Conductivity in Hydrated Proteins: No Signs of the Fragile-to-Strong Crossover

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Dielectric spectroscopy studies of hydrated protein demonstrate smooth temperature variations of conductivity. This observation suggests no cusplike fragile-to-strong crossover (FSC) in the protein's hydration water. The FSC at $T \sim 220$ K was postulated recently on the basis of neutron scattering studies [Chen *et al.*, Proc. Natl. Acad. Sci. U.S.A. **103**, 9012 (2006)] and was proposed to be the main cause for the dynamic transition in hydrated proteins. Following Swenson *et al.* [6,9], we ascribe the neutron results to a secondary relaxation. We emphasize that no cusplike solvent behavior is required for the protein's dynamic transition.

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Despite ages of detailed studies, the behavior of supercooled water still remains a mystery [1–3]. The main problem in the study of supercooled bulk water is inevitably crystallization below 235 K. Bulk water could be hyperquenched to a vitreous state at rates $>10^5$ K/s, but it crystallizes above 135 K on heating from this state. As a result, supercooled bulk water exhibits a ''no man's land'' in the temperature range from ~235 K down to ~135 K. One of the ways to study water in this temperature range is analysis of strongly confined water where crystallization is suppressed [4–9]. Water also does not crystallize in hydrated protein powders at hydration level of up to ~0.4 g water per 1 g of protein [10].

Recent investigations of confined water using quasielastic neutron scattering (QENS) spectroscopy reveal unusual behavior [5,7,8]. The relaxation time of hydrogen atoms' motions estimated from QENS experiments, $\langle \tau_H \rangle$, exhibits strongly non-Arrhenius temperature dependence (characteristic of so-called ''fragile'' glass-forming liquids) at high temperatures. However, at T below ~220 K, this dependence changes abruptly to a purely Arrhenius one (characteristic of so-called ''strong'' glass-forming systems) with very low activation energy. As a result, a sharp cusplike bend in the temperature dependence of $\langle \tau_H \rangle$ has been observed and was called fragile-to-strong crossover (FSC). The cusplike FSC has been clearly observed in water of protein lysozyme hydration [5,8] and was ascribed to a transition of water from high-density liquid to a low-density form upon cooling [7]. Moreover, FSC happens around the so-called protein dynamic transition temperature T_D, which marks sharp change in the temperature dependence of protein's mean-squared atomic displacement $\langle r^2 \rangle$ [11–13]. The authors then suggested that this transition in water of hydration causes the dynamic transition in proteins.

The proposed FSC has been challenged recently by Swenson and co-workers [6,9]. They suggested that a split of structural and secondary relaxations takes place in supercooled water in this temperature range. Moreover, they proposed particular mechanism of the secondary relaxation: motion of Bjerrum-type defects [9]. However, no definitive experimental results in favor of or against the proposed interpretations have been presented thus far. As a result, it remains unclear whether or not the structural relaxation of the protein's hydration water experiences some sharp changes at $T \sim 220$ K and whether this change is the main cause for the protein's dynamic transition.

In this Letter we present dielectric relaxation studies of hydrated lysozyme. We focus on the temperature dependence of conductivity σ. Our results demonstrate smooth variations of σ in the entire temperature range, with no sign of any sharp transition at $T \sim 220$ K. These results indicate that there is no cusplike FSC in structural relaxation of protein's hydration water. On example of lysozyme-glycerol sample, we demonstrate that no cusplike solvent's behavior is required for the protein's dynamic transition.

Hen egg white lysozyme (Sigma-Aldrich) was dialyzed and lyophilized, and then rehydrated isopiestically by exposing it to water vapor in closed chamber until desired hydration level $h \sim 0.4$ g of water per 1 g of protein was reached. In order to analyze the influence of hydration level and ion concentration, we additionally measured a sample hydrated to the level $h \sim 0.3$ and a sample with higher ion concentration (nondialyzed) hydrated to the level $h \sim 0.35$. For illustrative comparison, we also measured a lysozyme-glycerol sample with weight ratio 1:0.8. The sample was obtained by dissolving the proper amount of the dialyzed or lyophilized lysozyme and anhydrous glycerol (Fluka) in 0.1 μm filtered water (Sigma-Aldrich). The mixture was lyophilized to remove water. The sample was weighted at the end to verify that no significant amount of water was left. The dielectric relaxation spectra were measured in the frequency range from 10 mHz up to 3 MHz and the temperature range from 293 K down to 123 K using a Novocontrol Concept 80 system. Samples were placed in a parallel-plate capacitor with diameter 20 mm and a Teflon spacer. Weighting of hydrated lysozyme before and after measurements indicated no loss of water.

