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Phycobilisome core mutants of *Synechocystis* PCC 6803 ☆

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Abstract

Mutant strains of the cyanobacterium *Synechocystis* 6803 were constructed in which either the *apcABC* operon, encoding core subunits allophycocyanin α and β and a small linker L^8_C, or the *apcE* gene encoding the phycobilisome core-membrane linker was deleted. Phycobilisome assembly and energy transfer were studied in these mutants using both SDS gel analysis of phycobiliprotein complexes and low temperature fluorescence spectroscopy. Both mutants assembled phycocyanin rods but neither assembled a core complex. Although the mutants have no functional phycobilisomes, they grow photoautotrophically. No energy transfer between the remaining soluble phycobiliproteins and the photosystems was observed.

Keywords: Allophycocyanin; Cyanobacterium; Energy transfer; L_{CM}; Photosystem; Phycobiliprotein

1. Introduction

In cyanobacteria and red algae, light energy is harvested for Photosystem II (PS II) principally through a high molecular weight protein structure called the phycobilisome (PBS). Intact PBS can be isolated from most cyanobacteria using phosphate buffers of high ionic strength followed by differential centrifugation [1]. The PBS is comprised of both chromophore-containing phycobiliproteins (PBPs) and colorless linker polypeptides. Reconstitution experiments have shown that without the linker polypeptides, purified PBPs will not reassemble into PBS [2]. The linker polypeptides also influence the spectroscopic properties of the PBPs, enabling a large portion of the visible spectrum to be utilised. Energy transfer through the PBS is unidirectional, approaching an efficiency of 90% [3].

The physical structure of the PBS in *Synechococcus* and *Synechocystis* strains has been examined by electron microscopy. The PBS contains a central core from which six rods usually radiate. In *Synechococcus* 6301 the core is made of two cylinders [4] while in *Synechocystis* 6701 it contains three cylinders [5]. Each of the core cylinders is made up of four discs. The major protein within the discs is allophycocyanin (AP). The rods are comprised of phycocyanin (PC) and in some species, especially those which undergo chromatic adaptation, phycoerythrin (PE) or phycoerythrocyanin (PEC) is present at the distal end of the rods. Linker polypeptides join the rods to the core and the discs to each other, as well as joining the entire structure to the thylakoid membrane. Understanding PBS assembly and structure has been enhanced by mutational analysis of the PBPs and linker polypeptides of *Synechococcus* 7002 [6,7]

The structure of the wild-type *Synechocystis* 6803 PBS has been determined by electron microscopy. It consists of a three-cylinder core from which six rods, containing three disks each, radiate [8]. The cylinders at the base of the core are comprised of four disks each, with the following composition: (1) $(\alpha^{AP}\beta^{AP})_3$, (2) $(\alpha^{AP}\beta^{AP})_3$ L^8_C, (3) $(\alpha^{AP\text{-}B}\alpha_2^{AP}\beta_3^{AP})$ L^8_C, and (4) $(\alpha^{AP}\beta^{AP})_2\,\beta^{18.3}$ L_{CM}. Disc

Abbreviations: AP, allophycocyanin; KP, potassium phosphate buffer; PBP, phycobiliprotein; PBS, phycobilisome; PC, phycocyanin; L_{CM}, core-membrane-linker; Sm, streptomycin; Sp, spectinomycin; WT, wild type.

☆ Dedicated to Professor Yoshihiko Fujita on the occasion of his retirement from the NIBB.

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(1) contains three AP $\alpha\beta$ dimers. Disc (2), has the same composition as the first, except for the addition of the core linker, L_C^8, which holds the cylinder together by capping it. Disc (3) is similar to disc (2), having one α^{AP} subunit replaced by the $\alpha^{AP\text{-}B}$. Finally, in the fourth disc, one of the β^{AP} subunits is replaced by $\beta^{18.3}$ and one of the α^{AP} subunits is replaced by the core-membrane linker L_{CM}, the product of the *apcE* gene.This protein has both membrane-anchoring domains and AP-like domains, allowing it to replace one of the wedges in a disc and also insert into the thylakoid membrane [9].

Phycocyanin-deficient mutants of *Synechocystis* 6803 have been characterized by Elmorjani et al. [8]. These mutants can also grow photoautotrophically and their chlorophyll content is practically identical to that of wild-type cells. An *apcA*-defective mutant of *Synechocystis* 6803 was characterized by Su et al. [10] and an *apcE*-defective mutant of *Synechocystis* 6803 was characterized by Shen et al. [11] and we found some differences in the characterizations of these mutants compared to those we obtained and characterized in the present paper.

Starting with wild-type *Synechocystis* 6803 and PMB11, a phycocyanin-deficient mutant of *Synechocystis* 6803 [8], we isolated deletion mutants in which either *apcAB* or *apcE* were inactivated. Excitation energy transfer and the PBS polypeptide and chromophore composition of these mutants are presented and compared with those of the wild-type strain.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Cultures of wild-type *Synechocystis* 6803 were grown in liquid medium as described in [12] with twice the concentration of sodium nitrate, under 3000 lux illumination. Mutant strains were grown in the same medium supplemented or not with the antibiotics spectinomycin (Sp) (20 μg/ml; Sigma) and streptomycin (Sm) (10 μg/ml; Sigma). *Escherichia coli* strain DH5α was used to propagate pBluescriptSK$^-$ and the *apcABC* and *apcE* inactivation plasmids. SOC medium [13] supplemented with ampicillin (100 μg/ml), streptomycin (100 μg/ml), and spectinomycin (200 μg/ml) was used for selection and maintenance of the respective plasmids.

