Dynamics of Water in the Hydration Layer of a Partially Unfolded Structure of the Protein HP-36

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Atomistic molecular dynamics simulations of the folded native structure and a partially unfolded molten globule structure of the protein villin headpiece subdomain or HP-36 have been carried out with explicit solvent to explore the effects of unfolding on the dynamical behavior of water present in the hydration layers of different segments (three α-helices) of the protein. The calculations revealed that the unfolding of helix-2 influences the translational and rotational motions of water present in the hydration layers of the three helices in a heterogeneous manner. It is observed that a correlation exists between the unfolding of helix-2 and the microscopic kinetics of protein−water hydrogen bonds formed by its residues. This in turn has an influence on the rigidity of the hydration layers of the helices in the unfolded structure versus that in the folded native structure. These results should provide a microscopic explanation to recent solvation dynamics experiments on folded native and unfolded structures of proteins.

1. Introduction

It is known that water present near the surface of a protein in an aqueous solution behaves differently from bulk water.[1–3] This arises due to the dynamical coupling that exists between the protein and the water present in its hydration layer. These water molecules are generally termed "biological water", as they play vital roles in controlling the structure and dynamics of proteins and the mechanism of various biological processes.[1–4]

Protein folding−unfolding process is a complex problem and a subject of great interest at present. Due to the rugged nature of the free energy surface,[5–8] when a protein unfolds from its native state it generally goes to a nearby state in the configuration space with a partially unfolded conformation and a significant fraction of the secondary structures and hydrophobic contacts intact. An ensemble of such partially unfolded structures constitute what is commonly known as the molten globule (MG) state.[9]

Many experimental studies have been reported in the recent past on the coupled properties of native as well as non-native states of proteins and water present in their hydration layers.[10–23] Using femtosecond-resolved fluorescence spectroscopic techniques, Zewail, Zhong, and co-workers[12–14] have studied the solvation dynamics of different proteins. These studies indicate restricted environment of water at the protein surface and a bimodal distribution of solvation times. Recently, it is shown that terahertz (THz) spectroscopy can be used to estimate the size of the hydration layer of a protein and its dynamics.[15,16] Bhattacharyya and co-workers[17,18] have explored in detail the solvation characteristics of proteins in various non-native states. Importantly, they have shown that the solvation dynamics of the MG state of a protein can be faster or slower than the native state depending on the location of the probe molecule.[17] These studies further conclude that solvation dynamics can be used as a tool to characterize the intermediates along the folding pathway.[18] Peon et al.[19] have reported that the longest component of solvation dynamics increases significantly in the denatured state of monellin compared to its native state. Other experimental techniques such as dielectric relaxation,[3] NMR,[20,21] and quasi-elastic neutron scattering (QENS)[22] have also been used to study the properties of protein hydration water at different time and length scales.

A large number of theoretical and simulation studies on the dynamical coupling between proteins and water in their hydration layers are reported in the literature.[24–37] Molecular dynamics (MD) simulations have shown that water in the hydration layers of proteins exhibit restricted anomalous dynamics.[26,27] It is further observed that water near the surface of a protein has a higher density than bulk water.[28,29] The surface topography of the protein determines the alignment of these water molecules and hence controls the structure and dynamics of hydration water.[30,31] We have explored recently the origin of the slow dynamics of hydration water and its coupling with the residue side-chain dynamics.[33] It is found that the dynamics of protein−water (PW) hydrogen bonds are strongly correlated with the translational and rotational motions of water in the hydration layer and its density reorganization.[34] We have also shown that the polar solvation dynamics of the secondary structures of a protein are sensitive to the degree of exposure of the probes at the protein surface.[35] Similar position-dependent solvation dynamics of proteins have also been observed recently by Golosov and Karplus.[36] Only recently, using a combination of incoherent neutron scattering and MD simulations, Weik and co-workers[37] have studied the dynamical coupling between a protein and hydration water in a membrane environment.

A large number of simulation studies on protein folding have also been reported over the past several years.[38–48] These studies in general involve investigation of the kinetics and thermodynamics of folding from the denatured state to the native state as well as identifying the folding intermediates along the pathway. Recently, simple minimalistic models have been employed to understand the essential features of protein folding.[49] Attempts were also made to study the unfolding dynamics of proteins using MD simulations.[50–53] Using atomistic

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MD simulations, we have shown recently that an amino acid residue in a secondary structure of a protein can undergo a particular conformational transition, which may lead to an unfolding of the secondary structure.[52] English et al.[53] have recently used nonequilibrium MD simulations to study the effects of external fields on protein denaturation.

