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Mini-Review

Conformational Features and Hydration Dynamics of Proteins in Cosolvents: A Perspective from Computational Approaches

Rabiul Gazi, Sankar Maity, and Madhurima Jana*

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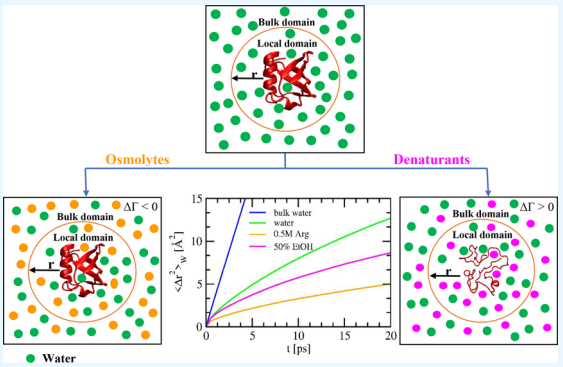
ABSTRACT: The importance of solvent in stabilizing protein structures has long been recognized. Water is the common solvent for proteins, and hydration is elemental in governing protein stability, flexibility, and function through various interactions. The addition of small organic molecules known as cosolvents may deploy stabilization (folding) or destabilization (unfolding) effects on native protein conformations. Despite exhaustive literature, the molecular mechanism by which cosolvents regulate protein conformations and dynamics is controversial. Specifically, the cosolvent behavior has been unpredictable with the nature and concentrations that lead to protein stabilizing/destabilizing effects as it changes in water content near the vicinity of proteins. With the massive development of computational resources, advancement of computational methods, and the availability of numerous experimental techniques, various theoretical and computational studies of proteins in a mixture of solvents have been instigated. The growing interest in such studies has been to unravel the underlying mechanism of protein folding and cosolvent/solvent−protein interactions that have significant implications in biomedical and biotechnological applications. In this mini-review, apart from the brief overview of important theories and force-field model-based cosolvent effects on proteins, we present the current state of knowledge and recent advances in the field to describe cosolvent-guided conformational features of proteins and hydration dynamics from computational approaches. The mini-review further explains the mechanistic details of protein stability in various popularly used cosolvents, including limitations of present studies and future outlooks. The counteracting effects of cosolvent on the proteins in the mixture of stabilizing and destabilizing cosolvents are also presented and discussed.



1. INTRODUCTION

Solvent effects direct the conformational stability and biological functionality of proteins. Within a certain range of thermodynamic states, i.e., temperature, pressure, solvent chemical potential, etc., proteins perform their biological activities. Further, the cellular processes are generally driven by the change in the solvent environment, e.g., the change in pH, ionic activity, etc. Besides, concentrations of solvents and small solutes present in cellular compartments steered the cellular functions. Water that remains within the small distance (∼5 Å) from the proteins' surface forms a thin layer of water well-known as the hydration layer. This water continuously exchanges with the bulk layer and maintains the structural integrity and dynamics of proteins. The hydration layer fluctuation governs the small-scale motions, and the large-scale motions are regulated by the fluctuations of bulk water.[1]

The presence of cosolvents in protein solution impart stabilization (folding) or destabilization (unfolding) effects on native protein conformations.[2−7] Cosolvents are generally small organic molecules whose addition affects the dynamics, stability, and solubility of proteins.[8] The folding equilibrium of proteins can be altered, and the equilibrium can be favored in either direction depending upon the cosolvent used. The behavior of the cosolvents becomes unpredictable based on their nature and concentrations; as a result, a change in water content around the protein surface leads to protein stabilizing/destabilizing effects. In order to elucidate conformational stability of proteins and its hydration dynamics in cosolvents, one has to look for the cosolvent abundance at the protein surface. Several experimental methods (not covered within this mini-review) determined the population of cosolvents around the protein surface; however, the microscopic measurement of the phenomenon is not straightforward and is model-dependent. Further, there is no general unifying theory in this regard. The molecular picture of conformational fluctuations, interactions, and hydration layer dynamics of

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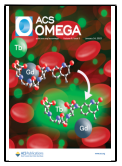

proteins in cosolvents can be obtained from computational studies. Computational approaches often provide significant information toward understanding the solvation phenomenon of a protein in solvent mixtures and the conformational preferences and associated time scales.

Water was the first solvent that was studied using computational techniques, and it has been used as a common solvent for computer simulations of biomolecules, including proteins. Different models of other solvents exist; however, studies of protein in mixed solvents are limited compared to those in pure water due to the complexity of modeling a mixture of solvents and the involvement of more computational power. During the last few decades, with the advancement of computational methods and massive development of parallel computation, many theoretical and computational studies of proteins in a mixture of different solvents have been instigated. The growing interest in such studies is to unravel the underlying mechanism of folding, change in proteins' conformations and structure, dynamical and functional properties, and cosolvent/solvent−protein interactions that have significant implications in biomedical and biotechnological applications.

The conformational stability of proteins involves entropic and enthalpic contributions from various parts of the systems that favor the folded form of the protein.[9] The free energy changes between the folded and denatured state of proteins are minimal. The nature of interactions between protein and solvent and the contributions arising from them on the overall free energy of folded and unfolded states of proteins depends on the solvent environment. Since protein flexibility and conformational stability depend on the properties of hydration water, it is obvious that cosolvents are likely to change the structure and dynamics of hydration water and alter the stabilization. The classes of solvent molecules that can stabilize the folded form of a protein structure are commonly known as osmolytes. Such solvents regulate osmotic balance in the cell and protect proteins even in harsh environmental conditions. On the other hand, a group of small organic molecules that induce a disruption in protein structure are called as denaturants. Apart from these, based on the action of cosolvents on the water structure, another two different classes known as chaotropes and kosmotropes exist. Those that instigate disorders in water structure are commonly known as chaotropes, and those that induce order in water are kosmotropes. The differential nature of water structure affects the protein conformation and properties.

In this mini-review, we discuss recent computational studies directed toward proteins' structure, dynamics, and hydration behavior in binary or ternary mixtures. The aim has been to understand the microscopic role of cosolvents in guiding proteins' conformational features through various interactions and the hydration properties of proteins in the presence of them. Many studies attempted to explore the mechanistic details of stabilizers with small solutes in cosolvents and extrapolated those effects to the relevance of proteins; however, the present mini-review does not cover such studies as the existing literature on the stability of proteins in cosolvents is colossal. Further, protein's conformational features and hydration dynamics are well-documented in pure water. However, in most of the existing studies, the protein's conformational features, highlighting its conformational flexibilities, structural dynamics, and, specifically, the dynamics of the hydration layer of proteins in the presence of cosolvents are addressed less. Here, we tried to discuss some of the recent advances, key issues, and findings over the last 15 years in this area. We focus on the effects of some of the popularly used osmolytes and denaturants to uncover the molecular basis of the fluctuation in protein conformations and hydration dynamics through computational approaches. This mini-review also covers some of the important statistical mechanics based theories of solutions that describe thermodynamics of protein−cosolvent interactions and the current state of knowledge. However, the main focus has been on important findings on proteins' structure, dynamics, interactions, and hydration properties in water−cosolvent mixed solutions as obtained from computational approaches.

2. PLAUSIBLE MECHANISM AND THEORIES OF PROTEIN−COSOLVENT INTERACTIONS

Protein−cosolvent interactions are generally explained through (i) direct interactions or (ii) indirect interactions mechanisms.[2,10] Apart from these, the concept of combined direct−indirect interactions exists. In general, the chemically heterogeneous nature of the protein backbone and amino acid side chains dictates the extent of hydrogen-bonded interactions between intraprotein and protein−cosolvent. According to the direct interaction mechanism, when proteins interact with the cosolvents through hydrogen bonds, it is believed that the protein−cosolvent hydrogen bonds compete with the intraprotein hydrogen bonds that are responsible for the stability of the secondary structural motifs of the proteins. Depending upon the nature and strength of such competitive hydrogen-bonded interactions conformational changes in protein structures occur. In the indirect interactions, cosolvents alter the hydration properties of a protein by perturbing the hydration layer and release water from the protein surface to establish direct interactions with the cosolvent.

