Accurate pKa Calculation of Titrable Groups in Ensembles of Protein Conformations with Application to pH Dependent Unfolding

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pH-dependent protein conformational change or structure unfolding occur in a variety of mechanisms of protein functioning. We have recently described such pH-dependent structure transformation of a water-soluble form of the Diphtheria Toxin Translocation (T) domain [1]. Acidification of the environment solution leads to the diphtheria toxin conformational reorganization and insertion into a cellular membrane. Partial unfolding and refolding of the T-domain in water with protonated histidine residues was observed in microsecond-long molecular dynamics simulations and supported by experiments [1]. Whether a specific protonatable residue drives modification of the structure depends on the difference in pKa values of such group in different conformations of a protein (for example, in a folded and unfolded state). Even in a stable protein that remains in a single conformation, fluctuations of the structure near equilibrium may affect a pKa. In order to estimate pKa values of the residues in different protein conformations it is necessary to account for structural fluctuations. In this work, Free Energy Perturbation (FEP) method using AMBER with the AMBER99SB force-field was implemented and used for calculating free energy change due to protonation of the titrable groups for proteins in different conformations. Accurate calculations of the method convergence and selection of the reference compounds will also be presented. We calculated the pKas of the histidine groups for different conformations that occur during the T-domain unfolding in MD simulations. We will also present calculated pKas of histidines in several pH-stable proteins and compare our results with experimental data.

1. Kurnikov, I. V., Kyrychenko, A., Flores-Canales, J., et al. (2013) pH-Triggered Conformational Switching of the Diphtheria Toxin T-Domain: The Roles of N-Terminal Histidines. Journal of Molecular Biology 425, 2752-2764