Water is expected to play an important role in protein folding.[54,55] Several simulation studies have been attempted in the recent past to understand such a role. Wolynes and co-workers[56] have shown that the water-mediated long-range interactions play important roles in facilitating the packing of secondary structures during folding. It is pointed out that the entropy gain by the water molecules surrounding a protein can be an important driving force for the protein to fold.[57] From MD simulations with explicit solvent it is shown that desolvation is an important step in the collapse of the hydrophobic core during protein folding.[58] Frauenfelder et al.[59] have shown recently that the large-amplitude conformational motions that occur during protein folding are correlated with the solvent fluctuations. Recently, we have studied in detail the solvation dynamics of a protein in a partially unfolded structure using atomistic MD simulations with explicit solvent.[60] It was found that the relative solvation behavior of different secondary structures of a protein can be different in a partially unfolded structure as against that in the folded native structure.

In this article, we have performed atomistic MD simulations with explicit water to study the dynamics of water in the first hydration layers of different segments of a partially unfolded MG structure of the 36-residue globular protein HP-36 (PDB ID: 1VII).[61] HP-36 is the thermostable helical subdomain present at the C-terminus of the water-soluble globular villin headpiece protein (PDB ID: 1QQV) containing 76 residues.[61,62] Villin is a unique protein that can both assemble and disassemble Actin structures.[63] In the absence of calcium ions, it can act as an Actin-bundling protein, while in the presence of calcium it becomes an Actin-severing protein. HP-36 contains one of the two F-Actin binding sites in villin necessary for the bundling activity.[63] The residues of HP-36 are numbered from 1 to 36, which correspond to residues 41−76 of villin protein.[61] The native structure of HP-36 contains three short α-helices which are connected by a few turns and loops. For convenience, 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).[33] The F-Actin binding activity of HP-36 is centered around helix-3, which contains 10 amino acid residues.[61] The article is organized as follows. In the next section we give a brief account of the system setup and the simulation methods employed. The results 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

We have employed the molecular dynamics code PINY-MD[64] to perform the calculations presented in this article. The CHARMM22 all-atom force field and potential parameters for proteins[65] were employed to describe the interaction between protein atoms, while the TIP3P model[66] which is consistent with the chosen protein force field was employed for water.

Two different simulations designated as S1 and S2 have been carried out. The folded native state structure of the protein has been studied at 300 K in simulation S1, while the unfolding of the protein was studied in simulation S2. The initial coordinates of the protein as obtained from NMR studies[61] were taken from the Protein Data Bank (PDB ID: 1VII) for simulation S1. The end residues (Met-1 and Phe-36) were capped, and the whole protein 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. At first, the system was equilibrated at constant temperature ($T = 300$ K) and pressure ($P_{ext} = 0$) (NPT) for about 500 ps. During this run, the volume of the simulation cell was allowed to fluctuate isotropically. At the end of this equilibration run, the volume of the system attained a steady value with a box edge length of 58.92 Å. At this point we fixed the cell volume, and the simulation conditions were changed from constant pressure and temperature (NPT) to constant volume and temperature (NVT). The NVT run was then continued at 300 K for about 3.5 ns.

The native configuration of the protein as obtained at the end of simulation S1 was taken to initiate the unfolding simulation, S2. An NPT simulation was first performed at high temperatures of 500−600 K for approximately 1.4 ns, during which the protein underwent a transformation from the native to a partially unfolded structure where the smallest α-helix (helix-2) unfolded into a coil.[52] This partially unfolded structure is a member of the large ensemble of structures forming the molten globule (MG) state. At this point with completion of the melting of the conformation of helix-2, the temperature of the system was lowered to 300 K, and the NPT run was continued for another 300 ps. The simulation conditions were then changed from constant pressure and temperature (NPT) to constant volume and temperature (NVT). The trajectory of the partially unfolded structure in NVT ensemble was then generated for about 2.5 ns at 300 K. The MD trajectories were stored for both simulations (S1 and S2) with a time resolution of 400 fs. To investigate the ultrafast properties, sections (∼600 ps) of the two equilibrated trajectories were also stored at a higher time resolution of 16 fs.

