The origin of the dynamic transition in proteins

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Despite extensive efforts in experimental and computational studies, the microscopic understanding of dynamics of biological macromolecules remains a great challenge. It is known that hydrated proteins, DNA and RNA, exhibit a so-called "dynamic transition." It appears as a sharp rise of their mean-squared atomic displacements $\langle r^2 \rangle$ at temperatures above 200–230 K. Even after a long history of studies, this sudden activation of biomolecular dynamics remains a puzzle and many contradicting models have been proposed. By combining neutron and dielectric spectroscopy data, we were able to follow protein dynamics over an extremely broad frequency range. Our results show that there is no sudden change in the dynamics of the protein at temperatures around ~200–230 K. The protein's relaxation time exhibits a smooth temperature variation over the temperature range of 180–300 K. Thus the experimentally observed sharp rise in $\langle r^2 \rangle$ is just a result of the protein's structural relaxation reaching the limit of the experimental frequency window. The microscopic mechanism of the protein's structural relaxation remains unclear. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2927871]

I. INTRODUCTION

Understanding the dynamics of biological macromolecules is one of the keys to understanding their function and knowledge of their structure alone is not sufficient. Despite significant experimental and computational efforts in analyzing the dynamics of biological macromolecules, our knowledge in this field remains rather limited. There are no well-developed concepts comparable to, for example, the Rouse and reptation models used for synthetic polymers. As a result, even the basic phenomena of biomolecular dynamics remain largely unexplained.

One of these phenomena is the so-called "dynamic transition" that has been observed in all hydrated proteins, DNA and RNA, as a sudden rise of their mean-squared atomic displacements $\langle r^2 \rangle$ at temperatures around $T_D \sim 200–230$ K.[1–7] The dynamic transition has usually been studied using neutron scattering, X-ray and Mössbauer spectroscopy.[1–7] The interest in this dynamic transition was stimulated by the fact that the measurable biochemical activity of proteins usually appears around the same temperature range,[4,8] although some exceptions have also been reported.[9] Despite almost two decades of studies, even the basic understanding of the mechanism underlying the observed sharp rise in $\langle r^2 \rangle$ remains controversial and various models have been proposed.[1,3,8,10,11] It is ascribed to a sudden change in an "effective elasticity" of the protein,[3] to motions of specific side groups, e.g., methyl group rotations,[8] to a dynamic crossover similar to the one predicted by Mode-Coupling Theory (MCT) of the glass transition,[1,5,10] to a specific fragile-to-strong crossover in dynamics of hydration water,[11] and simply to a relaxation process that enters the experimentally accessible frequency window.[9,12–16] Understanding the microscopic mechanism of the dynamic transition is an important step towards understanding the basics of protein motions, their specificity, and its connection to protein activity and stability.

In this work we combine neutron scattering and dielectric spectroscopy data to follow protein dynamics over a broad frequency and temperature ranges. We have been able to identify the main structural relaxation process of the protein. The characteristic relaxation time τ of this process exhibits a smooth slightly non-Arrhenius temperature variation. This behavior is characteristic for the structural relaxation in polymers and glass-forming liquids and emphasizes the similarity in dynamics between biological macromolecules and "complex fluids." Our results present clear evidence that there is no sudden change in the dynamics of the protein around T_D and that the observed sharp rise in $\langle r^2 \rangle$ just marks the temperature at which the protein relaxation process approaches the resolution of the spectrometer.

