Molecular dynamics simulation evidence of anomalous diffusion of protein hydration water

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The mean square displacement of protein hydration water has been found to increase nonlinearly in time, as observed for long times, by molecular dynamics simulations at low hydration levels and in proximity of the protein surface at full hydration. While such an anomalous diffusion is traced back to the more general properties of disordered media, some caution in the use of the self-diffusion coefficient D to characterize the water dynamics in these systems is suggested.

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A detailed knowledge of structure and dynamics of biological macromolecules, in connection also with the solvent behavior, is essential for a complete understanding of their functionality. Solvent plays a crucial role in the regulation of the protein properties; the spatial and temporal organization of water around a protein being strictly coupled to the dynamical response of the macromolecule. There is a general consensus about the fact that a minimum amount of water is required to activate the protein functionality [1–3]. Such an amount has been suggested to be in connection to a percolation threshold which assures a statistical joining of the H-bond network around the protein surface [4]. In addition, the protein hydration water properties (multiplicity of water states, dynamics of H-bond forming and breaking, amorphous state, and so on) have been indicated as contributing to the glassy character displayed by the protein macromolecules [5–9]; i.e., the existence of a huge amount of nearly isoenergetic conformational substates, minima of potential energy, regulating the kinetic response of the protein [10]. Above the glass transition, addition of water may be responsible for the onset of the protein dynamical behavior, which is reminiscent of that of amorphous disordered materials (characterized by α and β relaxations) [6,11,12] and makes transitions among conformational substates feasible [6]. However, the interplay between the local properties of the H-bond network and the protein dynamics, also in relationship to the hydration level, need to be fully clarified. In this respect, and since the water self-diffusion coefficient is a good reporter on the H-bond network properties [13,14], a detailed analysis of the hydration water diffusion coefficient, as a function of the hydration level and in proximity of the protein surface, may provide some insights into the mechanisms regulating the protein-solvent coupling.

An accurate and realiable description, with atomic resolution for both the protein and the surrounding water, is accessible by molecular dynamics (MD) simulation techniques. In this framework, the macromolecule and the solvent water are described as a classical many body system of atoms whose interactions are usually modeled by appropriate force fields [15–20]. Numerical integration of equations of motion provides a consistent picture of both protein and solvent dynamics on a time scale that, at present, covers times up to 1 nsec. While, on one hand, many experimental peculiarities of the protein-water systems may be reproduced (such as spectroscopic and thermodynamic data), on the other hand, some interesting nonlinear aspects [21,22], have been put into evidence by these techniques.

To calculate the water self-diffusion coefficient D, the following relationship is mainly used in MD simulations:

$$D = \lim_{\Delta t \to \infty} \frac{\langle \Delta r^2 \rangle_{t_0}}{6 \Delta t} \tag{1}$$

where $\langle \Delta r^2 \rangle_{t_0}$ is the mean square displacement registered for the trajectories of water oxygen atoms during the time interval Δt with t_0 as a starting time; the brackets $\langle\ \rangle$ indicate the average on both the water ensemble and the time origin t_0. Δt is to be large compared to the correlation time τ of the velocity autocorrelation function, so that any dynamical coherence in the motion of the molecule will be disappeared [23,24]. Since the presence of a solute might slow down the water dynamics, values of τ larger than those observed in bulk water (i.e., less than about 0.1 ps) could be expected for water surrounding a protein macromolecule [25]. Usually, in MD simulations dealing with the self-diffusion coefficient of the protein hydration water, Δt ranges from 1 to 20 ps [25–30]. Moreover, it should be remarked that to correctly use Eq. (1), a linear dependence of $\langle \Delta r^2 \rangle$ with time, at large times, has to be verified. Such a condition is generally obeyed by bulk water [31] and by water around a fully hydrated protein [28] where the values of D, as obtained by using different potentials for water, are in a good agreement with the experimental D value measured for pure water [32]. Conversely, when a local mapping of D is performed, some discrepancies in the different MD simulation results are observed. In particular, the plot of D, as a function of the distance from the protein surface, is sometimes found to be characterized by an increasing trend to reach the bulk value [25,33,34], while in some other cases it shows a maximum at intermediate distances from the protein surface [25–30]. The occurrence of this maximum, and also the values of D obtained close to the protein surface have been found to be dependent on the value employed for Δt [25]. This behavior was tentatively explained by taking into account that for dif-

