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SELECTED HEAVY METAL ION EFFECTS ON PHOTOSYNTHETIC ELECTRON TRANSPORT ACTIVITIES OF THE CYANOBACTERIUM *SPIRULINA PLATENSIS*

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ABSTRACT: In this investigation an attempt has been made to study the effect of selected heavy metals (Cd/Pb/Zn) on photosynthetic electron transport activities of the cyanobacterium *Spirulina platensis*. After incubation for 10 min in dark the heavy metal ions showed differential effects on various photochemical activities. Between two photosystems PS II seems to be more sensitive than that of PS I. Cd exhibits more inhibitory effects in all three photochemical activities than that of other two heavy metal ions. Light intensity measurements clearly demonstrated that alterations in light harvesting complex is mainly responsible for the observed inhibition in PS II activity in the cyanobacterium.
Key words: Electron transport activities, Heavy metal, Photosystem, *Spirulina platensis.*

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INTRODUCTION

Heavy metals inhibit photosynthetic electron transport at different target sites [1,2]. Majority of the studies are made in isolated systems. All these studies indicate that PS II catalyzed electron transport is more sensitive to heavy metals [3, 4,]. Several people showed that Zn and Cu induced inhibition is dependent on the illuminated light intensity. Compare to PS II, PS I catalyzed electron transport has been reported to be more resistant to the heavy metals [5, 6]. Cu and Pb are able to inhibit the PS I activity at much elevated concentrations. Hg is able to inhibit the PS I activity at multiple sites PCy [7], at the level of reaction center of PS I, P700 [8], Fd and FNR[3] and Fe-S centers. Most of the work has been made in higher plant chloroplast system. Very few studies have been made regarding the effect of heavy metals on cyanobacterial photosynthetic electron transport. Hence an attempt has been made to study the effect of heavy metal ions in a comparative manner on the photosynthetic electron transport activities in intact cells of *Spirulina platensis*. In this study the intact cells have been incubated for both short duration (10 min) and long term (12 h) in the presence of different concentrations of heavy metal ions. From the existing literature it is clear that, anion part of heavy metal (like Cl^-, NO_a^- , SO_4^-) is not having any influence on the inhibitory effect of heavy metal ions on electron transport activities. In our preliminary investigation we have studied the effect of Cl^- or SO_4 or NO_3^- salts of same metal ion and found the similar inhibitory pattern. In this study we have chosen three heavy metals, cadmium chloride ($CdCl_2$), lead ($Pb(No_3)_2$) and zinc sulphate ($ZnSO_4$) and studied their effect with different concentrations. Except copper all the heavy metals were incubated with cells for 10 min in the dark. Depending on the earlier reports of Cd, the incubation of cells was done in light in the case of Cd treatment.

MATERIALS AND METHODS

Spirulina platensis was grown axenically in the medium of Zarrouk's (1966) at 25 ± 2°C under continuous illumination (20 Wm^{-2}) [9]. Intact cells were harvested from mid log phase cultures by centrifuging at 9000 xg for 5 min, washed with the growth medium and suspended in it. The cells were incubated with or without 20 to 80 µM $CdCl_2$, Pb $(NO_3)_2$, $ZnSO_4$ for short duration (10 min). Whole chain electron transport was measured using 0.5 mM methyl viologen (MV) as terminal electron acceptor. Photochemical activity mediated by PS II was measured polarograghically with a Clark - type oxygen electrode. The reaction mixture for assaying the PS II catalyzed electron transport assay contained suspension buffer (25 mM HEPES buffer, pH. 7.5) and freshly prepared 0.5 mM pBQ. Electron transport activities were measured with Clarke – type oxygen electrode (Hanstech, UK). The reaction mixture of PS I contained above reaction buffer 2 mM ascorbate and DCPIP couple as donor and methyl viologen as acceptor. The electron transport assay were done under saturating light intensity (400 µ moles photon m^{-2} s^{-1} C according to Murthy *et al.,* (1989)[10]. By using neutral density filters the PS II catalyzed electron transport was measured at different light intensities (10 - 400 Wm^{-2}). Cells equivalent to 15µg of Chl was used for measuring electron transport assays.

