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Effect of Selected Heavy Metal Ions on the Photosynthetic Electron Transport and Energy Transfer in the Thylakoid Membrane of the Cyanobacterium, *Spirulina platensis*

[1]Nagendra G. Babu, [1]Phaninatha A. Sarma, [2,3]Idress H. Attitalla and [1]S.D.S. Murthy

[1]Department of Biochemistry, Sri Venkateswara University, Tirupati - 517 502, A.P., India
[2]Universiti Sains Malaysia, School of Biological Sciences, 11800, Pulau Pinang, Malaysia
[3]Omar Al-Mukhtar University, Faculty of Science, Botany Department, Box 919, Al-Bayda, Libya

Abstract: Addition of heavy metals (Cr and Ag) to intact cells of the cyanobacterium, *Spirulina platensis* caused alterations in the whole chain and photosystem II catalyzed electron transport activities preferentially in concentration dependent manner. The extent of inhibition of Hill activity was much higher under high intensity light than that under low intensity light. Heavy metals also suppressed the intensity of fluorescence emitted from phycocyanin at room temperature and induced blue shifts in the emission peak suggestive of changes in energy transfer within the phycobilisomes. Our results indicated that heavy metal ions depending on the concentration affect the electron transport at multiple sites and alter the energy transfer.

Key words: Cyanobacteria · *Spirulina* · Heavy metal stress · Electron transport · Energy transfer

INTRODUCTION

Heavy metals are phytotoxic and plants can easily assimilate heavy metals like Zn, Cd, Ag, Cr, Li [1-3]. These heavy metals are known to interfere with a variety of ways in the photosynthetic electron transport at multiple sites [4-8]. Majority of the observations of heavy metal ions effect on the partial electron transport activities have been made in isolated chloroplast systems (Ag: [9], Pb and Hg: [10, 11]; Cr: [12, 13]). These studies indicate that photosystem (PS) II is more susceptible to heavy metal ions induced damage compared to that of PS I. But these can inhibit the PS I activity at much higher concentrations [14, 15]. Studies related to heavy metal ions effect on cyanobacterial system and energy transfer properties are limited. Effluents form photography industry and leather industries are responsible for the accumulation of Ag and Cr respectively. These two metal ions in excess affect the primary process of the photosynthesis in aquatic organisms. Therefore an attempt has been made for comparison to study the effect of selected heavy metal ions (Ag, Cr) on the photochemical activities and spectral properties in the photochemical activities in *Spirulina platensis*. Our results indicate that among the selected metal ions, silver is a potent inhibitor of energy transfer and electron transport in this cyanobacterium.

MATERIALS AND METHODS

Spirulina plantensis is a non nitrogen fixing cyanobacterium, grown auto-tropically in the medium of Zarrouk [16] at 25± 2 °C under continuous illumination (20 Wm^{-2}). Intact cells of log phase cells were harvested by centrifugation at 9000 xg for 5min and washed twice with the 20mM HEPES-NaOH buffer (pH 7.5) contains 20 mM NaCl. It is centrifuged as above and the pellet was collected and suspended in the same buffer. Photochemical activities were measured polorographically with Clark type oxygen electrode. The reaction mixture suitable for assaying 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 PS II catalyzed electron transport contained suspension buffer and freshly prepared solution of 0.5 mM p-benzo quinone (pBQ). Cells equivalent to 15 µg chlorophyll (Chl) was used in all electron transport assays. The concentration of Chl was determines by the method of MacKinney [17]. Cells were incubated with or without heavy metal (Ag, Cr) and measurements were carried out at 25°C under saturatory irradiation by white light (≃ 480 Wm^{-2}).

The absorption spectra of a suspension of intact cells with and without heavy metal were made by using a Shimadzu UV-3000 double beam spectrophotometer

Corresponding Author: Dr. Nagendra G. Babu, Department of Biochemistry,
Dr. A.E.R. Educational Institutions, 160, Varadaraja Nagar, Tirupati-517507., A.P., India.

(Tokyo, Japan). Fluorescence emitted by whole cells was measured at room temperature with the excitation at 545 nm in a Perkin- Elmer Ls-5 spectrofluorimeter (Massachusetts, USA).

