Poster Note 64473

Improved Detection of Proteins in Complex Sample Matrices by Infusion Using FAIMS

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Overview

Purpose: Evaluate FAIMS for the improved detection of proteins by infusion.

Methods: Protein standards and samples (from cell lysates or digested plasma) were infused at 3 uL/min using FAIMS on a Thermo Scientific™ Orbitrap Velos Pro™ mass spectrometer. Compensation voltages were stepped to promote separation of analytes from high-intensity singly charged contaminant and other sample-related background ions.

Results: Detection of protein ions by infusion was drastically improved using FAIMS. Singly charged contaminant ions were preferentially excluded during mass detection, resulting in significantly improved signal-to-noise of over 50 proteins in a cell lysate. Detection of insulin was also improved versus no FAIMS, resulting in simplified, chromatography-free sample introduction.

Introduction

High-Field Asymmetric Ion Mobility Spectrometry (FAIMS) has long been demonstrated to improve signal-to-noise for targeting analytes in mass spectrometry by preferentially passing only ions of interest and reducing interfering chemical noise. While this technique will not overcome suppression at the ionization step, it can improve ion statistics for targets of interest at detection. This becomes increasingly important for highly charged ions, where ion concentration is diluted by the number of charges present on each ion, especially relative to singly charged contaminants. In addition, FAIMS' strength in separating desired analytes from chemical noise improves limits of detection of peptides within a complex sample matrix (digested cell lysates or plasma). Here, we evaluate its use without chromatographic separation for transmission of intact proteins relative to low charge contaminants in both simple and complex sample matrices.

Methods

Sample Preparation

Mixtures of peptides (PRTC Retention Time Standards, Pierce) or protein standards (myoglobin, carbonic anhydrase, BSA, enolase, trypsin inhibitor, cytochrome C, RNAse A, and insulin, Sigma) were prepared in solvent (30% acetonitrile/0.2% formic acid or 60% acetonitrile/ 0.2% formic acid, respectively). Complex sample matrices were prepared by cell lysis (for protein detection) or acetonitrile crash of plasma (for insulin detection). Cells were lysed in 50% formic acid,25% acetonitrile, 25% water for 1 minute. Then diluted with 50% acetonitrile for storage. 50uL of a 1:5 dilution were cleaned up (SPE) on Poros R1 spin plate. Sample was eluted with 60% acetonitrile 0.2% formic acid. Rat plasma was crashed 1:3 with cold acetonitrile and used as a sample matrix for insulin.

Instrumentation

Samples were infused at 1-10 uL/min and analyzed on a linear ion trap or Orbitrap Velos Pro MS in full scan MS mode. Complex sample matrices (undigested cell lysate) were analyzed using FAIMS with infusion compared to no FAIMS. FAIMS hardware was modified to sustain an analytical gap of 1.5 mm with a dispersion voltage setting of -5000 V. Compensation Voltage (V) was stepped in 2 V increments between -48 V and -10 V to determine the CVs for maximizing protein transmission and minimizing singly charged ions. Data were acquired for 30 sec at each CV. Experiments were repeated at different inner/outer FAIMS electrode temperatures (50/90, 70/90, 90/90, and 100/100) to determine the effect of temperature on resolution and transmission. Samples were also infused with the FAIMS hardware mounted, but the electronics turned off as a comparison.

Data Analysis

Protein data were analyzed in Qual Browser (Thermo Scientific) and protein charge states were deconvoluted to identify the number of proteins detected.

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Results

Application of FAIMS to Protein Detection by Infusion

High-field asymmetric waveform ion mobility spectrometry has shown great promise in the preferential transmission of desired analytes and minimization of chemical noise in targeted analyses. Recent development of FAIMS electrodes to redesign the cylindrical electrodes to better direct gas flow and to decrease the analytical gap has shown to be successful in improving ion transmission and gas-phase separation without the need for helium gas (Figure 1). Detection of proteins from complex sample matrices using trapping-based instruments can often cause suppression of highly charged ions due to the presence of abundant, singly charged contaminants. Salts, detergents, and other polymers can quickly dominate ion accumulation in the trap and prevent detection of more highly charged protein ions (Figure 2). Chromatographic separation, while useful, can be time consuming and a source of variability.

