

Time-resolved polarized absorption of C-phycoyanin from the cyanobacterium *Westiellopsis prolifica*

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Abstract

The energy transfer processes within C-phycoyanin monomers and trimers isolated from the cyanobacterium *Westiellopsis prolifica* were studied using picosecond polarized absorption techniques. In C-phycoyanin monomers, the fast depolarization time of about 52 ps was interpreted to be due to transfer from β_s to β_f in one β subunit. The long-lived anisotropic relaxation component in the range 2.4–4.6 ns was due to Brownian rotation of the chromophore–protein molecule. In C-phycoyanin trimers, two kinetic components of about 33 ps and 123–198 ps were observed and assigned to different isotropic relaxation processes. However, the α and β_f chromophores in adjacent $\alpha\beta$ monomers are expected to form electronic interaction, which results in pairwise delocalization of the excitation between the two chromophores (K. Sauer and H. Scheer, *Biochim. Biophys. Acta*, 936 (1988) 157–170). We attributed the shortest time constant of about 33 ps to a heterogeneous, directed relaxation from the upper exciton state to the ground state due to the site heterogeneity, which is suggested from hole-burning experiments (W. Köhler *et al.*, *Chem. Phys. Lett.*, 143 (1988) 169–173). The lifetime in the range 123–198 ps is probably due mainly to homogeneous energy transfer in the same monomer, such as $1\beta_s \rightarrow 1\beta_f$, and/or excitation equilibration between β_f chromophores in different monomers, such as $1\beta_f \leftrightarrow 2\beta_f$. These isotropic lifetimes in C-phycoyanin trimers are in agreement with the anisotropic relaxations also studied in this paper.

Keywords: C-Phycocyanin, Pump-probe, Anisotropy, Energy transfer

1. Introduction

Phycobiliproteins are the light-harvesting pigment–protein complexes of cyanobacteria and red algae in supramolecular aggregates, called phycobilisomes, at the surface of the thylakoid membranes [1]. They are optimized for light absorption and energy transfer to the photosynthetic reaction centre. C-Phycocyanin (C-PC), a pigment–protein complex, is one of the phycobiliproteins. It is composed of two types of polypeptide chains: a small α subunit containing one chromophore, and a larger β subunit containing two chromophores. These chromophores have been classified as sensitizing (s) and fluorescing (f), according to their

spectra [2]. Energy absorbed by the s chromophores is transferred to the f chromophores. The concept of the s and f chromophores is only relative, since f chromophores may change into s chromophores on formation of higher aggregates [3]. As a rule, *in vitro* C-PC occurs in monomeric ($\alpha\beta$), trimeric ($\alpha\beta$)₃ or hexameric ($\alpha\beta$)₆ aggregation states depending on the pH, ionic strength and pigment concentration [4].

We have studied the spectral properties and energy transfer processes in C-PC monomers and trimers isolated from the cyanobacterium *Westiellopsis prolifica* using steady state fluorescence, fluorescence polarization spectra, spectral deconvolution [5] and isotropic transient absorption spectroscopy on a picosecond time scale [6]. As a continuation of earlier work on the excited state

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processes in C-PC from the cyanobacterium *Westiellopsis prolifica*, we have investigated the excited state properties and energy transfer kinetics of C-PC monomers and trimers by picosecond time-resolved polarized absorption spectroscopy.

