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ENERGY TRANSFER KINETICS IN C-PHYCOCYANIN FROM CYANOBACTERIUM WESTIELLOPSIS PROLIFICA STUDIED BY PUMP-PROBE TECHNIQUES

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Abstract. The relaxation processes of C-phycocyanin at different aggregates have been investigated by pump-probe techniques. The lifetimes of ground state recovery measured at various wavelengths are analyzed by computer fitting of the kinetic data to a sum of three and four exponentials for monomers and trimers according to the nonlinear least-square principle, respectively. The shortest lifetime (about 56ps) is due to $\beta_s \rightarrow \beta_f$ transfer in one monomer, that decreases to 31ps in trimer due to the opening of new transfer channels. The second fastest component (about 151ps) in monomer is attributed tentatively to distribution of excitation energy between α and β_f chromophores, that decreases to about 117ps in trimer caused by redistribution of excitation energy between them. The two long-lived components (about 690ps and 1385ps for monomer, 620ps and 1320ps for trimer) from some kinds of heterogeneity in some chromophores, such as α and β_f chromophores which are emitting, show an equal amplitude ratio of 1:2 in both monomer and trimer.

Introduction. C-phycocyanin (hereafter called C-PC) is a light-harvesting phycobiliprotein in cyanobacteria and red algae (1). It is composed of two types of polypeptide chains, α subunit containing one phycocyanobilin chromophore, and β subunit containing two phycocyanobilin chromophores. One α and one β subunits form a C-PC monomer. The chromophores have been classified as s (sensitizing) and f (fluorescing) type according to their spectral differences (1). Therefore, this system provides a hierarchy of complexity, but it remains simple enough to permit exploration of the mechanism of excitation energy transfer among the chromophores in C-PC at various aggregates. As a rule, in vitro the C-PC trimer is known as the stable and minimum functional unit (2).

We have studied the spectral properties and energy transfer processes in C-PC monomer and trimer isolated from cyanobacterium Westiellopsis prolifica by using steady state fluorescence and fluorescence polarization spectra as well as spectral deconvolution (3). In this paper, we further study the excited state properties and energy transfer kinetics of C-PC monomer and trimer by using pump-probe techniques in ps scale. This techniques should provide some information about the excited state relaxation processes, such as the routing of excitation energy into radiative and nonradiative processes, which were more limited in our previous work.

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Materials and Methods. Cyanobacterial cultures for Westiellopsis prolifica are obtained from Indian Agricultural Research Institute, New Delhi, India, and maintained in liquid media under the prescribed condition of light and temperature, and grown in culture medium BG-11 (4) at 25°C + 2°C, with constant illumination (about $2W/m^2$) for 21 days. Cultures are agitated using the air pump. To obtain pure C-PC without colourless polypeptides, the C-PC is isolated from dissociation of phycobilisomes by column chromatography. Dissociated phycobilisomes are layered on DEAE-cellulose column (ϕ=1.5cm, l=15cm) equilibrated with 5mM K_2HPO_4/KH_2PO_4(pH=7.0) buffer. Fraction C-PC is eluted with 100mM K_2HPO_4/KH_2PO_4 (pH=7.0) buffer. The fraction C-PC is concentrated by polyglycol 6000, and centrifuged at 40,000xg for one hour, and fractionated again by the same column chromotography with the same concentration buffer. Three or four repetitions are needed to obtain purified C-PC, checked by absorption spectrum (A_{615}/A_{280}=4.2) and analytical ultracentrifugation ($S_{20,w}$=5.4)(Beckman L8-70Ti) as a trimeric aggregation state. To obtain monomeric C-PC, the C-PC trimer is dissociated by adding 1M $NaClO_4$ (5), a chaotropic agent in 5mM K_2HPO_4/KH_2PO_4(pH=7.0) and checked by the absorption spectrum (A_{610}/A_{280}=3.8) and analytical ultracentrifugation ($S_{20,w}$=2.6) as a monomeric state.

