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# Studies on the energy transfer among the rod-core complex from phycobilisome of *Anabaena variabilis* by time resolved fluorescence emission and anisotropy spectra

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**Abstract**

Picosecond time-resolved fluorescence emission and polarization spectra were recorded for the rod-core complex $(\alpha\beta)_6^{PC}L_{RC}^{27}(\alpha\beta)_3^{AP}L_C^{8.9}$ from cyanobacterium *Anabaena variablis*. Fluorescence decay was measured by single-photon timing and resolved to kinetic components by global data analysis with two different excitation wavelengths at 580 nm and 640 nm. Fluorescence anisotropies were recorded and analyzed at different excitation/emission pairs, such as, 590 nm/625 nm, 625 nm/640 nm, 635 nm/652 nm and 650 nm/665 nm. It was expected through this work to investigate the energy transfer processes between C-PC and APC, as well as those in a C-PC hexamer. Our data indicated that the rod-core complex acted as a functional entity with respect to excitation energy transfer. The kinetic component of 18 ps, observed with excitation at 580 nm and 640 nm, was interpreted as the excitation energy transfer from the three $\beta_{84}$ chromophores of terminal C-PC trimer to an $\alpha_{84}$ chromophore in the APC core. The 55 ps component, observed with excitation at 580 nm, most likely represent the transfer from $\beta_{155}$-PCB to $\beta_{84}$-PCB in a C-PC monomer. The component of 110 ps, observed with excitation at 640 nm, may originate from the transfer between $\alpha_{84}$- and $\beta_{84}$-PCB chromophores in a APC monomer. The 10 ps component was assigned to the energy transfer processes between the identical $\beta_{155}$-PCB or $\alpha_{84}$-PCB chromophores in two trimers while the $40 \pm 2$ ps and 45 ps components to those among the three $\beta_{84}$-PCB chromophores in a C-PC trimer and an APC trimer, respectively.

*Keywords:* Rod-core complex; Energy transfer; C-phycoayanin; Allophycocyanin; Anisotropy; Phycobilisome; *Anabaena variabilis*

## 1. Introduction

PBSs are superamolecular light-harvesting pigment-protein complexes of cyanobacteria and red algae and situate on the outer surface of thylakoid membrane. They collect light and funnel the energy towards the photosynthetic reaction centers (Photosystem I and Photosystem II). In PBS, the excitation energy transfer typically occurs in 100–200 ps with

Abbreviations: PBS, phycobilisome; PEC, phycoerythrocyanin; C-PC, C-phycocyanin; APC, allophycocyanin; PCB, phycocyaninbilin DEAE-, diethylaminoethyl-; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonylfluoride; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate; IRF, instrument response function; FWHM, full width at half maximum

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90% or higher efficiency over an array of several hundred chromophores. The PBS consists of a core and several rods. The core is directly connected to the thylakoid membrane by special linker polypeptide (anchor polypeptide) while the rods are bound to the core. In the cyanobacterium *A. variabilis*, used in this work, the PBS core consists of three cylinders, each with four trimeric units of mainly the biliprotein APCs. There are six or eight rods, each with one hexameric PECs and two or three hexameric C-PCs depending on the growth conditions. The energy transfer in phycobiliproteins, 18-S particles and PBSs has been studied extensively during the last decade by using both steady-state and time-resolved spectrum techniques [1,2]. From these studies, general pictures of excitation energy transfer in phycobiliproteins and PBSs have obtained. In principal, features of the energy transfer processes are: (1) In C-PC, PEC and APC, if the aggregates are larger than or equal to trimers, there will be a rapid process (approx. 500 fs) detected which originates from the energy transfer between an $\alpha_{84}$ and a $\beta_{84}$ in adjacent monomers [3–5], besides, a 10 ps component was detected in hexameric C-PC [6]. (2) The energy transfer from C-PC rods to the APC core is 2 or 3 times faster in rods with only one C-PC hexamer disk than in rods with three or four hexameric disks [7,8]. Thus, it appears that the comparatively long energy transfer from the rods to the core is controlled by the size of the rod [9]. (3) For 18-S particles [10,11], which consist of two separated C-PC trimers bound to a fragment of the allophycocyanin core in the same manner as that in PBS [11], 50 ps and 17 ps components were detected and assigned to the energy transfer from C-PC (rod) to APC (core) based on time-resolved absorption measurement. Recently, Suter and Holzwarth showed [12] that it is possible to account for the rod size effect if one assumes the energy transfer from the terminal C-PC trimer to the APC core is the rate-limiting step. This is an example of trap-limited excitation energy transfer in the model proposed by Pearestein [13]. The calculations showed that energy transfer time from the terminal trimer of C-PC to the APC core should be 20–25 ps, while a lifetime of 40–50 ps was observed in the PBS with each rod containing only one C-PC hexamer. There are two questions remaining to be answered. One of them is whether the 40–50 ps lifetime is determined by the excitation energy transfer within the hexameric unit or by that from C-PC to APC and another is whether hexameric C-PC is a prerequisite for the fast (10 ps) energy transfer and anisotropy relaxation in PBS [7,8]. To study rod-core complex $(\alpha\beta)_6^{PC}L_{RC}^{27}(\alpha\beta)_3^{AP}L_C^{8.9}$ experimentally may help to answer the questions, because this complex is a genuine constituent of the PBS and not an artificial product (the confirmation will be shown in discussion). In this work, time-resolved fluorescence emission and anisotropic spectrum techniques were applied to investigate the kinetics of energy transfer processes on the rod-core complex. The results show that the excitation energy transfer in rod-core complex is similar to the energy transfer in 18-S particles [10] except for the 10 ps component. Furthermore, there was only an 18 ps lifetime detected and assigned to the energy transfer from C-PC to APC in our work. Thus, the rod-core complex can be considered as the smallest functional unit of a PBS consisting of both C-PC and APC. In comparison, Sandstrom and coworkers [10] assigned the 55 ps component to the energy transfer from C-PC to APC [19,20] while it was assigned to the energy transfer from $\beta_{155}$ to $\beta_{84}$ in a monomer of C-PC in this work.

