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HIGH MOLECULAR WEIGHT IONS ANALYZED IN MS AND MS/MS MODE ON A QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETER

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OVERVIEW

- This poster demonstrates the ability to generate, transmit and detect large multiply charged molecular weight complexes and singly charged inorganic ion clusters on a Q-Tof Premier mass spectrometer.
- The extended oa-Tof mass range (up to m/z 100,000 in V-Optics mode) makes high molecular weight ion detection routine.
- The combination of the of the NanoFlow Z-Spray source with the T-Wave* ion guide and collision cell enable efficient desolvation and transmission of the high molecular weight compounds.
- A 32k amu high mass quadrupole option enables mass selection and fragmentation of ions with high m/z values.

INTRODUCTION

The transfer of non-covalently associated protein-protein complexes from solution to the gas phase generally results in the formation of ions possessing relatively few charges and therefore, often appear at m/z values above 4,000. Charged species as high as m/z 9,000-10,000 (1,2) and m/z 20,000 (3) have been reported. Recently however, a Q-Tof instrument has been used to demonstrate the separation of biomolecues utilising ion mobility (4). Such mass ranges would be unachievable on a standard triple quadrupole or ion trap style mass spectrometer. However, these mass range limitations are overcome by an extended mass range 32k amu quadrupole (5) and an orthogonal acceleration Time-of-Flight (oa-ToF) analyser possessing a an upper mass range of m/z 100,000—making this style of instrument the ideal platform for routine high molecular weight biomolecule analysis.

Samples are introduced into the Q-Tof™ Premier instrument (Fig.1) via the Z-Spray™ NanoFlow™ ESI Source. The source backing region and T-wave ion provide efficient transfer and desolvation of atmospheric gas phase ions into the vacuum of the mass spectrometer. Prior to the oa-Tof analyser, the ion traverse the quadrupole and T-Wave, collision cell.

** The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993)*

In the MS/MS mode, ions with high m/z values can be isolated in the quadrupole and subsequently fragmented in the T-Wave collision cell. The T-Wave collision cell can also provide additional ion desolvation if required.

Once the ion exits the gas cell, they are transferred to the oa-Tof analyser by a series of lenses. The ions are then orthogonally pulsed into the Tof analyzer where they are spatially and temporally resolved according to their m/z. The oa-Tof pusher frequencies may range from 4kHz to 8kHz depending on the mass range that data is acquired over.

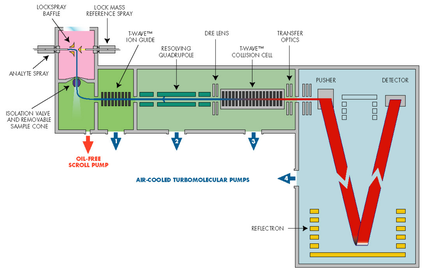

Figure 1. Schematic of the Q-Tof Premier Mass Spectrometer

EXPERIMENTAL METHODS

Protein samples were introduced into the mass spectrometer at approximately 20 nL/min via a borosilicate NanoFlow sample vial. Protein sample concentrations were in the pmol/µL concentration range, in an aqueous solution of 200mM ammonium acetate.
Instrument calibration was performed over the m/z range 1000-32,000, using a CsI solution. Alcohol dehydrogenase and caesium iodide were acquired from Sigma Co. Rabbit Proteasome 20S was obtained from CalBiochem. For each sample, continuum data was acquired in the V-optics mode, from m/z 1,000-32,000. Data was integrated every 2 seconds with an interscan delay of 0.1 second. Data was smoothed (40x 2, SG) and background subtracted (polynomial order 25, 5% below curve).

RESULTS - TOF-MS

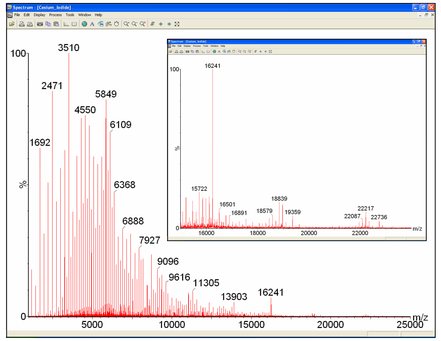

Figure 2.NanoFlow ESI spectrum of caesium iodide at a concentration of 10 µg/µL in a 50%(v/v) aqueous solution of isopropyl alcohol.

