.

Metabolite Extract Mixture—Pooled plasma samples were prepared from 40 individual samples by combining 25- μ L aliquots. Using a temperature-controlled microcentrifuge digital dry bath (Lanbnet International, Inc., Edison, NJ, USA), 400 μ L of 80 °C methanol is added to 100 μ L of pooled sample. The samples were vortexed and incubated first at 80 °C (5 minutes) and immediately transferred to ice (5 min). Proteins were pelleted using centrifugation (ThermoScientific, San Jose, CA, USA) at 13 000g for 20 min at 4 °C. The supernatant (~375 μ L) was combined with 300 μ L 18 M Ω H₂O:5% formic acid.

Both the tryptic digest and metabolite extract samples were delivered by a syringe pump (KD scientific Holliston, MA, USA) using a 500- μ L syringe (Hamilton, Reno, NV, USA) to a pulled-tip nanoelectrospray emitter (350 μ m o.d. $\times$ 75 μ m i.d.). The solution flow was ~300 nL·min⁻¹ and an electrospray bias of 2.2 kV was used.

Instrument

IMS theory,^{47–50} instrumentation,^{32,51–57} and techniques^{43,58–61} are described elsewhere in great detail. have been described in detail elsewhere. For these experiments, the IMS-MS instrument consists of a dual ion funnel/ion gate design coupled to a LTQ Velos (Thermo Electron, San Jose, CA, USA) mass spectrometer similar to that reported previously.^{29,42} Figure 1 shows the instrument schematic designating the locations of the ion funnels, ion gates and ion activation regions within the drift tube. The mass spectrometer has been modified by replacing the ion source and s-lens assembly with a stacked-ring ion drift region (Figure 1). Briefly, electrosprayed ions enter into a desolvation region and are focused through an hourglass ion funnel (F1 in Figure 1).^{13,62} Ions are trapped in F1 and periodically pulsed into the drift tube. The drift tube is filled with He buffer gas (~2.5 Torr at 300 K). Ions traverse the drift tube under the influence of a uniform electric field (~11 V·cm⁻¹) and separate based on differences in their mobilities. Upon reaching a selection gate (G2 in Figure 1) ions pass into a second ion funnel (F2) used to radially focus the ion cloud.^{34,35} The ions subsequently exit the drift tube through a conductance-limiting aperture and are focused by quadrupole and octopole rf guides before entering into the higher pressure linear ion trap followed by subsequent mass analysis in the lower pressure trap.

Recording Two-Dimensional (2D) t_D [mass-to-charge (m/z)] Distributions

Ion gates (Figure 1) are constructed using Ni gridded lens (90% transmittance mesh; Precision Eforming, Cortland, NY, USA). A single, gridded lens located immediately after the first funnel (F1 in Figure 1) serves as the first ion gate (G1 in Figure 1). Ions are pulsed into the drift region at G1 (–40 V) for 150 μ s every 20–30 ms. Data used to construct ion t_D distributions are collected using a time-delayed voltage drop (–3 V) applied to G2. The time

delay for opening G2 relative to the initiation pulse at G1 using a step resolution of 0.15 ms (tryptic digest sample) and 0.2 ms (metabolite extract sample) was scanned across the mobility distribution range. For the tryptic digest, this range was 4–12 ms resulting in 41 mobility-resolved mass spectra. For the metabolite sample, the range was 3.4–15 ms yielding 59 mobility-resolved mass spectra. The mass spectra are combined to create 2D t_D (m/z) data sets (see below). The introduction pulse at G1 and variable delay settings at G2 are controlled by 2 four-channel digital pulse generators (Stanford Research Systems, Sunnyvale, CA, USA).

Parallel Dissociation Methods

Parallel dissociation^{43,44} within a drift tube, (IMS-CID-MS, IMSⁿ-MS) has been previously described in detail^{36,38} and demonstrated for “omics” applications.⁷ Briefly, mobility separated ions are subjected to energizing collisions at the second ion activation region (IA2 in Figure 1). For dissociation, the voltage between the last two electrodes of the F2/IA2/G2 assembly (Figure 1) is elevated to ~220 V over a length of ~0.3 cm. This produces a field of ~700 V·cm⁻¹ to induce ion fragmentation prior to entering the mass analyzer. Thus, low-field settings (~11 V·cm⁻¹) and high-field settings (~700 V·cm⁻¹) are employed to transmit precursor and fragment ions, respectively. For neutral loss experiments (see below), more gentle activation conditions (~453 V·cm⁻¹) are employed in the collisional activation region.

