Dynamical Transition of Collective Motions in Dry Proteins

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Water is widely assumed to be essential for protein dynamics and function. In particular, the well-documented "dynamical" transition at ~200 K, at which the protein changes from a rigid, nonfunctional form to a flexible, functional state, as detected in hydrogenated protein by incoherent neutron scattering, requires hydration. Here, we report on coherent neutron scattering experiments on perdeuterated proteins and reveal that a transition occurs in dry proteins at the same temperature resulting primarily from the collective heavy-atom motions. The dynamical transition discovered is intrinsic to the energy landscape of dry proteins.

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Water is intimately involved in protein function [1–7]. In enzymes, for example, hydration enhances enzymatic rates, and noncatalytic water molecules can aid in transporting substrates and protons into the catalytic sites [1,8,9]. Numerous experiments have suggested that a minimum hydration level of $h \sim 0.2$ (g water/g protein) is required for enzymes to function [1,10–12]. Hydration may also enhance the internal motions required for the functional chemical steps [1,7,9,10]. Indeed, $h \sim 0.2$ has also been reported to be the minimum hydration level required for the widely studied dynamical transition in proteins at ~200 K, also named as the "glass" transition in analogy to the glass transition process in glass-forming systems, at which internal protein motions change from nonfunctional, glasslike, rigid, harmonic vibrations to incorporating liquidlike, flexible, anharmonic dynamics required for function [1,5,13–16]. A large body of experimental and simulation work has suggested that this transition is coupled to the activation of the translational motion of the hydration water on the protein surface [3,5,7,9,13,17–22]. However, the energy landscape of a globular protein is intrinsically complex and highly anharmonic, leading to the question as to whether the protein dynamical transition may be an intrinsic property of dry proteins.

Deuteration is key to the present observations. For simplicity, in what follows hydrogenated and perdeuterated samples are denoted using the prefixes of H and D. The neutron data collected on H proteins are mostly incoherent scattering signals [~90%, see Fig. S1(a) in the Supplemental Material [23]], resulting from self-correlations of the motions of hydrogen atoms. In contrast, the scattering from D proteins is primarily coherent [~90%, Fig. S1(b) in Ref. [23]], arising mostly from cross-correlations in motions between protein atoms [50–52], and is dominated by scattering signals from the heavy (non-H) atoms (~70%), especially those on the backbone [Fig. S1(c) in Ref. [23]].

Here, by performing neutron scattering and molecular dynamics (MD) simulation on lyophilized (hydration level, $h \sim 0.02$, Fig. S2 in Ref. [23]) perdeuterated cytochrome P450 (CYP) and green fluorescent protein (GFP) [Figs. 1(a) and 1(b)], we show that the motions of heavy atoms in an essentially dry protein themselves present a dynamical transition at ~200 K.

On the high-flux backscattering spectrometer HFBS at NIST, we measured $S(q, \Delta t)$, the intensity of the elastic peak of the dynamic structure factor, which is an estimate of the average amplitude of the atomic motions up to the temporal resolution time of the instrument, Δt, which is ~1 ns [53]. Figure 1 presents the temperature dependence of $S(q, \Delta t)$ for both dry ($h = 0.02$) H and D samples. The presence of a downward change in gradient in the temperature dependence of $S(q, \Delta t)$ indicates that an anharmonic dynamic process is activated at the corresponding temperature [54]. Figures 1(c) and 1(d) show that both H-CYP and H-GFP present a kink at ~125 K. This transition has been observed several times previously in various hydrogenated

