Glassy behavior of a percolative water-protein system

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We show that is possible to look at the glass transition as a percolation transition in phase space. This study has been carried out on a hydrated globular enzyme for which the thermodynamic transition and the percolative transition could have a functional significance. The approach adopted is based on the identity of roles played respectively by the glass transition temperature T_o and the critical hydration threshold h_c for the percolation of protons on the surface and through the protein, given that dynamical arrest is observed at temperatures and hydration below T_o and h_c. Theoretical predictions for temperature dependence of the nonexponentiality parameter, β_{KWW}, appearing in the KWW relaxation function, indicate that at high temperatures, β_{KWW} remains insensitive to temperature changes, whereas in the vicinity of the glass transition, β_{KWW} is linearly increasing with temperature. The low temperature limit of β_{KWW} is about $1/3$ and its temperature-independent behavior starts at $1.23T_g$: both predictions have been verified in the present study.

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Cluster concepts have been extremely useful in critical phenomena to elucidate the mechanism underlying a thermodynamic transition. In particular, the possibility to look at the glass transition as a percolation transition in phase space has attracted the interest of many researchers in recent years [1–5]. However, very few experimental studies testing this possibility are available in the literature, and none of them, to the best of our knowledge, has been carried out on a biological system where the thermodynamic transition and the percolative transition could have a functional role. Here we tackle this problem for the well known lysozime-water system, a protein-water system of particular interest due to the presence, at room temperature, of a percolative transition of the conductivity at a critical hydration level coinciding with the onset of enzymatic activity [6]. This transition is known to be due to proton displacements along hydrogen-bonded water molecules adsorbed on the protein surface, with ionizable groups as sources of migrating protons [7,8]. Recently, the dynamics of these migrating protons over the lysozyme surface has been investigated by means of dielectric spectroscopy techniques over a wide frequency and temperature range [9–11], providing compelling evidence of interesting analogies with the dielectric behavior of fragile proton glasses, the electric counterparts of magnetic spin glasses. In particular, three canonical features of a glassy system [12], that is the non-Arrhenius temperature dependence of the dielectric relaxation time, nonexponential relaxation processes, along with nonergodic behavior below a transition temperature, were observed. However, to find more analogies between the percolative behavior of the hydrated protein and that of more conventional glassy systems, in this paper we will focus our attention on one of the main features of a glass-forming system, namely, the nonexponential behavior of the relaxation function [12]. This characteristic feature is well accounted for, in the time domain, by the well known Kohlrausch-Williams-Watts (KWW) equation

$$\phi(t) = \exp[-(t/\tau_{\mathrm{KWW}})^{\beta_{\mathrm{KWW}}}] \tag{1}$$

where τ_{KWW} is a characteristic time scale and β_{KWW} the nonexponentiality parameter. It has been recently shown [13] that $\beta_{\mathrm{KWW}}(T)$ plays the role of a universal parameter for a wide array of glassy systems, especially with respect to its temperature dependence; namely, $\beta_{\mathrm{KWW}}(T)$ shows a crossover from a low temperature region where it increases with temperature to a high temperature region where it is essentially temperature independent. The crossover temperature is $T=1.23T_g$ where T_g is the real glass transition temperature, and, in the low temperature region, β_{KWW} shows a tendency to converge to a value close to $1/3$ at the temperature where the dielectric relaxation time τ diverges ($T=0.9T_g$). This limiting value of β_{KWW} seems to be ubiquitous in very different glassy systems, ranging from polymer melts to Ising spin glasses [1,13–15], while the scenario made up by the two dynamical regimes below and above the crossover temperature is consistent with the energy landscape picture [16,17] describing the temperature dependence of relaxation and transport properties of glass-forming materials.

In previous work, Lemke and Campbell [3] showed the influence of the phase space topology on the dynamical properties of a very simple spin-glass-like model, where a percolative transition was observed, the frustration was extreme and the topological character of the phase space played a central role. The dynamical behavior of this system could be accurately described using a stretched exponential function, and, as the author suggested, "the model may provide a use-

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ful representation of the evolution of phase space in complex systems when a glassy transition may be considered as a percolation transition in phase space." In this respect, the lysozyme-water system studied here appears to be the natural candidate to verify this hypothesis, given the glassy behavior of the protons percolating on the protein surface. Apart from being of interest in its own right, the possibility of looking at the percolation transition as a glassy transition might have far-reaching biological implications, as the proton mobility is closely linked to the dynamics of the water molecules adsorbed on the protein surface [11,18], and these water molecules are of crucial importance for protein structure, stability, and enzymatic activity [6,19–21]. The lysozyme powder sample used was prepared as described elsewhere [11] at *p*H 7 and hydration level $h=0.26$ g/g (expressed as grams of water over grams of dry protein). The sample was placed within a plane capacitor in a standard cryostat. Details on the sample holder and experimental setup can be found in Ref. [22]. Measurements were made in the temperature interval 230–320 K and in the frequency range 10^{-3}–10^{6} Hz.

