

Low-Frequency Vibrational Spectrum of Water in the Hydration Layer of a Protein: A Molecular Dynamics Simulation Study

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An atomistic molecular dynamics simulation has been carried out to understand the low-frequency intermolecular vibrational spectrum of water present in the hydration layer of the protein villin headpiece subdomain or HP-36. An attempt is made to explore how the heterogeneous rigidity of the hydration layers of different segments (three α helices) of the protein, strength of the protein–water hydrogen bonds, and their differential relaxation behavior influence the distribution of the intermolecular vibrational density of states of water in the hydration layers. The calculations revealed that compared to bulk water these bands are nonuniformly blue-shifted for water near the helices, the extent of shifts being more pronounced for water molecules hydrogen bonded to the protein residues. It is further noticed that the larger blue shift observed for the water molecules hydrogen bonded to helix 2 residues correlates excellently with the slowest structural relaxation of these hydrogen bonds. These results can be verified by suitable experimental measurements.

1. Introduction

It is now established that water near the surface of a protein behaves differently than water in bulk.^{1,2} These water molecules, often termed as “biological water”,^{2,3} are dynamically coupled with the protein and play crucial roles in controlling the protein’s structure, dynamics, and function.^{1,2,4}

Considering the importance, a molecular-level understanding of the microscopic mechanism of interaction between the protein and water solvating it has been an area of great interest. Many experimental studies have been reported over the past several years on the structural and dynamical couplings that exist between the protein and water in its hydration layer.^{5–14} Solvation dynamics of different proteins have been studied in great detail by Zewail, Zhong and co-workers^{5,6,9,10} using femtosecond-resolved fluorescence spectroscopic techniques. These studies in general indicate a restricted environment of water near the surface of the protein with a bimodal distribution of solvation times. The fast librational and reorientational dynamics of the surface water molecules that occur within a few picoseconds were attributed to the initial ultrafast component, while the slower component in the time scale of tens of picoseconds was believed to originate from the restricted slow dynamics of hydration water due to coupling with the protein residues. In contrary to these studies, Nilsson and Halle¹² have recently claimed that the hydration layer water molecules are not much retarded by the protein, and thus it is argued that the slower component observed in the solvation dynamics experiments should originate from the side-chain dynamics of the protein rather than from the coupled slow dynamics of water hydrating the protein surface. Solvation characteristics of proteins in various non-native states have also been studied recently.¹³ Only recently, Havenith and co-workers¹⁴ have shown that terahertz (THz) spectroscopy capable of probing the size of the hydration layer and its dynamical characteristics can be

employed as an effective tool to study “biological water”. Collective dynamics of aqueous protein solutions have also been studied using THz absorption spectroscopy.¹⁵ Besides, several other experimental techniques such as NMR,^{16,17} quasi-elastic neutron scattering (QENS),¹⁸ dielectric relaxation,^{3,19} nuclear magnetic relaxation dispersion (NMRD),²⁰ and so forth have also been used to study the properties of protein and hydration water at different time and length scales.

Molecular dynamics (MD) simulation is another important alternative tool that can provide direct microscopic information on protein–water interaction and hence the structural and dynamical coupling existing between them.^{21–36} It is shown that the surface topography of a protein plays a role in controlling the hydration water structure and dynamics.^{23,24} In fact, MD studies have shown that the water molecules at the surface of a protein are aligned parallelly with density higher than that in the bulk.^{25,26} Recently, Cannistraro and co-workers²⁷ have shown that water in the hydration layer of a protein exhibits restricted sublinear diffusion. Slower rotational dynamics of water around a protein molecule has also been shown by Marchi and co-workers.²⁹ We have studied recently the origin of the slow dynamics of hydration-layer water and its coupling with the side-chain dynamics of the protein residues.³¹ MD studies 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.³² Such heterogeneous position-dependent solvation dynamics of proteins have been confirmed further in a recent work by Golosov and Karplus.³³ They have shown that the slow components on the order of hundreds of picoseconds originate from the coupling between the dynamics of hydration water and the protein conformational dynamics. The correlation between the kinetics of protein unfolding and the water dynamics around the protein as well as the solvation dynamics of partially unfolded protein structures have also been studied recently using atomistic MD simulations.³⁴

