

Correlation between the Dynamics of Hydrogen Bonds and the Local Density Reorganization in the Protein Hydration Layer

Sudip Chakraborty and Sanjoy Bandyopadhyay*

Molecular Modeling Laboratory, Department of Chemistry, Indian Institute of Technology, Kharagpur-721302, India

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An atomistic molecular dynamics simulation of the protein villin headpiece subdomain or HP-36 has been carried out with explicit water to explore the microscopic inhomogeneity of local density reorganization of the hydration layers of the three α -helical segments of the protein. The density reorganization of the hydration layer of helix-3 is found to occur faster than that for the hydration layers of the other two helices. It is noticed that such inhomogeneous density reorganization at the surface of different secondary structures exhibits excellent correlation with the microscopic dynamics of hydrogen bonds between the protein residues and the hydration water. Further, it is observed that the reorientation of water molecules involved in the formation and breaking of protein–water or water–water hydrogen bonds plays an important role in determining the dynamics of local density of the hydration layer. The faster density reorganization of the hydration layer of helix-3 is also consistent with the functionality of HP-36, as helix-3 contains several active site residues.

1. Introduction

It is known that water plays a key role in governing the structure, stability, dynamics, and functionality of proteins.^{1–12} The properties of water molecules present in the first hydration layer of a protein (biological water) are of most importance due to the dynamical coupling that exists between these water molecules and the protein.^{4,13,14} Microscopic information of such coupling is necessary to understand the role played by water during biological processes, such as protein–substrate binding, folding–unfolding phenomena, etc.

Recently, several experimental studies have been reported on aqueous protein solutions with particular emphasis on exploring the structural and dynamical properties of water present in the hydration layer. Recent advances in ultrafast optical spectroscopy permit one to study the dynamics over a wide range of time scales, starting from femtoseconds to nanoseconds or even more.^{2,3,15–18} Using femtosecond-resolved fluorescence spectroscopy with tryptophan (Trp) as an intrinsic probe, Zewail, Zhong, and co-workers^{2,3,15,16} have studied in great detail the solvation dynamics at the surface of different proteins. They observed a bimodal distribution of solvation time scales. While the initial component within a few picoseconds was attributed to fast librational and reorientational motions of the hydration layer water molecules, the second component observed in the time scale of tens of picoseconds was attributed to the coupled slow dynamics of water molecules hydrating the protein surface. From site-specific mutation studies, it was concluded that the dynamics of the residue side chains do not have a major contribution toward the long-time component.^{15,16} In contrast, Nilsson and Halle¹⁷ have recently claimed that the dynamics of water at the protein surface is not much slower than that of bulk water, and hence the protein side chain relaxation contributes to the long-time components of the solvation dynamics. Bhattacharyya and co-workers¹⁸ have recently carried out solvation dynamics experiments to understand the solvent

behavior in the hydration layers of proteins in non-native states. Quasielastic neutron scattering (QENS) techniques have been used by Head-Gordon and co-workers¹⁹ to study the hydration water dynamics of proteins at different temperatures and concentrations. They have shown that the relaxation dynamics of the hydration layer water is non-exponential in nature, while the water translational dynamics exhibits a non-Arrhenius behavior over a wide range of temperatures. Recently, Havenith and co-workers²⁰ have demonstrated that terahertz spectroscopy can be used as an effective tool to directly investigate the dynamics of hydration water around biomolecules. Attempts have also been made to study the dynamics of proteins and hydration water at different time scales by using other experimental techniques such as NMR,^{21,22} dielectric relaxation,^{13,23} nuclear magnetic relaxation dispersion (NMRD),²⁴ etc.

Molecular dynamics (MD) simulations can provide valuable insight into the microscopic details of the dynamics of hydration layer water and its coupling with the residue side chain motions at the protein surface. This is due to the accessibility of the time scales associated with such dynamics by MD simulations. Rossky and Karplus⁷ and Levitt and Sharon⁸ in their early works used MD simulations to study the characteristic structure and dynamic properties of aqueous protein solutions. Since then several simulation studies have been reported on the structure and dynamics of water at the surface of proteins and their correlation with the structure and dynamics of the proteins themselves.^{9,10,12,25–35} Cheng and Rossky²⁵ have demonstrated that two different hydration structures can exist at the surface of a protein and the relative contributions of those depend on the topography of the protein surface. Using a combination of MD simulations and QENS measurements, Tarek and Tobias²⁶ have shown that the structural relaxation of a protein requires complete exchange of water molecules bound to the protein. Berne and co-workers⁹ have demonstrated that the kinetics of water–water hydrogen bonds and the diffusion of water in the hydration layer of a protein are much slower than that in bulk water. They have also shown that the local surface curvature and hydrophobicity influence the dynamics of water near the

