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Energy migration in allophycocyanin-B trimer with a linker polypeptide: analysis by the principal multi-component spectral estimation (PMSE) method**

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Abstract Energy migration processes in allophycocyanin-B trimer with a linker polypeptide were analyzed using the principal multi-component spectral estimation (PMSE) method, which does not require assumption of component number, decay function, or the spectral band shape. We determined the number of spectral components showing independent kinetic behavior by the eigenvalue of an auto-correlation matrix, and further the spectra of the components and their rise and decay curves. Two decay components were resolved at 20°C: one corresponded to the decay of one type of β-84 chromophore, and the other to the decay from the thermally equilibrated state between another type of β-84 chromophore and the α-allophycocyanin B chromophore. An additional slow decay process was resolved at −196°C. We also compared the component spectra obtained using the PMSE method with the decay-associated spectra obtained using the global analysis.

Key words: Allophycocyanin; Energy transfer; Photosynthesis; Picosecond spectroscopy; Spectral analysis; *Synechococcus elongatus*

1. Introduction

It is important that energy transfer processes in photosynthetic pigment systems are kinetically resolved to elucidate the transfer mechanism. Laser technology now allows time resolution to the femtosecond region for both transient absorption [1] and transient fluorescence [2] methods. There are several methods for the analysis of spectral components and their rise and decay, e.g. the global fit or decay-associated spectrum (DAS) [3]. We have used a deconvolution method to resolve the observed spectra into components [4–6]; but these analyses require certain assumptions. For example, DAS assumes the number of components and the decay function, usually an exponential decay, and our previous method assumed the number of components and the spectral band shape. To avoid the pitfalls of such assumptions, we sought an analytical method that does not require them. Several mathematical principles have been established for such a method, one of which, i.e. the principal multi- component spectral estimation (PMSE) method [7–9] is applied here to analyze energy transfer processes. The basic concept of this method is described elsewhere [10,11]. The present analysis focused on allophycocyanin (APC)-B trimers with a linker polypeptide [12].

APC-B trimer is one of four APC trimers found in the core complex of phycobilisomes [13,14]. In the case of *Synechococcus elongatus*, the APC-B trimer consists of two APC monomers, each containing α and β subunits, one special monomer containing α-allophycocyanin B (αAPB) and β subunit, and one colorless linker polypeptide [15]. The three-dimensional structure of the APC trimer is considered to be similar to that of the PC trimer, including the C_3 symmetry axis, since the primary structure of APC is similar to that of phycocyanin (PC) [16]. The α-84 chromophore in the PC trimer, which is covalently bound to the cysteine residue of the 84th amino acid of the α-subunit in one monomer, is located close to the β-84 chromophore in an adjacent monomer, and the center-to-center distance between the two chromophores is about 21 Å [17–19]. Since this geometry is analogous to the APC-B trimer, a strong interaction is expected between the α-84 and the β-84 chromophores, and the decay kinetics can be interpreted either by a simple transfer process or by coherent decay of the exciton state [20]. The degree of interaction can be discriminated by spectral resolution of the components and their kinetics. We applied the PMSE method to APC-B and compared the results with those obtained using the DAS method.

2. Materials and methods

Phycobiliproteins were isolated from the thermophilic cyanobacterium *Synechococcus elongatus* grown at 55°C, and an APC-B preparation was purified using three-step column chromatography (Hydroxylapatite (Bio-Rad), Sephacryl S-300 (Pharmacia), and DEAE-Sephacryl (Pharmacia)) in the presence of a protease inhibitor (PMSF, 1 mM) [21]. We detected α and β subunits, αAPB, and a linker polypeptide having a molecular mass of 8 kDa [15], in a molar ratio nearly equal

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**This paper is dedicated to Prof. H. Zuber, ETH, on the occasion of his retirement.

