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# UV-B RADIATION INDUCED ALTERATIONS IN THE THYLAKOID MEMBRANE LINKED PHOTOFUNCTIONS OF THE CYANOBACTERIUM *SPIRULINA PLATENSIS*

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**ABSTRACT**
Depending on the illumination, UV- B radiation (2-6 w $m^{-2}$) exerts multiple effects on photosynthetic electron transport. Between two photosystems, photosystem–II is highly susceptible to UV – B radiation. Light intensity measurements indicated that alterations in energy transfer from phycocyanin to Chl *a* is responsible for the alterations in photosystem II photochemistry of spirulina platensis. In addition UV – B radiation caused the lipid peroxidation in thylakoid membrane which could be one of the reason for the alteration in photosystem II catalyzed electron transport.

## INTRODUCTION

Stratospheric ozone exerts its beneficial effects by absorbing UV radiation in the 200-320 nm range with reducing amounts of radiation in the earth's surface. Depletion of the stratospheric ozone leads to the enhanced level of UV-B radiation in the earth's surface. Enhancement in UV- B region (280-315) is of particular interest in view of its adverse effect on biological systems. The impact of increasing UV- B irradiation on growth motility and pigmentation has been investigated in cyanobacteria and algae (Hader and Hader, 1989). UV- B radiation also affects DNA, proteins, lipids and photosynthetic pigments and alters the key physiological processes (Karentz *et al.,* 1991; Tyagi *et al.,* 1992; Adhikari, 2003) which regulate algae proliferation and development in aquatic ecosystems.

UV- B radiation predominantly effects the light reactions of photosynthesis. Besides inhibiting electron transport activity, UV- B radiation absorbed directly by the thylakoid membrane components and causes damage to the membrane organization (Bornmann, 1989). Some of the reports indicate that the Photosystem I activity was not affected by UV-B (Kulandaivelu and Noorudeen, 1983; Renger and Schreiber, 1986). UV- B stress damages PS II photofunction by formation of semiquinone anion formation at $Q_A$, the physiological stable acceptor of PS II (Melis *et al.*, 1992). UV-B radiation affects photosynthetic process in cyanobacteria (Kulandaivelu *et al.,* 1987). Studies related to UV-B effects on cyanobacteria are very limited. PBsomes are the potential targets for UV-B stress (Sah *et al.*, 1998). Kulandaivelu *et al.,* (1987) demonstrated that UV-B exposure of *Synechococcus* cells caused the inhibition of inhibited oxygen evolution and altered the spectral characteristics of phycobilin proteins (PBPs) due to partial uncoupling of energy transfer. Similar results were observed by Rajagopal and Murthy, (1996).Therefore, an attempt has been made to characterize the effect of UV-B on photosynthetic electron transport properties in the intact cells of the cyanobacterium *Spirulina platensis* cells.

## MATERIALS AND METHODS

*Spirulina platensis* was grown axenically in the medium of Zarrouk (1966) at 25 ± 2ºC. Under continuous irradiance of 40 $\mu$mol (photon) $m^2$ $S^1$. Cells from the late grown cultures were harvested by centrifuging at 6,000 Xg for 10 min. The collected cells were suspended in 25 mM HEPES- NaOH buffer (pH 7.5) at a Chl concentration of 200$\mu$g $mL^{-1}$. Samples were exposed to UV-B radiation from (2-6$wm^2$) for 10 minutes under constant stirring. The reaction mixture used for the assay of whole chain electron transfer ($H_2O$→methylviologen (MV) contained reaction buffer 25 mM HEPES-NaOH buffer, (pH 7.5), 0.5 mM MV and 1 mM Na- azide (Murthy, 1991). The reaction mixture for PS II catalyzed electron transfer ($H_2O$→p-benzoquinone (pBQ) contained the above mentioned reaction buffer and 0.5 mM pBQ (Murthy *et al.,* 1988). Thylakoid

membranes were prepared according to the method of Rajagopal *et al*.,1998). The reaction mixture of PS I catalyzed electron transfer ($DCPIPH_2 \rightarrow MV$) contained reaction buffer, 0.1 mM DCPIP, 5 mM ascorbate, 1 mM azide, 10$\mu$M DCMU, and 0.5 mM MV. In all assays cells equivalent to 15$\mu$g of Chl a were used. All the photochemical activities were measured at saturated light intensity of white light (410 $Wm^{-2}$) under continuous stirring. Low light intensity when ever required was provided by passing the light through calibrated neutral density filters. Phycocyanin fluoroscence emission spectra was measured using Shimadzu spectrofluoremeter by following the procedure of Murthy *et al.,* (1988). Lipid peroxidation of intact cells was measured by following the method of Heath and Packer (1968).

