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Temperature dependence of the internal dynamics of a protein in an aqueous solvent: Decoupling from the solvent viscosity

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ABSTRACT

We have recently observed decoupling of the dynamics of a protein from its aqueous solvent [Chu et al., JPCL 3 (2012) 380]; here we report the more detailed studies. We analyzed quasielastic neutron scattering data from a 40 mg/ml solution of lysozyme in $(D_2O)_8$(LiCl) and $(H_2O)_8$(LiCl). The internal dynamics of lysozyme exhibited super-Arrhenius temperature dependence with no crossover to a different regime down to at least 200 K. The decoupling of the internal protein dynamics from the viscosity of its aqueous solvent is evident. The temperature dependence of the protein dynamics indicates an apparent dynamic arrest at a temperature above 190 K, whereas the glass transition temperature for the solvent is around 135–140 K. The internal dynamics of the solvated protein is coupled to the dynamics of its hydration shell, not of the bulk solvent, which is qualitatively altered by the salt to defer the dynamic arrest to 135–140 K.

1. Introduction

It is well accepted that protein motions are largely governed by their "slow" conformational dynamics. These "slow" motions on the μs–ms (and longer) time scale are ultimately linked to the "fast" dynamics on the ps (and shorter) to ns time scale [1,2]. Neutron scattering is a powerful tool for studying "fast" dynamic processes in proteins, for several reasons. Firstly, the wavelength and energy of the thermal and cold neutrons closely match the interatomic/intermolecular distances and ps–ns time scale of the "fast" protein dynamics. To this close match is related the fact that the dependence of the neutron scattering signal on the momentum transfer, *Q*, uniquely provides information on the geometry of the "fast" motions. Secondly, the huge difference in the scattering cross-section between hydrogen and deuterium isotopes could potentially allow separate measurements of the dynamics of a protein and the protein's solvent. The major influence of the solvent on the solvated or hydrated protein dynamics is universally recognized, but the extent to which the latter is linked (or possibly even enslaved) to the former is hotly debated [3–10]. The term "solvent" is commonly used to describe any aqueous protein environment, whether in dilute or crowded aqueous solution, or in hydrated powders. However, the temperature dependence of the solute/solvent dynamics is customarily studied in the hydrated powders. While not representative of the environments that proteins encounter in biological systems, protein powders (typically hydrated from water vapors) host water that does not freeze and undergoes the glass transition instead, thereby enabling low-temperature measurements. The experiments on the proteins hydrated beyond the level attainable for hydrated protein powders (that is, proteins in bulk-like solvents) traditionally utilize cryo-protective solutions as solvents, e.g., of water with methanol, glycerol, glucose, ethylene glycol, dimethyl sulfoxide, etc. [11–19]. Unfortunately, such cryo-protective solutions are significantly different from pure water in terms of their properties, such as glass transition temperature, T_g. As an alternative, we have proposed [20,21] to use aqueous solutions of lithium chloride as the protein solvent for low-temperature studies. Such solutions are remarkably similar to pure water in their T_g [22], dynamic crossover temperature, T_c [23–25], and the vibrational density of states [26], even at very high salt concentrations. Using a backscattering neutron spectrometer with both high energy resolution and a wide accessible dynamic range, such as BASIS [27] at the Spallation Neutron Source (SNS) of the Oak Ridge National Laboratory (ORNL), it is possible to probe separately and simultaneously the relaxation dynamics of the aqueous solvent and protein solute down to ca. 200 K. The first quasielastic neutron scattering (QENS) study with a lithium chloride aqueous solution as a lysozyme solvent [20] yielded some unexpected result: while the temperature dependence of the relaxation times for the solvent exhibited a deviation from the super-Arrhenius behavior below ca. 220 K, the temperature dependence of the lysozyme relaxation times showed no such deviations. We explained this apparent decoupling on the basis of the fact that

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the dynamic crossover in aqueous systems at T_c of ca. 220 K does not affect the main structural, or α-relaxation [25,28]. Instead, a secondary relaxation process becomes apparent in aqueous systems below T_c. Therefore, the unchanged dynamics of the solvated protein could result from coupling to the α-relaxation of its aqueous solvent, which remains unchanged below T_c.

It should be noted that QENS studies of proteins in bulk-like solvents traditionally report not the temperature dependence of the protein relaxation times, but rather the mean-squared atomic displacements in the protein derived from the elastic intensities. These mean-squared displacements have been linked to the solvent viscosity [16,18]. Thus, notwithstanding the proposed explanation, the observation of the apparent decoupling in the temperature dependence of the relaxation times between the protein and its solvent below 220 K is unusual and may prompt questions about the data analysis procedure. Some of the simplifications of the data analysis [20] are obvious, such as using two Lorentzian components for the data fits, although this approach has been justified by a good match between the protein concentration in the solutions and the relative spectral weight of the narrower Lorentzian [20]. The use of the Q-averaged data, which relies on the assumed localization of the measurable dynamic processes in the system, can also be questioned. Likewise, it was pointed out [21] that the global protein dynamics was neglected in the data analysis, which probably could be justified only at the lower temperatures of the experiment. In this work, we attempted to address all these possible questions regarding the data analysis. What we have found is that the internal protein dynamics (relaxation times) indeed follow super-Arrhenius temperature dependence, which can be described by a Vogel–Fulcher–Tammann (VFT) fit, without any interruption near 220 K, in agreement with the early analysis [20]. Moreover, the temperature dependences of the solvent and solute relaxation times were decoupled in a much more profound way. For the internal dynamics of the protein, the critical temperature of the VFT fit, indicative of the dynamic arrest of the protein internal motions, was at least 50 K above the glass transition temperature of the solvent associated with the solvent's dynamic arrest. While the presence of the solvent (or at least the sufficient hydration level) is a prerequisite for the super-Arrhenius behavior of both the main relaxation in the solvent itself and the internal protein dynamics [21,29], the dynamics of the former and the latter are actually decoupled.