Abbreviations: αAPB, α subunit of allophycocyanin B; APC, allophycocyanin; DAS, decay-associated spectra; kDa, kilodaltons; PC, phycocyanin; PMSE, principal multi-component spectral estimation; PMSF, phenylmethylsulfonylfluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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to 2:3:1:1, which was estimated based on the HPLC elution pattern monitored by absorbance at 280 nm.

Time-resolved fluorescence spectra were measured as reported previously [4–6]. For low-temperature spectroscopy, 15% poly(ethylene glycol) 4000 was added to obtain homogenous ice, and samples in a 2-mm Lucite cuvette were immersed in liquid nitrogen. A commercially available computer program package (Component Spectral Separation) was purchased from System craft (Itami, Japan) and part of it was modified to fit an NEC PC-9801 Bp computer.

3. Results

Fig. 1A shows the time-resolved fluorescence spectra of the APC-B trimer with a linker polypeptide at 20°C. Upon excitation at 580 nm, fluorescence maximum was initially observed at 660 nm, with an apparent shoulder around 680 nm. A relative intensity around 680 nm increased with time, and became dominant after 58 ps, peaking at 681 nm. The spectrum became invariant after 200 ps with a shoulder at 660 nm, indicating equilibrium of the energy distribution among the pigments.

The PMSE method was applied to these time-resolved spectra. Seventy-two independent spectra, in the time range from −10 to 2330 ps, were given as an initial data set to estimate the number of components showing independent kinetic behavior, and the eigenvalue of the auto- correlation matrix was calculated [10,11]. The value (Fig. 1B) indicated two independent spectral components present in the series of time-resolved spectra. The resolved component spectra are shown in Fig. 1C; one shows the maximum at 661 nm with a tail in the long wavelength region (called C_1), and the other shows a dual-emissive component spectrum with the maxima at 662 and 681 nm (called C_2). The time-resolved spectra at any time could be simulated by a linear combination of these two component spectra, and their fractions for respective components were calculated by the least squares method. Fig. 1D shows the rise and decay curves of two spectral components. Lifetimes of the C_1 were 55 ps (97%) and 1700 ps (3%), and the C_2 arose with a time constant of 50 ps and decayed with a lifetime of 1700 ps. It was clearly shown that the long lifetime originated from the thermally equilibrated state, which was also supported by the decay analysis (data not shown).

At −196°C, the time-dependent changes in the time- resolved fluorescence spectra (Fig. 2A) were different from those observed at 20°C. Upon excitation at 580 nm, a 663-nm emission was dominant in the initial time region (up to 15 ps), and then it was replaced with the 683-nm emission. The 683-nm peak gradually shifted to the red, and finally reached 687 nm after 230 ps. No shoulder was observed in the shorter wavelength region of the maximum.

