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Cadmium induced changes in the photosystem II photochemistry of the thylakoid membranes of wheat (*TRITICUM AESTIVUM* L.) primary leaves

DR. JALARI RAMU*

Associate Professor of Biochemistry, School of Medicine, WSU Teaching and Referral Hospital, Ethiopia.

CORRESPONDING AUTHOUR
***DR. JALARI RAMU**
Email: ramujalari@gmail.com
Mobile No: +91-9492633090

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ABSTRACT

The treatment of Cd $(NO_3)_2$ to wheat plants caused inhibition photosystem (PS) II catalyzed electron transport in a concentration dependent manner. Cadmium induced inhibition is light illumination dependent indicatory the target for cadmium (250 – 750 µM) action is light harvesting complex of PS II. Chlorophyll (Chl) *a* fluorescence measurement also supported the above preposition by exhibiting the increase in F_o fluorescence. The other possible reaction for the alteration in PS II catalyzed activity is increased lipid peroxidation in thylakoid membranes under cadmium stress. Thus, cadmium alters the PS II catalyzed electron transport by affecting to the light harvesting complex II and thylakoid lipid organization in wheat primary leaves.

Keywords: Cadmium, chlorophyll *a* fluorescence, Light harvesting complex, Lipid peroxidation, PAM kinetics, Photochemistry, Photosystem II

ABBREVIATIONS

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Chl *a* - Chlorophyll *a*, DPC – Di phenyl carbazide, F_o – Initial fluorescence, LHC – Light Harvesting Complex, F_m – Maximal fluorescence, MDA – Melone di amine, HEPES - N-2-Hydroxy ethyl piperazine–N– ethanesulfonic acid, pBQ – Para Benzoquinone, PS I – Photosystem I, PS II – Photosystem II, PQ – Plastoquinone, PD – Phynylene di imine, PAM – Pulse Amplitude Modulation, Q – Quinone, F_v – Variable fluorescence.

INTRODUCTION

Cadmium (Cd) is one of the most toxic metals which affect the several biological processes in living organisms. Heavy metals such as cadmium have received wide attention because of its excess released into the environment as a result of industrial pollution (Vymazal, 1995). It is recognized extremely significant environment pollutant due to its higher solubility in water and toxicity (Pinto *et al.,* 2004). Several workers have shown that PS II photochemistry is highly susceptible to cadmium (Bazazz *et al.,* 1974; Krupa *et al.,* 1993; Baszynsky, 1993; Seildeck and Krupa, 1996). The studies conducted by several workers indicated that Diphenyl carbazide (DPC) is able to restore the PS II catalyzed electron transport activity in Maze plants indication that water oxidation complex is target is its action (Bazazz and Govindjee, 1974). So, to establish the molecular mechanism of cadmium action in this investigation an attempt has been made to correlate the electron transport activity of PS II with chlorophyll *a* fluorescence in wheat primary leaves. Our results clear the indication that cadmium exerts its effect on Chl *a* fluorescence and lipid peroxidation I wheat primary leaves.

MATERIALS AND METHODS

Health seeds of wheat (*Triticum aestevum*) were obtained from N.G. Ranga Agricultural College, Tirupati. The seeds were surface sterilized with 0.1% $HgCl_2$ for 2

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min and thoroughly washed with tap water and then with distilled water. The seeds were imbibed for 6 h and the seedlings were raised on Petri dishes on whatman 1 filter paper for 3 days under continuous white light (≈ 16 Wm^{-2}) at 25°C and half strength Hoagland solution was supplied at 4-day intervals to the seedlings (Narendranath Mohanty *et al.,* 1987). The seedlings were randomly placed in plastic trays and watered daily with half strength Hoagland nutrient solution and grown in growth chamber providing with fluorescent light (produced by neon lamps, Philips T-40 W/55) with a light intensity of 30 – 35 μ moles m^{-2} s^{-1} at 25 ± 1°C. Seedlings were treated with different concentration of cadmium as $Cd (NO_3)_2$ (250, 500, 750 μM) after 4th day of germination. Plants were harvested after 3 days (7th day old) of heavy metal treatment were used to isolation of thylakoid membranes.

