Deconvolution of C-Phycocyanin β-84 and β-155 Chromophore Absorption and Fluorescence Spectra of Cyanobacterium *Mastigocladus laminosus*

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ABSTRACT Absorption and fluorescence spectra of the C-phycocyanin β-subunit were quantitatively deconvoluted into component spectra of the β-84 and β-155 chromophores. The deconvolution procedure was based on a theoretical treatment of polarization properties. Four kinds of spectra (absorption, emission, emission polarization, and excitation polarization) measured on C-phycocyanin isolated from the cyanobacterium *Mastigocladus laminosus* were used as the experimental data set. Without any assumption of spectral shape, the absorption and fluorescence spectra of both chromophores were unambiguously resolved and their fluorescence quantum yields were evaluated. By combining the spectra of the α-subunit, independently measured, with the resolved spectra of the β-subunit, the fluorescence and fluorescence polarization spectra and the fluorescence quantum yield of the monomer were estimated; they agree with experimental values to within an acceptable error. Further, the matrix of energy transfer rates in the monomer was estimated; it gave a significantly different result (by up to 40%) from previously estimated ones.

INTRODUCTION

C-phycocyanin (C-PC) is one of biliproteins found in cyanobacteria, and it is one of the constituents of phycobilisomes, which funnel the excitation energy to chlorophyll *a* of photosystem II in thylakoid membranes. The C-PC is frequently used for experimental and theoretical analysis of energy transfer processes because its crystal structure has been determined precisely (Schirmer et al., 1985, 1987; Dürring et al., 1992). C-PC contains three kinds of chromophores, one binding to the α-subunit and two binding to the β-subunit of the monomer. The major functional unit of C-PC is a trimer consisting of three monomers. The chromophores are designated by the position of the cystein residues in the amino acid sequence to which they are covalently bound, i.e., α-84, β-84, and β-155. These chromophores are chemically identical, but their optical properties in the protein matrix differ because of their interaction with amino acids and/or the effect of electric/magnetic fields. Therefore, for the analysis of energy transfer it is crucial to examine the optical properties of individual chromophores.

There are several reports devoted to extraction of the optical properties of individual chromophores of C-PC isolated from the cyanobacterium *Mastigocladus laminosus* (Mimuro et al., 1986; Sauer et al., 1987; Fischer et al., 1988; Debreczeny et al., 1993.). So far, the deconvolution procedures were qualitative rather than quantitative. In our work, we introduce a new approach to the spectrum deconvolution procedure. On the basis of formulae derived by Demidov et al. (1994a, b) and experimental data reported by Mimuro et al. (1986), we make a new attempt to conduct a quantitative deconvolution of the absorption and fluorescence spectra of the C-PC β-subunits. Our study is based on the polarization properties of the β-subunit. Using the resolved spectra, we further calculate the fluorescence and polarization spectra of the monomer and estimate the fluorescence quantum yield and the matrix elements, which describe the rates of energy transfer among chromophores in the monomer.

THEORETICAL TREATMENT

In the following calculation, we consider that the C-PC β-subunit is a double-chromophore complex consisting of β-84 and β-155 chromophores with a mutual angle of $\theta = 47°$ between their transition dipole moments[1] (Schirmer et al., 1987). Further, we assume that each individual chromophore has "linear" absorption and emission transition dipole moments parallel to each other for their S_0–S_1 and S_1–S_0 transitions, respectively.

Demidov (1994a, b) has shown that the degree of fluorescence polarization in such complexes randomly distributed in space is equal to

$$P = \frac{3\cos^2(\theta) - 1 + 2A}{3 + \cos^2(\theta) + 4A},$$
$$A = \frac{\tau_2^{-1} + k_{21} + (\tau_1^{-1} + k_{12})\alpha\gamma}{\alpha k_{21} + \gamma k_{12}}. \quad (1)$$

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Abbreviations used: C-PC, C-phycocyanin; α(β)-84 or β-155, the chromophore that binds to the 84th of 155 cystein residues of α(β)-subunit of C-PC; PCB, phycocyanobilin.

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[1] The value $\theta = 47°$ is very favorable for our further calculations. Demidov (1994a, Fig. 1) has shown that degree of fluorescence polarization of the C-PC β-subunit is quite sensitive to the angle θ in this region.

Here k_{12} and k_{21} are the rates of energy transfer from the first (β-84) to the second (β-155) chromophores and vice versa; τ_1 and τ_2 are intrinsic lifetimes of chromophore de-excitation; $\alpha = \sigma_2(\lambda_{exc})/\sigma_1(\lambda_{exc})$; $\gamma = (\tau_1\eta_2 f_2(\lambda_{fl}))/(\tau_2\eta_1 f_1(\lambda_{fl}))$; σ_1 and σ_2 are the chromophore absorption efficiencies; λ_{exc} is the wavelength of excitation; f_1 and f_2 are the normalized spectra of the chromophore fluorescence ($\int f(\lambda)\, d\lambda = 1$); λ_{fl} is the wavelength of fluorescence detection; η_1 and η_2 are the fluorescence quantum yields. The degree of fluorescence polarization is defined as $P = (I_\parallel - I_\perp)/(I_\parallel + I_\perp)$, where $I_\parallel$ and $I_\perp$ are the fluorescence components parallel and perpendicular to the polarization of the incident light, respectively. The dependence of the parameter A on the excitation and detection wavelengths can be found from the spectrum of fluorescence polarization, as follows from Eq. 1

$$A = \frac{(3 - P)\cos^2(\theta) - 3P - 1}{4P - 2}. \tag{2}$$

Based on the two parameters in Eq. 1, that is, α and γ, which represent the ratios of absorption and fluorescence intensities of the β-84 and β-155 chromophores, respectively, we can quantitatively deconvolute the spectra of individual chromophores from their overlapped spectra.

