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Spectroscopic studies of phycobilisome subcore preparations lacking key core chromophores: assignment of excited state energies to the L_{cm}, β^{18} and $\alpha^{AP\text{-}B}$ chromophores

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Abstract

Chromophore absorption and emission characteristics of the $\alpha^{AP\text{-}B}$, β^{18} and L_{cm} (large core-membrane linker) chromopeptides within the phycobilisome core are investigated using genetically engineered strains of *Synechococcus* sp. PCC 7002. Steady-state and time-resolved emission were used to examine energy transfer in subcore preparations from the wild-type organism and two mutants. Low-temperature (77 K) emission spectra were also measured for intact phycobilisomes from the wild-type and five mutant strains. Mutants retaining either the $\alpha^{AP\text{-}B}$ subunit or the unaltered L_{cm} chromophore resulted in only small changes in the low-temperature emission spectra, while retention of only the β^{18} subunit resulted in blue-shifted emission spectra. The L_{cm} chromophore has a room-temperature absorption maximum at 675 nm. In phycobilisomes at 77 K the $\alpha^{AP\text{-}B}$ and L_{cm} chromophores emit at 682–683 nm, and they are the best candidates for long-wavelength emitters also at room temperature. Overlap of these emission spectra with the absorption of chlorophyll *a* in the associated thylakoid membrane plays a significant role in excitation transfer from the antenna complexes in cyanobacteria.

Key words: Energy transfer; Phycobilisome; Cyanobacterium; Fluorescence relaxation; Subcore particle, 18 S

1. Introduction

The phycobilisome (PBS) is a light-harvesting antenna complex found in cyanobacteria and red algae [1–4]. This water-soluble antenna assembly is located on the stromal side of the photosynthetic membrane. Calculations by Porter et al. [5] indicate that the PBS transfers light energy with greater than 95% efficiency to the reaction centers, with most of the energy going to Photosystem II (PSII) [6–9]. The cyanobacterium gains a competitive advantage from having the PBS, because this antenna absorbs light in the part of the visible spectrum where chlorophyll *a* (Chl *a*) absorption is minimal.

The PBS is composed of two macrostructures, rods and core, as shown by electron micrographs of the PBS [10,11]. A schematic representation of the PBS from *Synechococcus* sp. PCC 7002 is presented in Fig. 1a. The rods are composed of hexamers of chromoprotein held together with colorless linker polypeptides [12]. For *Synechococcus* sp. PCC 7002, the only chromoprotein found in the rods is phycocyanin (PC), $\lambda_{abs} = 620$ to 630 nm, $\lambda_F = 643$ to 652 nm [1]. The colorless linker polypeptide associated with each PC hexamer fine-tunes the spectral properties of the chromophores so that the PC at the rod periphery absorbs at a higher energy than the PC next to the core [13–15]. Using

Abbreviations: AP, allophycocyanin; Chl *a*, chlorophyll *a*; DAS, decay-associated spectra; fwhm, full-width at half maximum; λ_{abs}, absorption maximum; λ_F, emission maximum; PBS, phycobilisome; PC, C-phycocyanin; PS I, Photosystem I; PS II, Photosystem II; PMSF, phenylmethanesulfonyl fluoride; DCM, 4-dicyanomethylene-2-methylene-6-(*p*-dimethylaminostyrene)-4*H*-pyrane; HPLC, high-performance liquid chromatograpy.

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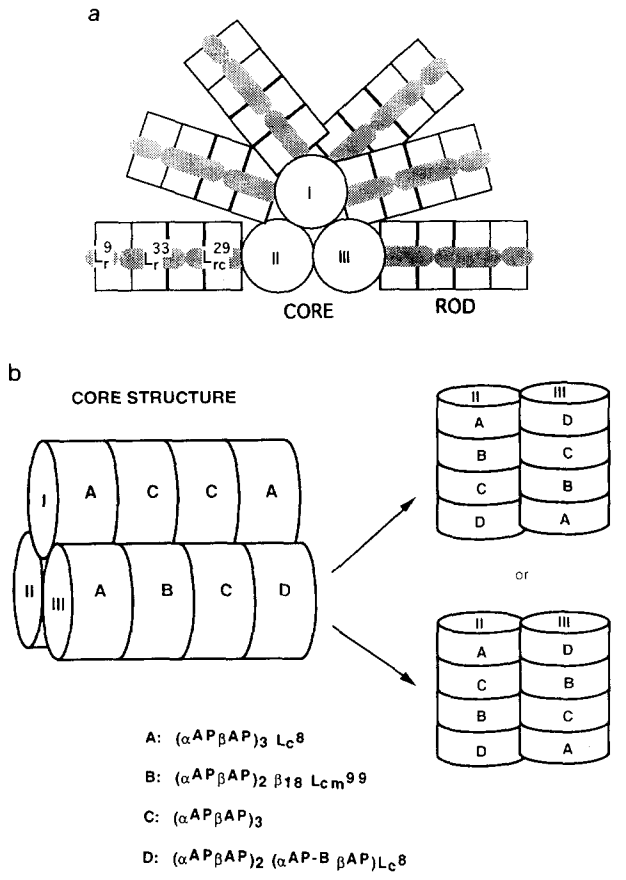

Fig. 1. (a) Schematic of a *Synechococcus* sp. PCC 7002 phycobilisome. The PBS is composed of two substructures, rods and core. The rods are made up of trimers of PC. The shading in the rods correspond to colorless linker polypeptides bound to the PC trimers. The core is made up of three cylinders with each cylinder containing four trimers of AP along with some minor phycobiliproteins. The schematic is based upon electron micrographs of PBS as described by Glazer [3]. (b) Composition of phycobilisome core. The molecular weight of the protein is given as a superscript with the location of the linker polypeptide given as a subscript. c, core; cm, core-membrane; PC, phycocyanin; AP, allophycocyanin; L, linker polypeptide. The core cylinders next to the membrane are labeled II and III, while the apical cylinder is labeled I. This model is from [1].

these small differences in the absorption of the PC chromophores, the rods are optimized for directional energy transfer to the PBS core.