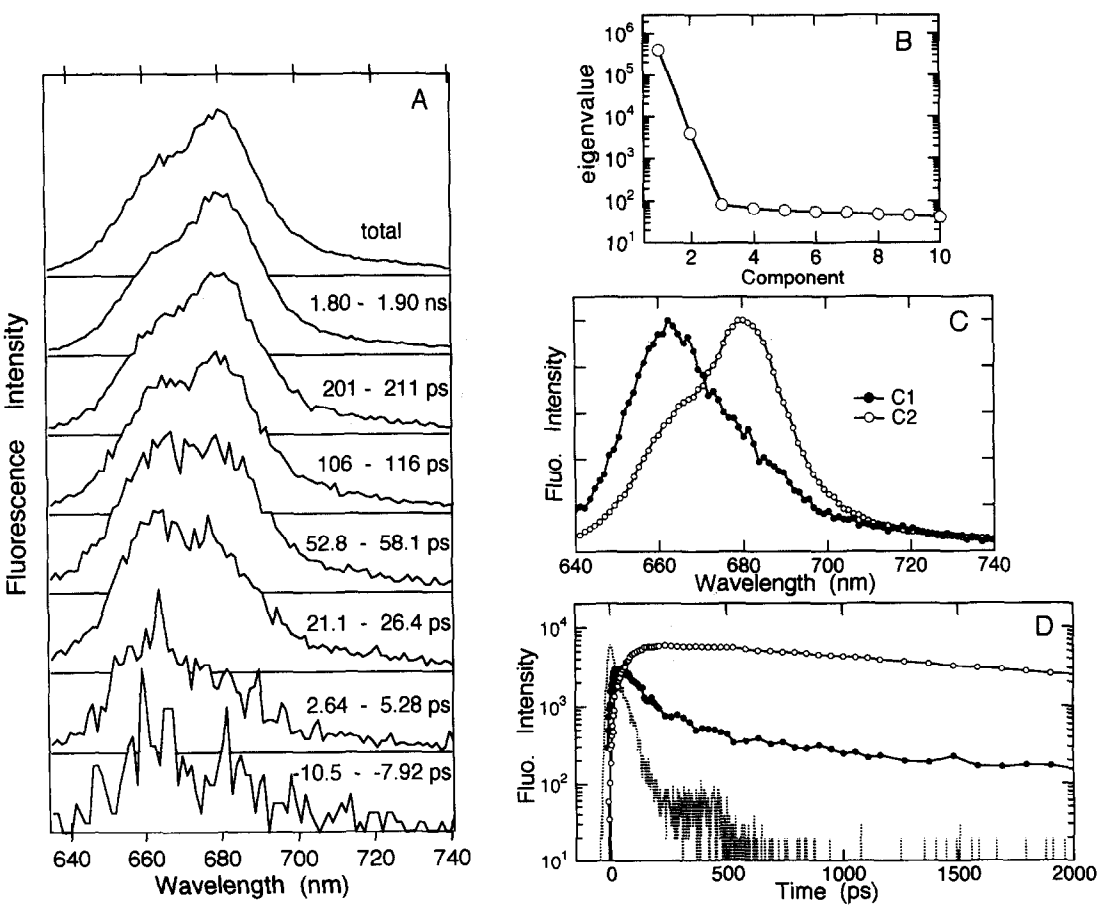


Fig. 1. Identification of components and their kinetics in APC-B with a linker polypeptide at 20°C. (A) Time-resolved fluorescence spectra; (B) the eigenvalue of an auto-correlation matrix; (C) component spectra (C_1 and C_2); and (D) the rise and decay curves of resolved components using the PMSE method. In C and D, corresponding components are represented by the same symbol; C_1 by open circles and C_2 by filled circles. In D, a dotted line indicates the profile of the excitation pulse. Samples were dissolved in a phosphate buffer (10 mM, pH 7.0) containing 20 mM NaCl and 0.05% NaN_3.