2.2. DNA preparation, Southern hybridization and transformation of Synechocystis 6803

Cloning and sequencing of the *Synechocystis* 6714 *apcE* and *apcABC* genes has been described previously [14]. Genomic DNA was prepared from wild-type and PBS core mutants of *Synechocystis* 6803 by the method of Marmur [15] adapted to cyanobacteria. Genomic DNA was prepared using either 5 ml of an exponentially growing liquid culture or a thick patch of cells scraped from plates. For Southern blots, DNA was transferred to nylon membranes (Gene-Screen Plus, DuPont). Probes were labeled by random priming [16] of DNA restriction fragments isolated from agarose gels and purified using silica gel chromatography (Gene-Clean, Bio101, La Jolla, CA).

Synechocystis 6803 was transformed according to the following protocol. Liquid cultures of *Synechocystis* 6803 were grown until 10^8 cells/ml. 10 ml of culture were centrifuged, the cell pellet was resuspended in 0.5 ml fresh liquid medium, and 1–2 μg (10–20 μl of a 0.1 mg/ml DNA solution) of linearized plasmid was added. After mixing, the cells were grown on a shaker for 12–16 h, then spread onto plates supplemented with antibiotics. Sp^r/Sm^r colonies were picked after 14 days.

2.3. Phycobiliprotein isolation

Phycobiliprotein (PBP) complexes were prepared by a procedure similar to that described in [8]. All steps were carried out at room temperature. 1 l cultures were harvested at $2 \cdot 10^8$ cells/ml and washed twice in 0.8 M potassium phosphate buffer, pH 7.0 (KP). The cells were resuspended in 10 ml KP, then passed twice through a French pressure cell at 20000 psi, or vortexed twice for 1 min with half the volume of glass beads (0.17 mm diameter). Triton X-100 was added to the broken cells to a final concentration of 2% (v/v). After incubation for 30 min with occasional shaking, unbroken cells and debris were removed by centrifugation at $15000 \times g$ for 20 min at 20°C. To reduce chlorophyll contamination, the supernatant was carefully removed from beneath the floating chlorophyll layer, leaving approx. 1–2 ml of supernatant on top of the greenish-grey pellet. 1 ml of supernatant was loaded onto sucrose step-gradients prepared as follows in 12 ml ultracentrifuge tubes: 1 ml of 2.0 M, 3 ml of 1.0 M, 2.5 ml of 0.75 M, 2.5 ml of 0.5 M, and 2 ml of 0.25 M sucrose solutions in 0.8 M KP. The gradients were spun 16–20 h in a Beckman SW41 rotor at 35000 rpm at 20°C. The blue-colored bands were isolated from the gradients and stored at 4°C as sucrose solutions. These samples were examined by SDS polyacrylamide gel electrophoresis and by spectroscopy within 48 h of isolation.

2.4. Electrophoresis of proteins

1 ml samples were diluted with KP to reduce the sucrose concentration of the sample to 0.2 M or less. The proteins were then precipitated by the addition of solid ammonium sulfate to a final concentration of 20%. After collection by centrifugation, the precipitates were dried, resuspended in cracking buffer (Tris, EDTA, SDS and mercaptoethanol), and then boiled for 3 min before being loaded on linear 10–20% acrylamide gradient gels (acrylamide/bisacrylamide = 30:0.8). The gels were run overnight at 15 V then stained with 1% Coomassie brilliant blue in acetic acid/methanol.

2.5. Preparation of thylakoid membranes

Cells were suspended at about 10^9 cells/ml in a medium containing 30 mM $CaCl_2$, 25% (v/v) glycerol and 20 mM Hepes (pH 7) and vortexed twice for one minute with half the volume of glass beads (0.17 mm diameter). Unbroken cells and glass beads were removed by centrifugation at $1000 \times g$, for 5 min and thylakoids were pelleted by centrifugation at $15\,000 \times g$ for 15 min and resuspended in the same medium as for disruption.

2.6. Spectroscopic analysis

Low temperature fluorescence spectra were recorded using a home-made apparatus. The exciting beam wavelength was selected by a monochromator (resolution of 9 nm); the fluorescence emitted by the sample, immersed in liquid N_2, was monitored by a photomultiplier tube through a monochromator with a resolution of 3 nm. The exciting beam and the fluorescence emission were passed through a Y-shaped flexible light guide, the sample being held at the common end with an appropriate holder. Soluble samples were placed in a flat cuvette 0.1 mm thick. Cell and thylakoid samples were filtered onto nitrocellulose discs. Cell samples were illuminated at 440 nm prior to freezing in order to adapt them to state 1 [17].

Room temperature Chl fluorescence inductions were performed as in [18].

Absorption spectra were recorded with a Varian Cary 2300 spectrophotometer in micro disposable cuvettes.

