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Excitation energy transfer in phycobilisomes at −196 °C isolated from the cyanobacterium *Anabaena variabilis* (M-3): evidence for the plural transfer pathways to the terminal emitters

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Excitation energy transfer in phycobilisomes at −196 °C was studied by means of the time-resolved fluorescence spectroscopy in the picosecond time-range. Supplemental data were obtained from the allophycocyanin-core complex. When the phycobilisomes were excited at 580 nm, at least nine fluorescence components were resolved by the time-resolved spectra and deconvolution of those spectra. Energy transfer among these components is not straightforward. At the phycocyanin level, two transfer pathways are probable; one may be among the β-155 chromophores, and another among α-84 chromophores along the long axis of the phycobilisome rods. The β-84 chromophores in intermediate discs of the rods might function as an energy pool by a fast equilibrium between α-84 and β-84 chromophores. The β-84 chromophores in the trimer next to the core-complex is the energy donor to the core-complex. At allophycocyanin level, two pathways of the energy transfer were also found; one from F_{660} to F_{686} through the F_{673}. This corresponds to the energy flow from β-84 chromophore without linker to 18.3 kDa polypeptide and finally to the 'anchor' polypeptide. The other pathway is from the F_{666} to F_{680}, i.e., from the β-84 with a linker polypeptide to the α-subunit of allophycocyanin B. Two independent pathways in the energy transfer shown in this study basically agree with the assembly model of the core components proposed by Glazer (cf. Biochim. Biophys. Acta 768 (1984) 29–51).

Introduction

A phycobilisome is a supramolecular light-harvesting pigment protein complex which facilitates the absorption and transfer of light energy to chlorophyll proteins in the thylakoid membranes [1,2]. In many species of cyanobacteria, phycobilisomes are hemidiscoidally shaped and consist of rods of phycoerythrin and/or phycocyanin (PC) and an allophycocyanin (APC)-core complex. The rods may be composed of two to four hexamer discs, while the core complex is usually composed of three dodecamers. Two basal dodecamers are adjacent to the thylakoid membrane. The energy flow in phycobilisome is assumed to occur from pigments at a higher energy level to pigments at a lower energy level.

The crystallographic structure of PC has been determined [3,4] and knowing the location of the chromophores allows us to determine that phycocyanobilin chromophore are attached to cysteine residues as follows; 84 on α and β polypeptides and an additional one at 155 on β polypeptide. The orientation and optical properties of individual chromophores have been well characterized [5–8] by steady-state spectrum. The energy levels of three types of chromophores in trimer have been determined in the PC trimer and assigned as follows; 598, 622 and 632 nm for the β-155, α-84 and β-84 chromophores, respectively. Excitation energy transfer from the chromophores at the outer side of trimer to the chromophores at the inner side has been established [5,8]. In hexamer, additional energy flows can be assumed among β-155s and among α-84s along the long axis of the rod structure [4].

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Abbreviations: α-APB, α-subunit of allophycocyanin B; PC, phycocyanin; APC, allophycocyanin; F_{623}, a representative expression of fluorescence component whose maximum is located at 623 nm; FWHM, full-width at half-maximum; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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The molecular assembly of the APC-core complex is more complicated, since there are several different molecular species which are present as four kinds of trimeric discs [2]. The optical characteristics of individual chromophores are still unclear, and the energy flow among the components is as yet not established. Two terminal emitters (α-allophycocyanin B (α-APB) [9] and 'anchor' polypeptide [10]) are located in the two central trimeric discs of the lower dodecamer [11]. They are adjacent to one another [11]; however, they are not connected in terms of energy transfer [12,13].

Extensive analysis of energy transfer in the picosecond time-range has been carried out on particular aggregation states of PC [14–16], and on whole phycobilisome [17–21]. Analysis involves either recovery of transient absorption or fluorescence decay at a fixed wavelength. Critical to analysis of such data is the exponential decay function. We, on the other hand, have measured the time-resolved fluorescence spectra in the picosecond time-range and clearly showed sequential energy flow in phycobiliproteins of cyanobacteria and red algae [22,23]. The rise and decay curves of the respective components were estimated by deconvolution of time-resolved spectra, and the energy transfer kinetics were described by the decay function proportional to the square root of time. In those analyses, the transfer between different molecular species was mainly taken into account; however, the critical point is the description of elementary process in energy transfer, that is, transfer between individual chromophores in PC and APC.

In the present study, time-resolved fluorescence spectra of phycobilisome at $-196\,^{\circ}$C were analyzed with a shorter time resolution (about 10 ps). This aims to characterize the spectral properties of fluorescence components and to estimate the number of components sufficient to deconvolute the fluorescence spectra. A possible pathway of energy transfer among components was then estimated based on their rise and decay kinetics. Analyses were done on PBS and isolated APC core complex which retained the capacity to form entire phycobilisome when incubated with PC rods. We could discriminate nine fluorescence components including two terminal emitters. Two independent pathways to the different terminal emitters were found in APC-core complex.

Materials and Methods

Growth of cyanobacterium and isolation of phycobilisome. Cells of *Anabaena variabilis* (M-3) were grown in C medium of Kratz and Myers [24] under continuous illumination of a red-light enriched fluorescent light. The air containing 2% CO_2 was continuously supplied. Cells were harvested from 4 day cultures for isolation of phycobilisome.

Phycobilisomes were isolated by the method described in Ref. 25 in high concentrations of phosphate buffer (0.75 M, pH 7.1). Phycobilisomes were released by Triton X-100 treatment (2%) and separation was performed on a sucrose linear gradient.

