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Photoinduced charge shift as the driving force for the excited-state relaxation of analogues of the Photoactive Yellow Protein chromophore in solution

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Abstract

Transient absorption spectroscopy with subpicosecond laser excitation is used to probe the primary photoinduced processes in two ester analogues (linear and cyclic) of the Photoactive Yellow Protein (PYP) chromophore in solution. The PYP chromophore is the thioester derivative of the deprotonated *trans*-4-hydroxycinnamic acid. The results found for the ester analogues are compared to those previously obtained for the deprotonated *trans*-4-hydroxycinnamic acid and its amide and thioester derivatives. Special attention is paid to the role of the electron donor–acceptor character of the chromophore substituents and of the molecular flexibility on the excited-state relaxation pathway and kinetics. Solvent viscosity and polarity effects on the kinetics are also analyzed. Two hypothetical relaxation pathways involving a one-bond flip mechanism are proposed to explain the observation of a transient species in the course of the excited-state relaxation of the analogues bearing the stronger electron-acceptor substituents. In the first one, the intermediate is described as a *perp* ground state, whereas the second one involves a twisted excited state where the conformation of the ethylenic bond deviates from 90°. In both cases, the relaxation of the transient state may lead or not to the *cis* isomer.

Keywords: 4-Hydroxycinnamic acid; PYP; Photoisomerization; Solvent effects; Ultrafast spectroscopy

1. Introduction

Photoactive Yellow Protein (PYP) is the photoreceptor for the known avoidance response to blue light exposure [1] of the bacterium *Halorhodospira halophila*. Upon optical excitation PYP undergoes a photocycle, the first overall step of which is the *trans-cis* isomerization of its chromophore, the deprotonated *trans*-4-hydroxycinnamic acid covalently linked to the side chain of the unique cysteine residue of the protein by a thioester bond (Fig. 1, PYP). *Trans-cis* isomerization was however either not observed [2–6] or found to have a very low quantum yield [7] for the deprotonated thioester-phenyl analogue of PYP in solution (Fig. 1: pCT^-). By subpicosecond transient absorption spectroscopy, we previously found that the excited state of pCT^- essentially relaxes back to the *trans* ground state via a populated intermediate state, characterized by a transient absorption band around 450 nm [2–6,8]. By contrast, the deprotonated *trans*-4-hydroxycinnamic acid (Fig. 1: pCA^{2-}) and its amide analogue with an NH_2 substituent at the α position of the carbonyl end group (Fig. 1: pCM^-) undergo *trans-cis* photoisomerization without any detectable intermediate [3–5,9]. The influence of the carbonyl substituent was previously rationalized in terms of electron donor–acceptor structure of the chromophore, which would control the excited-state deactivation pathway [6,10]. Solvent polarity and viscosity effects on the transient spectra were used to further stress the influence of this donor–acceptor character on the reaction free-energy profile or reaction coordinates [6]. The role of the electron donor–acceptor structure of the chromophore was also recently emphasized in a detailed comparison, by the fluorescence up-conversion technique, of ester (Fig. 1: pCE^-) and ketone analogues in their anionic and neutral forms, that is, for which the phenolic donor group is respectively deprotonated and protonated [11].

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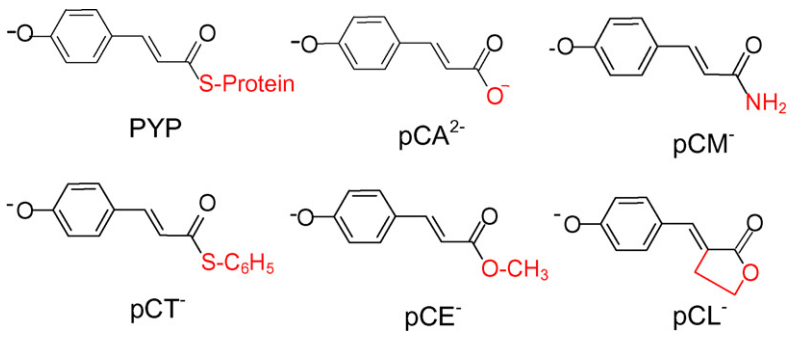

Fig. 1. Chemical structure of analogues of Photoactive Yellow Protein (PYP) chromophore. The substituent in α of the carbonyl group is varied. O^-: 4-hydroxycinnamic acid (pCA^{2-}); NH_2: amide derivative (pCM^-); SC_6H_5: thioester phenyl derivative (pCT^-); OCH_3: methyl ester derivative (pCE^-); OCH_2—CH_2—: cyclic ester derivative or lactone form (pCL^-).