3. Results

3.1. Construction of PBS core mutants of synechocystis 6803

The genomic DNAs of *Synechocystis* 6803 and *Synechocystis* 6714 are very similar. DNA from *Synechocystis* 6714 can be used to transform *Synechocystis* 6803 [19]. When mutated DNA is introduced on a non-replicating plasmid, a double-crossover event can occur in which the mutated DNA replaces the wild-type DNA in the chromosome. Gene-disrupted mutants are conveniently made by inserting a cassette encoding antibiotic resistance into the coding region of the target gene. Transformation followed by selection for antibiotic resistance usually yields the desired mutant in which the target gene is interrupted or deleted. Using this system, the *apcAB* and *apcE* genes in *Synechocystis* 6803 were inactivated using interruptions in the corresponding cloned genes from *Synechocystis* 6714 [14].

The *apcAB* deletion plasmid, pABS2, was constructed by replacing a 0.6 kb *Kpn*I fragment of the *Synechocystis* 6714 *apcABC* operon containing the 3′-end of *apcA*, the 5′-end of *apcB*, and the non-coding region between the two genes with the omega cassette isolated from pDW9 conferring Sm/Sp resistance [20]. Transformation of wild-type *Synechocystis* 6803 with a linearized non-replicating plasmid containing this construction, selecting for resistance to Sm and Sp, yielded a greenish transformant, ΔAB, which was homozygous for the deleted *apcAB* operon. The physical map of the *apcABC* operon in *Synechocystis* 6714 and the construction of the *apcAB* deletion plasmid are shown in Fig. 1A. Fig. 1B shows the Southern hybridization pattern of DNA from wild-type *Synechocystis* 6803, ΔAB and ΔE probed with a *Hin*dII fragment containing the *apcABC* operon, confirming the deletion/insertion in the ΔAB strain. The 1 and 0.4 kb *Hin*dIII bands seen in the wild type (and in ΔE) were replaced, as expected, by new 0.3 and 0.55 kb bands in the ΔAB mutant.

The *apcE* deletion plasmid, pES2, was constructed by replacing an internal 0.5 kb *Sma*I fragment of the *Synechocystis* 6714 *apcE* gene with the omega cassette. Trans-

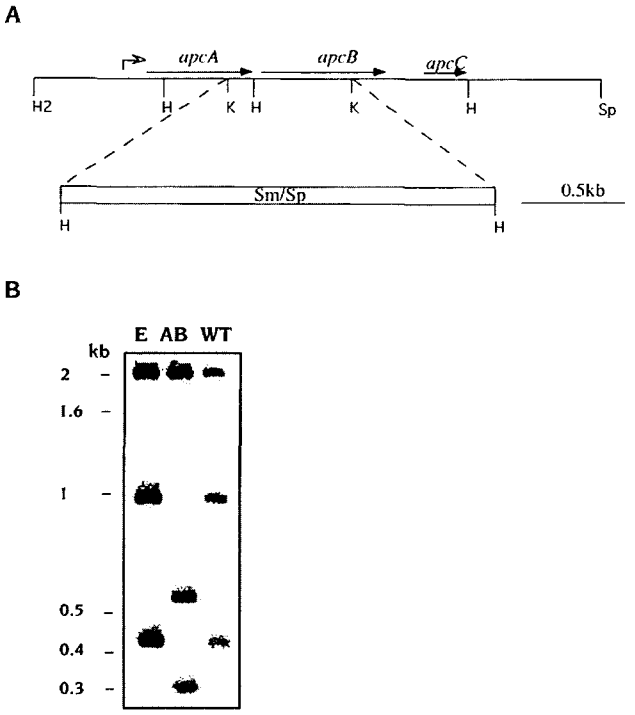

Fig. 1. (A) Physical map of a 2.6 kb *Hin*dII-*Sph*I fragment of DNA from *Synechocystis* 6714, carrying the *apcABC* genes and the transcription initiation site of the operon (bent arrow) [14]. The second line shows the position of the cassette in the deletion plasmid pABS2, in which the 580 bp *Kpn*I fragment containing most of *apcB* and the end of *apcA* is replaced by a Sm^r/Sp^r cassette. The cassette introduces new *Hin*dIII sites near its ends. (B) Southern hybridization of DNA from mutant ΔE (E), mutant ΔAB (AB) and from the wild-type *Synechocystis* 6803 (WT) cut with *Hin*dIII and probed with a *Hin*dII fragment containing the entire *apcABC* operon. Note that the mutant ΔAB retains none of the wild-type bands at 1 and 0.4 kb (lanes WT and E), which are replaced by new 0.3 and 0.55 kb bands (lane AB) due to the introduction of new *Hin*dIII sites by the inactivating cassette. This clean result is possible because *Synechocystis* 6714 and 6803 have the same *Hin*dIII sites in their *apcAC* genes. Abbreviations: H2 = *Hin*dII, H = *Hin*dIII, K = *Kpn*I, Sp = *Sph*I

formation of wild-type *Synechocystis* 6803 with this deletion plasmid yielded a greenish Sm and Sp resistant transformant, ΔE, which was homozygous for the deleted *apcE* gene. The physical map of the *apcE* gene from *Synechocystis* 6714 and the construction of the *apcE* deletion plasmid are shown in Fig. 2A. Fig. 2B shows the Southern hybridization of DNA from wild-type *Synechocystis* 6803, ΔAB and ΔE with an *apcE* probe, confirming the deletion/insertion in the mutant strain.

