Under Light Limiting Growth, CpcB Lyase Null Mutants of the Cyanobacterium *Synechococcus* sp. PCC 7002 Are Capable of Producing Pigmented β Phycocyanin but with Altered Chromophore Function

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ABSTRACT: Phycobilisomes are the major light-harvesting complexes for cyanobacteria, and phycocyanin is the primary phycobiliprotein of the phycobilisome rod. Phycocyanobilin chromophores are covalently bonded to the phycocyanin β subunit (CpcB) by specific lyases which have been recently identified in the cyanobacterium *Synechococcus* sp. PCC 7002. Surprisingly, we found that mutants missing the CpcB lyases were nevertheless capable of producing pigmented phycocyanin when grown under low-light conditions. Absorbance measurements at 10 K revealed the energy states of the β phycocyanin chromophores to be slightly shifted, and 77 K steady state fluorescence emission spectroscopy showed that excitation energy transfer involving the targeted chromophores was disrupted. This evidence indicates that the position of the phycocyanobilin chromophore within the binding domain of the phycocyanin β subunit had been modified. We hypothesize that alternate, less specific lyases are able to add chromophores, with varying effectiveness, to the β binding sites.

The phycobilisome (PBS),[1] a large phycobilin−protein complex associated with the cytoplasmic face of the thylakoid membrane, serves as the major accessory light-harvesting complex in cyanobacteria in a manner analogous to that of the family of intrinsic thylakoid membrane Chl *a*/*b*-containing light-harvesting complex polypeptides in higher plants (*1–4*). PBS are predominantly associated with photosystem II (PSII) (*1, 5*) yet also contribute significantly to the antennae of photosystem I (PSI) (*6, 7*). The typical hemidiscoidal PBS is composed of allophycocyanin (APC) containing core cylinders with hexameric disks of higher-energy phycobiliproteins radiating outward (*8*). The core-proximal disks always contain phycocyanin (PC), while subsequent disks may contain other phycobiliproteins such as phycoerythrocyanin. Colorless linker polypeptides serve to structurally link the phycobiliprotein subunits together and also to modify the energy levels of specific bilins to ensure proper energy flow toward the PBS core. The so-called terminal emitters (TE) of the core can then funnel this energy toward photosystem Chl *a* (*2, 6, 9*).

The PC monomer is composed of one α (CpcA) and one β (CpcB) subunit. The head-to-tail association of three monomers forms a trimer [$(\alpha\beta)_3$]. The face-to-face aggregation of two trimers forms a hexamer/disk [$(\alpha\beta)_6$], which is the functional oligomerization state. The PC-associated linker polypeptides of *Synechococcus* sp. PCC 7002 are CpcD/$L_R^{8.9}$, located at the distal end of the distal hexamer (terminates rod); CpcC/$L_R^{32.3}$, located between PC hexamer stacks; and CpcG/$L_{RC}^{28.5}$, positioned at the rod−core junction (*10, 11*).

Phycocyanobilin (PCB) is the phycobilin (bilin/chromophore) of PC and APC. PCB is bound to cysteinyl residues of the apoprotein via a single thioether linkage (*12*). The PC α subunit contains a single chromophore at position 84 (α84); the β subunit contains two chromophores, one at position 82 (β82) and one at position 153 (β153).

There are two main pathways for energy flow within the PC hexamer. Energy transfer occurs between β153 chromophores in adjacent trimers and between α84 chromophores in adjacent trimers, with the β82 chromophores functioning as an energy pool in rapid equilibrium with the α84 chromophores. Linker-associated β82 serves as an energy sink for individual hexamers and transfers this energy down the rod to the next PC hexamer or, for the core proximal hexamer, to APC of the core (*13–20*). Exclusive β153 energy transfer between adjoining hexamers is also probable (*17*).

The attachment of phycobilins to apoprotein has been shown to be mediated by lyases (*21–28*). It appears that specific enzymes are required for bilin attachment at specific cysteine residue positions. However, spontaneous addition of bilin to PC has been shown to occur in genetically engineered *Escherichia coli* with different reactivities described for each of the three bilin attachment sites in PC (*21*), and other studies have shown mesobiliverdin adducts capable of attaching to all three sites at high PCB concentrations (*23, 26*). The stereochemistry of β82- and α84-bound PCB is *R* (*R* configuration at C-3[1] of PCB), whereas the stereochemistry of β153-bound PCB is *S* (*29*), suggesting that at least two different enzymes would be required.

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[1] Abbreviations: PBS, phycobilisome(s); PSII, photosystem II; PSI, photosystem I; APC, allophycocyanin; PC, (C-)phycocyanin; TE, terminal emitters, the long wavelength chromophores of the PBS core; PCB, phycocyanobilin; PEC, phycoerythrocyanin; WT, wild-type.

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The enzyme mediating CpcA-84 bilin attachment has been identified in several cyanobacteria (*30, 22*). It is composed of two different subunits, CpcE and CpcF, which are often encoded on the PC operon (*31–33*). Similar genes have been found for attachment of phycoerythrobilin to the α84 site in phycoerythrocyanin (PEC) (*34, 35*). In *Nostoc* (*Anabaena*) sp. PCC 7120, CpeS (CpeS1) has been identified as a lyase for attachment of PCB to β82 in PC and PEC (*23–25*). However, in *Synechococcus* sp. PCC 7002, a heterodimer of CpcS (CpcS-I) and CpcU function together as the lyase for attachment of PCB to CpcB-82 (*27*). This difference in lyase protein may have a phylogenetic basis (*27*). The CpcS lyases also catalyze APC chromophorylation in these cyanobacteria (*25, 27*). Recently, a new class of bilin lyase, CpcT, has been identified as being able to specifically attach PCB to the CpcB-153 site (*26*). *cpcT*- and *cpeT*-like genes are present in the genomes of all sequenced cyanobacteria that contain β153 equivalent chromophore positions, suggesting that this class of lyase is responsible for β153 chromophorylation (*26*).

PCB:PC lyases may not be as site specific as elucidated from PCB addition assays completed either *in vitro* or in genetically engineered *E. coli* (*23, 25, 26*). *cpcEF* inactivation mutants in *Synechococcus* sp. PCC 7002 and *Synechococcus* sp. PCC 7942 were still capable of producing bilin-replete CpcA (*22, 32*), and fully chromophorylated CpcB could be detected in a *cpcSU* inactivation mutant of *Synechococcus* sp. PCC 7002 (*27*), in all suggesting a level of interchangeability between the targeted bilin lyase and additional bilin lyase(s) within the cyanobacterial cells. The lyases responsible for cysteine-82(84) bilin attachment are presumably somewhat interchangeable (*22, 26, 27*), because they both attach PCB to cysteine 82 in the *R* conformation (*29*).

In *Synechococcus* sp. PCC 7002, three genes have been identified as being primarily involved in CpcB bilin attachment. *cpcT* encodes a PC lyase for attachment of PCB to β153 (*26*); *cpcS* and *cpcU* encode a PC lyase for attachment of PCB to β82, which is also involved in attachment of PCB to APC subunits (*27*). Another member of the CpcS/CpeS protein family, CpcV, has also been identified in *Synechococcus* sp. PCC 7002 (*27*). CpcV has strong structural divergence from CpcS/CpcU, and its function is currently unknown. A *cpcV* single mutant had no visible phenotype, and its phycobiliprotein levels were unaffected (*27*).

