SPECTROSCOPIC CHARACTERISTICS AND ENERGY TRANSFER PROCESSES IN C-PHYCOCYANIN FROM CYANOBACTERIUM *Westiellopsis prolifica*

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Received May 25, 1991.

ABSTRACT

The spectroscopic characteristics and pathways of energy transfer in the C-phycocyanin monomer ($\alpha\beta$) and trimer $(\alpha\beta)_3$ isolated from cyanobacterium *Westiellopsis prolifica* have been investigated by absorption, excitation, fluorescence, fluorescence excitation polarization and fluorescence polarization spectra and the deconvolution of those spectra. The phenomenon of an energy-back-transfer within a C-phycocyanin trimer has been found, and a tentative model of the bidirectional energy transfer is proposed by us, which describes well our experimental results.

Keywords: C-phycocyanin, monomer, trimer, energy-back-transfer, strong coupling interaction.

I. INTRODUCTION

Cyanobacteria are a group of photosynthetic prokaryons which carry out plant type oxygenic photosynthesis. They contain abundant light-harvesting accessory pigment-protein complexes called phycobiliproteins. The phycobilisomes are composed of several types of phycobiliproteins and colourless linker proteins or polypeptides. In cyanobacterium *Westiellopsis prolifica*, the phycobilisomes consist of phycoerythrocyanin, C-phycocyanin (hereafter called C-PC) and allophycocyanin[1]. The light energy captured by phycobiliprotein transfers first among the chromophores within itself, then is transferred to the adjacent phycobiliproteins, and is finally transferred to chlorophyl a in the thylakoid membranes[1].

A lot of work has been done about the energy transfer in phycobilisomes, the

* Project supported by Academia Sinica and the Third World Academy of Sciences.

main pathways of energy transfer are almost clear. Unfortunately, the energy transfer processes and the spectroscopic characteristics in an individual phycobiliprotein are not understood yet. To study the nature of energy transfer in an individual phycobiliprotein in detail, it is necessary to analyze the energy transfer processes among the chromophores within an individual phycobiliprotein. For this purpose, we try to describe the spectroscopic characteristics and the energy transfer processes in the C-PC monomer ($\alpha\beta$) and trimer $(\alpha\beta)_3$ isolated from the cyanobacterium *Westiellopsis prolifica* by absorption, excitation, fluorescence, fluorescence excitation polarization and fluorescence polarization spectra, and obtain the available data about the electronic states of phycocyanobilin (hereafter called PCB) chromophores in C-PC by the deconvolution of these spectra. We explain well the experimental phenomena by suggesting a tentative model about the pathway of energy transfer in C-PC$(\alpha\beta)_3$.

II. Materials and Methods

Cyanobacterium cultures of *Westiellopsis prolifica* were 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 in medium BG-11[2] at 25±2℃, with constant illumination (2.5 W/m^2) for 21 days. Cultures were agitated using the air pump. The isolation of phycobilisomes was carried out according to [3], and C-PC was isolated and purified from dissociated phycobilisomes by a DEAE cellulose column and centrifugation. Dissociated phycobilisomes were laid on a DEAE cellulose colum (Carl-Schloicher & Schnell Co., U.S.A., ϕ1.5 cm, 1 15 cm) equilibrated with 5 mmol/L K_2HPO_4/KH_2PO_4 (pH = 7.0) buffer. The fraction of C-PC was eluted with 100 mmol/L K_2HPO_4/KH_2PO_4 (pH= 7.0) buffer, and concentrated with polyglycol 6000, and then centrifuged at 40,000 × g (Backman 70Ti rotor) for 1 h. The blue supernatant of C-PC was layered on the same DEAE cellulose column and eluted with 100 mmol/L K_2HPO_4/KH_2PO_4 (pH = 7.0) buffer, and then centrifuged at 40,000 × g once again. The C-PC was checked by the absorption spectrum as a trimer $(\alpha\beta)_3$(A_{614}/A_{280} = 3.6). To obtain the C-PC monomer ($\alpha\beta$), according to [4], the trimeric aggregates were dissociated by adding 1 mol/L $NaNO_3$, a chaotropic agent in phosphate buffer and adjusting pH to 3.9 with dilute HCl. It was expected that the aggregation states were monomeric and checked by the change in the absorption spectrum (A_{610}/A_{280} = 3.4). The sample concentrations for fluorescence measurements were always adjusted to less than 0.6 absorbance at its maximum in order to avoid the reabsorption of emission. All the buffer mentioned above contained 0.001 mol/L NaN_3 and 0.1 mol/L NaCl.