2. Materials and methods

2.1. Isolation of C-PC

Cyanobacterium cultures for *Westiellopsis prolifica* were obtained from the Indian Agricultural Research Institute, New Delhi, India, and maintained in liquid medium under the prescribed conditions of light and temperature. Cultures were grown in culture medium BG-11 [7] at 25 ± 2 °C, with constant illumination (about 2 W m^{-2}) for 21 days. Cultures were agitated using an air pump. Pure C-PC trimers of *Westiellopsis prolifica* were prepared according to the method given in ref. 6 without using $(\text{NH}_4)_2\text{SO}_4$. The homogeneity of this aggregation state was checked using analytical ultracentrifugation ($S_{20,w} = 5.4$) (Beckman L8-Ti70) and the absorption spectrum ($A_{615}/A_{280} = 4.2$). Monomers of C-PC were obtained by adding NaClO_4 up to 1 M to C-PC trimer solution [8]; under these conditions, C-PC is expected to be in the monomeric state, which was also checked using analytical ultracentrifugation ($S_{20,w} = 2.6$) and the absorption spectrum ($A_{610}/A_{280} = 3.8$). The absorption maxima are located at 615 nm and 610 nm for trimers and monomers respectively.

2.2. Picosecond polarized ground state absorption recovery

The picosecond polarized absorption measurements for C-PC ($\alpha\beta$) and $(\alpha\beta)_3$ were performed in a special quartz cell (optical path length, 1 mm). The absorbance of the trimers and monomers was adjusted to give 0.3–0.5 at the absorption maximum in a 1 mm cell. The picosecond polarized absorption measurements were performed using the pump-probe technique [6, 9]. Tunable picosecond light pulses were generated by a cavity-dumped dye laser (Spectra Physics 344), synchronously pumped by a mode-locked argon ion laser (Spectra Physics 171). This system typically produces about 10 ps light pulses at a repetition rate of 80 kHz. The light pulse width was determined by autocorrelation measurement (model 409 autocorrelator). Rhodamine 6G was used in the wavelength range 580–630 nm. The sample was excited by a strong pump pulse (about 1 mJ), and its ground state recovery was analysed with a weak probe pulse (about 1/15 of the pump power). A

lock-in amplifier (model 124A, EG&G) was used to improve the signal-to-noise ratio. Time resolution was obtained by varying the optical path length between the pump and probe pulses. The polarization of the probe beam was controlled by an appropriately oriented polarization rotator ($1/2\lambda$ plate) so that the ground state absorption recovery kinetics could be measured at right angles (90°) and parallel (0°) to the pump beam polarization. Measurements with parallel $I_{\parallel}(t)$ and perpendicular $I_{\perp}(t)$ polarization were used to monitor the decay of the induced anisotropy, $r(t) = [I_{\parallel}(t) - I_{\perp}(t)]/[I_{\parallel}(t) + 2I_{\perp}(t)] = D(t)/I(t)$. This $r(t)$ function cannot be evaluated directly in our case, since it contains contributions from two different origins; thus we calculated the difference function $D(t)$ (corresponding to anisotropic decay) and the sum function $I(t)$ (corresponding to isotropic decay) respectively. Therefore, the time-resolved polarized absorptions were analysed by a deconvolution procedure based on a non-linear least-squares method using an IBM computer for the estimation of the best fit of $I(t)$ and $D(t)$ to a sum of exponential decays

$$I(t) \text{ or } D(t) = \int_{-\infty}^{\infty} E(t-t') \left[\sum_{i=1}^n A_i \exp(-t'/\tau_i) \right] dt' \quad (1)$$

where $E(t)$ is the pump pulse profile, assumed to be a gaussian function. The quality of the fit was judged by the reduced χ^2 value and plots of the weighted residuals. For the ground state absorption recovery processes in C-PC, a fit with a small number of exponentials yields an essentially good fit for both $I(t)$ and $D(t)$.