Mutant strains CpcTV$^-$, CpcSU$^-$, and CpcSUT$^-$ are missing the lyases which mediate bilin attachment at β153, β82, and both β82 and β153, respectively (*26, 27*). Under high-light growth conditions (250 μmol m^{-2} s^{-1}), where photon flux is not limiting, these strains produce PC missing chromophore attachment at the targeted bilin-binding sites and show significant decreases in PC content (*26, 27*). However, in the study presented here, we found that substantial amounts of bilin-replete PC, including the β-linked chromophores, can be produced under low-light growth conditions. To characterize the chromophore functionality of such PC, a strictly *in vivo* spectroscopic approach was taken. Absorbance and emission were measured at low temperatures to look for changes in chromophore energy levels and energy transfer involving PC. Our results show that the β PC bilins in the CpcB lyase null mutants have altered energy levels and that the resulting PCs are characterized by decreased energy transfer efficiency both within rods and from rods to the APC core. In spite of this, the mutant cells are capable of autotrophic growth. We present a hypothesis based on chromophorylation of the CpcB bilin binding sites by alternate, less specific PCB lyases.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions. Synechococcus sp. PCC 7002 wild type (WT) and the derived mutant strains CpcTV$^-$, CpcSU$^-$, CpcSUT$^-$, and CpcC$^-$ were grown photoautotrophically in liquid A$^+$ medium (*36*). The CpcC$^-$ strain is lacking the structural PC linker polypeptides $L_R^{32.3}$ and $L_R^{8.9}$ (*10*). All mutant cultures were supplemented with 100 μg/mL kanamycin. Additionally, the CpcTV$^-$ and CpcSU$^-$ cultures were supplemented with 50 μg/mL gentamicin, and the CpcSUT$^-$ culture was supplemented with 50 μg/mL gentamicin and 50 μg/mL spectinomycin. Cultures were grown in clear glass tubes (3.5 cm diameter × 30 cm long), continuously bubbled with air, and continuously illuminated with light provided by fluorescent bulbs at an intensity of 60 μmol m^{-2} s^{-1}. A water bath was used to maintain cultures at a constant temperature of 38 °C.

Before collection of culture samples for experimental analysis, the cultures were maintained at mid-late log phase for at least 1 week (or 3 growth cycles) by continuously draining some culture from the growth tube and adding fresh growth medium. Cultures were not allowed to reach cell densities over an OD_{730} of ≈0.5 to minimize self-shading. OD_{730} measurements were taken with a Spectronic 20 spectrophotometer (Bausch and Lomb, Rochester, NY). All cells were collected at mid-late log phase for experimental analysis.

Room-Temperature Absorbance Spectroscopy and Pigment Content Analysis. Room-temperature absorption spectra were recorded on a DW-2 spectrophotometer equipped with computer-driven Olis data acquisition (Aminco, Silver Springs, MD) using a 1 cm path length quartz cuvette. A frosted glass plate was located directly behind the sample holder to compensate for light scattering of the sample. The slit width was set to 0.5 nm. Cell solutions were diluted with A$^+$ to a concentration at which OD_{440} equaled 0.7–0.8. Samples from six separate cultures were averaged. Chl *a* content and PCB content were calculated from whole cell absorption spectra using the method of Myers et al. (*37*); however, PCB concentrations instead of PC concentrations are reported, which was also done in refs *9* and *38*. Chl *a* and PCB concentrations were normalized to OD_{730} as an indicator of cell density (*39*). PC content and APC content were calculated from absorption spectra of methanol-washed cell extracts according to the method of MacColl and Guard-Friar (*40*).

Low-Temperature Absorbance Spectroscopy. Cell samples for low-temperature analysis had room-temperature absorption spectra within the error of the above-mentioned averaged spectra. A home-built absorption spectrometer was used to collect the data. It consisted of a 150 W halogen lamp as a light source and a spectrograph composed of a Triax 320 monochromator equipped with a liquid nitrogen-cooled Symphony CCD detector using SynerJY software for data acquisition (HORIBA Jobin Yvon Inc., Edison, NJ). A HC-2 compressed helium cryostat was used to cool samples to 10 K (APD Cryogenics Inc., Allentown, PA). Samples were held

between two polycarbonate disks sandwiching a silicon−rubber gasket. The distal (from the light source) polycarbonate disk was etched to compensate for light scattering of the sample. The spectrograph entrance slit width was 0.006 mm.

Steady State Fluorescence Emission Spectroscopy at 77 K. Dark-adapted (15 min) 0.5 mL cell pellet/A^+ suspensions with an OD_{730} of 1 were exposed to blue light (as defined by a Hoya glass B440 bandpass filter, HOYA Corp. USA, San Jose, CA) at an intensity of 50 μmol m^{-2} s^{-1} for 5 min to drive the cells to state 1. Ten milliliters of 1 M phosphate buffer (KH_2PO_4, pH 7.8) was then added to inhibit state transitions and stabilize the cells in state 1 (*41*). The addition of phosphate buffer served to increase the relative yield of phycobilin-sensitized PSII-associated Chl *a* fluorescence but did not change any emission or excitation band positions in either WT or mutant samples. Aliquots (0.2 mL) were then quickly transferred to sample tubes made from 5 mm outer diameter × 3.5 mm inner diameter borosilicate glass before being quickly frozen in liquid nitrogen. Samples were assayed for 77 K fluorescence emission with a purpose-built fluorescence spectrophotometer as previously described by Salehian and Bruce (*42*) except the emission side spectrograph was the same as that described in Low-Temperature Absorbance Spectroscopy. The excitation bandwidth was 2 nm, and the spectrograph entrance slit width was 0.1 mm. Samples were illuminated with light with an intensity of 3.5 μmol m^{-2} s^{-1} at all excitation wavelengths. The detector was protected with a Hoya glass R-60 long-pass sharp cut filter; for the collection of excitation spectra, the detector was further protected with a Hoya R-66 long-pass sharp cut filter. To compensate for the random scattering and heterogeneity associated with frozen samples, the sample tubes were continuously spun during data collection using a compressed air-powered rotor. Errors in fluorescence yield from the same sample in different tubes were within 3% using this method. The same cell captures used for the 10 K absorbance measurements were used for the 77 K emission measurements.

Spectral Fourth-Derivative Analysis. Fourth derivatives were taken of absorption and emission spectra using Synergy (HORIBA Jobin Yvon Inc.). For absorption spectra, differentiation intervals were varied according to chromophore type of interest; phycobilin absorbance peaks are characterized as having larger full widths at half-maximum than Chl *a* absorbance peaks.

