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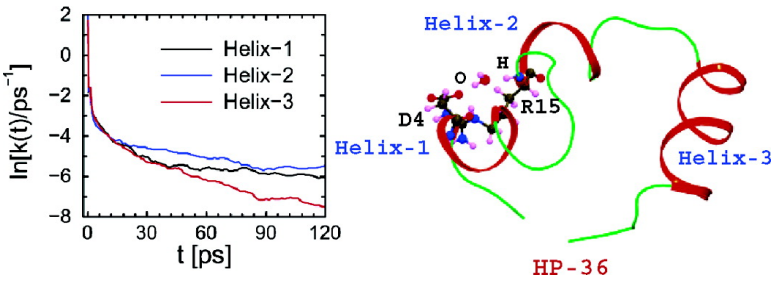
J. Am. Chem. Soc., **2005**, 127 (47), 16660-16667• DOI: 10.1021/ja054462u • Publication Date (Web): 03 November 2005



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Secondary Structure Sensitivity of Hydrogen Bond Lifetime Dynamics in the Protein Hydration Layer

Sanjoy Bandyopadhyay,*,† Sudip Chakraborty,† and Biman Bagchi*,‡

Contribution from the Molecular Modeling Laboratory, Department of Chemistry, Indian Institute of Technology, Kharagpur-721302, India, and Solid State and Structural Chemistry Unit, Indian Institute of Science, Bangalore-560012, India

Received July 6, 2005; E-mail: sanjoy@chem.iitkgp.ernet.in; bbagchi@sscu.iisc.ernet.in

Abstract: The heterogeneous nature of a protein surface plays an essential role in its biological activity and molecular recognition, and this role is mediated at least partly through the surrounding water molecules. We have performed atomistic molecular dynamics simulations of an aqueous solution of HP-36 to investigate the correlation between the dynamics of the hydration layer water molecules and the lifetimes of protein−water hydrogen bonds. The nonexponential hydrogen bond lifetime correlation functions have been analyzed by using the formalism of Luzar and Chandler, which allowed identification of the quasi-bound states in the surface and quantification of the dynamic equilibrium between quasi-bound and free water molecules in terms of time-dependent rate of interconversion. It is noticed that, irrespective of the structural heterogeneity of different segments of the protein, namely the three α-helices, the positively charged amino acid residues form longer-lived hydrogen bonds with water. The overall relaxation behavior of protein−water hydrogen bonds is found to differ significantly among the three helices of the protein. Study of water number density fluctuation reveals that the hydration layer of helix-3 is much less rigid, which can be correlated with faster structural relaxation of the hydrogen bonds between its residues and water. This also agrees excellently with faster translational and rotational motions of water near helix-3, and hence the lower rigidity of its hydration layer. The lower rigidity of the helix-3 hydration layer also correlates well with the biological activity of the protein, as several of the active-site residues of HP-36 are located in helix-3.

1. Introduction

The presence of an extended network of hydrogen bonds in liquid water is responsible for many exotic structural and dynamical properties of water.[1−3] The formation and breaking of hydrogen bonds play a crucial role in determining the dynamical properties of water.[4] Although a considerable effort has been made over the past several decades to study the hydrogen bond dynamics in liquid water,[4−10] a proper microscopic-level understanding of the problem is still far from being complete. The dynamics of hydrogen bonds can be probed indirectly and interpreted only qualitatively by different experimental techniques, such as Raman scattering, depolarized light scattering, inelastic neutron scattering, ultrafast IR spectroscopy, etc.[7,11−17] On the other hand, computer simulations in general, and molecular dynamics (MD) studies in particular, can provide quantitative information on hydrogen bond dynamics with atomistic resolution. The factors influencing the dynamics can be ascertained from MD trajectories by calculating different hydrogen bond time correlation functions, as proposed first by Stillinger[4] and developed further by Luzar and Chandler.[5,6] In recent years, there have been a number of simulation studies primarily focused on the relaxation behavior of hydrogen bonds in pure water as well as in aqueous solutions of electrolytes and micelles.[5,6,8−10,18−25] These simulation studies in general

† Indian Institute of Technology.
‡ Indian Institute of Science.

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10.1021/ja054462u CCC: $30.25

have shown that the relaxation behavior of hydrogen bonds in liquid water is nonexponential in nature. Recently, Luzar and Chandler[5,6] have proposed a simple model to describe the kinetics of hydrogen bonds in water. The model treats the hydrogen bond dynamics as an activated process, where the rate of relaxation is characterized by a reactive flux correlation function formalism. These studies revealed that the formation and breaking of hydrogen bonds in water are not simple processes with well-defined rate constants. They have also shown that the nonexponential relaxation behavior at long times arises due to the coupling of hydrogen bond dynamics and the diffusional motion of water.[5]

