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# trans−cis Photoisomerization of a Photoactive Yellow Protein Model Chromophore in Crystalline Phase

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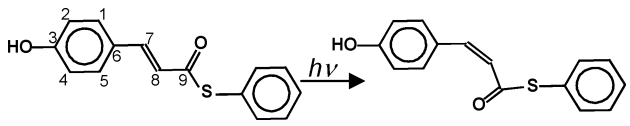
We have studied the photoinduced trans/cis isomerization of the protonated form of *p*-hydroxycinnamic thiophenyl ester, a model chromophore of the photoactive yellow protein (PYP), in crystalline phase, by both fluorescence and infrared spectroscopies. The conversion from trans to cis configuration is revealed by a shift of the fluorescence peak and by inspection of the infrared maker bands. The crystal packing apparently stabilizes the cis photoproduct, suggesting different environmental effects from the solvent molecules for this model chromophore in liquid solutions or from the amino acid residues for the PYP chromophore.

The photoactive yellow protein (PYP), a photoreceptor protein for a negative phototactic response found in the bacterium *Halorhodosphira halophila*, contains a chromophore, *p*-hydroxycinnamate thioester, covalently bonded to Cys69 via a thioester bond in the hydrophobic protein pocket. In the ground state, the chromophore is in the deprotonated form of the trans configuration.[1,2] Upon photon absorption, PYP undergoes a photocycle involving both chromophore and protein structural relaxation on time scales spanning from a few hundred femtoseconds to milliseconds.[1−4] The trans to cis photoisomerization in the primary stage is a key structural dynamic process of the overall photocycle. Detailed structural analyses of the intermediate $I_1$ and $I_2$ states (the cis structure with deprotonated and protonated phenolate group, respectively), which appear on time scales from nano- to milliseconds, have been obtained using time-resolved X-ray diffraction[5] and Fourier transform infrared (FTIR)[6] experiments. At low temperature, the PYP photocycle is known to involve additional intermediate formation that may be similar to those observed at room temperature. Although no direct correspondence has been established, the $PYP_{dark}$ and $PYP_M$ states at low temperature are related to the ground-state P and intermediate $I_2$ state of the room-temperature PYP photocycle, repectively.[3] Recent ultrafast time-resolved spectroscopic measurements on PYP in solution have revealed an early intermediate within 3 ps after excitation, named $I_o$, which already has a cis geometry without a relocation of the phenolate group.[7,8] The chromophore isomerizes by rotating the thioester bond after the photon absorption inducing apparently an intramolecular charge transfer. The trans−cis isomerization is then followed by a proton transfer from the side group of the protein pocket. Similar photoinduced trans−cis isomerization is also found in many types of photochromic switches and photosensor proteins.[9]

Photoexcitation dynamics of the PYP mutants[10] and isolated PYP model chromophores[11−14] in various solutions has been studied extensively to provide a better understanding of the photoisomerization mechanism and to find whether the isomerization is controlled by intrinsic properties of the chromophore or by the surrounding protein. Interactions of the chromophore with surroundings have been proven to affect both the excited-state structure and the couplings with other states that often lead to pronounced changes in the reaction dynamics and quantum yields. Determination of the structural dynamics of PYP and its model chromophores after photoexcitation remains an important issue in recent research topics.[7−16]

**SCHEME 1: Photoinduced trans−cis Isomerization of PCT**




In this Letter, we present a study of the photoinduced isomerization of *p*-hydroxycinnamic thiophenyl ester (PCT) (Scheme 1) in the crystalline phase. Studying the dynamics of the model compound of PYP in the packing structure would enhance the understanding of photophysics of PYP and the surrounding effects, because the packing structure which provides strong intermolecular interactions can be expected to give different effects from the surrounding environment compared with those of the buried chromophore in the protein or free model chromophores in liquid solutions.

