6-22-2005

Coupling Between Lysozyme and Glycerol Dynamics: Microscopic Insights from Molecular-Dynamics Simulations

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Recommended Citation

Coupling between lysozyme and glycerol dynamics: Microscopic insights from molecular-dynamics simulations

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We explore possible molecular mechanisms behind the coupling of protein and solvent dynamics using atomistic molecular-dynamics simulations. For this purpose, we analyze the model protein lysozyme in glycerol, a well-known protein-preserving agent. We find that the dynamics of the hydrogen bond network between the solvent molecules in the first shell and the surface residues of the protein controls the structural relaxation (dynamics) of the whole protein. Specifically, we find a power-law relationship between the relaxation time of the aforementioned hydrogen bond network and the structural relaxation time of the protein obtained from the incoherent intermediate scattering function. We demonstrate that the relationship between the dynamics of the hydrogen bonds and the dynamics of the protein appears also in the dynamic transition temperature of the protein. A study of the dynamics of glycerol as a function of the distance from the surface of the protein indicates that the viscosity seen by the protein is not the one of the bulk solvent. The presence of the protein suppresses the dynamics of the surrounding solvent. This implies that the protein sees an *effective* viscosity higher than the one of the bulk solvent. We also found significant differences in the dynamics of surface and core residues of the protein. The former is found to follow the dynamics of the solvent more closely than the latter. These results allowed us to propose a molecular mechanism for the coupling of the solvent-protein dynamics. © *2005 American Institute of Physics*. [DOI: 10.1063/1.1938191]

I. INTRODUCTION

The rate at which biological agents (e.g., proteins and DNA) denature is a determining factor for the shelf life of protein-enzyme-based pharmaceuticals. Thus, the dynamics of the protein has a direct influence on their shelf lives. In fact, through the use of neutron-scattering experiments, it has been suggested that there is an inverse relationship between the thermal stability and the dynamics of a protein powder.[1] In addition, a thorough understanding of protein dynamics at different length and time scales is essential not only from the stability point of view but also for an understanding of protein and enzyme reactions at the molecular level.[2]

Similar to glassy systems,[2] proteins are complex systems with many conformational substates that are related to local minima of the potential-energy surface.[3] The biological functions of proteins are affected by the structural fluctuations among these substates.[4] The solvent could play an essential role in the activation of these fluctuations.[3] For instance, it has been reported[5] that there is a correlation between the structural fluctuations of the protein and the thermal motion of water. It has been proposed[6] that these fluctuations are promoted by solvent water molecules through a hydrogen bond network in bulk water which occurs on the same picosecond time scale as the conformational fluctuations.

The biological function of a protein decreases with decreasing temperature, possibly due to the suppression of transitions between conformational substates.[7] Similar to the α

relaxation in glassy systems, proteins also exhibit a relaxation process that governs large scale motions.[2] This marks the separation of two kinds of molecular motions. At low temperature, the protein undergoes small-amplitude harmoniclike motions, while at high temperatures, large-amplitude anharmonic motions dominate the dynamics. The change of the dynamical behavior from harmoniclike to anharmonic motions is commonly called the dynamic transition.[8] This transition, observed by experimental[2] as well as molecular-dynamics (MD) simulation methods,[9] promotes the flexible motions in the protein that are widely accepted to be required for biological function.[7] Additionally, correlations have been found between the presence of anharmonic motions and protein activity.[10,11]

The protein dynamic transition observed at $T \sim 200-230$ K was suggested to originate from the solvent glass transition.[12] According to this scenario the solvent molecules slow down and trap the protein molecules in long-living conformations such that the conformational motions necessary for function are strongly hindered. This scenario was supported by the findings that the conformational relaxation of a protein can be suppressed by highly viscous solvents even at room temperature.[13] MD simulations by Vitkup *et al.*[12] showed that the magnitudes of the protein fluctuations are largely determined by solvent viscosity. Based on these results it was concluded that the protein motion below the dynamic transition is inhibited mainly by high solvent viscosity.[2,9,13]

This strong influence of the solvent on the protein dynamics has been described as the protein being a "slave" of

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the solvent or coupling of protein and solvent dynamics.[14–16] The ability of certain chemical agents utilized in biopreservation applications could be correlated to this dynamical coupling. Namely, the protein becomes slaved to the solvent molecules and its dynamics is suppressed resulting in a retardation of the denaturation process. The α relaxation of the solvent has been generally regarded as the most significant process for influencing protein dynamics.[16] However, Caliskan et al.[16] have suggested that fast conformational fluctuations of glass-forming systems that usually occur on a picosecond time scale may also influence protein dynamics. Using Raman and neutron spectroscopies they showed that, on a picosecond time scale, the solvent controls the dynamics of the protein through a coupling in both low-frequency vibrations and relaxations of the protein.

The main motivation behind this work is to bring a deeper understanding into the dynamics of preserving agent-protein mixtures in the freeze-dried form. The low level of hydration that can be achieved in freeze drying (less than 0.01-g water/g protein) causes the hydration shell to be virtually entirely removed.[17] Therefore, in this work we used MD simulations to investigate the dynamics of a dehydrated protein-solvent system: hen egg white lysozyme, a widely studied model protein, in glycerol, a biopreserving agent that is commonly used in low-temperature applications. We aimed to reproduce the dynamical transition of the protein and examine the extent of the dynamical coupling between the protein and the solvent. The former was characterized by the mean-square displacement (MSD) of the hydrogen atoms in lysozyme, $\langle u^2 \rangle (T)$, whereas the latter was elucidated using common experimental quantities such as incoherent intermediate scattering function $[S(q,t)]$ and dynamic structure factor $[S(q,\nu)]$ as computed from our MD simulation study. Moreover, the effect of the macromolecule on the solvent dynamics was studied. For this purpose, profile studies of various properties of the solvent molecules with respect to the distance from the protein surface were done. The role of hydrogen bond interactions on the dynamics of the protein and dynamical coupling is also discussed.

This article is organized as follows. In Sec. II we present the details of our simulation protocol including the details about the equilibration procedure and the values of temperature, pressure, and cooling rates. We present the results of our simulation study in Sec. III. In particular, we pay special attention to the validation of the simulation protocol employed in this work and the coupling of the protein and solvent dynamics. Afterwards, we mainly focus on correlations between hydrogen bond behavior and the dynamics of the protein. Section IV presents the discussion of our results. The conclusions of this work are formulated in Sec. V and we end with the appropriate acknowledgments.

