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Effects of protein conformational motions in the native form and non-uniform distribution of electrostatic interaction sites on interfacial water

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ABSTRACT

Protein–water interactions and their influence on surrounding water is a long-standing problem. Despite its importance, the origin of differential water behavior at the protein surface is still elusive. We have performed molecular simulations of the protein barstar in aqueous medium. Efforts have been made to explore how the conformational motions of the protein segments in the native form and the heterogeneous electrostatic interactions with the polar and charged groups of the protein affect the interfacial water properties. The calculations reveal that reduced dimension of the hydration layer on freezing the protein's degrees of freedom does not modify the heterogeneous water distributions around the protein. However, turning off the protein–water electrostatic contribution leads to non-preferential near-uniform water arrangements at the surface. It is further shown that with protein–water electrostatic interactions turned on, the local structuring of water molecules around the segments are correlated with their degree of exposure to the solvent.

1. Introduction

Biomolecules in general and proteins in particular are mostly inactive in their anhydrous states. Water molecules hydrating the surface of a protein in an aqueous environment critically influence the three-dimensional structure, dynamics, and function of the protein [1,2]. A molecular level dynamic correlation exists between the protein and its hydration layer water molecules, often known as the 'biological water' [3], with their properties different from that of water in pure bulk phase. A proper knowledge of protein–water (PW) interactions and the consequent dynamical correlations is essential not only from fundamental point of view, but also to understand the mechanisms of various biological processes involving proteins, such as protein–ligand binding, enzymatic catalysis, protein folding, etc. [2,4].

Considering the importance of various issues involved in protein hydration, it has been one of the most active areas in both experimental and theoretical research over past several years. However, it is often quite challenging to explore the dynamical correlations between a protein and water around it from a single experimental method due to wide range of time scales associated with various dynamical events involving such systems. Controversy exists in interpreting experimental results, as different methods often measure different quantities over a wide range of time scales [5,6]. In addition, separating the contributions of protein and water is often an issue in a solvated protein system. As a result, despite significant efforts, a quantitative picture of protein hydration has not yet emerged. Time-resolved fluorescence spectroscopy has been one of the most important tools over last one decade to study the time scale of protein solvation [7–14]. Using both intrinsic and extrinsic probes on different protein systems these studies in general demonstrate the presence of bimodal distribution of solvation time scales. The fast component occurring within a few picoseconds has been attributed to the presence of free or labile water molecules in the hydration layer. The second component in the range of tens of picoseconds has been identified with the restricted motions of water molecules hydrating the surface that are coupled with the local motions of protein residues. This component is sensitive to the location of the probe at the protein surface. The results obtained from such time-resolved studies are found to agree well with earlier nuclear magnetic relaxation (NMR) studies of nuclear Overhauser effect (NOE) [15,16], and recent neutron scattering experiments [17–19]. In contrast, Halle and co-workers [20,21] recently predicted from NMR dispersion (NMRD) studies that the hydration water molecules although slower than bulk water, but they exhibit much faster dynamics than that suggested by fluorescence measurements. With respect to water in bulk state, the protein surface water molecules are found to be only 2–5 times slower from these studies. It may be noted that such discrepency

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between NOE and NMRD experiments arise due to the fact that while NOE measurements offer spatial resolution, NMRD provides time resolution but no spatial resolution. Solvation dynamics experiments on the other hand have high degree of spatial resolution and therefore can capture appropriately the retarded dynamical picture of only first few hydration layers around a protein. It is shown recently that NMR studies can properly explore PW interactions by confining the protein in a nanoscale environment [22]. Neutron scattering is another important tool that has been used in recent times to understand the local structure and dynamics of water at biomolecular interfaces [23–29]. In an interesting recent work, Russo et al. [29] demonstrated the presence of low-density amorphous ice-like water molecules hydrating the surface of hydrophobic biomolecules at 200 K.

Molecular dynamics (MD) simulation is another powerful tool that has been widely used in recent times to obtain a microscopic understanding of PW interaction and its influence on structure and dynamics of water hydrating the proteins [30–48]. Simulation studies have shown that the surface topography of a protein controls the alignment of water molecules around it [30–33]. Strong dependence of structure and energetics of hydrophobic hydration on surface topography has been shown in an early work by Cheng and Rossky [34]. In an important study, Pizzitutti et al. [35] analyzed in detail the role played by the topological and energetic disorders at the protein surface in modulating the slow dynamics of hydration water. They showed that the presence of local motions of proteins reduce the anomalous behavior of surface water molecules. Restricted translational and rotational motions of water at the protein surface have been identified from several simulation studies [36–40]. Ordering of water molecules and their energetics around small peptides have been studied recently [41]. Another important issue in protein hydration is the rearrangement of regular water–water (WW) hydrogen bond network at the protein surface with the formation of PW hydrogen bonds. It is essential to probe such hydrogen bonds at the interface to understand the hydration behavior of proteins. Simulation studies at atomistic resolutions can directly probe formation of hydrogen bonds and their relaxation properties at the protein surface. In a systematic study, Xu and Berne [42] demonstrated that the presence of a polypeptide significantly slows down the kinetics of WW hydrogen bonds in its hydration layer. Using MD simulations along with neutron scattering measurements, Tarek and Tobias [43] showed that the structural relaxation of a protein is controlled by the PW hydrogen bond dynamics. Recently, we showed that the relaxation dynamics of hydrogen bonds around different segments of the same protein molecule can be noticeably different [39]. For small proteins, such heterogeneity is primarily noticed within the first hydration layer of the protein and correlated with the time scale of density reorganization within the layer [44,45]. A series of temperature dependent MD simulations have been performed recently to study the differential dynamical behavior of protein hydration water [46,47]. These studies revealed that the hydration water molecules not only perform localized motions but also participate in the global dynamics through long-range diffusion. In another recent study, Czapiewski and Zielkiewicz [48] systematically investigated the structural properties of water molecules present in the solvation shells around different conformations of simple polypeptides.