Fluorescence, excitation, fluorescence polarization and fluorescence excitation polarization spectra were performed with a Hitachi F-4000 fluorescence spectrophotometer, and the absorption spectra were obtained on a Shimazu UV-200 double-beam spectrophotometer, at room temperature. The degrees of polarization were calculated by $P = I_{//} - GI_{\perp}/I_{//} + GI_{\perp}$, where $G = i_{\perp}/i_{//}$ was a correction factor for the polarization due to the optics of the instrument.

The 4th-derivative spectra were measured on a Hitachi F-4000 fluorescence spectrophotometer by the method of Savitzky-Golay[5]. The spectral deconvolution was done by using a Model PDP-11/03 computer, based on the least-squares method. Each subband was set according to the result of the 4th-derivative spectra and assumed to have the Gaussian profile. The deconvolution was processed by changing the bandwidth, energy position and peak height to fit the experimental spectrum.

III. Results

1. *Monomer of C-PC*

The C-PC monomer consists of one α subunit and one β subunit. The former contained one, and the latter two PCB chromophores, which were covalently bound to cysteine residues of the apoprotein through thio-ether linkages[1]. The chromophores were classified as s (sensitizing) and f (fluorescing) types, according to their spectral difference[6].

Fig. 1 shows the absorption, excitation and fluorescence excitation polarization spectra of C-PC($\alpha\beta$). The absorption maximum of the monomer is located at about 610 nm. The excitation maximum is located at 618 nm approximately, as monitoring the fluorescence at 680 nm. The occurrence of energy transfer in C-PC ($\alpha\beta$) was confirmed by the fluorescence excitation polarization spectrum with the monitoring wavelength of 680 nm. The degree of polarization of C-PC($\alpha\beta$) was low (about 0.310) from 520 to 600 nm, and then stepwisely increased to 0.448 at 660 nm. Such a kind of increase in the degree of polarization is one of the characteristics of energy transfer, found by Teale and Date (1970)[7]. Meanwhile, the close resemblance of the excitation and absorption spectra of C-PC($\alpha\beta$) (as shown in Fig. 1) indicated that there was an efficient energy transfer among the PCB chromophores within C-PC($\alpha\beta$), i.e. energy transfer from the s type to the f type of the chromophore.

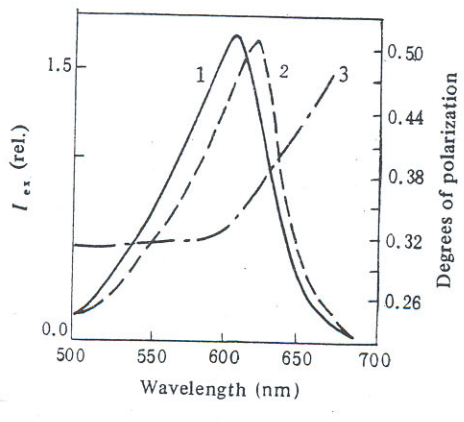

Fig. 1. Absorption (1), excitation (2) and fluorescence excitation polarization (3) spectra of C-PC($\alpha\beta$). The excitation and fluorescence excitation polarization spectra are measured with the monitoring wavelength of 680 nm.

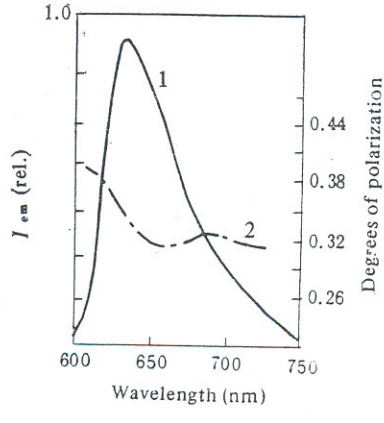

Fig. 2. Fluorescence (1) and fluorescence polarization (2) spectra of C-PC($\alpha\beta$) obtained by the excitation at 580 nm.

In comparison with M. Mimuro's[6] results about the spectroscopic characteristics of α and β subunits in C-PC($\alpha\beta$) isolated from cyanobacterium *Mastigocladus laminosus*, we considered that in our case, the energy transfer only occurred between two chromophores of the β subunit within C-PC($\alpha\beta$), i.e. from one chromophore (hereafter called β_s), which had an absorption maximum in the shorter wavelength region, to the other chromophore (hereafter called β_f), which absorbed and emitted the light energy in the longer wavelength region. There was no energy transfer process between α and β subunits in the C-PC monomer.