II. EXPERIMENT

We used the model protein, hen-egg white lysozyme (Sigma-Aldrich), hydrated to a level of $h \sim 0.4$ g water per gram of protein. This level of hydration is sufficient to restore the dynamics and activity of the protein, but is low enough to suppress water crystallization and globular diffu-

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sion. The protein was dialyzed to remove salts and lyophilized. The lyophilized powder was hydrated in D_2O for the neutron scattering measurements. Details of the sample preparation for neutron scattering measurements are presented in Ref. 15. Neutrons scatter mostly from hydrogen atoms and thus the neutron scattering spectra of a lysozyme/D_2O sample reflect mostly the motion of protein. The lyophilized lysozyme was hydrated with H_2O for dielectric measurements by isopiestic equilibration at 100% relative humidity until the desired hydration level $h \sim 0.4$ had been reached. In both neutrons and dielectric experiments, the mass of the samples was measured before and after experiments and no loss of water was detected. Lyophilized lysozyme with a residual hydration level of $h \sim 0.03-0.05$ was used as the "dry" sample. The hydration levels were determined by weighing the samples and by thermogravimetric analysis.

Neutron scattering spectra of hydrated and dry lysozyme at a few temperatures were measured using the high flux backscattering spectrometer[17] (HFBS) at the National Institute of Standards and Technology (NIST). The spectra were measured in the energy range $\Delta E = \pm 17$ μeV ($\approx$4 GHz) and scattering wave vector range 0.25 Å$^{-1} \leq Q \leq 1.75$ Å^{-1}. The spectral resolution was ≈ 1 μeV ($\approx$240 MHz). Neutron scattering data were corrected for scattering from the sample cell, and analyzed using the DAVE software provided by NIST.[18] The spectra were normalized by the mass of lysozyme. No correction for multiple scattering has been applied. A detailed description of the data treatment, including analysis of the mean-squared displacement $\langle r^2 \rangle$, has been presented in Refs. 15 and 19. The spectrum at $T=295$ K has been extended to higher frequencies using data from new backscattering spectrometer at the Spallation Neutron Source at the Oak Ridge National Laboratory[20] and from the time-of-flight disc chopper spectrometer at NIST (Ref. 21) (the latter have been published earlier in Ref. 15). A similar data treatment in the comparable Q range was applied to these data.

Dielectric spectra were measured using the Concept 80 system from Novocontrol. The samples were placed between two gold-coated electrodes with diameters of 20 mm for the low frequency ($\nu < 10$ MHz, Novocontrol alpha analyzer) measurements and 10 mm for the high frequency ($\nu \geq 1$ MHz, Agilent 4291B impedance analyzer) measurements. The samples were sealed with Teflon ring to minimize evaporation of water. The capacitor with the sample mounted in the header was placed in a cryostat. The temperature was stabilized to better than 0.1 K by a flow of nitrogen gas. The real and imaginary parts of the complex dielectric permittivity, $\varepsilon^* = \varepsilon' + i\varepsilon''$, were measured at frequencies between 10^{-2} and 10^9 Hz at different temperatures. Analysis of the data was performed using WINFIT software from Novocontrol.

III. RESULTS

A. Neutron scattering

The atomic mean-squared displacements of hydrated and dry lysozyme are shown in the inset of Fig. 1(a): They are very similar at $T < 200$ K, but hydrated lysozyme exhibits a sharp rise in $\langle r^2 \rangle$ above $T \approx 200$ K. This is the well-known dynamic transition. A detailed analysis of the quasielastic scattering (QES) spectra reveals no significant Q dependence of their spectral shape, consistent with earlier studies of lysozyme powders.[15,22] As a result, the data from all detectors were summed to increase the statistics of the spectra. The energy resolved spectra show a strong QES contribution that is almost identical in hydrated and dry protein at $T=200$ K [Fig. 1(a)] but that becomes much stronger in the hydrated protein at higher temperatures [Fig. 1(b)]. The QES contribution in dry proteins is ascribed mostly to methyl group rotations that activate at very low temperature and that are not sensitive to hydration.[15,23,24] The increase in the QES intensity in the hydrated protein at temperatures above T_D (Fig. 1) suggests the existence of an additional relaxation process.