3. Results and discussion

3.1. Kinetics of excitation energy transfer in C-PC monomers

The picosecond time-resolved polarized absorption measurements of C-PC monomers and trimers were performed at different wavelengths, *i.e.* 590, 615 and 630 nm. These wavelengths were chosen according to the deconvoluted absorption and fluorescence spectra [5, 8]. In Fig. 1(A), the ground state absorption recovery data of C-PC monomers at 590 nm are shown with the polarization of the pump light parallel $I_{\parallel}(t)$ or perpendicular $I_{\perp}(t)$ to the polarization of the probe light. Plots of the functions $I(t) = I_{\parallel}(t) + 2I_{\perp}(t)$ and $D(t) = I_{\parallel}(t) - I_{\perp}(t)$ of C-PC monomers, calculated

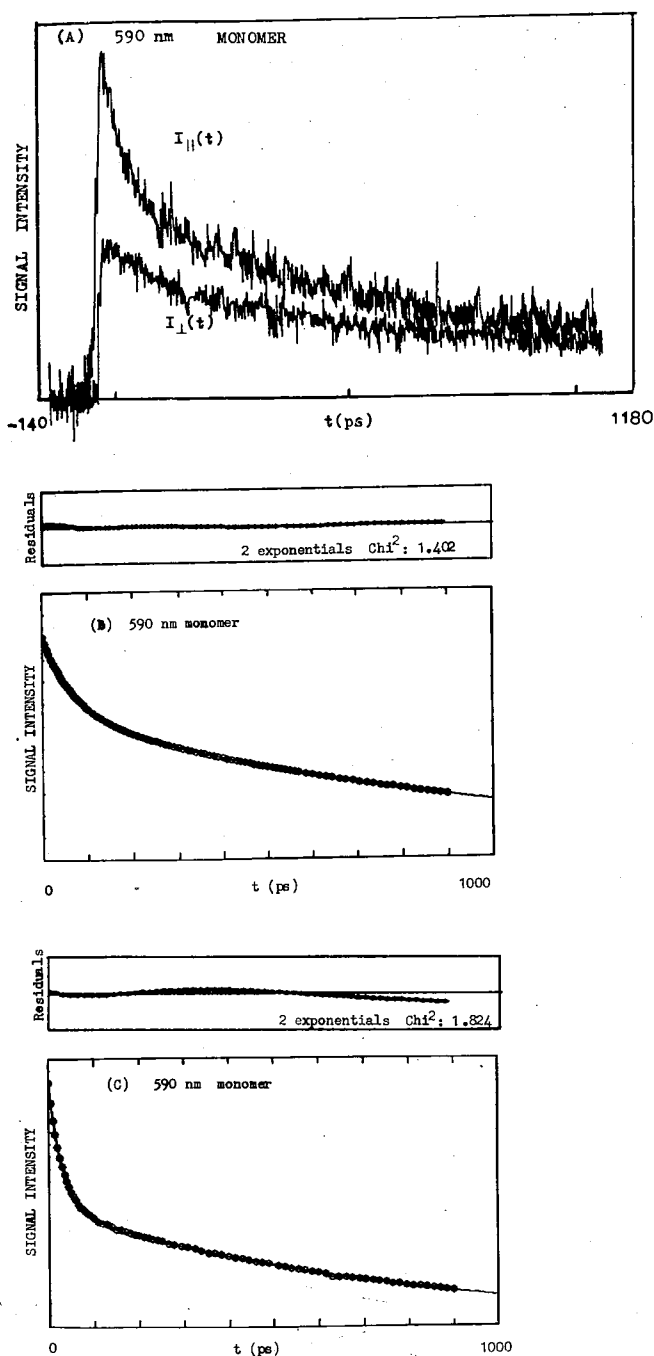


Fig. 1. (A) Transient absorption signals of C-PC monomers at 590 nm from the cyanobacterium *Westiellopsis prolifica* measured by the pump-probe technique. $I_{\parallel}(t)$ denotes probe pulse with polarization parallel to that of the pump pulse; $I_{\perp}(t)$ denotes probe pulse with polarization perpendicular to that of the pump pulse. (B) and (C) are the theoretical fits of functions $I(t)$ and $D(t)$, calculated from the traces of (A). Plots of the weighted residuals for the best fits of $I(t)$ and $D(t)$ are also shown at the top.