The present study is devoted to the comparison of the transient absorption spectroscopy of pCE^- with that of pCA^{2-}, pCM^- and pCT^-. In a preliminary report [10], we stressed that in pCE^- the acceptor character of the carbonyl substituent (O–CH_3) is larger than that of pCM^- (NH_2) and smaller than that of pCT^- (S–C_6H_5). Consequently, studying pCE^- provides the possibility to further test the role of the electron donor–acceptor character of the chromophore on the excited-state relaxation pathway. Special attention is paid to the coordinates involved in the deactivation of the chromophore in solution, in particular by comparing the transient spectroscopy of the cyclic ester, i.e. the lactone form (Fig. 1: pCL^-), to that of the linear ester pCE^-. Solvent viscosity effect on the kinetics is further analyzed and hypothetical relaxation mechanisms are discussed.

2. Experimental

2.1. Synthesis of the ester analogues of the PYP chromophore and sample preparation

Trans-p-hydroxycinnamic methyl ester (pCEH) was synthesized using the Fisher procedure [12] from *trans-p*-hydroxycinnamic acid, with methanol as the solvent and two equivalents of concentrated sulfuric acid. The product was purified by recrystallization in methanol (yield: 90%). The lactone form (pCLH) was synthesized according to the procedure reported by Zimmer and Rothe [13]: 4-hydroxybenzaldehyde (6.1 g, 50 mmol) and γ-butyrolactone (8.6 g, 100 mmol) were stirred in benzene (70 mL) and sodium methylate was slowly added (15 min). The mixture was warmed (50–60 °C, 4 h), then stirred during 18 h at room temperature. Aqueous sulfuric acid (1 mol L^{-1}) was added until pH ≈ 2 and, then stirring was continued for 1 h. The crude product was isolated by filtration and purified by recrystallization in water (m.p.: 181 °C, yield: 4.25 g, 42%).

The samples for transient absorption spectroscopy were prepared under conditions similar to those previously used for pCA^{2-}, pCM^- and pCT^- [2–6,9]. Aqueous samples of pCE^- and pCL^- were prepared in pH 10.2 buffer solutions (CAPS) to avoid hydrolysis. In alcohols, the compounds were dissolved with 5×10^{-4} M KOH in order to achieve 95% deprotonation and sufficient chemical stability [6]. The optical density of the samples was adjusted to 1 mm^{-1} at the maximum of absorption for steady-state and transient absorption measurements and to 0.1 cm^{-1} for steady-state fluorescence measurements. The solvents used were HPLC-grade water and ethanol (Merk Uvasol), 99%-pure 1-decanol (Acros Organics) and UV-spectroscopy-grade ethylene glycol (Fluka).

2.2. Subpicosecond transient absorption experiments

Transient absorption experiments were carried out with an unconventional 500-fs 10-Hz dye laser as the excitation source. The laser source was described elsewhere [14] but, in the present experiments, a thinner microcavity (15 μm) was however used. The pump pulses (50–100 μJ) were tuned at 355 nm and focused to a 2 mm-diameter spot in a recirculating 1-mm sample cell. High-power 570-nm pulses were simultaneously produced and used to generate a white-light probe in a 1-cm water cell. Pump and probe polarizations were set at the magic angle. The differential absorbance spectra were recorded in the 340–700 nm spectral range, through an imaging spectrograph (Jobin-Yvon 270M, entrance slit 64 μm), by a CCD camera (Roper Scientific RTE/CCD-128-H, 1024 × 128 pixels). They were averaged over 500 pump shots and corrected from group velocity dispersion of the probe pulse. Kinetics traces at selected wavelengths were fitted to a sum of exponentials convoluted to a Gaussian representing the instrumental response. The FWHM of this Gaussian was found to be about 1.5 ps.

3. Results and discussion

3.1. Time-resolved differential absorption spectra of the ester analogues in water

Fig. 2 shows the transient absorption spectra measured for the linear ester (pCE^-) and the lactone (pCL^-) derivatives in aqueous solution at pH 10.2 after 355-nm excitation. The steady-state absorption and fluorescence spectra are given in the lower part of the figure (in the explored spectral range) for the sake of comparison. The absorption and emission maxima of both compounds are respectively located around 350 and 450 nm. The upper part of the figure shows that, for both esters, an initial transient absorption between 370 and 400 nm, assigned to the