The thermodynamics of protein−water−cosolvent interactions can be acquired from different theories based on statistical mechanical approaches. Specifically, a detailed description of the protein folding/unfolding equilibria in cosolvent can be obtained through the Kirkwood−Buff (KB) theory of solutions.[11,12] For multicomponent solutions, using the KB method, it is possible to relate different experimentally derived quantities such as compressability, partial molar volumes, chemical potentials and osmotic pressures with the integrals of the radial distribution functions (g_{ij}) of several types of molecular pairs present in the solution. The KB integrals in terms of the corresponding radial distribution function can be expressed as $\approx 4\pi \int_0^{R_c} [g_{ij}^{NPT}(r) - 1] r^2 dr$, where R_c is the distance which defined correlation region, within which the local cosolvent and solvent density around the species of interest differs from the bulk density.[12] As per the integral correlation region exists within which the local cosolvent and solvent density around the species of interest differs from the bulk density. Beyond the correlation region, all $g_{ij}(r) \approx 1$. The correlation region can extend over many molecular solvation shells and therefore provides a potentially different representation of the cosolvent effect interrogating that the binding is usually limited to the protein surface. As the integral is directly related to molecular distributions, a series of models, each providing different approximations to the real KBIs can be developed to determine the resulting thermodynamic effects. Another thermodynamic framework that has been developed to relate the conformational stability of proteins with the preferences of the solvent of the protein

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surface is known as "preferential interaction theory."[2,13] The chemical potential of protein systems is perturbed in the presence of cosolvents due to their strong or weak association with the protein than water. Such interactions provide information on the relative preferences of the protein surface toward water or cosolvent. According to the concept, the local concentration of the ratio between cosolvent and water with respect to bulk is greater, smaller, or equal implying preferential solvation, preferential hydration, and neutral solvation, respectively. When a cosolvent, C, is added to an aqueous protein solution, it alters the chemical potential of the protein and the transfer free energy ($\Delta\mu_P^{tr}$) from pure water to the mixed solvent system occurs which can be expressed as

$$\Delta\mu_P^{tr} = \Delta\mu_P^{tr} = \int_0^{m_C}\left(\frac{\partial\mu_P}{\partial m_C}\right)_{m_P} dm_C = -\int_0^{m_C}\left(\frac{\partial\mu_C}{\partial m_C}\right)_{m_P}\left(\frac{\partial m_C}{\partial m_P}\right)_{\mu_C},$$

where m is molality, and subscripts C and P denotes the cosolvent and protein, respectively.[13] The transfer free energy at low cosolvent concentrations relates to the preferential binding coefficient, thermodynamically as $\Delta\mu_P^{tr} = -RT\Gamma_{CP}$. The relation between the thermodynamic definition and the intuitive notion of binding comes from statistical mechanics. The transfer free energy of whole protein as a sum of the transfer free energy of the groups it comprises (Δg_i^{tr}) can be expressed as $\Delta\mu_P^{tr} = \sum_i \alpha_i \Delta g_i^{tr}$, where α_i is the fractional change in solvent accessible area of the group due to structural transformation. Under statistical framework, the preferential interaction coefficient (Γ_{CP}) can be expressed as[3]

$$\Gamma_{CP} = \left\langle n_C^{II} - n_W^{II}\left(\frac{n_C^{I}}{n_W^{I}}\right)\right\rangle,$$

where n is the number of a specific type of molecule in a certain domain. Subscripts P, C, and W are for protein, cosolvent, and water, respectively. Superscripts I and II are for bulk and the local domain. The angle brackets denote an ensemble average. When the concentration of cosolvent is higher near the protein than in bulk, the $\Gamma_{CP} > 0$ and the chemical potential μ_P are lower in the presence of the cosolvent than in its absence. Depending upon the surface topography of the protein, there can be an ensemble of preferential solvation, preferential hydration, and neutral solvation near the protein surface; as a result, there can be a mosaic of solvation layers depending upon the differing nature of preferential interactions.

Experimentally, various spectroscopic techniques such as NMR, fluorescence, terahertz spectroscopy, etc. probe the solvation of protein and the size and character of hydration layer however these techniques are unable to quantify the local solvation preferences. The dialysis/densimetry technique can measure the preferential interaction coefficient experimentally for protein−cosolvent systems; however, these studies have limited insights into the preferences of the solvents toward the distinct location of the protein surface. In this direction, computational approaches involving computer simulation techniques are important to investigate protein conformations and solvation features. Next, we have highlighted the most widely used computational approaches in this regard.

3. COMPUTATIONAL METHODS

It is well-known that to study the behavior of chemical systems in a detailed microscopic way, computational studies such as computer simulation is an essential tool that relies upon the rules of statistical mechanics where macroscopic observables of a system are linked to microscopic atomic motion. Fundamentally, to know how proteins function requires knowledge of structure and dynamics. Molecular dynamics (MD) simulation is a powerful tool that helps to explore the conformational landscape accessible to proteins in the solvent phase.[14] The method is important since experimentally it may be sometimes difficult to capture the dynamics associated with the internal motion of proteins and the solvents. The first MD simulation of protein was reported in 1977 in a vacuum, and for the same protein, it took another 11 years to simulate in the aqueous phase.[14] With the significant improvement of computational power and potential energy models, one can simulate proteins in an aqueous medium and binary/ternary mixture solutions for a long time (>100 ns) with much accuracy. Solvent effects on the events of conformational changes in proteins and their free energy change landscape can be thoroughly studied by extensive MD simulation and advanced sampling techniques. Computational approaches further allowed one to obtain relevant thermodynamic information and explore the cosolvent molecules' behavior on a microscopic scale, gaining interesting insights into their interactions.

Replica-exchange molecular dynamics (REMD) simulation is a widely used enhanced sampling method to investigate protein conformationl space in explicit solvent. In this method, conventional MD simulation techniques are being used when a number of replicas of original systems are simulated independently at different temperatures. Although the bottleneck of the method is the high computational cost, in the presence of an explicit solvent, the difficulty that arises in crossing high energy barriers between the local energy minima from the conventional MD simulation can be overcome through this technique. It is important to note that obtaining the weight factor is not a trivial job for the systems when protein is dissolved in an explicit solvent. However, in REMD, the standard Boltzmann weight factor can be used; hence, the method gains popularity in analyzing protein conformations in an aqueous solution, including a mixture of solvents.[6,9,15,16]

Metadynamics is an another enhanced sampling method where one can reconstruct the free energy surface as a function of several degrees of freedom, commonly known as collective variables. In this method, an external history-dependent bias potential as a function of collective variables is applied where the potential can be decomposed into several Gaussian potentials within the collective variables space, and it forces the system to sample toward the unexplored configurations. The main limitation of this process is the proper choice of the collective variables; however, with the massive improvement of the technique, the current algorithm of metadynamics has been an effective method in the field of protein−ligand binding and in exploring the folding pathways of proteins in the mixed solvent.[17]

Researchers have adopted a few model-based studies to explore the cosolvent effects on proteins. The cosolvent-induced conformational swapping of proteins can be studied further by combining linear response path following (LRPF) method and the three-dimensional reference interaction site model (3D-RISM) theory that is known as LRPF/3D-RISM method.[18] The method consists of two MD simulation steps. The first step is the unbiased MD simulation, where the variance-covariance matrix elements of free protein atoms are calculated. This is followed by the 3D-RISM calculations, where the average structure was considered, and the forces were evaluated. In the second step, the biased MD simulation

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was performed. These two processes continue iteratively until the target structure is obtained.

The major requirements of the classical molecular dynamics simulation studies of biomolecular systems are the empirical molecular mechanics force field that elucidates the energies and forces acting on the systems. Mostly, the widely used popular force fields are nonpolarizable, additive force fields where the partial atomic charges of the systems are fixed. However, for the more realistic model to reproduce solvent environment effects and to quantify experimental observables more accurately, electronic polarization was incorporated.[19] The present mini-review considers different types of force fields in the corresponding MD studies of proteins in water−cosolvent mixtures to address several issues related to the topic.