## RESULTS AND DISCUSSION

After giving the UV-B radiation treatment (2-6 $Wm^{-2}$) for one hour, the photosynthetic parameters have been analyzed to identify the targets in the primary process of photosynthesis. The effect of UV-B radiation on the whole chain catalyzed electron transport in the cyanobacterium, *Spirulina platensis*. has been studied. The artificial acceptor MV which accepts electrons at the reducing side of PS I has been shown to have a free access to the thylakoid membranes in case of intact cells of *Spirulina* (Robinson *et al.,* 1982). Therefore, a study has been made on the effect of UV B radiation on the whole chain electron transport activity ($H_2O \rightarrow MV$). Control cells showed a high rate of oxygen consumption involved in the whole chain electron (199 $\mu$mole $O_2$ consumed mg $Chl^{-1}$ $h^{-1}$). When the cells were exposed to different doses of UV- B radiation, it caused dose dependent inhibition in the whole chain electron transport. At 4 $Wm^{-2}$, UV-B treatment, 44% inhibition was noticed in whole chain electron transport. Further raise in the dose to 6 $wm^{-2}$ induced 68% loss in the activity. The inhibition in whole chain electron transport could be due to alterations in either PS I or PS II. Therefore the effect of UV-B radiation has been studied on PS II catalyzed electron transport (Table 2). pBQ is being lipophilic in nature, it enters easily in to the intact cells of *Spirulina*. Control cells exhibited a high rate of oxygen evolution due to PS II activity (235 $\mu$mole $O_2$ evolved mg $Chl^{-1}$ $h^{-1}$).

The treatment of UV-B radiation brought 52% loss in pBQ supported Hill activity. At 6 $Wm^{-2}$ of UV B treatment, 74% inhibition was noticed. The reason for the inhibition of PS II activity could be alterations at the level of light harvesting pigment protein complex. To rule out this possibility, Hill activity has been measured at different light illuminating conditions (Table 3). The inhibition in the UV B treatment (4 $Wm^{-2}$) was more at light saturating conditions than that at light limiting conditions (Table 5). The reason for the inhibition at light limiting conditions could be alterations of light harvesting complex in PS II of the above cyanobacterium.

UV-B radiation caused marginal inhibition in PS I catalyzed electron transport mediated wit reduced DCPIP as donor. Even at 6 $Wm^{-2}$ only 31% loss in PS I catalyzed electron transport was observed (Table 4). From the above studies it is clear that PS II is more susceptible to UV B radiation than that of PS I. To identify whether the observed inhibition by UV-B radiation of PS II catalyzed electron transport activities is linked to the changes of LHC, the alterations at the level of pigment protein spectral properties have been measured. In control cells, excited at 545 nm, an emission peak at 653 nm emanating from phycocyanin (PC) was prominent in the spectrum ( Fork and Mohanty, 1986: Singhal *et al.,* 1981). Incubation under UV-B radiation (2-6 $Wm^{-2}$) caused 22% increase in the PC fluorescence intensity and caused blue shift in the peak position by 7 nm (Table 5). The decrease in the fluorescence intensity could be due to changes in the energy transfer from PC (phycocyanin) to Chl a in the intact cells. The shift in the peak position indicates the structural alterations in PC.

The changes under stress in the membrane organization can also affect the functional aspects of photosynthetic electron transport chain besides PS II. Therefore an attempt was made to study the effect of UV-B radiation on thylakoid membrane

**Table 1: Effect of UV-B radiation on the whole chain electron transport assay ($H_2O \rightarrow MV$) in the cyanobacterium, *Spirulina platensis*. Three ml of reaction mixture contains reaction buffer (25mM HEPES-NaOH (pH7.5) containing 20 mM NaCl), 0.5 mM MV, 1mM Na-azide and cells equivalent to 15$\mu$g of Chl. Cells were incubated in the presence and absence of UV B radiation (2, 4 and 6 $Wm^{-2}$). Other details were given in materials and methods. The SD is not more than 10%**

| UV-B radiation$W\ m^{-2}$ | Whole chain electron transport activity ($H_2O \rightarrow MV$) $\mu$ moles of $O_2$ consumed $mg^{-1}$ Chl $h^{-1}$ | Percentage Loss |
|---|---|---|
| Control | 199 ± 15 | 0 |
| 2 | 157 ± 11 | 21 |
| 4 | 111 ± 9 | 44 |
| 6 | 64 ± 4 | 68 |

