Protein Dynamical Transition Does Not Require Protein Structure

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Terahertz time domain spectroscopy shows that the protein dynamical transition, the rapid increase in protein dynamics occurring at ~200 K, needs neither tertiary nor secondary structure. Further, short chain alanine studies find a dynamical transition down to penta-alanine, with no transition observed for di-alanine or tri-alanine. These results reveal the temperature dependence arises strictly from the side-chain interaction with the solvent. The lack of a transition for shorter chain peptides may indicate a qualitative change in this interaction occurs at a specific peptide chain length.

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Among the more controversial phenomena of biomolecular dynamics is the existence of a strong temperature dependence of molecular flexibility near 200 K for proteins and polynucleotides hydrated above 30% by weight. The change in flexibility is characterized by a rapid increase in the mean square atomic displacement $\langle x^2 \rangle$ at a temperature $T_D \sim 180$–220 K for a variety of proteins and poly nucleotides as measured by x-ray Debye-Waller factors [1,2] neutron quasielastic scattering [3–6] and Mossbauer methods [7–9]. This strong change in flexibility requiring a minimal hydration level has been considered of fundamental importance to protein dynamics.

A number of mechanisms have been proposed for the rapid change in flexibility [3,9–12]. Some investigators have associated the transition with the distribution of energy barriers in the energy landscape arising from the precise 3D structure. However, the narrow temperature range observed for a wide variety of biomolecules and the minimum hydration requirement suggests the rapid increase in flexibility arises from a change in the dynamics of the solvent in direct contact with the biomolecule, the biological water [13]. As molecular motion requires the surrounding solvent to accommodate conformational changes, any rigidity in the solvent will constrain the motion and any rapid change in the solvent dynamics will be reflected in the biomolecular dynamics.

Among the proposed solvent mechanisms is a fragile to strong dynamical transition of the hydration water [6,14]. This type of transition is indicated by an Arrhenius dependence below T_D and fragile liquid dependence above T_D such as a Vogel Tammann Fulcher dependence $\sim \exp(-DT_o/[T - T_o])$ [15]. This transition has not been observed in bulk water due to crystal formation. Using nanoconfinement to suppress crystallization, the transition has been observed for water in nanoporous silicon and carbon nanotubes [16]. The protein surface also suppresses crystal formation and investigators have reported the fragile to strong transition in protein biological water [6]. Denaturing the protein alters the surface structure and the exposure of hydrophobic groups may possibly affect the presence or nature of the fragile to strong transition.

Another model proposes that there is no fragile to strong transition in the biological water, but rather activated diffusive motion of the water at the protein surface is the source of the apparent temperature dependence in $\langle x^2 \rangle$ [17–19]. Here the relaxation rate has an uniform temperature dependence through the 200 K region. As the temperature increases, the rate of relaxational motions increases, and $\langle x^2 \rangle$ appears to rapidly increase with temperature as the time scale of the motions moves into the frequency window of the measurement technique. For this model, the activated motion depends only on the weak bonding between the solvent and the solute. This weak bonding and the size of the motions are such that this activated motion should continue to the single peptide level.

Here we show that the protein dynamical transition is indeed independent of any polypeptide structure using terahertz time domain spectroscopy (THz TDS). Measurements on hen egg white lysozyme (HEWL) and poly lysine as a function of denaturing reveal the dynamical transition is independent of either tertiary or secondary structure. We observe the transition for short chain poly-alanine down to penta-alanine demonstrating that a quantitative predictive theory for the temperature dependence lies in the understanding of the interaction of the charged side chains of the poly peptide or poly nucleotide with the biological water. Molecular-dynamics calculations of the temperature dependent $\langle x^2 \rangle$ support the experimental observations. We find that a rapid temperature increase in the calculated $\langle x^2 \rangle$ appears at ~200 K for both α-helix and random coil hydrated structures.