The regular hydrogen bond network in pure water gets disrupted at the interface of self-organized assemblies and biomolecules, such as proteins. The nature of interactions between protein and water in aqueous solutions is an important issue, as it is believed that water plays a primary role in determining the structure, stability, and function of the protein. It is now known that a dynamical coupling exists between a protein molecule and the water present in its hydration layer (biological water).[26−28] It has been proposed that a quantitative description of this coupling can be obtained by assuming the presence of quasi-bound water molecules at the surface of the protein. These quasi-bound water molecules exist because of the long lifetime of the hydrogen bonds between polar amino acid residues on the surface and the water molecules. Dynamic equilibrium between these quasi-bound water molecules with the free ones is an important process occurring at the surface. A microscopic-level understanding of such dynamical coupling is crucial for many biological processes, such as protein−enzyme interactions, molecular recognition, and folding−unfolding phenomena. Because of the importance of the issues involved, this area has drawn the attention of many researchers over the past several years.[29−44] Time-resolved fluorescence spectroscopy and three-pulse photon echo measurements have been used recently by different researchers to study the dynamics of proteins and the interfacial water molecules.[30−32] These studies in general indicate a restricted movement of water close to the protein surface. Head-Gordon and co-workers[33] have recently used quasi-elastic neutron scattering (QENS) experiments to study the hydration water dynamics of model proteins as a function of temperature and concentration. They have shown that the relaxation dynamics of the hydration layer water is nonexponential in nature, while the water translational dynamics exhibited a non-Arrhenius behavior over a wide range of temperatures. Halle and co-workers[34] have studied in great detail the dynamics of the protein hydration layer using nuclear magnetic relaxation dispersion (NMRD) techniques. They have demonstrated that in most cases the water in the hydration layer is only weakly retarded by the protein.

Despite significant efforts, the origin and the extent of the sluggish dynamics of hydration layer water are still not properly understood. The formation of hydrogen bonds between protein and water and the dynamical coupling between them are primarily responsible for the slow dynamics of water in the hydration layer. Computer simulations provide a powerful tool to describe the kinetics of hydrogen bonds around a solvated protein molecule, and thus can help us gain a microscopic-level understanding of the time scale of such coupling. Since the early works of Rossky and Karplus,[35] and Levitt and Sharon,[36] many simulations have been attempted in the past decade or so to study the dynamical properties of proteins in aqueous solutions.[37−44] Tarek and Tobias[37] have reported water mobility for several proteins in solution as well as in their crystals, dry and hydrated powders, using a combination of MD simulations and QENS measurements. They have shown that a complete exchange of protein-bound water molecules is necessary for the structural relaxation of a protein. Xu and Berne[38] have shown that the kinetics of the water−water hydrogen bond formation and breaking in the first solvation shell of a polypeptide is slower than that in bulk water. Cheng and Rossky[39] have demonstrated that two different hydration structures can exist near a protein surface. Extensive MD studies have been carried out by Pettitt and co-workers[40] on the solvation behavior of proteins. In a recent study, Marchi et al.[41] found that the rotational dynamics of water in the vicinity of lysozyme is much slower than that in the bulk. It has been shown recently that besides exhibiting highly restricted mobility, water in the hydration layer of a protein also exhibits subdiffusive motion.[43] Recently, we studied in detail the correlation between the dynamics of the amino acid residues of a protein and the surrounding water molecules.[44] It was observed that the waters in the vicinity of the active-site residues are less structured and more mobile than those around the other residues. We also showed that the dynamics of protein

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solvation is sensitive to the details of the adjacent secondary structure of the protein, such as the relative exposure of probe residues at the protein surface.[44]

It has long been recognized that the molecular recognition of the specific regions of protein may be at least partly controlled by the structure and dynamics of water around various distinct structural moieties of proteins. It is expected that the water molecules present in the vicinity of polar/charged amino acid residues would be relatively less mobile, with residence times ranging from 10 to 100 ps[45] or even more in some cases, while water molecules around hydrophobic residues are expected to be more mobile. There are certainly several other factors which play important roles in determining the rigidity of water around specific regions/amino acids, hydrophobicity and charge being just two of them. For example, the solvent accessibility of a specific site in a protein is also expected to play a role in determining the water mobility around the site.[46] To the best of our knowledge, these aspects have not been studied in great detail and deserve more attention.

To probe the origin of the rigidity of the protein hydration layer, we have investigated in detail the microscopic dynamics of the hydrogen bonds formed by water molecules with different secondary structure segments present in a small 36-residue globular protein, HP-36. HP-36 is the thermostable subdomain present at the extreme C-terminus of the 76-residue chicken villin headpiece domain.[47] Villin is a unique protein which can both assemble and disassemble actin structures.[48] HP-36 contains one of the two F-actin binding sites in villin necessary for F-actin bundling activity.[48] In this work we number the residues from 1 to 36. Thus, residues 1−36 correspond to residues 41−76 in the NMR structure.[47] The primary sequence details of HP-36 are mentioned in our earlier work.[44] The secondary structure of the protein contains three short α-helices. These helices are connected and held together by a few turns and loops and a hydrophobic core. We denote the three α-helices as helix-1 (Asp-4 to Lys-8), helix-2 (Arg-15 to Phe-18), and helix-3 (Leu-23 to Glu-32).[44] The biological activity is believed to be centered around helix-3, which contains 10 amino acid residues.[47] This protein subdomain has been studied extensively in recent years, especially with respect to its folding.[49]

We employ atomistic MD simulations to study the dynamics of hydrogen bond lifetimes. The article is organized as follows. In the next section we describe the system setup and the simulation methods employed. The results obtained from our investigations are presented and discussed in the following section. 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 were taken from the Protein Data Bank (PDB ID 1VII) from the NMR structure of the villin headpiece subdomain, as reported by McKnight et al.[47] The two end residues (Met-1 and Phe-36) of the protein were capped appropriately, and the whole molecule was immersed in a large cubic box of well-equilibrated water. The system contained the 36-residue-long 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 ($P_{ext} = 0$) (NPT), followed by runs at constant temperature and volume (NVT). The MD trajectory was stored during the last 2.5 ns duration of the NVT production run with a time resolution of 400 fs. To investigate the ultrafast properties, a section (∼600 ps) of the equilibrated trajectory was also stored at a higher time resolution of 16 fs. The CHARMM22 all-atom force field and potential parameters for proteins[50] were employed to describe the interaction between protein atoms, while the TIP3P model[51] which is consistent with the chosen protein force field was employed for modeling water. The details of the simulation methods employed in this study are reported elsewhere.[44]

