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UV-B RADIATION INDUCED ALTERATIONS IN THE ELECTRON TRANSPORT ACTIVITIES OF THE THYLAKOID MEMBRANES ISOLATED FROM PRIMARY LEAVES OF BARLEY

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UV-B treatment (20-80 μ moles) for 60 min caused inhibition in whole chain electron transport and PS II catalyzed electron transport in a dose dependent manner. The reason for the inhibition in PS II catalyzed electron transport is alteration at oxidizing side as well as reducing side. UV-B radiation is able to inhibit PS I catalyzed electron transport marginally. Light intensity measurements indicated that LHC II is the main target for UV-B radiation. Thus, UV-B affects the photosynthetic electron transport at multiple sites in barley leaves.

Keywords: Barley leaves; Electron transport; Photosystems; UV-B radiation.

Introduction

UV-B radiation is known to influence photosynthetic processes at multiple sites[1] of the two photosystems, the PS II has been found to be highly susceptible to UV-B radiation is spinach[2,3], in peas[4,5], in spirodella[6], in *Dunaliella*[7] in pea and rice[8], *Vigna unguiculata* L., while PS I is some what resistant to UV-B radiation. Also, similar results were noted with the treatment of UV-B in cyanobacteria[8-10]. Only very high intensity of UV-B irradiation, may inhibit the PS I mediated cyclic electron transport and photophosphorylation[2,11]. However, this effect seems to be marginal as compared to the loss in PS II activity. UV-B induced damage to PS II has been monitored in chloroplast[2] and also in intact leaves[12-13].

Damage of PS II may be related to changes in the oxidation capacity of the reaction centre as suggested by Renger *et al.*[3]. They observed changes in the reaction centre it self and in the function of the oxygen evolving complex and P_{680}. Alterations on the reducing side of PS II have also been suggested by Greenberg *et al.*[14] who demonstrated changes in the rate of PS II-RC degradation and turnover of the D_1 polypeptide. They presented evidence that a UV-B, possibly the PS II semiquinone anion, radical, was involved in this process. Renger *et al.*[3] has suggested that UV-B radiation causes functional disconnection between LHC II and PS II reaction centre. Vass[15] has reviewed the adverse and damaging effect of UV-B light on photosystems and their components. Both structural and functional aspects of PS II mostly that of higher plants have been critically analyzed. We summarized the nature of inhibition of PS II electron transport by UV-B irradiance. UV-B affects the PS II quinone electron acceptor by causing the damage of Q_A via Tyr_Z in the D_1 protein. UV radiation seems to impair Q_A and Q_B functions on the acceptor side of PS II. Further, the effect of UV-B on reduction function of Tyr_{Z+} and Tyr_{D+} has been shown by use of EPR spectroscopy[15,16]. The quinones of photosynthetic electron chain in isolated chloroplast have been reported to be the targets of UV-B radiation[4]. In this paper we studied the effect of UV-B radiation on photosynthetic electron transport of the thylakoid membranes of barley primary leaves. For this purpose barley leaves were exposed by placing them in plates under UV-B radiation ranging from 20 μ moles to 80μ moles. After exposure the primary leaves were collected and thylakoids have been isolated to measure the photosynthetic electron transport activities by using oxygen electrode.

Materials and Method

Barley (*Hordeum vulgare*) seedlings were raised 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 (20-80 μ moles m^{-2} s^{-1}) for 60 min. After the treatment primary leaves of both control and UV-B treated seedling were sampled for thylakoid membranes isolation and assay of photochemical activities.

The thylakoids were used for measurement of photochemical activities by following the procedure of Sabat *et al.*[17] with slight modifications. The assay mixture for whole chain electron transport activity contained 0.5

Table 1. Effect of UV-B radiation on the whole chain electron transport activity of the thylakoids isolated from control and UV-B treated barley primary leaves. Three ml of reaction mixture contains reaction buffer 25mM HEPES-NaOH (pH 7.5) containing 20 mM NaCl, 0.5mM MV, 1 mM Na-azide and thylakoids equivalent to 40 μg of Chl. Other details were given in material and methods. The SD is not more than 10%.

UV-B radiation μ moles $m^{-2}s^{-1}$	Whole chain electron transport activity $H_2O \rightarrow MV$ μ moles of $O_2\downarrow$ mg^{-1} Chl h^{-1}	Percentage loss
Control	193 ± 19	0
20	152 ± 16	21
40	110 ± 7	43
60	68 ± 7	65
80	46 ± 6	76

Table 2. Time dependent effect of UV-B radiation on the whole chain electron transport activity of the thylakoids isolated from control and UV-B treated barley primary leaves. Three ml of reaction mixture contains reaction buffer (25 mM HEPES-NaOH (pH 7.5) containing 20 mM NaCl, 0.5mM MV, 1 mM Na-azide and thylakoids equivalent to 40 μg of Chl. Other details were given in material and methods. The SD is not more than 10%.