RESULTS

The CpcB lyase null mutations, $CpcTV^-$, $CpcSU^-$, and $CpcSUT^-$, and the PC linker mutation, $CpcC^-$, were disrupting to cell growth. The mutants grew very poorly under high-light conditions (150–250 μmol m^{-2} s^{-1}), where WT grows optimally. Although the mutants grew much better under low-light conditions (60 μmol m^{-2} s^{-1}), growth was still ~2.5 times slower than WT growth. To increase the magnitude of the PC "signal", when collecting whole cell absorption and emission spectra, low-light growth conditions (60 μmol m^{-2} s^{-1}) were employed throughout the study. Low-light growth conditions increase cellular PC content by increasing PBS rod length (*43*).

Pigment Content. The whole cell room-temperature absorbance spectra are shown in Figure 1. Cellular Chl *a* and

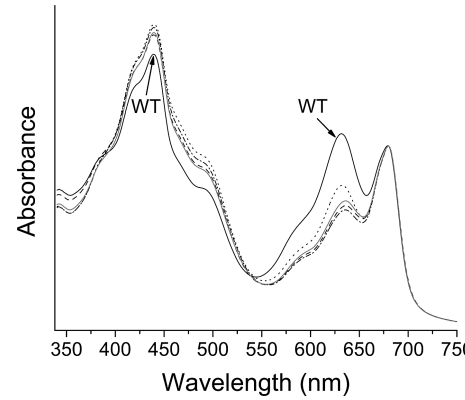

FIGURE 1: Whole cell room-temperature absorption spectra for WT (solid black line) *Synechococcus* sp. PCC 7002 and derived mutant strains $CpcTV^-$ (dashed line), $CpcSU^-$ (dotted line), $CpcSUT^-$ (dashed−dotted line), and $CpcC^-$ (solid gray line). Shown are the average spectra from six separate cultures. The standard error was within ±1.5% at absorbance peaks for all strains. Spectra are normalized to the Chl *a* Q_y absorption band at 680 nm.

Table 1: Pigment Content for *Synechococcus* sp. PCC 7002 Strains

	cellular pigment content[a] (M × OD_{730}^{-1})		% decrease in phycobiliprotein content[b]	
strain	Chl *a* (×10^{-5})	PCB (×10^{-5})	PC	APC
WT	2.50 ± 0.10	1.26 ± 0.07	−	−
$CpcTV^-$	2.59 ± 0.11	0.61 ± 0.05	49.3	1.74
$CpcSU^-$	2.53 ± 0.17	0.79 ± 0.07	24.1	6.79
$CpcSUT^-$	2.57 ± 0.13	0.55 ± 0.04	48.5	3.74
$CpcC^-$	2.59 ± 0.16	0.62 ± 0.04	46.8	0.00

[a] Chl *a* and phycocyanobilin (PCB) concentrations were calculated from whole cell absorption spectra according to the method of Myers et al. (*37*) and normalized to OD_{730} as an indicator of cell density. The standard error among six separate cultures is given. [b] Values are based on the percentage of the WT level and were calculated from absorbance assays on cellular phycobiliprotein extracts according to the method of ref *40*.

PCB concentrations for the various strains were calculated from the spectra (see Experimental Procedures) and are given in Table 1. The Cpc^- mutations induce significant decreases in PCB content but not in Chl *a* content. Spectra of the mutants clearly show a substantial decrease in phycobilin absorbance from 550 to 650 nm, and the PC absorbance peak at ~630 nm is also slightly red-shifted. The increase in absorbance from 425 to 530 nm in the mutant strains indicates an increase in carotenoid content. Absorbance assays with cellular phycobiliprotein extracts (Table 1; see Experimental Procedures for details) revealed that the majority of the PCB decrease in the Cpc^- strains is linked to PC.

Low-Temperature Absorbance. To identify the chromophores responsible for the altered phycobilin absorbance observed within the Cpc^- strains, 10 K absorbance measurements were taken. At low temperatures, absorbance bands are narrowed due to decreases in temperature-dependent random oscillation of the pigments' electrostatic field allowing room-temperature absorbance bands to be often split into their constituents (*44*). As seen in Figure 2A, 10 K absorption spectra show much more structure than those observed at room temperature (Figure 1). Absorbance from 660 nm outward is dominated by Chl *a*-containing protein complexes (the photosystems); phycobilins of the PBS dominate absorbance from 550 to 655 nm. The strong shoulder at ~651 nm largely belongs to APC of the PBS

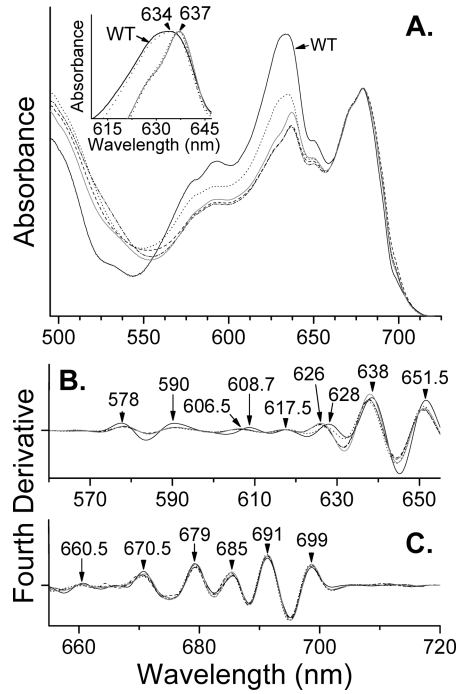

FIGURE 2: Absorbance at 10 K for WT (solid black lines) *Synechococcus* sp. PCC 7002 and mutant strains CpcTV$^-$ (dashed lines), CpcSU$^-$ (dotted lines), CpcSUT$^-$ (dashed−dotted lines), and CpcC$^-$ (solid gray lines). Whole cells were suspended in 2.5 M sucrose. (A) Absorption spectra normalized to the Chl *a* Q_y absorption band at 679 nm. The inset shows spectra normalized at PC absorbance peaks for ease of comparison. (B) Fourth derivative of spectra from 560 to 655 nm, which is dominated by phycobilin absorbance. Differentiation interval of 10 nm. (C) Fourth derivative of spectra from 655 to 720 nm, which is dominated by Chl *a* absorbance. Differentiation interval of 5 nm.

core, and the major peaks around ~630 nm belong to PC. It is clear that the Cpc$^-$ strains show less PC absorbance. The position and shape of the PC absorbance peaks from ~620 to 640 nm also varies among the strains (Figure 2A inset); the CpcTV$^-$, -SUT$^-$, and -TV$^-$ strains are characterized by a 3 nm red shift in absorbance maxima and a preferential loss of absorbance at ~625 nm relative to that of WT. Difference absorption spectra calculated by subtracting Cpc$^-$ strain absorbance from WT absorbance gave a strong peak at 631 nm for all the mutant strains (data not shown).

To identify peak shifts among individual phycobilin absorbance bands, fourth derivatives were taken of the spectra. Fourth-derivative analysis is sensitive to changes in spectral shape associated with changes in peak location (*44*). The CpcB lyase$^-$ and CpcC$^-$ strains show blue shifting in the location of several phycobilin-associated absorbance bands, particularly for those belonging to the β bilins: β153 at ~609 nm, L_{RC}-associated β82 at 638 nm (*11*), and the α84−β82 excitonic splitting band at 628 nm (*11*) (Figure 2B). The CpcB lyase$^-$ mutations do not directly target the α84 chromophore because there is no change in the position of the noninteracting α84 band at 617.5 nm (*15*). There are also no observable band shifts for the fourth-derivative analysis of the Chl *a* absorbance region (Figure 2C). Complete 10 K absorbance peak assignments are listed in Table 1 of the Supporting Information.

