Evaluation of Novel FAIMS Technology for Intact Protein Detection and Characterization by Infusion

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ABSTRACT

Purpose: A novel FAIMS (high-Field Asymmetric waveform Ion Mobility Spectrometry) technology was evaluated to determine if it provided benefits for improved intact protein detection by infusion-MS.

Methods: A novel FAIMS device was coupled to a Thermo Scientific™ Orbitrap™ Fusion™ Lumos™ Tribrid™ mass spectrometer, and protein samples ranging from simple mixtures to complex cell lysates were evaluated by infusion at nanoflow rates.

Results: FAIMS improved intact protein detection by infusion-based MS techniques by separating multiply charged ions of interest from singly charged species in the sample, resulting in greater detection of proteoforms.

INTRODUCTION

Detection of intact proteins from complex mixtures using mass spectrometry is of high interest, owing to the ability to directly detect analytes from the sample. There remain many challenges to this endeavor, due to the dynamic range of proteins in complex samples and the splitting of analytical signal caused by the presence of both proteoforms and charge state distributions in which proteins exist. In addition, infusion-based experiments are particularly challenging if the sample contains singly charged species, which can often fill trapping-based instruments and reduce ability for detection of more highly charged protein ions. Here we evaluate a novel FAIMS device for the detection of intact proteins by infusion.

MATERIALS AND METHODS

Sample Preparation

- Pierce Intact Protein Standard (PN A33526) was purchased from Fisher Scientific and dissolved in 500 uL Optima LC/MS grade water (Fisher Scientific). The sample was diluted by combining 100 uL with 300 uL of 66.6% Acetonitrile/0.2% Formic Acid (Optima LC/MS grade, Fisher Scientific).
- Lucky 7 sample was an equimolar mixture of cytochrome C, RNase A, myoglobin, trypsin inhibitor, carbonic anhydrase, enolase, and BSA.
- *E.coli* was grown on TSA (Trytone Soya Agar) plate over night at 37 °C. Colonies were harvested and lysed in 6M GHCl with 250mM Tris HCl pH 7.5 using Matrix Lysis B beads (MP Biologicals) for 2 minutes on Bead Mill Homogenizer (Thermo Fisher Scientific). Tubes were centrifuged at 12,000 rpm for 5 minutes at 10 °C and lysate transferred to tubes. Lysate protein concentration was quantified using the Pierce Coomassie Plus (Bradford) Protein Assay.
  - Protein solid phase extraction (SPE) was performed using the Supra-Clean™ WC4 SPE columns from PerkinElmer (200 mg/3 mL). Columns were wetted with 0.1%FA in acetonitrile, then conditioned with 0.1%FA in water. Five hundred micrograms of lysate was loaded onto SPE columns in 6M Guanidine HCl and then rinsed with 0.1%FA in water. Proteins were eluted off SPE columns with 0.1%FA in 50% acetonitrile, and sampled directly.
  - A portion of the *E. coli* lysate was retained before SPE, with a concentration of 5 ug/uL, and diluted 1:4 with 25% Acetonitrile/1% formic acid, and sampled directly.

Mass Spectrometry

- MS data were generated on an Orbitrap Fusion Lumos Tribrid MS by direct infusion using either a syringe pump and a Thermo Scientific™ EASY-Spray™ ion source with an EASY-Spray emitter (PN ES792), or a TriVersa NanoMate™ source (Advion).
- A prototype FAIMS interface with cylindrical electrodes was mounted to the MS with an adapter flange, which accommodated either the EASY-Spray ion source or the TriVersa NanoMate source.
- MS parameters were set to vary MS1 scan resolution in the Orbitrap mass analyzer at different resolutions: 7.5, 30, 60, and 120 K. AGC target: 2E6. Microscans 5–10. Max IT: 100 msec.
- FAIMS parameters: Inner and outer electrode temperatures: 100 °C. Dispersion voltage (DV) = -5000 V. Compensation voltage (CV) was scanned for optimization and infusion-based methods used 10 V CV steps between -100 and 60 V or -90 and 60 V.

Data Analysis

- Thermo Scientific™ FreeStyle™ v1.4 software was used for data interrogation and protein deconvolution.
- An in-house developed UI was used to generate "gel plots" and determine proteoform count and frequency.