## 2. Materials and methods

### *2.1. Preparation of the rod-core complex*

The rod-core complex was isolated using a modification of the method described by Glauser et al. [14]. About 50 ml of PBS solution (optical density $OD_{610\ nm} = 80–100$) was dissociated into phycobiliprotein-linker polypeptide aggregates by dialysis for 24 h in 5 mM potassium phosphate at pH = 7.0 (at temperature 4°C). All potassium phosphate buffers contained 2 mM EDTA, 1 mM $NaN_3$ and 1 mM PMSF. The dissociated PBS solution was concentrated to 10 ml by ultrafiltration. The concentrated solution was applied onto a DEAE-cellulose DE-32 (Whatman) column (3 cm × 35 cm), preequilibrated in 5 mM potassium phosphate at pH = 7.0 (dialysis buffer), and eluted with a linear gradient of 5–205 mM potassium phosphate of 1100 ml total volume and a constant flow rate of 1 ml/min. Five fractions were collected

separately for further purification by ultracentrifugation. Fraction 2 from DEAE-cellulose DE-32 column was dialyzed against 750 mM potassium phosphate buffer at pH = 8.0 and then concentrated. The obtained sample was distributed onto ultracentrifugation tubes in 24.5 ml linear sucrose gradients (0.2–0.5 M) in dialysis buffer of 750 mM and centrifugated with 43000 rpm for 16 h at a Backman XL-90 Ti-41 swing-out rotor. The fast migrated band in the ultracentrifugation tubes is the rod-core complex. The purity of this complex was judged by SDS-PAGE electrophoresis method.

### *2.2. Picosecond fluorescence measurement*

Tunable picosecond light pulses were generated by a cavity-dumped dye laser (3800, Spectra physics), synchronously pumped by an argon ion laser. This system produced pulses less than 1 ps duration with a pulses energy of 40 mW. The time-resolved fluorescence measurements were performed with a single photon timing apparatus described elsewhere [30]. The detector was MCP-PMT (R2809U- HAMAMATUS) and the instrument response function (IRF) was less then 35 ps (FWHM), and the resolution time was less then 2 ps. At each emission wavelength, an approximately 10 ns fluorescence decay window containing 8212 channels was acquired until 10000 counts had been accumulated in the peak channel. The isotropic fluorescence decay function was obtained by setting the analyzing polarizer at magic angle of 54.7° relative to the polarization of the excitation light. The anisotropic fluorescence signals were obtained from parallel and perpendicular polarizations, respectively. The anisotropy is defined and calculated as $r(t) = (I_{para} - I_{perp})/(I_{para} + 2I_{perp})$ in which the sum $I_{para} + 2I_{perp}$ is proportional to the isotropic signals. All measurements were carried out at room temperature with the optical density ($OD_{635}$) of the samples kept at 0.1 in a cell of 10 mm path length. The photon density through the sample was controlled no greater than $10^{11}$ per $cm^2$ per pulse which was proved far below that exciton annihilation might occur. We applied a deconvolution procedure based on a global optimization algorithm to analyze the fluorescence isotropic decay curves recorded at various emission wavelengths, in which the experimental signals at different wavelengths were analyzed simultaneously in a single run instead of analyzing individual decay curve separately. The algorithm is based on the assumption that for each decay component the decay constant (lifetime) keeps invariable while the preexponential factor varies with the detection wavelengths. The fluorescence decay was described by a sum of exponential functions, and the quality of the fits was judged by a global $\chi^2$ value and individual $\chi^2$ values (less than 2.0). The iteration procedure applied in our program is a semi-linear Marquardt algorithm [6]. Because the fluorescence anisotropy $r(t) = D(t)/I(t)$ was derived by a combination of the parallel and perpendicular decays, the deconvolution was applied on $I(t) = I_{para} + 2I_{perp}$ (corresponding to isotropic decay) and $D(t) = I_{para} - I_{perp}$ (corresponding to anisotropic decay), respectively. A semi-linear Marquardt algorithm was used for fitting the calculated $r(t)$ values at each wavelength, and then the depolarization decay times ($\tau_{ri}$) and fluorescence anisotropic values ($r_i(t)$) were obtained at different wavelengths.

## 3. Results

### *3.1. Steady-state spectra of the rod-core complex*

Absorption and fluorescence emission (at room temperature and 77 K) spectra of the isolated complexes from the PBS of *A. variabilis* are shown in Fig. 1. The fluorescence spectrum of the rod-core

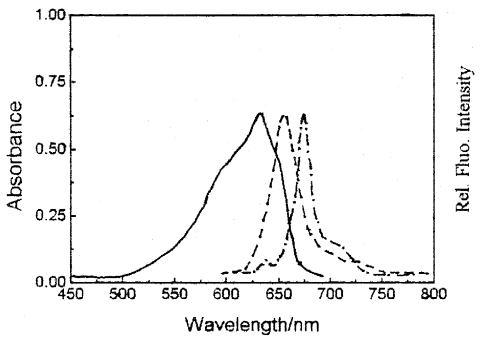


Fig. 1. The steady-state spectra of rod-core complex $(\alpha\beta)_6^{PC}L_{RC}^{27}$ $(\alpha\beta)_3^{AP}L_C^{8.9}$. (————) absorption spectra; (---) fluorescence emission at room temperature ($\lambda_{exci}$ = 580 nm); (— — —) fluorescence emission at 77 K ($\lambda_{exci}$ = 580 nm).

complex is very similar to that of APIII ($(\alpha\beta)_3^{AP}L_C^{8.9}$) of *A. variabilis* and shows a maximum at 665 nm (room temperature) independent of the excitation wavelength. This means that the energy transfer from C-PC to APC is very efficient in the rod-core complex and implies that the isolated rod-core complex can be considered as the smallest functional unit of a PBS consisting of both C-PC and APC.

### 3.2. Decay-associated fluorescence emission spectra

To investigate the coupling of C-PC to APC, decay-associated fluorescence emission spectra were recorded at two different excitation wavelengths 580 nm and 640 nm. The former was chosen to excite preferentially $\beta_{155}$-PCB chromophores of C-PC [28] while the latter to excite $\beta_{84}$-PCB chromophore in the C-PC [28] and the chromophores in APC [29].