4. PROTEINS IN OSMOLYTES AND DENATURANTS: CONFORMATIONS AND INTERACTIONS

4.1. Methylamines. Stability of protein conformations by methylamines such as TMAO, betaine, etc. is well-established through experiments and computational approaches. The chemical structure of the compounds suggests the possibility of forming hydrogen bonds through the oxygen atoms, and the methyl groups can participate in hydrophobic interactions with the side chain amino acids of a protein. A comparative atomistic MD study using an additive force field by Cremer et al.[4] suggests that although TMAO, betaine, and glycine stabilize the collapsed form of a polypeptide, the interactions between the TMAO−peptide and betaine/glycine−peptide are different. While betaine and glycine are strongly segregated away from the peptide−water interface and cause collapse, TMAO accumulates at the peptide−water interface. Nagaoka et al.[20] studied transfer free energy of apomyoglobin (AMb) from pure water into aqueous solution with TMAO by using combined atomistic MD simulation and KB integral method. They used radial, surficial, and elemental KB integrals. The radial KB (R-KB) integral, $G_{\alpha s} = \lim_{R\to\infty}\left(\frac{\langle N_{ks}^{\phi}(R,t)\rangle_T}{\rho_s} - (4\Pi R^3/3)\right)$ (where $N_{ks}^{\phi}(R,t)$ is the instantaneous coordination number of number density s in the bulk solvent phase ρ_s at time t inside the sphere $\phi_k(R,t)$ with the radius R centering on the given atomic site k) is based on the pair correlation function between one of the atomic sites of protein and the solvent composition, which is easy to calculate numerically. However, since the variable, radius, does not directly reflect the shape information on the protein, the solute shape-dependent KB integral, i.e., surficial KB integral (S-KB), $G_{\alpha s}^{S} = \lim_{R^v\to\infty}\left\langle \frac{N_{\alpha s}^{v}(R^v,t)}{\rho_s} - V_v\right\rangle_T$, where $N_{\alpha s}^{v}(R^v,t)$ is the instantaneous coordination number of s and Vv is the volume, was used. This integral includes the volume of the region as introduced by the authors. The elemental KB integral (E-KB) includes instantaneous number density and so $G_{\alpha s}^{E}(r) = \frac{\langle N_{\alpha s}^{\Delta r}(r,t)\rangle_T}{\rho_s} - \Delta x\Delta y\Delta z$, where $N_{\alpha s}^{\Delta r}(r,t)$ is the instantaneous coordination number of s inside the region Δr at position r whose volume is $\Delta x\Delta y\Delta z$. The angular brackets in each case is the time average properties obtained from MD trajectories. The elemental integral is convenient for determining three-dimensional distributions of the thermodynamic quantities around protein. With the aid of all different KB integrals, the time-resolved transfer free energy was calculated and its influence on the protein structure was monitored. Their calculations showed that there occurred preferential exclusion of TMAO in the vicinity of the protein; as a result, a positive transfer free energy was noticed. The important notification was that TMAO transiently stop to act as stabilizers for few nanosecond and makes the protein conformation less compact. The computed transfer free energy from simulation and preferential interaction parameters are in accordance with the experimental values. Garcia and co-workers[2] further discussed the model-dependent preferential exclusion of TMAO from the local domain of the protein surface. TMAO was found not to exhibit expected preferential exclusion from the protein surface and might act as denaturants in Kast models; however, scaling up charges and weakening Lennard-Jones potential could improve the model further. It was further demonstrated that the protein−urea interactions are more favorable in the unfolded ensemble than the folded ensemble, enabling enthalpy-driven unfolding phenomena of the protein in urea. Mukherjee and Mondal[15] performed free energy simulations and REMD simulations of mini-proteins over a temperature range of 280−540 K using an additive force field to investigate the folding mechanism in the presence of TMAO and glycine. A Markov state model was used to map the complete protein folding process statistically. The two-dimensional free energy landscape along the radius of gyration (r_g) and fraction of native contacts (NC) in all of the systems suggests that the primary conformational changes of the proteins mostly occurred along native contacts and that along the radius of gyration is minimal. Significantly, it was observed that the unfolded ensembles of the mini proteins were more unfavorable free energetically in aqueous TMAO solution than in neat water. A similar phenomenon was observed for the proteins in glycine solutions; while glycine excludes from the surface of both the proteins, TMAO exhibits contrasting behavior suggesting its preferential binding is protein specific. The study concludes that the conformational preferences of proteins toward folded state is driven by the relative preferential depletion of osmolytes from folded to unfolded states.

4.2. Amino Acid and Its Simple Derivatives. A few amino acids and their derivatives can function as protein conformation stabilizers. Early experimental works showed that proteins are stabilized in free amino acids such as proline, serine, alanine, and glycine. Among free amino acids, glycine is known to stabilize polypeptide/protein conformations.[4,15] Anumalla and Prabhu[21] investigated the interactions of free amino acids arginine, lysine, aspartic acid, and glutamine with RNase A and α-lactalbumin by performing atomistic MD simulations. Their study inferred that the conformational flexibilities of proteins by the means of root-mean-square deviation (RMSD) and solvent-accessible surface area (SASA) analysis are not large. They further observed that the extent of increase in density of water around the proteins is relatively less in the presence of arginine than in the other three amino acids, causing higher preferential interactions between protein and those amino acids. The effects of arginine concentrations on regulating the conformational properties of insulin monomer, and ubiquitin at ambient and elevated temperatures were explored by performing conventional atomistic MD simulations and REMD simulations using additive and polarizable force fields.[6,16] The studies identified the role of ion−π interactions in regulating protein conformational stability from both additive and polarizable force field models.

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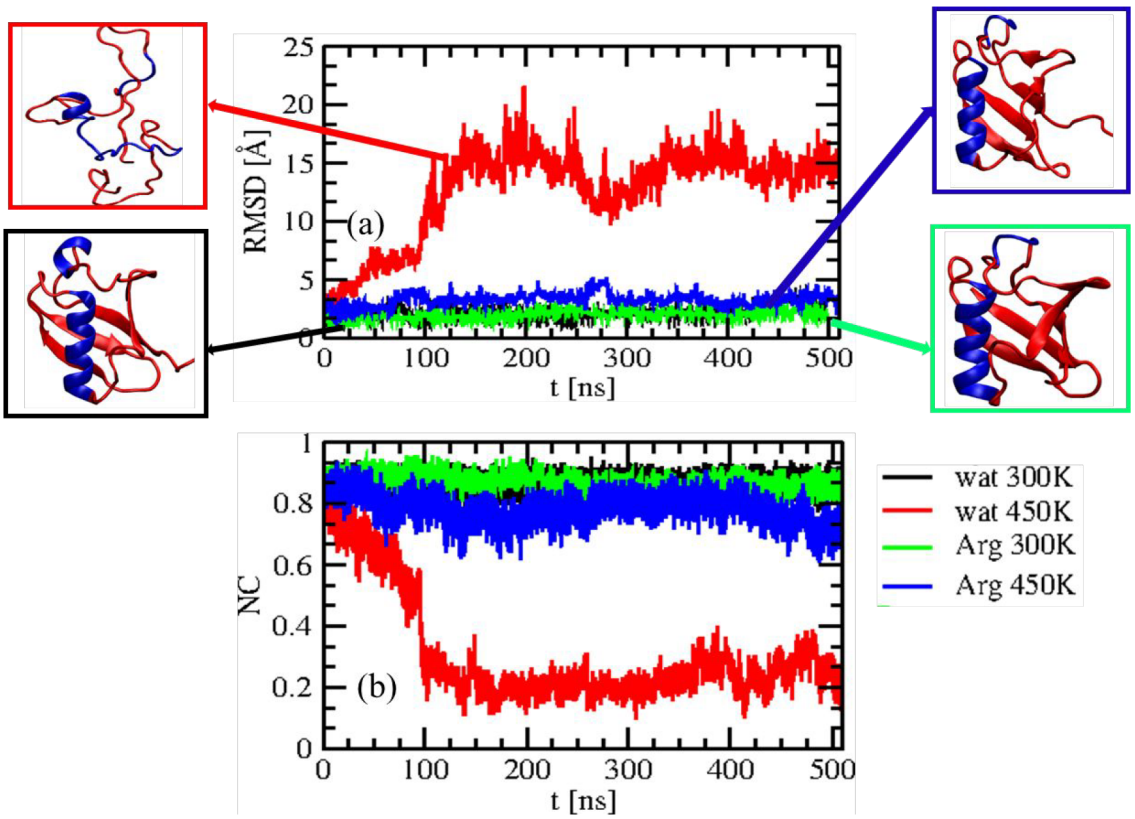

Figure 1. Time evolution of the (a) root-mean-square deviation (RMSD) and (b) fraction of native contacts (NC) of protein ubiquitin in aqueous and in 2 M arginine solution at 300 and 450 K. The representative conformations of the protein obtained from the simulated trajectories are also shown for reference. Adapted from ref 16. Copyright 2022 American Chemical Society.