Mass Spectrometry Measurements

Mass spectra are generated for all ions by deactivating the dual drift tube gates and setting the mass analyzer scan parameters over a m/z range of 80 to 2000 while enabling the automatic gain control (AGC). The AGC threshold is 1×10^6 ions. In this operational mode, the drift tube acts to transmit all ions into the mass analyzer. During t_D distribution generation, the gates are activated to transmit a specific mobility into the linear trap. For these analyses, the AGC is disabled and sample injection times of 400 ms (4 microscans) for the tryptic digest and 100 ms (1 microscan) for the metabolites are employed.

MSⁿ analysis is conducted by mobility-selecting and isolating (m/z) a precursor ion. For experiments employing CID in the ion trap, a m/z isolation window of ± 1.0 Da relative to the centroid m/z value results in the ejection of all other ions within the t_D window. Selected ions are collisionally activated by a resonant rf excitation waveform for 10 ms using a normalized activation energy of 35.0%. Fragment ions displaying sufficient signal-to-noise (S/N) levels are isolated in the linear ion trap and subsequently fragmented. For experiments employing ETD, a m/z isolation window of ± 1.0 Da relative to the centroid m/z value was utilized. The acquisition time was set at 100 ms. All precursor and fragment ion spectra were recorded using the XCalibur 2.2 software suite (ThermoScientific, San Jose, CA).

IMS-MS Data Sets

Two-dimensional IMS-MS data sets are generated by converting each t_D -selected mass spectrum (.RAW file) to a separate text file. Using software developed in house, the m/z values and intensities from all of the text files are then associated with the respective t_D selection time generating a three-column array text file containing (t_D , m/z , and intensity values). Intensity filters are applied to generate three-column array files of manageable sizes.

To generate t_D distributions for specific ions (including fragment ions from parallel dissociation), a separate program (in house) integrates all intensities within a user-defined m/z range for each t_D window. As mentioned above, this is termed a XIDTD.⁴²

Comparison of IMS-MS Data Sets

IMS-MS [$t_D(m/z)$] data sets (triplicate measurements) were compared across the two metabolite samples using intensity integration for user-defined pixels over a designated range in the $t_D(m/z)$ distribution. For these comparisons, each pixel represented m/z and t_D windows of 2 m/z units and 500 μs , respectively. Data normalization is performed by dividing all integrated pixels in the IMS-MS data set by the total ion intensity. Because of the high-reproducibility of the IMS measurements, alignment of data set pixels was not performed.

RESULTS AND DISCUSSION

Instrument Figures of Merit

For contextualization and discussion purposes several measurement capabilities of the new instrument are briefly presented. The resolving power of the drift tube (t_D/t_D at fwhm) can be determined for different types of ions. For $[M + H]^+$ peptide ions, the resolving power is determined to be 41 ± 4 which is similar to that reported previously for drift regions of the same length.³⁵ By spraying serial dilutions of single peptide samples (see Supporting Information) it is estimated that the detection limits for mobility-selected ions is tens of attomoles. This level of sensitivity is higher than that reported for IMS instrumentation employing an in-line gating operational mode.⁶³ The previous experiments employed a TOF mass analyzer and the dispersive nature of the ion detection may account for some of the difference in sensitivity. The dynamic range for a single IMS-MS data set of a tryptic digest can be estimated by comparing the integrated intensities of the lowest and highest signals for identifiable peptide ions. From these experiments, the dynamic range is estimated to be between 10^3 and 10^4 (see Supporting Information).

IMS-CID-ETD-MS Phosphopeptide Analysis

Figure 2A shows a heat plot of the 2D $t_D(m/z)$ data set of the cytochrome c digest containing the phosphorylated peptide standards. Tryptic peptide ions are observed to exist predominately as $[M + 2H]^{2+}$ and $[M + 3H]^{3+}$ ions exhibiting similar $t_D(m/z)$ trends as reported previously.¹ Most spectral features appear between ~5 and ~9 ms and are observed within the 400 to 1000 m/z range. The data in Figure 2A represents a number of ions exhibiting an intensity range of 10^3 .

