# Role of Protein-Water Hydrogen Bond Dynamics in the Protein Dynamical Transition

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The role of water in protein dynamics has been investigated using molecular dynamics simulations of crystals and a dehydrated powder. On the 100 ps time scale, the anharmonic and diffusive motions involved in the protein structural relaxation are correlated with the protein-water hydrogen bond dynamics. The complete structural relaxation of the protein requires relaxation of the hydrogen bond network via solvent translational displacement. Inhibiting the solvent translational mobility, and therefore the protein-water hydrogen bond dynamics, has an effect on the protein relaxation similar to dehydration.

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At low temperature proteins exist in a glassy state [1]. As the temperature is increased, the atomic motional amplitudes increase linearly, as in a harmonic solid. In hydrated proteins, at approximately 200 K, the amplitudes suddenly increase, signaling the onset of additional anharmonic and diffusive motion. This "dynamical" transition has been observed for atoms distributed throughout the protein over a wide range of length scales and time scales by Mössbauer spectroscopy [2], x-ray diffraction [3], and incoherent neutron scattering [4,5]. In analogy with supercooled liquids, the onset of additional motion at the dynamical transition has been associated with a structural relaxation, or $\alpha$ process [6]. The transition temperature and the amplitudes of the motion above the transition temperature are sensitive to the solvent environment of the protein. The amplitudes decrease as the protein is dehydrated [5,7], and the transition temperature increases with solvent viscosity [8]. These observations have been interpreted in terms of phenomenological models in which protein-solvent hydrogen bond dynamics play a central role [7].

Molecular dynamics (MD) simulations have revealed a correlation between the dynamical transition in a protein and a glasslike transition in the surrounding solvent [9], and have shown that room temperature atomic displacements in a protein could be significantly reduced by cooling the solvent or fixing the solvent coordinates [10]. In another simulation study, based on the observation that, at the dynamical transition temperature the average protein-water hydrogen bond lifetime decreases, and the number of different water molecules participating in protein-water hydrogen bonds increases, it was suggested that the transition is coupled to restructuring of the protein-water hydrogen bond network [11]. In this Letter we expand upon these previous simulation studies by presenting a detailed analysis of protein-water hydrogen bonds that distinguishes between fast and slow hydrogen bond dynamics. We firmly establish that slow relaxation of the protein-water hydrogen bond network is responsible for most of the additional motion involved in the protein structural relaxation above the dynamical transition temperature. Furthermore, by restraining the water molecules, we demonstrate that water translational diffusion is the primary mechanism of the protein-water hydrogen bond network relaxation, and show that inhibiting solvent displacements has an effect on the protein that is similar to dehydration.

The results presented here were obtained from constant pressure and temperature MD simulations of the globular protein Ribonuclease A (RNase) in a crystal at 100, 150, 200, 250, and 300 K, and a model for a powder at low hydration and 300 K. The simulations have been described in detail and validated by comparison with neutron scattering data elsewhere [12], so only a brief summary is given here. The crystal system consisted of one unit cell of the monoclinic crystal [13] containing two protein and 817 $D_2O$ molecules, corresponding to a hydration level, $h = 0.58$ g $D_2O$ per g protein. The powder contained eight protein molecules and 280 water molecules ($h = 0.05$). Three-dimensional periodic boundary conditions were applied, and the particle mesh Ewald sum was used to calculate the electrostatic interactions [14]. The CHARMM 22 force field was used for the protein [15] with the TIP3P model for water [16]. The Nosé-Hoover chain method [17] was used to control the temperature. The constant pressure simulations were carried out for $\approx$1 ns in a fully flexible cell using a multiple time step algorithm with a 4 fs time step [18].