Duration of the treatment, min	Whole chain electron transport activity $H_2O \rightarrow MV$ μ moles of $O_2\downarrow$ mg^{-1} Chl h^{-1}	Percentage loss
Control	196 ± 20	0
30	143 ± 15	27
60	94 ± 10	52
90	67 ± 7	66
120	45 ± 5	77

mM MV (Methyl viologen) and 1mM sodium azide in three ml of the 25 μM HEPES reaction buffer (pH 7.8). For PS II mediated oxygen evolution, the reaction mixture consisted of 0.5 μM pBQ in three ml reaction buffer. PS I catalyzed assay mixture contained 0.1 μM DCPIP (2, 6-Dichlorophenol-indophenol), 2μM azide, 1 μM MV and 5 μM DCMU. Inter system catalyzed electron transport assay contained all the chemicals as mentioned in PSI assay except 0.1μM DCPIP. In place of DCPIP, another donor duroquinone (DQH_2) is used.

Table 3. Effect of UV-B radiation on the photosystem II catalyzed electron transport activity of the thylakoids isolated from control and UV-B treated barley primary leaves. Reaction mixture (3 ml) for this assay contained reaction buffer, 0.5 mM pBQ and thylakoid equivalent to 40 μg of Chl. Other details were given in material and methods. The SD is not more than 10%.

UV-B radiation μ moles $m^{-2}s^{-1}$	PS II catalyzed electron transportactivity $H_2O \rightarrow pBQ$ μ moles of $O_2\uparrow$ mg^{-1} Chl h^{-1}	Percentage loss
ontrol	262±27	0
20	196±20	25
40	128±14	51
60	86±9	67
80	60±7	77

Table 4. Effect of UV-B radiation on the photosystem I catalyzed electron transport activity of the thylakoids isolated from control and UV-B treated barley primary leaves. Three ml of reaction mixture contained reaction buffer, 5 mM ascorbate, 0.5 mM DCPIP, 10 μM DCMU, 0.5 μM MV, 1 mM Na-azide and thylakoids equivalent to 40 μg of Chl. Other details were given in material and methods. The SD is not more than 10%.

UV-B radiation μ moles $m^{-2}s^{-1}$	PS I catalyzed electron transport activity $DCPIPH_2 \rightarrow MV$ μ moles of $O_2\uparrow$ mg^{-1} Chl h^{-1}	Percentage loss
Control	375±29	0
20	352±16	6
40	338±21	10
60	315±25	16
80	301±23	20

Table 5. Effect of UV-B radiation on the intersystem catalyzed electron transport activity of the thylakoids isolated from control and UV-B treated barley primary leaves. Three ml of reaction mixture contained reaction buffer, 5 mM ascorbate, 0.1 mM duroquinone, 10 mM DCMU, 0.5 mM MV, 1mM Na-azide and thylakoids equivalent to 40 μg of Chl. Other details were given in material and methods. The S.D. is not more than 10%.

UV-B radiation μ moles $m^{-2}s^{-1}$	Intersystem catalyzed electron transport activity DQH→MV μmoles of $O\downarrow_2$ mg^{-1} Chl h^{-1}	Percentage loss
Control	136±13	0
20	124±12	9
40	102±11	25
60	86±9	37
80	79±8	42

Table 6. Effect of different illuminated light intensities of UV-B radiation in inhibition of PS II activity in barley thylakoid membranes. The SD is not more than 10%.

Light intensity moles $m^{-2}s^{-1}$	PS II activity H_2O→pBQ μmoles of $0_2\uparrow$ mg^{-1} Chl h^{-1}		Percentage loss
	Control	UV- B treated	
4000	251±25	124±12	51
2100	163±15	85±8	48
1150	110±10	47±4	43
150	36±3	22±2	38

