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Free Radical Mediated Damage in the Thylakoid Photo Functions of Barley Thylakoid Membranes under UV-B Radiation: Analysis of Possible Protection Mechanisms

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Abstract: In the present study the effect of UV-B radiation (20-60 μmoles $m^{-2}s^{-1}$) has been studied on the thylakoid photochemical activity and alterations in the membrane organization of barley primary leaves. Our results clearly indicated that ultraviolet-B shows intensity dependent effect on photosystem II activity and induction in the enzyme activities of superoxide dismutase and catalase. The possible reason for the loss of photosystem II catalyzed electron transport could be from free radical mediated damage of thylakoids under ultraviolet-B radiation.

Key words: Antioxident enzymes · Ascorbate · Barley · Electron transport · Lipid peroxidation · Photosystem II

INTRODUCTION

Biological membrane contains high proportion of nonbilayer forming lipids in association with proteins to perform several physiological functions. In higher plants thylakoid membranes are rich in mono galactosyl diasyl glycerol, di galactosyl diasyl glycerol and sulfolipids. The nonbilyaer lipids are required for the packing of light harvesting units of photosystem (PS) II and PS I into thylakoid membranes [1]. Generally stress factors like, temperature and high light are known to cause the destabilization of polypeptide due to lipid peroxidation of thylakoid membrane. Since ultraviolet (UV) B stress is having interaction with oxygen and other biomolecules, it can result in the production of toxic intermediates of singlet oxygen and free radicals. There by they interact with biological membrane and cause peroxidation of membrane lipids. Several antioxidants such as glutathione, pyruvate and ascorbic acid accumulate during the stress and they protect the system form damage. Rijstenbil *et al.* [2] suggested that active oxygen may cause the growth inhibition of plants. Several organic and inorganic compounds present in the natural environment are known to play an additional role in the protection against UV-B radiation. Many antioxidants are recognized to have a protective effects mitigating UV-B radiation damage in higher plants [3]. The results produced by Tayagi *et al.* [4] show that reducing agents like ascorbic acid, glutathione, L-cystenine, L-tyrptophane were equally effective in the protection of cyanobacterial against UV-B induced damage. Biscof *et al.* [5] showed that reactive oxygen species (ROS) induced lipid peroxidation depended on the position and radiation conditions. Samples exposed to full solar spectrum were most effected, where as, samples exposed individually was exhibited partial damage. In addition ROS protection causes oxidative damage to photosynthetic machinery such as proteins and pigments [6]. UV-B treatment causes the increase of antioxidant defense enzymes like catalase (CAT) and superoxide dismutase (SOD) [7]. Studies related to the effect of UV-B radiation on thylakoid lipid peroxidation and antioxidant defense enzymes are scanty. Therefore, in this chapter we have studied effect of UV-B radiation on the lipid peroxidation of thylakoid membranes, induction of SOD, CAT activities and protection mediated by ascorbate (Asc).

MATERIALS AND METHODS

Growth Conditions and UV-B Treatment: Barley (*Hordeum vulgare*) seedlings were obtained from the market were surface sterilized with 30% ethanol and then

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kept in running water for 3 h. These seeds were further soaked in distilled water for 24 h in darkness. The well germinated seeds were grown in Petri plates under continuous white light (160 μ moles m^{-2} s^{-1}) at 25°C. Hoagland solution was supplied at 4 day intervals to the seedlings. 8-day-old seedlings were exposed to different doses of UV-B radiation (obtained from a Philips TL 20 type 05 in the spectral range 280 to 320 nm and with a peak at 312 nm) (20-80 μ moles $m^{-2}s^{-1}$) for 60 min. after the treatment primary leaves of both control and UV-B treated seedlings were sampled for various measurements.

Measurement of Photoelectron Transport Activities

Isolation of Thylakoid Membranes: Thylakoid membranes were isolated according to a procedure similar to that of Saha and Good [8] as described in Swamy *et al.* [9]. All operations were carried out at 4°C in dim light.

