BIOLOGIA PLANTARUM 38 (1): 53-59, 1996

Damages of photosynthetic apparatus in *Anacystis nidulans* by ultraviolet-B radiation

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Abstract

Changes in photosystem (PS) 2 activity were measured in *Anacystis nidulans* cells exposed to UV-B irradiation. The decrease in PS 2 activity was biphasic in cells exposed at the surface (0 cm) and at 2 cm depth in the water column, while gradual in those exposed at 10 and 30 cm depth. Addition of supplemental "white light" reduced the extent of UV-B damage. Decrease in photosynthetic activity was primarily due to the loss of energy transfer from phycobilisome to chlorophyll as the former in cyanobacteria acts as the primary light-harvesting complex. This was supported by the absorption and fluorescence excitation and emission spectral studies. All these changes were proportionally reduced by the thickness of the water column that reduced UV-B irradiance.

Additional key words: light-harvesting complex, photosystem 2, phycobilisome.

Introduction

UV-B radiation inhibits fundamental processes such as photosynthesis and plant growth (Teramura *et al.* 1980, Tevini *et al.* 1981, 1989, Bornman 1989, Kulandaivelu *et al.* 1989, Wilson and Greeenberg 1993). Several studies have shown that UV-B radiation adversely affects the function of PS 2 in chloroplasts (Van *et al.* 1977, Kulandaivelu and Noorudeen 1983, Nedunchezhian and Kulandaivelu 1991). This is manifested as low rates of PS 2 electron transport (Kulandaivelu and Noorudeen 1983) and low variable florescence yield (Tevini and Pfister 1985). Other investigations report loss of herbicide binding sites and damage to the PS 2 donor

Received 10 April 1995, *accepted* 21 June 1995.

Abbreviations: BQ - *p*-benzoquinone; Chl - chlorophyll; PBS - phycobilisoms; PS 2 - photosystem 2; UV-B - ultraviolet-B (280 - 320 nm) radiation.

Acknowledgements: This work was supported in part by a grant from the Ministerio de Education y Ciencia (ref SB 94-AM086772) to N. N.

side in UV-B treated thylakoids (Renger *et al.* 1989). Cyanobacteria are used extensively for studies on photochemical energy transfer reactions as they have phycobilisomes (PBS) as the major light harvesting complex. Besides, these unicellular forms are easy to manage, unlike the red and brown algae. The PBS, being protein chromophores, undergo denaturation on absorption of UV radiation. Hence, we studied in detail the nature of structural and photochemical changes occurring in PBS and energy transfer from PBS to Chl upon UV-B irradiation.

Materials and methods

Cultures of *Anacystis nidulans* strain IU 625 were developed phototrophically in 500 cm^3 culture tubes at 25 °C. The cultures were aerated with filtered air. For all experiments, cells were harvested at the mid log phase by centrifugation, washed once, and suspended in fresh cultivation medium.

UV-B treatment: Cell suspension at a Chl concentration of 1 g dm^{-3} was transferred into dialysis bags as a thin layer for UV-B treatment. These bags were placed at different depths of water column (0, 2, 10 and 30 cm) and exposed to UV-B radiation (4.5 mmol m^{-2} s^{-1} at the surface). Transmission of the bags increased linearly from 65 to 80 % in the region of 270 to 330 nm. UV-B irradiance at different depths of both the air and water columns declined exponentially from 4.5 (water surface level) to 1 mmol m^{-2} s^{-1} (30 cm). Although UV-B irradiance remained approximately the same in air and water, spectrally there was significant change in water (values not shown). Samples were collected at regular time intervals. To avoid sedimentation, the dialysis bags were agitated by using a glass rod.

The rate of photosynthetic O_2 evolution was measured in a *Hansatech* O_2 electrode under saturating "white light" at 25 °C. White light (0.8 mmol m^{-2} s^{-1}) was provided by a slide projector. Irradiance was measured using a *Li-Cor 188* quantum radiometer (*Li-Cor*, Lincoln, USA). Chl *a* content was determined by the method of Myers and Kratz (1955).

Absorption and fluorescence spectra: Room temperature absorption spectra of cells were measured using a *Hitachi 557* spectrophotometer (*Hitachi Ltd.*, Tokyo, Japan). Fluorescence excitation and emission spectra were measured in a *Hitachi MPF4* spectrofluorimeter. The spectra were corrected for the differences in the monochromator and photomultiplier sensitivity (S-20 response). The Chl content of the cell suspension was maintained at 1 g dm^{-3} to minimise self absorption.