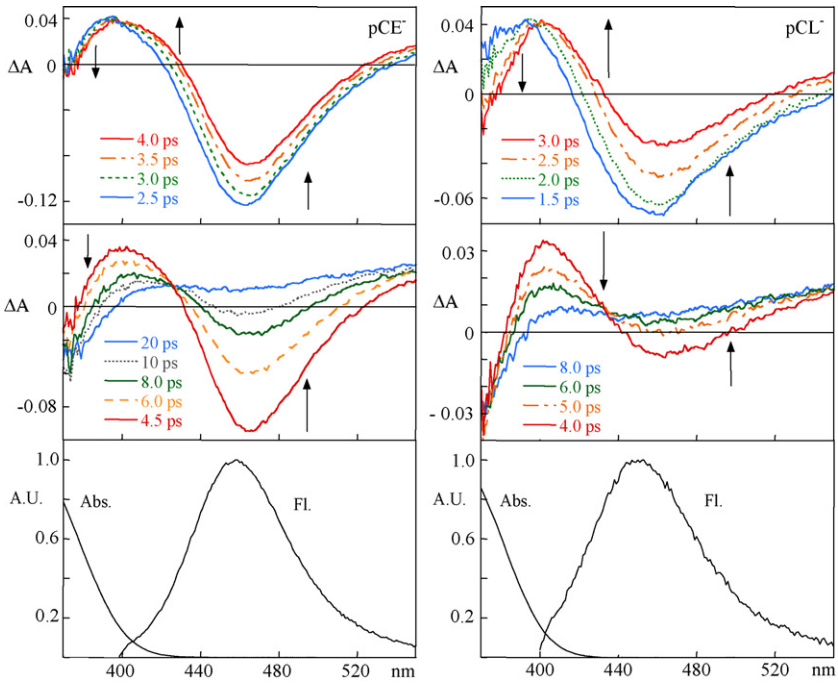

Fig. 2. Time-resolved transient absorption spectra of the linear ester pCE^- (left) and the lactone form pCL^- (right) in aqueous solution after subps excitation. Both compounds relax via the formation of a transient state absorbing around 400 nm. The normalized steady-state absorption (Abs.) and fluorescence (Fl.) spectra are given in arbitrary units (A.U.) in the lower part of the figure for comparison.

initial excited state, decays in a few picoseconds and reveals the bleaching band. Simultaneously, an absorption band rises above 400 nm and a temporary isosbestic point appears in the time-resolved transient absorption spectra around 400 nm. Such a temporary isosbestic point may be explained by the formation of a transient species from the initial excited state, like for the thioester derivative pCT^- [2–5]. The formation of a transient state was also proposed by Vengris et al. for pCE^- [15]. In the upper part of Fig. 2 one sees that the stimulated-emission band, which initially exhibits a maximum around 460 nm, shifts to the red while decaying. Such a shift was previously observed for pCA^{2-}, pCM^- and pCT^- and attributed to solvation dynamics [3,5,6] resulting from optically-induced charge redistribution in the solute [16]. In the middle part of the figure, it is seen that the 400-nm absorption band and the remaining stimulated-emission band keep decaying for several picoseconds.

The kinetics of the differential absorption signal at selected wavelengths are shown in Fig. 3. For the stimulated-emission band, the maximum amplitude of the band is plotted as a function of the delay, in order to avoid artifacts due to its time-resolved red shift. It is seen that, for both pCE^- and pCL^-, the decay of the UV absorption band is similar to that of the stimulated-emission band, which supports our above assignment of the UV band to the initially excited state. The curve showing the kinetics at 400-nm exhibits a flat top, as expected for the spectral region where a temporary isosbestic point is observed (Fig. 2, top) and a decay slower than that of the stimulated-emission band. This is an additional evidence for the presence of a transient species in the time-resolved spectra. The solvent-induced time-resolved red-shift of the stimulated-emission band contributes to the kinetics observed in the 400–440 nm so that the risetime of the transient species cannot be directly probed. Decay times of the stimulated-emission band and of the 400-nm band extracted from the fit of the kinetics shown in Fig. 3 are compared in Table 2. Studies in alcohols (see Section 3.3) further confirm these observations for both pCE^- and pCL^-. The primary photoinduced mechanism

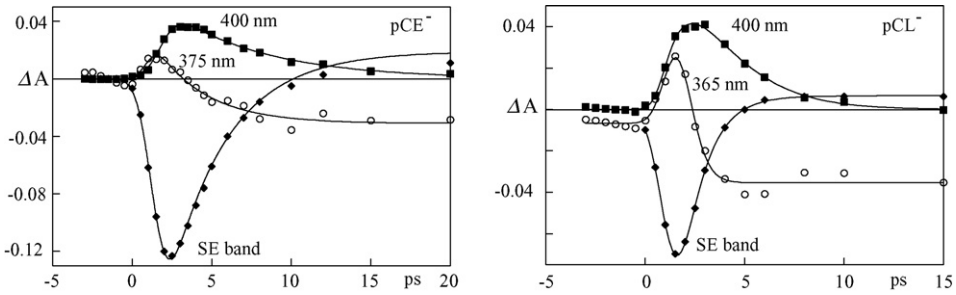

Fig. 3. Kinetics traces of the differential absorption at selected wavelengths for the linear ester pCE^- (left) and the lactone form pCL^- (right) in aqueous solutions. The decays of the UV transient-absorption band and of the stimulated-emission (SE) band are compared to that of the transient 400-nm transient band.