The fluorescence spectrum under the excitation of 580 nm (Fig. 2) showed the maximum at about 645 nm, with a bandwidth of about 50 nm. The fluorescence polarization spectrum (Fig. 2) excited by 580 nm showed a stepwise decrease toward the longer wavelength side and reached a minimum about 0.300 at about 650 nm. This indicates that there is an energy transfer process within C-PC($\alpha\beta$).

2. *Trimer of C-PC*

C-PC$(\alpha\beta)_3$ consists of three monomers with the C_3 symmetry arrangement around a central axis[9]. It has nine chromophores (three α, three β_s and three β_f chromophores). Compared with the results of C-PC($\alpha\beta$), the absorption maximum of C-PC$(\alpha\beta)_3$ was shifted to 614 nm and the excitation maximum was also shifted to 624 nm (monitoring 680 nm shown in Fig. 3). We considered that the red shifts in excitation and absorption spectra were due to the changes of conformations of C-PC$(\alpha\beta)_3$, which led to the difference in electronic state between ($\alpha\beta$) and $(\alpha\beta)_3$. The occurrence of energy transfer within C-PC$(\alpha\beta)_3$ was also comfirmed by the fluorescence excitation polarization spectrum monitored at 680 nm (Fig. 3). The degree of polarization showed a stepwise increase from a low value (about 0.135) at 520 nm toward a longer wavelength and reached a maximum value (about 0.191) at 640, and then turned to decreasing toward a longer wavelength above 640 nm. In

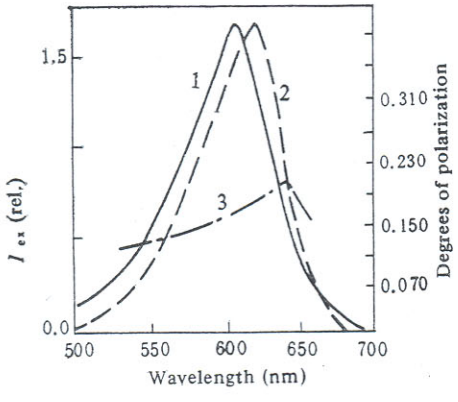

Fig. 3. Absorption (1), excitation (2) and fluorescence excitation polarization (3) spectra of C-PC$(\alpha\beta)_3$. The excitation and fluorescence excitation polarization spectra are measured with the monitoring wavelength of 680 nm.

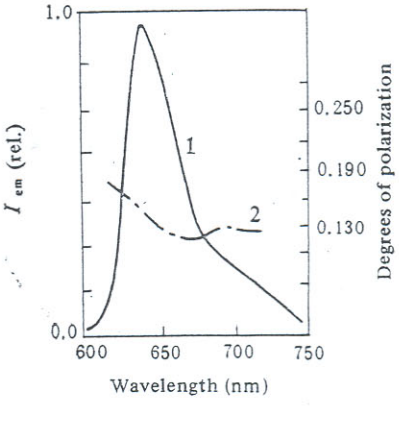

Fig. 4. Fluorescence (1) and fluorescence polarization (2) spectra of C-PC$(\alpha\beta)_3$ obtained by the excitation at 580 nm.

comparison with C-PC($\alpha\beta$), the degree of polarization of C-PC$(\alpha\beta)_3$ was always much lower than that of C-PC($\alpha\beta$). Meanwhile, the degree of polarization of C-PC $(\alpha\beta)_3$ above 640 nm decreased while that of C-PC($\alpha\beta$) continued to increase. These features indicated that there should be a new energy transfer process in C-PC $(\alpha\beta)_3$ which did not exist in C-PC($\alpha\beta$) (see Discussion), i.e. there was a bidirectional energy transfer within C-PC$(\alpha\beta)_3$.

On the excitation at 580 nm, the fluorescence maximum of C-PC$(\alpha\beta)_3$ was located at 647 nm approximately (Fig. 4), and the bandwidth was about 30 nm, about 20 nm narrower than that of C-PC($\alpha\beta$). The fluorescence polarization spectrum showed a monotonous decrease toward the longer-wavelength region, and reached a very low value (about 0.130) at the wavelength (about 660 nm) longer than the fluorescence maximum 647 nm. This implied that the energy is transferred to the energy sink of C-PC$(\alpha\beta)_3$. The 647 nm fluorescence thus must come from the energy sink and a little from energy-back-transfer (see Discussion).