Isolation of APC-core complex. APC-core complex (23 S APC) was purified from phycobilisome by the partial dissociation of phycobilisome and column chromatography (cf. Ref. 26). Phycobilisome dissolved in 10 mM potassium phosphate (pH 7.1) to a final concentration of $A_{620} = 100$ were applied to a hydroxyapatite column (Seikagaku Kogyo, Japan). The samples were eluted by increasing the ionic strength. APC-core complex was eluted in the ionic strength around 0.3 M phosphate. The eluate was immediately applied to sucrose density gradient (0.2 to 0.5 M in 0.75 M phosphate) and centrifuged in a Hitachi 40T rotor at 36000 rpm for 16 h at 20 °C. A fraction corresponding to 23 S APC was collected. Purity of the complex was checked by SDS-PAGE. A small amount of PC was bound to APC-core complex, which could not be separated. This fraction was used for experiment without further purification.

Measurements of steady-state fluorescence spectra. Steady-state fluorescence spectra at $-196\,^{\circ}$C were measured with a Hitachi 850 spectrofluorometer equipped with a laboratory made apparatus for low-temperature spectroscopy. Spectral sensitivities of the apparatus were numerically corrected by a microcomputer (HP model 216).

Measurement of time-resolved fluorescence spectra. Time-resolved fluorescence spectra in the picosecond time-range were measured with the apparatus reported previously [22,23,27]. The light source was composed of a mode-locked Ar^+ laser and a synchronously pumped and cavity-dumped dye laser. A pulse duration was 6 ps (FWHM). The pulse intensity at 580 nm was in a range of 10^8–10^9 photons/cm^2 per pulse, which was low enough to avoid singlet–singlet annihilation. The repetition rate of the cavity dumper was 800 kHz. Fluorescence from the samples was detected by a time-correlated single-photon counting method. A microchannel plate photomultiplier (R2809U-01, S-20 type photocathode, Hamamatsu Photonics, Japan) was adopted for a shorter time resolution. The half band-width of the scanning monochrometer was set to 2 nm. Spectral sensitivity of the detection system was corrected based on the radiation profile of the standard lamp. Time zero was set to the time when the excitation pulse was the strongest. Since the excitation pulse (6 ps, FWHM) was usually observed as a pulse of 30 to 40 ps (FWHM) due to a transit time in photocathode, the spectrum before time zero was obtainable.

Phycobilisome or APC-core complex in 0.75 M potassium phosphate (pH 7.1) was mixed with the same volume of poly(ethylene glycol) 4000 (30%, w/v) to

obtain homogeneous ice [28] and was frozen in the dark. Samples were always immersed into liquid nitrogen during measurements.

Deconvolution of the time-resolved fluorescence spectra. Fluorescence components in the time-resolved spectra were resolved by deconvolution of spectra. The component band-shape was assumed to be gaussian as a function of wavenumber, because absorption and fluorescence spectra of phycobiliproteins were well fitted by gaussian band shape [29]. The least-square method was adopted for the estimation of the best fit. A microcomputer (NEC 9801 VM2) and an HP computer (model 216) were used for data acquisition and analysis, respectively.

Results

Components of phycobilisome fluorescence

Fluorescence emission spectra, at steady-state or pulse-illuminated, are a combination of emissions originating from fluorescence components. Energy transfer among them has to be described on the base of their kinetics. The deconvolution procedure is usually adopted to separate the whole spectrum into component spectra, and because the identification of fluorescence component is critically important. Along this line, the fluorescence spectra of phycobilisome in the steady-state (Fig. 1) and the time-resolved spectra (Figs. 2 and 3) were analyzed in detail.

(i) Steady-state spectra: Fig. 1A shows fluorescence emission and excitation spectra of phycobilisome isolated from *Anabaena variabilis* (M-3) at −196 °C. The phycobilisome preparation is energetically well-coupled, as evidenced by a strong emission from terminal emitter(s) and a weak emission from PC and APC. In the excitation spectra, there were several component bands; the maxima deriving from PC were observed around 600, 618, 625 and 633 nm, and that from APC at 652 nm. A small shoulder around 656 nm suggests the presence of another component. In the wavelength region longer than 670 nm, another band(s) was clearly detected, probably corresponding to the terminal emitter(s).

(ii) Time-resolved spectra: Fig. 2 shows the three-dimensional expression of the time-resolved fluorescence spectra in phycobilisome at −196 °C. This expression enabled us to understand the energy flow conceptually. On excitation at 580 nm, which preferentially excited β-155 chromophores in PC, fluorescence was initially observed around 640 nm with a small but significant shoulder at 622 nm. PC emission was highest at 99.2 ps after the pulse. Fluorescence from APC was then detected around 665 nm in the time-range from 50 to 400 ps, with the maximum around 200 ps, and a rapid decay. The emission around 685 nm rose concomitant

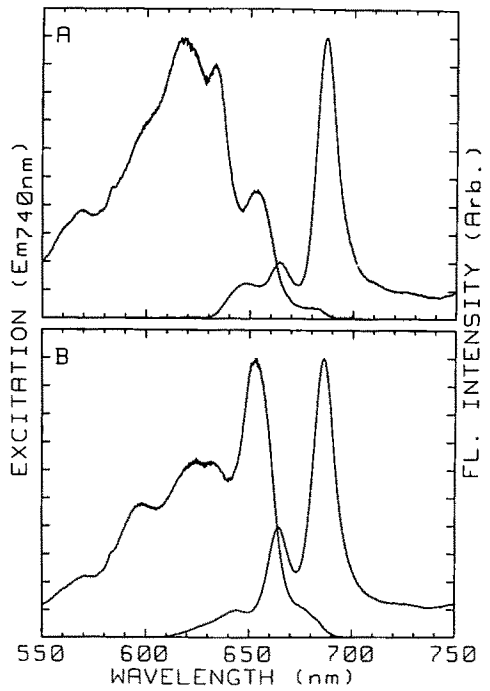

Fig. 1. Steady-state fluorescence spectra of phycobilisome (A) and APC-core (B) at −196 °C. In each figure, the spectrum on the left side was the excitation spectrum, and that on the right side the emission spectrum. Excitation was 580 nm for the emission spectrum, and the fluorescence was monitored at 740 nm for the excitation spectrum. For details see the test.

with the decay of APC emission, and its intensity was high during the next 2 ns.

