Ultraviolet-B Induced Damage to Photosystem II in Intact Filaments of *Spirulina platensis*

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Z. Naturforsch. **53c,** 369–377 (1998); received December 15, 1997

Fluorescence Transients, Photosystem II, Quinones, *Spirulina*, Ultraviolet-B

When *Spirulina platensis* filaments were exposed to 0.75 $mW.m^{-2}.s^{-1}$ of ultraviolet-B radiation (the ultraviolet-B radiation under clear sky condition is ~1.0 $mW.m^{-2}.s^{-1}$), an inhibition in photosystem II activity was observed, the inhibition being 90% after 90 min exposure. Upon exposure to ultraviolet-B, the room temperature emission characteristics of *Spirulina* cells were altered when excited with light primarily absorbed by chlorophyll *a* or phycobilisomes. When the cells were exposed for 3 h the emission at 685 nm (F_{685}), when excited at 440 nm (primarily chlorophyll *a* absorption), was enhanced compared to 715 nm (F_{715}) band of photosystem I suggesting a decrease in energy transfer from photosystem II to photosystem I. Similarly, when the cells were excited at 580 nm (primarily the phycobilisomes), the ratio of emission intensity at 685 nm (F_{685}) to that of 655 nm (F_{655}) was decreased in the exposed cells. This change in emission characteristics seems to be linked with the uncoupling of the energy transfer from allophycocyanin to chlorophyll *a* of photosystem II. A small shift in emission peak positions was also indicated when excited either at 440 nm or 580 nm. Analysis of the fast induction of chlorophyll *a* transients in the presence and absence of 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) indicated that ultraviolet-B exposure initially affects Q_A, the primary stable acceptor of photosystem II, and then the plastoquinone (PQ) pool. Our results on the loss in photosystem II-catalyzed Hill activity with p-benzoquinone or dichlorobenzoquinone as electron acceptors also supports the contention that ultraviolet-B, even at low dose, initially alters the Q_A of photosystem II and subsequently PQ pool. The analysis of functional pool size of *Spirulina* suggests a substantial decrease in the functional pool size after 2 h UV-B exposure. These results indicate that in *Spirulina* low intensity of ultraviolet-B initially damages the reaction centre of photosystem II.

Introduction

Spirulina biomass production outdoors is influenced by a variety of environmental factors. One of these factors which has drawn attention in recent years is ultraviolet (UV) radiation. There is an increase in UV radiation reaching the atmosphere from sun due to damage to stratospheric ozone layer by a number of pollutants (Frederick, 1993). This increase is more pronounced in case of UV-B (280–320 nm) as ozone selectively absorbs it (Bothwell *et al.*, 1994). This increased UV radiation is affecting all types of living organisms including plants. In plants, UV-B was shown to inhibit primarily photosynthesis (Caldwell *et al.*, 1982, Bornman, 1989, Tevini and Teramura, 1989, Teramura and Sullivan, 1994, Strid *et al.*, 1994). While some reports indicate that the photosystem I (PS I) activity was not affected by UV-B (Iwanzik *et al.*, 1983; Kulandaivelu and Noorudeen, 1983; Renger *et al.*, 1986) others indicated inhibitory effect on this activity (Brandle *et al.*, 1977; Van *et al.*, 1977). However, this effect seems to be marginal as compared to loss in photosystem II (PS II) activity (Strid *et al.*, 1990). UV-B induced damage to PS II has been monitored by measuring chlorophyll *a* (Chl *a*) fluorescence in isolated chloroplasts and in cell suspensions (Smillie, 1982; Iwanzik *et al.*, 1983) and also in intact tissue (Tevini *et al.*, 1991; Bornman and Vogelman, 1991; Middleton and Teramura, 1993; Naidu *et al.*, 1993; Ziska *et al.*, 1993). UV-B induced inhibition of PS II activity was shown to be associated with water oxidation complex of PS II by altering charge separation (Renger *et al.*, 1989) and the light harvesting complex of PS II (Renger *et al.*, 1986). Renger *et al.* (1989) have suggested that UV-B causes functional disconnection between LHC and PS II reaction centre. Also, it was shown in the cyano-

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bacterium *Synechococcus* that UV-B affects the energy transfer within the phycobilisomes (PBsomes) (Bala Krishna *et al.*, 1996) *in vivo* and from PBsomes to Chl *a in vivo* (Kulandaivelu *et al.*, 1989). Friso *et al* (1994, 1995) have shown that both the PS II reaction centre proteins (D2, D1) are degraded by UV-B. The quinones of the photosynthetic electron transport chain have been suggested to be the targets of UV-B radiation (Melis *et al.*, 1992). Strid *et al* (1994) have observed that UV-B down regulates all the photosynthetic genes, the damage to the nuclear-encoded ones being more rapid than the chloroplast encoded genes (Jordan *et al.*, 1991). Of the photosynthetic genes tested D1 was found to be more resistant to UV-B (Strid *et al.*, 1994).