The simulations utilized the Nosé−Hoover chain thermostat extended system method.[67] A recently developed reversible multiple time step algorithm, RESPA,[67] allowed us to employ a MD time step of 4 fs. Electrostatic interactions were calculated by using the particle-mesh Ewald (PME) method.[68] The PME and RESPA were combined following the method suggested by Marchi and co-workers.[69] The minimum image convention[70] was employed to calculate the Lennard-Jones interactions and the real-space part of the Ewald sum, using spherical truncations of 7 and 10 Å, respectively, for the short- and long-range parts of the force decomposition.

3. Results and Discussion

It is expected that the unfolding of a protein molecule will influence the correlated motion of the water molecules close to the protein surface. Also, due to the structural heterogeneity and varying degree of unfolding of different segments of a protein, the motion of water around the segments can be affected differently. As reported earlier,[52] simulation S2 led to the formation of a partially unfolded structure of HP-36, where the smallest α-helix (helix-2) present in the middle underwent complete unfolding. To explore the effect of unfolding on water mobility, we have compared the translational and rotational motions of water around the three helical segments of the partially unfolded and the native state structures of the protein molecule. To be specific, the calculations were carried out with those water molecules that are present within a layer of thickness (R) 5 Å from the residues of the three helices of the protein. This essentially corresponds to the first hydration layers of the helices. In Figure 1, we display the mean square displacement

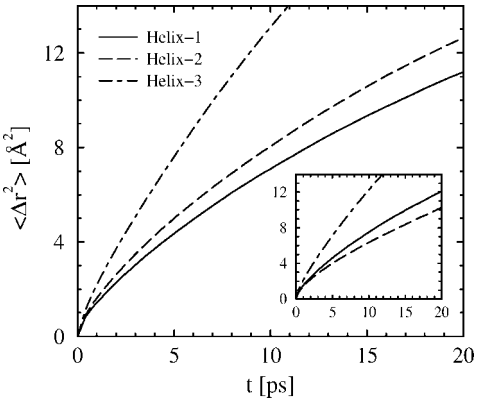

Figure 1. Mean square displacement (MSD) of water molecules present in the hydration layers of the three α-helices of the protein in the partially unfolded structure as obtained from simulation S2 at 300 K. The inset shows the corresponding data for the native structure as obtained from simulation S1 under identical conditions. Water molecules within a layer of thickness 5 Å from the helices are considered in the calculations.

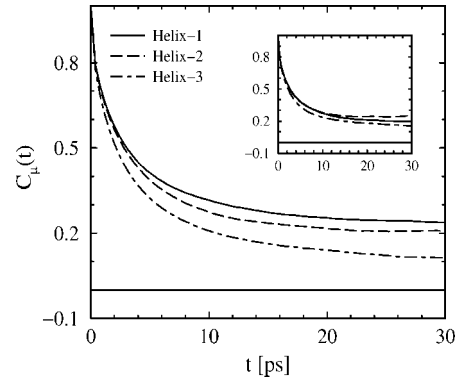

Figure 2. Reorientational time correlation function of the water dipoles, $C_\mu(t)$, for water molecules present in the hydration layers of the three α-helices of the protein in the partially unfolded structure as obtained from simulation S2 at 300 K. The inset shows the corresponding data for the native structure as obtained from simulation S1 under identical conditions. The definition of hydration layer is the same as that used for Figure 1.

of the hydration layer water molecules for the three helices in the unfolded structure as obtained from simulation S2 at 300 K. The inset shows the corresponding results for the native structure obtained from simulation S1 under identical conditions. It is clear that, as in the folded native structure, the hydration layer water molecules for the helices exhibit heterogeneous translational mobility in the unfolded structure, too. However, differences exist in the relative motions among these water molecules in the folded and unfolded structures. This is particularly noticeable for water around helix-2. Among the three helices the slowest translational mobility was observed for the hydration layer water molecules of helix-2 in the native structure. However, in the unfolded structure the mobility of water around helix-2 has been found to be higher than that around helix-1. It is further noticed that compared to the native structure the waters around helix-2 and helix-3 exhibit faster dynamics in the unfolded structure, with the effect being more pronounced for helix-2. This indicates that the unfolding of helix-2 is strongly correlated with the translational motion of the surrounding water molecules. In contrast, the motion of water around helix-1 has been found to have slightly slowed down in the unfolded structure. This is surprising as one would expect the unfolded structure of a protein to be more flexible with a relatively more mobile water layer around it compared to the native structure. We will discuss this again later.