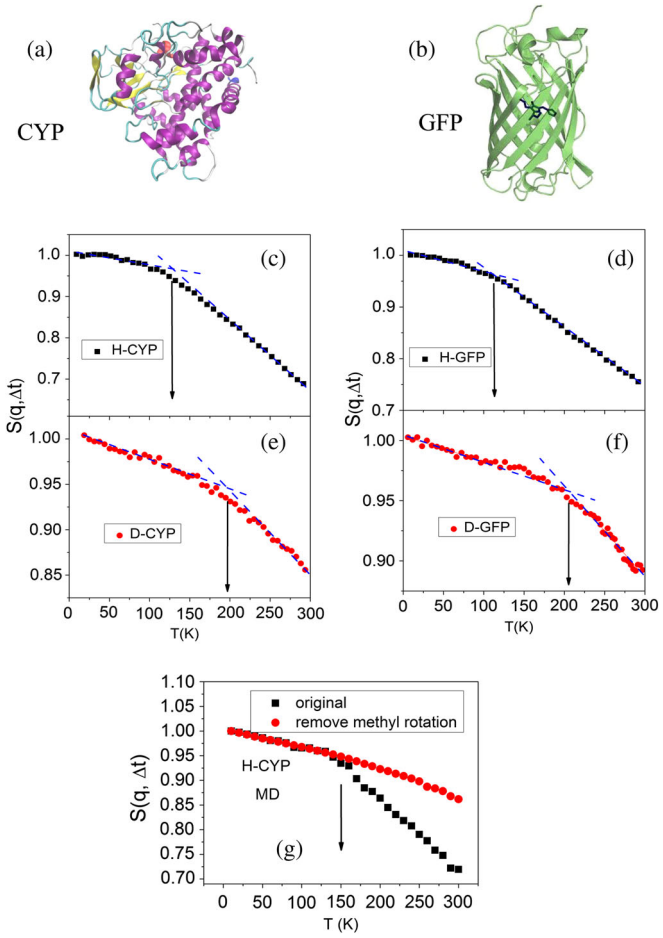

FIG. 1. Structures of (a) CYP and (b) GFP. Experimental $S(q,\Delta t)$, normalized to the lowest temperatures (~10 K) and summed over 15 values of q, ranging from 0.36 to 1.75 Å^{-1} for dry ($h = 0.02$) (c) H-CYP, (d) H-GFP, (e) D-CYP, and (f) D-GFP. The elastic-scan data of dry CYP in panels (c) and (e) have been reported in Ref. [52] in a different form. The experimental results of $S(q,\Delta t)$ are grouped in intervals of 8 K for clarity. The two dashed lines in each figure are linear fits in the low (5–110 K) and high (220–295 K) temperature regions, respectively, and the crossing point of the two fits determines the transition temperature. The same fitting procedure is also used in Fig. 2 to determine the transition temperature. In Fig. S3, we compared $S(q,\Delta t)$ scaled by the values at the lowest temperatures at each q with that scaled by the values collected on vanadium, exhibiting negligible differences. (g) Temperature dependence of incoherent $S(q,\Delta t)$ derived from MD simulation of dry ($h = 0.02$) H-CYP with or without methyl rotation being removed through postprocessing the MD trajectories. The detailed procedures for removing methyl rotations from the MD trajectories are presented in Ref. [23] (see Fig. S7). The MD-derived $S(q,\Delta t)$ is approximated as the value of the intermediate scattering function when decaying to the instrument resolution, $I(q,\Delta t)$ (see Eqs. S1 and S2 in the Supplemental Material [23], and Ref. [53]), the same as in Fig. S4 [23]. The MD-derived $S(q,\Delta t)$ is also normalized to the lowest temperature (10 K), being consistent with experiment.

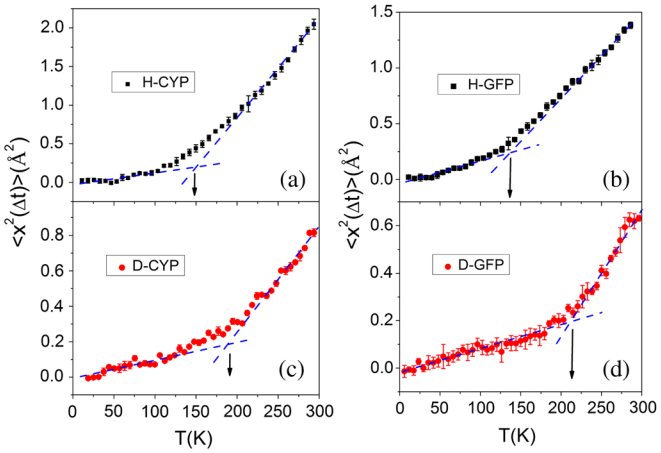

FIG. 2. Experimental mean-squared atomic displacements $\langle x^2(\Delta t)\rangle$ derived from dry ($h = 0.02$) (a) H-CYP, (b) H-GFP, (c) D-CYP, and (d) D-GFP. Detailed procedures to derive $\langle x^2(\Delta t)\rangle$ are presented in Ref. [23]. Error bars throughout the text represent 1 standard deviation.

proteins, and arises from the fact that methyl rotations start to enter the experimental time window (~1 ns) at that temperature, which is hydration independent [13,53–56]. This interpretation is confirmed in Fig. 1(g), where the incoherent $S(q,\Delta t)$ derived from the MD trajectory on dry ($h = 0.02$) H-CYP shows a transition at ~150 K that disappears when the methyl group rotations are removed by postprocessing the MD trajectories. The ~125 K transition is absent in the deuterated samples [Figs. 1(e) and 1(f)], as the neutron data are collected, is dominated by the coherent signal, which is insensitive to methyl group rotations. This is further verified in Fig. S4(b) in the Supplemental Material [23] in which the MD-derived coherent $S(q,\Delta t)$ remains intact when the methyl rotation in the protein is removed through postprocessing the MD trajectories.