The effects of temperature and hydration on the protein structural relaxation are illustrated by the incoherent intermediate scattering or single particle density correlation functions, $I(Q,t)$, shown in Fig. 1. The correlation functions were computed for the nonexchangeable protein hydrogen atoms, which are uniformly distributed throughout the protein, and powder averaged over several momentum transfer vectors with $|\mathbf{Q}| = 2$ Å$^{-1}$, which selects motions occurring on a length scale of approximately 3 Å. The time Fourier transform of $I(Q,t)$ is the incoherent dynamical structure factor, $S(Q,\omega)$, the quantity measured in incoherent neutron scattering experiments. The correlation functions reported here were averaged over multiple

  

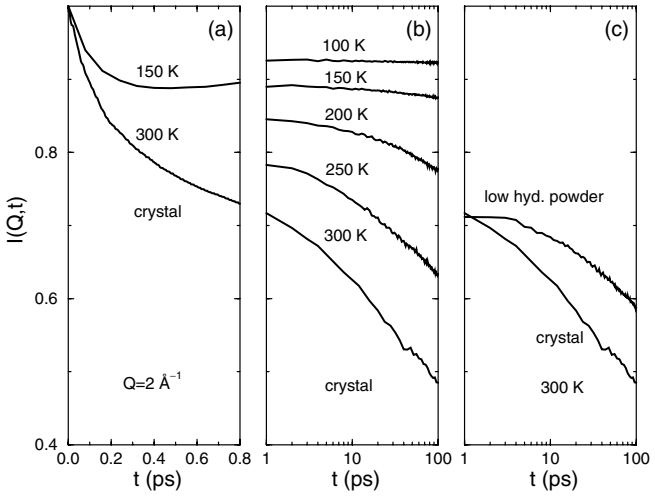


FIG. 1. Temperature dependence of the incoherent (self) intermediate scattering functions, $I(Q = 2\ \text{Å}^{-1}, t)$, for nonexchangeable hydrogen atoms computed from MD simulations of the RNase crystal: (a) 1 ps time scale; (b) 100 ps time scale. (c) Comparison of the intermediate scattering functions, $I(Q = 2\ \text{Å}^{-1}, t)$, in the crystal ($h = 0.58$) and a dehydrated powder ($h = 0.05$) at 300 K.

time origins out to 100 ps, which is roughly the time scale accessible to neutron time-of-flight and backscattering experiments. The $I(Q, t)$ display a fast, initial decay on the subpicosecond time scale (Fig. 1a) at both low (150 K) and high (300 K) temperatures in the well hydrated crystal. Below 200 K, following the subpicosecond initial decay, the $I(Q, t)$ are essentially flat, signaling the onset of structural arrest on the 100 ps time scale below the dynamical transition temperature (≈180 K). In contrast, above the dynamical transition temperature, the $I(Q, t)$ display a secondary decay, reflecting structural relaxation on the 100 ps time scale (Fig. 1b). The fast, initial decay is reminiscent of the $\beta$ process, and the secondary decay of the $\alpha$ process, in glasses. The secondary relaxation is diminished by about a factor of 2 in the dehydrated powder compared to the well hydrated crystal at 300 K (Fig. 1c). Comparing the results in Figs. 1b and 1c, it appears that the effect of dehydration on the decay of density correlations is roughly equivalent to a 50 K reduction in the temperature of the hydrated protein. This result is consistent with neutron scattering data [7], and underscores the crucial role of the solvent in protein structural relaxation.

The observation that protein structural relaxation is suppressed by dehydration suggests that water molecules participate in some sort of bond breaking process on the surface of the protein on a time scale that is shorter than that of the structural relaxation [7]. Specifically, we suppose that the surface of a dehydrated protein is rigidified by strong (electrostatic and hydrogen bonding) interactions between polar side chains. Protein-water hydrogen bonds break up these interactions, and water mobility is expected to facilitate the protein conformational fluctuations involved in the structural relaxation. To elucidate the role of water in the protein dynamical transition, we have analyzed the temperature dependence of protein-water hydrogen bond dynamics.