We have also investigated the rotational motion of water molecules present in the hydration layers (i.e., within 5 Å) of the three helices in the partially unfolded structure of the protein molecule. This is done by calculating the dipole−dipole time correlation function defined as

$$C_\mu(t) = \frac{\langle \hat{\mu}_i(t) \cdot \hat{\mu}_i(0) \rangle}{\langle \hat{\mu}_i(0) \cdot \hat{\mu}_i(0) \rangle} \quad (1)$$

where $\hat{\mu}_i(t)$ is the unit dipole moment vector of the ith water molecule at a time t, and is directed along the vector connecting the oxygen atom of the water molecule to the center of the line joining the two hydrogen atoms. The angular brackets denote that the averaging is carried out over the hydration layer water molecules at different reference initial times. The results are displayed in Figure 2. The inset shows the corresponding results obtained for the native structure of the protein. It is evident from Figure 2 that the hydration layer water molecules of the three helices exhibit heterogeneous rotational motion in the unfolded structure. Consistent with the translational motion, an inhomogeneity exists in the rotational motion of the hydration layer water for the three helices relative to each other in the two structures. Water molecules near helix-2 have been found to reorient faster than that around helix-1. This is in accordance with the translational mobility of the corresponding water molecules (see Figure 1), and indicates that the rotational motion of water close to the protein surface is also correlated with the protein's unfolding.

It is known that water molecules at the surface of a protein form strong protein−water (PW) hydrogen bonds.[34] The kinetics of the formation and breaking of the PW hydrogen bonds is correlated with the dynamics of the surface water molecules.[26,34,71−73] Such correlations play an important role in controlling the structure, stability, and function of proteins. Breaking of native tertiary contacts with partial or complete loss of secondary structures occurs during unfolding of a protein. Such structural changes are associated with the breaking of the PW hydrogen bonds and their rearrangement at the surface. Therefore, the kinetics of PW hydrogen bonds is likely to be modified in an unfolded structure. Again, the effect may be nonuniform among different segments of the protein due to their heterogeneous degree of unfolding.

We have investigated in detail the dynamics of PW hydrogen bonds formed between the amino acid residues of the three helices and the surrounding water molecules in the unfolded structure and compared the findings with those for the native structure of the protein. The calculations are based on two time correlation functions (TCFs), namely, the intermittent hydrogen bond TCF, $C(t)$, and the continuous hydrogen bond TCF, $S(t)$. The functions are defined as[74,75]

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

and

$$S(t) = \frac{\langle h(0)\, H(t) \rangle}{\langle h(0)\, h(0) \rangle} \quad (3)$$

where $h(t)$ and $H(t)$ are two hydrogen bond population variables. $h(t)$ is considered to be unity if a pair of sites is hydrogen bonded at time t according to the definition of the bond, and zero otherwise. $H(t)$, 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. The angular

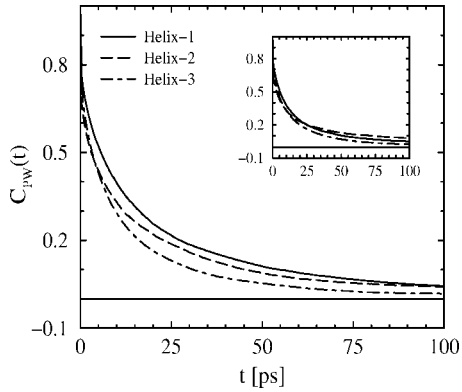

Figure 3. Intermittent hydrogen bond time correlation function, $C_{PW}(t)$, between the residues of the three α-helices of the protein in the partially unfolded structure and water around them as obtained from simulation S2 at 300 K. The inset shows the corresponding data for the native structure as obtained from simulation S1 under identical conditions.

brackets denote that the averaging is carried out over all the hydrogen bonds and at different reference initial times. We have adopted a geometric criterion from the literature to define a hydrogen bond.[76] The first condition for an atom of a protein (acceptor or donor) to form a hydrogen bond with water is that the distance (R_H) between the protein atom and the oxygen atom of the water molecule be within 3.3 Å. The second condition for an acceptor atom is that the angle between one of the OH bond vectors of the tagged water molecule and the vector connecting the hydrogen atom and the acceptor atom be within 30°, but for a donor atom the angle between one of the OH bond vectors of the water molecule and the vector connecting the water oxygen atom and the hydrogen atom attached with the donor atom should be within 80°−140° for a PW hydrogen bond to form. According to the definitions of $h(t)$ and $H(t)$, 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 re-formation 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 behavior of $C(t)$, one can obtain information about the structural relaxation of a particular type of hydrogen bonds. On the other hand, the function $S(t)$ describes the probability that a hydrogen bond formed between two sites at time zero remains bonded at all times up to t. Thus, $S(t)$ provides a strict definition of the lifetime of a tagged hydrogen bond.