3. *Spectral Deconvolution*

The electronic states of C-PC depended on the electronic states of PCB in C-PC. For a further understanding of the pathway of energy transfer in C-PC$(\alpha\beta)_3$, we deconvoluted the excitation and fluorescence spectra of C-PC$(\alpha\beta)_3$ and C-PC($\alpha\beta$). The reason why we studied the excitation spectrum but not absorption one was that the excitation spectrum was better to show directly the excitation energy in various energy levels involved in the energy transfer processes.

Fig. 5(a), (b) shows the deconvolution patterns of the excitation spectra for C-PC($\alpha\beta$) and C-PC$(\alpha\beta)_3$, respectively determined by their 4th-derivative spectra. The parameters obtained are listed in Table 1. Eight subbands were commonly found in two spectra. The position of each corresponding subband was almost identical, the change in bandwidth was also small. The spectral changes were only caused by the changes in the relative intensities of the subbands. There were three types of intensity changes in the two excitation spectra. The first type was the subbands at 652 nm, 624 nm and 598 nm in C-PC$(\alpha\beta)_3$ which were stronger than the corresponding subbands at 652 nm, 619 nm and 598 nm in C-PC($\alpha\beta$). The second type was the subbands at 605 nm and 576 nm which are stronger in C-PC($\alpha\beta$) but weaker in C-PC$(\alpha\beta)_3$. The other subbands at 632 nm, 566 nm and 550 nm remaining constant in intensity in the monomer and trimer belonged to the third type.

Fig. 6(a), (b) shows the deconvolution patterns of fluorescence spectra and the 4th-derivative spectra for C-PC($\alpha\beta$) and C-PC$(\alpha\beta)_3$, respectively. The parameters are listed in Table 2. The results showed that, except for a subband at 638 nm in C-PC($\alpha\beta$), five subbands were indentical for the monomer and trimer. The position of each corresponding subband was almost the same, too. The difference in bandwidth was also small. The difference was only the relative intensity of each subband. The important result was that there was a subband at 638 nm in C-PC($\alpha\beta$), which did not appear in C-PC$(\alpha\beta)_3$. In consideration of the same Stokes shifts between the excitation subbands and the fluorescence subbands, the fluorescence

