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Intensity fluctuations of the copper site resonant vibrational modes as observed by MD simulation in single plastocyanin molecule

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Abstract

The bond distance of the copper–sulfur in the plastocyanin (PC) active site has been followed along the molecular dynamics (MD) simulation trajectory, in a single molecule configuration. The power spectrum of the bond distance fluctuations, calculated for different time intervals, qualitatively reproduces the main spectral features of the experimental resonance Raman spectra of PC. The peak intensities of the vibrational modes appear significantly fluctuate during the dynamical evolution of the system. Such a variability, even if it occurs at an enormously different time scale, is consistent with the fluctuations observed in the experimental Raman spectra of similar systems when the single molecule limit is approached. © 2001 Elsevier Science B.V. All rights reserved.

1. introdution

Up to now, the spectroscopic techniques applied to study the structure and the dynamics of protein systems provide even detailed information but related to an ensemble of macromolecules. In such a way, stochastic fluctuations in the single molecule time trajectory, as well as the structural heterogeneity in a molecule population, are practically masked by the statistical average.

Recent advances in spectroscopic instrumentation have made possible the detection and the identification of signals arising from a single molecule [1–4]. This capability could provide new insight in the knowledge of fundamental processes regulating protein dynamics and functionality; actually, it allows one to investigate the dynamical behavior of a single biomolecule as a function of time. In this connection, we note that different spectroscopic approaches have put into evidence a time dependence of the spectral signals as obtained from various systems involving single molecules. Time-resolved fluorescence spectroscopy revealed that the emission of protein molecules undergoes abrupt changes in the intensity during the time analysis [4]. Furthermore, Raman spectra of dyes [1,2] and protein molecules [3] exhibit sudden spectral intensity changes with time; the origin of these variations having not been clarified.

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Molecular dynamics (MD) simulation, which provides an accurate and reliable description, at atomic resolution, of the single molecule dynamics from femto- to nano- or even micro-seconds, could represent a valid support to such a new experimental approach and offers the possibility to analyze the temporal fluctuations even from a portion of the macromolecule. In our previous MD simulation works, we have extensively investigated the structural and the dynamical behavior of plastocyanin (PC) at different hydration level and at various temperatures, by focusing on both the protein macromolecule and the surrounding water dynamical properties [5–9].

Here, we exploit the MD simulation capabilities to investigate the vibrational modes of the active site of PC which is a small copper containing protein (99 aminoacid residues) characterized by a globular β-barrel structure acting as an electron transport agent between cytochrome f to photosystem I in the photosynthetic process [10]. The active site, constituted by a redox copper coordinated to four ligands, is characterized by a strong electronic absorption at about 620 nm, arising from a charge transfer band between the copper ion and the cysteine (residue 84) ligand [11]. Moreover, excitation of this electronic transition, being strongly coupled to the vibrational modes of the active site, can be exploited to recover the resonance Raman spectrum of the active site vibrational modes.

Generally, vibrations of electron transfer proteins is of main concern for the functionality since they operate a fine tuning of the redox potential Cu(II)/Cu(I) and hence of the electron transfer process [12].

Vibrations of the PC active site have been investigated by normal mode analysis [13]. Moreover, information about the vibrations of the Cu–S(Cys84) bond can be inferred from the analysis of the temporal fluctuations of this bond distance as derived from classical MD simulations of all the macromolecule [14,15]. In the present study, we have calculated the power spectrum of the Cu–S(Cys84) bond distance fluctuations for a hydrated PC system by taking into account for different time intervals during the MD simulated dynamical trajectories. Such a power spectrum results to be closely reminiscent of the experimental resonance Raman spectra of PC and, in addition, reveals significant changes during the dynamical evolution of the system on the picosecond time scale. The last finding could be put into relationship to the spectral changes in the experimental Raman spectra of proteins when the single molecule limit is approached and observed on a time scale of seconds. The existence of these fluctuations might be traced back to a partial exploration of the complex energy landscape of the protein during its dynamics; the vibrational features of the protein active site possibly reflecting the entanglement of the macromolecule into local minima of the potential energy hypersurface.

2. Computational methods

Initial coordinates of PC including 110 crystallization water molecules (hydrated PC system) have been taken from the X-ray poplar PC crystal structure [16] at 0.133 nm resolution (1PLC entry of Brookhaven Protein Data Bank).

The MD simulation of the PC system has been performed by using the program DLPROTEIN [17]. The used force field is Charmm22 [18] including TIP3P model for water. The full connectivity and interaction terms of PC have been generated by using DLGEN utility included in the DLPROTEIN package.