The ~50% decrease in PC absorbance found in the CpcTV$^-$, -SUT$^-$, and -C$^-$ strains is accompanied by only small changes in APC absorbance (Table 1). To take into account this half-reduction in PC:APC stoichiometry, the dominant PBS populations within these strains would either (i) contain PBS having a rod length of one disk, as apposed to the standard rod length of two discs (*10*), or (ii) have standard size PBS missing roughly half of their PC chromophores. Though further biochemical analysis of isolated PBS would be required to completely distinguish between the two possibilities, the 10 K absorbance spectra tend to push toward scenario (i). Under low light levels, WT *Synechococcus* sp. PCC 7002 cells predominantly contain PBS with a rod length of two disks; conversely, the CpcC$^-$ strain has a phenotype of PBS rods of only one disk in length (*10*). Since the CpcTV$^-$ and -SUT$^-$ strains have a spectral shape identical to that of the CpcC$^-$ strain in the spectral region associated with PC disk absorbance maxima (Figure 2A inset) and exhibit the same shifting in PC absorbance bands (Figure 2B), we stipulate that the CpcTV$^-$ and -SUT$^-$ strains also predominantly contain PBS of one disk rod length. For further evidence, the preferential decrease in the absorbance of the outer disks, associated with $L_R^{32.3}$, at 625 nm relative to the absorbance of the inner disks, associated with $L_{RC}^{28.5}$, at 637 nm (*10, 11*) suggests that there is a specific loss in outer disk PC in the CpcC$^-$, -TV$^-$, and -SUT$^-$ strains (Figure 2A inset).

Low-Temperature Fluorescence Emission. Steady state fluorescence emission spectra were collected at 77 K from whole cells with preferential excitation of both Chl *a* (Figure 3A) and phycobilins (Figure 3B) to characterize energy transfer among the PBS chromophores and thylakoid Chl *a*. Three major emission peaks are visible with excitation of Chl *a* with 435 nm excitation (Figure 3A). The 715 nm peak (F715) originates from the long wavelength Chl *a* of PSI (*45*); the 695 nm peak (F695) originates from the long wavelength Chl *a* of the CP47 protein of PSII (*46*), and the 685 nm peak (F685) is largely attributed to Chl *a* of the PSII core (*46*). Since all strains contain equal amounts of Chl *a*, relative photosystem stoichiometry can be estimated from the 435 nm excitation–emission spectra by comparing the fluorescence intensity at F695 to the fluorescence intensity at F715 (*47*). An increase in the relative amount of PSII is a typical response to a decreased effective PBS antenna size in *Synechococcus* sp. PCC 7002 (*26, 27, 39*). We found that two of the Cpc$^-$ strains exhibited a small increase in PSII and two a small decrease, relative to the WT value. Fourth-derivative analysis of the spectra revealed no shifts in emission band positions between the strains (Figure 3C), again indicating that the Cpc$^-$ mutations do not seem to directly affect Chl *a* chromophore function.

Upon PC excitation, the phycobilins are the major contributors to cell emission (Figure 3B) either by direct fluorescence or from energy transfer to photosystem Chl *a*. PC fluorescence components are visible from ~620 to 650 nm and non-TE APC fluorescence peaks at 665 nm. With phycobilin excitation, F685 is dominated by the TE of the PBS core. F715 values seem to be directly correlated with the PSI levels estimated from Figure 3A. The effects of the Cpc$^-$ mutations on the spectra are quite visible; there are significant increases in β153 emission at 624 nm and a loss in both APC emission at 665 nm and TE emission at 685 nm in relation to PC emission at 650 nm (Figure 3B). Recalling that the CpcTV$^-$, -SUT$^-$, and -C$^-$ strains have PC:APC levels decreased by approximately half (Table 1),

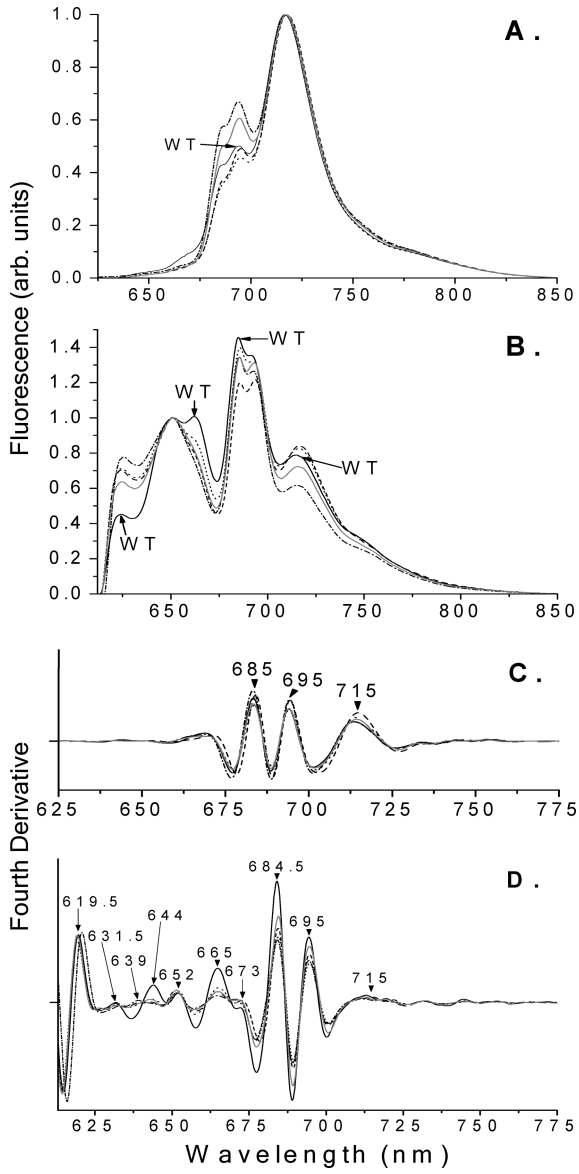

FIGURE 3: Whole cell 77 K steady state emission spectra for WT (solid black lines) *Synechococcus* sp. PCC 7002 and mutant strains CpcTV$^-$ (dashed lines), CpcSU$^-$ (dotted lines), CpcSUT$^-$ (dashed−dotted lines), and CpcC$^-$ (solid gray lines). Cells were suspended in 1 M phosphate buffer prior to being frozen. (A) Spectra collected after preferentially exciting Chl *a* at 435 nm. Spectra are normalized to the PSI emission peak at 715 nm for ease of comparison. (B) Spectra collected after preferentially exciting PC at 575 nm. Spectra are normalized to the PC emission peak at 651 nm for ease of comparison. (C and D) Fourth derivatives of panels A and B, respectively. Differentiation intervals of 7.5 nm.

we find that an increase in PC:APC emission over that of WT would be indicative of a substantial loss of energy transfer from PC to PBS core acceptor chromophores. Furthermore, an increase in β153 emission at 624 nm relative to linker-associated β82 emission at 650 nm (Figure 3B) suggests a loss in the level of transfer of energy away from β153 within the PC disk.