Photosystem II Activity: p-benzoquinone (p-BQ) was used to measure the PS II catalyzed electron transport in thylakoid membranes. 2 mL reaction mixture contained 50 mM HEPES-NaOH (pH.7.5), 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl, 0.5 mM freshly prepared p-BQ and thylakoid membranes equivalent to 40 μg of Chl. PS II mediated electron transport was studied in terms of molecular oxygen evolution.

Lipid Peroxidation: Lipid peroxidation (LPO) has been measured according to the method of Heath and Packer [10].

Determination of Antioxidant Enzyme Activity: The activity of SOD was determined according to the procedure of Van Rossun *et al.* [10]. One SOD unit was defined as the amount of enzyme required to inhibit 50% of the NBT photoreduction in comparison with tubes lacking the plant extract and expressed as units of enzyme activity (IU) g^{-1} FW min^{-1}. CAT activity was determined according to the method of Havir and McHale [12]. Enzyme activity was calculated using the molar extinction coefficient 36 x 10^3 mM^{-1} m^{-1} and expressed as μmole H_2O_2 oxidized g^{-1} FW min^{-1}.

Statistical analysis: The experiments were done in triplicate. Data are expressed as the means±SD (standard deviation).

RESULTS

Effect of UV-B Radiation on Lipid Peroxidation: UV-B radiation affects photosynthetic electron transport at multiple sites in the thylakoid membranes. Since the thylakoid membranes are made up of lipids, lipid protein interaction plays a key role in the characterization of electron transport activity. To analyze the changes of lipids in the thylakoid membrane regarding the effect of UV-B radiation, we have measured the LPO in terms of malonyldialdehyde (MDA) formation. In control thylakoids 39 nmole MDA g^{-1} FW was observed (Table 1). The treatment of UV-B radiation from 20 μmoles to 60 μmoles gradually caused enhancement in the LPO. At 60 μmoles UV-B treatment 67% enhancement in the LPO was noticed.

Asc Mediated Protection in Lipid Peroxidation: To analyze the possible scavenging mechanisms of lipid peroxidation, a study has been made using Asc (3 mM) as a protecting agent. For this study cells were incubated at 40 and 60 μmoles of UV-B radiation, in the presence and absence of Asc. In the absence of Asc at 40 μmoles of UV radiation, there was 52% increase in the lipid peroxidation. When the Asc was incubated along with UV-B radiation almost the lipid peroxidation was negligible (Table 2). Even with 60 μmoles UV-B treatment in the absence of Asc 68% enhancement where as in the presence of Asc it is almost equal to that of control thylakoids. Thus, Asc protects the peroxidation of lipids under UV-B radiation stress.

Comparison of PS II Photochemistry with Lipid Peroxidation: To study the comparative effect of UV-B radiation on PS II catalyzed electron transport in relation to lipid peroxidation of thylakoid membranes, an attempt has been made. For this thylakoids were isolated from control and UV-B treated samples were used for measurement of PS II catalyzed electron transport as well as lipid peroxidation. The increase in the UV-B radiation brought almost 50% inhibition at 40 μmoles of UV-B treatment. Under the same conditions an increase in the lipid peroxidation by 53% were noticed (Table 3). Thus there is an inverse relationship between the enhancement of lipid peroxidation and inhibition of PS II photochemistry.