The dynamical transition in unstructured polypeptides has not been reported before, most likely due to the difficulty in performing such measurements. THz TDS is a table top measurement with small sample requirements (~2 mg) and ideal for examining picosecond protein dynamics on solution phase samples. The observation of the dynamical transition in the THz dielectric response for cytochrome c solutions was previously reported [20]. THz TDS measures the complex dielectric response, both the absorption coefficient α and the index n, with $n + i\alpha c/2\omega = \sqrt{\varepsilon}$ where c, ω and ε are the speed of light,

angular frequency and permittivity, respectively. Starting HEWL solutions were prepared by dissolving HEWL powder (Sigma Aldrich L6876) in Trizma buffer (pH 7.0, 0.1M) to a concentration of 200 mg/ml. The denatured solutions (D-HEWL) were prepared by adding guanidinium hydrochloride (GdmHCl) to the starting HEWL solution to a GdmHCl concentration of 6 M. The solutions were clear and without precipitates. GdmHCl is an excellent denaturant, and has been demonstrated at these high concentrations to inhibit aggregation and fibril formation [21]. The extent of unfolding was characterized by UV fluorescence, circular dichroism, and attenuated total reflection Fourier-transform infrared measurements [22], which show the tertiary and secondary structure entirely absent for D-HEWL. The appearance of the dynamical transition without the presence of secondary structure was verified with random coil poly-L-lysine solutions. Poly-L-lysine hydrobromide powder (Sigma Aldrich P7890) was dissolved in 30% methanol solution (by volume) to a concentration of 100 mg/ml. Poly-L-lysine was found to have a random coil structure for this methanol concentration [23]. THz TDS measurements were also made of di-alanine (A2), tri-alanine (A3), penta-alanine (A5), short chain alanine peptide (A7–A10), and poly-DL-alanine (PA). Poly-alanine solutions were prepared by dissolving poly-alanine powder (A2, A3, A5, A7–A10: Biomer Technology; poly-DL-alanine: Sigma Aldrich P9003) in Trizma buffer (pH 7.0, 0.1 M) to a concentration of 100 mg/ml. The temperature dependent THz TDS measurements of solutions follow the same procedure as discussed previously [20].

Molecular-dynamics simulations for two simple models—α-helix and random coil (with same amino acids sequence: ELLKKLLEELKG) were done to compare to the experimental results. The AMBER 9 molecular-dynamics package with PARM99 was used in the molecular-dynamics simulations. Molecular-dynamics simulations were performed for several temperatures in the 80–260 K range for the α-helix without water and with 456 water molecules ($\sim$5.65 g water/g), and for the random coil without water and with 395 water molecules ($\sim$ 4.89 g water/g). The peptides were solvated using a solvent box with periodic boundary conditions. For each temperature the solvated system is heated and equilibriated for 700 ps. The $\langle x^2 \rangle$ for the peptides for each temperature was determined from a subsequent production run of 100 ps. The squared displacement for each atom relative to the initial minimized structure was averaged over the 100 ps production run. These time averaged atomic displacements are averaged over the molecule to give $\langle x^2 \rangle$. For all dynamics simulations, the α-carbons were restrained to retain the backbone conformation and all solvent and side-chain atoms were unrestrained. This backbone restraint method used in docking and interaction simulations did not interfere with the appearance of the dynamical transition [24].

The frequency dependent absorption coefficient α and refractive index n for N-HEWL and D-HEWL solutions for several different temperatures are shown in Fig. 1. The dielectric response $\varepsilon(\omega)$ is related to resonant and relaxational processes through

$$\varepsilon(\omega) = \varepsilon_o + \int \frac{f(\omega')g(\omega')}{(\omega'^2 - \omega^2) + i\gamma(\omega')\omega} d\omega' + \varepsilon_r \int_0^\infty \frac{h(\tau)d\tau}{1 + i\omega\tau}, \quad (1)$$

where ε_o is the dc dielectric constant. The second term on the right-hand side is the under damped resonant response with density of states $g(\omega)$, oscillator strength $f(\omega)$ and damping coefficient $\gamma(\omega)$. The third term is the relaxational response, assuming Debye relaxation for a distribution of relaxation times $h(\tau)$. The broad response seen in Fig. 1 is typical of large biomolecules in this frequency range and likely arises from overdamping of structural vibrational modes and relaxational response of surface side chains and water. As seen in Fig. 1, the overall magnitude of α and n increases with denaturation. Figure 2(a) shows the absorption coefficient for the denaturant alone is small and thus cannot account for the increase. An increase in THz response with denaturing agrees with an increased flexibility of the denatured system with lower relaxational barriers and redshifted structural mode frequencies [25,26].

