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ALTERED THYLAKOID MEMBRANE ORGANIZATION AND PHOTOCHEMICAL FUNCTIONS IN WHEAT THYLAKOID MEMBRANES UNDER THE INFLUENCE OF CADMIUM

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Electron transport measurements of thylakoid membranes isolated from Cd treated eight day old wheat primary leaves indicated that there is a concentration dependent loss of PS II activity and 50% inhibition was noticed at 0.2 mM $CdCl_2$. Chlorophyll fluorescence measurements demonstrated the existence of inhibitory site at water oxidation complex through the decrease of fluorescence intensity. Lipid Peroxidation measurements of wheat thylakoid membranes clearly gave an indication that free radical mediated lipid Peroxidation of thylakoid membranes is one of the reason for the loss of PS II activity.

Keywords: Cadmium stress; Electron transport; Lipid peroxidation; Spectral properties; Wheat leaves.

Abbreviations: pBQ; Para benzoquinone; Chl, chlorophyll; PS; Photosystem; MDA, Malondialdehyde.

Introduction

Cadmium is strongly phytotoxic and causes growth inhibition and even plant death by some studies using different plant species[1, 2] however, the mechanisms involved in Cd toxicity are still not completely understood. The plant growth inhibition induced by Cd was probably correlated to its reduced photosynthetic rate, detrimental effects on chloroplast function, cell division[3], chloroplast structure[4], as well as the water splitting apparatus of photosystem (PS) II and photosynthetic electron transports[5]. Chlorophyll fluorescence is an indicator which gives information about the absorption, distribution and utilization of energy in photosynthesis[6, 7]. Room temperature Chl a fluorescence is associated with the functioning of PS II. Any inhibition of electron flow to the PS II reaction center lowers the Chl fluorescence yield where as block at acceptor site of PS II increases fluorescence[8]. In addition to this, the over production and rapid accumulation of reactive oxygen species (ROS), which is considered as. one of the important mechanisms of heavy metal toxicity, are the early responses of plant to heavy metals[9,10]. Therefore, a comparative study has been made regarding the effect of Cd on PS II electron transport activity, Chl fluorescence and lipid peroxidation of thylakoids isolated from wheat primary leaves. Our results indicate that Cd exerts inhibition in PS II catalyzed electron transport activity by causing lipid peroxidation in thylakoid membranes.

Material and Methods

Wheat *(Triticum vulgare)* seedlings were rised on 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. Eight day old seedlings were exposed to different concentrations of $CdCl_2$ (O.l mM - 0.5 mM) for 24 hr After the treatment, primary leaves of both control and heavymetal treated seedling were sampled for thylakoid membranes isolation and for the assay of photochemical activities.

The thylakoids were used for measurement of photochemical activities by following the procedure of Sabat *et al.*[11] with slight modifications. The assay mixture for whole chain electron transport activity contained 0.5 mM MV (Methyl Viologen) and 1mM sodium azide in three ml of the 25 mM HEPES reaction buffer (pH 7.8). For PS II mediated oxygen evolution, the reaction mixture consisted of 0.5 mM pBQ in three ml reaction buffer. The fluorescence emission spectra was measured by exciting the thylakoid membranes with 440 nm light. The slit width for both excitation and emission was 5 nm. Samples were kept in dark for 5 min before measurement of the spectra. Lipid peroxidation has been measured according to the method of Heath and Packer[12]. The Malondialdehyde (MDA) calculations were made by using the extinction coefficient 155 mM^{-1} cm^{-1}. The amount of MDA was expressed as

Table 1. Effect of Cd on the whole chain electron transport activity of the thylakoids isolated from control and Cd treated wheat 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%.

Concentration of $CdCl_2$, mM	Whole chain electron transport activity $H_2O \rightarrow MV$ µ moles of $O_2\downarrow$ mg^{-1} Chl h^{-1}	Percentage loss
Control	195±19	0
0.1	141±13	28
0.2	102±9	48
0.3	65±7	67
0.4	45±4	77
0.5	35±3	82

Table 2. Effect of Cd on the photosystem II catalyzed electron transport activity of the thylakoids isolated from control and Cd treated wheat 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%.