In general, the dielectric response of many hydrated systems, when plotted as permittivity versus frequency, shows an anomalous low frequency dispersion (LFD), which occurs mainly in samples with large densities of low mobility charge carriers [23]. One interpretation of such LFD suggests that it could be due, at least partially, to the sample-electrode interface. In this case, it can be represented in terms of lumped circuit elements, by a constant phase angle (CPA) element [24], whose frequency-dependent complex permittivity ϵ_{CPA} can be written as

$$\epsilon_{\mathrm{CPA}}(\omega) = C(j\omega)^{d-1} \tag{2}$$

where C and d are positive constants with $d \leqslant 1$. Another possible contribution to LFD may arise from processes associated with the hopping of charge carriers between localized sites in the sample [23]. The frequency dependence of this contribution cannot be distinguished from that indicated in Eq. (2), but it is possible to state that if d is in the range of $0.8 \leqslant d \leqslant 1$, then the LFD response may be ascribed to charge carrier hopping [25]. Broadband dielectric measurements were performed on a lysozyme sample, as shown in Fig. 1, where real and imaginary components of the total measured electric modulus $M^{\star}=1/\epsilon^{\star}$ are shown. This way of representing the data is totally equivalent to the more conventional permittivity versus frequency one, but it offers the advantage of being more sensitive to conductivity processes, visible as a low frequency relaxation, and less sensitive to polarization phenomena and LFD in the same frequency range (compare Fig. 1 with Fig. 1 of Ref. [11]). Following the fitting procedure described in previous papers [11,22], which gives the solid lines shown in Fig. 1, the frequency dependence of individual contributions to the measured dielectric response can be isolated. Figure 2 shows the value of d appearing in Eq. (2) as a function of temperature. This fitting parameter is constantly above 0.95 for temperatures larger than 240 K, suggesting the likely contribution of percolating protons to the LFD. The relaxation term due to proton displacements on the protein could be isolated from interfacial and conductivity phenomena contributing to the

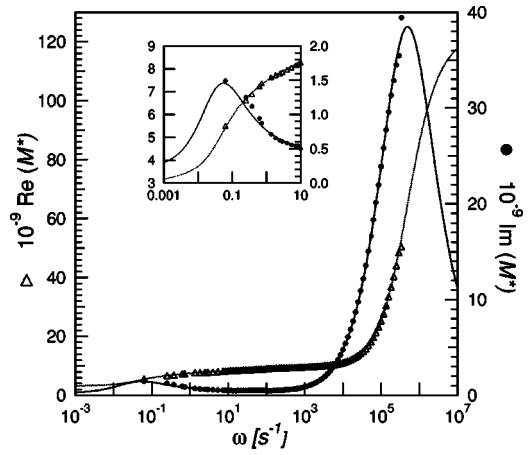

FIG. 1. Angular frequency (ω) dependence of the real (left axis) and imaginary (right axis) components of the measured electric modulus for a lysozyme sample at $T=270$ K, $h=0.26$ g/g. The lines through the data are the result of a fit procedure that takes into account the complex admittance of low frequency dispersion and sample relaxations. The inset shows a detail of the low frequency peak due to sample conductivity.

measured spectra, and the Havriliak-Negami (HN) parameters characterizing it could be obtained [11]. In particular, the dielectric relaxation time τ and the coefficients α and β were estimated as a function of temperature over the range extending from above the glass transition to above room temperature. As shown in Fig. 3, the relaxation time $\tau(T)$ follows closely the Vogel-Fulcher-Tammann (VFT) law (solid line) over the entire frequency and temperature range investigated, and this is typical of glassy systems where the dynamics becomes frozen approaching low temperatures:

$$\tau(T) = \tau_0 \exp\left(\frac{B_T}{T - T_0}\right) \tag{3}$$

with T_0, the temperature at which the relaxation time diverges, equal to 193 K. Evidence linking the glass and percolation transition for a hydrated protein sample is provided by the hydration dependence of the proton relaxation time at constant temperature (see Fig. 4 of Ref. [10]). In that case it was possible to fit experimental data with a VFT modified function where the variable temperature in Eq. (3) has been