probed for the esters is thus similar to that of the thioester derivative, which further supports that the presence of a strong electron-acceptor in α of the carbonyl group is a key element which controls the excited state relaxation pathway.

By analogy with our previous studies on pCA^{2-}, pCM^{-} and pCT^{-} [3,5,6,9], the residual broad absorption band observed above 400 nm in Fig. 2, at 20 ps for pCE^{-} and 8 ps for pCL^{-}, is assigned to the biphotonic formation of solvated electrons. Such a two-photon ionization process was also reported in studies of other analogues [8]. According to results obtained for thioester derivatives [5,8], the radical cation photoproduct is expected to absorb around 350 nm, which is out of the spectral window probed in the present experiments. The observed residual bleaching below 400 nm is thought to arise from the ground-state depopulation that results from the two-photon ionization process. It was not observed in experiments carried out with a 310 nm excitation wavelength, where two-photon ionization does not occur [15].

One can thus conclude that the primary photoinduced processes in pCE^{-} and pCL^{-} seem to be comparable to those in the thioester derivative pCT^{-}. In addition, the evolution of the transient spectra is found to be faster for the bridged form (pCL^{-}) than for the linear form (pCE^{-}) of these ester analogues. This indicates that the rigidity of the electron-acceptor substituted carbonyl group does not hinder the observed process.

3.2. Role of the electron donor–acceptor character of the carbonyl end group on the excited-state decay time

In our previous reports [2–5], we emphasized a major effect of the chemical structure of the chromophore on the light-induced process in this class of compounds. The present results confirm this trend.

Table 1 gives the excited-state lifetime of the five analogues in aqueous solution and the Hammett parameter (σ_p) of the corresponding carbonyl substituent, which measures its electron acceptor character [17]. The lifetimes were obtained from the fit of the stimulated-emission decay with a monoexponential function. The Hammett parameter of the $O–(CH_2)_2$-substituent of the lactone pCL^{-} is not available but we expect it to be similar to that of the $–O–CH_3$ substituent of pCE^{-}, thus smaller than that of the –S–Ph substituent of pCT^{-}. pCL^{-} is however found to have the shortest lifetime of all studied analogues, which suggests that motions hindered by the lactone ring provide channels for the excited population to temporarily explore the excited-state potential surface out of the reactive channel. One may hypothesize that removing these degrees of freedom forces the system into a more efficient reaction pathway. Alternatively, one might presume that the lactone ring is in fact a better electron acceptor than the linear form. Charge-transfer reactions in the femtosecond regime were reported for other lactones even in apolar solvents, the parameter controlling the dynamics being assigned to intramolecular vibrational motions involving the CO bond in the lactonic ring [18].

Table 1
Excited-state lifetime of five PYP analogues in aqueous solution

Analogue	pCA^{2-}	pCM^{-}	pCE^{-}	pCT^{-}	pCL^{-}
τ_{S1} (ps)	10 ± 1	4.0 ± 0.5	3.0 ± 0.5	2.4 ± 0.4	1.0 ± 0.2
σ_p	−0.81	−0.66	−0.27	0.07	

Hammett parameters σ_p of the substituent in α of the carbonyl group (see Fig. 1) from [17]. The error given on the lifetimes indicates the range of values obtained in different experiments.

The present study definitely demonstrates that both the excited-state deactivation pathway and the decay kinetics of the studied analogues of the PYP chromophore are controlled by the electron-acceptor strength of the carbonyl end group. The deactivation pathway involves a detectable intermediate only for the analogues bearing a strong electron-acceptor group such as pCE^{-}, pCT^{-} and pCL^{-}. The photoisomerization yield of pCT^{-} is negligible [2–6] or less than 5% [7] whereas steady-state photolysis of pCE^{-} and pCL^{-} leads to a photoproduct absorbing in the blue edge of the absorption spectrum of the initial *trans* isomer. The photoproduct has been attributed [11] to the *cis* isomer by comparison with spectra reported for *trans-p*-coumaric acid but the photoisomerization yield has not been quantified. Possible reaction mechanisms will be examined in Section 3.4.

