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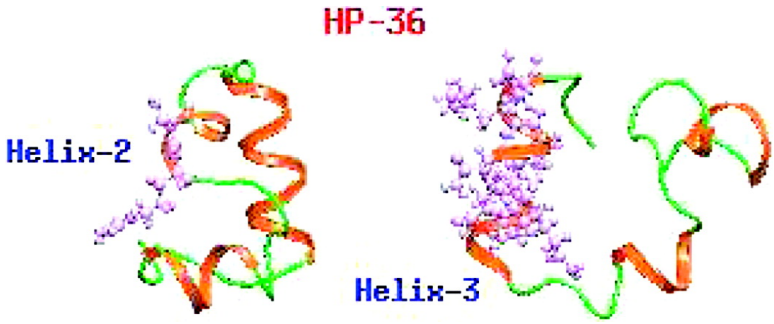

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Sensitivity of Polar Solvation Dynamics to the Secondary Structures of Aqueous Proteins and the Role of Surface Exposure of the Probe

Sanjoy Bandyopadhyay,*,† Sudip Chakraborty,† Sundaram Balasubramanian,‡ and Biman Bagchi*,§

Molecular Modeling Laboratory, Department of Chemistry, Indian Institute of Technology, Kharagpur-721302, India, Chemistry and Physics of Materials Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore-560064, India, and Solid State and Structural Chemistry Unit, Indian Institute of Science, Bangalore-560012, India

Received November 28, 2004; E-mail: sanjoy@chem.iitkgp.ernet.in; bbagchi@sscu.iisc.ernet.in

Abstract: The structure and dynamics of water around a protein is expected to be sensitive to the details of the adjacent secondary structure of the protein. In this article, we explore this sensitivity by calculating both the orientational dynamics of the surface water molecules and the equilibrium solvation time correlation function of the polar amino acid residues in each of the three helical segments of the protein HP-36, using atomistic molecular dynamics simulations. The solvation dynamics of polar amino acid residues in helix-2 is found to be faster than that of the other two helices (the average time constant is smaller by a factor of 2), although the interfacial water molecules around helix-2 exhibit much slower orientational dynamics than that around the other two helices. A careful analysis shows that the origin of such a counterintuitive behavior lies in the dependence of the solvation time correlation function on the *surface exposure of the probe*—the more exposed is the probe, the faster the solvation dynamics. We discuss that these results are useful in explaining recent solvation dynamics experiments.

1. Introduction

It is now widely accepted that the natural dynamics of proteins play a crucial role in their biological activity, such as ligand binding. As most of the proteins function in aqueous solution, it is expected that the water surrounding the protein would participate in the dynamical events that lead to activity. Understanding of the dynamical coupling between the protein and surrounding water molecules is a complex problem that has drawn the attention of many researchers over the past several years.[1−15] More recently, time-dependent fluorescence Stokes shift (TDFSS) and three-pulse photon echo techniques have been used to study the dynamics of proteins and of the water molecules at the surface of the proteins.[3] It has been found that the measured time correlation function (generally known as the solvation time correlation function) exhibits a slow component which is absent in bulk water. Fleming and co-workers[3] used three-pulse photon echo measurements on an external probe (eosin) to study its solvation dynamics at the surface of aqueous lysozyme. Several slow components, including one of the order of 500 ps, were observed, in addition to the usual ultrafast component which is present in bulk water. Bhattacharyya and co-workers[4] have recently reported studies of solvation dynamics of a probe covalently bound to protein GlnRS, in both its native and molten globule states. Solvation dynamics was found to have two components, a major component of 40 ps and a minor one of 580 ps. Using tryptophan as a natural probe, Zewail and

† Indian Institute of Technology.
‡ Jawaharlal Nehru Centre for Advanced Scientific Research.
§ Indian Institute of Science.

