The use of chip-based FAIMS-MS for the analysis of intact proteins

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Overview: A prototype chip-based FAIMS device has been evaluated for the analysis of intact protein ions in combination with time-of-flight (ToF) mass spectrometry. This has permitted the study of protein ion behaviour with electric fields and frequencies higher than those previously reported and extends chip-based FAIMS utility to the analysis of biological macromolecules.

1. Introduction

High field asymmetric waveform ion mobility spectrometry (FAIMS) separates ions on the basis of differential mobility under alternating low field and high field conditions. Complementary to *m/z* separation, FAIMS has been shown to improve mass spectral analysis of proteins by improving signal to noise ratios, separating different charge states and separating isobaric species such as multi-conformer detection.

A chip-based FAIMS device[1] (Owlstone Ltd.) has been interfaced to an Agilent 6230 series ToF MS with a Jet Stream ESI source (Figure 1). Previously the hyphenated system has been shown to enhance the mass spectral detection of peptides, pharmaceuticals and metabolites[2-4].

The chip-based FAIMS consists of multiple parallel planar electrode channels, 35 µm wide, allowing higher electric field intensities (60 kV cm^{-1}) to be applied. Interleaved electrode pairs enable multiple, simultaneous ion transmission through the electrodes, reducing charge capacity constraints and improving transmission.

Figure 1 Prototype chip-based FAIMS device installed on Agilent TOF, with close-ups of the FAIMS chip

2. Methods

Insulin B chain, insulin, ubiquitin, cytochrome c and myoglobin (molecular weight 3.5 – 18 kDa) were prepared at 10 pmol $µL^{-1}$ in 50/50 (*v/v*) methanol/water containing 0.1 % formic acid.

Table 1: Experimental conditions

Nebulizer voltage	1.5 kV
Spray shield , nozzle and counter electrode voltage	400V
Sheath gas temperature and flow	250°C, 12 L/min
Nebulizer pressure	25psi
Drying gas temperature	150°C, 10 L/min
Capillary voltage	-15V
Fragmentor voltage	175-225 V
Acquisition rate	2 spectra/sec
Solvent flow rate	0.05 ml/min
CV sweep range	-1 to +5 V
CV sweep rate	0.5V/s
DF settings	59kV/cm

References

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3. Brown, L.J., Smith, R.W., Reynolds, J.C., Toutoungi, D., Bristow, A.T., Ray, A., Sage, A., Wilson, I.D., Weston, D.J., Boyle, P., Creaser, C.S., *Anal Chem*,, 2012, **84**, 4095
4. Smith R.W., Toutoungi D.E., Reynolds J.C., Bristow A.W.T., Ray A., Sage A., Wilson I.D., Weston D.J., Boyle B., Creaser C.S., *J. Chrom. A*., 2013, **1278**, 76

3. Results

The electrospray ionisation generated multiply charged ions of each protein. This allowed the differential mobility behaviours of ions of different mass and charge to be studied.

Multiple charge states of each protein were separated by differences in differential mobility. In general, higher charge states were transmitted at a higher compensation voltage (CV). Higher charge states of ubiquitin were found to be transmitted at similar CVs, consistent with the theory that the chip-based FAIMS device was separating ions on the basis of 3D structure; higher charge states are likely to be unfolded and therefore more similarities in structure than lower charge states (Figure 2). Multiple peaks observed at the same *m/z* suggested the presence of different protein conformers (Figure 3). A summary of all proteins studied is displayed in Figure 4.

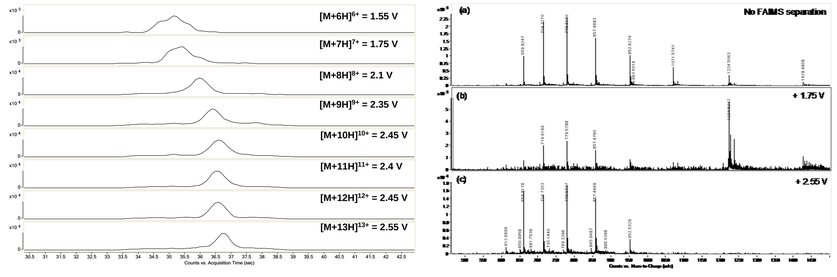

Figure 2 Extracted ion chromatograms (EIC) showing FAIMS-MS separation of multiple charge states of bovine ubiquitin and ubiquitin mass spectra (a) No FAIMS (b) + 1.75 V CV showing preferential transmission of the +7 charge state (*m/z* 1224) and (c) +2.55 V CV showing simultaneous transmission of +9 to +13 charge states at the expense of lower charge state species.

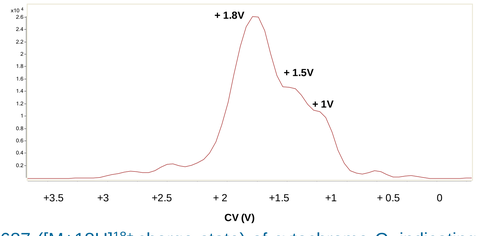

Figure 3 EIC for *m/z* 687 ($[M+18H]^{18+}$ charge state) of cytochrome C, indicating the presence of at least 3 isobaric species of differing differential mobility.