from Fig. 1(A), are shown in Figs. 1(B) and 1(C) in which the use of two exponentials is normally sufficient to obtain good fits. Table 1 lists the

TABLE 1. Lifetimes and relative amplitudes of the functions $I(t)$ and $D(t)$ of C-PC monomers measured by the pump-probe technique at different wavelengths

Wavelength (nm)	Function	τ_1 (ps)	R_1 (%)	τ_2 (ps)	R_2 (%)
590	$I(t)$	69	45	1074	55
	$D(t)$	29	51	749	49
615	$I(t)$	98	43	1190	57
	$D(t)$	35	42	887	58
630	$I(t)$	170	26	1050	74
	$D(t)$	40	48	856	52

lifetimes and relative amplitudes of the functions $I(t)$ and $D(t)$ at different wavelengths. The corresponding depolarization times and relative amplitudes are collected in Table 2 (see below) for C-PC monomers at various wavelengths.

From Table 1, it can be seen that the expressions $I(t)$ of C-PC monomers at different wavelengths commonly show two exponentials, with τ_1 in the range 69–170 ps and τ_2 of about 1.1 ns, and the relative amplitude of the short-lived component decreases at longer wavelengths. On the basis of the similar X-ray structure of C-PC from *Mastigocladus laminosus* [10, 11], the centre-to-centre distance between β_s and β_f in monomers can be considered to be about 4 nm, with an orientation factor ($k=0.83$) which is more favourable for resonance energy transfer than that between β_s and α or α and β_f in monomers (which has an unfavourable orientation and wide spatial separation). Therefore the short-lived τ_1 is attributed mainly to $\beta_s \rightarrow \beta_f$ energy transfer. The long-lived τ_2 is due to both radiative and non-radiative decay from terminal emitting chromophores, such as f chromophores in C-PC monomers. This interpretation is supported by the depolarization lifetimes. Let us consider the difference function $D(t)$, which is related to the anisotropic decay signals. The difference functions $D(t)$ of C-PC monomers at different wavelengths are commonly fitted by a biexponential with a short lifetime τ_1 of about 35 ps and a long lifetime τ_2 of about 887 ps. Thus from the convolution results of the functions $I(t)$ and $D(t)$, we can obtain the depolarization times (τ_{dep}) from the relation [12]

$$\frac{1}{\tau_{\text{dep}}} = \frac{1}{\tau} - \frac{1}{\tau'} \quad (2)$$

where τ' is the isotropic decay time from the functions $I(t)$ and τ is the orientation relaxation time from the difference function $D(t)$ according to the analysis of Hefferle *et al.* [12]. Therefore

we can obtain the depolarization times at different wavelengths, which are summarized in Table 2.

From Table 2, it can be seen that the fast depolarization time of about 52 ps does not depend on the wavelength and the slow anisotropic decay seems to increase from 2.4 to 4.6 ns when the wavelength is changed from 590 to 630 nm. Such slow anisotropic decay was also found by Sandström *et al.* [13]. The observation that the fast depolarization time of about 52 ps in C-PC monomers does not depend on the wavelength indicates that the depolarization is caused by energy transfer in the same species; the small change in amplitude relative to the total amplitude at different wavelengths is probably caused by the different distribution of excitation energy for chromophores in C-PC monomers at different excitation wavelengths. The slow anisotropic decay with a lifetime in the range 2.4–4.6 ns, for C-PC monomers, may be due to excitation energy transfer to β_f chromophores with different orientations of their transition dipole moments, caused by Brownian rotation of whole pigment proteins in solution. This long lifetime of depolarization in C-PC monomers indicates that the Brownian rotation motion of such a large protein-pigment molecule will not produce complete depolarization on the time scale of ground state absorption recovery.