The core cylinders of the PBS are mostly allophycocyanin (AP), with $\lambda_{abs} = 650$ to 653 nm, $\lambda_F = 660$ to 662 nm, but the cores contain longer wavelength chromoproteins in smaller amounts [3]. The AP in the core is arranged in trimers, with four trimers forming each core cylinder. Three core cylinders are found in PBS from *Synechococcus* sp. PCC 7002, but the number of core cylinders per PBS can differ from two to four depending on the species of origin [1].

Three longer wavelength chromoproteins, L_{cm}, $\alpha^{AP\text{-}B}$ and β^{18}, are located in the core of the PBS along with the AP. While an AP trimer emits at 660 nm, trimers containing the L_{cm} and β^{18} chromoproteins or the $\alpha^{AP\text{-}B}$ chromoprotein emit at 675–680 nm. Two copies of each of the three long wavelength chromoproteins are present in the PBS core. The exact arrangement of these proteins is still under investigation. One likely arrangement of the core, shown in Fig. 1b, is based on work from several laboratories [16–22].

The isolated L_{cm} and $\alpha^{AP\text{-}B}$ chromoproteins display red-shifted absorption spectra compared with isolated AP subunits [23–25]. We will present evidence that the chromophores of these two proteins, and to a lesser extent the β^{18} chromophore, are responsible for the red-shifted emission in intact PBS. The L_{cm} and $\alpha^{AP\text{-}B}$ chromophores have been assigned the role of transferring energy from the PBS core to the Chl *a* of the reaction centers. The large core polypeptide L_{cm} also participates in the binding of the core trimers [26,27]. The $\alpha^{AP\text{-}B}$ subunit has been proposed to transfer energy to PSI when the cells are in the physiological State II [28]. The role of the β^{18} subunit is unclear at this time, although it appears to play a part in the transfer of energy to PSII [28].

To elucidate the roles played by the long wavelength chromoproteins in the core, we have examined the spectroscopic properties of preparations from several phycobilisome mutants [29]. Mutations affecting core components of *Synechococcus* sp. PCC 7002 were created using interposon and/or site-directed mutagenesis. The mutants examined in this paper include the *apcF* mutant which is missing the β^{18} subunit, the *apcD* mutant which is missing the $\alpha^{AP\text{-}B}$ subunit, the *apcE / C186S* mutant in which the chromophore-binding cysteine at position 186 on the L_{cm} protein has been changed to serine, and the double mutants *apcDapcF* and *apcDapcE / C186S*.

We also examine subcore preparations from the *apcF* and *apcE / C186S* mutants. Our subcore preparation is similar to the 18 S preparation that Gingrich, Lundell and Glazer isolated from *Synechocystis* sp. PCC 6701 [30]. They found the relative ratios of $\alpha^{AP}:\beta^{AP}:\alpha^{PC}:\beta^{PC}:L_{cm}$ to be approx. 6:6:3:3:1 in the *Synechocystis* sp. PCC 6701 preparation, with no $\alpha^{AP\text{-}B}$ present. Based on an analogous study with *Synechococcus* sp. PCC 6301, the β^{18} subunit should be present in the subcore preparation at the same concentration as L_{cm} [18,19].

As reported earlier, we found that the PBS from the *apcE / C186S* and the *apcDapcE / C186S* strains contain an unusual chromophore noncovalently bound to the L_{cm} polypeptide [31]. This chromophore which emits at 715 nm is probably 3(*Z*)-phycocyanobilin, identified as the immediate precursor to the bound pigment [32]. Since it has increased π-electron conjugation, the 3(*Z*)-phycocyanobilin chromophore has a lower excited state energy than that of the covalently bound phycocyanobilin chromophore. This altered

chromophore, while retained physically in the PBS from the mutant, is lost from the subcore preparations from the *apcE / C186S* and *apcDapcE / C186S* strains.

In addition to the spectroscopy at room temperature on the 18 S subcore preparations, we present low-temperature emission spectra for all of the PBS and subcore preparations described above. The room-temperature steady-state and time-resolved emission spectra of the intact PBS from all mutants, with the exception of the *apcDapcE / C186S* strain, have been reported in earlier papers from our laboratories [31,33]. Details of the spectroscopy carried out on the *apcDapcE / C186S* PBS and on intact cells from all strains are described elsewhere [34].

2. Materials and methods

2.1. Culture conditions

The mutant strains of *Synechococcus* sp. PCC 7002 were constructed as described previously [29]. All of the strains were grown using Medium A containing 1 mg mL^{-1} $NaNO_3$ [33] under 200 $\mu E\ m^{-2}s^{-1}$ of white fluorescent light at approx. 32°C. The cultures were aerated with a 5% (v/v) CO_2/N_2 gas mixture with constant stirring. Continuous stock cultures used to provide starting stock were grown in 125 mL Erlenmeyer flasks under the same temperature and light conditions as above but without addition of CO_2(g). The stock cultures were grown in A^+ medium supplemented with the appropriate antibiotics to maintain the genetic markers in the mutant strains. The concentration of each of the antibiotics used was as follows: kanamycin (100 $\mu g\ mL^{-1}$), erythromycin (20 $\mu g\ mL^{-1}$), ampicillin (5 $\mu g\ mL^{-1}$) [29,31]. The *apcE / C186S* culture required ampicillin and the *apcDapcE / C186S* culture required both erythromycin and ampicillin.