With the exception of the copper ligands (Hi37, Cys84, His87), all the ionizable residues are assumed to be in their fully charged state, according to the value of $pH = 6$.

The copper ion in PC is coordinated to the side chain nitrogen of His37 and His87 and to the side chain sulfurs of Cys84 and Met92. Even if a complete description of the active site in PC, as well as in other metallo-proteins, has not been yet reached, different semiempirical and ab initio methods have been developed to parameterize the metal–ligand interactions in metallo-proteins [19]. In our previous works, we have first described the Cu–ligand interactions in PC by a nonbonded approach based on an electrostatic model [5], successively covalent bonds between the copper ion and the four ligands have been introduced [7].

Here, we have extended our last approach to include the modellization of the PC copper site developed, within the Charmm force field, by Ungar et al. [15]; such an approach having provided a satisfactory description of the vibrational spectroscopic features of the PC system. The copper is considered to be bound to three ligands (His37, Cys84 and His87), while the much weaker interaction of copper with Met92 has been treated by a nonbonded approach. The partial charges for the copper and its ligand residues have been modified by a linear interpolation of the standard Charmm charges between the protonated and the unprotonated residues to match the redistribution of the charges in the residues as due to the reduction of the copper charge from +2 to +0.98 (see [15, Table 2]).

The solvated protein system, in a single molecule configuration without any periodic boundary conditions, was simulated in the microcanonical ensemble. Initial atomic velocities have been assigned from a Maxwellian distribution corresponding to 300 K. The Shake constraint algorithm has been used throughout the simulation to keep the protein bond lengths involving hydrogen atoms rigorously fixed at their equilibrium values [20]. A cut-off radius of 10 Å for both the electrostatic and the van der Waals interactions has been used. The MD simulation consists of 300 ps for equilibration, followed by 800 ps of data collection. The time step was 1 fs and the data were collected every 5 fs. To assess the stability of the simulation and to check that the protein structure has properly equilibrated, a collection of dynamical properties as a function of simulation time was monitored. The gyration ratio of the protein fluctuates around a mean value of 1.258 nm, while the mean square displacements of C_α are characterized by a value of 0.142 nm. Other details about the statistical analysis of the simulation are reported in [28].

3. Results and discussion

The trend of the Cu–S(Cys84) bond distance for the PC system is shown for a time interval of 800 ps in Fig. 1. Fast oscillations, to which less evident slower oscillations are overimposed, ranging from about 1.82–2.40 Å around an average value of 2.12 Å with a standard deviation of 0.07 Å, appear throughout all the analyzed time interval. Temporal fluctuations, covering a wide frequency range, are commonly observed when some

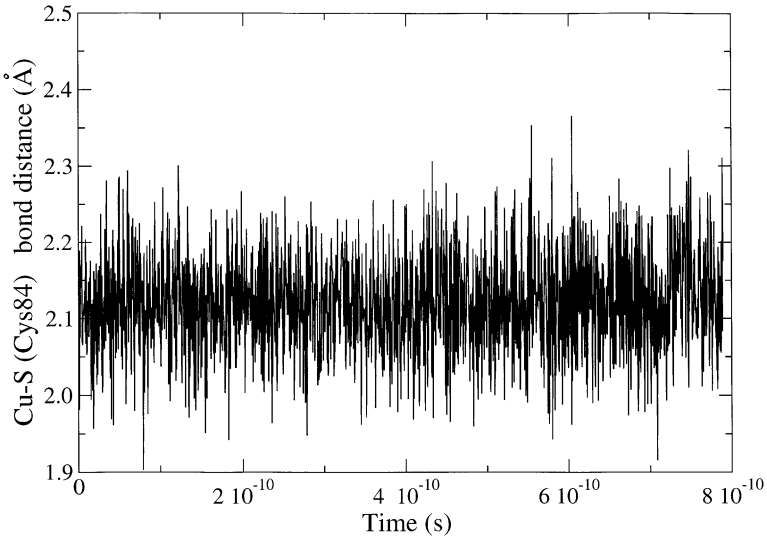

Fig. 1. The bond distance between the copper ion and the sulfur of the residue cysteine 84 (Cu–S(Cys84)), as a function of time, for the PC sample during 800 ps, successive to 300 ps of equilibration (time step 5 fs); for clearness not all the points have been plotted.

dynamical parameters of protein systems are analyzed; as for example, the dihedral angles, the mean square displacements, the conformational potential energy, and so on [21,22]. In general, these fluctuations can be traced back to the large variety of motions, spanning a wide range of time scale [8,21], characterizing the protein dynamics [23,24]. Such a heterogeneity of motions is intimately connected to the complexity of the protein energy landscape in which several local minima are present and become explored by the macromolecule during its dynamical evolution [25].