3.3. Solvent properties and relaxation dynamics

We previously compared the influence of the solvent properties on the excited-state deactivation pathway of pCA^{2-} and pCT^{-}, which respectively bear the weaker and the stronger electron-acceptor end groups of the linear series [6], as well as the influence of the negative charge on the electron-donor phenol end group for pCE^{-} and a ketone analogue [11]. The excited-state lifetimes of the anionic forms were found to decrease with increasing polarity (ε) and increase with increasing viscosity (η). pCT^{-} was however found to exhibit a larger sensitivity to viscosity and polarity than pCA^{2-}. An empirical analysis of the S_1 lifetime in terms of the power law η^a showed that parameter "a" is two to three times larger for pCT^{-} (0.75 versus 0.28) [6]. A similar power law was found for the fluorescence lifetime of pCE^{-} and a ketone analogue in water–glycerol mixtures with $a = 0.64$, whereas only a very weak viscosity effect could be observed for the protonated forms [11]. This power law is tested again here for pCE^{-} and pCL^{-} in aqueous and alcoholic solutions. The excited-state lifetimes obtained from the decay of the stimulated-emission band (τ_{se}) are reported in Table 2 with the

Table 2
Decay-time (τ_{es}) of the stimulated-emission band and of the transient state (τ_{tr}), of the ester analogues pCE^{-} and pCL^{-} in aqueous and alcoholic solutions

Solvent	η (cP)	ε	pCE^{-}		pCL^{-}	
			τ_{se} (ps)	τ_{tr} (ps)	τ_{se} (ps)	τ_{tr} (ps)
Water	0.89	78.3	3.0	4.5	1.0	3.5
Ethanol	1.07	24.6	10*	34	1.4	37*
Decanol	10.9	7.2	15*	30*	2.9*	29*
Ethyleneglycol	16.1	37.7	24	36*	6	17*

For nonexponential decays, the average lifetime is given. Values of the solvent viscosity (η) and dielectric constant (ε) at 25 °C were taken from [19].
* Nonexponential decays.

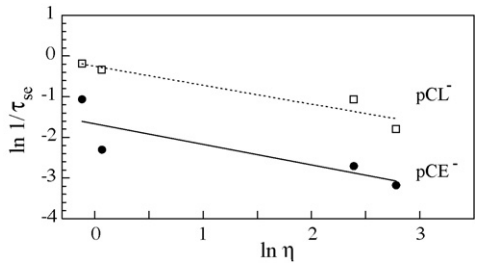

Fig. 4. Decay-time of the stimulated-emission band (τ_{se}) of pCE^- (●) and pCL^- (□) as a function of the solvent viscosity η, in aqueous and alcoholic solutions. The two esters exhibit similar power-law dependence (η^a), with a ≈ 0.5.

corresponding solvent polarity and viscosity taken from reference [19]. The decay times of the transient state are also given for comparison. The plots of $\ln(1/\tau_{se})$ as a function of $\ln \eta$ (Fig. 4) indicate that the two esters experience similar viscosity effects. This rough analysis leads to a smaller "*a*" value for pCE^- than that found from fluorescence up-conversion measurements in water–glycerol mixtures [11] (0.5 instead of 0.64). It is nevertheless still noticeably larger than that found for pCA^{2-} (0.28). The larger viscosity effect observed for the analogues bearing the stronger electron-acceptor substituent is considered as further evidence that the relaxation pathway of these analogues depends on the electron donor–acceptor structure of the chromophore.

Let us now consider the relaxation of the transient state. We previously reported that the lifetime of the transient state of the thioester derivative pCT^- was 3.3 ps in water [6]. Although the decay time of the transient state is difficult to measure with precision due to the small amplitude of its absorption band, one sees in Table 2 that this value is about the same as the transient-state lifetime of the lactone pCL^- (3.5 ps) and somewhat smaller than that of pCE^- (4.5 ps). Comparison between pCE^- and pCT^- seems thus to indicate that not only the formation rate but also the decay rate of the transient state increases with the electron-acceptor character of the carbonyl substituent. Comparison between pCE^- and pCL^- seems to indicate again that removing of some internal degrees of freedom forces the system into a more efficient relaxation pathway. We previously reported that the decay of the transient state of pCT^- was slowed down in viscous solvents [6]. The viscosity effect on the transient state decay rate was even found larger than that on its formation rate [6]. One observes a similar effect for pCE^- and pCL^- when the solvent is changed from water to ethanol (Table 2). However, when the solvent viscosity is further increased, the transient state lifetime remains of the same order of magnitude so that it cannot be analyzed in terms of the power law η^a.

