

ELECTROSPRAY IONIZATION MASS SPECTROMETRY (ESI-MS) AS A PROBE OF PROTEIN CONFORMATION

Stephen J. Watt¹, Pavel Prosselkov², Nicholas E. Dixon², Jennifer L. Beck¹ and Margaret M. Sheil¹.

¹Department of Chemistry, University of Wollongong, NSW 2522, Australia; ²Research School of Chemistry, Australian National University, ACT 0200, Australia

ESI-MS has been used to study conformational changes of proteins either by deuterium exchange experiments¹ or by observation of changes in abundances of protein molecules with particular numbers of charges.² In most of the latter types of study, unfolding of proteins has been achieved by decreasing the pH, thereby increasing the number of exposed charges on protein molecules.

In gas phase studies of non-covalent interactions (and conformational changes), the relationship with solution behaviour of biomolecules remains to be established. In previous work, the increase in thermal stability in solution with respect to folding resulting from cyclization of the N-terminal domain of DnaB (helicase from *E. coli*) was measured. The melting temperature for unfolding of linear DnaB-N measured by differential scanning calorimetry was 39.8°C, and for cyclized DnaB-N was 53.4°C.³ Under different buffer conditions, the corresponding values for the DnaB-N(F102E) mutant proteins were 37.9 and 48.5°C, respectively. This system offers an excellent opportunity to compare gas and solution phase behaviour of protein folding/unfolding.

Positive and negative ion ESI mass spectra of linear and cyclized DnaB-N(F102E) were acquired under different pH and desolvation conditions as a probe of the stabilities of these proteins in the gas phase. When maintaining pH levels at pH 7.6 and adjusting desolvation temperatures over the range 20–240°C, positive and negative ion spectra of cyclized DnaB-N(F102E) showed only one (folded) conformation. In contrast, spectra of linear DnaB-N(F102E) showed that the protein was folded when the desolvation temperature was low (<60°C), but gradually unfolded as the temperature was increased. These preliminary results suggest that both linear and cyclized proteins were more stable to unfolding in the gas phase than in solution. Finally, we also found some interesting differences between stabilities of positive and negatively charged complexes. The possibility of using ESI-MS as a sensitive indicator of protein conformation will be discussed.

References:

1. Last A.M. Robinson C.V. (1999) *Curr. Opin. Chem. Biol.* 3(5), 564-557.
 2. Konermann L. Douglas D.J. (1998) *J. Am. Soc. Mass Spectrom.* 9(12), 1248-1254.
 3. Williams N.K., Prosselkov P., Liepinsh E., Line I., Sharipo A., Littler D.R., Curmi P.M.G., Otting G. and Dixon N.E. (2002) *J. Biol. Chem.* 277, 7790-7798.
-