When we consider the initial $r(0)$ values (see Table 2), we find a very high anisotropy of about 0.42 at $t=0$ and a high constant anisotropy of about 0.28 after $t > 50$ ps, which is similar to steady state polarization measurements [5, 6] within experimental error. The anisotropic decay to a high value of about 0.28 at longer times may be explained by the fact that the final distribution of the transition dipoles of the terminal emitting chromophore molecules is anisotropic, and the Brownian rotation of pigment-proteins in solution does not produce complete depolarization on the time scale of decay of f chromophores. However, we consider the order parameter S of C-PC monomers according to [14]

$$r(\infty) = r(0)S^2 \quad \text{where } S = \langle (3 \cos^2 \theta - 1)/2 \rangle$$

TABLE 2. Anisotropic lifetimes and relative amplitudes (± 0.03) of C-PC monomers at different wavelengths

Wavelength (nm)	τ_{depl} (ps)	$r_1(0)$	$\tau_{\text{depl}2}$ (ps)	$r_2(0)$	$r(0)$	$r(\infty)$
590	50	0.15	2475	0.28	0.43	0.27
615	54	0.15	3424	0.27	0.42	0.28
630	52	0.12	4632	0.28	0.40	0.28

where θ is the angle between the transition dipoles of the chromophore molecules involved in energy transfer. With $r(\infty) = 0.28$ and $r(0) = 0.42$, we obtain $S = \pm 0.82$ and $\theta = 160^\circ$ (or 20°) which is in good agreement with previous results [10, 11]. The value is very favourable for energy transfer, and thus the depolarization may largely be produced by the first step of energy transfer, such as $\beta_s \rightarrow \beta_f$ transfer with a relative amplitude of about 0.15. A high anisotropy value $r(\infty)$ of about 0.28 indicates that the rotation of protein molecules is slow compared with the rotation correlation time in aqueous solution.

3.2. Picosecond polarized absorption kinetics of C-PC trimers

In Fig. 2(A), we show the results of a ground state absorption recovery experiment of C-PC ($\alpha\beta$)₃ at 590 nm with the polarization of the pump light parallel ($I_{\parallel}(t)$) and perpendicular ($I_{\perp}(t)$) to the polarization of the probe light. The expression $I(t)$ of C-PC trimers at different wavelengths can also be satisfactorily fitted with two exponentials. The lifetimes and relative amplitudes obtained for two-exponential analysis at different wavelengths are summarized in Table 3. From Table 3, it can be seen that the shorter lifetime of about 46 ps remains constant at 590 nm and 615 nm, and increases to about 75 ps at 630 nm. The amplitude of this short-lived component shows a wavelength-dependent change, and decreases from about 60% at 590 nm to 38% at 630 nm. The long-lived (about 1.0 ns) component only changes slightly, corresponding to the ground state population recovery time of the f chromophores in C-PC trimers. The increase in the amplitude of the longer lifetime from about 40% at 590 nm to 62% at 630 nm is due to the increase in the excited state population in the f chromophores at longer excitation wavelengths. However, the change in the relative amplitude of the short-lived component with a time constant of about 46 ps seems to be due to excitation energy transfer between different chromophores. In C-PC trimers, there is a significantly closer contact between the bilin chromophores in different monomers (according to the available X-ray structure of C-PC [10, 11]), such as the $1\alpha/2\beta_f$ pair (1 and 2 represent different monomers in trimers) with spatial separation of about 2.0 nm and a very favourable orientation factor k of about -1.34 ; the interaction between these two monomers is larger than that between any others; the $1\beta_f/2\beta_f$ distance is also relatively short (about 3.5 nm) and has an orientation factor k of about 0.67. These features do not exist in monomers. Thus

