

Glasslike dynamical behavior of the plastocyanin hydration water

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The dynamical behavior of water around plastocyanin has been investigated in a wide temperature range by molecular dynamics simulation. The mean square displacements of water oxygen atoms show, at long times, a t^α trend for all temperatures. Below 150 K, α is constant and equal to 1; at higher temperatures it drops to a value significantly smaller than 1, and thereafter decreases with increasing temperature. The occurrence of such an anomalous diffusion matches the onset of the dynamical transition observed in the protein. The intermediate scattering function of water is characterized, at high temperature, by a stretched exponential decay evolving, at low temperature, toward a two step relaxation behavior, which becomes more evident on increasing the exchanged wave vector q . Both the mean square displacements and the intermediate scattering functions show, beyond the ballistic regime, a plateau, which progressively extends for longer times as long as the temperature is lowered, such behavior reflecting trapping of water molecules within a cage formed by the nearest neighbors. At low temperature, a low frequency broad inelastic peak is observed in the dynamical structure factor of hydration water; such an excess of vibrational modes being reminiscent of the boson peak, characteristic of disordered, amorphous systems. All these features, which are typical of complex systems, can be traced back to the glassy character of the hydration water and suggest a dynamical coupling occurring at the macromolecule-solvent interface.

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I. INTRODUCTION

The question of the glassiness of the hydration water surrounding a protein macromolecule is still an open problem which has recently received great attention in connection also with protein dynamics and functionality [1–5]. With decreasing temperature, crystallization of water belonging to the shells close to the protein surface can be suppressed and an amorphous state for the hydration water can be envisaged [6,7]. On the other hand, the structural and dynamical properties of protein hydration water, markedly deviating from those of the bulk, exhibit a number of interesting and unusual phenomena [2,5,8–11], whose occurrence can be ascribed to the unusual interactions with solvent exposed protein atoms, possibly modulated by the hydrogen bond network dynamics at the interface. In particular, a sublinear trend with an anisotropic character characterizes the molecular dynamics (MD) simulated mean square displacements (MSD's), at room temperature, of water moving in the proximity of the protein surface [3,12]. Such a phenomenon, experimentally supported by neutron scattering [3], is clear evidence for the occurrence of anomalous diffusion of hydration water near the protein surface. In addition, a nonexponential decay in the relaxation of the survival time correlation function in the first hydration layer can be observed [8,9,13]. All these features, which may relate to the unusual organization of solvent water at the interface, are reminiscent of those of other amorphous systems [4].

Furthermore, a MD simulation approach has allowed us to show the existence of an excess of low frequency vibrational modes, over the estimated Debye level, in the density of states of hydration water around plastocyanin (PC) [5]; an experimental confirmation of this result was provided very recently by neutron scattering [14]. Such a vibrational

anomaly, sometimes called the boson peak, is a signature of the disordered, amorphous state, whose origin is still amply debated [15–18] and might be connected to the low temperature thermal anomalies in these systems [19]. Indeed, the presence of a low frequency peak in the dynamical structure factor $S(q, \nu)$, generally located in the 0.1–5 meV range, has been detected, by different experimental and MD simulation approaches, in a large variety of glassy systems [15,20], and, remarkably, in several proteins [21–26]. The simultaneous occurrence of such a vibrational anomaly in both the hydration water and the protein has led us to speculate about a possible interplay between the macromolecule and the surrounding solvent vibrational features [5,14].

On the other hand, there is ample evidence of a significant influence exerted by the hydration water on the macromolecule dynamics. A minimum amount of water (about 0.40 g of water per g of protein) is required to fully activate the protein dynamics and functionality [1,27]. The dynamics of the hydration shells around a macromolecule, characterized by a continuous forming and breaking of hydrogen bond patches and involving the presence of a multiplicity of water states at the macromolecular surface [6], has been hypothesized to be responsible for the existence of conformational substates (CS's) [6], local minima in the potential energy landscape of the protein [28]. Sampling of the CS, which is coupled to the onset of anharmonic protein motions [29], occurs above the dynamical transition temperature [30,31], whose value has been found to be “slaved” to the solvent composition [32]. Generally, such a dynamical transition in proteins is closely reminiscent of that occurring in glasses [33], and it has been suggested that the surrounding water may act as a trigger by injecting its own motions into the protein lateral chains, inducing fast dynamical processes which, in turn, are precursor of slower, collective macromole-

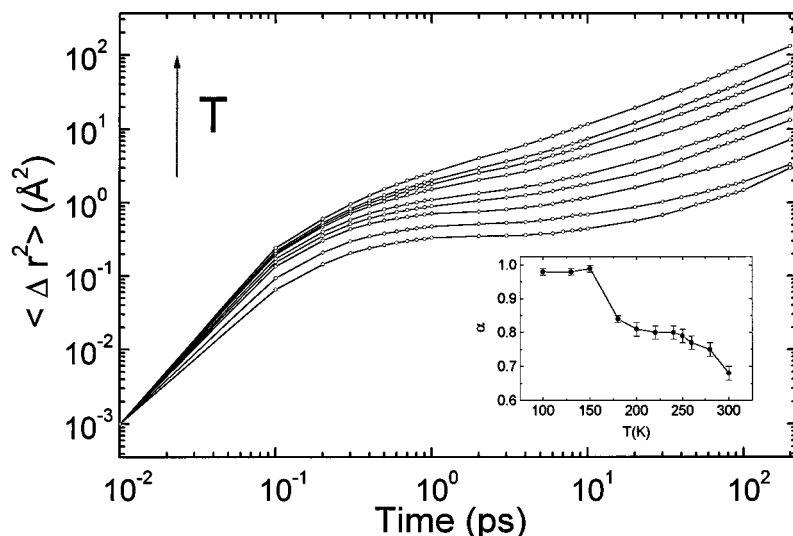


FIG. 1. Mean square displacement $\langle \Delta r^2 \rangle$ as a function of time of water oxygen atoms around PC, for different temperatures: 100, 150, 180, 200, 220, 240, 250, 280, and 300 K from bottom to top. All the data have been obtained by averaging over a 400 ps time interval. Inset: α exponent, as a function of temperature, derived from a fit to Eq. (1) in the 100–200 ps time interval. Solid lines are a guide to eye.