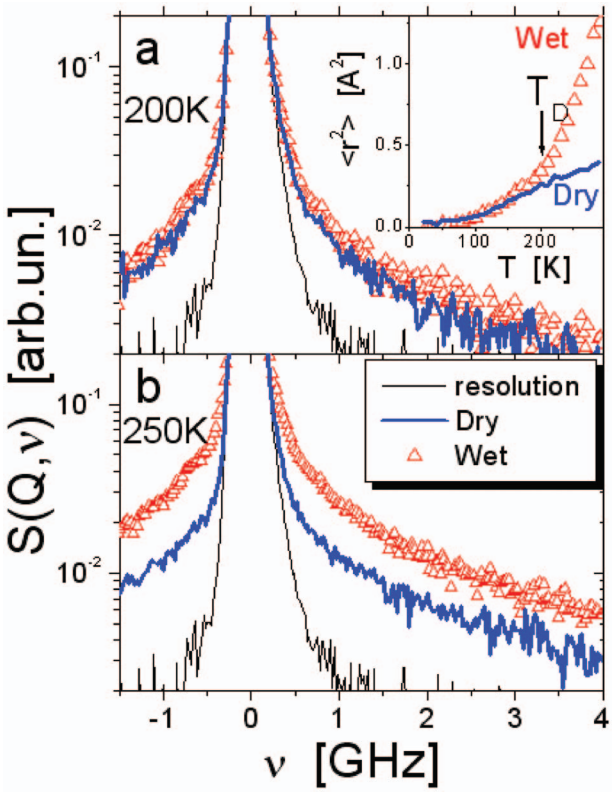

FIG. 1. (Color) Dynamic structure factor of dry (thick solid line) and hydrated (symbols) lysozyme at $T=200$ K (a) and at $T=250$ K (b). Thin solid lines show the resolution function of the spectrometer. The inset shows temperature variations of the mean-squared displacements $\langle r^2 \rangle$. The sharp rise in $\langle r^2 \rangle$ of the hydrated protein at T above $\sim$200 K is called the dynamic transition. The strong QES scattering observed in these spectra indicates an active relaxation processes. The QES intensity is almost the same in the dry and the hydrated protein at $T=200$ K and is ascribed primarily to methyl group dynamics (Refs. 15 and 23). QES spectra become significantly different at higher temperatures indicating the appearance of an additional relaxation process in the experimentally accessible frequency range in the spectra of the hydrated protein.

To analyze the spectra of the additional relaxation process, we subtracted the spectra of the dry protein from the spectra of the hydrated protein measured at the same temperature. In this way, we essentially corrected the spectra for the methyl group contribution that is not sensitive to

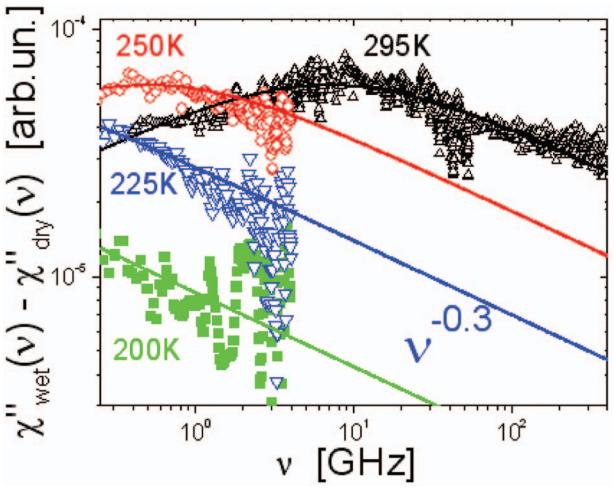

FIG. 2. (Color) Neutron scattering susceptibility spectra of the structural relaxation process in hydrated lysozyme at different temperatures. The spectra are extremely stretched and the high frequency tail can be approximated by a power law $\chi''(\nu) \propto \nu^{-a}$ with the exponent $a=0.3\pm0.05$.