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co-workers[5] have recently detected a slow component in the range of 20−40 ps at protein surfaces. This is slower by more than an order of magnitude than the slowest component observed in bulk water (about 1 ps). The slower solvation dynamics observed in protein solutions is believed to reflect the sluggish dynamics of hydration water. Pierce and Boxer[16] had earlier observed that solvation dynamics in a protein environment was nonexponential with a long time component of 10 ns. The origin of slow components observed in photon echo experiments and also in the TDFSS studies of Bhattacharyya et al.[4] may be partly due to the protein side-chain motions, or due to the self-motion of the probe.

Although the study of the role of water dynamics in the stability and function of a protein has a long history, the origin and the extent of the slow component in the solvation dynamics of a polar probe placed at the surface of a protein are still not clear and are subjects of current research. Many simulations,[9−15] starting from the early work of Levitt and Sharon,[9] have investigated the dynamics of water at the surface of proteins. These studies primarily focus on the translational motion of the interfacial water, which is now known to be slowed by at most a factor of 2.[14] Rotational motion, being a faster process, is affected significantly. Note that the solvation dynamics is controlled mostly by rotational motion of water molecules.[17] An explanation for the slowing of rotation of surface water molecules has been put forward in terms of a dynamic exchange between bound and free water molecules.[5] The bound water molecules are slowed by hydrogen-bonding to the polar or charged groups on the protein surface. However, this model cannot explain the presence of the ultraslow component, with time constants more than few hundred picoseconds (often observed in experiments). Clearly, long time simulation studies can help answer many of the detailed questions regarding the time scale of solvation and the nature of the dynamics near a protein surface at a microscopic level.

In addition to the issues related to the observed solvation time correlation function, it seems plausible that TDFSS can be used to selectively probe dynamics of secondary structures (along with the dynamics of the adjacent water molecules). It is highly likely that such specific information is already present in the experiments discussed above. Such experiments can provide rich information about the water dynamics around specific secondary structures and also of the motion of the secondary structure itself.

To probe the origin of the slow time scale and its relationship to the protein surface's exposure to solvent and solvent dynamics, we have investigated the solvation dynamics of 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.[18] Villin is a unique protein which can both assemble and disassemble actin structures.[19] HP-36 contains one of the two F-actin binding sites in villin necessary for F-actin bundling activity.[19] In this work we number the residues from 1 to 36. Thus, residue numbers 1−36 correspond to residues 41−76 in the NMR structure.[18] 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).[15] The biological activity is believed to be centered around helix-3, which contains 10 amino acid residues.[18] This protein subdomain has been studied extensively in recent years, especially with respect to its folding.[20]

We employ atomistic molecular dynamics (MD) simulations to study the solvation dynamics of the protein molecule. Solvation dynamics (SD) is an important time-resolved technique that is used to study various dynamical processes that take place when a solute molecule is introduced in a polar solvent. It provides quantitative information on the time-dependent response of the solvent reorganization around a solute probe. SD can be studied by characterizing the decay of the equilibrium solvation time correlation function (TCF), $C_S(t)$, which according to linear response theory is related to the solvation energy[21] as

$$C_S(t) = \frac{\langle \delta E(t) \delta E(0) \rangle}{\langle \delta E(0) \delta E(0) \rangle} \quad (1)$$

where $\delta E(t)$ corresponds to the fluctuation in the polar part of the potential energy of the probe molecule at time t with respect to the average equilibrium value. The angular brackets denote averaging over the probe molecules and over different reference initial times. We have calculated $C_S(t)$ by measuring the polar part of the interaction energy between all the atoms (backbone as well as side chain) of only the polar amino acid residues of each of the three helices and the rest of the simulation system. Thus we have used the polar residues as intrinsic probes to measure the solvation TCF. The use of such intrinsic probes is much akin to the method employed by Zewail and co-workers.[5]

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.[18] 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 (∼400 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[22] were employed to describe the interaction between protein atoms, while the TIP3P model,[23] which is consistent with the chosen

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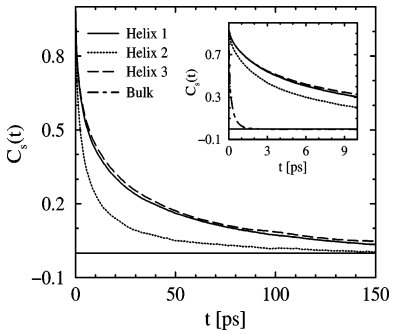

Figure 1. Solvation time correlation function, $C_S(t)$, for the three helices of the protein. The TCFs are calculated by averaging over the polar amino acid residues present in the helices. The calculations are carried out at a time resolution of 400 fs. The inset shows the same curves calculated with data at a high resolution of 16 fs, as well as the corresponding correlation function of a water molecule in bulk water.

