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Photoinhibition Induced Alterations in Energy Transfer Process in Phycobilisomes of PS II in the Cyanobacterium, *Spirulina platensis*

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Exposure of algae or plants to irradiance from above the light saturation point of photosynthesis is known as high light stress. This high light stress induces various responses including photoinhibition of the photosynthetic apparatus. The degree of photoinhibition could be clearly determined by measuring the parameters such as absorption and fluorescence of chromoproteins. In cyanobacteria and red algae, most of the photosystem (PS) II associated light harvesting is performed by a membrane attached complex called the phycobilisome (PBS). The effects of high intensity light (1000-4000 μmol photons m^{-2} s^{-1}) on excitation energy transfer from PBSs to PS II in a cyanobacterium *Spirulina platensis* were studied by measuring room temperature PC fluorescence emission spectra. High light (3000 μmol photons m^{-2} s^{-1}) stress had a significant effect on PC fluorescence emission spectra. On the other hand, light stress induced an increase in the ratio of PC fluorescence intensity of PBS indicating that light stress inhibits excitation energy transfer from PBS to PS II. The high light treatment to 3000 μmol photons m^{-2} s^{-1} caused disappearance of 31.5 kDa linker polypeptide which is known to link PC discs together. In addition we observed the similar decrease in the other polypeptide contents. Our data concludes that the *Spirulina* cells upon light treatment causes alterations in the phycobiliproteins (PBPs) and affects the energy transfer process within the PBSs.

Keywords: Energy transfer, Fluorescence, High light stress, Photosystem I, Photosystem II, Phycobilisome, *Spirulina platensis*

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Introduction

The cyanobacteria photosynthetic apparatus is very similar to those of higher plants, except its antenna pigment complexes. The cyanobacterial photosynthetic apparatus principally consist of three types of macromolecular complexes: PS I, PS II and PBSs. PS I and PS II are intrinsic complexes where as PBSs arranged on the outer surface of thylakoid membrane in the form of beads (Gantt, 1981; Liu *et al.*, 2005). The PBS serves as a harvester of light energy in the spectral gap between the major chlorophyll absorbing bands (500-680 nm) allowing the species bearing these antennas to utilize the entire visible range of sunlight. All PBS complexes have been shown to contain two substructures: a core structure found closest to the membrane surface, and a series of rods emanating out from the core (Glazer 1984; Glazer 1989; Huber 1989; MacColl 1998). The PBSs, which biochemically consists of water-soluble phycobiliproteins such as APC, C-PC and PE together with linker polypeptides. PBPs, primarily composed of α, β polypeptides (in some phycoerythrins, there is a special type of subunit, the γ subunit), are a brilliantly colored group of disc shaped proteins bearing covalently attached open chain tetrapyrrolle known as phycobilins (Liu *et al.*, 2005). Linker polypeptides located between PBSs and thylakoid membranes are thought to play two roles in the PBS: (1) provide structural connection between adjacent PBPs and stabilize the PBS structures, and (2) modulate the absorption and fluorescence properties to facilitate or directly participate in energy transfer from the rod to the core and eventually to the chlorophyll containing thylakoid membrane of the photosynthetic cells. Different linker polypeptides at distinct sites contribute to different functions of the PBS. For example, the small PC rod linker polypeptide is thought to limit the number of hexamers assembled in a PBS and by preventing the addition of other hexamers. Increasing proportions of $L_R^{8.2}$ in *Synechococcus* 6301 inhibited the elongation of the rods, and therefore it has been named rod-terminating linker (Lorimier *et al.*, 1990). *In vitro* assembly studies indicate that 30 and 33 kDa linker polypeptides from the same cyanobacteria could stimulate

long rod growth whereas the 27 kDa polypeptide could nucleate the formation of discs and terminate rod assembly (Lundell *et al*., 1981).