The chloroplast isolation and the polarographic measurement of the partial photochemical activities were done as described earlier (Sabat *et al.,* 1986) with slight modification. Chlorophyll was estimated before measuring photochemical activities by following Arnon (1949). The leaves were homogenized in 25 mM HEPES isolation buffer (pH 7.5) containing 400 mM sucrose 10 mM $MgCl_2$ and 5 mM KCl. The assay mixture for the measurement of PS II mediated oxygen evolution activity contained 0.3 mM FeCN and 0.2 mM phenylene di imine (PD). Thylakoid membranes were isolated according to Saha and Good (1970) as described in Swamy *et al.,* (1995) with some modifications. PS II catalyzed electron transport activity was measured as O_2 evolution in thylakoid membranes according to Mohanty *et al.,* (1989). PS II catalyzed electron transport activity was measured at different light intensity ranges from 13 - 410 μ moles irradiance. The fluorescence emission spectra were measured by exciting the thylakoid

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membranes with 440 nm light by following the procedure of Mohanty *et al*, (1989). The slit width for both excitation and emission was 5 nm. Samples were kept in dark for 5 min before measurement of the spectra. Chlorophyll *a* fluorescence induction kinetics was measured in PAM Chl fluoremeter which was developed by Schreiber (1986). The intensity of weak modulated light was 1 m wm^{-2} with a modulation frequency of 100 kHz and the intensity of red actinic light (>689 nm) was 60 m wm^{-2}. Cell suspension equivalent to 20 µg of Chl was used for kinetic measurements. Lipid peroxidation has been measured according to the method of Heath and Packer (1968). The malondialdehyde (MDA) calculations were made by using the extinction coefficient 155 mM^{-1} cm^{-1}. The amount of MDA was expressed as nmole MDA per mg protein.

RESULTS AND DISCUSSIONS

Cd effect on Photosystem II catalyzed electron transfer activity

To identify the target photosystem, a measurement of the partial electron transfer reaction mediated by individual photosystems was made. Hence an attempt has been made to study the effect of Cd on PS II catalyzed electron transport activity. The study was made to study effect on PS II catalyzed pBQ Hill reaction in the thylakoid membranes. pBQ is an artificial electron acceptor ($H_2O \rightarrow pBQ$) and it accepts electron from PQ pool (Trebst, 1974). Control thylakoid membranes exhibited a high rate of PS II dependent 312 µ moles of O_2 evolved mg^{-1} Chl h^{-1}, in the absence of Cd heavy metal. But in the presence of Cd at a concentration 250 µM there was about 45 % inhibition in the PS II catalyzed electron transport activity in treated samples. The increase in the concentration of Cd from 250 to 500 µM caused 69 % inhibition of PS II catalyzed

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electron transfer activity. But, at a high concentration of about 750 µM, Cd caused 82 % inhibition Hill activity (Table 1).

Effect of illuminated light intensity on Cd induced Potosystem II catalyzed electron transfer

To study the maximal alterations in photosynthetic electron transport in terms of spectral features, wheat plants were treated with Cd in a particular concentration. To study whether the inhibition by Cd on Hill activity is linked to the Cd induced alterations in energy transfer with in chlorophylls. Therefore, a measurement was made regarding the extent of inhibition caused by Cd toxicity at different intensities of light. For this study, concentration of Cd of about 250 µM was selected. 250 µM of Cd was able to cause nearer to 50 % inhibition in Hill activity ($H_2O \rightarrow pBQ$). Under the light limiting condition i.e. 13 µ moles irradiance of photons m^{-2} s^{-1} caused 40 % inhibition by Cd in the PS II catalyzed electron transport activity, whereas increase in the light intensities of about 120 µ moles and 230 µ moles irradiance caused 46 % and 47 % inhibition respectively under the presence of Cd at 250 µM concentration. The increase in the saturating intensity of light above 410 µ moles irradiance of photons $m^{-2}s^{-1}$ did not change the extent of inhibition significantly with 250 µM of Cd. But, with the higher intensity of about 410 µ moles irradiance photons m^{-2} s^{-2} the inhibition with 250 µM of Cd was around 48 % (Table 2).

Effect of Cd on room temperature chlorophyll fluorescence under presence and absence of DCMU

In control thylakoid membranes, the ratio of fluorescence intensity in the presence and absence of DCMU was 1.6 in control thylakoid membranes of wheat primary leaves.

