ELSEVIER

Biophysical Chemistry 106 (2003) 221–231

Biophysical Chemistry

www.elsevier.com/locate/bpc

Excited state charge-transfer dynamics study of poplar plastocyanin by ultrafast pump-probe spectroscopy and molecular dynamics simulation

Tiziana Cimei[a], Anna Rita Bizzarri[a], Giulio Cerullo[b], Sandro De Silvestri[b], Salvatore Cannistraro[a,*]

[a]*Biophysics & Nanoscience Group, INFM, Dipartimento di Scienze Ambientali, Università della Tuscia, I-01100 Viterbo, Italy*
[b]*National Laboratory for Ultrafast and Ultraintense Optical Science-INFM, Dipartimento di Fisica, Politecnico di Milano, Piazza Leonardo da Vinci 32, I-20133 Milano, Italy*

Received 3 July 2003; received in revised form 18 July 2003; accepted 22 July 2003

Abstract

We have applied ultrafast pump-probe spectroscopy to investigate the excited state dynamics of the blue copper protein poplar plastocyanin, by exciting in the blue side of its 600-nm absorption band. The decay of the charge-transfer excited state occurs exponentially with a time constant of approximately 280 fs and is modulated by well visible oscillations. The Fourier transform of the oscillatory component, besides providing most of the vibrational modes found by conventional resonance Raman, presents additional bands in the low frequency region modes, which are reminiscent of collective motions of biological relevance. Notably, a high frequency mode at approximately 508 cm^{-1}, whose dynamics are consistent with that of the excited state and already observed for other blue copper proteins, is shown to be present also in poplar plastocyanin. This vibrational mode is reproduced by a molecular dynamics simulation involving the excited state of the copper site.

Keywords: Poplar plastocyanin; Ultrafast pump-probe spectroscopy; Charge-transfer excited state; High frequency mode; Duschinsky rotation; Molecular dynamics simulation

1. Introduction

The application of femtosecond pump-probe spectroscopy to study the ligand-to-metal charge-transfer (LMCT) dynamics of several blue copper proteins [1–4], has very recently gained large popularity, particularly because it gives information on the excited state nuclear dynamics [2,5–8] and in addition circumvents the experimental difficulties of Resonance Raman (RR) spectroscopy in discriminating the low frequency modes [5]. Moreover, the dynamics following the optical preparation of the LMCT band may provide useful information on the processes associated with the thermal electron transfer (ET) reaction in blue proteins, even if the charge-transfer that occurs is

*Corresponding author. Tel.: +39-076-135-7136; fax: +39-076-135-7136.
E-mail address: cannistr@unitus.it (S. Cannistraro).

0301-4622/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0301-4622(03)00215-1

not precisely equivalent to the physiological ET [2,9].

Femtosecond pump-probe studies on poplar and spinach plastocyanin [1–3], human ceruloplasmin [2] and azurin [4], have shown a close similarity in the photophysics of these cupredoxin proteins. In most of the blue copper proteins investigated, it has been found that the charge-transfer state, populated by pump pulses exciting their LMCT bands, decays to the ground state in a single relaxation step with a time constant of approximately 300 fs. Only for spinach plastocyanin, Edington et al. [1] found that the charge-transfer state decays non-radiatively, into an intermediate *d* state with a 125-fs lifetime, from which a return to the ground state with a time constant of 285 fs occurs.

In a previous study on copper azurin [4], upon excitation with a 10-fs laser pulse centred at 550 nm, we have measured a charge-transfer state lifetime of approximately 270 fs with the excited state deactivation appearing essentially non-radiative. Frequency domain analysis of the oscillatory component superimposed to the signal decay and due to the pump-induced vibrational coherence in both ground and excited states, not only revealed almost all the bands characterising the conventional RR spectrum of azurin but, in addition, put into evidence the presence of other vibrational modes. The most striking vibrational feature appearing in the Fourier spectrum of the protein is a relatively high frequency mode peaking at approximately 500 cm^{-1} [4]. The linear-prediction singular-value decomposition (LPSVD) analysis in azurin [4] provided for such a mode, a time consistent with the excited state dynamics. This band had been found also in spinach plastocyanin and human ceruloplasmin by Book et al. [2], who assigned it to an excited state mode consistently with a Duschinsky rotation and suggested the presence of this mode as being a general characteristic of blue copper proteins, although it was not found in poplar plastocyanin.

In this context, due to our strong interest in the spectroscopic, molecular dynamics (MD) and electron transfer properties of poplar plastocyanin (pPC) (see Fig. 1) [10–16] and by taking advantage of an unprecedented high resolution due to 10 fs pulse duration, we have revisited the excited state charge-transfer dynamics of pPC by pump-probe spectroscopy, exciting the sample protein in the blue side of its strongest LMCT absorption band (see Fig. 2).

In this paper, we report on the results of the pump-probe spectroscopy measurements on pPC and discuss them in connection also with those concerning the other blue copper proteins investigated with the same technique [1–4]. We observe an exponential fast excited state deactivation occurring within approximately 280 fs. The Fourier Transform (FT) analysis of oscillatory pattern superimposed to the decaying signal reveals some vibrational frequencies characterising the conventional RR studies of this protein, in addition to a new intense mode at approximately 508 cm^{-1} whose dynamics is consistent with that of the excited state. Some low frequency modes are observed between 20 and 80 cm^{-1}.