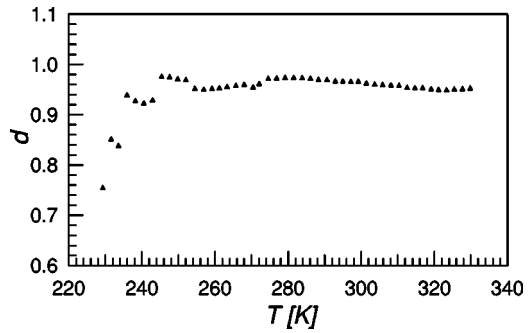

FIG. 2. Temperature dependence of the parameter d appearing in Eq. (2).

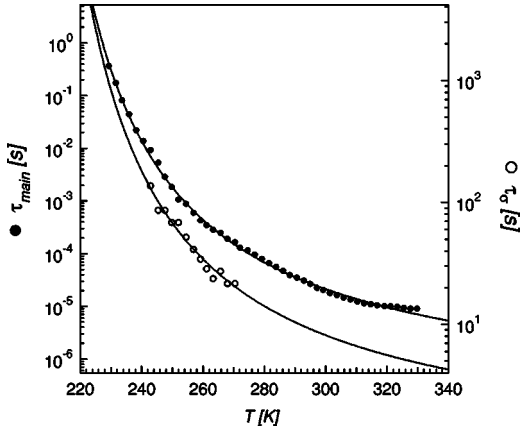

FIG. 3. Temperature dependence of the main relaxation time $\tau_{\text{main}}(T)$ (closed symbols). The solid line through the data represents the fit with a VFT equation ($T_0=193$ K, $\tau_0=1.33\times10^{-7}$ s, and $B_T=544$ K). On the right axis, the temperature dependence of the conductivity relaxation time $\tau_\sigma(T)$ is shown (open symbols). The conductivity relaxation time is defined as the inverse of the angular frequency where a maximum appears in the imaginary component of the electric modulus (see inset in Fig. 1). The limited data range for the conductivity relaxation time is due to the simultaneous presence in the measured spectra of sample dielectric relaxations, interfacial relaxation, and polarization electrode effects that at high temperature does not allow an easy and unambigous determination of the conductivity relaxation time.

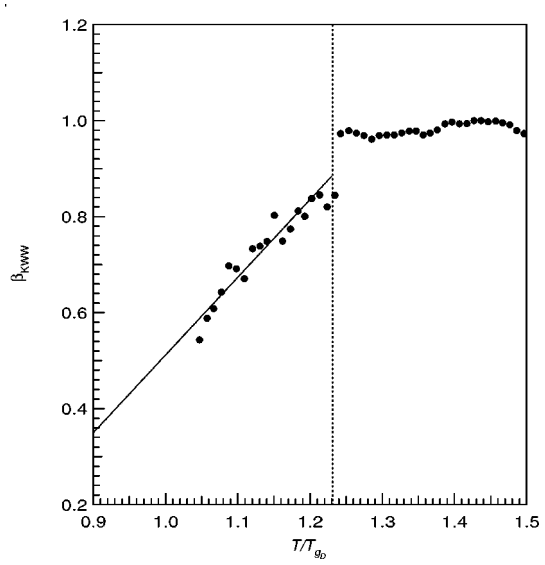

FIG. 4. Temperature dependence of the nonexponentiality parameter β_{KWW}. The solid line is a linear fit of the data in the interval (1.0,1.2). Dashed vertical line is drawn at $T=1.23T_{g_D}$ representing the crossover temperature between two distinct dynamical regimes.