II. SIMULATION PROTOCOL

The AMBER molecular-dynamics package[18] with ff99 (Ref. 19) (a common Amber force field for proteins) and GAFF (Ref. 20) (general Amber force field for general organic molecules) were used in this study to model lysozyme and glycerol, respectively. The structure of glycerol[21] in its crystalline state was optimized and the electrostatic potentials on atom surfaces were calculated using the software package GAUSSIAN 03.[22] The Gaussian calculation was done using (ground state) the Hartree–Fock method with 6-31G basis set. Point charges on the atomic nuclei were then fitted by restrained electrostatic potential (RESP). The structure of the hen egg white lysozyme was obtained from the Protein Data Bank (193L). The Lennard-Jones parameters for the lysozyme-glycerol interactions were derived from those of lysozyme-lysozyme and glycerol-glycerol using the standard Lorentz–Berthelot combination rules.

Rectangular parallelepiped periodic boundary conditions were used. Long-range electrostatic interactions were calculated using the particle-mesh Ewald (PME) method, while van der Waals interactions were calculated using the 6-12 Lennard-Jones potential. The cutoff distance for nonbonded van der Walls interactions was set to 8 Å. However, in the case of the electrostatic interactions this cutoff is used for the evaluation of Ewald's standard direct sum; corrections are taken into account via the reciprocal sum. The hydrogen bonds in this all-atom potential function are represented by a balance between electrostatic and van der Waals interactions. Random initial velocities were assigned to all atoms after minimization of the initial structure. The equations of motion were integrated using leap-frog Verlet algorithm with a step size of 1 fs. Constant temperature and pressure were satisfied by a weak coupling algorithm.[23]

A simulation box of glycerol was preequilibrated at 300 K and the energy-minimized structure of lysozyme was placed at the center. The glycerol molecules within proximity of 2 Å to lysozyme were removed from the simulation box. The resulting protein-solvent mixture with 1118 glycerol molecules was then equilibrated first under constant volume conditions for 50 ps and then under isobaric conditions for 300 ps at 500 K and 0.1 MPa. During this step of the equilibration we put harmonic restraints on the protein atoms. Following an additional equilibration at 300 K for 300 ps where the restraints were removed, the data collection run was performed in isobaric conditions for 2 ns and the coordinate sets were saved for every 0.1-ps intervals for subsequent analysis. For the simulations at 250, 200, and 150 K the system was first annealed to 50 K below the current temperature at a cooling rate of 0.1 K/ps followed by an equilibration in the N-P-T ensemble at this temperature before the data collection run. The trajectories for pure glycerol were taken from our previous work.[24]

III. RESULTS

We start the presentation of our results with a verification of the simulation protocol employed in this work. For this purpose, we present direct comparisons between some of our simulation results and the corresponding experimental observations. Perhaps, the most widely studied property of protein-solvent systems is the dynamic transition temperature T_d which is the temperature at which the conformational component, using the terminology of Fenimore et al.,[25] of the atomic mean-square displacement of the atoms ($\langle u^2 \rangle$) in the protein departs from zero. The $\langle u^2 \rangle$ employed throughout
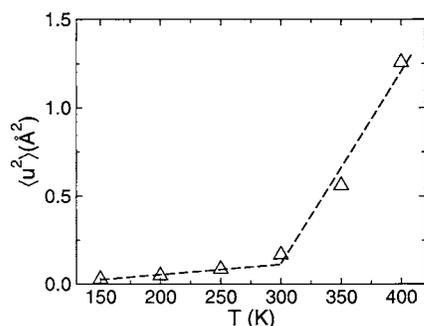
FIG. 1. Temperature dependence of the mean-square displacement of the hydrogen atoms in lysozyme immersed in glycerol.

FIG. 2. Incoherent intermediate scattering function of the hydrogen atoms in lysozyme ($\times$) and pure glycerol molecules ($\blacktriangle$) at 150, 200, and 250 K.

this article is the one computed from the hydrogen atoms only. This selection is based on our interest in comparing the results with the experimental data from neutron spectroscopy. T_d can be estimated from the simulation data as the intercept between two straight lines: the first line is a fit of $\langle u^2 \rangle$ as a function of temperature at low temperatures and the second one is the same fit but at high temperatures. Figure 1 shows a plot of $\langle u^2 \rangle$ for the *hydrogen* atoms in lysozyme as a function of temperature. $\langle u^2 \rangle$ was derived from the MD trajectories through the relationship

$$\langle u^2 \rangle = \langle (r(t + t_0) - r(t_0))^2 \rangle, \tag{1}$$

where $r(t_0)$ and $r(t+t_0)$ are the coordinates of atoms at reference time t_0 and after time t. The brackets represent averaging over hydrogen atoms and reference time. The MSD for each temperature was obtained after averaging over 800 ps. The two regimes traditionally observed in protein-solvent systems are clearly visible in Fig. 1. At low temperatures, $\langle u^2 \rangle$ increases linearly with increasing temperature up to $\sim$250 K. Above this temperature the dynamics is largely nonlinear. The low-temperature behavior shown in Fig. 1 is typical of a harmonic oscillator. Thus, in this regime the atoms in the protein can be visualized as *vibrating* in a harmoniclike potential around their equilibrium positions. At high temperatures, the deviation of $\langle u^2 \rangle$ from the linear, low-temperature, behavior becomes clear and is due to the activated transitions between different substates. These transitions give rise to anharmonic motions. The intercept between both straight lines occurs around 300 K implying that T_d for our system, which contains 12% of lysozyme by weight, is located close to 300 K. Tsai *et al.*[26] have reported the values of T_d for 80% and 50% (by weight) lysozyme in glycerol to be 330 and 270 K, respectively. Our estimate agrees with these results on a quantitative level providing some experimental support to the simulation protocol described in Sec. II and the force field employed in this study.

