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EFFECT OF UV-B RADIATION ON LIPID PEROXIDATION OF THYLAKOID MEMBRANES OF CYANOBACTERIUM *SYNECHOCOCCUS* 6301

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ABSTRACT
The objective of the present study was to characterize the effect of UV-B radiation on thylakoid lipid peroxidation in the cyanobacterium *Synechococcus* 6301. The UV-B radiation caused gradual enhancement in the lipid peroxidation of thylakoid membrane. To analyze the protective mechanism against lipid peroxidation 2 mM ascorbate was used as reducing agent in reaction mixture before exposed to UV-B radiation. The studies explained that ascorbate acted as potent reducing agent during peroxidation of thylakoid membranes. To correlate the lipid peroxidation with photosystem II catalyzed electron transport, the results indicated the inverse relationship between photosystem II photochemistry and lipid peroxidation.

INTRODUCTION

The UV-B radiation is potential hazard for the aquatic organisms, because they don't possess the protective UV absorbing epidermal layers like those of higher plants. UV-B radiation inhibits fundamental processes such as photosynthesis and plant growth

(Tevini and Teramura, 1989; Bornman, 1989). The impact of increasing UV-B radiation on higher plants has been investigated recently. In higher plants reduction of leaf area, fresh and dry weight occurs by UV-B exposures. Decrease in lipid content and loss in photosynthetic activity have been reported in a number of UV-B sensitive plant species (Iwanzik *et al*., 1983). Further it has been also reported that UV-B radiation affects many metabolic processes pigment biosynthesis, and community composition of biological systems other than higher plants (Dohler *et al*., 1986; Tyagi *et al*., 1991).

In thylakoid membrane always lipid protein interactions play a key role during the transfer of electrons from photosystem II to photosystem I. Among the different lipids present in the thylakoid membrane mainly monogalactosyl diacylglcerol being affected more than digalactosyl diacylglcerol (Tevini and Teramura, 1989). Since the lipids are involved in bilayer formation of thylakoid any alteration in lipid could affect the photosynthetic electron transport activities of thylakoid membranes (Rai *et al*., 1995; Mackerness *et al*., 1998).

Hence an attempt was made to study the effect of UV-B radiation on lipid bilayer in the cyanobacterium *Synechococcus* 6301. For this study, analysis was carried out on the thylakoid lipid peroxidation of thylakoid membranes.

MATERIALS AND METHODS

Synechococcus 6301 was obtained from Department of Plant Science, Madurai Kamaraj University, Tamilnadu, India. Culture was grown axenically in BG-11 (Stanier *et al*., 1971) at 25 ± 2ºC under continuous illumination (20 W m^{-2}). The spheroplasts were prepared by incubating the intact cells at 37ºC in the presence of lysozyme (1mg/mL) for 3 hours according to Newman and Sherman (1978).

Treatment of UV–B radiation

Synechococcus 6301 spheroplasts were exposed to UV–B irradiance (5 Wm^{-2}) for 0 to 45 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.

Lipid peroxidation

Lipid peroxidation was measured according to method of Heath and Packer (1968). The thylakoids were homogenized in 0.1% trichloroacetic acid. The homogenized suspension was centrifuged at 10,000 g for 5 minutes. After adding 4 mL of 0.5 mM of thiobarbituric acid (TBA) and mixture was boiled at 95°C for 30 minutes. Then it was cooled and centrifuged at 10,000 g for 15 minutes. The absorbance of supernatant was measured at 532 nm and at 600 nm. The malondialdehyde (MDA) concentration was calculated using an extinction coefficient 155 $mm^{-1}cm^{-1}$. The amount of lipid peroxidation was expressed as nmoles of malondialdehyde per mg wet weight of cells.

RESULTS AND DISCUSSION

The lipid peroxidation was measured in terms of malondialdehyde formed in control thylakoid membranes. The lipid peroxidation was equal to 42 nmoles malondialdehyde formed per mg protein (Table 1). The increase in the incubation period from 5 to 20 minutes caused the gradual enhancement in lipid peroxidation. After 20 minutes of UV-B exposure, 71% enhancement was noticed in lipid peroxidation of UV-B treated samples.

Table 1: Effect of UV-B radiation (1 Wm^{-2}) on lipid peroxidation of thylakoid membranes

Duration of exposure (min)	Lipid peroxidationnmoles malondialdehyde/mg protein	Percent of increase
Control	42 ±4	0
5	52 ±5	23
10	65 ±6	54
15	69 ±7	64
20	72 ±7	71

Table 2: Effect of UV-B radiation (1 Wm^{-2}) on lipid peroxidation of thylakoid membranes in presence and absence of ascorbate

UV-B Exposure	Lipid peroxidation nmoles malondialdehyde/mg protein	Percent of increase
Control	46 ±5	0
UV-B(absence of ascorbate)	70 ±8	52
UV-B(presence of ascorbate)	47 ±4	1

Table 3: Comparative effect of UV-B radiation (1 Wm^{-2}) on lipid peroxidation and photosystem II catalyzed electron transport activity in thylakoid membranes

Duration of exposure (min)	PS II catalyzed electron transport ($H_2O \rightarrow$ Dichlorobenzoquinoe) μmoles of $O_2\uparrow$mg $chl^{-1}h^{-1}$	Lipid peroxidation nmoles malondial-dehyde/mg protein
Control	104 ±10	43 ±4
10	63 ±7	66 ±6
15	48 ±5	70 ±7
20	28 ±3	74 ±7

To analyze the possible protection mechanism adapted by spheroplasts, a study was made by using 2 mM ascorbate as proecting agent against stress. Thylakoids has been exposed for 10 min to UV-B radiation (1 Wm^{-2}) in the presence and absence of ascorbate (Table 2). In the absence of ascorbate UV-B radiation cause 52% enhancement in the lipid peroxidation. When ascorbate is included while giving the UV-B stress almost there is negligible rise in lipid peroxidation the malondialdehyde formation is almost equal to that of sample. Thus ascorbate protects the formation of lipid peroxides from thylakoid membrane during UV-B radiation treatment. To correlate the lipid peroxidation to photosynthetic electron transport of photosystem II, both these parameters were studied in a comparative manner. For this purpose, 1Wm^{-2} of UV-B radiation was selected and exposed the thylakoids for 10 minutes then both the parameters are analyzed (Table 3). The increase in the UV-B exposure caused the loss of photosystem II catalyzed electron transport by 55%. The same sample exhibited the 53% enhancement in the lipid peroxidation activity. Thus the inhibition of photosystem II very much related to the increase of lipid peroxidation under UV-B treatment. Thus UV-B radiation may induce toxic oxy radicals, which in turn affect the photosystem II activity by altering the lipid protein interaction. From this study it is clear that UV-B stress causes the alteration of thylakoid lipid environment around PS II there by impair the PS II activity.

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