

Hydration Water Dynamics and Instigation of Protein Structural Relaxation

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Until a critical hydration level is reached, proteins do not function¹. This critical level of hydration is analogous to a similar lack of protein function observed for temperatures below a dynamical temperature range of 180-220K², that also is connected to the dynamics of protein surface water^{1,2}. Restoration of some enzymatic activity is observed in partially hydrated protein powders, sometimes corresponding to less than a single hydration layer on the protein surface, which indicates that the dynamical and structural properties of the surface water is intimately connected to protein stability and function³⁻¹⁵. Many elegant studies using both experiment and simulation have contributed important information about protein hydration structure and timescales^{3,4,6-14,16-19}.

The molecular mechanism of the solvent motion that is required to instigate the protein structural relaxation above a critical hydration level or transition temperature has yet to be determined. In this work we use experimental quasi-elastic neutron scattering (QENS) and molecular dynamics simulation to investigate hydration water dynamics near a greatly simplified protein system. We consider the hydration water dynamics near the completely deuterated N-acetyl-leucine-methylamide (NALMA) solute, a hydrophobic amino acid side chain attached to a polar blocked polypeptide backbone, as a function of concentration between 0.5M-2.0M under ambient conditions. We note that roughly 50-60% of a folded protein's surface is equally distributed between hydrophobic and hydrophilic domains, domains whose lengths are on the order of a few water diameters²⁰, that justify our study of hydration dynamics of this simple model protein system.

The QENS experiment was performed at the NIST Center for Neutron Research, using the disk chopper time of flight spectrometer (DCS). In order to separate the translational and rotational components in the spectra, two sets of experiments were carried out using different incident neutron wavelengths of 7.5Å and 5.5Å to give two different time resolutions. All the spectra have been measure at room temperature. The spectra were corrected for the sample holder contribution and normalized using the vanadium standard. The resulting data were analyzed with DAVE programs (<http://www.ncnr.nist.gov/dave/>). The AMBER force field²¹ and SPCE water model²² were used for modelling the NALMA solute and water, respectively. For the analysis of the water dynamics in the NALMA aqueous solutions, we performed simulations of a dispersed solute configuration consistent with our previous structural analysis²³, where we had primarily focused on the structural organization of these peptide solutions and their connection to protein folding^{23,24}. Further details of the QENS experiment and molecular dynamics simulations are reported elsewhere²⁵.

The QENS data arising from translational water dynamics of these biological solutions are analyzed in a first approximation with a jump diffusion model. At the highest solute concentrations,

corresponding to a (shared) single layer of water, the hydration dynamics is significantly suppressed and characterized by a long residential time and a slow diffusion coefficient, similar to supercooled water at -10°C, and a rotational relaxation time of about 2.2ps. The analysis of the more dilute concentration solutions, corresponding to approximately 2-3 hydration layers of water per solute, has been performed taking in account the results of the 2.0M solution as a model of the first hydration shell. Subtracting the first hydration layer based on the 2.0M spectra, the translational diffusion dynamics is still suppressed, although the rotational relaxation time and residential time are converged to bulk-water values.

The experimental “elastic incoherent structure factor” (EISF) can be interpreted as a measure of the fraction of “immobile” or localized hydrogen rotational dynamics that are faster or slower than our experimental resolution of 1-5.5ps^{26,27}. The EISF shows significant evolution between 0.5M-2.0M; the EISF for the 0.5M solution measures 37% of immobile hydrogens, whereas only 17% of the protons are not observed for the 2.0M concentrations. Figure 1 presents the hydration water EISF variation as a function of NALMA concentration.

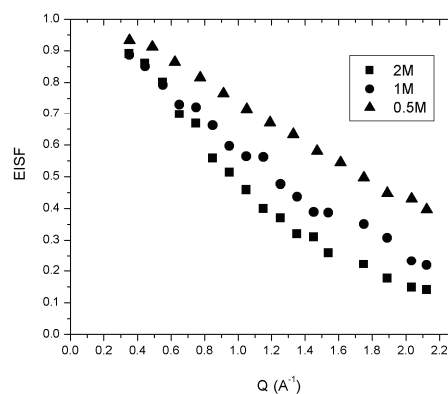


Figure 1 EISF of hydration water plotted versus Q^2 , for 0.5M, 1M and 2.0M NALMA concentration.