The changes in the spectra were much more clearly observed after normalization (Fig. 3). The 623 nm emission was clearly detected at 0 ps and it disappeared soon. The presence of several other bands was also evident. The emission at the wavelength region longer than 700 nm might be vibrational bands. Changes in the relative intensities of fluorescence components directly show the energy transfer from PC to the terminal emitters.

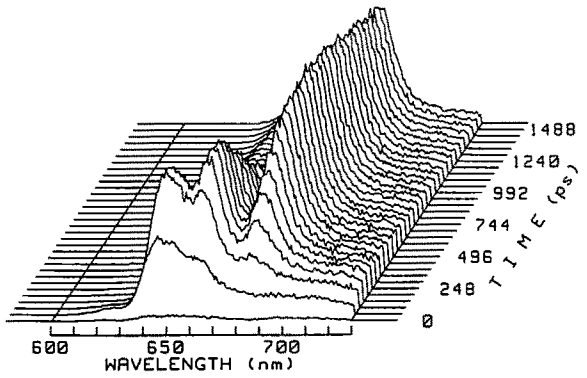

Fig. 2. Excitation energy transfer in phycobilisome at −196 °C expressed by three-dimensional time-resolved fluorescence spectra. Time-resolution of time-to-amplitude converter was 49.6 ps. Excitation was done at 580 nm. For details see the text.

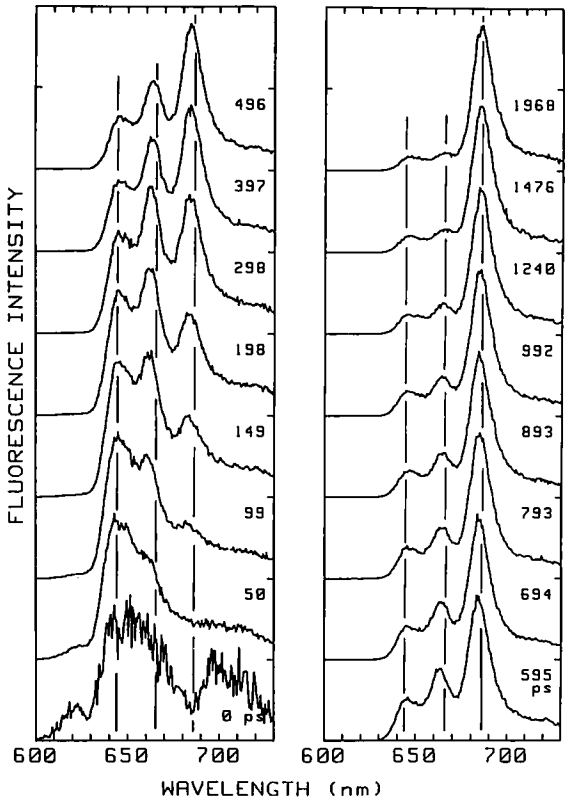

Fig. 3. Normalized time-resolved fluorescence spectra of phycobilisome at −196 °C. Each spectrum was shown after normalization to the maximum intensity. Numbers in the figure show the time in picoseconds after the excitation pulse. For details see the text.

One typical feature in the spectral change is the shift of the maximum of two fluorescence bands; one around 665 nm and the other at 685 nm. Vertical reference lines

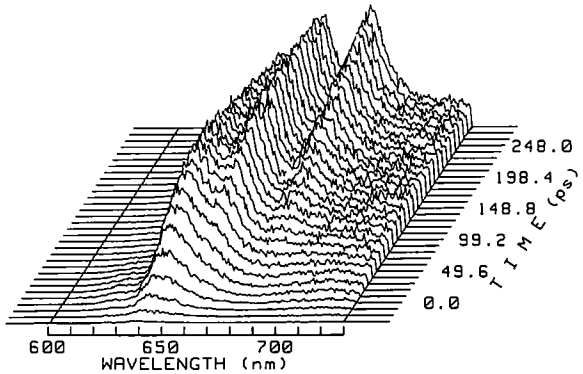

Fig. 4. Excitation energy transfer in phycobilisome at −196 °C expressed by three-dimensional time-resolved fluorescence spectra. Time-resolution of one channel in time-to-amplitude converter was 9.92 ps. The other conditions were the same as Fig. 2. For details see the text.

in Fig. 3 indicate the locations of the maxima, 1.97 ns after the initial pulse. Initially the peaks were blue-shifted, but with time both shifted simultaneously towards the red, i.e., from 660 and 680 to 666 and 686 nm. This shift was slow, compared with other changes in the spectrum. This shift pattern indicates the presence of plural components in each wavelength region (two components in APC and two components in the terminal emitters). It further suggests that the component located at 666 nm is not the energy donor to both terminal emitters. If it were the donor to both, then the fluorescence rise of the terminal emitter should have occurred when the maximum shifted to 666 nm.

