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Ultraviolet-B induced changes in ultrastructure and D1/D2 proteins in cyanobacteria *Synechococcus* sp. PCC 7942

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Abstract

Effects of ultraviolet-B (UV-B) irradiation on ultrastructure, total cellular protein, and PS2 proteins D1 and D2 of *Synechococcus* sp. PCC 7942 cells was studied. The scanning electron micrographs showed UV-B radiation induced bending of the cells. The transmission electron micrographs revealed disorganization and shift in thylakoid lamellar structure to one side of the cell. The cellular phycocyanin/chlorophyll ratio decreased with increasing UV-B treatment and due to this the colour of cells turned light-green. No apparent change in total cellular proteins was evident, but the contents of two major proteins of PS2, D1 and D2, showed decline due to UV-B irradiation, although to different extent.

Additional key words: carotenoids; cell shape, chlorophyll, phycocyanin, thylakoid.

Introduction

UV-B radiation affects various processes of photosynthesis, including thylakoid membrane structure and pigment contents (for review see Murthy and Rajagopal 1995). The reduction of stratospheric ozone concentration and resultant increase in UV radiation reaching the planet surface negatively affect phytoplankton primary productivity (Smith *et al.* 1992). This increased solar UV-B affects the marine food web and biogeochemical cycles (Merchant and Davidson 1991, Häder 1993, Holm-Hansen and Lubin 1993, Tevini 1993) because UV-B can penetrate water to significant depths (Smith *et al.* 1992).

Cyanobacteria are photosynthetic communities capable of extremely high rates of

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Abbreviations: Car, carotenoids; Chl, chlorophyll; PC, phycocyanin; PS2, photosystem 2 complex; SEM, scanning electron microscopy; TEM, transmission electron microscopy; UV-B, ultraviolet B (280-320 nm) radiation.

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photosynthesis and contribute significantly to the very high primary productivity of shallow and clear water environments (Zedler 1980). Bebout and Garcia-Pichel (1995) report that in a mat community the dominant cyanobacterium *Microcoleus chthonoplastes* migrates in response to UV-B. These microorganisms migrating within the mat apparently sense UV-B directly and respond in order to limit their exposure to UV-B. The microbial mats become visibly lighter green concomitantly with rapid reduction in gross photosynthetic rate. Motile organisms may orient by utilizing various complex strategies to optimize their vertical position (Liu *et al.* 1990, Eggersdorfer and Häder 1991) while others drift with the currents.

UV irradiation induces a decrease in phycocyanin (PC) and total protein contents of the cyanobacterium *Aulosira fertilissima* (Banerjee and Häder 1996). Quantitative study of the photodestruction of the cyanobacterial (*Anabaena* sp. PCC 7120) phycobilisomes by UV radiation of 285-305 nm has been done by Lao and Glazer (1996). Nedunchezhian *et al.* (1996) used absorption and fluorescence excitation and emission spectra to show that UV-B induced decrease in photosynthetic activity in *Anacystis* is due to loss of energy transfer from phycobilisomes to Chl which in turn leads to dissociation of phycobilisomes. Photoinhibition and resistance to photoinhibition appear to be centred on PS2 and D1 turnover (Samuelsson *et al.* 1987, Greenberg *et al.* 1989, Wunschmann and Brand 1992, Jansen *et al.* 1996, Rajagopal and Murty 1996).

Not much is known about the effect of UV-B on the ultrastructure of *Cyanobacteria*. In order to assess the possible consequences of UV-B irradiation on *Synechococcus* sp. PCC 7942, a mechanistic approach using an artificial source of UV-B was used to examine the UV-B induced ultrastructural changes and the effect on D1/D2 proteins (the two most important proteins of photosystem 2).

