Effect of Light Quality on Phycobilisome Components of the Cyanobacterium Spirulina platensis

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ABSTRACT

Phycobilisomes from the nonchromatic adapting cyanobacterium Spirulina platensis are composed of a central core containing allophycocyanin and rods with phycocyanin and linker polypeptides in a regular array. Room temperature absorption spectra of phycobilisomes from this organism indicated the presence of phycocyanin and allophycocyanin. However, low temperature absorption spectra showed the association of a phycobiliviolin type of chromophore within phycobilisomes. This chromophore had an absorption maximum at 590 nanometers when phycobilisomes were suspended in 0.75 molar K-phosphate buffer (pH 7.0). Purified phycocyanin from this cyanobacterium was found to consist of three subparticles and the phycobiliviolin type of chromophore was associated with the lowest density subparticle. Circular dichroism spectra of phycocyanin subparticles also indicated the association of this chromophore with the lowest density subparticle. Absorption spectral analysis of α and β subunits of phycocyanin showed that phycobiliviolin type of chromophore was attached to the α subunit, but not the β subunit. Effect of light quality showed that green light enhanced the synthesis of this chromophore as analyzed from the room temperature absorption spectra of phycocyanin subparticles and subunits, while red or white light did not have any effect. Low temperature absorption spectra of phycobilisomes isolated from green, red, and white light conditions also indicated the enhancement of phycobiliviolin type of chromophore under green light.

Phycobiliproteins are the major accessory light harvesting pigments present in cyanobacteria, red algae, and cryptomonads. These proteins are broadly classified into three groups based on the spectroscopic properties: PE, λmax, 540–570 nm; PC, λmax, 610–620 nm; and APC, λmax, 650–655 nm (12, 14). A few cyanobacteria possess a fourth type of biliprotein, PEC, λmax, 568 nm, 585 nm(s) in place of PE (7). Each of these phycobiliproteins are comprised of two subunits, α and β, to which linear tetrapyrroles are covalently attached by a cysteine thioether bond (15). The absorption characteristics of these tetrapyrroles in acidic aqueous solutions group them into four types: PCβ, λmax, 660 nm; PXβ, λmax, 590 nm; phycocerythobilin λmax, 555 nm; and phycourein bilin λmax, 495 nm (14, 22). The chromoporic proteins along with some of the colorless linker polypeptides form an organized structure called phycobilisome. Phycobilisomes are situated on thylakoid membranes and function as light harvesting antennae to channel the absorbed light energy to PSII (25). Some cyanobacteria containing PE exhibit a phenomenon called complementary chromatic adaptation by modulating the synthesis of PE alone or both PC and PE under specific light qualities (5, 23). Thus, enhancement in the synthesis of PC under red light and PE under green light was observed in complementary chromatic adapting cyanobacteria (3, 10, 11, 13).

The present communication describes the effect of light quality on the synthesis and regulation of phycobiliproteins in the nonchromatic adapting cyanobacterium Spirulina platensis. We report here the presence of a PXB type of chromophore in this cyanobacterium and modulation in its level in response to the light quality.

MATERIALS AND METHODS

Organism

The cyanobacterium Spirulina platensis was obtained from the Microbiology Division, Indian Agricultural Research Institute, New Delhi, India.

Culture Conditions

The organism was cultivated in modified Zarrouk medium (21, 26) at 28 ± 1°C. White light grown cells were illuminated using a combination of cool white fluorescent lamps and tungsten bulbs. Green and red light grown cells were exposed to a similar light filtered through layers of appropriate colored cellophane papers wrapped around the culture flasks. However, in all three cases, the light intensity inside the culture flasks was maintained at ≈ 8 W m−2. The transmission spectra of colored cellophane papers are given in Figure 1.

Phycobiliprotein Quantitation

PC and APC estimations were done using the equations of Bennett and Bogorad (3).

