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Title of the thesis: Structural, Functional and Thermodynamic Studies of the pH Dependence of the Protein Folding Abilities of the Ionizable Chemical Chaperones

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Abstract

Osmolytes are water-soluble compounds of relatively low molecular mass and found in micro-organisms, plant and animal cells. They stabilize the native protein structure at increased temperatures, pH changes, and at high pressure in deep-water fishes and at high salt concentrations in plants. Their effects seem to be general for all proteins. It is important to note the effect that many osmolytes have on pH, as this change in pH itself may critically change the stability of proteins. The charge of osmolytes is of great importance for their interactions with proteins. In fact many other osmolytes are zwitterionic yet charged *in vivo*, and charging these osmolytes by pH could lead to undesired effects. Hence, charge plays an important role in the stability of protein. Charged osmolytes have different effect on the stability of protein depending on the pK_a value of the compound. Thus pK_a plays an important role in the stabilization and destabilition of protein. Taking in to account all these point it will be interesting to study that is there any destabilizing effect of osmolytes above and below their pI values.

The objective of this research work is to understand the question: do pI of the compounds play the role in the stabilization of protein and is this stability is pH-dependent or not? To answer the above question we have carried out thermal denaturation studies of RNase-A in the presence of various osmolytes at different pH values above and below the pI value of osmolytes. Although the effects of osmolytes on protein stability, folding and the activity of protein and enzyme have been widely studied, the relationship between protein stabilization by osmolytes and its consequent effects on

the activity of enzymes has not been examined. Thus we intentionally selected different groups of osmolytes that come under different class and which do not differ much in their pI values (pI range 6.0 to 7.0). The osmolytes are glycine and proline (amino acid and amino acid derivatives) and sarcosine, dimethylglycine and betaine (methylamines compounds). They all have approximately same pI values and we expect that there will be no difference in the stabilizing ability above and below their pI values. Alongwith all osmolytes, dimethylglycine, a methyl derivative of glycine, and inorganic osmolyte such as NaCl is also selected for the comparative studies.

The findings from this study are:

(i) The stabilizing effects of all osmolytes and NaCl were found to be pH-dependent with the stabilizing effect increasing with the decrease in pH from 8.0 to 2.0. (ii) Osmolytes and NaCl have no effect on the structural characteristics of N (native) and D (denatured) states of denaturation equilibrium of RNase-A. (iii) Protein stability and activity are coupled in the presence of osmolytes. (iv) Amino acids and its derivatives neither perturb the denaturation equilibrium nor affect the functional activity under native condition. However, they have the ability to protect proteins from denaturing stresses by raising T_m. (v) Methylamines have significant effects on ΔG_D^o and functional activity (K_m and k_{cat}) thus refold the denatured protein to a more active state under native conditions. (vi) There is perfect enthalpy-entropy compensation in the protein stabilization by amino acids and its derivatives, whereas stabilization of protein by methylamines and NaCl is entropically driven.