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BRIEF COMMUNICATION

High temperature induced alterations in energy transfer in phycobilisomes of the cyanobacterium *Spirulina platensis*

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Abstract

Exposure of intact cells of *Spirulina* to high temperature (HT) stress (40–60 °C) caused decrease in absorption spectrum and fluorescence emission spectrum. Low temperature emission spectra were altered at phycocyanin (PC) level. Room and low temperature emission spectra of intact phycobilisomes showed that PC was the main target in this cyanobacterium for the altered energy transfer under HT.

Additional key words: absorption spectra; fluorescence emission spectra at room and low temperature.

Cyanobacteria are oxygenic photosynthetic prokaryotes whose thylakoid organization is almost similar to that of higher plants. The major pigments of *Spirulina platensis* are chlorophyll (Chl) *a* and the phycobiliproteins, phycocyanin (PC) and allophycocyanin (APC). These pigments are associated with the photosynthetic apparatus in the thylakoid membranes (Bryant 1991). Phycobiliproteins are pigmented constituents of phycobilisomes (PBSs) which contain a water soluble light-harvesting complex attached to the thylakoid membranes (Glazer 1984). The efficiency of energy transfer from PC to Chl *a* in intact cells is influenced by various environmental factors such as high temperature, HT (Schreiber 1979), nitrogen stress (Yamanaka *et al*. 1980), and heavy metals (Hg: Pecci and Fujimori 1967, Murthy and Mohanty 1991; Cu: Park and Sauer 1991). Singhal *et al*. (1981) made a preliminary investigation on the spectral properties of intact cells under HT stress in *Synechococcus*. Babu *et al.* (1991) studied under HT the photosynthetic electron transport and spectral properties of intact cells of *Spirulina*. In this work we checked the effect of HT on spectral properties of PBSs both *in vivo* and *in vitro*.

Spirulina platensis was grown in a defined medium at 25±2 °C under continuous "white light" (15 W m^{-2}) as described in Venkataramanaiah *et al*. (2003). The culture was continuously bubbled with filtered air. The mid-log-phase culture was harvested by centrifugation at 12 000×*g* for 10 min and washed with fresh growth medium and finally suspended in the medium by maintaining 0.5 kg(Chl) m^{-3}. Separate aliquots of intact cells were exposed to different temperatures (35–50 °C) for 30 min in dark at Chl *a* concentration of 2 kg m^{-3}. For *in vitro* studies, PBSs were isolated from intact cells by following the procedure of Gantt *et al.* (1979). Similarly, PBSs equivalent to 30 g(protein) m^{-3} were taken separately and exposed to temperatures of 30–40 °C for 10 min in the dark. Chl amount was estimated according to Mackinney (1941). Absorption spectra of intact cells and PBSs were recorded using *Hitachi U-2000* spectrophotometer. Fluorescence emission spectra at room temperature and 77 K were recorded on a *Perkin-Elmer LS-5* spectrofluorimeter according to Murthy *et al.* (1991). The spectra were not corrected for the spectral efficiency of the equipment. Protein content was estimated by the procedure of Lowry *et al.* (1951).

The *Spirulina* cells treated with 40 °C showed no large changes in the absorption spectrum (Fig. 1*A*). The increase in temperature to 50 and 60 °C caused a decrease in the peak absorption of 618 nm, which is emanating from PC. The cells treated with 60 °C showed a drastic decrease in the PC absorption with a shift in the peak position towards longer wavelength (618 to 625 nm) (Fig. 1*A*) while peak positions of Chl *a* (~680 nm) and carotenoids (~440 nm) remained unchanged. This decrease in the PC absorption could be due to the bleaching of phycobiliproteins. These results indicate that depending on the extent of temperature there was a decrease in the PC/Chl *a* ratio.

