

Optimization of parameters for Protein and Peptide Analysis Using High Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS)

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Overview

Purpose: Optimization of FAIMS parameters for proteins and peptides

Methods: Direct infusion and LC-MS/MS separation using the FAIMS interface

Results: Charge state separation on proteins and peptides at different compensation voltages (CV)

Introduction

FAIMS has emerged as an important tool for the study of chemical and biological targets in the presence of polymers and other interfering background ions. One of the main motivations for these studies is optimizing FAIMS parameters for protein and peptide analysis in the presence of polymers and other background ions. Our previous work has highlighted the role of FAIMS for the separation of singly charged polymers which can cause ion suppression in traditional LC-MS/MS analysis. In this work, we compare LC-MS/MS runs using a single compensation voltage versus multiple compensation voltages followed by several data dependent MS/MS scans for protein and peptide identification.

Methods

Sample Preparation: Standard enzymatic peptide solutions; Phosphorylase B (Sigma-Aldrich, MO), Bovine 6-protein mix (Michrom Bioresources, Auburn, CA) were prepared at a concentration of 3 pmol/μL. Standard protein solution, Ubiquitin (Sigma-Aldrich, MO) was prepared at a concentration of 10 pmol/μL. RnaseA and RnaseB (Sigma-Aldrich) proteins were prepared at concentrations of 5 pmol/μL and 1 pmol/μL respectively.

LC-MS/MS:

Mass Spectrometer: LTQ Orbitrap™ Discovery

HPLC: Accela™ pump with Surveyor™ Autosampler

Column: 50x2.1, 1.9μm Hypersil Gold™ column (Thermo Fisher Scientific)

FAIMS and MS conditions:

DV: +5000V

Outer Bias Voltage: -35V

Inner Electrode: 70°C; Outer Electrode: 90°C

FAIMS gas: 100% N₂ at 3.0 L/min

Positive HESI

Vaporizer Temperature: 300°C

Data Processing: Data was processed using Proteome Discoverer software.

FIGURE 1. Schematic showing the asymmetric waveform and the separation of ions in FAIMS. FAIMS separates ions based on differences in mobility at very high vs. low electric fields.

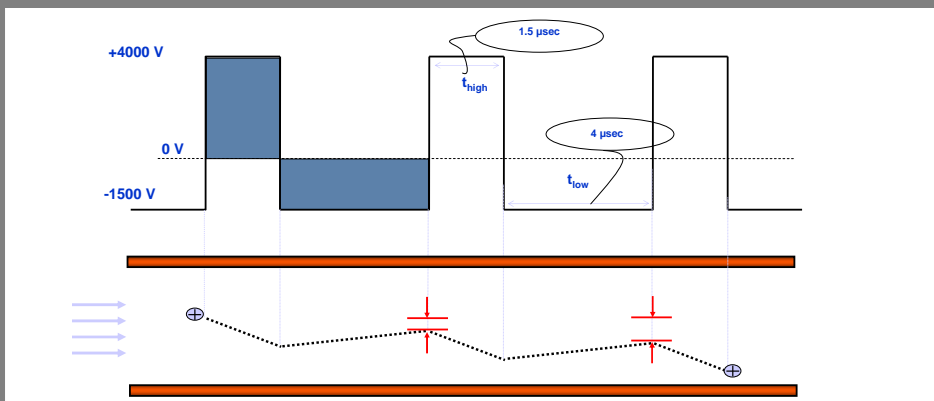
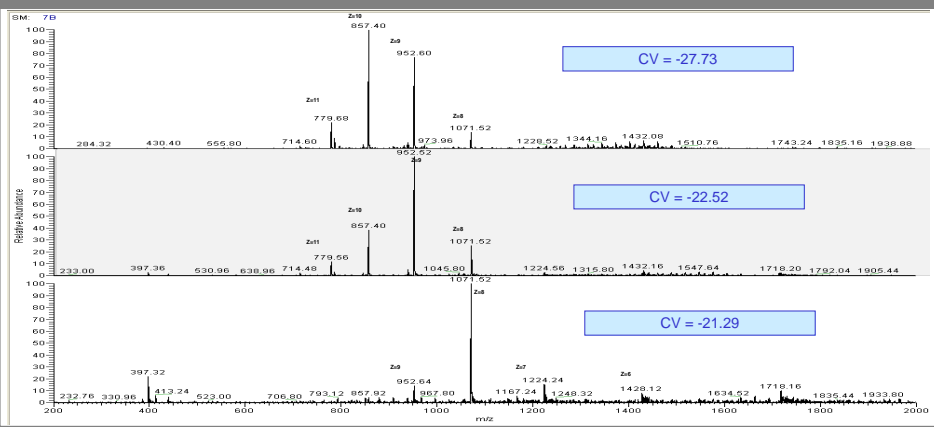