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Hydrogen bonding at the surface of a protein is another important issue. The regular hydrogen-bond network in pure bulk water gets disrupted near the surface of a protein in an aqueous solution with the formation of the protein–water (PW) hydrogen bonds. In a recent MD study,³⁶ we showed that the PW hydrogen bonds are of higher strength than the water–water (WW) hydrogen bonds, which led to longer lifetimes of the PW hydrogen bonds. Interestingly, heterogeneous dynamics of the PW hydrogen bonds among different segments of the same protein molecule has also been noticed. It is also revealed from MD studies that the kinetics of WW hydrogen bonds in the hydration layer of a protein is much slower than that in bulk water.^{35,36}

Modification of water hydrogen bonds in the hydration layer of a protein should influence the low-frequency intermolecular vibrational spectrum of water in the terahertz (THz) and far-infrared (far-IR) regions.^{37–39} Such influence can in principle be monitored and analyzed experimentally using Raman, IR, and THz spectroscopy as well as inelastic incoherent neutron scattering (IINS) techniques. From ultrafast optical Kerr-effect spectroscopy studies on protein solutions, Giraud and Wynne⁴⁰ have shown that the shift of the broad Raman band originates from PW hydrogen bonding. Results from IINS measurements have recently revealed that the intermolecular translational and librational motions of hydration water of biomolecules are strongly perturbed.⁴¹

From simulated MD trajectories, one can compute different time correlation functions and the corresponding spectral densities, which can be associated with different dynamical events. In this article, we have performed an MD simulation to study the low-frequency intermolecular vibrational spectrum of water present in the hydration layers of three α -helical segments of HP-36, a small globular protein containing 36 amino acid residues. In particular, the calculations are carried out by the Fourier transformation of the velocity autocorrelation function (VACF) of the hydration-layer water molecules, which is defined as

$$C_v(t) = \frac{\langle \vec{v}_i(t) \cdot \vec{v}_i(0) \rangle}{\langle \vec{v}_i(0) \cdot \vec{v}_i(0) \rangle} \quad (1)$$

where $\vec{v}_i(t)$ is the velocity vector of the atom of type i (O or H) in a water molecule at time t . The angular brackets denote averaging over all atoms of the particular type present in the hydration layer and over different reference initial times. This is a routine approach to calculate vibrational density of states from MD simulations.^{38,42,43} In an earlier report,³⁶ we showed that the heterogeneous relaxation dynamics of the PW hydrogen bonds for the three helices of HP-36 are correlated with the rigidity of the hydration layers. In the present work, we explore the correlation between the vibrational spectrum of water in the hydration layers of the helices and the heterogeneous dynamics of the PW hydrogen bonds and the rigidity of the hydration layers. HP-36 is the thermostable subdomain present at the extreme C-terminus of the 76-residue villin protein⁴⁴ and contains one of the two binding sites of villin necessary for F-actin bundling activity.⁴⁵ The residues of HP-36 are numbered from 1 to 36, which actually correspond to residues 41–76 of villin protein.⁴⁴ The primary sequence details of HP-36 are listed in our earlier work.³¹ The three short α helices present in it are connected by a few turns and loops and a hydrophobic core. For convenience, the three α helices are denoted as helix 1 (Asp-4 to Lys-8), helix 2 (Arg-15 to Phe-18), and helix 3 (Leu-23 to Glu-32).³¹ The function of the protein is centered around

helix 3, which contains 10 residues.⁴⁴ The article is organized as follows. In Section 2, we give a brief description of the system setup and the simulation methodologies employed. The results are presented and discussed in Section 3. In the last section we summarize the important findings and the conclusions reached from our study.

2. System Setup and Simulation Details

The initial coordinates of the protein as obtained from NMR studies⁴⁴ were taken from the Protein Data Bank (PDB ID: 1VII). 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.

The CHARMM22 all-atom force field and potential parameters for proteins⁴⁶ were employed to describe the interaction between protein atoms, while the TIP3P model,⁴⁷ which is consistent with the chosen protein force field, was employed for water. The simulation was carried out for over 3.5 ns duration with a MD time step of 4 fs, using the RESPA algorithm.⁴⁸ The simulation was first performed at constant temperature ($T = 300$ K) and pressure ($P_{\text{ext}} = 0$) (NPT), followed by runs at constant temperature and volume (NVT). The MD trajectory was stored during the last 2.5 ns duration of the NVT run with a time resolution of 400 fs. The further details of the simulation methods employed in this study have been reported earlier.³¹ The VACFs were calculated by averaging over segments of the trajectory, each of duration 15 ps with the positions and velocities of all of the atoms stored at every time step.