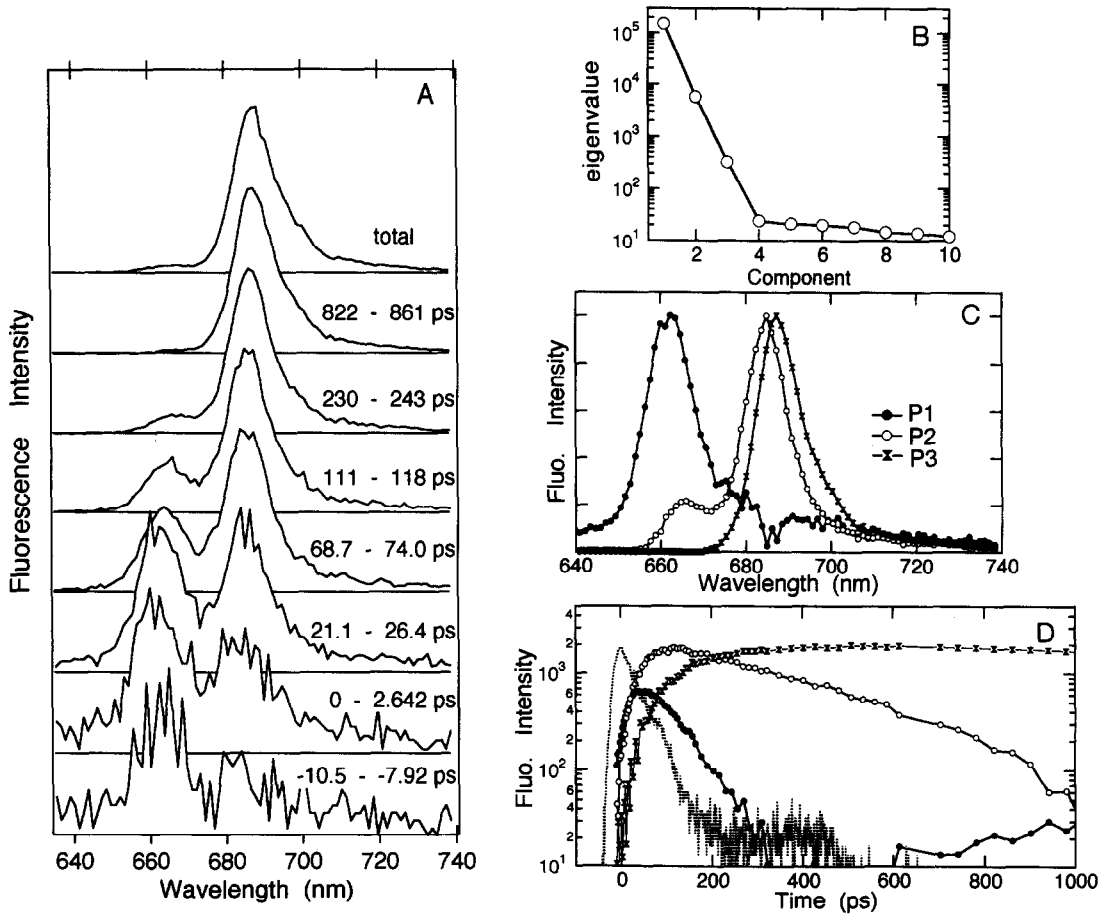


Fig. 2. Identification of components and their kinetics in APC-B with a linker polypeptide at −196°C. (A) Time-resolved fluorescence spectra; (B) the eigenvalue of an auto-correlation matrix; (C) component spectra (P_1, P_2, and P_3), and (D) the rise and decay curves of resolved components using the PMSE method. In C and D, corresponding components are represented by the same symbol; P_1 by open circles, P_2 by filled circles and P_3 by filled combined triangles. In D, a dotted line indicates the profile of the excitation pulse. Samples were dissolved in a phosphate buffer (10 mM, pH 7.0) containing 20 mM NaCl, 0.05% NaN_3 and 15% poly(ethylene glycol).

Fig. 2C shows component spectra obtained using the PMSE method. Three resolved components were noted at −196°C, based on the eigenvalue of an auto-correlation matrix (Fig. 2B). The three components, P_1, P_2, and P_3, showed the maxima at 662, 685, and 687 nm, respectively. The P_1 possessed a tail in the long wavelength region, similar to the C_1 found at 20°C. The P_2 showed a side band at 665 nm, similar to the C_2. The third component was unique in the low-temperature spectra, showing a single maximum at 687 nm. The rise and decay curves of these three components were rather simple (Fig. 2D); the progression of curves was observed in the order P_1, P_2, and P_3. The decay curve of P_1 was simulated with a time constant of 53 ps. This constant matched the rise time of the P_2, and its decay time was 170 ps. The P_3 had a rise time of 140 ps and a decay time of 1600 ps. These results clearly indicate the sequential energy migration in the order P_1, P_2, and P_3 in the APC-B trimer with a linker, even though there was a small deviation in the kinetic parameters.