Table 1. Multiexponential Fitting Parameters for the Solvation Time Correlation Function, $C_S(t)$, of the Polar Amino Acid Residues Present in the α-Helices of the Protein

segment	time constant (ps)	amplitude (%)	$\langle\tau_S\rangle$ (ps)[a]
helix-1	0.07	11.4	26.2
	2.2	26.7	
	12.9	28.6	
	65.8	33.3	
helix-2	0.06	16.5	11.0
	1.9	35.2	
	9.5	33.7	
	48.6	14.6	
helix-3	0.06	12.2	29.4
	2.5	27.2	
	18.5	33.9	
	84.0	26.7	

[a] Average time constant.

protein force field, was employed for modeling water. The details of the simulation methods employed in this study are reported elsewhere.[15]

3. Results and Discussion

The calculated solvation TCFs, $C_S(t)$, for the three helices of the protein are displayed in Figure 1. The calculations are carried out by averaging over the polar probe residues present in each of them. The inset of the figure shows the relaxation of $C_S(t)$, as obtained from the high-resolution data. For comparison, the inset also shows the corresponding correlation function of a water molecule in bulk water. The decay curves clearly show the presence of a significaltly slow component compared to bulk water for all the three helices. To understand the different solvation time scales, we have fitted the decay curves for the three helices to multiexponentials. The parameters for best fit are shown in Table 1. Broadly, four different solvation time scales are identified for the helices, starting from ultrafast to slow components. We noticed an ultrafast component with time constant within 60−66 fs, followed by a fast component of around 1.9−2.4 ps. Two slower components with time constants in the ranges of 9.5−18.5 and 48.6−84.0 ps are also noticed. The ultrafast time constants are calculated from the high-resolution data as shown in Figure 1 (inset). Such different solvation time scales arise from the presence of different types of water molecules near the protein surface, ranging from bound to quasi-bound to free or bulklike water molecules. The initial ultrafast relaxation arises from the high-frequency librational (hindered rotation) and intermolecular vibrational (hindered translation) motions of the free or bulklike water molecules.[24] Damped rotational motions of these water molecules contribute to the fast relaxation (∼1−2 ps). The presence of such free water molecules in the hydration layer has been indicated from ultrafast fluorescence studies.[5] However, these fluorescence studies were unable to resolve the ultrafast component for protein solvation. It is well known that water molecules present near the surface of a protein often form strong hydrogen bonds with the polar residues. Such water molecules contribute little to the solvation process as long as they remain in the bound state. The slowest component observed in our case (48−84 ps) is likely to correspond the contribution arising from such water molecules which are bound to the amino acid residues of the protein molecule by hydrogen bonds.[25] Interestingly, we have also noticed the presence of a not-so-slow (9−18 ps) component of the dynamics. This might reflect the dynamics of the quasi-bound water molecules. These water molecules are those for which the hydrogen bonds with the protein residues are just broken, but they have not diffused away to the bulk. These water molecules are expected to exhibit a dynamics that is somewhat intermediate between the bound and free bulklike water molecules, and are believed to exist in a dynamical equilibrium with both. However, much more work is necessary for a microscopic level understanding of the dynamics of water molecules in the quasi-bound state as opposed to that in bound or free states.