The model chromophore in the deprotonated form of the phenolate group ($PCT^-$) has been studied previously in various solvents by UV/vis and IR pump−probe spectroscopies.[12,14] The existence of a nonfluorescent intermediate and a photoproduct cis configuration with very low quantum yields (not in every case) has been discussed. Recently, it was pointed out that the electron-donating nature of the hydroxy group strongly affects the photoisomerization channel.[12c,17] Despite the fact that the protonated hydroxy group of the model chromophore in the present study is expected to have a less-strong donor ability, which affects the charge mobility upon excitation compared with those of the deprotonated form,[12c,d] its effects on photoisomerization remain a subject of investigation. Notably, when the

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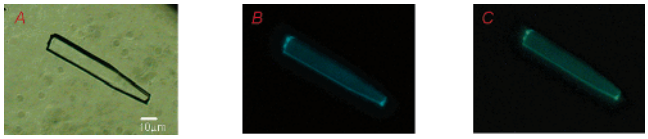


**Figure 1.** Image of a PCT microcrystal (A). Fluorescence images of the same crystal after a few seconds of irradiation (405 nm, 8 mW) (B) and after 125 s of irradiation (C).

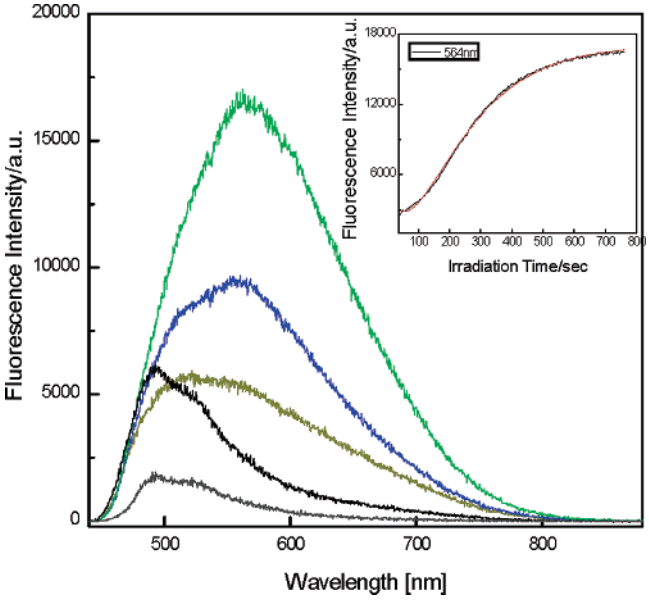


**Figure 2.** Fluorescence spectra of a PCT microcrystal after UV light irradiation ($\lambda$ = 405 nm, 1.9 mW) at 3.8 s (gray), 38 s (black), 150 s (dark yellow), 250 s (blue), and 750 s (green). Inset: the time-dependent fluorescence intensity of the new band having a peak at 564 nm and the calculated time profile (red curve) with two exponential rise components (100 and 160 s).

model chromophore isomerizes to the cis form, it resembles the later stage of the chromophore, $I_2$ or $PYP_M$ intermediate state, in the PYP photocycle. We investigate here the trans−cis isomerization in crystalline phase by monitoring fluorescence dynamics and static FTIR spectra. The new emission in fluorescence spectra is associated to the excitation of a new species, and the spectral changes in the IR-active marker modes provide details on structural deformations of the molecular configuration, as this technique is highly sensitive to minor changes in bond lengths, bond angles, protonation, and hydrogen bond interactions.

The PCT chromophore was synthesized according to the procedure reported earlier.[18] Needlelike microcrystals were prepared using a slow evaporation of solvent from 20 mM of PCT in dichloromethane solution on quartz plate substrates. After complete drying, the microcrystals were used for fluorescence spectroscopic measurements using an inverted microscope (Olympus) equipped with a Hg lamp as an excitation light source. A dichroic mirror and ND filters were used to select a monochromatic light and to adjust the intensity power, respectively. Steady-state IR spectroscopy before and after UV irradiation was performed on samples of a microcrystal aggregate using a Horiba FT720 spectrophotometer separated from the fluorescence microscope.