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ferent flying times Δt, the water molecules might explore different regions around the protein where the H-bond network can be, in some way, perturbed by the presence of the protein [25,35]. It should alternatively be conceived that these discrepancies might arise from an anomalous diffusion process to which the water molecule random walkers undergo close to the disordered surface of the biopolymer [36]. Actually, the peculiar disorder and the roughness of the protein surface (quite often modeled as fractal surface [36–38]) could influence the water mobility, not only by affecting the proportionality constant of $\langle \Delta r^2 \rangle$ with time, but also the very diffusional process, which could be no longer described by a Brownian dynamics [39,40]. Additional support to this hypothesis is provided by a neutron scattering investigation on partially hydrated myoglobin that showed a nonlinear trend for the mean square displacements of water during a time interval of 10 ps [41]. On such a ground, we have deeply investigated the mean square displacements of water as a function of time for a fully hydrated protein at different distance from the surface and for a hydrated protein at different hydration levels, crossing the hydrogen bond percolation threshold.

The trajectories of all atoms of plastocyanin (PC), a copper containing protein involved in the phosynthetic process, and their surrounding water were determined by the GROMOS program package [15] including the single point charge/extended (SPC/E) model for water [42]. Cutoff radii of 0.8 nm for the nonbonded interactions and of 1.1 nm for the long-range charged interactions were used. The protein molecule was centered in a truncated octahedral obtained from a cube of edge 5.986 57 nm filled with 3103 water molecules. Lower hydrated systems (230 and 680 waters) were obtained from the 3103 water system by selecting, according to the method reported in Ref. [43], waters whose oxygen atom was put from the protein solute at a distance less than 0.330 nm and 0.587 nm, respectively. A protein system with only waters from crystalline structure (110 waters) was also considered. The motion of the system, followed for 600 ps, was performed on the canonical ensemble set at the temperature of 300 K [44]. Other details on the simulation procedure are reported elsewhere [45].

Figure 1 shows the mean square displacement $\langle \Delta r^2 \rangle$, calculated for all the water molecules in a time interval Δt of 10 ps, for each of the four differently hydrated protein systems. At very short times (less than approximately 0.7 ps), before the diffusive regime is established, the behavior of $\langle \Delta r^2 \rangle$ for the different systems is almost indistinguishable. The free flight region ($\langle \Delta r^2 \rangle$ is proportional to t^2) occurs for times less than 0.05 ps in agreement with other results [28]. After the break, occurring at about 0.7 ps, the $\langle \Delta r^2 \rangle$ trends appear linear with time but with different slopes in the log-log plot. These curves have been fitted, in the time interval from 1 to 10 ps, to a law

$$\langle \Delta r^2 \rangle \sim t^{\alpha}. \tag{2}$$

The extracted α values are reported in Fig. 1 where, for comparison, a line increasing with time as t^1, and representing normal diffusion [40], has been also plotted. At the highest hydration level, α is very close to one (0.96); such a fact pointing out that, at full hydration, the water diffusion can be correctly described by a Brownian process. In addition, the value of the diffusion coefficient (0.26 Å^2/ps), obtained from the limiting slope of the $\langle \Delta r^2 \rangle$ plot, is in agreement with that estimated for bulk SPC/E water [42]. The reduction of the hydration level determines a progressive decrease of the α values. In particular, for the two systems whose hydration level is close or below the percolation threshold, values of 0.66 and 0.62, respectively, are registered for α. This behavior is in a qualitative agreement with the results obtained by neutron scattering in partially hydrated myoglobin [41] in which, however, a smaller value (0.44) was obtained for α.