The highly tunable nature of the IMS-CID process^{36,37} can be combined with the efficient mobility separations^{64,65} to characterize phosphorylated peptides in tryptic digests. Because the bond dissociation energy of the phosphate modification is lower than that of backbone amide fragmentation, an IMS-CID approach that employs gentle activation ($\sim 450 \text{ V}\cdot\text{cm}^{-1}$) can be utilized. Here a goal is to increase the activation voltage in IA2 (Figure 1) until neutral loss (H_3PO_4) is observed while preserving much of the other tryptic digest peptide

ions. This allows for a more straightforward analysis of precursor and product ions similar to ion trap experiments described previously.^{40,41}

For matching the precursor and neutral loss fragment ions, it is useful to evaluate the XIDTDs. Figure 2A shows the XIDTD of the [KRpQRHGSKY-NH₂ + 3H]³⁺ (m/z 475.2) ions as an inset. This peptide exhibits a broad mobility profile containing more compact (t_D = 5.2 ms) and more elongated (t_D = 6.4 ms) species. Figure 2B shows t_D (m/z) data generated from IMS-CID of the digest mixture. The m/z difference between the precursor ion and the neutral loss product ion is 32.6, which is consistent with the loss of H₃PO₄ for the triply charged ion. The XIDTD of the neutral loss product ion is shown as an inset in Figure 2B revealing a slightly broader distribution with conformational maxima matching the precursor XIDTD profile (5.2 and 6.4 ms). A difference in the distributions is the higher intensities observed for more elongated species in the IMS-CID data set. That said, the t_D matching of the dominant features in the XIDTDs helps confirm the origin for much of the ions. The differences in the distributions may arise from the presence of overlapping lower-intensity fragment ions.

Both the intact precursor and neutral loss ions are present in the same t_D (m/z) distribution (Figure 2B) and either can be mobility-selected and m/z isolated for further characterization by ETD. Figure 2C and D show the ETD spectra for the intact precursor ions and neutral loss fragment ion, respectively. Here the z -ion series is most informative for observing the site of phosphorylation. That is, for the precursor ETD fragments, the z_8 ion at m/z 1025.4 reveals residue-specific phosphorylation. Comparatively, in the neutral loss product ion spectrum, the same z_8 ion has a m/z value of 946.4. As expected, lower z -series ions for both the precursor and neutral loss ions demonstrate the same m/z values. For larger ions ($>z_8$ ion), those originating from the precursor species are higher in m/z due to the addition of the phosphate moiety. Also noticeable in the spectra are the ETD charge-reduced species that further confirm the neutral loss product ion. In the case of the $[M + 3H]^{2+}$ ions, the precursor m/z is 712.4, while the product ion m/z is 663.4. The m/z difference (49) is consistent with the neutral loss of H₃PO₄ for the doubly charged ion.

The use of ion trapping instruments without ETD capabilities often requires at least MS³ to determine the site of phosphorylation.^{66,67} As a result, the technique requires relatively higher ion signal levels for informative higher-order MSⁿ results. The use of IMS-CID-ETD-MS for PTM peptide precursor scans is unique in that neutral loss ions can be uniquely matched back to the precursor based on XIDTDs; this can be followed by ETD-MS of the intact precursor for site specific PTM determination. Such a technique also represents an enhanced scanning approach compared to that employing CID and ETD in an ion trap^{40,41} as the mobility matching and selection would help to identify precursor ions and improve their isolation for PTM site determination.

Comparative IMS-MS Analysis of Metabolomics Samples

2D IMS-MS data sets have been recorded in triplicate for the two metabolite extract samples. Figure 3A, shows the t_D (m/z) heat plot of metabolites obtained from a single IMS-MS analysis of the EXP24 pooled sample. For all data sets, spectral features are first observed at ~3.4 ms and extend to ~15.0 ms; the highest intensity metabolite features exist

between ~8.0 ms to ~9.0 ms. In general, data set features are observed over a wide m/z range from ~80 to ~1200 with the ions exhibiting the greatest intensity being observed over a m/z range of ~500 to ~650. Using the pixel integration sizes mentioned above, data set pixel intensities for 19 000 separate t_D (m/z) regions range from 0 to $>10^4$.