lecular motions [6]. These fast and slow relaxations have often been related to the β and α relaxation, respectively, in the framework of mode coupling theory (MCT), which accounts for the dynamics of glasses [2,34].

In the present paper, the capabilities of MD simulation, which has been shown to be a rewarding tool in providing an accurate and reliable description of the dynamics of protein systems, have been exploited to study the dynamical behavior of the protein hydration water. To avoid the contribution arising from the bulk phase, the hydration level has been restricted to the first hydration shell, at which, however, a complete activation of protein dynamics is reached [27,35]. The MSD, the intermediate scattering function, and the dynamical structure factor of hydration water have been investigated in a wide temperature range, by crossing the temperature at which the dynamical transition of the PC macromolecule takes place [36]. Surprisingly enough, hydration water diffusion, which is found normally below 150 K, becomes anomalous at the temperature at which the onset of anharmonic protein motions takes place and remains anomalous up to room temperature. Such behavior has been analyzed in connection with the spatial and temporal disorder at the protein-solvent interface. The intermediate scattering function and the dynamical structure factor, analyzed as a function of temperature, reveal dynamical anomalies that can be related to the unusual structural and topological organization of the solvent around the macromolecule. Moreover, the overall results point out that the dynamical behavior of hydration water, on one hand, is reminiscent of that of other complex systems, and, on the other, appears to be strictly coupled to the dynamics of the macromolecule.

The paper is organized as follows. In Sec. II, the MD simulated computational methods are briefly reported. Section III is divided into three subsections where the results are presented. In the first one, the MSD and the diffusive properties of hydration water are analyzed. The second one deals with the intermediate scattering function and its relaxation properties. In the third, an analysis of the dynamical structure factor, and in particular of the inelastic region, is presented. In Sec. IV, a discussion of the results is reported in connection also with the protein dynamical behavior. Finally, conclusions are drawn in Sec. V.

II. COMPUTATIONAL METHODS

The MD simulated trajectories of PC, an electron transfer copper protein that takes part in the photosynthetic process, and of the surrounding water molecules, have been computed by the GROMOS87 program package [37], including the single point charge/extended (SPC/E) potential for hydration water [38]. A modified force has been employed to describe the protein-solvent interactions [39]. A covalent bond between the copper ion and each of its four ligands has been introduced to preserve the x-ray structure [40]. PC has been hydrated with 230 water molecules corresponding to 0.39 g of water per g of protein. The energy of such a protein-water system has been minimized by using the steepest descent method. A cutoff radius of 0.8 nm for nonbonded interactions and of 1.4 nm for the long range charged interactions has been applied.

Simulations at several temperatures (100, 130, 150, 180, 200, 220, 240, 250, 260, 280, and 300 K) have been performed by assigning to each atom Maxwellian velocities at the corresponding temperature. A decreasing positional restraining force with a constant ranging from 4000 kJ/(mol nm²) to 250 kJ/(mol nm²) was used during the first 10 ps. The system was coupled to an external temperature bath with a relaxation time of 0.1 ps, a separate coupling for the protein and the solvent being used. After an initial period of 100 ps during which all the systems reach thermal equilibrium, a production run of 400 ps was performed. Configurations of all trajectories and energy values have been saved every 0.1 ps (for other details see Refs. [36,40]).

III. RESULTS

A. Mean square displacements

Figure 1 shows the MSD $\langle \Delta r^2(t) \rangle$ of water oxygen atoms around the PC macromolecule, in the 0–200 ps time interval, for different temperatures in the 100–300 K range. At very short times, a t^2 trend, indicative of a ballistic regime, is registered for the MSD at all the temperatures analyzed. At the lowest temperatures, the MSD curves show a plateau, such a trend pointing out that the motion of the particles is