Most of the above observations on the effect of UV-B on photosynthesis were made with exposure to relatively high intensities of the ultraviolet radiation than naturally observed in solar radiation. Although UV-B exposure seems to induce damages at multiple sites, it is important to estimate the extent of the damage to photosystems and also to ascertain the early event of the damage. Thus, in this communication, we have attempted to characterize the damage caused to PS II activity by moderate intensity (0.75 $mW.m^{-2}.s^{-1}$) of UV-B radiation in the cyanobacterium *Spirulina platensis* under laboratory conditions since cultivation of this organism outdoors is likely to be affected specifically by exposure to UV-B. Our results suggest that PS II reaction centres are damaged at low to moderate intensities of UV-B. Also, the energy transfer between phycobilisomes (PBsomes) and PS II, and between PS II and PS I gets altered. We also show that UV-B exposure alters the intersystem PQ pool of the electron transport chain in *Spirulina*.

Materials and Methods

Spirulina platensis was obtained from National Facility for Algae, New Delhi. The culture was grown at 30 °C in continuous light of 75 $\mu E.m^{-2}.s^{-1}$ to mid-exponential phase according to Murthy and Mohanty (1995) in Zarrouk's medium (Zarrouk, 1966) consisting of 215 mM $NaHCO_3$, 29 mM $NaNO_3$, 17 mM NaCl, 5.7 mM K_2SO_4, 2.8 mM K_2HPO_4, 0.75 mM $MgSO_4.7H_2O$, 0.35 mM $FeSO_4.7H_2O$, 0.3 mM $CaCl_2.2H_2O$, 0.22 mM EDTA, 46 μM H_3BO_3, 9.2 μM $MnCl_2$, 1.6 μM $NaMoO_4.2H_2O$, 0.77 μM $ZnSO_4.7H_2O$, 0.32 μM $CuSO_4.5H_2O$ and 0.17 μM $Co(NO_3)_2.6H_2O$ (pH 9.0). The cells were harvested and were resuspended in fresh Zarrouk's medium at 5 mg $Chl.l^{-1}$ concentration. The cells were subjected to UV-B exposure (0.75 $mW.m^{-2}.s^{-1}$) for different intervals of time at 30 °C with the help of UV-B tubes from Fotodyne Inc. (USA) having maximal emission at 300 nm with 40 nm bandwidth at 50% intensity. This intensity was found to be within the range of UV-B observed (1 $mW.m^{-2}.s^{-1}$) on a sunny summer afternoon under a clear sky in Delhi.

Chl (Chl) was determined according to MacKinney (1941) with methanol as solvent.

Absorption spectra were recorded on a dual wavelength double beam spectrophotometer (Hitachi 557). Room temperature fluorescence emission spectra of the whole cells were recorded on a Perkin-Elmer LS-5 spectrofluorimeter. The cells were suspended at 5 μg $Chl.ml^{-1}$ in Zarrouk's medium. The excitation and emission slit widths were 15 and 5 nm, respectively. Corning 4–96 filter was used with the excitation beam.

Fast induction of Chl *a* fluorescence were recorded on a pulse-amplitude modulated (PAM) fluorimeter (Walz, Germany) following Tiwari and Mohanty (1996). The cells (5 μg $Chl.ml^{-1}$ Zarrouk's medium) were dark incubated for 3 min. The spectra were recorded at $F > 655$ nm with the help of a yellow measuring light (590 nm) after fluorescence induction by red actinic light (650 nm). 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was used where indicated.

The capacity of photosynthetic electron transport chain components to reduce PS I was also measured using the PAM fluorimeter following Schreiber *et al.* (1988) as described in Tiwari and Mohanty (1996). After UV-B exposure the cells were resuspended at 95 μg $Chl.ml^{-1}$ in Zarrouk's medium. Since in cyanobacteria respiratory electron flow and cyclic electron flow around PS I are coupled to intersystem electron chain (Scherer *et al.*, 1988) the *Spirulina* cells were dark-incubated for 10 min at 25 °C prior to measurement to deplete the respiratory substrates. Also 0.3 mM $HgCl_2$ was added 10 min prior to measurement to inhibit electron donation mediated by NAD(P)H dehydrogenase from respiratory and cyclic electron flow to the intersystem chain (Mi *et al.*,

1992a,b) which may otherwise wrongly overestimate the intersystem electron carriers. For measurement, P700 was oxidized by application of far-red light (approx. intensity of 100 μE.m^{-2}.s^{-1}) for 10 s prior to turning the measuring light on. A single turnover saturating flash (6 μs) was given with a xenon single turnover lamp (XST-103, Walz, Germany) and the decrease in absorbance around 830 nm was recorded with an emitter-detector unit (ED 800T, Walz, Germany) after a multiple turnover saturating flash (50 ms) from Xenon multiple turnover lamp (XMT-103, Walz, Germany). The area above the curve gives the intersystem pool size.

PS II activity was measured polarographically by monitoring the amount of O_2 evolved with H_2O as electron donor and either *p*-benzoquinone (*p*BQ, 1 mM) or dichlorobenzoquinone (DCBQ, 0.1 mM) as electron acceptors (Srivatsava *et al.*, 1994). The measurements were made in a clark type O_2 electrode from Hansatech, U. K. (model DW2).

Light intensity was measured with Li-Cor radiometer (model LI-189, Li-Cor, USA). UV-B irradiance was measured with the help of VLX-312 UV meter (Vilber-Lourmat, France). The detector of UV meter was protected with UV-B interference filter with 100% transmittance at 312 nm and a half bandwidth of 5 nm at half the maximum intensity.