3.4. Influence of the electron donor–acceptor character on the relaxation pathway

If the electron-acceptor character of the end group is weak (pCA^{2-} and pCM^-), *trans-cis* photoisomerization occurs without any detectable intermediate [3–6,9]. If it is strong (pCE^-, pCL^- and pCT^-), the excited-state relaxation pathway involves a detectable intermediate and, in the case of pCT^-, does not lead to the *cis* isomer [2–6]. An interpretation of those observations is to consider that, in fact, an intermediate state is reached for all molecules. Depending on the electron-acceptor character of the end group, this intermediate is either very short-lived, hence non-observed, and partially leads to the *cis* isomer or else it is longer-lived, hence observed, and either totally or partially leads back to the *trans* isomer. In this section we will focus our attention on the influence of the electron donor–acceptor character on the escape of the excited-state population from the Franck–Condon state and formation of the intermediate state. We will then hypothesize different photoinduced relaxation

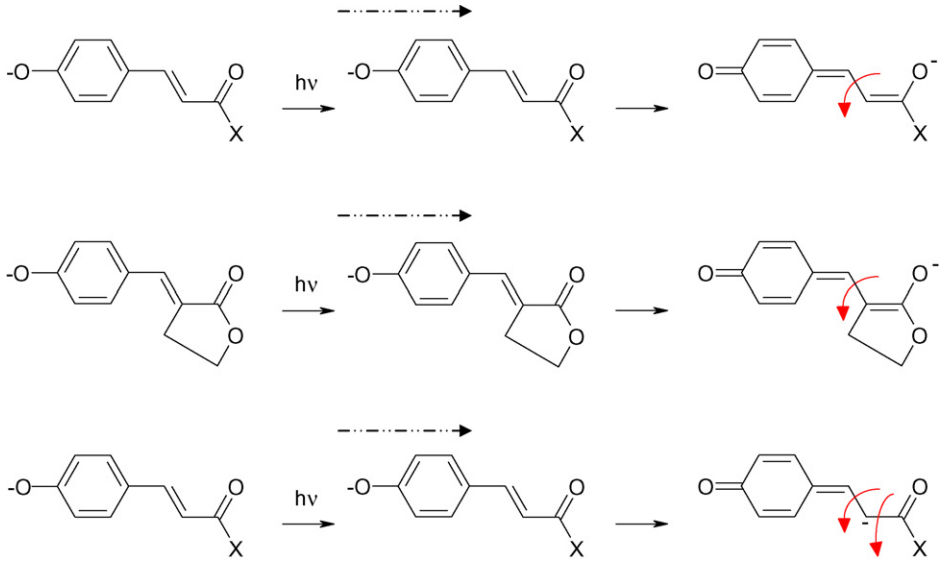

Fig. 5. Hypothetical role of the electron-acceptor character of the carbonyl end group on the extent of the photoinduced charge shift and associated skeleton flexibility (top: X = strong electron-acceptor, middle: lactone form, bottom: X = weak electron-acceptor). Arrows drawn in dashed line illustrate the optically induced charge shift. Red arrows show potential sources for geometrical changes associated to the electronic redistribution.

mechanisms by considering that the intermediate state is either a ground state species or a non-emissive excited species.

3.4.1. Photoinduced charge transfer

The fact that the excited-state decay rate (i) increases with the electron-acceptor character of the carbonyl substituent (Table 1) and (ii) increases with the solvent polarity [6,11] is a strong indication that the production of the intermediate state from the initial excited state involves a charge transfer. From electrooptical measurements [16], it is expected that optical excitation leads to a dipole moment change corresponding to shifting the negative charge from the oxygen of the phenolate donor-group to the phenol ring or even to the ethylenic bond. We thus propose that this charge shift proceeds further in the excited state, giving rise to an even more charge-shifted state, as illustrated in Fig. 5. The driving force of this reaction could be a better localization of the charge, hence a larger solvation energy of this product state. In the case of analogues with a strong electron-acceptor end group (pCT^-, pCE^-), the charge can be thought to go all the way to the carbonyl oxygen, with a complete reversal of the single and double bond pattern as shown in the upper part of Fig. 5. This analysis also holds for the lactone pCL^-, the intramolecular bridge of which does not affect the photoinduced change in the bond pattern (Fig. 5, middle). However, the presence of a weak electron-accepting end group, or even a repulsive one in the case of pCA^{2-}, is expected to lead either to a charge shift of a lesser extent (Fig. 5, bottom) or else to a complete shift of a partial charge, and then to a less rigid structure at the carbonyl-end site.