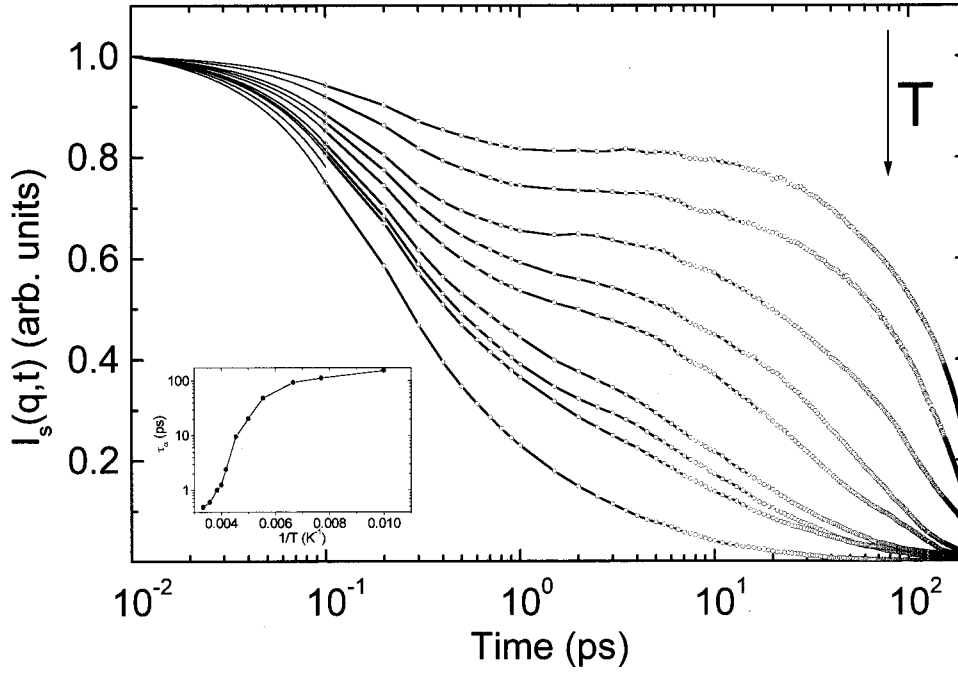


FIG. 2. Intermediate scattering function $I_s(q,t)$ as a function of time of water oxygen atoms around PC, at a fixed q value ($q = 2 \text{ \AA}^{-1}$) for different temperatures: 100, 150, 180, 200, 220, 240, 250, 280, and 300 K from top to bottom. All the curves have been normalized to the one at $t = 0$. Inset: τ_α relaxation time as a function of $1/T$. Solid lines are a guide to eye.

essentially frozen in the corresponding temporal window. The onset of the plateau occurs at about 1 ps, while its temporal extension increases as long as the temperature is lowered, reaching a value of about 10 ps at 100 K. Beyond the plateau, the curves attain a linear trend in the log-log plot at progressively longer times as long as the temperature is decreased. To check that the system has really equilibrated in the temporal window considered, we have extended the analysis up to 1 ns for 100 and 300 K (not shown); the results reveal that no changes in the α values are obtained in agreement with those previously observed by some of the authors [41]. From 100 ps, a power law is able to describe the long time behavior of the MSD at all the investigated temperatures:

$$\langle \Delta r^2(t) \rangle = 4D_{eff}t^\alpha, \quad (1)$$

where D_{eff} is an effective diffusion coefficient which, for $\alpha \neq 1$, depends on the distance traveled; such behavior accounts for the large spread in D values observed in the literature when different temporal windows are considered [9]. On the other hand, Eq. (1) becomes, for $\alpha = 1$, the usual Einstein relationship $\langle \Delta r^2(t) \rangle = 4Dt$ where D is the diffusion coefficient. The corresponding values of the α exponent, extracted from the slopes of the curves by a best fit procedure, are plotted as a function of temperature in the inset of Fig. 1. At temperatures below 150 K, α is constant and practically equal to 1, such behavior being indicative of normal, Brownian, diffusion. The corresponding diffusion coefficient ranges from 0.005 to 0.020 $\text{\AA}^2/\text{ps}$ for temperatures from 100 K to 150 K. We note that these values are much smaller than that obtained, at room temperature, when all the water molecules in a fully hydrated PC system are taken into account [12]. On the other hand, the possibility that the heterogeneity of the water population could have some relevance on the diffusive properties cannot be ruled out. Upon increasing the temperature, a rapid drop followed by a progressive lowering of α is observed. We remark that

an α exponent smaller than 1 is indicative of the occurrence of anomalous diffusion and implies that a diffusion coefficient value cannot be extracted in an unequivocal way [12]. It is worthy of note that anomalous diffusion is not a peculiarity of low hydrated systems; actually, it takes place also in a fully hydrated PC system, at room temperature, when water molecules moving close to the macromolecular surface are taken into account. Indeed, an α value less than 1 was also obtained for myoglobin hydration water, at room temperature, by neutron scattering spectroscopy [3].

B. Intermediate scattering function

The self-intermediate scattering function $I_s(q,t)$, which corresponds to the self part of the density-density autocorrelation function or, equivalently, to the spatial Fourier transform of the Van Hove correlation function, has been directly calculated from the MD trajectories of the water oxygen atoms through the relationship

$$I_s(q,t) = \frac{1}{3}N \left\langle \sum_{i=1}^N \exp\{i\mathbf{q} \cdot [\mathbf{R}_i(t) - \mathbf{R}_i(0)]\} \right\rangle, \quad (2)$$

where N is the total number of water oxygen atoms in the sample, $\mathbf{R}_i(t)$ is the position vector of the i th atom at time t , and the angular brackets $\langle \rangle$ denote averaging over both the water ensemble and the exchanged momenta \mathbf{q} having the same modulus q , to take into account the anisotropic effects. A q value of 2 \AA^{-1} , approximately corresponding to the first maximum in the structure factor $S(q)$, has been used. Such a function has the advantage that it can easily be measured by neutron scattering experiments.

The self-intermediate scattering function $I_s(q,t)$, obtained from Eq. (2), for all the investigated temperatures is shown in Fig. 2. At high temperature, $I_s(q,t)$ is characterized, after the initial ballistic regime, by a single step time relaxation behavior. Upon decreasing the temperature, a small shoulder begins to appear at intermediate times this effect becoming

TABLE I. Stretched exponential fitting parameters of the intermediate scattering function of water oxygen atoms around PC as extracted by a fit to Eq. (3) in the 100–200 ps time interval.