It is well known that the temperature of the dynamic transition is greatly affected by the surrounding solvent.[2] The role of solvent has been demonstrated in many experimental studies on different proteins such as lysozyme and myoglobin.[14,25,27–30] The aforementioned studies have found that the dynamics of the protein is a *slave* of the dynamics of the solvent. Also MD simulations of myoglobin in water[12] found that solvent mobility is a crucial parameter in the determination of the atomic fluctuations in the protein which,
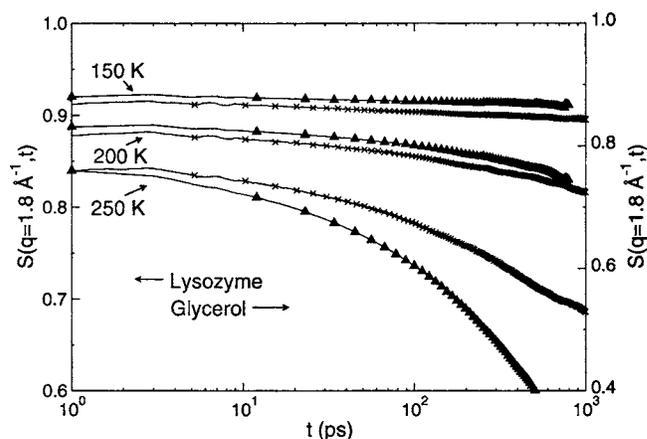
in turn, determine T_d. Other MD simulation studies, e.g., ribonuclease A in water[31] and copper plastocyanin in water,[32] have provided additional support for the strong coupling of protein and solvent dynamics. In order to investigate the presence of this coupled dynamic behavior we analyzed the incoherent intermediate scattering function $S(q,t)$ and the corresponding dynamic structure factor $S(q,\nu)$ of the hydrogen atoms in the pure solvent and in the protein; here q is the scattering wave vector, t is the time, and ν is the frequency. A comparison between $S(q,t)$ of the pure solvent and, separately, $S(q,t)$ of the protein gives some insight into the coupling of their dynamics within the accessible time window of 1 ns. A comparison of the frequency of the low-energy vibration mode, the so-called boson peak in $S(q,\nu)$, yields additional information about the collective molecular vibrations in the solvent and collective vibrations of residues in the protein.

First, we look at $S(q,t)$ for lysozyme in glycerol (lysozyme) and pure glycerol. $S(q,t)$ was calculated from the MD trajectories using the formula

$$S(q,t) = 1/N \left\langle \sum_{i=1}^{N} e^{[i\mathbf{q} \cdot (\mathbf{R}_i(t) - \mathbf{R}_i(0))]} \right\rangle, \tag{2}$$

where $\mathbf{R}_i(t)$ is the position of the ith hydrogen atom at time t and N is the total number of hydrogen atoms. The brackets indicate average over time origins. Figure 2 shows the results for lysozyme and pure glycerol. At 150 and 200 K the time dependence of $S(q,t)$ for lysozyme and glycerol is approximately the same over the whole time window studied (except a prefactor) indicating that there is a strong coupling between the dynamics of glycerol and lysozyme up to 1 ns. This coupling could be a consequence of both types of molecules being trapped in a glassy state at very low temperatures as well as molecular interactions between the two. This result implies that practically *all* the modes with characteristic time scales between 1 ps and 1 ns are strongly coupled. On the other hand, $S(q,t)$ for lysozyme and glycerol differ at 250 K: it decreases in glycerol faster than in lysozyme. Therefore, at these temperatures and for the time window mentioned above, lysozyme does not show the same dynamics as pure
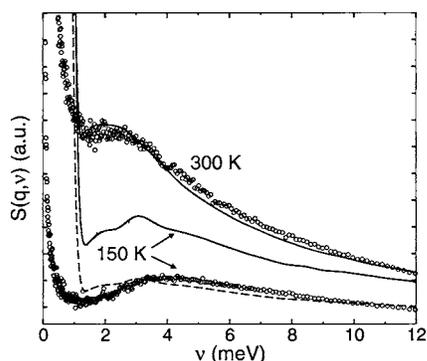
FIG. 3. Dynamic structure factor for lysozyme in glycerol from MD simulation (lines) and from neutron-scattering spectra (symbols) from Caliskan *et al.* (Ref. 16). The experimental data were multiplied by the frequency and Bose factor. The upper and bottom curves/symbols correspond to temperatures of 300 and 150 K, respectively. The meaning of the continuous and dashed lines are explained in the text.

glycerol. Only *some* molecular motions in lysozyme and glycerol are coupled.

In order to further evaluate the conformity between experiments and our simulations, we compare the dynamic structure factor $S(q,\nu)$ obtained from our MD simulations to the experimental neutron-scattering data obtained for lysozyme in glycerol, as shown in Fig. 3.[16] $S(q,\nu)$ was calculated from $S(q,t)$ by Fourier transformation,

$$S_{inc}(q,\nu) = \frac{1}{2\pi} \int_{-\infty}^{+\infty} e^{(-2i\pi\nu t)} S_{inc}(q,t) dt, \qquad (3)$$

after multiplication with a Gaussian function that takes into account the experimental resolution of the spectrometer. The resolution was set to 300 μeV which corresponds to a Gaussian function with a full width at half maximum (FWHM) of 700 μeV. We also tried higher resolutions (200 and 100 μeV), however, a reduction in the value of this parameter led to some small fluctuations on top of the curves shown in Fig. 3. Similar fluctuations have also been observed in simulations of other proteins like azurin.[33] The origin of these fluctuations is the lack of enough structural inhomogeneity which can be resolved by using not a single lysozyme but a cluster of many lysozymes, e.g., four or five proteins in the simulation,[34] or by running various MD simulations where the glassy phases are prepared differently and averaging the results.[35] Our main interest, however, is in the behavior of $S(q,\nu)$ in the vicinity of the boson peak and not in the low-frequency (quasielastic) region. Thus, a resolution of 300 μeV is a reasonable choice because it removes the small oscillations allowing us to resolve the boson peak clearly. The price we pay for this resolution is a disagreement at low frequencies (quasielastic spectrum). In addition, the experimental data in Ref. 16 were obtained after averaging over values of q ranging from 0.25 to 1.5 Å^{-1}, so we averaged $S(q,t)$ over 20 randomly chosen wave vectors with moduli between the aforementioned values. Finally, numerical inaccuracies appeared in the Fourier transformation at 150 K. Since the origin of these inaccuracies was traced to the truncation of $S(q,t)$, we extrapolate the intermediate scattering function at times beyond 1 ns using a stretched exponential

