THE USE OF ION MOBILITY SPECTROMETRY (IMS) COUPLED WITH TIME-OF-FLIGHT MASS SPECTROMETRY FOR THE STUDY OF GAS PHASE PROTEIN CONFORMATION

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INTRODUCTION

The transfer of high molecular weight ions and non-covalently associated protein/protein complexes from solution to the gas phase generally results in the formation of ions which possess relatively few charges. Therefore, these ions often appear at m/z values well above 4000. It has been previously possible to detect charged species as high as m/z 9,000-10,000 [1, 2] and m/z 20,000 [3] on an orthogonal acceleration time-of-flight mass spectrometer.

We have recently described a novel quadrupole/TWIMS/oa-Tof mass spectrometer, operated with an electrospray ion source that provides for the separation of ions based both upon their mobility and subsequently their m/z [4].

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, through the TWIMS device and subsequently mass analysed using the oa-TOF analyser.

We have investigated the use of the hybrid ion mobility/ time-of-flight system for the analysis of intact proteins. We have looked at proteins of different molecular masses and examined the IMS-MS data for the information that we can obtain. Here we discuss this work with examples of how IMS coupled with time-of-flight MS can be used for the systematic analysis of protein structure shown.

EXPERIMENTAL

All data was acquired on a Waters Synapt HDMS system, **Figure 1**.

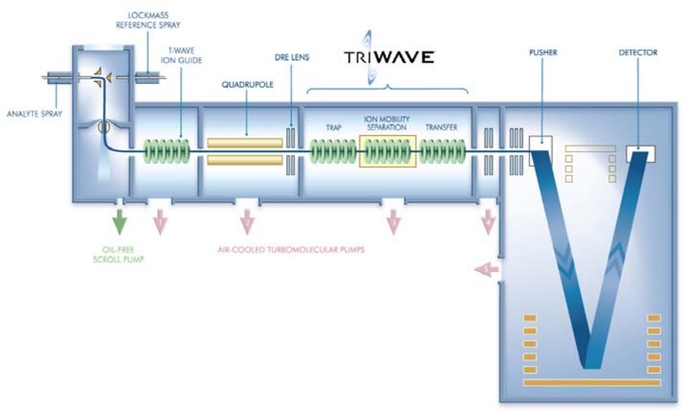

Figure 1: *Schematic diagram of the Waters Synapt HDMS instrument used in this study.*

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 used in resolving mode to select a particular m/z or in the rf only mode to transmit a substantial mass range. The TWIMS comprises three T-Wave devices [5] shown in **Figure 2**. The first T-Wave device 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.

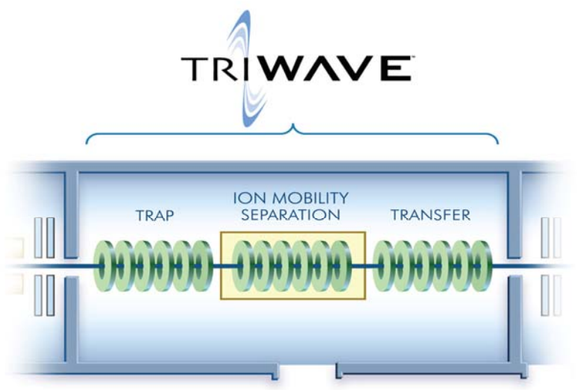

Figure 2 *: Schematic diagram of the Waters Tri Wave device used in the waters Synapt HDMS system.*

Samples were introduced into the source at a flow rate of 1µL min^{-1}. The sample used in the study was a standard protein obtained from Sigma—Hen Egg Lysozyme. Samples were prepared to a concentration of 10pmol/µL in a solution of either Ammonium Acetate to maintain their native conformation or in a solution of 50/50 acetonitrile / water + 0.1% formic acid to assess the mobility changes due to the protein being in a more open configuration.

RESULTS

The Tof MS spectrum of a native lysozyme infusion, displaying the expected charge state distribution from 6^+ to 9^+ is shown in Figure 3. The rectangles will be used to identify these ions on the corresponding DriftScope data plot in Figure 4 below.

