COMPUTATIONAL STUDIES OF CO-SOLVENT EFFECTS ON PROTEIN STABILITY

By

Deepak R. Canchi

A Thesis Submitted to the Graduate

Faculty of Rensselaer Polytechnic Institute

in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: CHEMICAL ENGINEERING

Approved by the
Examining Committee:

_____
Prof. Angel E. García , Thesis Adviser

_____
Prof. George I. Makhatadze, Member

_____
Prof. Steven M. Cramer, Member

_____
Prof. Shekhar Garde, Member

_____
Prof. Patrick T. Underhill, Member

Rensselaer Polytechnic Institute
Troy, New York

November 2011
(For Graduation December 2011)

ABSTRACT

Proteins are biopolymers that fold into a well-defined, stable structure in solution, and the diverse array of functions carried out by proteins in cells is intimately connected to it's structure and dynamics. The stability of proteins is marginal, and the folding/unfolding equilibrium of proteins in solution can be altered by the addition of small organic compounds called co-solvents. Co-solvents that shift the equilibrium towards the folded state are known as protecting osmolytes while those that favor the unfolded states are termed denaturants. Understanding the molecular mechanism of these phenomena is a problem of great fundamental interest, with potential applications in biotechnology.

Urea is a commonly used chemical denaturant in protein folding studies, the molecular mechanism of its denaturing ability has been a subject of considerable debate. Previous Molecular Dynamics (MD) simulation studies have sought to elucidate the mechanism of urea denaturation by focusing on the pathway of denaturation rather than examine the effect of urea on the folding/unfolding equilibrium, which is commonly measured in experiment. We have simulated the reversible folding/unfolding equilibrium of a model system - the Trp-cage miniprotein - in presence of urea and over a broad range of urea concentrations, using all-atom Replica exchange MD (REMD) simulations. The simulations capture the experimentally observed linear dependence of the unfolding free energy on urea concentration. We find that the denaturation is driven by favorable direct interaction of urea with the protein through both electrostatic and van der Waals forces, with the contribution of the van der Waals interaction being larger. We also find that hydrogen bonding of urea to peptide backbone does not play a dominant role in denaturation. Increasing urea concentration favors conformations with larger solvent exposure in the unfolded ensemble, in agreement with theoretical predictions and FRET experiments. Fitting the equilibrium data to a Hawley-like free energy surface, we find that the process is governed by enthalpy and predict the m-value to increase with temperature, and more strongly so with pressure.

The interaction of urea with various protein moieties has been studied experimentally through various approaches, but there is no consensus on the contribution of these

groups to the free energy of unfolding in presence of urea. We have reported the first computation of the changes in preferential interaction coefficient of the protein upon urea denaturation to examine the contribution from the backbone and the sidechain groups in the process. The preferential interaction coefficient, an experimentally measured quantity, is obtained from extensive REMD simulations of Trp-cage in presence of urea. The increase in preferential interaction upon unfolding is dominated by the side-chain contribution, rather than the backbone. Similar trends are observed in simulations using two different force fields, Amber94 and Amber99sb, for the protein. The magnitudes of the side-chain and backbone contributions differ in the two force fields, despite containing identical protein-solvent interaction terms. The differences arise from the unfolded ensembles sampled, with Amber99sb favoring conformations with larger surface area and lower helical content. These results emphasize the importance of the side-chain interactions with urea in protein denaturation, and highlight the dependence of the computed driving forces on the unfolded ensemble sampled. Our results are in contrast to the widely held view that urea destabilizes proteins mainly by forming hydrogen bonds with the protein backbone.

Despite its denaturing effect on proteins, many marine organisms accumulate urea to counter environmental stress. They simultaneously build up concentration of a 'protecting osmolyte', Trimethylamine-N-oxide (TMAO), to offset the effect of urea on protein structure, typically in a 2:1 concentration ratio of urea and TMAO. Since current MD forcefields for TMAO unable to capture this phenomenon, we have developed a new model for TMAO using a Kirkwood-Buff inspired approach. This was achieved by matching the osmotic pressure of TMAO solutions and urea-TMAO solutions computed from simulations to the experimental measurement. The new model captures the experimentally observed preferential exclusion of TMAO from the protein surfaces and is expected to enable detailed simulations of the protecting effect of TMAO on protein stability. This study also provides insight into the connection between osmolyte-osmolyte interaction and the preferential interaction of osmolytes with proteins.