3. Results and Discussion

The dynamics of interfacial water molecules and their structural organization are correlated with the network of hydrogen bonds formed between them and the amino acid residues of the protein molecule.[43,52−54] The formation and breaking of these hydrogen bonds play an important role in determining the functionality of the protein. Generally, either a geometric[43,53,55] or an energetic[56,57] criterion is used to define a hydrogen bond. In this work, we have employed a purely geometric criterion to define a hydrogen bond.[55]

The dynamics of hydrogen bonds formed between water and the amino acid residues of the protein, as well as among the water molecules themselves, have been characterized in terms of two time correlation functions (TCFs), namely, the continuous hydrogen bond time correlation function, $S(t)$, and the intermittent hydrogen bond time correlation function, $C(t)$.[4,18] These TCFs are defined as

$$S(t) = \frac{\langle h(0)H(t)\rangle}{\langle h\rangle} \tag{1}$$

and

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

These definitions are based on two hydrogen bond population variables, $h(t)$ and $H(t)$. The variable $h(t)$ is unity when a particular pair of sites (protein−water or water−water) is hydrogen bonded at time t according to the definition used and zero otherwise. The variable $H(t)$, on the other hand, is defined as unity when the tagged pair of sites remain continuously hydrogen bonded from time $t = 0$ to time t, and zero otherwise. Thus, $S(t)$ describes the probability that a hydrogen bond formed between two sites at time zero remains bonded at all times up to t. In other words, $S(t)$ provides a strict definition of the

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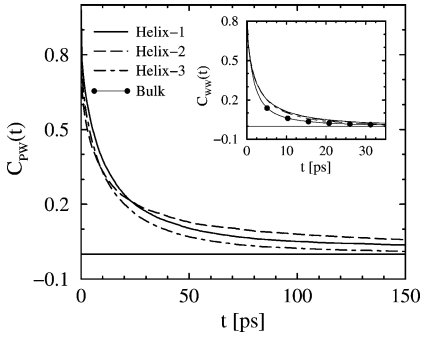


Figure 1. Intermittent hydrogen bond time correlation function, $C_{PW}(t)$, between the amino acid residues of the three helices of the protein and the hydration layer water molecules. The inset shows the corresponding correlation function $C_{WW}(t)$ for hydrogen bonds between water molecules present in the hydration layers of the helices as well as that in pure bulk water.

lifetime of a tagged hydrogen bond. The correlation function $C(t)$, on the other hand, describes the probability that a particular tagged hydrogen bond is intact at time t, given it was intact at time zero. Thus, $C(t)$ is independent of possible breaking of hydrogen bonds at intermediate times and allows re-formation of broken bonds. In other words, it allows recrossing the barrier separating the bonded and nonbonded states, as well as the long-time diffusive behavior. Therefore, the relaxation of $C(t)$ provides information about the structural relaxation of a particular hydrogen bond.

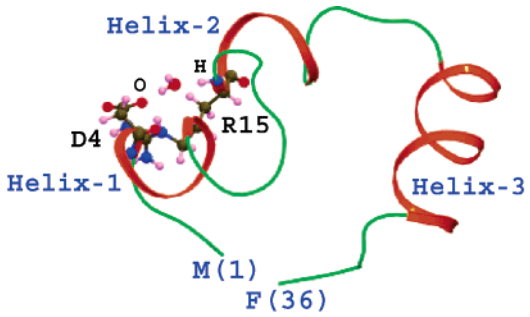
We have calculated the time correlation function $C_{PW}(t)$ for the hydrogen bonds formed between the amino acid residues and the water molecules separately for the three helices of the protein molecule. These are displayed in Figure 1. The inset of the figure shows the corresponding function $C_{WW}(t)$ for the hydration layer water of the three helices as well as that for pure bulk water. The results for bulk water have been obtained from a MD simulation of pure TIP3P water under identical conditions. It is apparent from the figure that the structural relaxation of the protein−water (PW) hydrogen bonds is much slower than that of the water−water (WW) hydrogen bonds. Most interestingly, we find that the relaxation behavior of $C_{PW}(t)$ is significantly different for the three helices. The structural relaxation of the hydrogen bonds formed between the residues in helix-3 and the hydration layer water molecules is faster than that for the other two helices. The inset shows that the relaxation of $C_{WW}(t)$ for water in the hydration layer of the helices is also slower than that for pure bulk water. The decay curves clearly show the presence of slow components for all the cases. Such slow long-time decay cannot be described by a single exponential law. It is a common practice to use multiexponentials to fit such functions because one can then directly obtain the time constants associated with different motions.[25,44] We have used a sum of four exponentials to fit the $C_{PW}(t)$ and $C_{WW}(t)$ decay curves for the three helices, while three exponentials are used for pure bulk water. The amplitude-weighted average time constants, $\langle\tau_c^{PW}\rangle$ and $\langle\tau_c^{WW}\rangle$, obtained from the fits are listed in Table 1. It may be noted that the $\langle\tau_c^{PW}\rangle$ values are about 5−15 times longer than the $\langle\tau_c^{WW}\rangle$ values for pure bulk water. We also note that the $\langle\tau_c^{WW}\rangle$ values for the hydration layer water are 30−70% longer than those for pure bulk water. In our earlier studies,[44] we observed that the average rotational time constants for the hydration layer water molecules were 8−16 times longer than that for pure bulk water. It is also noticed that the $\langle\tau_c^{PW}\rangle$ value for helix-3 is 2−3 times shorter than that for the other two helices. Further, we find small but noticeable differences among the $\langle\tau_c^{WW}\rangle$ values for the hydration layers of the three helices. The $\langle\tau_c^{WW}\rangle$ for helix-3 hydration layer is ∼30% shorter than that for the other two helices. Thus, the differential relaxation behavior of $C_{WW}(t)$ for the hydration layers of the three helices is consistent with that of the corresponding $C_{PW}(t)$ functions. Again, the translational diffusion and rotational motions of water in the hydration layer of helix-3 were approximately 2 times faster than those for the hydration layer water of helix-1 and helix-2.[44] Thus, we observe an excellent correlation between the relaxation behavior of intermittent protein−water and water−water hydrogen bond TCFs ($C_{PW}(t)$ and $C_{WW}(t)$) and the dynamics of the hydration layer water molecules. These results also indicate contrasting rigidity of the hydration layers of the three helices. These are extremely important findings and agree well with the biological functionality of the protein, as most of the active-site residues in HP-36 are centered in helix-3. It may be noted that the $C_{PW}(t)$ decay curves for helix-1 and helix-2 are nearly flat at long times, compared to that for helix-3. This is in accordance with slow reorientational relaxation of the hydration layer water for helix-2, observed earlier.[44] Such slow decay of $C_{PW}(t)$ is a signature of the presence of a small fraction of strongly bound motionally restricted water molecules near helix-1 and helix-2. We noticed that, in most cases, an interfacial bound water molecule forms one hydrogen bond with an amino acid residue site. Only a few water molecules present near helix-1 and helix-2 have been found to form two hydrogen bonds with two different residue sites. We identified one such location at the protein surface, where a water molecule forms a bridged structure by simultaneously forming hydrogen bonds with residues Asp-4 in helix-1 and Arg-15 in helix-2. As a result, these water molecules are motionally restricted with long residence times of 300 ps or more within the hydration layers of helix-1 and helix-2. Figure 2 displays a snapshot obtained from the simulation showing the location of such a water molecule. These water molecules should have a higher propensity to re-form hydrogen bonds, as they are doubly hydrogen bonded to the protein residues. The slower decay of $C_{PW}(t)$ curves (Figure 1) for helix-1 and helix-2 partly originates from such motionally restricted, strongly bound water molecules.