Materials and methods

Growth conditions: Cultures of *Synechococcus* sp. PCC 7942 (a kind gift from Dr. Susan Golden) were grown autotrophically in BG-11 medium (Stanier *et al.* 1971) with occasional shaking. They were irradiated with cool white fluorescent lamps (photon flux density 20 μmol m^{-2} s^{-1}) at 28 °C. The A_{550} of the culture taken for experiments was adjusted to 0.7 by diluting it with fresh medium as and when desired. This relatively low and constant cell density minimized the *in situ* variation of incident irradiance resulting from self-shading of the cells; they were subjected to UV-B radiation (280-320 nm) from lamps (*VL-215 M, Vilber Lourmat*) with maximal emission at 312 nm for different time periods giving a total irradiance of 50 μmol m^{-2} s^{-1}.

Scanning electron microscopy (SEM): The cells were fixed in 2 % glutaraldehyde (EM-grade, *LAAD Research Industry*) followed by fixation in 2 % osmium tetraoxide made in 100 mM cacodylate buffer, pH 6.2. The cells were dehydrated in graded series of ethanol and acetone, and coated with analytical grade silver using a vacuum

splutter coater on copper grids (Gupta and Singhal 1996). The grids were viewed under SEM model *501 Phillips* operated at 15 kV.

Transmission electron microscopy (TEM): The control and UV-B irradiated cells were collected by centrifugation at 6000×*g* for 10 min. The prefixation was carried out in 2 % glutaraldehyde solution made in 100 mM cacodylate buffer, pH 6.2 at 4 °C. Following pre-fixation, the cells were washed 2-3 times in cacodylate buffer and post-fixed in 2 % osmium tetraoxide, for one hour in ice bath in a fume hood. The cells were washed 5-6 times with cacodylate buffer and dehydrated in graded series of ethanol solution (15 %, 80 %, 90 %, 2 changes for 10 min each). The cells were then embedded in araldite, thin sections (1 μm) were cut and mounted on copper grids. The sections were stained with both uranyl acetate (saturated solution in 60 % ethanol) and lead citrate (2 % in 0.2 M NaOH) and examined under transmission electron microscope model *CM12 Phillips* operated at 30 kV.

Absorption spectra of intact cells treated with different doses of UV-B were taken at room temperature by a *UV-160A* spectrophotometer (*Shimadzu*, Kyoto, Japan) using an opal glass assembly.

Cell proteins were isolated using a slight modification of protocol of Clarke *et al.* (1993). Cells were harvested by centrifugation at 4000×*g* for 5 min and resuspended at a Chl concentration of 5 g m^{-3} in fresh BG-11 medium. Total cellular proteins were extracted by centrifuging samples at 14 000×*g* for 5 min and then suspending the cell pellet in 2.5 cm^3 of 100 mM Tris.HCl, pH 8.6/150 mM sucrose/30 mM dithiothreitol/1 mM EDTA/1 mM phenylmethylsulfonyl fluoride/2 % *n*-lauroyl sarcosine (sodium salt). Cells were disrupted by sonication with 4 bursts of 30 s each at 18 Ω, with an intermittent cooling of 30 s after each burst. Insoluble material was removed by centrifugation at 14 000×*g* for 5 min. Protein samples containing equal amounts of Chl (5 μg) were electrophoresed on Tricine SDS-PAGE (10 % T, 3 % C) (Schagger and Jagow 1987). Proteins were transferred electrophoretically to nitrocellulose (Towbin *et al.* 1979) and immunoblot analysis was done as described in Chauhan and Singhal (1996).

Results and discussion

Comparison of scanning electron micrographs of control (grown under cool white fluorescent lamps only) and 2 h-UV-B-treated cells (Fig. 1, *A* to *D*) showed a bending of majority of cells induced by UV-B. Transmission electron micrography of ultrathin sections revealed in the control rod shaped (longitudinal section) and round (cross section) cells, prominent thylakoids arranged in 3-4 concentric layers with attached bead-like phycobilisomes (Fig. 1*E*). In the UV-B irradiated cells, the thylakoid lamellae shifted to one side of the cell, and the thylakoid membrane structure at the side which received UV-B was drastically reduced with only a single membrane pairing evident (Fig. 1*F*).