FIGURE 1. Diagrams of the cylindrical Coanda FAIMS electrodes. The analytical gap between the inner and outer electrodes is 1.5 mm. (A) Gas flow dynamics of the new electrodes showing minimal gas departure from the inlet aperture. (B) Simion modeling of ion motion from the inlet (left), around the inner electrode, and exiting to the mass spectrometer ion transfer tube (right).

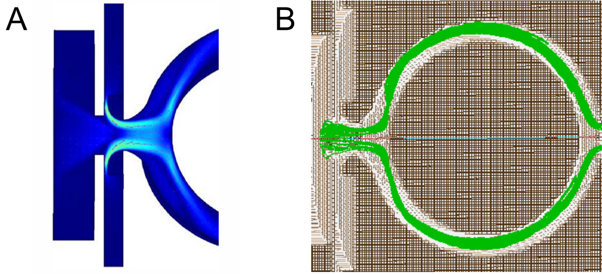


FIGURE 2. Mass Spectrum of 7 Protein Standards Infused Without FAIMS.

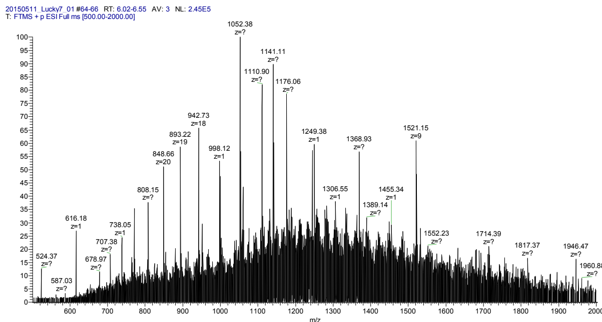
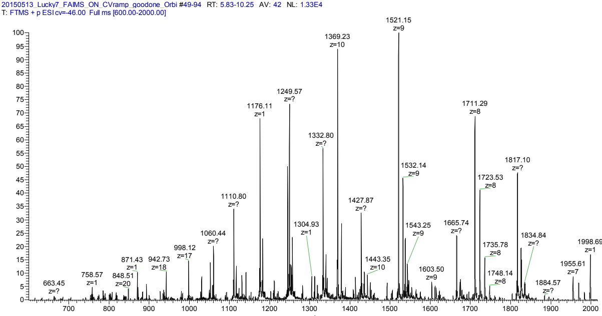


FIGURE 3. Mass Spectrum of 7 Protein Standards Infused With FAIMS.



Cellular extracts of E. coli grown on MacConkey Agar were infused in 60% acetonitrile/0.2% formic acid to generate high-quality MS specta for detection of proteins. MacConkey Agar contains several additives that facilitate cell growth, but cause intense, singly charged contaminant ions to dominate the mass spectrum (Figure 2). With FAIMS installed, the CV voltage was ramped from -50 to -10 V in 2 V increments and the full MS spectrum (m/z 600-2000) was monitored (Figure 3A). The singly charged contaminant ions were preferentially transmitted through FAIMS between CVs -18 and -16 (Figure 3B). With the CV range limited to -50- to -20V, the singly charge contaminants are not transmitted, allowing for better quality protein detection by infusion without the need for further sample purification.

