BIOLOGIA PLANTARUM 37 (1): 79-84, 1995

Action of selected heavy metal ions on the photosystem 2 activity of the cyanobacterium *Spirulina platensis*

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Abstract

Addition of different concentrations of heavy metal ions (Hg^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+}) inhibited the photosystem 2 catalyzed electron transport activity ($H_2O \rightarrow$ *p*-benzoquinone) of the cyanobacterium *Spirulina platensis*. Hg^{2+} caused the inhibition in electron transport activity in very low concentrations compared to the other metal ions. Hg^{2+} at this low concentration specifically altered the spectral properties of phycocyanin of the phycobilisomes in the intact cells of *Spirulina*, whereas other heavy metal ions were ineffective in this sense.

Key words: copper, lead, mercury, nickel, phycocyanin

Introduction

Heavy metals like Zn, Cd, Cu, Co, Hg and Pb inhibit the photosynthetic electron transport at multiple sites (Clijsters and Van Assche 1985, Mohanty and Mohanty 1988, Murthy and Mohanty 1991). Majority of the observations of heavy metal ions effect on the partial electron transport activities have been made in isolated chloroplast systems (Pb^{2+}: Bazzaz and Govindjee 1974a, Cd^{2+}: Bazzaz and Govindjee 1974b, Tripathy and Mohanty 1981, Hg^{2+}: Katoh and Takamiya 1964, Honeycutt and Krogmann 1972, Samson and Popovic 1990, Cu^{2+}: Cedeno-Maldonado *et al.* 1972, Mohanty *et al.* 1989a, Ranganathan and Bose 1989, Lindon and Henriques 1993, Zn^{2+}: Tripathy and Mohanty 1980, Mohanty *et al.* 1989b). These studies indicate that photosystem (PS) 2 is more susceptible to heavy metal ions induced damage compared to that of PS1. Studies related to heavy metal ions effect on cyanobacterial electron transport and energy transfer properties are scanty. We have, therefore, made a comparative study of selected heavy metal ions (Hg^{2+},

Received 12 May 1994, *accepted* 1 July 1994.
Abbreviations: Chl - chlorophyll; ETC - el.ectron transport chain; MV - methylviologen; PBQ - *p*-benzoquinone; PC - phycocyanin; PS - photosystem.
Acknowledgements: Supported by grant No. J. 12017/70/82ENI from Department of Environment, Government of India, to Prasanna Mohanty.

Cu^{2+}, Ni^{2+} and Pb^{2+}) effects on the photochemical activities and spectral properties in the intact cells of the cyanobacterium *Spirulina platensis*. Our results indicate that among the selected metal ions, mercury is a potent inhibitor of energy transfer and electron transport in this cyanobacterium.

Materials and methods

Spirulina platensis was grown axenically in the medium of Zarrouk (1966) at 25 ± 2 °C under continuous irradiation (20 W m^{-2}). Intact cells were harvested from the late log grown cultures by centrifuging at 9000 *g* for 5 min, washed twice with the 20 mM HEPES-NaOH buffer (pH 7.5) that contained 20 mM NaCl, and centrifuged as above. The resulting pellet was suspended in the same buffer. Photochemical activities were measured polarographically with a Clark-type oxygen electrode. The reaction mixture for asssaying the whole-chain electron transport contained in addition to suspension buffer, 0.5 mM methyl viologen (MV) and 1 mM sodium azide. The assay mixture of the PS2 catalyzed electron transport contained suspension buffer and a freshly prepared solution of 0.5 mM *p*-benzoquinone (PBQ). Cells equivalent to 15 μg chlorophyll (Chl) was used in all electron transport assays. The concentration of Chl was determined by the method of MacKinney (1941). Cells were incubated with or without heavy metal for 5 min in dark except Cu, which needed incubation in light for its action. The measurements were carried out at 25 °C under saturating irradiation by "white light" (≅ 480 W m^{-2}).

The absorption spectra of a suspension of intact cells with and without heavy metal were made using a *Shimadzu UV-3000* double beam spectrophotometer. Fluorescence emitted by whole cells was measured at room temperature with the excitation at 545 nm in a *Perkin-Elmer LS-5* spectrofluorimeter. Emission spectra were not corrected for the spectral sensitivity of the photomultiplier. Cells equivalent to 5 μg Chl were used in spectral measurements.