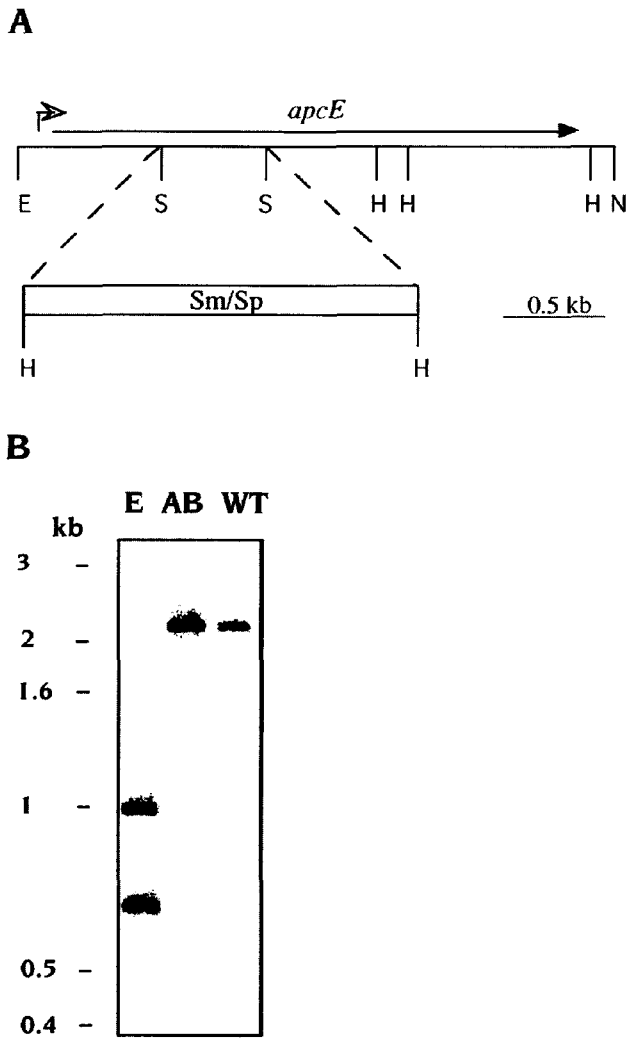

Fig. 2. (A) Physical map of a 3 kb *Eco*RV-*Nae*I fragment of DNA from *Synechocystis* 6714 containing the *apcE* gene and its transcription initiation site (bent arrow) [14]. The second line shows the position of the cassette in the deletion plasmid pES2, in which the internal 535 bp *Sma*I fragment is replaced with the same cassette used previously. (B) The same blot as in Fig. 1. B was probed with the 1.9 kb *Eco*RI-*Hin*dIII fragment that contains the left part of *apcE*. This probe recognizes a *Hin*dIII fragment at 2.2 kb in both wild type and ΔAB DNA (lanes WT and AB) and two fragments of 1.0 and 0.6 kb in the ΔE mutant DNA (lane E). The former correspond to a *Hin*dIII fragment to the left of the cassette and the latter *Hin*dIII fragment to the right of the cassette, in A above. The 2.2 kb fragment that contains most of the *apcE* gene in the wild-type is completely replaced by two smaller fragments, due to the introduction of new *Hin*dIII sites by the inactivating cassette. Abbreviations: same as in Fig. 1, but also S = *Sma*I, E = *Eco*RV, N = *Nae*I.

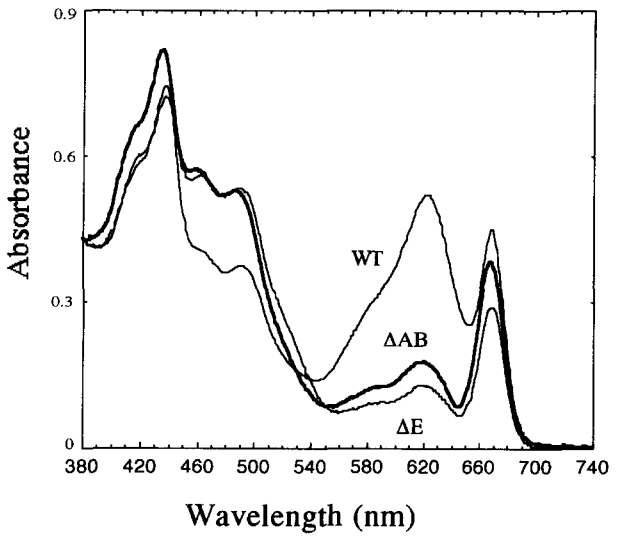

Fig. 3. Absorption spectra of cell-free lysates of wild-type *Synechocystis* 6803 and PBP-deficient mutants. Lysates were prepared as described in the text from suspensions at $5 \cdot 10^7$ cells per ml.

The same procedure was used to inactivate the *apcE* gene in PMB11 [9] (Southern not shown). The resulting mutant was called PΔE.