In order to rationalise the presence of the novel 500 cm^{-1} mode, which is common to the excited state of several blue copper proteins, we have also performed a classical MD simulation study of the excited state of pPC reproducing this mode.

2. Experimental and computational methods

pPC is a copper-binding protein of only 99 amino acids in size (see Fig. 1), belonging to the cupredoxin family. It is localised in the thylakoid lumen of the chloroplast and functions in photosynthesis as mobile electron carrier, transferring an electron from cytochrome *f* in photosystem II to chlorophyll P700 in photosystem I.

In the ‘type I’ active site of pPC the copper atom is ligated to a sulphur atom from a cysteine residue, two nitrogens from histidine residues and another sulphur atom from a methionine residue (a schematic representation of pPC active site structure is shown in Fig. 1). This site exhibits unique spectral features that result from the unusual geometric and electronic structures imposed on the metal ion in the protein environment [17,18]; the most striking one being are its intense blue colour due to the strong absorption at approximately 600 nm (see Fig. 2). This absorption is two orders of magnitude greater than that found

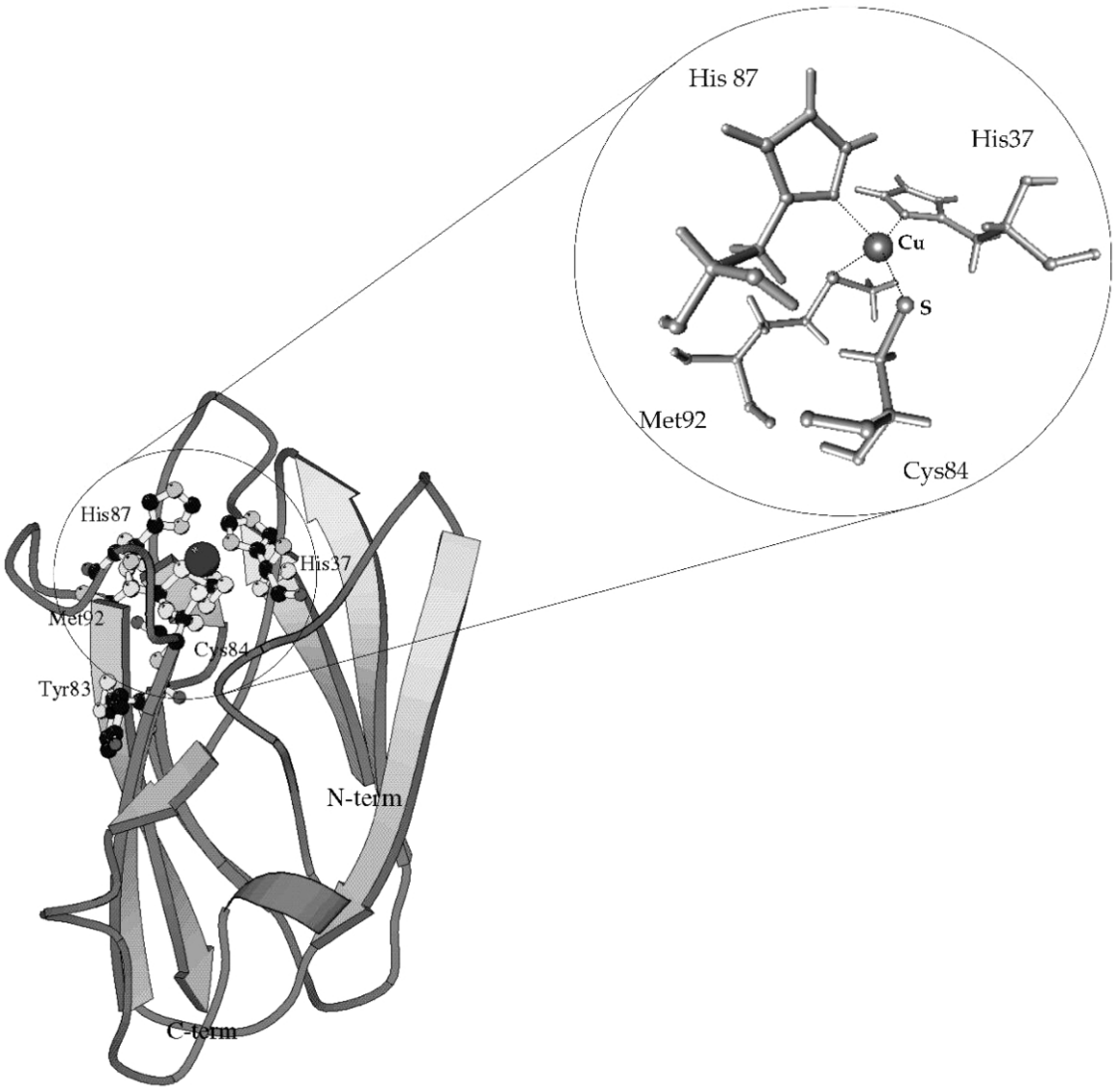

Fig. 1. Three-dimensional structure of pPC showing the copper atom at the top (left); on the right a schematic picture of the pPC active site is reported. These drawings were generated using coordinates from 1 plc file (Brookhaven Protein Data Bank).

with normal tetragonal complexes ($\varepsilon = 5000\ M^{-1}\ cm^{-1}$ vs. $\varepsilon < 100\ M^{-1}\ cm^{-1}$) in the same spectral region.