Quantification of RMSD, fraction of native contacts, solvent accessible surface area, and percent helicity of the proteins suggested that the conformational features of the proteins depend on arginine concentrations, and among all the tested solutions, 2 M arginine solutions that mimic the cellular environment was noted to preserve the native folded form of the proteins in an excellent manner. For the ubiquitin protein, a remarkable change in RMSD and fraction of native contact values in pure water at 450 K was noted compared to that in arginine solution at this temperature (Figure 1).[16] The significant jump in the RMSD value of the protein in pure water at 450 K occurred near 100 ns. Further, the fraction of native contacts monitored throughout the simulation time suggested a significant breakdown of internal contacts of the protein at an elevated temperature beyond 100 ns in pure water. Arginine solution, in this aspect, at elevated temperature (450 K) was found to conserve the initial native interactions of the protein, similar to pure water at 300 K. This inferred that in arginine solution, retention of proteins' native-like folded form at elevated temperature occurred, whereas in pure aqueous solution, the protein lost its folded native form. The RMSD distribution plots for the insulin monomer (Figure 2a) in different arginine concentrations show the efficacy of 2 M arginine solution in retaining native-like conformation compared to that in other solutions at both ambient and elevated temperatures.[6] This in general demonstrated that concentration of cosolvent plays an important role in governing the protein's conformations. The nearly similar average fraction of native contact values (0.84−0.77) of the same protein as obtained from REMD simulations (Figure 2b) in 2 M arginine solution at different temperatures is the strong signature of the osmotic efficacy of arginine in preserving the protein conformation at a wide range of temperatures (300−420 K). The temperature-dependent preferential hydration phenomenon (Figure 2c) with variable values of r (0−10 Å) signifies the exclusion of arginine (preferential binding coefficient values, $\Gamma_{AP} < 0$) from the local domain of folded protein ensembles. Basic amino acid solutions (arginine, histidine, and lysine) and aromatic amino acid (phenylalanine, tryptophan, and tyrosine) solutions at ambient temperature were observed to restrict the conformational motions and configurational entropy of an insulin monomer compared to pure aqueous solution from conventional MD studies.[22,23] The comparative study revealed that conformationally the protein was more stable in arginine solution compared than in histidine or lysine solution. Among the aromatic amino acid solutions,[23] in tryptophan and tyrosine, the protein exhibited relatively lesser flexibility and was more compact than in pure water and phenylalanine solution. It was demonstrated that the aromatic amino acids present in the solutions interacted with insulin and stabilized its native folded conformation through cation/anion−π and π−π stacking, and partly through hydrogen-bonded interactions. Among the three, tryptophan was prone to interact through cation− π interactions with the protein while phenylalanine and tyrosine interacted through π−π stacking with insulin. Considering the important improvement in reproducing the experimental ion−π pair distances in protein, polarizable force field model for both protein and water was further used.[22] Saladino et al.[17] used metadynamics, solute tempering metadynamics, and conventional MD techniques to study the stability of β-hairpin in 1 M glycine betaine−water mixed solvent. They observed that in

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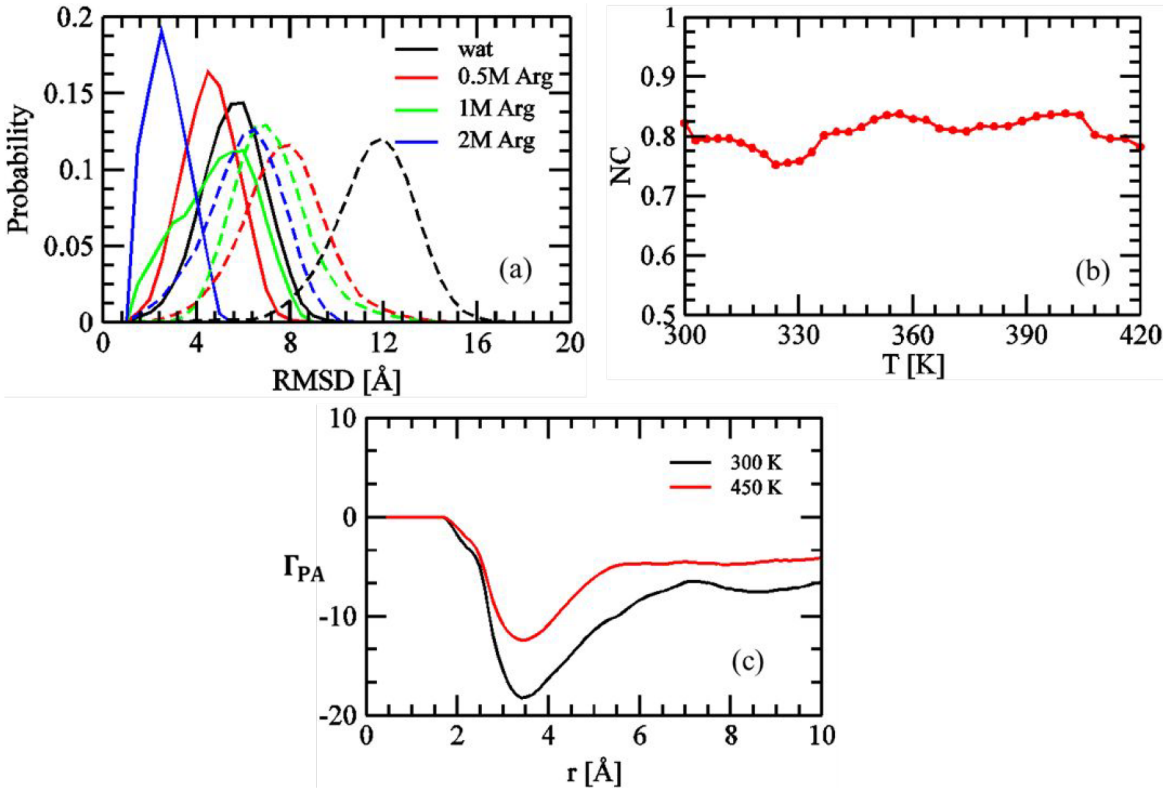

Figure 2. (a) Probability distribution of RMSDs of insulin monomer in aqueous solution, in 0.5, 1, and 2 M arginine solutions as obtained from the simulations performed at 300 K (solid line) and 400 K (dashed line). (b) Temperature-dependent average remaining fraction of native contact values of insulin monomer as obtained from the REMD simulations performed in 2 M arginine solution. (c) Apparent preferential binding coefficient as a function of the cutoff distance (r) between the local and bulk domains for equilibrated trajectories of ubiquitin in 2 M arginine solution at 300 and 450 K. Adapted from ref 16 (copyright 2022 American Chemical Society) and ref 6 (copyright 2020 American Chemical Society).

this solution, the native minimum was considerably stable, and the exclusion-coefficient calculation strongly suggests that the protecting effects of glycine betaine are consistent with the indirect mechanism hypothesis.