decay with parameters estimated from the fit of the simulation data obtained from the last 997 ps. The Fourier transformation showed in Eq. (3) was applied to this *extended* function where the first 1 ns of the data was taken from the MD simulation and, the fitted stretched exponential was used after 1 ns. The data at 300 K did not need this correction because $S(q,t)$ decayed fast enough during the 1-ns time window. Figure 3 shows two quantitative comparisons of the simulation and experimental results. The simulation data obtained at 300 K (line) were scaled and shifted *vertically* to maximize the quantitative agreement between both results. This is justified due to the arbitrary units used in the experimental $S(q,\nu)$. The results of our simulations reproduce well the experimental data in the frequency range of interest (Fig. 3). Namely, the presence of the inelastic boson peak and the increase of the quasielastic-scattering intensity (QES) with decreasing frequency are captured by our simulations. The simulation data obtained at 150 K were treated in two different ways. First, we used the same scaling and vertical shifts employed with the 300-K data. This result is shown as a continuous line in Fig. 3. Second, we optimized the vertical scaling and shift to obtain the best quantitative agreement with the experimental data (dashed line). As expected, the first treatment of the simulation data leads to a curve above the experimental data. The origin of this vertical displacement is the different cooling rates used in typical MD simulations (about 1 K/ps) and experimental studies. However, when the second approach is used our simulation study reproduces the experimental data quite well in the frequency window (1 and 10 meV) of interest. However, the maximum of the boson peak in the simulation appears at slightly lower frequency (the difference is about 0.6 meV). This has also been reported for other systems and many of which were studied using different force fields and MD simulation packages (see Ref. 36 and references therein). The difference in the frequency of the boson peak has been rationalized in terms of the softness of the potential force field. However, another, perhaps more physical, origin of the disagreement might be found in the preparation of the system at low temperatures. In particular, it is experimentally known that systems quenched into the glassy state have boson peaks with lower frequencies and higher amplitudes than systems annealed into the glass.[37] Thus, the systems studied using MD simulations will always have the boson peak shifted to lower frequencies when compared with the experimental results. Other possible origin of the discrepancy between simulation and experiment might be the concentration of lysozyme: it was 50% by weight in the experimental studies while it was 12% in our case.

Let us now elaborate further on the behavior of $S(q,\nu)$ and the coupling of the protein and solvent dynamics. For this purpose we do not average different values of q because we will not be comparing with the experimental data. We employ only one value, $q=1.8$ Å^{-1}.[33] The resulting $S(q,\nu)$ curves (not shown) clearly exhibited the boson peak in both systems at all temperatures except for glycerol at 300 K. The physical origin of this peak for glassy systems as well as proteins is still a subject of discussion.[38] However, for the case of proteins, Tarek and Tobias[39] showed that the boson
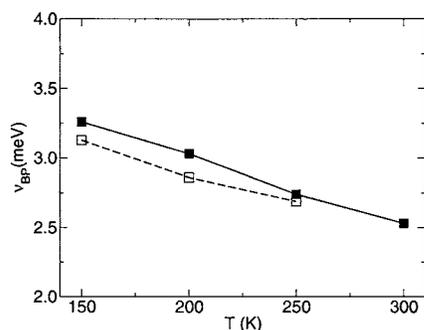
FIG. 4. Temperature dependence of the frequency of the boson peak from MD simulation. Lysozyme (■) and pure glycerol (□).

FIG. 5. Mean-square displacement of the hydrogen atoms in glycerol as a function of the distance from the surface of lysozyme at 300 K.

peak vibrations involve the protein as a whole. Strong similarities in the spectral shape of $S(q, \nu)$ for lysozyme and pure glycerol were observed, the only difference being the higher scattering intensity of glycerol, as expected. The frequencies of the boson peak for lysozyme and pure glycerol are very similar. This is clearly shown in Fig. 4 where we plot the frequency of the boson peak (ν_{BP}) as a function of temperature for both systems. ν_{BP} was extracted from the simulation data using the extrapolation formula

$$S(q,\nu) = \frac{A\nu_0}{\nu_0^2 + \nu^2} + B \exp\left\{-\frac{[\ln(\nu/\nu_{BP})]^2}{2[\ln(W/\nu_{BP})]^2}\right\}, \quad (4)$$

that comprises two terms: the first one approximates the QES part with a Lorentzian function of width ν_0 and height A/ν_0, whereas the second term (long-normal function) fits the boson peak of width W.[40] The similar temperature dependence of the frequencies of the boson peak for lysozyme and pure glycerol clearly indicates that the low-frequency collective vibrations are coupled. This has been reported experimentally[16] and is now reproduced well in our simulations. This result implies that our simulation protocol is also capable of reproducing the coupling of the protein-solvent dynamics thus, putting our simulation approach on a stronger foundation.

QES is due to the relaxationlike dynamics such as overdamped vibrations or activated processes.[41] Quasielastic broadening is negligible at low temperatures because of significant slowing down of relaxation processes. The QES intensity increases with temperature and dominates the spectra at $T = 300$ K. As a result, the inelastic contribution to the spectra (e.g., boson peak) becomes undetectable in our simulation study of glycerol.

As we have discussed before, the influence of solvent on the dynamics of the protein is well established. However, few studies have been done to investigate the effect of the protein on the dynamics of the surrounding solvent.[42–44] Yet, it has been shown[43] that the dynamics of water molecules near the surface of the protein is more restricted than in bulk water. The restricted mobility of water near the protein surface has been attributed[43] to the following three factors: the decrease of the dimensionality of the space at the interface, solute surface roughness, and solvent structuring. In order to explore if this result is applicable to glycerol we calculated the profile of $\langle u^2 \rangle$ for the hydrogen atoms in glycerol as a function of the distance from the surface of lysozyme for
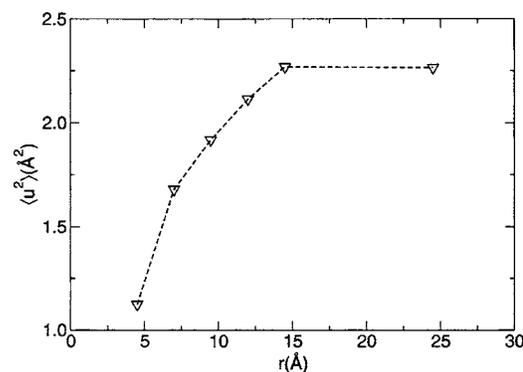
four temperatures. This distance was computed following the work of Makarov *et al.*[43] The average positions of solvent hydrogen atoms were sorted into six shells with respect to the distance from their nearest protein atom. The first shell comprised the hydrogens within a distance of 4.5 Å from the protein surface, the following four shells were created with thicknesses of 2.5 Å, and the last shell consisted of hydrogens between 14.5 and 24.5 Å from the surface of the protein. $\langle u^2 \rangle$ for each of these shells was calculated and the results are shown in Fig. 5 for 300 K. From the figure it is clear that the dynamics of glycerol is suppressed near the surface of the protein for all the temperatures studied. $\langle u^2 \rangle$ increases with increasing distance from the surface until it plateaus around 10–15 Å and reaches the bulk value. The magnitude of $\langle u^2 \rangle$ near the surface can differ from the one in the bulk by a factor as large as two at 300 K. Figure 6 shows $S(q,t)$ computed for the six shells around the protein at 300 K. The plot clearly shows that the further the solvent molecules are from the surface the faster $S(q,t)$ decays. This result shows that the dynamics of the glycerol molecules in close proximity to the protein surface is significantly affected by the presence of the protein. However, the effect of the protein on the dynamics of the solvent vanishes for distances longer than ~10 Å. This is observed in the rate of decay of the curves and in amplitude of $\langle u^2 \rangle$ which remains virtually unchanged beyond 10 Å. Similar results as shown in Figs. 5 and 6 were observed at temperatures 150, 200, and 250 K.