Deconvolution of absorption spectra

In the β-subunits, the rates of energy exchange, k_{12} and k_{21}, are much greater than the rates of intrinsic excitation decay, τ_1^{-1} and τ_2^{-1} (Demidov and Borisov, 1993; Sauer et al., 1988). This leads to a simplified formula for A

$$A = \frac{k_{21} + k_{12}\,\alpha\gamma}{\alpha k_{21} + \gamma k_{12}}. \tag{3}$$

Let us consider one specific wavelength of excitation, λ^0_{exc}—such a wavelength where both chromophores have equal absorption efficiencies: $\sigma_1(\lambda^0_{exc}) = \sigma_2(\lambda^0_{exc})$ and, thus, $\alpha = 1$. Under such a condition, Eq. 3 yields $A = 1$ for any values of k_{ij} and γ. Thus, if one excites the double-chromophore complexes at wavelength λ^0_{exc}, the resulting fluorescence polarization emission spectrum must be stable throughout the range of fluorescence wavelengths. In the case of the β-subunits, substituting $A = 1$ and $\theta = 47°$ in Eq. 1 provides $P = 0.325$. The latter value coincides with the experimental data by Mimuro et al. (1986) at $\lambda^0_{exc} \approx 618$ nm. Thus, we can draw a conclusion that at $\lambda \approx 618$ nm the absorption coefficients of the β-155 and β-84 chromophores are equal.

Obviously, the problem may be reversed. Consider, for example, that one has the task of estimating the angle between two chromophores, with no information about the energy transfer rates and absorption efficiencies of individual chromophores. This task, which at first glance looks intractable, can be solved. To do this, the experimenter has to find such a wavelength of fluorescence excitation that the degree of fluorescence polarization remains stable for any wavelength of fluorescence detection. Then it can be claimed that at the wavelength of excitation so determined the parameter α is equal to 1; being sure that $k_{ij} \gg \tau_{i,j}^{-1}$, it can then be claimed that $A = 1$. Thus, from knowledge of the latter value and the measured degree of polarization (P), it is possible to calculate the angle θ using Eq. 1.

Calculation of the whole spectra of the individual chromophores is more complicated. According to Förster (1948), the rate of energy migration from a donor D to an acceptor A is equal to

$$k_{DA} = \frac{C\chi^2\eta_D}{\tau_D R^6} S_{DA}. \tag{4}$$

In this equation, C is a constant, χ^2 is the orientation factor, η_D is the donor fluorescence quantum yield, τ_D is the donor intrinsic lifetime of excitation; R is the distance between the donor and acceptor, and S_{DA} is the overlap integral, which is equal to

$$S_{DA} = \int f_D(\nu)\, \sigma_A(\nu)\, \nu^{-4}\, d\nu. \tag{5}$$

Introducing new parameters $s = S_{21}/S_{12}$ and $f = f_2\tau_1/f_1\tau_2$, one can obtain from (1)

$$A = \frac{s + f\alpha}{s\alpha + f}. \tag{6}$$

Consequently, it follows from this equation that A and P are sensitive to the chromophore fluorescence and absorption spectra, but they are not dependent on the fluorescence quantum yields.

For calculation of the absorption spectra, we modify (6) relative to $\alpha(\lambda_{exc})$

$$\alpha(\lambda_{exc}) = \frac{\sigma_{\beta155}(\lambda_{exc})}{\sigma_{\beta84}(\lambda_{exc})} = \frac{(s/f) - A}{(s/f)A - 1} \simeq \frac{1}{A(\lambda_{exc})}. \tag{7}$$

The latter simplification follows from the inequalities $(S_{21}/S_{12}) > 1$ and $(f_2/f_1) < 1$, thus, $(s/f) \gg 1, A$. The wavelength of fluorescence detection λ_{fl} was chosen equal to 670 nm, as in the work by Mimuro et al. (1986). In our calculations, we used the values $\tau_1 = \tau_2 = 1.5$ ns as reported by Sauer et al. (1987, 1988). The proportionality $\alpha(\lambda_{exc}) \approx 1/A(\lambda_{exc})$ is a rough assumption, and for a precise solution it is preferable to use the complete form of Eq. 7, but in this case it should be linked with Eqs. 13 and 14 for f_1 and f_2. The last approach is significantly more complicated, while the deconvoluted spectra calculated by both methods, at the same positions of the absorption peaks, differ in magnitude by only about 10–15%. Thus, the final system of equations can be adopted in the form

$$\begin{aligned} \frac{\sigma_{\beta155}(\lambda_{exc})}{\sigma_{\beta84}(\lambda_{exc})} &= \frac{1}{A(\lambda_{exc})} \\ \sigma_{\beta155}(\lambda_{exc}) + \sigma_{\beta84}(\lambda_{exc}) &= \sigma_{\beta\text{-sub.}}(\lambda_{exc}). \end{aligned} \tag{8}$$

Here $\sigma_{\beta\text{-sub}}$ is the absorption by β-subunits. One can find spectra of the β-subunits excitation $P(\lambda_{exc})$ and $A(\lambda_{exc})$ in Fig. 1, and in Fig. 2 the deconvoluted absorption spectra of the β-84 and β-155 chromophores calculated using (8).