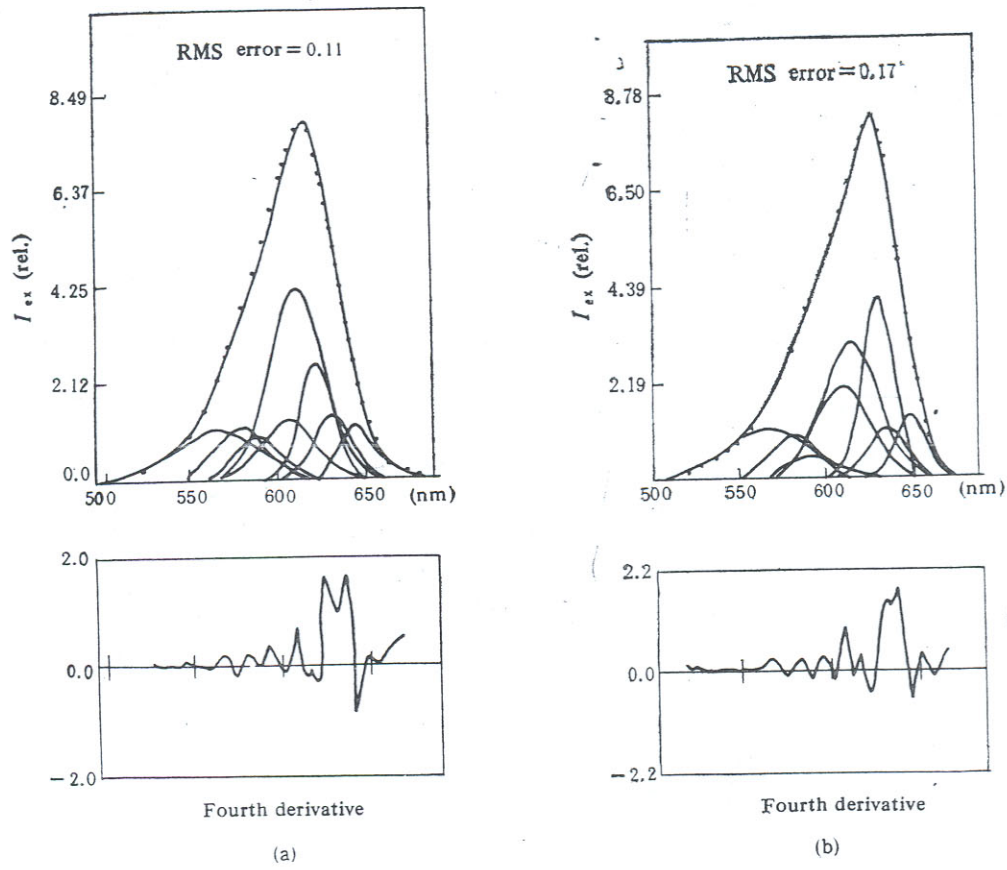

Fig. 5. Deconvolution patterns and the 4th derivative of excitation spectra monitored at 680 nm. ……, Experimental data; ——, theoretic data. (a) Monomer, (b) trimer.

subbands at 666 nm, 646 nm and 621 nm might be attributed to the corresponding subbands in the excitation spectrum at 652 nm, 632 nm and 598 nm, respectively. Since the fluorescence subband at 638 nm corresponding to the excitation subband at 619 nm in C-PC($\alpha\beta$) did not appear in C-PC($\alpha\beta$)$_3$, the fluorescence subband at 638 nm corresponding to the excitation subband at 624 nm in C-PC($\alpha\beta$)$_3$ might transfer the energy from 638 nm to lower energy levels and resulted in the disappearance of the fluorescence subband at 638 nm and the enhancement of the 646 nm subband. Thus, it was not difficult to explain why the bandwidth of the fluorescence spectrum of C-PC($\alpha\beta$)$_3$ was about 20 nm narrower than that of C-PC($\alpha\beta$) by these features mentioned above.

X-ray structure analysis[3] showed that there are three PCB chromophores which were covalently linked to cysteine residues at three different positions called β_s, α and β_f chromophores in C-PC. Thus there were at least three different electronic states in C-PC. Compared with the M. Mimuro's results[8] about the spectroscopic characteristics of various subunits in C-PC, we assigned the energy levels for various subbands at 598 nm, 619 nm (624 nm in C-PC($\alpha\beta$)$_3$) and 632 nm to the electronic energy levels of β_s, α, β_f chromophores, respectively. Meanwhile, the existence of

Table 1

Parameters of Subbands of Excitation Spectra of C-PC Monomer and Trimer

	Position (nm)	Width (nm)	Height	Area	Area (%)
C-PC ($\alpha\beta$)	550.0	59.17	1.10	2283.28	0.14
	566.0	35.21	1.12	1309.04	0.08
	576.0	34.37	0.95	1046.64	0.06
	598.3	44.98	1.26	1683.24	0.10
	605.6	48.89	4.15	5879.74	0.35
	619.7	30.55	2.51	2124.09	0.13
	632.5	36.12	1.35	1296.20	0.08
	651.8	34.52	1.20	1037.20	0.06
C-CP $(\alpha\beta)_3$	550.0	59.17	1.10	2283.28	0.14
	566.0	33.67	1.00	1117.69	0.07
	576.0	34.30	0.50	549.80	0.03
	598.3	44.37	2.00	2635.62	0.16
	605.6	48.71	3.05	4324.49	0.26
	624.2	31.35	4.09	3500.35	0.21
	632.5	36.12	1.10	1056.16	0.06
	651.5	29.72	1.40	1043.18	0.06