We obtained the DAS of APC-B trimer using a custom-made computer program [22] applied to the fluorescence decay curves measured at 5 nm intervals. At 20°C (Fig. 3A), one component with a lifetime of 60 ps showed a positive band around 660 nm and a negative band at 680 nm. The second component spectrum had two maxima at 660 and 680 nm, with a lifetime of 1735 ps. At −196°C (Fig. 3B), three components were required for the best fit. The first one (τ = 53 ps) showed a positive band (658 nm) and a negative band (682 nm), and was similar to the first component at 20°C. The second component (τ = 139 ps) showed two positive maxima (665 and 680 nm) and one negative maximum (690 nm). The third component was long lived (1562 ps), having a maximum around 685 nm. The kinetic parameters obtained using DAS were consistent with those obtained using the PMSE method.

4. Discussion

4.1. Energy migration in the APC-B trimer

In the APC-B trimer, the energy levels of three types of chromophores are postulated as follows: α-84, shorter than 615 nm [23]; β-84, at 650 nm; and αAPB, at 655 nm [12]. Recently, femtosecond spectroscopy on the APC trimer $(\alpha\beta)_3$ revealed that the energy transfer between the α-84 and β-84 chromophores occurs with a rate constant in the 500-fs range, and the emission from the α-84 chromophore was located at a wavelength significantly shorter than that for the β-84 chromophore [23]. Since the time resolution of our apparatus was 3 ps, we

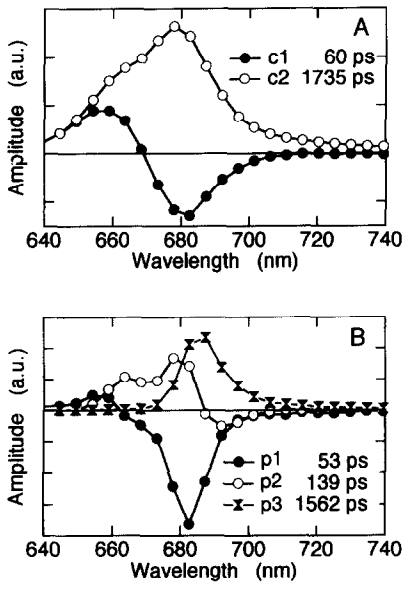
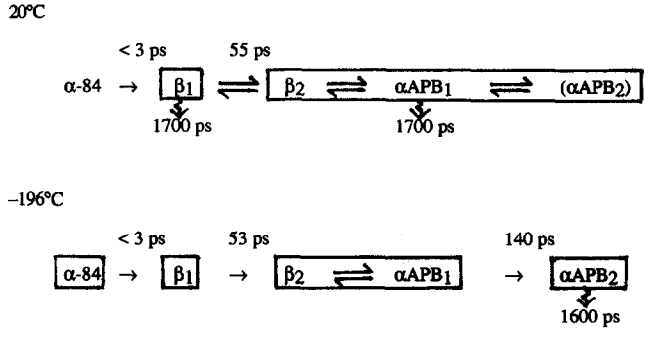


Fig. 3. DAS of APC-B with a linker polypeptide at 20°C (A) and at −196°C (B). The same number of components was assumed as for the PMSE method. Components with the letters in lower case correspond to those resolved by the PMSE method (Figs. 1C and 2C).

probably missed the primary transfer process. We did not observe the emission component peaking at a wavelength shorter than 660 nm in the APC-B trimer at 20°C (Fig. 1A), indicating there was no emission from the α-84 chromophores in our preparation. Accordingly we assigned the 660-nm component to the β-84 chromophore. However, two types of β-84 chromophores are expected in APC-B, depending on the counterpart of interaction: one interacting with the α-84 chromophore, and the other with αAPB. In fact, two kinds of β-84 chromophore were resolved in APC-B: one found in the component spectrum C_1, and the other in C_2. Based on these results, we assigned the resolved component spectrum C_1 to the β-84 chromophore interacting with the α-84 chromophore, and C_2 to the thermally equilibrated state between the other β-84 and αAPB chromophores.