In all cases drawn in Fig. 5 the charge-shifted state displays a single bond instead of the initial ethylenic bond. We thus think that its geometry tends toward a 90° twist of the ethylenic bond, because this rotation enhances the decoupling of the two halves of the molecules and localizes better the negative charge on the carbonyl side. This structure, comparable to the so-called excited-state *perp* minimum involved in the photoisomerization of stilbene [20], would similarly be thought to be the gateway toward a possible *trans-cis* isomerization.

According to Grote and Hynes' theory [21], the larger constraint exerted by the solvent viscosity on the stimulated-emission band decay of pCT^-, pCE^- and pCL^-, as compared to pCA^{2-} is an indication that the formation of the twisted charge-shifted state involves the crossing of a lower barrier, with a smaller frequency, which implies a larger sensitivity to low-frequency fluctuations of the solvent. It may also mean that the photoinduced process involves a motion of larger amplitude of the molecular site bearing the localized charge, that is, more displacement of the localized negative charge in the solvent and thus more solvent reorganization with an apparent viscosity effect. This would be consistent with the fact that the excited-state lifetime of pCT^- keeps increasing with the average solvation time (which also contains diffusional dielectric responses) when that of pCA^{2-} levels off [10]. In previous reports [6,11], we proposed that the photoinduced internal torsion may change from a process involving concerted motions to a one-bond flip (torsion around the ethylenic bond) depending on the electron donor–acceptor structure of the chromophore, the stronger donor–acceptor structure undergoing a one-bond flip. For protonated ester and ketone analogues [11], which have the weakest electron donor–acceptor structure, motions like the hula-twist [22] or bicycle-pedal [23] mechanisms have been suggested [11]. These different mechanisms have indeed been proposed as possible photoisomerization pathways of different types of compounds. At this stage of the discussion one has to explain why, in the present study, the deprotonated analogues bearing the weaker electron-acceptor carbonyl substituents lead to a net *trans-cis* isomerization whereas the others do not, or do less. We will thus focus on the formation of the twisted charge-shifted intermediate proposed above and discuss relaxation mechanisms involving such an intermediate either in the ground state or in the excited state.

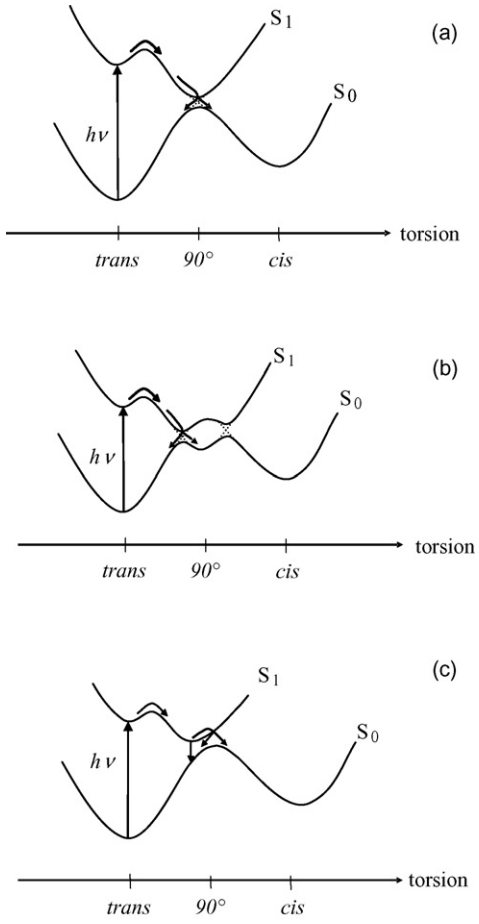

Fig. 6. Tentative relaxation pathways describing: (a) photoisomerization of analogues bearing the weaker electron-acceptor substituents, (b) formation of a transient ground state (adapted from reference [6]) or (c) of a transient excited state in the relaxation pathway of analogues bearing the stronger electron-acceptor substituents.

3.4.2. Relaxation via a twisted ground state intermediate

In a previous comparison of pCA^{2-}, pCM^- and pCT^- [6], we considered that a fully 90° twisted charge-shifted *perp* state is reached in all cases. For the less rigid analogues bearing the weaker electron acceptor (pCA^{2-}, pCM^-), this *perp* configuration would be reached by a mechanism involving concerted motions like in stilbene (see Fig. 6a) [24–26]. For the rigid analogue bearing the stronger electron acceptor, pCT^-, the *perp*