RESULTS AND DISCUSSION

The addition of Cr showed a concentration dependent inhibition in whole chain electron transport (WCE) activity after 48 h. 25 µm of Cr caused 17% inhibition in whole chain electron transport activity. Further increase in the concentration to 50 and 100 µm brought 49% and 62% inhibition respectively. In the case of Ag, 51% inhibition was noticed at 10 µm of Ag concentration which further rises at 15 µM Ag concentration causing 66 % inhibition (Table 1). The reason for the loss of whole chain electron transport activity could be due to either decreasing level of PSII catalyzed reaction center or that of level of LHC or both. To confirm the target, the effect of Ag and Cr on pBQ support PS II activity was measured. The PSII activity declined with increasing concentration of Cr and Ag. An 46% inhibited was noticed at 50 µM of Cr and 49% inhibition was noticed at 10 µM of Ag (Table 1). The time dependent effect was studied for these two metals by selecting 50 µM Cr and 10 µM of Ag concentrations. Following 24h and 48h incubation times, 46% and 48% inhibition of PSII activity was obtained (data not shown). The reason for the loss of PSII catalyzed electron transport could be either alterations at PS II reaction centre or at light harvesting complex (LHC) as suggested by Murthy [5] and Ranjani [7].

To find out the exact target site of heavy metals in PSII reaction center, PSII activity was measured at different light intensities. The PSII activity was measured at both light saturating and limiting conditions (Table 2). The difference of inhibition between light saturating and light limiting conditions in case of Cr was 13%. Similar results (12 % inhibition) are also observed in the case of Ag. These inhibitory effects of both metal ions under light limiting condition indicate alterations in LHC of PS II. This inhibition may be linked to alterations in the energy transfer systems [5].

The absorption and fluorescence emission of phycobiliproteins i.e., phycocyanin (PC) were drastically decreased with increasing concentrations of Cr and Ag, respectively. In case of Cr (25-100 µM) a 5 nm shift of peak in PC absorption was noticed. Similarly, Ag (5-15 µM), caused an 7 nm peak shift in PC absorption (Fig. 1 and 2). The decrease in PC absorption and alterations in peak position due to alteration in the apoprotein and chromophore interaction in PBPs.

Table 1: Effect of heavy metals (chromium and silver) on wholechian and photosystem II catalyzed electron transport activities. Other details were described in materials and methods

		Electron transport activity	
Heavy metal	Concentration of metal (µM)	WCE ($H_2O \rightarrow MV$)	PS II
Cr	0	253 ± 21 (0)	369 ± 28 (0)
	25	211 ± 20 (17)	325 ± 26 (12)
	50	128 ± 10 (49)	201 ± 24 (46)
	100	95 ± 8 (62)	143 ± 17 (61)
Ag	0	251 ± 20 (0)	376 ± 29 (0)
	5	203 ± 17 (19)	310 ± 27 (18)
	10	123 ± 11 (51)	191 ± 21 (49)
	15	85 ± 6 (66)	171 ± 18 (55)

Table 2: Effect of heavy metals (chromium and silver) on photosystem II catalyzed electron transport activities under different light intensity measurements. Other details were described in materials and methods

	PSII catalyzed electron transport activity (µ moles O_2 evolved mg Chl^{-1} h^{-1})			
Light intensity µmoles	Control	Cr treated (50 µM)	Control	Ag treated (10 µM)
105	43 ± 4	25 ± 3 (41)	43 ± 4	24 ± 2 (41)
1100	106 ± 11	57 ± 6 (46)	102 ± 12	56 ± 6 (45)
2050	185 ± 21	91 ± 10 (51)	190 ± 22	95 ± 9 (50)
3000	370 ± 35	170 ± 18 (54)	365 ± 34	172 ± 18 (53)

