Protein Folding at Constant pH: Salt Concentration and pH Changing Protein Stability

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***Short Abstract* — The understanding of electrostatic interactions in protein folding process and, in native state stability is important to molecular biophysics area. The charge interactions are affected by changes in pH and salt concentration of the environment which may alter the protein stability. Here it is implemented the Structure-Based Models with a Constant pH Molecular Dynamics (SBM-CpHMD) to investigate the protein folding in different pH and salt concentration conditions. The simulations present qualitative and quantitative results that agree with experimental data and these studies help in the understanding of pH and salt dependence effects in protein stability.**

***Keywords* — Constant pH Molecular Dynamics, Structure-Based Models, pH Dependence, Ionic Strength**

I. Introduction

An understanding of factors which affect the stability and the folding of proteins is of fundamental importance in the comprehension of principles that govern the behavior of these macromolecules as well as in the development of biotechnological applications. Charged residues may play an essential role in protein stability and function, especially when they are related to binding site recognition. Electrostatic interactions during the protein folding process have been extensively investigated by experiments and theory. pH and ionic strength play a central role in these electrostatic interactions, as well as in the arrangement of ionizable residues in each protein-folding stage.

Most of the computational studies a fixed charge has been adopted to investigate the contribution of the electrostatic interaction. In biological systems, The charge distribution in a protein may change following the protonation/deprotonation of the charged residues based on the ionic strength and the pH of the environment.

In this study, a Molecular Dynamics simulation with Constant pH was implemented in the Structure-Based Model (CpHMD-SBM) to investigate the pH and Salt concentration effects on the folding of different proteins[1–3].

II. Methods

The proteins were modeled using a Structure-based model (SBM) in which the protein is coarse-grained in a Cα atom level of simplification. The residues are represented by beads in α carbon positions, and the Hamiltonian that gives the protein energy interaction is based on the geometry of its native state[4].

The electrostatic interactions were introduced through the inclusion of point charges at the center of the beads representing basic/acidic residues and treating the electrolyte solution according to the Debye-Hückel theory. Thus, a Coulomb screening potential gave the charge-charge interaction[5].

The constant-pH molecular dynamic method (CpHMD) adopted in this study combines a standard molecular dynamics simulation with the Metropolis Monte Carlo method for sampling protein protonation states[6]. Basically, at each given number of molecular dynamics steps, a titratable residue is randomly chosen, and the changing of its protonation state is accepted or rejected according to the Metropolis criterion.

III. Conclusion

The simulations were performed for different proteins capturing the essential features of the pH and salt concentration dependence. The protein stability calculation shows a good agreement between simulations and experiments.

The implementation of CpHMD-SBM reveals to be a powerful tool which helps in the evaluation of salt and pH effects throughout the folding process.

IV. References

[1] Coronado MA et al. Cold Shock Protein A from Corynebacterium pseudotuberculosis: Role of Electrostatic Forces in the Stability of the Secondary Structure. Protein Pept Lett. 2017;24: 358–367.

[2] Contessoto VG and Oliveira VM et al.. NTL9 Folding at Constant pH: The Importance of Electrostatic Interaction and pH Dependence. J Chem Theory Comput. 2016;12: 3270–3277.

[3] Oliveira VM and Contessoto VG et al. Effects of pH and Salt Concentration on Stability of a Protein G Variant Using Coarse-Grained Models. Biophys J. 2018;114: 65–75.

[4] Clementi C et al.. Topological and energetic factors: what determines the structural details of the transition state ensemble and “en-route” intermediates for protein folding? An investigation for small globular proteins. J Mol Biol. 2000;298: 937–953.

[5] Azia A, Levy Y. Nonnative electrostatic interactions can modulate protein folding: molecular dynamics with a grain of salt. J Mol Biol. 2009;393: 527–542.

[6] Baptista AM et al.. Constant-pH molecular dynamics using stochastic titration. J Chem Phys. 2002;117: 4184–4200.

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