Expression and purification of pPC were carried out as reported elsewhere [10]. The purity ratio A_{280}/A_{597} is 1.1. The absorption spectrum of the protein, recorded at room temperature by a double beam Jasco V-550 UV/visible spectrophotometer, using 1 cm path length quartz cuvettes, is shown in Fig. 1.

For pump-probe experiments, 50 μl of sample at a concentration of 0.2 mM were kept in a 0.5-mm-thick cuvette. The sample is first excited by a pump pulse and, successively, the transmission changes induced in the sample are investigated by a suitably delayed probe pulse. The change in the

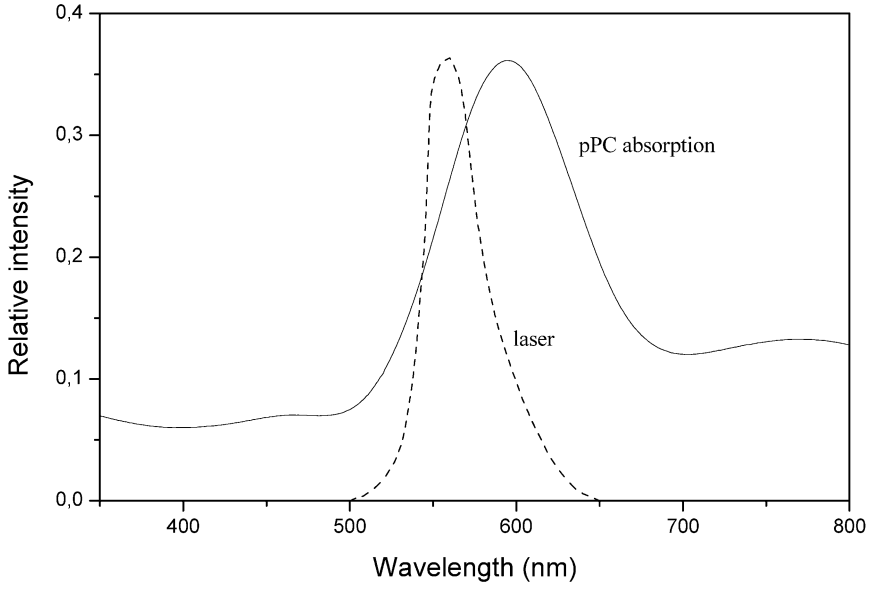

Fig. 2. Absorption spectrum of pPC (70 μM in 20 mM sodium phosphate) in the visible region (solid line). The laser spectrum used in the pump-probe measurements is also shown (dashed line). The pPC absorption band peaking at approximately 600 nm corresponds to the S(Cys-π)→Cu LMCT transition (see text).

transmitted probe pulse energy induced by the pump as a function of the time delay between the pump and the probe pulses is recorded. The pump-probe experiments were performed using a non-collinear visible optical parametric amplifier, pumped by the second harmonic of a Ti/sapphire laser at 1 kHz. The system generates pulses with ultrabroad bandwidth, extending from 500 to 650 nm (the laser spectrum is shown in Fig. 2), compressed to near transform-limited, sub-10-fs duration by multiple reflections onto chirped dielectric mirrors [19]. Since the main focus of this study is vibrational dynamics of pPC, we chose to work with the highest possible temporal resolution. To this purpose we selected a pulse spectrum peaking at approximately 550 nm, that allows to achieve the shortest pulses [19]. Time resolved measurements at specific wavelengths are obtained by selecting, after the sample, spectral components of the broadband probe pulse with 10-nm bandwidth interference filters and combining differential detection with lock-in amplification. Absorption spectra of pPC were taken immediately before and immediately after the pump-probe measurements to check that no photodegradation had occurred.

The pump-probe signal, recorded as the differential optical transmission $\Delta T/T$ as a function of time delay between the pump and probe pulses, consists of an exponential decay with superimposed an oscillatory pattern. The decay has been fitted by using a single exponential with a constant offset ($y(t)=A+B\cdot e^{-t/\tau}$). In order to analyse the oscillatory component of the signal (residual), we have subtracted the fitting exponential decay from the experimental data. The residual was firstly analysed in the frequency domains, by a Fourier Transform [20], to obtain the frequencies of the single vibrational components. Successively, the LPSVD [21] routine provides, amplitude (A), phase (ϕ) and exponential decay constant (τ) for the vibrational components at frequency ν_i of the residual by fitting it to a function of the form:

$$S(t)=\sum_i\left[A_i\cos(\nu_i t+\phi_i)\exp(-t/\tau_i)\right] \quad (1)$$

The frequencies (ν_i) extracted from the Fourier spectrum were used as initial parameters.

The molecular dynamics (MD) simulations of hydrated pPC have been performed by using the CHARMM package [22] with Charmm27 as force field including TIP3 model for water. Details of simulations are reported elsewhere [16]. By such an approach, the main RR experimental features approximately 400 cm^{-1} have been reproduced by calculating the Fourier transform of the Cu–S(Cys84) distance autocorrelation functions [16,23].

