Picosecond-Time-Scale Fluctuations of Proteins in Glassy Matrices: The Role of Viscosity

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Through elastic neutron scattering we investigated the fast dynamics of lysozyme in hydrated powder form or embedded in glycerol-water and glucose-water matrices. We calculated the relaxational contribution to the mean square displacements of protein hydrogen atoms. We found that the inverse of this quantity is linearly proportional to the logarithm of the viscosity of the solvent glassy matrix. This relationship suggests a close connection between the picosecond-time-scale dynamics of protein side chains and the solvent structural relaxation.

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The protein internal dynamics is central to biological activity [1–4], to stability through residual entropy [5], and to protein folding [3]. Internal motions occur over a wide time window, ranging from the picosecond window of rapid librations and vibrations, up to the microsecond and millisecond slower motions of protein subunits and subdomains [6]. Such an outstanding dynamical variety results from the structural complexity of these biomolecules. In fact, proteins may assume a huge number of different conformations, also called conformational substates [3], which are points of the $\sim 3N$-dimensional protein potential energy hypersurface, where N is the number of atoms of protein and hydration shell. Within this picture, structural fluctuations are depicted as jumps between conformational substates. Fast relaxations in the picosecond time scale at ambient temperature seem to be crucial for biological activity [1,2,7], as they guarantee a prompt response of side chains to biological events such as, for instance, the approaching of a substrate molecule through the milieu toward the protein surface. Both the onset and the amplitude of these protein picosecond fluctuations, which can be described in terms of rearrangements of side chains to substates of nearly equal energy [2,8], have been proved to be driven by the glassy behavior of the molecular environment just around the protein surface [1,9]. Actually, it is still unclear by what mechanisms such an environment affects the protein dynamics. Several experimental and theoretical investigations have shown that the solvent viscosity plays a pivotal role in affecting both functional, i.e., reaction rates, and dynamical protein properties, such as characteristic vibrational features and relaxation times [10,11]. There exists also evidence that coupling between solvent viscosity and diffusive motions within a protein becomes weaker at elevated viscosity, and as increasingly local or interior protein motions are considered [12].

On these grounds, with the aim at better understanding the intimate relationship between protein dynamics and solvent viscosity, we have investigated via elastic incoherent neutron scattering (EINS) the dynamics of lysozyme, a simple model enzyme, when it is embedded in a different kind of glassy matrices. In this Letter we draw attention to the existence of a noticeable relationship between the amplitude of the mean square displacements (MSD) related to protein picosecond-time-scale fluctuations and the viscosity of the solvent glassy matrix. As the molecular environment we chose simple water, glycerol-water, and glucose-water mixtures, thus measuring, respectively, lysozyme hydrated powders at $0.3h$, $0.4h$ (h denotes grams of D_2O/g of protein), lysozyme in deuterated glycerol-D_2O glassy mixtures (percentage in weight 1:1:h) at $0h$, $0.2h$, $0.42h$, $0.83h$, lysozyme in deuterated glucose-D_2O glassy mixtures (percentage in weight 1:1:h) at $0h$, $0.15h$, $0.41h$, $0.59h$, $0.71h$. In all the samples the water or water-additive matrix forms an amorphous molecular shell around the protein surface. Both dialyzed salt-free chicken egg white lysozyme and solvents have been purchased by SIGMA (St. Louis, MO). Lysozyme was previously dissolved in D_2O to allow the substitution of all the exchangeable hydrogen atoms, which are essentially located at the protein surface. Because of the high incoherent cross section of hydrogen atoms, which are abundantly distributed throughout the biomolecule, EINS allows estimating the atomic MSD of protein nonexchangeable H atoms [5,9], thus sampling internal protein fluctuations. Deuterated solvents were used to minimize their contribution to the revealed signal [13]. Samples were lyophilized and then hydrated in the presence of a saturated NaCl solution of D_2O by varying the equilibration time and determining the D_2O content by weighting the sample before and after the hydration process. All the samples were measured in the temperature range 20–320 K, except for the sample lysozyme in glucose $0h$ for which the highest temperature was 380 K. The measurements were done at the IN13 backscattering spectrometer (Institut Laue-Langevin, Grenoble), with an energy resolution of $\Gamma_R = 4.5$ μeV (half-width at half-maximum) in the wide Q range 0.3–4.4 Å^{-1}. An amount of about 0.5 g of the sample was held in a standard flat aluminum cell with internal spacing of 0.5 mm, placed at an angle of 120°

with respect to the incident beam. The data were corrected to take into account for incident flux, cell scattering, self-shielding, and detector response. Then, the intensity of each sample has been normalized with respect to the corresponding lowest measured temperature. An average transmission of 90%–93% was obtained, then multiple scattering processes have been neglected.

