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BRIEF COMMUNICATION

# Short term effect of ultraviolet-B radiation on photosystem 2 photochemistry in the cyanobacterium *Synechococcus* 6301

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## Abstract

Exposure of intact cells of the *Synechococcus* 6301 to UV-B radiation induced a loss in photosystem 2 (PS 2) electron transport activity prior to the alteration in pigment complexes. Thus the degradation of PS 2 was not directly related to pigment alteration.

*Additional key words*: absorption spectra; chlorophyll; energy transfer; fluorescence emission and excitation spectra; phycocyanin.

Ultraviolet radiation can be classified into three different regions (for review see Häder and Tevini 1987): UV-C (< 280 nm), UV-B (280 - 320 nm) and UV-A (320 - 400 nm). Since the absorption coefficient of ozone is nearly zero at 330 nm, mainly UV-B radiation reaches the earth due to the reduction in ozone level. UV-B radiation affects plant productivity mainly by influencing photosynthesis (Bornman 1989, Tevini and Teramura 1989); it predominantly attacks PS 2 (Noorudeen and Kulandaivelu 1982, Nedunchezhian and Kulandaivelu 1993) at multiple sites, *i.e.*, water oxidation complex, charge separation due to the structural alterations in $D_1/D_2$ polypeptides (Renger *et al.* 1989), and photosynthetic pigments, namely those of the light-harvesting complex (Renger *et al.* 1986, Lingakumar and Kulandaivelu 1993). We tried to characterise the short-duration effect of UV-B radiation on PS 2 photochemistry and energy transfer in the cyanobacterium *Synechococcus* 6301.

The cyanobacterium was grown axenically in BG-11 medium (Stanier *et al.* 1965) at 25 ± 2 °C under continuous irradiance (15 W $m^{-2}$). Cells in the late logarithmic

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*Abbreviations:* Chl - chlorophyll; PBQ - parabenzoquinone; PC - plastocyanin; PS - photosystem.

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phase were harvested by centrifuging at 10 000 *g* for 10 min, washed with fresh growth medium and suspended in it. Exposure of the samples (10 g chlorophyll (Chl) $m^{-3}$ of cell suspension) to UV-B irradiation (5 W $m^{-2}$) was performed in Petri dishes under constant stirring. Photochemical activity was assayed polarographically with a Clark type oxygen electrode according to Murthy *et al.* (1989). The 3 $cm^3$ reaction mixture used for the assay of PS 2 contained 25 mM Hepes buffer (pH 7.5), 0.5 mM PBQ, and cells equivalent to 15 μg Chl. The Chl concentration was determined by spectrophotometrically by the method of MacKinney (1941). The electron transport activity was measured at 25 °C under saturating irradiance by 'white light' (450 W $m^{-2}$). The absorption spectra of intact cells were recorded using a *Hitachi*-557 spectrophotometer by following the procedure of Murthy *et al.* (1989). Fluorescence emitted by the cells was recorded at room temperature by exciting phycocyanin (PC) at 365 nm in a *Perkin-Elmer* LS-5 spectrofluorimeter. Cells equivalent to 5 μg Chl were used for measuring the fluorescence spectra.

Table 1. Short-term effect of UV-B radiation on photosystem 2 (PS 2) electron transport activity ($H_2O \longrightarrow$ PBQ) of *Synechococcus* 6301 intact cells. Cells were exposed to UV-B radiation (5.0 W $m^{-2}$) for different intervals. The reaction mixture contained 25 mM Hepes buffer (pH 7.5), 20 mM NaCl, 0.5 mM PBQ, and cells equivalent to 15 μg of chlorophyll (Chl) *a*. The values are means of three separate experiments ± S.E.

| Duration of exposure [min] | PS 2 activity [mmol($O_2$ evolved) $kg^{-1}$(Chl) $s^{-1}$] | Inhibition [%] |
|---|---|---|
| 0 (control) | 112 ± 5.8 | 0 |
| 15 | 86 ± 5.0 | 23 |
| 30 | 48 ± 2.8 | 57 |
| 45 | 31 ± 2.5 | 72 |
| 60 | 19 ± 2.0 | 83 |