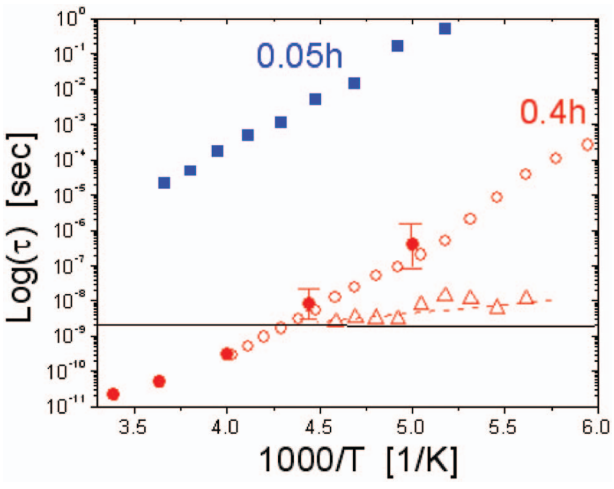

FIG. 3. (Color) Temperature dependence of the relaxation times in hydrated and dry lysozyme: (●) Protein's structural relaxation from neutron scattering data, large error-bars at lower temperatures are related to a weak signal; (○) the main dielectric relaxation process and (△) the fast dielectric relaxation process that splits from the main process at $T<220$ K (Fig. 4); (■) main dielectric relaxation process in dry lysozyme. The dashed line shows the relaxation times of hydration water from neutron experiments presented in Ref. 11. The solid line marks the resolution function of the neutron scattering spectrometer (~2 ns).

hydration.[15] We emphasize that this correction is vital for the interpretation of neutron scattering[15] and molecular dynamics (MD) simulation[25] data. In order to analyze the spectral shape and the characteristic relaxation time of the additional relaxation process, we represent the neutron scattering data (Fig. 2) as the imaginary part of the susceptibility $\chi''(Q,\nu) = S(Q,\nu)/n(\nu)$, where $S(Q,\nu)$ is the dynamic structure factor and $n(\nu)=[\exp(h\nu/kT)-1]^{-1}$ is the temperature Bose factor. The susceptibility representation allows the estimation of characteristic relaxation times from the frequency of the peaks, $\tau=(2\pi\nu_{\max})^{-1}$, and of the stretching parameters from the slopes of the peaks $\chi''(\nu) \propto \nu^{a}$. Moreover, this presentation of scattering data is analogous to the dielectric and mechanical loss spectra.

The additional relaxation process appears to be strongly stretched and can be described by a Cole–Cole (CC) function with a stretching parameter of $a \approx 0.30 \pm 0.05$ (Fig. 2). A maximum in the relaxation peak is clearly visible in the spectra measured at $T=250$ K and above, providing direct estimates of the relaxation time $\tau=(2\pi\nu_{\max})^{-1}$. The maximum, however, disappears from our experimental window at temperatures lower than 250 K and only the high frequency tail of the relaxation peak is visible. Assuming that the amplitude of the process and its spectral shape do not change significantly with temperature (this is the case, at least, for temperatures between $T=295$ and 250 K in Fig. 2), τ has been estimated using the shift factor for the high frequency tail of the susceptibility spectra (Fig. 2). The so-obtained relaxation times are plotted in Fig. 3 as a function of temperature. τ shows a slightly non-Arrhenius temperature dependence.

B. Dielectric spectroscopy

Dielectric relaxation spectra of hydrated and dry lysozyme samples exhibit a number of relaxation processes, as can be discerned from Fig. 4. The data were fitted using a few (up to three) peaks approximated by Cole–Cole distribution functions and a conductivity tail,

$$\varepsilon^* = \varepsilon_\infty + \sum_j \frac{\Delta\varepsilon_j}{1+(i\omega\tau_j)^{a_j}} - i\frac{A}{\omega^s}. \quad (1)$$

Here, A is the tail amplitude, $\omega=2\pi\nu$ is the angular frequency, s is the exponent describing the tail slope, τ_j is the relaxation time, $\Delta\varepsilon_j$ is the dielectric strength, and a_j is the stretching parameter for j process. At temperatures above 223 K, the spectra were fitted with a single Cole–Cole distribution function to describe the main relaxation peak and a power law to describe the low frequency tail that includes conductivity and a slower relaxation process. Because a faster process emerges at lower temperatures, as can be seen