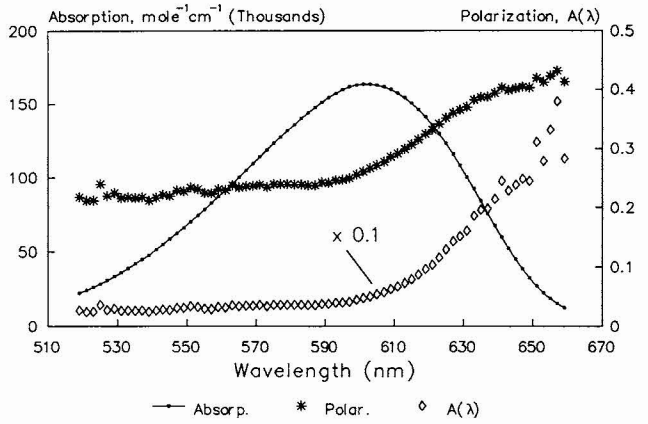

FIGURE 1 The β-subunit spectra for absorption and fluorescence polarization excitation measured by Mimuro et al. (1986), and the calculated dependence of the parameter A on the wavelength of excitation λ_{exc} (Eq. 2). The wavelength of fluorescence detection is λ_{fl} = 670 nm.

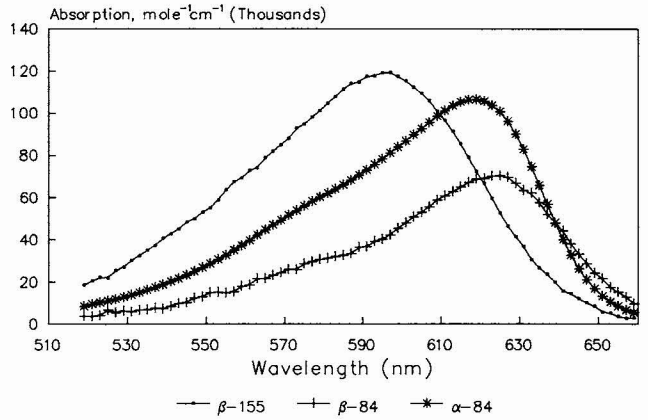

FIGURE 2 Absorption spectra of β-84, β-155, and α-84 chromophores. The first two are deconvoluted spectra.

As expected, at the wavelength $\lambda \approx 618$ nm the absorption efficiencies of both β-chromophores are equal. Our calculations provide the following positions and values of β-84 and β-155 absorption peaks: β-84 − $\lambda_{max} \approx 625$ nm, $\sigma_{\beta\text{-}84} \approx 70{,}500$ mole^{-1} cm^{-1}; β-155 − $\lambda_{max} \approx 596$ nm, $\sigma_{\beta\text{-}155} \approx 119{,}300$ mole^{-1} cm^{-1}. In Fig. 2, the α-84 chromophore absorption spectrum is also presented to complete the information about the absorption spectra of the C-PC chromophores. The absorption spectrum of the α-84 chromophores has a maximum at λ_{max} = 618 nm, with $\sigma_{\alpha\text{-}84}$ = 106,000 mole^{-1} cm^{-1} (Mimuro et al., 1986).

Deconvolution of fluorescence spectra

The presence of the ratio $\gamma = (\eta_2\tau_1 f_2(\lambda_{fl}))/(\eta_1\tau_2 f_1(\lambda_{fl}))$ in the parameter A (see Eq. 1) allows one to conduct the quantitative deconvolution of fluorescence spectra. For this purpose, one has to fix the excitation wavelength λ_{exc} and to measure the isotropic and polarized fluorescence emission spectra. The procedure of fluorescence spectra deconvolution entails the same idea as for the deconvolution of absorption spectra, but it is more complicated for at least three reasons. 1) The overlapped fluorescence spectra are not simply sums of the fluorescence spectra of the two chromophores. 2) The fluorescence spectra are measured in arbitrary units. 3) Generally, the overlapped fluorescence spectrum should depend on the chromophore quantum yields and energy equilibrium between chromophores.

The strategy for deconvolution of the fluorescence spectra was as follows: 1) solution of the system of balance equations for the distribution of energy between chromophores at some particular wavelength of excitation; and 2) derivation of a formula for determination of the β-subunit fluorescence spectrum versus the individual spectra of the chromophores—the latter formula could be considered to be equivalent to the second formula in system (8)—then 3) calculation of $f_2(\lambda_{exc})/f_1(\lambda_{exc})$ from parameter A (see Eq. 6). The latter parameter must be determined from Eq. 2. Because the fluorescence is measured in relative units, the deconvoluted spectra must be calibrated; in our case, the calibration procedure involves $\int f(\lambda)\, d\lambda = 1$.