#### 3.2.1. Excitation at 580 nm

The decay-associated spectra with predominant excitation of $\beta_{155}$-PCB chromophores at 580 nm have been recorded from 620 nm to 700 nm in 10 nm intervals. Typical residual plots and $\chi^2$-values from the global data analysis are shown in the *left* and the *right* of Fig. 2 with three- and four-exponential model functions, respectively. These data show that four exponentials are necessary for satisfactorily fitting the fluorescence decay profiles. The Fig. 3 and Fig. 4 show two examples of the global analysis for fluorescence emission 630 nm and 670 nm, respectively. The decay-associated spectra of this complex contain two short-lived components with $\tau = 18$ ps and $\tau = 55$ ps as well as two long-lived components with $\tau = 1100$ ps and $\tau = 1710$ ps (see Fig. 5A). The 18 ps component possesses positive amplitudes at short wavelengths with the maximum at around 640 nm and negative amplitudes at long wavelengths with the

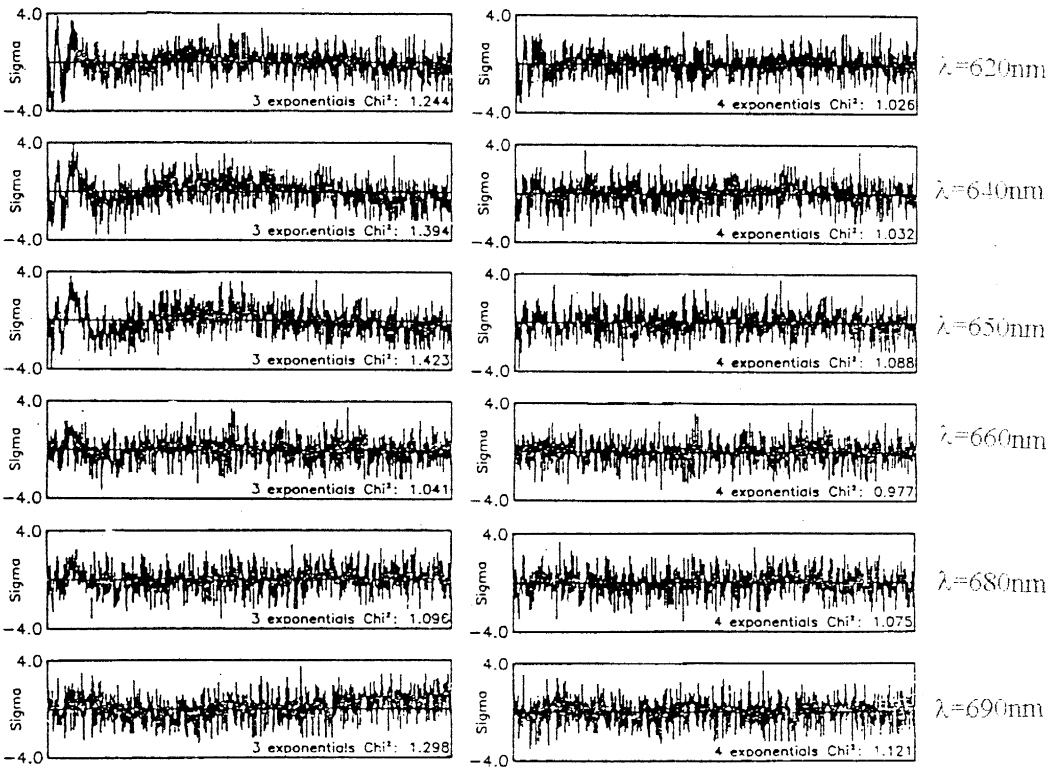


Fig. 2. Weighted residual plots from the global data analysis of the fluorescence decays from the rod-core complex for a three-exponential (left) and a four-exponential (right) model function at 620, 640, 650, 660, 680 and 690 nm. The $\chi^2$-values at emission wavelengths mentioned above are given for each decay. Note the strong systematic deviations from a random distribution in the case of the three-exponential model function.

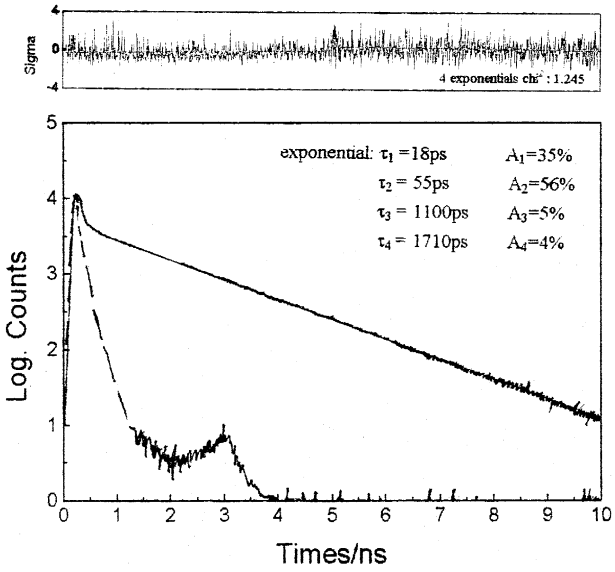


Fig. 3. Global analysis of the fluorescence decay kinetics of rod-core complex; $\lambda_{ex} = 580$ nm, $\lambda_{em} = 630$ nm. Lower panel: excitation function (dashed line) and fluorescence decay (full line) on a semilogarithmic scale. Upper panel: weighted residuals plot for model function of four exponentials.

minimum around 665 nm. The negative amplitudes correspond to a fluorescence rise term of the energy acceptor.

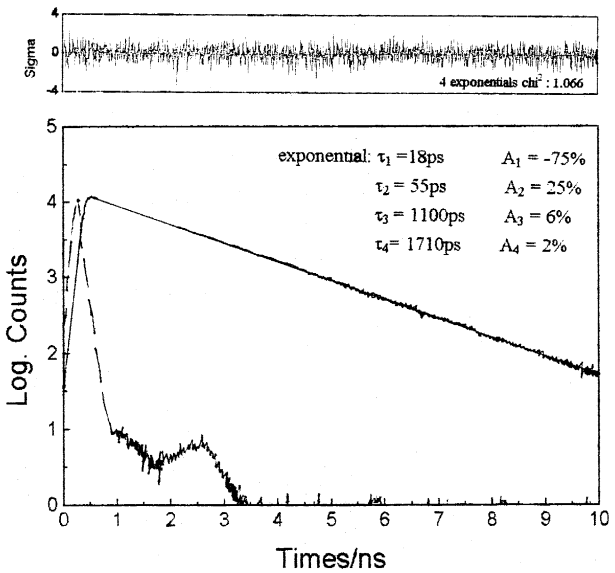


Fig. 4. Global analysis of the fluorescence decay kinetics of rod-core complex, $\lambda_{ex} = 580$ nm, $\lambda_{em} = 670$ nm. Lower panel: excitation function (dashed line) and fluorescence decay (full line) on a semilogarithmic scale. Upper panel: weighted residuals plot model function of four exponentials.