The phycobilisomes absorb light in the wavelength range of 500-650 nm and transfer energy to the chlorophyll for photosynthesis. The kinetics of energy transfer with the isolated PBS typically occurs in 100-200 ps with 90% or more efficiency over an array of several hundred chromophores. The light is harvested by the PBS and transferred to the reaction center (RC) in the following sequence: PC → APC → APCB → Chl *a* (Bald *et al*., 1996). The energy transfer with in the PBS can be influenced by several environmental factors like low temperature, high temperature (Li *et al*., 2001; Sacina *et al*., 2001; Murthy *et al*., 2004; Wen *et al*., 2005), heavy metals (Hg: Murthy, 1991) salt stress (Sudhir *et al*., 2005) and state transition (Li *et al*., 2006). High light intensity causes alterations in the light harvesting pigment protein complexes in the green alga *Dunaliella salina* (Smith *et al*., 1990). Telfar *et al*., (1990) showed that prolonged illumination causes destruction of chlorophyll pigments and leads to the inhibition of PSII photochemistry. Fluorescence analysis indicated that Chl *b* is the main target in photosystem comparative to the other photosynthetic pigments (Lischanthaler *et al*., 1992; Lambreva *et al*., 2005). Studies with reference to the effect of high light on cyanobacterial system with reference to the energy transfer process are scanty. Here in this paper an attempt has been made to study the effect of high light on spectral properties and energy transfer under both *in vivo* and *in vitro* conditions. For *in vivo* studies cells were treated with high light intensity for 30 min before measuring the spectra. For *in vitro* studies the treatment has been given for the same period of different intensities of light and the PBSs were isolated.

In this study, we have investigated the changes in room temperature absorption and PC fluorescence spectroscopy in the cyanobacterium *Spirulina platensis* during light stress. The objective of this study is to understand the mechanism of high light stress induced change in energy transfer from PBSs to PSII in this alga. Our results clearly indicate that PC is the main target for photoinhibition and there is a loss of linker polypeptides as well as other PBPs depending on the extent of photoinhibition.

Materials and Methods

Plant material and high light treatment. *Spirulina platensis* cells were grown at 30°C in Zarrouk's medium (Zarrouk, 1966) under continuous illumination (100 µmol photons m^{-2} s^{-1}). The culture was bubbled with filtered air. The mid log phase cyanobacterial cells (5 µg Chl *a*/ml) were taken in fresh culture medium and high light treatment was given by varying the photons flux area density (PFD) from 1000-4000 µmol for 30 min under controlled experimental conditions. The spectral measurements were taken immediately after giving the treatment.

Isolation of phycobilisomes. The PBSs were isolated from *Spirulina* cells according to the method of Gantt *et al*. (1981) with some modifications. After harvesting, *Spirulina* cells were washed twice with 1 M potassium phosphate (K-PO_4) buffer at pH 7.0. The cells were resuspended in 10 ml of 1 M K-PO_4 buffer at pH 7.0 containing 1 mM PMSF, 1 mM sodium azide, 2 mM EDTA. The PBSs have been isolated by treating with triton X-100 and layering the resulted supernatant on sucrose density gradient by following the procedure of Murthy (1991). The PBSs were recovered from the 1.0 M region as an intense blue band. Sucrose was removed from the PBSs by passing them through a sephadex G-25 column, equilibrated with 1 M phosphate buffer pH 7.0. After removing sucrose, these PBSs were used for spectral measurements.

Absorption and room temperature PC fluorescence emission spectra. Absorption spectra were recorded on Jasco UV- Vis spectrophotometer. The cells were suspended in the reaction buffer (25 mM HEPES-NaOH, pH 7.5) at the concentration of 6 µg of Chl *a* per ml. In the case of PBSs, they were suspended in the 0.75 K-PO_4 buffer pH 7.0 at the concentration of 30 mg protein per ml. This cell suspension was taken for scanning the absorption spectra from 400 nm to 750 nm in the visible region. All these absorption spectra were taken at the room temperature and they were not corrected for spectral sensitivity. Hitachi spectrofluorimeter was used to record fluorescence emission and excitation spectra. Sample preparation was done in the same way as described above. The reaction mixture contained reaction buffer and the intact cells equivalent to 5 µg Chl *a* concentration. 5 nm slit width were maintained for recording both the excitation and emission spectra. The intact cells or PBSs or pigment proteins were excited at 545 nm to excite PC specifically since this organisms contain PXB chromophore which absorbs light at 580 nm (Babu *et. al*, 1991).