The fluorescence of double-chromophore complexes like β-subunits is dependent on the energy balance between the excited chromophores. This balance is described by a system of two equations:

$$\begin{aligned} -n_1(\tau_1^{-1} + k_{12}) + n_2 k_{21} + C_1\sigma_1 I_0 &= 0 \\ n_1 k_{12} - n_2(\tau_2^{-1} + k_{21}) + C_1\sigma_2 I_0 &= 0, \end{aligned} \tag{9}$$

where n_1 and n_2 are the densities of excited chromophores of the first and second spectral types (β-84 and β-155, respectively), C_1 is a constant, and I_0 is the intensity of incident light. The solution of this system provides the fluorescence intensity emitted from complexes at the wavelength λ_{fl}:

$$\begin{aligned} I_{fl} &= C_2 I_0(\eta_1\tau_1^{-1} f_1(\lambda_{fl}) n_1 + n_2\tau_2^{-1} f_2(\lambda_{fl}) n_2) \\ &= C_2 I_0 \frac{\mathcal{A}}{(\tau_2^{-1} + k_{21})(\tau_1^{-1} + k_{12}) - k_{12}k_{21}}; \end{aligned} \tag{10}$$

where

$$\begin{aligned} \mathcal{A} = (\eta_1\tau_1^{-1} f_1(\lambda_{fl}))((\tau_2^{-1} + k_{21})\sigma_1 + k_{21}\sigma_2) \\ + \eta_2\tau_2^{-1} f_2(\lambda_{fl})(k_{12}\sigma_1 + (\tau_1^{-1} + k_{12})\sigma_2). \end{aligned}$$

Here C_2 is a constant. According to Eq. 4, $k_{ij} \sim \eta_i S_{ij}$; then if $k_{12,21} \gg \tau_{1,2}^{-1}$, Eq. 10 can be simplified:

$$I_{fl} = C_2 I_0 \frac{\eta_1\eta_2(\sigma_1 + \sigma_2)}{\eta_1 S_{12} + \eta_2 S_{21}} (\tau_1^{-1} f_1(\lambda_{fl}) S_{21} + \tau_2^{-1} f_2(\lambda_{fl}) S_{12}), \tag{11}$$

i.e., the spectral shape of the β-subunit fluorescence is linearly proportional to the sum: $\tau_1^{-1} f_1(\lambda_{fl}) S_{21} + \tau_2^{-1} f_2(\lambda_{fl}) S_{12}$, and does not depend on the fluorescence quantum yields. At first glance, such behavior might seem strange, but this fact can be simply explained on a physical basis as follows: the intensity of fluorescence emitted from some chromophores is linearly proportional to the density of their excited states and their fluorescence quantum yield, whereas this excitation

density is inversely proportional to the rate of energy escape. Because the energy migration rate is proportional to the fluorescence quantum yield ($k \sim \eta$), then the fluorescence intensity and spectral shape are independent of quantum yield.

Now we come to the conclusion that in complexes with the dependence $k \sim \eta S$ the shape of the fluorescence spectrum does not carry information about the fluorescence quantum yields of the chromophores! Thus, for the estimation of fluorescence quantum yields it is necessary either to measure independently k_{12} and k_{21} or to use some theoretical evaluation of radiative lifetimes (Birks, 1970). The radiative lifetime is associated with excitation deactivation via the emission of fluorescence quanta.

Equation 11 can be modified by using the parameters $s = S_{21}/S_{12}$ and $f = f_2\tau_1/f_1\tau_2$:

$$I_{fl}(\lambda_{fl}) = \left[C_2 I_0 \frac{\eta_1 \eta_2(\sigma_1 + \sigma_2)}{\eta_1 + \eta_2 s} \tau_2^{-1}\right] f_2(\lambda_{fl})\left(\frac{s}{f} + 1\right). \quad (12)$$

then involving Eq. 6 one can obtain

$$I_{fl}(\lambda_{fl}) = F f_2(\lambda_{fl})\left(1 + \frac{\alpha - A(\lambda_{fl})}{\alpha A(\lambda_{fl}) - 1}\right)$$

or

$$f_2(\lambda_{fl}) = F^{-1} I_{fl}(\lambda_{fl})\left(1 + \frac{\alpha - A(\lambda_{fl})}{\alpha A(\lambda_{fl}) - 1}\right)^{-1}. \quad (13)$$

The parameter F^{-1} can be estimated from the calibration procedure $\int f_2(\lambda)\, d\lambda = 1$:

$$F^{-1} = \left(\int I_{fl}(\lambda_{fl})\left(1 + \frac{\alpha - A(\lambda_{fl})}{\alpha A(\lambda_{fl}) - 1}\right)^{-1} d\lambda_{fl}\right)^{-1}.$$

Thus, calculation of $f_2(\lambda_{fl})$ involves the following: 1) measurement of the β-subunit fluorescence spectrum $I_{fl}(\lambda_{fl})$ at some excitation wavelength λ_{exc}; 2) measurement of the fluorescence polarization emission spectrum $P(\lambda_{fl})$ at the same wavelength of excitation; 3) calculation of $A(\lambda_{fl})$ using Eq. 2; 4) calculation of $f_2(\lambda_{fl})$ using Eq. 13 and the calibration procedure $\int f_2(\lambda)\, d\lambda = 1$.