3.2. Growth and spectroscopic analysis of the phycobilisome core mutants

An immediate indication of the changed PBP composition in ΔAB and ΔE is their color: both are olive or greenish. Absorption spectra of cell-free lysates, prepared by cell disruption in a low salt phosphate buffer followed by incubation in 2% Triton X-100 (to avoid scattering) show that both mutants have a similar amount of chlorophyll but less phycocyanin and more carotenoid compared to the wild type (Fig. 3). In addition to the change in appearance, both strains grew autotrophically 6- to 7-fold slower than the wild type, i.e., with a doubling time of 48 h, under light intensities used here (3000 lux).

Chlorophyll fluorescence induction experiments in the presence of 10^{-5} M DCMU with 440 nm light, sensitising the chlorophylls, were performed with the mutants and the wild-type cells, at 10^7 cells/ml. All samples presented variable fluorescence (ΔF) reflecting a functional PS II. The maximal amplitude of ΔF were higher in the mutants than in the wild type. As the half time of rise (reflecting the PS II Chl antenna) were identical in the mutants and in the wild type, the increase of ΔF amplitude must be due to an increased PS II amount in the mutants compared to the wild type.

Low temperature (77 K) fluorescence emission spectra of whole cell *Synechocystis* 6803 wild-type, ΔAB, ΔE, PMB11 and PΔE were recorded. Cell samples were excited at 440 nm, which preferentially excites chlorophyll (Fig. 4A) or at 570 nm, which preferentially excites phyco-

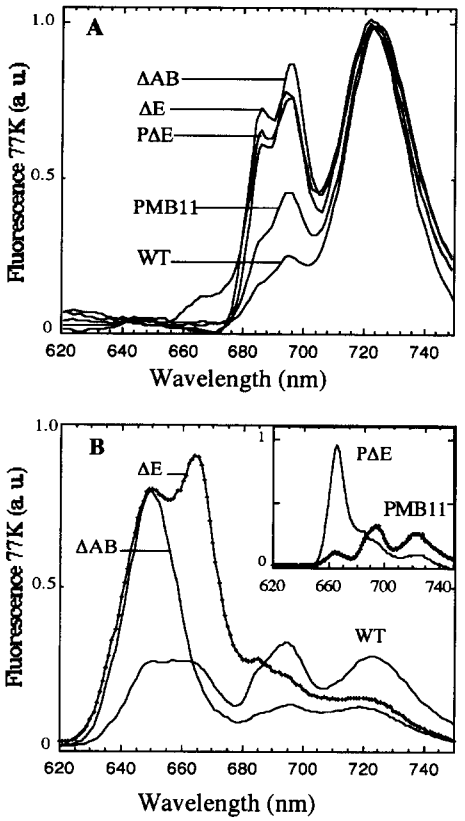

Fig. 4. 77 K fluorescence emission spectra of whole cell WT and PBP-deficient mutants. (A) Excitation at 440 nm, sensitising chlorophyll preferentially. Spectra are normalised at 720 nm. (B) Excitation at 570 nm, sensitising PBPs preferentially. The same cell amount was used for each sample. For better viewing, two mutant spectra were put into an inset.

biliproteins (Fig. 4B). Excitation at 440 nm induced PS II (685 and 695 nm) and PS I fluorescence (720 nm) in the wild type and all the mutants. This is consistent with the fact that all mutants are capable of photoautotrophic growth and that PS II is normally assembled in the absence of PC, AP or L_{CM}. All mutants present a greater PS II/PS I fluorescence ratio than the wild type. The same phenomenon was observed with isolated thylakoids (data not shown). Excitation at 570 nm (Fig. 4B) produced, in the wild-type, the emission bands that have been assigned to PC (650 nm), AP (665 nm), PS II (685 nm and 695 nm), and PS I (720 nm). The PMB 11 spectrum is shown for comparison (insert Fig. 4B): the PC band was absent as expected. In these two strains, the PBP fluorescence bands are low and PS II and PS I bands high. In contrast, ΔAB shows very high fluorescence emission from PC, ΔE very high fluorescence emission from both PC and AP and PΔE very high fluorescence emission from AP only. In these three mutants, there is no indication of energy transfer from PBPs to PS II. The emission bands from PS II and PS I are hardly visible, their amplitude corresponding to direct Chl excitation, similar to that obtained in thylakoids totally devoid of PBPs.

3.3. Analysis of wild-type and mutant phycobiliproteins

PBPs were isolated from wild-type *Synechocystis* 6803, ΔAB, and ΔE. Profiles of sucrose gradients containing material from the three strains are shown in Fig. 5A. The location of the bands in the sucrose gradients from wild-type *Synechocystis* 6803 are similar to those found in other published PBS preparations [8,18]. The sucrose gradients of preparations from ΔAB and ΔE did not contain a pigmented band at the 1.0 M sucrose level. However, three blue bands were seen in the ΔAB and four in the ΔE preparations at other sucrose concentrations: one band in the 0.25 M sucrose layer and two bands in the 0.5 M sucrose layer for ΔAB, two bands in the 0.25 M sucrose layer and two bands in the 0.5 M sucrose layer for ΔE.

