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Title of Thesis: Relation Between Stability and Functional Activity of Proteins in the Presence of Sugar Osmolytes

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Abstract

Newton's 3rd Law states that "For every action there is an equal and opposite reaction". Similarly for any physical stress (extreme of temperature, pressure, salt concentration, pH, and presence of denaturants) there is a stress tolerance mechanism in living organisms to acclimate in extreme environmental conditions. Accumulation of small organic molecules, i.e., osmolytes, is the part of stress-tolerance strategy adopted by organisms during stress conditions. During extrinsic stress conditions, to sustain the stability of protein remains a major issue for organisms. Osmolyte–induced protein stabilization always attracts protein chemist, and they try to unfold the mechanism behind this increased stabilization of proteins in the presence of osmolyte. Thus, to understand the mechanism of osmolyte-induced protein stabilization, we have investigated the effect of sugar osmolytes on structure, function and stability of proteins. We studied different aspect of sugar–induced protein stabilization such as the effect of concentrations of sugar osmolytes, effect of mixture of sugar, and the effect of their shape and size on thermodynamic stability of proteins. Since, the excluded volume by a mole of osmolyte to the centre of mass of protein surface results in their increased stability, and it depends upon two important factors, shape and size of osmolyte as well as of the protein. Therefore, osmolytes having large size will exclude relatively more volume and leads to more change in free energy per mole of a protein in comparison to that of the small size osmolytes. To check the assumption of size–dependent osmolyte–induced protein stabilization, we performed thermal denaturation of two proteins, apo α–lactalbumin (α–LA) and lysozyme, in the absence and presence of seven different sugar osmolytes of various sizes, i.e. mono– (glucose, galactose and fructose), di– (sucrose and trehalose), tri– (raffinose) and tetra– (stachyose) at different pH values.

Thermal denaturation curves measured under all experimental conditions were analysed by two-state reversible non-linear equation to get the values of T_m (mid-point of thermal denaturation curve) and ΔH_m.(enthalpy change at T_m). Slop of the plot of ΔH_m versus T_m, was obtained at different pH values under a given experimental conditions yielded ΔC_p (heat capacity change at constant pressure). Values of thermodynamic parameters (T_m, ΔH_m and ΔC_p) and obtained under a given experimental condition was used to estimate the value of ΔG_D^0 (Gibbs free energy change at 25 °C) at that experimental conditions. Excluded volume (α), an important thermodynamic parameter that determines protein stability in a crowded environment created by osmolytes, was also estimated for both the protein in the presence of each sugar at different pH values.

There are three main observations were found from our results obtained from thermal denaturations of proteins in the presence of different saccharides individually and in the presence of mixture of monosaccharides at different pH values, (i) Change in free energy per mole or degree of stabilization of both the proteins increases as we increase the size of saccharides from mono- to tetra- saccharides, (ii) Change in free energy per mole of both the proteins increases as we deviate from their isoelectric point (pI) in the presence of a particular sugar, and (iii) Change in free energy per mole of proteins obtained in the presence of mixture of monosaccharides in the appropriate stoichiometric ratio was greater than that is obtained in the presence of their respective oligosaccharides. To understand the effect of sizes of different saccharides, pH of the solution and also to explain the increased stabilization of proteins in the presence of mixture of monosaccharides, we introduced simple structural models based upon excluded volume theory. Since, increased stabilization of protein in the presence of osmolytes may bring the rigidity in protein that perturbed the functional activity of proteins. Hence, functional activity of lysozyme was measured in the presence of sugar osmolytes and developed a relation between functional activity and stability of protein in the presence of each sugar. This work will help to understand the mechanism behind osmolyte-induce stabilization of proteins and provide a mechanistic view about various aspects of osmolyte-induced protein stabilization such as shape and size, pH, and mixture of osmolytes.