Interfacial Properties and Conformational Equilibria of Heat-Stressed Proteins in Aqueous Systems

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An experimental study is presented on the thermal stability of globular proteins in the presence of water-miscible organic additives. The additives investigated include alcohols, glycols, sugars and amino acids. Ultraviolet difference spectroscopy was used to monitor the influence of these compounds on the protein's conformational changes induced by heating. Spectroscopic measurements performed at constant wavelength allowed for evaluation of the fraction of unfolded protein molecules as a function of temperature. The results obtained showed that the effect of the various additives on protein stability does not rely on any special property of these compounds but rather on their ability to perturb the surface tension of water. In particular, for all of the proteins examined the melting temperature of the protein was found to be linearly related to the bulk surface tension of the mixed solvent. To provide a theoretical framework to this result, a molecular thermodynamic model based on the additive-induced perturbation of the equilibrium between the folded and the unfolded states was developed. The model accounts for conformational changes, ideal mixing effects and interaction of the protein with the surrounding medium. It is shown that under particular limiting conditions the analytical relationships derived from the Gibbs equilibrium criterion correctly predict the observed dependence of the melting temperature on surface tension. This result suggests that the primary factor governing protein stability be the interfacial free energy between the macromolecule and the surrounding medium. Since the protein-solvent interface increases during denaturation, additives increasing the interfacial free energy should make the transition to the denatured form less thermodynamically favorable than in water, that is, stabilize the protein. The interfacial free energy can be expressed as the sum of two contributions: the bulk surface tension of the solvent and a residual term. The first of these is fully nonspecific, while the second is dependent on both the protein and the additive, being related to the free energy of adhesion. As long as the residual term can be considered temperature- and additive-independent, an increase in surface tension should cause a parallel increase in the interfacial free energy. Under these conditions the melting temperature of the protein should vary linearly with the bulk surface tension of the medium. The model proposed can be used for interpretation and correlation of thermal unfolding data. Moreover, useful indications can be derived for devising efficient strategies of thermostabilization by solvent engineering.