We have calculated the function $C_{PW}(t)$ for the hydrogen bonds formed between the residues of the three helices and the corresponding hydration layer water molecules in the partially unfolded structure of the protein. The results are displayed in Figure 3. For comparison, the corresponding results for the native structure are shown in the inset of Figure 3. Inhomogeneous relaxation behavior of $C_{PW}(t)$ among the three helices is noticed in both structures. The hydrogen bonds between the helix-2 residues and the surrounding water molecules exhibit interesting dynamics with respect to the other two helices in the two structures. It can be seen that the structural relaxation of PW hydrogen bonds is slowest for helix-2 in the native structure. This is particularly true at long times when the contribution arising from the slow diffusion of water around helix-2 becomes important. However, in the unfolded structure the function $C_{PW}(t)$ for helix-2 relaxes faster than that for helix-1. Thus, the unfolding of helix-2 has led to faster dynamics of PW hydrogen bonds formed by its residues. This also agrees

TABLE 1: Average Relaxation Times as Obtained from Intermittent ($\langle\tau_C^{PW}\rangle$) and Continuous ($\langle\tau_S^{PW}\rangle$) Protein−Water Hydrogen Bond Time Correlation Functions for the Hydration Layers of the Three α-Helices of the Protein in the Folded Native and Partially Unfolded Molten Globule (MG) Structures

	$\langle\tau_C^{PW}\rangle$ (ps)		$\langle\tau_S^{PW}\rangle$ (ps)	
segment	native	MG	native	MG
helix-1	28.68	19.10	0.53	0.51
helix-2	45.48	15.86	0.54	0.53
helix-3	14.13	11.41	0.73	0.73

well with the enhanced translational and rotational motions of the hydration layer water of helix-2 in the unfolded structure, as shown earlier (see Figures 1 and 2). The calculations reveal that a microscopic correlation exists between the unfolding of a secondary structure of a protein, the dynamics of the corresponding PW hydrogen bonds, and the mobility of water around it. The average relaxation time constants (τ_C^{PW}) obtained from multiexponential fitting of the $C_{PW}(t)$ decay curves are listed in Table 1. A large decrease in the τ_C^{PW} value for the helix-2 residues in the unfolded structure is evident versus that for the other two helices.

In Figure 4, we display the relaxation of the continuous hydrogen bond TCF, $S_{PW}(t)$, for the hydrogen bonds between the residues of the three helices and water around them in the partially unfolded structure. The inset shows the corresponding results for the native structure. Inhomogeneous relaxation behavior of the function is once again noticed among the three helices in both cases. However, unlike the function $C_{PW}(t)$, no noticeable differences are observed in the relaxation behavior of $S_{PW}(t)$ and its degree of inhomogeneity among the helices in the two structures. This is also reflected in the identical average PW hydrogen bond lifetimes (τ_S^{PW}) for the respective helices in the two structures, as obtained from the $S_{PW}(t)$ decay curves and listed in Table 1. It would be interesting to explore the microscopic origin of the differential influence of unfolding on the relaxation behavior of $C_{PW}(t)$ and $S_{PW}(t)$. As discussed before, the contributions arising from the breaking of PW hydrogen bonds, diffusion of the freed water molecules, and the subsequent re-formation of those bonds are present in the function $C_{PW}(t)$. This explains the reason behind the influence of unfolding on the characteristics of the function $C_{PW}(t)$. The function $S_{PW}(t)$, on the other hand, provides information only about the hydrogen bond breaking. Thus, instead of the diffusional behavior of hydration layer water, the relative

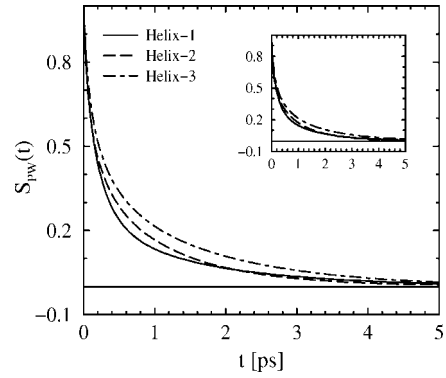

Figure 4. Continuous hydrogen bond time correlation function, $S_{PW}(t)$, between the residues of the three α-helices of the protein in the partially unfolded structure and water around them as obtained from simulation S2 at 300 K. The inset shows the corresponding data for the native structure as obtained from simulation S1 under identical conditions.