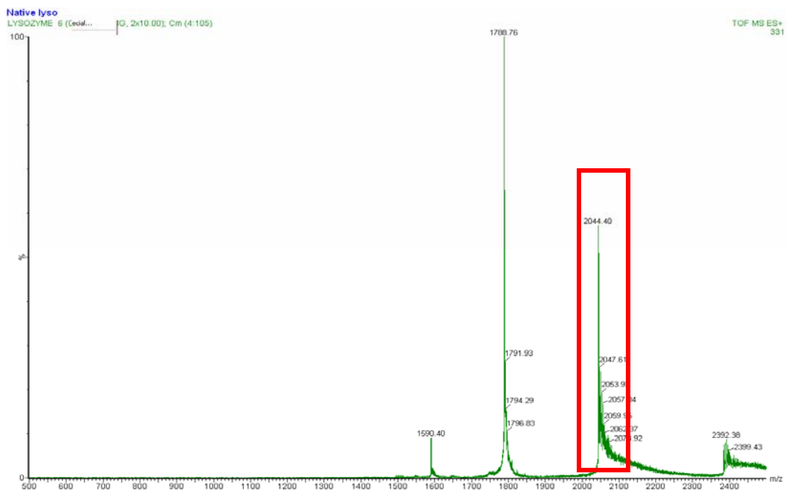

Figure 3 *ToF MS spectrum of Lysozyme obtained over the m/z range 500 to 2500.*

This plot displays both the drift time and m/z ratio of the multiply charged ions of the intact lysozyme. The horizontal peaks represent ions of a given m/z ratio, as read from the y axis, and the corresponding drift time, as read off the x axis. The red and white rectangles associates these ions with the 7+ charge state indicated in the spectrum. The mobility characteristics of these ions indicate a wider arrival time distribution than many of the higher charge state ions (which are present at lower m/z values) of lysozyme.

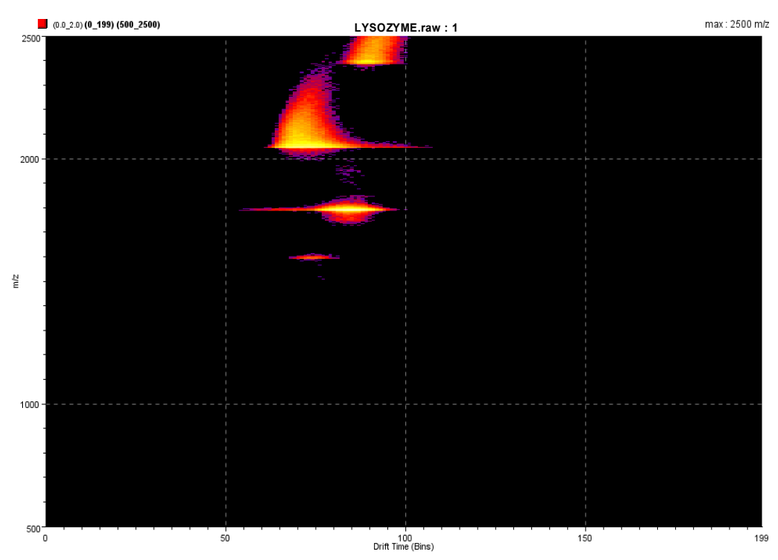

Figure 4 Driftscope display of native Lysozyme at 10pmol/ul in ammonium acetate showing both drift time and m/z ratio.

Figure 5, displays a plot of mass: charge against drift time where the Lysozyme sample has been reduced and acidified. From the associated TIC spectrum, Figure 6, it can be seen that many more charged states now exist and the mobility plot of these species is different as some of the molecules have a more open conformation than those in the native state. The mobility

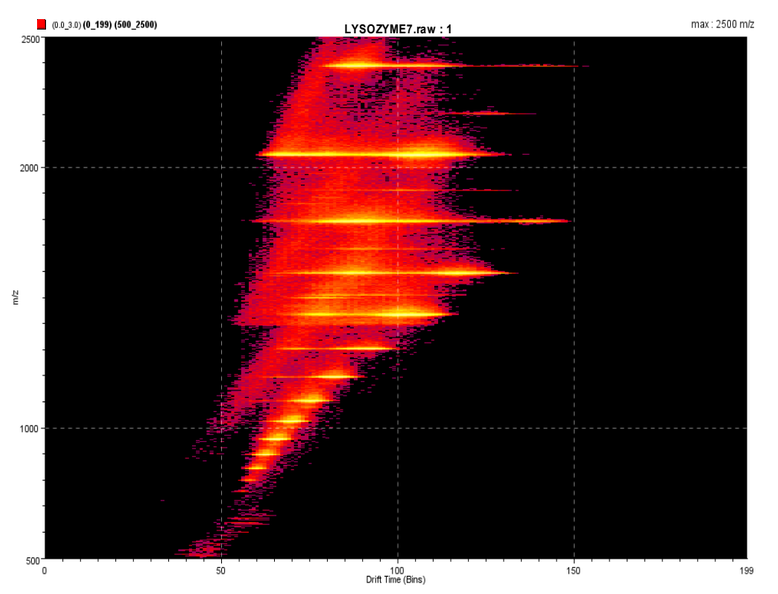

Figure 5 Driftscope display of reduced and acidified Lysozyme, at 10pmol/ul showing both drift time and m/z ratio.

