

Electrospray Ionisation Mass Spectrometry of Gas Phase Macromolecular Complexes: Progress, pitfalls and prospects

Margaret M. Sheil,¹ Jennifer L. Beck,¹ Rajesh Gupta,¹ Amit Kapur,¹ Stephen Watt,¹ Susan E. Brown² and Nicholas E. Dixon.²

1. Institute for Biomolecular Science and Department of Chemistry, University of Wollongong, NSW, 2522, AUSTRALIA
2. Research School of Chemistry, Australian National University, Canberra, ACT, 0200, AUSTRALIA

Elucidation of the genome of an organism and the corresponding studies of expressed proteins (*i.e.* proteome) are the first steps in understanding biological processes at the molecular level. Equally as important as the determination of the primary structure of the protein or DNA, however, is characterisation of the higher order structures of these biopolymers and the way in which biopolymers come together to form large macromolecular assemblies (e.g. protein-receptor complexes, ribosomes and viruses).

The enormous advances in the capabilities and performance of analytical instrumentation for elucidation of primary structures over the past decade has been paralleled by both major improvements in the methodology for traditional higher order structural methods *i.e.* X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy and the advent of a variety of new techniques including surface plasmon resonance (SPR) and electrospray ionisation mass spectrometry (ESI-MS). Together these have provided exciting new capabilities for probing the molecular basis of cellular processes.

ESI-MS has been used, for example, to probe interactions between proteins and RNA of the ribosome¹ and the MtGimC chaperone complex.² The technique has advantages in terms of both sensitivity and accuracy in determination of stoichiometry of large biological complexes. Furthermore, observation of equilibria is possible without the perturbation of the equilibrium position that can occur with other methods (*e.g.*, filter binding), where removal of free or bound components of a mixture is necessary for analysis. Despite these advantages, however, there are reasons why data obtained from ESI-MS need to be interpreted with caution. First, the ionization process itself might perturb equilibria. Second, there is a paucity of information available concerning changes in the strength or specificity of non-covalent interactions that occur on transfer from the condensed to the gas phase during the ionization process. The stabilities of complexes between biological macromolecules involve contributions from ionic, H-bonding, hydrophobic and van der Waals interactions. Several ESI-MS studies support the proposal that electrostatic interactions are strengthened *in vacuo*, while hydrophobic interactions are unaffected or weakened through loss of water during desolvation and/or ionization.³

In order to improve our understanding of the limitations and advantages of studying such complexes in the gas phase, we have studied a variety of different complexes important in DNA replication in *E. coli*, each with different binding modes. These include: (i) complexes of Tus protein (35,652 Da), with its double-stranded 21 mer DNA recognition sequence, *Ter B*,⁴ and (ii) the complex formed between the α and β subunits of DNA polymerase III (*i.e.* a multisubunit enzyme that is the major replicative polymerase of *E. coli*. Domain.

In this paper data from these studies will be presented and compared, together with a summary of the work of others in this area to present an overview of the pitfalls, progress and prospects for the use of ESI-MS as a complementary tool for analysis of macromolecular complexes.

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