The vibrational modes associated to Cu–S(Cys84)-ligand bond can be determined, in the frequency domain, by taking the power spectrum of the temporal evolution of the corresponding bond distance as derived from the MD simulated trajectories. The power spectrum $P(f)$ of a function $x(t)$ can be numerically calculated by performing the Fourier transform of the autocorrelation function of $X(T)$. Numerically, the Fourier transform can be derived by performing the fast Fourier transform (FFT) or, alternatively, by the maximum entropy method (MEM) [26]. In the framework of the latter method, the power spectrum can be expressed as a finite sum of $2M + 1$ terms [26]:

$$P(f) = \sum_{j=-M}^{M} \phi_j \exp(2\pi i f j \Delta), \tag{1}$$

where Δ is the sampling interval in the time domain, ϕ_j is the autocorrelation component j of the function $x(t)$, and M is the number poles in the approximation (M cannot exceed the total number of data N); smaller is the number of poles, more smoothed is the resulting curve.

Fig. 2 shows the power spectrum of the PC Cu–S(Cys84) ligand bond as obtained by sampling eight different time intervals, each one of 5 ps,

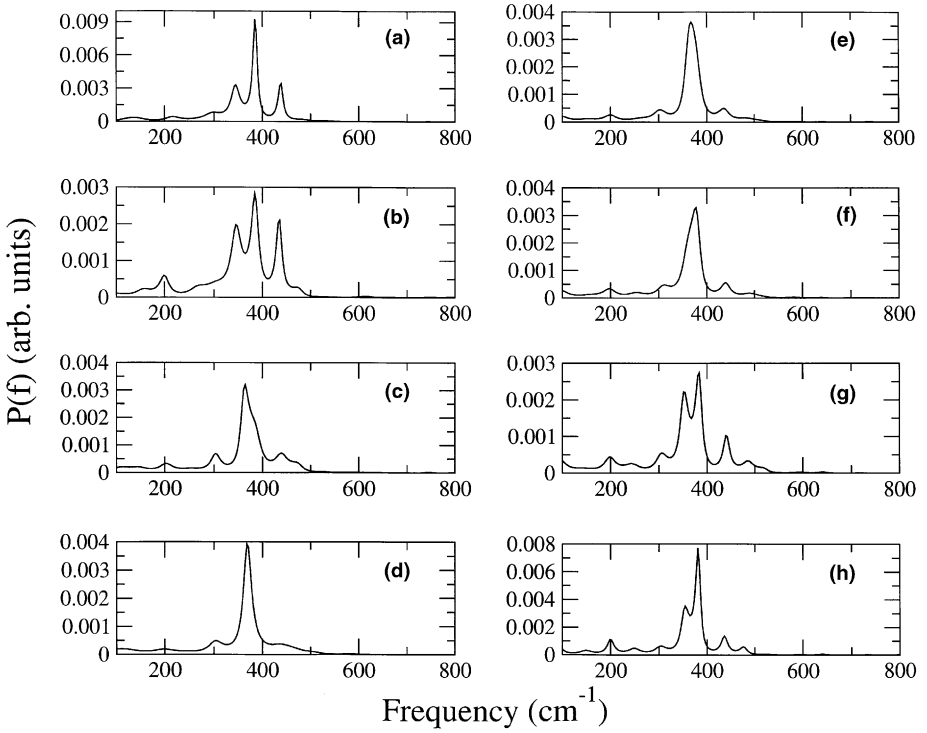

Fig. 2. The power spectrum $P(f)$, as a function of frequency, of the Cu–S(Cys84) bond distance for the PC sample as obtained by Eq. (1) from eight different time interval, each one of 5 ps. All the curves have been obtained by the MEM approach implemented in the TISEAN (2.0) package [27] by using 128 poles and with 1024 frequencies.

randomly selected in the 800 ps interval. According to the analysis reported in [15], a time interval of 5 ps is expected to be sufficient to reproduce the vibrational features of the PC Cu–S(Cys84) bond, since the corresponding vibrational relaxation was observed to be largely over within 0.5 ps [15]. In all the spectra of Fig. 2, the presence of a major band, located at about 350–450 cm^{-1}, in which different peaks can be distinguished; minor bands at about 50 and 200 cm^{-1} and, in addition, some infinitesimal peaks at about 620 and 750 cm^{-1} can be detected. From Fig. 2, we can also observe that the peak locations and, more markedly, the corresponding peak intensities significantly vary in the power spectra as calculated from differently selected 5 ps intervals. Such a trend suggests that the sampling of vibrational modes may change during the dynamical evolution of the system.

