TOP DOWN SEQUENCE ANALYSIS OF INTACT PROTEIN SPECIES USING ION MOBILITY COUPLED WITH TIME OF FLIGHT MASS SPECTROMETRY

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INTRODUCTION

In bottom-up proteomics, intact proteins are subjected to a proteolytic enzyme, for example trypsin, to produce a mixture of peptides. These species are then usually separated by nanoscale reversed phase chromatography and introduced into a mass spectrometer where fragment ion information is generated using collision induced dissociation. Each peptide intact mass and associated fragment ion spectrum is then compared to the predicted fragment ions of tryptic peptides from proteins in a sequence database. Protein identification is accomplished by locating the protein in the database that best matches the observed tryptic peptides and / or their fragment ions. A potential drawback with bottom up proteomics is that information about the intact mass of the protein is lost during the digestion procedure and hence the determination of some Post Translational Modifications (PTMs) may be difficult if the peptide on which a PTM resides does not ionise well.

Top down proteomics is the identification and characterization of intact proteins from tandem mass spectrometry experiments and can enable the identification of PTMs that may not be identified in the bottom up approach. This is because the intact protein mass can be measured directly prior to any fragment ion information being generated. However, even with a small to medium sized protein the range of charge states produced during the fragmentation of a single charge state leads to a spectrum that can be extremely complex and challenging to interpret.

In this presentation, we have used ion mobility separation prior to Time of Flight mass analysis to enable the separation of different charged state fragments and by post processing of the data, produced a simplified top down fragment spectra containing ions of mainly one charge state. These ion mobility separations and region selections also reveal low abundance species that are masked by larger species in a spectrum where no mobility separation is used.

METHODS

Instrumentation

The Mass Spectrometer used in these studies was a Synapt HDMS (Waters Corporation), **figure 1**. Briefly, ions produced by an ESI probe are sampled by a Z-spray source. They pass through a quadrupole which may be set to transmit a substantial mass range or to select a particular m/z. The Tri-Wave comprises three T-Wave devices [1]. The first device (trap T-Wave) accumulates ions and releases them in a short pulse (100µs) every 20 ms into the next device (IMS T-Wave) in which the mobility separation is performed, the final device (transfer T-Wave) is used to transport the separated ions into the oa-ToF for subsequent analysis. Ions may be fragmented on entrance to the accumulation T-Wave and/or in the transfer T-Wave. The pressure in the accumulation and transfer T-Wave regions was ~ 10^{-2} mbar of Argon and the pressure in the IMS T-Wave was 0.5 mbar of N_2. The T-Wave pulse velocity and voltage were optimised to provide ion mobility separation.

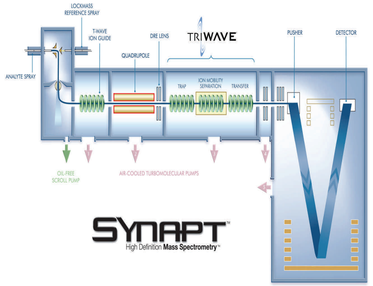

Figure 1 Schematic diagram of the Waters Synapt HDMS instrument.

Sample

Bovine Beta Lactoglobulin contains two disulphide bonds and was analysed in both the non-reduced and reduced (DTT treated) form. The samples were prepared in a solvent composition of 50/50 acetonitrile / water + 0.1% formic acid and the solution was introduced to the mass spectrometer at a flow rate of 400nL/min.

Experiment

Intact protein ToF and Mobility mass spectra were acquired in order to determine the mass of the protein and the m/z of the different charge states. The quadrupole was then set to transmit a single charge state and the trap collision energy raised to produce a fragment ion spectrum, **Figure 2**. Different charge states were subsequently isolated and further fragment ion spectra generated.
From m/z vs drift time plots, regions were selected and exported to produce new datafiles of simplified spectra of mainly one charge state. For z>1, these spectra were deconvoluted using Maximum Entropy algorithms. Fragment ion stretches were used to identify the amino acid sequence of the protein.

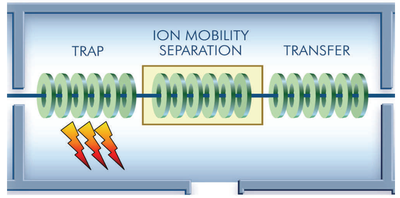

Figure 2 Synapt Trap T-Wave Fragmentation. Fragment ions produced before the Ion Mobility device are separated based on charge state, size and shape. They are subsequently passed to the oa-ToF analyser for mass analysis. Typical collision energies used in this study were 35-55V.

RESULTS

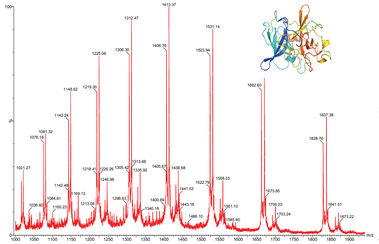

Figure 3 ToF mass spectrum of Bovine Beta Lactoglobulin (disulphides intact). The mass spectrum shows both the A and B variants with G→D (pos. 64) and A→V (pos. 118) amino acid changes.