This seemingly puzzling result was analyzed by molecular dynamics simulation, in which the residence times of water molecules near the NALMA solute were monitored at the hydrophobic side chain and the polar backbone separately. To analyze the EISF results, we evaluated the rotational dynamics of water molecules that maintained a distance of 4.0Å or less from the branching carbon center of the hydrophobic side chain, and within 4.0Å of one of the backbone carboxyl oxygens of the NALMA molecule, as well as an average residence time of that subset of water molecules.

Figure 2 presents the orientation autocorrelation function, $P_2(t)$, of water molecules with these residence times. The 0.5M $P_2(t)$ data are well fit with two exponentials, and show populations with very slow rotational timescales (\sim 6-8 ps) and fast rotational timescales (\sim 1ps). The total 2.0M $P_2(t)$ data is best fit with one exponential, which arises from a slow rotational timescales (\sim 4-5ps) near the hydrophilic site, and faster rotational timescales (\sim 2 ps) near the hydrophobic site. A stretched exponential model also provided a good fit to the autocorrelation function of the 2M data, with a β -exponent value between 0.4-0.6. This complementary analysis confirms that the 2M NALMA concentration shows a distribution of rotational time scales.

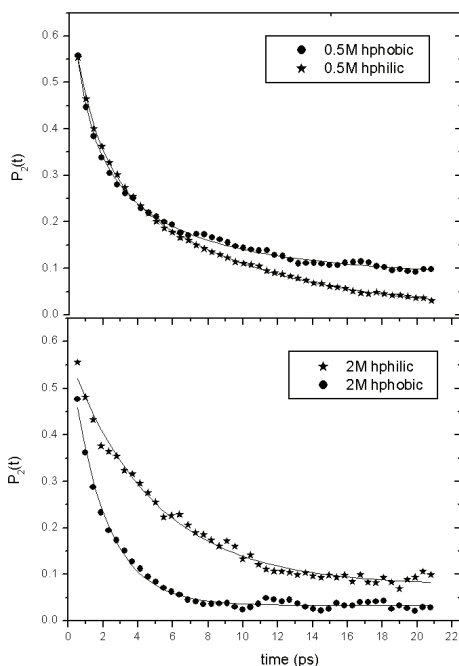


Figure 2 The rotational dynamics were analyzed using the orientational autocorrelation function: $P_2(t) = \langle 0.5[3\cos^2\theta(t) - 1] \rangle$ where $\theta(t)$ measures the angle between the dipole vector of the water molecule at times t and 0. To analyze the EISF results, we evaluated the rotational dynamics of water molecules that maintained a distance of 4.0Å or less from the branching carbon center of the hydrophobic side chain, and within 4.0Å of one of the backbone carboxyl oxygens of the NALMA molecule, as well as an average residence time of that subset of water molecules.

The MD simulations provide an interpretation of the EISF results, in which the 0.5M solute concentration shows the presence of long rotational relaxation times near both the hydrophilic and hydrophobic side chains, while the 2.0M solute concentration shows more water protons near the hydrophobic side chain whose dynamics become faster, and therefore resolvable by the QENS experiment.

The MD simulations also measured first layer water residence times near hydrophobic and hydrophilic sites. We find that at 0.5M the water residence times are largely equal near both sites: \sim 8.5-9.0ps; however at 2.0M we find that the water residence times are very different between the two sites: \sim 3.5-4.0ps near the hydrophobic side chain, whereas it is \sim 10.0-10.5ps near the hydrophilic site. Qualitatively we attribute the higher percentage of localized hydrogens seen at 0.5M as arising from the better ability to form more idealized hydrogen-bonded networks around the hydrophobic side chain. By contrast the reduced levels of immobile hydrogens at higher NALMA concentrations results in a more strained-non-optimal network that breaks more easily to permit faster motions.

We focus our QENS and MD results of hydration dynamics near a model protein surface on the issue of how enzymatic activity is restored once a critical hydration level is reached, and provide a hypothesis for the molecular mechanism of the solvent motion that is required to trigger protein structural relaxation when above the hydration transition. Below the critical hydration level, water dynamics near hydrophobic sites is much faster and incommensurate with that near the hydrophilic sites; it is *too fast* to effectively solvate the hydrophobic side chains, and the hydrogen-bonded water network across the protein surface is dynamically unstable. At a sufficient level of hydration, the hydration dynamics become spatially homogeneous, with restoration of a water network that can support hydrophobic hydration over the surface with sufficient time scales that are *slow* enough to couple to protein conformational transitions to realize the structural plasticity necessary for protein function.

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