PROTEIN STABILIZATION BY POLYOLS: A THERMODYNAMIC STUDY OF PROTEIN FOLDING/UNFOLDING

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Thermal denaturation curves of lysozyme and ribonuclease-A were determined by measuring their far-UV circular dichroism (CD) spectra in the presence of different concentrations of five polyols (sorbitol, glycerol, mannitol, xylitol and adonitol) at various pH values in the range 7.0–1.9. The denaturation curve at each polyol concentration and pH was analyzed to obtain values of T_m (midpoint of denaturation) and ΔH_m (enthalpy change at T_m), and these ΔH_m and T_m values obtained at different pH values were used to obtain ΔC_p (constant-pressure heat capacity change) at each polyol concentration. Using values of ΔH_m, T_m and ΔC_p in the Gibbs-Helmholtz equation, ΔG_D^o (Gibbs energy change at 25 ^{o}C) was determined at a given pH and polyol concentration. Main conclusions of this study are that polyols have no significant effect on ΔG_D^o at pH 7.0, and they stabilise proteins in terms of ΔG_D^o against heat denaturation at lower pH values. Other conclusions of this study are: (i) T_m at each pH increases with increasing polyol concentration, (ii) ΔH_m remains, within experimental error, unperturbed in the presence of polyols, and (iii) ΔC_p depends on polyol concentration. Since the estimation of ΔG_D^o of proteins from heat-induced denaturation curves requires a large extrapolation, the reliability of this procedure for the estimation of ΔG_D^o is always questionable, and so are conclusions drawn from such studies. This led us to measure ΔG_D^o of ribonuclease-A and lysozyme using a more accurate method, i.e., from their isothermal (25 ^{o}C) guanidinium chloride (GdmCl)-induced denaturations. We show that our earlier conclusions drawn from heat-induced denaturation studies are correct. Since the extent of unfolding of heat- and GdmCl-induced denatured states of these proteins is not identical, the extent of stabilization of the proteins by polyols against heat and GdmCl denaturations may also differ. We report that in spite of the differences in the structural nature of the heat- and GdmCl-denatured states of each protein, the extent of stabilization by a polyol is same. We also report that the functional dependence of ΔG_D of proteins in the presence of polyols on denaturant concentration is linear through the full denaturant concentration range. Furthermore, polyols do not affect the secondary and tertiary structures of the native and GdmCl-denatured states. Furthermore, measurements of the far- and near-UV CD spectra suggested that secondary and tertiary structures of both proteins in their native and denatured states

are not perturbed on the addition of polyols.