* Author to whom correspondence should be addressed. E-mail: sanjoy@chem.iitkgp.ernet.in.

surface.²⁷ Marchi and co-workers²⁸ found that the rotational dynamics of water near the surface of a protein is much slower than that in the bulk. Bizzarri and Cannistraro¹⁰ have shown recently that water exhibits restricted subdiffusive mobility in the hydration layer of a protein. Extensive MD studies have also been carried out by Pettitt and co-workers²⁹ to understand the solvation behavior of proteins. Recent MD studies have shown that the density of water in the hydration layer is higher than bulk density with parallel alignment of the water molecules.^{30,31} Only recently, Golosov and Karplus³² have demonstrated that the polar solvation dynamics of proteins are position-dependent and heterogeneous. They showed that the slow components of the order of hundreds of picoseconds originate from coupled hydration and protein conformational dynamics. We studied in detail the origin of the slow dynamics of hydration layer water and its coupling with the residue side chain dynamics of proteins.¹² A heterogeneity in the dynamics of hydrogen bonds between the amino acid residues and hydration layer water was observed among different segments of a protein.³³ We also showed that the solvation dynamics of different secondary structures of a protein are sensitive to the location of the probes and their exposure at the protein surface.³⁴ Recently, we have also explored the kinetics of protein unfolding and its correlation with the hydration water dynamics.³⁵

In this article, we have performed an atomistic MD simulation to investigate the microscopic details of the inhomogeneity in the local density reorganization of the hydration layers of different secondary structures present in a small globular protein containing 36 amino acid residues, HP-36. The correlation between the kinetics of hydrogen bonds formed by the hydration layer water molecules, either among themselves or with the protein residues, and the time scale of density reorganization have been explored. HP-36 is the thermostable α -helical subdomain present at the extreme C-terminus of the 76-residue villin protein.³⁶ It contains one of the two F-actin binding sites of villin necessary for F-actin bundling activity.³⁷ As an easy convention, we number the residues of HP-36 from 1 to 36, which actually correspond to residues 41 to 76 of villin protein.³⁶ The primary sequence details of HP-36 are mentioned in our earlier work.¹² It contains three short α -helices, which are connected and held together by a few turns and loops and a hydrophobic core. The three α -helices are denoted as helix-1 (Asp-4 to Lys-8), helix-2 (Arg-15 to Phe-18), and helix-3 (Leu-23 to Glu-32).¹² The functionality of the protein is centered around helix-3, which contains 10 residues.³⁶ The rest of the article is organized as follows. In Section 2 we describe the setup of the simulation system and the methodologies employed. The results obtained from our investigations are presented and discussed in Section 3. In the last section we summarize the important findings and the conclusions reached from our study.

2. System Setup and Simulation Details

The initial coordinates of the protein as obtained from NMR studies³⁶ were taken from the Protein Data Bank (PDB ID 1VII). The end residues (Met-1 and Phe-36) of the protein were capped and the whole molecule was immersed in a large cubic box of equilibrated water. The system contained the protein molecule (596 atoms) in a 61 Å cubic box containing 6842 water molecules.

The simulation was carried out for over 3.5 ns duration with a MD time step of 4 fs. It was first performed at constant temperature ($T = 300$ K) and pressure ($P_{\text{ext}} = 0$) (NPT), followed by runs at constant temperature and volume (NVT). The MD trajectory was stored during the last 2.5 ns duration

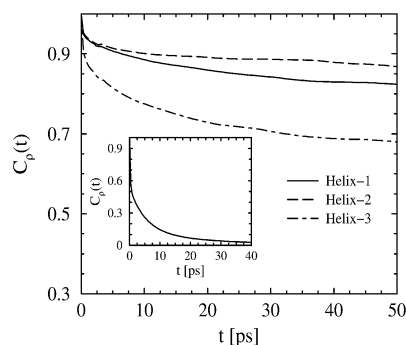


Figure 1. Local density autocorrelation function, $C_\rho(t)$, for the hydration layers of the three α -helices of the protein. The inset shows the corresponding function for pure bulk water.

of the NVT production run with a time resolution of 400 fs. The CHARMM22 all-atom force field and potential parameters for proteins³⁸ were employed to describe the interaction between protein atoms, while the TIP3P model,³⁹ which is consistent with the chosen protein force field, was employed for water. Further details of the simulation methods employed in this study are reported elsewhere.¹²