In order to distinguish between the fast (≈1 ps) formation and break up of hydrogen bonds due to water libration/rotation and the slower (tens of ps) relaxation of the protein-water hydrogen bond network due to diffusion of water molecules between sites on the protein surface and/or exchange with bulk water, we employ two measures of hydrogen bond lifetime, following a recent analysis of fast and slow hydrogen bond dynamics in supercooled water [19]. The fast hydrogen bond lifetime, $\tau_{\text{HB}}$, is simply defined as the average time that a given protein-water hydrogen bond remains intact. We use a geometric criterion for hydrogen bonding, according to which a hydrogen bond donor (D) and acceptor (A) are considered hydrogen bonded if the D-A distance is less than a cutoff value depending on the identity of the D-A pair, and the D-H-A angle is greater than 150°. The slow hydrogen bond network relaxation time is defined in terms of the decay of the bond correlation function, $c(t) = \langle h(0)h(t)\rangle/\langle h\rangle$ [20]. Here $h(t)$ is a hydrogen bond population operator, which is equal to one if a given D-A pair is hydrogen bonded at time $t$, and zero otherwise, and the angular brackets denote an average over all D-A pairs. The function $c(t)$ is the probability that a random D-A pair that is hydrogen bonded at time zero is still bonded at time $t$, regardless of whether or not the bond was broken at intermediate times. Thus, beyond an initial transient period, the decay of $c(t)$ is not determined by fast hydrogen bond breaking by water rotation/libration, but rather by rearrangement of the protein-water hydrogen bond network. The hydrogen bond network relaxation time, $\tau_{\text{R}}$, is defined as the time at which $c(t)$ decays to $1/e$, i.e., $c(\tau_{\text{R}}) = e^{-1}$ [19].

The $c(t)$ computed for protein-water hydrogen bonds at four temperatures spanning the protein dynamical transition are shown in Fig. 2a. All of the $c(t)$ display an initial subpicosecond transient decay. At longer times, the temperature dependence of the decay of the $c(t)$ is similar to that of the protein $I(Q, t)$ shown in Fig. 1b. Above the protein dynamical transition temperature (≈180 K), the $c(t)$ display a clear secondary decay, reflecting relaxation of the protein-water hydrogen bond network, on the 100 ps time scale. Comparing Figs. 1b and 2a, it is evident that the protein-water hydrogen bond correlation function decays somewhat faster than the protein single particle density correlation function at a given temperature. Below the transition temperature (e.g., at 150 K), following the transient decay, the $c(t)$ is essentially flat.

In order to determine $\tau_{\text{R}}$ below 300 K, the $c(t)$ were fit to stretched exponential functions and extrapolated. Because of the lack of decay of $c(t)$, a reliable fit could not be obtained at 150 K. The temperature dependence of the fast hydrogen bond lifetime, $\tau_{\text{HB}}$, and slow network relaxation time, $\tau_{\text{R}}$, of protein-water hydrogen bonds is shown in Fig. 2b. The lifetime $\tau_{\text{HB}}$ shows a smooth variation