Results and Discussion

Effect on spectral properties

The absorption spectra of control and UV-B treated *Spirulina* is given in Fig. 1. The *Spirulina* filaments showed typical absorption peaks at 620 nm and 680 nm pertaining to the absorption by pigments of PBsomes and Chl *a*, respectively (Fork and Mohanty, 1986). Kulandaivelu *et al.* (1989) have reported a decrease of 10% in PBsomes absorption peak intensity when cells of the cyanobacterium *Anacystis* were exposed to 5 W.m^{-2} UV-B. The absorption spectra of UV-B treated and control cells did not show significant change after exposure to 0.75 mW.m^{-2}.s^{-1} of UV-B intensity. Thus, the decrease in PBsomes absorption is probably dependent on the dose of UV-B to which the cells are exposed. At low dose of UV-B (0.75 mW.m^{-2}.s^{-1}) the absorption profile of cells did not alter.

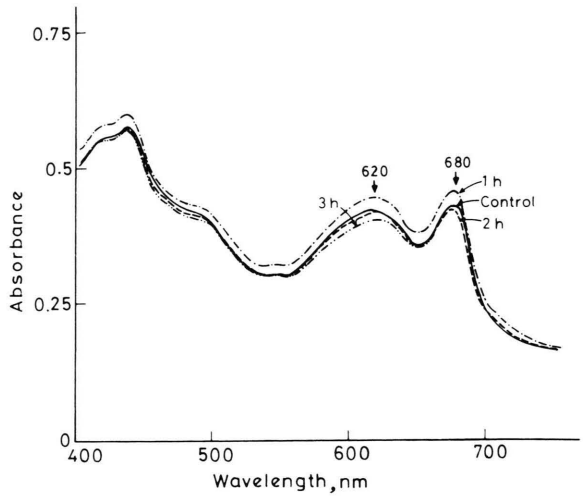

Fig. 1. Absorption spectra of *Spirulina* cells exposed to 0.75 mW.m^{-2}.s^{-1} of UV-B for different durations. The arrows indicate absorption peaks due to phycobilisomes (620 nm) and chlorophyll *a* (680 nm). —, control; –·–, 1 h; ----, 2 h; – – ··– –, 3 h UV-B treatment.

With excitation at 440 nm, absorbed primarily by Chl *a*, the room temperature emission spectra of both the control and UV-B treated cells of *Spirulina* (Fig. 2a) showed emission peaks at 685 nm, emission originating from Chl a of PS II, and at 715 nm emanating from Chl *a* of PS I, and a shoulder at 660 nm emanating from allophycocyanin (APC) of PBsomes (Fork and Mohanty, 1986). The F_{685} emission peak of the *Spirulina* cells was quenched by approximately 8% after 1 h exposure to UV-B. However, when the cells were exposed for 3 h, the same F_{685} emission was enhanced by 7% to that in control. We consider this increase is due to uncoupling induced enhanced emission of APC contribution to F_{685} peak (Mohanty *et al*, 1985). Similarly, with 2 h exposure the F_{660} emission due to APC was enhanced by 9.5%. However, these changes are less than 10% and hence are considered insignificant. Also, cross-over of the spectra of 2 and 3 h UV-B exposed cells to that of the control occurred at around 665, 695 nm which is indicative of a shift of F_{685} peak emission band that emanates from both APC and Chl *a* (Mohanty *et al*, 1985). The ratio of F_{715}/F_{685} with 440 excitation indicated that there is a drop in the ratio from 0.83 in control to 0.77 in 3 h UV-B treated

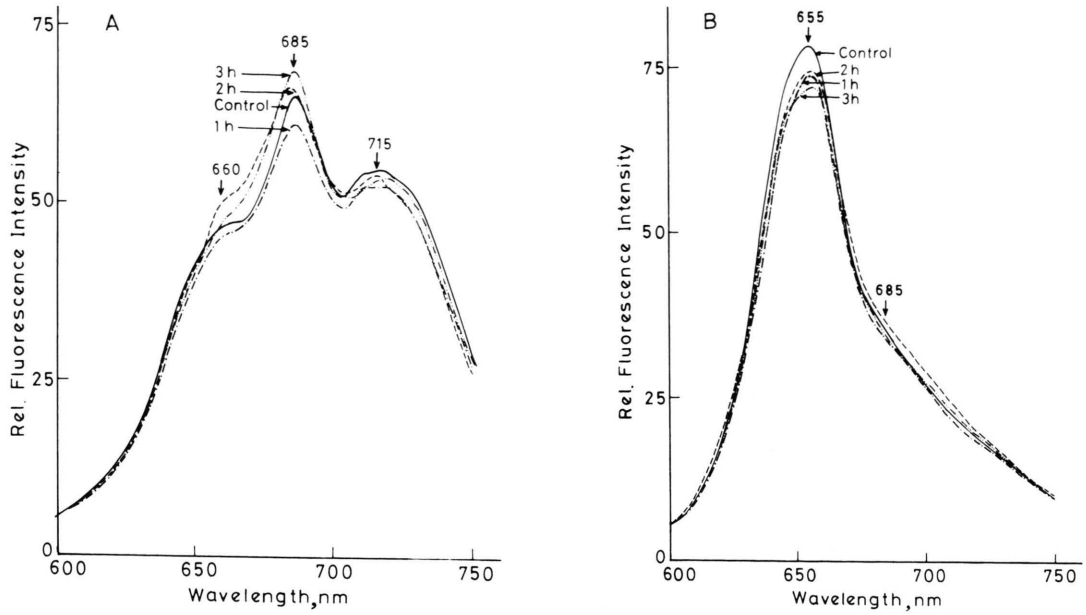

Fig. 2. Fluorescence emission spectra of *Spirulina* cells exposed to 0.75 $mW.m^{-2}.s^{-1}$ of UV-B for different durations. Emission spectra were recorded with cells (5 μg $Chl.ml^{-1}$) on a spectrofluorimeter with slit widths of 15/5 nm (excitation/emission). Cells were excited at (A) 440 nm where primarily Chl *a* absorbs and (B) at 580 nm where primarily phycocyanin absorbs. —, control; – – · – –, 1 h; ----, 2 h; – – · · – – –, 3 h UV-B treatment. The spectra are typical of atleast three independent measurements whose standard deviations at the peak positions are less than 5%.