Although, protein hydration has received immense attention in recent past, but several aspects of the problem still remain elusive. In particular, the effects of conformational and energetic heterogeneities of proteins on the microscopic properties of the surface water molecules have not been explored much. Recently, we carried out MD simulations to study how the heterogeneous PW electrostatic interactions and the local motions of the secondary structures of the protein barstar influence the microscopic dynamics and hydrogen bond properties of water molecules hydrating its surface [49]. The calculations revealed that enhanced confinement at the protein surface on freezing its local motions restricts water mobility around the secondary structures. Further, it is found that the inability of the surface water molecules to form hydrogen bonds with the protein residues in absence of PW electrostatic interactions is compensated by enhanced WW hydrogen bonds around the protein. In this work, we explore the roles played by differential local motions of barstar in its native form and the non-uniform distribution of PW electrostatic interaction sites on the structure and ordering of the surface water molecules. Barstar is a protein with 89 residues and is formed by bacterium bacillus amyloliquefacien. The same organism produces an extracellular ribonuclease, namely barnase, that is potentially lethal to the cell. Barstar acts as an intracellular polypeptide inhibitor of barnase [50]. The solution structure of the native form of barstar as obtained from NMR studies [51] is displayed in Fig. 1. The regular secondary structural segments of the protein consist of three parallel α-helices packed onto a three-stranded parallel β-sheet. It also has an additional α-helix linking the second central strand and the fourth α-helix. The amino acid sequence of the protein is K(1)KAVINGEQIRSISDLHQTLKKELALPEYYGENLDALWDCLTGWVEYPLVLEWRQFEQSKQLTENGAESVLQVFREAKAEGCDITIILS(89), with the N-terminus residue K(1) and the C-terminus residue S(89). For convenience, we denote these segments as helix-1 (Ser-14 to Ala-25), helix-2 (Asn-33 to Gly-43), helix-3 (Phe-56 to Thr-63), helix-4 (Glu-68 to Gly-81), and β-sheet (Lys-1 to Asn-6, Leu-49 to Arg-54, and Asp-83 to Ser-89). These segments are connected by several loops, which are denoted as loop-1 (Gly-7 to Ile-13), loop-2 (Leu-26 to Glu-32), loop-3 (Trp-44 to Pro-48), and loop-4 (Glu-64 to Ala-67). Barstar inhibits the function of barnase by sterically blocking its active site by helix-2 and the loop connecting that to helix-1 (loop-2).

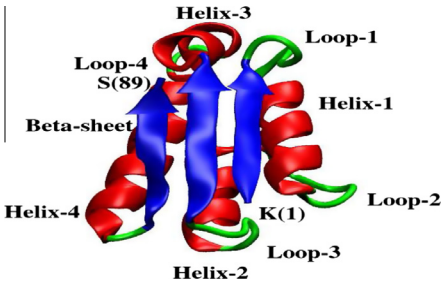

Fig. 1. The NMR structure of the 89 residue protein barstar [51]. It contains four α-helices (drawn in red) packed onto a three-stranded parallel β-sheet (drawn in blue). There are several loops interconnecting the secondary structures as shown in green. The amino acid sequence of the protein is given in the text. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. System setup and simulation protocols

We have carried out three simulations, designated as S1, S2, and S3 with the protein barstar in aqueous medium at room temperature. The fully flexible protein molecule in equilibrium with the solvent was studied in simulation S1. In the second simulation (S2), the protein molecule was kept frozen, while in addition to freezing the protein matrix, the electrostatic interactions between the protein and the solvent water molecules were turned off (by removing the protein atom charges) in simulation S3.

The initial coordinates of barstar were taken from the Protein Data Bank (PDB code: 1BTA), as obtained from NMR studies [51]. Two end residues (Lys-1 and Ser-89) of the protein were taken as ammonium and carboxylate ionic forms and the whole molecule was immersed in a large cubic box containing well-equilibrated water molecules. We followed the standard practice to avoid