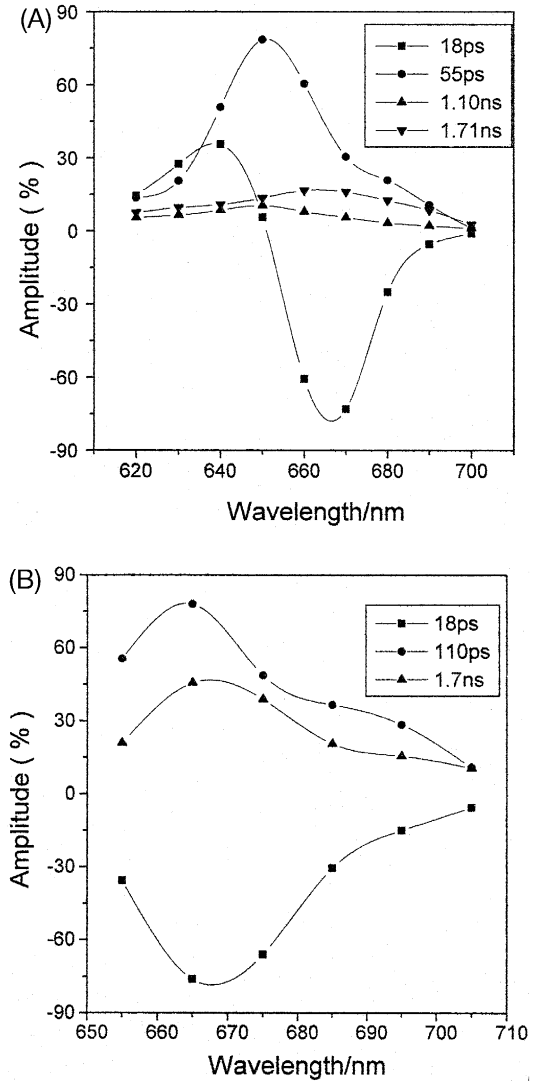


Fig. 5. A: the decay-associated fluorescence spectra at different probe wavelengths at excitation wavelength 580 nm. B: the decay-associated fluorescence spectra at different probe wavelengths at excitation wavelength 640 nm.

### *3.2.2. Excitation at 640 nm*

The excited light was chosen for dominantly exciting the $\beta_{84}$-PCB chromophores in the C-PC and APC of this complex [28,29]. The decay-associated fluorescence was recorded from 655 nm to 705 nm in 10 nm intervals. For the complex, the fluorescence decay kinetics at all the detection wavelengths could be fitted very well with a sum of three exponential functions (the residual plots are not shown). The

results are shown in Fig. 5B. Two short-lived components with $\tau = 18$ ps and $\tau = 110$ ps are derived. The 18 ps component possesses negative amplitudes with the minimum at around 665 nm but no positive amplitudes at all detection wavelengths. It should be mentioned that the exactly same component of 18 ps was derived at both excitation wavelengths 580 nm and 640 nm. However, excited at 640 nm, the component of 55 ps was replaced by the one of 110 ps, that could not be ascribed to the experimental error but might imply that the two components originated from different energy transfer processes. Furthermore, excited at 640 nm, only one long lifetime component ($\tau = 1700$ ps) was observed while the component of 1100 ps disappeared.

### 3.3. Time-resolved fluorescence anisotropy

Some different excitation/emission pairs were chosen for probing the energy transfer kinetics between symmetrically equivalent chromophores in the rod-core complex. The 590 nm/625 nm, 625 nm/640 nm, 635 nm/652 nm and 650 nm/665 nm pairs were chosen in this work.

#### 3.3.1. Excitation / emission pair at 590 nm / 625 nm

This excitation/emission pair was chosen for obtaining the kinetic information of energy transfer among the $\beta_{155}$-PCB chromophores in the hexameric C-PC unit of the rod-core complex [18]. The results are shown in Fig. 6A,B and listed in Table 1. Two depolarization times: $\tau_{r1} = 10$ ps and $\tau_{r2} = 53$ ps were derived and implied decay of the excited state of $\beta_{155}$-PCB chromophores through two pathways.

Table 1
The anisotropy values ($r$) and anisotropy decay times ($\tau_r$) at different excitation/emission pairs

| $\lambda_{ex}$ (nm) | $\lambda_{em}$ (nm) | $r_0$ | $\tau_{r1}$ (ps) | $r_1(t)$ | $\tau_{r2}$ (ps) | $r_2(t)$ | $r(\ )$ |
|---|---|---|---|---|---|---|---|
| 590 | 625 | 0.40 | 10 | 0.35 | 53 | 0.09 | 0.07 |
| 625 | 640 | 0.28 | 9 | 0.20 | 42 | 0.10 | 0.10 |
| 635 | 652 | 0.28 | 16 | 0.18 | 38 | 0.12 | 0.12 |
| 650 | 665 | 0.40 | 45 | 0.09 | / | / | 0.08 |