A transition does appear in the dry D proteins at ~200 K [Figs. 1(e) and 1(f)]. A transition around this temperature has been widely reported in H proteins, and to be measurable requires hydration to at least 0.2 g water/g proteins: namely, the "dynamical" or glass transition [5,10,15,57,58]. Once dehydrated, this transition should disappear in H samples, and this is indeed evident in Figs. 1(c) and 1(d). To confirm that the 200 K transition occurs in a dry deuterated protein, we also estimated the mean-squared atomic displacement, $\langle x^2(\Delta t)\rangle$, of the protein. Consistent with $S(q,\Delta t)$ for the D proteins [Figs. 1(e) and 1(f)], one unambiguous transition for both deuterated dry proteins occurs in $\langle x^2(\Delta t)\rangle$, at ~200 K [Figs. 2(c) and 2(d)], while the hydrogenated counterparts present a transition at 125–150 K [Figs. 2(a) and 2(b)]. Therefore, both $S(q,\Delta t)$ and $\langle x^2(\Delta t)\rangle$ demonstrate that the dry proteins possess a ~200 K dynamical transition that is visible as long as the neutron signal is dominated by coherent scattering. This result is likely to be quite general for protein systems, as the two proteins studied here differ considerably in both their secondary and tertiary structures

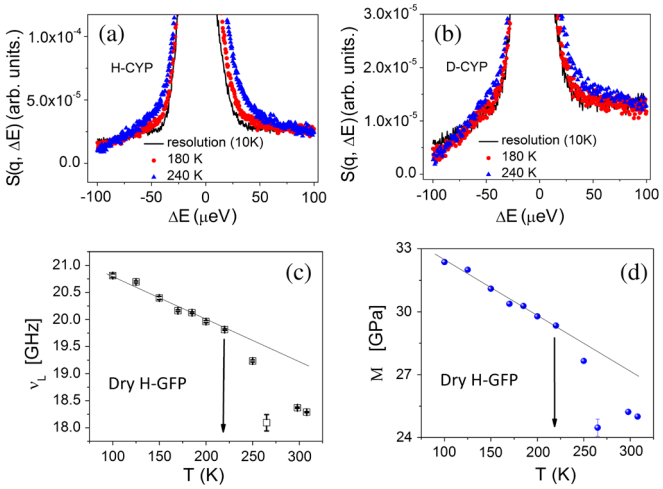

FIG. 3. Quasielastic neutron scattering spectra measured at 180 and 240 K for dry ($h = 0.02$) (a) H-CYP and (b) D-CYP. To improve statistics, the spectra measured over the experimental q window from 0.4 to 1.8 Å^{-1} were summed up. For comparison, the resolution function measured at 10 K is also presented. (c) The frequencies of longitudinal sound waves ν_L measured using Brillouin light scattering on lyophilized ($h = 0.02$) H-GFP (the results are taken from Ref. [53]). (d) Longitudinal modulus estimated using the result of (c), with the density and refractive index of the protein assumed to be 1.4 g/cm^3 [59] and 1.4 [60], respectively. Arrows in (c) and (d) mark the kinks in the temperature dependence of the elastic properties.

[Figs. 1(a) and 1(b)]; whereas GFP consists of mostly β sheets wrapped into a barrel-like structure, CYP consists of comparable amounts of β sheets and α helices and forms three closely packed domains [52].

In addition to the elastic scans, quasielastic neutron spectra were also determined for dry ($h = 0.02$) H-CYP and D-CYP, using the backscattering spectrometer, BASIS at ORNL (see Ref. [23]). The experimentally measured quantity is the dynamic structure factor $S(q, \Delta E)$ (Eqs. S4 and S5 in the Supplemental Material [23]), which reveals the distribution of dynamic modes in the material over the time window from ~3 to ~300 ps. The resolution functions of both samples were measured at 10 K for comparison.

For H-CYP, the quasielastic component of $S(q, \Delta E)$ at 180 K is clearly broader than the resolution function [Fig. 3(a)], indicating that at this temperature anharmonic motions have been activated. These results are consistent with the $S(q, \Delta t)$ measured for H proteins [Figs. 1(c) and 1(d)] as the methyl rotation enters the experimental time window already at ~125 K. The quasielastic component is further enhanced by increasing the temperature to 240 K.