Plural component bands were also detected in PC (Figs. 4 and 6) only when the spectra of the intact

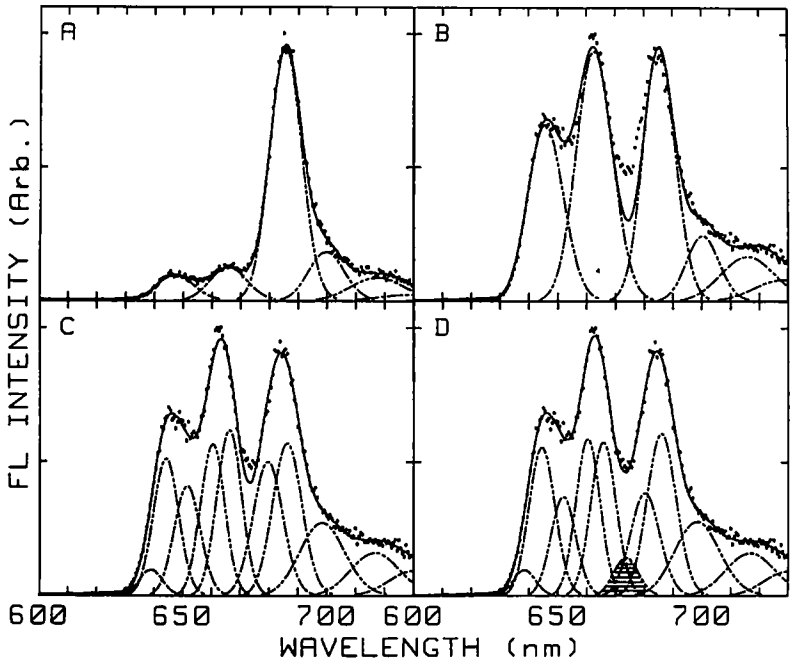

Fig. 5. Procedure for obtaining parameters for deconvolution of time-resolved fluorescence spectra. In each figure, observed spectrum (------), component band (----) and sum of component bands (———). In (D), a shaded band represents the F_{673}. For details, see the text.

phycobilisome were measured with a short time resolution (9.92 ps). This was simply due to a fast shift of the maxima. When the time-resolved fluorescence spectra were expressed as bird's-eye view, the rise of individual components was clearly detected (Fig. 4). The maximum of PC was seen around 70 ps and that of APC around 150 ps. Compared with the decay of PC emission, the rise of APC seemed faster. The time difference between two peaks was about 80 ps. The peak of the emission from terminal emitter(s) was around 250 ps. By the spectra with this short time resolution, the component bands could be identified. Just after the excitation, a plateau was observed between 640 and 655 nm, indicating the presence of plural components. The normalized spectra (Fig. 6) indicate that those components were located at 623, 639, 644 and 652 nm. This is the first demonstration of the presence of four fluorescence components in PC. This is partly due to the measurement at low temperature where high spectral resolution is obtainable. The first of the four components may correspond to the 620 nm component proposed by Holzwarth et al. [13,15].

Identification of component bands by deconvolution of time-resolved spectra

Fluorescence components in the time-resolved spectra were resolved by deconvolution of spectra attributing intensity changes to certain component bands. Gaussian band-shape was assumed as a function of wavenumber. This treatment should be regarded as a first-order approximation. We assumed three parameters to describe a component band; location of its maximum, its height and band-width. In setting the parameters as objectively as possible, we adopted the following procedure. First, we obtained the parameters of the three main components (apparent maxima at 644, 666 and 686 nm) by the deconvolution of the spectrum in which one band is predominant, i.e., for the 686 nm component, the spectrum at 1.97 ns (Fig. 5A) or for the 666 nm component, that at 248 ps (Fig. 5B). Second, those parameters were modified a little to assume plural components in PC, APC and terminal emitters, as suggested by the shift of the maxima. We assumed four for PC, two for APC, and two for terminal emitters. The band-widths usually became narrower than those in a previous treatment for the best fit. The band-width obtained by single component simulation was set to the maximum width for the following simulation. Third, when we deconvoluted the spectra, peak height was preferentially changed with the fixed location and band-width. The maximum magnitude for a shift of the location was set to shorter than 0.5 nm and it was applied only when the changes in the other parameters did not give a better fit.

First we used eight components for deconvolution, which were identified in the time-resolved fluorescence

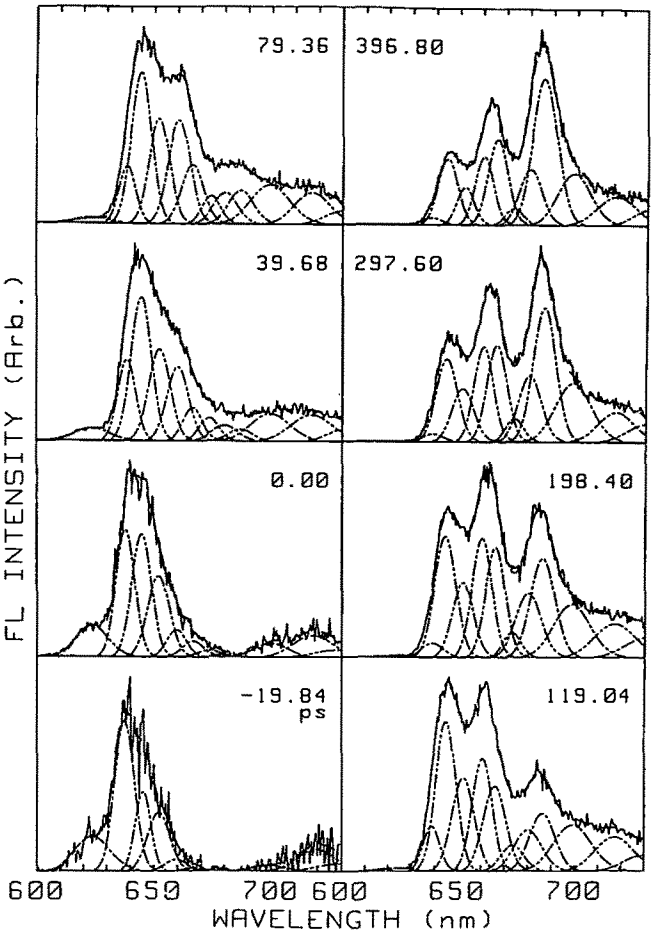

Fig. 6. Deconvolution pattern of time-resolved fluorescence spectra. Deconvolution was done on the spectra measured with a time-resolution of 9.92 ps. Gaussian band shape was assumed as a function of wavenumber. In each figure, observed data (———), component band (----) and sum of component bands (------). For details see the text.

spectra. With these components we could not obtain a better fit in a particular wavelength region, i.e., around 670 nm (Fig. 5C) where there is a trough in the spectrum. Although there is no reason to suggest the presence of a component, the best fit has never been achieved unless an additional component was assumed. The spectrum was corrected in terms of spectral sensitivity; thus, an introduction of one additional component is not due to artificial error in the measurements. Therefore, we assumed the presence of a new component at 673 nm to obtain the best-fit (a shaded band in Fig. 5D, hereafter, it is called F_{673}).