In Figure 3, we display the relaxation of the continuous hydrogen bond time correlation function, $S_{PW}(t)$, for the hydrogen bonds between the amino acid residues of the three helices and water. The inset shows the corresponding function $S_{WW}(t)$ for pure bulk water as well as that for water in the hydration layers of the three helices. The calculations are carried out by averaging over the hydrogen bonds that are formed at

Table 1. Average Relaxation Times of Intermittent ($\langle\tau_c^{PW}\rangle$) and Continuous ($\langle\tau_s^{PW}\rangle$) Protein−Water Hydrogen Bond Time Correlation Functions, $C_{PW}(t)$ and $S_{PW}(t)$, between the Three α-Helices and Water Molecules[a]

segment	$\langle\tau_c^{PW}\rangle$ (ps)	$\langle\tau_s^{PW}\rangle$ (ps)	$\langle\tau_c^{WW}\rangle$ (ps)	$\langle\tau_s^{WW}\rangle$ (ps)
helix-1	28.68	0.53	4.70	0.65
helix-2	45.48	0.54	4.98	0.63
helix-3	14.13	0.73	3.78	0.64
bulk water			2.93	0.29

[a] Corresponding average times ($\langle\tau_c^{WW}\rangle$ and $\langle\tau_s^{WW}\rangle$) for hydrogen bonds between water molecules present in the hydration layers of the helices as well as that for pure bulk water are also listed for comparison.



MLSDEDFKAVFGMTRSAFANLPLWKQQNLKKEKGLF

Figure 2. Snapshot of a representative configuration of the protein, highlighting the location of a strongly bound water molecule, which is doubly hydrogen bonded to the residues Asp-4 in helix-1 and Arg-15 in helix-2. The helices are drawn as red ribbons, while the coils are in green. The atoms of the residues Asp-4 and Arg-15 and the bound water molecule are drawn using a ball-and-stick model. The water molecule is hydrogen-bonded with the side-chain O atom of Asp-4 and the backbone NH group of Arg-15. The primary sequence of the protein is also displayed in one-letter code, with the N-terminus residue M(1) on the left and the C-terminus residue F(36) on the right.

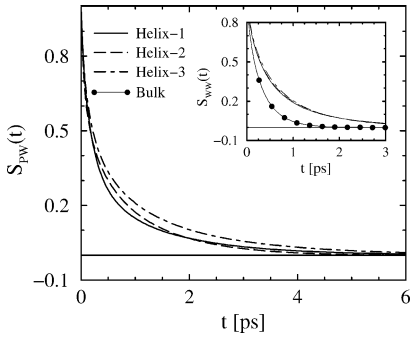


Figure 3. Continuous hydrogen bond time correlation function, $S_{PW}(t)$, between the amino acid residues of the three helices of the protein and the hydration layer water molecules. The inset shows the corresponding correlation function, $S_{WW}(t)$, for hydrogen bonds between water molecules present in the hydration layers of the helices as well as that in pure bulk water.

