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Dynamic Transition in tRNA is Solvent Induced

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All hydrated proteins undergo a dynamic transition in the temperature range of $T_D \sim 200-230$ K that is usually observed as a sharp increase in the mean-squared displacement $\langle x^2 \rangle$ of the atoms.[1−4] This dynamic transition correlates with the appearance of measurable biochemical activity in proteins.[3,5] There is much experimental and computational evidence that solvent dynamics control the dynamic transition in proteins.[4,6,7] However, some authors ascribe this transition to intrinsic properties of proteins, including methyl groups of side chains.[8]

To assess if a universal mechanism drives the dynamical transition in biomolecules, we measured the dynamics of tRNA using neutron scattering. Despite differences in the architecture and chemical backbones of proteins and RNA, hydrated tRNA undergoes the dynamic transition at nearly the same temperature as hydrated lysozyme. The similarity of the dynamic transition in RNA and proteins supports the idea that it is solvent induced. Our results also suggest that specific side chains are not the main contributor of the dynamic transition in biological macromolecules, although they may explain differences in the dynamics of tRNA and lysozyme at temperatures below T_D.

Wheat germ tRNA was extracted with phenol, dialyzed, freeze-dried in D_2O, and then freeze-dried in 10 mM deuterated Na-cacodylate and 10 mM $MgCl_2$. Hen egg white lysozyme was dialyzed and freeze-dried twice in D_2O. This preparation replaced virtually all exchangeable protons in both tRNA and lysozyme. Samples were hydrated at 98% relative humidity to 0.59 g of D_2O/g tRNA and 0.45 g of D_2O/g protein. Incoherent neutron scattering probes the dynamics of H atoms because their scattering cross-section is significantly larger than that of any other atoms. H atoms are distributed throughout tRNA and lysozyme, which provides information on their overall motions. Neutron scattering measurements were done on the High-Flux Back-Scattering instrument[9] (resolution ~ 1 μeV) at the National Institute of Standards and Technology Center for Neutron Research. Elastic scans were measured at a heating/cooling rate of 0.7 K/min. An energy window -17 to $+17$ μeV was used for quasi-elastic scattering (QES) measurements.

We estimated $\langle x^2 \rangle$ from analysis of elastic scattering intensity as a function of the scattering wave vector.[1,2] Figure 1 shows $\langle x^2 \rangle$ of tRNA and lysozyme in dry and hydrated states. In both hydrated molecules, $\langle x^2 \rangle$ increases sharply above $T \sim 200-220$ K. Our results agree with a decrease in the elastic neutron scattering intensity of RNA at 180 K observed earlier.[10] $\langle x^2 \rangle$ of dry tRNA and dry lysozyme differ significantly above $T \sim 100$ K; dry tRNA exhibits

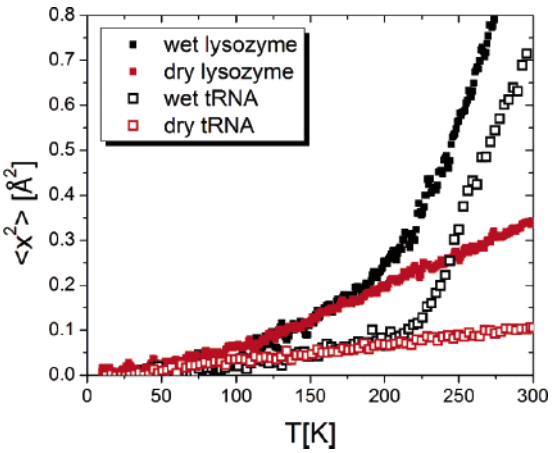

Figure 1. Mean-squared displacement as a function of temperature for lysozyme (solid symbols) and tRNA (open symbols) in hydrated (black) and dry (red) forms. Error bars are less than ± 0.02 Å^2.

linear increase in $\langle x^2 \rangle$ (harmonic behavior) over the entire temperature range, while dry lysozyme shows the onset of anharmonicity (stronger temperature dependence) at $T \sim 100$ K (Figure 1).

Significant broadening of the spectra (relative to the spectrometer resolution function) is observed at 300 K in both hydrated tRNA and hydrated lysozyme (Figure 2). This quasi-elastic contribution is due to a relaxation process in the 1−20 μeV energy range (~1 ns to 50 ps). However, wet tRNA at 200 K and dry tRNA at $T =$ 300 K do not show any broadening outside the resolution of the spectrometer, consistent with the harmonic behavior of $\langle x^2 \rangle$ (Figure 1). In contrast, a QES contribution is visible in the spectra of dry lysozyme at $T = 320$ K and of wet lysozyme just below the dynamic transition ($T = 200$ K; Figure 2b). This indicates that an additional relaxation is active in dry and hydrated lysozyme at temperatures below 200 K, consistent with anharmonic behavior of $\langle x^2 \rangle$ (Figure 1). This process is absent in the spectra of tRNA.