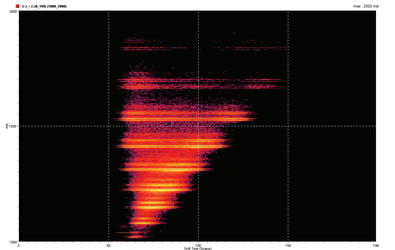

Figure 4 m/z vs drift time plot for mobility acquisition of Bovine Beta Lactoglobulin (disulphides intact . The mobility characteristics of the lower charge state ions indicate a wider arrival time distribution than many of the higher charge state ions at lower m/z. This is presumed to be due to the lower charge state ions displaying a range of differing conformations.

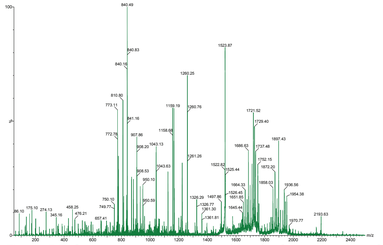

Figure 5 Tof mass spectrum produced by the fragmentation of the 13+ ion at m/z 1523.9amu. Even for this protein with a mass of approx 18277amu, the fragment spectrum contains species of many different charge states and can be challenging to interpret.

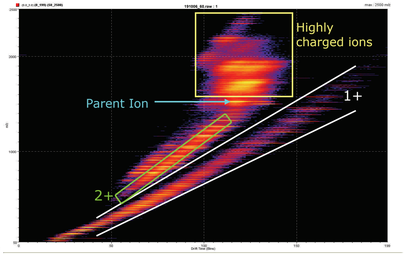

Figure 6 Fragment Ion Mobility Separation. The m/z vs drift time plot of the trap T-Wave fragmentation of the 13+ ion at m/z 1524amu exhibits distinct regions and bands. These regions are found to contain species of the same charge state.

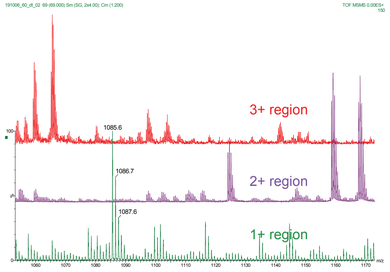

Figure 7 Selection of different regions of the m/z vs drift time plot. Selecting horizontal bands from the plot produces spectra with the same mass scale but different charge states.

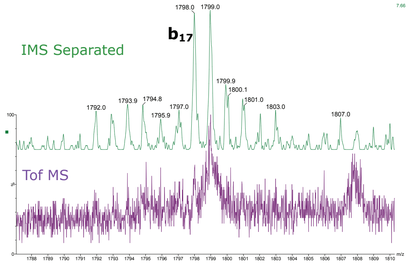

Figure 8 Selection of different regions of the m/z vs drift time plot. Low abundant species completely hidden in the equivalent ToF MS scan (bottom) can be revealed by selection of specific regions. This example shows the emergence of the b_{17} fragment by selecting and exporting the Z=1 band.

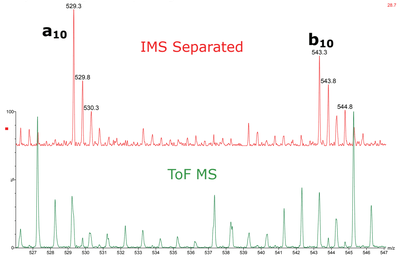

Figure 9 Selection of different regions of the m/z vs drift time plot. This example shows the enhancement of the doubly charged fragments a_{10} and b_{10}, both of which are partially hidden by singly charged background ions in the ToF MS scan

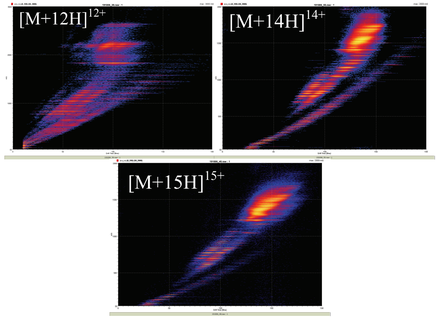

Figure 10 M/z vs drift time plots resulting from the trap fragmentation of charge states, 12+, 14+ and 15+.

Figure 11 Sequence coverage resulting from the combination of data from the isolation and trap fragmentation of four different charge states. The cysteine residues on which the two disulphide bonds reside are indicated in yellow and green (the bond indicated green is alternate) and sequence coverage can be assigned up to the residue at position 66. The sequence coverage from the Ion Mobility separated fragments represents a 25% increase over the coverage obtained without IMS. Further analysis of the Beta Lactoglobulin with the disulphide bonds reduced using DTT leads to additional sequence assignments, specifically y''_2 to y''_{12} and y''_{43} to y''_{52} (box outlined).

CONCLUSION

- A novel quadrupole/Tri-Wave oa-Tof mass spectrometer (Synapt HDMS) was used to mobility separate and analyse intact protein species.
- Different charged states of the protein were isolated in the quadrupole and fragmented in the trap T-Wave. These fragments were subsequently separated by ion mobility to produce interpretable spectra containing ions of mainly one charge state.
- The ion mobility separation of fragment ions enables the identification of low abundance species otherwise masked by overlapping species in the normal Tof analysis.
- For the small to medium sized protein analysed, a sequence coverage increase of 25% is observed when comparing mobility separations with normal Tof data.

REFERENCES

1. "Travelling Wave Ion Propulsion in Collision Cells" K. Giles, S.Pringle, K. Worthington and R. Bateman— Presented at the 51st ASMS Conference, Montreal, Canada 2003. The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993).

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