Rapid comparisons of percent differences in pixel intensity across multiple data sets can reveal features that are significantly different even when they are of much lower intensity. Figure 4 shows the XIDTDs of ions of m/z 518.3 for the CNTRL24 and EXP24 samples. Data set replicates of the EXP24 sample show the presence of a higher abundance feature at ~7.2 ms. The average normalized pixel intensities for this region in the EXP24 replicates is 0.64 ± 0.02 compared to 0.11 ± 0.01 for the CNTRL24 replicates. Although the features in the XIDTD cannot be ascribed to different gas-phase conformers or isobaric ions, Figure 4 shows the ability to rapidly compare distinguishing data set features that may otherwise not be considered to be significantly different between samples by MS analysis alone. Additionally, the comparison reveals the high-reproducibility of the measurement. Without having to employ peak (pixel) alignment techniques, the coefficients of variation for this low-intensity feature are ~3% (EXP24 replicates) and ~9% (CNTRL24 replicates).

IMS-MSⁿ Experiments for Metabolomics Analyses

The low-pressure drift tube allows trapping of electrosprayed ions increasing the overall sensitivity and enabling MSⁿ experiments for mobility-selected ions. To demonstrate this capability, metabolite ions exhibiting a t_D of ~8.2 ms and having m/z 518.3 have been selected for MSⁿ analysis. A precursor mass search of the Human Metabolome Database (HMDB)⁶⁸ presented the sodiated Lysophosphatidylcholines [LysoPC (16:0)] as the top match. MS² of this ion yields essentially one fragment ion at m/z 459.2 as shown in Figure 3B. This ion could indicate the loss of a methylated ammonium headgroup (m/z 59) that is characteristic to many LysoPCs.^{69,70} Fragments that displayed the highest S/N ratios have been used in subsequent fragmentation experiments. For example, activation of the m/z 459.2 ion produced from the mobility-selected m/z 518.3 precursor yields several peaks including those at m/z 313.2, 335.2, and 415.2. Isolation of m/z 313.2 ions allows for MS⁴ analysis that produces several features. Although not possible to assign all fragment ions, the peak at m/z 257.2 is consistent with the alkyl chain and ester region of a LysoPC molecule. When compared to the feature at m/z 257.2, the product ion with m/z 239.2 indicates a loss of water. Additionally, a number of low m/z fragments differing by 14 Da is consistent with fragmentation of the aliphatic chain of a LysoPC molecule. The inset in Figure 3B shows a schematic representation of the sodiated LysoPC(16:0) molecule and the observed fragments that are consistent with those obtained from the precursor ion of m/z 518.3. The tentative molecular assignment obtained from the MS⁴ experiments is further supported by accurate mass matches (± 0.5 ppm) obtained by direct electrospray of the sample into a Q-Exactive Orbitrap (ThermoScientific) mass spectrometer (see Supporting Information). Such an assignment is still presented as tentative as biomarker discovery efforts would require exact mass and ion fragmentation matches to molecular standards. That said, the IMS-MS instrumental approach is presented here as a means to rapidly provide candidate molecules for which confirmatory experiments can be conducted.

IMS-CID-MS Measurements

Not requiring the isolation and fragmentation of each individual ion, shotgun proteomics experiments have demonstrated that parallel dissociation⁴³ of ions in a drift tube offers significant advantages in terms of speed.^{7,71} Such a speed increase using the current IMS-MS instrument can be advantageous for rapid comparative metabolomics analyses as well. Figure 5A shows a 2D IMS-CID-MS data set for the EXP24 metabolite extract sample. Here, increased ion intensity is observed in high- and low- m/z regions indicating ion dissociation.