Although the dimensions of the three helices as well as the number and types of the residues present in them are different, it would be interesting to investigate the dynamics exhibited by the individual residues of the three helices. In Figure 2, we display the solvation TCFs for each of the polar probe residues belonging to the three helices of the protein. The presence of a slow component for each of the residues is clearly evident from the figure. However, the most striking feature to note from this figure is that the individual probe residues, whether identical or not, can exhibit significant differences in their solvation behavior within a particular secondary structure. This is more prominent for the polar residues in helix-1 and helix-3. For example, the solvation TCFs of identical residues, such as Asp-4 and Asp-6 in helix-1 and Lys-30 and Lys-31 in helix-3, are significaltly different. While the polar probe residues in helix-2 exhibit homogeneous dynamics, indicating that they are exposed to nearly identical environments, the probe residues in helix-1 and helix-3 exhibit heterogeneous dynamics, with some showing really slow solvation (for example, Asp-6 in helix-1 and Glu-32 in helix-3). This suggests that, unlike in helix-2, the *amino acid residues in helix-1 and helix-3 are exposed to different environments.* As will be discussed later, there is a microscopic explanation for such differential dynamics.

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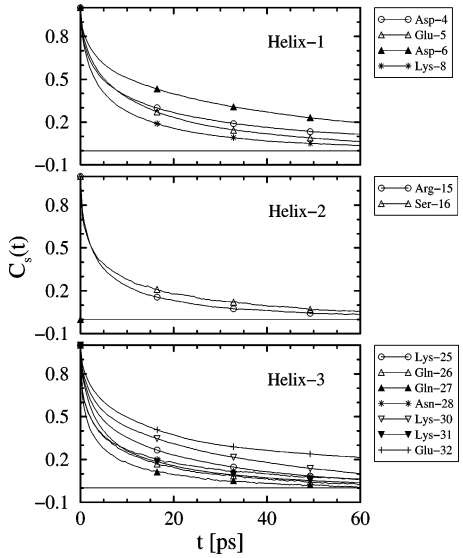

Figure 2. Solvation time correlation function, $C_S(t)$, for the individual polar amino acid residues of the three helices of the protein. The polar amino acid residues are used as intrinsic probes, and the calculations are carried out at a time resolution of 400 fs.

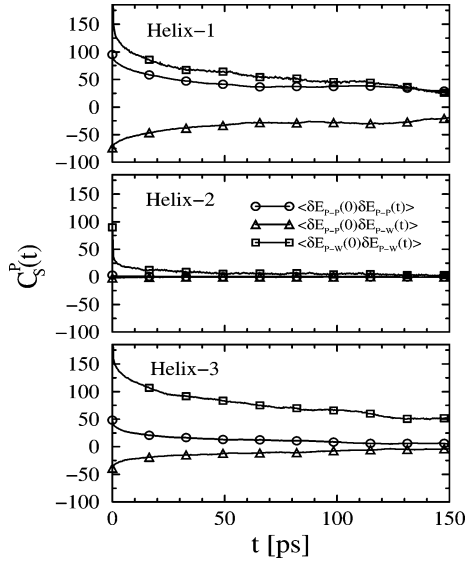

Figure 3. Partial solvation time correlation function, $C^P_S(t)$, for the three helices of the protein. All the contributions are shown as indicated in the figure. It is clear that the most dominant contribution arises from the interaction between the polar amino acid residues and water molecules. These calculations are carried out at a time resolution of 400 fs.

