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UV-B STRESS INDUCED ALTERATION IN THE PHOTOSYNTHETIC APPARATUS OF THE CYANOBACTERIA *SYNECHOCOCCUS* 6301

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ABSTRACT
Exposure of spheroplasts of the cyanobacteria *Synechococcus* 6301 to UV-B irradiance (5 Wm^{-2}) stress for 0 to 60 minutes caused inhibition in various partial photochemical reactions. Compare to two photosystems (PS), PS II seems to be more susceptible to UV-B radiation than the PS-I. The results indicated that light haversting complex of PS II is the primary target for UV-B irradiation. It causes inhibition in Hill reaction by inducing the structural alterations in the spectral properties of phycobiliproteins.

INTRODUCTION

In recent years stratospheric ozone depletion by pollution was led to the enhanced UV radiations on earth (Smith *et al*., 1992; Frederick, 1993). UV-B radiation is considered to be one of the environmental stresses which affect the light reactions of photosynthesis. Stunted growth of plants, decrease in the length of interposed and reduction of leaf size is typical responses of plants to UV-B radiation (Teramura and Sullivan, 1994; Liu-Li-Xia *et al*., 2005). Plants exposed to supplementary UV-B radiation exhibit an overall decrease in photosynthetic pigments, like chlorophylls and carotenoids, per unit dry mass of leaves was observed in *Pisum sativum* (Strid *et al*., 1990), in *Vigna sinesis* (Lingakumar and Kulandaivelu, 1993), in *Vigna unguiculate* (Nedunchezhian and Kulandaivelu, 1997). UV-B radiation also causes damage to thylakoid membrane (Bornman, 1989). In cyanobacteria, UV-B radiation affects a number of physiological and biochemical processes such as growth, survival, pigmentation and total protein profile (Kulandaivelu *et al*., 1989; Tyagi *et al*., 1991; Sinha *et al*., 1995; Banerjee and Hader,1996). Compare to two photosystems the photosystem II has been found to be highly susceptible to UV radiation in spinach, (Iwanzik *et al*., 1983; Renger *et al*., 1989), in peas (Melis *et al*., 1992; Jansen *et al*., 1996), in *Spirodella* (Hermann *et al*., 1997), in *Dunaliella* (He *et al*., 1993), in barley (Barbato *et al*., 2000). While photosystem I is some what resistant to UV-B radiation similar observations were also observed in cyanobacteria (Kolli *et al*., 1998; Rajagopal, 1999).

Studies related to the effect of UV-B radiations on the spheroplasts of the cyanobacteria are scanty. Therefore an attempt has been made to study the UV-B induced alterations in the photosynthetic electron transport in cyanobacteria *Synechococcus* 6301 spheroplasts were exposed to UV–B irradiance (5 Wm^{-2}) for 0 to 60 minutes.

MATERIALS AND METHODS

Synechococcus 6301 was grown axenically in BG-11 (Stanier *et al*., 1971) at 25 ± 2ºC under continuous illumination (20 Wm^{-2}). The spheroplasts were prepared by incubating the intact cells at 37ºC in the presence of lysozyme (1mg/mL) for 3 hour according to Newman and Sherman (1978).

Spheroplast suspension was taken from the culture flasks and was subjected to centrifugation at 9,000Xg for 5 minutes.The pellet was washed twice with reaction buffer (25 mM N-(2-hydroxyethyL)-piperzine-N'-2-ethaonesulfonicacid (HEPES) – NaOH buffer (pH – 7.5) containing 20 mM NaCl) and suspended in the same buffer.The spheroplasts were exposed to UV–B irradiance (5 Wm^{-2}) for 0 to 60 minutes in petri dishes under constant stirring at 25 ±2ºC. UV-B tubes having maximal emission at 300 nm with 40 nm half band width were used to give the UV-B irradiance source. Whole chain electron transport assay (H_2O →methylviologen) was studied in terms of O_2 consumption due to photoreduction of methylviologen and its subsequent auto oxidation. The reaction mixture contained reaction buffer (25mM HEPES–NaOH (pH–7.5), 20mM NaCl), 0.5mM methylviologen, 1mM sodium azide and spheroplasts equivalent to 12-15μg chlorophyll *a*. (Murthy, 1991).