Isolation of Phycobilisomes

Phycobilisomes were isolated using the procedure described earlier (20). Cells grown to exponential phase in the media were incubated with 0.01% of the cationic detergent, hexadecyl trimethyl ammonium bromide for 40 s at 20°C and
Figure 1. Transmission spectra of colored cellophane papers.

Isolation of PC Subparticles

Phycobilisomes recovered from 1 mM sucrose layers were diluted four times in 0.75 M K-phosphate buffer (pH 7.0) containing 0.1 mM PMSF. Diluted samples were centrifuged at 20,000g for 30 min, at 20°C in a Sorvall RC-5 centrifuge, using an SS-34 rotor. The resultant blue supernatant was precipitated with a 20% saturated solution of ammonium sulfate for 30 min, at 20°C and pelleted at 30,000g for 30 min. Phycobilisome pellets were resuspended in 10 mM K-phosphate buffer (pH 7.0) containing 0.1 mM PMSF with a total protein concentration of 10 mg/mL and dialyzed against the same buffer for 6 h at 4°C. The dialyzed phycobilisome samples were loaded onto a hydroxyapatite column equilibrated with 10 mM K-phosphate buffer (pH 7.0) containing 0.1 mM PMSF. PC was eluted with the same buffer, while APC absorbed to the top of the column. PC-containing fractions were immediately applied to linear sucrose density gradients of 0.2 to 0.5 M sucrose dissolved in 0.3 M K-phosphate buffer (pH 7.0) and centrifuged in a Beckman preparative ultracentrifuge at 110,000g for 15 h at 20°C, using an SW-41Ti rotor. The resolved blue colored PC subparticles were determined separately and examined for their spectral properties.

Isolation of PC Subunits

Isolation of PC subunits was done according to Glazer and Fang (17). About 20 mL of purified PCs from a hydroxyapatite column (10 mg protein) were mixed with an equal volume of 24% formic acid. The solution was applied to a column of Bio-Rex 70 minus 400 mesh (2.4 × 12 cm) preequilibrated with 12% formic acid. The column was then successively washed with 25 mL of 12% formic acid, 30 mL of 2 mM urea, 20 mL of 4 mM urea, and 50 mL of 5 mM urea. These urea solutions were adjusted to pH 2.0 with 1 N HCl. The column was then developed with a linear gradient (140 mL total volume) of acidic urea (5–10 mM). The α and β subunits of PC were eluted as two separate peaks. Fractions of the two peaks were separately pooled and dialyzed against 5 mM urea for 6 h followed by dialysis against 50 mM K-phosphate buffer (pH 7.0) containing 1 mM NaCl. PC subunits isolated in this manner were used for spectrophotometric analysis.

Absorption Spectroscopy

Absorption spectra were recorded at room temperature on a Shimadzu UV 260 spectrophotometer with a 1-cm light path and a slit width of 2 nm. Low temperature absorption spectra in liquid nitrogen (−196°C) were recorded on a Shimadzu MPS 5000 spectrophotometer. For the low temperature absorption spectra, samples were mixed with an equal volume of 75% potassium glycerophosphate.

CD Spectra

CD spectra of PC subparticles suspended in 0.3 M K-phosphate buffer (pH 7.0) were recorded, at room temperature, on a JASCO J-20 CD-ORD spectropolarimeter in a 5-nm pathlength cell, with a sample absorbance of 1/cm at λmax.

RESULTS

Light quality effects on the phycobilisome components were observed by harvesting S. platensis cells at exponential growth phase. Changes that may occur in PC and APC levels in this cyanobacterium were measured using the equations of Bennett and Bogorad (3). The values taken as an average of