2.2. Preparation of the PBS and 18 S core preparations

Intact PBS were prepared as previously described [33,35]. The following procedure, a composite of several different procedures [18,33,36], was used for the 18 S core preparation. Cells are harvested near the end of the exponential phase of growth and are concentrated by centrifugation at $12000 \times g$. The cells are resuspended using a minimum of growth medium and centrifuged a second time at $17000 \times g$ for 5 min to obtain an accurate cell wet weight. The cells are resuspended to 0.12 g mL^{-1} in 50 mM Tris buffer (pH 7.8) with 0.8 M Na_2SO_4, 1 mM NaN_3 and 20 μM PMSF (Buffer A).

The cells are homogenized and passed through a French Pressure Cell four times at 140 MPa (20000 psi) and 4°C. The suspension is incubated with 1.2% (w/w) Triton X-100 for 30 min at room temperature with vigorous stirring. The suspension is then centrifuged for 30 min at $30600 \times g$ at 15°C. The blue supernatant is loaded onto a sucrose step-gradient. The sucrose step-gradient is made up of 3 mL of 2.0 M, 5 mL of 1.0 M and 7 mL each of 0.75 M and 0.5 M sucrose in buffer A. The gradients are ultracentrifuged for 2–4 h at $190000 \times g$ and 15°C. The intact PBS are found in the 0.75 and 1.0 M sucrose layers. A significant amount of chlorophyll is also found in the 1.0 M sucrose layer, but it is precipitated during the subsequent steps.

After the intact PBS are removed from the gradients they are exchanged into 50 mM Tricine buffer (pH 7.8) with 5 mM $CaCl_2$, 10% (v/v) glycerol, 1 mM NaN_3 and 20 μM PMSF (Buffer B) in which the PBS immediately dissociate. The PBS can be exchanged into the Tricine buffer using either 18 h of dialysis or Bio-Rad 10-DG desalting columns (Bio-Rad, Richmond, CA). The dissociated proteins are concentrated using an Amicon PM-30 ultrafiltration membrane (Amicon Company, Danvers, MA) at $3.7 \cdot 10^5$ Pa (55 psi) pressure until the absorbance is 10–15 cm^{-1} at 630 nm.

Approx. 3 mL aliquots of concentrated protein are loaded onto linear sucrose gradients composed of 0.3 M to 0.9 M sucrose in buffer B. The gradients are ultracentrifuged for 11–12 h at $160000 \times g$ and 15°C. The 18 S subcore preparation is located in the lower, narrow band; the top, broad band contains mostly PC.

Linker-free PC hexamers and AP trimers used in the low-temperature emission spectroscopy were prepared as described in [37].

2.3. High performance liquid chromatography (HPLC)

To determine the composition of the 18 S preparations, HPLC was carried out on wild-type and *apcF* samples using the protocol and equipment described in Swanson and Glazer [38]. The polypeptides came off the C_4 analytical column resolved with base-line separation. The eluted polypeptides were identified using SDS-PAGE.

2.4. Sample preparation

For absorption spectroscopy, samples were taken directly from the sucrose gradients. The preparations were diluted to less than 1 cm^{-1} at the absorption maximum using Buffer B containing 1 M sucrose. The high sucrose concentration was used because the preparation was found to be more stable in this solution.

Samples used for room-temperature (19 ± 2°C) fluorescence spectroscopy were diluted to an absorbance of less than 0.12 cm^{-1} at the excitation wavelength using Buffer B with 1.0 M sucrose. Samples for low-tempera-

ture fluorescence spectroscopy were prepared by diluting a minimum volume, less than 10% (v/v), of sample with 2.0 M sucrose (Scheer, H., personal communication). The sample was placed under vacuum for approx. 10 min before freezing to remove most of the gases present. The sample was frozen to a clear glass in a 1 cm polystyrene cuvette by immersing the cuvette in a square optical dewar filled with liquid N_2.

2.5. Spectroscopy

Absorption spectroscopy was carried out on an AVIV 14DS spectrophotometer (AVIV, Lakewood, NJ). Steady-state fluorescence spectra were taken on a Spex Fluorolog fluorimeter (Spex Industries, Edison, NJ) with right angle detection.

Time-resolved fluorescence spectroscopy was carried out using a reverse-timing, single-photon counting system with microchannel plate detection [31,39]. The samples were excited with light produced by a dye laser [DCM dye: 4-dicyanomethylene-2-methylene-6-(*p*-dimethylaminostyrene)-4*H*-pyrane; (Exciton)] with pulses of approx. 6 ps full width at half maximum (fwhm). The instrument response function was approx. 55 ps at fwhm. The time-to-amplitude converter was set to 11.6 ps per channel for each of the 1024 channels, and 10000 counts were collected in the peak channel. The fluorescence decays were fit to a sum of exponentials using a global analysis software program kindly provided by A. Holzwarth (Mülheim, Germany).

3. Results

HPLC was used to determine the polypeptide composition of the 18 S preparations from the wild-type and *apcF* strains. The wild-type preparation contained five chromoproteins and two colorless polypeptides. The order of protein elution was: L_{cm} at 28 min, β^{AP} at 28.5 min, α^{PC} along with a colorless 33 kDa polypeptide at 29 min, β^{PC} at 30.5 min, α^{AP} at 33 min and β^{18} at 36 min. A second colorless polypeptide of approx. 29 kDa eluted at about 31.5 min. The elution profile of the wild-type preparation is presented in [34]. The *apcF* 18 S preparation did not contain the β^{18} polypeptide. The $\alpha^{AP\text{-}B}$ polypeptide was absent from the wild-type and from all of the mutant subcore preparations. Because the L_c^8 polypeptide is apparently missing from the preparation, we conclude that only the interior core trimers are present (trimers B and C in Fig. 1a) along with PC hexamers.