Colour of the *Synechococcus* cells turned during the 4-h UV-B irradiation from dark blue green to light green. Absorption spectra of the cells grown under "white light" showed three peaks: those at 622 and 680 nm corresponded to the absorption of PC and Chl *a*, respectively. The total pigment ratio of Car/Chl (490/680 nm) did not change significantly with increasing UV-B treatment, whereas the cellular PC/Chl (622-680 nm) ratio decreased with the increasing UV-B treatment (Table 1).

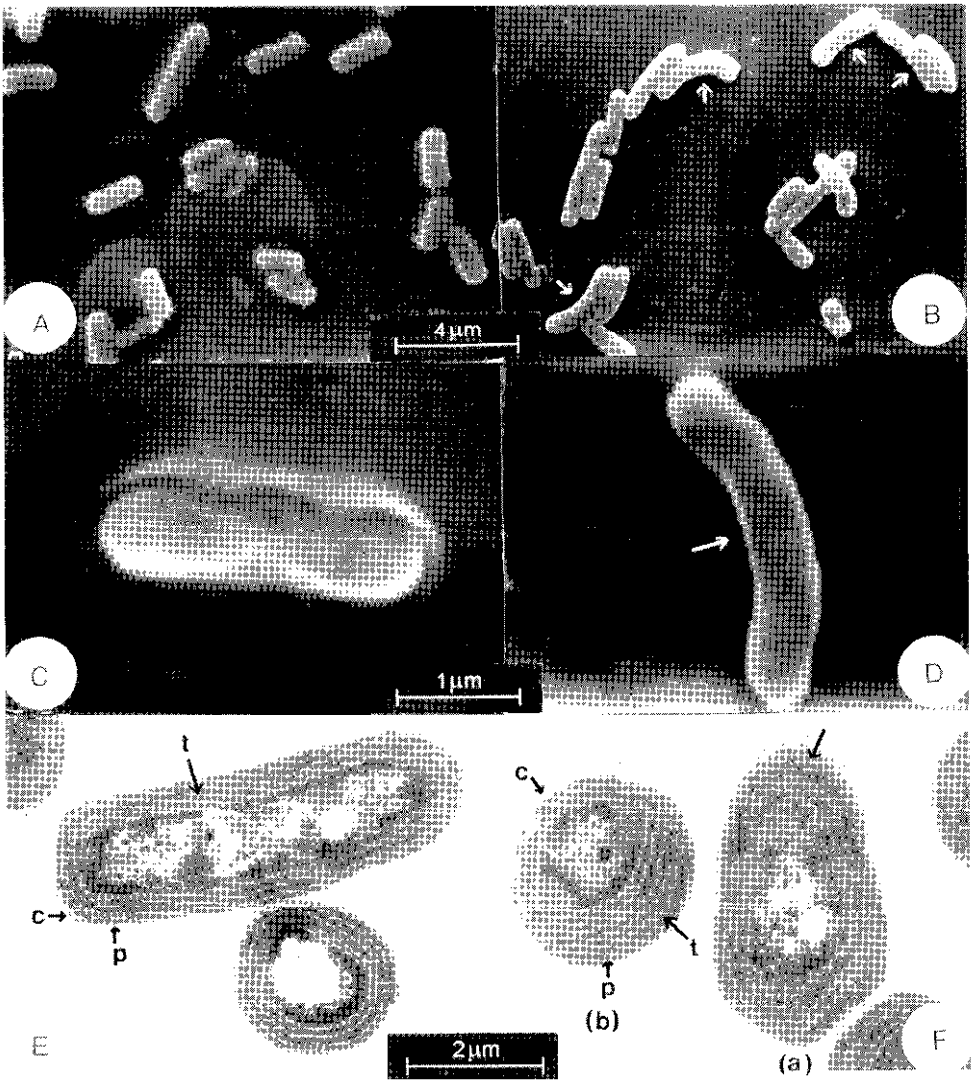

Fig. 1. Scanning electron micrographs (*A*, *B*, *C*, *D*) and transmission electron micrographs (*E*, *F*) of *Synechococcus* cells. The rod shaped cells with intact cell walls (*A*, *C*) bend due to UV-B treatment (*B*, *D*). Control cells contain 3-4 concentric layers of thylakoid lamellae (*E*); they shift in UV-B treated cells (*F*). c, cell wall; p, phycobilisome; t, thylakoid.