In order to identify proteins present in the colored bands

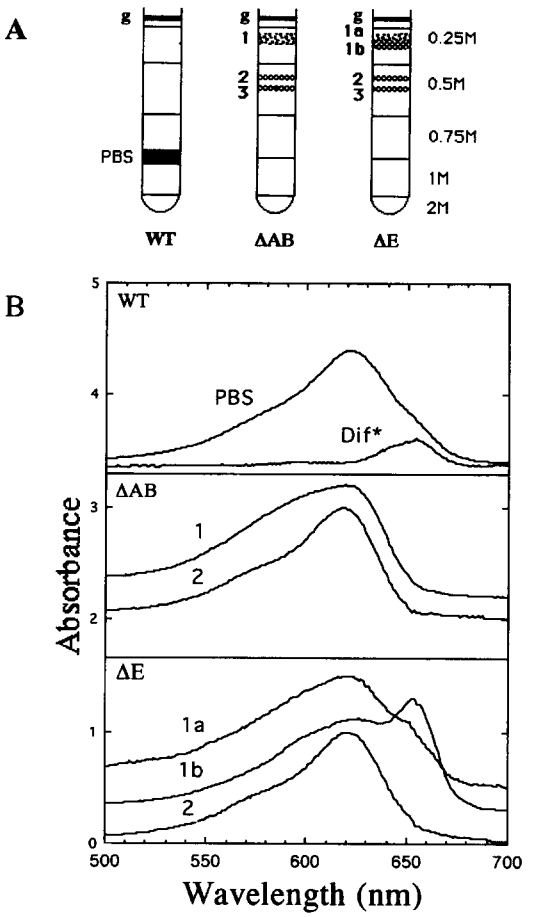

Fig. 5. (A) Sucrose gradient profiles of phycobiliprotein complexes from ΔAB, ΔE and wild-type *Synechocystis* 6803. Numbers on the left indicate the bands described in the text, PBS corresponds to phycobilisomes and g to a green band. Major polypeptide components of each layer are indicated in Table 1. (B) Absorption spectra of the sucrose gradient bands normalised at the absorption maxima. Numbers indicate the sucrose gradient bands shown in A. The spectrum called Dif* is the difference spectrum between the PBS and the band 2 spectra, showing a maximum at 650 nm. Band 3 spectra from both mutants are identical to those of band 2. The zero level of the upper curves was shifted for better viewing.

Table 1
Major polypeptide components of the sucrose gradient bands shown in Fig. 5 analyzed by SDS polyacrylamide gel electrophoresis

Strain	Band	Polypeptides							
		L_{CM}	L_R^{33}	L_R^{35}	L_{CR}^{27}	α^{PC}	β^{PC}	α^{AP}	β^{AP}
WT	PBS	+	+	+	+	+	+	+	+
ΔAB	1					+	+		
	2		+	+		+	+		
	3		+	+		+	+		
ΔE	1a					+	+		
	1b							+	+
	2		+	+		+	+		
	3		+	+		+	+		

The polypeptides listed are described in the text.

recovered from the ΔAB and ΔE gradients, an aliquot of each band (plus the 1.0 M band recovered from the wild-type *Synechocystis* 6803 sucrose gradient) was analysed by SDS polyacrylamide gel electrophoresis. The results are summarised in Table 1. The various proteins seen in wild-type PBS are assigned to the L_{CM} polypeptide (94 kDa), the L_R35 rod linker (35 kDa), the L_R33 rod linker (33 kDa), the $L_{CR}27$ core-rod linker (27 kDa), the α and β subunits of PC (17.6 kDa and 19.8 kDa), and the α and β subunits of AP (16.2 kDa and 18.8 kDa). Small polypeptides (< 10 kDa) were not resolved in our gel. A band seen at 48 kDa, present in all of our PBS preparations, has no known photosynthetic function and may or may not be an integral component of the PBS [8].

Table 1 also lists the proteins contained in the sucrose gradient bands isolated from ΔAB and ΔE. The α and β protein subunits of PC and the two linker polypeptides L_R33 and L_R35 were present in the two lower bands isolated from both mutant strains. The proteins found in the upper bands, however, were different: AP and PC were found in ΔE while only PC was found in ΔAB. The $L_{CR}27$ linker and the L_{CM} polypeptide were not seen in any of the colored complexes isolated from the mutant strains.

Room temperature absorption spectra of the gradient bands are shown in Fig. 5B. The bands 2 and 3 of both mutants had identical spectra, similar to wild-type PBS spectrum except the shoulder at 650 nm corresponding to the AP of the core. This is shown by the spectrum Dif * (PBS spectrum minus band 2 spectrum of ΔAB). The difference of absorption between 'free' PC (band 1) and assembled PC with linkers (bands 2 and 3) is clear in ΔAB, bands 2 and 3 are narrower than band 1. About 38% of the PC were assembled (24% in band 2 and 14% in band 3) the remaining 62% were unassembled (band 1), estimated by the 620 nm absorption of each band. In ΔE, bands 1a and 1b are slightly mixed, so there is some AP in the PC band (giving a shoulder at 650 nm) and some PC in the AP band increasing the 620 nm shoulder of AP.

Low temperature fluorescence emission spectra of the bands were also recorded. In ΔAB, 'free' PC (band 1) or assembled PC (band 2) have similar spectra, with a maximum at 650 nm ± 2 nm. In ΔE, AP (band 1b) has a maximum at 665 nm with a shoulder at 650 nm due to PC contamination. In the wild type, the PBS spectrum has a maximum at 682 nm due to the fluorescence of the terminal emitter(s) of the PBS, L_{CM} and/or $\alpha^{AP\text{-}B}$.