3.1. Absorption spectroscopy of the 18 S preparations

The absorption spectra for the wild-type, *apcF* and *apcE / C186S* 18 S subcore preparations are shown in Fig. 2. Absorption spectra from at least four different preparations of each strain were averaged. The absorption maxima of the spectra are at 651 to 653 nm, 20 nm further to the red than the absorption maximum for intact PBS. The subcore preparations are enriched in the lower energy chromophores of the PBS.

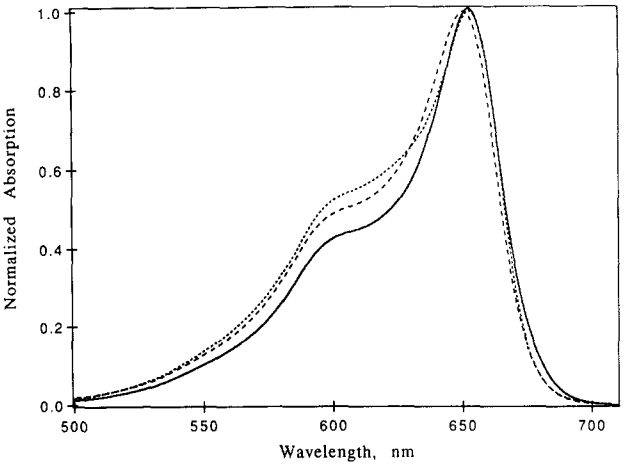

Fig. 2. Absorption spectra of 18 S subcore preparations. Averaged spectra from four different wild-type preparations, five *apcE / C186S* preparations, and four *apcF* preparations. Samples were taken directly off sucrose gradients without dilution. The spectra were taken at room temperature and normalized at their absorption maxima. wild-type ———, *apcF* - - - - - -, *apcE / C186S* · · · · · ·.

The *apcE / C186S* and wild-type preparations have absorption maxima at 653 nm. The *apcE / C186S* preparation absorbs relatively less than that of the wild-type at approx. 675 nm. The absorption maximum of the *apcF* preparation is blue-shifted by 2 nm to 651 nm. The *apcF* preparation also appears to absorb less than the wild-type preparation on the red edge of the spectrum. All absorption spectra from the subcore preparations contain shoulders at approx. 600 nm. The relative height of the shoulder differs slightly with preparation, and the preparations from the mutants have consistently higher 600 nm shoulders.

3.2. Room-temperature emission

The room-temperature emission spectra for the 18 S subcore preparations are given in Fig. 3. The wild-type preparation gives a broad emission spectrum centered at 668 nm. The emission spectrum from the *apcE / C186S* subcore preparation is narrower, with an emission maximum at 666 nm. The *apcF* spectrum is also narrower than that of the wild-type, and its emission maximum is blue-shifted to 662 nm. In the *apcE / C186S* and *apcF* preparations relatively more fluorescence originates from higher energy chromophores than from 680 nm emitters.

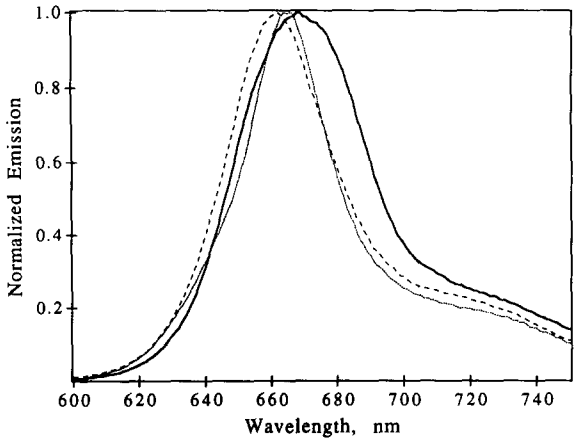

Fig. 3. Room-temperature emission spectra of 18 S subcore preparations. The samples were excited at 590 nm, and emission was collected at 90° to excitation. The excitation monochromator resolution was 3 to 6 nm, while the emission monochromator resolution was from 0.4 to 0.8 nm. The spectra were collected within two hours after the preparations were removed from the gradients. The samples were diluted to give absorbance less than 0.1 cm^{-1} at the excitation wavelength using 1.0 M sucrose in 50 mM Tricine with 5 mM $CaCl_2$ and 10% (v/v) glycerol (pH 7.8). The spectra were taken at room temperature and normalized at the emission maxima. wild-type ———, *apcF* - - - - - -, *apcE/C186S* ·····.

3.3. Low-temperature emission

The 77 K emission spectra for the intact PBS are given in Figs. 4a and 4b. The locations of the emission maxima are listed in Table 1. The original room-temperature emission spectrum is regained upon thawing in each case. We found PC hexamers to emit at 651 nm and AP trimers to emit at 659 nm at 77 K.

The emission spectra from the wild-type, *apcD*, *apcDapcF* and *apcF* PBS, Fig. 4a, contain one main emission band centered at 683 nm for the wild-type and apcD PBS, and at 681 nm for the *apcDapcF* and *apcF* PBS. The 77 K emission spectra from the *apcDapcE/C186S* and *apcE/C186S* PBS, Fig. 4b, are more complicated, with each spectrum containing three major emission bands. In the *apcE/C186S* spectrum, the bands are located at 653, 682 and 715 nm. In the *apcDapcE/C186S* spectrum the bands are found at 648, 678 and 713 nm. The relatively broad 715 nm emission band is attributed to the non-covalently bound 3(*Z*)-phycocyanobilin found in the *apcE/C186S* and *apcDapcE/C186S* PBS [31] while the 653 nm emission is ascribed to PC.