SDS-PAGE analysis. Total soluble proteins of PBS samples were analyzed on SDS-PAGE according to the method of Laemmli (1970). The stacking gel was 4% polyacrylamide and the running gel was 12.5% polyacrylamide used for the separation of polypeptides. Samples containing 1 mg/ml protein was solubilised by boiling for 2 min in sample buffer containing 5% glycerol 0.1 M Tris-HCl, (pH 6.8), 2% SDS and 100 µM mercaptoethanol along with 0.1% of bromophenol blue. The electrophoresis was conducted by adopting the procedure of Murthy (1991). The molecular mass of the resolved proteins was calculated by using Bio-Rad markers (phosphorylase B 97,400; Bovine albumin 66,200; Ovalbumin 42,700; Carbonic anhydrase 31,000; Trypsin inhibitor 21,500; Lysozyme 14,400 daltons).

Results and Discussion

The present study has demonstrated the effects of high light intensities on spectral properties using intact cells and isolated PBSs in both control and treated (1000-4000 µmol photons m^{-2} s^{-1}) *Spirulina* cells. The typical absorption spectra of the *Spirulina* cells having the five partially resolved peaks could be assigned to Chl *a* (418,436 and 680 nm), carotenoids (490 nm) and phycobilin (620 nm) respectively. Fig. 1A shows the

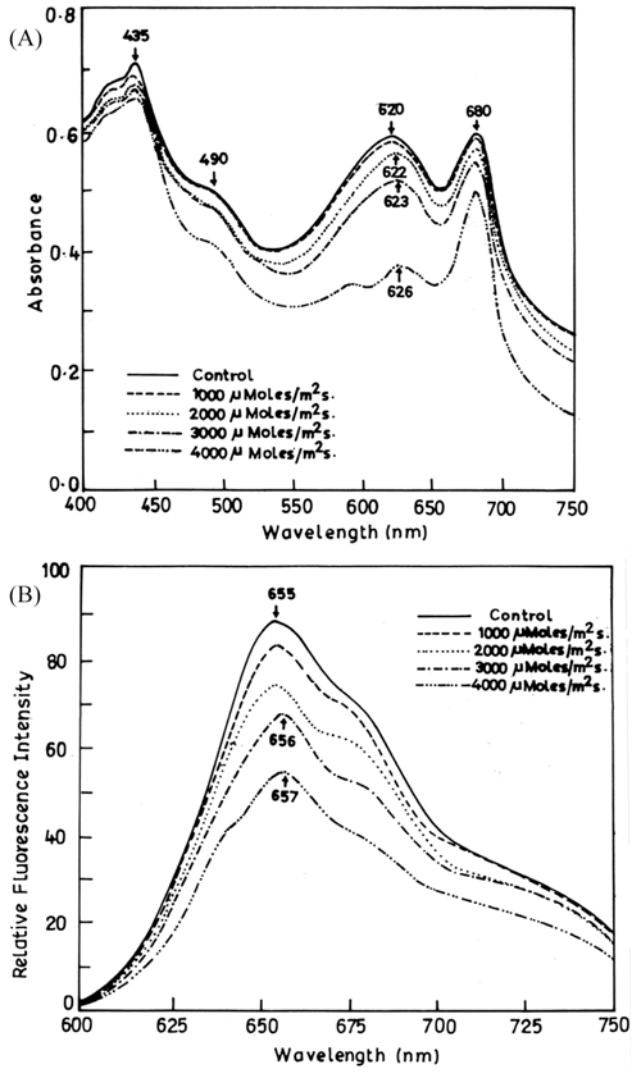

Fig. 1. (A) Effect of different intensities of high light on the absorption spectra of the intact cells of the cyanobacterium *Spirulina platensis*. (B) Effect of different intensities of high light on the fluorescence emission spectra of the intact cells of the cyanobacterium *Spirulina platensis*. Cells were excited with 545 nm light. Cells equivalent to 6 μg of Chl *a* were used. Slit width for both excitation and emission was 5 nm.

absorption spectra of the intact *Spirulina* control and high light treated cells measured at room temperature. The cells at 1000 μmol photons m^{-2} s^{-1} intensity did not influence the absorption characteristics. However, an increase in the light intensity to 3000 μmol photons m^{-2} s^{-1} caused decrease in the PC absorption and minor changes were observed in Chl *a* and carotenoids absorption (Fig. 1A). In addition it caused a red shift by 3 nm under 3000 μmol photons m^{-2} s^{-1} high light treatment. In control sample the PC/Chl ratio is 1.2, whereas after 30 min of treatment with 3000 μmol photons m^{-2} s^{-1} light intensity the ratio decreased to 0.7 (see Table 1). Since the absorption properties are related to light harvesting in PSII, we have measured fluorescence emission spectra of PC.