RESULTS

FAIMS Operation

FAIMS is a technology that separates ions based on their mobility in the gas phase at high and low electric fields.[1] The asymmetric waveform is applied to the inner, cylindrical electrode, and the outer electrode is grounded. The amplitude of the waveform is the DV (Figure 1). A separate DC voltage, the CV can be applied to the inner electrode to filter ions for transmission into the MS (Figure 2). When the CV is stepped through a series of voltages during sample infusion, different ions are transmitted into the MS over time, while others discharge on the electrode surfaces. In this way, ions can be filtered in the gas phase to improve detection by the mass spectrometer.

Figure 1. Cylindrical FAIMS Electrode Assembly illustrating the ion path between the electrodes, from the nESI emitter to the heated metal capillary of the MS.

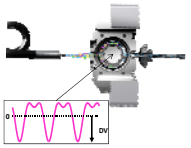
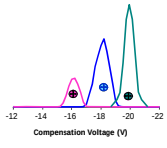


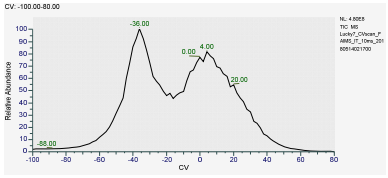
Figure 2. Illustration of a CV plot for different ions being transmitted through the FAIMS electrodes at different compensation voltages.



Evaluation of FAIMS for Simple Protein Mixtures

Two simple protein mixtures (7 proteins or less in a mixture of water, acetonitrile, and 0.2% formic acid) were infused into the Orbitrap Fusion Lumos Tribrid MS coupled to a FAIMS device and the CV of the FAIMS was stepped through a series of voltages to determine optimal transmission of protein ions over the CV range (Figure 3).

Figure 3. Lucky 7 Protein Mix Standard (L7). The CV was ramped in 2 V increments to determine the best CV ranges for multiply charged ion transmission. Here, the total ion current is plotted as a function of CV, indicating a CV range between -80 and +60 V that transmits multiply charged protein ions .



Protein Detection by Infusion is Improved with FAIMS

Method optimization for protein infusion

After reviewing the CV scan data for L7 (Figure 1), a range of CVs were chosen in 10 V increments between -100 and 60 V (Figure 4). The method was set up to begin MS acquisition with the FAIMS DV and CV = 0 V for 1 minute, then step through each CV at 1 minute intervals to acquire MS scans (5–10 microscans). This infusion method was repeated at four resolving powers: 7.5, 30, 60, 120 K. Injection time was set to 100 msec and ion target was set to 2E+6. The sample was infused using an EASY-Spray emitter with the EASY-Spray ion source and a syringe pump. Charge states were deconvoluted to generate intact protein molecular weight, and the data are summarized in Table 1.

Figure 4. L7 Analysis with a FAIMS Method. The L7 sample was infused at 0.5 uL/min and data were acquired at 120K in MS1. The first minute of the method, FAIMS voltages were set to 0, and for each subsequent minute, the CV was changed in 20 V increments and MS1 data were acquired for 1 minute. The protein gel plot demonstrates the deconvoluted intact protein masses that were calculated from the charge states detected. Each MS spectrum illustrates the different multiply charged protein species detected with FAIMS voltages off, and at each discrete CV during the 9 minute method. Overall, more proteins were detected with FAIMS enabled, and the CV steps allowed different ions to be detected, resulting in more proteins detected vs. FAIMS voltages off.

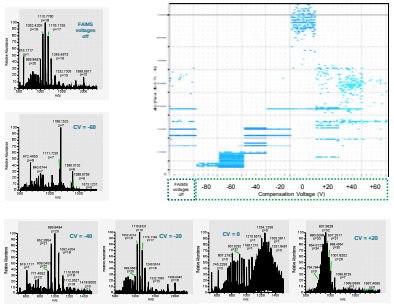


Table 1. Protein composition of L7 sample, molecular weight, and indication of detection with FAIMS voltages off, or with FAIMS voltages on and the corresponding CV at which it was observed at 120K.

