Osmolyte solutions and protein folding

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Abstract

In this brief review we discuss the evolution of recent thought regarding the role and mechanism of osmolytes with respect to protein stability. Osmolytes are naturally occurring intracellular compounds that change the protein folding landscape. Contributions from experiments are considered in the context of current theory and simulation results.

Introduction and context

The driving forces and resulting pathways governing the transition of proteins from their unfolded state to their native state remain unanswered fundamental questions for biophysics. As the misfolding of proteins has been associated with a number of diseases [1,2], the understanding of the folding process has important potential therapeutic implications. While a great deal of experimentation has been, and still needs to be, done, there is an increasingly important role for theory and computation to provide unique insight into the reversible process of folding as it may occur in the cell.

Nature rarely uses temperature to stabilize or renature proteins; most commonly, biological systems utilize concentration changes of various intracellular components, including electrolytes and osmolytes. A recurring issue is whether such components of the solution affect the proteins directly or indirectly [3]. By this we mean, whether the components of the solution serve to change only the global properties of the solution and thus the proteins within it, or whether they directly interact with the proteins through local correlations. Recent advances in our theoretical descriptions of multicomponent aqueous solutions and their effects on proteins are changing the way we understand such systems [4-7]. Complementary to theory is the use of computer simulations, which brings its own vivid picture about

such solutions into focus [8-14]. We will discuss how such techniques are leading to testable hypotheses in this area.

Major recent advances

In order to accurately describe protein folding in the native environment of the cellular milieu, we should take into account all of the possible interactions between all of the possible molecular species. This is not feasible at present. Yet, fundamentally we understand that because of different solubilities and binding affinities these systems may only accurately be modeled as thermodynamically non-ideal solutions. That is, they are neither dilute nor ideal solutions. By recognizing the importance of the influence of cosolvents on protein stability, a more accurate understanding of protein folding is being achieved. Recent computational and theoretical descriptions of non-ideal behavior have begun to provide accurate descriptions of experimentally observed behaviors of such systems [4,10,15].

A specific class of cosolvents known as osmolytes is particularly interesting because of their usage as protein stabilizers in all kingdoms of life [16]. Such compounds play a role in regulating the cell volume in the presence of stresses like high external salinity, extreme temperatures, and desiccation. There are two classes of osmolytes, delineated by their effect on protein

stability: protecting osmolytes and denaturing osmolytes. Protecting osmolytes are known to increase protein stability, whereas denaturing osmolytes shift the reversible process of folding towards the unfolded state. The exact mechanisms by which osmolytes affect protein stability, direct or indirect, are a matter of current debate [3,17].

Knowledge gained by experimentally studying the effects of osmolytes on proteins have provided an emerging paradigm-shift in the understanding of protein folding [18]. Thermodynamic measurements carried out on proteins and osmolyte aqueous solutions are consistent with the notion that the driving force behind osmolyte activity is the interactions of the osmolyte with the peptide backbone [19,20]. This is in contrast to the view that Tanford has left us with [21,22]. His famous decomposition of the thermodynamics of protein stability (transfer free energies) into backbone and side chain contributions was important pedagogically, but the results as a function of concentration were not activity corrected and mislead one into thinking that the side chains are a dominating driver [21].

We now understand quantitatively that osmolyte-induced stability is brought about by unfavorable correlations with the protein backbone (the osmophobic effect), whereas denaturation occurs by favorable interactions of the osmolyte with the protein backbone [23]. The result is a de-emphasis of the role of the amino side chains in the folding collapse, and a refocusing on the importance of the protein backbone for protein folding, as envisioned at the onset of protein structural biophysics [24]. Simulations show a striking correlation with this emerging view [3,8,13].

Though the relatively small binding constants associated with the interaction of osmolytes and proteins [25] initially obscured the mechanisms behind osmolytes and protein stability, it is clear that framing the effect of osmolytes on protein folding as purely a change in water structure is far too simple [9,26]. This model, whereby osmolytes affect protein stability by altering local water structure, does not take into account binding or exclusionary events that are known to occur. Therefore, once these interactions are explicitly considered, the mechanisms for osmolyte-affected protein stability become consistent with the known data.

Among denaturants, urea is special; it is the only molecule belonging to the class of biological denaturing organic osmolytes. Interestingly, despite the denaturing effects of urea, it is still accumulated in high concentrations in several species [27]; for instance, in mammalian kidneys [28,29], marine elasmobranchs [28] and

amphibians [28]. The protein destabilizing ability of urea is well established, however, the mechanism by which urea denatures proteins is still debated [8-10,13]. As mentioned previously, there are two competing prevailing mechanisms by which urea is viewed to denature proteins: the direct and indirect mechanisms. The direct mechanism states that urea directly interacts with the protein, thereby destabilizing the native state. The indirect mechanism involves a weakening of the protein stabilizing hydrophobic effect caused by a decrease in the structure of water. Whether the action is direct or indirect, a coupled aspect of this problem is whether the action of the solvent is on the entire protein, predominantly on the side chains, or predominantly on the backbone. Recent data suggest that the mechanism for urea denaturation is due to its direct interaction with the protein backbone [30-32]. Some authors continue to regard the side chains as equally important [3,13], although the activity-corrected data do not appear to support that idea [30,31].

Mounting evidence from theoretical, simulation, and experimental studies have demonstrated that urea does not appreciably perturb water structure, contradicting the notion that urea denaturation is caused by a weakening of the water structure, thus disturbing the hydrophobic effect [33]. Some have suggested that water is the initial denaturant of proteins [34]. However, a recent theoretical analysis has proved that this cannot be the mechanism by which denaturing osmolytes unfold proteins [4,5]. Using a rigorous framework, equations for protein stability and chemical activity are found to be completely devoid of terms describing water self-hydration [5]; water structure is not part of the picture. This clearly indicates that the fundamental origin of protein stability effects by osmolytes is not dictated by the minor changes in the structure of water seen in some simulations [5].

Future directions

Knowledge gained by studying the interactions between osmolytes and proteins have provided an impetus for a paradigm shift in the understanding of protein folding [18]. This change in thinking is due in part to new theoretical tools [7] for re-analyzing both new [30] and venerable [21] experimental data. It yields a mechanistic picture that now must be further tested in its ability to predict protein folding-related data. This picture has other consequences. Protein folding stability is coupled to ligand binding equilibria, which are notoriously sensitive to solution conditions. Understanding the influence of osmolyte action on binding may enable the design of solution conditions that better mimic the conditions within cells, or even specific organelles, to

better determine *in vivo* binding constants of drug candidates to their target proteins.

Competing interests

The authors declare that they have no competing interests.

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