Results and discussion

The PS 2 activity of *Anacystis* cells exposed to UV-B showed a biphasic loss in cells exposed at the water surface and at 2 cm depth of the water column, while those at 10 and 30 cm showed a gradual loss (Fig. 1). Complete loss of photosynthetic activity occurred after 45 min of UV-B irradiation at the water surface, while the cells at 30 cm depth retained as much as 80 % of initial activity during the same period. Addition of supplemental "white light" reduced the extent of UV-B damage

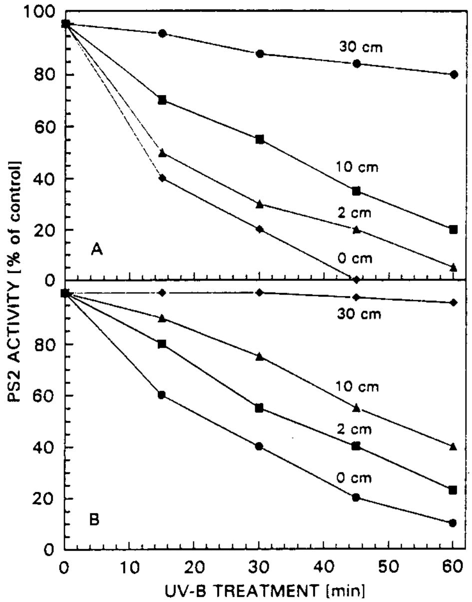
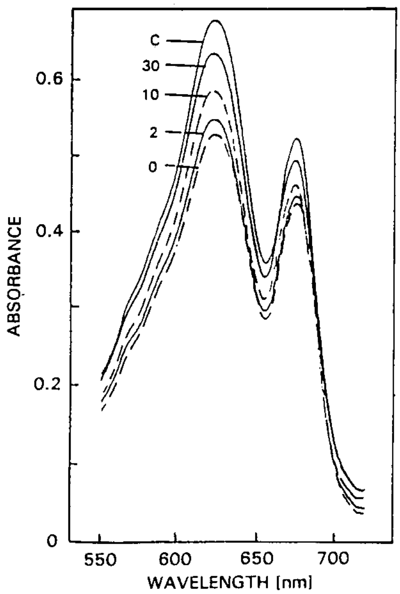


Fig. 1 . Changes in PS 2 activity (rate of benzoquinone mediated Hill reaction) as a function of UV-B (*A*) and UV-B + "white light" (*B*) irradiation of *Anacystis* cells at different depths of water column. The 100 % value was 35 nmol(O_2) g^{-1}(Chl) s^{-1}.

and such reduction was proportional to irradiance up to a certain level. Loss of O_2 evolution capacity in *Anacystis* could be either due to inhibition of photosynthetic electron transport by the inactivation or alteration of the electron transport intermediates or by a block in the energy transfer between the primary light-harvesting PBS and the reaction centres. UV-B radiation may predominantly attack the charge separation reaction sequence Z-680-Pheo-QA $\rightarrow$ Z^+-680-Pheo-QA^- (Noorudeen and Kulandaivelu 1982, Renger *et al.* 1986, Renger and Eckert 1991), so that the centres once blocked get transformed into dissipative sinks for excitation energy (Iwanzik *et al.* 1983) and are no longer capable of supporting normal electron flow. According to Renger *et al.* (1989) UV-B irradiation deteriorates primarily water oxidation.

Anacystis cells possess a complex light-harvesting system. In fully developed cells 80 % of the available radiation is captured by PBS. Hence we followed how the energy transfer from PBS to Chl was altered on UV-B treatment. We measured the changes in room temperature absorption spectra of control and UV-B treated *Anacystis* cells in order to find out if any correlation exists between the loss of photosynthetic activity and damage of thylakoid membrane. At room temperature, the control cells revealed absorption maxima at 625 and 678 nm in the red region of the spectrum, corresponding to PBS and Chl. UV-B radiation decreased both absorption maxima (Fig. 2). The maximum change was noticed in cells exposed at 0 cm. The decrease in Chl absorption could be due to the effect of UV-B radiation on the membrane and to the destruction of Chl (pheophytinisation). The decrease of the phycobilin absorption band is due to conformational changes in the PBS proteins and bleaching of the chromophore (Scheer and Hufer 1977, Laczko and Barabas 1981).



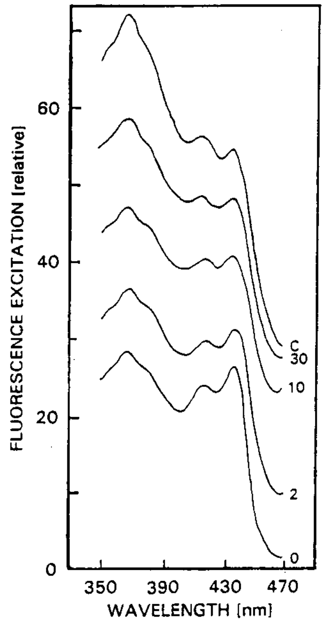


Fig. 2. Effect of UV-B irradiation on the room temperature absorption spectra of *Anacystis* cells. Numbers along with trace indicate depths [cm] of the water column. C = control.

Fig. 3. Changes in the excitation spectra for F682 in *Anacystis* cells treated with UV-B radiation for 60 min at different depths [cm] of the water column. C = control.