cells. This drop may be due to changes in the energy transfer from PS II to PS I. However, the ratio of F_{685}/F_{660}, which reflects the energy transfer between APC of PBsomes and Chl *a* of PS II, decreased by 7.2% after 2 h of exposure and subsequently increased by 4.1% after 3 h of exposure suggesting uncoupling of energy transfer from APC to Chl *a*.

The room temperature emission spectra of both the control and UV-B exposed *Spirulina* cells when excited at 580 nm, which primarily excites chromophores of PBsomes, showed a peak at 655 nm and a shoulder at 685 nm (Fig. 2b). Intensity of the peak at 655 nm decreased steadily on exposure to UV-B by ~8% after 3 h of UV-B treatment and the decrease is considered insignificant upto the time measured. Cross-over of the spectra between control and UV-B exposed cells were observed at 665 and 695 nm again indicating a small peak shift. The ratio of F_{685}/F_{655} enhanced by about 6% after 1 h and 11% after 2 h. The increase in F_{685}/F_{655} ratio seems to indicate uncoupling of energy transfer between PBsome antenna and the reaction centre. Thus, analyses of emission characteristics of *Spirulina* cells suggest that UV-B induced spectral changes arise because of alterations in energy transfer processes.

Changes in PS II activity

Table I shows the PS II activity of intact cells of *Spirulina* with either *p*BQ or DCBQ as electron acceptors. It is known that *p*BQ accepts electrons from the PQ pool of electron transport chain (Satoh *et al.*, 1992) and DCBQ mostly from Q_A, the stable primary acceptor of electrons in PS II (Cao and Govindjee, 1990; Satoh *et al.*, 1992). Thus, an assay of the PS II activity with *p*BQ and DCBQ is likely to provide information regarding the relative extent of UV-B induced impairment in electron transport chain. Both the *p*BQ-supported and DCBQ-supported PS II activities were inhibited with increasing duration of exposure to UV-B (Table I). Up to 1 h of UV-B exposure, the *p*BQ-supported O_2 evolution was more suppressed than the DCBQ-supported one after which the difference between them became negligible. This initial difference in loss in PS II activity with *p*BQ and

Table I. Changes in photosystem II activity of *Spirulina* cells exposed to 0.75 $mW.m^{-2}.s^{-1}$ intensity of UV-B. The samples were drawn at different intervals during the exposure and the activities were assayed as amount of O_2 evolved with the help of O_2 electrode immediately with either *p*-benzoquinone (*p*BQ, 1 mM) or dichlorobenzoquinone (DCBQ, 0.1 mM) as electron acceptor. The values in the parentheses indicate SEM.

Duration of exposure [min]	Photosystem II activity (μmol O_2 evolved.mg^{-1} Chl.h^{-1})			
	$H_2O \rightarrow$ *p*BQ	% Inhibition	$H_2O \rightarrow$ DCBQ	% Inhibition
0	407 (0.057)	0	279 (0.061)	0
20	345 (0.008)	15	251 (0.011)	10
40	204 (0.007)	50	153 (0.033)	45
60	93 (0.174)	77	84 (0.05)	70
90	46 (0.276)	89	24 (0.259)	91

DCBQ as electron acceptors is due to the fact that initially Q_A, which is less in amount than PQ (Kok and Cheniae, 1966) was affected by UV-B. Loss in 50% activity of PS II activity was noted within 40 min of exposure to UV-B with either *p*BQ or DCBQ as electron acceptors (Table I). In higher plants, Kulandaivelu *et al* (1991) have also observed a loss in PS II activity when they were exposed to 5 $W.m^{-2}$ UV-B. In *Anacystis* Kulandaivelu *et al.* (1989) have shown that when the cells were exposed to UV-B (5 $W.m^{-2}$) the photosynthetic O_2 evolution was inhibited by 50% within 30 min. Thus, the loss in PS II activity seems to exhibit dose-dependence of UV-B and *Spirulina* cells seem to be quite sensitive to UV-B.