The third component spectrum (P_3) resolved using the PMSE method at −196°C showed the lowest energy level. When we consider a single trimer unit, this component (P_3) can only be explained by the heterogeneity of the trimers. However, the kinetics of resolved components indicate the sequential energy flow to this component. Therefore it is reasonable to assume a hexameric component in APC-B in which a lower energy level of the αAPB chromophore was found under the low-temperature condition. The P_2 spectrum was a little unusual because it showed the dual-emissive character at both 20°C and at a low temperature (Fig. 2C). Since the corresponding spectrum was also resolved using DAS (Fig. 3A), this property may be intrinsic and independent of the analytical method used. The P_2 spectrum might be ascribed to the stronger interaction between the β-84 and αAPB chromophores, even if the actual state responsible for such a spectrum is unclear.

The above results led us to make the assignments listed below. There are five kinds of chromophores in APC-B: α-84, β-84 interacting with α-84 (called β_1), β-84 interacting with αAPB (called β_2), αAPB in a higher energy level (called αAPB_1), and αAPB in the lowest energy level (called αAPB_2). The last component was observed only at low temperatures and was not resolved at 20°C, probably due to a small energy difference between αAPB_1 and αAPB_2. Energy transfer from the α-84 to the β_1 chromophore was not observed due to limited time-resolution. At 20°C, an equilibrium was established among the β_2, αAPB_1, and αAPB_2 chromophores. Only two components were resolved for the β_1 and the equilibrated state. The 55-ps lifetime is the transfer time from the β_1 chromophore to the β_2 chromophore, which is in the equilibrated state with the αAPB_1 and αAPB_2chromophores. A long-lived emission (1700 ps) arises from the equilibrated state. The kinetic parameters are nearly the same as those reported for room temperature [24,25], however, the thermally equilibrated state was shown by the spectrum for the first time. At −196°C, matching of the kinetic parameters for the resolved components clearly indicated the sequential energy flow among them. The 140-ps transfer time to the αAPB_2 chromophore was long for the single transfer step between trimers, compared with that predicted for the process between PC trimers [26]. This might be due to an unfavorable geometry of the hexameric unit of APC-B. Transfer schemes with forward transfer time constants under two temperature conditions were summarized in Scheme 1.



Scheme 1.

4.2. Validity and applicability of the PMSE method

The PMSE method offers several unique advantages, including: (i) no need to assume the number of spectral components, (ii) no need to assume the decay function, (iii) no need to assume the model of energy migration, and (iv) definitive resolution into individual component spectra with a high S/N ratio. We applied the DAS method to the same data set as that used for the PMSE method to compare the two methods (Fig. 3A and B). Using the same number of components, DAS gave essentially the same kinetic parameters. However, an exponential decay is implicitly assumed in the DAS method, but is not always applicable. Further, it is not necessarily easy to obtain component spectra from DAS. In the analysis of APC-B, component spectra were well reproduced by DAS when the kinetic parameters of exponential decay obtained by the PMSE method were used (data not shown). However, this is not generally the case. The PMSE method is applicable to any system, without requiring assumptions.

Our previous method [4–6], which employed deconvolution of spectra into components, also required assumptions of band shape and component number, and the number of components can be affected by the band shape. This introduces arbitrariness

in to the parameters, including band width and band locations, problems which are absent with the PMSE method. Deconvolution, however, is useful when the resolved component spectrum contains plural spectral components, as shown by the emission from the thermally equilibrated state (C_2 in Fig. 1C).

The PMSE method can be applied to time-resolved spectra and to any multi-component system in which the observed spectra are linear combinations of components. Each component is not necessarily a single element, but that showing the same behavior as a function of time or some other variable(s). We have applied this method to several other systems and expect it to become a fundamental analytical tool in the biological sciences.

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