For complex mixtures, the data in Figure 5A show the challenge in associating fragment and precursor ions by IMS-CID due to the presence of many overlapping species. One method that may help alleviate this problem is comparison of XIDTDs. The utility of this approach for distinguishing precursor ions with overlapping mobilities has been shown previously in IMS-photodissociation (PD)-MS experiments.⁴² Figure 5B shows an example comparison of the XIDTD for the precursor ion of m/z 534.2 and the XIDTD for the fragment ion of m/z 475.2. Overall the two distributions are very similar. Each exhibits a peak maximum at a t_D value of ~ 6.8 ms. The major peak in each is observed at a t_D of ~ 8.2 ms and both exhibit peaks at a t_D of ~ 9.2 ms. Differences are observed in that the XIDTD of the precursor ion exhibits a small feature at a t_D ~ 7.2 ms as well as slightly increased intensities above a t_D of ~ 9.2 ms. A semiquantitative assessment can be performed to rank the comparison of two XIDTDs. Figure 5C shows the percent differences for comparisons of the XIDTD for the fragment ion of m/z 475.2 and the XIDTDs of two different precursor ions (m/z 496.3 and 534.2) observed in the precursor ion 2D IMS-MS data set (Figure 3A). Here the percent difference between the distributions is observed to be ~ 2 times smaller (integrated intensities in Figure 5C) for the larger precursor ion. Accurate mass matching (Supporting Information) of the precursor ion of m/z 534.2 is consistent with potassiated LysoPC(16:0). The IMS-CID-MS product ion of m/z 475.2 suggests the loss of the methylated ammonium in the choline headgroup for this tentative assignment.

Instrumental Implications for Comparative Metabolomics Analyses

Although the data provided here present a proof-of-principle demonstration of instrumentation capabilities, several improvements are envisioned to yield more effective comparative analyses. Increased IMS resolving power would better disperse the overlapping (t_D dimension) precursor ions (Figure 2A) and allow for higher accuracy association of fragment ions to precursor ions in parallel dissociation experiments. Additionally, adaptations in drift tube design would allow for fragmentation of mobility-selected precursor ions followed by parallel dissociation and mobility resolution of fragment ions as demonstrated previously.^{30,38} An additional enhancement can be obtained by coupling off-line LC fractionation with the IMS-MS analysis. Because the IMS separation occurs after the ionization step, ion suppression contributes to the observation of the very high-signal LysoPC species observed in the middle range of the mass spectrum (Figure 3A) along with the observation of much lower intensity species in other spectral regions. Future comparative studies using this instrument will combine LC fractionation with enhanced IMS separation (2-m drift tube).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful for financial support from the Eberly College of Arts and Sciences at West Virginia University for providing laboratory research startup funds as well as the Brodie Discovery and Innovation Award.

References

1. Valentine SJ, Counterman AE, Hoaglund CS, Reilly JP, Clemmer DE. *J Am Soc Mass Spectrom.* 1998; 9:1213–1216. [PubMed: 9794086]
2. Gillig KJ, Ruotolo B, Stone EG, Russell DH, Fuhrer K, Gonin M, Schultz AJ. *Anal Chem.* 2000; 72:3965–3971. [PubMed: 10994952]
3. Ruotolo BT, Gillig KJ, Stone EG, Russell DH. *J Chromatogr B: Anal Technol Biomed Life Sci.* 2002; 782:385–392.
4. Wu C, Siems WF, Klasmeier J, Hill HH Jr. *Anal Chem.* 2000; 72:391–395. [PubMed: 10658335]
5. Clowers BH, Dwivedi P, Steiner WE, Hill HH, Bendiak B. *J Am Soc Mass Spectrom.* 2005; 16:660–669. [PubMed: 15862767]
6. McLean JA, Ruotolo BT, Gillig KJ, Russell DH. *Int J Mass Spectrom.* 2005; 240:301–315.
7. Valentine SJ, Plasencia MD, Liu X, Krishnan M, Naylor S, Udseth HR, Smith RD, Clemmer DE. *J Proteome Res.* 2006; 5:2977–2984. [PubMed: 17081049]
8. Becker C, Fernandez-Lima FA, Russell DH. *Spectroscopy.* 2009; 24:38–42.
9. Dwivedi P, Wu P, Klopsch SJ, Puzon GJ, Xun L, Hill HH. *Metabolomics.* 2008; 4:63–80.
10. Isailovic D, Kurulugama RT, Plasencia MD, Stokes ST, Kyselova Z, Goldman R, Mechref Y, Novotny MV, Clemmer DE. *J Proteome Res.* 2008; 7:1109–1117. [PubMed: 18237112]
11. McLean JA. *J Am Soc Mass Spectrom.* 2009; 20:1775–1781. [PubMed: 19646898]
12. Kliman M, May JC, McLean JA. *Biochim Biophys Acta Mol Cell Biol Lipids.* 2011; 1811:935–945.
13. Baker ES, Clowers BH, Li F, Tang K, Tolmachev AV, Prior DC, Belov ME, Smith RD. *J Am Soc Mass Spectrom.* 2007; 18:1176–1187. [PubMed: 17512752]
14. Dwivedi P, Schultz AJ, Hill HH. *Int J Mass Spectrom.* 2010; 298:78–90. [PubMed: 21113320]
15. Zinnel NF, Pai PJ, Russell DH. *Anal Chem.* 2012; 84:3390–3397. [PubMed: 22455956]
16. Counterman AE, Hilderbrand AE, Barnes CAS, Clemmer DE. *J Am Soc Mass Spectrom.* 2001; 12:1020–1035.
17. Kurulugama RTIK, Taylor L. Agilent Technologies. 2014
18. Hernandez DR, DeBord JD, Ridgeway ME, Kaplan DA, Park MA, Fernandez-Lima F. *Analyst.* 2014; 139:1913–1921. [PubMed: 24571000]
19. Waters, 2014
20. Steiner WE, Clowers BH, English WA, Hill HH. *Rapid Commun Mass Spectrom.* 2004; 18:882–888. [PubMed: 15095357]
21. Hoaglund CS, Valentine SJ, Sporleder CR, Reilly JP, Clemmer DE. *Anal Chem.* 1998; 70:2236–2242. [PubMed: 9624897]
22. Scheubert K, Hufsky F, Rasche F, Bocker S. *J Comput Biol.* 2011; 18:1383–1397. [PubMed: 22035289]
23. Cao M, Fraser K, Rasmussen S. *Metabolites.* 2013; 3:1036–1050. [PubMed: 24958264]
24. Prien JM, Ashline DJ, Lapadula AJ, Zhang H, Reinhold VN. *J Am Soc Mass Spectrom.* 2009; 20:539–556. [PubMed: 19181540]
25. Jiao J, Zhang H, Reinhold VN. *Int J Mass Spectrom.* 2011; 303:109–117. [PubMed: 21686090]
26. Bluhm BK, Gillig KJ, Russell DH. *Rev Sci Instrum.* 2000; 71:4078–4086.