RESULTS

Robinson *et al*. (1982) [11] showed that MV is having a free access to the thylakoid membranes in intact cells of *Spirulina*. Therefore whole chain electron transport activity has been measured in intact cells using MV as terminal acceptor ($H_2O \rightarrow$ MV). Control cells without heavy metals exhibited a high rate of consumption (260µmol oxygen consumed/mg Chl/h). Cd treatment caused 55% inhibition in electron transport activity at 60 µM concentration of $CdCl_2$. By increasing the concentration to 80 µM it brought 70% inhibition (see Table 1). The studies made by the earlier workers in higher plant chloroplasts also indicated that light reactions are more sensitive to Cd [11]. But in the case of Pb 80 µM concentration is required to bring the 48% inhibition in whole chain electron transport (see Table 2). Zn is able to inhibit the whole chain electron transport by 35% at high concentration of heavy metal (80µM) (see Table 3). The inhibition in Cd treated samples could be due to alterations at the level of PS II as reported by the earlier workers [12]. The partial inhibiton of whole chain electron transport activity in the case of Zn is due to its inhibitory effects of PS I reaction centers as reported earlier by Tripathy and Mohanty (1980) [13]. Pb induced inhibition in whole chain electron transport activity may be due to the alterations at the level of P700 as has been observed by Wong and Govindjee (1976) [14]. As heavy metals inhibited the whole chain electron transport, to find out whether the inhibition is due to alterations in PS II or PS I an attempt has been made to study the heavy metal ions effect on PS II catalyzed pBQ Hill reaction, pBQ accepts electrons from PQ pool [15]. pBQ is lipophilic in nature and easily enters into the intact cells of *Spirulina*. Control cells exhibited a high rate of oxygen evolution activity (392 µmol of oxygen evolved/mg Chl/h (see Table 1). In the presence of low concentrations of Cd (40 µM) 38% of inhibition was observed. The increase in the concentration to 80µM brought 61% of inhibition in pBQ supported Hill activity. In the case of Pb 30% inhibition in PS II catalyzed electron transport was observed only at 80 µM concentrations (see Table 2). The addition of Zn at high concentrations (80 µM) brought 32% inhibition in the Hill activity of intact cells (see Table 3). The inhibition in PS II catalyzed electron transport activity by Cd may be due to the alterations in the reaction center of PS II as suggested by Bazzaz and Govindjee, (1974a) [12] or at the level of Q_B protein. The inhibition observed with Pb and Zn at medium concentrations was not significant. The observed inhibition in the case of Cd could be due to altered spectral properties as suggested by earlier workers. Artificial electron donors of PS I could not enter readily into the intact cells of *Spirulina*. Therefore there is a need to go for the isolation of PS I rich thylakoid membranes to study the effect of heavy metal ions on PS I catalyzed electron transport activity. These thylakoid fragments could not exhibit any oxygen evolution with pBQ as an acceptor. Table 1 shows the effect of various concentrations of Cd on PS I catalyzed electron transport activity. Control thylakoid fragments exhibited the maximum activity of PS I (700 µmol of oxygen cosumed/mg Chl/h). The increase in the concentration to 60 µM brought 34% inhibition in PS I activity. Further increase of concentration to 80 µM could not influence the extent of inhibition. Pb treatment also showed similar inhibitory pattern as that of Cd (see Table 1and 2). Zn even at high concentrations (80 µM) is able to inhibit the PS I activity by 25% only (Table 3).To examine whether the inhibition induced by heavy metals (Cd, Pb and Zn) in Hill activity is linked to the alterations in the energy transfer or not we have measured the inhibition caused by heavy metals at different light intensities (see Table 4). For this study 60 µM concentrations of Cd have been chosen. The electron transport measurements of PS II indicate that the inhibition was more at light saturating conditions (400 µmole photons m^{-2} s^{-1}) than at light limiting conditions (10 Wm^{-2}) under Cd treatment.