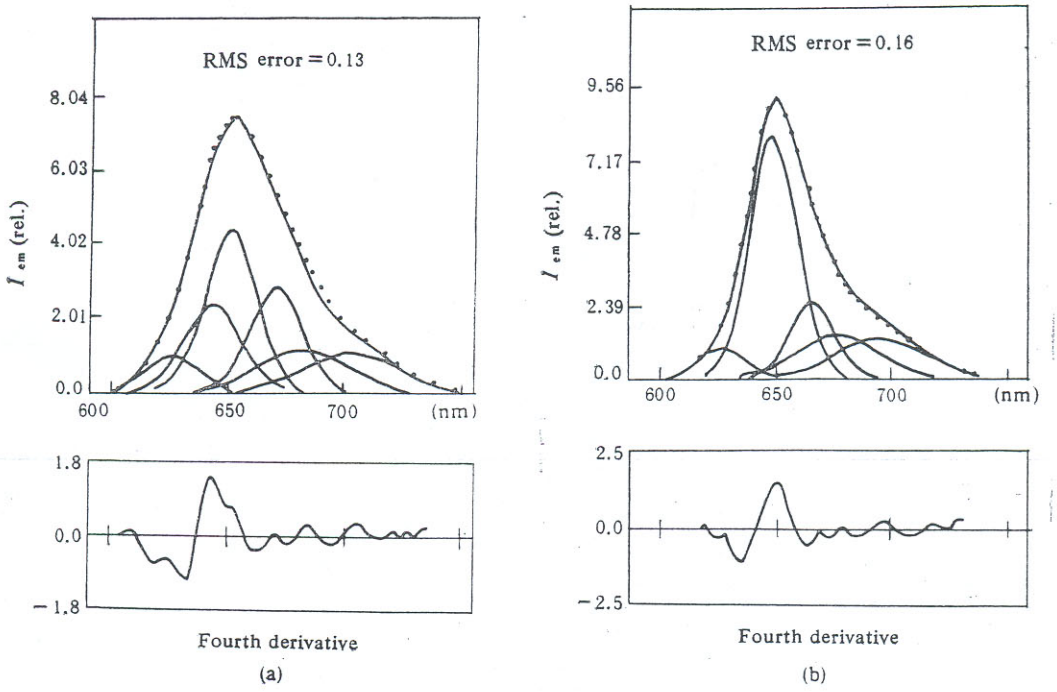

Fig. 6. Deconvolution patterns and the 4th derivative of fluorescence spectra obtained by the excitation at 580 nm.
……, Experimental data; ——, theoretic data. (a) Monomer, (b) trimer.

the fluorescence subbands at 623 nm, 639 nm, 646 nm, 666 nm, 680 nm and 703 nm were pointed out by other workers[10] who studied the excitation energy transfer

Table 2

Parameters of Subbands of Fluorescence Spectra of C-PC Monomer and Trimer

	Position (nm)	Width (nm)	Height	Area	Area
C-PC ($\alpha\beta$)	621.9	24.84	0.96	656.05	0.06
	638.2	29.63	2.38	1841.80	0.18
	646.2	27.78	4.38	3100.47	0.30
	666.4	29.49	2.84	2007.33	0.20
	679.9	46.99	1.08	1258.80	0.12
	703.0	60.41	1.07	1384.51	0.14
C-PC $(\alpha\beta)_3$	621.9	24.84	0.96	656.05	0.06
	645.9	27.63	7.83	5517.62	0.51
	666.3	27.19	2.45	1596.06	0.15
	679.9	49.87	1.32	1513.29	0.14
	703.0	60.41	1.25	1622.95	0.15

at −196℃ by means of the time-resolved fluorescence spectrum in the ps scale. We attributed the fluorescence subbands at 622 nm, 639 nm and 646 nm to β_s, α, β_f chromophores, respectively. The others were from the β_f chromophore with a linker to various molecular weight polypeptides. The emission at the wavelength 703 nm might be due to the vibrational level.

According to the results mentioned above, since the α chromophore participated in the energy transfer processes in C-PC$(\alpha\beta)_3$, besides the pathway from β_s to β_f in a single monomer, a possible new pathway of energy transfer in C-PC$(\alpha\beta)_3$ might be from β_s to β_f in the same monomer via the α chromophore in an adjacent monomer, with an energy-back-transfer from the β_f to the α chromophore on an adjacent monomer.

IV. Discussion

The energy transfer among these chromophores depends on the association states of C-PC. The energy transfer in monomer is only from the β_s to β_f (center-to-center distance is 4 nm[9]) in one β subunit. The lack of energy transfer from the β_s to α (center-to-center distance is 5.2 nm[9]) or from α to β_f (center-to-center distance is 7 nm[9]) in a monomer is due to the larger distance between them. As shown in Figs. 1 and 3, in C-PC$(\alpha\beta)_3$, the degree of fluorescence excitation polarization is always lower than that in C-PC$(\alpha\beta)$, both spectra show a stepwise increase from 520 nm toward the longer wavelength region, but turns to decrease for C-PC$(\alpha\beta)_3$ while that continues to increase for C-PC$(\alpha\beta)$ above 640nm. The increase of the polarization degree is the characteristic of excitation energy transfer from s chromophore to f chromophore. While in the region above 640 nm, the decrease of the polarization degree expresses an energy-back-transfer from f chromophore to s chromophore. Based on the deconvolution data, the disappearance of the fluorescence subband of the α chromophore at 638 nm in C-PC$(\alpha\beta)_3$ indicates that the α chromophore has transferred its energy to other chromophores, which does not occur in C-PC$(\alpha\beta)$. Besides the energy transfer from β_s to β_f in a single monomer within a trimer, it is clear that there should be another possible energy transfer