3. Results and Discussion

The structural inhomogeneity of a protein molecule is likely to influence the local reorganization of solvent around it in a differential manner. To verify this, we have characterized the instantaneous number density, $\rho(t)$, of water in the hydration layers of the three helices of HP-36. The hydration layer volume of a particular helix is considered to be the volume of a shell with a 5 Å width from the non-hydrogen atoms of the respective helix. We have used the method as proposed by Smolin and Winter³¹ to calculate the volumes. The program MOL_VOL-UME⁴⁰ was used for the calculations. The instantaneous density $\rho(t)$ is then obtained by dividing the number of water molecules present in the calculated instantaneous volume by the corresponding volume. The dynamics of density reorganization of the hydration layers of the three helices are then monitored through the local density fluctuation autocorrelation function, $C_\rho(t)$, defined as

$$C_\rho(t) = \frac{\langle \delta\rho(t)\delta\rho(0) \rangle}{\langle \delta\rho(0)\delta\rho(0) \rangle} \quad (1)$$

where, $\delta\rho(t) = \rho(t) - \langle \rho \rangle$, and $\rho(t)$ is the instantaneous number density of water in the hydration layer at time t and $\langle \rho \rangle$ is the average number density. The angular brackets denote averaging over the hydration layer water molecules and over different reference initial times.

We have calculated the function $C_\rho(t)$ for the hydration layers of the three helices of HP-36, as displayed in Figure 1. The inset shows the corresponding function for pure bulk water. The bulk water results are computed from a MD simulation of pure TIP3P water under identical conditions. The instantaneous number density $\rho(t)$ around a central water molecule in bulk water is measured by dividing the number of water molecules present within a sphere of radius 5 Å with respect to the central water by the volume of the sphere. It is clear from the figure that compared to bulk water the relaxation of $C_\rho(t)$ occurs much more slowly in the protein hydration layers. This indicates the rigid nature of the hydration layer water molecules and is consistent with their slower dynamical behavior, as reported earlier.¹² However, most interestingly, a heterogeneous relaxation behavior is observed among the hydration layers of the

TABLE 1: Average Local Density Reorganization Times (τ_ρ) and the Intermittent Protein–Water (τ_C^{PW}) and Water–Water (τ_C^{WW}) Hydrogen Bond Time Constants Corresponding to the Hydration Layers of the Three α -Helices of the Protein Along with the Corresponding Times for Pure Bulk Water for Comparison

segment	τ_ρ (ps)	τ_C^{PW} (ps)	τ_C^{WW} (ps)
helix-1	83.4	28.7	4.7
helix-2	87.2	45.5	5.0
helix-3	68.8	14.1	3.8
bulk water	4.2		2.9

three helices. The density reorganization of the hydration layer of helix-3 occurs faster than that for the hydration layers of the other two helices. The time integral of the function $C_\rho(t)$ describes the average time, τ_ρ , required for the density of the hydration layers to undergo significant changes. τ_ρ is often known as the local density reorganization time.^{41,42} We have estimated the τ_ρ values for the hydration layers of the three helices, which are listed in Table 1. It may be noted that the densities of the hydration layers take an order of magnitude longer to reorganize than bulk water. It is also noticed that the τ_ρ value for the hydration layer of helix-3 is $\sim 20\%$ shorter than that for the other two helices. The results clearly indicate differential rigidity of hydration layers among the three helices. Earlier, we showed that the mobility of water in the hydration layer of helix-3 is faster than those for the other two helices.¹² Thus a correlation clearly exists between the local density reorganization of the hydration layers of the three helices and the differential dynamics of water in the layers. Relatively faster density reorganization of the hydration layer of helix-3 also correlates well with the biological activity of HP-36. It may be noted that although the time scale of a binding process is much longer than the time scale of the simulation, it is expected that for a successful binding, the density of water around the active site residues should fluctuate faster. This is exactly what we observe for helix-3, which contains several active residues. However, much more work is needed to quantitatively establish such correlation with biological activity.

The inhomogeneous density fluctuations of the hydration layers of different secondary structures of a protein are likely to be correlated with the dynamics of hydrogen bonds formed by the hydration layer water. Faster hydrogen bond dynamics should result in faster reorganization of water density and vice versa. To explore such a correlation, if any, we have characterized the dynamics of hydrogen bonds formed by water in the hydration layers separately for the three helices. It is known that the regular water–water (WW) hydrogen bond network is modified at the surface of a protein molecule with the formation of protein–water (PW) hydrogen bonds. The PW hydrogen bonds are generally stronger and hence have longer lifetimes.³³ Here, we study the microscopic dynamics of both PW and WW hydrogen bonds formed by the hydration layer water of the three helices to verify their degree of influence on the inhomogeneous density reorganization of the hydration layers.