different time origins. In all cases a rapid initial decay in the correlation function arising primarily due to the fast librational and vibrational motions of the hydrogen-bonded sites has been observed. We notice that the relaxation of the functions for the protein−water hydrogen bonds, $S_{PW}(t)$, as well as for the water−water hydrogen bonds, $S_{WW}(t)$, in the hydration layers is significantly slower than that of the water−water hydrogen bonds in pure bulk water. We have used a sum of three exponentials to fit the $S_{PW}(t)$ and $S_{WW}(t)$ decay curves for the three helices, while a double exponential is used for pure bulk water. The estimated amplitude-weighted average hydrogen bond lifetimes, $\langle\tau_s^{PW}\rangle$ and $\langle\tau_s^{WW}\rangle$, are listed in Table 1. We notice that the $\langle\tau_s^{PW}\rangle$ and $\langle\tau_s^{WW}\rangle$ values for the hydration layers of the helices are approximately 2 times longer than that estimated for pure TIP3P water. It may be noted that the calculated average water−water hydrogen bond lifetime of 0.29 ps for pure TIP3P water is closer to the lower end of the experimental values of the characteristic hydrogen bond time constant, which vary between 0.3 and 0.7 ps.[12] The interaction between the protein residues and the bound water molecules, or the strength of the protein−water hydrogen bonds, might be responsible for their long lifetimes. The average energy between

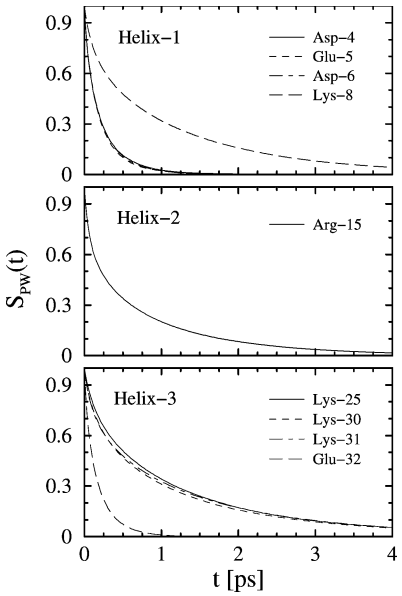


Figure 4. Continuous hydrogen bond time correlation function, $S_{PW}(t)$, between the individual charged amino acid residues of the three helices of the protein and the hydration layer water molecules.

a water molecule and the amino acid residue with which it is hydrogen bonded has been found to be in the range of −7 to −12.6 kcal mol^{-1}, which is much lower than the average hydrogen bond energy of water molecules in pure water (−4.1 kcal mol^{-1}). Thus, the waters present at the protein surface form stronger hydrogen bonds with the amino acid residues, and hence have longer lifetimes. The longer lifetimes of the hydrogen bonds formed between the water molecules in the hydration layers are consistent with the corresponding $\langle\tau_c^{WW}\rangle$ values (Table 1) and agree once again with the overall slow dynamics of the hydration layer water molecules.[44] It may, however, be noted that although the $S_{WW}(t)$ curves for the hydration layers decay much more slowly than those for pure bulk water, no noticeable difference in the relaxation behavior is observed among the hydration layers of the helices.

Interestingly, a differential relaxation behavior is once again noticed for the $S_{PW}(t)$ decay curves among the three helices. Surprisingly, an opposite trend is observed for $S_{PW}(t)$ as compared to the corresponding $C_{PW}(t)$ curves (Figure 1). Among the helices, the relaxation of $S_{PW}(t)$ is slowest for helix-3. The average lifetime of the hydrogen bonds between the residues in helix-3 and water is about 35% longer than those for the other two helices. To understand such complex behavior of protein−water hydrogen bond lifetimes among the helices, we investigated the relaxation of $S_{PW}(t)$ for the individual amino acid residues of the three helices of the protein. It is expected that the ability to form hydrogen bonds with water would be higher for charged residues. In Figure 4, we display the relaxation of $S_{PW}(t)$ for each of the charged residues of the helices. The results obtained are extremely interesting. It can be noticed that the $S_{PW}(t)$ curves decay differently for positively and negatively charged residues. The function decays faster for the negatively charged residues (Asp-4, Glu-5, and Asp-6 for helix-1; Glu-32 for helix-3), while for the positively charged residues (Lys-8 for helix-1; Arg-15 for helix-2; Lys-25, Lys-30, and Lys-31 for helix-3) the relaxation is much slower. Interestingly, this appears to be true for all three helices of the protein, irrespective of their size and composition, as well as

Table 2. Average Interaction Energy (in kcal mol^{-1}) between the Charged Residues (Positive or Negative) of the Three α-Helices and the Hydrogen-Bonded Water Molecules

segment	positive	negative
helix-1	−13.2	−13.0
helix-2	−9.4	
helix-3	−13.6	−12.3

the spatial orientation and the side-chain dynamics of the individual residues present in them.[44] To understand the reason behind such behavior, for each of the helices we have estimated the average interaction energy between a charged residue and a water molecule with which it is hydrogen bonded. The calculated values for the positively and negatively charged residues are listed in Table 2. Clearly, the positively charged residues form stronger and hence longer-lived hydrogen bonds with water than the negatively charged residues. This is particularly true for the residues in helix-3. Besides, as there are more positively charged residues in helix-3 than in the other two helices, the effect is more prominent in the former, which is manifested in the slower relaxation of $S_{PW}(t)$ (Figure 3) and the corresponding longer average lifetime of a protein−water hydrogen bond in helix-3 (Table 1). Formation of stronger hydrogen bonds by positively charged residues also correlates well with the higher hydrophilicity of these residues, as evident from the relative hydropathy scale of the amino acid residues.[58] It may also be noted from Figure 3 that, unlike in the case of the relaxation of $C_{PW}(t)$ (Figure 1), the presence of a few motionally restricted, strongly bound water molecules appears to have little or no influence on the relaxation of the function $S_{PW}(t)$ for helix-1 and helix-2.