Figure 1 shows fluorescence images of a PCT single microcrystal showing emission color changes upon UV light irradiation. Fluorescence spectra of the single microcrystal at different irradiation times are shown in Figure 2. In a few seconds of irradiation, the intensity of the fluorescence band at 490 nm from the trans configuration of PCT increases without

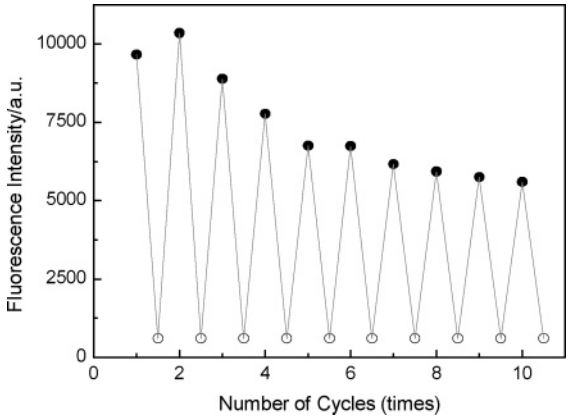


**Figure 3.** Fluorescence intensity at 605 nm against the number of alternating UV and visible irradiations; the filled (●) and open (○) data points are the intensity after UV (125 s, $\lambda$ = 405 nm, 8 mW) and visible radiations (125 s, $\lambda$ = 546 nm, 17.7 mW), respectively.

any change in peak position. Assignment of the increase is still unclear, but we consider that, during one or a few cycles of excitation and relaxation processes, molecular conformation and intermolecular interactions (such as hydrogen bonding) in the crystal could be slightly changed before a twisted conformation occurs, resulting in an increase of fluorescence efficiency. Upon continuous irradiation, the fluorescence intensity reaches a maximum at around 40 s, and then, the fluorescence peak shifts toward a longer wavelength. This is followed by the rise of a new band having a peak at 564 nm. This new emission band is associated to fluorescence from distinctly different species, apparently the cis photoproduct. An interesting experimental observation is that the reverse process can be induced by a subsequent visible light irradiation as shown in Figure 3. By repeated UV and visible irradiations, we demonstrated that the fluorescence from the initial trans configuration is reproducible, although there is a sign of degradation. The degradation suggests that only a partial amount of the trans form is recovered upon visible excitation, and hence, the photochromic process involves a branched pathway. It is noted that both trans and cis configurations were thermally stable, and after UV irradiations, their fluorescence intensities increased under dark condition, indicating a recovery from intermediates between trans and cis forms. The possibility of photodimerization which occurs upon UV irradiation of several trans cinnamic acid derivatives should be excluded because the dimerization process is irreversible.

The two main fluorescence bands with peaks at 490 and 564 nm strongly overlap without any isoemissive point. As shown in Figure 2, the rise of intensity at 564 nm involves an induction period, and overall data could be fitted to double exponential time constants. This also strongly suggests that the photoinduced trans−cis isomerization involves a complex pathway upon relaxation and is not a simple two-state photochromism. An intermediate species is considered to be involved as a gateway toward cis form. The intermediate may be related to a configuration of 90° twist of the ethylenic bond so-called ground-state perp minimum. Formation of such an intermediate has been proposed by Martin's group[12] in femtosecond UV−vis spectroscopic studies on the deprotonated $PCT^-$ and by Rettig's group[17] for other PYP model chromophores (4-hydroxycinnamate ester analogues) in liquid solutions.

Conversion from the trans to cis structural configuration was revealed by inspection of IR vibrational marker modes similar to those in the trans/cis isomerization of the PCT in liquid solutions[14] or the chromophore in PYP.[7,8,15,16,19−21] The bands of $\gamma$CH═CH hydrogen out-of plane bending and $\nu C_8$—$C_9$