The analogous procedure for the $f_1(\lambda_{fl})$ calculation provides

$$f_1(\lambda_{fl}) = \tilde{F}^{-1} I_{fl}(\lambda_{fl})\left(1 + \frac{\alpha A(\lambda_{fl}) - 1}{\alpha - A(\lambda_{fl})}\right)^{-1}$$
$$\tilde{F}^{-1} = \left(\int I_{fl}(\lambda_{fl})\left(1 + \frac{\alpha A(\lambda_{fl}) - 1}{\alpha - A(\lambda_{fl})}\right)^{-1} d\lambda_{fl}\right)^{-1}. \quad (14)$$

The fluorescence and fluorescence polarization emission spectra of the β-subunits and the deconvoluted spectra of the β-84 and β-155 chromophores are presented in Figs. 3 and 4, respectively. We found that the deconvoluted spectra have maxima at the wavelengths $\lambda_{max} \approx 625$ and 645 nm for the β-155 and β-84 chromophores, respectively. The fluorescence spectrum of the α-84 chromophores (α-subunits) is also presented in Fig. 4. It has a maximum at the wavelength $\lambda_{max} \approx 639$ nm.

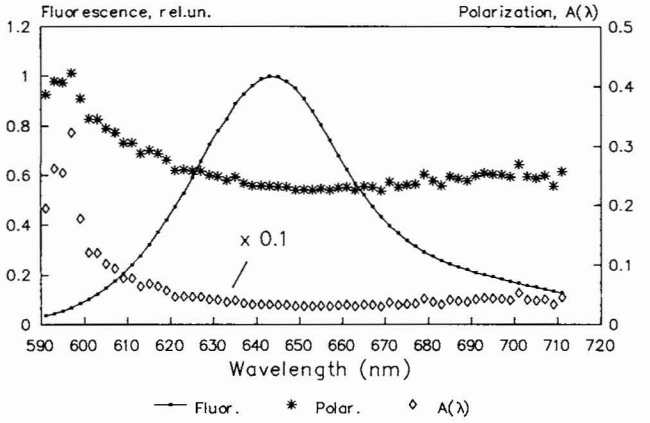

FIGURE 3 The β-subunit spectra for fluorescence and fluorescence polarization emission measured by Mimuro et al. (1986), and the calculated dependence of the parameter A on the wavelength of emission λ_{fl} (Eq. 2). The wavelength of fluorescence excitation is $\lambda_{exc} = 550$ nm.

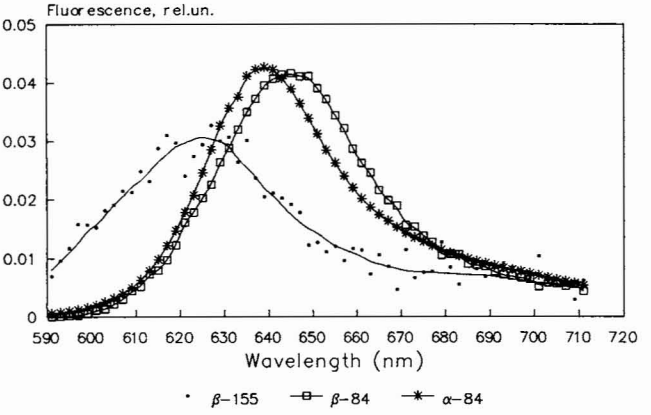

FIGURE 4 Normalized fluorescence spectra of the β-84, β-155, and α-84 chromophores: $\int f(\lambda)\, d\lambda = 1$. The first two are deconvoluted spectra. Points represent the result of calculations. Lines are Harvard Graphics approximations.

EXTENSION OF ANALYSIS

Using the above results, we extended our analysis to calculation of a few optical properties and derivation of parameters related to energy transfer processes in C-PC.

Evaluation of fluorescence quantum yields

By definition, the fluorescence quantum yield is equal to the ratio $\eta = \tau/\tau_{rad}$, where τ is the fluorescence decay time and τ_{rad} is the radiative lifetime. The radiative lifetime is proportional to the integral of the absorption spectrum (Birks, 1970):

$$\frac{1}{\tau_{rad}} = C_4 C_\nu \int \frac{\sigma(\nu)\, d\nu}{\nu} = C_5 C_\lambda \int \frac{\sigma(\lambda)\, d\lambda}{\lambda}, \quad \nu \sim \lambda^{-1},$$

where

$$C_\nu = \frac{\int f(\nu)\, d\nu}{\int f(\nu)\nu^{-3}\, d\nu}, \qquad C_\lambda = \frac{\int f(\lambda)\lambda^{-2}\, d\lambda}{\int f(\lambda)\lambda\, d\lambda}.$$

The constants C_4 and C_5 comprise only universal constants. The radiative lifetimes τ_{rad} of the respective chromophores were estimated from their deconvoluted spectra (Figs. 1 and 2). The relative quantum yields were obtained, with the assumption that $\tau_{\beta\text{-}84} = \tau_{\beta\text{-}155} = \tau_{\alpha\text{-}84} = 1.5$ ns (Sauer et al., 1987), and were found to be

$$\frac{\eta_{\beta 155}}{\eta_{\alpha 84}} = \frac{\tau_{rad}^{\alpha 84}}{\tau_{rad}^{\beta 155}} \simeq 1.33, \qquad \frac{\eta_{\beta 84}}{\eta_{\alpha 84}} = \frac{\tau_{rad}^{\alpha 84}}{\tau_{rad}^{\beta 84}} \simeq 0.58.$$

Thus, if $\eta_{\alpha\text{-}84}$ is equal to 0.72 (Sauer et al., 1987), then $\eta_{\beta\text{-}84} = 0.42$ and $\eta_{\beta\text{-}155} = 0.96$. Sauer et al. (1987) determined the values $\eta_{\beta\text{-}84} = 0.48$ and $\eta_{\beta\text{-}155} = 0.72$.