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In the presence of Cd at a concentration of 250 µM brought to (1.4) decrease in the ratio of the Chl *a* fluorescence intensity under the absence and presence of DCMU. Whereas increase in the concentration of Cd to 500 µM brought the ratio to 1.2 and with the high concentration of Cd at 750 µM it was changed to 1.07 (Table 3). The metal toxicity caused the decrease in the ratio of Chl fluorescence from 1.6 to 1.07 by the Cd at room temperature. These studies indicate that there is an existence of another inhibitory site at reducing side of PS II near PQ. Therefore to identify the alterations in the LHC II, Chl fluorescence kinetic (PAM) measurements were made.

Effect of Cd on Chl *a* fluorescence kinetics in thylakoid membranes:

These studies indicate that there is an existence of another inhibitory site at reducing side of PS II near PQ. Therefore to identify the alterations in the LHC (Light Harvesting Complex) II, Chl fluorescence kinetic (PAM) measurements were made. The inhibition at the acceptor side of PS II caused by diuron abruptly raises the yield of variable fluorescence to the maximal level (Butler, 1977). However, during impairment of electron flow from donor side of PS II, the fluorescence yield remains at low level (Butler, 1977). In dark adapted thylakoid membranes Chl *a* fluorescence transient was observed upon illumination (Papageorgious, 1975).

The fluorescence emission increases from an initial level called, F_o, to a maximal level, F_m. This fluorescence rise from F_o to F_m is called variable fluorescence, F_v, because of its variable nature associated with redox reaction of PS II stable acceptor Q_A. A portion of absorbed light is lost and appears as fluorescence or initial fluorescence level, F_o (Mathis and Paillotin, 1981) (Fig 1). The true F_o can be observed at the onset of illumination when the Q_A is in fully oxidized state (dark adapted samples) or with a very

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weak modulated light (1m wm^{-2}) which is incapable of causing PS II photochemistry (Schreiber, 1986). After dark adaptation thylakoid membranes were excited with low modulated light to measure F_o followed by red actinic and strong additional white light to measure the F_m. The difference between F_m and F_o is F_v (Fig 1).

In control spectrum, weak modulated light caused a rise upon excitation which is nothing but F_o (2.0). Further illumination with strong light caused enhancement in the signal to F_v (4.5), the maximum fluorescence variable was, $F_{m,}$ 6.5 (Table 4). But in the Cd treated Chl *a* fluorescence kinetics of thylakoid membranes weak modulated light caused enhancement of F_o excitation to 2.2. Then illumination with strong light caused increase in F_v to 4.1, the fluorescence maximum value was 6.3, (F_m) under the presence of Cd at 250 µM concentration. When increase in the concentration of Cd to 500 µM caused increase in F_o (2.5), F_v value was 3.2 which leads to F_m value was about 5.7. Cd caused alterations in fluorescence kinetics of 750 µM treated sample brought F_o value to 3.2, F_v value to 1.8, the maximal value, $F_{m,}$ was 5.0. There was a significant change of values of fluorescence kinetics of Chl *a* as F_o value (2.0 to 3.2), F_v value (4.5 to 1.8) and F_m value (6.5 to 5.0). The above results indicate that the alterations by Cd may be due to the inhibition at the donor side, since the decrease in the F_v was observed. It clearly indicates that the loss in the F_v and F_m are responsible for the inhibition of the PS II activity. The increase in F_o indicates the damage at LHC II in PS II photochemistry (Campbell *et al.,* 1998). Thus, Cd is able to cause alterations in the PS II photochemistry i.e., electron transport activity under this toxic conditions. The alterations in PS II photochemistry is related to changes in water oxidation complex and PHC II of PS II.

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Thus, Cd exerts multiple effects on photosynthetic electron transport activities depending on its concentrations.

Characterization of effect of different concentrations of Cadmium on lipid peroxidation of thylakoid membranes

The heavy metal toxicity may cause membrane damage and changes lipid hyper fluidity, which alters lipid-protein interactions (Pali *et al.,* 2003). It is suggested that the membrane permeability increases during metal toxicity, which results in a decrease in the proton gradient formation across the thylakoid membrane and a suppression of the linear electron flow. The change in the membrane viscosity could be due to the variation of grana and stroma thylakoids ratio. The interaction between the xanthophylls molecules and the membrane lipids leads to a decrease in the membrane fluidity, an increase the membrane thermo stability and a lowered susceptibility to lipid peroxidation (Havaux, 1998).