pathway in the trimer, that is, the bidirectional energy transfer between the α chromophore in one monomer and the β in an adjacent monomer (center-to-center distance is 2.2 nm)[9]. A lot of work has laid emphasis on this point[11]. In fact, for the very short distance (2.2 nm) and very small excitation energy difference ($\Delta E = E_\alpha - E_{\beta f} = 202.86\ cm^{-1}$, calculated by deconvolution data) between the two (α and β_f) chromophores in different monomers within a trimer, the interaction energy U between α and β_f chromophores is given by Eq. (1)[12].

$$U = K^2 |\mu_\alpha \mu_{\beta f}| / n^2 R^3, \tag{1}$$

where μ_α and $\mu_{\beta f}$ are the dipole transition matrices of the chromophores α and β_f, respectively; n is refractive index of solution; K is the orientation factor; R is the center-to-center separation of the chromophores α and β_f in different monomers. Let A_{ml} be the self-radiative coefficient of the excited state of α chromophore, we have

$$A_{ml} = \frac{1}{\tau_\alpha} = 64\pi^2 \nu_\alpha^3 n |\mu_\alpha \mu_{\beta f}| / 3hc^3, \tag{2}$$

where, $\lambda_\alpha = c/\nu_\alpha$.

Therefore, $$U = 3hK^2/64\tau_\alpha \pi^4 \lambda_\alpha^{-3} n^3 R^3. \tag{3}$$

Taking $\tau_\alpha = 0.4$ ns[11], $K^2 = 2/3$, $R = 2.1$ nm[2], $\lambda_\alpha = 638$ nm, $n = 1.43$. Thus we calculate the value of U as 256.54 cm^{-1}. The excitation energy difference (ΔE) between α and β_f is 202.86 cm^{-1}. $U > \Delta E$[13] indicates that the interaction between α and β_f chromophores is a strong coupling interaction. Generally the relaxation lifetime of the molecular vibration state is about 10^{-11}s, but the energy transfer time between α and β_f is faster than 10 ps[15]. So in the strong coupling case, the energy transfer can occur before the energy relaxes to the lowest excited state. Meanwhile, energy transfer occurs mainly in vibrational states. Thus, energy transfer should be bidirectional between α and β_f chromophores, i.e. $2\alpha \rightleftharpoons 1\beta_f$ (1, 2 represent different monomers). Furthermore, there is no strong dependence of the orientation between chromophores because energy transfer takes place in vibrational states. This ensures that energy transfer between chromophores is efficient.

To explain the reason of energy-back-transfer, we think that the complexes of pigments and proteins in photosynthetic membrane play not only the role of harvesting-light, but also the role of adjusting and assigning light energy among difference chromophores.

Fig. 7 is the suggested model that shows the bidirectional energy transfer caused by the strong coupling interaction between 2α and $1\beta_f$ through excitation energy exchange by means of the vibrational processes. The depolarization region above 640 nm may be due to the unsuitable orientation of the 2α and $1\beta_f$. But depolarization does not affect further energy transfer because of the strong coupling interaction which is comfirmed by the fluorescence polarization spectrum (Fig. 4) in C-PC$(\alpha\beta)_3$. The degree of the polarization shows a monotonous decrease toward