Figure 4. Mass Spectrum of lysate from *E. coli* grown on MacConkey Agar (infusion). Masses shown are singly charged contaminant ions, causing suppression of ion statistics for detection of protein ions in the sample

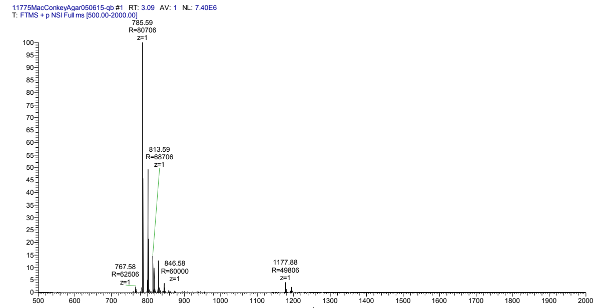
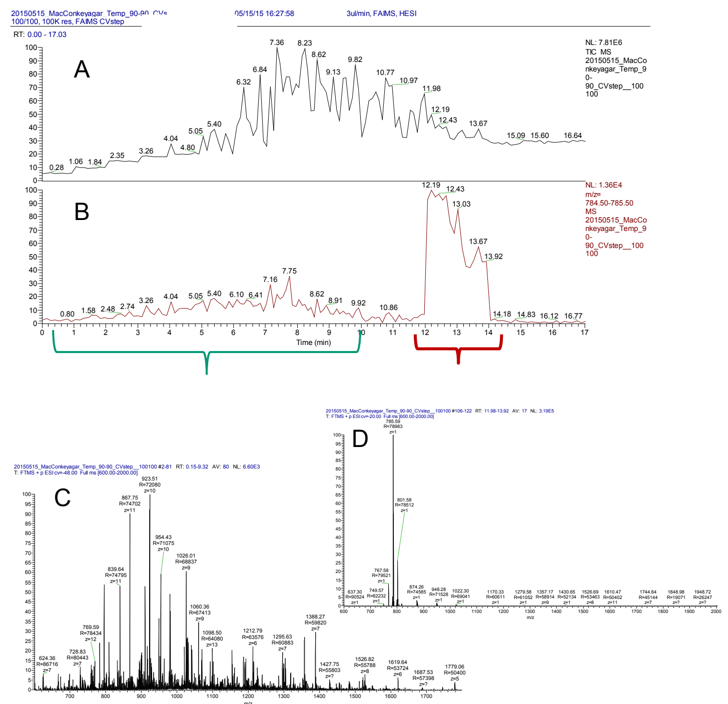


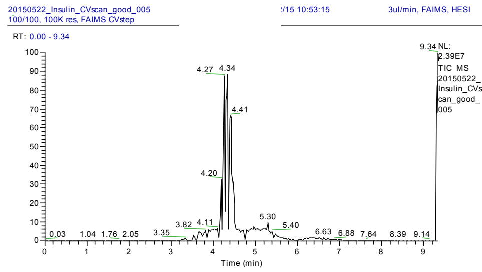
Figure 5. MacConkey Agar cell lysate with FAIMS CV stepping throughout infusion. (A) TIC showing total signal during infusion. (B) Extracted ion chromatogram showing the CV regions where *m/z* 785 contaminant transmits through FAIMS. (C) Averaged mass spectrum showing detection of protein ions over CV range -48 to -24 V. (D) Mass spectrum from CV range -18 to -16 V, showing optimal transmission of singly charged contaminant ions.



Application of FAIMS to Insulin Detection in Crashed Plasma by Infusion

Insulin is of interest for detecting in plasma samples to differentiate variants such as human insulin and Humalog, a synthetic version of insulin with an amino acid sequence swap near the C-terminus of the B-chain. Here, we optimized FAIMS for insulin (Figure 4) and then infused 3 samples of crashed plasma spiked with human insulin (Figure 5). No further sample preparation or chromatography was used. The 5+ charge state was detected in the 1 ug/mL plasma sample.

Figure 4. Compensation Voltage Ramp for Optimizing Insulin Transmission with FAIMS. CVs were ramped from -80 to -10 V.