Protein	MW (Da)	Observed	
		FAIMS off	FAIMS on (CV)
Cytochrome C	12350.3	√	√ (-40)
RNAseA	13673.3		√ (-40)
Myoglobin	16941.0	√	√ (-40)
Trypsin Inhibitor	19985.0	√	√ (-40)
Carbonic Anhydrase	29005.8	√	√ (+20)
Enolase	46670.7		√ (+40)*
BSA	66370.8		√(0)

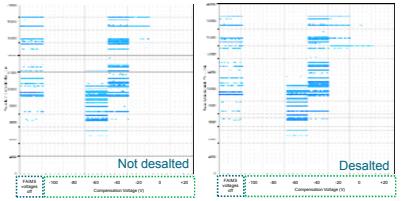
*only detected at 30K resolving power or lower

FAIMS Enables Detection of More Proteins in Cell Lysate

FAIMS evaluation for protein detection from *E. coli* cell lysates

Cell lysates are a complex mixture of proteins over a wide range of concentrations, which often makes MS detection of individual proteins difficult without some form of separation. Here we evaluated an *E. coli* lysate both before and after SPE cleanup to determine if FAIMS could not only improve protein detection versus not using FAIMS, but also detect similar proteoforms from the lysate sample before and after SPE (Figure 5).

Figure 5. Protein gel plots of the FAIMS analysis of *E. coli* lysates both before (left) and after (right) desalting. Each sample was infused with a TriVersa NanoMate at ~300 nL/min and began acquisition with FAIMS voltages off, then stepped through a CV range between -100 and +60 V in 20 V increments. Scans were acquired at each CV for 1 minute. The gel plots show the molecular weights of proteoforms detected after deconvolution of the MS spectra.



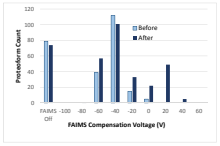


Figure 6. The Proteoform count detected during each minute of the method is plotted for the *E. coli* lysate "before" and "after" SPE cleanup. A larger variety of proteoforms are detected over the FAIMS CV range vs. the "FAIMS voltages off". While SPE cleanup did not seem to drastically affect the overall number of proteoforms in the "FAIMS off" scans, SPE cleanup did improve protein detection, particularly at the more positive CV values using FAIMS.

Figure 7. The "FAIMS Voltages off" portion of the infusions are shown for comparison between the *E. coli* lysate before (top) and after (bottom) SPE cleanup. While similar highly charged ions are present, there appear to be more 1+ ions in the "before" sample, which can make protein detection more difficult.

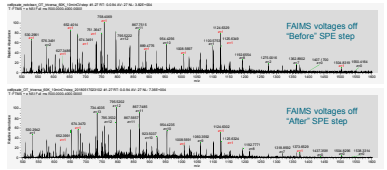
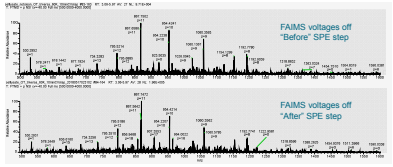


Figure 8. The mass spectra for the FAIMS CV range between -60 and -20 V were summed to observe the charge states detected for both the Before and After SPE *E. coli* lysate samples. Both samples have very similar spectra for this CV range, implying that SPE cleanup may not be required after cell lysis (using the conditions mentioned in the Methods section) to detect adequate numbers of intact protein while FAIMS is in use as a gas-phase filter.



This experiment (Figures 5–8) was designed to evaluate if there was a significant difference between "clean" and "not clean" cell lysate samples without FAIMS, and if FAIMS could show similar results between the two samples. Here we demonstrated that the cell lysate that was not desalted had a higher incidence of 1+ ions detected in the MS without FAIMS, and when FAIMS was in use, similar proteoforms were detected, suggesting FAIMS can be used to minimize sample prep steps. However, the desalted sample was found to have more proteoforms detected with FAIMS than the non-desalted sample, indicating that the SPE-cleanup step is still good practice for cell lysate analysis by MS.

CONCLUSIONS

- FAIMS improves intact protein detection in cell lysates when compared to no FAIMS for infusion methods.
- A variety of proteins are transmitted at different CVs, but there does not appear to be a relationship between CV and protein molecular weight.
- While SPE cleanup of this *E. coli* cell lysate is still considered best practice, FAIMS can be used to analyze the sample prior to SPE, and detects a similar number of proteoforms as in the SPE cleaned-up sample.

REFERENCES

1. Purves, R. W.; Guevremont, R. *Anal. Chem.* **1999,** *71*, 2346–2357.

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TRADEMARKS/LICENSING

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