Absorption spectra are measured on a shimazhu UV-200 double-beam spectrophotometer. Corrected fluorescence and fluorescence polarization spectra are recoreded on a Hitachi F-4000 fluorescence spectrophotometer. Fluorescence quantum yields are obtained relative to rhodamine B in ethanol (0.94) (6). The concentration of the standard (rhodamine B in absolute ethanol) and the C-PC monomer and trimer are adjusted so that their absorption at the excitation wavelength 580nm is 0.150±0.003. Picosecond absorption recovery measurements are performed using the pump-probe techniques (7). Tunable picosecond light pulses are generated by a cavity-dumped dye laser (Spectra physics 344), synchronously pumped by a mode-locked argon ion laser (Spectra physics 171-18). This system produces light pulses of typically 3-10ps(FWHM) at a repetition rate of 80 KHz. The pulse widths are determined by autocorrelation measurement (Model 409 Autocorrelator). Rhodamine 6G covers the wavelength range of 580-640nm in the dye laser with an average laser power of 0.5-1.0mW as pump power. The probe power is about 1/15 times of the pump power. No singlet-singlet annihilation effects have been observed by slightly varying the pump power. The probe beam is polarized at the magic angle (54.7°) relative to the pump beam in order to obtain the isotropic decay. To avoid the reabsorption, the picosecond measurements are made in a special quartz cell of 1 mm optical pathlength, and absorbances for monomer and trimer are always adjusted to give as 0.3-0.5 (at their absorption maxima in 1 mm cell). To exclude any possibility for light induced changes of samples, the samples flow slowly through the cell during the recording. The lifetimes of ground state recovery are calculated by computer fitting of the kinetic data to a sum of exponential decays ($I(t)=\sum_{i=1}^{2} R_i \exp(-t/\tau_i)$) on an Apple-II computer. The nonlinear least-square method is adopted for the estimation of the best fit. The maximum errors in the short lifetimes are about 10-15%, and about 5% in the long lifetimes.

Results. The absorption, fluorescence and fluorescence polarization spectra of C-PC monomer and trimer are given in Fig.1. It can be seen that the absorption and emission maxima are 610nm and 645nm for C-PC monomer, respectively, and shift to 615nm and 647nm for trimer. The fluorescence quantum yields are about 0.61 for C-PC monomer, and 0.82 for C-PC trimer relative to rhodamine B at their excitation wavelength 580nm. The fluorescence bandwidth of C-PC trimer is about 30nm, about 20nm narrower than that of C-PC monomer. The fluorescence polarization spectra of C-PC trimer shows a monotonous decrease toward the longer-wavelength region and reachs a constant low value (about 0.130) at the wavelength (660nm) longer than the fluorescence maximum (647nm), where that of C-PC monomer reach a constant value (about 0.300) at the wavelength (650nm) nearby its fluorescence maximum (645nm).

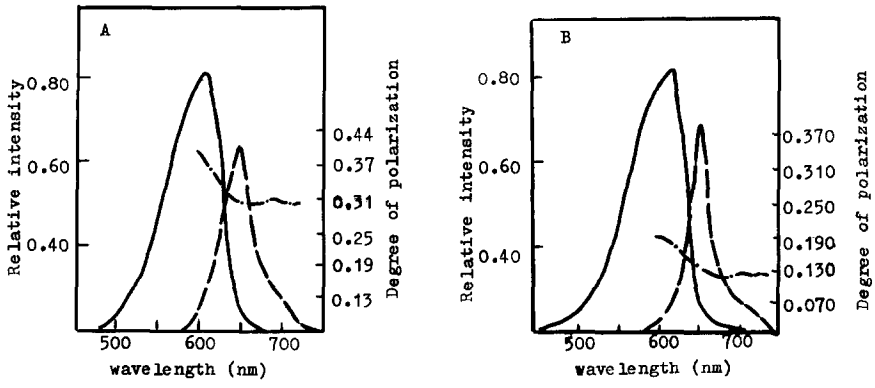

Fig. 1. Absorption (———), corrected fluorescence (- - -) and fluorescence polarization (—·—·—) spectra of C-PC. (A) monomer, (B) trimer. Fluorescence and fluorescence polarization spectra are measured at the excitation wavelength 580nm.

This has also been pointed out by other (5). It is possible that there must be considerable back-transfer processes in C-PC trimer (see discussion section).

Picosecond absorption recovery experiments on C-PC monomer and trimer are performed at different wavelengths 590nm, 615nm and 630nm. These wavelengths are chosen according to the absorption, fluorescence spectra and deconvolution data (3) for the individual chromophores of C-PC, as well as the data from (5).