The data shown in Figs. 5 and 6 can be interpreted from
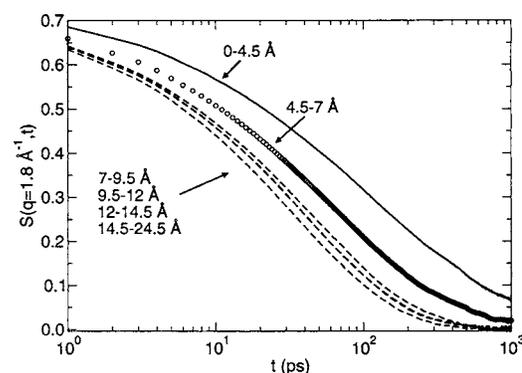

FIG. 6. Incoherent intermediate scattering function for glycerol molecules within 0–4.5 Å (line), 4.5–7 Å (○), and larger distances (dashed lines) from the surface of lysozyme at 300 K.

a different perspective using the concept of *local* viscosity of the medium surrounding the protein. It is intuitively clear that the viscosity of a fluid should increase monotonically with decreasing MSD of the molecules. This is clearly the case for a simple fluid that satisfies the Stokes–Einstein relationship,

$$\eta = \frac{k_b T}{6\pi r D},\qquad(5)$$

where η is the viscosity of the liquid and D is the diffusion coefficient that can be expressed in terms of the atomic MSD as follows:

$$D = \lim_{t\to\infty} \frac{\sum_n [x(t) - x(0)]^2}{6tn},\qquad(6)$$

where t is the time, x is the atomic position of the center of mass, and n is the number of molecules. From Eqs. (5) and (6) we can compute η from the MSD data obtained from MD simulations. However, the linear dependence of the MSD on time must be satisfied.[45,46] Similarly, a monotonic dependence of the viscosity on the MSD has been reported for melts of glass-forming polymers.[47] Following this line of reasoning an interesting observation can be made about the data shown in Fig. 5. The suppression of the dynamics of glycerol near the surface of the protein can be also interpreted as the protein being immersed in an environment of higher viscosity than the bulk viscosity of the solvent. Thus, the protein sees an effective *local* viscosity higher than the viscosity of bulk solvent.

The results presented thus far motivated us to explore possible physical origins for the aforementioned properties. Some previous studies on glycerol-trehalose[24] and protein-water[3,12,31,32] mixtures suggest that the hydrogen bond network plays an important role in the dynamical behavior of these kinds of systems. Thus, we studied the behavior of the hydrogen bonds present in our systems using a geometric criterion based on the distance between the donor and the acceptor oxygen atoms, and the angle formed by the donor oxygen, the acceptor hydrogen and the acceptor oxygen atoms.[48] The cutoff distance between oxygen atoms was set to 3.4 Å which is about the location of the minimum after the first peak of the radial distribution function; the cutoff for the angle was set to 120 deg. Using this geometric criterion we characterized the hydrogen bonding network using the hydrogen bond correlation function defined by the following equation:

$$c(t) = \frac{\langle h(t)h(0)\rangle}{\langle h\rangle},\qquad(7)$$

where $h(t)$ is the hydrogen bond population operator which is equal to one when a donor-acceptor pair satisfies the hydrogen bond criterion at time t and zero otherwise. Therefore, $c(t)$ is the probability that a hydrogen bond originally formed at $t=0$ between a randomly chosen donor-acceptor pair exists at time t.

Figure 7 shows the hydrogen bond correlation functions for the hydrogen bonds between glycerol and lysozyme for the following five temperatures: 150, 200, 250, 300, and
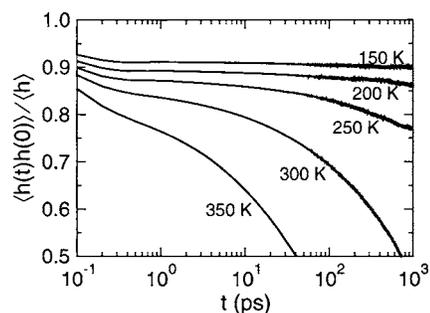

FIG. 7. Hydrogen bond correlation function for the hydrogen bonds between glycerol and lysozyme at five temperatures.

350 K. At 150 and 200 K, the correlation functions show an initial decay for time scales shorter than 1 ps and then remain approximately constant for the time window explored in this study (1 ns). At 250 K, the hydrogen bond correlation function shows the initial decay in the subpicosecond regime and the beginning of a second decay at times close to 1 ns. At higher temperatures, the hydrogen bond correlation function decays to small values within the time window studied in this work. The existence of two decays, one in the subpicosecond regime and the other one at long times, show the existence of two types of hydrogen bonds: fast and slow.[31] The fast hydrogen bonds correspond to rotation and libration of the solvent molecules, and affect the fast dynamics of the protein. Clearly, we are not interested in these types of hydrogen bonds because their lifetimes are short thus, they do not affect the *long-time* dynamics of the protein significantly. However, the dynamics of the slow hydrogen bonds is important because the structural relaxation of the protein (i.e., changes in the conformational substrates) requires the relaxation of the protein-solvent hydrogen bonding network. This is achieved via solvent translational displacement.[31] Therefore, long-living hydrogen bonds have an effect on the dynamics of the protein thus affecting T_d and other properties. In addition, Tarek and Tobias[31] have demonstrated that the hydrogen bond network relaxation time correlated to the dynamics of the protein as opposed to the fast hydrogen bonding lifetime which did not exhibit such a relationship. Therefore, we will focus on the slow hydrogen bonds.