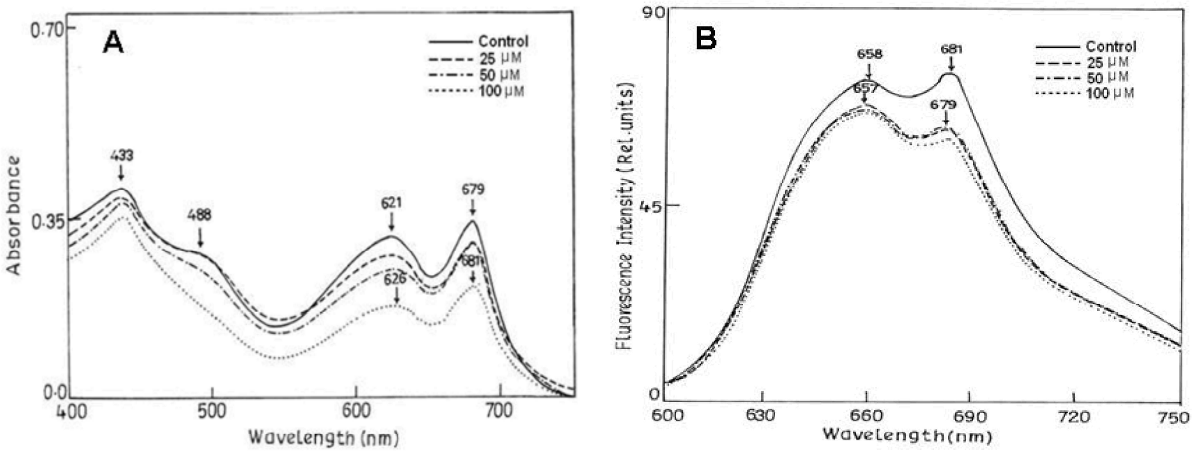

Fig. 1: Effect of chromium on spectral properties A) Absorption spectra B) fluorescence emission spectra of control and chromium treated intact cells of *Spirulina platensis*

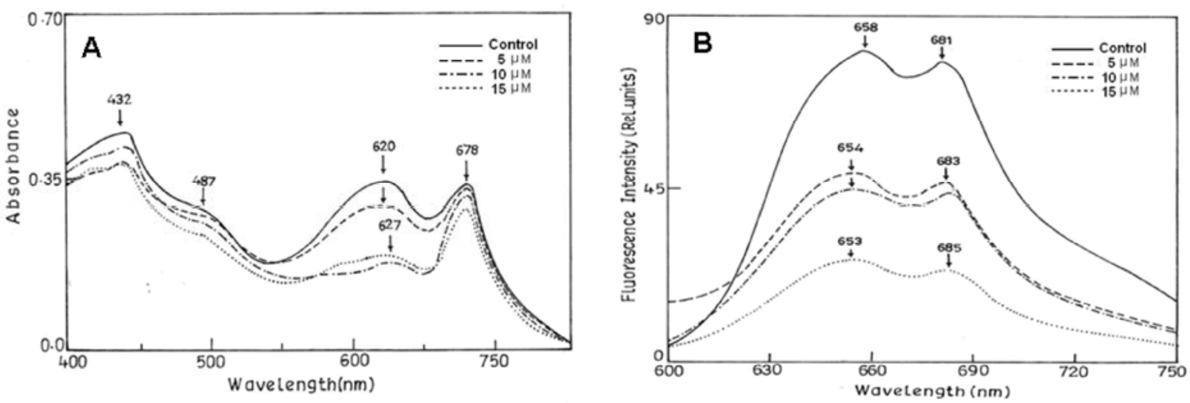

Fig. 2: Effect of silver on spectral properties A) Absorption spectra B) fluorescence emission spectra of control and silver treated intact cells of *Spirulina platensis*

In fluorescence emission, control cells excited with 545 nm exhibited two peaks at 658 nm and 681 nm which shows energy transfer from PC to Chl *a*. In the Cr treated cells the concentrations between 25-100 μM caused a drastic decrease in emission intensity of Chl rather PC (Fig.1) and 2 nm shift in Chl *a* and 1 nm shift in PC (Fig. 1B., 2 nm shift in Chl *a* and 1 nm shift in PC fluorescnce). In case of Ag (5-15 μM), maximum decrease in fluorescence intensity was noticed in the Chl *a* and PC. Furthermore, 15 μM of Ag causes 5 nm shift in PC towards blue region and 4 nm shift in Chl *a* towards red region.

This spectral alterations suggest changes in energy transport from PC to Chl *a*. Similar findings were obtained by Ranjani [7]. Thus depending on the concentration, heavy metals like Cr and Ag ions induce changes not only in absorption of light and energy transfer in PBSomes but also in photochemical activities close to PS II in *Spirulina platensis*.

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