$T(K)$	β	A
300	0.33	0.90
280	0.34	0.95
260	0.36	0.93
250	0.39	0.93
240	0.40	0.91
220	0.50	0.94
200	0.62	0.93

more and more pronounced until a plateau is established at the lowest temperatures. In the long time limit, all the curves eventually decay to zero.

A characteristic relaxation time τ_α can be singled out as the time at which $I_s(q, t)$ has decayed to $1/e$ of its initial value [42]. The calculated τ_α values, plotted as $\ln(\tau_\alpha)$ vs $1/T$ in the inset of Fig. 2, reveal a non-Arrhenius behavior. However, for temperatures above 180 K, the almost linear trend could give some hints about the activation energy for the corresponding process.

For times longer than τ_α , and for temperatures above 200 K, a stretched exponential or Kohlrausch-Williams-Watts function

$$I_s(q, t) = A e^{-(t/\tau_\alpha)^\beta} \quad (3)$$

can reliably describe the temporal evolution of $I_s(q, t)$. The A and β values, as extracted by a best fit procedure from the curves of Fig. 2, are reported in Table I. The preexponential parameter A is characterized by a slight dependence on temperature, while the β exponent increases as long as the temperature is lowered, almost doubling its initial value at the lowest temperature. This indicates that the stretched relaxation behavior becomes more marked as long as the temperature is increased.

At the lowest temperatures, the $I_s(q, t)$ curves, from the end of the plateau, can be appropriately described by the power law, usually called the von Schweidler law,

$$I_s(q, t) = B - C \left(\frac{t}{\tau_\alpha} \right)^b, \quad (4)$$

where B, C , and b are fitting parameters [3,34] (the fitting curves practically superimpose on the experimental ones and are not shown in Fig. 2). These parameters are reported in Table II and reveal a weak temperature dependence; in particular, all these values, with the exception of the C parameter at 130 K, show a slight increasing trend with decreasing temperature.

At low temperatures, slight oscillations, clearly distinct from the noise background, can be observed in the $I_s(q, t)$ plots, starting from about 2.5 ps. Oscillatory behavior in $I_s(q, t)$ has been recently interpreted as a time domain manifestation of the boson peak [43,44]. Nevertheless, great caution has been suggested in attributing a physical relevance to such oscillations, since they could arise from some simulation artifacts; e.g., a finite size effect arising from a distur-

TABLE II. Power law fitting parameters of the intermediate scattering function of water oxygen atoms around PC as extracted by a fit to Eq. (4) in the 1–100 ps time interval.

$T(K)$	B	C	b
180	0.70	0.36	0.69
150	0.77	0.40	0.73
130	0.78	0.38	0.76
100	0.82	0.46	0.77

bance that propagates through the system leaving and reentering the boundaries of the periodic box at the sound velocity [45]. It should be remarked, however, that in the present study we do not apply any kind of boundary conditions.

To get some hints on the dynamical behavior over different spatial scales, the intermediate scattering function $I_s(q, t)$ has been calculated, at 200 K, as a function of q and it is shown in Fig. 3. From this figure, it can be remarked that the decay to zero of the $I_s(q, t)$ curves becomes slower when smaller q values are taken into account; i.e., when progressively more extended spatial regions are considered. Moreover, the presence of a plateau becomes more and more evident with increasing q . Again, for times longer than the relaxation time τ_α (calculated by the same procedure reported above), the $I_s(q, t)$ curves follow a stretched exponential decay and they can be properly fitted by Eq. (3). The inset of Fig. 3 shows the extracted β exponent as a function of q . This parameter is almost 1 at the smallest q value, while it continuously decreases on increasing q up to 2 \AA^{-1} ; thereafter it remains almost constant.

C. The dynamical structure factor

The incoherent dynamical structure factor $S(q, \nu)$ of PC hydration water can be directly derived by the temporal Fourier transform of $I_s(q, t)$:

$$S(q, \nu) = \frac{1}{2\pi} \int_{-\infty}^{+\infty} dt \exp(-2\pi i \nu t) I_s(q, t). \quad (5)$$

In calculating $S(q, \nu)$ we used numerical fast Fourier transform, and the slowly decaying tail of $I_s(q, \nu)$ was multiplied by a Gaussian damping envelope, in agreement with Ref. [46], to overcome spurious effects due to truncation. $S(q, \nu)$, at a $q = 2 \text{ \AA}^{-1}$ wave vector, is shown in Fig. 4 for the different temperatures analyzed. The spectra of Fig. 4 can be generally interpreted in terms of three main components, i.e., the elastic, the quasielastic, and the inelastic ones, whose relative intensity depends on the temperature. By restricting our analysis to the inelastic region, we note that, at temperatures up to 180 K, a broad inelastic bump appears clearly visible in the low frequency region, peaking at about 1.3 meV. By increasing the temperature, this peak becomes less and less distinct due to the increasing intensity of the quasielastic contribution. The inelastic nature of this peak is supported by an observed q^2 variation of its intensity [5].

