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 harnessing 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 ± 2°C under continuous illumination (20 Wm^{-2}). The spheroplasts were prepared by incubating the intact cells at 37°C 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)piperazine-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 ± 2°C. 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_{2}O → 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-Benzooquinone was used to measure the photosystem II catalyzed electron transport (H_{2}O → 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 1989/002Ethylalcohol diethylamine were prepared according to the method of Rajagopal (1999). The reaction mixture of PS I catalyzed electron transfer (DCPiPH₂ → MV contained reaction buffer, 5mM ascorbate, 0.1mM DCPIP, 10μg 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 A₄ 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 O₂ 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 O₂ mg chl⁻¹ h⁻¹) (Table 3).

By exposure of spheroplasts to UV irradiance, caused time dependent 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 transport 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)
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.

REFERENCES