The low-temperature emission spectra of the 18 S subcore preparations from the wild-type, *apcF* and *apcE/C186S* strains obtained under conditions similar to those for the spectra of intact PBS are shown in Fig. 5. The emission spectrum obtained from the wild-type 18 S subcore preparation is virtually identical to the spectrum obtained from the wild-type PBS except for the addition of a second emission band at 653 nm. This probably owes to the presence of some contaminating rod complexes. The emission spectrum obtained from the *apcF* subcore preparation is significantly blue-shifted from that obtained from the *apcF* PBS. The *apcF* subcore preparation has an emission maximum at 676 nm while the *apcF* PBS have a maximum at 681

Table 1
Phycobiliprotein fluorescence emission components

Strain	Sample [a]	Long-wavelength chromophores present [b]	Emission maxima (nm)	
			77 K	RT
WT	PBS	$\alpha^{AP\text{-}B}$, β^{18}, L_{cm}	683	665, 678 [c]
apcF	PBS	$\alpha^{AP\text{-}B}$, L_{cm}	681	664 [d]
apcD	PBS	β^{18}, L_{cm}	683	665, 678sh [c]
apcE [e]	PBS	$\alpha^{AP\text{-}B}$, β^{18}	682, 653, 715	660, 715 [d]
apcD/F	PBS	L_{cm}	681	664 [d]
apcD/E [e]	PBS	β^{18}	678, 648, 713	653, 662sh, 712 [f]
WT	AP-B-L^8_c	$\alpha^{AP\text{-}B}$		680 [g]
WT	SC	β^{18}, L_{cm}	682.5, 653	668, 678sh
apcF	SC	L_{cm}	676, 652	662
apcE [e]	SC	β^{18}	671, 647.5	666

[a] PBS, phycobilisomes; SC, subcore preparation.
[b] Preparations also contain allophycocyanin and sometimes phycocyanin.
[c] Ref. [33].
[d] Ref. [31].
[e] apcE = apcE/C186S.
[f] Ref. [34].
[g] Ref. [46].

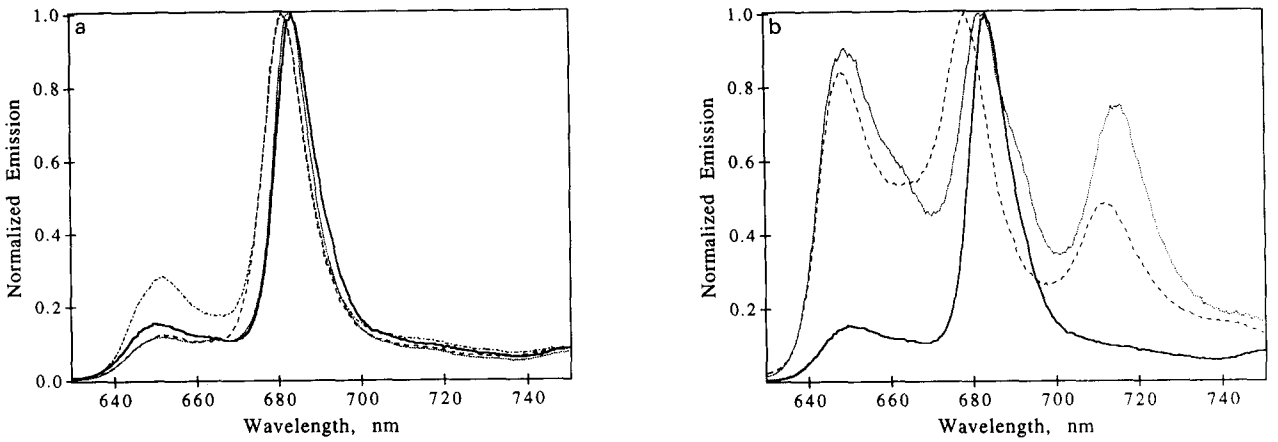

Fig. 4. Low-temperature emission spectra of intact phycobilisomes. The PBS were excited at 590 nm, and emission was collected 90° to excitation. The excitation monochromator resolution was 6 to 7 nm, while the emission monochromator resolution was from 0.1 to 0.4 nm. The spectra were collected within eight hours after the PBS were removed from the gradient. The samples were diluted using 2.0 M sucrose to give absorbance less than 0.1 cm^{-1} at the excitation wavelength. The diluted samples were vacuum-degassed for 10 min and frozen at 77 K. The wavelengths of the emission maxima are given in Table 1. Spectra are normalized at the emission maxima (a) or arbitrarily at the emission maximum near 680 nm (b). (a) wild-type ———, *apcD* ·····, *apcF* - - - - -, *apcDapcF* ·–·–· (b) wild-type ———, *apcE/C186S* ·····, *apcDapcE/C186S* - - - - -.

nm. As with the wild-type 18 S spectrum, the *apcF* subcore preparation has an additional emission band at 652 nm. Also, the width of the main emission band in the *apcF* 18 S spectrum is 1.5 times broader than the band in the wild-type spectrum.

The low-temperature emission spectrum from the *apcE/C186S* subcore preparation is also significantly altered from that of the *apcE/C186S* PBS. Instead of the three emission bands present in the *apcE/C186S* PBS spectrum, only one major band is present, at 671 nm, in the *apcE/C186S* subcore preparation. The 715 nm emission ascribed to the altered chromophore is absent, and the 653 nm emission attributed to PC is greatly decreased from that of the *apcE/C186S* PBS spectrum.