Figure 8 shows a semilogarithmic plot of the average lifetime (τ_R) of the slow hydrogen bonds as a function of
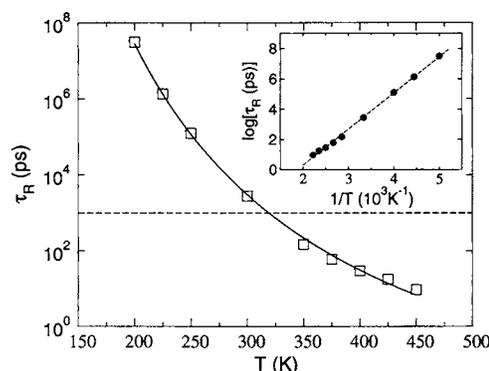

FIG. 8. Relaxation time of the hydrogen bond network (τ_R) as a function of temperature. The inset shows the same data but presented as $\log(\tau_R)$ vs T^{-1} (Arrhenius form).
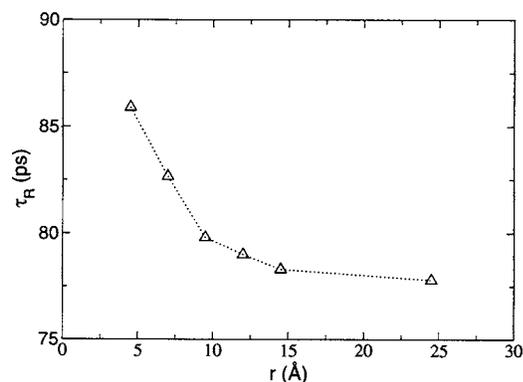
FIG. 9. Relaxation time of the hydrogen bond network (τ_R) for glycerol-glycerol hydrogen bonds plotted as a function of the distance from the surface of the protein at 300 K.

temperature. The dashed line corresponds to 1 ns which is the time window of our simulation study. The inset is a plot of the same data but as a function of T^{-1} (Arrhenius form). τ_R is the relaxation time of the *slow hydrogen bonds* and was extracted from the data as follows: we fitted a stretched exponential function to the data collected during the last 997 ps and extracted τ_R from the fit. Figure 8 shows that the values of τ_R vary from the picosecond time scale at high temperatures to the microsecond time scale at low temperatures. At temperatures close to 300 K the dynamics of the hydrogen bonding network enters the time window accessible to our MD studies. A comparison of Figs. 1 and 8 clearly suggests a correlation between the lifetime of the slow hydrogen bonds and the dynamics of the protein. In other words, when the dynamics of the hydrogen bonding network enters the time window of our simulation study, $\langle u^2 \rangle$ increases rapidly indicating the presence of the dynamic transition. Further discussion is presented in Sec. IV. The inset shows that the behavior of the relaxation time follows an Arrhenius law (E_a=46 kJ/mol) implying that we have only one type of dynamical process. In other words, the dynamical transition does not involve a transition between two different relaxation processes. This result is in agreement with a recent study by Fenimore *et al.*[25]

The possible connection between τ_R and the dynamics of the protein led us to compute the behavior of τ_R for the intermolecular hydrogen bonds between glycerol molecules as a function of the distance from the surface of the protein. The calculation method was similar to the calculation of the $\langle u^2 \rangle$ profile. Namely, hydrogen bonds were sorted into the various shells using the distance of the acceptor and donor oxygen atoms from the surface of the protein. When, for a particular hydrogen bond, both acceptor and donor atoms were found to be in the same shell, the hydrogen bond contributed to the hydrogen bond correlation function of that shell. If the acceptor and donor atoms were in different shells, then the hydrogen bond contributed to both shells. Figure 9 shows this profile for 300 K. τ_R decreases with distance implying that the slow hydrogen bonds break more rapidly as we move away from the surface. This suggests that glycerol is less constrained the further away it is from the surface. This behavior correlates to the increase in the MSD showed in Fig. 5.
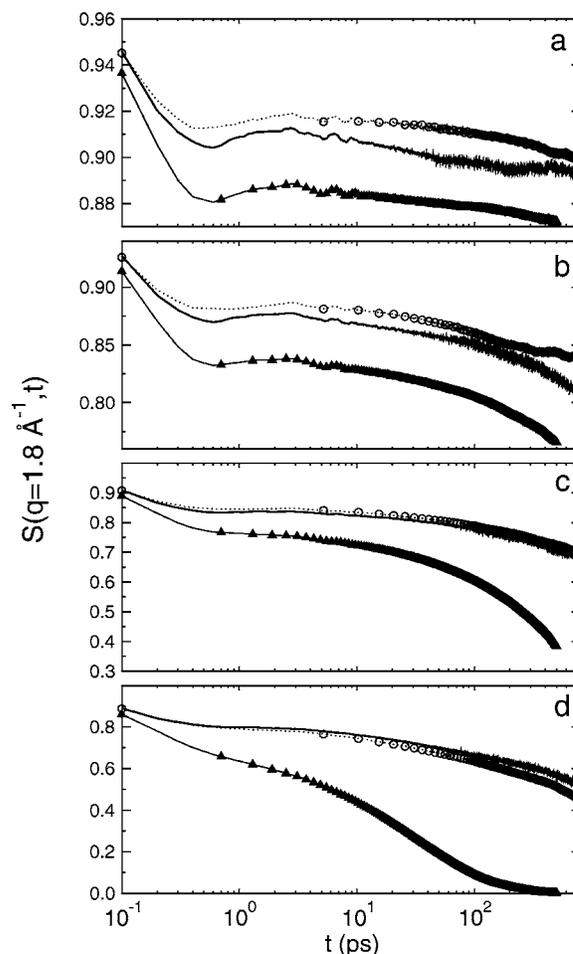

FIG. 10. Incoherent intermediate scattering function for glycerol hydrogen atoms (▲), the surface (○), and the core (continuous line) hydrogen atoms in lysozyme at (a) 150, (b) 200, (c) 250, and (d) 300 K.