3. Results and Discussion

We have calculated the velocity autocorrelation function (VACF) of 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 layer around the helices. The VACF for the oxygen ($C_v^O(t)$) and the hydrogen ($C_v^H(t)$) atoms of the water molecules are computed separately, which are displayed in Figure 1. For comparison, the corresponding results for bulk water as obtained from a separate MD simulation of pure TIP3P water under identical conditions are also included in the figure. It can be noticed from Figure 1a that small but subtle differences exist in the relaxation behavior of $C_v^O(t)$ among the first hydration-layer water molecules of the three helices and bulk water. The function for bulk water exhibits an initial small bump followed by a negative dip. This is a characteristic of back-scattering of the oxygen atoms due to their collisions with neighboring molecules forming cages around them. $C_v^O(t)$ for the hydration-layer water exhibit deeper minima compared to bulk water for all the three helices, indicating enhanced rigidity of the water layer close to the protein surface. A closer examination further reveals that among the three helices, $C_v^O(t)$ exhibits a slightly deeper minimum for water around helix 2. This indicates heterogeneous rigidity of the first hydration layers of the three helices. The VACF for the hydrogen atoms ($C_v^H(t)$) of the water molecules that are within 5 Å from the three helices exhibit almost identical behavior among themselves and that of bulk water, as shown in Figure 1b. The presence of a deeper minimum and oscillatory nature of the function indicates stronger caging effects, which arise because of the librational motions of the water molecules. This will be discussed again later.

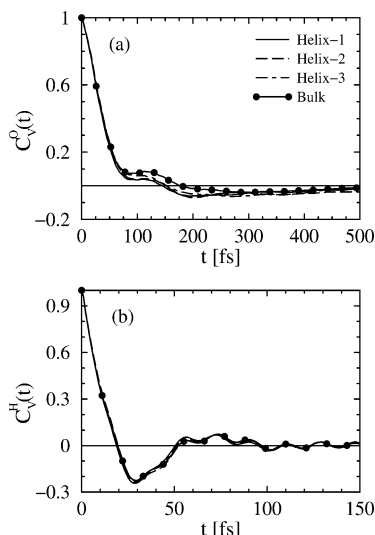


Figure 1. (a) Velocity autocorrelation functions $C_V^O(t)$ for the oxygen atoms of the water molecules that are present within a region of distance $R = 5$ Å from the residues of the three α helices of the protein. The corresponding functions $C_V^H(t)$ for the hydrogen atoms of these water molecules are shown in b. The results for pure bulk water are included for comparison.

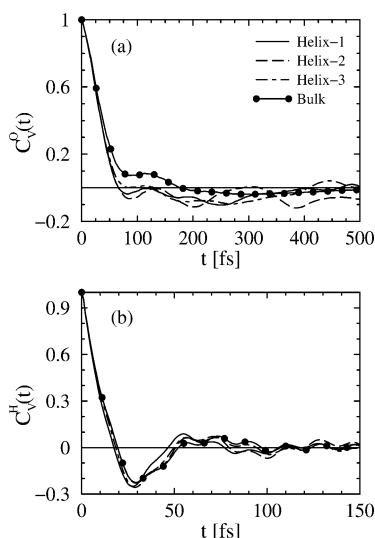


Figure 2. (a) Velocity autocorrelation functions $C_V^O(t)$ for the oxygen atoms of the water molecules that are hydrogen bonded to the amino acid residues of the three α helices of the protein. The corresponding functions $C_V^H(t)$ for the hydrogen atoms of these water molecules are shown in b. The results for pure bulk water are included for comparison.