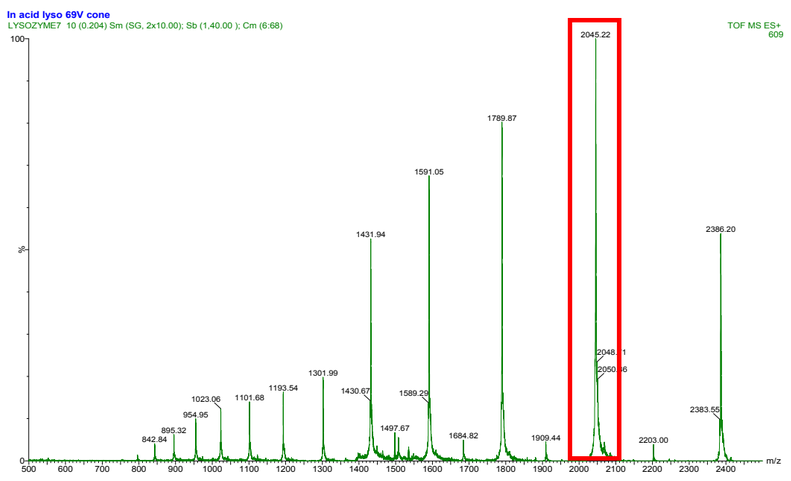

Figure 6 *ToF MS spectrum of reduced and acidified Lysozyme.*

of the differing charged state species can be profiled as shown in Figure 7 where the drift time associated with the 7^+ charge of the native species i.e. the tightly bound conformer in the top box is between 60 and 90 msec. The drift time of the reduced and acidified Lysozyme i.e. in the open conformation is show in the bottom box from 60 to 140 msec. This suggests that there are a range of conformers, including the native form, present in the reduced and acidified sample.

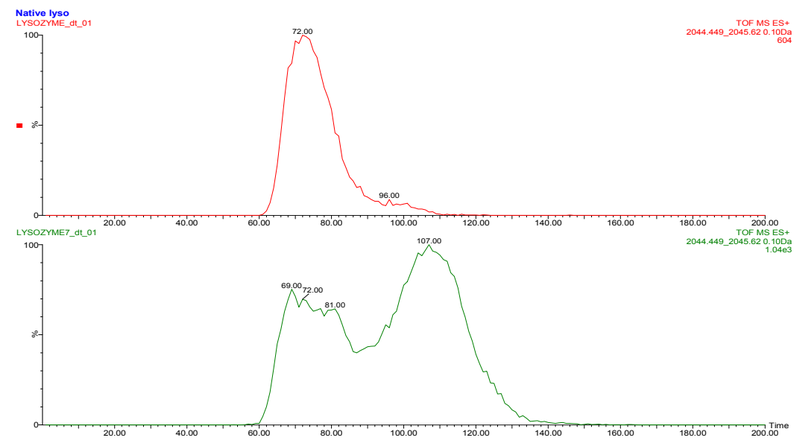

Figure 7 *Mobility profiles of the 7+ charged state of Lysozyme in the native form (top) and in the reduced form (bottom) showing drift time versus intensity.*

Top Down Sequencing

The Tri-Wave device affords the ability to perform fragmentation either before or after the IMS separation. The Driftscope plot shown in Figure 8 was obtained by isolation of the 7^+ ion in the quadrupole and performing fragmentation in the TRAP T-Wave. The resulting product ions were separated by ion mobility and several nested distributions related by their m/z and drift time can be observed.

