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Coincidence of Dynamical Transitions in a Soluble Protein and Its Hydration Water: Direct Measurements by Neutron Scattering and MD Simulations

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The coupling between protein dynamics and hydration-water dynamics is of fundamental importance for understanding protein function in a cellular context. A close correlation between solvent and protein dynamics has been proposed based on experimental and simulation evidence.[1−12] In this context, a functionally relevant protein dynamical transition, evidenced as a change in slope of atomic mean square displacements as a function of temperature at around 220 K,[13−15] has served as a paradigm to monitor the correlation of protein and solvent motions. The transition is absent in dehydrated proteins, and the transition temperature and amplitudes of protein motions above the transition temperature depend sensitively on solvent viscosity.[5,8,16−19] Here we show directly by incoherent neutron scattering experiments, which separately probed protein and solvent dynamics, that the dynamical transitions of a soluble protein (maltose binding protein, MBP) and its hydration water occur at the same temperature. Specifically, a deuterated MBP powder hydrated by H_2O, which primarily reflects the hydration water dynamics, revealed a broad transition at around 220 K, and a sample of hydrogenated MBP in D_2O, which reports the protein dynamics, exhibited a dynamical transition at the same temperature. Molecular dynamics simulations carried out on the same system reproduced that dynamical transitions in both the protein and the hydration water occur at the same temperature and identified a relaxation in the protein-water hydrogen-bonding network due to water translational diffusion as the origin of both transitions. This study, the first to apply a combination of neutron scattering, perdeuteration, and MD simulations to the same system, firmly establishes and explains the direct coupling between soluble protein and water dynamics suggested by many previous experimental and theoretical investigations.

Elastic incoherent neutron scattering (EINS) is a particularly well-suited tool to explore separately protein and solvent dynamics on the ns−ps time scale. EINS predominantly probes atomic motions of hydrogen atoms, which have an incoherent scattering cross section at least 40 times greater than other atoms (including deuterium) in biological samples.[20] Consequently, water and protein motions can be probed separately by measuring perdeuterated-protein samples hydrated in H_2O and natural-abundance proteins in D_2O, respectively. MBP, a soluble, monomeric protein[21] of 41 kDa, was fully deuterated at the ILL-EMBL-Deuteration-Laboratory in Grenoble, France. About 200 mg of deuterated and natural-abundance powders of MBP were hydrated in H_2O and D_2O, respectively, at a level corresponding to about one hydration layer per MBP molecule (see Supporting Information). The incoherent scattering cross sections of the D−MBP−H_2O and the H−MBP−D_2O samples correspond to 73 and 98% from hydration-water and protein moieties, respectively, if H/D exchange of labile protons and deuterons is taken into consideration (see Supporting Information). The elastically scattered neutron intensities of both samples were measured in the temperature range from 20 to 300 K on the backscattering spectrometer IN16 at the ILL. The instrumental energy resolution of 0.9 μeV (FWHM) implies that only motions in the ps−ns time scale are probed. Figure 1 shows atomic mean square displacements (MSD) that were extracted from the Q-dependence of the elastic intensity according to the Gaussian approximation (see the Supporting Information). The MSD of both samples are very similar up to ∼220 K, above which their rates of increase change, signaling dynamical transitions.

MBP exhibits a dynamical transition in the same temperature range (180−220 K) as other soluble proteins.[13,14] The use of perdeuterated MBP hydrated in H_2O has enabled us to simultaneously probe hydration-water dynamics on the same type of sample. These data show that a soluble protein (MBP) and its hydration water undergo a dynamical transition at the same temperature, supporting the hypothesis of an intimate coupling between protein and solvent dynamics. The microscopic details of this coupling were explored by MD simulations.

The MD simulations were carried out at constant temperature and pressure on a model powder consisting of four MBP molecules and 3460 water molecules, corresponding closely to the hydration level used in the experiments, over a range of temperature spanning the dynamical transitions. The powder model was constructed based on the crystal structure (PDB entry 1JW4[22]) as described elsewhere.[23,24] Details of the simulation setup, protocols, and analysis are given in the Supporting Information.

