Commun. Comput. Phys.
doi: 10.4208/cicp.290711.121011s

Vol. **13**, No. 1, pp. 90-106
January 2013

Modeling the Influence of Salt on the Hydrophobic Effect and Protein Fold Stability

Mihir S. Date and Brian N. Dominy*

Department of Chemistry, Clemson University, Clemson SC 29631, USA.

Received 29 July 2011; Accepted (in revised version) 12 October 2011

Available online 12 June 2012

Abstract. Salt influences protein stability through electrostatic mechanisms as well as through nonpolar Hofmeister effects. In the present work, a continuum solvation based model is developed to explore the impact of salt on protein stability. This model relies on a traditional Poisson-Boltzmann (PB) term to describe the polar or electrostatic effects of salt, and a surface area dependent term containing a salt concentration dependent microscopic surface tension function to capture the non-polar Hofmeister effects. The model is first validated against a series of cold-shock protein variants whose salt-dependent protein fold stability profiles have been previously determined experimentally. The approach is then applied to HIV-1 protease in order to explain an experimentally observed enhancement in stability and activity at high (1M) NaCl concentration. The inclusion of the salt-dependent non-polar term brings the model into quantitative agreement with experiment, and provides the basis for further studies into the impact of ionic strength on protein structure, function, and evolution.

PACS: 87.15.K-, 87.15.-v

Key words: Electrostatic stability, hydrophobic effect, halophile, cold shock protein, HIV-1 protease.

1 Introduction

It has long been understood that salts have a significant impact on the stability and activity of proteins and nucleic acids, which constitute a foundation underlying cellular function. The intracellular and extra cellular salt concentration varies with the organism and environment, but fluctuates typically in the range of 100-200 mmol/L for organisms living within physiological conditions consistent with mesophiles. Salts can significantly influence the stability of biomolecules by screening electrostatic interactions. For example, the repulsion between negative charges on the phosphate backbone of nucleic acids

*Corresponding author. *Email addresses:* mdate@clemson.edu (M. S. Date), dominy@clemson.edu (B. N. Dominy)

is screened by salt, contributing to the stability of biologically relevant DNA and RNA conformations [1]. The effect of salts on proteins has been demonstrated using numerous systems including multimeric complexes that have been shown to disintegrate into separate monomers upon changes in the environmental salt concentration. Some proteins within halophilic organisms have even adapted to function specifically within high salinity environments, destabilizing under physiological salt concentrations consistent with mesophilic environments [2, 3]. The development of accurate physical models describing the thermodynamic impact of salts on macromolecules could lead to a broader understanding of biomolecular structure and function.

Within a cellular environment, biomolecules are solvated in an aqueous environment containing salt ions and numerous other solutes. The influence of ions on biomolecular interactions is mediated through electrostatic screening, site-specific binding, and preferential hydrophobic effects or Hofmeister effects. The role of salt concentration in protein stability can be determined by measuring the unfolding transition as a function of salt concentration, but interpreting the mechanism of action is a non-trivial problem. Some ions stabilize proteins by binding to specific sites. This ligand-induced ion-specific stabilization is usually observed below 0.2 mol/L ionic strength [4]. Bulk ionic strength results in the screening of surface charge-charge interactions primarily at lower salt concentrations. Hofmeister effects, which are dominant at higher salt concentrations, strengthen the hydrophobic effect by increasing the surface tension of the solvent, or by stabilizing peptide dipoles through specific ionic interactions [5]. Theoretical modeling can provide a basis from which the different mechanisms associated with salt effects may be assessed and compared.

The electrostatic screening effect (and the effect on self polarization energies), primarily related to bulk ionic strength, can be studied through continuum electrostatic models such as those based on a Poisson-Boltzmann (PB) formalism [6,7]. The PB equation can be used to describe the electrostatic potential from the reaction field of a system containing a solute with a fixed charge distribution and a surrounding mobile charge distribution representing the salt. Mobile charges are modeled by a Boltzmann distribution with respect to the electrostatic potential generated by the fixed charges of the solute [8]

$$\begin{aligned}&\vec{\nabla}\cdot[\epsilon(\vec{r})\vec{\nabla}\varphi(\vec{r})]\\&=-4\pi p^f(\vec{r})-4\pi\sum_i c_i^\infty Z_i\lambda(\vec{r})\cdot e^{-Z_i\varphi(\vec{r})/\kappa_\beta T}.\end{aligned}\tag{1.1}$$

Eq. (1.1) is the Poisson-Boltzmann equation, which describes electrostatic interactions between solute and solvent molecules where p^f is charge density of fixed charges. c_i^∞ is the concentration of ion i and Z_i is the charge of the ion. The second term on right hand side represents mobile charges typically restricted to the solvent region. It contains the ionic strength and charge density associated with the mobile charges. The distribution of mobile charges around solute's fixed charges is modeled by the Boltzmann factor ($e^{-Z_i q\varphi(\vec{r})/\kappa_\beta T}$). This special case of Debye-Hückel theory with a 1:1 electrolyte accounts