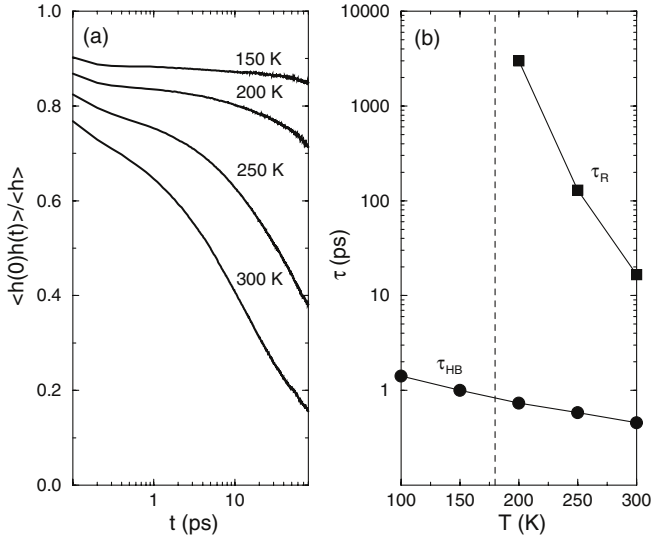


FIG. 2. Temperature dependence of the bond correlation function computed for protein-water hydrogen bonds (a), and the average protein-water hydrogen bond lifetime, $\tau_{HB}$, and network relaxation time, $\tau_R$ (b). The dynamical transition temperature (180 K) is indicated by the vertical broken line.

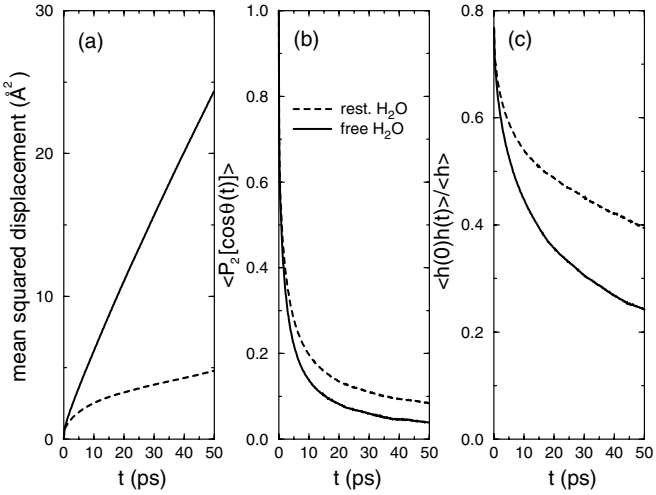


FIG. 3. Comparison of water dynamical properties at 300 K during simulations in which the water oxygen atoms were free (solid curves) and harmonically restrained (broken curves): (a) mean-squared displacements of water oxygen atoms; (b) OH bond orientational correlation functions, where $\theta(t)$ is the angle between an OH bond at time 0 and at time $t$, and $P_2(x)$ is the second Legendre polynomial of argument $x$; (c) protein-water hydrogen bond correlation function.

with temperature over a range of temperature that includes the protein dynamical transition. In contrast, as the temperature is decreased from 300 K, the relaxation time $\tau_R$ appears to diverge at the protein dynamical transition temperature. The coincidence of structural arrest in the protein and the protein-water hydrogen bond network at the same temperature suggests a role for the onset of restructuring of the protein-water hydrogen bond network in the protein dynamical transition.

To investigate the role of water translational diffusion in protein structural relaxation, we have performed a simulation of the RNase crystal at 300 K in which the positions of the water O atoms were restrained by a harmonic potential with a force constant of 0.6 kJ/(mol · Å$^2$). The effects of the restraints on the water dynamics are illustrated in Fig. 3. The water oxygen mean-squared displacements plotted in Fig. 3a show that, on average, in 50 ps water molecules diffuse ≈5 Å in the unrestrained ("free") simulation, and ≈2.2 Å (i.e., less than the diameter of a water molecule) in the restrained simulation. The OH bond orientational correlation functions plotted in Fig. 3b show that the restraints have only a small effect on the water rotational motion. Thus, the restraints had the desired effect of inhibiting water translational diffusion, while preserving nearly complete librational/rotational freedom.

In light of the small impact on water rotational motion, it is not surprising that the restraints hardly affect the protein-water hydrogen bond lifetime, $\tau_{HB}$, which is 0.45 ps in the free simulation and 0.47 ps in the restrained simulation. Moreover, as expected, by inhibiting water translational diffusion the restraints significantly slow the relaxation of the protein-water hydrogen bond network. This is illustrated by the bond correlation functions plotted in Fig. 3c, from which we obtain a network relaxation time, $\tau_R = 65$ ps in the restrained simulation, which is about 3.6 times longer than the 18 ps obtained from the free simulation. This result clearly establishes the role of water translational diffusion on the relaxation of the protein-water hydrogen bond network.