2. Experiment

Two aqueous solvents, of $(D_2O)_8(LiCl)$ and $(H_2O)_8(LiCl)$ composition ($m \approx 6.25$ mol/kg and $m \approx 6.94$ mol/kg, respectively), were prepared using deionized distilled water and anhydrous, ultra-dry lithium chloride powder (99.995% purity) available commercially from Alfa Aesar. We report the mol ratios in the units of the number of water molecules (either H_2O or D_2O) per LiCl. Thus, $(H_2O)_8(LiCl)$ composition matches $(D_2O)_8(LiCl)$ composition. For these compositions the concentrations are as follows: $M \approx 6.94$ mol/l, $m \approx 6.94$ mol/kg for $(H_2O)_8(LiCl)$ and $M \approx 6.9$ mol/l, $m \approx 6.25$ mol/kg for $(D_2O)_8(LiCl)$ at ambient temperature.

The 40 mg/ml protein solutions were then prepared by dissolving lyophilized Hen egg white lysozyme (Sigma Aldrich L4919; 98% purity) in the $(H_2O)_8(LiCl)$ and $(D_2O)_8(LiCl)$ solvents. For the latter solution, the labile hydrogen atoms in the lysozyme were at first exchanged for deuterium atoms by dissolving the protein in D_2O followed by the lyophylization, which was repeated 3 times before the final dissolution in $(D_2O)_8(LiCl)$. Circular dichroism spectra were recorded using a Jasco 810 CD spectropolarimeter from 190 to 240 nm at 298 K. The CD spectrum of the lysozyme remains qualitatively unchanged in the presence of high concentration of LiCl in H_2O and D_2O solutions, as indicated in Fig. 1, thus giving good confidence that the protein is in a folded state. The CD spectra were analyzed using the CDSSTR program [30] available through DICHROWEB [31] to determine the secondary structure of the protein in different solvents. The relative amounts of α-helix and β-sheet in the protein were slightly different in the absence and presence of LiCl, which may be due to the electrostatic interaction of the protein and the salt (see Table 1).

QENS measurements were performed on a backscattering spectrometer BASIS [27] operated as in the previous experiment [20], with an energy resolution of 3.4 μ eV (full-width at half-maximum for the Q-averaged resolution value) and a useful dynamic range of accessible energy transfer of ±100 μ eV. In departure from the earlier experiment setup [20], different aluminum sample holders were used: 0.1 mm thick annular cylinders with the outer diameter of 29 mm. The data collected between 240 K and 200 K with a fine 5 K step and the resolution data set collected at 5 K were divided into separate Q-bins, from 0.3 $Å^{-1}$ to 1.7 $Å^{-1}$.

3. Results and discussion

Let us start with evaluation of various contributions to the scattering signal. We will use the numbers from the previously published MD simulations studies of hydrated lysozyme powders [32]. For a single lysozyme molecule with labile hydrogen atoms exchanged for deuterium, with approximately 710 H, 257 D, 150 N, 300 O, and 544 C atoms, the total neutron scattering cross-section is about 66,200 barn. Because of the Q-range of the experiment, perhaps the more relevant is the incoherent neutron scattering cross-section, which is about 57,600 barn per a lysozyme molecule. Assuming approximately 340 D_2O molecules in the immediate hydration level (a rather generous allowance based on the MD result for the fully hydrated lysozyme powder at $h = 0.42$ [32]), we have 6600 barn and 1360 barn for the total and incoherent neutron scattering cross-section, respectively, for the D_2O molecules in the first hydration layer per hydrated lysozyme molecule. We note that that scattering strength ratio of the hydration D_2O molecules (1360 barn incoherent) and the hydrated lysozyme molecules (57,600 barn incoherent) is low; this is what helps successful studies of hydrogenated protein powders hydrated with D_2O that use only one fit component (for the protein, but not D_2O dynamics).

At a solution concentration of 40 mg/ml, per milliliter of the solution there is ca. 2.73×10^{-6} mol of lysozyme, 0.05 mol of

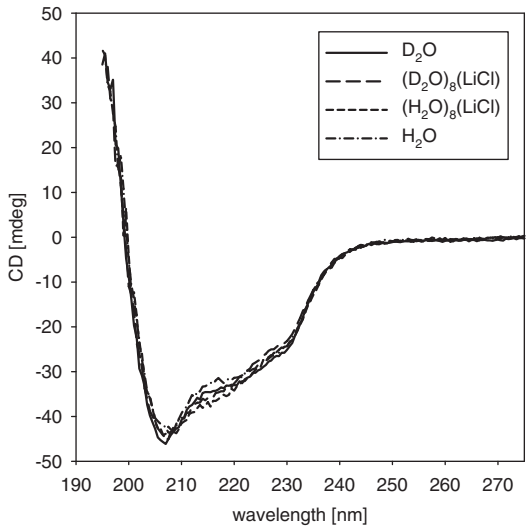

Fig. 1. Circular dichroism analysis of lysozyme samples in different solvents.