To elucidate the rise and decay curves of individual fluorescence components, we deconvoluted the time-resolved spectra measured with a time resolution of 9.92 ps (Fig. 6). Nine components were used for simulation, and each spectrum was reasonably fitted only by changes in the relative intensities. Three additional bands (F_{700}, F_{717} and F_{730}) were also used for the simulation, which were ascribed to vibrational bands. At −19.84 ps, F_{623},

F_{639}, F_{644} and F_{652} were observed, but the intensities of the other bands were almost insignificant. On the other hand, intensities at wavelengths longer than 700 nm were clearly observed. These might originate from vibrational bands of PC. The intensity of the F_{644} became high, giving rise to a plateau at 0 ps. The relative contribution of the component bands of APC became significant with time. After 40 ps, the emission from the F_{680} was evident, and at the same time the F_{686} was discernible. The shift of the maxima around 665 and 685 nm was clearly explained by the changes in the relative intensities of two component bands (F_{660} and F_{666} for APC, and F_{680} and F_{686} for terminal emitters).

Rise and decay curves of individual fluorescence components

Based on the relative intensities of individual bands and absolute photon counts in each time-resolved fluorescence spectrum, rise and decay curves of individual fluorescence components were obtained (Fig. 7). In PC,

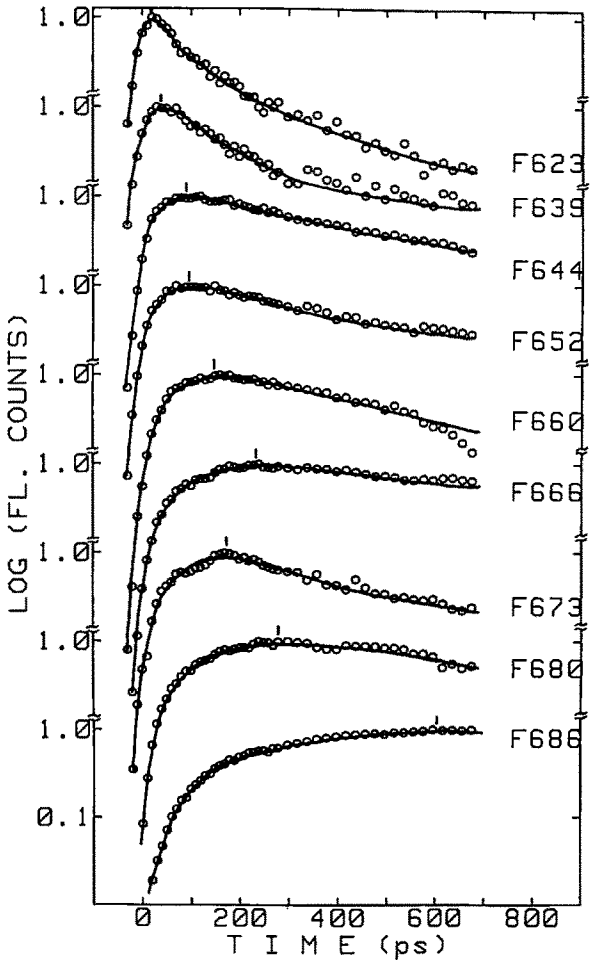

Fig. 7. Rise and decay kinetics of individual fluorescence components in phycobilisome. Each curve was obtained by the relative intnsities of individual bands shown in Fig. 6 and actual absolute photon numbers. Bars over rise and decay curves indicate the peak of the curves. For details see the text.

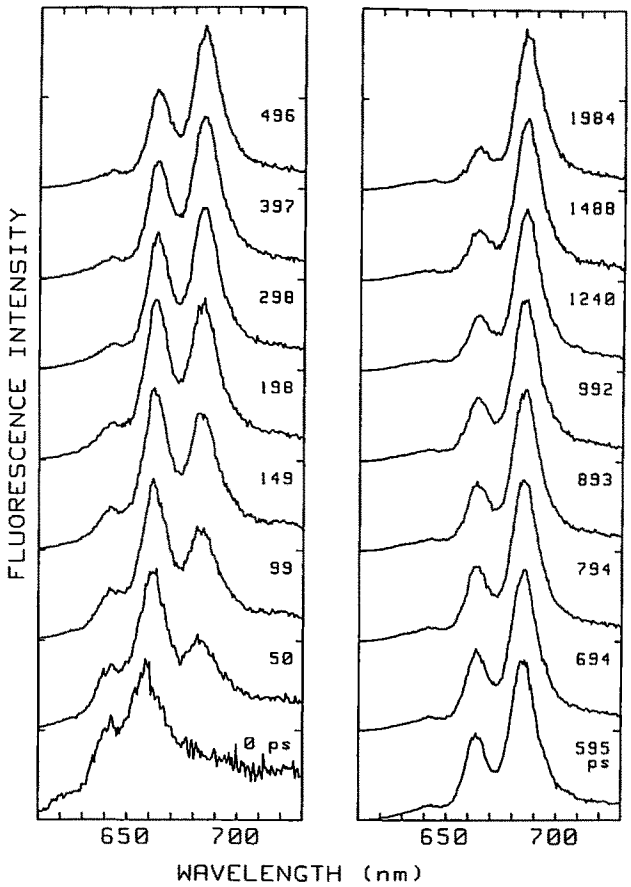

Fig. 8. Time-resolved fluorescence spectra of APC-core complex at −196 °C. Each figure was normalized to the maximum intensity. Numbers reprsent the time in picoseconds after the excitation pulse. For details see the text.

the F_{623} rose quickly and its maximum was observed 20 ps after the excitation. It decayed also quickly (the shortest lifetime was estimated to be about 40 ps with the assumption of exponential decay). Compared with this, the rise and decay profile of the F_{639} was a little slower (the maximum was at 30 ps, and the lifetime 50 ps). The rise and decay curves of the F_{644} and F_{652} were substantially the same within experimental error.

