Mitigating fragmentation of peptides with controlled clustering

J.C. Yves LeBlanc[1]; Brendon Seale[1,2]; Bradley B. Schneider[1]; Aaron Wheeler[2]

[1]SCIEX, 71 Four Valley Drive, Concord, ON, L4K 4V8 Canada; [2]University of Toronto, Department of Chemistry, 80 St. George St. Toronto, ON, M5S 3H6, Canada

ABSTRACT

In protein analysis, the stability of peptides is essential for proper identification and sequencing. But the introduction of peptide ions subjects them to highly energetic conditions which can result in pre-collision cell fragmentation. In this poster, we utilize a SelexION® differential mobility separation technology device equipped mass spectrometer to introduce acetonitrile (ACN) to the curtain gas. The ACN shelters the incoming ions and a) prevents fragmentation, b) increases signal intensity, c) decreases chemical noise. This facile technique is well suited to samples containing fragile molecules as it does not change the resulting MS results.

INTRODUCTION

Peptide detection by MS is integral in the analysis of protein biopharmaceuticals. But certain peptides are prone to fragmentation before they reach the mass spectrometer. In-source peptide fragmentation could account for up to 60% of the peaks in a protein digest mass spectrum.[1] These fragments can confound the analysis by reducing the intensity of the peptide signal, complicating the MS1 spectrum by the addition of extraneous peaks and causing additional triggers of information dependent acquisition techniques which do not lead to appropriate data. Furthermore, background noise in the system can have the same results. This means a technique which could limit the background ion signal as well as reduce the fragmentation of desired peptides is warranted.

It has been previously shown that the introduction of polar organic compounds to the curtain gas of the mass spectrometer can limit the fragmentation of proteins.[2] The addition of ACN or methanol (MeOH) kept fragile protein complexes together while undergoing the transition to the mass spectrometer. It is hypothesized that the polar compounds in the curtain gas associate with the proteins and cluster around them. These clusters protect the protein from the energetic conditions experienced when enter the mass spectrometer from atmospheric pressure ionization sources.

In this poster, we extend the application of polar organic compounds to present fragmentation to the field of peptides. We introduce organic modifiers to the curtain gas via the SelexION® technology on a SCIEX TripleTOF® 5600+ LC/MS system. We show that for certain charge states of fragile peptides the introduction of ACN to the curtain gas a) dramatically reduces the fragmentation of the peptide, b) increases the signal intensity of that charge state, and c) limits the chemical noise of the spectra. We explore this through direction injection analysis as well as fragile peptides spiked into bovine serum albumin (BSA) digests.

MATERIALS AND METHODS

Samples

Standard preparations of peptides were made in 50% MeOH/50% Water. KGAILKGAILR (KGAIL) was prepared at 1 pmol/µL for infusion analysis at a flow rate of 10 µL/min. Angiotensin I and III were added at various concentrations to 1 pmol/µL trypsin digest of BSA in water with 0.1% formic acid.

Mass Spectrometry

Modifiers were introduced to the curtain gas by SelexION® technology at 1.5% (low) of the total gas flow. Parameters were set below except: 'DMS on' experiments had fixed separation voltages (SV) of 3500 V and ramping compensation voltages (with intervals of 0.2 V) while declustering potential (DP) experiments ramped the DP from 0 to 225 V.

LC-MS

LC-MS was performed using a Shimadzu Prominence LC system with a Phenomenex Aeris 2.6 µm PEPTIDE XB-C18, 100 x 2.1 mm column using a gradient elution of eluent A (Water/ACN/FA 97.9/2/0.1%) and eluent B (ACN/Water/FA 97.9/2/0.1%) over 20 minutes with 10 minutes of equilibration post run at a conserved flow rate of 0.5 mL/min. 10 µL of sample was injected on column and the MS was operated in IDA mode.

Infusion MS conditions	
GS1	30
GS2	15
CUR	20
ISVF	5500
ITC	40
DP	100
CE	10

LC-MS conditions	
GS1	50
GS2	50
CUR	30
ISVF	5000
ITC	100
DP	100

Tables (left): Additional instrument parameters for each kind of experiment performed.

RESULTS

Ion clustering

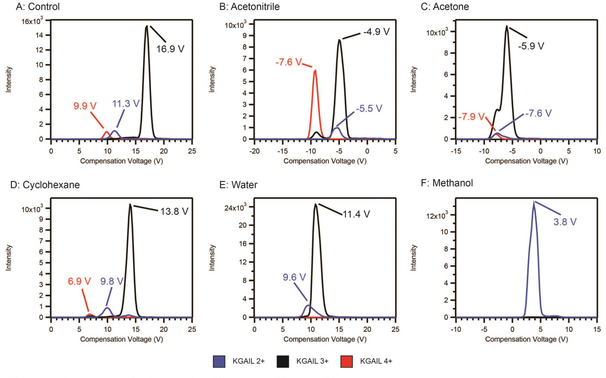

Figure 1: CoV ramps of KGAIL under different modifier conditions.

