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ANALYSIS OF INTACT PROTEIN SPECIES USING A NOVEL TRAVELLING WAVE ION MOBILITY DEVICE COUPLED WITH A TIME OF FLIGHT MASS SPECTROMETER

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INTRODUCTION

The transfer of high molecular weight species from solution to the gas phase results in the formation of ions which possess relatively few charges. These ions often appear at m/z values well above 4000 in the case of non-covalently bound species. It has been previously possible to detect charged species as high as m/z 20,000 [1] on an orthogonal acceleration time-of-flight mass spectrometer.

In this presentation we will show data acquired using a novel hybrid Quadrupole/TWIMS/oa-ToF mass spectrometer, operated with an ESI source. The TWIMS is a stacked-ring ion guide, operated at elevated pressure, with opposite phases of an rf voltage applied to adjacent plates to provide radial ion confinement. A continual sequence of dc pulses is superimposed on the confining rf to provide 'waves' which propel ions through the gas.

Protein species were ionised and the resulting ions separated based upon their ion mobility, or collision cross section and subsequently mass analysed using the oa-ToF analyser.

We have investigated the use of IMS where a protein is highly contaminated with PEG, and have investigated the potential use of IMS as a means to allow rapid separation of proteins from these polymeric species.

We have also examined IMS-MS data from protein samples for the amino acid sequence information that we can obtain. We have conducted MS/MS of a small to medium molecular weight protein, followed by IMS separation of the protein fragment ions.

EXPERIMENTAL

The hybrid quadrupole/TWIMS/oa-ToF used was a Waters Synapt HDMS system, **figure 1**. Briefly, ions produced by an ESI probe are sampled by a Z-spray source where they may be activated/fragmented by applying a potential to the sample cone. They pass through a quadrupole that may be set to select a particular m/z or transmit a substantial mass range.

The TWIMS comprises three T-Wave devices [2]. The first device (accumulation 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 the transfer T-Wave. The pressure in the accumulation and transfer T-Wave regions was ~ 10^{-2} mbar of Ar 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.

Samples were introduced into the source at a flow rate of 5µL min^{-1}. The samples used in the study were standard proteins obtained from Sigma—Horse Heart Myoglobin and Bovine Beta-Lactoglobulin. The samples were prepared to a concentration of 1pmol/µL in a solution of 50/50 acetonitrile / water + 0.1% formic acid.

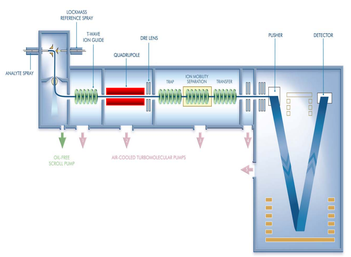

Figure 1 Schematic diagram of the Waters Synapt HDMS instrument.

RESULTS

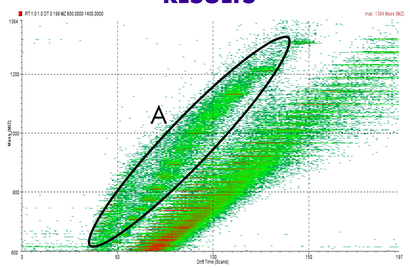

Figure 2 IMS m/z vs drift time plot for myoglobin in PEG

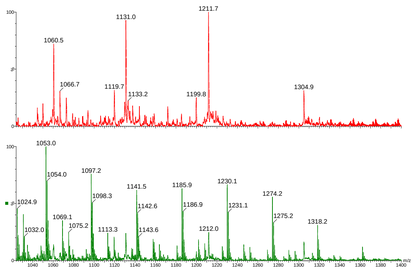

Figure 3 Selection of region A in fig.2 leads to the top spectrum. At the bottom is the spectrum resulting from selection of the entire m/z v dt plot, equivalent to the TIC from ToF MS

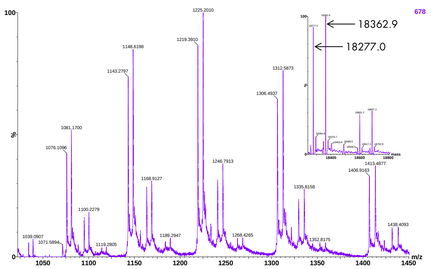

Figure 4 ToF MS spectrum of Beta Lactoglobulin. Inset is the MaxEnt1 deconvoluted spectrum, showing both the A and B variants. The 86Da mass difference is consistent with G→D (pos. 64) and A→V (pos. 118) amino acid changes from the B to A variants.

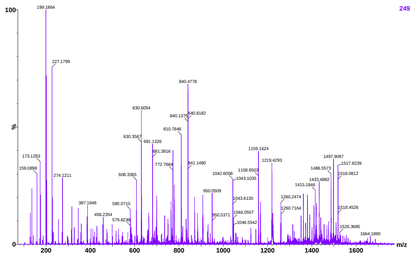

Figure 5 Beta Lactoglobulin ToF MS/MS spectrum resulting from quadrupole selection of the 15⁺ charge state at m/z 1219.4.

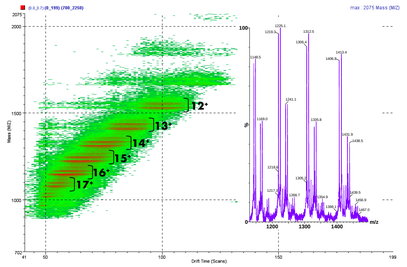

Figure 6 IMS m/z vs drift time plot for Beta Lactoglobulin. The charge states for the two variants are indicated. Inset is an expanded region of the spectrum produced by combining the whole of the m/z vs drift time plot.

