

**EFFECTS OF SIZE OF POLYOLS ON THE PROTEIN FOLDING/UNFOLDING  
EQUILIBRIUM UNDER PHYSIOLOGICAL CONDITION**

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**ABSTRACT**

Intracellular osmolytes protect native proteins from denaturation and some of them behave as chemical chaperones by promoting the correct proteins refolding *in vitro* and in the cell. In this thesis we investigated the effect of size of sugar osmolytes on the thermodynamic stability and function of RNase-A. To investigate the effects of sugar osmolytes on protein stability we have carried out thermal denaturation measurements of RNase-A in the absence and presence of six sugars (glucose, fructose, galactose, sucrose, raffinose and stachyose) in the pH range of 6.0-2.0. We discovered that oligosaccharides have more stabilizing effect than the individual monosaccharide constituent, and that, on the molar scale, the order of stabilization is: stachyose > raffinose > sucrose > glucose, fructose, and galactose. We also observed that the extent of stabilization of RNase-A is pH dependent, that is, stabilization by a sugar increases with decreasing from 6.0 to 2.0. It has been argued that the effect of osmolytes is predominantly on the denatured molecules, for it is very little on the native molecule. If this is the case, the stabilization of the protein by a sugar will be more at lower pH, for lowering the pH will increase the number of denatured molecules.

We again investigated the effects of mixture of sugar osmolytes on RNase-A. Interestingly, we found that on the molar scale sucrose is better stabilizer than its individual constituents. However,  $\Delta\Delta G_D^\circ$  of the protein in a given concentration of sucrose is less than  $\Delta\Delta G_D^\circ$  of the protein obtained in the presence of 1:1 mixture of glucose and fructose. Thus, mixture of the monomers provides better stability to the

protein in comparison to disaccharide cosolute. This suggests that mixture behaves differently than two monomers individually as an osmolyte. It is interesting to note that the observed  $\Delta\Delta G_D^\circ$  in the presence of 1:1 molar mixture is, within experimental errors, equal to the algebraic sum of  $\Delta\Delta G_D^\circ$  of the protein obtained in the presence of individual monomer osmolyte.

Similarly, we found that (i) trisaccharide (or tetrasaccharide) has more stabilizing effect than each individual constituent, (ii) the sum of  $\Delta\Delta G_D^\circ$  values in the presence of a mixture of equimolar constituents is larger than that in the presence of the trisaccharide (or tetrasaccharide), and (iii) in a mixture, the stabilizing effect of one monomer on the stability is independent of the effect of the other monomer(s). This observation is found to be similar with the earlier report that two osmolytes have independent effect on the protein stability. Moreover, the stabilizing effect of the mixtures of monosaccharide constituents of each oligosaccharide was found to be pH dependent with the stabilizing effect increasing as the pH decreases from 6.0 to 2.0.

Functional activity measurement indicates that sugar polyols at lower concentrations were found to be minimal perturbation on the kinetic parameters,  $K_m$  and  $k_{cat}$ . However, at higher concentrations, sugar polyols as well as their mixtures of monosaccharides constituents of each oligosaccharide were found to decrease the kinetic parameters,  $K_m$  and  $k_{cat}$  of the protein. Moreover, both  $K_m$  and  $k_{cat}$  of RNase-A were found to decrease with the increasing sizes of co-solvents. Our results suggest that crowding of cosolvents favor more number of N states of enzyme and this leads to increase of enzyme-substrate affinity but at the same time it decreases the catalytic rate of the enzyme. This shows that crowding of cosolvent effects on global protein stability may be distinct from effects on mobile regions of the protein that undergo conformational changes during catalysis.