Effect of UV-B on Antioxidant Enzymes: Generally under stress conditions of UV-B there will be a generation of superoxide and peroxide radicals [5,6]. To scavenging

Table 1: Effect of UV-B radiation on lipid peroxidation of thylakoid membranes. The values are average of three separate experiments

UV-B radiation μmoles $m^{-2}s^{-1}$	Lipid peroxidation (nmole MDA/g FW	Percentage of increase
Control	39±2.8	0
20	51±4.6	30
40	60±5.4	53
60	65±5.9	67

Table 2: Effect of ascorbate on lipid peroxidation mediated by UV-B radiation in thylakoid membranes of barley. The values are average of three separate experiments

UV-B radiation μmoles $m^{-2}s^{-1}$	Lipid peroxidation (nmole MDA/ g FW	Percentage of loss
Control (-UV-B)	40±3.5	0
20 (-Asc)	61±5.7	52
20 (+Asc)	41±3.6	3
40 (-Asc)	67±5.9	68
40 (+Asc)	42±3.7	5

Table 3: Comparative effect of UV-B radiation on PS II catalyzed electron transport ($H_2O \rightarrow pBQ$) and lipid peroxidation of thylakoid membrane of barley primary leaves. The values area average of three separate experiments

UV-B radiation μmoles $m^{-2}s^{-1}$	Activity of PS II O_2 ↑ mg^{-1} Chl h^{-1}	Lipid peroxidation (nmole MDA/ g FW
Control	262±27	39±2.8
20	196±20	51±4.6
40	128±14	60±5.4
60	86±9	65±5.9

the generated toxic oxygen species there will be an enhancement in the activities of enzymes like CAT and SOD which help in detoxification of generated toxic superoxy and peroxy radicals. To verify this we have measured the enzyme activity of SOD. Table 4 shows the effect of UV-B radiation in the activity of SOD and CAT. In control sample the SOD activity is equal to 65.2 units X 10^{-2} g^{-1} FW. The increase in UV-B radiation from 20 to 60 μmoles gradually caused the enhancement in the enzyme activity to 52%. The other antioxidant defense enzyme, CAT activity in the control sample is equal to 13.2 μmoles H_2O_2 oxidized g^{-1} FW (Table 4). UV-B treatment gradually caused the increase in the CAT activity and at 60 μ moles of UV-B treatment 94% enhancement in the enzyme activity was noticed. This enhancement of enzyme activity Cleary shows that UV-B radiation mainly promotes the generation of peroxy radicals rather than superoxy radicals.

DISCUSSION

In this investigation we have made an attempt to characterize the effect of UV-B radiation on the peroxidation of lipids in thylakoid membrane, role of Asc and antioxidant enzymes like SOD and CAT. Stress factors are known to influence the photochemical reactions by altering the membrane organization by keeping the about points in mind we have measured the UV-B mediated lipid peroxidation and its relation to the PS II activity and enzyme induction particularly catalase and SOD. To analyze the changes of lipids in thylakoid membrane under UV-B stress we have measured the peroxidation of lipids in terms of MDA formation. The raise in the UV-B radiation caused the enhancement in lipid peroxidation which leads to the formation toxic oxyradicals. These radicals may intern cause damage to the electron transfer near PS II and affect the primary process of photosynthesis. To verify this we have made a comparative study regarding the UV-B effect on lipid peroxidation and PS II catalyzed electron transport. There is an inverse relationship between the inhibition of PS II and an enhancement of lipid peroxidation.These free radicals are scavenged by antioxidant enzymes. Hence, we have measured the above two enzyme activities to establish the relation ship between free radical production UV-B radiation and enzyme induction. This shows a rapid induction of both enzyme activities under UV-B stress. This induction indirectly indicates the formation of

Table 4: UV-B radiation induced changes in antioxidant enzymes (SOD and CAT) activities in barley leaves. The values are average of three separate experiments

UV-B radiation μmoles $m^{-2}s^{-1}$	SOD activity units x 10^{-2} g^{-1} FW	Percentage of increase	CAT activity μmole H_2O_2 oxi g^{-1} FW	Percentage of increase
Control	65±5.8	0	13±1.1	0
20	82±7.7	26	19±1.4	44
40	94±8.9	45	23±1.9	77
60	99±8.7	52	25±2.4	94

superoxide radicals under UV-B stress. When compare to the SOD activity, the activity of CAT is almost doubled under UV-B treatment. Thus, UV-B stress seems to cause more production peroxy radicals when compare to superoxide radicals.

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