Changes in Chl a fluorescence transients

Charge transfer at PS II reaction centres can be analyzed by fast Chl *a* fluorescent transient rise (Joshi and Mohanty, 1995). To further characterize the damage caused to PS II reaction centre by UV-B, we have recorded Chl *a* fluorescence transients using PAM fluorimeter. When dark-adapted photosynthetic samples are illuminated, the Chl *a* fluorescence yield follows a time course rising from minimal fluorescence, F_o to maximum fluorescence, F_m (Govindjee, 1995). The extent of this rise of fluorescence from F_o to F_m, termed the variable fluorescence (F_v), is an indicator of the redox state of the stable primary acceptor of PS II (Duysens and Sweers, 1963; Butler and Kitajima, 1975; Butler, 1978; Joshi and Mohanty, 1995). *Spirulina* cells without exposure to UV-B showed a typical O-I-D-P transient (Munday and Govindjee, 1969) before attaining the maximal fluorescence (M) (curve 1 in Fig. 3). But the exposure to

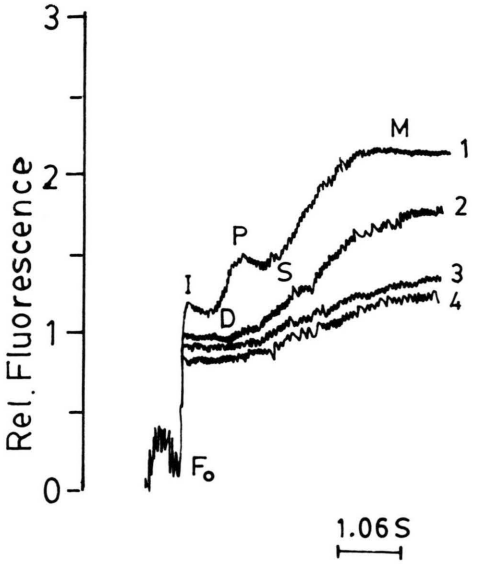

Fig. 3. Chlorophyll *a* fluorescence transients of UV-B treated *Spirulina* cells. The cells were dark adapted for 3 min. Chl *a* fluorescent transients were recorded on pulse-amplitude modulated (PAM) fluorimeter as described in Materials and Methods. Red actinic light (83 μmol.$cm^{-2}.s^{-1}$) was provided by PAM 102. F_o was recorded with a weak measuring light. The transients have been normalized for F_o. Curve 1, control; Curves 2, 3 and 4, Cells treated with UV-B for 1, 2 and 3 h, respectively.

1 h or more of UV-B resulted in the loss of dip (D), and the P peak was also lowered. The dip (D) has been claimed to be associated with the oxidation of reduced Q_A (Munday and Govindjee, 1969; Satoh and Katoh, 1981). The loss of dip in *Spirulina* exposed to UV-B, thus reflects changes on the oxidation of Q_A- and subsequent reduction of Q_B and damage to PQ (pool).

Table II. Changes in Chl *a* fluorescence transient parameters on UV-B (0.75 $mW.m^{-2}.s^{-1}$) treatment of *Spirulina* cells. The cells (5 μg $Chl.ml^{-1}$) were dark adapted for 3 min. Chl *a* fluorescent transients were recorded on pulse-amplitude modulated (PAM) fluorimeter as described in Materials and Methods. Red actinic light (83 $\mu mol.cm^{-2}.s^{-1}$) was provided by PAM 102. F_o was recorded with a weak measuring light. The values in the parentheses indicate SEM.

Duration of exposure [min]	F_o (relative units)	F_p (relative units)	$F_v = F_p - F_o$
0	1.216 (0.006)	1.328 (0.005)	0.112
20	1.221 (0.009)	1.333 (0.016)	0.112
40	1.24 (0.014)	1.325 (0.015)	0.085
60	1.261 (0.01)	1.32 (0.009)	0.059
90	1.339 (0.026)	1.397 (0.027)	0.058

The variable Chl *a* fluorescence, $F_v = (F_p - F_o)$, reflects the quantum yield of photochemistry (Duysens and Sweers, 1963; Butler, 1978, Joshi and Mohanty, 1995). Variable fluorescence of the *Spirulina* cells decreased when exposed to UV-B (Table II) from 0.112 in control to 0.058 after 90 min exposure. It is observed that 20 min exposure did not affect the F_v and there was no change between 60 and 90 min. There was an increase in F_o values upon exposure to UV-B (Table II) and this value further increased with increase in exposure time, the increase being 10% after 90 min exposure, indicating a possible energy uncoupling between the light harvesting antenna and the reaction centre (Schreiber and Berry, 1977). However, the yield of fluorescence when all the reaction centres are closed (F_p) did not change much up to 60 min exposure to UV-B. Up to 20 min of exposure to UV-B, the extent of variable fluorescence (F_v) was not altered while there was an increase in F_o. This reflects that the initial UV-B effect is due to suppression of energy flow from PS II antenna to reaction centre. This drop in F_v also is in conformity with the inhibition of PS II activity (Table I).

The Chl *a* transients recorded in the presence of DCMU which blocks electron flow at Q_A (Duysens and Sweers, 1963), showed a decrease in F_v from 0.219 in control to 0.141 (51% decrease) after 60 min exposure of *Spirulina* filaments to UV-B (Table III). After 90 min exposure the loss in F_v increased to 72%. These changes in F_v corresponds to the changes in PS II activities.

Thus, a comparison of the F_v/F_o ratios recorded both in the absence and the presence of DCMU (Tables II and III) indicate that up to 60 min of exposure of *Spirulina* cells to UV-B the ratios were of same magnitude (0.50 and 0.56, respectively). However, after 90 min exposure the corresponding values were 0.53 and 0.75, respectively indicating that as the dose of UV-B increased the proportion of quinone pool got reduced.