Table 1. Effect of UV-B treatment on absorption properties of intact cells of *Synechococcus* PCC 7942. Car - carotenoids; Chl - chlorophyll; PC - phycocyanin.

UV-B treatment	PC/Chl (622/680 nm)	Car/Chl (490/680 nm)
control	1.06	0.72
1 h	0.76	0.80
2 h	0.65	0.79
3 h	0.61	0.80
4 h	0.47	0.78

The polypeptide profiles of the total protein produced by PAGE of control and UV-B treated samples were very similar (Fig. 2*A*). When the gels were electroblotted and immunodecorated with anti-D1 and D2 antibodies, the blots showed that within 1 h of UV-B treatment the D1 protein was completely degraded, whereas the D2 protein was comparatively less degraded; it was faintly visible even after 4 h of UV-B treatment (Fig. 2*B*).

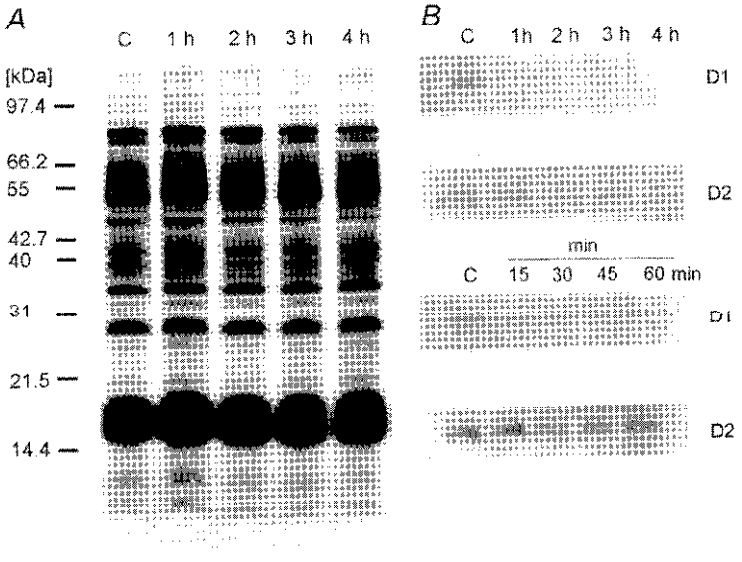

Fig. 2. (*A*) Coomassie stained polypeptide profile of total protein of *Synechococcus* cells after UV-B treatment for different time periods as compared to control. (*B*) The relative amounts of D1 and D2 proteins present in *Synechococcus* cells irradiated with UV-B for different time periods or under control conditions measured immunologically.

The used UV-B treatment intended to maximize response in *Synechococcus*, even if it was not comparable to long-term UV-B treatments in nature. Bending of *Synechococcus* cells as seen in SEM could be due to a short term UV-B acclimation strategy employed by the cyanobacteria. Although cyanobacteria sense radiation stress and respond by producing certain sun-screen pigments (Protean *et al.* 1993), in

our experiments the duration of UV-B exposure was short and we did not check the immediate production of these pigments in the cells. Some dinoflagellates orient themselves to optimize their vertical position, while some drift with the currents (Liu *et al.* 1990, Eggersdorfer and Häder 1991). The TEM showed that the UV-B exposed cells tried to reorient the thylakoid membrane structure probably to save the light-harvesting phycobilisomes and the rest of the photosynthetic apparatus. The 2-h UV-B treatment partially destroyed the thylakoid lamellar structure (Fig. 1*F*, cell *a*), whereas in cell *b* the reorientation of the lamellar structure was evident. The side facing the UV-B source contained the most disorganized thylakoids.

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