J Proteomics Bioinform 2017, 10:10 (Suppl)
DOI: 10.4172/0974-276X-C1-104

Stabilization of proteins by co-solute engineering: Soft interactions vs volume exclusion

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The macromolecular crowding is gaining attention in recent years as it acts as a sword with double-edge on protein stability and folding, i.e., showing assorted results of having both stabilizing and destabilizing effects. Organisms acclimate in extreme stress conditions by accumulating small molecules called osmolytes, which induces stabilization in proteins. We investigated the role of sugar osmolytes on structure, function and stability of proteins. The excluded volume of osmolytes varied with shape and size of osmolyte. Most of the studies to understand protein folding have been done in dilute solutions. However, the cellular environment is crowded with macromolecules of different sizes, shapes and compositions, such as DNA, RNA, proteins, ribosomes and cytoskeletal elements. To understand the consequences of such crowded environment, we investigated the effect of macromolecular crowding on the stability and activity of hen egg white lysozyme and ribonuclease A. The results indicate that owing to volume exclusion, proteins are stabilized; however, the stabilization of the protein is more at lower pH where the effect of exclusion is more. Most of the studies have demonstrated that volume exclusion plays major role in crowded environment, however, we also investigated the role of soft interactions, in the case of poly ethylene glycols of high molecular mass. The tertiary structure of myoglobin was perturbed in the presence of polyethylene glycol, whereas, the secondary structural content remained constant. It was observed that polyethylene glycol induces molten globule state in myoglobin. Moreover, ITC showed strong binding between myoglobin and PEG at the physiological pH. We have taken care while binding of protein with crowder and other soft interactions need to be gravely well thought-out when studying macromolecular crowding.