It is known that the presence of a protein molecule modifies the regular water–water (WW) hydrogen bond network in bulk water with the formation of protein–water (PW) hydrogen bonds. The PW hydrogen bonds are expected to influence the VACF of the water molecules at the protein surface. To explore that, we have calculated the functions $C_V^O(t)$ and $C_V^H(t)$ for the water molecules forming hydrogen bonds with the amino acid residues of the three helices. The results are shown in Figure 2. For comparison, the corresponding functions for pure bulk water are also included in the figure. It can be seen that compared to bulk water the function $C_V^O(t)$ exhibits distinctly different behavior for the water molecules hydrogen bonded to the residues of the helices. All three decay curves exhibit deeper minima with frequent oscillations than bulk water. The oscillations are also more pronounced than that noticed in Figure 1a, where all of the water molecules present in the first hydration

layers (whether hydrogen bonded or not with the protein residues) were considered. The oscillatory nature of $C_V^O(t)$ for the water molecules hydrogen bonded to the residues of the helices indicates stronger caging effects for these molecules in a more pronounced rigid environment. This is a direct consequence of stronger PW hydrogen bonds and their slower dynamics as reported earlier.³⁶ Interestingly, we observe a distinct differential relaxation pattern of $C_V^O(t)$ among the three helices. It is noticed that the function for water molecules hydrogen bonded to the residues of helix 2 is more oscillatory in nature with deeper first minimum than the other two helices. Such heterogeneous behavior indicates significant differences in the spatial environments and dynamics of PW hydrogen bonds among the three helices of the protein molecule. We observed earlier that the PW hydrogen bonds formed by the three helices exhibit differential dynamics.³⁶ It was demonstrated that the structural relaxation of PW hydrogen bonds formed by helix 2 residues is much slower than that for the other two helices. Thus, we observe an excellent correlation between the differential oscillations in $C_V^O(t)$ for the water molecules hydrogen bonded to the residues of the three helices and the dynamics of the corresponding PW hydrogen bonds. Unlike for all of the water molecules present in the first hydration layer, the function $C_V^H(t)$ for the water molecules hydrogen bonded to the helices exhibits relatively more pronounced oscillations than bulk water. This is again consistent with enhanced caging effects for the water molecules hydrogen bonded to the protein residues as compared to the other molecules present at the surface. The heterogeneous influence of the secondary structures of the protein on the VACF of the surrounding water molecules should also affect the low-frequency intermolecular vibrational modes of those water molecules. We discuss this next.

Several experiments using Raman,³⁷ infrared (IR),³⁹ and IINS^{49,50} techniques have shown two broad low-frequency bands in the vibrational spectrum of water at ~ 50 and ~ 200 cm^{-1} . However, controversy exists regarding the microscopic origin of these two intermolecular vibrational modes. This is particularly so for the band around 50 cm^{-1} . Walrafen and co-workers³⁷ assigned the band around 50 cm^{-1} to the $\text{O}\cdots\text{O}\cdots\text{O}$ bending mode arising from triplets of hydrogen-bonded water molecules, and the band around 200 cm^{-1} to the $\text{O}\cdots\text{O}$ stretching mode between pairs of hydrogen-bonded water molecules. Although there is a general consensus about the origin of the band around 200 cm^{-1} , ambiguity exists with the interpretation of the band around 50 cm^{-1} . By demonstrating the presence of the ~ 50 cm^{-1} band in the spectrum of non-hydrogen-bonded liquids, Padro and co-workers^{38,51} concluded from their MD simulations that this band may not necessarily be associated with hydrogen bonds; rather, it may originate from restricted transverse translations along all directions in the local environment around each molecule. However, the presence of hydrogen bonds in a liquid like water can alter the position of this frequency band.

One can note from the above discussion that the network of hydrogen bonds in liquid water does play a role behind both of the low-frequency bands in its vibrational spectrum. We have already discussed that in an aqueous protein solution the regular WW hydrogen bonds of water are perturbed with the formation of PW hydrogen bonds. Thus, rearrangement of the hydrogen-bonding environment occurs at the protein surface, which should influence the low-frequency bands of water around the protein. To explore this, we have calculated the power spectra for the oxygen ($S_O(\omega)$) and hydrogen ($S_H(\omega)$) atoms of water molecules by Fourier cosine transformation of the corresponding VACFs. The results for the water molecules present in the first hydration