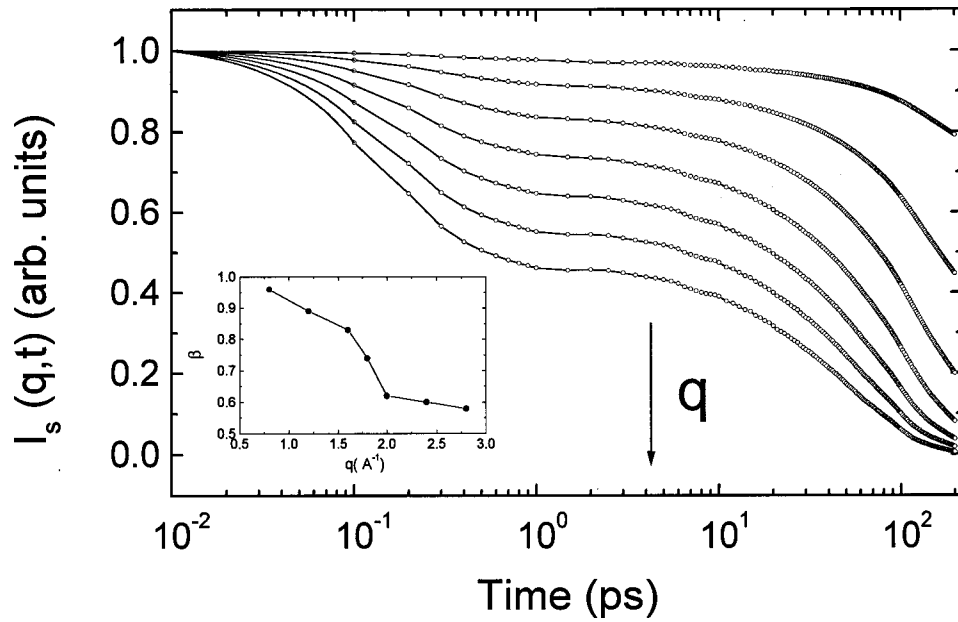


FIG. 3. Intermediate scattering function $I_s(q,t)$ as a function of time of water oxygen atoms around PC, for different q values (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8 \AA^{-1} , from top to bottom) at 200 K. Inset: β exponent, as a function of q extracted from a fit to Eq. (3), in the long time tail (10–200 ps) of $I_s(q,t)$. Solid lines are a guide to eye.

Generally, the frequency dependence of the dynamical structure factor in the inelastic region can be cast in the form $\sim (1/\nu)n(\nu,T)g(\nu)$, where $n(\nu,T)$ is the Bose factor and $g(\nu)$ is the density of states. In the Debye approximation and at low frequency, $g(\nu)$ turns out to be proportional to ν^2 and $n(\nu,T)$ is approximated by $k_B T/h\nu$; accordingly, a constant trend as a function of frequency is expected for the dynamical structure factor. Therefore, the observed peak represents an excess of vibrational states over the flat Debye level (boson peak).

To confirm this finding, the density of states $g(\nu)$ of PC hydration water has been derived from the spectral density $C_{vv}(\nu)$ of the velocity autocorrelation function $C_{vv}(t)$:

$$g(\nu) \sim C_{vv}(\nu) = \frac{1}{2\pi} \int_{-\infty}^{\infty} dt \exp(-2\pi i \nu t) C_{vv}(t). \quad (6)$$

The ratio $g(\nu)/\nu^2$ of the PC hydration water, shown for some temperatures in the inset of Fig. 4, reveals at 100 K a bump located around 1.3 meV which shifts to a slight lower frequency at 180 K; conversely, at higher temperatures (220 and 300 K), this peak essentially disappears. Therefore, the presence of such a peak in the density of the states, which is located almost at the same position observed in $S(q,\nu)$, provides further support for the presence of a boson peak in the hydration water below the dynamical transition of the protein.

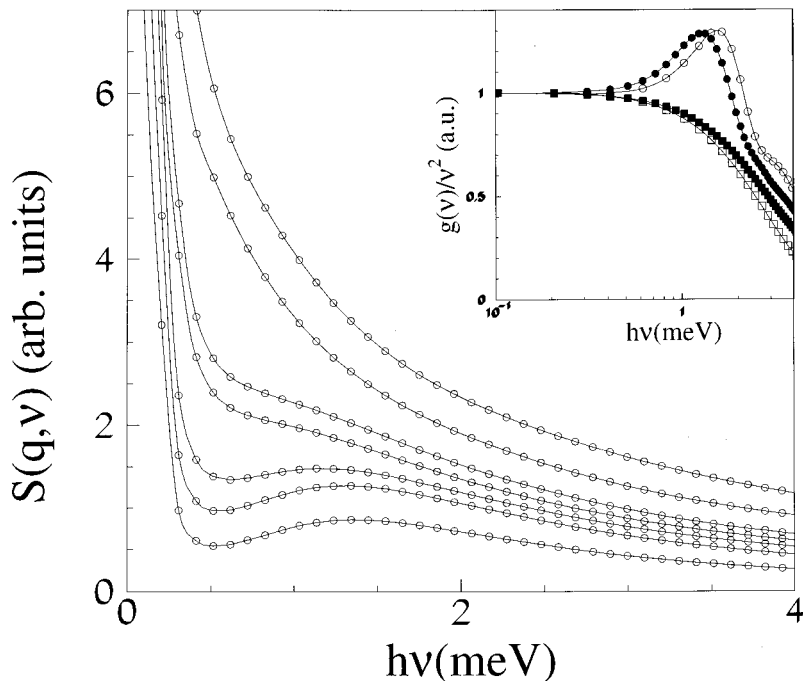


FIG. 4. Incoherent dynamical structure factor $S(q,\nu)$ as a function of energy at a fixed q value ($q=2 \text{ \AA}^{-1}$) of the water oxygen atoms around PC for different temperatures: 100, 150, 180, 200, 220, 260, and 300 K from bottom to top. Inset: Density of states [Eq. (6)] divided by ν^2 as a function of energy at 100 K (white circles), 180 K (black circles), 200 K (black squares), and 220 K (white squares). Solid lines are a guide to eye.