Measurements of PS 2 electron transport ($H_2O \rightarrow$ PBQ) indicated that among the tested UV-B irradiance only 5 W $m^{-2}$ induced a 50 % loss in photochemical activity during short exposures (data not shown). This UV-B irradiance was used for further studies. UV-B radiation inhibited PS 2 catalysed $O_2$ evolution in a time-dependent manner (Table 1). A 50 % loss in photochemical activity was observed after exposing cells to 5 W $m^{-2}$ of UV-B radiation for less than 30 min. Further prolongation of the exposure caused additional loss in the photochemical activity. A similar inhibition was observed in *Vigna* chloroplasts after exposure to UV-B radiation (Noorudeen and Kulandaivelu 1982). The observed alteration in PS 2 activity could be either due to the alterations in reaction centre or to the changes in the antenna pigment-protein complexes.

To examine the latter possibility, we measured the spectral properties of UV-B treated cells. After 30 and 60 min of UV-B irradiation we found a partial loss in both Chl and PC absorption (Fig. 1, *left*). In control cells excited at 365 nm a fluorescence emission peak at 640 nm was prominent in the spectrum (Fig. 1, *middle*). The cells exposed to UV-B for 30 min showed a partial loss in the intensity without exhibiting

any shift in the peak position. Further exposure caused larger decrease in biliprotein fluorescence. This effect was even more evident when fluorescence excitation spectra were measured for spectral region corresponding to the absorption of the chromophore of PC (Fig. 1, *right*). A typical excitation spectrum of cyanobacterial

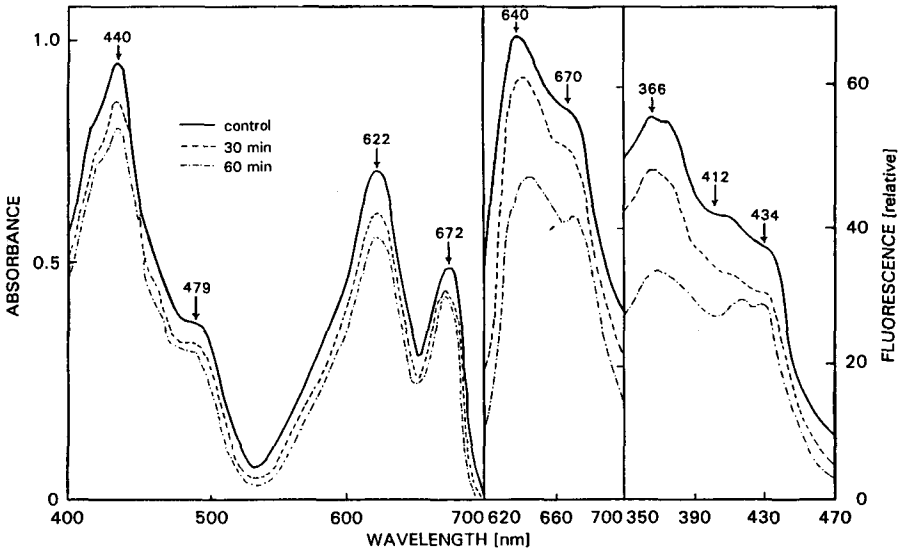


Fig. 1. Short-term effects of UV-B radiation on the absorption (*left*), fluorescence emission (*middle*) and excitation (*right*) spectra of intact *Synechococcus* cells measured at room temperature. Cells were exposed for 30 and 60 min to UV-B radiation (5 W $m^{-2}$) before measuring the spectra. $\lambda_{exc}$ = 365 nm (*middle*); $\lambda_{em}$ = 682 nm (*right*). Slit width for both excitation and emission was 5 nm.

cells (Fork and Mohanty 1986) with maximum at 366 nm was drastically changed by prolonged UV-B irradiation (Fig. 1, *right*). The most affected were the antenna pigments. Hence the observed inhibition of PS 2 electron transport in *Synechococcus* cells during the early period incubation (30 min) preceded the degradation of pigment apparatus and was probably due to either alteration of oxidising or reducing side of PS 2 or of its reaction centre.

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