Fourth-derivative analysis of the emission spectra obtained with 575 nm excitation exposed no obvious shifts in the emission bands from photosystem Chl *a* at 695 and 715 nm, the TE ApcE at 684.5 nm, and APC at 665 nm among the strains (Figure 3D). However, there were several shifts in PC emission peak positions for the Cpc$^-$ strains. Most notably, the α84 emission band at 639 nm (*15*) is resolved in the CpcB lyase$^-$ strains, which is suggestive of a decrease in the level of transfer of energy away from the α84 bilin. Efficient α84-to-β82 energy transfer would result in little emission from the α84 chromophore, to such an extent that the fourth derivative would not detect the α84 emission peak, which was seen in WT. Since CpcC$^-$ is lacking the PC linker polypeptide $L_R^{32.3}$ (*10*), it would be expected to be missing the β83-$L_R^{32.3}$-attributed emission band at 644 nm (*11, 15*). In concert, there is an emission band at 642 nm in CpcC$^-$, as opposed to 644 nm, corresponding to non-linker-associated β83 emission (*13*). The highly resolved ∼620 nm β153 emission band (*15, 48*) is red-shifted by 1 nm in CpcSUT$^-$, and there may also be a 0.8 nm blue shift in the β82-$L_{RC}^{28.5}$ (652 nm) emission band (*11, 15*) in CpcC$^-$ and CpcTV$^-$. Assignments for all of the emission bands identified by fourth-derivative analysis, from both 435 and 575 nm excitation, are given in Table 2 of the Supporting Information.

To further investigate energy transfer pathways specifically involving PC-absorbed excitation energy, emission spectra were collected with excitation encompassing that of the PC/APC yellow/orange absorbance region. F685, F695, and F715 were monitored to determine what wavelengths of PC-absorbed light energy were reaching TE, PSII, and PSI, respectively (Figure 4A−C). The major loss in the contribution of PC-absorbed excitation energy to TE, PSII, and PSI emission within the mutant strains is linked to their losses in PC content. PC-absorbed excitation energy, as apposed to APC-absorbed excitation energy, preferentially excites PSI. This is clearly revealed by the enhancement in F715 when it is excited with light primarily absorbed by PC at ∼630 nm. The F715 excitation spectral peaks (Figure 4C) correspond to the PC absorbance peaks in Figure 2A. To detect which specific wavelengths of absorbed light energy were not reaching the terminal energy acceptors in the Cpc$^-$ strains, difference spectra were calculated by subtracting the excitation spectra of the Cpc$^-$ strains from that of WT (Figure 4D−F). The excitation difference peaks for F685 (Figure 4D) are at ∼625/626 nm for CpcTV$^-$, -SUT$^-$, and -C$^-$ and at ∼627/628 nm for CpcSU$^-$. The excitation difference peaks for F695 (Figure 4E) are at ∼625/626 nm for CpcTV$^-$, -SUT$^-$, and -C$^-$ and at ∼627/628 nm for CpcSU$^-$. The excitation difference peaks for F715 (Figure 4F) are at ∼625 nm for CpcTV$^-$, -SUT$^-$, and -C$^-$ and at ∼627 nm for CpcSU$^-$. Absorbance at 625–628 nm corresponds to the α84−β82 splitting bands within the mutant strains (Figure 2B). The slight red shift in the difference peaks for the CpcSU$^-$ strain corresponds to the red shift in the position of its α84−β82 band position (Figure 2B). The excitation difference peaks for all the mutant strains are blue-shifted over their absorbance difference peaks (631 nm, data not shown), suggesting that the loss of PC-absorbed excitation energy reaching the terminal acceptors is not simply due to the loss of PC absorbance induced by the mutations. Because the losses in excitation contribution are nondiscriminating on the terminal acceptor, whether it be TE, PSII, or PSI (Figure 4D−F), the disruption in energy transfer is occurring within the PBS.

DISCUSSION

If the CpcB lyase null mutations inhibited chromophorylation at the targeted bilin binding sites, corresponding

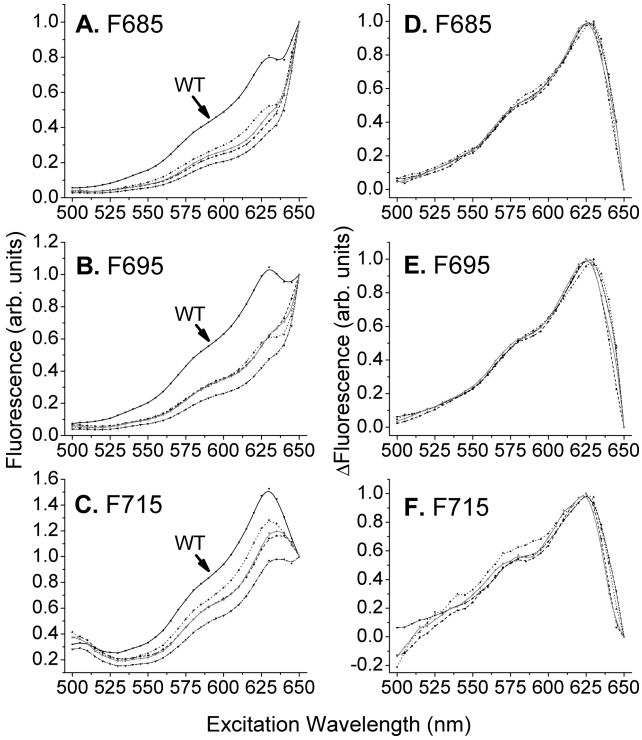

FIGURE 4: Differential phycobilin excitation contribution to terminal acceptor emission at 77 K. The fluorescence intensity was monitored from whole cells at the terminal acceptor emission peaks of F685 (A), F695 (B), and F715 (C) with excitation from 500 to 650 nm for WT (solid black lines) *Synechococcus* sp. PCC 7002 and mutant strains CpcTV$^-$ (dashed lines), CpcSU$^-$ (dotted lines), CpcSUT$^-$ (dashed−dotted lines), and CpcC$^-$ (solid gray lines). Spectra are corrected for excitation light intensity and are normalized to emission from 650 nm excitation to correct for the number of PBS cores. (D−F) Corresponding difference spectra obtained from subtracting Cpc$^-$ strain fluorescence intensity from that of WT (ΔFluorescence) at the emission peaks of F685, F695, and F715, respectively. Difference spectra are normalized to their maxima for ease of comparison and are representative of loss of PC excitation contribution to terminal acceptor emission within the Cpc$^-$ strains. Cells were suspended in 1 M phosphate buffer prior to being frozen.

absorbance and emission bands should be missing; however, all mutants still show the same bands as in WT, albeit with some shifting (Figure 2B and 3D). Thus chromophorylation is still viable. If we were witnessing spontaneous bilin addition, mesobiliverdin would be the most likely PCB adduct and PC absorbance bands would be red-shifted by more than 30 nm (*26*), instead of the blue shift of 0.8–2.2 nm seen in this study. Enzymatic activity must be involved to account for the significant level of PC chromophorylation observed [the lyase$^-$ strains produced at least approximately half the PC as WT (Table 1)]. We believe that less specific, substitute lyases are catalyzing the addition of PCB to the CpcB apoprotein. However, these alternate lyases are not as effective at attaching the PCB tetrapyrole in the correct orientation within the binding domain. We assume that changes in chromophore orientation are dominantly responsible for the perturbed PC energy transfer observed in the CpcB lyase$^-$ strains, as opposed to changes in chromophore conformation. If the substitute lyases were attaching the β bilins in a different conformation, we would expect to see larger shifts in energy levels.