The APC-core complex showed characteristic rise and decay curves of five components. The rise of all the five components was slow, compared with those of PC. Of these five, the F_{660} and F_{673} showed a faster rise and decay, compared with those of the F_{666} and F_{680}, as is clearly shown by the appearance of the maximum at an earlier time. The rise of the F_{686} was very slow. These rise and decay curves reflect heterogenous energy transfer among these components. Sequential transfer among these five is not possible, because the rise and decay curve of the acceptor should be slower than that of the donor. A progressive shift of the rise and decay curves is what is required for such a sequential energy flow. The presence of a fast decaying component (F_{673}) in the intermediate of two slow decaying components (F_{666} and F_{680}) suggests that the F_{666} is not an energy donor

of the F_{673}. The energy transfer in core complex does not seem simple (see Discussion).

Fluorescence properties of APC-core complex

Next we checked the fluorescence properties of the APC-core complex because more of the PC was removed and this would cause little perturbation of emission. We used 23 S APC-core complex which retained the capacity to form intact phycobilisome when incubated with PC rods in high concentration of phosphate buffer (0.75 M) [25,26]. The optical properties of the fraction at steady-state are shown in Fig. 1B. The APC-core was energetically well-coupled, since on excitation at 580 nm the main emission originated from terminal emitter(s) at 686 nm. A relatively strong emission at 665 nm indicates a partial uncoupling between APC and terminal emitter(s) or a contamination of free APC. A significant difference from the fluorescence of phycobilisome was the appearance of fluorescence around 630 nm due to contamination of PC which was not coupled with the APC-core. In the excitation spectrum, the maximum was observed at 652 nm with a clear shoulder around 658 nm. Minor bands at 620 and 632 nm were probably due to contamination of PC. There were a few additional minor bands around 670 and 683 nm, corresponding to terminal emitter(s). These bands have already been reported in the phycobilisome of *Nostoc* sp. (MAC) [12] and in another species [30].

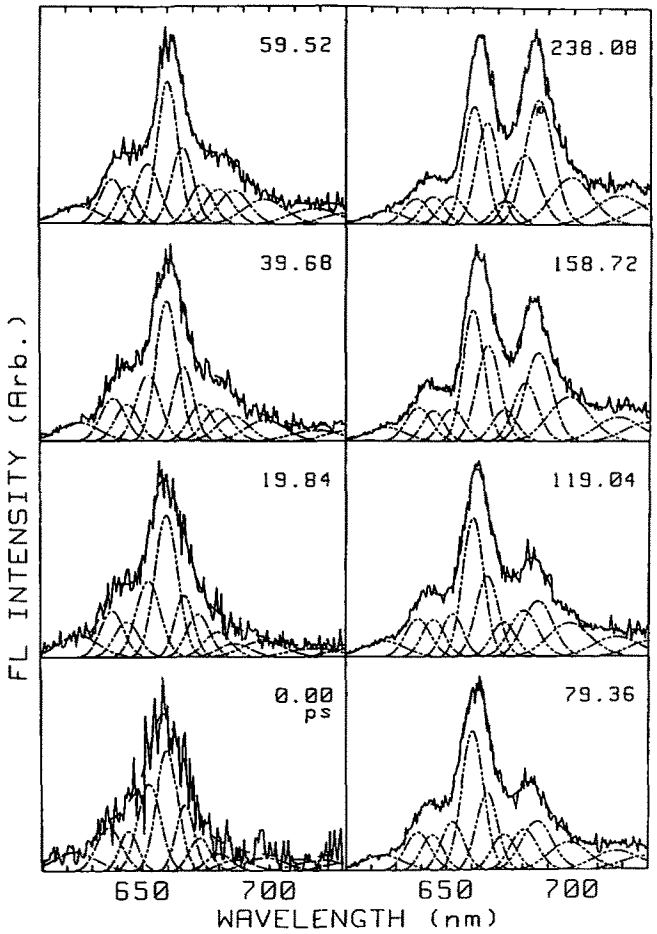

Fig. 9. Deconvolution patterns of time-resolved spectra in APC-core complex at −196 °C. The same procedures and parameters were used for deconvolution as in the case of phycobilisome (Fig. 6). In each figure, observed data (———), component band (----) and sum of component bands (------). For details see the text.