Here we focus our attention to the excited state. According to experiments, indicating that no net motion of charge occurs upon excitation [24,25], the excited state of pPC has been described by changing the equilibrium distance of the Cu–S bond [23]. On such a ground, the potential energy difference between the excited and ground states results to be proportional to Cu–S distance $x = |\mathbf{r}_{Cu} - \mathbf{r}_S|$. Furthermore, a previous study has suggested that the related force constant k assumes the same value for both the ground and the excited states [23]. In order to reproduce the vibrational features characterising the excited state, we have extended this model to include the possibility that the force constant for the excited state might be characterised by a different value from that of the ground state. On such a basis, the potential energy difference ΔV of the excited state has been expressed by:

$$\Delta V = (k_e - k_g)x^2 - 2(k_e x_{0,e} - k_g x_{0,g})x + k_e x_{0,e}^2 - k_g x_{0,g}^2 + \varepsilon \quad (2)$$

where $x_{0,g}$ and $x_{0,e}$ are the minima for the Cu–S distance in the ground and excited state, respectively, k_g and k_e are the force constants in the ground and the excited state, respectively, and ε is a constant electronic energy gap. The values of k_g and $x_{0,g}$ have been fixed equal to those previously used in Ref. [16] [$k_g = 110$ kcal/(mol Å^2) and $x_{0,g} = 2.10$ Å]. The two parameters $x_{0,e}$ and ε of Eq. (2) have been estimated to reproduce the absorption spectra and the values obtained are 2.35 Å and 13060 cm^{-1}, respectively [23]. The $k_e$value in Eq. (2) has been adjusted to reproduce the excited state frequencies of pPC and finally it was fixed at 290 kcal/(mol Å^2).

The vibrational modes associated to the 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 of the excited state. The power spectrum $P(f)$ of a function $x(t)$ has been numerically calculated by performing the Fourier transform of the autocorrelation function of $X(T)$ by the maximum entropy method (MEM) ([26] and Refs. therein).

3. Results and discussion

Fig. 3a shows the time evolution of the pPC pump-probe signal resolved at a wavelength of 560 nm. The signal is characterised, as well as those obtained at other wavelengths, by a large-amplitude feature at zero time delay (removed in the figure). The inset in Fig. 3a, that shows the pump-probe signal of the buffer only, indicates that this spike is due to the buffer solution. Such a spike has been found also by Book et al. [2] in their pump-probe experiments on other blue copper proteins and has been attributed to non-resonant absorption of the buffer solution. Although the solvent contribution covers the protein signal until a delay time of approximately 100 fs, no oscillations are observed beyond this time delay.

The differential optical transmission (Fig. 3a) decays from a positive value to the baseline with superimposed oscillations. The exponential decay is due to the recovery of the ground state, while the oscillatory component corresponds to vibrational coherence induced by the ultrashort pump pulse in both ground and excited states [2,5–8]. The solid line in Fig. 3a, represents the best fit of the differential optical transmission signal obtained by using a sum of a decaying exponential with a constant offset (all the fitting parameters are reported in the legend of Fig. 3). The data fit was performed starting at the delay time for which the buffer response has ceased and a time constant of approximately 280 fs has been obtained; this value is very similar to that obtained for pPC, spinach plastocyanin and ceruloplasmin by Book et al. [2], and for azurin by the authors [4]. This rapid decay, due to non-radiative relaxation processes, indicates that a fairly strong coupling exists between the

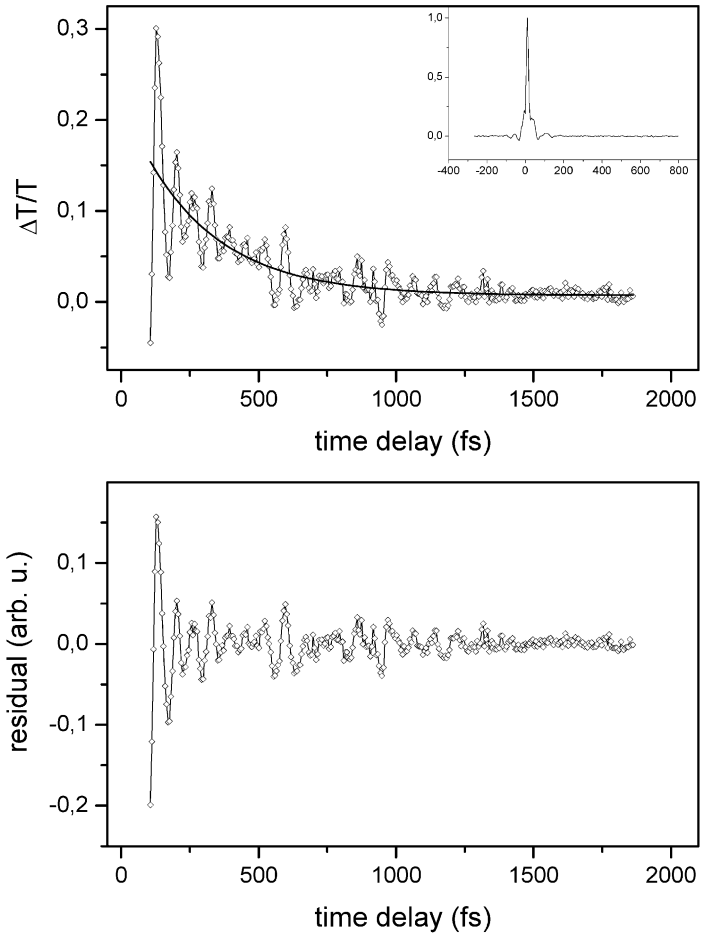

Fig. 3. (a) Differential optical transmission, as a function of pump-probe delay, analysed at 560 nm for pPC. The diamonds represent the experimental data (the line is a guide to the eye) (data below 100 fs have been removed, see text) while the solid line is the best fit obtained by a sum of a single exponential decay with a constant offset: $y(t)=A+B\cdot e^{-t/\tau}$. The best-fit values are: $A=0.007\pm0.002$; $B=0.215\pm0.016$; $\tau=280.2\pm26.7$. Inset: wavelength-resolved pump-probe signal of bare buffer solution. (b) Oscillatory component (residual) obtained after subtraction of the exponential fit from experimental data of Fig. 1a (the line is a guide to the eye).