4.3. Polyols and Sugars. The ability of small carbohydrates to preserve proteins has been well-documented. Polyols such as glycerol and small sugars such as glucose, trehalose, and sucrose are used as lyoprotective or cryopreservatives. It was demonstrated by protecting the hydrophobic interactions at low temperature glycerol can protect proteins from cold denaturation.[24] Trout and co-workers[3] performed atomistic MD simulations and developed a methodology to investigate the molecular anatomy of preferential interactions of protein lysozyme in water−glycerol mixture. The main objective of their study was to investigate the effects of protein conformational changes on preferential interaction coefficient. Their study revealed that although the preferential interaction coefficient is positive in the solvent region that forms hydrogen bonds with protein, the overall preferential interactions are negative. Laage and co-workers[25] studied the preferential solvation phenomenon of protein in the water-glycerol mixture at 50% glycerol in volume to understand the stability of protein conformation and cryopreservation of the solution. They used three different force-field combinations for the protein/glycerol/water components. Their study based on a theoretical model and atomistic simulations reveals that with the change in solvent composition, temperature, and nature of protein the differences between local and bulk compositions exhibit dramatic changes. Their study demonstrated that glycerol depletion occurs from the protein's solvation shell due to entropic factors at high concentrations. MD study of chymotrypsin inhibitor 2 (CI2) in different polyols and sugars such as glycerol, xylitol, sorbitol, trehalose, and sucrose suggests that protein stability by these cosolvents is positively correlated with the molecular volume and the fractional polar surface area, and the former contributes more significantly to the protein's stability.[26] The study further suggests that the preferential exclusion of the polyols from protein surface is size-dependent. Katyal and Deep[27] attempted to investigate the effects of trehalose on the native, partially unfolded and denatured states of lysozyme from atomistic MD study under different temperatures and concentration regimes. From the principal component analysis, the authors demonstrated that trehalose molecules slowed down protein dynamics and helps protein to get trapped in the native-like folded conformation. However, they do not alter the principal direction of protein's motion and offer an on-pathway stabilization. Their study further reports that the relative stabilization of lysozyme's native state can be attributed due to the favorable interactions of trehalose with the polar flexible side chain residues of the protein.

4.4. Urea, Guanidium Chlorides, and DMSO. Recent studies using computational and experimental approaches provide a converging view of the protein-denaturant interactions by which protein structure gets disrupted from its native folded form. A bunch of computational studies were performed to explore the effects of these solvents and folding-unfolding pathways of proteins. Several researchers explored

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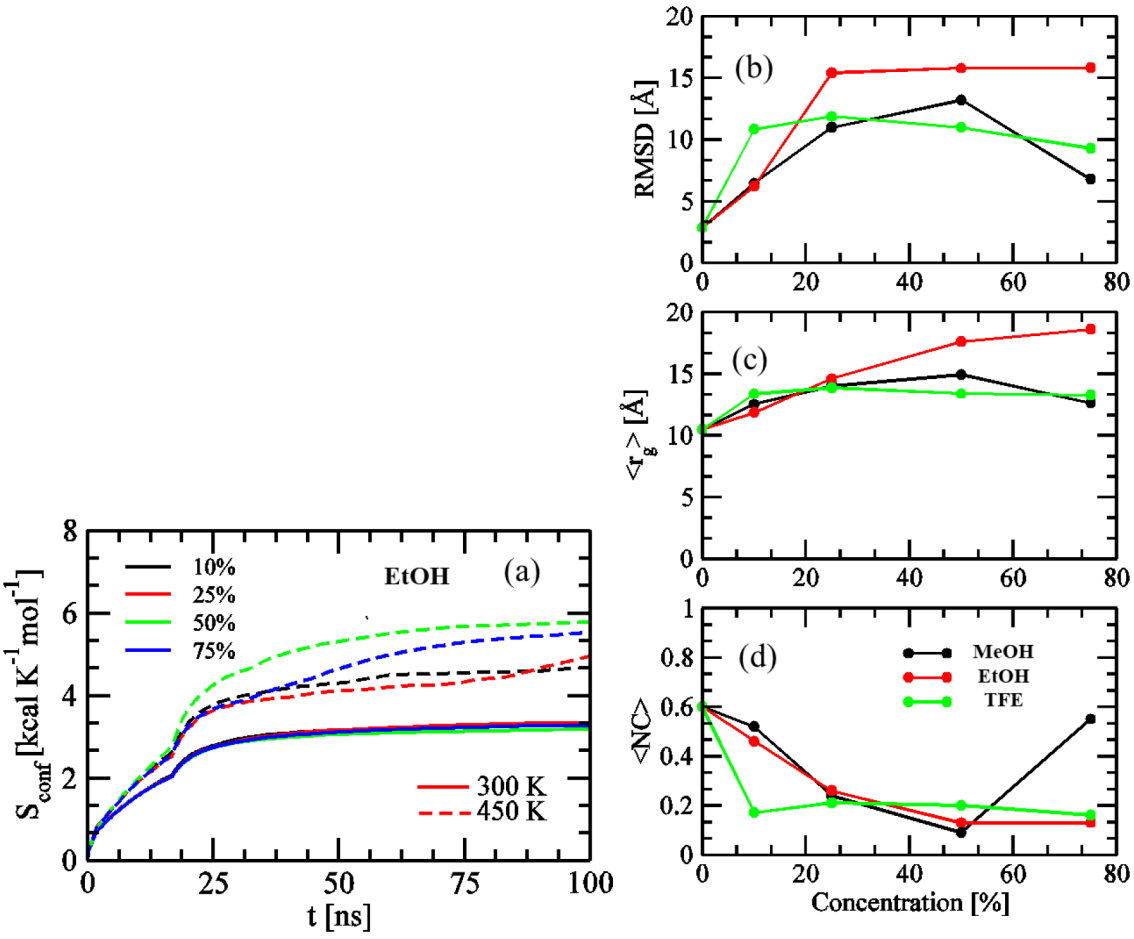

Figure 3. (a) Cumulative configurational entropy (S_{conf}) of the folded chymotrypsin inhibitor 2 (CI2) protein as obtained from the simulation performed at 300 K (dotted line) and that of the unfolded protein as obtained from the simulation performed at 450 K (solid line) at different ethanol concentrations. The average (a) RMSD (in Å), (b) radius of gyration, (r_g), and (c) remaining fraction of native contacts (NC) as a function of increasing methanol (MeOH), ethanol (EtOH), and 2,2,2-trifluoroethanol (TFE) concentrations as obtained from the equilibrated trajectories generated at 450 K. Panel (a) adapted with permission from ref 31a. Copyright 2018 Royal Society of Chemistry. Panel (b) adapted with permission from ref 31b. Copyright 2017 Royal Society of Chemistry.

protein stability in urea/guanidium chloride (GdmCl)/DMSO solution from MD techniques in great detail. Canchi et al.[9] studied the protein denaturation in urea with the change of urea concentrations from the combined atomistic MD and REMD studies. They computed Gibbs free energy of unfolding from the knowledge of fraction folded concept and observed that with increasing urea concentration, the equilibrium shifted toward the unfolded ensemble, which was reflected in the decrease of the melting temperature from 430 K for 0 M to 304 K for 5.8 M urea. A metadynamics study of a β-hairpin was carried out in urea−water mixed solvent to study its conformational stability.[17] The study reports the free energy change in glycine betaine was 9.78 kJ/mol, reproducing the osmolyte stabilizing effect. On the other hand, the simulation in urea reported a negative free energy change of −18.5 kJ/mol, in agreement with the denaturing effect of urea. Reddy and Thirumalai[28] carried out MD simulations of ubiquitin folding in guanidinium chloride and urea solutions by using coarse-grained model and molecular transfer model. They constructed a two-dimensional folding landscape as a function of the radius of gyration and a measure of similarity to the folded state. This reveals that the native state assembly is preceded by collapse. It was found that ubiquitin becomes compact as the denaturant concentration is decreased, and the extent of compactness depends on the folded state's stability. Tanimoto et al.[18] applied LRPF/3D-RISM method to the urea-induced denaturation process of ubiquitin and suggested that the method can simulate the early stage of the denaturation process within the limited simulation time than the standard MD simulation technique to explore the mechanism of unfolding of the protein. The detailed MD study of chemical unfolding of HP-36 and lysozyme in aqueous DMSO solution by Roy et al.[5] identified a sequence of structural changes in proteins on increasing DMSO concentration and identified the partial unfolded intermediate states of proteins. In these works, the authors used the united atom model of protein to perform simulations. It was found that at low concentration (around 5%) of DMSO, lysozyme's conformation was considerably suppressed, while at concentration range of 10−15% partial unfolding of the protein occurred which increased both conformational fluctuation and solvent accessible surface area (SASA). Importantly, conformational fluctuation and SASA decreased suddenly at a concentration range of 15−20%, causing an intermediate collapse state. These findings agreed with circular dichroism (CD) and fluorescence studies.