The dynamics of hydrogen bonds are characterized by calculating the intermittent hydrogen bond autocorrelation function, $C(t)$, defined as^{43,44}

$$C(t) = \frac{\langle h(0)h(t) \rangle}{\langle h(0)h(0) \rangle} \quad (2)$$

where the hydrogen bond population variable $h(t)$ is considered to be unity if a pair of sites (PW or WW) is hydrogen bonded at time t according to the definition of the bond, and zero

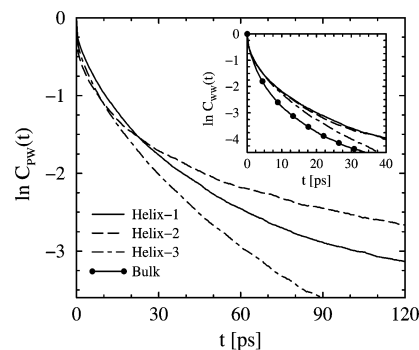


Figure 2. Intermittent hydrogen bond time correlation function, $C_{PW}(t)$, between the residues of the three α -helices and the hydration layer water molecules. The inset shows the corresponding function, $C_{WW}(t)$, for the water molecules themselves in the hydration layers of the helices as well as that for pure bulk water.

otherwise. We have employed the same geometrical criterion to define a hydrogen bond as in our previous study.³³ The function $C(t)$ describes the probability that a hydrogen bond is intact at time t , given that it was intact at $t = 0$. Thus, $C(t)$ is independent of possible breaking of hydrogen bonds at intermediate times and allows reformation of broken bonds. Alternatively, $C(t)$ allows recrossing the barrier between the bonded and free states and the long-time diffusive behavior. Therefore, by monitoring the relaxation of $C(t)$ one can obtain information about the structural relaxation of a particular type of hydrogen bonds.

We have calculated the correlation functions for the hydrogen bonds between the amino acid residues and water molecules ($C_{PW}(t)$) as well as those between the water molecules themselves ($C_{WW}(t)$) in the hydration layers of the three helices separately. These are displayed in Figure 2. It is apparent from the figure that the relaxations of $C_{PW}(t)$ and $C_{WW}(t)$ for the hydration layer water are slower than that for bulk water. However, more importantly, it can be noted that an inhomogeneity exists in the relaxation behavior of $C_{PW}(t)$ and $C_{WW}(t)$ among the three helices. The degree of inhomogeneity is more pronounced for the PW hydrogen bonds. The dynamics of hydrogen bonds formed between the helix-3 residues and water in the surrounding hydration layer is significantly faster than that for the other two helices. The WW hydrogen bonds formed among the helix-3 hydration layer water molecules also relax slightly faster than those formed between water molecules near the other two helices. The average relaxation time constants (τ_C^{PW} and τ_C^{WW}) as obtained from the decay curves are listed in Table 1. It can be seen that an excellent correlation exists between the heterogeneous dynamics of hydrogen bonds and that of the local density reorganization of the hydration layers of the three helices. The correlation is particularly evident for the PW hydrogen bonds. Thus, the density reorganization of water in the immediate vicinity of a particular segment of a protein is primarily controlled by the formation of PW hydrogen bonds by the segment and their dynamics. Demonstration of such a correlation is an important finding, particularly due to the fact that helix-3 contains several active residues of HP-36.

It is known that the dynamics of hydrogen bonds between a pair of sites at long times is coupled with the diffusion of the pair.^{45,46} Faster diffusion will lead to faster dynamics of hydrogen bonds and vice versa. Since the self-diffusion of water is orders of magnitude faster than that of the protein, the dynamics of both PW and WW hydrogen bonds formed by the hydration layer water will be essentially correlated with their self-diffusion. As discussed earlier, the intermittent hydrogen

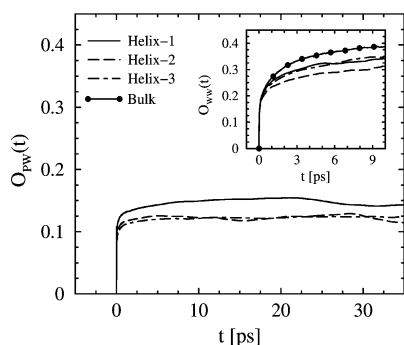


Figure 3. Time-dependent probability of the breaking of protein–water hydrogen bonds without the water molecules being diffused away from the residues, $O_{PW}(t)$, for the three α -helices of the protein. The inset shows the corresponding function, $O_{WW}(t)$, for the water molecules themselves in the hydration layers of the helices as well as that for pure bulk water.

bond correlation function ($C(t)$) contains information about the long time pair diffusion. Thus the correlation between $C_\rho(t)$ and $C_{PW}(t)$ or $C_{WW}(t)$ at long times (Figures 1 and 2) indicates the role of diffusion of hydration layer water in substantial variation of the density of the layer.