Concentration of $CdCl_2$ mM	Whole chain electron transport activity $H_2O \rightarrow pBQ$ µ moles of $O_2\uparrow$ mg^{-1} Chl h^{-1}	Percentage loss
Control	271±27	0
0.1	200±19	26
0.2	127±10	53
0.3	91±8	66
0.4	58±6	79
0.5	48±5	82

Table 3. Effect of Cd on chlorophyll fluorescence emission properties of thylakoid membranes. Three ml of reaction mixture contains reaction buffer 25mM HEPES - NaOH (pH. 7.5) containing 20 mM NaCl and thylakoids equal to 8 µg of Chl a Slit width for both excitation and emission was 5 nm.

Concentration of $CdCl_2$, mM	Chlorophyll fluorescence (Rel. units)	Percentage loss
Control	72± 7	0
0.1	52±4	28
0.2	36±3	50
0.3	29±3	60
0.4	25±2	65
0.5	23±2	68

Table 4. Effect of Cd on lipid Peroxidation of thylakoid membranes of wheat plants.

Concentration of $CdCl_2$, mM	Lipid peroxidaiton n mol MDA mg $protein^{-1}$	Percentage enhancement
Control	42 ± 4.1	0
0.1	50±4.9	19
0.2	62±5.8	47
0.3	75±6.8	78
0.4	78±6.9	85
0.5	74±6.7	75

nmol of MDA per mg protein.

Results and Discussion

Cadmium induced alterations in whole chain Electron transport- Methyl Viologen (MV) is known to accept the electrons from A_0 in photosynthetic electron transport chain[13]. Therefore, whole chain electron transport activity has been measured in thylakoid membranes using MV as terminal electron acceptor ($H_2O \rightarrow MV$). Control thylakoids without heavymetal treatment exhibited a high rate of oxygen consumption (195 µ moles $O_2\downarrow$ mg^{-1} Chl h^{-1}). Increase in the Cd concentration from 0.1 mM to 0.5 mM brought enhancement in the inhibition of whole chain electron transport. Almost 50% loss was noticed above 0.2 mM of Cd treatment (Table-I). 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[13,15,5]. Thus, Cd stress induced inhibition of whole chain electron transport could be due to either alterations at PS II or PS I. To identity the target photosystem, we have measured the partial electron transport reactions mediated by individual photosystems.

Inhibitory effect of Cd on photosystem II catalyzed electron transport - Since Cd 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 Cd effect on PS II catalyzed p-benzoquinone (PBQ) supported Hill reaction (Tabel-2). pBQ is known to accept the electrons ftom PQ pool[13]. Being lipophilic in nature pBQ can easily enter into thylakoid membrane and reach PQ pool. Control thylakoids exhibited a rate of

oxygen evolution activity (271 μ moles of O_2 ↑mg^{-1} Chl h^{-1}) Cd treatment caused gradual increase in the inhibitory pattern and maximum loss was observed after giving the treatment with 0.5 mM of $CdCl_2$. 50% loss was noticed at 0.2 mM of $CdCl_2$. 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 site of PS II[15-17].

Identification of the target site for Cd action in photosystem II: Chlorophyll fluorescence is an indicator of photo system II photochemistry therefore an attempt has been made to identify the target site in PS II photochemistry using ch fluorescence as a tool (See Table 3). Control thylakoid membranes upon excitation with 440 nm light exhibited a single peak at 685 nm. The fluorescence intensity at this peak position was observed to be 65 rel. units. The increase in the concentration of Cd brought gradual decrease in the fluorescence intensity and 50% decrease was noticed at 0.2 mM concentration of Cd. The decrease in the chl fluorescence clearly indicate the existence of inhibitory site at the donor site of PS II as suggested by Butler[8]. Thus, the reason for the observed inhibition in PS II activity could be due to changes induced by Cd in the water oxidation complex. Since thylakoid membranes are the sites for PS II photochemistry, any change in the thylakoid lipid profile can also lead to the loss of PS II activity. To verify this, a study has been made to study the lipid peroxidation in control and treated samples.

Effect of Cd on the lipid Peroxidation of thylakoid membranes: Table 4 shows the lipid Peroxidation pattern of control and Cd treated thylakoid membranes. The increase in the Cd concentration from 0.1 to 0.4 mM caused gradual increase in the malondialdehyde (MDA) fractions by 85 %. Further increase in the concentration to 0.5 mM could not cause further enhancement in the lipid peroxidation. In this way, alterations in the thylakoid membrane lipids and changes in the water oxidation complex are together responsible for the altered photochemistry in PS II under Cd stress.

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