ground and the excited states in all copper proteins [2].

It is interesting to note that the addition of a relatively small offset (3% of the initial fit amplitude) just above the noise level improves remarkably the fit of pump-probe signal decay. As suggested in similar studies on spinach plastocyanin [2] and azurin [4], this offset that appears stationary on the time scale of the measurements, could be an evidence of a small amount of population becoming trapped on the excited state surface and preventing the reestablishment of the equilibrium on the few picosecond time scale of the pump-probe experiments.

In order to analyse the oscillatory component superimposed to the probe transmission decay, the exponential fit was subtracted from the experimental data to produce the residual shown in Fig. 3b. Successively, a careful Fourier transform [20] of the residual was performed and the spectrum

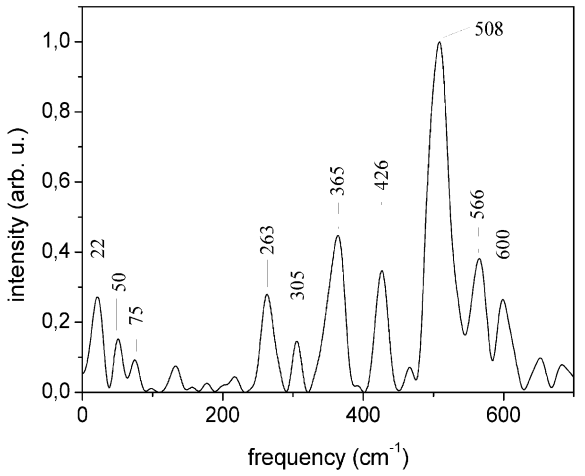

Fig. 4. Fourier spectrum of the residual shown in Fig. 1b. Most of the bands appearing in the figure are typical of the conventional RR spectra of pPC. Three low frequency modes are observed between 20 and 80 cm^{-1}. The band at 508 cm^{-1} is that attributed to an excited state mode.

obtained is shown in Fig. 4. Some of the major bands that appear in Fig. 4 are typical of the pPC RR spectrum enhanced via excitation of the protein in its strong 600 nm absorption band [27,28]. The RR spectra of this protein are quite complex and approximately five bands between 350 and 500 cm^{-1} arise from mixing of Cu–S stretching with multiple heavy atom bending modes involving the cysteinate ligand and adjacent residues [27,28]. A band at approximately 263 cm^{-1} has previously been assigned to the symmetric Cu–N stretch from the histidine ligands [27,28]. In the Fourier spectrum of Fig. 4, the bands at 426 and 263 cm^{-1} could correspond to the bands at the same frequency in the RR spectra of the protein, while the bands at 390 and 365 cm^{-1} could be tentatively associated to the RR bands positioned at 382 and 375 cm^{-1}, respectively, although slightly shifted [27,28]. While some RR bands (as those at 402, 441 and 480 cm^{-1} [27,28]) do not appear in the Fourier spectrum of pPC (Fig. 4), other bands are visible in the latter. A band with low intensity appears at approximately 305 cm^{-1} and two relatively strong bands are visible at approximately 565 and 600 cm^{-1}. These two last vibrational features can be overtones of another band situated at lower frequency.

Three low frequency modes can be observed between 20 and 80 cm^{-1}. Also Book et al. [2] found low frequency modes in the 35–55 cm^{-1} region in pPC, spinach plastocyanin and ceruloplasmin, and assigned these vibrational features to protein phonon-like modes coupled to the optical excitation. In azurin three vibrational features between 30 and 80 cm^{-1} have been observed by pump-probe [4] and RR spectroscopies [29]. Nakashima et al. [3], by studying the charge-transfer dynamics of *Synechococcus* plastocyanin by ultrafast pump-probe spectroscopy, focused the attention just on these low frequency modes and attributed the strong and quite broad band observed at approximately 30 cm^{-1} to a delocalised mode involving the protein skeleton motion. On such a ground and on the basis of previous inelastic neutron scattering results [30] obtained by some of the authors, we attribute the low frequency bands shown in Fig. 4 to collective modes involving large biomolecule regions. The presence of these low frequency bands might deserve some biological relevance: actually quite recently, it has been shown that collective modes, with large amplitude around the active site, function as a mechanism for an enzyme to achieve substrate recognising specificity [31].

However, the most prominent feature in the Fourier spectrum of the protein is the presence of the vibrational band at 508 cm^{-1}. As mentioned in the Introduction, this band that has been found in the pump probe spectra of spinach plastocyanin [2], human ceruloplasmin [2] and azurin [4] was not observed in poplar plastocyanin [2].