Fig. 3 shows the power spectra of the Cu–S(Cys84) ligand bond corresponding to two successive time intervals, each one of 400 ps, and to the total interval of 800 ps. Again, a major band with different peaks in the 350–450 cm^{-1} region can be observed. All the spectra appear to be quite similar; such a result pointing out that within 400 ps the convergence of the spectra is reached. In other words, an exhaustive exploration of the vibrational modes of the Cu–S(Cys84) bond is assessed in such a time interval. A similar trend was observed when trajectories obtained by different initial configurations were taken into account [28].

Our results should be compared with the experimental data. The experimental resonance Raman spectrum of PC, arising from a charge transfer between the copper ion and the sulfur of the Cysteine 84 residue, exhibits an intense Raman band between 350 and 450 cm^{-1} which has mainly been attributed to the Cu–S(Cys84) stretch, and a weaker band at 220–270 cm^{-1} assigned to Cu–N stretches coupled to the Cu–S(Cys84) bond [29]. The spectrum does not change if the Raman

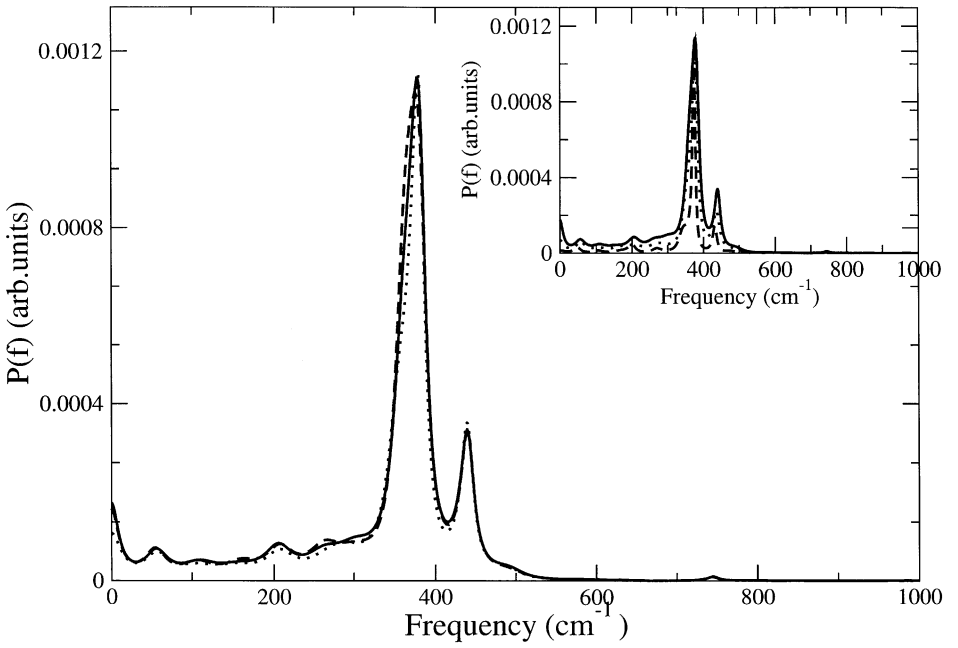

Fig. 3. The power spectrum $P(f)$, as a function of frequency, of the Cu–S(Cys84) bond distance for the PC as obtained by Eq. (1) from a time interval of 800 ps (continuous line), and two successive intervals, each one of 400 ps (dashed and dotted lines). All the curves have been obtained by the MEM approach by using 128 poles and 1024 frequencies. *Inset*: The power spectrum $P(f)$, as a function of frequency, of the Cu–S(Cys84) bond distance for the PC as obtained by Eq. (1) from time intervals of: 10 ps (dashed line), 100 ps (dotted line), 800 ps (continuous line).

experiment is carried out on hydrated powered of PC [11,29]. Such results finds a correspondence with the fact that no significant changes on the dynamical properties of PC were observed, by MD simulations, at different hydration levels [5,15]. We note that even if the positions of the peaks – as well as their relative intensity – registered in the MD simulated power spectra for the various time intervals do not completely match those of the experimental resonance Raman spectrum, the main experimental spectral features are qualitatively well described by our classical approach in agreement with what obtained in [15] in which similar force fields and simulation conditions have been used with an interval of 2.5 ps. Indeed, the calculated spectrum could be improved by including a quantum mechanical treatment [30,31].