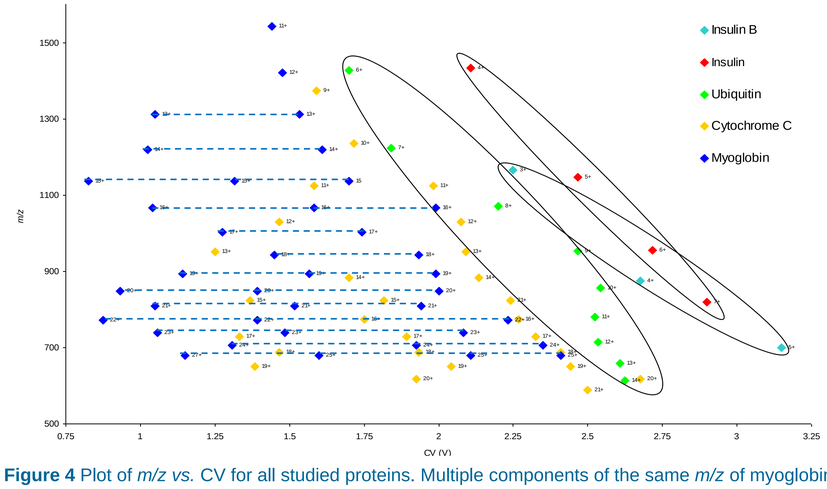

Figure 4 Plot of *m/z vs.* CV for all studied proteins. Multiple components of the same *m/z* of myoglobin are observed demonstrating the broad CV distribution of suspected conformations.

A reproducibility study was performed to determine the CV stability for different charge states of different proteins. Intra and inter day repeats gave good % relative standard deviations (%RSD) (n = 6) of 0.9-2.9% (Table 2).

Table 2: Intra and inter day reproducibility

		Intra Day		Inter Day	
Protein	Charge State	CV	% RSD	CV	% RSD
Insulin B Chain	3+	2.25	0.9	2.25	1.41
	4+	2.65	1.19	2.7	1.02
	5+	3.25	2.53	3.15	1.74
Insulin	4+	2.1	0.97	2.1	2.33
	5+	2.45	1.65	2.45	1.05
	6+	2.75	1.63	2.7	1.5
	7+	2.95	1.66	2.9	1.54
Ubiquitin	6+	1.65	1.91	1.7	2.63
	7+	1.8	2.08	1.85	2.04
	8+	2.15	2.55	2.2	2.04
	9+	2.4	2.04	2.45	2.09
	10+	2.5	1.7	2.55	1.93
	11+	2.45	1.29	2.5	2.43
	12+	2.5	1.51	2.5	2.04
	13+	2.55	1.47	2.6	1.88
	14+	2.55	2.01	2.65	2.89

A study of the transmission behaviour of protein ions in the high field was also performed. Ions of higher *m/z* ratio suffered fewer ion losses, and, in the case of the highest molecular weight proteins, transmission appeared enhanced compared to no FAIMS conditions (Figure 5).

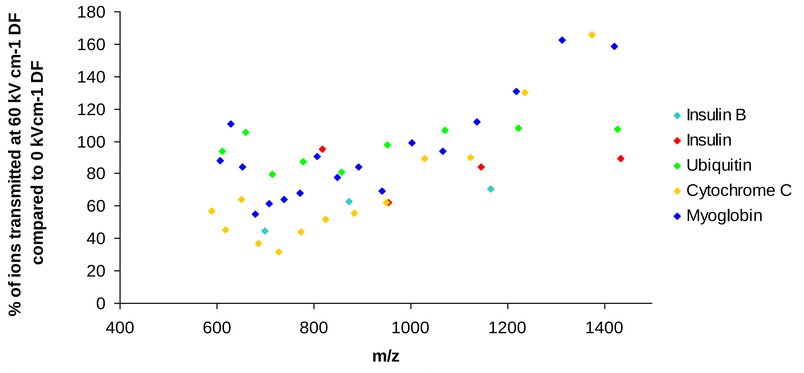

Figure 5 A plot of *m/z vs*. protein transmission. Enhanced transmission is observed for higher molecular weight proteins of lower charge state.

4. Conclusions

- Multiple charge states of intact proteins were separated by differences in differential mobility with protein ions of higher charge state requiring higher CVs for transmission.
- Higher charge states were more likely to be transmitted at similar CVs supporting the hypothesis that the chip-based FAIMS device was separating ions on the basis of 3D structure; higher charge states are likely to be unfolded and therefore more similarities in structure than lower charge states.
- Several species could be observed at the same *m/z* suggesting the presence of different protein conformers. This added weight to the argument that the chip-based FAIMS device separated ions based on differences in 3D structure.
- Ions of higher *m/z* ratio suffered fewer ion losses, and showed enhanced transmission in the case of the highest molecular weight proteins, when analysed by the FAIMS-MS technique when compared to MS analysis. The hyphenated technique could therefore offer a further advantage over analysis by MS alone.

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