The ground state recovery signal of C-PC monomer at 590nm is shown in Fig. 2A. Fig. 2B shows a fit of the experimental traces to a sum of three exponential functions. A good fit is obtained by varying the ratio of residuals. Table I lists the decay lifetimes and relative amplitudes of excited-state C-PC monomer at different wavelengths.

Fig. 3A and B show the results of a pump-probe measurement and a fit of the experimental trace to a sum of four exponential functions, respectively at 590nm for C-PC trimer. The lifetimes and relative amplitudes of C-PC trimer from thus analysis at various wavelengths are collected in Table II.

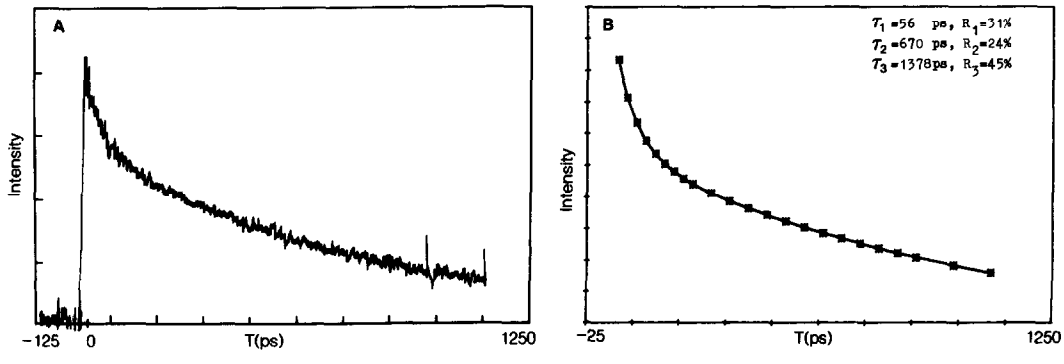

Fig. 2. (A) Ground state recovery kinetic of C-PC monomer at 590nm. (B)The theoretical fit (——) of a sum of three exponential functions to the experimental trace (* *) from Fig. 2(A)
The best fit of the lifetimes and amplitudes is shown on top.

Table I. Lifetimes and relative amplitudes of excited state C-PC monomer measured by the pump-probe techniques

wavelength (nm)	τ_1 (ps)	R_1 (%)	τ_2 (ps)	R_2 (%)	τ_3 (ps)	R_3 (%)
590	56	31	670	24	1378	45
615	134	33	708	21	1395	46
630	194	30	690	26	1385	44

Discussion. The energy transfer processes among the various chromophores in the different C-PC aggregates will now be discussed on the basis of the known spectral differences of the various chromophores (3). C-PC is composed of two kinds of subunits, α and β containing one and two phycocyanobilin chromophores, respectively, where the detailed spectral studies have been made as following: the absorption maximum of α chromophore is located at about 619nm, two β chromophores absorb at 598nm and 632nm for β_s and β_f chromophores, respectively, according to the deconvolution data (3). M. Mimuro et al have obtained similar results for C-PC from Mastigocladus laminosus (5).

C-PC monomer. The absorption recovery experiments on C-PC monomer at 590nm, 615nm and 630nm show commonly a three-exponential decay with various lifetimes (ps) and relative amplitudes (%) listed in Table I. The shortest lifetime seems to increase from 56ps to 194ps while the wavelength changes from 590nm to 630nm with a constant amplitude about 31%. The other two kinetic components almost have constant lifetimes (about 690ps and 1385ps) at different wavelengths. Their amplitudes do not change too much). Our explanation of these data is as following. At 590nm, we excite mainly β_s chromophore which absorbs the light at about 598nm, a little is absorbed by other two chromophores (α and β_f). According to the structural data (8), the center-to-center distance between β_s and β_f in monomer is about 4nm, with an orientation factor (k=0.83) which is relatively favorable for resonance energy transfer between them. The center-to-center distance between β_s and α in monomer