It is well-known that the dynamics of hydrogen bonds between two molecules is strongly coupled with the diffusion of the molecules.[5,6,10,38] Luzar and Chandler[5] have demonstrated that such coupling is the physical origin of the nonexponential relaxation of hydrogen bond TCFs. Faster diffusion will result in faster hydrogen bond relaxation and vice versa. In this case, as the diffusion of the protein molecule is several orders of magnitude slower than that of water, it is expected that the dynamics of protein−water hydrogen bonds will be correlated with the self-diffusion of hydration layer water molecules. Slower diffusion of water is expected to allow re-formation of broken hydrogen bonds, and hence will result in slower relaxation of protein−water hydrogen bonds. To eliminate the contribution arising from the diffusion of hydration layer water molecules, we calculate the time correlation function,[5,6,10,38,59]

$$N(t) = \frac{\langle h(0)(1 - h(t))H'(t)\rangle}{\langle h\rangle} \qquad (3)$$

for protein−water hydrogen bonds. $H'(t)$ is unity if the tagged pair of sites is closer than a cutoff distance, R_H (3.3 Å for protein−water and 3.5 Å for water−water hydrogen bonds), at time t, and zero otherwise. Thus, a nonzero value for $N(t)$ indicates that the tagged pair of sites is no longer hydrogen bonded, but the sites remain in the vicinity of each other (i.e., within R_H). A value zero suggests that the two sites are either in the bonded state or separated by a distance larger than R_H. Thus, $N(t)$ describes the time-dependent probability that a particular hydrogen bond between a pair of sites is broken at time t, but the two sites have not diffused away and remain as nearest neighbors. Thus, it provides a quantitative measure of free water in the hydration layers.[26] The relaxation of $N(t)$ can occur due to re-formation of the broken hydrogen bonds or due to diffusion (mainly rotational) of the two sites.[5]

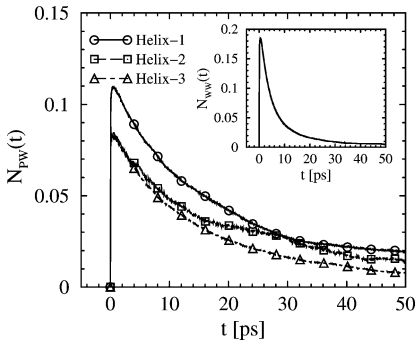


Figure 5. Time-dependent probability that a protein−water hydrogen bond is broken but the water molecule remains in the vicinity of the residue (i.e., within R_H), $N_{PW}(t)$, for the three helices. The inset shows the corresponding function, $N_{WW}(t)$, for pure bulk water.

In Figure 5, we display the relaxation of $N_{PW}(t)$ for the hydrogen bonds formed between the amino acid residues and water molecules separately for the three helices of the protein molecule. The corresponding function $N_{WW}(t)$ for pure bulk water is displayed in the inset for comparison. The figure shows that the relaxation of $N_{PW}(t)$ is much slower (particularly for helix-1 and helix-2) than that of $N_{WW}(t)$. This is a signature of the rigidity of the protein hydration layer, which is in accordance with slow translational and rotational motions of interfacial water molecules.[44] Interestingly, a differential relaxation behavior of $N_{PW}(t)$ is observed for the three helices, which indicates that the rigidity of the hydration layer is sensitive to the secondary structures of the protein molecule. It is apparent that the hydration layer of helix-3 is less rigid than those for the other two helices. This is an important observation and agrees well with the differential dynamics of hydration layer water molecules observed earlier.[44] It also correlates well with the biological activity of the protein. This is because helix-3 contains several active-site residues, and such contrasting rigidity among the hydration layers of the helices is likely to help the initial recognition and subsequent binding of action with HP-36.

To further investigate the rigidity of the hydration layer and its sensitivity to the local secondary structures of the protein molecule, we looked into the kinetics of breaking and re-formation of protein−water hydrogen bonds in further detail. We adopt the simple model proposed by Luzar and Chandler[5,6] to describe the kinetics of breaking and formation of protein−water hydrogen bonds as

$$\mathrm{B} \rightleftharpoons \mathrm{QF} \qquad (4)$$

with B the bound state, where a water molecule is hydrogen bonded with protein residue, and QF the quasi-free state, where the hydrogen bond is broken but the water molecule remains within the first coordination shell of the residue site (i.e., within distance R_H). As per the definitions, the probabilities $C_{PW}(t)$ and $N_{PW}(t)$ correspond to local populations of states B and QF, respectively, which can interconvert according to eq 4.[5,6] For a rigid hydration layer, where the diffusion is slow, the populations $C_{PW}(t)$ and $N_{PW}(t)$ can individually change by interconversion,

(58) Kyte, J.; Doolittle, R. F. *J. Mol. Biol.* **1982**, *157*, 105−132.

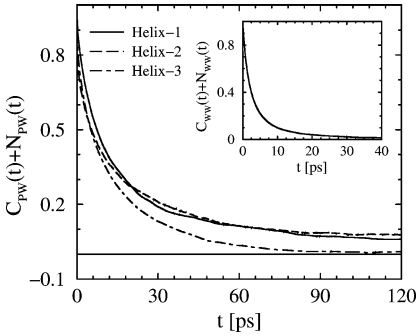


Figure 6. Relaxation of the function $C_{PW}(t) + N_{PW}(t)$ for the three helices of the protein molecule. The inset displays the corresponding function, $C_{WW}(t) + N_{WW}(t)$, for pure bulk water.