**Table 2: Effect of UV-B radiation (2,4, and 6 $Wm^{-2}$) on photosystem II catalyzed electron transport activity ($H_2O \rightarrow pBQ$) in the cyanobacterium, *Spirulina platensis*. Reaction mixture (3 mL) for this assay contained reaction buffer, 0.5 mM pBQ, and intact cells equivalent to 15 $\mu$g of Chl a. Cells were incubated in the presence and absence of UV B radiation (2, 4 and 6 $Wm^{-2}$). Other details were given in materials and methods. The values are average of 3 separate experiments. The SD is not more than 10%.**

| UV-B radiation$W\ m^{-2}$ | PS II catalyzed electron transport activity $H_2O \rightarrow pBQ$ $\mu$ moles of $O_2$ evolved $mg^{-1}$ Chl $h^{-1}$ | Percentage Loss |
|---|---|---|
| Control | 235 ± 18 | 0 |
| 2 | 181 ±15 | 23 |
| 4 | 113 ± 9 | 52 |
| 6 | 61 ± 4 | 74 |

**Table 3: Effect of illuminated light intensity on UV B radiation induced inhibition of photosystem II catalyzed electron transport activity ($H_2O \rightarrow pBQ$) Reaction mixture (3 mL) for this assay contained reaction buffer, 0.5mM pBQ and intact cells equivalent to 15 $\mu$g of Chl a. Cells were incubated in the presence and absence of UV B radiation for different intervals. Other details were given in materials and methods. The values are average of 3 separate experiments. The SD is not more than 10%**

| Light intensity $\mu$ moles | PSII catalyzed electron transport activity ($\mu$ moles of $O_2$ evolved $mg^{-1}$ $h^{-1}$) | | Percentage Loss |
|---|---|---|---|
| | Control | UV B treated (4 $Wm^{-2}$) | |
| 105 | 42 ± 4 | 25 ± 2 | 39 |
| 1100 | 70 ± 8 | 41 ± 4 | 42 |
| 2050 | 121 ± 19 | 63 ± 6 | 48 |
| 3000 | 234 ± 22 | 112 ± 13 | 52 |

**Table 4: Effect of UV-B radiation (2,4, and 6 $wm^{-2}$) on photosystem II catalyzed electron transport activity ($DCPIPH_2 \rightarrow MV$). Thylakoid fragments were isolated from the cells grown under different illuminations of UV B radiations. Other details were given in materials and methods. The values are average of 3 separate experiments. The SD is not than 10%**

| UV-B radiationW $m^{-2}$ | PS I catalyzed electron transport activity $DCPIPH_2 \rightarrow MV\mu$ moles of $O_2$ consumed $mg^{-1}$ Chl $h^{-1}$ | Percentage Loss |
|---|---|---|
| Control | 411 ±36 | 0 |
| 2 | 349 ± 30 | 15 |
| 4 | 308 ± 28 | 25 |
| 6 | 284 ± 25 | 31 |

**Table 5: Effect of UV-B radiation (2,4 and 6 $Wm^{-2}$) on the PC fluorescence emission properties of the intact cells of *Spirulina* at room temperature. Cells were excited at 545 mm to excite the PBsomes specifically (slit width for both excitation and emission was 5 mm. Intact cells equivalent of 5 $\mu$g of Chl a were suspended in 3 mL of reaction buffer for measuring spectral characteristics. The values are average of 3 separate experiments**

| UV – B radiationW $m^{-2}$ | Phycocyanin fluorescence emission Intensity (relative units) | Peak position, nm | Percent decrease |
|---|---|---|---|
| Control | 58 ± 4 | 653 | 0 |
| 2 | 52 ± 3 | 650 | 10 |
| 4 | 47 ± 2 | 647 | 19 |
| 6 | 45 ± 2 | 646 | 22 |