p-Benzoquinone was used to measure the photosystem II catalyzed electron transport (H_2O → *p*-benzoquinone) in the

spheroplasts. Being a lipophilic compound *p*-benzoquinone enters into spheroplasts and accepts electron at plastoquinone position (Trebst, 1974). The reaction mixture contained reaction buffer (25mM HEPES–NaOH (pH–7.5), 20mM NaCl), 0.5mM freshly prepared *p*-benzoquinone and spheroplasts equivalent to 12-15μg chlorophyll *a*. (Murthy *et al*., 1988 and 1989)002EThylakoid membranes were prepared according to the method of Rajagopal (1999). The reaction mixture of PS I catalysed electron transfer ($DCPIPH_2 \rightarrow$ MV contained reaction buffer, 5mM ascorbate, 0.1mM DCPIP, 10μM DCMU, 0.5mM methylviologen, 1mM sodium azide and thylakoid membrane fragments equivalent to 10-15μg of chlorophyll *a*.

RESULTS AND DISCUSSION

In the present investigation to characterize the alterations in photosynthetic electron transport, initially the effect of UV-B stress on whole chain photosynthetic electron transport was studied. MV is known to accept the electron from A_o in the photosynthetic electron transport chain (Trebst, 1974). Therefore the electron transport has been measured by using MV as terminal acceptor. Control spheroplasts without UV-B radiation showed a high rate of oxygen consumption 232 μmoles of $O_2\downarrow$ mg $chl^{-1}h^{-1}$, (Table 1). The increase in the UV-B radiation from 1 to 7 Wm^{-2} caused gradual increase in the inhibition. Almost 50% inhibition was noticed at 5 Wm^{-2}. Further raised UV-B radiation to 7 Wm^{-2} brought 58% loss in the electron transport activity. Hence 5 Wm^{-2} has been selected for study of UV-B stress on photosynthetic electron transport in spheroplasts of *Synechococcus* 6301.

Table 2 shows the time dependent effect of UV-B radiation on whole chain electron transport activity. After treatment with 5 Wm^{-2} for 15 minutes the inhibition was only 28%. The increase in the incubation from 30-60 minutes brought gradual enhancement in the inhibition pattern. After one hour of incubation only 20% activity remained. The reason for loss of whole chain electron transport could be alteration either at photosystem II reaction center level or at photosystem I reaction center level. These results are in agreement with the observations of Kulandaivelu *et al.,* (1989).

Since UV-B radiation affected the whole chain electron transport, its effect on photosystem II and photosystem I catalyzed electron transport individually was studied. *p*-Benzoquinone is an artificial electron acceptor and accepts electron from plastoquinone pool (Trebst, 1974). Being lipophilic in nature it easily enters through the membrane and reach plastoquinone. Control spheroplasts exhibited a high rate of photosystem II dependent oxygen evolution (352 μmoles of $O_2\uparrow$mg $chl^{-1}h^{-1}$) (Table 3).

By exposure of spheroplasts to UV irradiance, caused time dependant inhibition in photosystem II catalyzed electron transport. After 15 minutes of exposure to UV-B irradiance brought 20% loss in oxygen evolution. Further increase in the incubation period from 30-60 minutes brought 79% inhibition in photosystem II catalyzed electron transport. The inhibition in photosystem II catalyzed electron transport by exposure of UV-B ($5Wm^{-2}$) in *Synechococcus* spheroplasts was time dependent. A similar type of inhibition was observed in *Vigna* chloroplasts after exposure of UV-B radiation (Noorudeen and Kulandaivelu, 1982). Loss in photosystem II catalyzed electron in *Synechococcus* spheroplasts could be either due to inactivation of photosynthetic electron carrier (or) by suppression of energy transfer between light harvesting complex to the reaction center (Kulandaivelu *et al*., 1989; Renger *et al*., 1989; Kolli *et al*., 1998).

