# ELECTROSPRAY IONIZATION MASS SPECTROMETRY OF NON COVALENT PROTEIN-PROTEIN COMPLEXES- DNA POLYMERASE III SUBUNITS

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There have been numerous reports of non-covalent biomolecular complexes detected using electrospray ionization mass spectrometry (ESI-MS)[1]. ESI-MS has been used to characterize various features of protein-protein, protein-DNA, protein-RNA, and DNA-DNA complexes including macromolecular and ligand binding stoichiometry and solution binding affinities. In some of these studies, however, the question as to whether the gas phase behavior of these complexes correlates with solution behavior has not been rigorously addressed. Previously, we compared the relative binding affinity of a DNA recognition sequence for a replication terminator protein (Tus), with its binding affinity for Tus mutants.[2] In this case, the relative order of binding affinity in the gas phase was the same as in solution. The X-ray structure of the native complex shows there are substantial polar and electrostatic contacts between binding partners.[3] ESI mass spectra showed that the complex dissociated when treated with ammonium acetate concentrations over the range 1-2 M, but was stable to a cone voltage ≥100 V.

We have recently examined the complex of the $\theta$ and $\varepsilon$ 186 subunits of *E. coli* DNA polymerase III using ESI-MS. DNA polymerase III (pol III) is one of the most extensively studied replication enzymes. Three subunits constitute the core of the enzyme: (1) $\theta$ (130 kDa) encoded by the *dnaE* gene contains the 5'-3' DNA polymerase activity; (2) $\varepsilon$ (28 kDa) encoded by the *dnaQ* gene is a 3'-5' proofreading exonuclease; and (3) $\alpha$ (9 kDa) encoded by the *holE* gene and has no known discrete function. The N-terminal fragment of $\varepsilon$ containing amino acids 2-186 ($\varepsilon$186) bears the exonuclease active site while the C-terminal domain containing amino acids 187- 243 contains $\alpha$ binding sites. The structure of this complex is not known. ESI mass spectra show that $\theta$ - $\varepsilon$186 complex remains intact up to at least 9 M ammonium acetate, suggesting that binding might involve hydrophobic interactions. To test this possibility, ESI mass spectra of the complex treated with a range of alkanols were acquired. The propensity of the alkanols to dissociate the complex decreased with increasing polarity in the order: 1-butanol, 1-propanol, ethanol and methanol. These results are consistent with proposals based on chemical shift mapping experiments (NMR) that a hydrophobic sequence (AAAGVAFKE) of $\theta$ might associate with $\varepsilon$186.[4] The complex was stable at low cone voltage, however, as cone voltage was increased to more than 30V some dissociation occurred, with almost complete dissociation at a cone voltage of 60V.

### *References:*

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