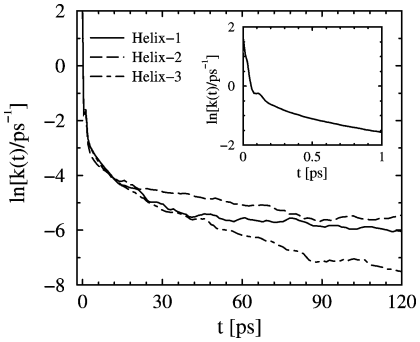


Figure 7. Protein−water hydrogen bond reactive flux, $k(t)$ (semilog plot), for the breaking and re-formation of hydrogen bonds between the amino acid residues and water molecules in the hydration layer of the three helices. The inset shows the corresponding function for pure bulk water.

but $C_{PW}(t) + N_{PW}(t)$ should remain constant.[5] In Figure 6, we plot $C_{PW}(t) + N_{PW}(t)$ for the protein−water hydrogen bonds separately for the three helices. The inset shows the decay of the corresponding function for pure bulk water. It is clear that, compared to bulk water, the function relaxes much more slowly for the hydration layer of the helices. Once again, the hydration layers of the three helices exhibit different relaxation behavior. For helix-1 and helix-2 the function decays very slowly, attains a plateau value, and remains almost constant. This clearly demonstrates slow diffusion of hydration layer water and significant interconversion between B and QF states for these two helices, resulting in rigid hydration layers for them. The helix-3 curve, on the other hand, decays steadily to zero, indicating that the re-formation of broken hydrogen bonds is less significant in this case, and relaxation of $N_{PW}(t)$ occurs mainly by diffusion. This makes the hydration layer of helix-3 less rigid.

Luzar and Chandler[5,6] proposed a simple model to describe the hydrogen bond kinetics in liquid water. Following their work, we attempt to connect the microscopic description of protein−water hydrogen bond dynamics and phenomenological reaction kinetics of their breaking and re-formation, as shown in eq 4. If k_1 and k_2 are the forward (breaking) and backword (re-formation) rate constants, then a simple rate equation for the "reactive flux" can be written as

$$k(t) = -\frac{dC_{PW}(t)}{dt} = k_1 C_{PW}(t) - k_2 N_{PW}(t) \qquad (5)$$

The relaxation of $k(t)$ to equilibrium occurs by transitions from reactants to products, i.e., from state B to state QF (eq 4). We have calculated $k(t)$ from the derivative of the simulated results of intermittent hydrogen bond TCF, $C_{PW}(t)$, separately for the three helices. This is displayed in Figure 7. The inset shows the corresponding function for pure bulk water. At short times (transient period), $k(t)$ relaxes fast for all the helices, which arises due to fast librational and vibrational motions involving the hydrogen bonded sites. The duration of this transient period for the protein−water hydrogen bonds (∼0.3 ps) is almost of the same order as that for pure bulk water (∼0.2 ps), as shown in the inset of Figure 7 and also reported in the literature.[5] Interestingly, beyond the transient period, significant differences in the relaxation behavior of $k(t)$ have been observed for the three helices. Although the function decays monotonically for helix-3, it remains almost constant and attains a plateau for helix-1 and helix-2. This agrees well with the relaxation of hydrogen bond TCFs as discussed earlier (Figures 1, 5, and 6). Because of the slow diffusion and rigid nature of the first hydration layer, the bond-breaking and re-formation equilibrium (eq 4) is established quickly for helix-1 and helix-2, as evident from the nature of the corresponding $k(t)$ decay curves. We have used the least-squares fit approach[10,59] for $t > 1$ ps to obtain the forward and backward rate constants (k_1 and k_2) that best satisfy eq 5 for the helices. These are listed in Table 3. The inverse of the forward rate constant ($1/k_1$), which corresponds to the average hydrogen bond lifetime, is also included in the table. It may be noted that the values of $1/k_1$ have been found to be larger than the average lifetime of protein−water hydrogen bonds ($\langle\tau_s^{PW}\rangle$) obtained from $S_{PW}(t)$. This is expected, as $S_{PW}(t)$ essentially provides information about the dynamics of hydrogen bond breaking due to librational and vibrational motions, while the quantity $1/k_1$ additionally includes contributions from slower diffusional motion of hydration layer water molecules.[59]

Table 3. Forward (k_1) and Backward (k_2) Rate Constants for Protein−Water Hydrogen Bond Breaking and the Average Hydrogen Bond Lifetime ($1/k_1$) as Obtained from a Least-Squares Fit of Eq 5 to the Simulation Results

segment	k_1 (ps^{-1})	k_2 (ps^{-1})	$1/k_1$ (ps)
helix-1	0.43	2.16	2.32
helix-2	0.26	1.40	3.85
helix-3	0.77	5.08	1.30

The hydrogen bond dynamics at the surface of a protein in aqueous solution and the degree of rigidity of its hydration layer will be reflected in the fluctuation in the number of waters present in the hydration layer. We have calculated the number fluctuation correlation function, $\langle\delta N(0)\delta N(t)\rangle$, for the water molecules present in the hydration layer of the three helices. Here, $\delta N(t) = N(t) - \langle N\rangle$, where $N(t)$ is the number of water molecules present in the hydration layer at time t and $\langle N\rangle$ is the average number of water molecules present in the layer. Those water molecules which reside within 5 Å from any atom of the residues of a helix are considered to be in the hydration layer of that particular helix.[44] Figure 8 shows the decay of the correlation function for the three helices. The function decays rapidly (within ∼100 ps) and fluctuates around zero for helix-3. This is in accordance with the lower rigidity of the helix-3 hydration layer and the higher diffusional motion of water present in it.[44] In comparison, the function decays much more slowly for helix-1 and helix-2. This is particularly noticeable

(59) Paul, S.; Chandra, A. *Chem. Phys. Lett.* **2004**, *386*, 218−224.