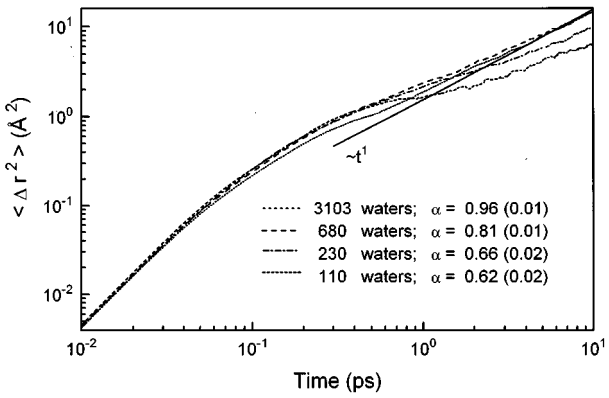

FIG. 1. Mean square displacements of water vs time for differently hydrated plastocyanin systems. Each curve was obtained by averaging over 20 different time origins and over the water molecule ensemble. The values of α reported in the figure were extracted by a fit of the curves, in the time interval from 1 to 10 ps, to a law $\langle \Delta r^2 \rangle \sim t^{\alpha}$. Standard deviations are reported in parentheses. The heavy line indicates a trend of $\langle \Delta r^2 \rangle$ as $\sim t^1$.

A similar deviation of α from 1 is observed in the fully hydrated protein system, when the $\langle \Delta r^2 \rangle$ analysis is restricted to waters whose trajectories are confined within a region put at a limited distance from the protein surface. We have analyzed three different distances from the protein sur-

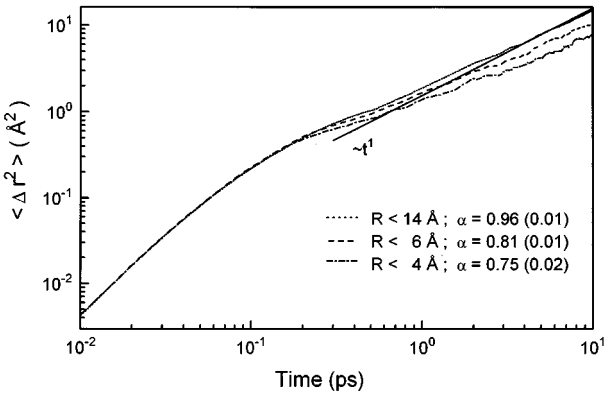

FIG. 2. Mean square displacements of water vs time for the fully hydrated plastocyanin system by restricting the analysis to water molecules moving within regions characterized by different distances from the protein surface. Each curve was obtained by averaging over 20 different time origins and over the corresponding water ensemble. The values of α reported in figure were extracted by a fit of the curves, in the time interval from 1 to 10 ps, to a law $\langle \Delta r^2 \rangle \sim t^{\alpha}$. Standard deviations are reported in parentheses. The heavy line indicates a trend of $\langle \Delta r^2 \rangle$ as $\sim t^1$.

faces: 14 Å, 6 Å, and 4 Å (see Fig. 2); the largest region (14 Å) including almost all the water molecules around the protein system. The three $\langle \Delta r^2 \rangle$ curves of Fig. 2 are almost indistinguishable at short times and show, after the break occurring at about 0.2 ps, a linear trend in the log-log plot characterized by a decreasing α exponent as long as smaller distances from the protein surface are considered. For water diffusing in the largest region ($R<14$ Å), α is found to be very close to 1 (0.96). For waters moving closer to the protein surface ($R<4$ Å), a significant deviation of α from 1 is registered (see Fig. 2), while $\alpha=0.81$ is obtained for $R<6$ Å.