replaced with the variable h, indicating the water content of the sample. Surprisingly, the hydration level at which the relaxation time diverges was found to be equal to 0.16 g/g, i.e., coinciding with the percolation threshold for protons. This result strongly resembles the finding of Lemke and Campbell (see Fig. 6 of Ref. [3] showing the divergence of the relaxation time at the percolation threshold), giving us a strong indication of the link between percolation and glassy behavior. When we replace the variable temperature with hydration level we are still able to induce a kind of "topological" glass transition in the phase space; in other words, $\tau(h)$ diverges when $h\sim h_c$ because the proton dynamics is frozen due to lack of long range connectivity among hydration water molecules. At hydration below h_c, the dynamics of the system of charges over the protein surface becomes nonergodic, in analogy with the behavior of glasses below T_0. Second evidence linking the glass and percolation transitions can be obtained looking at the temperature dependence of the conductivity relaxation time τ_σ (Fig. 3), defined as the inverse of the angular frequency where a maximum in the imaginary component of the electric modulus appears (see inset in Fig. 1) [26]. This temperature dependence can be fitted by the VFT equation, with the same transition temperature as the main sample relaxation time, confirming that the processes of glassy dielectric relaxation and percolative conductivity are indeed closely related. Finally, following the theoretical indication given by Lemke and Campbell [3], we studied the temperature dependence of the nonexponentiality parameter β_{KWW} appearing in Eq. (1). The measurements described here were taken in the frequency domain; while the KWW relaxation is in the time domain; therefore it is not possible to get a direct estimate of β_{KWW} from the data described in the present report. To overcome this problem, it has been shown that the time-domain KWW relaxation function and the frequency-domain HN functions are interconnected [27]. In particular, the relationship among the HN parameters α and β for the main dispersion and the β_{KWW} is given by [27]

$$\beta_{\text{KWW}}(T)=\sqrt[1.23]{\alpha_1(T)\beta_1(T)}. \tag{4}$$

Adopting the procedure used in Ref. [13], we determined, for the hydrated protein investigated, a dielectric glass transition temperature T_{g_D}, as $\tau(T_{g_D})=100$ s. We then plotted $\beta_{\text{KWW}}(T)$ as a function of T/T_{g_D} in Fig. 4. Remarkably, not only is the low temperature limit (extrapolated down to $T=0.9T_g$) of β_{KWW}, 0.35 close to the expected value of 1/3, but the crossover temperature occurs exactly at $T=1.23T_{g_D}$ also as expected in the framework of the energy landscape picture. The gap in the β_{KWW} values at the crossover temperature might be due to a subtle change of shape of the main relaxation: additional data above and below the crossover temperature are probably needed to clarify this point. Nevertheless, the agreement of our findings with both theoretical predictions [3,14,15] and experimental results obtained with other glassy systems [13] not only points to the analogies between a glass and a percolation transition, but also indicates that the approach based on configuration space morphology provides a simple but general description to a wide class of disordered systems. The cluster percolation model, used to explain the dielectric response of hydrated proteins, describes a stochastic system with self-similarity on at least two levels of scale, which is associated with the random walk of protons through the hydrogen-bonded network of water molecules. An approximation for the fractal

dimension D_f of the random walk of protons may be obtained from β_{KWW} [25]:

$$D_f = 3\beta_{\mathrm{KWW}}. \quad (5)$$

Below the crossover temperature, D_f is in the range 1.2–2, while above the crossover temperature D_f is very close to 3, indicating a three-dimensional (3D) Euclidean system, in good agreement with previous studies on the same hydrated protein [25,28]. In particular, the percolative protonic conductivity has been shown to be described as a 2D process with the hydration level close to the critical threshold h_c, and as a 3D process at larger water content. As far as the possible biological implication of these findings is concerned, we notice that a transition between two dynamical regimes around $T=1.23T_{g_D}$ has been observed for the glutamate dehydrogenase enzyme with neutron scattering technique [29] and with Raman optical activity measurements on the lysozyme enzyme [30]. These observations may stress once more the interplay between water dynamics, protein flexibility, and proton transfer as measured by dielectric techniques. This interplay is particularly important in the context of the search for a functional link between the dynamics of the hydrated protein and enzymatic activity. The standard view of the origin of catalytic properties of enzymes focuses on the binding energy differences between the ground state and the transition state arising from arrangements of residues in the active site. There is an alternative view, however, that suggests that protein dynamics might play a role in catalysis [31]: given the interplay between solvent and protein dynamics, the glassiness of migrating protons along the hydrogen bond network at the protein surface might have been envisaged to couple with the intrinsically fast proton transfer process (occurring on the picosecond time scale [32,33]) with the much slower process governing enzymatic activity.

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