4.5. Monohydric Alcohols. Alcohols in solutions have remarkable effects on the cellular and enzymatic proteins to

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regulate their complex biological activities. Monohydric alcohols including halogen-substituted one can alter the conformations of proteins. However, such effects are alcohol specific and concentration-dependent. Ghosh et al.[29] used united atom force field to simulate protein in water−ethanol binary mixtures to explore the sensitivity of the protein conformational dynamics with varying ethanol concentration to unravel the pathway of conformational changes. They adopted a theoretical scheme that combines the aspects of Bryngelson-Wolynes theory with Marcus theory of electron transfer to identify the unfolding pathway through the partially stable/metastable intermediates of the protein. The simple theory developed by them following Smoluchowski equation

$$\frac{\partial}{\partial t}P(\{R_i\}, \eta, t) = \sum_{i=1}^{N_{sp}} D_i \frac{1}{R_i^2}\frac{\partial}{\partial R_i} e^{-\beta V_i(\{R_i\})}\frac{\partial}{\partial R_i} e^{\beta V_i(\{R_i\})} P - \Gamma_\eta \frac{\partial V(\eta)}{\partial \eta}$$

where D_i is the mutual diffusion coefficient of the two groups involved in the contact pair, $\beta V_i(\{R_i\})$ is the potential functions, R_i is the separation values, and the fraction of native contacts, η at time, t, suggested that the dynamical evolution in the structure of protein can be significantly altered by tuning solvent concentration. Their study identified that at low (mole fraction ∼0.05−0.1) and high ethanol concentrations, the structural changes of the protein is significant and adopted unfolding states, while at an intermediate concentration (mole fraction ∼0.25) the native contacts came closer to form a native-folded-like conformation of the protein and at high concentration of ∼0.4 mole fraction. Such an unusual phenomenon was probably due to the self-association of ethanol molecules through hydrogen bonds. It was reported that the ethanol reaction pathway changes considerably due to the entropy-enthalpic competition and preferential solvation of the intermediate states. The distribution of water and ethanol around the dimer forming region reveals that in the presence of ethanol, the two antiparallel sheet forming regions can experience a long-range interaction and stabilize the intermediate state where the number of contacts is close to zero, but the intermonomeric distance is not too small (∼3 nm). Mohanta and Jana studied the effects of different monohydric alcohols, namely methanol, ethanol, and 2,2,2-trifluoroethanol, on a protein CI2 from atomistic MD study.[30,31] The conformational behavior of CI2 toward the change in ethanol concentrations ranging from 0 to 75% (v/v) at different temperatures was investigated.[30] In pure water while the native structure of CI2 remains unaffected at elevated temperature for a certain simulation time scale, partial unfolding in 10% ethanol solution followed by complete unfolding of the protein at ethanol concentrations above 25% was observed, which was supported by RMSD and radius of gyration calculations. Ethanol addition increases the exposed surface of helix by ∼5−20% and that for sheets is ∼2−13%. The conformational disorder of the protein in different ethanol solutions as computed by calculating configurational entropy from Schlitter's equation, $S_{conf} = \frac{1}{2}k\ln\det\left[1 + \frac{kTe^2}{\hbar^2}\boldsymbol{M}^{1/2}\sigma\boldsymbol{M}^{1/2}\right]$ (here S_{conf} is configurational entropy, e is Euler's number, $\mathbf{M}$ is the $3N$-dimensional diagonal matrix containing N atomic masses of the solute, and σ is the elements of the covariance matrix), showed the concentration-dependent conformational disorderness of the protein (Figure 3a). The ethanol concentration-dependent cumulative configurational entropy of the protein exhibited initial buildup in entropies before attaining almost steady values. Heterogeneity in protein conformational disorderness was prominent in 50% ethanol. Notable differences in the average values of structural parameters, such as RMSD, r_g, and NC of protein in methanol-, ethanol-, and fluorine-substituted alcohols such as trifluoroethanol (TFE) at various concentrations compared to pure water indicated that the structural transformation of the protein at any experimental TFE solutions are almost similar while it differed for other alcohol solutions (Figure 3b−d).

Among these alcohols, at low concentration TFE was shown to accelerate the unfolding time scale, while, the influence of ethanol and methanol becomes prominent at concentration.[31] Computation of molecular contact frequency which is a measure of transfer free energy between protein and alcohol follows the trend, methanol < ethanol < 2,2,2-trifluoroethanol at low concentrations, whereas the trend becomes methanol ∼ ethanol > 2,2,2-trifluoroethanol at more concentrated solutions. The comparative unfolding pattern of the protein in methanol, ethanol, and trifluoroethanol from atomistic simulation suggests that the unfolding phenomenon significantly depends on the concentration and the nature of the alcohol used.

4.6. Proteins in the Ternary Mixtures of Osmolytes and Denaturants. Mixtures of organic solvents such as osmolytes and denaturants in cells of many organisms, question on their action on protein's conformational stability. In living organisms, specifically marine animals, urea-induced protein denaturation is protected by TMAO. Similarly, several other osmolytes were detected to preserve protein conformation in the presence of denaturants. The counteracting effect of osmolytes can be explained as the unfolded conformation of a protein is counter balanced by the presence of osmolytes in order to minimize surface area in contact with water molecules and finally the osmolyte reduces the denaturant−protein interactions. Here, some of the aspects of different pairs of osmolyte−denaturant effects on protein conformational stability have been discussed.

Although direct interactions of urea with proteins cause destabilization of protein structure, denaturation effect of urea can be counterbalanced by the osmolyte such as TMAO. The counteracting effects of TMAO on urea to stabilize proteins is controversial. Shea and co-workers[12] studied the conformational changes of biologically relevant polypeptide and R2 fragment of the intrinsically disordered Tau protein in pure and mixed urea−TMAO solutions. To model urea, they used the KB derived Smith force field, while TMAO was simulated with the Netz, Hölzl, and Kast models.[12] Their study showed that TMAO inhibited the protein−urea preferential interactions, enhanced the propensity of the salt-bridge formation, and counteracted the denaturing effects of urea and the extent of counteraction depending heavily on the amino acid composition of the peptide. The counteracting effects of trehalose against urea-induced protein denaturation are well-documented.[10,32] It is observed that while in urea-water solution, the proteins denature completely, the introduction of trehalose in the solution maintained the native structure of the peptide satisfactorily.[10] Study reports that in the ternary mixture of water−urea−trehalose, a considerable amount of peptide−trehalose hydrogen bonds was formed; as a result, urea was excluded from the peptide surface and interacted less

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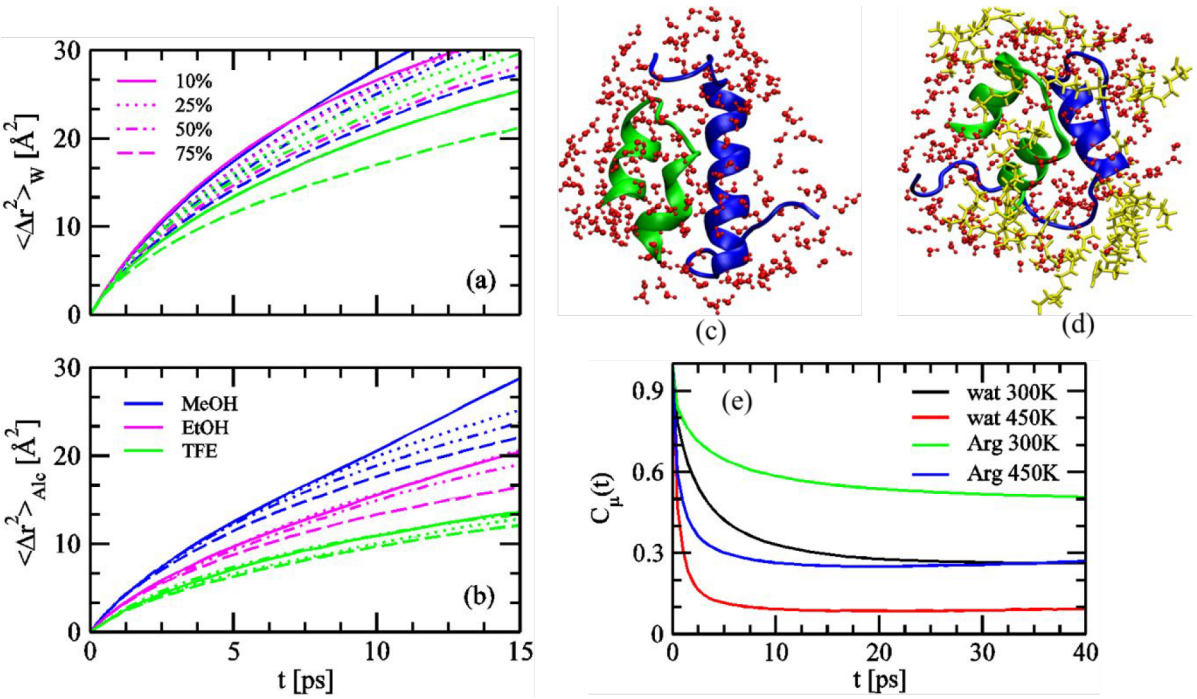