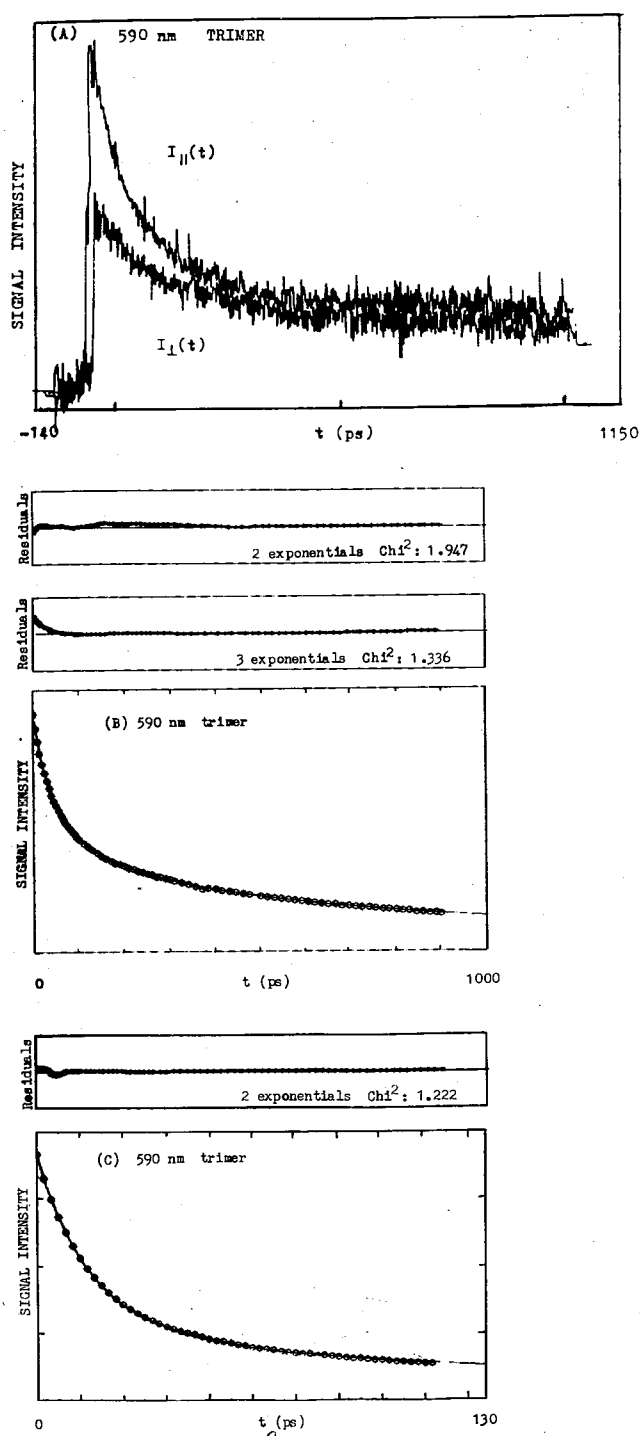


Fig. 2. (A) Transient absorption signals of C-PC trimers at 590 nm from the cyanobacterium *Westiellopsis prolifica* measured by the pump-probe technique. $I_{\parallel}(t)$ and $I_{\perp}(t)$ have the same meaning as in Fig. 1. (B) and (C) are the theoretical fits of functions $I(t)$ and $D(t)$, calculated from the traces of (A). Plots of the weighted residuals for the best fits of $I(t)$ and $D(t)$ are also shown at the top.

many new transfer channels are opened with a substantially increased number of coupled chromophores in C-PC trimers, *i.e.* there are only three

TABLE 3. Lifetimes and relative amplitudes obtained for a two-exponential analysis of $I(t)$ in C-PC ($\alpha\beta$)₃ at different wavelengths

Wavelength (nm)	τ_1 (ps)	R_1 (%)	τ_2 (ps)	R_2 (%)
590	46	60	970	40
615	43	51	943	49
630	75	38	1020	62

TABLE 4. Lifetimes and relative amplitudes of the functions $I(t)$ and $D(t)$ of C-PC trimers measured by the pump-probe technique at various wavelengths