It is necessary to note that, contrary to our previous calculations, the fluorescence quantum yield is sensitive to the values of the fluorescence lifetimes τ. We can check the calculated fluorescence quantum yields by using the experimental data by Mimuro et al. (1986). In their work, they estimated the ratio of β- and α-subunit quantum yields: $\eta_{\beta\text{-sub}}/\eta_{\alpha\text{-}84} = 0.65$. By a routine calculation procedure, this ratio is equal to

$$\frac{\eta_{\beta\text{-sub.}}}{\eta_{\alpha 84}} = \frac{\int I_{fl}^{\beta\text{-sub.}}(\lambda_{fl})\, d\lambda_{fl}}{\int I_{fl}^{\alpha 84}(\lambda_{fl})\, d\lambda_{fl}} \frac{\sigma_{\alpha 84}}{(\sigma_{\beta 84} + \sigma_{\beta 155})}. \qquad (15)$$

Using Eq. 11, one can then obtain

$$\frac{\eta_{\beta\text{-sub.}}}{\eta_{\alpha 84}} = \frac{1}{\eta_{\alpha 84}}\left(\eta_{\beta 84}\frac{k_{21}}{k_{12}+k_{21}} + \eta_{\beta 155}\frac{k_{12}}{k_{12}+k_{21}}\right) = \frac{\eta_{\beta 155}}{\eta_{\alpha 84}}\frac{1+s}{1+s(\eta_{\beta 155}/\eta_{\alpha 84})}. \qquad (16)$$

After substituting in Eq. 16, the values of $s = S_{21}/S_{12}$ (values S_{ij}, see below) and the values $\eta_{\beta\text{-}84} = 0.42$ and $\eta_{\beta\text{-}155} = 0.96$ found above, we obtain $\eta_{\beta\text{-sub}}/\eta_{\alpha\text{-}84} = 0.72$. This value differs by 10% from those measured by Mimuro et al. (1986). Analogous calculations based on the data presented by Sauer et al. (1987) provide $\eta_{\beta\text{-sub}}/\eta_{\alpha\text{-}84} = 0.73$. The discrepancy between our calculations and the experimental data is very small, within the limit that the current evaluation is sensitive to the ratio of the fluorescence decay times of the free chromophores, which are assumed to be equal (1.5 ns).

Calculation of spectral overlap integrals and energy migration rates

In the theory of inductive resonance energy transfer (Förster, 1948), the energy migration rate (4) is proportional to the overlap integral of the donor fluorescence and acceptor absorption (5). We calculated the α- and β-chromophore overlap integrals normalized to the overlap integral of the α-chromophores $S_{3,3} = S_{\alpha\text{-}84,\alpha\text{-}84}$:

$$S_{ij} = \begin{matrix} 0.71 & 0.42 & 0.81 \\ 0.95 & 1 & 1.33 \\ 0.85 & 0.54 & 1 \end{matrix}.$$

Here the position of the indices "i" and "j" are assigned to the donor fluorescence and acceptor absorption spectra, respectively, whereas the index values i and $j = 1$, 2, and 3 are assigned to the β-84, β-155, and α-84 chromophores, respectively. The above data differ slightly from those calculated by Sauer et al. (1987), whereas the β-chromophore quantum yields differ more significantly from those used by Sauer et al. (1987, 1988).

The major parameters used in analysis of the energy migration processes in C-phycocyanin aggregates are the energy transfer rates between chromophores of different types. In the works by Sauer et al. (1988) and Demidov and Borisov (1993), these rates were calculated on the basis of spectral data provided by Sauer et al. (1987, 1988). Taking the current calculations into account, we can offer the following matrix for converting the "old" data produced by Sauer et al. (1988) and Demidov and Borisov (1993) to the "new" ones:

$$M_{ij} = \begin{matrix} 0.85 & 1.09 & 0.74 \\ 1.25 & 1.69 & 1.15 \\ 1.12 & 1.59 & 1 \end{matrix}.$$

Thus, the new rates k_{ij}^{new} are given by $k_{ij}^{new} = M_{ij}k_{ij}^{old}$. For example, the updated rates of energy transfer in the C-PC monomer, based on the data obtained by Demidov and Borisov (1993), are equal to

$$k_{ij}^{new}\,[ns^{-1}] = \begin{matrix} 0 & 8.22 & 7.77 \\ 42.6 & 0 & 2.35 \\ 13.9 & 0.71 & 0 \end{matrix},$$

whereas the old data (Demidov and Borisov, 1993) were equal to

$$k_{ij}^{old}\,[ns^{-1}] = \begin{matrix} 0 & 7.54 & 10.5 \\ 34.1 & 0 & 2.04 \\ 12.4 & 0.445 & 0 \end{matrix}.$$

We can check the reliability of the data so obtained by their application to C-PC monomers, comparing the calculated spectra with those measured by Mimuro et al. (1986).

