Abstract

Proteins in their natural environment experience two phenomena which are absent in in vitro buffer solutions: crowding and confinement. On the one hand, proteins are surrounded by a multitude of different dissolved molecules, such as other proteins, nucleic acids as well as small organic and inorganic compounds (crowding). On the other hand, available space can be restricted to small pores by membranes (confinement). Crowding and confinement phenomena are commonly understood in terms of an volume exclusion effect. Cosolute molecules are either treated as inert molecules considering hard-core interactions only or by including chemical interactions between cosolute and protein. Chemical interactions involving the solvent are often neglected. This thesis tackles the question to what extent protein stability is affected by crowding and confinement with particularly focussing on the role of the solvent. For that purpose, protein stability is quantified as the free energy change of unfolding which is dissected into its enthalpic and entropic contributions to get mechanistic insights into the underlying protein-(co-)solvent interactions. In addition, protein stability data are complemented by measurements of hydration dynamics to focus on the role of the solvent. The combined results provide clear evidence that excluded volume effects are not the main driving force of protein stability in crowding and confinement. An understanding of protein stability in these systems requires a careful analysis of chemical interactions explicitly involving the solvent.

The results of this thesis are divided into five chapters (chapters 2.1-2.5). The first project describes the stability of of the globular protein ubiquitin in the presence of the artificial crowding agents dextran, poly(ethylene glycol) (PEG), glucose and potassium chloride. The thermal stability of ubiquitin in the various cosolute solutions was measured using far-ultraviolet circular dichroism (CD) spectroscopy and differential scanning calorimetry (DSC). In this project, the importance of the temperature dependence of the heat capacity change upon unfolding was identified in order to precisely estimate the excess enthalpy and entropy induced by the addition of the cosolute. In contrast to the predicted entropic stabilization by the established
excluded volume model, ubiquitin is stabilized through enthalpy rather than entropy indicating a possible role of interstitial hydration water molecules.

The second project describes the cosolvent effect of salts and ionic liquids on the stability of Ribonuclease A. The experimental results obtained from DSC measurements are discussed in the light of the classical Hofmeister series. A temperature dependence of the Hofmeister series was identified, which is capable of explaining many outliers of this series discussed in the literature. A comparison of the cosolvent effect of ions with the cosolvent effect of nonelectrolytes revealed an important role of the hydrophobicity of the cosolvent in the cosolvent-mediated protein (de-)stabilization. A water-mediated mechanism of water molecules hydrating these hydrophobic groups is presented.

In a third project the stability of the N-terminal Src homology 3 (SH3) domain of the Drosophila signal transduction protein drk encapsulated in cetyltrimethylammonium bromide reverse micelles and reverse micelles made from decyl-1-rac-glycerol plus lauryldimethylamine-N-oxide was measured by nuclear magnetic resonance spectroscopy. In contrast to expectation from hard-core excluded volume theory, encapsulation of SH3 in these two different types of ionic reverse micelles did not stabilize the protein entropically. For both reverse micelle systems tested, the excess enthalpy was stabilizing and the excess entropy destabilizing, indicating that other interactions dominate hard-core repulsions.

Measurements of hydration dynamics of water inside ionic reverse micelles using gigahertz and terahertz dielectric relaxation spectroscopy revealed a strong depolarization of the water pool. Despite of the low relative permittivity, this global depolarization is not the main driving force of protein stability inside reverse micelles. The dominating interactions are rather local interactions at the hydrated protein-surfactant interface.

In a last project, protein charge dependent stability measurements of SH3 in various cosolute solutions could identify a role of charged amino acids in the cosolute-mediated protein (de-)stabilization. Positively charged proteins appear to be more hydrophobic than negatively charged proteins. This gives rise to an increase of both the stabilizing excess enthalpy and the destabilizing excess entropy. A dissection of both into protein and solvent terms indicates that changes in (co-)solvent-(co-)solvent interactions play an important role to explain the differences of protein stability at positive and negative protein net charge.