TABLE 2: Average Interaction Potential Energy between the Residues of the Three α-Helices of the Protein and the Water Molecules Hydrogen Bonded to Them in the Folded Native and Partially Unfolded Molten Globule (MG) Structures

segment	native (kcal mol^{-1})	MG (kcal mol^{-1})
helix-1	−12.6	−13.0
helix-2	−7.0	−6.6
helix-3	−9.9	−9.8

strengths of the PW hydrogen bonds formed by the residues of the three helices will determine the relaxation pattern of the $S_{PW}(t)$ decay curves and the lifetimes of these bonds.

We have calculated the interaction potential energy between the amino acid residues and the water molecules with which they are hydrogen bonded. The average potential energy values for the three helices in both structures are listed in Table 2. Due to heterogeneous distribution of residues and their types, the interaction potentials are different for the three helices. However, the nearly identical energy values for a particular helical segment in the two structures indicate that the local geometric arrangements of the residues and the water molecules hydrogen bonded to them in the unfolded structure are similar to that in the native structure. We believe that this is an important result and may be general, too, and suggests that the average lifetime of a PW hydrogen bond is the same for different structures of a protein molecule along the folding−unfolding pathways. However, this needs to be verified for other proteins with more complex structures and by exploring multiple pathways.

It is known that the kinetics of hydrogen bonds is coupled with the diffusion of the molecules.[77–80] Such coupling is believed to be the microscopic origin of the nonexponential relaxation behavior of the hydrogen bond TCFs.[77] As the diffusion of water is much faster than that of the protein molecule, the kinetics of PW hydrogen bonds should be correlated with the self-diffusion of hydration layer water molecules. Faster diffusion will lead to faster hydrogen bond relaxation and vice versa. We have shown that the mobility of water around helix-2 of the protein undergoing unfolding is significantly influenced (see Figures 1 and 2). This in turn has been found to affect the overall structural relaxation of PW hydrogen bonds (see Figure 3). We attempt to eliminate the contribution arising from the diffusion of hydration layer water molecules on hydrogen bond kinetics by calculating the time correlation function[77–81]

$$N(t) = \frac{\langle h(0)\,(1 - h(t))\,H'(t)\rangle}{\langle h(0)\,h(0)\rangle} \tag{4}$$

for the PW hydrogen bonds. The variable $H'(t)$ is unity if the tagged pair of sites is closer than the PW hydrogen bond cutoff distance (R_H) of 3.3 Å, at time t, and zero otherwise. A nonzero value for $N(t)$ indicates that the tagged pair of sites are no longer hydrogen bonded, but remain close to each other (i.e., within R_H). A value of zero suggests that the two sites are either hydrogen bonded to each other or separated by a distance larger than R_H . Thus, $N(t)$ describes the time-dependent probability that a particular PW hydrogen bond is broken at time t, but the water molecule has not diffused away. The relaxation of $N(t)$ can occur due to re-formation of the broken PW hydrogen bond or due to diffusion of the water molecule.[77]

In Figure 5, we display the relaxation of $N_{PW}(t)$ for the hydrogen bonds between the residues of the three helices and water in the partially unfolded structure. The inset shows the corresponding results obtained for the native structure. Heterogeneous relaxation behavior of $N_{PW}(t)$ among the three helices indicates differential rigidity of the hydration layers for both structures. Slightly faster relaxation in the unfolded structure is noticed versus that in the native structure for helix-2 and helix-3. This is expected as the structure of a protein molecule is generally more flexible in the unfolded structure with lowering of rigidity of the surrounding hydration layer. Interestingly, however, compared to the native structure the function relaxes slowly in the unfolded structure for helix-1. This is an indication of enhanced rigidity of the hydration layer of helix-1 in the unfolded structure and is consistent with relatively slower mobility of the corresponding water molecules as discussed before. This is an important observation which suggests that the unfolding of a segment of a protein molecule may often lead to the formation of new structural motifs around other segments which may be more rigid with slower mobility of the surrounding water molecules. This may be more evident for larger proteins with more complex secondary structures. However, this needs to be verified.