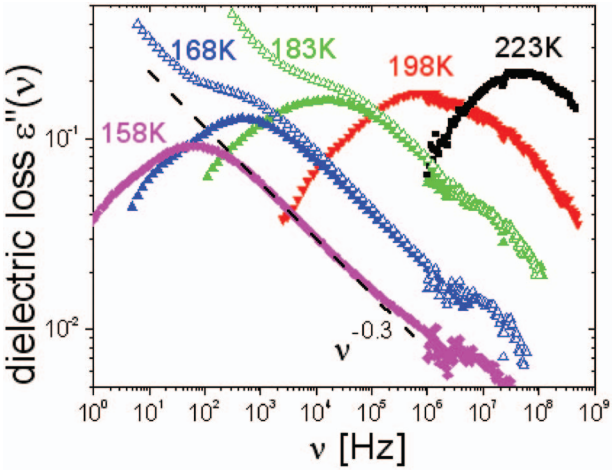

FIG. 4. (Color) Dielectric loss spectra of the hydrated sample at different temperatures. Open symbols show the experimentally measured spectra (only for $T=183$ K and 168 K). Closed symbols show the spectra after subtraction of the contribution of other processes at lower frequencies. The dashed line shows the high frequency slope of the spectrum $\varepsilon''(\nu) \propto \nu^{-0.3}$. The spectra show the development of the second relaxation peak at $T<200$ K.

in Fig. 4, we fit the spectra measured at temperatures between 203 and 223 K with two CC distribution functions and a low frequency tail. At $T<203$ K, the main relaxation peak moves into the lower frequency range and the presence of the process between conductivity and the main relaxation process becomes apparent. We used both real and imaginary parts of the permittivity to analyze the temperature variations of the three relaxation peaks. Details of the fit will be presented elsewhere.[26]

Analysis of the main relaxation process that is clearly visible in the dielectric loss spectra indicates a strongly stretched relaxation process with a stretching parameter of a between 0.3 and 0.45 (Fig. 4). This process splits into two processes at lower temperatures. Characteristic relaxation times for these two processes are presented in Fig. 3. The main dielectric process shows a smooth and slightly non-Arrhenius temperature dependence through the entire range from 250 down to $T\sim180$ K, while the faster process seems to exhibit an Arrhenius temperature dependence with a rather low activation energy.

The faster process observed in the dielectric spectra at $T<220$ K has relaxation times and a temperature dependence similar to the process observed in neutron scattering by Chen *et al.* for the relaxation of hydration water of lysozyme.[11] The authors specifically used H_2O hydration to detect this process in neutron scattering spectra. We also ascribe the faster process to a particular relaxation of water because this process was not observed in our neutron scattering measurements that used D_2O hydration. Since this process is not the main focus of our work, we will not engage into the controversy associated with the microscopic interpretation of this fast process.[11,27–29]

IV. DISCUSSION

A comparison of the dielectric and neutron scattering data for hydrated lysozyme (Figs. 2–4) indicates that the main relaxation processes observed by the two techniques have (i) similar relaxation times (ii) with the same temperature dependence and (iii) similar spectral shape (stretching parameter). We emphasize that the neutron scattering experiment probes the motion of lysozyme and not the water of hydration, due to the hydrogen (in the protein)-deuterium (in D_2O) contrast. The similarities between the neutron and dielectric relaxation spectra suggest that the main dielectric process is the same structural relaxation of the protein, or, at least, should be strongly coupled to it. This interpretation differs from the assignment proposed in Ref. 27 where a similar dielectric relaxation in hydrated myoglobin was ascribed solely to the dynamics of the water of hydration. The microscopic nature of the protein's relaxation process remains unknown although it might be directly related to the protein's biochemical activity.[15,23] Detailed analysis of neutron scattering data suggests that this process involves significant part of the protein, but has, however, limited atomic displacements of the order of 3 Å.[15] One of the interpretations relates it to motions of secondary structures.[30]