In contrast, $S(q, \Delta E)$ for D-CYP at 180 K does not differ significantly from the resolution function [Fig. 3(b)], indicating the absence of detectable anharmonic motions in the corresponding time window. However, at 240 K a quasielastic component is clearly present and broadened. Therefore, Fig. 3(b) confirms the results of $S(q, \Delta t)$ in Figs. 1(e) and 1(f), that the anharmonic motion can be observed only above the transition temperature (~200 K) in D proteins. Moreover, the broadening of $S(q, \Delta E)$ indicates that the dynamical transition discovered in the dry deuterated protein results from the thermal activation of anharmonic relaxation modes on the pico-to-nanosecond time scale.

The scattering from D proteins arises mostly from cross-correlations in motions between heavy atoms, especially those of the backbone (see Fig. S1 in Ref. [23]). The question thus arises as to whether the activated motions are local, or global and collective, i.e., distributed over the protein. Brillouin light scattering data suggest a transition in the mechanical properties of dry proteins over the corresponding temperature range [53]: the frequency of longitudinal sound waves in dry GFP (lyophilized, $h = 0.02$) drops significantly as the temperature is increased to ~220 K [Fig. 3(c)], indicating a strong reduction of the elastic modulus [Fig. 3(d)] of the protein; i.e., the protein becomes softer. This elastic modulus has been found to be inversely proportional to the amplitude of collective atomic motions spanning the entire protein molecule [53]. Therefore, the reduction of the elastic modulus at ~220 K [Figs. 3(c) and 3(d)] suggests that the dynamical transition discovered in the deuterated dry proteins [Figs. 1(e), 1(f), 2(c), and 2(d)] arises primarily from the activation of collective, global protein motions. The Brillouin light scattering results are consistent with a recent finding of softening of bovine serum albumin around 250 K [61].

Reference [54] reported a weak transition, i.e., a small deviation from linear temperature dependence of mean-squared atomic displacement, at ~150 K in a hydrated ($h = 0.2$) homopolymer, polyphenylalanine, ascribed as the thermal activation of phenyl-group motions. However, this will not contribute significantly to the present observed signal due to the low populations of phenyls in the proteins studied, containing only 10% aromatic amino acids. More detailed discussion about why the phenyl-group motion is not the major contribution to the dynamical transition found here in dry deuterated protein can be found in supplemental material [23].

Taken together, the above results permit the conclusion that dry proteins present a dynamical transition at ~200 K, resulting primarily from the thermal activation of collective, anharmonic motions on the pico-to-nanosecond time scales. This dynamical transition is an intrinsic property of the internal protein energy landscapes, as its presence does not require water. The transition was unobservable previously in neutron scattering from dry H proteins [13,53,55], because the incoherent scattering from rotations of methyl hydrogens, which themselves enter the experimental time window at ~125 K, swamp the neutron signal, masking the ~200 K heavy-atom transition. In contrast, the coherent signal dominating the neutron data from deuterated proteins is weighted more heavily by the motions of

heavy atoms, and especially collective modes [52], while at the same time being insensitive to the rotations of methyl groups. Therefore, the transition at ~200 K becomes visible in neutron scattering on dry deuterated proteins.

We cannot yet confirm that the dynamical transition observed here in the dry deuterated protein is the same process as the one widely reported in the hydrated hydrogenated proteins (H proteins) [5,10,15,57,58]. However, we expect these two might be strongly correlated. First of all, they occur at similar temperatures. Second, the transition identified in H proteins is crucial for protein function, which requires activation of collective protein atomic motion and global softening of the protein molecule around 200 K [53,62,63]. The transition found here in dry D proteins using neutron scattering is indirect evidence demonstrating the activation of collective protein motion at 200 K, as the measured neutron data are dominated by coherent signals resulting primarily from protein heavy atoms, especially the backbone heavy atoms (Fig. S1 [23]). Further, the Brillouin light scattering data [Figs. 3(c) and 3(d)] are a strong indication that the dry protein molecules are globally softened around the transition temperature.

By taking advantage of fundamental differences between coherent and incoherent neutron scattering, we have demonstrated that dry proteins exhibit a dynamical transition in heavy-atom dynamics that is activated at ~200 K. The transition involves an increase in protein flexibility involving the activation of anharmonic, collective heavy-atom motions. The absence of water is consistent with fluorescence measurements on Zn-cytochrome C peroxidase, which reveal that the rate of quenching of conformational transitions around 200 K is independent of the composition of the solvent [64]. Moreover, the presence of a dynamical transition in dry proteins may provide an explanation for the recent observation of residual enzyme function in almost dry conditions [65], as the essential functional dynamical modes may be already present.

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