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Computational investigation of the interaction of denaturing and protecting agents on folded and unfolded proteins

Hishara Kesani Gallage and Samantha Weerasinghe*

Department of Chemistry, University of Colombo, Colombo 00300, Sri Lanka

Proteins are the most abundant biological macro molecules that show great diversity of functions from differentiation of cells to crucial enzymatic reactions. The unique three dimensional structure of proteins lead to specific biochemical functions. Oxygen binding protein like hemoglobin, specific enzymes that catalyze thousands of reactions, milk proteins, feathers, and genetic expression are a few functional roles of them. The wide range of functional groups and specific 3D structures of proteins are some key features that lead to this wide range of functions. Unfolding of proteins could change their functionality. A denaturing agent, urea, unfolds proteins, while glycine betaine (GB), a protecting agent, resists the unfolding of proteins. To elucidate binding modes of urea and GB with a protein, molecular dynamics (MD) studies were conducted for the RNA binding domain 2 protein (PDB ID: 2U1A) in aqueous urea and aqueous GB solutions of different concentrations. First, 2U1A was unfolded with MD at high temperature. After that, a series of MD simulations were conducted with native and the unfolded protein separately with different concentrations of urea and GB in aqueous medium using GROMACS software package. The interaction patterns were investigated in terms of preferential interaction parameter (PIP), root mean square deviations (RMSD), radius of gyration (RG), interaction energy and solvent accessible surface area (SASA). PIP values are given in the Table below

	Concentration (mol/dm^3)	PIP of proteins	
		Native	Unfolded
Urea	1.20	-4.20	5.69
Urea	2.30	5.51	9.38
Urea	3.60	5.53	14.76
Urea	8.00	-0.33	8.02
GB	1.28	-1.03	-1.83
GB	1.93	-2.91	-3.57

Results show enrichment of urea and depletion of water from all the concentrations in unfolded state and moderate concentrations in folded state. The magnitude of affinity of urea is higher for unfolded state. The exclusion behavior ofGB from the protein backbone is prominent in this study. It is interesting to note that protein-GB interaction is stronger with unfolded protein than with folded protein.It is assumed that the folded state needs not to be protected while the unfolded protein may be turning towards the folded state with relatively stronger interaction with GB. The hydrophobic SASA of the unfolded protein in GB solution showed some reduction of SASA with increasing GB concentration. This may be due to the folding up of the protein in GB solution. These observations have been further strengthened by the reduction of Rg of the unfolded protein with increasing GB concentration.

weera@chem.cmb.ac.lk +94 112503367