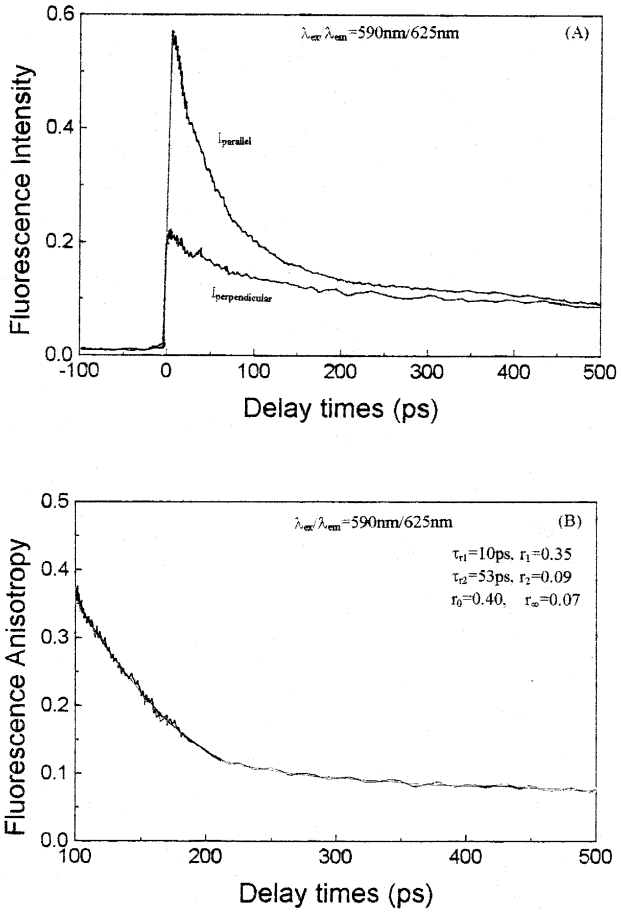


Fig. 6. A: the decay of fluorescence polarized parallel and perpendicular at excitation/emission pair 590 nm/625 nm on the isolated rod-core complex. B: the fluorescence anisotropy decay calculated from the parallel and perpendicular traces in Fig. 4A (pointed curve) and two-exponential fitted curve (straight line) on the isolated rod-core complex.

#### 3.3.2. Excitation / emission pairs at 625 nm / 640 nm and 635 nm / 652 nm

According to the absorption maxima and fluorescence spectra [18], at these excitation/emission pairs, it is expected that the depolariztion kinetic information about the $\alpha_{84}$-PCB and $\beta_{84}$-PCB chromophores in the C-PC could be obtained. The fluorescence anisotropy curves were fitted well by sum of two exponentials with decay time constants 9 ps and 42 ps, respectively. The shortest time constant of 9 ps was similar to that for the excitation/emission pair 590 nm/625 nm within the experimental error. However, the 42 ps constant is shorter than that for the

excitation/emission pair 590 nm/625 nm, in which the large difference can not be due to experimental error but indicate different energy transfer routes. At excitation/emission pair 635 nm/652 nm, the time constants were changed to 16 ps and 38 ps, respectively (see Fig. 7A,B). The decay time of 42 ps and 38 ps could reflect the same process within experimental error, but those of 9 ps and 16 ps represented different transfer routes.

### *3.3.3. Excitation / emission pair at 650 nm / 665 nm*

The excitation/emission pair was chosen for obtaining the depolarization kinetic information in APC trimer of the rod-core complex [29]. From Fig. 8A,B, a single decay time $\tau_r = 45$ ps was observed, this implies a new pathway for fluorescence depolarization in the APC trimer.

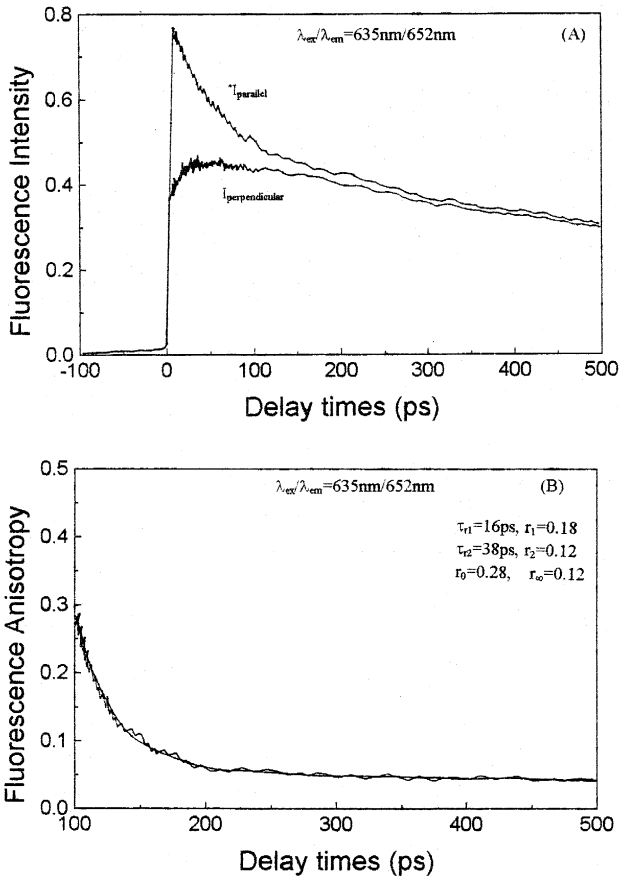


Fig. 7. A: the decay of fluorescence polarized parallel and perpendicular at excitation/emission pair 635 nm/652 nm on the isolated rod-core complex. B: the fluorescence anisotropy decay calculated from the parallel and perpendicular traces in Fig. 5A (pointed curve) and two-exponential fitted curve (straight line) on the isolated rod-core complex.

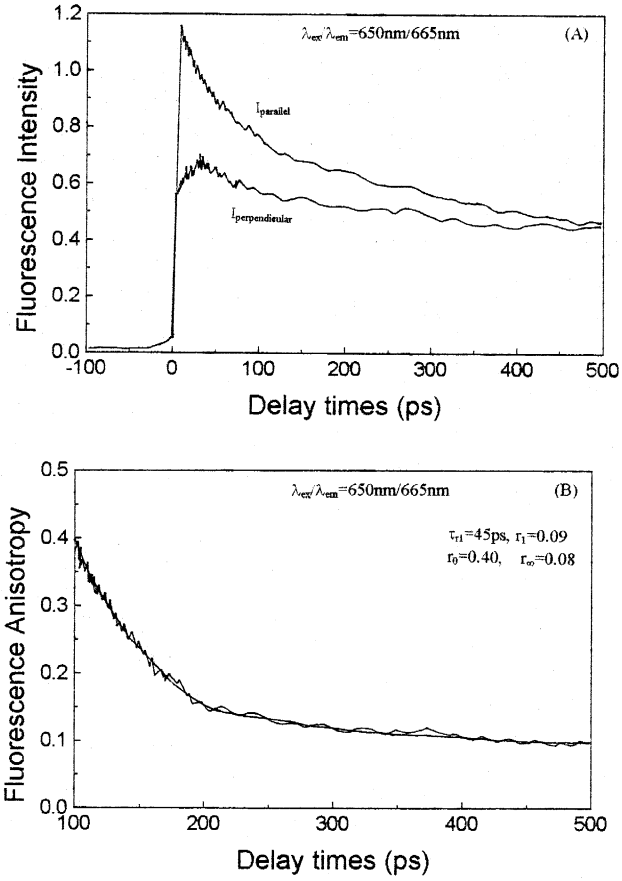


Fig. 8. A: the decay of fluorescence polarized parallel and perpendicular at excitation/emission pair 650 nm/665 nm on the isolated rod-core complex. B: the fluorescence anisotropy decay calculated from the parallel and perpendicular traces in Fig. 6A (pointed curve) and one-exponential fitted curve (straight line) on the rod-core complex.