Wavelength (nm)	Function	τ_1 (ps)	R_1 (%)	τ_2 (ps)	R_2 (%)	τ_3 (ps)	R_3 (%)
590	$I(t)$	33	39	123	24	1040	37
	$D(t)$	15	67	60	33		
615	$I(t)$	29	46	140	13	970	41
	$D(t)$	13	70	80	30		
630	$I(t)$	56	24	198	18	1060	60
	$D(t)$	19	76	112	24		

bilin chromophores per monomer but nine per trimer. The variation in the amplitude of the shorter lifetime component at different wavelengths seems to be due to the superposition of at least two components, especially when pumped at the shortest wavelength of 590 nm, where most chromophores are populated and very short-lived species (excited) are expected to be produced. The decrease in the relative amplitude of the shorter lifetime component is more pronounced when pumped at longer wavelengths. According to theoretical calculation [15] and the available X-ray structure of C-PC [10, 11], several short-lived species, such as $2\alpha \rightarrow 1\beta_f$ and $1\beta_s \rightarrow 1\beta_f$ (or $1\beta_f \leftrightarrow 2\beta_f$), should be expected in C-PC trimers. Therefore an accurate best fit with three exponentials may be needed to interpret the complicated excited state relaxation processes in C-PC trimers.

Figure 2(B) shows the fitted results of the function $I(t)$, calculated from Fig. 2(A), to a sum of three exponentials. The lifetimes and relative amplitudes of the function $I(t)$ for such an analysis at different wavelengths are collected in Table 4. From Table 4, it can be seen that the longest lifetime τ_3 of about 1.0 ns only changes slightly at different wavelengths, but the relative amplitude increases from about 37% at 590 nm to about 60% at 630 nm, which is similar to the variation observed in C-PC monomers. We can therefore interpret it as the "free" decay of the fluorescing

chromophores in C-PC trimers. The most interesting features are the two shorter lived components. From Table 4, it can be seen that the fastest component τ_1 remains constant at about 33 ps at 590 nm and 615 nm, and increases to about 56 ps at 630 nm. The relative amplitude is larger (about 40%) at 590 nm and 615 nm, but then decreases to about 24% at 630 nm. The variations in the lifetimes and relative amplitudes of this short-lived decay component may be due to the different excited state populations of the chromophores pumped at different wavelengths. The second fastest component shows similar changes in lifetimes and relative amplitudes as those of C-PC monomers: the lifetime increases from about 123 ps at 590 nm to about 198 ps at 630 nm. We cannot observe decay lifetimes of less than 1 ps, expected by theoretical calculation [15], due to instrument limitation (less than 10 ps). Since the shortest distance is between 2α and $1\beta_f$ in C-PC trimers, the interaction energy about 56 cm^{-1} [15] produces a localized exciton pair (upper and lower exciton states) between 2α and $1\beta_f$ chromophores. Therefore there should be at least two possible energy transfer pathways between chromophores with different ground state absorption spectra, *i.e.* $1\beta_s \rightarrow 1\beta_f$ (or $1\beta_s \leftrightarrow 2\beta_f$) and $2\alpha \rightarrow 1\beta_f$. The two paths would share the same excitation profile when pumped at shorter wavelengths, where the donor excited states would transfer their population through the two paths in parallel. The first path(s) is believed to exhibit a decay similar to that in C-PC monomers. However, for the second transfer path, the relaxation process is complicated. Recently, a hole-burning experiment at low temperature [16] has provided evidence for inhomogeneous broadening in C-PC. Köhler *et al.* [16] observed sharp zero phonon holes and interpreted them to be due to an inhomogeneous broadening mechanism as a result of microscopic disorder. The microscopic heterogeneity of chromophore conformations in C-PC ($\alpha\beta$)₃ should affect the interaction between 2α and $1\beta_f$ chromophores: a dual path relaxation from the upper exciton state would be expected, *i.e.* in addition to the transfer path between upper and lower exciton states, the upper exciton state also relaxes directly to the ground state. Therefore the transfer time constant between upper and lower exciton states is expected to be less than 1 ps, as calculated by Sauer and Scheer [15], and heterogeneous relaxation from the upper exciton state directly to the ground state should be observed. We thus attribute the shortest isotropic lifetime of about 33 ps to the relaxation process from the

upper exciton state to the ground state in C-PC ($\alpha\beta$)₃.