We now investigate whether on eliminating the contribution arising from the diffusion of water, similar correlation exists between the inhomogeneous reorganization of hydration layer density and the dynamics of hydrogen bond breaking for the three helices. For that, we calculate another hydrogen bond time correlation function

$$O(t) = \frac{\langle h(0)(1 - h(t))H(t) \rangle}{\langle h(0)H(t) \rangle} \quad (3)$$

where $H(t)$ is unity if the tagged pair of sites is closer than a cutoff distance, R_H (3.3 Å for PW and 3.5 Å for WW hydrogen bonds) at time t , and zero otherwise. A value of zero for $O(t)$ indicates that the tagged pair of sites (PW or WW) are either in the bonded state or separated by a distance greater than R_H . A nonzero value, on the other hand, signifies that although the two sites remain within the first neighboring shell, they are not hydrogen bonded. Thus, $O(t)$ describes the time-dependent probability of breaking a hydrogen bond without the two sites being diffused away from each other's neighboring shells. As the hydrogen bond breaking and reformation within the first neighboring shell primarily occur due to the relative reorientation of the pair of bonded sites, $O(t)$ therefore represents the breaking and reformation of PW or WW hydrogen bonds due to reorientation of the involved pair of sites.

We have calculated the function $O_{PW}(t)$ for the hydrogen bonds formed by the hydration layer water molecules with the amino acid residues of the three helices, as shown in Figure 3. The inset of the figure shows the corresponding function $O_{WW}(t)$ for the hydrogen bonds formed by the hydration layer water molecules among themselves as well as that for water in bulk. The figure shows that the PW hydrogen bonds break much more slowly than the WW hydrogen bonds in the hydration layers and in bulk water. More interestingly, a degree of inhomogeneity is noticed for both PW and WW hydrogen bonds among the three helical segments. We observe that the PW hydrogen bonds break relatively faster for helix-1 residues. On the other hand, the WW hydrogen bonds in the hydration layer of helix-2 break more slowly than those in the hydration layers of the other two helices. It can be seen that the differential behavior exhibited by $O_{WW}(t)$ for the hydration layers of the helices is consistent with that observed for the density reorganization of the hydration

layers. This is an important observation which suggests that not only diffusion, but also the breaking of hydrogen bonds due to reorientation of water molecules involved in PW or WW hydrogen bonds also govern the degree of inhomogeneity of the local density reorganization of the hydration layers of different segments of a protein.

4. Conclusions

In this work, we have presented results obtained from an atomistic MD simulation study of an aqueous solution of the 36 residue helical subdomain of villin headpiece (HP-36). In particular, we have explored the dynamics of reorganization of local density of the hydration layers of the three α -helical segments of the protein. An attempt has been made to establish whether a correlation exists between the dynamics of density reorganization and that of the hydrogen bonds formed by the hydration layer water molecules.

The calculations revealed the existence of an inhomogeneity in the time scale of density reorganization of the hydration layers of the three helices of the protein molecule. It is noticed that the density of water in the hydration layer of helix-3 fluctuates faster than that for the other two helices. We have observed an excellent correlation between the differential relaxation behavior of the intermittent PW and WW hydrogen bond correlation functions among the three helices and that of the water density reorganization in the respective hydration layers. To the best of our knowledge, this is the first report where such a microscopic correlation has been established. It is also shown for the first time that by eliminating the contribution arising from pair diffusion, the breaking and formation of hydrogen bonds in the hydration layer due to relative reorientation of the two sites involved in bonding can influence the dynamics of density reorganization. The relatively faster time scale of density reorganization in the hydration layer of helix-3 and its correlation with the dynamics of corresponding PW and WW hydrogen bonds may have important biological significance. The active amino acid residues of HP-36 are located in helix-3. Although the exact mechanism of the binding process is not known and the time scale associated with it is much longer than the present simulation time scale, the rapid fluctuation of water density in the hydration layer of helix-3 and faster dynamics of hydrogen bonds are likely to facilitate the binding process. However, this needs to be verified further. Currently, we are investigating whether similar correlation exists for other proteins with more complex secondary structures.

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