Analysis of the neutron scattering spectra clearly demonstrates that the observed sharp rise of $\langle r^2\rangle$ at $T>T_D$ is related to the rise of the QES intensity, i.e., it is caused by a protein's relaxation process that appears in the measured neutron scattering spectra of hydrated protein (as seen from Figs. 1 and 2). Analyzing the neutron scattering data alone, we cannot follow the detailed evolution of the protein's structural relaxation process at lower temperatures. Quantitative analysis of the spectra, especially below $T\approx225$ K, requires additional assumptions about amplitude and spectral shape of the relaxation peak (see the previous section). However, combining the neutron and dielectric spectroscopy data reveals that the structural relaxation of the protein varies smoothly with temperature down to $T\sim180-170$ K (Fig. 3). No sudden and/or unusual variations in τ are observed around the dynamic transition temperature $T_D\sim230-200$ K. These results provide clear experimental evidence of the absence of any peculiar behavior in the protein's relaxation around T_D. They show that the observed sharp rise in $\langle r^2\rangle$ is merely related to the appearance of the protein's relaxation process in the experimentally accessible frequency window (Fig. 3). This interpretation has already been proposed in earlier papers.[9,12–16] It suggests that changing the resolution function of the spectrometer will result in a shift of T_D. This shift has indeed been reported in Ref. 12. Our results, however, provide clear experimental observation of the temperature dependence of the protein's relaxation time in the temperature range across the dynamic transition.

The dielectric measurements identified a similar relaxation process in dry lysozyme. However, it is significantly (five to six orders) slower than the relaxation process observed in the hydrated protein (Fig. 3). Given this time scale, this process will be outside the neutron scattering time window at any temperature of our measurements. This observation explains the absence of the dynamic transition in the neutron scattering measurements of the dry protein: The structural relaxation is too slow for the neutron scattering spectrometer even at the highest temperatures of our measurements.

We want to emphasize that the presented results and analysis do not exclude the existence of a MCT-like dynamic crossover in biological macromolecules. MCT has been successfully applied to the analysis of high-temperature dynamics in various glass-forming systems,[31] including polymers.[32,33] It has been shown that, in general, the dynamics of proteins and DNA follow the high-temperature MCT scenario.[10,34] It is known that structural relaxation time in glass-forming systems varies smoothly through the crossover temperature range,[31,35] which is consistent with the temperature dependence of the protein's relaxation time observed here (Fig. 3). However, regardless of the existence of such a crossover, our results clearly demonstrate that the observed sharp rise of $\langle r^2\rangle$ is related to the appearance of the protein's relaxation process in the experimental frequency window. The process itself has smooth slightly non-Arrheinus temperature variation of its relaxation time, similar to behavior of structural relaxation in glass-forming liquids.

V. CONCLUSIONS

By combining neutron and dielectric spectroscopy data we were able to identify the structural relaxation process in a

hydrated protein that is responsible for the observed sharp rise in $\langle r^2 \rangle$ at $T > 200$ K. Characteristic relaxation time of this process shows a smooth slightly non-Arrhenius temperature dependence in the entire temperature range studied (295–180 K). Temperature variation of this process is similar to the behavior of glass-forming systems. No peculiar variations are observed around the dynamic transition temperature $T_D \sim 200-230$ K. The results provide clear experimental evidence that there are no sudden changes in the dynamics of the protein in this temperature range. Instead, its structural relaxation reaches the resolution limit of the experimentally accessible frequency window of the neutron scattering spectrometer at T_D. This leads to the observed sharp rise of the measured $\langle r^2 \rangle$ with temperature above T_D. However, the microscopic mechanism of the protein's structural relaxation still remains unclear and is the next important step in our basic understanding of the protein dynamics.

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