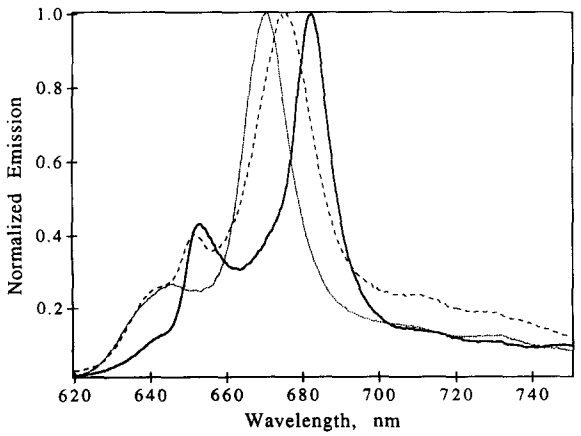

Fig. 5. Low-temperature fluorescence emission spectra of 18 S subcore preparations. The spectra were collected under the conditions described in Fig. 4 and normalized at the emission maxima. The wavelengths of the emission maxima are given in Table 1. wild-type ———, *apcF* - - - - - -, *apcE/C186S* ·····.

3.4. Time-resolved emission

The time-resolved emission from the 18 S core preparations was collected, and the decays were fit to a sum of exponentials using global analysis, where a single set of lifetimes was used at all wavelengths. Decay-associated spectra (DAS) were calculated for the wild-type, *apcF* and *apcE/C186S* subcore preparations. A minimum of three lifetime components was required to fit the wild-type and *apcF* decays, while four components were required for the *apcE/C186S* decays. Typical DAS are displayed in Figs. 6a, 6b and 6c.

The decays for the 18 S preparations from the wild-type required lifetime components of 2.7, 1.4 and 0.08 ns for a good fit, as judged by chi-squared values. The 2.7 and 1.4 ns components have positive amplitudes across the entire spectrum, while the 0.08 ns component has a positive amplitude below 660 nm and a negative amplitude from 665 to 720 nm. A negative amplitude in the DAS indicates a delay in the appearance of the emission at that wavelength, evidence of energy transfer.

The DAS calculated for the *apcF* preparation are virtually identical to those calculated for the wild-type preparation within the error of the experiment. Three lifetime components of 2.6, 1.3 and 0.2 ns are required to fit the time-resolved emission data. The spectral dependences of the three components are similar to those found in the wild-type DAS, except that the

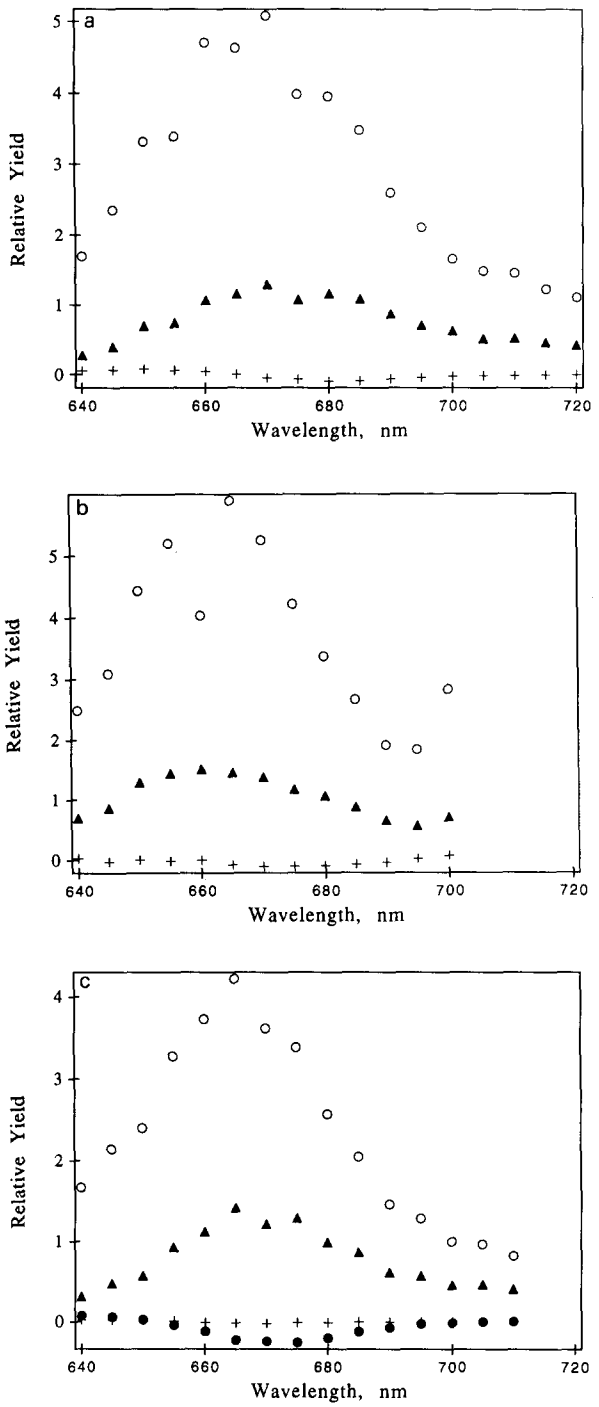

Fig. 6. Decay-associated spectra of the 18 S subcore preparations. The samples were excited at 633 nm at room temperature. The fluorescence decays were collected using the single-photon counting instrument described in [31,39]. The DAS were obtained from global analysis. (a) wild-type; ▲2.7 ns, ○ 1.4 ns, +0.08 ns (b) *apcF*; ▲ 2.6 ns, ○ 1.3 ns, +0.2 ns (c) *apcE/C186S*; ▲ 2.3 ns, ○ 1.2 ns, ● 0.5 ns, +0.05 ns.

yields of the two longer lifetimes are slightly decreased at 680 nm in the *apcF* preparation.