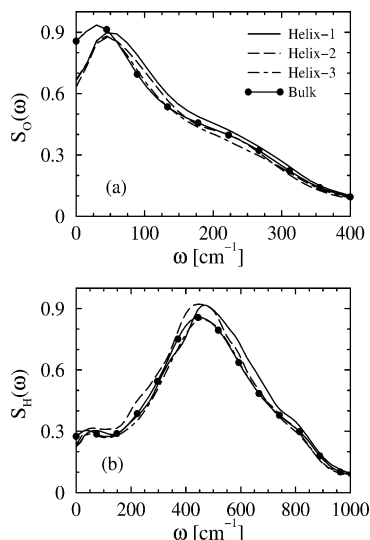


Figure 3. (a) Power spectra $S_O(\omega)$ obtained by the Fourier transformation of the velocity autocorrelation function $C_V^O(t)$ for the oxygen atoms of the water molecules that are present within a region of distance $R = 5$ Å from the residues of the three α helices of the protein. The corresponding power spectra $S_H(\omega)$ for the hydrogen atoms of these water molecules are shown in b. The results for pure bulk water are included for comparison.

layers of the three helices are shown in Figure 3. For comparison, the corresponding results for pure bulk water are also included in the figure. It can be seen (Figure 3a) that compared to bulk water there is a clear blue shift by ~ 25 cm⁻¹ in $S_O(\omega)$ for the band corresponding to the O···O···O bending or restricted translations in molecular cages for water around the three helices. Importantly, small differences in the blue shifts of this band have been noticed among the hydration layers of the three helices. This is consistent with the trend observed in the relaxation pattern of $C_V^O(t)$ (Figure 1a) and confirms heterogeneous rigidity of the first hydration layers of different segments of the protein molecule. Alternatively, the band corresponding to the O···O stretching or longitudinal translations (~ 200 cm⁻¹) seems to remain unaffected. Similar behavior was observed earlier for aqueous protein and micellar solutions.^{27,42} Such contrasting results on the two low-frequency bands in the vibrational spectrum of the water molecules near the protein surface are likely due to the varying degree of influence of the protein on the orientational and translational degrees of freedom of these water molecules. However, more studies are necessary to further understand this. The intensity of $S_O(\omega)$ at zero frequency ($\omega = 0$) provides a measure of the diffusion coefficient of water. Importantly, we observe the lowering of zero-frequency intensities for water molecules near the protein surface as compared to bulk water. This indicates the restricted translational mobility of water around the protein molecule. We have calculated the diffusion coefficients (D_S) of the water molecules present in the first hydration layers of the helices from the zero-frequency intensities of the spectra, as listed in Table 1. For comparison, the diffusion coefficient values (D_E) obtained from the mean square displacements of these water molecules as reported earlier³¹ are also listed. It can be seen that though the absolute values of D_S and D_E differ, both exhibit similar trends among the hydration layers of the helices. It may be noted that water molecules near the surface of a protein exhibit sublinear trends in their mean square displacements.²⁷ As a result, the calculated D_E values for these water molecules may not be very accurate and thus differ from the D_S values calculated from the oxygen atom power spectra.

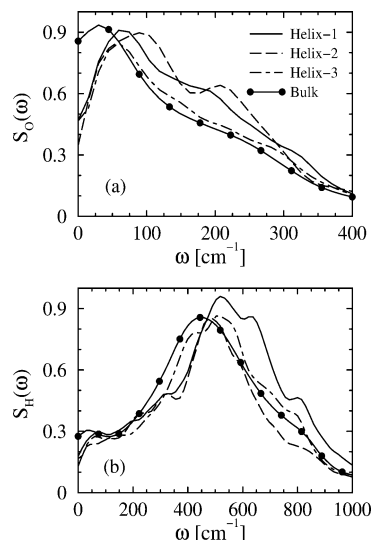


Figure 4. (a) Power spectra $S_O(\omega)$ obtained by the Fourier transformation of the velocity autocorrelation function $C_V^O(t)$ for the oxygen atoms of the water molecules that are hydrogen bonded to the amino acid residues of the three α helices of the protein. The corresponding power spectra $S_H(\omega)$ for the hydrogen atoms of these water molecules are shown in b. The results for pure bulk water are included for comparison.