Intersystem pool analysis

We have further measured the photosynthetic electron transport chain for its ability to reduce PS I. This reduction results from both the activity of PS-II dependent electron transfer as well as the intersystem pool size comprising plastoquinone, cytochrome b_6/f and plastocyanin. The quantification of P_{700} reduction and its subsequent oxidation by far-red light was carried out according to Schreiber *et al.* (1988). PS I was oxidized by far-

Table III. Changes in Chl *a* fluorescence transient parameters on UV-B (0.75 $mW.m^{-2}.s^{-1}$) treatment of *Spirulina* cells in the presence of DCMU. The cells (5 μg $Chl.ml^{-1}$) were dark adapted for 3 min. DCMU (10 μmol) was added and the transients were recorded with red actinic light (83 $\mu mol.cm^{-2}.s^{-1}$) provided by PAM 102. F_o was measured with a weak measuring light. For details see Materials and Methods. The values in the parentheses indicate SEM.

Duration of exposure [min]	F_o (relative units)		F_m (relative units)		F_v	
		% Increase		% Inhibition		% Inhibition
0	1.149 (0.046)	0	1.438 (0.058)	0	0.289	0
60	1.274 (0.053)	10.9	1.415 (0.041)	1.6	0.141	51.2
90	1.278 (0.09)	11.2	1.359 (0.067)	5.5	0.081	72

Table IV. Intersystem pool measurement of UV-B (0.75 $mW.m^{-2}.s^{-1}$) treated *Spirulina* cells. After exposure for different durations, cells were harvested and resuspended in Zarrouk's medium at 95 μg $Chl.ml^{-1}$. Cells were incubated in dark for 10 min after addition of 0.3 mM $HgCl_2$. Baseline was recorded after 10 sec illumination with far-red light (0.04 $\mu mol.cm^{-2}.s^{-1}$). Single turnover (ST) saturating flash was given from XST-103 and decrease in absorbance at 830 nm was recorded after a 50 ms multiple turnover (MT) saturating flash with XMT-103. MT flash was given after 1 min of ST flash. Red light was kept on throughout the measurement. The area above the curve was determined by cutting the curves and weighing them. Values in the parentheses indicate SEM.

Duration of exposure [min]	% Area above the curve
0	100 (0.018)
60	54.1 (0.074)
120	21.6 (0.053)

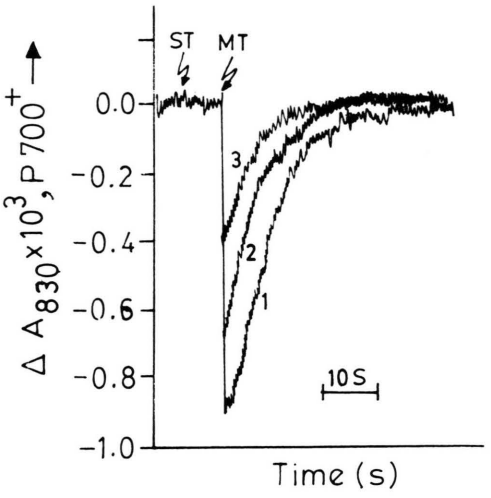

Fig. 4. Determination of intersystem pool size upon exposure of cells to UV-B by following the p700 redox changes at 820 nm. Cells after exposure to UV-B were suspended at 95 μg $Chl.ml^{-1}$ in Zarrouk's medium and were dark-incubated for 10 min after addition of 300 μM $HgCl_2$. The cells were illuminated with far-red light (approx. 100 $\mu E.m^{-2}.s^{-1}$) for 10 s and the absorbance changes were recorded using the emitter-detector unit (ED 800T) of PAM fluorimeter. Single (ST) and multiple (MT)-turnover saturating flashes were given as indicated in the fig. Curve 1, Control cells, Curves 2 and 3, cells exposed for 1 h and 2 h to UV-B.

red light. Then, a multiple turnover flash subsequent to a single turnover flash reduced all P_{700} which was reoxidized by the continuous far-red light kept on during the entire measurement essentially as described by Schreiber *et al* (1988). The time taken to re-oxidize P700 will be proportional to the number electrons stored in the PQ-pool and, therefore, the reduction area (Fig. 4) is a measure of intersystem pool size (Schreiber *et al.*, 1988). This area decreased continuously as the duration of exposure of *Spirulina* to UV-B increased. Within 1 h of exposure of *Spirulina* filaments to UV-B the damage to this functional pool was estimated to be 50% of that of the control which decreased to 70% after 2 h of exposure (Table IV). Taken together with the PS II activity (Table I) and fast Chl *a* fluorescence transient analysis (Table II) these results suggest that PS II reaction centre as well as quinone pool are the major sites for damage by UV-B (Melis *et al.*, 1992).

In summary, 0.75 $mW.m^{-2}.s^{-1}$ ultraviolet-B radiation caused alterations in emission spectral characteristics and these changes seem to be linked to alterations in the efficiency of energy transfer between PBsomes and PS II and between PS II and PS I in *Spirulina*. Also, UV-B caused inhibition of the PS II photochemistry as a result of damage to both PS II reaction centres and the intersystem PQ pool. The proportion of Q_A-linked PS II damage was more in extent than that of PQ pool and was an early event in UV-B induced damage to photosynthetic membranes of *Spirulina*.

Acknowledgments

This investigation was carried out with the grant 09/374/EMR-II from Council of Scientific and Industrial Research, India to PM. The authors wish to acknowledge the support from Department of Atomic Energy, Government of India (grant No. 4–3-95/R&D-II) to PM. We thank Prof. N. Murata, National Institute of Basic Biology, Okazaki, Japan for gift of DCBQ.

