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Effect of monomeric and oligomeric sugar osmolytes on ΔG_D, the Gibbs energy of stabilization of the protein at different pH values: Is the sum effect of monosaccharide individually additive in a mixture?

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ABSTRACT

Thermal denaturation curves of ribonuclease-A were measured by monitoring changes in the far-UV circular dichroism (CD) spectra in the presence of different concentrations of six sugars (glucose, fructose, galactose, sucrose, raffinose and stachyose) and mixture of monosaccharide constituents of each oligosaccharide at various pH values in the range of 6.0-2.0. These measurements gave values of T_m (midpoint of denaturation), ΔH_m (enthalpy change at T_m), ΔC_p (constant-pressure heat capacity change) under a given solvent condition. Using these values of ΔH_m, T_m and ΔC_p in appropriate thermodynamic relations, thermodynamic parameters at 25 °C, namely, ΔG^o_D (Gibbs energy change), ΔH^o_D (enthalpy change), and ΔS^o_D (entropy change) were determined at a given pH and concentration of each sugar (including its mixture of monosaccharide constituents). Our main conclusions are: (i) each sugar stabilizes the protein in terms of T_m and ΔG^o_D, and this stabilization is under enthalpic control, (ii) the protein stabilization by the oligosaccharide is significantly less than that by the equimolar concentration of the constituent monosaccharides, and (iii) the stabilization by monosaccharides in a mixture is fully additive. Furthermore, measurements of the far- and near-UV CD spectra suggested that secondary and tertiary structures of protein in their native and denatured states are not perturbed on the addition of sugars.

1. Introduction

When it needs to maintain the osmotic pressure of living cells, nature has (through evolutionary selection) opted to do this by incorporating a number of compounds known as osmolytes into the cells. It is remarkable that the small numbers of these compounds span cellular organisms, plants, and animal vertebrates and invertebrates [1–3]. These compounds comprise polyols, sugars, methylamines, amino acids and their derivatives, and in some cases urea in combination with methylamines [2]. Among these chemical categories, carbohydrates are usually dominant solutes accumulated in organisms to protect the proteins in terms of loss of activity [4,5] and chemical [6,7] and thermal denaturations [8–12]. They have also been found to be effective stabilizers of proteins and biological assemblies when added at high concentrations [13–18].

There are various mechanisms that have been used to explain the observation on the effect of sugars on the protein denaturation equilibrium, native (N) state ↔ denatured (D) state [19–23]. According to one mechanism sugars stabilize N state because they are preferentially excluded from the protein surface, for the preferential exclusion increases the chemical potential of the protein proportionately to the solvent exposed surface area. Thus, by Le Chatelier's principle, sugar osmolytes favor more compact state, i.e., the N state over the structurally expanded state, i.e., D state. Hence according to this mechanism ΔG_D, the Gibbs free energy change associated with the denaturation process, N state ↔ D state, should increase in the presence of osmolytes, for $\Delta G_D = -RT\ln([D]/[N])$, where square bracket represents concentration. According to the most recent mechanism of sugar osmolytes stabilization of proteins, Bolen and colleagues [24] used apparent water-to-osmolyte solution transfer free energies for side-chain and backbone models to interpret the increase in stability. They concluded that unfavorable interactions between the fully unfolded protein backbone and the osmolyte solution drive folding. That is, the decreased exposure of the backbone on folding is the major driving force for osmolyte-induced stabilization.

A few studies have reported the effect of these osmolytes, singly and in combination on the denaturation equilibrium of proteins [25 and Refs. therein, 26]. The main conclusion of these studies is that all osmolytes act independently on the protein, i.e., none of the osmolytes alters the efficacy of the other in forcing the protein to fold or unfold. However, yet it is difficult to predict the effects of mixture of osmolytes in protein

Abbreviations: ΔG_D, Gibbs free energy change; ΔG^o_D, Gibbs free energy change at 25 °C; ΔC_p, constant-pressure heat capacity change; T_m, midpoint of thermal denaturation; ΔH_m, enthalpy change at T_m; CD, circular dichroism; $[\theta]_{222}$, mean residue ellipticity at 222 nm; RNase-A, ribonuclease-A; Glc, glucose; Fru, fructose; Gal, galactose.