The β82 site was evidently more accessible to alternate lyase activity than the β153 site. The CpcSU$^-$ strain assembled more chromophorylated PC than the CpcTV$^-$ strain (Table 1), and the resulting PC was functionally more similar (smaller shifts in bilin energy levels and less perturbed energy transfer) to WT. This difference in binding pocket accessibility may stem from the differences in the stereochemistry of the two sites (*29*).

To account for the observed decrease in PC bilin absorbance (Table 1), either there is less PC protein assembled into disks or there are large amounts of bilin-deplete PC protein present, which could account for some of the loss of the level of energy transfer. Without more in-depth biochemical analysis of the mutants' PBS, we cannot rule out the possibility that there are small populations of bilin-deplete PC, missing PCB attachment at one or both of the β attachment sites. However, we believe that the majority of the PC protein assembled into PBS is fully bilin-replete. Bilin-deplete biliprotein subunits are highly susceptible to degradation and are less likely to be integrated into PBS during the PBS assembly pathway (*49, 50*). Additionally, the spectral shape of the PC absorbance region of the CpcB lyase$^-$ strains very closely matches the spectral shape of one of the previously characterized control strains: CpcSU$^-$ resembles WT, CpcTV$^-$ and -SUT$^-$ resemble CpcC$^-$ (Figure 2A inset).

When the CpcB lyase$^-$ strains are grown under high-light conditions (250 μmol m^{-2} s^{-1}), they produce bilin-deplete PC (*26, 27*); however, our study shows that they produce predominantly bilin-replete PC when grown under low-light conditions (60 μmol m^{-2} s^{-1}). Not only is there a difference in the chromophorylation state of PC from high- and low-light growth environs, but there is also a difference in the amount of PC within the mutant cells. When the CpcSU$^-$ and CpcSUT$^-$ strains were grown under high-light conditions, they retained only 25 and 12.5% of the phycobiliprotein content, respectively, of WT cells (*27*). Additionally, the CpcSU$^-$ mutant cells contained only ~27% of WT levels of ApcB protein, and no intact PBS could be isolated from CpcSUT$^-$ cells (*27*). In contrast, this study shows at most an ~50% decrease in PC content and only small reductions in APC (Table 1).

Under light limiting conditions, PC levels are increased in *Synechococcus* sp. PCC 7002 (*43*) to increase the antennae size of PBS. Regulatory pathways activate the synthesis of PC by increasing cellular levels of *cpcBA* transcripts (*43*). It is not surprising then that there would be more PC in the mutant cells when they are grown under low-light conditions. If the cells need to positively shift phycobiliprotein assembly equilibria, they may not only increase the extent of apoprotein synthesis but also decrease the levels of active proteases. If PC apoprotein is not being rapidly degraded, slower, less efficient lyases (i.e., alternate lyases) would have more time to act on the PCB attachment sites, thereby increasing the likelihood of chromophorylation. The increase in APC levels in the *cpcSU* mutants, when grown under low-light conditions, could be similarly explained. CpcEF is probably the alternate lyase at work in the *Synechococcus* sp. PCC 7002 CpcB lyase$^-$ strains. It is not known yet if, or how, phycobiliprotein lyases are controlled in response to PBS synthesis. One reason why the CpcB lyase$^-$ strains grew faster under low-light conditions could be because they did not needlessly waste cellular energy supplies through the futile process of synthesizing and degrading large amounts

of apophycobiliprotein, which was probably occurring in the cells grown under high-light conditions (*27*).

Interestingly, all the CpcB lyase$^-$ mutations lowered the energy levels of both β bilins and induced a blockage in the rate of transfer of energy out of the PC rod at β82. Because chromophorylation is a prerequisite for subunit folding (*49, 50*), altering the positioning of a chromophore could potentially change the surrounding protein's secondary and tertiary structures. The two CpcB chromophores are located near interacting surfaces of their polypeptide and would be especially sensitive to changes in protein structure. Affecting one of the β bilins could feasibly induce a conformational change in the entire CpcB polypeptide, as such disturbing the other bilin, as we observed with the CpcTV$^-$ and CpcSU$^-$ strains. The PC linker polypeptide mutation also affected both of the β bilins. Linker polypeptides occupy the central cavity of the PC hexamer (*51, 52*), where the β82 bilins are positioned, and interact directly with the β82 bilins. Interaction of the linker with the β82 bilins is significantly strong enough to increase the chromophore's absorptivity (*53, 54*) and can perturb the coupling interaction of the α84$-\beta$82 chromophore pair (*11*). The experimental data showed that disrupting the α84$-\beta$82 excitonic splitting interaction was mainly responsible for the disruption in energy transfer within the mutant strains. This would stem from either a loss of interaction of the β82 chromophore with linker protein, as in the CpcC$^-$ strain, or improper alignment of the β82 chromophore as a consequence of alternate lyase activity, as observed in the CpcTV$^-$, -SU$^-$, and -SUT$^-$ strains.

To summarize, the CpcB lyase$^-$ strains were able to cope with their potentially costly mutations. The contribution of the β PC chromophores to the PBS absorbance cross section increases the potential photosynthetic yield within cells, especially when grown under less than optimal light conditions; therefore, functioning PC is beneficial to the cells. Under light limiting conditions, regulatory pathways activate the synthesis of PC (*43*), so within the CpcB lyase$^-$ strains, less than optimal methods of chromophorylating PC would be possible and even favored over the complete loss of PC. The CpcB lyase$^-$ mutants were able to produce chromophorylated PC; albeit, the resultant PC was a poorer light-harvesting partner within the PBS than its WT counterpart. The spectroscopic evidence implies that the chromophores were attached within the correct β PC binding domains yet were in energy transfer unfavorable orientations. The differential loss of PBS antenna function among the mutant strains is likely attributed to the binding site specificities of the substitute lyases; the β82 site was evidently more accessible than the β153 site.