Results and Discussion

UV-B radiation induced alterations in whole chain electron transport: Methyl viologen (MV) is known to accept the electrons from A_0 in photosynthetic electron transport chain[18]. Therefore, whole chain electron transport activity has been measured in thylakoid membranes using MV as terminal electron acceptor (H_2O → MV). Control thylakoids without UV-B treatment exhibited a high rate of oxygen consumption (193 μ moles $O_2\downarrow$ mg^{-1} Chl h^{-1}). Increase in the UV-B treatment from 20 to 80 μmoles brought enhancement in the inhibition of whole chain electron transport. Almost 50% loss was noticed above 40μ moles of UV-B treatments (Table 1). The reason for the loss of whole chain electron transport could be either alterations at the level of PS II or PS I catalyzed electron transport[8]. This UV-B radiation induced inhibition of whole chain electron transport is found to be time dependent (Table 2). 40μ moles of UV-B exposure was selected and leaves were exposed to different time intervals. After 30 min of exposure only 27% loss was noticed in whole chain electron transport. Further raise in the incubation period to 60 min brought 52% loss in the whole chain electron transport. A raise in incubation period to 120 min brought maximum loss (77%) in whole chain catalyzed transport. Thus, UV-B radiation induced inhibition of whole chain electron transport is not only dose dependent but also time. To identify the target photosystem, the partial electron transport reactions mediated by individual photosystems were measured.

Inhibitory effect of UV-B radiation on photosystem II catalyzed electron transport: Since UV-B radiation inhibited the whole chain electron transport, to find out whether the alterations are due to changes in PS II or PS I, an attempt has been made to study the UV-B effect on PS II catalyzed p-benzoquinone (pBQ) supported Hill reaction (Tabel 3). pBQ is known to accept the electrons from PQ pool[18,19]. Being lipophilic in nature pBQ easily enters into thylakoid membrane and reaches PQ pool. Control thylakoids exhibited a rate of oxygen evolution activity (262 μ moles of $O_2\uparrow$ mg^{-1} Chl h^{-1}). UV-B treatment caused gradual increase in the inhibitory pattern and maximum loss was observed after giving the treatment with 80 μ moles of UV-B radiation. 50% loss was noticed at 40 μ moles of UV-B treatment. The reason for the loss of PS II catalyzed electron transport could be either due to alterations at water oxidation complex or due to changes in D_1 and D_2 polypeptides or due to alteration at the level of reducing side of PS II[3,4].

Effect of UV-B radiation on PS I catalyzed electron transport: Artificial donor like ascorbate + DCPIP is known to donate electrons near Cyt b_6/f during the measurement of PS I catalyzed electron transport. To examine the susceptibility of PS I to UV-B radiation, the PS I catalyzed electron transport was studied by using reduced DCPIP as donor system and MV as an electron acceptor (Table 4). Control thylakoid membranes exhibited a high rate of oxygen consumption in PS I catalyzed electron transport (375 μ moles $O_2\downarrow$ mg^{-1} Chl h^{-1}). The increase in the UV-B radiation treatment from 20-80 μ moles caused the increase of inhibition from 6% to 20%. At the end of 80 μ moles UV-B treatment only 20% inhibition was noticed in PS I

catalyzed electron transport. The reason for the loss of marginal inhibition in PS I catalyzed electron transport might be due to alteration either at the level of P_{700} or alteration at reducing side of PS I catalyzed electron transport.

UV-B radiation induced alterations in the intersystem catalyzed electron transport of barley thylakoids: To rule out the possibility of the presence of target site in the intersystem catalyzed electron transport, the PS I catalyzed electron transport activity was measured by using reduced duroquinone as electron donor and methyl viologen as electron acceptor. Reduced duroquinone is known to donate the electrons near PQ[18]. In control thylakoid membranes the activity of intersystem catalyzed electron transport was equal to 136 μ moles of oxygen-consumed mg^{-1} Chl h^{-1} (Table 5). UV-B radiation treatment caused gradual enhancement in the inhibitory pattern. 42% loss was noticed after the treatment of leaves with of 80 μ moles of UV-B radiation. This clearly shows that PQ is partially susceptible to UV-B radiation and causes inhibition in the PS I mediated electron transport.

Characterization of possible inhibitory sites in PS II catalyzed electron transport under UV-B radiation stress: To examine whether the inhibition induced by UV-B radiation is linked to the alterations in the energy transfer or not, we have measured UV-B (40 μ moles) induced inhibition at different light intensities (Table 6). For this study samples were initially given 50 μ moles of UV-B treatment and then electron transport activities of PS II were measured at different illuminated light intensities. The inhibition at light saturating conditions (4000 μ moles) was more that than at light limiting conditions 150 μ moles photon m^{-2} s^{-1}). Variation in light intensity was done by using neutral density filters. The difference of inhibition induced by UV-B radiation between light limiting and light saturating conditions was 13%. The possible reason for the increase of the extent of inhibition under light saturating conditions suggests that, there may be an additional site inhibition of PS II, in addition to the alterations of light harvesting complex. Similar observations have been made by Rajagopal[20] in the thylakoids of *Spirulina platensis*. Thus, UV-B exerts multiple effects on photosynthetic electron transport of barley thylakoids.

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