TABLE 1: Diffusion Coefficients of the Water Molecules in the First Hydration Layers ($R = 5$ Å) of the Three α Helices of the Protein, as Obtained From the Power Spectra $S_O(\omega)$ at Zero Frequency (D_S), and From the Mean Square Displacements (D_E)

segment	D_S (10^{-5} cm ² s ⁻¹)	D_E (10^{-5} cm ² s ⁻¹)
helix 1	2.57	0.76
helix 2	2.44	0.65
helix 3	3.61	1.48

The hydrogen atom power spectrum $S_H(\omega)$ for the water molecules in the first hydration layers of the three helices are shown in Figure 3b. The $S_H(\omega)$ band corresponds to the librational motions of water and is peaked around 500 cm⁻¹ in the vibrational spectrum of liquid water.³⁹ Our calculations do not show any significant differences in the peak positions of this band for the hydration-layer water molecules. A slight blue shift is noticed for water around helix 1. The results indicate that the librational motions of the water molecules near the protein surface are not affected much.

Furthermore, we have calculated the power spectra $S_O(\omega)$ and $S_H(\omega)$ for the water molecules that are hydrogen bonded to the amino acid residues at the protein surface. This is done to investigate the effect of the PW hydrogen bonds on the vibrational spectrum of these water molecules. The results for the three helices are shown in Figure 4 along with that for pure bulk water. Figure 4a shows that the low-frequency band corresponding to the O···O···O bending or local restricted translations is considerably blue-shifted by ~ 25 –60 cm⁻¹ for the water molecules hydrogen bonded to the helices as compared to pure bulk water. This is in accordance with the differences in the relaxation of the function $C_V^O(t)$ between bulk water and the water involved in the PW hydrogen bonds with the helices (Figure 2a). Importantly, we notice significant nonuniform blue shifts of this band for water molecules hydrogen bonded to different helices. It can be seen that the shift is much more pronounced for the water molecules hydrogen bonded to helix 2 as compared to those bonded to the other two helices. This is consistent with the relaxation pattern of the corresponding VACF (Figure 2a) and correlates excellently with the structural

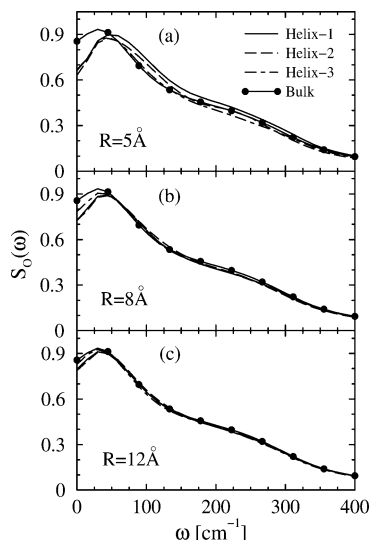


Figure 5. Power spectra $S_O(\omega)$ obtained by the Fourier transformation of the velocity autocorrelation function $C_V^O(t)$ for the oxygen atoms of the water molecules that are present within regions characterized by distances (a) $R = 5 \text{ \AA}$, (b) $R = 8 \text{ \AA}$, and (c) $R = 12 \text{ \AA}$ from the residues of the three α helices of the protein. The corresponding spectrum for pure bulk water is included for comparison.

and dynamical heterogeneity of the PW hydrogen bonds among the three helices.³⁶ Severely restricted translational mobility of the water molecules strongly bound to the residues of the helices by hydrogen bonds is clearly evident from further lowering of intensity at zero frequency in Figure 4a. Although the position of the $\text{O}\cdots\text{O}$ stretching band at $\sim 200 \text{ cm}^{-1}$ is not affected much for the water molecules involved in the PW hydrogen bonds, a noticeably higher intensity of this band is observed, particularly for helices 1 and 2. This is likely to arise from a fraction of water molecules that are doubly hydrogen bonded to these two helices³⁶ and thus exhibit restricted longitudinal translations. The band corresponding to the librational mode of the water molecules hydrogen bonded to the helices has also been found to be affected heterogeneously, as evident from the corresponding hydrogen atom power spectrum ($S_H(\omega)$) in Figure 4b. Compared to bulk water, this band is blue-shifted by $\sim 25\text{--}75 \text{ cm}^{-1}$ for the three helices. The water molecules hydrogen bonded to helix 1 residues suffered maximum shift. The results observed here are in good agreement with recent IINS studies on interfacial water of DNA and proteins in aqueous solutions.⁴¹ The differential shifts in the librational bands for the water molecules hydrogen bonded to the residues of the three helices again confirm their heterogeneous structural rigidity.