Dielectric loss spectra $\varepsilon''(\nu)$ for all the samples exhibit conductivity contribution at low frequencies (Fig. 1). The

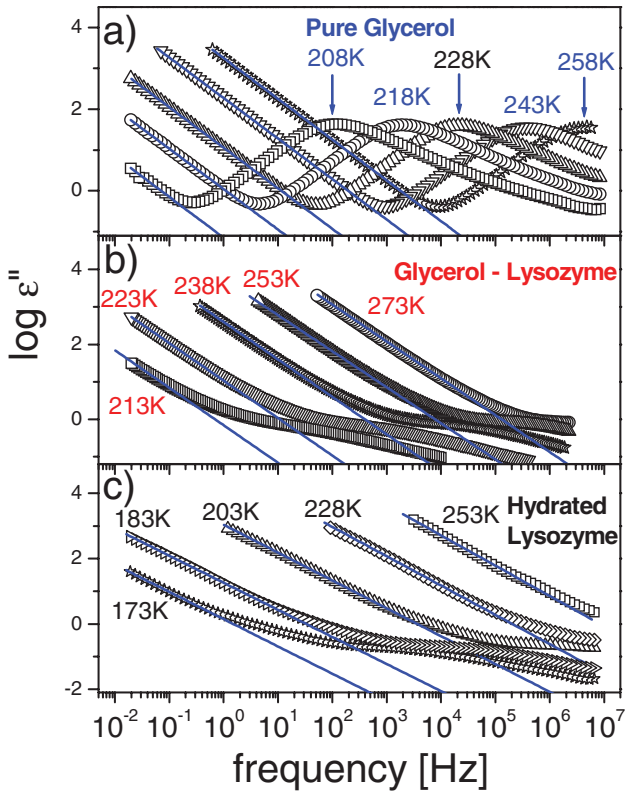

FIG. 1 (color online). Dielectric loss spectra of pure glycerol (a), lysozyme-glycerol (b), and hydrated lysozyme with $h \sim 0.4$ (c) samples at a few representative temperatures. Straight lines present fit of conductivity contribution [Eq. (1)].

spectra of protein-containing samples were fit by a sum of two relaxation peaks approximated by the Havriliak-Negami function and a conductivity term:

$$\varepsilon^* = \varepsilon_\infty + \sum_j \frac{\Delta\varepsilon_j}{[1 + (i\omega\tau_j)^{\alpha_j}]^{\beta_j}} - i\frac{\sigma}{\varepsilon_0\omega^s}, \qquad j = 1, 2, \tag{1}$$

where $\omega = 2\pi\nu$ is the angular frequency, s is the exponent describing conductivity slope, ε_0 is the vacuum permittivity, and τ_j, $\Delta\varepsilon_j$, α_j, and β_j are the relaxation time, the dielectric strength, and shape parameters of the jth process, respectively. A single Havriliak-Negami function plus the conductivity term was enough to describe the spectra of bulk glycerol.

Figure 1 shows that the conductivity contribution can be easily distinguished from the relaxation processes at lower frequencies. The best fit in the case of pure glycerol and lysozyme-glycerol samples gives conductivity slope $s = -1$, while the best fit for hydrated lysozyme gives $s = -0.88 \pm 0.05$. Usually a conductivity term should have the slope $s = -1$. Our analysis shows that fixing the slope to -1 in the fit of the spectra does not affect results for conductivity in any significant way.

Conductivity in liquids is usually related to a diffusion of ions, $\sigma \propto D$, where D is the ions' diffusion coefficient [14]. Traditional Debye-Stokes-Einstein (DSE) relationship connects temperature dependence of dc conductivity to the structural relaxation time of the liquid: $\sigma(T) \propto D(T) \propto 1/\tau(T)$ [14]. Indeed, the DSE relationship has been observed in many glass-forming materials [15–17], although some liquids exhibit its breakdown upon approaching the glass transition. The so-called fractional DSE relationship, $\sigma(T) \propto 1/\tau(T)^x$ with the exponent $x < 1$, has been observed for these materials [17,18]. Thus conductivity in glass-forming liquids usually varies with temperature as the inverse structural relaxation time $1/\tau(T)$, or a bit slower.