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stabilizing. To investigate the effects of mixture of osmolytes on protein, we have carried out measurements of thermal denaturation of RNase-A in the absence and presence of six sugars (glucose, fructose, galactose, sucrose, raffinose and stachyose) and mixtures of monosaccharide constituents of each oligosaccharide at different pH values in the range 6.0–2.0. In this paper, we report values of thermodynamic parameters, T_m (midpoint of thermal denaturation), ΔH_m (enthalpy change at T_m), ΔC_p (constant-pressure heat capacity change), and ΔG_D^o (value of ΔG_D at 25 °C) obtained from these measurements. It has been observed that an equimolar mixture of monosaccharide constituents has more effect on

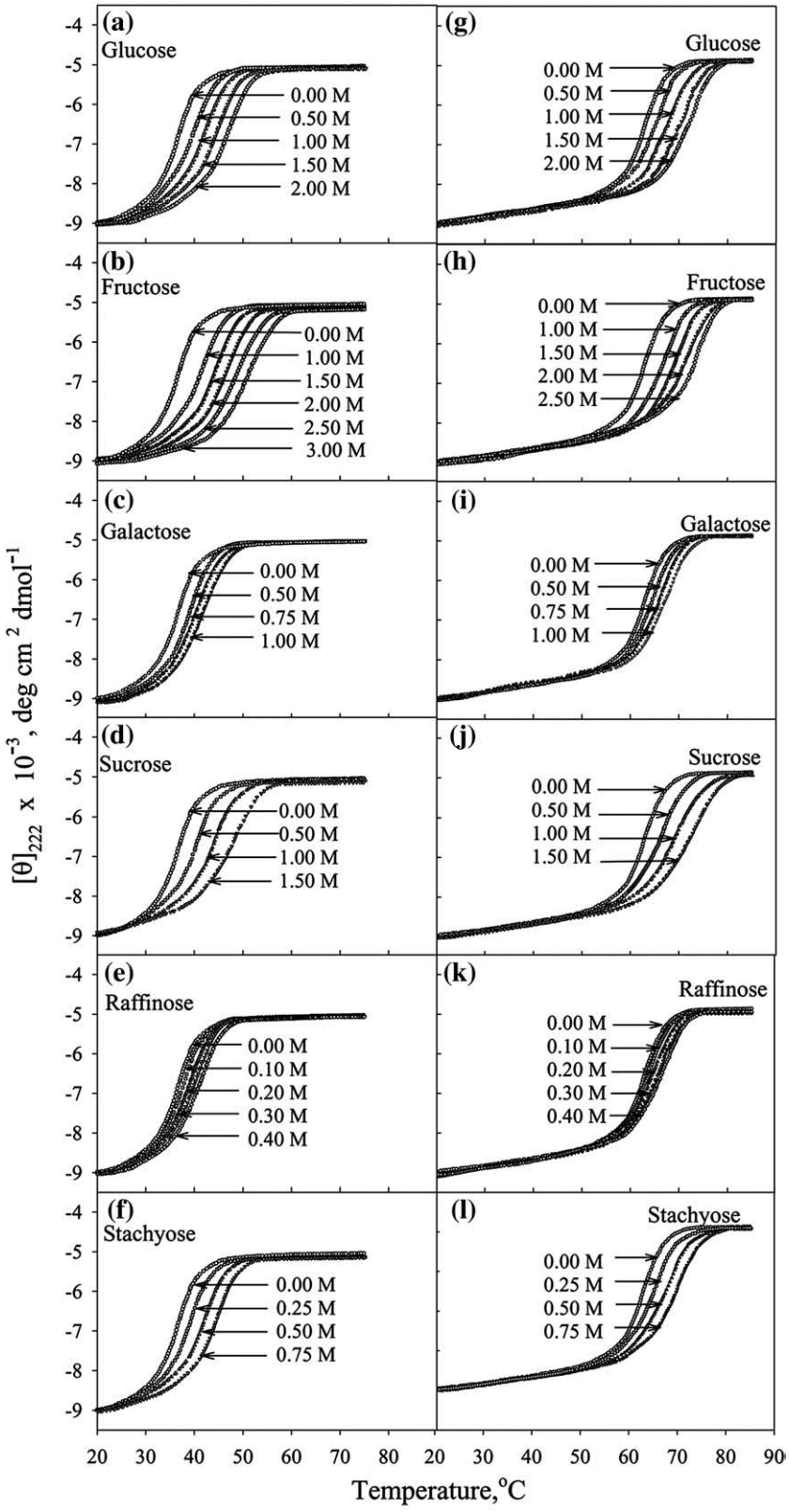

Fig. 1. Representative thermal denaturation profiles of RNase-A in the presence of different indicated concentrations of sugar(s) at pH 2.0 (a–f) and pH 6.0 (g–l). In order to maintain clarity all data points are not shown.