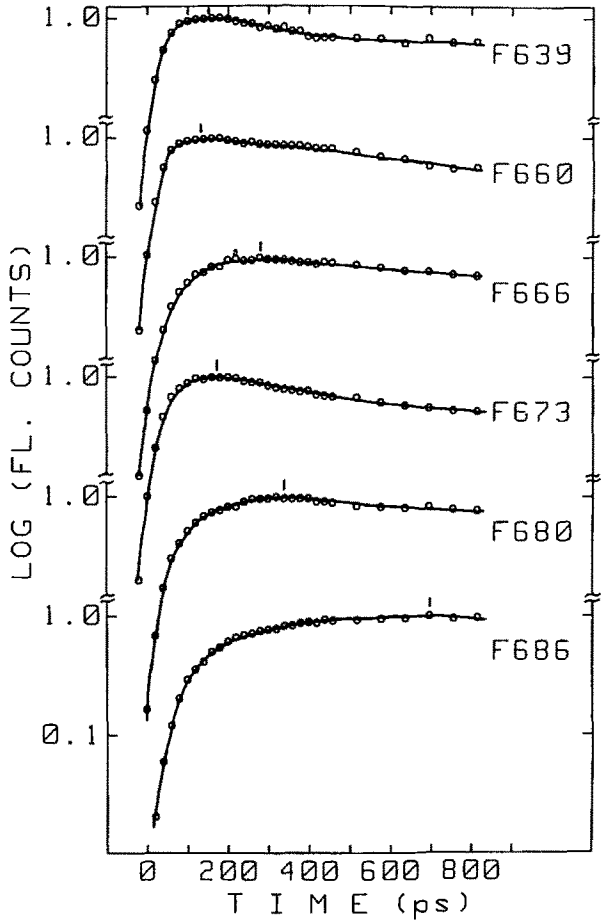

Fig. 10. Rise and decay curves of individual fluorescence components in APC-core complex at −196 °C. Each curve was obtained based on the relative intensity and absolute photon counts. Bars over the curves represent the peak of each curve. For details see the text.

The time-resolved fluorescence spectra of APC-core complex are shown in Fig. 8 with a time resolution of 49.6 ps. When excited at 580 nm, emissions at 642 and 660 nm were observed. A minor shoulder around 622 nm was also detected. In the wavelength region longer than 660 nm, significant intensities were apparent. The emission around 685 nm increased, accompanying the shift of the maximum from 680 to 686 nm, as in the case of whole phycobilisome (Fig. 3). Compared with phycobilisome, the fluorescence intensity around 640 nm was significant even a long time after excitation, indicating inefficient energy transfer to terminal emitter(s). The spectral feature was almost the same as in phycobilisome, thus deconvolution was done on the spectra using the same parameters as those for phycobilisome.

Fig. 9 shows deconvolution patterns of time-resolved spectra in APC-core complex. All the components found in phycobilisome were detected even in the spectrum at 0 ps, probably due to the direct excitation of APC. The F_{660} was dominant in the initial time range, and the components around 685 nm became significant with time. Accompanying the shift of the maxima around 665 and 685 nm, the relative intensities of two components varied as in phycobilisome.

Rise and decay kinetics of individual components in APC-core complex

Rise and decay curves of individual fluorescence components (Fig. 10) were obtained by the same treatment as those in phycobilisome (cf. Fig. 7). The rise and decay of the fluorescence components in PC was slow, as shown by F_{639}, unlike in phycobilisome. This indicates the PC in this core-complex was probably only loosely bound to the complex. The essential profile of the rise and decay curves of the components in APC-core complex were the same as those in phycobilisome. The rise and decay of the F_{660} and F_{673} were faster than those of the F_{666} and F_{680}. Again the rise of the F_{686} was slowest, as in the case of phycobilisome. These results clearly indicate that the heterogeneous energy transfer sequence is a general properties of APC-core complex.

Discussion

Identification of component bands by time-resolved fluorescence spectra

In our previous study on cyanobacteria containing PC and APC, we identified only three fluorescence components (PC, APC and far emitting APC) [22,23,27], and energy transfer kinetics were discussed only on these three components. Now we found at least eight components and there is probably a ninth. This is partly due to better time resolution and partly to measurements at low temperature (−196 °C). Reasonable assumption of such a large number of components is not necessarily easy in the global analysis which is adopted by other groups [14–16]. In this sense, the time-resolved fluorescence spectrum is a useful method for identifying components and describing the energy flow among them. Some of the components we found that they correspond to those reported by other groups. Their components weremainly determined by lifetime and thus should be regarded as decay components. At −196 °C, the lifetime is essentially different from those at room temperature, thus direct comparison of our fluorescence components with their decay components is not straightforward.

Presence of the F_{673}

In this study, we found one new fluorescence component, that is F_{673}. The main reason for the assumption of this component is a worse fit in deconvolution of time-resolved fluorescence spectra (Fig. 5). This component has never been observed as a clear maximum. It could be detected as a shoulder in the time-resolved spectra of APC-core complex only in the initial time-range after the excitation (at 39.68 ps in Fig. 9). The relative contribution in intensity was not high and positive evidence is weak. However, in the steady-state measurements, there are some indications for the presence of this component. In the fluorescence excitation spectra of APC-core complex, minor but significant bands were detected around 670 and 683 nm. This is also the case in phycobilisome preparation isolated from *Nostoc* sp. (MAC) (668 and 678 nm) [12]. These two bands can be ascribed to a 18.3 kDa polypeptide and the overlapping band of α-APB and the anchor polypeptide, respectively (cf. Ref. 12). The degree of polarization of the former was around 0.2, while that of the latter was nearly 0.5. This indicates that the 668 nm component is an energy donor of the longer component(s). Considering a Stokes shift, the F_{673} emission can be ascribed to the 668 nm component, most probably the 18.3 kDa polypeptide. The isolated 18.3 kDa polypeptide shows a fluorescence maximum that is a little longer when compared with the α- and β-subunits of APC (cf. Fig. 4 in Ref. 31). This also supports evidence for the presence of the F_{673}.

Assignment of individual fluorescence components and energy transfer among them

There are nine fluorescence components in the time-resolved fluorescence spectra of phycobilisome. Of these, four components belong to PC and the remaining five to APC-core complex, as is clearly seen in the spectra of this complex. The origins of these fluorescence components can be assigned based on the steady-state measurements of individual chromophores.

In the PC trimer, the energy levels of three types of chromophore are in the following order; β-155 (598 nm), α-84 (624 nm) and β-84 (632 nm) chromophores. Their fluorescence maxima at room temperature are reported to be at 622, 638 and 642 nm, respectively [5]. The β-84 chromophores with particular linker polypeptides show characteristics fluorescence maxima at 643, 648 and 652 nm, respectively, with 30, 32 and 27 kDa linker polypeptides [2]. Changes in the optical properties of the β-155 and α-84 chromophores by linker polypeptides might be small. Based on these data, the origins of the individual fluorescence components in the time-resolved spectra can be assigned as follows: F_{623} from β-155, F_{639} from α-84, and F_{644} from β-84 without linker or β-84 with a 30 kDa linker. The F_{652} might originate from the β-84 in the disc proximal to the APC-core complex.

