PHOTOSYNTHETICA 36 (4): 617-620, 1999

BRIEF COMMUNICATION

Protection of energy transfer process in isolated phycobilisome by "white light" from ultraviolet-B induced damage in the cyanobacterium *Spirulina platensis*

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Abstract

Effect of UV-B (1.9 W m^{-2}) alone or in combination with supplemental "white light", WL (20 W m^{-2}) exposure was studied on the energy transfer process of intact phycobilisomes isolated from *Spirulina platensis*. Exposure of UV-B or supplemental irradiation induced a decrease in room temperature fluorescence intensity and caused a shift towards shorter wavelengths. The low temperature fluorescence measurements showed that UV-B impairs energy transfer from phycocyanin to allophycocyanin B and the extent of damage may be reduced by the exposure to supplemental WL.

Additional key words: allophycocyanin; chlorophyll fluorescence; phycocyanin.

UV-B radiation which results from the depletion of ozone layer affects plant productivity mainly by influencing the most important biological process, *i.e.*, photosynthesis (Bornman 1989, Teramura and Sullivan 1994, Murthy and Rajagopal 1995, Nedunchezhian *et al.* 1996). It predominantly attacks photosystem 2 (PS2) (Noorudeen and Kulandaivelu 1982, Nedunchezhian and Kulandaivelu 1993) at multiple sites, *i.e.*, water oxidation complex, charge separation due to structural alterations in D_1/D_2 polypeptides (Renger *et al.* 1989), and photosynthetic pigments such as the light-harvesting complex (Lingakumar and Kulandaivelu 1993). Recent studies of the cyanobacterial system indicate that in addition to alterations in the reaction centre of

Received 9 April 1998, *accepted* 27 July 1998.
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Abbreviations: APC, allophycocyanin; Chl, chlorophyll; PBS, phycobilisome; PC, phycocyanin; PS, photosystem; UV-B, ultraviolet-B.
Acknowledgements: The authors thank the Dean, School of Life Sciences, JNU, New Delhi for facility under DSA-UGC link activity. Financial support by the DST-YS project of S.D.S. and the DST B6.9 (PM) are gratefully acknowledged. SR is thankful for UGC for providing fellowship.

PS2, impairement in energy transfer from phycobilisome (PBS) to Chl is also responsible for the loss in PS2 photochemistry (Balakrishna *et al.* 1996, Nedunchezhian *et al.* 1996, Rajagopal and Murthy 1996, Lorenz *et al.* 1997, Sah *et al.* 1998). UV-B exposure causes impairement of energy transfer in PBS (Zündorf and Häder 1991) and disintegration of low molecular mass phycobilin monomers (Sinha *et al.* 1995, Banerjee and Häder 1996). Due to depletion of ozone layer, plants under natural conditions receive UV-B radiation along with WL. The effect of UV-B in combination with PAR on cyanobacteria has been studied very rarely. This is why we studied the energy transfer process in isolated PBS from the cyanobacterium *S. platensis*.

S. platensis was axenically grown in Zarrouk's (1966) medium at 25±2 °C under continuous irradiance (25 W m^{-2}). PBS were isolated on saccharose density gradient according to Gantt *et al.* (1979). Equal amounts of purified PBS (2 kg m^{-3}) in quartz cuvettes were irradiated for different intervals in the presence of UV-B (1.9 mW m^{-2}) alone or with supplemental WL (20 W m^{-2}+UV-B). Fluorescence emission spectra of PBS at room temperature and 77 K were recorded using the *Perkin-Elmer LS-5* spectrofluorimeter. The emission and excitation monochromators were protected by band-pass filters *Shimadzu 0-52* and *Corning CS 4-96,* respectively. The obtained spectra were not corrected for instrument sensitivity. For spectral measurements, equal protein concentrations at room (30 g m^{-3}) and 77 K (10 g m^{-3}) temperature were maintained with 1.0 M phosphate buffer. Protein content of PBS was measured by the procedure of Lowry *et al.* (1951).

Table 1. Characteristics of room temperature and 77 K fluorescence emission of *Spirulina platensis* phycobilisomes exposed to UV-B and supplemental "white light" (WL). The phycobilisomes were suspended in 1.0 M PO_4 buffer pH 7.0 in an equal protein basis (30 or 10 g m^{-3}). The excitation and emission slits were 5 nm. In columns of relative fluorescence at peak position (F_{rel}) the values in brackets give % of quenching. For other details see Materials and methods. The values are averages of three separate experiments and SD is lower than 11 %.