Absorption and fluorescence spectra of the C-PC monomer

Each C-PC monomer consists of one α-subunit and one β-subunit. We found that the monomer absorption spectrum is equal to the sum of the α-84, β-84, and β-155 absorption spectra as presented in Fig. 5. It is clear that the calculated absorption spectrum is a good match for the measured one. The calculation of the fluorescence spectrum was based on the system of balance equations similar to system (9), but now with three equations instead of two. The calculated fluorescence spectrum is presented in Fig. 6. It also well matches the measured one. In these calculations, we used the matrix of energy migration rates k_{ij}^{new} presented above. We investigated the influence of chromophore fluorescence quantum yields on the shape of fluorescence and fluorescence polarization spectra of the monomer and found that they are not sensitive to the fluorescence quantum yields when calculations are based on S_{ij} instead of k_{ij}. This result agrees with the

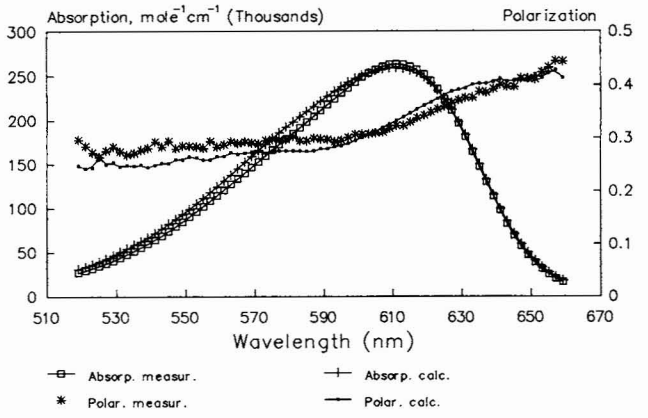

FIGURE 5 Absorption and fluorescence polarization excitation spectra of C-PC monomers: (□, *) Measured; (+, •) calculated. The wavelength of fluorescence detection is λ_{fl} = 670 nm.

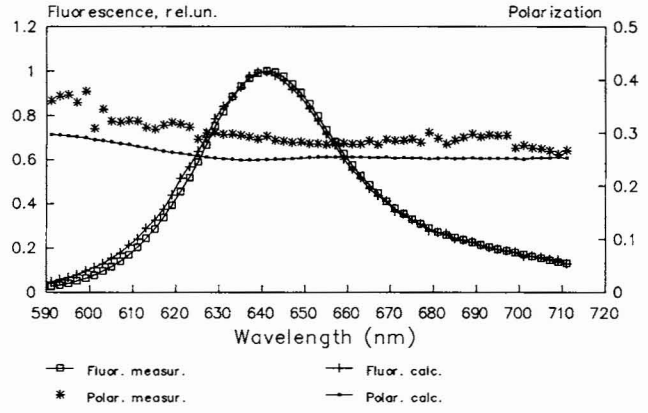

FIGURE 6 Fluorescence and fluorescence polarization emission spectra of C-PC monomers: (□, *) Measured; (+, •) calculated. The wavelength of fluorescence excitation is λ_{exc} = 550 nm.

analysis made for a β-subunit as shown in Deconvolution of Fluorescence Spectra.

Fluorescence polarization spectra of the C-PC monomer

For calculation of fluorescence polarization excitation and emission spectra, we have to use another formula for their determination, because the monomer is a triple-chromophore complex (Demidov, 1994b):

$$P = \frac{3(q_{12}\cos^2\theta_{12} + q_{13}\cos^2\theta_{13} + q_{23}\cos^2\theta_{23}) - 1 + 2A^\circ}{3 + (q_{12}\cos^2\theta_{12} + q_{13}\cos^2\theta_{13} + q_{23}\cos^2\theta_{23}) + 4A^\circ},$$

$$q_{12} + q_{13} + q_{23} = 1. \quad (17)$$

This equation is more complicated than (1), but again the parameters q_{ij} and A^0 are dependent on the same spectroscopic data as in Eq. 1: intrinsic lifetimes τ_k (k = 1, 2, 3), relative absorption $\alpha_{k1} = \sigma_k(\lambda_{exc})/\sigma_1(\lambda_{exc})$ and fluorescence $\gamma_{k1} = (\tau_1\eta_k f_k(\lambda_{fl}))/(\tau_k\eta_1 f_1(\lambda_{fl}))$ parameters (k = 1, 2, 3), and rates of energy transfer k_{km}. The angles θ_{ij} are those between chromophore transition dipole moments. These angles have the values $\theta_{12} = \theta_{\beta\text{-}84,\beta\text{-}155} = 47°$, $\theta_{13} = \theta_{\beta\text{-}84,\alpha\text{-}84} = 16°$, and $\theta_{23} = \theta_{\beta\text{-}155,\alpha\text{-}84} = 62°$ (Schirmer et al., 1987). In the work by Demidov (1994b), it was shown that Eq. 1 is a particular case of Eq. 17.

The fluorescence polarization excitation and emission spectra calculated on the basis of Eq. 17 are presented in Figs. 5 and 6, respectively. These spectra well match the spectra measured by Mimuro et al. (1986) and, again, they are not sensitive to fluorescence quantum yields when calculations are based on the S parameters instead of the rates k.