**Table 6: Effect of UV-B radiation (2,4, and 6 $Wm^{-2}$) on the lipid peroxidation and PSII activity 1of thylakoid membranes. Thylakoid membranes equivalent to 15 $\mu$g / mL of Chl a were used for the estimation of MDA levels. Other details were given in materials and methods.**

| UV-B radiationW $m^{-2}$ | Lipid peroxidation(n moles MDA $mg^{-1}$ protein) | PS II catalyzed electron transport activity$H_2O \rightarrow pBQ$ $\mu$ moles of $O_2$ evolved $mg^{-1}$ Chl $h^{-1}$ |
|---|---|---|
| Control | 49 ± 3 | 235 ± 18 |
| 2 | 63 ± 6 | 181 ± 15 |
| 4 | 69 ± 7 | 113 ± 9 |
| 6 | 74 ± 7 | 61 ± 4 |

organization in the cyanobaterium *Spirulina platensis*. For this purpose cells were given two different doses of UV-B radiation (2-6 $Wm^{-2}$) and lipid peroxidation was measured in terms of MDA formed (Table 6).

In control cells 49 n moles of MDA/mg protein was observed. UV-B treatment caused an enhancement in the lipid peroxidation and at 6 $Wm^{-2}$ almost 51% enhancement was noticed. To verify whether the lipid peroxidation is linked to the loss of PS II activity or not, a comparative study has been made between lipid peroxidation and PS II photochemistry (Table 6). There is a inverse relationship between enhancement of lipid peroxidation and loss of PS II photochemistry. Thus changes in LHC (PBsomes), alterations at the oxidizing side of thylakoid membrane and lipid peroxidation are responsible for the inhibition of PS II photochemistry under UV-B radiation.

UV- B radiation treatment caused the inhibition of both whole chain electron transport and PS II catalyzed electron transport (Table 1 and 2). The effect of UV B on the electron transport is intensity dependent (Table 3). The reason for the inhibition of PS II catalyzed electron transport could be altered at the level of either water oxidation complex or LHC (Noorudeen and Kulandaivelu, 1982; Strid *et al.*, 1990; Friso *et al.*,1995). our results are in agreement with the observations of Nedunchezhian and Kulandaivelu (1993) who showed that between two photosystems, PS I is less susceptible to UV-B radiation stress (Table 4).

The studies of light intensity measurements indicated that the inhibition is more at light saturating conditions than at light limiting conditions (Table 3). Similar observation was also made in the case of copper treatment in the same cyanobacterium (Ranjani, 2003). Fluorescence spectral measurements also indicated that among the other photosynthetic pigments, PC is the main target for UV-B action (Table 4, 5). Murthy (1991) also reported that mercury at low concentration (6 $\mu$M) causes the inhibition of energy transfer from PC to Chl a in the cyanobacterium, *Spirulina platensis*. These results are in agreement with the observations made in fluorescence emission properties of PC under UV-B stress. UV- B induced

decrease in the PC fluorescence intensity and shift in the peak position towards the blue region of the spectrum indicates the uncoupling of energy transfer with in the PBsome (Table 5). Since all the primary processes are occurring in the thylakoid membrane, any alteration in the lipid component of thylakoid membrane could be responsible for the alterations of PS II activity. Therefore an attempt has been made to study the effect of UV B on thylakoid membrane organization by measuring the lipid peroxidation. UV B treatment caused the enhancement of lipid peroxidation which is linked to the loss of PS II activity (Table 6).Thus the lipid peroxidation and the existence of the inhibitory site near oxidizing site are responsible for the altered PS II photochemistry under UV- B stress in *Spirullina platensis*.

## REFERENCES


**Adhikari, S. P. 2003.** Heat shock protein in the terrestrial epilithic cyanobacterium *Tolypothrix byssoidea*. *Biol. Plant.* **47:** 125-128.

**Bornmann, J. F. 1989.** Target sites of UV B radiation in photosynthesis of higher plants. *J. Photochem. Photobiol. B.* **4:** 145-158.

**Fork, D. C. and Mohanty, P. 1986.** Fluorescence and other characteristics of blue –green algae (Cyanobacteria), red algae and Cryptomonads. In: Light emission by plants and bacteria (Govindjee, Amsez, j. and Fork, D.C. ed.) pp.451-496. *Academic press, New York*.

**Friso, G., Vass, I., Spetea, C., Barber, J. and Barbato, R. 1995.** UV B induced degradation of the $D_1$ protein in isolated reaction centers of Photosystem II. *Biochim. Biophys. Acta.* **1331:** 41-46.

**Hader, D. P. and Hader, M. A. 1989.** Effects of enhanced solar radiation on aquatic ecosystems. *Photochem. Photobiol.* **53:** 717-725.