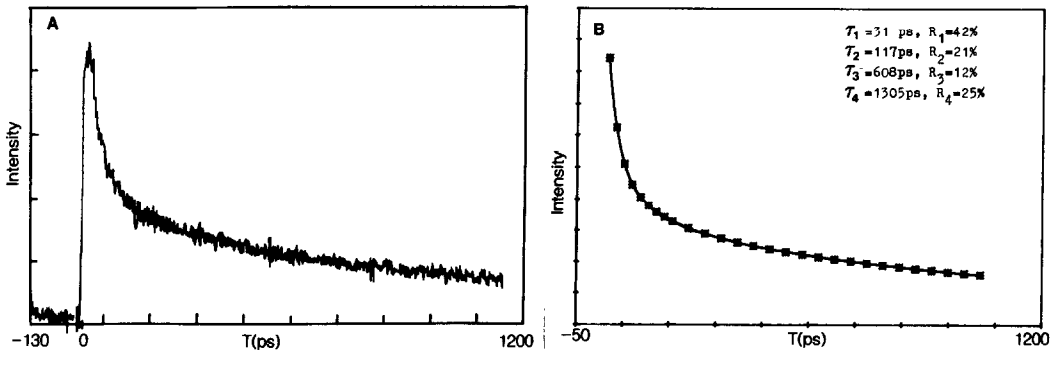

Fig.3. (A) Ground state recovery kinetic of C-PC trimer at 590nm. (B)The theoretical fit (——) of a sum of four exponential functions to the experimental trace (* *) from Fig.3(A). The best fit of the lifetimes and amplitudes is shown on top.

Table II. Lifetimes and relative amplitudes of excited state C-PC trimer measured by the pump-probe techniques

Wavelength (nm)	τ_1 (ps)	R_1 (%)	τ_2 (ps)	R_2 (%)	τ_3 (ps)	R_3 (%)	τ_4 (ps)	R_4 (%)
590	31	42	117	21	608	12	1305	25
615	33	38	201	29	620	10	1320	23
630	41	43	268	30	635	9	1315	18

is about 5.46nm, and the orientation factor of k=-0.13 for their interaction is particularly unfavorable. Therefore, we attribute the 56ps lifetime (τ_1) at 590nm to the energy transfer from β_s to β_f. As to the lifetime (τ_1) increases to 134ps at 615nm, 194ps at 630nm, in fact at 615nm or 630nm, we excite mainly the β_f and α chromophores, and a little photons are captured by β_s chromophore. So the another dominant process in this case should be between α and β_f, which has a very large orientation factor k=1.73, even if the distance is too far between them (about 5.76nm). Thus we can resolve the lifetime (τ_1) into two components as 61ps and 151ps at 615nm with the intensity ratio about 1:5, 65ps and 208ps at 630nm with the amplitude ratio about 1:10, respectively. The latter therefore represents the transfer time from α to β_f chromophore while the former is due to the $\beta_s \rightarrow \beta_f$ energy transfer. The largest amplitude (about 45%) carries by a component with a long lifetime about 1385ps. Another component about 680ps carries an amplitude about 24% to the total amplitude. They almost remain constant at different wavelengths. These two longer lifetimes might be due to the both radiative and nonradiative processes from the terminal emitting chromophores, such as α and β_f chromophores in monomer. Such behavior has been described already in reference (9). These two longer lifetime components are attributed to the presence of a heterogeneity in the chromophore conformations. This heterogeneity seems to be characteristic for the lower aggregation state of C-PC, even for α subunit (10). However, a more detailed analysis based on further experimental data seems to be required to clarify this point.

C-PC trimer. All kinetic trace for C-PC trimer obtained at various wavelengths 590nm, 615nm and 630nm could be fitted well with four exponentals. In comparison to the monomer, the two long-lived components (about 620ps and 1320ps) almost remain constant at different wavelengths. Their amplitude ratio R_3/R_4 is about 1:2 as that in the case of the C-PC monomer. These two long-lived components in C-PC trimer are due to the same processes as in monomer. The fastest component remains constant (about 31ps) at 590nm and 615nm, and increases to 41ps at 630nm. Its amplitude does not change much. The second fastest component increases from 117ps at 590nm, to 201ps at 615nm, and to 268ps at 630nm. The amplitude of the component also increases from 21% at 590nm, to 29% at 615nm, and 30% at 630nm. These increases are due to the large number of transfer processes involved the availability of more favorable acceptor chromophores in C-PC trimer, i.e. many new transfer channels are opened as compared to monomer, mainly such as $2\alpha \rightarrow 1\beta_f$ (1,2 represent different monomer in trimer). In fact, from the similar structural data available from (11),