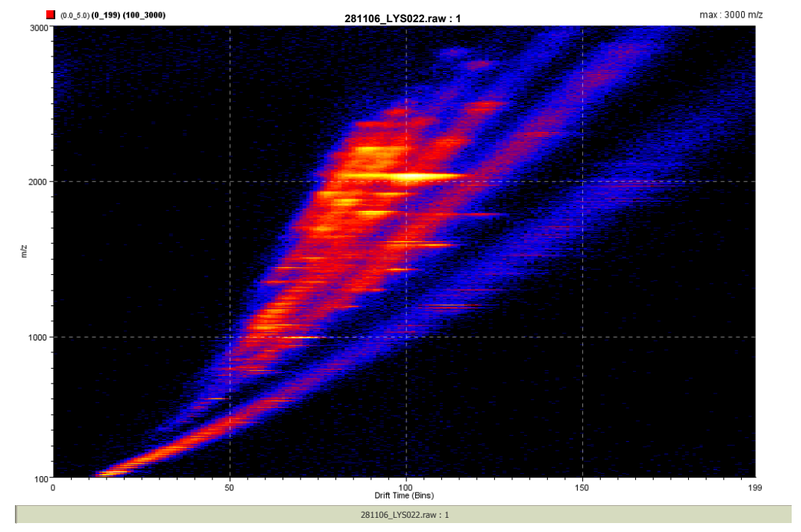

Figure 8. DriftScope data, displaying both the drift time and m/z ratio of the product ions of $[M+7]^{7+}$ of Lysozyme. The ions can be distinguished by their charge state.

The mass spectra, from the regions indicated, predominantly separated by their charge state, are shown in Figure 9 below. Without separation by ion mobility many of these product ions would be indistinguishable from one another or from the general background. IMS separation enables more of the product ions to be visualised. This is shown dramatically in Figure 10 whereby the product ion y_{11}" ion is indistinguishable from the background in the Tof MS/MS data .

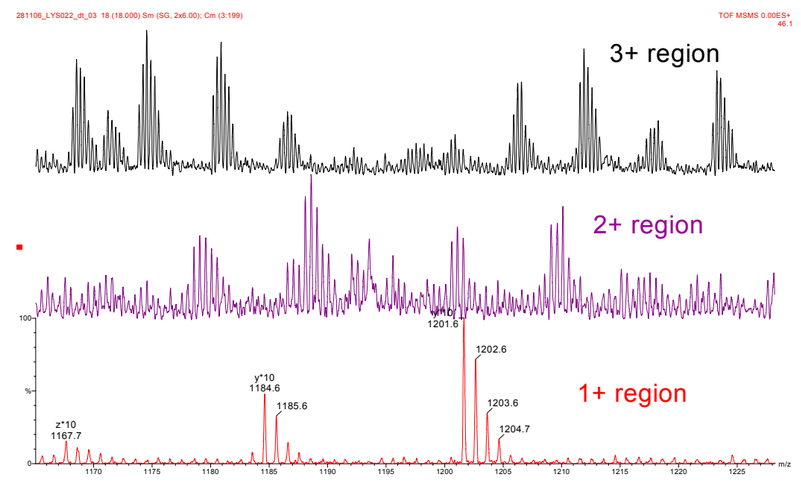

Figure 9 Charge state separation by Ion mobility

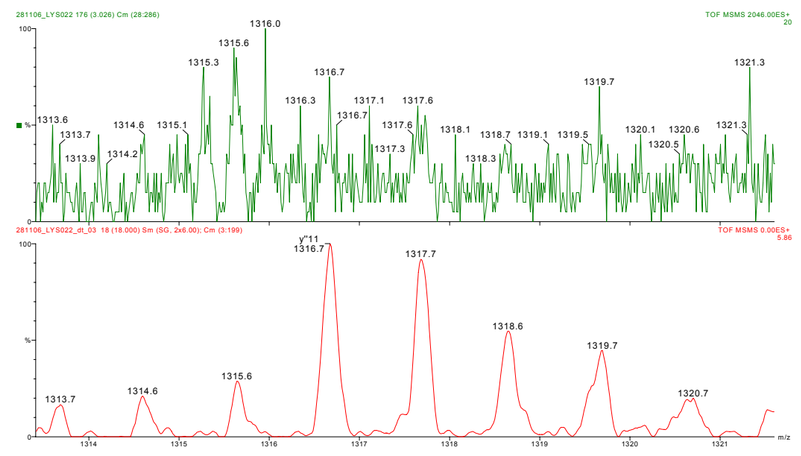

Figure 10 Comparison of standard Tof MS/MS data, i.e. no IMS separation in the top trace, with IMS separated product in the bottom trace.

CONCLUSIONS

- Different conformers of Lysozyme can be separated by Ion Mobility
- Using ion mobility spectrometry (IMS) to separate fragment ions adds another, orthogonal, dimension of separation to the MS experiment and provides additional information that is often hidden in the TOF MS experiment
- This Information content and hence coverage of the amino acid sequence is increased using IMS post fragmentation

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