Four lifetime components were required to fit the time-resolved emission from the *apcE/C186S* subcore preparation. Three of the lifetimes are similar to those obtained from the wild-type DAS, including the 2.3, 1.2 and 0.05 ns components. The 2.3 and 1.2 ns components are comparable to the 2.7 and 1.4 ns lifetimes found in the wild-type DAS, while the 0.05 ns lifetime is similar to the 0.08 ns wild-type component. The fourth lifetime component of 0.5 ns has a spectral dependence similar to that of the 0.08 ns wild-type component.

4. Discussion

The objective of this study is to characterize the absorption and emission spectra of the L_{cm}, β^{18} and $\alpha^{AP\text{-}B}$ chromophores within intact PBS. Because of the inherent stability and symmetry of phycobiliprotein trimers, a question arises as to the structural consequences of mutations that result in the deletion of one or more subunits. In the *apcF* strain, the β^{18} polypeptide and chromophore have been deleted from the PBS core. One possible result of the deletion is the substitution of a β^{AP} subunit for the missing β^{18}. The subunits have similar amino acid sequences, and the absorption spectra of the purified subunits are very similar [23]. On structural grounds it seems likely that deletion of a subunit from a trimer would be replaced by substitution during assembly of the core. HPLC carried out on the wild-type and *apcF* subcore preparations was not sufficiently reproducible, however, to determine whether such a substitution occurred within the *apcF* core, because replacement would lead to only a small percentage increase in the β^{AP} content. Other substitutions within the PBS core could also occur in the other deletion mutants; i.e., α^{AP} could replace $\alpha^{AP\text{-}B}$ in the *apcD* mutant.

Unlike the *apcF* mutant, the *apcE/C186S* strain is missing only the L_{cm} chromophore covalent attachment site. The L_{cm} apoprotein is present in the 18 S subcore preparations as shown by SDS-PAGE (data not shown). Structural reorganization resulting from the empty chromophore binding pocket on the L_{cm} polypeptide could modify the spectral properties of adjacent chromophores within the PBS core, but the polypeptide content of the *apcE/C186S* 18 S subcore is expected to be identical to that of the wild-type preparation.

4.1. Absorption spectroscopy

A small but significant difference between the absorption spectra of wild-type and *apcE/C186S* phycobilisomes is found at 675 nm [31]. The *apcE/C186S* spectrum exhibits a decrease at 675 nm that can be attributed to the absence of the L_{cm} chromophore. Assignment of an absorption maximum of 675 nm for

the L_{cm} chromophore within the PBS core is consistent with earlier work carried out on the isolated L_{cm} polypeptide [40].

Differences between the absorption spectra of subcore preparations from wild-type and *apcF* mutant strains are more difficult to interpret. Like the *apcE / C186S* preparation, the *apcF* preparation appears to have an increased PC content. Unlike the *apcE / C186S* preparation, the absorption maximum from the *apcF* preparation is blue-shifted 2 nm from that of the wild-type. The *apcF* preparation has a chromophore composition different from that of the wild-type. Even though the isolated β^{18} and β^{AP} subunits have similar absorption spectra [23], the spectrum of the β^{18} chromophore incorporated into an AP trimer could be significantly different from that of a β^{AP} chromophore. For example, interaction of β^{18} and L_{cm} could produce alteration of the absorption properties of β^{18}. Part of the change in the absorption spectrum may be caused by the substitution of β^{AP} for the missing β^{18} subunit, but the blue shift in the absorption maximum cannot be adequately explained at present.

4.2. Room-temperature emission spectroscopy

The steady state emission spectra from the *apcE / C186S* and *apcF* subcore preparations indicate that relatively less of the total emission originates from low energy chromophores in the preparations from the mutants. The changes in the emission spectra can be explained by a decrease in either the number or the fluorescence yield of 680 nm emitters in the subcore preparations from the mutants.

The lifetime components extracted from the time-resolved emission of the *apcF* preparation are virtually identical to those determined for the wild-type except that the relative yield at 680 nm from the mutant is decreased. The decrease in yield is consistent with the steady state emission spectra.

The time-resolved emission from the *apcE / C186S* subcore preparation is more difficult to interpret (Fig. 6c). The requirement of an additional lifetime component indicates that at least one energy transfer step within the *apcE / C186S* complex is significantly altered from that of the wild-type. One explanation for the additional lifetime component, involving negative amplitude between 660 and 700 nm, is that an energy transfer step within the core has been slowed sufficiently that it can now be resolved using single-photon timing.

Some important results obtained from the time-resolved emission need to be emphasized. Virtually all time-resolved emission studies carried out on intact PBS using the technique of single-photon timing report two long-lifetime components of 1–1.5 and 2–3 ns that are attributed to the L_{cm} and $\alpha^{AP\text{-}B}$ chromophores [41–45]. We find that the deletion of the $\alpha^{AP\text{-}B}$ and/or β^{18} chromophores from the PBS does not change the 1–1.5 ns and 2–3 ns components, although their relative yields at 680 nm decrease [31]. Modification of the L_{cm} chromophore to a longer wavelength non-covalently bound emitter does decrease the duration of the two longest lifetimes; the noncovalently bound chromophore acts to quench the antenna complex [31]. Global analysis of the time-resolved emission from each of the mutants discussed in this paper yields two long lifetime components. We cannot attribute any long lifetime component from the time-resolved fluorescence data preferentially to the $\alpha^{AP\text{-}B}$, β^{18}, or L_{cm} chromophores.

4.3. Low-temperature emission

At 77 K, excitation transfer leads to preferential population of the lowest energy excited states, because uphill energy transfer becomes less likely at the low temperature. Only the lowest energy chromophores in the coupled antenna complex should emit. At 77 K, the emission bands shift to longer wavelength and decrease in width, because the energy distribution of populated states at 77 K narrows relative to that at room temperature. PC or AP chromophores which are no longer coupled to lower energy chromophores emit at wavelengths well resolved from the lowest energy chromophores in the core. A mixture of PC hexamers and trimers emits at 651–653 nm, while AP trimers emit at 659 nm. The amount of uncoupled PC can be estimated from the height of the 653 nm emission in the low-temperature spectra.

