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Molecular-dynamics simulation evidences of a boson peak in protein hydration water

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Molecular-dynamics (MD) simulations of a hydrated protein system, performed at different temperatures, allowed us to point out anomalies in the low-frequency spectral features of hydration water. The dynamical structure factor calculated from the water MD trajectories shows, below 180 K, a broad inelastic peak in the low-frequency region (~ 1.3 meV) reminiscent of the so-called boson peak observed in amorphous disordered materials. Additional evidence of this boson peak is provided by the calculated vibrational density of states. The behavior of the simulated dynamical susceptibility at various temperatures was found to be very similar to that recently obtained by scattering experiments in similar systems. Possible implications of these anomalies in the protein-solvent coupling mechanisms are briefly discussed. [S1063-651X(98)50806-9]

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A full understanding of the interplay among the structure, the dynamics, and the functionality of proteins is a crucial problem in molecular biophysics; this debated aspect being, in turn, tightly joined to the mechanisms governing the coupling of the biomolecule to the solvent. In fact water, which is the natural environment to biological molecules, appears to be essential, at least in a minimum amount, in determining the biological activity [1,2]. Therefore the attention of most recent studies has been drawn by the protein-water interface dynamical features, where a number of interesting and unusual phenomena occurs [3]. In particular, crystallization at usual freezing temperatures is suppressed in hydration water [4,5], which, on the other hand, seems to show an amorphous character [5]. Moreover the solvent mobility near the protein surface is somewhat restricted and shows an anomalous and anisotropic behavior [3]. Conversely, hydration water properties have been indicated as contributing to the complex character displayed by protein macromolecules [5,6]; i.e., the existence of a huge amount of nearly isoenergetic conformational substates [6]. Above a critical temperature, where a departure of the atomic mean-square displacements from the harmonic temperature dependence occurs, addition of water may be responsible for the onset of the protein dynamical behavior, which is characterized by fast β and slow, collective, α relaxations [7,8]. In particular, hydration water dynamics has been hypothesized to be coupled to the dynamics of the protein polar lateral chains through the injection into the latter of fast excitations which could, in turn, trigger more extensive collective motions of the protein [7,9]. Therefore, a detailed knowledge of the hydration water dynamical features is essential for a complete understanding of the mechanisms regulating the coupling between the temporal and spatial behavior of both the protein and the surrounding water. Molecular-dynamics (MD) simulation could be a rewarding tool to both providing accurate microscopic information on the macromolecule-solvent system and making accessible properties that sometimes cannot be measured with current experimental techniques. In the present study, MD simulation capabilities have been exploited to get some insight about the low-frequency behavior of the hydration water of a widely studied protein [3]. Our results are consistent with the presence, in the protein hydration water, of a typical spectral glassy anomaly that can be reconducted to the so-called boson peak [10–12].

The main contributions to the self-intermediate scattering function, the dynamical structure factor, the dynamical susceptibility, and the vibrational density of states were calculated at different temperatures directly from the MD trajectories of the water molecules forming the hydration layer of plastocyanin (PC), an electron transfer copper protein that takes part in the photosynthetic process. In order to achieve the desired hydration degree of $h=0.40$ g water/g protein, 230 water molecules were extracted from the fully hydrated system. The Groningen molecular simulation (GROMOS) force field [13] was employed to describe the protein system interactions including the single point charge/extended (SPC/E) model for the hydration water [14]. Cutoff radii of 8 Å for the nonbonded interactions and of 14 Å for the long-range charged interactions were used. After an initial period of 100 ps used to equilibrate the system, a 500 ps production run was performed. Other details on the simulations are reported in Ref. [3].

The main contribution to the self-intermediate scattering function $I_s(\mathbf{q},t)$ has been directly calculated from the MD trajectories of the involved hydrogen atoms through the relationship

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

where N is the total number of water hydrogen atoms in the sample; $\mathbf{R}_i(t)$ is the position vector of the ith atom at time t, and the brackets $\langle\ \rangle$ denote an averaging over both the water ensemble and the exchanged momenta $\mathbf{q}$ having the same modulus q, to take into account for anisotropic effects.