Figure 4. (a) Mean square displacements (MSDs) of hydration water ($\langle \Delta r^2 \rangle_W$) and (b) MeOH, EtOH, TFE molecules ($\langle \Delta r^2 \rangle_{Alc}$) that are present within the first solvation layer of the CI2 at 450 K. (c) Representative snapshots of the hydration layer water present around the insulin monomer in aqueous solution at 300 K and (d) in 2 M arginine solution at 400 K. Water molecules are shown in red and the arginine in yellow. (e) Reorientational time correlation function of the dipoles of water present in the hydration shell of ubiquitin as obtained from the equilibrated trajectories generated in aqueous solution and in 2 M arginine solutions at ambient temperature and at 450 K. Adapted with permission from ref 16 (copyright 2022 American Chemical Society) and ref 31 (copyright 2018 Royal Society of Chemistry).

with the peptide. Another study by Zhang et al.[32] on CI2 in water−urea−trehalose mixture inferred that while the protein partially unfolds in 8 mol/L urea, due to the complete disappearance of β-strands, it retains its native folded form in a ternary mixture of 8 mol/L urea and 1 mol/L trehalose by keeping both the α-helix and β-strands intact. The study further demonstrates that due to the formation of hydrogen bonds between trehalose and urea in ternary solutions, urea molecules also expelled from the protein surface along with the preferential exclusion of trehalose. The exclusion of trehalose from protein surface is the origin of its counteracting effects. Further, they concluded that in absence of trehalose, the denaturation of the protein in urea occurs through the direct Lennard-Jones mechanism rather than the direct electrostatic mechanism between urea and protein. The counteraction of trehalose on the conformational instability of lysozyme induced by GdmCl as studied from combined atomistic MD simulations and spectroscopic analysis suggests significant activity loss along with secondary and tertiary content of lysozyme in the presence of GdmCl, whereas the addition of trehalose in GdmCl reverses the effect.[33] In presence of GdmCl in solutions, the protein surface is accommodated by water rather Gdm^+ and the addition of trehalose improves the water content more near the active site region of the protein. The study demonstrates that trehalose induced remarkable changes in the solvent environment, electrostatic, and Lennard-Jones interactions between the protein and GdmCl are the important factor in the counteraction activity as well as structural change of lysozyme instigated by GdmCl.

5. HYDRATION PROPERTIES OF PROTEINS IN OSMOLYTES AND DENATURANTS

The hydration properties of biomolecules such as proteins have differential features than the bulk water, which challenges the scientific communities to explore the properties by means of experiments and theories or models. Near the biomolecular surface, the characteristic features of interfacial water molecules change significantly over the bulk, causing differential dynamics over a wide range of time scales. Computation of residence time, rotational correlation, mean-square displacements, hydrogen bonding dynamics involving water in protein's hydration shell provides differential time scale of relaxations of water around the vicinity of proteins. The presence of cosolvent further modifies the properties of water present in the hydration layer of proteins. It is worth to mention that there exists uncertainty in measuring the thickness of hydration shell of proteins, however, in MD simulation by employing geometric cutoff it is possible to differentiate the local and bulk domain. Although exhaustive computational studies were performed to explore the conformational stability of proteins in different cosolvents, the atomistic study to probe hydration dynamics is less.

Johnson et al.[34] simulated two and three-component solution systems that contained peptide and water-glycerol, peptide and water-DMSO, peptide and water-glycerol-DMSO systems and explored the effects of the cosolvent chemistry on the hydration water dynamics and compared them with the experimental findings. Their detailed analysis was based on the computation of translational diffusion coefficient of water computed from Einstein relation $D_t = \frac{1}{6}\lim_{t\to\infty}\frac{d}{dt}\langle |r(t) - r(0)|^2 \rangle$ (where $r(t)$ is the position vector of each atomic center at time