## 4. Discussion

### *4.1. Energy transfer kinetics in hexamer C-PC of the complex*

Up to now, the crystallographic structure of C-PC has been determined [15,16] and the optical properties of individual chromophore have been well characterized by steady-state [17] and time-resolved fluorescence or transient absorption spectra [18]. Based on these, the energy transfer in monomeric and

trimeric C-PC has been studied extensively by many investigators [6,3,19,20].

In this work, when the rod-core complex was excited at 580 nm, the shortest lifetime detected by the time-resolved fluorescence was 18 ps, which was much longer than that (10 ps) observed in C-PC hexamer of S*ynechococcus 6301* [6,7], and amplitudes of the component changed from positive to negative at about 650 nm with the maximum at 640 nm and the minimum at 665 nm. However, when it was excited at 640 nm, the same component was also observed but the amplitudes were negative at all detected wavelengths with the minimum at about 665 nm (see Fig. 5B). Judging from the spectral composition, the lifetime should originate from the energy transfer between C-PC and APC because it has never been observed in any aggregates of C-PC [6,19,20].

Furthermore, from the time-resolved fluorescence anisotropy (see Fig. 6), a 16 ps component was observed with a residual anisotropy $r_1(t) = 0.20$ and most probably was the same component as what mentioned above [20]. The energy transfer from C-PC to APC (18 ps) competes efficiently with that of $\tau = 55$ ps in a hexamer [20], therefore, in such a case, the energy transfer within a C-PC hexamer becomes less probable way, which can be seen from the partial depolarization in a C-PC hexamer of the rod-core complex.

A strong support for this point can be found from the large anisotropy $r_1(t) = 0.20$ at $\tau_{r1} = 16$ ps observed from fluorescence emission at 652 nm in the rod-core complex (see Fig. 6 and Table 1). Compared with that in a C-PC monomer or a trimer [17,21], in the rod-core complex, the relatively high anisotropy may originate from the competing transfer from an excited C-PC chromophore to one of APC before a complete redistribution of the excitation energy over the C-PC chromophores. Further, In the view of the structure, the $\beta_{84}$-PCB chromophores occupy the central positions in a C-PC hexamer and approximate to the APC core most, therefore, they are most likely the candidates to mediate energy to the APC core. Bittersmann [22] and Sandstrom [10] observed similar lifetimes of about 20 ps and 17 ps in intact cells of cyanobacterium *Synechocystis sp*. pcc *6803* and in an isolated 18-S complex from AN 112 (a mutant of *Synechococcus 6301*) separately, which agree well with our results. This implied that the rod-core complex, obtained by us, was a genuine constituent of the PBS and not an artificial product.

For structure reasons, the three $\beta_{84}$-PCB chromophores of the terminal trimer in the rod approximate the binding site of the rod with the core. Based on the crystal structure of APC trimer [31] and the time-resolved spectrum measurement [32,33], it can be seen that the structure of APC is quite similar to that of C-PC and the $\alpha_{84}/\beta_{84}$ pairs in adjacent monomers of APC could also be strongly coupled. Up to now, there is very little information about detailed structure of APC attached to C-PC rod, however, generally, it is well known that APC is attached to the terminal C-PC trimer at a right angle and its molecular plane lies on the $C_3$-axis of C-PC. Furthermore, in this work, there was only one component observed for energy transfer from the rod to the core, that means the energy transfer paths from the three $\beta_{84}$-PCB chromophores of the C-PC trimer to the chromophores in APC must be equivalent. Therefore, based on available structure information and especially on the homogeneous feature of the energy transfer kinetics from the rod to the core measured in this work, it can be deduced that an $\alpha_{84}$-PCB chromophore of APC must locate at the threefold symmetrical axis of the terminal C-PC trimer so that $C_3$ symmetry is well kept otherwise a multiple component kinetics should have been observed.

A 55 ps kinetic component was observed in fluorescence isotropy measurement while a 53 ps component in anisotropy detection for the excitation/emission pair 590 nm/625 nm (see Fig. 6 and Table 1). In fact, 52 and 50 ps time constants were ever reported by Debrecenzy et al. [19,20] from time-resolved fluorescence anisotropy for the monomeric and trimeric C-PC of *synechococcus* sp. PC 7002. Obviously, at the excitation wavelength of 590 nm, it is the $\beta_{155}$-PCB chromophore that is excited dominantly [18], therefore, we assign this component to the energy transfer from $\beta_{155}$-PCB to $\beta_{84}$-PCB in a C-PC monomer. Besides, a high anisotropy $(r_0) = 0.40$) was observed at time zero and changed to 0.09 at 53 ps, while similar polarization values were reported by time-resolved and steady-state polarization studies [17,20] separately on C-PC monomers and trimers of *Mastigocladus laminosus*. Based on crystal structures [15,16] and optical properties of the chromophores in C-PC, a $49 \pm 8$ ps time constant was

derived by calculation [23]. That means our assignment is reasonable. On the other hand, this component did not show any negative amplitudes which might be duo to the energy transfer rates from the $\beta_{84}$-PCB to other chromophores are much faster than the 55 ps process therefore the accepted energy could not reside on the $\beta_{84}$ chromophore such a long time.

Now, we consider the $40 \pm 2$ ps component observed in the fluorescence decay of the rod-core complex at the excitation/emission pair 625 nm/640 nm and 635 nm/652 nm (see Fig. 7 and Table 1). These excitation/emission pairs were chosen to probe the kinetic information mainly on $\alpha_{84}$- and $\beta_{84}$-PCB chromophores in hexameric C-PC unit of the rod-core complex [18]. From the crystal structure of C-PC, one can predict that the two chromophores in the pair $\alpha_{84}^{1}$-$\beta_{84}^{2}$ (and also the pairs of $\alpha_{84}^{2}$-$\beta_{84}^{3}$ and $\alpha_{84}^{3}$-$\beta_{84}^{1}$) of C-PC should be most strongly coupled because they possess shortest center-to-center distance and preferable space orientation. For this pair, the energy transfer time between the two chromophores was proved less than 1 ps (about 500 fs) [3–5]. However, due to the 2 ps resolution time in our instrument, this energy transfer processes could not be observed.