<table>
<thead>
<tr>
<th>Light Source</th>
<th>PC mg/g</th>
<th>APC mg/g</th>
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<tbody>
<tr>
<td>Green</td>
<td>180</td>
<td>75</td>
</tr>
<tr>
<td>Red</td>
<td>220</td>
<td>74</td>
</tr>
<tr>
<td>White</td>
<td>220</td>
<td>75</td>
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a Values indicated are an average of four experiments.
four separate experiments are represented in Table I. It was observed that the ratio of PC to APC in the crude extracts was in the order of 2.4, 2.9, and 2.9 for green, red, and white light grown cells, respectively. It was found that PC underwent partial modulations under light while APC remained constant under all three light qualities used. Absorption spectra of phycobilisomes at 20°C isolated from green, red, and white light grown cultures showed no significant differences (Fig. 2A). The spectra showed a peak at 615 nm and a shoulder at 652 nm for all three samples of phycobilisomes. Absorption spectra at liquid nitrogen temperatures (−196°C) showed an additional peak at 590 nm in all three samples (Fig. 2B). Further characterization of the 590 nm absorbing component was done by the isolation of PC subparticles and its subunits and analyzing their spectral characteristics. Purified PC from white light grown cells, when subjected to linear sucrose density gradients was resolved into three distinct subparticles: PC I, PC II, and PC III. PC III was on top of the sucrose density gradient, followed by PC II and PC I (data not shown). The absorption spectra of PC subparticles from white light grown cells showed a peak at 615 nm for all three subparticles, while only PC III exhibited an additional shoulder at 590 nm (Fig. 3A). The presence of 590 nm band in PC III was further confirmed from the CD spectra of PC subparticles (Fig. 3B).

The α and β subunits of PC in the renatured form exhibited an absorption band at 615 nm, while only the α subunit of PC contained an additional peak at 590 nm (Fig. 4A). Similarly, both α and β subunits of PC in their denatured form showed a common peak at 665 nm, but again only the α subunit of PC showed an additional peak at 595 nm (Fig. 4B). We considered the possibility of PEC being complexed to C-PC in the case of α subunit of PC for the origin of 595 nm peak. However, this possibility appears unlikely, as a complex of PEC and PC from Anabaena variabilis exhibited absorption bands at 508 and 568 nm with a shoulder at 608 nm in 0.1 M Na-phosphate buffer (pH 6.0) (1). Similarly, this complex in the presence of 9 M urea (pH 3.0) gave peaks at 565 and 595 nm with a shoulder at 665 nm. These findings clearly indicate that the absorption bands at 508 and 568 nm (in 0.1 M Na-phosphate buffer, pH 6.0) are characteristic of PEC and this spectral profile is different from that of α subunit of PC (suspended in 0.05 M K-phosphate buffer, pH 7.0) isolated from S. platensis. In addition, organisms containing PEC exhibited a 620 nm characteristic fluorescence emission band at 77K when excited at 580 nm, while those which did not contain PEC lacked the 620 nm emission band (19). In the present study, S. platensis cells when excited at 545 nm.
Thus, these results enhanced the levels of (n)

Because PC III subparticles and α subunits of PC possessed the 590 nm absorbing component, further studies on the effect of light quality were carried out isolating these components. The absorption spectra of PC III subparticles isolated from green, red, and white light grown cells is shown in Figure 5A. The absorption spectra indicated that the 590 nm absorbing component was increased in the cells grown under green light, while red and white lights did not have any significant effect on this component. A similar observation was made from the absorption spectra of α subunits of PC (both renatured and denatured forms) isolated from the cultures grown under green, red, and white light conditions (Fig. 5, B and C). Thus, these results suggest that the 590 nm absorbing component was under the light quality control and green light enhanced the levels of this component.

**DISCUSSION**

In the present communication, we describe the presence of a PXB type of chromophore in the cyanobacterium *S. platensis* and that this chromophore undergoes light quality dependent modulations.

Phycobiliprotein levels in the crude extracts of *S. platensis* exhibited partial modulations in the levels of PC but not APC under the light qualities mentioned (Table I). It has been reported that in complementary chromatic adapting cyanobacteria, only the inducible PC gene set modulates its gene expression depending upon the light quality (8), while the constitutive PC gene set expresses itself both under green and red light (19). The synthesis of PC and PE in such cyanobacteria is photoreversible (10, 11, 18) and it has also been postulated that the reversible nature of these biliproteins is under the control of photoreversible photoreceptors (5, 24). Because, in the present investigation, PC did not undergo major changes in its synthesis, we assume that neither photoreversible photoreceptors mentioned above nor inducible PC gene sets are present in the nonchromatic adapting cyanobacterium *S. platensis*. Thus, nonchromatic adapting cyanobacteria may contain only constitutive PC gene sets, which are expressed both under green and red light conditions.