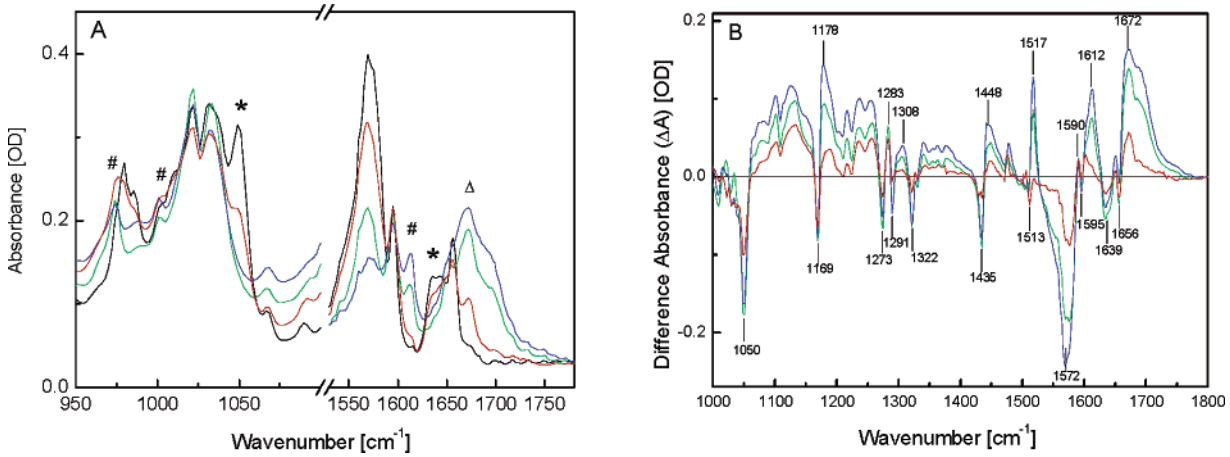


**Figure 4.** (A) IR spectrum of PCT before (black) and after UV irradiation (1.9 mW) at 60 s (red), at 500 s (green), and at 750 s (blue). The asterisk (*), pound (#), and triangle (△) denote the disappearing, newly raised, and frequency-shifting bands, respectively. (B) The difference IR spectra between before and after irradiations.

stretching vibrational modes around 1050 $cm^{-1}$ of the *trans*-PCT[14] disappear upon UV light irradiation. A clear band at 973 $cm^{-1}$ and a tiny band at 994 $cm^{-1}$ (Figure 4) which have been assigned to the $\gamma$CH═CH and $\nu C_8$—$C_9$ modes, respectively,[19,20] of the cis configuration arise after the irradiation. In comparison to PYP, the conversion from $PYP_{dark}$ to $PYP_M$ clearly exhibited similar frequency downshift of the $\nu C_8$—$C_9$ of the chromophore from 1058 to 994 $cm^{-1}$.[15,19−21] On the other hand, for the deprotonated $PCT^-$ in solution, the cis photoproduct state arises after 30 ps in the 980−995 $cm^{-1}$ region. Owing to the low quantum yield, this new band is extremely small, and since no other bands could be assigned to the cis form, its accurate structure determination was hampered.[14] The isotropic solvent molecules surrounding the free chromophore and the strong electron donor character of $-O^-$ are responsible for the very unstable cis ground state or for different twisting channels and conical intersections.[17]

The ethylenic double-bond bridge ($C_7$═$C_8$) is another important feature and an indicator of the trans−cis isomerization of the PYP chromophore. In this study, we observe the clear disappearance of the mode mainly assigned to the trans $\nu$C═C($A_g$) at 1639 $cm^{-1}$, which is followed by the appearance of a new band at 1613 $cm^{-1}$. This latter band has been assigned to the $\nu$CC(8a) + $\nu$C═C($A_1$) mode of cis configuration.[20] This mode, however, is observed at 1599 $cm^{-1}$ in the $PYP_M$ due to a difference in contributions or degrees of vibrational couplings.[19,20] Accordingly, the change in ethylenic mode from the trans $\delta C_7H$═$C_8H(B_u)$ to a coupled mode of cis $\gamma C_3$–O and $\delta C_7H$═$C_8H(A_1)$ is exhibited by shifting down an IR band from 1291 to 1283 $cm^{-1}$. A recent IR study on *trans*-4-hydroxycinnamic acid shows the related pair of bands at 1294/1288 $cm^{-1}$ upon isomerization.[20] In addition, the cis $\delta C_7H$═$C_8H(A_1)$ vibrational mode in the $PYP_M$ is assigned to the band at 1286 $cm^{-1}$.[21] We note that, although the phenolic moiety of PCT is structurally unchanged, the IR band at 1273 $cm^{-1}$ assigned to coupling vibrations between $\nu C_3$—O and the phenolic ring skeleton modes in the trans form is frequency-upshifted to 1283 $cm^{-1}$ upon the isomerization. This new frequency position is assigned to a coupling between $\nu C_3$—O vibration and the $\delta$CH═CH($A_1$) rocking motion of the cis configuration.[20]