Table 1. High light exposure induced alterations in the spectral properties of photosynthetic pigments in intact cells (column 2) and phycobilisomes (column 3) the values are average of three separate experiments. Other details were given in the legends of Fig. 1 and 2

Light intensity μmoles m^{-2} s^{-1}	Spectral characteristics Absorption ratio between PC fluorescence PC and Chl emission intensity (rel. units)	
Control	1.2	65
1000	0.95	60
2000	0.9	52
3000	0.89	45
4000	0.7	33

The room temperature fluorescence emission spectra of intact cells and isolated PBSs exhibit various emission bands at different wavelengths depending on the excitation wavelength. Due to the presence of PXB chromophore in PBS, we have excited the cells with 545 nm light. When we excite the cells in the PC absorbing region at 540 nm, an emission peak is observed mainly at 655 nm due to PC (Gomez-Lojero *et al*., 1997; Li *et al*., 2004), and also a hump is observed at 680 nm due to Chl *a* emission (Fig. 1B). The cells treated with high light intensity caused gradual decrease in the PC fluorescence and induced 2 nm peak shifts towards the red region of the spectrum. 4000 μmol photons m^{-2} s^{-1} of high light treatment caused almost 40% decrease in the fluorescence intensity. The decrease in the fluorescence intensity indicated that energy transfer from PBS to the photosystems was suppressed. The other possibility is uncoupling of energy transfer between PC and APC.

To understand the alterations in PBS structure and functions, PBSs were isolated from the intact cells after giving high light treatment with different intensities. The PBSs isolated by ultracentrifugation in sucrose density gradients. The intact PBSs were collected at 1 M region of sucrose gradient and were characterized spectrophotometrically. The PBS shows a wide absorption band in range of 500-700 nm with a distinct major peak at 615 nm and a shoulder at 650 nm. The PBSs isolated from the high light treated cells exhibited large decrease in the absorption capacity with 2 nm peak shift towards the red region of the spectrum (Fig. 2A). This shift indicates the selective bleaching of chromophore attached to the PC. The decrease in the absorption intensity suggests that there could be variation in the chromophore and protein interaction of PC.

The room temperature fluorescence emission spectra of PBSs excited specifically at 545 nm light used to evaluate the changes in PC (Fig. 1B). High light treatment caused decrease in PC fluorescence intensity in dose dependent manner (Table 1). In PBSs fluorescence emission spectra of 3000 μmol photons m^{-2} s^{-1} treated sample there was a 50% drop in the fluorescence intensity with huge 8 nm red shift when compared to that of