FIGURE 2. Separation of the different charge states of intact Ubiquitin at different compensation voltages.



Results: Protein Analysis

Figure 2 shows the separation of individual charge states of the intact protein, Ubiquitin, with high signal to noise at different compensation voltages. At a particular compensation voltage, a single charge state and associated post-translational modifications are rendered the highest signal in the spectrum with no prior separation. This can enable further fragmentation or other interrogation of the 'purified' protein without interference from external sources.

FIGURE 3. (a) Mixture of intact proteins Rnase A and Rnase B with microspray.

(b) Rnase A; predominantly at CV = -17.73V

(c) Rnase B; predominantly at CV = -21.99V

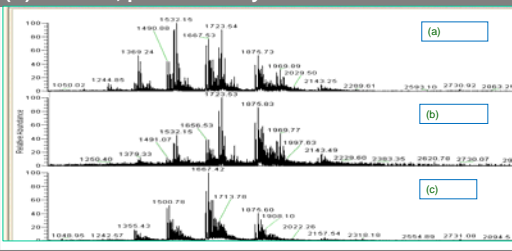


FIGURE 4. (a) Xtract deconvolution, Figure 3(b); Rnase A

(b) Xtract deconvolution, Figure 3(c); Rnase B

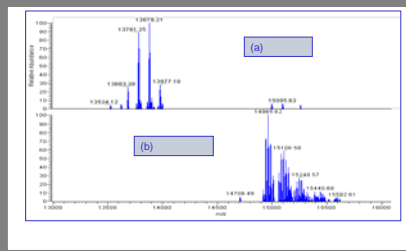


Figure 3 (a) shows a mixture of Rnase A and its glycosylated form, Rnase B without FAIMS. The two different forms [Figures 3(b) and 3(c)] share amino acid sequences in common; as a result, they do not show complete separation. The main benefit for separating out individual proteins can enable a better understanding of the modifications present on the protein with high signal to noise ratio.

Figure 4 (a) and (b) shows MW of the two proteins and possible modifications from their individual spectra

PEPTIDE ANALYSIS

Figure 5 shows a direct infusion experiment on a mixture of phosphopeptides at a flow rate of 3 μl/min. By switching to the optimum CV for the phosphopeptide we are able to render it the most intense ion in the mass spectrum. We obtained full MS spectrum for the mono-phosphopeptide at m/z 1031 at CV (-10.52) and the tetraphosphopeptide at m/z 1375.56 at a different CV (-12.69) without chromatographic separation.

FIGURE 5. Synthetic Monophosphopeptide (FQpSEEQQTEDELQDK) and Tetraphosphopeptide(RELEELNVPGEIVePslpSpSpSEESITRINKKIEKF) separated from mixture at different compensation voltages.

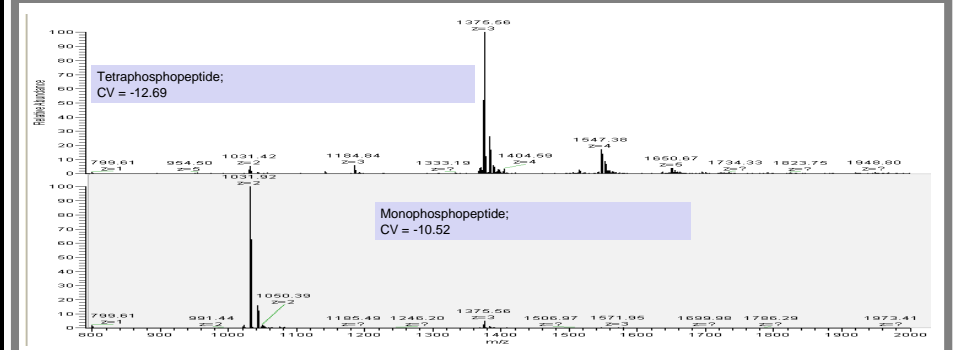


FIGURE 6. Comparison of 1 CV versus 2CV versus 3CV for enzymatic digest of Phosphorylase B.



