Effect of Crowding Agent on the Thermal Stability of Ribonuclease-A

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The process of protein refolding *in vitro* has been studied extensively as a mean of understanding how proteins fold inside cells. These experiments are, mainly for practical reasons, commonly carried out in simple buffer system of 20-50 mM with low concentrations of protein (~ 1-2 mg/ml) in order to avoid aggregation during the refolding reactions. A major difference between these idealized conditions and those encountered within cells is that the intracellular environment is highly crowded (300-400 g/l) due to the presence of high concentrations of soluble and insoluble macromolecules in the cytoplasm. This has major thermodynamic and kinetic consequences on the properties of macromolecules present in the cell. These effects can be orders of magnitude different from those in the typical dilute solution used to study proteins in vitro. Biochemical equilibrium in a living cell may be quite different from those under idealized conditions. It is therefore surprising that the effects of macromolecular crowding on protein refolding have been mostly neglected with a few exceptions. In this study we have created an artificially crowded environment around ribonulease-A (RNase-A) through the use of dextran, an inert artificial molecular crowding agent. We have used near-UV absorbance to study the effect of macromolecular crowding on the stability of the protein at pH 2.0. We found that the high concentration of dextran stabilizes the protein. We heated RNase-A at 287 nm in the presence of various concentrations of dextran. We found that there was no change in T_m on addition of dextran from 0-150 g/l. However, there was a slight increase in T_m, 2.3 °C and 3 °C, in the presence of 190 and 250 g/l of dextran, respectively. We found an increase of 5 °C of T_m at 350 g/l which happens to be *in vivo concentration* (300-400 g/l) of crowding agents in the cell. We propose that the high concentration of crowding agent plays an important role in the stability of proteins; however, further experiments are required to generalize the hypothesis.