Biomolecular mass spectrometry

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Introduction

The main focus of our research is the development and application of mass spectrometric techniques to investigate the tertiary and quaternary structures of biomolecules. We use non-covalent electrospray ionisation mass spectrometry (ESI-MS) and tandem mass spectrometry (MS/MS) to determine the mass, conformational properties, stoichiometry, stability, and binding characteristics of proteins and protein complexes. We are also pioneers of ion mobility spectrometry-mass spectrometry (IMS-MS), which offers a unique opportunity to separate co-populated biomolecular entities on the basis of their physical shape and to measure their mass and cross-sectional area (Ω) in a single, rapid (≤ 2 mins) experiment.

Results

In collaboration with Prof. Christian Cambillau (Université de Marseilles, France), we have used ESI-(IMS)-MS to study the mass and stoichiometry of the intact, non-covalently bound baseplate complexes of two siphophages, p2 and TP901-1, as well as several significant structural sub-complexes generated by a "block cloning" strategy. Both p2 and TP901-1 infect different strains of the gram+ lactic acid bacterium *Lactococcus lactis*. They possess a large multi-protein organelle (1-2 MDa) at their distal tail end, termed the baseplate, which is responsible for specific host recognition, attachment and initiation of infection. However, despite X-ray crystallography and/or electron microscopy of the final baseplate, the pathways leading to these large complexes remain ill-defined.

The masses of the intact phage baseplates, and a range of associated sub-complexes, were etermined within an error of ≤0.2 %. Thus, the stoichiometries of the intact baseplates were confirmed and the identities of the multi-protein sub-complexes assigned unambiguously. For example, the stoichiometry of the intact TP901-1 baseplate (mass 1.769 MDa) was confirmed as [(6 x Dit) + (18 x BppU) + (54 x BppL] subunits (Figure 1).

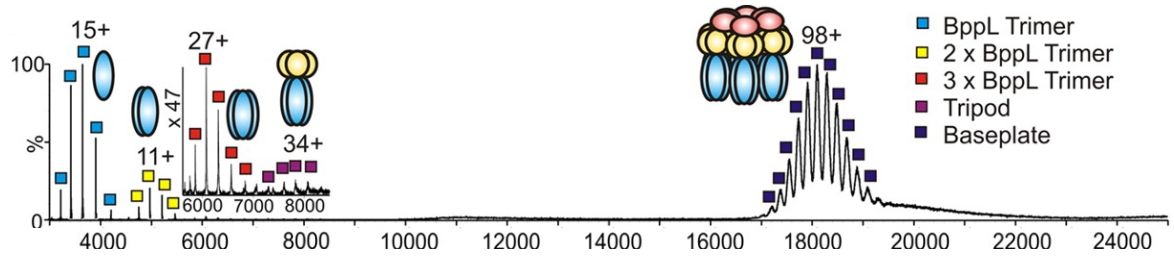

Figure 1: ESI-MS spectrum of the intact TP901-1 lactococcal phage baseplate (mass 1.769 MDa; m/z ca 18,000) and associated sub-complexes. Key: pink = distal tail protein (Dit); yellow = baseplate protein upper (BppU); blue = baseplate protein lower (BppL).

The characterisation of these sub-complexes has provided valuable insights into the assembly of the organelles and we have been able to propose plausible baseplate assembly pathways for the p2 and TP901-1 lactococcal phages (Figure 2). The collision cross-sectional areas measured by ESI-IMS-MS were compared with solution-phase dynamic light scattering data to support the notion that the structure of a protein complex can be maintained in the gas phase. Together the data illustrate the value of ESI-(IMS)-MS for studying heterogeneous, megaDalton, macromolecular baseplate complexes.

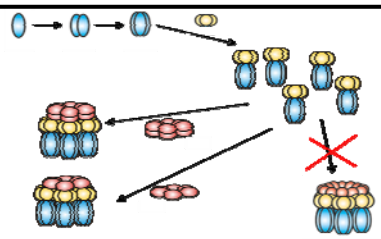

Figure 2: proposed TP901-1 baseplate assembly pathway from baseplate complex and sub-complexes characterised by ESI-MS and ESI-IMS-MS. Key: pink = distal tail protein (Dit); yellow = baseplate protein upper (BppU); blue = baseplate protein lower (BppL).

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Collaborators

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Leeds: S. Harris, E. Hewitt, S. Homans, I. Manfield, P. Stockley and S. Warriner.