In the inset of Fig. 1 we show the elastic intensity as a function of Q^2 at low and room temperature for a typical sample (lysozyme + glucose and D_2O at $0.15h$). The easiest way to describe in the whole temperature range the elastic intensity behavior is to use the double-well model [8,9], where the protein hydrogen atoms, assumed to be dynamically equivalent, can jump between two sites of different free energy separated by a distance d. Within such a simplified description of the protein energy landscape, the elastic scattering intensity is given by the following equation:

$$I_{\mathrm{el}}(Q) = I_0 e^{-\langle x^2\rangle_G Q^2}\left[1 - 2p_1p_2\left(1 - \frac{\sin Qd}{Qd}\right)\right]. \quad (1)$$

The first term denotes the Gaussian Debye-Waller-like vibrational contribution to I_{el}, and the term in square brackets represents the elastic incoherent structure factor of the two-state model. I_0 is a normalization factor; p_1 and p_2 are the probabilities of finding the hydrogen atom in the ground and excited states, respectively. The experimental data relative to all the samples are fitted quite well by applying Eq. (1), with a value of $d = 1.1 \pm 0.1$ Å. The double-well method allows one to directly calculate the hydrogen total MSD $\langle u^2\rangle = 6\langle x^2\rangle_G + 2p_1p_2d^2 = \langle u^2\rangle_G + \langle u^2\rangle_{2w}$ [8,9]. Figure 1 shows that a linear temperature dependence for $\langle u^2\rangle$ can be found at low T, where the main contribution is provided by the term $\langle u^2\rangle_G$ which is taken into account for the purely vibrational MSD. However, at around 100 K a gradual departure from this vibrational behavior takes place. We can suppose that methyl-bearing side chains are related to such a low-temperature departure. In fact, a recent NMR study indicates that, despite the dynamical onset of a large fraction of methyl groups taking place at ~180 K, a significant portion of methyls contributes to the protein internal dynamics on the subnanosecond time scale already at 100 K [14]. The deviation of MSD from the vibrational trend is due to the emerging contribution related to jumps between the two wells $\langle u^2\rangle_{2w}$, i.e., relaxations faster than experimental energy resolution. The MSD of glass former materials show a similar behavior [15–18]. In these systems $\langle u^2\rangle$ may be decomposed as the sum of a vibrational and a relaxational term, which are analogous, respectively, to $\langle u^2\rangle_G$ and $\langle u^2\rangle_{2w}$. In some glassy systems, such as selenium [15], poly-butadiene [17], glycerol [18], and sugar-water mixtures [18], it has been found that a remarkable relationship exists between the viscosity and the relative relaxational MSD $\langle u^2\rangle_{\mathrm{rel}}$:

$$\log(\eta/\eta_0) = b^2/\langle u^2\rangle_{\mathrm{rel}}. \quad (2)$$

This relation, which has been verified over a wide range of temperatures above the glass transition temperature T_g, has been predicted as well by theoretical models [15,19,20]. Flow in viscous fluids is in general schematized as a sequence of sudden flow events involving several molecules [2]. Equation (2) can be derived on the basis of the key idea that the effective force constant localizing a particle is inversely proportional to the relaxational MSD and directly proportional to the energy barrier height, in analogy with Ref. [20]. The slope b^2 is just the average square distance between the minima that are involved in

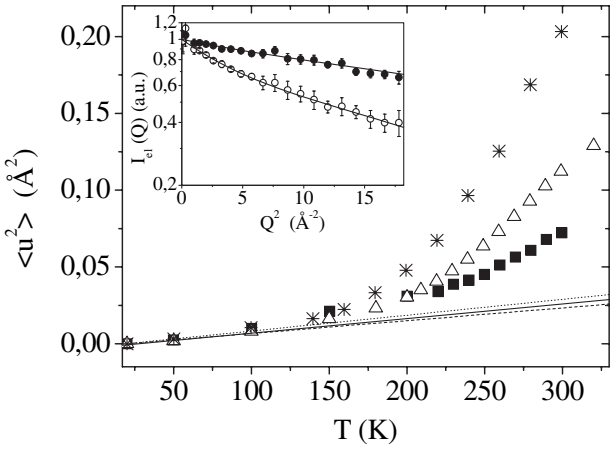

FIG. 1. Total MSD (symbols) and Gaussian contribution [lines, see Eq. (1)] for lysozyme $0.4h$ (stars and dotted line), lysozyme in glycerol $0.2h$ (open triangles and solid line), and lysozyme in glucose $0.15h$ (solid squares and dashed line). Inset: elastic intensity for lysozyme in glucose $0.15h$ at 200 K (solid circles), 310 K (open circles), and corresponding fit to Eq. (1) (solid line).