Absorption spectra of phycobilisomes (at 20°C) isolated from green, red, and white light grown cells did not show any

**Figure 4.** Absorption spectra of PC subunits (at 20°C) isolated from cells grown under white light. α Subunit (α-PC) and β subunit (β-PC). A, samples were suspended in 50 mM K-phosphate buffer (pH 7.0). B, Samples were suspended in 8 M acidic urea (pH 2.0).

**Figure 5.** Absorption spectra of (A) PC III subparticles, (B) α subunits of PC suspended in 50 mM K-phosphate buffer (pH 7.0), and (C) α subunits of PC suspended in 8 M acidic urea, pH 2.0, isolated from cells grown under green, red, and white light. PC III subparticles were isolated as described in "Materials and Methods."
changes in the quality of their spectra (Fig. 2A). Thus, phycobilisomes under these light qualities may not have undergone drastic structural changes. On the other hand, the additional 590 nm absorbing band seen in the low temperature absorption spectra of phycobilisomes (~196°C) isolated from green, red, and white light grown cultures (Fig. 2B) may be attributed to the presence of a PXB type of chromophore, which has been identified only in a few cyanobacteria (4, 6). Low temperature absorption spectra of all three samples of phycobilisomes also indicated that the absorption units of PXB was higher in green light relative to red and white light conditions. Because there were partial modulations in PC levels when S. platensis was grown under green, red, and white lights and the synthesis of PXB chromophore was higher under green light, we have presumed that this chromophore should be associated with PC in this cyanobacterium. Room temperature absorption and CD spectra of all three PC subparticles isolated from white light grown cultures indicated that the slowest migrating component (PC III) was associated with the PXB chromophore (Fig. 3, A and B). Studies with the α and β subunits of renatured PC demonstrated that α subunit of PC was associated with PXB chromophore but not β subunit (Fig. 4, A and B). Thus, the 590 nm absorption band in renatured α subunit of PC indicated the presence of PXB bound polypeptide in S. platensis. Similarly, in the denatured state (in presence of 8 M acidic urea) where only chromophores exhibit their properties, the α subunit of PC showed an absorption band at 595 nm (Fig. 4B). These results again indicated that α subunits of PC contained both PXB and PCB chromophores, while β subunits of PC had only PCB chromophores. Effect of light quality on the synthesis of PXB chromophore showed that green light enhanced the levels of this chromophore in S. platensis (Figs. 2A and 5A). Absorption spectra of α subunit of PC in the renatured form also indicated that the PXB containing polypeptide of PC increased its levels under green light conditions (Fig. 5B). When the properties of chromophores alone were analyzed, it was again confirmed that PXB chromophore was enhanced in the α subunit of PC isolated from green light grown culture (Fig. 5C). A similar observation was made earlier by Beguin et al. (2), where induction of PXB chromophore under green light occurred in a mutant of Fremyella diplosiphon lacking PE, and this chromophore was also associated with α subunits of PC.

To conclude, our results suggest that PXB chromophore is under the control of light quality and green light enhanced the levels of this chromophore in S. platensis. It may be hypothesized that in green light some kind of structural changes occur in some of the native apoproteins of PC which in turn are exposed to isomerases responsible for the transformation of PCB chromophores into PXB chromophores. These kinds of changes were suggested earlier by Glazer (16) and Beguin et al. (2). Further, the presence of both 590 and 615 nm absorbing bands on the α subunits of PC and from the fact that α subunits of polypeptides carry only one chromophore (15), it is quite possible that in this cyanobacterium S. platensis, some of the α subunit polypeptides of PC carry PCB chromophores and the others PXB chromophores.

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