Results and discussion

The addition of mercury (6 μM) to the intact cells caused a 48 % inhibition of whole ETC activity. The increase in the concentration to 15 μM brought approximately 71 % loss in the activity (Table 1). In the case of Cu^{2+} and Ni^{2+}, higher concentrations (18 μM) were required to bring partial (26 %) inhibition. At the same concentration, Pb^{2+} was able to inhibit the whole ETC by 35 %. The significant inhibition of whole ETC activity by Hg^{2+} could be due to the inactivation of plastocyanin (Katoh and Takamiya 1964) in the intersystem ETC as has been suggested earlier for chloroplasts. Pb^{2+} induced loss in the whole ETC activity may be due to the alteration at the level of P700 as has been observed by Wong and Govindjee (1976). The possible reasons for the partial inhibition in the ETC activity by Cu^{2+} and Ni^{2+} might be due to the impairement at the level of PS2 (Cu^{2+}:

Cedono-Maldonado *et al.* 1972, Shioi *et al.* 1978, Mohanty *et al.* 1989a, Ranganathan and Bose 1989, Mohanty *et al.* 1989b).

Table 1. Effect of various concentrations of heavy metal ions on (*1*) whole chain electron transport (ETC) assay ($H_2O \rightarrow MV$) [mmol(O_2 consumed) kg^{-1}(Chl) s^{-1}] and (*2*) photosystem 2 activity ($H_2O \rightarrow PBQ$) [mmol(O_2 produced) kg^{-1}(Chl) s^{-1}]. Three cm^3 of reaction mixture contained cells equivalent to 15 μg Chl, reaction buffer (25 mM Hepes-NaOH, pH 7.5 containing 20 mM NaCl) and (*1*) 0.5 mM MV and 1 mM Na-azide, or (*2*) 0.5 mM PBQ. Cells were incubated in the presence and absence of heavy metal ions for 5 min in dark except with Cu^{2+}. The incubation of cells with Cu^{2+} was done in the light. The values are averages of 3 separate experiments.

Heavy metal salt	Concentration [μM]	Whole ETC	Inhibition [%]	Concentration [μM]	PS2	Inhibition [%]
Control	0	79.1 ± 4.2	0	0	112.5 ± 5.8	0
$HgCl_2$	6	41.7 ± 2.8	48	6	94.4 ± 6.9	16
	15	23.1 ± 2.2	71	18	53.9 ± 4.2	52
$CuCl_2$	6	75.0 ± 3.9	5	6	104.2 ± 4.7	7
	18	58.3 ± 3.1.	26	18	56.5 ± 4.3	50
$Pb(NO_3)_2$	6	62.8 ± 2.8	21	18	105.6 ± 4.2	6
	18	45.0 ± 2.2	35	36	95.0 ± 6.4	16
$NiSO_4$	6	73.9 ± 4.2	7	18	108.6 ± 5.3	4
	18	59.7 ± 3.3	25	36	106.9 ± 4.4	5

Since these heavy metals inhibited the whole ETC, we have investigated their effect on PS2 catalyzed PBQ supported Hill reaction (Table 1). 6 μM Hg^{2+} caused approximately 16 % loss in the PS2 activity, at 18 μM the loss was 52 %. Cu^{2+} at low concentrations (6 μM) showed only 7 % in the PS2 activity, while at 36 μM the loss was 50 %. Pb^{2+} and Ni^{2+} were unable to bring appreciable inhibition in the PS2 activity even at 18 μM. The inhibition in PS2 activity by Hg^{2+} could be most probably due to the alteration at the oxidising side of PS2 as has been shown by Honeycutt and Krogmann (1972) in a higher plant system and by Samson and Popovic (1990) in a green alga *Dunaliella*. The loss in the PS2 activity by Cu^{2+} may be due to the alterations in the reaction centre as suggested by Ranganathan and Bose (1989) or at the level of Q_B protein as reported by Mohanty *et al.* (1989a).

To examine whether the observed inhibition in PS2 activity by Hg^{2+} is linked to alterations in the pigment-protein complexes, we measured the spectral properties of phycobilisomes in this cyanobacterium. 15 μM Hg^{2+} caused a partial decrease in the PC absorption (Fig. 1A). At this or higher concentration, none of the other heavy metal ions (Cu^{2+}, Ni^{2+} and Pb^{2+}) affected PC absorption ratios of the pigment-protein complexes (Table 2). Since Hg^{2+} affected the PC absorption extensively, we have measured the room temperature PC fluorescence emission spectra of cells with or without heavy metal ions (Fig. 1B). In control cells, excited at 545 nm, an emission peak at 652 nm emanating from PC was prominent in the spectrum (*cf.* Fork

and Mohanty 1986). Incubation of cells with Hg^{2+} (9 μM) caused increase in the fluorescence intensity and induced a peak shift of 12 nm to a shorter wavelength. Further increase in the concentration of Hg^{2+} not only shifted the peak further, but also induced further enhancement in the fluorescence intensity (Table 2).