Bala Krishna K., Sah J. F. and Mohanty P. (1996), Ultraviolet-B induced alteration in energy transfer in phycobilisomes of *Synechococcus* PCC 7942. Indian J. Exp. Biol. **34**, 1133–1137.

Bornman J. F. (1989), Target sites of UV-B radiation in photosynthesis of higher plants. J. Photochem. Photobiol. B 4, 145-158.

Bornman J. F. and Vogelmann T. C. (1991), The effect of ultraviolet-B radiation on leaf optical properties measured with fiber optics. J. Exp. Bot. **42**, 547–554.

Bothwell M. L., Sherbot D. M. J. and Pollock C. M. (1994), Ecosystem response to solar UV-B radiation: Influence of trophic level interactions. Science, **265**, 97-100.

Brandle J. R., Campbell W. F., Sisson W. B. and Caldwell M. M. (1977), Net photosynthesis, electron transport capacity and ultrastructure of *Pisum sativum* L. exposed to ultraviolet-B radiation. Plant Physiol. **60**, 165–168.

Butler W. L. (1978), Energy distribution in the photochemical apparatus of photosynthesis. Annu. Rev. Plant Physiol. **29**, 345–378.

Butler W. L. and Kitajima M. (1975), Energy transfer between photosystem II and photosystem I in chloroplasts. Biochim. Biophys. Acta. **396**, 72–85.

Caldwell M. M., Robberecht R., Nowak R. S. (1982), Differential photosynthetic inhibition by ultraviolet radiation in species from the arctic-alpine life zone. Arctic Alpine Res. **14**, 195-202.

Duysens W. L. N. M. and Sweers H. E. (1963), Mechanism of two photochemical reactions in algae as studied by means of fluorescence. In: Studies on Microalgae and Photosynthetic Bacteria. (Japanese Society of Plant Physiologists eds.). University of Tokyo Press, Tokyo, pp. 353–372.

Fork D. C., Mohanty, P. (1986), Fluorescence and other characteristics of blue-green algae (cyanobacteria), red algae, and cryptomonads. In: Light Emission by Plants and Bacteria. (Govindjee, Amesz, J., Fork, D. C. eds.) Academic Press, Orlando, pp. 451–496.

Frederick J. E. (1993), Ultraviolet sunlight reaching the earth's surface: a review of recent research. Photochem. Photobiol. **57**, 175-178.

Friso G., Barbato R., Giacometti G. M. and Barber J. (1994), Degradation of D2 protein due to UV-B irradiation of the reaction centre of photosystem II. FEBS Lett. **339**, 217–221.

Friso G., Vass I., Spetea C., Barber J. and Barbato R. (1995), UV-B-induced degradation of the D1 protein in isolated reaction centres of photosystem II. Biochim. Biophys. Acta. **1231**, 41–46.

Govindjee (1995), Sixty-three years since Kautsky chlorophyll *a* fluorescence. Aust. J. Plant Physiol. **22**, 131–146.

Iwanzik W., Tevini M., Dohnt G., Voss M., Weiss W., Graber P. and Renger G. (1983), Action of ultraviolet-B radiation on photosynthetic primary reactions in spinach chloroplasts. Physiol. Plant. **58**, 401-407.

Jordan B. R., Chow W. S., Strid A. and Anderson J. M. (1991), Reduction in *cab* and *psb* A RNA transcripts in response to supplementary ultraviolet-B radiation. FEBS Lett. **284**, 5–8.

Joshi M. K. and Mohanty P. (1995), Probing photosynthetic performance by chlorophyll *a* fluorescence: Analysis and interpretation of fluorescence parameters. J. Sci. Ind. Res. **54**, 155–174.

Kok B. and Cheniae G. M. (1966), Kinetics and intermediates of the oxygen evolution step in photosynthesis. Current Topics in Bioenergetics. **1**, 1–47.

Kulandaivelu G., Gheetha V. and Periyanan S. (1989), Inhibition of energy transfer reactions in cyanobacteria by different ultraviolet radiation. In: Photosynthesis – Molecular Biology and Bioenergetics. (Singhal G. S., Barber J., Dilley R. A., Govindjee, Haselkorn R. and Mohanty P. ed.). Narosa Publishing House, New Delhi, pp. 305–313.

Kulandaivelu, G., Nedunchezhian N. and Annamalainathan, K. (1991), Ultraviolet-B (280–320 nm) radiation induced changes in photochemical activities and polypeptide components of C_3 and C_4 chloroplasts. Photosynthetica. **25**, 333–339.

Kulandaivelu G. and Noorudeen, A. M. (1983), Comparative study of the action of ultraviolet-C and ultraviolet-B radiation on photosynthetic electron transport. Physiol. Plant. **58**, 389-394.

MacKinney G. (1941), Absorption of light by chlorophyll in solutions. J. Biol. Chem. **140**, 315-322.

Melis A., Nemson J. A. and Harrison M. A. (1992), Damage to functional components and partial degradation of photosystem II reaction centre proteins upon chloroplast exposure to ultraviolet-B radiation. Biochim. Biophys. Acta. **1100**, 312-320.

Mi H., Endo T., Schreiber U. and Asada K. (1992a), Donation of electrons from cytosolic components to the intersystem chain in the cyanobacterium *Synechococcus* sp. PCC 7002 as determined by the reduction of $P700^+$. Plant Cell Physiol. **33**, 1099–1105.