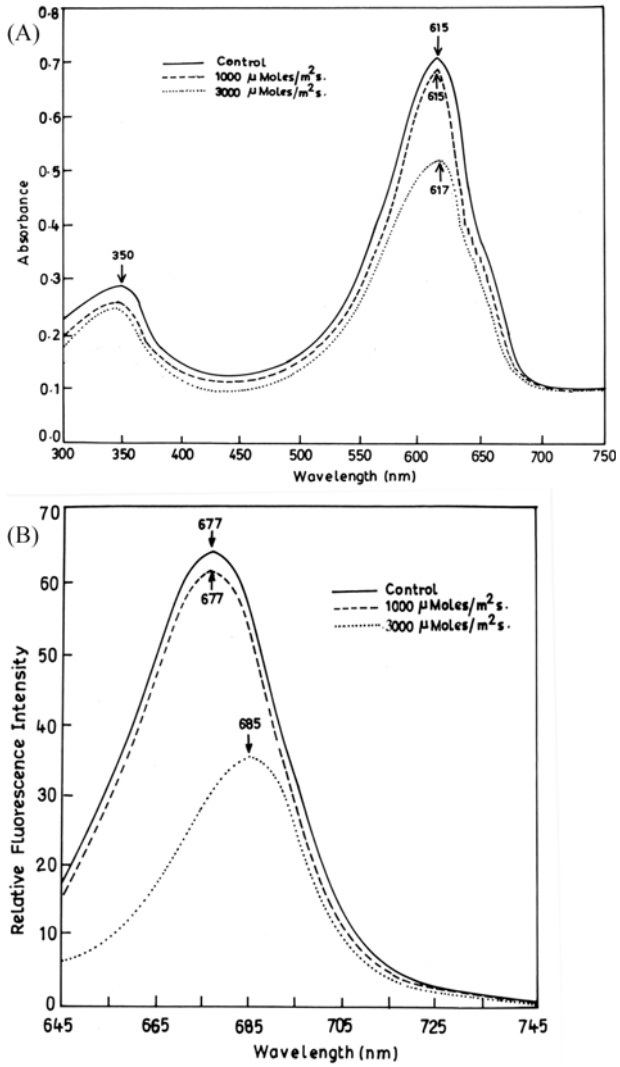

Fig. 2. (A) Effect of different intensities of high light on the absorption spectra of PBSs isolated from high light treated *Spirulina platensis* cells. PBSs equivalent to 15 μg of protein were suspended in 0.75 M phosphate buffer (p^H 7.0). (B) Effect of different intensities of high light on the fluorescence emission spectra of PBSs isolated from high light treated *Spirulina platensis* cells. PBSs were excited with 545 nm light. PBSs equivalent to 15 μg of protein were suspended in 0.75 M phosphate buffer (p^H 7.0). Slit width for both excitation and emission was 5 nm.

control PBSs. Thus it is clear that the high light affects the spectral properties of PBSs in short term incubations and also induces uncoupling of energy transfer in PBS.

To identify the target protein of PBSs towards high light, we performed a similar study to investigate the effect of high light on PBSs in *Spirulina* on the polypeptide analysis of isolated PBSs from control and high light treated samples (Fig. 3). SDS-PAGE profile of PBSs of untreated *Spirulina* cells resolved its components in to the range of 97 kDa to 14 kDa. The PBPs of PBS were in the molecular range of 16-22

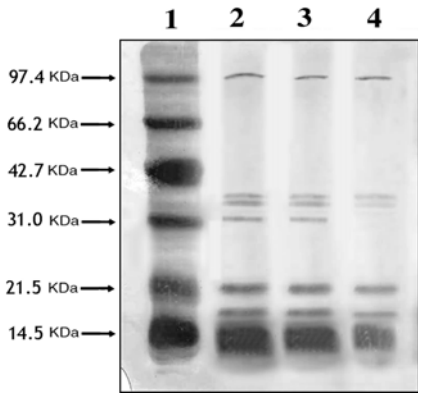

Fig. 3. SDS-PAGE poly acrylamide gel profile of the phycobilisomes isolated from control and high light treated *Spirulina platensis* cells. Lane 1 indicates the molecular masses of standard protein marker. Lane 2 indicates the polypeptide composition of control PBSs. Lane 3&4 indicates the polypeptide composition of the treated PBSs samples of 1000 and 3000 μmol photons m^{-2} s^{-1} respectively.

kDa due to the presence of chromophores these polypeptides can be observed on the gel prior to staining with coomassie brilliant blue (Fig. 3, lane 2). The polypeptide at 97 kDa is the anchor polypeptide, which links PBS to the thylakoid membrane. The polypeptide in the range of 31.5-37 kDa are helpful in the attachment of PC-PC rods. The major bands in the region of 16-22 kDa are the a and b subunits of PBPs (both PC and APC) since 70% of the total proteins in *Spirulina platensis* is made up by PBPs. Appearance of single band as anchor polypeptide indicates that no proteolysis has taken place during PBS isolation. In Fig. 3 lane 3 is the polypeptide profile of 1000 μmol photons m^{-2} s^{-1} of high light treated samples, and further increase in the high light treatment to 3000 μmol photons m^{-2} s^{-1} caused the complete disappearance of 31.5 kDa linker polypeptide with similar decrease in the other polypeptide contents (Fig. 3, lane 4). 31.5-kDa protein is known to link PC discs together. The homologous red shift in the spectral absorption maximum and a modification of the PC induced in 30-33 kDa linker polypeptides has also been reported (Lundell *et al*., 1981).

In summary, our results suggest that exposure of *Spirulina platensis* to high light brings on a complete disappearance of the specific 31.5 kDa linker polypeptide and phycobiliproteins α and β subunits and also affects the general coordination and stabilization and influences spectral changes of PBPs related to the energy transfer in PBsomes.

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