As already observed by Book et al. [2] the assignment of this high frequency band is not straightforward. At first, such a mode is not present in the conventional RR spectrum of pPC. Furthermore, it is not due to the solvent contribution because water does not have any peak in its Raman spectrum at this frequency and a pump-probe experiment on the pure solvent did not reveal any oscillation. In order to better investigate the origin of this mode, we analysed the residual of Fig. 3b by LPSVD method [21]. For the 508-cm^{-1} mode we obtained a time of approximately 300 fs,

consistent with the excited state lifetime. This finding, which is found in agreement with those on other blue copper proteins [2,4], suggested that it is a vibrational feature typical of the excited state nuclear dynamics. This interpretation would, however, be in contrast with the expectation that the optical promotion of an electron to an orbital with less bonding character causes a decrease in the excited state vibrational frequency. Actually, Book et al. [2] proposed a Duschinsky rotation as a possible source for a frequency increase in the excited state of spinach plastocyanin and ceruloplasmin.

The Duschinsky effect arises when an excited state has a vibrational normal mode coordinate system that is rotated and translated relative to that of the ground state [2]. Excited state normal coordinates are, therefore, formed from linear combination of the ground state normal coordinates of the same symmetry and the resulting mode frequencies can be remarkably different from the corresponding ground state frequencies. Book et al. [2] suggested that if a Duschinsky mixing occurs among the spinach plastocyanin Raman-active modes (six modes between 370 and 480 cm^{-1}), involving the Cu–S(Cys84) stretch, it is possible that a mode at approximately 500 cm^{-1} appears in the excited state. A further indication that a Duschinsky rotation could take place in pPC, as well as in the other blue copper proteins, is the presence of an intense band at approximately 800 cm^{-1} in its RR spectrum composed of the overtones and combinations of the plastocyanin vibrations between 350 and 500 cm^{-1} [32].

An aspect that needs further comment is connected with the fact that Book et al. [2], who observed the 500 cm^{-1} mode in spinach plastocyanin and ceruloplasmin, by exciting at 770 nm, corresponding to three *d–d* transitions, did not find it in pPC. These authors, aiming at obtaining information on the dynamics of the charge-transfer state, excited the samples just in correspondence to the *d–d* transitions by taking advantage from the coupling existing between these latter transitions and the Cu–S charge-transfer transition. Actually, the dominant vibrational modes probed in their pump-probe experiments were effectively those resulting from the optical excitation of the Cu–S(Cys84) bond. Therefore, the observation that the 500 cm^{-1} mode appears in pPC when the protein is directly excited in the charge-transfer band, could be an indication that a different coupling between the Cu–S and the *d–d* transitions exists in pPC with respect to the other blue copper proteins studied.

Sando et al. [33] have recently shown that the Duschinsky mixing can affect sensitively the electron transfer rate by influencing some relevant parameters such as the reorganisation energy. Because the $\sim$500 cm^{-1} band is a characteristic of all the blue copper protein so far studied, it is conceivable that the Duschinsky effect could play a fundamental role in the electron transfer properties of this class of proteins.

In order to obtain further insights relative to the 508 cm^{-1} mode, we tried to reproduce this vibrational band by a classical MD simulation approach involving the excited state of the copper site. The MD simulation capabilities to investigate the vibrational character of the pPC ground state have been recently demonstrated by some of the authors [16] by following the approach originally proposed by Ungar et al. [23]. Very recently, in this work it has been shown that the main vibrational modes of the experimental RR spectrum of pPC can be reproduced by taking the power spectrum of the temporal evolution of the Cu–S(Cys84) bond distance of pPC during the MD simulation run.

To our purpose, here we have modelled the excited state of pPC active site by increasing the equilibrium bond length in the harmonic Cu–S(Cys84) interaction [23]. In addition, on the basis of the new information provided by ultrafast pump-probe spectroscopy on the excited state and according with the hypothesis of a Duschinsky rotation as source of the 508 cm^{-1} band, we also changed the force constant of the same bond from 110 kcal/(mol Å) of the ground state up to 290 kcal/(mol Å).

Fig. 5 shows the power spectrum of the Cu–S(Cys84) bond distance temporal fluctuation for pPC in the excited state; such a spectrum having been obtained from a time interval of 800 ps. A main peak at 511 cm^{-1} appears and represents a vibration mode of the excited state of pPC obtained by increasing the force constant of the Cu–