In the calculations based on Forster mechanism, the excitation is thought to be localized on a distinct chromophore at any given time. However, on the other hand, because the energy transfer within the $\alpha_{84}^{1}$-$\beta_{84}^{2}$ pair is so fast that the pair can be treated as excitonically excited couple. Therefore, we assigned the decay time of $40 \pm 2$ ps to the energy transfer among the three energetically degenerate pairs ($\alpha_{84}^{1}\beta_{84}^{2}$, $\alpha_{84}^{2}\beta_{84}^{3}$ and $\alpha_{84}^{3}\beta_{84}^{1}$) around the trimer ring. In a trimer of C-PC, the three $\beta_{84}$-PCB chromophores, separated by a center-to-center distance of 36 A, are most likely the candidates for energy transfer among the three monomers. In the localized model, the observed decay constant of $40 \pm 2$ ps should then originate from the energy transfer among the three $\beta_{84}$-PCB chromophores instead of the three $\alpha_{84}$-PCB chromophores. Since isotropic fluorescence decay is not sensitive to energy transfer between identical chromophores, this decay time of $40 \pm 2$ ps was not observed in time-resolved isotropic fluorescence experiment. Debreczeny [20] and Zhao and coworkers [21] found this lifetime $40 \pm 2$ ps by using different methods, their results support our assignment.

The Forster calculations indicate that two main processes are responsible for this mode of depolarization: energy transfer between $\beta_{84}$-PCB chromophores on adjacent monomers and energy transfer between $\beta_{84}$- and $\alpha_{84}$-PCB chromophores on the same monomer [23]. The observed and predicated rate constants for these processes are in close agreement.

The decay time of 10 ps and 9 ps at excitation/emission pairs 590 nm/625 nm and 625 nm/640 nm (see Fig. 6 and Table 1) might originate from the energy transfer between $\beta_{155}$-PCB chromophores or $\alpha_{84}$-PCB chromophores in different C-PC trimers of a hexameric unit. Because these chromophores ( $\beta_{155}^{1}$–$\beta_{155}^{6}$, $\beta_{155}^{3}$–$\beta_{155}^{5}$ and $\beta_{155}^{2}$–$\beta_{155}^{4}$ and/or $\alpha_{84}^{1}$–$\alpha_{84}^{4}$, $\alpha_{84}^{2}$–$\alpha_{84}^{5}$ and $\alpha_{84}^{3}$–$\alpha_{84}^{6}$) separate by a center-to-center distance of 28.7 Å and/or 25.7 Å, the energy transfer between them was proved to be quite fast [23]. At excitation/emission pair 590 nm/625 nm, it gave high anisotropy $r_0 = 0.43$ at time zero, which come from $\beta_{155}$-PCB chromophores [19], however, the anisotropy decreased to 0.30 at decay time $\tau_{r1} = 10$ ps (see Table 1). The high residual anisotropy may be caused dominantly by $\beta_{155}$-PCB chromophores [18,19], which indicated that the decay component of 10 ps came from the energy transfer between the $\beta_{155}$-PCB chromophores in different trimers of the C-PC disk. However, when excitation/emission pair was chosen at 625 nm/640 nm, the anisotropy $r_0 = 0.28$ at time zero was dominantly from the $\alpha_{84}$-PCB chromophores [18,19], and the anisotropy decreased to 0.20 when the depolarization time reached 9 ps. We assigned the 9 ps component to the energy transfer among the $\alpha_{84}$-PCB chromophores in different trimers of the C-PC disk.

In fact, we thought what Holzwarth A.R. [6] observed a 10 ps process in C-PC hexamer was not the energy transfer between the f-f chromophore pairs, but that between the chromophores in $\beta_{155}^{1}$–$\beta_{155}^{6}$, $\beta_{155}^{3}$–$\beta_{155}^{5}$ and $\beta_{155}^{2}$–$\beta_{155}^{4}$ chromophore pairs or $\alpha_{84}^{1}$–$\alpha_{84}^{4}$, $\alpha_{84}^{2}$–$\alpha_{84}^{5}$ and $\alpha_{84}^{3}$–$\alpha_{84}^{6}$ chromophore pairs as we did in the C-PC hexamer of the rod-core complex. Mimuro and coworkers [24] thought that the energy transfer has two pathways in the C-PC rods of PBS: one of them may be among $\beta_{155}$-PCB chromophores, and the other may be among $\alpha_{84}$-PCB chromophores along the long axis of the PBS rods. This statement and the calculation results [23] support our assignment of 10 ps or 9 ps component.

*4.2. Energy transfer within the APC*

When the excitation wavelength was chosen at 640 nm, at which APC chromophores was dominantly excited, fluorescence decay kinetics of this complex could be fitted well by sum of three exponentials (see Fig. 5B). The 18 ps component could be assigned to the energy transfer from the $\beta_{84}$-PCB chromophores of ''terminal C-PC trimer to an $\alpha_{84}$-PCB chromophore of APC as mentioned above''.

The 110 ps component might due to the energy transfer between the $\alpha_{84}$- and $\beta_{84}$-PCB chromophores in the same monomer of APC. Gillbro and coworkers [25] observed the lifetime of 144 ps in APC monomers isolated from *M. laminosus*, and assigned it to the $\alpha_{84}$-$\beta_{84}$ path. Besides a component of $70 \pm 10$ ps had been observed in the APC by Sauer et al. [26].