Thylakoid membranes contain MGDG, DGDG, sulpholipids and phospholipids. These lipids are necessary for package of LHC units in the photosystems of thylakoid membranes (Kurisu *et al.,* 2003). Any stress factor has ability to influence the organization of lipids with different polypeptides of two photosystems. Therefore, an attempt has been made to verify whether the heavy metal toxicity mediated alterations of electron transport are related to the lipid changes or not. The lipid peroxidation has been measured in terms of MDA formation. For this purpose, the wheat plants were treated with selected heavy metal ions, Cd and Pb, to measure the lipid peroxidation.

In control thylakoid membranes, 41 µ moles of MDA in mg per protein was released without heavy metal treatment such as Cd and Pb. When the Cd was present at

250 μM concentration there was a 22 % enhancement of the MDA in mg per protein in the Cd treated thylakoid membranes of wheat primary leaves. The increase in the concentration of Cd to 500 μM caused 56 % enhancement of the MDA mg protein^{-1}, whereas 87 % enhancement of the MDA mg protein^{-1} was observed during the presence of Cd at a high concentration of about 750 μM in the Cd treated thylakoid membranes of wheat primary leaves (Table 5).

STATISTICAL ANALYSIS

All the data are represented as the mean ± SE of four replications. Students' "l" test was performed to identify the different concentration at which the mean for Cd different from the mean of the corresponding control. Values were considered significant at (<0.05).

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Table 1: Effect of Cd $(NO_3)_2$ on PS II [μ moles of O_2 evolved mg^{-1} Chl h^{-1}] catalyzed electron transport activities in wheat primary leaves.

Parameter	Concentration (μM)	PS II electron transfer activity $H_2O \rightarrow$ pBQ, μ moles of O_2 evolved mg^{-1} Chl h^{-1}	Percent Inhibition
Control		312 ± 4	0
$Cd(NO_3)_2$	250	172 ± 8	45
	500	97 ± 9	69
	750	56 ± 3	82

Table 2: Effect of illuminated light intensity on Cd $(NO_3)_2$ induced PS II electron transfer activity in the wheat primary leaves.

Irradiance μM Photons m^{-2} s^{-1}	PS II catalyzed electron transport activity $H_2O \rightarrow$ pBQ μM of O_2evolved mg^{-1} Chl h^{-1} Control	$Cd(NO_3)_2$ treated(250μM)	Percent inhibition
13	55 ± 5	33 ± 3	40
120	130 ± 13	70 ± 6	46
230	180 ± 17	95 ± 10	47
410	300 ± 26	154 ± 14	48

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Table 3: Effect of Cd $(NO_3)_2$ on room temperature Chl *a* fluorescence in the presence and absence of DCMU.

Concentration $Cd(NO_3)_2$ (μM)	Fluorescence intensity (rel. units)		Ratio +/-
	F685 (- DCMU)	F685 (+ DCMU)	
Control	60 ± 3.1	100 ± 7.1	1.6
250	56 ± 4.1	79 ± 6.1	1.4
500	48 ± 3.1	58 ± 5.2	1.2
750	40 ± 2.3	43 ± 4.3	1.07

Table 4: Effect of Cd $(NO_3)_2$ on Chl *a* fluorescence kinetics of wheat thylakoid membranes. The samples were excited with very low light and then increased the light intensity after the initial fluorescence (F_o) is reached. Variable fluorescence (F_v) and maximum fluorescence (F_m) measurement were taken.

Concentration $Cd(NO_3)_2$ (μM)	Fluorescence parameter in terms of distance, cm		
	F_o	F_v	F_m
Control	2.0	4.5	6.5
250	2.2	4.1	6.3
500	2.5	3.2	5.7
750	3.2	1.8	5.0

Table 5: Effect of Cd $(NO_3)_2$ on lipid peroxidation of thylakoid membranes of wheat primary leaves.

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Concentration $Cd(NO_3)_2$ (μM)	Lipid peroxidation n mole MDA mg protien^{-1}	Percent enhancement
Control	41 ± 3.9	0
250	50 ± 4.8	22
500	64 ± 5.7	56
750	77 ± 6.9	87

Fig 1: Fluorescence kinetics of Chl *a* in control and Cd treated wheat primary leaves.