IV. DISCUSSION

The dynamical behavior of hydration water around PC reveals some properties which, if on the one hand they markedly deviate from those of the bulk, on the other are closely reminiscent of those observed in many disordered systems. Moreover, the dynamics of the PC hydration water appears to evolve with temperature in a way that suggests an interplay with the protein dynamics. Actually, at low temperature, at which only localized vibrational motions of the macromolecule are active, the diffusion of hydration water is almost normal [$\alpha = 1$ in Eq. (1)]. Conversely, at higher temperature, and in particular just at the glass transition temperature of PC [36], at which the onset of anharmonic stochastic fluctuations takes place [47], anomalous diffusion ($\alpha < 1$) appears and persists up to room temperature. Such behavior is particularly relevant also in connection with the fact that MD simulations of supercooled bulk water, based on the same potential model for water, do not reveal any diffusive anomaly in a wide temperature range [44,48]. We note that, as a direct consequence of the presence of anomalous diffusion, an unequivocal value for the diffusion coefficient cannot be determined and some caution in the analysis of water mobility around biomolecules is required, as remarked in Ref. [12]. The presence of anomalous diffusion was previously reported to occur in PC hydration water at room temperature [12]. At that time, it was suggested that the spatial disorder connected to the roughness of the protein surface, as well as the temporal disorder due to a distribution of jumping times, or to a concerted diffusion of particles trapped in the cages formed by the nearest neighbors, could be in some way responsible for such a phenomenon [4,9]. The present results, which extend the previous analysis to cover a wider temperature range spanning well below and above the protein dynamical transition temperature, can provide us with insight about the mechanisms underlying the diffusive processes.

Generally, normal diffusion can be described by a Gaussian shape for the probability distribution $P(r,t)$ of finding a tagged particle at r at the time t , when starting at the origin at $t=0$, and the propagator takes the form

$$P(r,t) = \left(\frac{1}{4\pi Dt} \right)^{d/2} e^{-dr^2/2Dt}, \quad (7)$$

where d is the dimensionality of the spatial region in which the diffusion process occurs and D is the diffusion coefficient of the particles. A Gaussian propagator results in the well-known Einstein relationship which connects the long time limit MSD and D . For particles diffusing on a fractal surface, such as the solvent accessible surface of many globular proteins, including PC [49], the propagator $P(r,t)$ can be expressed by [9,50]

$$P(r,t) = t^{-d_s/2} \xi^\beta e^{-a_1 \xi^\gamma}, \quad (8)$$

where d_s is the spectral dimensionality of the fractal surface; $\xi = rt^{-d/2d_f}$ is the scaling variable assumed to be greater than 1, and d_f is the fractal dimensionality of the surface; and β and γ are

$$\beta = \frac{d_f(d_f - d_s)}{2d_f - d_s}, \quad \gamma = \frac{2d_f}{2d_f - d_s}, \quad (9)$$

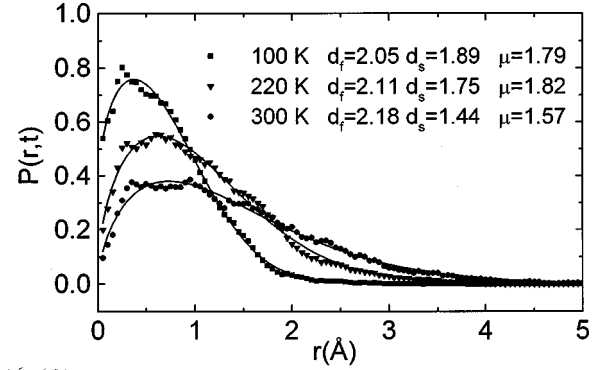


FIG. 5. Probability distribution $P(r,t)$ as a function of the distance traveled at the fixed time $t=3$ ps, calculated from the MD simulated trajectories of water oxygen atoms around PC for three different temperatures: 100, 220, and 300 K. The continuous lines are the best fit curves obtained at the same level of accuracy (as evaluated by χ^2 test) by using Eqs. (8) and (11).

from which, by assuming $d_s \neq d_f \neq d$, it comes out that $\beta \neq 0$ and $\gamma \neq 2$ and then the propagator of Eq. (8) is no longer Gaussian [50]. In the framework of this model, the following expression for the long time limit MSD can be worked out:

$$\langle \Delta r^2 \rangle \sim t^{d_s/d_f}. \quad (10)$$

According to this expression, the observed trend of the α exponent, shown in the inset of Fig. 1, can be traced back to an evolution with temperature of the d_s and/or d_f parameters. Conversely, a spread of the waiting time between successive jumps of the diffusing particles could give rise to a type of temporal disorder which can be described by the following form for the propagator [9,50]:

$$P(r,t) = t^{-(\mu-1)/2} \xi^\beta e^{-b_1 \xi^\gamma}, \quad (11)$$

where μ is a parameter related to the time distribution, $\xi = rt^{-(\mu-1)/2}$ is the scaling variable, larger than 1, and β and γ are given by

$$\beta = \frac{2-\mu}{3-\mu}, \quad \gamma = \frac{2}{3-\mu}. \quad (12)$$

Again, the propagator of Eq. (11) deviates from a Gaussian for $\mu \neq 2$. In this framework, the long time limit MSD can be expressed by

$$\langle \Delta r^2 \rangle \sim t^{\mu-1}. \quad (13)$$

Accordingly, the trend of the α exponent shown in the inset of Fig. 1 would involve a corresponding evolution of the μ parameter with temperature.