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SUPPORTING INFORMATION AVAILABLE

Detailed fourth-derivative band assignments for panels B and C of Figure 2 and panels C and D of Figure 3 (Tables 1 and 2, respectively). This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Glazer, A. N. (1984) Phycobilisomes: A Macromolecular Complex Optimized for Light Energy Transfer. *Biochim. Biophys. Act. 768*, 29–51.
2. Glazer, A. N. (1989) Light Guides. Directional Energy Transfer in a Photosynthetic Antenna. *J. Biol. Chem. 264*, 1–4.
3. Zilinskas, B. A., and Greenwald, L. S. (1986) Phycobilisome Structure and Function. *Photosynth. Res. 10*, 7–35.
4. Mullineaux, C. W., Bittersmann, E., Allen, J. F., and Holzwarth, A. R. (1990) Picosecond Time-Resolved Energy Transfer from the Phycobilisome to Photosystem II in Light-State 2 in the Cyanobacterium Synechococcus 6301. *Biochim. Biophys. Acta 1015*, 231–242.
5. Bald, D., Kruip, J., and Rögner, M. (1996) Supramolecular Architecture of Cyanobacterial Thylakoid Membranes: How is the Phycobilisome Connected with the Photosystems. *Photosynth. Res. 49*, 103–118.
6. Mullineaux, C. W. (1992) Excitation Energy Transfer from Phycobilisomes to Photosystem I in a Cyanobacterium. *Biochim. Biophys. Acta 1100*, 285–292.
7. Mullineaux, C. W. (1994) Excitation Energy Transfer from PBS to PSI in a Cyanobacterial Mutant Lacking PSII. *Biochim. Biophys. Acta 1184*, 71–77.
8. MacColl, R. (1998) Cyanobacterial phycobilisomes. *J. Struct. Biol. 124*, 311–334.
9. Ashby, M. K., and Mullineaux, C. W. (1999) The Role of ApcD and ApcF in Energy Transfer from Phycobilisomes to PSI and PSII in a Cyanobacterium. *Photosynth. Res. 61*, 169–179.
10. de Lorimier, R. M., and Bryant, D. A., Jr. (1990) Genetic Analysis of a 9 kDa Phycocyanin-Associated Linker Polypeptide. *Biochim. Biophys. Acta 1*, 29–41.
11. Pizarro, S. A., and Sauer, K. (2001) Spectroscopic Study of the Light-Harvesting Protein C-Phycocyanin Associated with Colorless Linker Peptides. *Photochem. Photobiol. 73*, 556–563.
12. Glazer, A. N., and Fang, S. (1973) Chromophore Content of Blue-Green Algal Phycobiliproteins. *J. Biol. Chem. 248*, 659–662.
13. Mimuro, M., Füglistaller, P., Rümbeli, R., and Zuber, H. (1986) Functional Assignment of Chromophores and Energy Transfer in C-Phycocyanin Isolated from the Thermophilic Cyanobacterium *Mastigocladus laminosus*. *Biochim. Biophys. Acta 848*, 155–166.
14. Mimuro, M., Lipschultz, C. A., and Gantt, E. (1986) Energy Flow in the Phycobilisome Core of *Nostoc* Sp. (MAC): Two Independent Terminal Pigments. *Biochim. Biophys. Acta 933*, 478–486.
15. Mimuro, M., Yamazaki, I., Tamai, N., and Katoh, T. (1989) Excitation Energy Transfer in Phycobilisomes at−196 °C Isolated from the Cyanobacterium *Anabaena variabilis* (M-3): Evidence for the Plural Transfer Pathways to the Terminal Emitters. *Biochim. Biophys. Acta 973*, 153–162.
16. Padyana, A. K., and Ramakumar, S. (2006) Lateral Energy Transfer Model for Adjacent Light-Harvesting Antennae Rods of C-Phycocyanins. *Biochim. Biophys. Acta 1757*, 161–165.
17. Stec, B., Troxler, R. F., and Teeter, M. M. (1999) Crystal Structure of C-Phycocyanin from *Cyanidium caldarium* provides a New Perspective on Phycobilisome Assembly. *Biophys. J. 76*, 2912–2921.
18. Zhang, J., Zhao, J., Jiang, L., Zheng, X., Zhao, F., and Wang, H. (1997) Studies on the Energy Transfer among the Rod-Core Complexes from Phycobilisome of *Anabaena variabilis* by Time Resolved Fluorescence Emission and Anisotropy Spectra. *Biochim. Biophys. Acta 1320*, 285–296.
19. Debreczeny, M. P., Sauer, K., Zhou, J., and Bryant, D. A. (1995) Comparison of calculated and experimentally resolved rate constants for excitation energy transfer in C-Phycocyanin. 2. Trimers. *J. Phys. Chem. 99*, 8420–8431.
20. Xie, J., Zhao, J.-Q., and Peng, C. (2002) Analysis of the Disk-to-Disk Energy Transfer Processes in C-Phycocyanin Complexes by Computer Simulation Technique. *Photosynthetica 40*, 251–257.
21. Arciero, D. M., Bryant, D. A., and Glazer, A. N. (1988) In Vitro Attachment of Bilins to Apophycocyanin. *J. Biol. Chem. 263*, 18350–18357.
22. Swanson, R. V., Zhou, J., Leary, J. A., Williams, T., de Lorimier, R., Bryant, D. A., and Glazer, A. N. (1992) Characterization of Phycocyanin Produced by cpcE and cpcF Mutants and Identification of an Intergenic Suppressor of the Defect in Bilin Attachment. *J. Biol. Chem. 267*, 16146–16154.
23. Zhao, K.-H., Su, P., Li, J., Tu, J.-M., Zhou, M., Bubenzer, C., and Scheer, H. (2006) Chromophore Attachment to Phycobiliprotein β-Subunits: Phycocyanobilin:Cysteine-β84 Phycobiliprotein Lyase