27. Clowers BH, Hill HH. *Anal Chem.* 2005; 77:5877–5885. [PubMed: 16159117]
28. Tang X, Bruce JE, Hill HH Jr. *Rapid Commun Mass Spectrom.* 2007; 21:1115–1122. [PubMed: 17318922]
29. Zucker SM, Lee S, Webber N, Valentine SJ, Reilly JP, Clemmer DE. *J Am Soc Mass Spectrom.* 2011; 22:1477–1485. [PubMed: 21953250]
30. Li HL, Bendiak B, Siems WF, Gang DR, Hill HH. *Anal Chem.* 2013; 85:2760–2769. [PubMed: 23330948]
31. Knorr FJ, Eatherton RL, Siems WF, Hill HH. *Anal Chem.* 1985; 57:402–406. [PubMed: 3977072]
32. Clowers BH, Siems WF, Hill HH, Massick SM. *Anal Chem.* 2006; 78:44–51. [PubMed: 16383309]
33. Szumlas AW, Ray SJ, Hieftje GM. *Anal Chem.* 2006; 78:4474–4481. [PubMed: 16808456]
34. Tang K, Shvartsburg AA, Lee HN, Prior DC, Buschbach MA, Li FM, Tolmachev AV, Anderson GA, Smith RD. *Anal Chem.* 2005; 77:3330–3339. [PubMed: 15889926]
35. Koeniger SL, Merenbloom SI, Valentine SJ, Jarrold MF, Udseth HR, Smith RD, Clemmer DE. *Anal Chem.* 2006; 78:4161–4174. [PubMed: 16771547]
36. Valentine SJ, Koeniger SL, Clemmer DE. *Anal Chem.* 2003; 75:6202–6208. [PubMed: 14616002]
37. Koeniger SL, Valentine SJ, Myung S, Plasencia M, Lee YJ, Clemmer DE. *J Proteome Res.* 2005; 4:25–35. [PubMed: 15707354]
38. Merenbloom SI, Koeniger SL, Valentine SJ, Plasencia MD, Clemmer DE. *Anal Chem.* 2006; 78:2802–2809. [PubMed: 16615796]
39. Syka JEP, Coon JJ, Schroeder MJ, Shabanowitz J, Hunt DF. *Proc Natl Acad Sci USA.* 2004; 101:9528–9533. [PubMed: 15210983]
40. Beausoleil SA, Jedrychowski M, Schwartz D, Elias JE, Villen J, Li J, Cohn MA, Cantley LC, Gygi SP. *Proc Natl Acad Sci USA.* 2004; 101:12130–12135. [PubMed: 15302935]
41. Schroeder MJ, Shabanowitz J, Schwartz JC, Hunt DF, Coon JJ. *Anal Chem.* 2004; 76:3590–3598. [PubMed: 15228329]
42. Lee S, Li ZY, Valentine SJ, Zucker SM, Webber N, Reilly JP, Clemmer DE. *Int J Mass Spectrom.* 2012; 309:154–160. [PubMed: 22518092]
43. Hoadlund-Hyzer CS, Li JW, Clemmer DE. *Anal Chem.* 2000; 72:2737–2740. [PubMed: 10905301]
44. Stone E, Gillig KJ, Ruotolo B, Fuhrer K, Gonin M, Schultz A, Russell DH. *Anal Chem.* 2001; 73:2233–2238. [PubMed: 11393846]
45. Nurkiewicz T, Porter D, Hubbs A, Cumpston J, Chen B, Frazer D, Castranova V. *Part Fibre Toxicol.* 2008; 5:1. [PubMed: 18269765]
46. Knuckles TL, Yi JH, Frazer DG, Leonard HD, Chen BT, Castranova V, Nurkiewicz TR. *Nanotoxicology.* 2012; 6:724–735. [PubMed: 21830860]
47. Revercomb HE, Mason EA. *Anal Chem.* 1975; 47:970–983.
48. Shvartsburg AA, Jarrold MF. *Chem Phys Lett.* 1996; 261:86–91.
49. Mesleh MF, Hunter JM, Shvartsburg AA, Schatz GC, Jarrold MF. *J Phys Chem.* 1996; 100:16082–16086.
50. Wyttenbach T, vonHelden G, Batka JJ, Carlat D, Bowers MT. *J Am Soc Mass Spectrom.* 1997; 8:275–282.
51. Wittmer D, Luckenbill BK, Hill HH, Chen YH. *Anal Chem.* 1994; 66:2348–2355.
52. Clemmer DE, Hudgins RR, Jarrold MF. *J Am Chem Soc.* 1995; 117:10141–10142.
53. von Helden G, Wyttenbach T, Bowers MT. *Science.* 1995; 267:1483–1485. [PubMed: 17743549]
54. Hoaglund-Hyzer CS, Clemmer DE. *Anal Chem.* 2001; 73:177–184. [PubMed: 11199963]
55. Merenbloom SI, Glaskin RS, Henson ZB, Clemmer DE. *Anal Chem.* 2009; 81:1482–1487. [PubMed: 19143495]
56. Kurulugama RT, Nachtigall FM, Lee S, Valentine SJ, Clemmer DE. *J Am Soc Mass Spectrom.* 2009; 20:729–737. [PubMed: 19195909]
57. Blase RC, Silveira JA, Gillig KJ, Gamage CM, Russell DH. *Int J Mass Spectrom.* 2011; 301:166–173.
58. Stlouis RH, Hill HH. *Crit Rev Anal Chem.* 1990; 21:321–355.