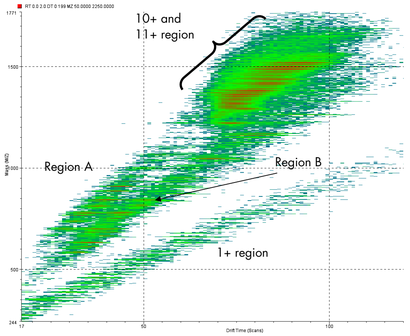

Figure 7 MS/IMS/MS for parent at m/z 1219.4. Distinct regions are observed and can be selected for further analysis.

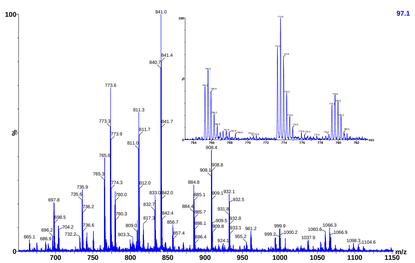

Figure 8 Mass spectrum resulting from the selection of region A indicated in figure 7. The species here are triply charged (inset)

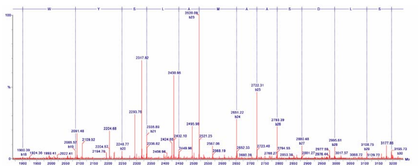

Figure 9 MaxEnt3 deconvoluted spectrum from figure 8. This region results in the sequence b_{18} to b_{30} being identified.

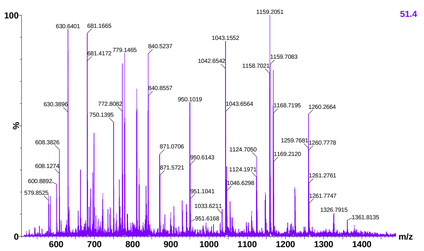

Figure 10 Mass spectrum resulting from the selection of region B indicated in figure 7. The species in this region appears to be a combination of doubly, triply and quadruply charged species

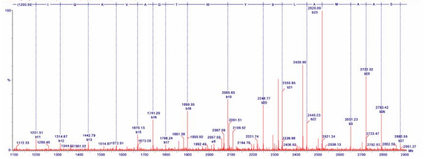

Figure 11 MaxEnt3 deconvoluted spectrum from figure 9. This region results in the sequence b_{11} to b_{27} being identified.

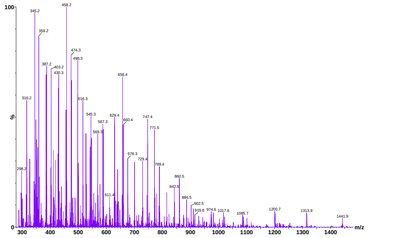

Figure 12 Mass spectrum resulting from the selection of the 1+ region indicated in figure 7.

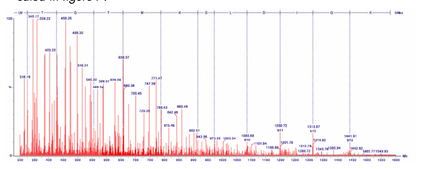

Figure 13 MaxEnt3 deconvoluted spectrum from figure 12. This region results in the sequence b_3 to b_{13} being identified.

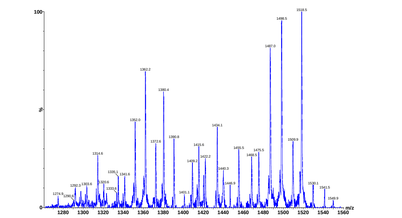

Figure 14 Mass spectrum resulting from the selection of region 10⁺ and 11⁺ indicated in figure 7.

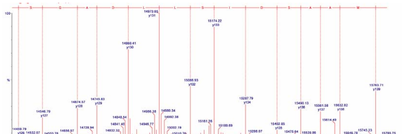

Figure 15 MaxEnt1 deconvoluted spectrum for the 10⁺ and 11⁺. Series y_{126} to y_{139} is identified.

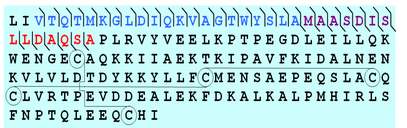

Figure 16 Sequence of Beta Lactoglobulin (variant B) with fragments identified in this study indicated. The disulphide bond from position 106 to 119 and 121 is alternate.

CONCLUSIONS

- **Employing Ion Mobility Spectroscopy for the analysis of protein species that are highly contaminated with polymeric species allows enhancements in signal to noise of the protein m/z envelope.**
- **MS/IMS/MS of protein species allows the selection of distinct regions which can be individually interrogated for sequence information.**
- **In this example, we have achieved 22% coverage of Beta Lactoglobulin using a two minute infusion experiment.**
- **Future work to increase the coverage obtained could involve the reduction of the two disulphide bonds present in Beta Lactoglobulin.**

REFERENCES

1. Rostom, Fucini, Benjamin, Juenemann, Nierhaus, Hartl, Dobson & Robinson, *Proc. Natl. Acad. Sci*, **97**, 5185-5190, 2000
2. "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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