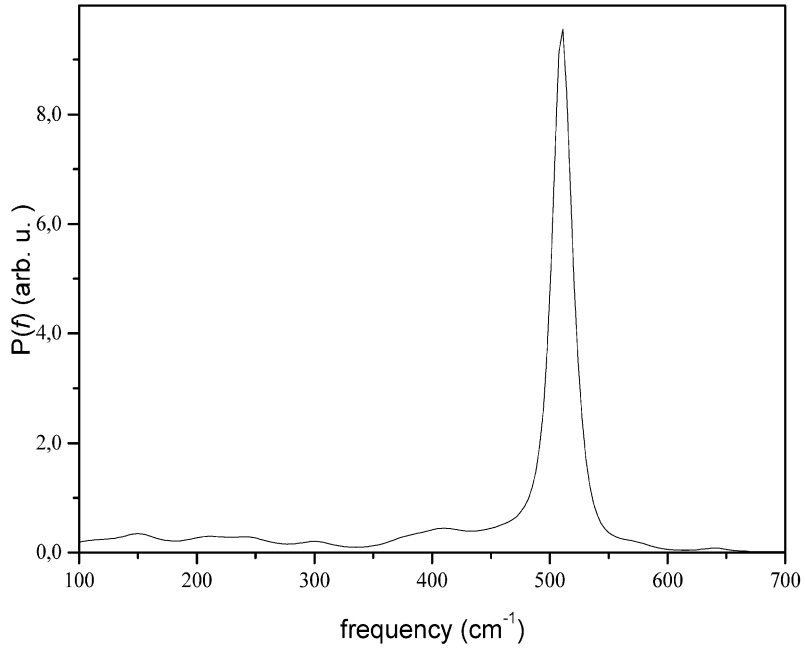

Fig. 5. Power spectrum $P(f)$, as a function of frequency, of the pPC Cu–S(Cys84) bond distance fluctuations as obtained by eq. 3 and analysed for 800 ps. The main vibrational feature, consistent with the 508 cm^{-1} mode of the experimental spectrum of Fig. 4, has been reproduced by modelling the excited state of pPC by increasing the equilibrium bond length and the constant force of the Cu–S(Cys84) bond (see text). The curve has been obtained by the MEM approach implemented in the TISEAN (2.0) package [34] by using 128 poles and with 1024 frequencies.

S(Cys84) bond. Such an increase is able to reproduce the increase in the mode frequency as expected from a Duschinsky rotation. Some other bands, with very low intensity, are visible at approximately 200–400 cm^{-1}. The fairly good agreement between the MD and the experimental results indicates the reliability of the approach here presented to modellise the excited state of pPC and gives some support to the hardening of the excited state modes as resulting from a Duschinsky rotation.

4. Conclusions

The results of the present pump-probe measurements on pPC are in quantitative agreement with the previous measurements made by Book and coworker [2], showing that the deactivation of the pump-induced excited state takes place in a nonradiative fashion with a time constant of approximately 280 fs. At variance with these authors, in the Fourier spectrum of pump-probe signal oscillatory component, we observe the 500 cm^{-1} mode already found for some other blue copper proteins [2,4]. A classical MD study of the excited state of pPC copper site confirms that this high frequency mode is typical of the excited state dynamics and strengthens the hypothesis of a Duschinsky rotation as source of this vibrational feature. Duschinsky phenomenon could play an important role facilitating the electron transfer process mediated by blue copper proteins.

Acknowledgments

This work has been partially supported by the EC Project SAMBA (V Frame FET) and the ‘Molecular Nanodevices’ FIRB-MIUR project. Thanks are due to L. Andolfi for preparation of pPC samples and to Dr G. Costantini for preliminary MD runs.

References

[1] M.D. Edington, W.M. Diffey, W.J. Doria, R.E. Riter, W.F. Beck, Radiationless decay from the ligand-to-metal charge-transfer state in the blue copper protein plastocyanin, Chem. Phys. Lett. 275 (1997) 119–126.

[2] L.D. Book, D.C. Arnett, H. Hu, N.F. Scherer, Ultrafast pump-probe studies of excited-state charge-transfer dynamics in blue copper proteins, J. Phys. Chem. A 102 (1998) 4350–4359.

[3] S. Nakashima, Y. Nagasawa, K. Seike, T. Okada, M. Sato, T. Kohzuma, Coherent dynamics in ultrafast charge-transfer reaction of plastocyanin, Chem. Phys. Lett. 331 (2000) 396–402.

[4] T. Cimei, A.R. Bizzarri, S. Cannistraro, G. Cerullo, S. De Silvestri, Vibrational coherence in azurin with impulsive excitation of the LMCT absorption band, Chem. Phys. Lett. 362 (2002) 497–503.

[5] A.E. Johnson, A.B. Myers, A comparison of time- and frequency-domain resonance Raman spectroscopy in triiodide, J. Chem. Phys. 104 (1996) 2497–2507.

[6] M.J. Rosker, F.W. Wise, C.L. Tang, Femtosecond relaxation dynamics of large molecules, Phys. Rev. Lett. 57 (1986) 321–324.

[7] J.M.Y. Ha, H.J. Maris, W.M. Risen, J. Tauc, C. Thomsen, Z. Vardeny, Observation of molecular vibrations in real time, Phys. Rev. Lett. 57 (1986) 3302.

[8] K.A. Nelson, L.R. Williams, Femtosecond time-resolved observation of coherent molecular vibrational motion, Phys. Rev. Lett. 58 (1987) 745.

[9] J.R. Winkler, H.B. Gray, Electron transfer in ruthenium-modified proteins, Chem. Rev. 92 (1992) 369–379.

[10] L. Andolfi, S. Cannistraro, G.W. Canters, P. Facci, A.G. Ficca, I.M.C. Van Amsterdam, et al., A poplar plastocyanin mutant suitable for adsorption onto gold surface via disulfide bridge, Arch. Biochem. Biophys. 339 (2002) 81–88.

[11] L. Andolfi, B. Bonanni, G.W. Canters, M.PH. Verbeet, S. Cannistraro, Scanning probe microscopy characterisation of gold-chemisorbed poplar plastocyanin mutant, Surf Sci 530 (2003) 181–194.