The temperature dependence of the α and n are shown in Fig. 2 for several frequencies. In Fig. 2(a), we see a rapid increase in the absorption coefficient for both of N-HEWL ($T_D \sim 200$ K) and D-HEWL ($T_D \sim 210$ K) with the tran-

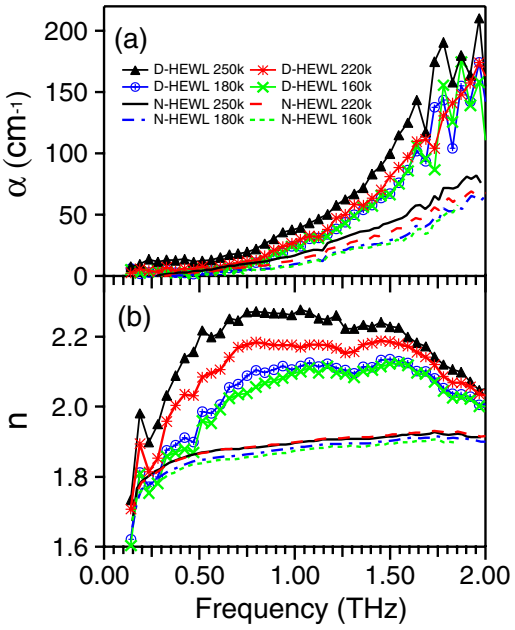

FIG. 1 (color online). The frequency dependent absorption coefficient α (a) and index of refraction n (b) for N-HEWL and D-HEWL solutions at several different temperatures.

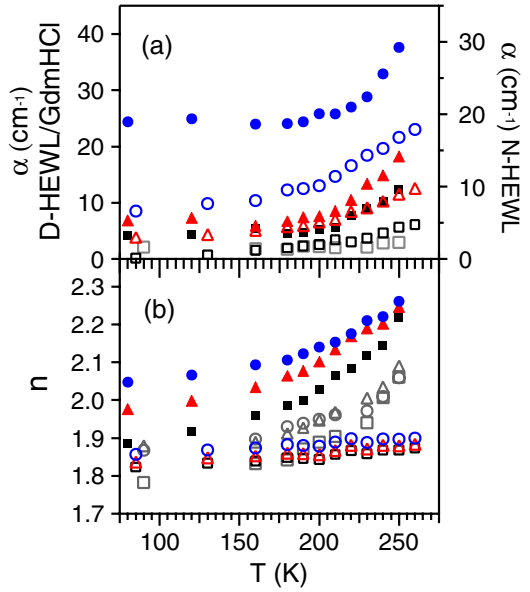

FIG. 2 (color online). The temperature dependent THz absorption coefficient α (a) and index of refraction n (b) for N-HEWL (unfilled symbols) and D-HEWL (filled symbols) and neat GdmHCl solutions (gray symbols). Symbol notation: ■: 0.51 THz; ▲: 0.70 THz; ●: 0.98 THz.

sition temperatures nearly constant over the full frequency range demonstrating that structure is not necessary for the transition. The α for pure buffer and 6 M GdmHCl show no temperature dependence. We expect this temperature dependence should also be seen in the index, however in Fig. 2(b) the THz index is nearly temperature independent for N-HEWL, while it increases with temperature for D-HEWL. The same temperature dependencies are found for the index of pure buffer and 6 M GdmHCl solvents as seen in Fig. 2(b) and Ref [20] indicating the index is dominated by bulk solvent here. Recent studies of minimally hydrated powders (0.3 gm H_20/gm protein) with no bulk solvent present find a transition in both α and n, as expected for temperature dependent relaxation [27]. The lack of structural dependence of the dynamical transition is confirmed by the random coil poly-L-lysine measurements. Figure 3 shows the frequency and temperature dependence of absorption coefficient for the random coil poly-L-lysine. An obvious transition in absorption coefficient ~200 K is observed in Fig. 3. Again, no transition is observed for pure solvent.

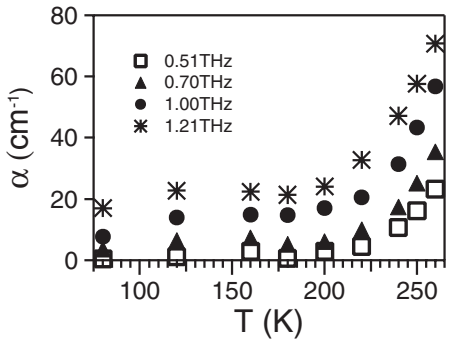

FIG. 3. The temperature dependent absorption coefficient α for random coil poly lysine.