The self-intermediate scattering functions obtained from Eq. (1), for different temperatures, are shown in Fig. 1. At low temperature, $I_s(q,t)$ is characterized by a two-step relaxation behavior, in which the fast relaxation is distinctly

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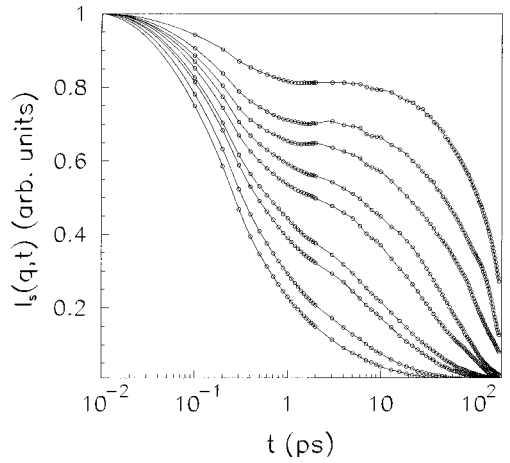

FIG. 1. Intermediate scattering function $I_s(q,t)$ at a fixed q value ($q=2$ Å^{-1}) for different temperatures. The temperatures are 100, 150, 180, 200, 220, 240, 250, 280, and 300 K from top to bottom. Solid lines are a guide to eye.

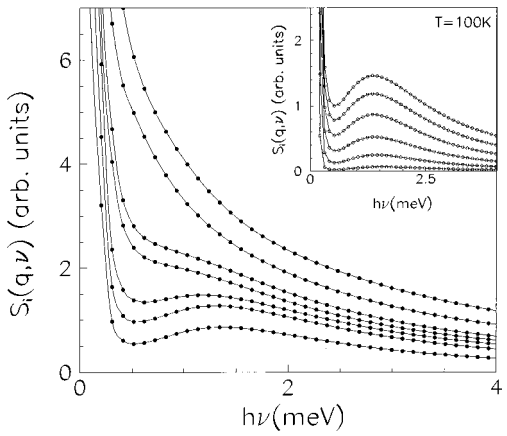

FIG. 2. Incoherent dynamical structure factor $S_i(q,v)$ at a fixed q value ($q=2$ Å^{-1}). The temperatures are 100, 150, 180, 200, 220, 260, and 300 K from bottom to top. Inset: $S_i(q,v)$ at $T=100$ K for various wave vectors: $q=n\times 0.5$ Å^{-1} with $n=1,...,6$, n increasing from bottom to top.

separated from the slow one by a plateau, such a distinction being gradually less evident as the temperature increases. These features are in a qualitative agreement with previous MD studies on supercooled bulk water [15], where the two relaxations have been termed β and α, respectively, within the mode-coupling theory. Moreover, our simulated $I_s(q,t)$ at 300 K shows some similarities with the experimental $I_s(q,t)$ at 320 K reported for myoglobin hydration water [16] where this function has been extensively analyzed at different q. However, in comparison with these previous results [15,16], our $I_s(q,t)$ exhibits some differences in the temporal extension of the plateau and in the behavior of the early and late decay rates [17].

At low temperatures, a slightly oscillatory effect, well distinct from the noise background, can be observed in $I_s(q,t)$, starting from about 2.5 ps. Such an oscillatory behavior in $I_s(q,t)$, as well as in mean square displacements, has been recently interpreted as a time domain manifestation of the boson peak [18]. Nevertheless, great caution has been suggested in attributing a physical significance to such oscillations, since they could originate from some simulations artifacts; e.g., a finite size effect arising from a disturbance that propagates through the system leaving and reentering the boundaries of the periodic box at the sound velocity [19]. It should be remarked, however, that in the present study we do not apply any kind of boundary conditions.