A direct insight into the trans−cis isomerization is also provided by frequency upshift of the carbonyl $\nu$C═O stretching mode from 1656 $cm^{-1}$ to a broad band at 1672 $cm^{-1}$, where the twisting and isomerization sets the carbonyl relatively free and less substantial couplings with other nuclear vibrations resulting in higher-frequency position of the $\nu$C═O mode. A similar frequency upshift of the carbonyl stretching mode from 1631 $cm^{-1}$ in $PYP_{dark}$ to 1653 $cm^{-1}$ in $PYP_M$ was also observed, assigning the trans−cis isomerization of the chromophore in PYP.[19−21] In addition, intermolecular hydrogen bonds in the crystal packing involving the hydroxyl group are disrupted upon molecular twist as indicated by a frequency upshift of the OH stretching mode by ∼38 $cm^{-1}$ at 3358 $cm^{-1}$. This is in agreement with the increase of 490-nm fluorescence intensity after a few seconds of irradiation. In the IR spectra, in addition to the isomerization maker modes, the frequency positions of several bands assigned to coupled vibrational motions of the phenolic moiety[14,19,20] slightly shift from 1169, 1435, 1513, and 1595 $cm^{-1}$ to 1178, 1448, 1517, and 1590 $cm^{-1}$, respectively, whereas localized vibrational modes of the thiophenyl moiety ($SC_5H_5$)[14] at 1067, 1108, and 1476 $cm^{-1}$ remain unchanged. These provide conclusive evidence that the vibrational motions of these two moieties are not significantly altered upon the flipping and isomerization, although the detailed mechanism is still an open question.

Finally, from the fluorescence spectra and the inspections of the IR vibrational marker modes, we believe that the photoinduced isomerization of trans PCT occurs in the crystalline phase with the photoproduct being consistent with the cis geometry. This is the first unequivocal demonstration of the conversion from the trans to the cis state of PCT. In the tight crystal packing, due to its steric hindrances, the trans−cis photoisomerization should be considered to involve volume-conserving conformational movements. The movements could be a concerted twisting of the ethylenic double bond and one or two adjacent bonds such as the bicycle pedal or hula twist mechanism.[22] Similar mechanisms have also been proposed recently by Martin's group for protonated forms of *p*-hydroxycinnamic ester analogues, other PYP model chromophores, where their excited-state relaxations are weakly viscosity-dependent and dramatically decreased compared with those of their deprotonated forms.[12d] As mentioned above, the stability and long lifetime of the cis photoproduct of PCT in the crystalline phase is in contrast to $PCT^-$ in liquid solutions and to the chromophore in PYP, further evidence of effects from the surrounding environment. Those can be attributed to possible hydrogen bonds or other intermolecular interactions during the photoisomerization which could stabilize the cis photoproduct, although the crystal lacks anisotropic binding pockets and amino acid residues that stabilize the efficient photoprocess in the PYP. By taking the

intermolecular interactions into account, a more complicated mechanism for the photoisomerization of PCT in the crystalline phase is a subject of further research, and for this purpose, an analysis of single-crystal crystallographic data which would provide finer details on available space and hydrogen bond interactions is required. In addition, this crystalline-state trans−cis photoisomerization is a very rare case (yet only a few molecules show such phenomena); therefore, PCT could become an important crystalline-state photochromic material in the future.

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