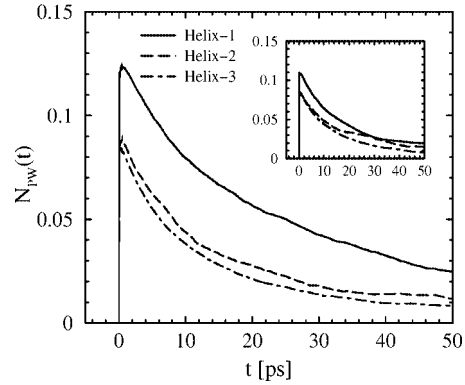

Figure 5. Time-dependent probability that a protein−water (PW) hydrogen bond is broken, but the water molecule remains in the vicinity of the residue, $N_{PW}(t)$. The calculations are carried out for the three α-helices of the protein in the partially unfolded structure as obtained from simulation S2 at 300 K. The inset shows the corresponding data for the native structure of the protein as obtained from simulation S1 under identical conditions.

To further understand the inhomogeneous influence of unfolding on the rigidity of the hydration layers around the helices, we have investigated the kinetics of breaking and re-formation of the PW hydrogen bonds in the two structures. We have employed the model proposed by Luzar and Chandler[77,78] to describe the kinetics as

$$B \rightleftharpoons QF \tag{5}$$

where B designates the bound state with a water molecule hydrogen bonded to a protein residue, and QF is the quasi-free state, where the hydrogen bond is broken, but the water molecule remains within the first neighboring 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 the populations of the states B and QF, respectively, which can interconvert according to eq 5.[77,78] Therefore, 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

$$k(t) = -\frac{dC_{PW}(t)}{dt} = k_1 C_{PW}(t) - k_2 N_{PW}(t) \tag{6}$$

$k(t)$ relaxes to equilibrium by transitions from state B to state QF (eq 5), i.e., from reactants to products.

In Figure 6, we display the relaxation behavior of $k(t)$ obtained from the derivative of the simulated $C_{PW}(t)$ data for the three

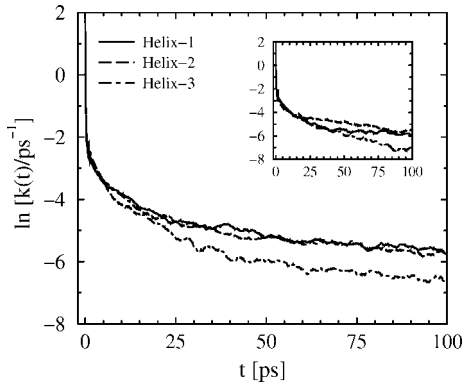

Figure 6. Reactive flux, $k(t)$ (semilog plot), for the breaking and re-formation of protein−water (PW) hydrogen bonds. The calculations are carried out for the three α-helices of the protein in the partially unfolded structure as obtained from simulation S2 at 300 K. The inset shows the corresponding data for the native structure of the protein as obtained from simulation S1 under identical conditions.

TABLE 3: Forward (k_1) and Backward (k_2) Rate Constants for Protein−Water Hydrogen Bond Breaking and the Average Hydrogen Bond Lifetime ($1/k_1$ (ps)) for the Three α-Helices of the Protein in the Folded Native and Partially Unfolded Molten Globule (MG) Structures As Obtained from Least-Squares Fit of Eq 6 to the Corresponding Simulation Results

	k_1 (ps^{-1})		k_2 (ps^{-1})		$1/k_1$ (ps)	
segment	native	MG	native	MG	native	MG
helix-1	0.43	0.46	2.16	2.24	2.32	2.17
helix-2	0.26	0.50	1.40	2.86	3.85	2.00
helix-3	0.77	0.81	5.08	5.74	1.30	1.23