For the 640 nm/665 nm excitation/emission pair, the anisotropy decreased quite fast with a time constant of 45 ps and then to a constant value of about 0.09 at $t > 50$ ps (see Fig. 8). This indicates that excitation energy transfer occurs among the chromophores with identical spectra but different orientations in the APC trimer. Considering the structure, this process most probably originate from the transfer among the three $\beta_{84}$ chromophores of the APC trimer. In fact, the two chromophores in an $\alpha_{84}/\beta_{84}$ pair of adjacent monomers of APC trimer are strongly coupled just like that in C-PC. When excitation energy is transferred to an $\alpha_{84}$ of APC, it will be transferred in fs level (300–1000 fs) forward and back many times

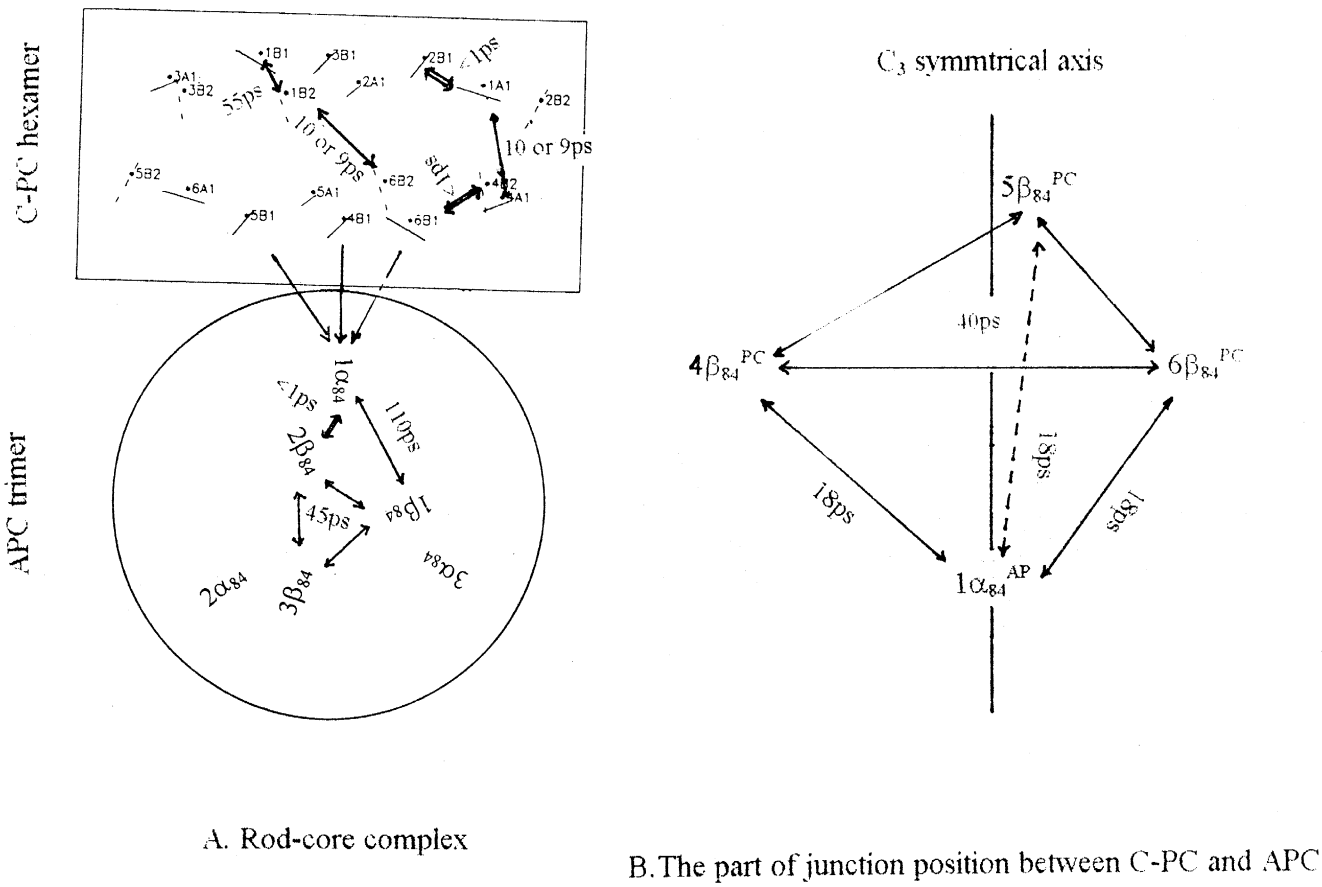


Fig. 9. Schematical drawing for the energy transfer paths and the time constants in the rod-core complex (A) while (B) for the three identical paths and the time constant of the energy transfer from the three $\beta_{84}$ chromophores of the terminal C-PC trimer to an $\alpha_{84}$ chromophores of the APC trimer. 1A1 means the $\alpha_{84}$ in the number 1 monomer, B1 and B2 stands for $\beta_{84}$ and $\beta_{155}$, respectively, while the numbers 2–6 stand for the number 2 to number 6 momomer in the hexamer.

between the strongly coupled chromophores, then occasionally it may be transferred to another strongly coupled pair through the $\beta_{84} \rightarrow \beta_{84}$ path (45 ps) at which the ultrafast processes will be repeated. Yeh [27] measured the steady-state polarization of APC trimers from *Anabaena variabilis*, and found a polarization value of about 0.08 independent of excitation wavelength, which agreed well with the constant value of the anisotropy obtained in this work.

## 5. Conclusions

From the current work, it can be seen that the rod-core complex from *Anabaena variabilis* is a functional entity of the PBS in terms of the energy transfer. From the results, some conclusions can be drawn and are schematically shown in Fig. 9. The Fig. 9a shows the schematic structure of the rod-core complex while Fig. 9b shows the three identical paths for the energy transfer from the three $\beta_{84}$ chromophores of 'terminal' C-PC trimer to an $\alpha_{84}$ chromophore of the APC trimer.

The 10 ps component, observed in the isotropic decays at both excitation/emission pairs (590 nm/625 nm, 625 nm/640 nm),is assigned to the energy transfer between $\beta_{155}$-PCB chromophores (or between $\alpha_{84}$-PCB chromophores) in different C-PC trimers of a hexameric C-PC. The 18 ps (or 16 ps) component observed in the isotropic and anisotropic decays must reflect the energy transfer from the three $\beta_{84}$-PCB chromophores of the ''terminal C-PC trimer to an $\alpha_{84}$-PCB chromophore of APC core''. The 55 ps lifetime observed in the isotropic decays with excitation wavelength at 580 nm is tentatively attributed to the energy transfer between the $\beta_{84}$- and $\beta_{155}$-PCB chromophores in a monomer of C-PC. Forster calculation [23] and experimental results [19,20] support the assignment. The $40 \pm 2$ ps and 45 ps lifetimes, observed in anisotropic decays at different excitation/emission pairs, are assigned to energy transfer between the identical $\beta_{84}$-PCB chromophores in a C-PC and an APC trimer, respectively. The 110 ps lifetime observed in isotropic decays at excitation wavelength 640 nm, is tentatively attributed to the energy transfer between $\alpha_{84}$- and $\beta_{84}$-PCB chromophores in the same monomer APC.

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