Environmental Dependence of the Dynamics of Protein Hydration Water

Mounir Tarek[†] and Douglas J. Tobias*[,‡]

NIST Center for Neutron Research
National Institute of Standards and Technology
Gaithersburg, Maryland, 20899-8562
Department of Chemistry, University of California
Irvine, California, 92697-2025

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The function of some proteins appears to require the activation of anharmonic vibrational and diffusive motions on picosecond time scales.[1,2] These motions are supressed in dry proteins,[2,3] and this underscores the influence of hydration on fast structural relaxation processes in proteins. Indeed, water has been called "the lubricant of life" because of the role it plays in promoting extremely fast protein conformational fluctuations that may be important in protein folding and function.[4] The characterization of the dynamics of water molecules intimately associated with proteins ("protein hydration water") has accordingly been the subject of numerous recent experimental and theoretical investigations.[2,3,5−8]

We report a molecular dynamics (MD) simulation study that addresses the question: does the picosecond dynamics of hydration water depend on the protein environment? We compare water mobility in a crystal, dry and hydrated powders, and a protein/water "cluster" model commonly employed in MD simulations. Our principal findings are: (1) the overall water mobility on the time scale of tens of picoseconds is essentially identical in the crystal and hydrated powders; (2) water mobility is significantly higher in a cluster compared to the crystal and powder at a given hydration level. These results suggest that experiments performed on powder samples are appropriate for discussing water dynamics in native protein environments and that simulations of clusters do not give a quantitatively correct picture of water dynamics near protein surfaces.

Here we focus on incoherent neutron scattering experiments,[6,7] which probe the ps dynamics of protons, and related MD simulations. Powder samples are commonly employed in neutron experiments, but very little is known about the organization and interactions of the protein and water molecules in these powders. Neutron diffraction data on C-phycocyanin exhibits broad diffraction peaks characteristic of amorphous solids.[6] Spectroscopic measurements generally indicate that lyophilization results in an increase of β at the expense of α structure, which suggests aggregation by partially denatured proteins.[9] Therefore, it is conceivable that the water dynamics may be different in powder and native environments, and hence dynamical data taken on powder samples may not be completely relevant to native proteins. On the theoretical front, several simulations of protein hydration have been performed on "cluster" systems in which a single protein molecule is surrounded by a finite hydration shell.[3,8] In this case, the absence of a condensed environment might also lead to different water behavior compared to a native environment.

We simulated the protein ribonuclease A (RNase) at 300 K in hydrated cluster, crystal, and three different model powder environments: an "ordered" powder (OP) at low and high hydration, and a "random" powder (RP) at high hydration (Figure 1). The initial OP configuration was prepared from a $2a \times 2b \times c$ lattice (8 RNase molecules) of the monoclinic crystal[10] by removing all of the waters and heating to 500 K in a MD run to produce non-native disordered protein configurations. This was followed by a constant pressure (NPT) MD run at 300 K during which the system contracted, enabling the protein molecules to interact with their neighbors and periodic images. The low ($h =$ 0.05 g of D_2O per g of protein) and high ($h = 0.42$) hydration systems were hydrated by adding water molecules, respectively, to early and late configurations from the NPT run. The two hydration levels were chosen to correspond with neutron scattering experiments.[6,7] The RP system was prepared by the same procedure starting from eight RNase molecules randomly rotated and then repacked before heating.

The *PINY_MD*[11] program was used to carry out MD simulations at constant temperature and zero pressure (except the cluster, which was simulated in a large box at constant volume) with reversible, multiple time step algorithms,[12] and a time step of 4 fs. The CHARMM 22 protein force field[13] and TIP3P water model[14] were employed, and the particle mesh Ewald method[15] was used for the electrostatics. The root-mean-squared deviations (RMSDs) of the α carbon positions from the crystal structure were 1.4 and 1.3 Å for the two RNase molecules in the unit cell during the last 300 ps of the 1.5 ns crystal simulation. The maximum deviation of less than 3% from the experimental unit cell parameters corroborates that the potential is reasonable for the systems under study. The RMSDs averaged over the 8 RNase molecules were 2.4 ± 0.2 in the OP and 2.8 ± 0.4 in the RP, indicating a substantially larger deviation from the crystal structure in the model powders at high hydration.

In Figure 2 we show incoherent intermediate scattering functions, $I(Q,t)$, computed from the trajectories (3 ns cluster, 1.5 ns crystal, 600 ps powders) of the water hydrogen atoms.

[†] National Institute of Standards and Technology.
[‡] University of California, Irvine.