On the other hand, knowledge of the MD simulated trajectories allows us to determine this probability distribution $P(r,t)$ and therefore to compare it with those corresponding to the theoretical models [Eqs. (8) and (11)]. Such a probability distribution, calculated at a fixed time $t=3$ ps, as a function of the traveled distance r is shown in Fig. 5, for three different temperatures (100, 220, and 300 K). The broadening with temperature of the $P(r,t)$ curves indicates that the mobility of water molecules is enhanced by increasing the temperature. These data can be reproduced, at almost

the same level of accuracy, by both Eqs. (8) and (11) (see the continuous lines and the legend of Fig. 5). This means that, from a formal point of view, both the models can adequately describe the diffusive process and its evolution with temperature in our system. The values of the fitting parameters, for both cases, are also reported in Fig. 5. We note that, while d_f decreases, both d_s and the μ parameters show an increasing trend on lowering the temperature. If we look at these values in connection with the previous analysis, it appears that, for both the models, the propagator closely approaches a Gaussian at the temperature of 100 K, while it markedly deviates from it at 300 K; such behavior implies that the physical mechanism underlying the diffusive process can be deeply affected by the temperature. If we assume that the diffusion is driven by the spatial disorder at the interface, a variation of both the d_f and d_s parameters with temperature should be conceived. However, this is not consistent with the fact that both the fractal and spectral dimensionalities of systems similar to ours are expected to be substantially independent of temperature [51,52]. On the other hand, under the assumption that the temporal disorder could be at the basis of the observed diffusive process, the evolution of the parameter μ with temperature should be taken into account. In this respect, we remark that an analysis of the residence times of water, at room temperature, around PC [13] showed a power law distribution of these times; such a distribution having been described in terms of the above mentioned temporal disorder model [4]. Generally, this behavior reflects a spread of the times that water molecules spend at the various protein sites, possibly arising from the heterogeneity of the environment, which, in addition, undergoes stochastic fluctuations. While the spatial heterogeneity, which represents an intrinsic property of the protein system, should not change with temperature, the fluctuations connected to the dynamical behavior of the macromolecule could be drastically affected by the temperature. Actually, above the glass transition temperature, the enhancement of the macromolecular flexibility, involving larger atomic displacements, could determine a larger spread in the position of water molecules if compared to that occurring at low temperature at which only harmonic, small amplitude, motions take place in the macromolecule. On such a basis, the temporal disorder could be a more likely candidate to describe the occurrence of anomalous diffusion in our system. The fact that anomalous diffusion is not registered in supercooled bulk water [44,48], as already mentioned, is strongly indicative of a modulation of the diffusive behavior by the interaction of water molecules with the protein atoms at the interface.

Additional insights into the dynamics of the hydration water can be obtained from analysis of the intermediate scattering function. We have shown that, at high temperature, this quantity follows a stretched exponential relaxation decay, which is a common feature of disordered systems [34]. Such relaxation behavior is particularly remarkable when it is considered in connection with the nonexponential relaxation trend of other related chemophysical parameters observed in proteins. For example, the kinetics of CO rebinding to myoglobin (1 of its biological substrates) is nonexponential in time and it deviates from Arrhenius behavior [47]. This feature, which reflects a dynamics ruled by a non-Markovian statistics and characterized by several time scales [53], can

be traced back to a rugged energy landscape and then to the existence of a huge amount of CS, whose topological organization depends on the solvent [32,47]. A similar description can be invoked for the energy landscape of the solvent in close contact with the protein macromolecule. Such a picture is supported by a recent MD simulation study on a glass-forming liquid, which suggests that the appearance of a stretched exponential relaxation could arise from the existence of many nearly isoenergetic local minima whose exploration is strongly dependent on temperature [54]. In light of these results, and in connection with the fact that the protein and the solvent exist in a sort of symbiosis, the energy landscape of both systems should be conceived as a global entity [55].

At low temperature, the $I_s(q,t)$ curves reveal a two step relaxation behavior and the appearance of a plateau, depending on the temperature, reflects a slowing down of the water dynamics. Such behavior, which has also been observed in supercooled bulk water [44], could be due to a sort of trapping of the molecules in a cage formed by the nearest neighbors. In other words, the water molecule could be rattling in the cage until it reaches sufficient energy to overcome the energy barrier or to find a vacancy outside the cage [34]; such a hopping process of a single molecule may require a simultaneous rearrangement of a large number of particles surrounding it. An estimation of the cage radius r can be obtained, in the framework of the MCT [34], from the A preexponential factor in Eq. (3) through the expression $A = e^{-r^2 q^2/3}$. Actually, if we use for q a value of 2 \AA^{-1} , we obtain a cage radius r of $\sim 0.3 \text{ \AA}$, a value which is consistent with the effective hard core diameter of water [3,4,44]. Moreover, such a finding is in agreement with the behavior observed at different length scales. Actually, when low q values, corresponding to a length scale larger in comparison with the cage dimension, are taken into account, the plateau disappears and the relaxation converges toward simple exponential decay ($\beta=1$); conversely, for shorter length scales, the stretched exponential behavior persists.

On the other hand, the dynamics that characterizes the escape from the cage, occurring before the complete establishment of the long time relaxation, can be described by the van Schweidler law [see Eq. (4)] [34]. Such an approach allows us to independently evaluate the cage dimension. Actually, the B parameter in Table II can be related to the cage radius through the expression $B = (1 - q^2 r^2)$ from which we can extract r values ranging from 0.25 to 0.28 \AA , in satisfactory agreement with the previous estimate. Concerning the b parameter, whose values are also reported in Table II, we note that it essentially does not depend on temperature, such behavior being consistent with the hypothesis that b is a characteristic quantity of the system, related to its spatial organization [34]. The fact that, in PC, b is found to be larger than in the myoglobin hydration water ($b=0.55$) [3] could reflect the different spatial arrangement of the solvent around the two macromolecules characterized by a different secondary structure.