Figure 6 is a comparison of a Data Dependent™ LC-MS/MS run on a single protein digest, Phosphorylase B. Results using a single compensation voltage gave better protein coverage versus using 2 or 3 different compensation voltages. This could be a result of efficient cycle time for ionization of the predominantly +2 charged peptides from the enzymatic digest.

For Figure 7, the effect of high concentrations of buffers that causes ion suppression in an ionspray experiment is shown. FAIMS effectively diverts all the +1 charged buffers/impurities away from the ion source; protein coverage in this case is similar to the FAIMS experiment on this enzymatic digest with no additional buffers added.

FIGURE 7. Base peak chromatogram of enzymatic digest of Phosphorylase B for (a) No additional buffer added; 2 CV FAIMS proteomics experiment, (b) 10mM PBS, 2%TFA, 10mM TCEP, 100mM Urea buffer added to enzymatic digest for FAIMS experiment, (c) 10mM PBS, 2%TFA, 10mM TCEP, 100mM Urea buffer added to enzymatic digest for Microspray experiment

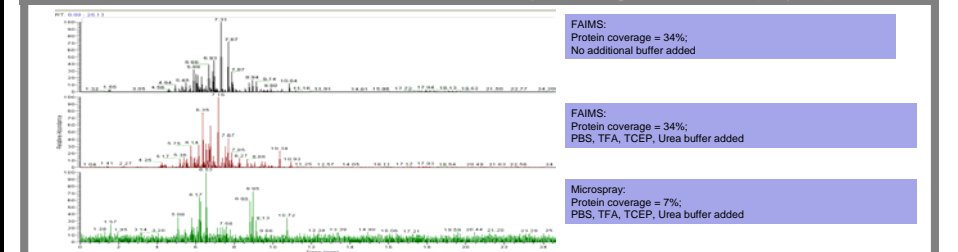
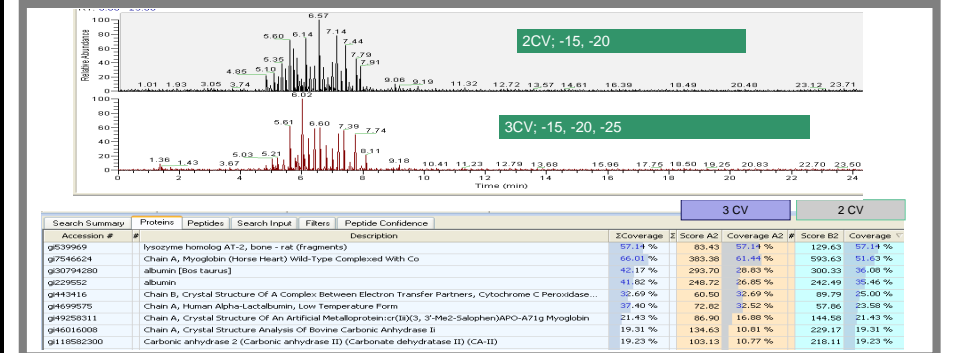


FIGURE 8. Comparing protein sequence coverage for Bovine 6-protein mix at 2 compensation voltages versus 3 compensation voltages.



Enzymatic digest from Bovine 6-Protein mix is used to compare cycle times for FAIMS with two versus three compensations voltages for Figure 8. Using 3 different compensations voltages gives more sequence coverage across multiple proteins compared with 2 different CVs. In addition, if enzymes other than trypsin are used, using multiple CVs will render multiple charge states the highest intensity for further data dependent fragmentation experiments.

Conclusions

- Separation of protein and peptide charge state can be successfully performed using FAIMS with high signal to noise ratios
- Data Dependent LC-MS/MS can be successfully performed on samples in high buffer concentrations by FAIMS without prior clean up which are not amenable with ionspray experiments
- For an individual proteolytic digest, depending on the number of charge states on the peptide, one CV for FAIMS experiment is optimal
- For multiple proteins digest; 2 or even 3 CVs work better for added sequence coverage.

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