Mi H., Endo T., Schreiber U. and Asada K. (1992b), Electron donation from cyclic and respiratory flows to the photosynthetic intersystem chain is mediated by pyridine nucleotide dehydrogenase in the cyanobacterium *Synechocystis* PCC 6803. Plant Cell Physiol. **33**, 1233–1237.

Middleton E. M. and Teramura A. H. (1993), The role of flavonol glycosides and carotenoids in protecting soybean from ultraviolet-B damage. Plant Physiol. **103**, 741–752.

Mohanty P., Hoshina S. and Fork D. C. (1985), Energy transfer from phycobilins to chlorophyll *a* in heat stressed *Anacystis nidulans*: Characterization of low temperature 683 nm band. Photochem. Photobiol. **41**, 597–603.

Munday J. C. Jr. and Govindjee (1969), Light-induced changes in the fluorescence yield of chlorophyll *a in vivo*. III. The dip and the peak in the fluorescence transients of *Chlorella pyrenoidosa*. Biophys. J. **9**, 1–9.

Murthy S. D. S. and Mohanty P. (1995), Action of selected heavy metal ions on the photosystem 2 activity of the cyanobacterium *Spirulina platensis*. Biologia Plantarum. **37**, 79–84.

Naidu S. L., Sullivan J. H., Teramura A. H. and DeLucia E. H. (1993), The effects of ultraviolet-B radiation on photosynthesis of different needle age classes in field-grown loblolly pine. Tree Physiol. **12**, 151–162.

Renger G., Volker M., Eckert H. J., Fromme R., Hohm-Veit S. and Graber P. (1989), On the mechanism of photosystem II deterioration by ultraviolet-B radiation. Photochem. Photobiol. **49**, 97-105.

Renger G. Voss M., Graber P. and Schulze A. (1986), Effect of ultraviolet radiation on different partial reactions of the primary processes of photosynthesis. In: Stratospheric Ozone Reduction, Solar Ultraviolet Radiation and Plant Life. (Worrest R. C. and Caldwell M. M., ed). Springer-Verlag, Berlin, pp. 171-184.

Satoh K., Koike H., Ichimura T. and Katoh S. (1992), Binding affinities of benzoquinones to the Q_B site of photosystem II in *Synechococcus* oxygen-evolving preparation. Biochim. Biophys. Acta. **1102**, 45–52.

Scherer S., Almon H. and Boger P. (1988), Interaction of photosynthesis, respiration and nitrogen fixation in cyanobacteria. Photosynth. Res. **15**, 95–114.

Schreiber U. and Berry J. A. (1977), Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of photosynthetic apparatus. Planta. **136**, 233–238.

Schreiber U., Klughammer C. and Neubauer C. (1988), Measuring P700 absorbance changes around 830 nm with a new type pulse modulation system. Z. Naturforsch. **43C**, 686–698.

Smillie R. M. (1982), Chlorophyll fluorescence *in vivo* as a probe for rapid measurement for tolerance to ultraviolet radiation. Plant Sci. Lett. **28**, 283–289.

Srivatsava M., Mohanty P. and Bose S. (1994), Alterations in the excitation energy distribution in *Synechococcus* PCC 7942 due to prolonged partial inhibition of photosystem II. Comparison between inhibition caused by (a) presence of PS II inhibitor, (b) mutation in the D1 polypeptide of PS II. Biochim. Biophys. Acta. **1186**, 1–11.

Strid A., Chow W. S. and Anderson J. M. (1990), Effects of supplementary ultraviolet-B radiation on photosynthesis in *Pisum sativum*. Biochim. Biophys. Acta. **1020**, 260–268.

Strid A., Chow W. S. and Anderson J. M. (1994), Ultraviolet-B damage and protection at the molecular level in plants. Photosynth. Res. **39**, 475-489.

Teramura A. H. and Sullivan J. H. (1994), Effects of ultraviolet-B radiation on photosynthesis and growth of terrestrial plants. Photosynth. Res. **39**, 463-473.

Tevini M., Braun J. and Fieser F. (1991), The protective function of the epidermal layer of rye seedlings against ultraviolet-B radiation. Photochem. Photobiol. **53**, 329–333.

Tevini M. and Teramura A. H. (1989), Ultraviolet-B effects on terrestrial plants. Photochem. Photobiol. **50**, 479-487.

Tiwari S. and Mohanty P. (1996), Cobalt induced changes in photosystem activity in *Synechocystis* PCC 6803: Alterations in energy distribution and stoichiometry. Photosynth. Res. **50**, 243-256.

Van T. K., Garrard L. and West S. H. (1977), Effects of 298 nm radiation on photosynthetic reactions of leaf discs and chloroplast preparations of some crop species. Environ. Exp. Bot. **17**, 107–112.

Zarrouk C. (1966), Contribution a l'etude d'une cyanophyce influence de diverse facteurs physiques et chimiques sur la croissance et la photosynthese de *Spirulina maxima* (setch et Gardner) Geitler, Ph. D. thesis, Univ. Paris, Paris, 4-5.

Ziska L. H., Teramura A. H., Sullivan J. H. and McCoy A. (1993), Influence of ultraviolet-B (UV-B) radiation on photosynthetic and growth characteristics in field-grown cassava (*Manihot esculentum* Crantz). Plant Cell Environ. **16**, 73–79.