Time	UV-B					UV-B + supplemental WL				
[min]	Peak [nm]	F_{rel}	F_{650}	F_{684}	F_{684}/F_{650}	Peak [nm]	F_{rel}	F_{650}	F_{684}	F_{684}/F_{650}
0	670	52±5 (0)	26±2	39±4	1.50	670	52±5 (0)	26±2	39±4	1.50
20	668	31±3 (40)	36±3	19±2	0.52	668	31±3 (41)	40±4	24±2	0.60
40	665	20±2 (60)	35±3	10±1	0.28	667	26±2 (51)	37±3	18±2	0.48
60	662	12±1 (76)	33±3	8±1	0.24	665	20±2 (63)	35±3	15±1	0.43

In order to check the possible site of alteration in the energy transfer characteristics of the PBS complex, fluorescence emission spectroscopy was used. The emission peak intensity of PBS at 670 nm was reduced by about 40 and 76 % when irradiated with UV-B for 20 and 60 min, respectively, whereas in PBS exposed to supplemental WL the peak intensity was decreased by *ca.* 41 and 63 % for 20 and 60 min (Table 1). Accordingly, the PBS emission peak shift from 670 to 662 nm was observed after exposure to UV-B. Under a supplemental irradiation a similar shift from 670 to 665 nm was noted. Generally, the absorbed energy is efficiently transferred in few pico-

seconds from phycocyanin (PC) to allophycocyanin (APC) B, the terminal emitter of energy from PBS. A depreciation in fluorescence emission from APC-B was either due to decreased absorption or due to inefficient energy transfer from (PC) to APC-B (Fork and Mohanty 1986). Similar observations have been made in rice field cyanobacteria (Sinha *et al.* 1995, Banerjee and Häder 1996). When PBS was exposed to supplemental WL there was loss in emission intensity but the extent was less than that under UV-B alone. The radical change in emission characteristics of PBS after exposure to UV-B and supplemental WL was due to both bleaching of chromophoric proteins and changes in energy flow to APC-B from PC. This differential decrease in PBS emission may be due to a WL mediated protective mechanism.

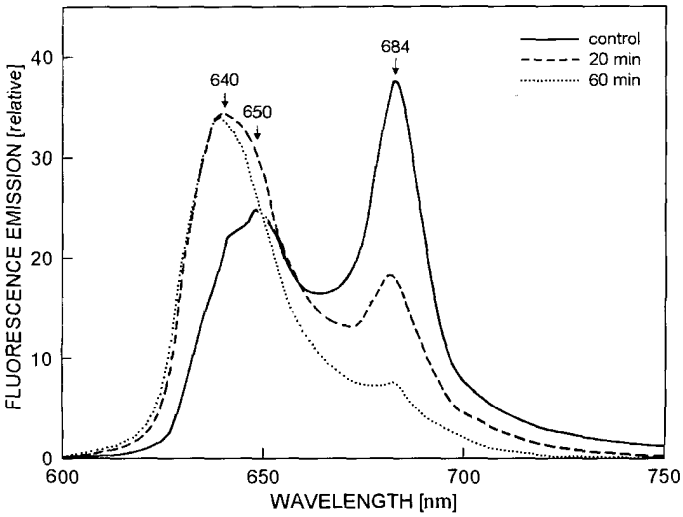

Fig. 1. 77 K fluorescence emission spectra of *Spirulina platensis* phycobilisomes exposed for 20 or 60 min to 1.9 mW m^{-2} UV-B at 20 °C. The phycobilisomes were suspended in 1.0 M PO_4 buffer (pH 7.0). Samples were mixed with glycerol (in 1:1 ratio) before freezing the PBS. Slit width for both excitation and emission was 5 nm. For other details see Materials and methods.

To indentify the target chromoprotein, low temperature fluorescence emission spectra of PBS were measured. In control spectra of PBS, two peaks appeared (F_{650} due to PC and F_{684} due to APC, see Fig. 1). UV-B radiation caused the alteration of F_{684}/F_{650} ratio: it decreased from 1.50 (control) to 0.52 or 0.24 in PBS exposed to UV-B for 20 or 60 min, respectively (Table 1). In PBS exposed to supplemental WL, F_{684}/F_{650} decreased from 1.50 (control) to 0.60 (20 min) and 0.43 (60 min). Thus UV-B exposure leads to the alteration in energy transfer from PC to APC-B in a time-dependent manner. Similar observations in *Synechococcus* PBS during UV-B treatment (Balakrishna *et al.* 1996, Sah *et al.* 1998) indicate that the PC to APC-B energy transfer is the main target. Our results also indicate that PC is the sensitive chromoprotein that is affected by UV-B radiation, since there is a blue shift in the peak position. The new aspect in our attempt is the comparison of UV-B and supplemental WL effects on energy transfer. In PBS exposed to supplemental WL

there was also inhibition in energy transfer (from PC to APC-B) and the extent of inhibition was lower when compared to UV-B alone. Thus supplemental WL protects against UV-B.

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