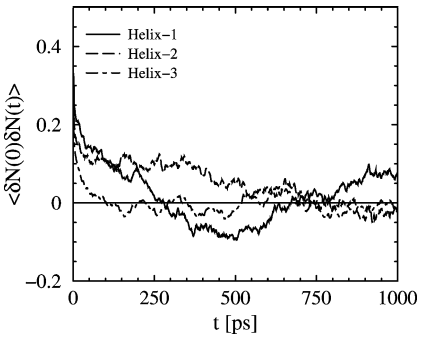


Figure 8. Fluctuation in the number density of water molecules present in the hydration layer of the three helices.

for helix-2, where the number of hydration layer water molecules fluctuates about 6−7 times more slowly than that for the helix-3 hydration layer. This agrees well with the slower structural relaxation of protein−water hydrogen bonds (Figure 1) and slower diffusion of water present in the hydration layers of helix-1 and helix-2,[44] and once again shows the higher rigidity of the corresponding hydration layers. This result may have important biological significance as well. It is expected that for a protein in aqueous solution, the water within the hydration layer of the active-site residues should fluctuate rapidly to facilitate the binding process. This is exactly what we observe for helix-3, which, as mentioned before, contains several active residues that take part in the function of the protein HP-36.

4. Conclusions

In this paper, we have explored in detail the hydrogen bond lifetime dynamics in the solvation shell of a protein in aqueous solution by means of extensive atomistic MD simulations. In particular, we have studied the dynamics of protein−water hydrogen bonds around different secondary structures of an aqueous chicken villin headpiece subdomain containing 36 amino acid residues (HP-36). We have used the analysis of Luzar and Chandler[5,6] to obtain the time-dependent rate constant which quantifies the dynamic equilibrium between the quasi-bound and free water molecules in the hydration layer. To the best of our knowledge, this is the first such analysis to quantify the protein−water hydrogen bond lifetime dynamics at any protein surface. In addition, we believe that this is also the first analysis to quantify the states of water molecules in the hydration layer on the basis of hydrogen bond lifetime correlation functions. The results are compared with the properties of water−water hydrogen bonds in pure bulk water.

The present calculations revealed that the water molecules in the hydration layer of the protein form strong hydrogen bonds with it, and hence the relaxation of protein−water hydrogen bond TCFs is much slower than that of those corresponding to pure bulk water. The average time constants for the protein−water hydrogen bond lifetime were 2−3 times longer than the corresponding value for pure bulk water, in agreement with QENS data.[33] Irrespective of the structural and dynamical heterogeneity of the three helices, it is noticed that the positively charged amino acid residues interact strongly with water and form hydrogen bonds with them with longer lifetimes. The long lifetime of these hydrogen bonds allows these bonded water molecules to be classified as "quasi-bound". Equally interestingly, significant differences in the dynamical behavior of protein−water hydrogen bonds have been noticed among the different secondary structures, namely the three α-helices of the protein molecule. The structural relaxation of the hydrogen bonds formed between the helix-3 residues and water in their hydration layer has been found to be faster than those for the other two helices. We observed an excellent correlation between the differential relaxation behavior of the intermittent protein−water hydrogen bond TCFs ($C_{PW}(t)$) among the helices and the translational and rotational motions of the corresponding hydration layer water molecules.[44] To the best of our knowledge, this is the first report on the existence of such a microscopic-level correlation. It has been noticed that the heterogeneous nature of the protein surface influences the rigidity of the hydration layer near different secondary structures. Faster relaxation of protein−water hydrogen bonds and diffusion of water molecules make the hydration layer of helix-3 less rigid than the hydration layers of the other two helices. The lower rigidity of the helix-3 hydration layer was clearly evident from the rapid fluctuation of water number density in the layer. Adopting the simple model proposed by Luzar and Chandler,[5,6] we studied the kinetics of hydrogen bond breaking and re-formation between the amino acid residues and water. It is found that the re-formation of broken protein−water hydrogen bonds is more significant for helix-1 and helix-2, as compared to helix-3. Such rapid re-formation of hydrogen bonds is also responsible for the higher rigidity of the hydration layers of helix-1 and helix-2.

Thus, in this work we have attempted to establish a correlation between the dynamics of protein−water hydrogen bonds and the rigidity of the hydration layer of three α-helices of HP-36. The lower rigidity of the hydration layer of helix-3 is an interesting observation which may have important consequences for the biological functionality of HP-36. Helix-3 of HP-36 contains several active amino acid residues. Although the detailed molecular mechanism of the binding process is not known, the faster relaxation of hydrogen bonds between helix-3 residues and water, and the lower rigidity of its hydration layer, are likely to facilitate the actin binding process of HP-36. The relatively greater rigidity of the hydration layers around helix-1 and helix-2 can also play an important role during the initial stages of actin recognition. However, this needs to be verified further. It would also be interesting to compare the protein−water hydrogen bond energetics and the solvent accessibility of different secondary structures of the protein. Some of these aspects are under extensive investigation in our laboratory. We also plan to extend such analyses to several different proteins, such as lysozyme and myoglobin.

Acknowledgment. This study was supported in part by grants from the Department of Biotechnology (DBT), Council of Scientific and Industrial Research (CSIR), and the Department of Science and Technology (DST), Government of India. S.C. thanks CSIR for providing a scholarship. We also thank Prof. S. Balasubramanian for many interesting discussions on this problem.

Supporting Information Available: Complete ref 50. This material is available free of charge via the Internet at http://pubs.acs.org.

JA054462U