Inset of Fig. 3 shows the normalized power spectra of the Cu–S(Cys84) ligand bond derived from 10, 100 and 800 ps intervals, respectively. Besides the slight variability on the peak position and intensity, it is worth of note that a progressive broadening of the linewidths is revealed by increasing the time intervals. Such a finding is consistent with the broadening observed in the Raman experimental spectra of proteins. Actually, the usual spectra of proteins obtained by Raman, as well as by other spectroscopic techniques, are a collection of signals from a large number of macromolecules and, therefore related to spectroscopic properties averaged over a heterogeneous population. Accordingly, the lines reveal an inhomogeneous broadening which can be described in terms of signals arising from a variety of molecules arranged in different conformational substates [29]. As due to the extensive dynamical coupling of the active site to the overall dynamics of the protein [11,29], it is reasonable to imagine that the spectral features in the Raman spectra might be modulated by the entanglement of the macromolecule in different conformational local minima as long as different time intervals, in its dynamical trajectory, are considered. Accordingly, the changes in the Cu–S bond vibrational modes of PC with the sampling time could be connected to the richness of protein motions which results in a temporal variability of the vibrational features of the active site as long as the macromolecule explores the configurational space along the dynamic trajectory [23]. On the other hand, the inhomogeneous line broadening detected in the EPR spectra at low temperature of PC has been traced back to the existence of a large number of macromolecules frozen in different conformational substates giving rise to different arrangements of the ligand–copper bonds and, then, to a spread of spin Hamiltonain parameters describing the paramagnetic site [32].

We remark that standard experimental Raman spectra of proteins appear to be stable in time. Conversely, sudden spectral changes are revealed in the Raman spectra of different systems including proteins in the single molecule limit [1–3]. The origin of these intensity fluctuations has not been clarified, even if it has been ascertained that is connected to the approaching of the single molecule limit detection [2,3]. We wonder if the fluctuations of the signals coming from single molecule Raman (surface enhancement Raman scattering, SERS) experiment might be, in some way, linked to the temporal fluctuations observed in the power spectrum derived from our MD simulations for short time intervals.

By exploiting the dramatic enhancement of the Raman cross section (up to 10^{14} times) as induced by the absorption of the active Raman centers to metal particles (SERS), single molecule detection has been recently achieved [1,3,33]. It should be noticed that in a SERS experiment on single molecule, a reliable signal is collected by sensitive, cooled CCD detectors, for times of the order of seconds [1,3]. During such a time, taking into account the standard features of a Raman detection apparatus (the electronic and the optical collection system, the detection solid angle etc.) between 50 to 500 photons are counted [34]. Since Raman spectroscopy provides a picture of atomic motions on the scale of femtoseconds [35], it turns out that the counted photons correspond to a molecule sampling time ranging from 0.5 to 5 ps. Such a time is strikingly close to that we have used to extract, from the MD simulation trajectories, the power spectra of the Cu–S bond fluctuations. This means that both our MD simulation and the Raman spectroscopic experiments give information about the vibrational modes on the same temporal window. However, it should be remarked that the

local minima explored by the macromolecule in the two cases can be different. By MD simulation, we continuously follow the macromolecule for a time interval of 5 ps during which the system explores adjacent local minima. On the contrary, by single molecule Raman spectroscopy, a sort of random sampling of the vibrational modes during trajectories of one second is accomplished. Nevertheless, in both the cases, the resulting temporal variability can be assumed to be a direct consequence of the intrinsic dynamical fluctuations of the system when analyzed at the single molecule level.

4. Conclusions

MD simulation which generally provides a reliable description, at atomic resolution, of the classical dynamics of a biomolecule, including the surrounding solvent, is also an useful tool to investigate the features emerging from single molecule spectroscopic approaches. The power spectrum of the Cu–S(Cys84) bond distance fluctuations of the PC in a single molecule configuration, closely reminiscent of the resonance Raman spectra of PC, has revealed a strong dependence on the time interval sampling over the MD simulation trajectories. This behavior is put into relationship to the spectral fluctuations observed in the experimental resonance Raman spectra of various systems, in the single molecule limit, and traced back to the entanglement of the system in the local minima of its trajectory.

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