We focus first on analysis of the protein glycerol mixture, because the dynamics of glycerol is well-known for the entire temperature range. Analysis shows that the temperature dependences of conductivity in pure glycerol and in lysozyme-glycerol samples are very similar and follow well the expected behavior: $1/\sigma(T) \propto \tau(T)$ (Fig. 2). Here $\tau(T)$ is the structural relaxation time of bulk glycerol estimated from the dielectric spectra [Fig. 1(a)]. These results suggest that the conductivity in the protein-glycerol mixture is controlled mostly by the structural relaxation of glycerol (Fig. 2).

Although the conductivity in three different samples of hydrated lysozyme differs by more than tenfold, its temperature dependence appears to be the same. Comparison of the temperature dependence of conductivity in hydrated lysozyme to the relaxation time $\langle\tau_H\rangle$ from the QENS measurements reveals significant difference (Fig. 3). Conductivity varies smoothly through the entire temperature range, while $\langle\tau_H\rangle$ exhibits sharp change at $T \sim 220$ K. It is not possible that such a dramatic change

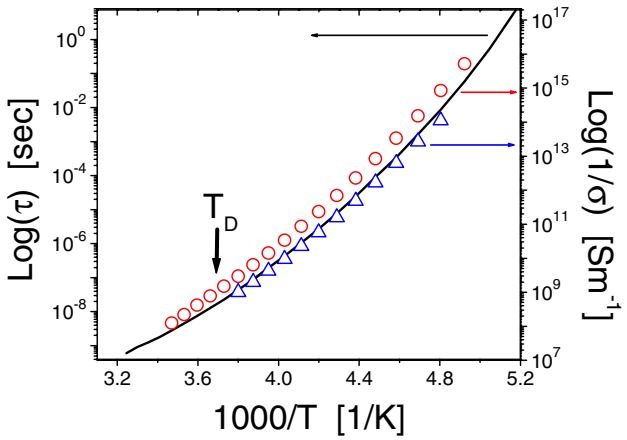

FIG. 2 (color online). Temperature dependence of the structural relaxation time τ in bulk glycerol (line), reciprocal conductivity in bulk glycerol (△), and lysozyme-glycerol sample (open circles). The data for the protein-containing sample were shifted slightly for clarity. Arrow indicates the protein's dynamic transition temperature in glycerol $T_D \sim 270$ K [12].

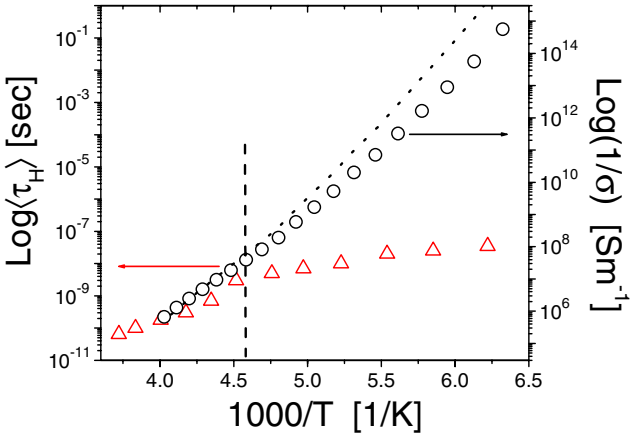

FIG. 3 (color online). Temperature dependence of the relaxation time $\langle \tau_H \rangle$ measured by QENS (△) (data from [5]) and of reciprocal conductivity (open circles) in hydrated lysozyme. The dotted line presents expected temperature dependence of the structural relaxation time calculated assuming the fractional DSE relationship. The dashed line marks the temperature 220 K.

in the temperature dependence of the main structural relaxation would not appear in the conductivity. The conductivity does not provide direct measure of the structural relaxation time due to, in particular, possible fractional DSE relationship. The recent simulations indeed found the fractional DSE relationship in water [19], with translational diffusion $D \propto (\tau/T)^{-x}$, and $x \sim 0.83$–0.84. Assuming that the same relationship can be applied to the conductivity data, we estimated the expected temperature dependence of the structural relaxation time in hydrated lysozyme (Fig. 3). The difference with $\langle \tau_H \rangle$ became even stronger. Thus, even if there is a fractional DSE relationship for conductivity in hydrated lysozyme, it does not affect the main result presented here—no cusplike behavior in the temperature dependence of the structural relaxation in protein hydration water.