In the *apcDapcE / C186S* PBS, the $\alpha^{AP\text{-}B}$ chromoprotein has been deleted, and the L_{cm} chromophore is altered such that it emits at 715 nm (Fig. 4b). Therefore, the emission at 678 nm must originate from the β^{18} chromophore, while the 653 nm peak is assigned to PC, and the 713 nm peak is assigned to the altered L_{cm} (Table 1). An alternative explanation for the 678 nm emission in the *apcDapcE / C186S* spectrum is that it originates from the interaction of the AP trimers in the core with the binding domains of the L_{cm} polypeptide. In this case the spectral characteristics of the β^{18} chromophore would be very similar to that of the β^{AP} chromophore. The extent to which the absorption band positions of the long wavelength chromophores may depend on excitonic coupling with nearby chromophores is difficult to ascertain in the absence of measurements on smaller, well-defined sub-core complexes.

PBS from the *apcDapcF* mutant contain neither the $\alpha^{AP\text{-}B}$ nor the β^{18} chromophores. The 77 K emission spectrum from the *apcDapcF* PBS exhibits two emission bands, at approx. 653 nm and 681 nm, respectively (Fig. 4a). We attribute the 653 nm emission to uncou-

pled PC and the 681 nm emission to the L_{cm} chromophore.

The absence of the $\alpha^{AP\text{-}B}$ chromoprotein from the PBS of *apcD* or *apcDapcF* has virtually no effect on the low-temperature emission spectrum. The *apcD* spectrum is very similar to that of the wild-type, while the *apcDapcF* spectrum is identical to that of the *apcF* strain. In *apcE/C186S* PBS, the L_{cm} chromophore is altered such that it emits at 715 nm. Three emission bands are present at 653 nm, 682 nm and 715 nm, respectively, in the *apcE/C186S* PBS spectrum (Fig. 4b). The 653 and 715 nm bands are attributed to PC and to the altered L_{cm} chromophore, respectively. The third band at 682 nm is tentatively assigned to the $\alpha^{AP\text{-}B}$ chromophore, because the β^{18} chromophore was assigned an emission maximum of 678 nm from the *apcDapcE/C186S* PBS spectrum. A consequence of this assignment is that the emission from the L_{cm} and $\alpha^{AP\text{-}B}$ chromophores in wild-type are essentially indistinguishable at 77 K.

The 77 K wild-type long-wavelength emission cannot be assigned to a single chromophore. The wild-type emission maximum is found at 683 nm, but removal of either the $\alpha^{AP\text{-}B}$ chromophore or the L_{cm} chromophore results in a shift of only 1 nm in the emission maximum. These two chromophores may interact to give the 683 nm emission.

The 77 K emission spectra from the 18 S preparations were also examined (Fig. 5). The emission spectrum from the wild-type 18 S subcore preparation, which is missing $\alpha^{AP\text{-}B}$, is virtually identical to that of the wild-type PBS. One possible explanation for this similarity is that the L_{cm} and $\alpha^{AP\text{-}B}$ chromophores are essentially equivalent emitters. Each is capable of quenching (collecting) the emission from the entire set of PBS pigments. When both are present, each is responsible for a significant portion of the long-wavelength emission. When one is missing, the other in effect takes over the whole task (thus the similarities in the emission maxima in Figs. 4a and 4b), at least at 77 K. At room temperature the loss of $\alpha^{AP\text{-}B}$ causes some leakage of emission at shorter wavelengths, but the long wavelength component is still present [33].

The *apcF* subcore preparation that contains only the L_{cm} chromophore, aside from AP and PC, emits at 676 nm. The *apcE/C186S* subcore preparation that contains only the β^{18} chromophore emits at 671 nm. The 5–7 nm blue shift in the 77 K emission spectra of either the L_{cm} or β^{18} chromophore within the subcore preparations, relative to the spectra of the PBS from the double mutants containing the same chromophores, could be due to the instability of the subcore preparations. A second interpretation of the *apcE/C186S* subcore emission spectrum is that the 671 nm emission is due to AP trimers bound by a linker domain of the L_{cm} protein. As discussed earlier, the emission spectrum of phycobiliprotein is very dependent upon the environment of the chromophore. It is possible that the AP chromophores within the intact PBS core have a spectrum significantly different from that of the isolated trimer. It is not possible at present to develop a clear picture of the properties of the β^{18} chromophore that applies to both intact PBS and subcore preparations measured at room temperature and at 77 K. There is no evidence from our studies that supports a role for β^{18} as a long-wavelength emitter, by contrast with the situation for the $\beta^{AP\text{-}B}$ and L_{cm} chromoproteins.

4.3. Conclusions

The L_{cm} and $\alpha^{AP\text{-}B}$ chromophores appear to have very similar emission spectra, with wavelengths of maximum emission at 682–3 nm at 77 K and 680 nm at room temperature for PBS from *Synechococcus* sp. PCC 7002. Neither chromophore can be assigned specifically to one of the fluorescence lifetimes obtained from the global analysis and decay-associated spectra. The emission spectrum of the β^{18} chromophore is somewhat blue-shifted, with a maximum at 678 nm at 77 K, although the 678 nm emission could be assigned to AP trimers interacting with the L_{cm} polypeptide. Thus, the energetically downhill flow of excitation in the PBS proceeds from PC to AP to the β^{18} and finally to the L_{cm} or the $\alpha^{AP\text{-}B}$ chromoproteins prior to transfer to Chl *a* in the associated thylakoid membrane.

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