All the properties analyzed show that the dynamics of PC hydration water, whose features appear to be strongly affected by the dynamics of the macromolecule, displays close analogies to that of other amorphous systems. In this respect,

we remark that our data on the intermediate scattering function show a close similarity with those obtained by MD simulation on supercooled bulk water whose dynamics was also described in the framework of the MCT [44,48,56].

A further support for the glassy character of protein hydration water is provided by the occurrence of the low frequency peak in both $S(q, \nu)$ and the density of states. Such a peak, preliminarily presented in Ref. [5], is closely reminiscent of the low frequency vibrational anomaly typical of glassy materials, usually called the boson peak [57,15]. It is worthy of note that the existence of such a peak in protein hydration water was first put into evidence by MD simulation [5], and recently confirmed by a neutron scattering study [14]. By the way, the MD analysis revealed the presence in the dynamical susceptibility of the water translational band in the hydration water around PC [5]; such a band appears similar to that observed in MD simulated bulk water [56] as well as in myoglobin hydration water as investigated by neutron scattering [3].

The origin of the boson peak observed in glasses has often been attributed to the topological disorder of the system [16]. It has been hypothesized that it could originate from structural correlations over an intermediate range scale, associated with localized excitations which give rise to a strong scattering of acoustic phonons. In other words, it is believed that density fluctuations on domains of medium order, due to the intrinsic heterogeneity of glassy systems, could be responsible for the strong scattering of acoustic localized excitations and then lead to the observed enhancement of low frequency modes [16,51,58]. It could then be speculated that the concerted motions of particles trapped in the cages, which may involve a large region, might be connected to the low frequency vibrational excess of modes appearing in the dynamical structure factor. Actually, in the Debye approximation, a relationship between the peak frequency ν_0 and the correlation length ξ characterizing this intermediate range order has been postulated [16]:

$$\nu_0 = \frac{v_s}{2\xi}, \quad (14)$$

where v_s is the sound velocity. Even if it is not easy to get a reliable estimate of the acoustic mode propagation velocity in a hydrated protein system, a value for v_s ranging between 2000 and 4000 m/s for proteins at different hydration levels has been suggested [6,59]. By using such values, together with a boson peak energy $h\nu_0 \sim 1.5$ meV, Eq. (14) provides a correlation length ξ between 7 and 14 Å. This value could be consistent with the presence of some collective motions whose spatial extension covers a large part of the protein macromolecule. We would like to focus our attention on the fact that a boson peak, centered at about the same frequency, was detected by MD simulation in both the protein macromolecule and the surrounding solvent [5,60], this result having been confirmed by recent neutron scattering results [60,14]. As already mentioned, the presence of such an excess of modes appears to be a sort of universal feature of proteins, of their structure or folding. Our results suggest that a similar universality might also be invoked for the boson peak of hydration water around proteins. The intimate con-

tact between the solvent exposed protein residues and the water molecules could give rise to a sort of vibrational coupling between the two systems. The density fluctuations of the whole system, shown to be of long range and collective character and of biological relevance, would primarily arise from the fact that solvent water could “inject” its dynamics into the protein sidechains [2], whose librations have been shown to be strictly dependent on the hydrogen bond network restructuring dynamics occurring at the protein-solvent interface [61].

The strict interplay between the protein and the solvent is further supported by the presence of $1/f^\alpha$ noise in these systems. Actually, we have shown the appearance of $1/f^\alpha$ noise, with the same value for the α exponent, in the potential energy fluctuations of both PC and the surrounding solvent [62]. Generally, $1/f^\alpha$ noise, a feature of dissipatively coupled, nonlinear systems [63], is a manifestation in the temporal domain of complexity and it could be connected in some way to the density of states [64]. In this framework, it can be suggested that a sort of exchange of modes between the protein and the surrounding solvent could occur. On the other hand, since $1/f^\alpha$ noise has been related to the existence of a multiplicity of metastable states [63,65], an interplay between the macromolecular and solvent energy landscape could again be envisaged. All these aspects, which should be analyzed also in connection with the functional role played by the macromolecule, require further investigation.

V. CONCLUSIONS

Hydration water around PC exhibits dynamical properties whose features are closely reminiscent of those observed in glassy systems. The unusual interactions with protein atoms are expected to be responsible for the observed dynamical behavior of the solvent. The occurrence of hydration water anomalous diffusion, which has been traced back to the presence of a temporal disorder at the interface, is observed at the same temperature at which the protein dynamical transition takes place; such behavior is probably correlated to the enhanced macromolecular stochastic motions above the glass transition temperature. Further evidence of a close connection between the solvent and the protein dynamics is provided by the stretched exponential relaxation of the water intermediate scattering function above the protein dynamical transition temperature. In addition, the dynamical behavior of hydration water at the interface indicates the presence of structural correlations involving different length scales. At low temperature, hydration water molecules appear to be trapped in cages formed by the nearest neighbors, and a simultaneous rearrangement of several particles is required to activate the hopping events at the basis of the diffusive and relaxation processes. Moreover, concerted motions of particles, characterized by length scales spanning a quite large portion of the system, have been hypothesized to be at the origin of the low frequency vibrational excess of modes observed in the hydration water. The presence of such an excess of modes, when considered in connection with the similar vibrational anomaly shown by the protein, might suggest a possible, intriguing, dynamical coupling between the macromolecule and the surrounding solvent.

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