4. Discussion

In this work mutant strains were constructed and characterized in *Synechocystis* 6803 for the *apcAB* and *apcE* genes. In these mutants which lack the PBS, the 30–40 Chl associated with the PS II reaction center must assume the light-harvesting responsibility allowing photoautotrophic growth. It was also the case for similar mutants constructed in *Synechococcus* 7002 [7] and *Synechocystis* 6803 [10,11] in contrast to what was suggested for a *Synechococcus* 7942 mutant inactivated in the *apcE* gene [21].

4.1. Energy transfer

In the wild-type cells, light energy absorbed by PC is transferred to the PS II reaction center along the following pathway: PC to AP to the terminal emitters (L_{CM} or $\alpha^{AP\text{-}B}$) to Chl in PS II. At 77 K, the bands seen in the fluorescence emission spectrum of wild-type *Synechocystis* 6803, excited at 570 nm, have been assigned to PC (650 nm), AP (665 nm), PS II (685 nm and 695 nm) and PS I (720 nm) (Fig. 4B). PC and AP fluorescence intensities are low because of efficient energy transfer to the terminal emitters, the fluorescence of which is invisible due to energy transfer to PS II. In PMB 11 were the PBS has no PC rods but an intact core [8] the PC band is missing but AP is still transferring energy to PS II (Fig. 4B, inset).

When excited at 570 nm, ΔAB cells show a high fluorescence emission peak from PC, ΔE cells show high fluorescence emission peaks from PC and AP and PΔE cells show a high fluorescence emission peak from AP. The three mutants show very low emission from PS II and PS I. Therefore, we conclude that the PBPs do not transfer excitation energy to PS II nor to PS I in these mutants. The remaining small bands seen at 685, 695 and 720 nm are due to direct chlorophyll excitation since similar small bands were obtained with isolated thylakoids devoid of PBPs. This is in agreement with results obtained by Shen et al. [11] with an *apcE* deficient mutant of *Synechocystis* 6803. We were surprised to find some differences with an *apcA*-defective mutant of *Synechocystis* 6803 [10] which must be similar to our ΔAB mutant. Whereas the sucrose gradients of the isolated PBPs and the SDS polyacrylamide gel electrophoresis analyses were similar in both mutants, the emission spectra at 77 K were quite different: excitation at 570 nm produced much higher PS II and even more PS I emission fluorescence in the *apcA*-defective mutant

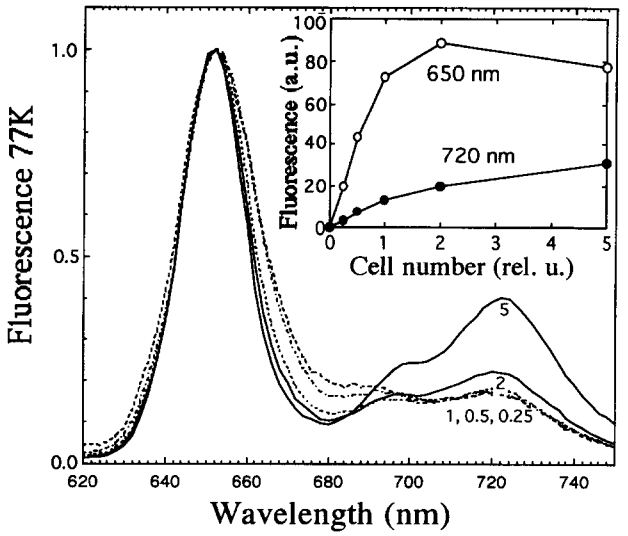

Fig. 6. 77 K Fluorescence emission spectra recorded with increasing amounts of ΔAB cells and normalised at 650 nm, the emission maximum. The insert shows a plot of the fluorescence intensity at 650 nm and 720 nm as a function of cell number in relative units. Assuming a cell diameter of about 2 μm [18], a monolayer corresponds to $8 \cdot 10^7$ cells per cm^2 (1.3 in relative units employed).

described in [10] than in our ΔAB mutant. The authors concluded that PS I was sensitised by PC in the mutant. This contradiction might be explained by the phenomenon of fluorescence reabsorption: fluorescence emission bands can be quenched by reabsorption in samples possessing absorption bands at the same wavelengths, this occurs specially when samples are concentrated. To explore this possibility, we recorded several spectra with various cell amounts of the ΔAB mutant. Fig. 6 shows the spectra, normalised at 650 nm, and the insert shows the relationship between fluorescence intensity and cell number for the two emission wavelengths 650 nm and 720 nm. The short-wavelength bands are reabsorbed more than higher wavelength bands, producing, at high cell concentration, spectra identical to that presented by Su et al. [10]. In their device, the samples were placed in tubes where cells, even diluted, can aggregate during freezing, whereas in our apparatus, cells were deposited as a monolayer unless we deliberately put a larger number of cells. The fluorescences at 650 and at 720 nm are proportional to cell number only below a monolayer. Furthermore, we show that in the emission spectrum of the ΔAB mutant excited at 570 nm, there is a large contribution from the PC peak at 720 nm, not due to PS I emission. As shown for other PBP mutants [11,22], a PBP band in the excitation spectrum for 720 nm emission does not automatically imply energy transfer from PBPs to PSI.