[12] C.X. Wang, A.R. Bizzarri, Y.W. Xu, S. Cannistraro, Molecular dynamics of copper plastocyanin: simulation of structure and dynamics as a function of hydration, Chem. Phys. 183 (1994) 155–166.

[13] A. Ciocchetti, A.R. Bizzarri, S. Cannistraro, Long term molecular dynamics simulation of copper Plastocyanin in water, Biophys. Chem. 69 (1997) 185–198.

[14] C. Arcangeli, A.R. Bizzarri, S. Cannistraro, Concerted motions in copper plastocyanin and azurin: an essential dynamics study, Biophys. Chem. 90 (2001) 45–56.

[15] C. Arcangeli, A.R. Bizzarri, S. Cannistraro, MD simulation and essential dynamics study of mutated plastocyanin: structural, dynamical and functional effects of a disulfide bridge insertion at the protein surface, Biophys. Chem. 92 (2001) 183–199.

[16] A.R. Bizzarri, S. Cannistraro, Intensity fluctuations of the copper site resonant vibrational modes as studied by MD simulation in single plastocyanin molecule, Chem. Phys. Lett. 349 (2001) 503–510.

[17] E.I. Solomon, J.W. Hare, D.M Dooley, J.H. Dawson, P.J. Stephens, H.B. Gray, Spectroscopic studies of stellacyanin, plastocyanin, and azurin. Electronic structure of the blue copper sites, J. Am. Chem. Soc. 102 (1980) 168–178.

[18] A.A. Gewirth, E.I. Solomon, Electronic structure of plastocyanin: excited state spectral features, J. Am. Chem. Soc. 110 (1988) 3811–3819.

[19] M. Zavelani-Rossi, G. Cerullo, S. De Silvestri, L. Gallmann, N. Matuschek, G. Steinmeyer, et al., Pulse compression over a 170-THz bandwidth in the visible by use of only chirped mirrors, Opt. Lett. 26 (2001) 1155–1157.

[20] P. Carlini, A.R. Bizzarri, S. Cannistraro, Temporal fluctuations in the potential energy of proteins: $1/f^{\alpha}$ noise and diffusion, Physica D 165 (2002) 242–250.

[21] H. Barkhuijsen, R. de Beer, W.M. Bovee, J.H. Creyghton, D. van Ormondt, Application of linear prediction and singular value decomposition (LPSVD) to determine NMR frequencies and intensities from the FID, Magn. Reson. Med. 2 (1985) 86–89.

[22] C.L. Brooks, R.E. Bruccoleri, B.D. Olafson, D.J. Statos, S. Swaminathan, M. Karplus, CHARMM: a program for macromolecular energy, minimisation and dynamics calculations, J. Comp. Chem. 4 (1983) 187–217.

[23] L.W. Ungar, N.F. Scherer, G.A. Voth, Classical molecular dynamics simulation of the photoinduced electron transfer dynamics of plastocyanin, Biophys. J. 72 (1997) 5–17.

[24] J.A. Guckert, M.D. Lowery, E.I. Solomon, Electronic structure of the reduced blue copper active site: contributions to reduction potentials and geometry, J. Am. Chem. Soc. 117 (1995) 2817–2844.

[25] S. Larsson, A. Broo, L. Sjölin, Connection between structure, electronic spectrum, and electron-transfer properties of blue copper proteins, J. Phys. Chem. 99 (1995) 4860–4865.

[26] W.H. Press, B.P. Flannery, S.A. Teukolsky, W.T. Vetterling, Numerical Recipes: The art of scientific computing, Cambridge University Press, New York, 1986.

[27] G.R. Loppnow, E. Fraga, Proteins as solvents: The role of amino acid composition in the excited-state charge-transfer dynamics of plastocyanins, J. Am. Chem. Soc. 119 (1997) 896–905.

[28] D. Qiu, S. Dong, J.A. Ybe, M.H. Hecht, T.G. Spiro, Variation in the type I copper protein coordination group: resonance raman spectrum of ^{34}S-, ^{65}Cu, and ^{15}N-labelled plastocyanin, J. Am. Chem. Soc. 117 (1995) 6443–6446.

[29] T.M. Loehr, J. Sanders-Loehr, in: R. Lontie (Ed.), Copper proteins and copper enzymes, I, CRC Press, Boca Raton, FL, 1984, p. 116.

[30] A.R. Bizzarri, A. Paciaroni, C. Arcangeli, S. Cannistraro, Low frequency vibrational modes in proteins: a neutron scattering investigation, Eur. Biophys. J. 30 (2001) 443–449.
[31] H.X. Zhou, S.T. Wlodek, J.A. McCammon, Conformational gating as a mechanism for enzyme specificity, Proc. Natl. Acad. Sci. USA 95 (1998) 9280–9283.
[32] D.E. Morris, W.H. Woodruff, Detailed aspects of Raman scattering. Overtone and combination intensities and prescriptions of determining excited-state structure, J. Phys. Chem. 89 (1985) 5795–5798.
[33] G.M. Sando, K.G. Spears, J.T. Hupp, P.T. Ruhoff, Large electron transfer rate effects from the Duschinsky mixing of vibrations, J. Phys. Chem. A 105 (2001) 5317–5325.
[34] R. Hegger, H. Kantz, T. Schreiber, Practical implementation of non-linear time series methods: the TISEAN package, Chaos 9 (1999) 413–435.