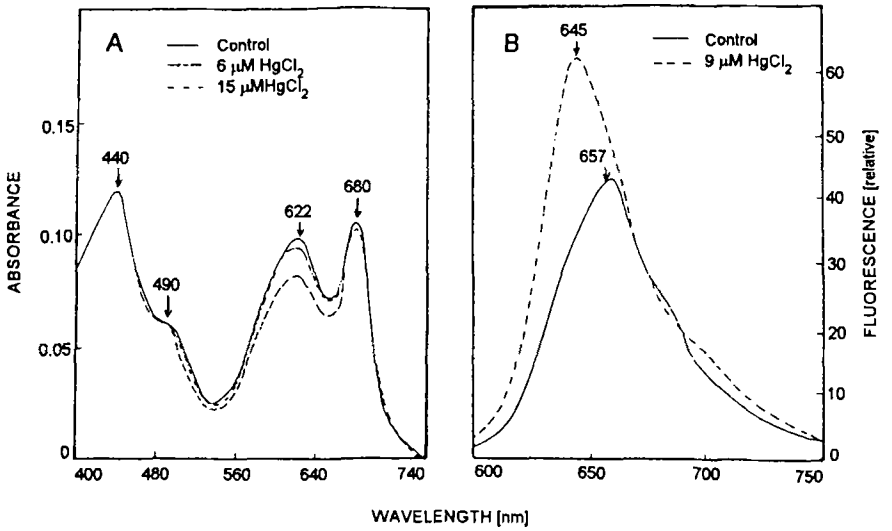

Fig. 1. Absorption (A) and fluorescence (B) spectra of phycobilisomes of *Spirulina platensis* as affected by $HgCl_2$ addition. Cells equivalent to 5 μg Chl were incubated for 5 min in the dark in the presence and absence of $HgCl_2$. For details see Materials and methods.

Table 2. Effect of various concentrations of heavy metal ions on absorption and fluorescence properties of intact cells of *Spirulina platensis* at room temperature. Cells were incubated in the presence and absence of heavy metals for 5 min in dark except Cu^{2+}. The incubation of cells with Cu^{2+} was done in light before measuring the spectra. Intact cells equivalent of 6 μg Chl were suspended in 3 cm^3 of reaction buffer for measuring spectral properties. Specific excitation of phycobilisomes was done by 545 nm (slit width 5 nm).

Heavy metal salt	Concentration [μM]	Absorption ratios			PC fluorescence emission		
		440/680	494/680	622/680	Relative intensity	Increase [%]	Peak position [nm]
Control	0	1.16 ± 0.08	0.58 ± 0.04	0.96 ± 0.07	42 ± 4	0	652
$HgCl_2$	6	1.14 ± 0.05	0.59 ± 0.05	0.93 ± 0.05	75 ± 5	79	638
	9	1.15 ± 0.04	0.58 ± 0.02	0.89 ± 0.04	82 ± 3	95	635
	15	1.17 ± 0.06	0.58 ± 0.03	0.80 ± 0.03	46 ± 4	10	635
$CuCl_2$	6	1.12 ± 0.07	0.59 ± 0.05	0.94 ± 0.06	41 ± 2	0	652
	18	1.14 ± 0.04	0.57 ± 0.05	0.93 ± 0.07	42 ± 3	0	651
$Pb(NO_3)_2$	18	1.15 ± 0.03	0.58 ± 0.06	0.96 ± 0.03	44 ± 4	5	652
	36	1.13 ± 0.05	0.56 ± 0.03	0.94 ± 0.04	41 ± 1	0	652
$NiSO_4$	18	1.14 ± 0.02	0.59 ± 0.02	0.95 ± 0.05	43 ± 3	0	652
	36	1.16 ± 0.04	0.57 ± 0.04	0.94 ± 0.03	42 ± 2	0	651

In *Scenedesmus quadricauda*, 1 μM $HgCl_2$ partially inhibited ETC between Q_A and Q_B, while 100 μM $HgCl_2$ affected mainly the donor side of PS2 (Prokowski 1993). Unlike Hg^{2+}, other heavy metal ions (Cu^{2+}, Ni^{2+} and Pb^{2+}) did not induce alterations in the PC emission properties even at high concentrations (Table 2). The increase in the emision in the presence of Hg^{2+} could be due to the uncoupling of energy transfer from PC to Chl *a*. Thus among the selected heavy metal ions only Hg^{2+} was able to bring inhibition of PS2 photochemistry by specifically affecting the pigment-protein(s) in the light-harvesting pigment-protein complexes of this cyanobacterium.

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