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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 200,000 [1] on an orthogonal acceleration time-of-flight mass spectrometer. In this presentation we will show data acquired using a novel hybrid Quadrupole/TIMS/oa-ToF mass spectrometer operated with an EI source. The TIMS is a stacked-ring ion guide, operated at elevated pressures, with opposite phases of an rf-voltage applied to adjacent plates to provide radial ion confinement. A circumferential sequence of rf 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/ or the transfer T-Wave. The pressure in the accumulation device (accumulation T-Wave) may be fragmented on entrance to the accumulation T-Wave. The pressure in the accumulation T-Wave may be fragmented on entrance to the accumulation T-Wave.

**EXPERIMENTAL**

The hybrid quadrupole/TIMS/oa-ToF used was a Waters Synapt HDMS system. Figure 1. briefly, ions produced by a ES probe are sampled by a 25grip source where they may be ionised/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 TIMS consists three Tiwave devices [2]. The first device (accumulation Tiwave) accelerates ions and releases them in a short pulse (100µs) every 20 ms into the next device (IMS Tiwave) in which the mobility separation is performed, the final device (transfer Tiwave) is used to transport the separated ions into the oa-ToF for subsequent analysis. The TIMS may be fragmented on entrance to the accumulation Tiwave and/or the transfer Tiwave. The pressure in the accumulation and transfer Tiwave regions was ~ 10 Torr of Ar and the pressure in the IMS Tiwave was 0.3 mtorr of N2. The Tiwave pulse voltage and velocity were optimised to provide mobility selectivity.

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 Serum Albumin. The samples were prepared in a concentration of 0.5% acetonitrile / water + 0.1% formic acid.

**RESULTS**

Figure 2: IMS m/z v drift time plot for myoglobin in PEG 10000. The charge 10+ region is highlighted.

Figure 3: IMS/MS/IMS of protein species allows the selection of protein species that are highly contaminated with fragment ions. The disulphide bond from position 106 to 110 was covalently linked and can be selected for further analysis.

Figure 4: MaxEnt3 deconvoluted spectrum from figure 3. This region results in the sequence A is b11 to b27 being identified.

Figure 5: MaxEnt3 deconvoluted spectrum from figure 9. This region results in the sequence b11 to b27 being identified.

Figure 6: IMS m/z v drift time plot for Beta Lactoglobulin. The charge 10+ region is highlighted.

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: Tof MS spectrum of Beta Lactoglobulin. Inset is the MaxEnt3 deconvoluted spectrum, showing that the MALDI mass difference is countered with Q=10 (see Ar) and Q=11 (see 110) minor signal changes from the B to A variants.

Figure 9: MaxEnt3 deconvoluted spectrum from figure 8. This region results in the sequence B is y126 to y139 is identified.

**DISCUSSIONS**

Employing ion Mobility Spectrometry for the analysis of protein species that are highly contaminated with fragment ions allows enhancements in the sensitivity of the protein m/z envelopes.

**REFERENCES**

[1] Rostom, Fucini, Benjamin, Juenemann, Nierhaus, Hartl, Dobson & Robinson, 2005

[2] Townley, Christopher, Giles, Chris Hughes, Iain Campuzano; Therese McKenna; Johannes Vissers; Steven Pringle; Jason Wildgoose; Kevin Giles and Robert Bateman

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