state would be reached by a one-bond flip mechanism. However, as a result of the large stabilization of this state due to the strong electron-acceptor character of the thioester-phenyl end group, we proposed that an avoided crossing with the ground state occurs at the 90° geometry [6]. As shown in Fig. 6b, such an avoided crossing leads to a minimum at the 90° configuration in the ground state. This type of situation has previously been described for the zwitterionic *perp* state of olefins in polar solvents [27]. From the present study one would thus conclude that the other two rigid analogues bearing a strong electron acceptor, pCE^- and pCL^-, would follow a relaxation mechanism similar to that of pCT^-. The relative height of the barriers from this ground-state minimum respectively to the *cis* and *trans* ground state configurations would thus control the photoisomerization yield. One might expect a higher barrier to the *cis* isomer for more rigid structures, i.e. for compounds bearing the strongest electron acceptors such as pCT^-.

3.4.3. Relaxation via a twisted excited state intermediate

In a second approach, since pCA^{2-} and pCM^- are found to photoisomerize without any detectable intermediate, i.e. according to a stilbene-like mechanism, one can hypothesize that reaching the complete 90° twist of the *perp* state in the excited state requires the concerted motion of other coordinates. As a matter of fact, for pCA^{2-} and pCM^-, one can see in Fig. 5 bottom that the carbonyl end group can rotate freely. Such flexibility would also be kept for a complete shift of a partial charge. This flexibility could thus be thought to stabilize the *perp* state, hence to favor isomerization. Although it is not obvious at first sight why such a molecular flexibility would be needed, one can suppose that, in the *perp* geometry, the torsion of the carbonyl end group releases internal constraints, possibly due to steric hindrance and/or electronic repulsion between the substituted carbonyl end group and the phenol ring moiety. For the analogues with stronger electron acceptors, pCT^-, pCE^- and pCL^-, such flexibility is not available, as shown in Fig. 5 top and middle, and the twist angle of the ethylenic bond at the minimum energy of the S_1 state would thus be thought to deviate from 90° in order to avoid the above-mentioned constraints. This scenario is illustrated in Fig. 6c. The twisted excited species at the minimum of the excited state potential would thus be the non-emissive transient state that we observe. Its picosecond lifetime would result from a fast internal conversion (the S_1–S_0 energy gap is indeed likely to be small for geometries close to the *perp* configuration and to be smaller for analogues bearing stronger electron acceptor) and/or from an activated process necessary to reach an hypothetical conical intersection at the *perp* geometry. Internal conversion would be favored in polar solvents because of a lower energy gap and up-hill motion to the conical intersection would experience viscosity effect. Fast internal conversion down to the *trans* ground state would thus be the dominant process in the case of pCT^-.

Although a detailed description of the mechanism is still an open question, the present study gives a clear demonstration of the influence of the electron donor–acceptor structure on the photophysics of the analogues of the PYP chromophore. We can thus confirm our previous statement that within the natural PYP photoreceptor, photoisomerization of the thioester chromophore does occur because the expected large photoinduced charge shift is counteracted by the presence of a positive charge on the nearby Arginine52 residue [6]. The role of this positive charge had previously been stressed by Groenhof et al. [28], who however proposed a reversed photoinduced charge-transfer mechanism.

4. Conclusion

From ultrafast spectroscopy experiments carried out on two ester analogues of the Photoactive Yellow Protein chromophore, and by comparison with previously published data on three other analogues (coumaric acid, its amide and thioester derivatives) [2–6,9,10], we have further demonstrated that the photoinduced behavior of these compounds is strongly dependent on their electron-donor–acceptor structure. Compounds for which the carbonyl group is substituted with a strong electron acceptor exhibit a relaxation pathway involving a transient state that may preferably relax to the initial *trans* ground state. In particular the thioester derivative, which models the native chromophore of PYP, does not isomerize in aqueous and alcoholic solutions. We have tried to rationalize those facts in terms of the extent of a photoinduced charge shift and of molecular flexibility. The excited-state decay rate of these analogues is sensitive to the solvent viscosity. The fact that analogues bearing good electron-acceptor substituents experience larger viscosity effect is interpreted by the crossing of a lower barrier and/or by the involvement of different intramolecular coordinates as well as a solvent coordinate. We have hypothesized two relaxation mechanisms to explain the observation of a transient state in the relaxation pathway of the analogues substituted with a strong electron acceptor. In the first one, the intermediate is described as a *perp* ground state, whereas the second one involves a twisted excited state where the conformation of the ethylenic bond deviates from 90°. Although the mechanism is still an open question, the present study allows us to confirm our previous statement that within the natural PYP photoreceptor, photoisomerization of the thioester chromophore does occur because the expected large photoinduced charge shift is counteracted by the presence of a positive charge on the nearby Arginine52 residue.

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