Figure 2(C) shows the fitted result of the difference function $D(t)$ of C-PC ($\alpha\beta$)₃ at 590 nm with two exponentials. The lifetimes and relative amplitudes of $D(t)$ for C-PC ($\alpha\beta$)₃ at various wavelengths are collected in Table 4. The difference function $D(t)$ of the C-PC trimers at various wavelengths can be fitted by a biexponential with two short-lived components (τ_1 , about 15 ps; τ_2 , in the range 60–112 ps). As mentioned above, the $D(t)$ and $I(t)$ functions of C-PC trimers can be combined to yield the depolarization time τ_{dep} via eqn. (2). The anisotropic relaxation times and relative amplitudes of C-PC trimers at different wavelengths are listed in Table 5. A very fast anisotropic decay lifetime of about 27 ps is observed which does not depend on the wavelength; a second fast anisotropic decay is also found with a lifetime which increases from 117 ps to 257 ps when the wavelength is changed from 590 nm to 630 nm. We believe that these two fast depolarization times in C-PC trimers are mainly due to energy transfer as analysed above. The constant depolarization lifetime τ_{dep1} of about 27 ps is due to the heterogeneous, directed ground state recovery process from the upper exciton state. Such a relaxation process is sufficient to produce large depolarization with an initial $r(0)$ value of about 0.19 (see Table 5). The second fast depolarization time τ_{dep2} in the range 117–257 ps is attributed to homogeneous energy transfer between the chromophores in one β subunit, such as $1\beta_s \rightarrow 1\beta_f$ transfer, and/or excitation energy delocalization among the β_f chromophores in different monomers; this, in turn, causes depolarization, where the equilibrium of the excitation energy transfer in each $1\beta_s/1\beta_f$ pair with different orientations will partly contribute to the fast anisotropic decay, and/or the excitation energy delocalization among β_f chromophores in different monomers is the immediate cause of depolarization. Furthermore, from Table 5, it can be seen that the anisotropy of about 0.10 of trimers is much smaller than that of monomers (about 0.28) at longer times ($t \rightarrow \infty$). Thus, as a result

TABLE 5. Anisotropic lifetimes and relative amplitudes (± 0.03) of C-PC trimers at various wavelengths

Wavelength (nm)	τ_{dep1} (ps)	$r_1(0)$	τ_{dep2} (ps)	$r_2(0)$	$r(0)$	$r(\infty)$
590	27	0.20	117	0.13	0.37	0.09
615	24	0.21	186	0.11	0.38	0.10
630	29	0.17	257	0.10	0.37	0.12

of depolarization by energy transfer in C-PC trimers, the anisotropic relaxation processes are mainly caused by two transfer pathways, with a high relative anisotropy $r(0)$ of about 0.20 for the fastest depolarization processes (heterogeneous relaxation) and a value of about 0.13 for the second fastest anisotropic relaxation ($1\beta_s \rightarrow 1\beta_f$ and/or $1\beta_f \leftrightarrow 2\beta_f$). These results are in good agreement with previous steady state measurements [5, 6].

In this study, due to the limitation of the laser pulse width, we could not obtain the fast component (less than 1 ps) expected by Sauer and Scheer [15]. Such a subpicosecond component is also suggested by Monte Carlo computer simulation of the energy transfer kinetics in C-PC [17] in which all possible interactions among the chromophores have been taken into account. Further studies of the energy transfer kinetics of C-PC on the femtosecond time scale are under way in our laboratory.

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