The striking similarity in T_D of hydrated tRNA and lysozyme (Figure 1) indicates that the dynamic transition is not an intrinsic property of the macromolecule. Extensive measurements on various hydrated proteins revealed that the dynamic transition is invariably around 200−230 K.[1−4] The T_D of hydrated DNA was estimated to occur in the same temperature range.[11] Despite differences in backbone chemistry and three-dimensional structure, the T_D for all these molecules including tRNA appears in the same temperature range. It has been noted[11] that the dynamic transition of hydrated biomolecules occurs at the dynamic crossover temperature of water, $T_C \sim 220-230$ K.[12] This transition from liquid-like to solid-like dynamics ("dynamic arrest"[13]) of water on a molecular time and length scales was argued to be the main cause of the dynamic transition observed in hydrated biomolecules.[11] The observation that

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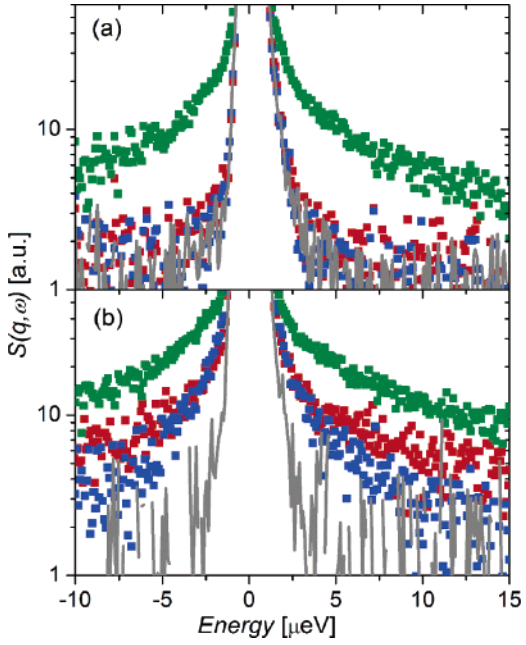

Figure 2. Quasi-elastic scattering spectra of wet tRNA at 300 K (green) and 200 K (blue), and dry tRNA at 300 K (red) (a). Similar data for wet lysozyme at 295 K (green) and 200 K (blue), and dry lysozyme at 320 K (red) (b). In both figures, solid gray lines indicate the vanadium spectra, which define the resolution of the spectrometer used. Error bars are less than ±3 au.

the T_D of lysozyme in glycerol (~270 K)[4] coincides with T_C of bulk glycerol (~280−300 K)[14] further supports the interpretation that the dynamical transition in biomolecules is solvent induced.

Using simulations, Smith and co-workers[7] showed that the protein does not undergo the dynamic transition up to 300 K when the temperature of the surrounding water is held constant. If the protein temperature is fixed at 300 K, then a dynamic transition occurs when the solvent temperature crosses 200 K. According to these simulations, at 200 K, the water hydrating the protein undergoes qualitative changes in translational motion. In another simulation,[6] the protein dynamics resembled that of the dehydrated state when the translational motion of the surrounding water molecules was artificially restricted. Thus, restricted translation of the solvent causes the hydrated protein to behave as if it is below the dynamic transition, even at $T = 300$ K. These results suggest that arrest of the translational diffusion of the solvent molecules blocks motion in the macromolecules.

On the basis of the similarity of the temperature dependence of $\langle x^2 \rangle$ and NMR-determined orientational order parameter for methyl-bearing side chains, it has been proposed[8] that freezing of rotational relaxation causes the dynamic transition in proteins. The paucity of methyl groups in tRNA suggests that their rotational motion cannot cause the transition at T_D. Instead, the dynamic transition is related to another relaxation process that enters the energy range of the spectrometer at temperatures above T_D and appears as a strong QES scattering in hydrated biomolecules (Figure 2). The microscopic nature of this process is known neither for proteins nor for DNA. Using MD simulations, it has been suggested[15] that motion of α-helices in myoglobin becomes active above T_D. If this is correct, this type of motion would require translational motion of water molecules. In any case, we can relate this relaxation to the "solvent slaved" processes according to the classification proposed in ref 16.

The most significant difference in the dynamics of tRNA and proteins appears at low T (Figure 1). The anharmonicity observed in lysozyme and other proteins[17−19] around 100 K is absent in tRNA. The QES contribution in dry lysozyme and in wet lysozyme below T_D (Figure 2b) indicates that an additional relaxation process becomes active in proteins at temperatures below T_D. It was proposed recently[19] that this low-temperature relaxation in proteins might be ascribed to methyl group dynamics. About 25% of nonexchangeable hydrogen atoms in proteins are located on methyl groups, and their dynamics should be a significant contribution to the neutron scattering spectra. This idea is consistent with our results showing that tRNA lacks this relaxation component (Figure 2a) and low-temperature anharmonicity (Figure 1). However, these differences may be also related to the different flexibility of polypeptide and polynucleotide backbones. Protein relaxation below T_D has been discussed in ref 20 and ascribed to solvent independent processes.

The present analysis demonstrates that the 3D structure of biological macromolecules and their chemical backbone do not significantly affect the temperature of the dynamic transition. This conclusion supports the idea[6] that unfreezing the translation of solvent molecules is the main cause of the dynamic transition in biomolecules. Our results also suggest that the rotation of methyl groups is not the main cause of the dynamic transition, although they may cause the low-temperature anharmonicity observed in proteins.[19] Understanding the microscopic nature of the motions that activate in the nanosecond−picosecond time range in biological macromolecules above T_D remains an important challenge as these motions are intrinsically linked to biochemical interactions.

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