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t, and the angled brackets indicate an average over the ensemble of configurations at the simulated volume), the self-intermediate scattering function, ($F_s^H(Q,t) = \langle \exp\{iQ[r_H(t) - r_H(0)]\}\rangle$), where Q is the momentum transfer and $r_H(t)$ is the position vector of each hydrogen center) for the water hydrogen atoms and the evaluation of hydrogen-bonding population relaxation between waters and water−cosolvent molecules. The qualitative trends of the results for all evaluated systems were consistent with the experimental results. However, certain discrepancies between simulation and experimental results were observed.[34] Importantly, while the contact between water and the hydrophilic peptide backbone was maintained in the experiment, some of the contacts were disrupted in water−DMSO mixture. The probable reason for such discrepancy in simulation vs experimental data could be due to the parametrization strategy of cosolvents. The strong directionality and hydrogen-bonding lifetime between water and cosolvent were detected as an important factor in slowing down the diffusion constants of the solutions in the present of cosolvent. The study further demonstrated that glycerol as protein stabilizer retains the peptide surface hydrated and reproduces the dynamics signatures observed in the pure water solvent, while the denaturant DMSO limits the access of water to the peptide and disrupts the dynamics property of water. From atomistic MD simulations of a protein in different alcohol mixtures Mohanta and Jana[30,31] found that the translational motions computed from the mean-square-displacement of the water and alcohols (sublinear curve, Figure 4a,b) present in the solvation layer of an unfolded protein in different water−alcohol mixed solutions are restricted and nonuniform in nature. In presence of fluorinated alcohol, TFE, the translational mobility of hydration water was more restricted than the monohydric methanol or ethanol (Figure 4a,b).[31] The study demonstrated that in concentrated solutions the water mobility was more restricted than in relatively dilute solutions, which is particularly true for TFE solutions. The possibility for such restricted dynamics is the formation of strong protein−water and water-mediated protein−alcohol hydrogen bonds that prevent the water molecules from diffusing away from the unfolded protein surface. Another possibility is that, in concentrated solutions, the relatively crowded solvation layer of TFE traps the water molecule by forming strong TFE−water hydrogen bonds at both the -O and -F sites. Additionally, the bulky $-CF_3$ groups prevent the trapped water from moving. Particularly, with the increase in ethanol concentrations, the diffusion coefficient of hydration water around protein decreases by a factor 2. Gervasio and co-workers[35] studied the mechanistic details of the effects of protecting osmolytes glycine betaine and TMAO as well as denaturant urea on a villin headpiece HP-35 protein by performing bias exchange molecular dynamics simulations (BEMD). They used additive force-field parameters consist of backbone corrections. The protein adopted more helical conformations in osmolytes while in urea partial disruption of the hydrophobic core takes place causing destabilization of one of the helices of the protein (helix 3). It was reported that the osmolytes affect the rotational dynamics of water molecules in the bulk and protein domain, and the main driving force for the native state protection is due to the significant slow rotational diffusion of solvent in the protein domain. The results are in agreement with both NMR and IR observations. The effects of amino acids as osmolytes on the hydration properties of proteins were studied from atomistic MD simulations.[6,16,22,23] Studies showed that the diffusivity of hydration water for protein insulin monomer in a pure water system, which is ∼4.7 times slower than that of pure bulk water, becomes more restricted with the presence of arginine as a cosolvent.[6] Unfolded protein conformations form fewer hydrogen bonds with water than the folded protein when arginine was added to the solutions, and the percentage of protein−water hydrogen bonds in the concentrated arginine solution of 2 M was similar to the protein in the pure aqueous medium. In fact, at higher arginine concentration the less structuration of arginine around the protein leaves the protein surface free and promotes the hydration water to be structured more and conserved the protein's folded native form efficiently as they do when the protein remains in pure water at ambient temperature. Figure 4c,d represents the hydration layer of a protein in the presence and absence of cosolvents, respectively. Further, arginine solution exhibited heterogeneous influence on the hydration dynamics of helices and strands of a protein.[16] It was reported that the rotational motion of the hydration water computed from dipole−dipole time correlation function, $C_\mu(t) = \frac{\langle \hat{\mu}_i(t)\cdot\hat{\mu}_i(0)\rangle}{\langle \hat{\mu}_i(0)\cdot\hat{\mu}_i(0)\rangle}$, (where $\vec{\mu}_i(t)$ is the unit dipole moment vector of the i-th hydration layer water molecule at time t. The angular brackets denote that the averaging is carried out at different reference initial times over all the identified water molecules) around each helices and strands of the folded protein in arginine solution exhibits hindered motion compared to the unfolded protein due to the nonuniform distribution of cosolvents around them. Such a hindrance obstructs the reorientation of water dipoles and causes slower structural relaxation of the hydrogen bonds formed by the secondary structural segments of the protein and water. Figure 4e represents the relaxation patterns of rotational motions of hydration water in the presence and absence of arginine in the solution. The appearance of the plateau region of the curves indicates a longer time for the water to reorient at protein surface in the presence of arginine. The study revealed that with the presence of arginine, the water molecules reorient about 2−10 times slowly around the secondary segments of the protein than in around the unfolded protein in pure water. It was also identified that the arginine molecules primarily form bifurcated or simultaneous multiple hydrogen bonds with the same or different secondary structural segments of the protein and stabilize its native folded form. A comparative study on protein conformation stability in basic amino acid solutions such as arginine, histidine, and lysine revealed that the hydration layer around protein is enriched when arginine is present in the system compared to histidine and lysine.[22] The effects of hydration layer water entropy (S^{wat}) on the conformational dynamics of insulin in these basic amino acid solutions was studied by using two-phase thermodynamic (2PT) model, as $S^{wat} = k_B[\int_0^\infty d\omega S^{solid}(\omega)W^{solid}(\omega) + \int_0^\infty d\omega S^{gas}(\omega)W^{gas}(\omega)]$, where $W^{gas}(\omega)$ and $W^{solid}(\omega)$ are the weighting functions for gas-like and solid-like components of translational motion, respectively. $S^{solid}(\omega)$ and $S^{gas}(\omega)$ are the entropy of vibrational solid-like and vibrational gas-like, respectively. Compared to pure bulk water, the entropy of hydration layer water around insulin in the presence of basic amino acids is at least ∼16 J/mol/K less, suggesting that ordered hydration water in the presence of these classes of amino acids around protein plays an important role in restoring the native-like conformation of insulin. The dynamics of hydration shell of SNase protein in different

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cosolvent solutions containing various concentrations of urea and glycerol as studied from atomistic MD simulation reveals that water dynamics at the protein interface exhibited fast formation and breakup of hydrogen bonds due to water rotation/libration.[36] The slower relaxation of the protein−water hydrogen bond network due to diffusion of water molecules between sites on the protein surface was evident. The addition of both cosolvents leads to a marked increase of the H-bond network relaxation time at the protein interface. Computation of residence time of water and cosolvent such as glycerol molecules that are present around the local domain of a protein lysozyme as carried out by calculating survival functions characterize the different class of glycerol and water molecules as per their characteristics residence time.[35] It was observed that the glycerol molecules of two classes could have residence times of 0.5−1 and 2−7 ns, while water molecules were divided into three classes with a residence time of <0.2, 0.4−1, and >5 ns. The study concluded that the differences in residence times were due to the location near the protein surface and bonded through multiple hydrogen bonds, which causes conformational changes of the protein and hence the preferential interaction coefficients in different simulations.

6. CONCLUDING REMARKS AND OUTLOOK

Understanding conformational features and hydration dynamics of proteins in cosolvent is essential to control proteins' stability in a mixture of solvents. This is an important biophysical problem related to the applications in the field of biotechnology. In this mini-review under a single umbrella, we have highlighted model based preferential interaction phenomenon of cosolvents and some of the key issues of protein structure, dynamics, interactions, and hydration dynamics in various popularly used cosolvents, osmolytes, and denaturants from the computational approaches such as conventional MD simulation, metadynamics, replica exchange MD techniques with the adaptation of all-atoms additive force fields, united atoms force fields, and all-atom polarizable force fields. The combined effects of the osmolytes and denaturants in mixed solution of water−osmolyte−denaturant are further discussed. The probable reasons for stabilizing and destabilizing phenomena have been highlighted and conferred. Studies inferred that few osmolytes could stabilize proteins conformations in stressed conditions such as elevated temperatures. Further, the unfolding phenomenon of proteins in various denaturants at different environmental conditions is evident. The studies importantly suggest that the structural evolution of proteins can be significantly altered by tuning the cosolvent concentrations.

The discussions clearly infer that by using computer simulation techniques, it is possible to probe the conformational features, hydration dynamics of proteins and the mechanistic details of interactions between model proteins and cosolvents in various water−cosolvent mixed solutions in an atomistic resolution. However, the task is challenging, and the significant issues in this regard are the inadequate sampling, modeling of real physical systems using all-atom interactions which are often large and computationally expensive and the choice of force field model that usually plays an important role. Empirical force fields based on nonpolarizable additive force fields for proteins and peptides are widely used to understand the structure, dynamics, and solvent properties. The explicit treatment of electronic polarizations yields a more realistic and consistent model. However, important issue for protein−cosolvents interactions is that the empirical force field for proteins and the cosolvents are mostly derived from the individual protein−water or cosolvent−water solutions. Such combination without introducing certain modifications to direct protein−cosolvent interactions sometimes might lead to disagreements with experimental observations related to the topic of interest.[12,24,34] However, mostly the results obtained from different computational studies are in excellent agreement with experiments and in accordance with the available experimental findings, which further proves the reliability of the existing force field in manifolds. It is doubtless that experiments are wonderful and provide a wide range of information on the dynamics and associated time scale for protein−cosolvent systems, however it can be enumerated that accurate computer models can provide information which is difficult to obtain directly from different experimental approaches.

AUTHOR INFORMATION

Corresponding Author

Madhurima Jana − *Molecular Simulation Laboratory, Department of Chemistry, National Institute of Technology, Rourkela 769008, India;* orcid.org/0000-0002-6567-4490; Email: janam@nitrkl.ac.in

Authors

Rabiul Gazi − *Molecular Simulation Laboratory, Department of Chemistry, National Institute of Technology, Rourkela 769008, India*

Sankar Maity − *Molecular Simulation Laboratory, Department of Chemistry, National Institute of Technology, Rourkela 769008, India*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c08009

Notes

The authors declare no competing financial interest.

Biographies

Rabiul Gazi received his Master's degree in Chemistry from Presidency University, Kolkata, India in July 2017. Currently, he is a Senior Research Fellow and exploring protein structure and dynamics in mixed solvents by using computational techniques.

Sankar Maity received his Master's degree in Chemistry from Vidyasagar University, Midnapore, West Bengal, India in October 2020. He is currently working as a Junior Research Fellow. He is exploring saccharide effects on lipid bilayers under alcohol-induced stress from atomistic MD simulations.

Madhurima Jana is currently an Associate Professor at the Department of Chemistry, National Institute of Technology Rourkela, Odisha, India. She completed her doctoral research from Indian Institute of Technology Kharagpur in 2012. Her current research focused in understanding cosolvent guided conformational stability of proteins, model membrane dynamics, protein-glycan interactions. Her research group is also exploring the effects of anion receptors as additives for the Li-ion battery electrolytes.

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