To identify the target pigment protein in photosystem II, the affect of UV-B radiation under different illumination conditions (Table 4) was measured. The inhibition observed with UV-B radiation was more at light saturating conditions (400 Wm^{-2})

Table 1: Effect of UV-B radiation on whole chain electron transport activities in spheroplasts of *Synechococcus* 6301. ($H_2O \rightarrow$methylviologen)

UV-B radiation (Wm^{-2})	Whole chain electron transport activity($H_2O \rightarrow$ methylviologen) μmoles of $O_2\downarrow$ mg $chl^{-1}h^{-1}$	Percent of loss
0	232 ±21	0
1	201 ±19	14
3	185 ±17	21
5	121 ±13	48
7	98 ±10	58

Table 2: Time dependant effects of UV-B irradiance (5 Wm^{-2}) on whole chain electron transport activity in the spheroplasts of *Synechococcus* 6301. ($H_2O \rightarrow$methylviologen)

Duration of exposure minutes	Whole chain electron transport activity ($H_2O \rightarrow$ methylviologen) μmoles of $O_2\downarrow$ mg $chl^{-1}h^{-1}$	Percent of loss
Control	228 ±23	0
15	165 ±17	28
30	115 ±11	50
45	75 ±9	67
60	45 ±5	80

Table 3: Short term effect of UV-B ($5Wm^{-2}$) irradiance on photosystem II catalyzed electron transport activity the spheroplasts of *Synechococcus* 6301

Duration of exposure minutes	Photosystem II catalyzed electron transport ($H_2O \rightarrow$*p*-benzoquinone) μmoles of $O_2\uparrow$ mg $chl^{-1}h^{-1}$	Percent of Inhibition
Control	352 ±35	0
15	281 ±29	20
30	193 ±20	45
45	142 ±16	60
60	75 ±8	79

Table 4: Effect of different illuminated light intensities on UV-B (5 Wm^{-2}) induced inhibition of photosystem II catalyzed electron transport activity the spheroplasts of *Synechococcus* 6301

Light Intensity Wm^{-2}	Photosystem II catalyzed electron transport ($H_2O \rightarrow$ *p*-Benzoquinone) μmoles of $O_2\uparrow$ mg $chl^{-1}h^{-1}$		Percent of Inhibition
	Control	Treated	
400	361 ±37	186 ±14	51
205	204 ±21	104 ±11	49
110	102 ±12	57 ±6	45
15	37 ±4	22 ±2	40

Table 5: Short term effect of UV-B ($5Wm^{-2}$) radiation on photosystem I catalyzed electron transport activity the spheroplasts of *Synechococcus* 6301

Duration of exposure minutes	Photosystem I catalyzed electron transport ($DCPIPH_2 \rightarrow MV$) μmoles of O_2 consumed mg $chl^{-1}h^{-1}$	Percent of loss
Control	552 ±55	0
15	541 ±53	2
30	532 ±52	4
45	509 ±16	8
60	492 ±49	11

than at light limiting conditions (15 Wm^{-2}). The inhibition at light limiting condition clearly indicating the alteration light harvesting complex of photosystem II, the inhibition at saturating condition shows the existing of additional site of inhibition in photosystem II other than light harvesting complex.

Table 5 shows the effect of UV-B radiation on photosystem I catalyzed electron transport. The increase in the incubation period from 15-60 minutes brought only 10% lose in the electron transport activity.

This clearly shows that compared to photosystem II, photosystem I seems to be resistant to UV-B radiations. These results are in agreement with the observation of Kulandaivelu *et al*., (1989). Thus UV-B radiation specially affects photosystem II catalyzed electron transport without affecting the photosystem I to a lesser extent.

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