59. Clemmer DE, Jarrold MF. *J Mass Spectrom.* 1997; 32:577–592.
60. Collins DC, Lee ML. *Anal Bioanal Chem.* 2002; 372:66–73. [PubMed: 11939214]
61. Bohrer BC, Mererbloom SI, Koeniger SL, Hilderbrand AE, Clemmer DE. *Annu Rev Anal Chem.* 2008:293–327.
62. Shaffer SA, Tang KQ, Anderson GA, Prior DC, Udseth HR, Smith RD. *Rapid Commun Mass Spectrom.* 1997; 11:1813–1817.
63. Kurulugama RT, Valentine SJ, Sowell RA, Clemmer DE. *Journal of Proteomics.* 2008; 71:318–331. [PubMed: 18590839]
64. Ruotolo BT, Verbeck GF, Thomson LM, Woods AS, Gillig KJ, Russell DH. *J Proteome Res.* 2002; 1:303–306. [PubMed: 12645885]
65. Ruotolo BT, Gillig KJ, Woods AS, Egan TF, Ugarov MV, Schultz JA, Russell DH. *Anal Chem.* 2004; 76:6727–6733. [PubMed: 15538797]
66. Palumbo AM, Tepe JJ, Reid GE. *J Proteome Res.* 2008; 7:771–779. [PubMed: 18181561]
67. Palumbo AM, Smith SA, Kalcic CL, Dantus M, Stemmer PM, Reid GE. *Mass Spectrom Rev.* 2011; 30:600–625. [PubMed: 21294150]
68. Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu YF, Djoumbou Y, Mandal R, Aziat F, Dong E, Bouatra S, Sinelnikov I, Arndt D, Xia JG, Liu P, Yallou F, Bjorndahl T, Perez-Pineiro R, Eisner R, Allen F, Neveu V, Greiner R, Scalbert A. *Nucleic Acids Res.* 2013; 41:D801–D807. [PubMed: 23161693]
69. Fang N, Yu S, Badger TM. *J Agric Food Chem.* 2003; 51:6676–6682. [PubMed: 14582959]
70. Barroso B, Bischoff R. *J Chromatogr, B.* 2005; 814:21–28.
71. Liu XY, Valentine SJ, Plasencia MD, Trimpin S, Naylor S, Clemmer DE. *J Am Soc Mass Spectrom.* 2007; 18:1249–1264. [PubMed: 17553692]