We have varied the light intensity by using neutral density filters. These results indicated that the inhibition is PS II electron transport activity under light limiting conditions could be due to the alterations in the energy transfer to LHC II in PS II. The possible reason for the increase of the extent of the inhibition under light saturating conditions suggests that there may be an additional site near PS II reaction center in addition to the altered energy transfer. Thus among the tested heavy metals Cd seems to be more potent inhibitor of photosynthetic electron transport in comparision with others.

Table 1: Effect of various concentrations of $CdCl_2$ on whole chain, PS II and PS I electron transport activities in the cyanobacterium, *Spirulina platensis*. The values are average of three separate experiments and SD is not more than 10%

Cd (μM)	(H_2O→MV)(μ mol O_2 ↓ mg Chl-^{1} h^{-1})	% loss	(H_2O→pBQ)(μ mol O_2 ↑ mg Chl-^{1} h^{-1})	% loss	($DCPIPH_2$→ MV)(μ mol O_2 ↓ mg Chl-^{1} h^{-1})	% loss
0 (control)	260 ± 24	0	392 ± 37	0	700 ± 68	0
20	232 ± 20	11	360 ± 34	8	668 ± 63	5
40	157 ± 13	40	242 ± 22	38	545 ± 51	22
60	118 ± 9	55	173 ± 15	56	460 ± 43	34
80	79 ± 5	70	151 ± 12	61	420 ± 38	40

Table 2: Effect of various concentrations of Pb $(NO_3)_2$on whole chain, PS II and PS I electron transport activities in the cyanobacterium, *Spirulina platensis*. The values are average of three separate experiments and SD is not more than 10%

Pb (μM)	(H_2O→MV)(μ mol O_2 ↓ mg Chl-^{1} h^{-1})	% loss	(H_2O→pBQ)(μ mol O_2 ↑ mg Chl-^{1} h^{-1})	% loss	($DCPIPH_2$→ MV)(μ mol O_2 ↓ mg Chl-^{1} h^{-1})	% loss
0 (control)	260 ± 24	0	392 ± 37	0	700 ± 68	0
20	240 ± 22	8	372 ± 35	5	680 ± 65	3
40	196 ± 16	25	312 ± 28	20	600 ± 58	14
60	159 ± 13	39	290 ± 26	26	520 ± 49	26
80	136 ± 10	48	275 ± 24	30	457 ± 42	35

Table 3: Effect of various concentrations of $ZnSO_4$ on whole chain, PS II and PS I electron transport activities in the cyanobacterium, *Spirulina platensis*. The values are average of three separate experiments and SD is not more than 10%

Zn (μM)	(H_2O→MV)(μ mol O_2 ↓ mg Chl-^{1} h^{-1})	% loss	(H_2O→pBQ)(μ mol O_2 ↑ mg Chl-^{1} h^{-1})	% loss	($DCPIPH_2$→ MV)(μ mol O_2 ↓ mg Chl-^{1} h^{-1})	% loss
0 (control)	260 ± 24	0	392 ± 37	0	700 ± 68	0
20	248 ± 21	5	376 ± 35	4	686 ± 65	2
40	210 ± 18	20	350 ± 33	11	660 ± 62	6
60	186 ± 16	28	315 ± 27	20	619 ± 58	12
80	170 ± 14	35	268 ± 24	32	526 ± 49	25

Table 4: Effect of illuminated light intensity on Cd induced inhibition of PS II catalyzed electron transport activity.

Light intensity (Wm^{-2})	PS II catalyzed electron transport activity ($H_2O \rightarrow pBQ$)(mol O_2 evolved mg Chl^{-1} h^{-1})	
	Control	Cu (60 μ M)
10	36 ± 3	22 ± 2
100	99 ± 8	55 ± 5
200	198 ± 18	99 ± 9
400	390 ± 37	175 ± 15

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