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Abbreviations: APC, allophycocyanin; Chl, chlorophyll; HT, high temperature; PBSs, phycobilisomes; PC, phycocyanin.
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Since HT affected the PC spectral absorption properties, an attempt was made to measure the room temperature fluorescence emission spectra of *Spirulina* cells with and without HT treatment. In control cells excited at 545 nm, an emission peak at 642 nm from PC was prominent in the spectrum. Incubation of cells at HT (40–60 °C) caused decrease in fluorescence intensity and induced blue shift by 3 nm (Fig. 2*A*).

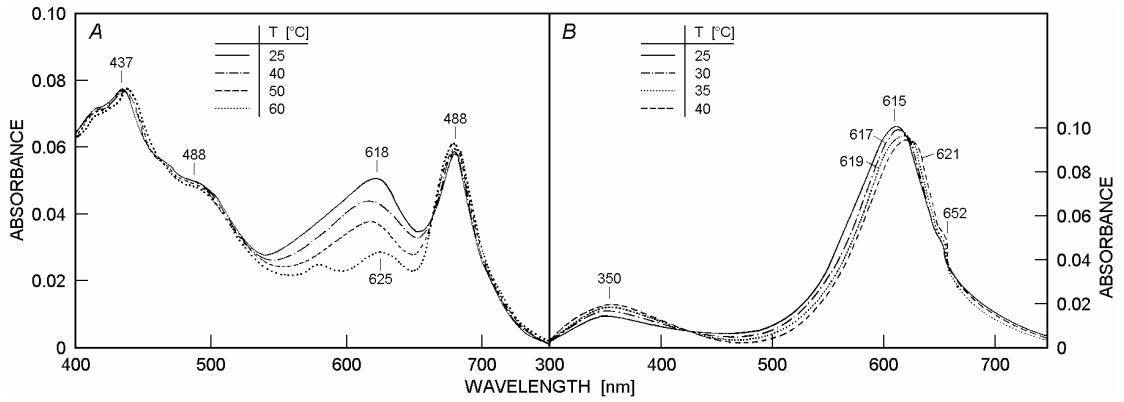

Fig. 1. Effect of high temperature (HT) on absorption spectra of intact cells (*A*) and isolated PBSs (*B*) of *Spirulina platensis*. Cells were given 30 min HT treatment (PBSs 10 min) and then were kept in dark for 5 min before the measurements. Intact cells equivalent to 6 μg chlorophyll (Chl) *a* were suspended in 3 cm^3 of 20 mM HEPES-NaOH buffer, pH 7.5. PBSs equivalent to 15 μg Chl *a* were suspended in 1 cm^{-3} of 0.75 M phosphate buffer, pH 7.0.