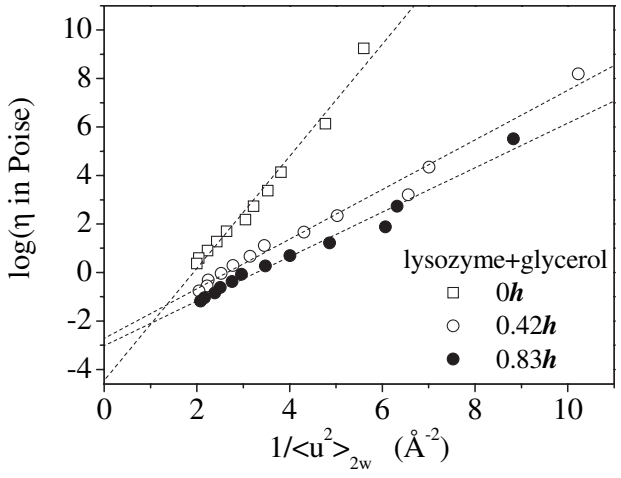

FIG. 2. Logarithm of solvent viscosity vs the inverse of the double-well (relaxational) contribution to total MSD. Dashed lines are fits to Eq. (2).

flow rearrangements. Equation (2) holds for a wide class of amorphous materials, among which the glassy matrices that enclose the protein in the present study. Recently we have seen that the MSD of the protein embedded in a glassy matrix are strictly correlated with those of the pure enclosing environment [9]. Then we may wonder if Eq. (2) can be extended to protein molecules. In Fig. 2 we represent the logarithm of the bulk viscosity of glycerol-water mixtures vs the inverse of the relaxational MSD $\langle u^2 \rangle_{2w}$ of lysozyme in the corresponding glycerol-water matrices. $\langle u^2 \rangle_{2w}$ refers to the protein dynamics on the finite time scale of observation of the experimental energy resolution. Actually, we may expect that the parameters b and η_0 in Eq. (2) can change when we change the experimental resolution. Then one should be aware that the results we show in the present study are all relative to dynamical processes faster than $h/\Gamma_R \approx 150$ ps. We see that a striking linear relationship is observed in the whole investigated temperature range. In this temperature range neither does the viscosity follow a simple Arrhenius-like behavior nor is the $\langle u^2 \rangle_{2w}$ trend linear, then the result we found is highly nontrivial. The meaning of such a relationship, rather surprising for a quite complicated system such as protein in glassy environments, is different with respect to the case of glass materials mentioned above, where viscosity and dynamics of the same system correlate. The protein relaxational MSD show a temperature critical behavior that may be tightly linked with that of bulk solvent viscosity [21], with crucial changes just in proximity of the glass transition, as it happens in many glass-forming systems [22]. There is a general consensus on the fact that it is just the molecular network immediately around the protein surface to drive the fast fluctuations in proteins [1,2]. Indeed, the internal dynamics of proteins is strongly determined by the ability of the surface protein side chains to move [23]. When surface side chains sense a liquidlike molecular environment, they can move and activate protein internal fluctuations. Conversely, when the protein surface is surrounded by a glassy- or solid-state-like molecular matrix, the entire dynamics is locked [23]. If we describe the picosecond-time-scale motions of a particle (a solvent molecule or a protein side chain exposed to solvent) in terms of Brownian diffusion, then the Stokes-Einstein law leads to an inverse relationship between the relevant MSD and bulk viscosity $\langle u^2 \rangle \approx \eta^{-1}$, for a fixed experimental temporal window. Actually, the validity of Eq. (2) for glass formers and proteins in glassy matrices implies that $\langle u^2 \rangle \approx (\log \eta)^{-1}$. This weaker dependence by the solvent viscosity can be due to different reasons. When temperature is lowered, continuous diffusion is replaced by single particle hopping processes, already well above the glass transition temperature [24], and the zero-shear viscosity affects less and less the protein dynamics, which depends increasingly on the experienced short-time nonadiabatic friction [25]. In addition, one has to consider that it is the microviscosity sensed by the particle, possibly different from bulk viscosity, which is related to the corresponding dynamics [26]. In the case of protein side chains such a microviscosity is also strongly affected by preferential hydration effects, by which cosolvents such as glucose and glycerol are preferentially excluded from the protein domain [27], thus giving rise to a surface viscosity with a much weaker increase than bulk viscosity [12]. Besides the quite entangled physical relationship between the protein dynamics and solvent bulk viscosity, the evidence that both solvent and protein MSD are related to bulk solvent viscosity by the same functional dependence, i.e., Eq. (2), indicates that the protein local dynamics is closely coupled with that of the host. This result is in agreement with previous MD simulations [23] and experimental [28] findings.