IV. DISCUSSION

Probably, the most important question to ask about the lysozyme-glycerol system is how the coupling of the solvent and protein dynamics occurs. Clearly, the solvent-protein interface contains crucial information needed to answer this question. In an attempt to enhance our understanding of the surface protein dynamics we compared the dynamics of the hydrogen atoms that are on the protein surface to the dynamics in the core of the protein. The hydrogen atoms in the protein that are the closest ones to any hydrogen atom in any solvent molecule were defined as surface hydrogen atoms and the rest were considered as core atoms. In other words, we calculated the distances between a particular hydrogen atom in a solvent molecule and all hydrogen atoms in the protein. The hydrogen atom in the protein that was the closest one to the solvent hydrogen atom was considered to be on the surface of the protein. This method was repeated for all the hydrogen atoms in all the solvent molecules present in the system. This provided us with a list of those protein hydrogens that are the closest ones to the solvent, that is, the surface hydrogens. Using this definition, we found 440 hydrogens on the surface of the protein and 537 in the core. Figure 10 shows $S(q,t)$ for glycerol and the surface and core hydrogen atoms of lysozyme. For all the temperatures stud-
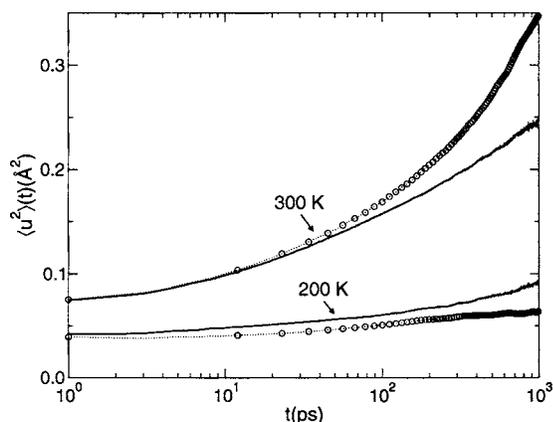
FIG. 11. Mean-square displacement for the surface (○) and the core (continuous line) hydrogen atoms in lysozyme at 200 and 300 K.
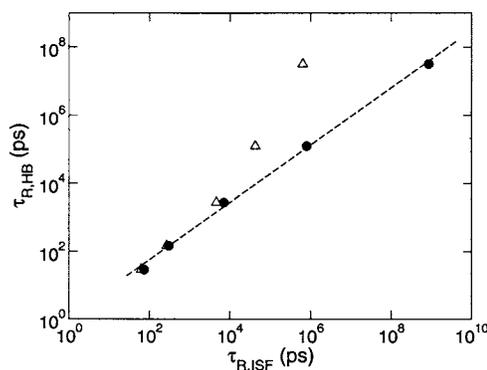

FIG. 13. Plot of the relaxation time for the hydrogen bonds between the protein and the solvent as a function of the relaxation time of $S(q,t)$ of lysozyme considering the methyl group hydrogen atoms (△) and neglecting them (●).

ied, $S(q,t)$ shows a first fast decay in the subpicosecond regime. However, the behavior of the dynamics at long times is substantially different. For example, at temperatures below T_d (150 and 200 K), $S(q,t)$ for both the surface and the core atoms does not show any indication of a second decay at long times and remains approximately constant (note the scale). It is interesting to notice that the curve for the core atoms is below the one for the surface atoms indicating that the atoms in the core of the protein are more mobile than the ones on the surface. This result implies that glycerol reduces the size of the cage around the surface residues. However, at 250 and 300 K, $S(q,t)$ for the surface atoms starts to follow the decay observed for glycerol and crosses the curves that correspond to the core atoms. This implies that the protein atoms on the surface become more mobile than the core atoms at long enough times. The core atoms also follow the decay in $S(q,t)$ of glycerol but the effect is less pronounced than for the surface atoms. Thus, we can say that their dynamics are more shielded from the effects of the solvent than the ones of the surface atoms. However, the figure clearly shows an effect of the solvent properties on the core residues. Similar conclusions can be drawn from the behavior of $\langle u^2 \rangle(t)$ shown in Fig. 11. At low temperatures (200 K), the core residues of the protein have larger cage sizes than the surface residues. Meanwhile, at high temperatures (300 K), the MSD of the hydrogen atoms on the surface crosses the one of the core atoms at times close to 20 ps and surpasses it throughout the rest of the time window of this study. This suggests that the dynamical coupling between the protein and the solvent is translated through the surface of the protein. Basically, the influence of the solvent dynamics is con-

veyed to the surface atoms by means of hydrogen bond interactions (see below) and, afterward, the surface atoms translate that effect onto the core atoms through intramolecular interactions. This inference is further supported by the MD simulations of Walser and van Gunsteren.[49]

Additional evidence that the properties of the protein-solvent interface have a major role in the dynamics of the protein is shown in Figs. 12 and 13. It was emphasized in Ref. 50 that methyl group rotation in lysozyme appears in the nanosecond-picosecond time window at $T \sim 100$ K and gives significant contribution to the dynamic structure factor at higher temperatures. Thus we paid particular attention to the methyl group contribution in our simulations and its implications will be clarified below. Figure 12 shows $S(q,t)$ for lysozyme with and without the hydrogen atoms in the methyl groups, and the hydrogen bond correlation function, Eq. (7). $S(q,t)$ without methyl groups decays slower for all temperatures. We emphasize the remarkable similarity between $S(q,t)$ without methyl groups and hydrogen bond correlation function. In particular, the effect of temperature on both functions is practically the same, e.g., both functions show very similar decays at long times for all the temperatures. Moreover, at 300 K both functions have very similar values at 1 ps and reach the value of 0.6 at similar times (the difference is a multiplicative factor of two). However, the hydrogen bond interactions that determine the hydrogen bond correlation function occur within *a shell with a thickness of 3.4 Å around the protein*, while $S(q,t)$ is determined by *the dynamics of the whole protein*. Therefore, hydrogen bonds formed on the surface affect the dynamics of the protein as whole. The resemblance of these plots emphasizes how
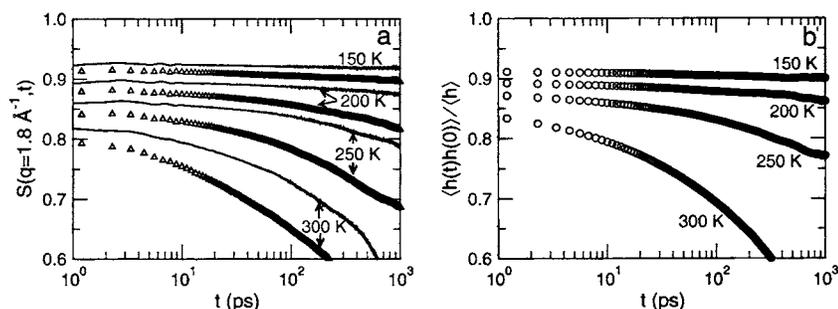