in C-PC trimer there are significantly close contacts between 2α and $1\beta_f$ of 2.1nm, with a very favorable orientation factor of K=1.46. Meanwhile, the excitation energies of α and β_f do not differ much ($\Delta E=E_\alpha-E_\beta=202.86cm^{-1}$, as calaulated by deconvolution data (3)). In this case, the exciton interaction between 2α and $1\beta_f$ needs to be considered in addition to Forster resonance transfer. As calculated by (12), a strong exciton interaction energy ($V\pm$) in C-PC trimer is about $56cm^{-1}$ ($2\alpha/1\beta_f$). Thus, the spectroscopic splitting ($2V\pm=112cm^{-1}$) expected from this interaction is comparable with the separation ($202.86cm^{-1}$) of 2α and $1\beta_f$. Therefore, the excited states should be considered to be delocalized over the 2α and $1\beta_f$. Assuming that the exciton coupling is limited to pairwise interaction, this will result in two electronic excited states with energy levels $E\pm V\pm$ for each $2\alpha/1\beta_f$ pair. The relaxation from the upper to the lower exciton states would be expected to mix by vibrational coupling, probably involving interactions with phonons associated. That is to say, this relaxation takes place before or during the vibration state relaxed. The process is therefore very fast. It seems therefore that the lifetime (about 31ps) is a strong candidate by this $2\alpha\rightarrow1\beta_f$ transfer processes. In comparison of the $\alpha\rightarrow\beta_f$ transfer within one monomer, the lifetime (τ_2) increases from about 117ps at 590nm to 201ps at 615nm, and 268ps at 630nm with an amplitude increase from 21% to 30%. These increases reflect the shift of the equilibrium of excitation energy between α and β_f (such as back and forth transfer). Thus, it is possible in C-PC trimer, that both chromophores are in equilibrium and are emitting as in one monomer. The equilibrium time (τ_2) is shorter in C-PC trimer than that in C-PC monomer. Therefore, it can explain the reason that there are a energy back transfer processes between 2α and $1\beta_f$ chromophores in C-PC trimer, which results in that its fluorescence polarization spectrum decrease to the lowest value at the longer wavelength than its fluorescences maximum. The opening of the new transfer channel ($2\alpha\leftrightarrow1\beta_f$) results in that the most of energy absorbed by α chromophore is transferred to β_f chromophore and emitting, a little is emitting from α chromophore itself. The fluorescence bandwidth of C-PC trimer is therefore 20nm narrower than that of monomer.

Conclusions. The picosecond absorption made it possible to investigate the excitation energy transfer kinetics of C-PC in monomeric and trimeric aggregation states. A sum of three or four exponentials are necessary to fit the data for monomer or trimer, respectively. The lifetime τ_1 (about 56ps) is tentatively attributed to the transfer from the distant β_s to β_f chromophore in C-PC monomer. The fact that τ_1 decreases to about 31ps in C-PC trimer reflects there are much more numbers of efficient acceptors available for a donor such as s chromophores in C-PC trimer than that in C-PC monomer, for instance, the change might be due to the opening of the transfer channel ($2\alpha\leftrightarrow1\beta_f$) according to the exciton model. The

lifetime 31ps is due mainly to the transfer $2\alpha \rightarrow 1\beta_f$. Direct evidence for the $\alpha \rightarrow \beta_f$ transfer in C-PC monomer has been obtained when we resolve the τ_1 into two components at 615nm and 630nm, where most of the light energy are captured by both α and β_f chromophores, and the lifetime is about 151ps between them in monomer. That process ($\alpha \rightarrow \beta_f$) shows a lifetime about 117ps in the trimer and increases to 201ps at 615nm, 268ps at 630nm with an amplitude increase from 21% to 30%, a likely explanation of this changes is a redistribution of the excitation energy between 1α and $1\beta_f$ in trimer because of the opening of the new transfer channel, as in one monomer. The two long-lived components (about 620ps and 1320ps for trimer, 690ps and 1385ps for monomer) are from some kind of heterogentity in some of the chromophores, such as α and β_f, which are emitting. Their amplitude ratio is about 1:2 in the cases of the monomer and trimer.

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