The relationship we found holds quite well for all the samples we studied, irrespective of the composition of the molecular matrix around the protein, as is shown in the master plot of Fig. 3. This suggests that the similar mechanism couples the motion of protein side chains to flow events in all the molecular matrices around the protein surface. Motions of polar side chains at the protein surface require the shoving of the surrounding molecules, which move via jumplike diffusive motions in the cages of the neighboring solvent molecules [29]. An estimate of the characteristic length of such jumplike diffusive dynamics in different solvents is provided by the b values reported in Fig. 4. The b lengths calculated for lysozyme in glassy matrices are consistent with those estimated for pure solvents, where the MSD vs T trend is available, i.e., glycerol [30], glucose water at 1:0.25 percentage in weight [31], and disaccharides water at 1:1 percentage in weight [18], thus confirming that the b parameter is determined by the solvent dynamical behavior. For low water contents, the b of lysozyme embedded in both glycerol and glucose is higher than for simple hydrated lysozyme powders. Glucose or glycerol molecules have to jump over distances

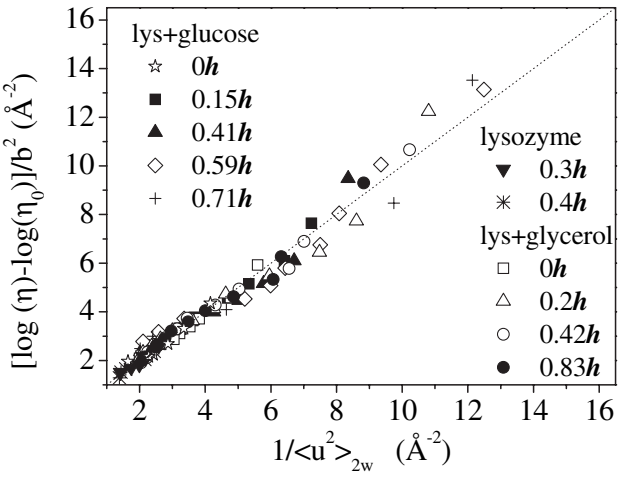

FIG. 3. Check of the validity of Eq. (2) (dotted line) for all the measured samples.

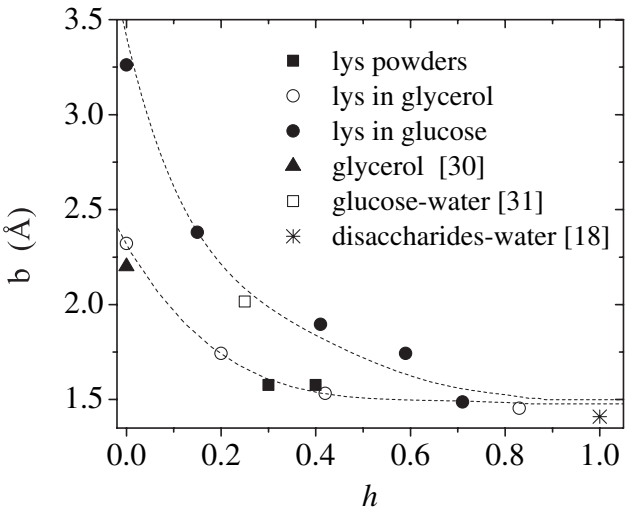

FIG. 4. b values as a function of h. In the case of glucose-water and disaccharides-water mixtures, h refers to the water content with respect to the cosolvents' weight. Dashed lines are guides for the eye.

higher than simple water to allow protein side chains to move. As the energy barrier to be overcome is proportional to b^2 [19,20], when the protein is embedded in glucose or glycerol matrices higher temperatures are necessary to activate the solvent, i.e., protein side chains, dynamics compared to a simple hydration shell. The constrained dynamics in sugars or polyols matrices has already been put in a relationship with their ability to protect biological molecules and cells against stresses induced by potentially detrimental freezing, drying, and heating processes [32,33]. When water content in glassy matrices increases, the viscosity dependent protein dynamics is activated in a similar fashion as in hydrated protein powders, as b for all the systems approaches a common value of 1.5 ± 0.1 Å, which is typical of hydrated protein powders. This behavior is consistent with the well-studied plasticizing action performed by water molecules [2,34].

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