When wild-type cells were excited at 440 nm, PS II (685 and 695 nm) and PS I (720 nm) fluorescence were observed, PSI fluorescence being much higher than PS II (Fig. 4A).

In ΔAB, ΔE and PΔE, fluorescence emission spectra excited at 440 nm show PS II and PS I fluorescence bands. PS II band intensities relative to PS I band intensity are higher than in the wild type. The same phenomenon was observed for PMB11 but with a lower amplitude because this PC-deficient mutant still has an intact core. Fluorescence spectra of isolated thylakoids showed PS II/PS I ratio similar to those found with whole cells. Room temperature fluorescence induction experiments, in the presence of DCMU, show that the Chl antenna size in the wild type and the mutants were similar whereas the ΔF maximum amplitude were higher in the mutants. We therefore conclude that there is an increased amount of PS II centers, relative to PS I, in the PBP deficient mutants, in order to compensate for the decreased size of the PS II antenna. This is in agreement with results obtained in previously studied PBP deficient strains, *Cyanidium caldarium* [23] and *Synechocystis* sp. BO 8402 [24]. A PS II/PS I increase was observed by Fujita and Murakami [25] in *Synechocystis* 6714 grown under chlorophyll light (red light) preferentially sensitising PS I compared to cells grown under PBS light (orange light) preferentially sensitising PS II. Our cultures were grown under white light, but we consider that the mutants are grown under Chl light preferentially sensitising PS I in contrast to the wild type where both PS II and PS I were sensitised This regulation phenomenon was not observed in the *apcE* defective mutant studied by Shen et al. [11] where PS II/PS I (measured by either herbicide binding or oxygen evolution) was reported to be unchanged. This discrepancy might be due to the fact that their mutant was grown in the presence of glucose. Under photoheterotrophic conditions, glucose might diminish the need for a fully active PS II by providing reducing equivalents to PS I through the cytochrome b_6f complex.

4.2. PBS assembly

The proteins contained in the 1.0 M sucrose layer isolated from wild-type *Synechocystis* 6803 (Fig. 5 and Table 1) are identical to those observed by Elmorjani et al. [8]: the α and β protomers of PC and AP, the L_{CM} polypeptide, the linker polypeptides of the PC rods L_R^{33} and L_R^{35}, responsible for PC rod organisation and the L_{CR}^{27} polypeptide, which attaches the PC rods to the core [26,27].

Intact PBS are not produced in either mutant strain. However, in preparations from both ΔAB and ΔE, the lower two bands (0.5 M sucrose) recovered from the gradients contain PC and the rod linker polypeptides, L_R^{33} and L_R^{35} (Table 1). Analysis of the absorption spectra of these bands (Fig. 5B) showed that they lack the 650 nm absorption shoulder corresponding to the core in the intact PBS spectrum (Dif * corresponding to the difference between the two spectra). The fluorescence emission spectrum of these PC rods has a maximum at 650 nm typical of PC, showing that no AP (fluorescing at 665 nm), $\alpha^{AP\text{-}B}$ or L_{CM} (fluorescing at 680 nm–683 nm) were associated with

them. Renaturation experiments performed on highly purified PC from *Synechococcus* 6301 showed that fractionation of a mixture containing PC and the L_R^{33} and L_R^{35} linker polypeptides in a sucrose gradient yielded two high molecular weight bands [28]. The upper band was found to contain short rods and discs, while the lower band was enriched in long rods. Therefore, we believe that the two lower bands observed in the ΔAB and ΔE preparations in the 0.5 M sucrose layer contain complexes similar to those observed in the *Synechococcus* 6301 renaturation experiments. The same results and conclusion were reached by Su et al. for an *apcA*-defective mutant of *Synechocystis* 6803 [10].

Bands found in the 0.25 M sucrose layer contain unassembled PBPs. This can be explained by the fact that highly purified PBPs do not assemble into structures larger than trimers or hexamers in the absence of linker proteins. They have low sedimentation rates in sucrose gradients. The SDS polyacrylamide gels, summarised in Table 1, as well as absorption and fluorescence spectra show that there is free PC in ΔAB band 1, and free PC and AP in ΔE band 1a and 1b, respectively.

Neither the L_{CM} polypeptide nor the L_{CR}^{27} linker polypeptide was observed in any of the colored bands isolated from ΔE and ΔAB. Su et al found hardly any L_{CM} in cell extracts from an *apcA* mutant, using antibodies [10]. It seems likely that the linker polypeptides are degraded when they are not assembled into PBS.

In conclusion, both ΔAB and ΔE mutants are able to assemble PC rods, but are unable to assemble a core. For ΔAB, this is obvious since AP is a major component of the core cylinders. For ΔE, L_{CM} is a minor component but it has a key role for the core assembly as well as the terminal energy acceptor. While in the absence of the PC (PMB11 mutant) AP is still assembled into cores and transfers energy to PS II, in the absence of either the AP or the L_{CM} the remaining PBPs (even assembled into rods) do not transfer excitation energy to any of the photosystems.

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