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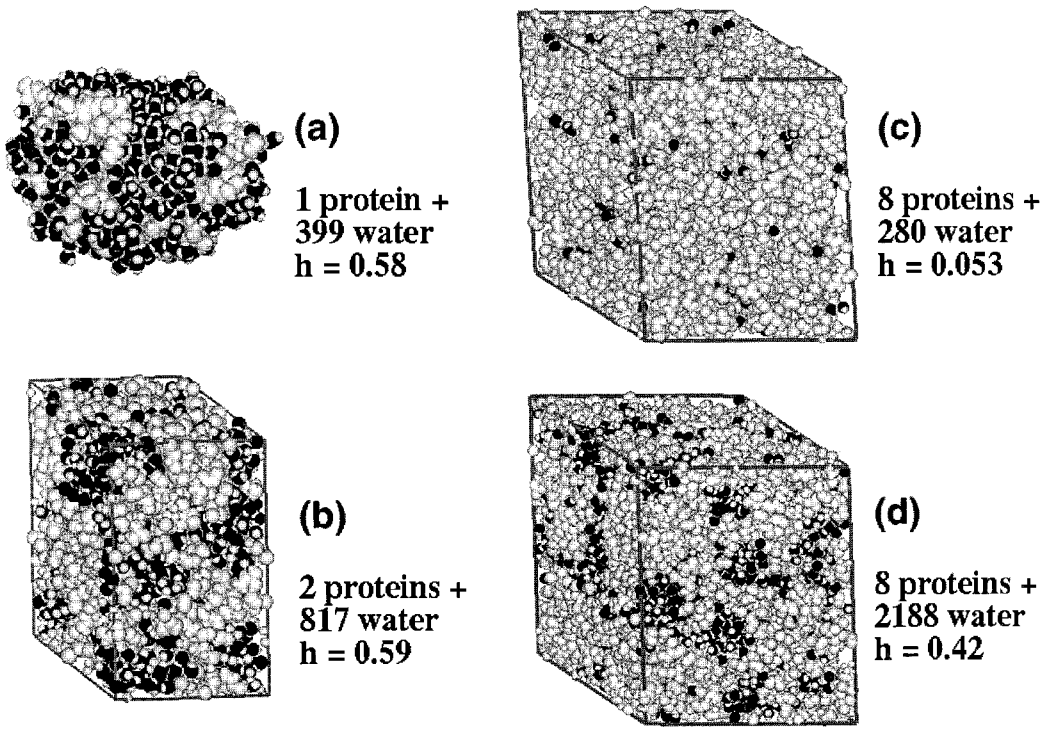

Figure 1. Snapshots from MD simulations of hydrated RNase. Water O atoms are black, water H white, and protein atoms light gray. (a) "Cluster." (b) One unit cell of the crystal containing two RNase molecules. (c) Dry "ordered" powder (OP) and (d) hydrated "random" powder (RP) models each containing eight RNase molecules, which are replicated by periodic boundary conditions.

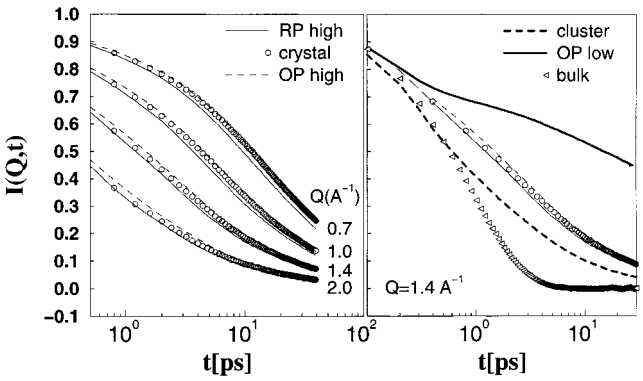

Figure 2. Incoherent intermediate scattering functions, $I(Q,t) = \langle \exp[\imath \mathbf{Q}(\mathbf{r}_j(t) - \mathbf{r}_j(0))] \rangle$, of the water hydrogens. At left, dependence of $I(Q,t)$ for the crystal and high hydration powders on the scattering vector, Q. At right, comparison of $I(Q = 1.4\ \text{Å}^{-1},t)$ for the RNase cluster, crystal, and powders, and bulk TIP3P water.

Although there is mounting evidence that single trajectories do not adequately sample protein fluctuations in the basin of the folded state,[16] we are confident that our proton $I(Q,t)$, averaged over many water molecules and time origins, provide reasonable estimates of true ensemble averages. $I(Q,t)$ probes the single particle density correlations of the hydrogens, and its time Fourier transform is proportional to the double differential incoherent neutron scattering cross section.[17] The $I(Q,t)$ of the water hydrogens are very similar for a range of Q values (length scales, $d = 2\pi/Q$) over the time scale of their complete decay for the crystal and the two distinct high hydration powders. Thus, on the time scale of tens of picoseconds, the overall water motion is insensitive to the details of the protein packing at high hydration. This suggests that the neutron experiments performed on powders with $h \approx 0.4$ are indeed relevant to describe the water dynamics near proteins in nativelike environments such as crystals. In contrast, the cluster result shows that the water moves much too freely on the protein surface and demonstrates that cluster simulations are not appropriate for quantitative characterization of the dynamics of protein hydration water. In the low hydration powder, where the water is highly confined, the water motion is strongly retarded. All of the $I(Q,t)$ for the protein hydration waters decay more slowly than the pure water $I(Q,t)$, in a stretched exponential fashion, in agreement with previous investigations.[7,8]

In conclusion, our results show that picosecond water dynamics in hydrated protein powders is similar to that in nativelike environments (e.g., crystals), and that some care is needed to properly model water dynamics in these environments by using MD simulations. These conclusions are significant for intensive ongoing experimental and theoretical investigations into the details of the dynamics of protein hydration water.

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