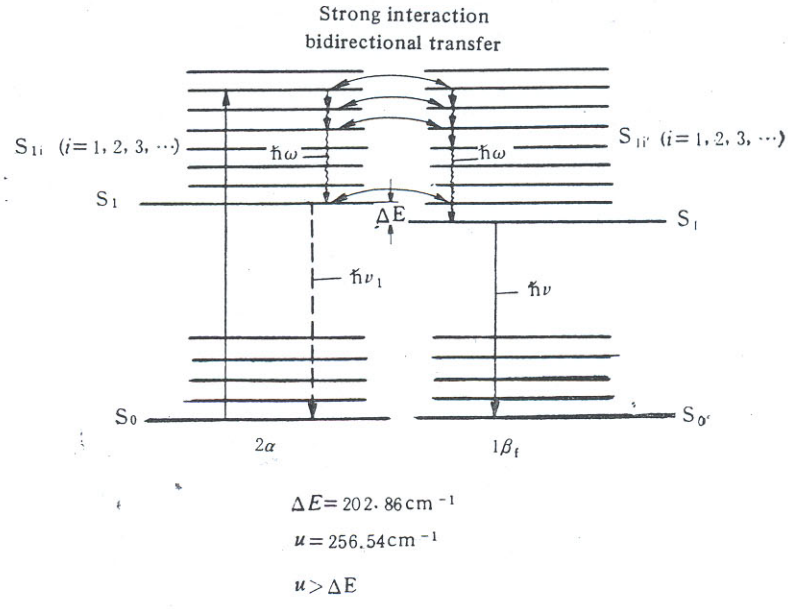

Fig. 7. Proposed model of energy level for strong coupling interaction in the C-PC trimer.

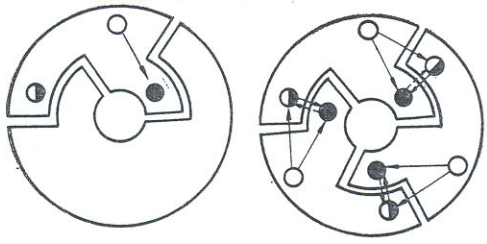

Fig. 8. Diagram of the energy transfer pathways in C-PC monomer and trimer. ○, β_s; ◐, α; ●, β_f.

the longer wavelength region, and reaches a low value (about 0.130) at the wavelength about 660 nm longer than the fluorescence maximum at 647 nm. Glazer et al. estimated the transfer time to be 10—100 ps in C-PC$(\alpha\beta)_3$ and attributed it to the backtransfer process[14]. In our results, the feature that the fluorescence bandwidth of C-PC$(\alpha\beta)_3$ is 20 nm narrower than that of C-PC$(\alpha\beta)$ may be due to the fact that α chromophore participates in the energy transfer processes in C-PC$(\alpha\beta)_3$. We classify the α chromophore into s type chromophore. Excitation energy is transferred from α to β_f in different monomers within C-PC$(\alpha\beta)_3$. Thus, the new energy transfer in C-PC$(\alpha\beta)_3$ occurs between α and β_f (i.e. 2α and $1\beta_f$), which is not observed in C-PC$(\alpha\beta)$.

One possible energy transfer pathway in the trimer is from $1\beta_s$ to 2α (center-to-center distance is also 4 nm[3]). Both 2α and $1\beta_s$ in C-PC $(\alpha\beta)_3$ are located at the edges of monomers out of the cyclic trimer with a C_3 symmetry. The distance between 2α and $1\beta_s$ is the same as that between $1\beta_s$ and $1\beta_f$, with isotropic absorption and the equal possibility of transfer in C-PC$(\alpha\beta)_3$. Therefore, the pathway of energy transfer from $1\beta_s$ to 2α might exist. Fig. 8 is the diagram of the pathways of energy transfer in the monomer and trimer.

The pathways of energy transfer are as follows:

Monomer: $1\beta_s \rightarrow 1\beta_f$;

Trimer: $1\beta_s \rightarrow 1\beta_f$,

$1\beta_s \text{——} 2\alpha \rightleftharpoons 1\beta_f$

.....

and so on.

The three-dimensional structure of C-PC$(\alpha\beta)_3$ obtained from the X-ray structure analysis[7] shows that the β_f lies at the edge opposite to the α chromophore, but inside the cyclic trimer. Thus the energy transferred from the outside ($1\beta_s$, 2α) to the inside ($1\beta_f$) of the trimer might be transferred to the energy sink (β_f-) in C-PC$(\alpha\beta)_3$ with a linker polypeptide and finally reaches the allophycocyanin-core. The fact that the α chromophore is thought to be an s-type chromophore, with the function as an "intermediate" state agrees with our results shown in this paper.

The results mentioned above have produced some understanding of the energy transfer processes in C-PC$(\alpha\beta)$ and $(\alpha\beta)_3$. Further studies by time-resolved fluorescence spectroscopy in ps scale are under way in our laboratory.

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