FIG. 12. Comparison of (a) incoherent intermediate scattering function for lysozyme in glycerol by considering the methyl hydrogen atoms (△) and neglecting them (continuous line) and (b) hydrogen bond correlation function for the hydrogen bond interactions between lysozyme and glycerol.

strongly the hydrogen bonding behavior and the dynamics of the protein are related. Figure 13 shows a plot of the relaxation time for the slow hydrogen bonds ($\tau_{R,\mathrm{HB}}$) as a function of the protein's relaxation time obtained from $S(q,t)$ ($\tau_{R,\mathrm{ISF}}$) with and without considering methyl groups. $\tau_{R,\mathrm{ISF}}$ was obtained in the same way as $\tau_{R,\mathrm{HB}}$. When the methyl hydrogens are neglected, $\tau_{R,\mathrm{HB}}$ and $\tau_{R,\mathrm{ISF}}$ show a power-law relationship with exponent and prefactor equal to 0.858 and 0.809, respectively. The correlation coefficient for these data is 0.999. On the other hand, this simple relationship breaks down when methyl groups are included, especially at low temperatures (200 and 250 K). These are the temperatures where the dynamical transition has not been reached yet while the methyl group rotations are active. In addition, note that the methyl group rotations do not contribute in a significant manner to the structural relaxations in the protein. Therefore, it is expected that the hydrogen bond relaxation time correlate to the relaxation time of $S(q,t)$ when the motions of methyl groups are omitted. This shows that the relaxation of the slow hydrogen bonds on the surface of the protein determines the structural relaxation of the protein, at least in the time window accessible to our MD simulation study. In fact, based on our current and recent findings,[24] we argue that the hydrogen bonding network is a major factor controlling the dynamics of the protein.

It is widely accepted that one very important property of the solvent that controls the protein dynamics is its viscosity.[29,30,51] For instance, it has been demonstrated using MD simulations[49] that the dynamics of the protein is considerably slower in a high viscosity solvent. However, an atomistic picture of this viscosity effect is still not available. Our results show that the viscosity felt by the protein is not the bulk viscosity. Indeed, Figs. 5 and 9 show that the MSD of the hydrogen atoms in glycerol decreases and the relaxation time of hydrogen bonds increases as the distance from the surface decreases. These variations can be understood as an increase in the effective viscosity felt by the protein and could be a consequence of the decrease of the dimensionality of the space around the protein, surface roughness, strong interactions with the surface of the protein (i.e., hydrogen bonds), or combinations of all these effects. This dependence of the MSD on distance together with the analysis of the dynamic behavior of the surface and core atoms in the protein discussed before provides clues about the physical origin of the effect of viscosity on the protein dynamics. Namely, the inherently different dynamics of the protein and the solvent merge together by two means: first, the solvent molecules close to the protein surface modify their dynamics in a gradual manner due to the presence of the protein (the closer to the surface the greater the similarity between the dynamics of the solvent and the surface of the protein) and, second, the surface atoms that are hydrogen bonded to the solvent molecules translate the dynamics imposed by the solvent to the core atoms via intramolecular interactions, thereby modifying the dynamics of the protein. Since the solvent molecules near the surface move less than in the bulk and form hydrogen bonds with the protein, we conclude that the effective viscosity of the solvent on the surface of the protein is increased. Therefore, the protein dynamics should be slowed down due to the increase in the effective solvent viscosity.

V. CONCLUSIONS

In this article we have explored possible physical mechanisms behind the coupling of the dynamics of lysozyme and the dynamics of glycerol, the surrounding solvent. We found that the dynamics of the interface between the solvent and the protein plays a fundamental role in the determination of the dynamics of the protein. In particular, we have provided evidence that the dynamics of the hydrogen bond network between the protein and the first shell of solvent molecules controls the structural relaxation of the protein as a whole. This is clearly shown by the power-law relationship displayed by the structural relaxation time of the protein and the relaxation time of the slow hydrogen bonds between the protein and the solvent. Moreover, our study suggested a molecular-level mechanism that leads to the coupling between the dynamics of the protein and the one of the solvent. First, the hydrogen bonds between the solvent and the surface atoms of the protein couple the dynamics of the surface of the protein to the one of the solvent. This coupling is propagated into the core atoms via intermolecular interactions, i.e., van der Waals, electrostatic, bonded, etc. However, this propagation shields the core atoms and their dynamics is not affected by the solvent as strongly as the one of the surface atoms.

Further analysis of the relaxation of the hydrogen bond network showed a correlation between the temperature dependence of the hydrogen bond relaxation time and the one of the MSD of the hydrogen atoms in lysozyme. This gives a stronger support to the effect of the hydrogen bonds on the dynamics of the protein. Moreover, the temperature dependence of the hydrogen bond relaxation time was found to follow an Arrhenius law leading to the conclusion that during the dynamic transition nothing special happens to the dynamics, there is only one dynamical process in the system above and below the dynamic transition temperature. Simply, the relaxation process enters the accessible time range and this leads to strong increase in MSD.

We also addressed the effect of the protein on the dynamics of the solvent. We found that the solvent dynamics in proximity to the protein surface is strongly suppressed. Indeed, the MSD of the hydrogen atoms in glycerol shows a decrease as the molecules get close to the surface of the protein. This implies that the solvent molecules move less as they get closer to the surface or, in other words, their dynamics is suppressed. This was also corroborated with the calculations of the incoherent intermediate scattering function and interpreted using the concept of viscosity. The protein sees a viscosity higher than the one of the bulk solvent. In addition, we also found that the behavior of the MSD correlates with the relaxation time of the hydrogen bonds between solvent molecules. Indeed, this relaxation time was found to increase as the molecules get closer to the surface of the protein. Thus, the dynamics of the hydrogen bonds controls the dynamics of the solvent molecules.

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