Most interestingly, we find that the solvation TCF can be significantly different for different secondary structures. In this case, it is noticed that the $C_S(t)$ curve decays much faster for helix-2, compared to the other two helices (see Figure 1). It may be noted that the average solvation time constant ($\langle\tau_S\rangle$) for helix-2 as obtained from our simulation is 2−3 times faster than those corresponding to the other two helices. Recently, we observed that the water molecules near helix-2 are more structured and exhibit noticeably slower translational and rotational motions compared to those around the other two helices.[15] In particular, the water molecules around helix-2 have been found to exhibit a very slow reorientational dynamics with a long time component (>500 ps), which we could not determine due to the limitation of the time scale of our simulation.[15] Thus, on the basis of those findings, one would expect the solvation TCF for helix-2 to exhibit slower dynamics than those corresponding to the other two helices. Contrary to this expectation, the present result shows an opposite solvation behavior for helix-2. To understand the reason behind such an anomalous solvation behavior, we have estimated the contributions from different partial solvation time correlation functions for the three helices, as shown in Figure 3. It is clear from the figure that, within the time scale of our simulation, the most dominant contribution arises from the interaction between the polar amino acid residues and the water molecules (P−W interactions). This is more clearly evident for helix-1 and helix-3, which exhibit almost identical behavior. The large, slow decay of this component for these two helices is clearly reflected in the corresponding total solvation time correlation functions. We notice that the partial solvation TCFs for all the components are significantly different for helix-2 compared to the other two helices. It is expected that both interfacial (W_I) and bulk (W_B) water molecules would contribute to the P−W interactions. The relative exposure of the polar probe residues in different helices to bulk water might play a crucial role in determining whether the contribution from the interfacial water or the bulk water is playing the dominant role.

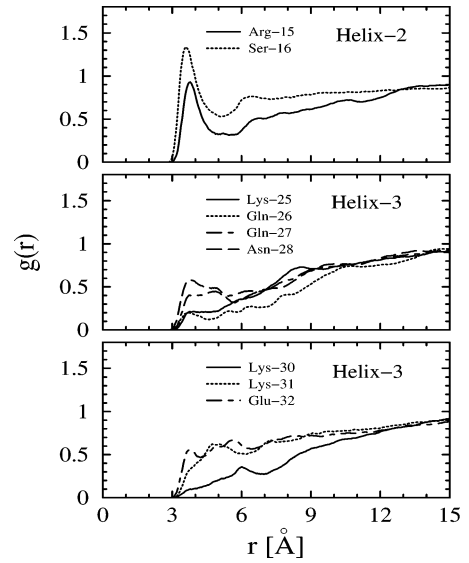

Figure 4. Pairwise correlation function, $g(r)$, of water molecules as a function of distance from the C_β atoms of the polar amino acid residues of helix-2 and helix-3.

As most of the active-site residues are located in helix-3, it would be interesting to investigate the origin of the differential solvation behavior between helix-2 and helix-3. We have calculated the pairwise correlation function, commonly known as the radial distribution function, $g(r)$, of the C_β atoms of the polar residues of helix-2 and helix-3 with the water molecules to investigate the degree of exposure of these residues to bulk water. The result is displayed in Figure 4. The presence of a distinct, high-intensity first peak for each of the polar residues in helix-2 (Arg-15 and Ser-16) clearly indicates that the side chains of these residues are exposed to bulk solvent. On the other hand, most of the polar residues present in helix-3 do not exhibit a distinct first peak. In fact, the first peak has almost disappeared for a few polar residues in helix-3. This suggests that the polar residues of helix-3 are either very close to the

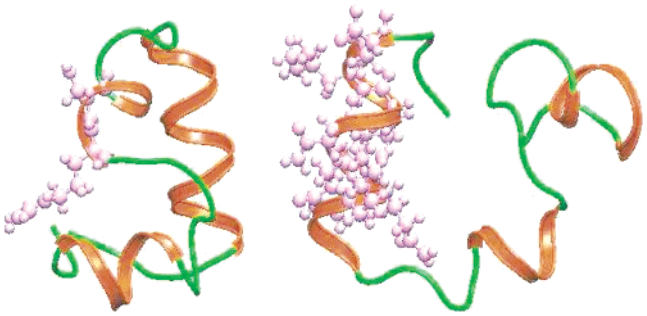

Figure 5. Representative configuration of the protein, highlighting the polar amino acid residues of helix-2 (left) and helix-3 (right). The α-helices are drawn as red ribbons, while the coils are in green. The polar residues in the two helices are drawn using ball-and-stick model (light blue). The water molecules are not shown for visual clarity. Note the opposite orientation of the side chains of the polar residues in the two helices.