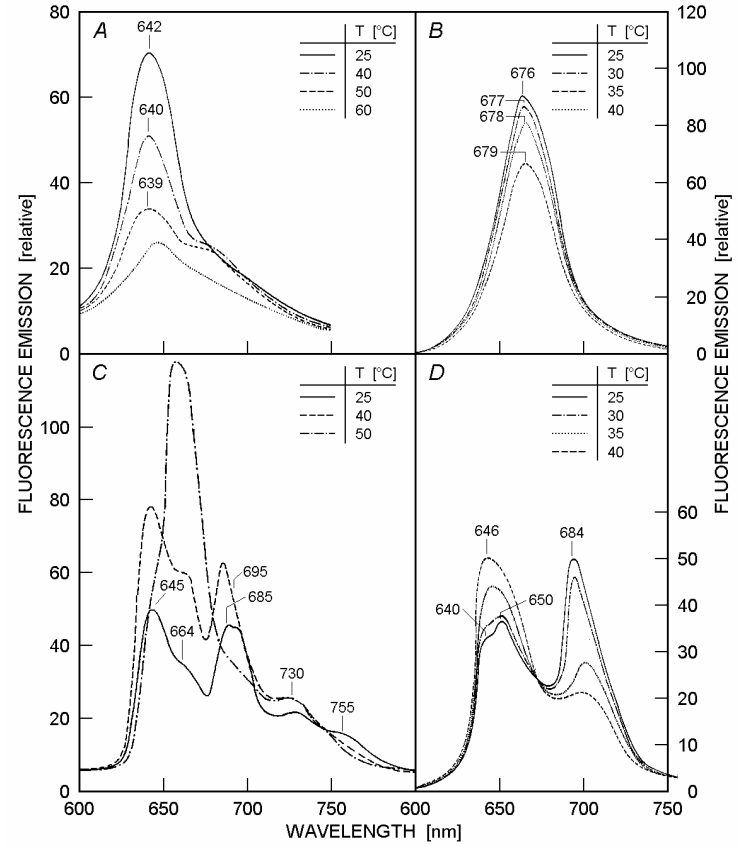

Fig. 2. Effect of high temperature (HT) on room (*A, B*) and low temperature (77 K) (*C, D*) fluorescence emission spectra of intact cells (*A, C*) and isolated PBSs (*B, D*) of *Spirulina platensis*. Cells were given 30 min of HT treatment (PBSs 10 min) and then kept in dark for 5 min before the measurements. In *A* and *B*, intact cells equivalent to 6 μg chlorophyll were suspended in 3 cm^3 of 20 mM HEPES-NaOH buffer, pH 7.5. In *C*, cells were suspended in 50 % glycerol. In *D*, PBSs equivalent to 10 or 30 g m^{-3} chlorophyll were suspended in 1 cm^3 of 0.75 M PO_4 buffer, pH 7.0. Excitation wavelength was 545 nm, slit width for measurements of both excitation and emission was 5 nm.

This decrease in fluorescence intensity could be due to alteration in the PBSs organization, which in turn caused an interruption of energy transfer. Our results are in agreement with the observations of Babu *et al*. (1991) who showed alteration at the level of phycobiliproteins in intact cells of *Spirulina*. Similar results were obtained under mercury ion stress in *S. platensis* (Murthy *et al.* 1989, Murthy and Mohanty 1991) and in *Synechococcus* (Singhal *et al.* 1981). To identify the target protein of energy transfer in intact cells, low temperature emission spectra were measured. HT caused increase in the intensity of PC and APC-β fluorescence emission at 40 °C. Further increase to 50 °C caused maximum enhancement of PC emission and shift in the peak position towards longer wavelengths. This indicates HT is able to cause impairment of energy transfer by affecting the level of PC (Fig. 2*C*).

To correlate the *in vitro* observations with *in vivo* studies, we isolated the PBSs. The room temperature absorption spectra of intact PBSs isolated from control cells showed a peak at 615 nm due to PC absorption with a conspicuous shoulder at 652 nm (APC absorption) and another peak at 350 nm due to transitions of chromophores (Fig. 1*B*). The HT treatment caused in PBSs a 2-nm red shift in peak position and a small increase in the absorption at 350 nm. The shift indicates disorganization of PC and the increase in the 350 nm absorption peak is due to altered PC chromophore and apoprotein interaction. Incubation of PBSs at HT (40 °C) caused decrease in fluorescence intensity and induced a 3-nm red shift (Fig. 2*B*). The results of *in vitro* studies suggest that isolated PBSs are very sensitive to HT treatment which supports the *in vivo* observations.

To establish the target pigment protein in the PBSs, fluorescence spectra of PBSs were measured after freezing the sample at liquid nitrogen temperature (77 K) (Fig. 2*D*). In control spectra two peaks were seen, one at 650 nm having a hump at 640 nm and another one at 684 nm due to the presence of APC-β. After HT treatment (35 and 40 °C) there was a raise in PC fluorescence along with decrease in APC fluorescence intensity. In addition a shift of 4 nm towards the blue region of PC emission was also observed. The increase in intensity of PC fluorescence was due to uncoupling of energy transfer between PC and APC. The blue shift indicated the disorganization in the pigment protein interaction of PC. Rajagopal *et al.* (1998) reported similar alterations in PC under UV-B stress in the above cyanobacterium.

Thus our results suggest that HT treatment causes the uncoupling of energy transfer within the PBSs by selectively affecting PC and thereby causing impairment of photosystem 2 photochemistry.

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