Optical properties of PC crystal [7] and of trimers in solution [5,8] indicate that the primary energy transfer takes place between α-84 and the nearest β-84 chromo-

phores. The transfer time between these two chromophores has been calculated to be shorter than 1 ps, which is based on the Foerster kinetics and orientation factors between the two [32]. The observed fastest decay component was the F_{623}, originating from β-155. We interpret from these results that the decay of the F_{639} reflects the shift of the equilibrium between the α-84 and β-84 chromophores (difference between back and forth transfer), and the decay of the F_{623} corresponds to the energy transfer between β-155 and β-84 chromophores or between β-155s in an adjacent trimer disc. The actual energy transfer between α-84s and β-84s might not be detected by the apparatus we used due to a limited time resolution (9.92 ps).

The rise and decay curves of the F_{644} and F_{652} were almost the same. This suggests that the energy transfer between these two components is not probable, because if transfer occurs then the transfer time should be very short, giving rise to the same kinetics. A predicted rate constant for transfer time between β-84 in one trimer and the β-84 in the other trimer in the same hexamer is long (9 ns^{-1}) [32]. Thus F_{644} might not be the component mediating the energy transfer to the F_{652}. Probably the energy transfer among the α-84 chromophores, and among β-155 chromophores along the long axis of rod structure, is the main energy flow. The β-84 might function as a pool of energy by a fast equilibrium between β-84 and α-84. At the site adjacent to the APC-core complex, the energy is transferred from the chromophores (β-155 and α-84) in the outer position of the trimer to the β-84 on the inner side. The energy donor to APC-core is supposed to be the β-84 chromophore in the trimer next to the core complex.

In APC, there are five components; F_{660}, F_{666}, F_{673}, F_{680} and F_{686}. Of these, the latter three originate from the 18.3 kDa polypeptide, the α-APB and 'anchor' polypeptide, respectively. The origins of the F_{660} and F_{666} are not necessarily clear. However, they probably do not originate from the α-chromophores of APC, even if we take the intermediate interaction into account (cf. Ref. 33). The predicted interaction energy between α-84 and β-84 in PC is 64 cm^{-1} [32], and this value is also applicable to the case of APC because the primary structure of APC is very similar to those of PC [34]. This also suggests that the tertiary structure of APC, and thus the arrangement of chromophores, is similar to that of PC. This interaction energy gives rise to band-splitting of the magnitude of 120 cm^{-1} [32]. This is very close to the band-splitting between the F_{660} and F_{666} (136 cm^{-1}). However, the observed lifetimes of these two components are longer than the expectable rate constants for energy migration between two transitions of the exciton bands. Thus, these two components can be assumed to be the fluorescence from the lowest excited states of two different chromophores. In general, the energy level of the β-84 chromophore with a linker polypeptide is lower than that of the β-84 without a linker polypeptide, as is seen in the case of PC [2,32]. Therefore, the origins of the F_{660} and F_{666} are assigned to be the β-84 without the 10 kDa linker polypeptide in the trimer and the β-84 with the 10 kDa linker polypeptide in the different trimer, respectively.

The observed rise and decay curves of the component bands in APC-core complex did not support the idea for a sequential energy flow among the components. A flow from the F_{666} to F_{673} is not possible. Judged from the kinetics, the energy flow in the order of $F_{660} \rightarrow F_{673} \rightarrow F_{680} \rightarrow F_{686}$ and $F_{666} \rightarrow F_{680} \rightarrow F_{686}$ is possible. However, the F_{680} and F_{686} are independent terminal emitters of phycobilisome, as is clearly shown in our previous study [12]. Furthermore, the rise of the F_{686} corresponds well to the decay of the F_{673}, compared with the rise of the F_{680}. Thus, we propose the following energy flow in the APC-core; $F_{660} \rightarrow F_{673} \rightarrow F_{683}$ and $F_{666} \rightarrow F_{680}$. The former corresponds to the flow from β-84 without a linker polypeptide to anchor polypeptide through 18.3 kDa polypeptide, and the latter from β-84 with a linker polypeptide to α-APB. In both cases, the energy donor to the F_{660} and F_{666} is assumed to be the β-84 chromophores in the trimer next to the core complex. A recent proposal [21] that the β-155 might be an energy donor to core-complex is not supported by our analysis.

Molecular assembly in APC-core complex

There are two models for the molecular assembly of APC-core complex; one is proposed by Glazer's group [11,35] and the other, by Anderson and Eiserling [36]. The critical difference in these two models is the location of the trimer with 10 kDa linker polypeptide. The basal dodecamer in the core complex consists of four trimers; (1) $(\alpha\beta)_3$, (2) $\alpha_2\beta_2 \cdot 18.3$ kDa $\cdot$ anchor, (3) α-APB $\cdot \alpha_2\beta_3 \cdot 10$ kDa and (4) $(\alpha\beta)_3$ 10 kDa. According to Glazer's model, these four are arranged in the order of (1,2,3,4). Contrary to this, Anderson and Eiserling proposed the order of (4,1,2,3). Our kinetical data indicate the energy flows from β-84 without a linker to the 'anchor' polypeptide through the 18.3 kDa polypeptide, and from β-84 with linker to α-APB. This scheme is compatible with the model proposed by Glazer et al. [11,35]. The energy level of the final emitter in the APC trimer with a linker is lower than that of the trimer without linker, thus we can expect a long lifetime component when Anderson's model is correct. We could not find such a long lifetime component in the F_{666}, thus according with the kinetics, our data support the model for molecular assembly of APC-core complex proposed by Glazer et al.

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