The low-frequency behavior of the protein hydration water can be characterized by the incoherent dynamical structure factor $S_i(q,\nu)$, which can be directly derived by temporal Fourier transform of $I_s(q,t)$. In calculating $S_i(q,\nu)$ 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. [20], to overcome spurious effects due to truncation. $S_i(q,\nu)$, calculated for various temperatures at the fixed wave vector $q=2$ Å^{-1}, is shown in Fig. 2. At temperatures up to 180 K, a broad inelastic peak appears well visible in the low-frequency region, centered at about 1.3 meV (~ 11 cm^{-1}). The position of this peak is found to be practically independent on the q value while a small dependence of its intensity on the exchanged momentum is registered (see the inset of Fig. 2), such a result being in agreement with the experimental results on a molecular glass [21]. By increasing the temperature, this peak becomes less and less distinct due to the raising intensity of the quasielastic contribution, whose linewidth has been verified to vary as q^2 [22]. The features of the bump revealed in the $S_i(q,\nu)$ are reminiscent of the low-frequency vibrational anomalies typical of glassy materials [21,23], where such an inelastic contribution has been termed a ''boson peak.'' The latter is an excess of quasilocal vibrational modes, or group of modes, whose origin is still under discussion [11]. The boson peak, which might be related to both structural correlations at intermediate range scale [24] and topological disorder [25], could probably be connected to certain low-temperature anomalies in the specific heat and thermal conduction of glasses [26].

In order also to test the reliability of our MD results, we have calculated the imaginary part of the dynamical susceptibility $\chi''(q,\nu)=\nu S_i(q,\nu)$, which can be directly compared with recent neutron scattering experimental data on the hydration water of another protein (myoglobin [16]). The calculated χ'' is shown in Fig. 3 for three different temperatures. At 100 K, four principal peaks are evident: the water librational one near 60 meV, a translational intermediate peak approximately at 24 meV, a broad translational peak at 4 meV, and the slow motions peak (α peak) at very low frequencies. A slight dependence on temperature characterizes the behavior of the dynamical susceptibility in the high-energy region (>1 meV), a small downwards shift of the peaks being registered as the temperature increases. On the contrary, a strong dependence on T occurs in the lowest energy region, as it can be especially inferred from the large variability of the lowest frequency minimum. Such a behavior, also observed experimentally [16], can be ascribed to a temperature modulation of the locally diffusive motions. The overall trend of χ'' PC hydration water is in a good qualitative agreement with that obtained by neutron scattering at 270 and 180 K for myoglobin hydration water (see the inset of Fig. 3) [16]. All the four peaks observed for PC hydration water are also registered in the χ'' of myoglobin hydration water even if at slightly different positions [16], the broad translational peak being significantly sharper in myoglobin

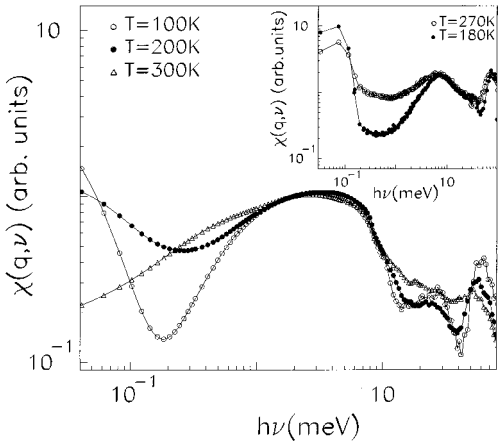

FIG. 3. Dynamical susceptibility $\chi''(q,v)$, calculated in an extended frequency range ($q=2$ Å^{-1}): $T=100$ K (white circle), 200 K (black circle), 300 K (white triangle). Inset: experimental dynamical susceptibility of myoglobin at 270 K (white circle) and at 180 K (black circle) (data from Ref. [16]).

with respect to PC hydration water. Finally, it should be remarked that the boson peak, expected to be localized around 1.0–1.5 meV, is hidden by the broad translational peak [27] and might be responsible for the higher intensity, observed below 1 meV, with respect to the experimental curve in the same region.

The presence of the boson peak could also be put into evidence as an excess (or bump) of low-frequency modes in the vibrational density of states $g(\nu)$ [23,28]. Actually, Fig. 4, where the ratio $g(\nu)/\nu^2$ of the PC hydration water is shown for various temperatures, reveals the presence of a bump, located at about 1.3 meV for temperatures below 180 K, while, at higher temperatures (220 and 300 K), a Debye-like behavior is registered in the low-frequency region. Such