The effects of prohibiting water translational diffusion on protein structural relaxation at 300 K are manifested in Fig. 4. The intermediate scattering functions plotted in Fig. 4a show that the secondary ($\alpha$) relaxation of the protein in the crystal is significantly reduced when the water molecules are restrained. Indeed, after the initial ($\beta$) relaxation occurring in the first few picoseconds, the protein $I(Q, t)$ for the restrained crystal closely tracks that of the dehydrated powder. Thus, it appears that the effect on the protein density fluctuations of eliminating solvent translational diffusion on and away from the protein surface is analogous to removing the solvent.

To gain more insight into specific protein motions affected, we have calculated the mean-squared fluctuations (msfs) of protein heavy atoms on the 100 ps time scale. It is evident from Figs. 4b and 4c that inhibiting water translational motion reduces the protein atomic fluctuations throughout the protein, both in the backbone and side chains, and that the extent of the reduction is similar to that of the dehydrated system. Averaged over all the protein residues, the backbone msfs in the restrained crystal and dehydrated powder, 0.12 and 0.13 Å$^2$, respectively, are 20% to 25% lower than the 0.16 Å$^2$ in the unrestrained crystal, and the side chain msfs in the restrained crystal and dehydrated powder, 0.22 and 0.23 Å$^2$, are about 30% lower than the 0.32 Å$^2$ in the unrestrained crystal. Overall, the

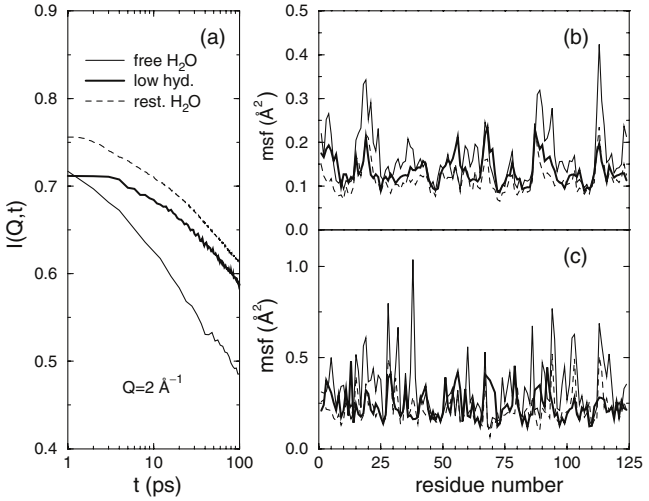


FIG. 4. Comparison of protein dynamical properties at 300 K during simulations of the hydrated crystal in which the water oxygen atoms were free (solid curves) and harmonically restrained (broken curves), and the dehydrated powder (heavy solid curves): (a) intermediate scattering functions, $I(Q = 2\ \text{Å}^{-1}, t)$, and mean-squared fluctuations (msf) averaged over 100 ps of protein backbone (b) and side chain (c) heavy atoms. The msfs were computed for several blocks of 100 ps and averaged over blocks and protein molecules.

reduction of the motion of the side chains is significantly greater than that of the backbone. The effects appear to be greatest in the more mobile regions of the protein structure (i.e., loops and solvent exposed side chains).

The results presented here firmly establish a correlation between solvent mobility on the surface of globular proteins and (possibly functionally relevant) anharmonic and diffusive motions involved in structural relaxation throughout the protein molecule. By separately characterizing both fast and slow protein-water hydrogen bond breaking processes, we have gained new insight into the role of solvent dynamics in protein structural relaxation at the microscopic level. We have shown that complete structural relaxation requires relaxation of the hydrogen bond network via solvent translational displacement, and that inhibiting solvent translational diffusion is dynamically analogous, on the 1 to 100 ps time scale, to dehydrating the protein.

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