When a concentrated aqueous solution of caesium iodide is infused into the Q-Tof Premier, very intense clusters of caesium iodide (based on the empirical formula $Cs_{(n+1)}I_n$ can be observed up to m/z 25,000 (Figure 2). The intense singly-charged ion at m/z 16,241, represents $Cs_{63}I_{62}$+. This is a typical spectrum, used to calibrate the Q-Tof Premier, up to m/z 25,000.

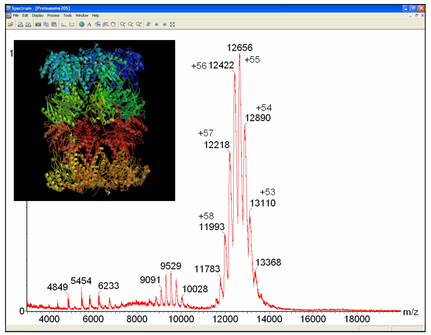

Figure 3. NanoFlow ESI spectrum of the rabbit Proteasome 20S complex, analyzed in an aqueous solution of 200 mM ammonium acetate.

Proteasome 20S is composed of 28 subunits arranged in four heptameric, tightly stacked rings (α7, ß7, ß7,α7) to form a cylindrical structure. The multiply-charged series (+58,+57, etc.) between m/z 12,000 and 15,000 represents the Proteasome 20S multimer with a molecular weight of 685 kDa.

RESULTS - TOF-MS/MS

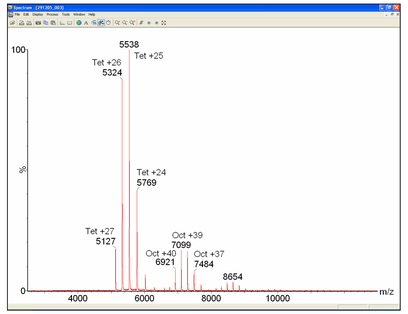

Figure 4. NanoFlow ESI spectrum of a tetrameric protein/protein complex in an aqueous solution of 200 mM ammonium acetate. Tet = Tetramer, Oct; = Octamer.

When the protein shown in Figure 4 was infused into the Q-Tof Premier, under native conditions, it was observed primarily in its tetrameric form with an intact molecular.
weight of 138,425Da. There is also evidence of an octameric species (276,849Da) and a dodecameric species with a molecular weight of 415,313Da.

Figure 5 shows the MS/MS spectrum of *m/z* 5537 fragmented with an applied collision energy of 95eV. Fragmentation results in the production of a highly charged monomeric species (+17,+16,+15, centred around *m/z* 2000) with a mass 34,619Da. Also produced is a trimeric species with a molecular weight of 103,785Da, which possesses relatively few charges (+8,+9) and as a result appear very high in the *m/z* scale (*m/z* 12,000 to 13,000).

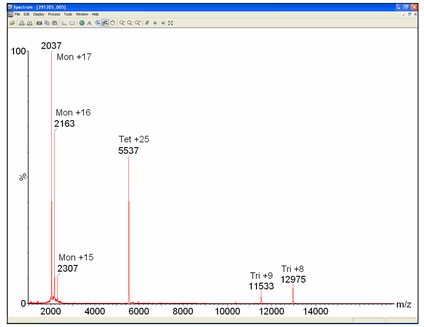

Figure 5. NanoFlow MS/MS spectrum of the ion m/z 5537 (+25).

CONCLUSIONS

- The high mass range of the oa-Tof analyzer enables the detection of the large biomolecular complexes, such as the Proteasome 20S shown here, which has an intact molecular weight of 685kDa.
- Analysis of a large protein complex via MS/MS, (m/z 5537), results in the production of highly charged monomeric species and a trimeric species possessing relatively few charges.
- The high mass quadrupole option allows selection of precursor ions of up to m/z 32,000. These mass ranges would be unobtainable on a standard quadrupole or ion trap instrument.

REFERENCES

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