The room temperature excitation spectra for F682 in control and UV-B treated *Anacystis* cells showed three major peaks in the blue region at 365, 415 and 435 nm (Fig. 3). UV-B irradiation drastically decreased the 365 nm excitation band. This indicates a reduced energy transfer from PBS to Chl a which is partially due to chromophore bleaching and also to the confirmational changes in the phycobilin tetrapyrrole ring. Spectral analysis of absorption (Fig. 2) also indicates that chromophores in UV-B treated cells undergo degradation.

The relative fluorescence yields for F682 after excitation at 365, 415 and 435 nm in *Anacystis* cells exposed to UV-B radiance at different depth of water column was decreased (Table 1). In control cells, the ratio of F365/F435 was 1.72, while in UV-B treated cells it decreased with increasing UV-B irradiance indicating loss of Chl energy transfer.

Table 1. Changes in the relative fluorescence yield after excitation with 365, 415 and 435 nm for F682 in *Anacystis* cells exposed to UV-B irradiation at different depths of the water column. Values were calculated from the spectra in Fig. 3. All spectra were normalised at 435 nm.

Treatment	Excitation wavelength [nm] 365	415	435	F365/F435
Control	43	27	25	1.72
30 cm	37	25	25	1.45
10 cm	35	23	25	1.40
2 cm	32	24	25	1.28
0 cm	26	22	25	1.04

To check the UV-B induced changes in the efficiency of the PBS to Chl *a* energy transfer and also the PS 2 mediated electron transfer, fluorescence emission spectra were measured at 365, 415 and 435 nm excitation. When 365 nm was used as excitation wavelength, then emission spectrum showed two major peaks at 652 and 682 nm (Fig. 4). At 30 and 10 cm depths, the 652 nm emission showed a marginal

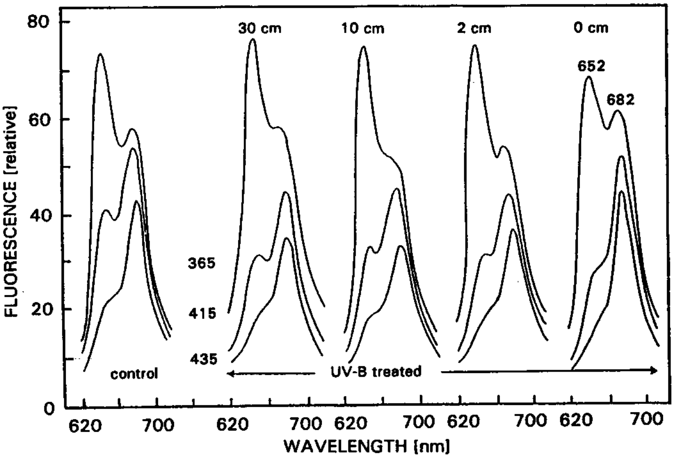


Fig. 4. Changes in the fluorescence emission spectra for *Anacystis* cells treated with UV-B radiation for 60 min. Fluorescence was excited at 365, 415 and 435 nm.

increase over the control. However, the cells irradiated at 0 cm showed drastic decrease in the level of 652 nm (PBS) emission. The level of 682 nm was not significantly altered. With 365 nm excitation the F652/F682 fluorescence ratio in control cells was approximately 1.4. In contrast to this, at 0 cm depth, the ratio was 1.1 (Table 2). This was due to drastic reduction of F682 (PBS) emission by UV-B radiation. In cells irradiated at 30 and 10 cm the ratio increased over the control because under these conditions fluorescence emission from PBS increased due to a block in the energy transfer from PBS to Chl. With 415 nm excitation, this ratio was gradually decreased with decrease of the water column. In contrast to this, at 435 nm the F682/F652 ratio was 3.09 in control cells, but in cells under high UV-B irradiance the ratio increased to 4.55. This could be partially due to the loss of PBS when more radiant energy was used directly for excitation of Chl.

Table 2. Changes in relative fluorescence emission at 652 and 682 nm and the ratios of F652/F682 and F682/F652 in *Anacystis* cells treated with UV-B irradiance. Excitation wavelengths were selected based on the peaks observed in the excitation spectra (see Fig. 4.). For 365 and 415 nm excitation, the 684 nm peak was adjusted in all samples, while for 435 nm excitation the 652 nm peak was adjusted approximately to the same level in all samples.

Treatment	Excitation wavelenght [nm]								
	365			415			435		
	F652	F682	652/682	F652	F682	652/682	F652	F682	682/652
Control	38	27	1.41	16	23	0.79	11	34	3.09
30 cm	42	27	1.56	16	23	0.70	11	29	2.64
10 cm	40	27	1.48	15	23	0.65	11	30	2.73
2 cm	36	27	1.33	15	23	0.65	11	35	3.18
0 cm	30	27	1.11	10	23	0.43	11	50	4.55

Our observations show that UV-B radiation does not bring about major structural damage to the thylakoids but induces dissociation of PBS and also of certain phycobiliproteins, thereby affecting the overall photochemical efficiency.

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