The only rational explanation for this observation is that the $\langle \tau_H \rangle$ measured by QENS at low temperatures presents some secondary relaxation process (as has been already proposed in [6,9]). This process decouples from the main structural relaxation at $T \sim 220$ K and does not have significant impact on conductivity. The experiment designed in [5] probes motions of H atoms in water of lysozyme's hydration. Water molecules exchange H atoms and thus the motion of protons does not necessarily reflect the diffusion of the whole water molecule. More important, sharp change in the wave vector Q dependence of $\langle \tau_H \rangle$ has been observed at the same $T \sim 220$ K [5]: It changes from a slightly subdiffusive regime at high T to essentially a localized one (Q independent) at $T < 220$ K. This is a clear sign of changes in the character of the measured relaxation process. Strong localization is usual for a secondary relaxation but is not observed for the main structural relaxation in liquids. Moreover, Arrhenius temperature dependence with rather low activation energy, observed for $\langle \tau_H \rangle$ at low T, is also the usual sign of a secondary relaxation. We emphasize that similar results (a transition from Q dependent to Q independent τ associated with sharp change in its temperature dependence from non-Arrhenius to Arrhenius behavior) have been observed in QENS measurements of a glass-forming system, polybutadiene [20]. Interpretation of this observation is well-known (because relaxation processes in polybutadiene are well studied): QENS probes structural relaxation at high temperatures and a secondary relaxation at lower temperatures [20]. Mode-coupling theory (MCT) dynamic crossover temperature T_{MCT} of water has been estimated around the same temperature ~220 K [21–23]. However, it is known that glass-forming liquids do not exhibit sharp change in the temperature dependence of their structural relaxation around T_{MCT}, and many of them show split of the structural and secondary relaxations at T_{MCT} [17]. Thus a secondary relaxation in water, if it exists, is expected to split from the main structural relaxation at temperatures around $T_{\mathrm{MCT}} \sim 220$ K. This is an additional argument that a secondary relaxation could have been measured by QENS in water of lysozyme hydration at $T < 220$ K.

Recently a confirmation of the cusplike behavior of proton diffusion has been reported from NMR studies of hydrated lysozyme [24]. We cannot comment on these NMR results due to the absence of any experimental details in [24], but the results clearly contradict the behavior of conductivity. The NMR data also contradict the results of recent simulations of the diffusion of water of lysozyme and DNA hydration presented in [25]: no cusplike behavior has been observed in the molecular dynamics simulations. Simulations show smooth temperature variations of the water diffusion coefficient, in agreement with our conductivity data (Fig. 3).

The above discussion leads to the following scenario. The main structural relaxation in protein hydration water shows non-Arrhenius temperature dependence that is observed in neutron scattering and is also reflected in the temperature dependence of conductivity (Fig. 3). A secondary relaxation splits from the main structural relaxation at $T \sim T_{\mathrm{MCT}} \sim 220$ K. It is clearly observed in neutron scattering as the process with much stronger localization (much weaker Q dependence). However, it does not affect the conductivity that still follows the main structural relaxation. Because of the limits of QENS resolution (~10 nsec) the main structural relaxation cannot be detected in these experiments at $T < 220$ K (Fig. 3) and only secondary relaxation is observed [5].

Finally, we want to add a comment on the dynamic transition in proteins. There are no doubts that solvents control the protein's dynamic transition. It is known that proteins placed in glycerol also exhibit the dynamic transition (sharp rise of $\langle r^2 \rangle$) but at temperature ~270–280 K [12], significantly higher than $T_D \sim 200$–230 K characteristic for hydrated biomolecules [11–13] and close to the

T_{MCT} of glycerol [22]. As is obvious from Fig. 2 and vast literature data, no sharp changes in the structural relaxation of glycerol happen at this temperature. Thus no sharp (e.g., cusplike) change in the solvent behavior is required for the protein's dynamic transition. This picture is consistent with the idea that the sharp rise in protein's $\langle r^2 \rangle$ simply reflects the fact that relaxation of protein (most probably controlled by the solvent viscosity) enters the experimentally accessible frequency window [26–28].

As a conclusion, analysis of temperature dependence of conductivity in hydrated lysozyme does not find any cusplike transition in behavior of structural relaxation around $T \sim 220$ K. The result is in strong contradiction with the interpretation of neutron scattering data presented in [5]. Conductivity in wet protein exhibits smooth non-Arrhenius temperature dependence indicating no cusplike fragile-to-strong crossover in protein hydration water. Instead, the data of [5] can be explained by a split of a secondary relaxation from the main structural relaxation at $T \sim 220$ K. Arrhenius temperature dependence of the secondary relaxation is observed in QENS experiment at $T < 220$ K, while behavior of τ_α (according to the conductivity data) remains non-Arrhenius. We suggest that measurements of oxygen diffusion and/or detailed simulations at $T < 220$ K can resolve this controversy. On example of lysozyme-glycerol sample we also emphasize that no sharp change in behavior of solvent is required for the protein's dynamic transition.

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