Another important issue in protein solvation is the thickness (R) of the hydration layer up to which water molecules exhibit correlated dynamics. Because of the heterogeneous distribution of the amino acid residues, the thickness of the hydration layers around different secondary structures of a protein can be different. To explore this, in addition to $R = 5 \text{ \AA}$, we have calculated the oxygen atom power spectra $S_O(\omega)$ for the water molecules present within $R = 8 \text{ \AA}$ and $R = 12 \text{ \AA}$ from the residues of the three helices of the protein. The results are displayed in Figure 5 along with the data for pure bulk water. The figure shows that with increase in the thickness of the hydration layers of the three helices, the power spectra of the water molecules appear similar to that of bulk water. The blue shift in the $\text{O}\cdots\text{O}\cdots\text{O}$ bending mode as observed for $R = 5 \text{ \AA}$ disappears as the thickness is increased. Besides, there appears to be no distinction in the distribution of the low-frequency modes for water around the helices at larger distances. This is

particularly true for $R = 12 \text{ \AA}$. This indicates that the thicknesses of the hydration layers of the three helices are almost similar. Currently, we are investigating the effects of the variation of thickness on different properties of the hydration layer water molecules of HP-36 and other protein molecules with more complex secondary structures.

4. Conclusions

In this work, we have studied in atomistic detail the vibrational spectrum of water present near the surface of a protein in an aqueous solution using MD simulations. The calculations are carried out with the 36 residue helical subdomain of villin headpiece or HP-36. An attempt has been made to understand how the heterogeneous rigidity of the hydration layers, the strength of the PW hydrogen bonds, and their structural relaxation are correlated with the low-frequency intermolecular vibrational modes of water around the secondary structures (α helices) of the protein molecule.

The calculations reveal several interesting results. We have observed clear blue shifts of $\sim 25 \text{ cm}^{-1}$ in the low-frequency band corresponding to the $\text{O}\cdots\text{O}\cdots\text{O}$ bending or the oscillatory caging motion of the water molecules in the first hydration layer. It is further noticed that the extent of the shift ($> 25 \text{ cm}^{-1}$) is more pronounced for the water molecules that are hydrogen bonded with the protein residues. This result is consistent with our earlier report³⁶ that the PW hydrogen bonds are much stronger with longer lifetimes, and thus the water molecules involved in the PW hydrogen bonds exhibit drastically restricted local motions. However, interestingly we observe nonuniform blue shifts of the band for water molecules around the three helices. As expected, such nonuniform behavior is more prominent for PW hydrogen bonds. The larger shift for the water molecules hydrogen bonded to helix 2 residues has been found to correlate rather well with the slowest structural relaxation of the corresponding PW hydrogen bonds.³⁶ To the best of our knowledge, this is the first report where it is shown that the heterogeneous rigidity of the hydration layers of different segments of a protein molecule and the properties of PW hydrogen bonds are directly correlated with the distribution of the low-frequency bands for water around those segments. Raman, IR, THz spectroscopic measurements, and IINS experiments are capable of identifying these low-frequency modes and thus can be used to verify such correlations. It may be noted that although THz measurements provide the power spectra corresponding to the dipole moment autocorrelation function the shifts in the spectral densities may be similar to that observed in the present study. Therefore, these experiments in combination with MD simulations can provide a direct understanding of the heterogeneous properties among different segments of a biomolecule, which in turn can be useful in exploring the molecular mechanism of various biological processes.

Surprisingly, the other low-frequency band corresponding to the $\text{O}\cdots\text{O}$ stretching due to longitudinal translations has not been found to suffer any shift for the first hydration layer water molecules. This is an important finding that indicates a nonuniform influence of the protein on the orientational and translational degrees of freedom of water around it. We have shown that the strong PW hydrogen bonds and their heterogeneous characteristics among the three helices also influence the blue shifts of the band corresponding to the librational motion of water molecules hydrogen bonded to the helices in a nonuniform fashion.

It is further noticed that with increasing the thickness of the hydration layer the distribution of the low-frequency intermo-

lecular vibrational modes of water around the three helices gradually approaches that of pure bulk water. This indicates that the thickness of the hydration layers of the helices are almost identical. It would be interesting to study whether similar properties of the hydration layer water are observed for proteins with more complex secondary structures. This is under extensive investigation in our laboratory.

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