Evaluation of the fluorescence quantum yield of the C-PC monomer relative to the quantum yield of the α-subunits provides

$$\frac{\eta_{mon.}}{\eta_{\alpha 84}} = \frac{\int I_{fl}^{mon.}(\lambda_{fl})\,d\lambda_{fl}}{\int I_{fl}^{\alpha 84}(\lambda_{fl})\,d\lambda_{fl}} \frac{\sigma_{\alpha 84}}{(\sigma_{\alpha 84} + \sigma_{\beta 84} + \sigma_{\beta 155})} = 0.8.$$

Here we used the same method as described above, but applied to a three-chromophore complex like the C-PC monomer. The calculated ratio fits the value measured by Mimuro et al. (1986): $\eta_{mon}/\eta_{\alpha\text{-}84} = 0.78$. The calculations based on the data by Sauer et al. (1988, 1987) yield $\eta_{mon}/\eta_{\alpha\text{-}84} = 0.84$.

DISCUSSION

The evaluation of deconvoluted spectra

For the estimation of optical properties of individual chromophores, we adopted a theoretical consideration of their polarization properties and did not make any assumptions about the shapes of the chromophore spectra. Hence, our method could be recognized as a quantitative method compared with the previous qualitative methods, which assumed that the shapes of either the absorption or fluorescence spectra of some chromophores are identical (Siebzehnrübl et al., 1987; Fischer et al., 1988; Sauer et al., 1988). Thus, the reliability of our method is higher, and it correctly provides deconvoluted spectra consistent with the vibrational structure reported by Mimuro et al. (1986). The earlier attempt (Demidov, 1994b) to accommodate the previously determined spectra of chromophores (Sauer et al., 1988) yielded a poorer match between the polarization spectra calculated and measured (Mimuro et al., 1986) than the match obtained in our current study.

Recently, Debreczeny et al. (1993) reported the deconvolution of C-PC monomer spectra based on the steady-state absorption and time-resolved fluorescence spectra of a wild type and mutant strains of *Synechococcus sp.* PCC 7002. Even if they adopted procedures different from ours, the results were essentially identical to ours, especially in 1) the order of the locations of the spectrum peaks for individual chromophores and their extinction coefficients, β-155, α-84, and β-84, and 2) the order of the fluorescence quantum yields, β-155, α-84, and β-84. The resolved locations were different from those in our study by 4–6 nm; however, this difference could be attributed to the difference in the species used for analysis, *Mastigocladus laminosus* and *Synechococcus sp.* PCC 7002.

Validity and applicability of the current analysis

Energy transfer processes in the C-PC monomer are basically treated by the Förster model based on a weak interaction between chromophores. In this case, the spectral shapes of individual chromophores are crucial for estimation of the energy transfer rates. The other parameters involved are the distances between the chromophores and their mutual orientations. We estimated a new matrix of transfer rates, and this estimation was based on the calculated shapes of spectral bands. In particular, it yields a higher rate of energy transfer between the β-155 and β-84 chromophores. The newly calculated rates will give us a more precise model for energy migration. However, our estimation provides rate constants that are faster to some extent than those estimated by others (Holzwarth et al., 1987; Gillbro et al., 1988; Xia et. al., 1991, Debreczeny et al., 1993).[2] This takes place even if the ratios of energy transfer rates for forward and backward energy flow were consistent. The C-PC monomer can be treated as a sum of two subunits and, thus, it was reasonable to expand our optical analysis to the C-PC monomers. Thus, it was found that the monomer fluorescence quantum yield, its fluorescence and absorption spectra, and the polarization properties predicted by our model agree well with the experimental data (Figs. 5 and 6). This result also confirms the credibility of the method used.

Our deconvolution method can be applied to other systems, for example, allophycocyanin having exciton interaction between chromophores (Maxson et al., 1988) and phycoerythrocyanin, for which the optical properties of the individual chromophores are still unknown (Dürring et al., 1991). Those will be subjects of another study.

CONCLUSION

We believe that our study of the spectroscopic features of the C-PC chromophores is more reliable and accurate than the previous estimations based on simple deconvolution procedures. This is the first and successful application of a new theory of fluorescence polarization in molecular complexes with energy transfer (Demidov, 1994a, b). We found that the locations and extinction coefficients of individual chromophores are: α-84 $-$ λ_{max} = 618 nm, σ = 106,000 mole^{-1} cm^{-1}; β-84 $-$ λ_{max} = 625 nm, σ = 70,500 mole^{-1} cm^{-1}; and β-155 $-$ λ_{max} = 596 nm, σ = 119,300 mole^{-1} cm^{-1}. The positions of the fluorescence peaks and fluorescence quantum yields are: α-84 $-$ λ_{max} = 639 nm, η = 0.72; β-84 $-$ λ_{max} = 645 nm, η = 0.42; and β-155 $-$ λ_{max} = 625 nm, η = 0.96. The complete set of spectra are shown in Figs. 2 and 4.

Our calculation shows that the spectra, both for absorption and fluorescence, of C-PC monomers can be described as a linear combination of α- and β-chromophore spectra. The calculated spectra are in good agreement with those obtained experimentally.

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[2] In particular, in the case of the C-PC monomer the fast component of fluorescence decay time measured in the referred works is about 50–57 ps. Our theoretical evaluation yields two fast components with decay times equal to 18 and 46 ps.