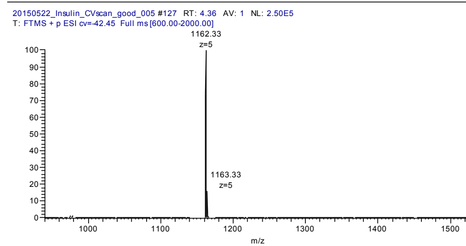
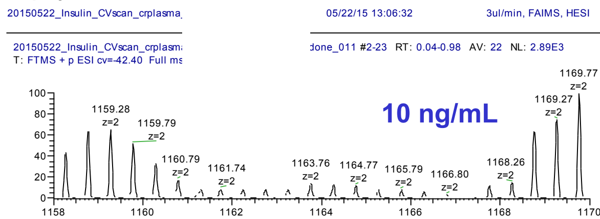
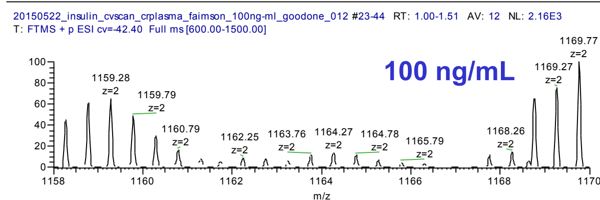
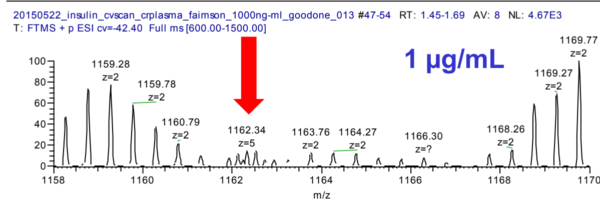


Figure 5. Insulin spiked into crashed plasma MS detection with FAIMS by infusion. The precursor m/z 1162.3 (5+) was visible at the 1 ug/mL spike level when FAIMS was employed.







Practical Application of FAIMS to Protein Detection

FAIMS is an orthogonal separation method to both liquid chromatography and mass spectrometry. Its strengths are that it can separate ions in the gas phase, based on their mobility between alternating electric fields, and preferentially allow desired ions to pass into the mass spectrometer while limiting transmission of "noise" ions contributed by the sample matrix. Here, we demonstrate the use of FAIMS for protein detection by infusion, without chromatographic separation.

While FAIMS will not overcome suppression due to ionization during sample introduction to the FAIMS source, it can enhance analyte detection when using an ion trap mass spectrometer. Ion traps are limited in the total number of ions they can collect and detect with appropriate m/z precision. Many trapping instruments utilize an upper limit ion target (based on current, and hence total charge) to prevent the incidence of space charging, which would occur when too many ions are trapped and cause significant mass shift in the analysis. FAIMS can limit the total number of ions in the trap to those targeted ions of interest, and preferentially exclude highly abundant contaminant ions that would quickly fill the trap before the lesser abundant ions of interest can be collected in enough quantity for accurate measurements. Normally, liquid chromatography is employed to separate the introduction of all ions in time, but can be time consuming, require significant method development, and is another analytical instrument to maintain and ensure is working properly.

Sample introduction by infusion suffers from ion suppression, as all ions are being generated simultaneously. However, as long as the target ion is being generated, FAIMS has the ability to separate it from background ions, and selectively introduce it to the MS for detection.

Conclusions

- FAIMS has shown promise for detection of proteins by infusion in complex sample matrices.
- Singly-charged contaminant ions are excluded from transmission to the MS by use of FAIMS over a range of compensation voltages without the need for chromatography.
- Use of FAIMS on trapping instruments improves ion statistics to allow detection of lesser-abundant ions when contaminants are excluded from transmission.
- Further improvements in signal to noise are possible with the use of liquid chromatography and are under investigation.

References

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2. Prasad, S.; Belford, M. W.; Dunyach, Jean-Jacques. Control of gas flow in high field asymmetric waveform ion mobility spectrometry. US Patent 8,664,593 B2, March, 4, 2014.

Acknowledgements

We would like to thank Dr. Nathan Yates for his collaboration and the use of his FAIMS source.

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