helices in the partially unfolded structure. The inset shows the corresponding results obtained for the native structure. Within the transient period (<0.5 ps)[77,78] when the librational and vibrational motions dominate, $k(t)$ relaxes equally fast for all the helices in both structures. However, inhomogeneous relaxation of $k(t)$ has been observed for the three helices beyond the transient period in both structures. Although for helix-3 the function decays monotonically, and more quickly, the decay is much slower for helix-1 and helix-2. This indicates that the re-formation of broken PW hydrogen bonds is more frequent for helix-1 and helix-2, which led to the establishment of the corresponding equilibrium (eq 5) relatively quickly. The re-formation of PW hydrogen bonds is less significant for helix-3 residues. This seems to be true whether the protein molecule is folded or not and is consistent with faster motions of water around helix-3 versus those around the other two helices in both structures. A closer look at the decay curves reveals that partial unfolding of the protein has some influence on the function. This is particularly true for helix-1 and helix-2. It can be seen that compared to the native structure the function relaxes faster in the unfolded structure for helix-2. An opposite trend is observed for helix-1, for which the function relaxes slowly in the unfolded structure. Such differential influence of unfolding on the relaxation behavior of $k(t)$ for helix-1 and helix-2 agrees well with the corresponding PW hydrogen bond dynamics and the mobility of water around them in the two structures (see Figures 1−3). We have calculated the PW hydrogen bond breaking and re-formation rate constants (k_1 and k_2) using the least-squares fit approach[79,81] for $t > 1$ ps data for the helices. These are listed in Table 3 for both structures of the protein. The inverse of the forward rate constants ($1/k_1$) which correspond to the average lifetimes of the PW hydrogen bonds are also included in Table 3. It can be seen that the rate constants associated with the breaking and re-formation of hydrogen bonds between the residues of helix-2 and the surrounding water molecules have been significantly affected due to its unfolding. The kinetics associated with the hydrogen bonds between the residues of the other two helices and water around them remain mostly unaffected. These are important findings and provide a microscopic understanding of the kinetics of PW hydrogen bonds in an unfolded structure of a protein versus that for the native structure.

4. Conclusions

In this work we have carried out atomistic MD simulations at room temperature to investigate the dynamic properties of water present in the first hydration layer of the native structure and a partially unfolded molten globule (MG) structure of the 36-residue helical subdomain of villin headpiece protein or HP-36 in aqueous solution. The calculations reveal that the unfolding of helix-2 of the protein has a strong influence on the dynamics of water surrounding it. As a result, inhomogeneities in the relative translational and rotational motions among the hydration layers of the three helices have been observed for the folded and unfolded structures of the protein. It is noticed that the water molecules around helix-3 exhibit faster motions than those around the other two helices for both structures. However, compared to the native structure, a reverse trend in the relative mobility of water around the segments corresponding to helix-1 and helix-2 has been observed in the unfolded structure. This is largely due to faster mobility of water around helix-2 in the unfolded structure versus that in the folded native structure. The dynamics of protein−water (PW) hydrogen bonds formed between the residues of helix-2 and water have been found to be much faster in the unfolded structure versus that in the folded native structure. The average PW hydrogen bond relaxation time has been found to be about 3 times shorter for helix-2 in the unfolded structure. Similar effects have also been noticed for the other two helices, but the extent of influence is much smaller compared to that for helix-2. Thus we observe that the unfolding of a segment of a protein molecule is strongly correlated with the dynamics of hydrogen bonds formed by the segment with water. The calculations further reveal that the rigidity of the hydration layers of the helices are influenced in a nonuniform heterogeneous manner due to unfolding. We have studied the kinetics of breaking and re-formation of hydrogen bonds between the residues of the helices and water using the formalism proposed by Luzar and Chandler.[77,78] It is found that the kinetics of hydrogen bond breaking and re-formation between the residues of helix-2 and water have been influenced due to its unfolding.

Thus, in this article we have compared the microscopic dynamics of water present in the hydration layers of the secondary structural segments of the folded native structure and a partially unfolded structure of a protein molecule. It is found that the unfolding of helix-2 of the protein is correlated with the mobility of water in its hydration layer and the kinetics of PW hydrogen bonds formed by its residues. To the best of our knowledge, this is the first report where the existence of such a microscopic correlation has been demonstrated. However, more studies are necessary to further quantify such a correlation. It would also be interesting to explore whether such a correlation exists for other proteins with more complex secondary structures as well as for different unfolded structures of the same protein. That will enable us to obtain a general understanding of protein unfolding and its correlation with the properties of water

surrounding the protein. Some of these aspects are under investigation in our laboratory.

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