The lack of structural dependence for the transition suggests it arises strictly from either the solvent or the side-chain diffusive motion and not from protein structural collective motions. If the origin is strictly side-chain relaxations, then the temperature dependence should continue as we reduce the polymer length. Measurements on a short chain peptide series allow us to determine the minimum chain length needed for the transition. The THz absorption coefficient measurements for the alanine peptides are shown in Fig. 4. The dynamical transition does not appear for A2 and A3, while the transition is clearly observed for A5, A7–A10, and PA. If the transition simply arises from diffusive behavior of the side chains, this should still be present for A2 and A3 [28,29]. Molecular-dynamics simulations have suggested that the transition originates in the biological water [30]. Our results for alanine peptides suggest that possibly the water-protein interactions that give rise to the temperature dependence have a minimum chain length dependence.

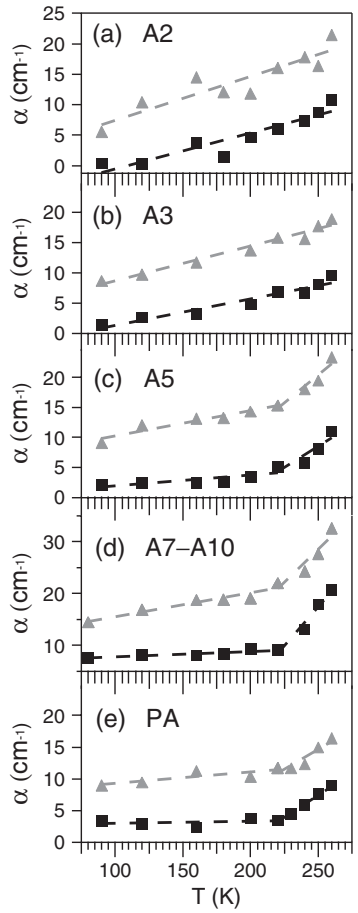

FIG. 4. The temperature dependent absorption coefficient for A2 (a), A3 (b), A5 (c), A7–A10 (d) and PA (e) at frequency 1.00 THz (■) and 1.21 THz (▲).

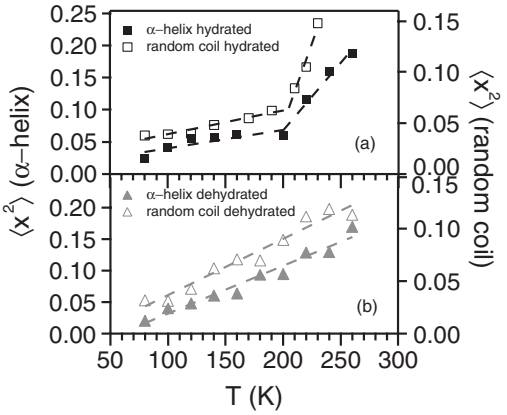

FIG. 5. Temperature dependent calculated $\langle x^2 \rangle$ for a peptide in random coil and α-helix conformations: (a) fully hydrated, and (b) dehydrated.

Figure 5 shows the calculated temperature dependence of $\langle x^2 \rangle$ for a peptide with α-helix and random coil conformations. The $\langle x^2 \rangle$ for both dry α-helix and random coil has a linear temperature dependence, while for both hydrated α-helix and random coil there is an obvious transition at ~200 K, consistent with the experimental results. Many measurements have verified the hydration dependence for native proteins, and recent THz studies on hydration controlled powders also show this [27].

These results impact the continuing discussion of the origin of the so-called protein dynamical transition. As discussed earlier, measurement of the fragile to strong transition requires suppression of water crystallization. If the fragile to strong transition is the origin of the protein dynamical transition, then the suppression of crystallization appears independent of surface structure. The data also suggest that the water-peptide interaction changes with peptide length and crystallization suppression ceases below a specific chain length. For the alternative model with a continuous temperature dependent relaxation rate and the appearance of a transition dependent on the frequency window of the measurement, our data surprisingly suggest that there is a sudden size dependence to this temperature dependent rate.

The measurements and calculations presented here are consistent with the peptide influencing the dynamics of the adjacent water either by preventing crystallization and allowing the fragile to strong transition, or by forming bonding resulting in activated diffusive motions. Our dynamical transition studies in the 100–400 mg/ml range do not show any concentration effects, however more extensive concentration measurements may reveal the extent the peptide influences the solvent as seen in room temperature studies [31,32]. Further measurements and calculations are required to determine how the peptide influences solvent dynamics and how surface hydrophilicity may effect the transition [33]. However these future measurements need not focus on complex structured polypeptide chains. It is clear that the protein dynamical transition does not require a protein.

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