backbone or buried within the core. In Figure 5, we display a representative configuration obtained from the simulation highlighting the polar amino acid residues of helix-2 and helix-3. The exposure of the side chains of the polar residues of helix-2 (Arg-15 and Ser-16) to the solvent is clearly evident from the figure. These side chains are oriented away from the core of the protein toward the bulk solvent. On the other hand, the side chains of most of the polar residues of helix-3 are oriented in the opposite direction, mainly toward the core of the protein. This figure provides direct evidence for the relative differences in surface exposure of the polar residues in the two helices. We have also calculated the number of water molecules that are nearest neighbors (coordination number) to the C_β atoms of the polar residues in these two helices. This is done by integrating the $g(r)$ curves up to 5 Å. We found that, on average, there are approximately 9.1 and 4.5 water molecules per C_β atom of helix-2 and helix-3, respectively. This further confirms that the polar residues in helix-2 are significantly exposed to the solvent. The presence of such buried polar residues in helix-3 is in agreement with NMR data.[18] Because of the exposure of the polar residues in helix-2, the interactions with bulk water ($P-W_B$ interactions) play an important role and dominate over the interactions with interfacial water molecules ($P-W_I$ interactions). Due to the much faster dynamics of bulk water molecules, the $P-W_B$ interactions fluctuate more rapidly than the $P-W_I$ interactions. This results in faster relaxation of the solvation TCF for helix-2 (see Figure 1). The reverse is the case for helix-3. As the polar residues in helix-3 are either close to the protein surface or buried in the core, $P-W_I$ interactions play the major role in this case and dominate over $P-W_B$ interactions. It is indeed true that the interfacial water molecules exhibit noticeably faster dynamics for helix-3, as compared to those corresponding to helix-2.[15] Nevertheless, the interfacial water molecules are always much slower than bulk water. In summary, the solvation TCF exhibits a faster relaxation for helix-2, as compared to that for helix-3, due primarily to its larger exposure to water. It may be noted that, although the relaxation of the solvation TCF for helix-2 is faster compared to that for the other two helices, it is much slower than that in bulk water (see Figure 1). Such slowness arises from the favorable interaction of interfacial water molecules with the protein surface.

The relative dimensions of the interface as well as the strength of the protein−water hydrogen bonds and the rate of exchange between the bound and free water molecules might have some influence on the solvation behavior of the secondary structure segments of a protein molecule. Currently, we are investigating in detail the kinetics of protein−water and water−water hydrogen bonds formed at the interface of the protein.

4. Conclusions

In this work, we have presented results obtained from an extensive MD simulation study of an aqueous chicken villin headpiece subdomain containing 36 amino acid residues (HP-36). The calculations revealed that the solvation time correlation function is significantly different for polar residues in helix-2 segment as compared to those in the other two helices. Within the time scale of our simulation, the most dominant contribution arises from the interactions between the polar amino acid residues and the water molecules. The solvation dynamics in helix-2 has been found to be faster than that of the other two helices. This is an extremely interesting and surprising result, as the interfacial water molecules exhibit slower dynamics for helix-2 as compared to those for the other two helices.

Another potentially important result of this work is the dependence of solvation time on the relative exposure of the polar probe residues to water—this exposure (which is quantifiable from the protein structure) plays a major role in determining the solvation dynamics of a particular secondary structure. The polar residues of helix-2 are more exposed to water and exhibit significantly faster solvation dynamics, even though the interfacial water molecules are slow near helix-2.

The observed sensitivity of the probe to surface exposure can be used to understand some recent experiments. Zewail and coworkers[5] found that the solvation dynamics of tryptophan in the protein Subtilisin Carlsberg has a long time component equal to 38 ps. On the other hand, for the sweet protein Monellin, with more surface-exposed tryptophan residues, the same time constant is 16 ps. Although the proteins studied were different, these time constants are of the same order as the ones obtained in the present study. Thus, in this work we have been able to establish a correlation between the exposure of polar probe residues and the solvation dynamics of different secondary structures of a protein molecule. To the best of our knowledge, this is the first report on such a correlation. The existence of such a correlation is highly significant to study the solvation dynamics of a protein with different secondary structures and its likely influence on the biological activity of the protein molecule.

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