The temperature dependence of mean square displacements computed from the simulations for protein nonexchangeable H atoms (i.e., those probed by incoherent neutron scattering), plotted in Figure 2a, exhibits a dynamical transition at 240 K, qualitatively consistent with the neutron data. Also plotted in Figure 2a is the temperature dependence of the protein-water hydrogen-bond relaxation rates, $1/\tau_R$, where τ_R is the average relaxation time determined from a hydrogen-bond correlation function[25] (see Supporting Information for details). The H-bond relaxation rate increases rapidly with temperature at the dynamical transition of the protein, consistent with the previous suggestion that the protein transition is associated with relaxation of the network of protein-water H-bonds.[25] The time evolution of the MSD of the O atoms

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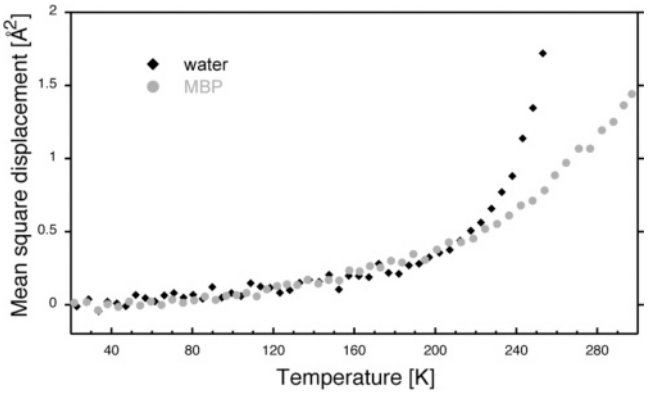

Figure 1. Mean square displacements of ns − ps motions in maltose binding protein (H−MBP−D_2O sample; gray circles) and in its hydration water (D−MBP−H_2O sample; black diamonds). Dynamical transitions (changes in slope of temperature-dependent mean square displacements) in the protein and in its hydration water take place at similar temperatures (∼220 K).

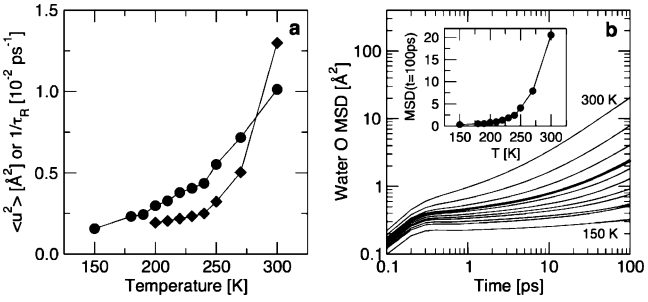

Figure 2. (a) Temperature dependence of mean-square displacements of nonexchangeable H atoms in MBP computed as 1 ns averages over the MD simulation trajectories (●), and protein-water H-bond network relaxation rates (♦). (b) Time evolution of mean-square displacements of water O atoms over a range of temperature from 150 to 300 K (bottom to top: 150, 180, 190, 200, 210, 220, 230, 240, 250, 270, 300 K; the curve at 240 K, the temperature of the dynamical transition in the simulations, is indicated by a heavy line). (Inset) Temperature dependence of the water MSDs at 100 ps.

of the water molecules is plotted in Figure 2b for each temperature. Well below the protein dynamical transition T (∼240 K) in the MD simulations, following the initial, subpicosecond rise, corresponding to ballistic motion, the MSD are essentially flat, indicating that the water molecules are in a structurally arrested, glass-like state. At higher T, the MSD begin to curve upward after a few ps, indicating the onset of translational diffusion, with a diffusion rate that increases with temperature. The extent of translational diffusion on a given time scale (e.g., $t = 100$ ps) begins to increase rapidly with temperature above the dynamical transition at ∼240 K (see inset to Figure 2b). Thus, the simulations show that dynamical transitions in the protein and water motional amplitudes, as well as the protein-water H-bond relaxation rate, occur at roughly the same T. We therefore conclude that the protein dynamical transition is correlated with relaxation of the protein H-bond network, which, in turn, is associated with the onset of water translational diffusion.

The temperature dependencies of experimental and simulated dynamics of MBP and its hydration water, probed individually on the same sample, affirm the existence of a dynamical coupling between protein and solvent motions on the ns−ps time scale. Below the protein dynamical transition, water translational motions on the protein surface are suppressed, and the protein is structurally arrested in a glassy solvent cage, as shown by MD simulations. At around 220 K, the protein dynamical transition and the onset of hydration-water translational diffusion occur concomitantly, and the lifetime of the protein-water hydrogen-bond network drastically decreases. Consequently, the hydration-water MSD become much larger than protein MSD above the dynamical transition. In the terminology of Frauenfelder and co-workers,[1] fast motions on the ns−ps time scale in soluble proteins are thus hydration-shell coupled. In contrast, a similar study on a biological membrane recently revealed a difference of ∼50 K in the dynamical transition temperatures of membrane (protein and lipid) and hydration-water motions.[26,27] Membrane proteins appear to be controlled to a lesser extent by the dynamics of hydration-water than are soluble proteins. Future experiments and simulations on membrane components separately and on other soluble proteins are required to complete the emerging picture of the functionally relevant dynamical coupling of protein molecules to their environment in a realistic biological context.

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Supporting Information Available: Details on deuteration and sample-preparation protocols, neutron scattering experiments, and MD simulations. This material is available free of charge via the Internet at http://pubs.acs.org.

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