Figure 1.

Schematic representation of the IMS-MS instrument showing the funnels (F), gates (G), and ion activation regions (IA). The boxed portion of the schematic shows the LTQ Velos (ThermoScientific) mass spectrometer consisting of rf focusing devices, the linear ion trap mass analyzer, and the ETD source.

Figure 2.

(A) Two-dimensional (2D) t_D (m/z) heat-plot of a cytochrome *c* tryptic digest containing the phosphorylated peptide standard [KRPQRHGSKY-NH₂ (*m/z* 475.2)]. The color map for the plot is shown on a logarithmic scale ranging from -2 to 3 to show the low-intensity $[M+H]^+$ ions. (B) Expanded region of a 2D t_D (m/z) heat-plot for the same digest sample upon performing IMS-CID. The same color scale is used. The XIDTDs of the precursor and neutral loss product ions are shown as insets on the respective 2D plots. An asterisk denotes the location of the precursor ion both 2D plots. (C and D) Mobility-selected ETD spectra of the precursor and neutral loss ions, respectively. Assigned fragment ions are labeled. The z_8^* label denotes the fragment ion corresponding with the S4 residue for neutral loss of H₃PO₄ by IMS-CID.

Figure 3.

(A) 2D t_D (m/z) heat-plot of one replicate analysis of the EXP24 metabolite sample. The color map for the plot is shown on a logarithmic scale ranging from -2 to 3 in order to show low-intensity features. (B) Multistage tandem mass spectrometry (MS^n) of the precursor ion of m/z 518.3. The MS^3 spectrum (top) is obtained upon activation of the major product ion (m/z 459.2) generated during MS/MS (data not shown). The bottom spectrum shows MS^4 fragments produced by selecting the ion of m/z 313.2 from MS^3 experiments. The peak marked with an asterisk indicates the m/z selected for subsequent MS analysis.

Figure 4.

Single replicate XIDTDs of the ion of m/z 518.3 for the CNTRL24 (solid trace) and the EXP24 (dashed-line trace) samples. Each distribution is normalized such that the total area under the curve is unity. The arrow indicates an intensity difference in spectral features between the two samples that was recognized originally in the pixel-by-pixel intensity comparison of the 2D IMS-MS data sets (see text for details).

Figure 5.

(A) 2D t_D (m/z) heat-plot of the EXP24 sample obtained from an IMS-CID-MS experiment. The color map for the plot is shown on the same scale. (B) XIDTDs for the precursor (top) and fragment (bottom) ions of m/z 534.2 and 475.0, respectively. Dashed lines in panel A show the XIDTD regions. (C) Percent difference plots obtained upon comparison of the XIDTD for the fragment ion of m/z 475.0 and the XIDTDs of precursor ions of m/z 534.2 (solid trace) and 496.3 (dashed-line trace).