A. Control shows separation of each ion.

B. Acetonitrile produces a strong -ve CoV shift, large increase in 4+ ion intensity.

C. Acetone has a strong -ve CoV shift, no intensity improvements.

D. Cyclohexane has limited clustering as seen in a minimal CoV Shift.

E. Water demonstrates 'charge stripping' , 4+ ion is eliminated from the ionogram.

F. Methanol exhibits extreme charge stripping, only 2+ ion remains.

Fragmentation reduction

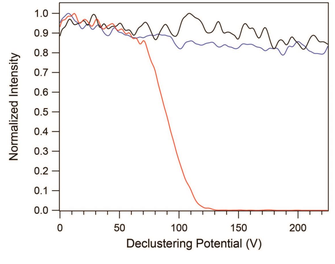

Figure 2: Normalized XIC of a declustering potential ramp of 4+ KGAIL.By ramping DP, fragmentation of the 4+ ion is induced resulting in loss of intensity with increasing DP.

- No modifier (red): ion intensity drops rapidly, approximately 30% at default operating DP of 100 V.
- With modifiers (blue, black): intensity drop is far more gradual.

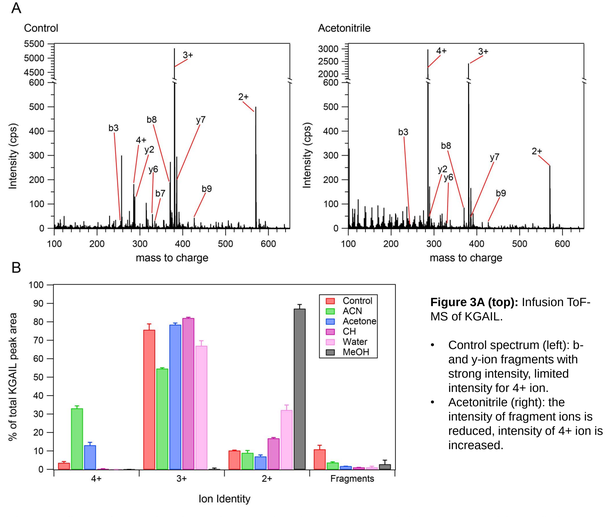

Figure 3A (top): Infusion ToF-MS of KGAIL.

- Control spectrum (left): b- and y-ion fragments with strong intensity, limited intensity for 4+ ion.
- Acetonitrile (right): the intensity of fragment ions is reduced, intensity of 4+ ion is increased.

Figure 3B (bottom): % total peak area for KGAIL ions (4+, 3+, 2+, sum of all fragment areas) for studied modifiers.

- Acetonitrile and acetone are the most effective modifiers for preserving 4+ ion intensity and limiting fragment ions.
- Methanol is effective at limiting fragment ions but limits all KGAIL ions to the 2+ charge state.

High charge state detection of fragile peptides in protein digest

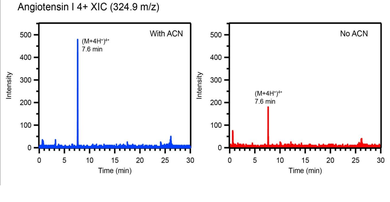

Figure 4. Representative chromatogram of human angiotensin I (6.7 ng/mL) in digest sample.

- ACN (blue): peak area improvement of more than 2x over control (red).

Ion	Conc. (ng/mL)	Increase w/ ACN
KGAIL 4+	13.6	5.6 x
Angl 4+	6.7	2.2 x
AngIII 3+	16.7	4.3 x
Bradykinin 3+	6.7	1.7 x

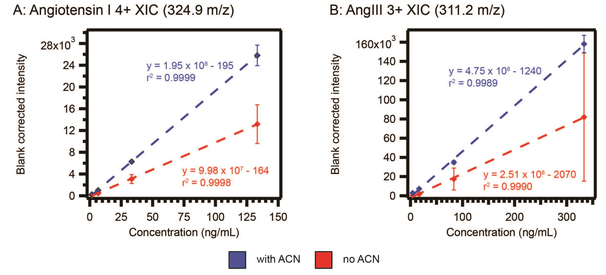

Figure 5: Calibration curves of fragile peptides in BSA digest.
The signal improvement and noise reduction from the addition of ACN increases the sensitivity of a standard IDA method for the highest charge state of fragile peptides.

- A. Human angiotensin I: limit of detection 5.5 ng/mL without modifier vs. 3.5 ng/mL with ACN.
- B. Human angiotensin III: limit of detection 6.5 ng/mL without modifier vs. 1.1 ng/mL with ACN.

CONCLUSIONS

The addition of organic modifiers to the curtain gas is easy to accomplish using SelexION® technology and an effective method of sheltering fragile ions. All organic modifiers were shown to reduce fragmentation across a wide range of declustering potentials due to their clustering ability, demonstrated by differential mobility spectrometry. Acetonitrile had a strong charge preserving effect, increasing the intensity of high charge state ions while methanol had a strong charge stripping effect reducing all charges down to the lowest state observed.
The ion sheltering of ACN was exploited to improve the detection and quantitation of highly charge fragile peptides in standard protein digests, reducing the limits of detection by 1.5-5x. We believe this will have a great impact on the detection of peptides in complex samples by eliminating confounding data and preserving fragile targets from harsh detection conditions.

REFERENCES

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(2) DeMuth, J. C.; Bu, J.; McLuckey, S. A. *Rapid Commun. Mass Spectrom.* **2015**, *29* (10), 973–981.

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