**Heath, R. L. and Packer, L. 1968.** Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arciv. Biochem. Biophys.* **125:** 189-198.

**Karentz, D., Cleaver, J. E. and Mitchell, D. L. 1991.** Cell survival characteristics and molecular responses of antartic phytoplankton to ultraviolet-B radiation. *J. Phycol.* **27:** 326-341

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

**Kulandaivelu, G. Geetha, V. and Lingakumar, K. 1987.** Possible inhibition of water oxidizing complex of Photosynthesis by ultraviolet-A (365nm) radiation. *Proc. Ind. Natl. Sci. Acad.* Part B (*Biological sciences)*. **53:** 421-423.

**Melis, A., Nemsom, J. A. and Harrison, M. A. 1992.** Damage to functional components and partial degradation of PS II reaction center protein upon chloroplast exposure to ultraviolet-B radiation. *Biochim. Biophys. Acta.* **1109:** 312-320.

**Murthy, S. D. S. 1991.** Studies on bioenergetic processes of cyanobacteria: Analysis of the selected heavy metal ions on energy linked process. Ph.D thesis, Jawaharlal Nehru University, New Delhi.

**Murthy, S. D. S., Sabat, S. C. and Mohanty, P. 1988.** Mercury induced inhibition of photosystem II activity and changes in the emission of fluorescence from phycobilisomes in intact cells of cyanobacterium, *Spirulina platensis*. *Plant Cell physiol.* **30:** 153-1157.

**Nedunchezhian, N. and Kulandaivelu G. 1993.** Increased stability of thylakoid components in *Vigna sinensis L.* seedlings grown under ultraviolet-B enhanced radiation. *Photosynthetica* **29:** 369-375.

**Noorudeen, A. M. and Kulandaivelu, G. 1982.** On the possible site of inhibition of photosynthetic electron transport by ultraviolet-B radiation. *Physiol. Plant.* **55:** 161-166.

**Rajagopal, S. and Murthy, S. D. S. 1996.** Short term effect of ultraviolet B radiation on Photosystem II Photochemistry in the cyanobacterium *Synechococcus* 6301. *Biol. Plant.* **38:** 129-132.

**Rajagopal, S., Murthy, S. D. S. and Mohanty, P. 1998.** Effect of UV-B radiation on intact cells of the cyanobacterium, *Spirulina platensis:* characterization of the alterations in thylakoid membrances. *J. Photochem. Photobiol. (B) Biol.* **54:** 61-66.

**Ranjani, R. 2003.** Characterization of heavy metal ions induced damage in photochemical functions of the cyanobacteria. Ph.D. Thesis, Sri Venkateswara University, Tirupati, India.

**Renger, G. and Schreiber, U. 1986.** Practical applications of fluorometric methods to algae and higher plant research. In: Light emission by plants and Bacteria (Govindjee, Amesz, *J. and Fork, D.C. ed.) pp. 587-619,* Academic press, New York.

**Robinson, S. J., Deroo, C. S. and Yocum, C. F. 1982.** Photosynthetic electron transfer in preparation of the cyanobacterium Spirulina plantesis. *Plant Physiol.* **70:** 154-161.

**Sah, J.F., Kolli, B .K., Srivastava, M. and Mohanty, P. 1998.** Effects of ultraviolet-B radiation on phycobilisomes of Synechococcus 7942 alteration in confirmation and energy transfer characteristics.*Biochem. Mol. Biol. Int.* **44:** 245-247.

**Singhal, G. S.. Mohanty, P. and Govindjee, 1981.** Effect of preheating intact cells on pigments revealed by absorption and fluorescence spectra. Z. *pflanzen-Physiol*. 103:217-228.

**Strid, A. Chio, W. S. and Anderson, J. M. 1990.** Effects of supplementary UV B radiation on photosynthesis in *Pisum sativum*. *Biochim. Biophys. Acta.* **1020:** 260-268.

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

**Tyagi, R. Srinivas, Gvyas, D., Kumar, A. and Kumar, H. D. 1992.** Differential effect of Ultra violet B radiation on certain metabolic process I a chromatically adapting Nostoc. *Photochem Photobiol.* **55:** 401-407.

**Zarrouk, C. 1966**. Contribution a l'etude d'unn cyanophyce. Influence de diverse facteurs physique et chimiques sur la croissance et photosynthese le *Spirulina maxima* Geitler, Ph.D. Thesis, University of Paris.