Activity of CpeS-Like Protein from *Anabaena* sp. PCC 7120. *J. Biol. Chem. 281*, 8573–8581.
24. Zhao, K.-H., Wu, D., Zhang, L., Zhou, M., Böhm, S., Bubenzer, C., and Scheer, H. (2006) Chromophore Attachment in Phycocyanin. Functional Amino Acids of Phycocyanobilin-α-Phycocyanin Lyase and Evidence for Chromophore Binding. *FEBS J. 273*, 1262–1274.
25. Zhao, K.-H., Su, P., Tu, J.-M., Wang, X., Liu, H., Plöscher, M., Eichacker, L., Yang, B., Zhou, M., and Scheer, H. (2007) Phycobilin:cystein-84 biliprotein lyase, a near-universal lyase for cysteine-84-binding sites in cyanobacterial phycobiliproteins. *Proc. Natl. Acad. Sci. U.S.A. 104*, 14300–14305.
26. Shen, G., Saunée, N. A., Williams, S. R., Gallo, E. F., Schluchter, W. M., and Bryant, D. A. (2006) Identification and Characterization of a New Class of Bilin Lyase. The cpcT Gene Encodes a Bilin Lyase Responsible for Attachment of Phycocyanobilin to Cys-153 on the β-Subunit of Phycocyanin in *Synechococcus* sp. PCC 7002. *J. Biol. Chem. 281*, 17768–17778.
27. Shen, G., Schluchter, W. M., and Bryant, D. A. (2008) Biogenesis of Phycobiliproteins. I. cpcS-I and cpcU Mutants of the Cyanobacterium *Synechococcus* sp. PCC 7002 Define a Heterodimeric Phycocyanobilin Lyase Specific for β-Phycocyanin and Allophycocyanin Subunits. *J. Biol. Chem. 283*, 7503–7512.
28. Saunee, N. A., Williams, S. R., Bryant, D. A., and Schluchter, W. M. (2008) Biogenesis of Phycobiliproteins. II. CpcS-I and CpcU comprise the hetrodimeric bilin lyase that attaches phycocyanobilin to Cys-82 of β-phycocyanin and Cys-81 of allophycocyanin subunits in *Synechococcus* sp. PCC 7002. *J. Biol. Chem. 283*, 7513–7522.
29. Ritter, S., Hiller, R. G., Wrench, P. M., Welte, W., and Diederichs, K. (1999) Crystal Structure of a Phycourobilin-Containing Phycoerythrin at 1.90 Å Resolution. *J. Struct. Biol. 126*, 86–97.
30. Schluchter, W. M., and Glazer, A. N. (1999) Biosynthesis of Phycobiliproteins in Cyanobacteria, in *The Phototrophic Prokaryotes* (Peschek, G., Löffelhardt, W., and Schmetterer, G., Eds.) pp 83–95, Kluwer Academic/Plenum Press, New York.
31. Bhalerao, R. P., and Gustafsson, P. (1994) Factors Influencing the Phycobilisome Rod Composition of the Cyanobacterium *Synechococcus* sp. PCC 7942: Effects of Reduced Phycocyanin Content, Lack of Rod-Linkers, and Over-Expression of the Rod-Terminating Linker. *Physiol. Plant. 90*, 187–197.
32. Bhalerao, R. P., Lind, L. K., and Gustafsson, P. (1994) Cloning of the cpcE and cpcF Genes from *Synechococcus* sp. PCC 6301 and their Inactivation in *Synechococcus* sp. PCC 7942. *Plant Mol. Biol. 26*, 313–326.
33. Zhou, J., Gasparich, G., Stirewalt, V. A., de Lorimier, R., and Bryant, D. A. (1992) The cpcE and cpcF Genes of *Synechococcus* sp. PCC 7002: Construction and Phenotypic Characterization of Interposon Mutants. *J. Biol. Chem. 267*, 16138–16145.
34. Fairchild, C. D., Zhao, J., Zhou, J., Colson, S. E., Bryant, D. A., and Glazer, A. N. (1992) Phycocyanin α-Subunit Phycocyanobilin Lyase. *Proc. Natl. Acad. Sci. U.S.A. 89*, 7017–7021.
35. Fairchild, C. D., and Glazer, A. N. (1994) Oligomeric Structure, Enzyme Kinetics, and Substrate Specificity of the Phycocyanin α Subunit Phycocyanobilin Lyase. *J. Biol. Chem. 269*, 8686–8694.
36. McConnell, M. D., Koop, R., Vasil'ev, S., and Bruce, D. (2002) Regulation of the Distribution of Chlorophyll and Phycobilin-Absorbed Excitation Energy in Cyanobacteria. *Plant Physiol. 130*, 1201–1212.
37. Myers, J., Graham, J.-R., and Wang, R. T. (1980) Light Harvesting in *Anacystis nidulans* Studied in Pigment Mutants. *Plant Physiol. 66*, 1144–1149.
38. Emlyn-Jones, D., Ashby, M. K., and Mullineaux, C. W. (1999) A Gene Required for the Regulation of Photosynthetic Light-Harvesting in the Cyanobacterium *Synechocystis* 6803. *Mol. Microbiol. 33*, 1050–1058.
39. Zhao, J., Gaozhong, S., and Bryant, D. A. (2001) Photosystem Stoichiometry and State Transitions in a Mutant of the Cyanobacterium *Synechococcus* sp. PCC 7002 Lacking Phycocyanin. *Biochim. Biophys. Acta 1505*, 248–257.
40. MacColl, R., and Guard-Friar, D. (1987) *Phycobiliproteins*, CRC Press, Inc., Boca Raton, FL.
41. Joshua, S., and Mullineaux, C. W. (2004) Phycobilisome Diffusion is Required for Light-State Transitions in Cyanobacteria. *Plant Physiol. 135*, 2112–2119.
42. Salehian, O., and Bruce, D. (1992) Distribution of Excitation Energy in Photosynthesis: Quantification of Fluorescence Yields from Intact Cyanobacteria. *J. Lumin. 51*, 91–98.
43. de Lorimier, R. M., and Smith, R. L., Jr. (1992) Regulation of Phycobilisome Structure and Gene Expression by Light Intensity. *Plant Phys. 98*, 1003–1010.
44. LeClerc, J. C., Hoarau, J., and Remy, R. (1979) Analysis of Absorption Spectra Changes Induced by Temperature Lowering on Phycobilisomes, Thylakoids, and Chlorophyll-Protein Complexes. *Biochim. Biophys. Acta 547*, 398–409.
45. Shen, G., and Bryant, D. A. (1995) Characterization of a *Synechococcus* sp. Strain PCC 7002 Mutant Lacking Photosystem I. Protein Assembly and Energy Distribution in the Absence of the Photosystem I Reaction Center Core Complex. *Photosynth. Res. 44*, 41–53.
46. van Amerongen, H., and Dekker, J. P. (2003) Light-Harvesting in Photosystem II, in *Light-Harvesting Antennas in Photosynthesis* (Green, B. R., and Parson, W. W., Eds.) pp 219–251, Kluwer Academic Publishers, Dordrecht, The Netherlands.
47. Murakami, A. (1997) Quantitative Analysis of 77K Fluorescence Emission Spectra in *Synechocystis* sp. PCC 6714 and *Chlamydomonas reinhardtii* with Variable PS I/PS II Stoichiometries. *Photosynth. Res. 53*, 141–148.
48. Demidov, A. A., and Mimuro, M. (1995) Deconvolution of C-Phycocyanin β-84 and β-155 Chromophore Absorption and Fluorescence Spectra of Cyanobacterium *Mastigocladus laminosus*. *Biophys. J. 68*, 1500–1506.
49. Anderson, L. K., and Toole, C. M. (1998) A Model for Early Events in the Assembly Pathway of Cyanobacterial Phycobilisomes. *Mol. Microbiol. 30*, 467–474.
50. Toole, C. M., Plank, T. L., Grossman, A. R., and Anderson, L. K. (1998) Bilin Deletions and Subunit Stability in Cyanobacterial Light-Harvesting Proteins. *Mol. Microbiol. 30*, 475–486.
51. Yu, M.-H., and Glazer, A. N. (1982) Cyanobacterial Phycobilisomes: Role of the Linker Polypeptides in the Assembly of Phycocyanin. *J. Biol. Chem. 257*, 3429–3433.
52. Mörschel, E., and Rhiel, E. (1987) Phycobilisomes and Thylakoids, in *Electron Microscopy of Proteins* (Harris, J. R., and Horne, R. W., Eds.) pp 209–254, Academic Press, London.
53. Klotz, A. V., and Glazer, A. N. (1985) Characterization of the Bilin Attachment Sites in R-Phycoerythrin. *J. Biol. Chem. 260*, 4856–4863.
54. Scheer, H. (2003) The Pigments, in *Advances in Photosynthesis and Respiration* (Green, B. R., and Parson, W. W., Eds.) pp 55, Kluwer Academic Publishers, Dordrecht, The Netherlands.

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