The results from the different analyzed systems are consistent with the fact that anomalous diffusion takes place when the water molecules are confined in the proximity of the protein surface; such anomalies becoming less pronounced when the contribution to $\langle \Delta r^2 \rangle$ of waters moving far from the surface, as in the fully hydrated system, is predominant. Since in the presence of anomalous diffusion the slope of $\langle \Delta r^2 \rangle$ changes with the time, an improper use of Eq. (1) could lead to both incorrect and Δt-dependent values for D. This fact might be at the origin of the above mentioned discrepancies found in the diffusion coefficient when analyzed as a function of the distance from the protein surface.

A variety of mechanisms may lead to anomalous diffusion, involving the geometrical complexity of molecular surfaces (i.e., the surface roughness), broad distribution of jump times and/or lengths, or strong correlation in diffusive motion [40].

Actually, the surface of many different proteins have been described as fractal surfaces with a fractal dimension of 2.2 [37,38]; in particular, for PC, a value of 2.18 was calculated [38]. Such a property of the protein surface could have a relevant impact on the biological functionality: a fractal surface dimension larger than 2, while, on the one hand, accelerates the capture of the substrate from the bulk, on the other, determines a slowing down of the migration of the substrate along the protein surface [36,37]. On a fractal surface, the α exponent in Eq. (2) can be expressed, as it was first proposed by Alexander and Orbach [46], by

$$\alpha = \frac{\tilde{d}}{d} \tag{3}$$

where $\bar{d}$ is the surface fractal dimension, and $\tilde{d}$ is the spectral dimension which can be connected to the low frequency behavior of the vibrational state density of the system [46] and characterizes, in some way, the connectivity of the fractal structure [46]. For a given $\tilde{d}$ value, lower α values are obtained as long as larger fractal dimensions are considered; such a fact suggests that a slowing down of the water dynamics can occur on a protein surface. If we use the values reported for the fractal dimension d of PC and the α values extracted from the fit of the $\langle \Delta r^2 \rangle$ curves, $\tilde{d}$ can be determined by Eq. (3). In particular, restricting our analysis to water molecules moving close to the protein surface (within a distance $R<4$ Å), the following values for $\tilde{d}$, 1.63, 1.60, 1.44, and 1.30, respectively, were obtained as far as the hydration level is reduced. These results suggest that the connectivity of the protein surface is significantly dependent on the total amount of water present. While at low hydration level, the roughness of the surface causes a slowing down of the water exploration of protein, at higher hydration, water can move faster across the solvent accessible protein area. Therefore, the hydration level could influence the capability of a substrate to reach the active site; such a fact being in agreement with the existence of a percolation threshold to activate the protein functionality [1,4]. Furthermore, it is interesting to observe that as far as the hydration level is reduced, $\tilde{d}$ becomes closer to the value (1.25) some of us estimated for the PC spectral density by electron paramagnetic resonance (EPR) relaxation measurements at low temperature [47].

As we have mentioned, other mechanisms could be responsible for the onset of anomalous diffusion in our systems. In this connection, it should be remarked that a wide variability in the water residence times of the different sites of the protein surface was detected by NMR investigations [48] and also observed in MD simulations [49]. In addition, neutron scattering measurements put into evidence the occurrence of diffusion jumps with different lengths for water close to the protein surface [50]. Finally, a strong dynamical correlation between water molecules and some specific amino-acid residues could occur. Further investigations (some of which are in progress) are however required to fully clarify the mechanisms responsible for the observed behavior of the water mean square displacements as a function of time.

Our results point out that the water diffusion process on the analyzed protein surface, as investigated by MD simulation in the 10 ps temporal window, is characterized by an anomalous behavior which can be influenced by the hydration level. Even if, at present, only some hypothesis can be put forward about its origin, some caution is highly suggested in the use of the self diffusion coefficient D to characterize the local dynamical properties of water around the protein surface.

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