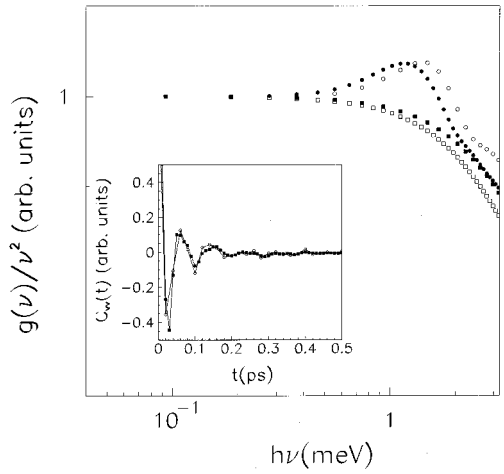

FIG. 4. Density of states divided by v^2 at 100 K (white circle), 180 K (black circle), 200 K (black square), and 220 K (white square). The 0 *frequency* value was subtracted from $g(\nu)$ to eliminate a possible offset error due to an incorrect integration over the $C_{vv}(t)$ rapidly oscillating queue. $C_{vv}(t)$ has been multiplied by a Gaussian envelope to overcome truncation effects and a spherical average has been performed to take into account anisotropic effects. Inset: hydration water hydrogen velocity autocorrelation function $C_{vv}(t)$ at $T=100$ K (black circle) and $T=200$ K (white circle).

a bump, which is located almost at the same position observed in the $S_i(q,\nu)$, provides additional evidence for the presence of the boson peak in the hydration water of PC. The $g(\nu)$ of PC hydration water was derived from the spectral density $C_{vv}(\nu)$ of the velocity autocorrelation function $C_{vv}(t)$ (shown in the inset of Fig. 4),

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

where $C_{vv}(t)$ has been obtained by considering only the hydration water hydrogen atoms that provide the largest contribution to $g(\nu)$.

The MD quantities analyzed in the present study reveal some interesting features of the protein hydration water. The intermediate scattering functions $I_s(q,t)$ are characterized, at low temperature, by a peculiar two-step relaxation behavior, reminiscent of that observed in supercooled bulk water [15], such a fact indicating a close similarity between the dynamical behavior of supercooled bulk water and that of protein hydration water. Actually both systems show a complex behavior that manifests itself in marked anomalies in the water diffusional properties. It should be kept in mind, however, that in our system it is the peculiarity of the protein-water interface that is at the origin of the observed anomalous behavior at 300 K [3].

Moreover, our results put into evidence peculiar anomalies in the low-frequency region of the PC hydration water. Actually, both $S_i(q,\nu)$ and $g(\nu)$ show a bump, centered at about 1.3 meV, which is clear evidence for the so-called boson peak. The presence of such a bump may represent a fingerprint of the amorphous character of the system [23]. In addition, the fact that the peak disappears for temperatures above 180 K is consistent with a ''fragile'' character of the hydration water according to the Angell nomenclature [18]. Actually, in fragile liquids, which consist of molecules interacting through nondirectional, noncovalent interactions, with a non-Arrhenius behavior, the boson peak is present only below the glass-temperature T_g, strong glasses being characterized by the presence of the boson peak in all the temperature range.

It should be remarked that the $S_i(q,\nu)$ of the PC protein itself shows a low-frequency peak at about 1.3–1.5 meV, which persists up to higher temperatures [17]. Such a peak is located at lower energy with respect to the boson peak observed by neutron scattering in hydrated myoglobin, centered at about 3 meV [29,18]. In this connection, we note that a similar low-frequency peak, but at lower energy, has been detected in the $S_i(q,\nu)$ calculated from MD simulation of myoglobin [30]. Such a downward shift in the simulated peak with respect to the experimental one seems to be recurrent in protein systems, and could be, in some way, connected to a too soft water-protein interaction in the MD simulations [9,30]. In this respect, it would be interesting to compare our MD simulation results with experiments.

On the other hand, it has been suggested that the protein low-frequency excess of modes could be linked to that of hydration water through the motions involving protein cross chain interactions, such an effect being strongly modulated by the solvent dynamics. In other words, solvent water could

inject its dynamics into polar side chains whose librations have been shown to be strictly dependent on the H-bond network restructuring dynamics occurring at the protein-solvent interface [9].

All these observations outline a scenario in which the protein-solvent coupling seems to play a crucial role in determining the dynamical features of the protein hydration water. We can therefore speculate about a possible connection between the presence of the boson peak in the hydration water and the excess of low-frequency anomalies in protein macromolecules, this aspect deserving a further experimental investigation, which is in progress.

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