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STUDIES ON THE ALTERATIONS IN PHOTOSYNTHETIC ELECTRON TRANSPORT AND SPECTRAL PROPERTIES OF THE CYANOBACTERIUM, *SPIRULINA PLATENSIS* UNDER COPPER STRESS

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ABSTRACT: *Spirulina platensis* is a filamentous and photosynthetic blue green algae. Heavy metals are toxic when their levels in the environment exceed. They cause damage to living organisms including plants. Prolonged incubation (48 h) of *Spirulina platensis* cells in the presence of copper (Cu) (20 and 80 µM) caused inhibition of Photosystem (PS) II catalyzed electron transport activity ($H_2O \rightarrow pBQ$). The loss observed by 50% in PS II catalysed electron transport at 60µM. At this concentration it also alters the absorption spectra of *Spirulina platensis*. The inhibition at PS II could be due to either alterations at the water oxidation complex or changes in light harvesting complex. Unlike PSII, PSI catalysed electron transport (DCPIPH→MV) is less sensitive to Cu treatment. This loss in PSI catalysed electron transport could be due to alterations in the PSI reaction centre. Thus Cu affects both PSII and PSI in preferential manner.
Key words: Absorption spectra, Copper, Cyanobacteria, electron transport, photosystem, *Spirulina platensis*.

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INTRODUCTION

Heavy metals are known to interfere with a variety of photosynthetic functions at multiple sites [1]. Majority of the observations related to heavy metal ions effect on partial electron transport activities have been made in isolated chloroplast system under short incubations (5-10 min). [2,3,4,5 & 6] Those studies indicate that mainly photosystern II (PS II) is more susceptible to heavy metal ions induced damage compared to that of photosystem I (PSI). Studies related to heavy metal ions effect on cyanobacterial system and energy transfer properties are limited. Copper in the form of cupric ions at more than 1µM has been showed to inhibit the photosynthetic functions in isolated chloroplasts of higher plants [7,8,9,10 and11] and in green algae [12]. Copper usually accumulates in the chloroplast and inhibits PS II catalyzed electron transport [7] and also it requires light for binding to chlorophyll protein and also to bring maximal inhibition in PS II catalyzed electron transport activity. Among two photosystems, PS II is more sensitive to Cu toxicity. Therefore an attempt has been made to study the effect of Cu on electron transport and energy transfer of *Spirulina*, by incubating the cells in the presence of 20 and 80 µM of $CuCl_2$ for long duration (48 h). Our results indicate that copper is a potent inhibitor of energy transfer and electron transport of PS II in this cyanobacterium. All the electron transport activities have been employed to assess the Cu toxicity in *Spirulina platensis.*

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}). 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 and 80 µM $CuCl_2$ for long duration (48 hr). 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. Cells equivalent to 15µg of Chl was used for measuring electron transport assays. Electron transport activities were measured with Clarke – type oxygen electrode (Hanstech, UK). 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). The assay mixture contained reaction buffer, freshly prepared 0.5 mM of MV and pBQ for whole chain electron transport and PS II activity respectively. The absorption spectra of a suspension of intact cells with or without heavy metal ions were made using Shirnadzu UV-190 Double beam spectrophotometer. Lipid peroxidation (LPO) has been measured according to the method of Heath and Packer (1968) under copper stress.

RESULTS AND DISCUSSION

In this investigation a study has been made to characterize the effect of heavy metal (Cu: 20-80µM) on intact cells of cyanobacterium, *Spirulina platensis* which is having economically importance. During the investigation, polorography has been used to analyse electron activity and spectrophotometry has been used to characterize spectral alterations in pigment proteins under the influence of heavy metal (Cu) stress. When Cu (60µM) is applied there is a 55% loss in the whole chain electron transport activity (Table 1). This shows the effect of Cu stress on whole chain electron transport. The measurement of Hill activity catalyses by PS-II clearly demonstrates that there is a concentration dependent inhibition of Cu on PS-II activity and 64% loss was noticed after treating with 60µM Cu. The reason for the loss of PS-II activity could be due to the effect of Cu at Q_B protein or reaction centre as suggested by earlier workers [5] (Table 2). So from these above studies it is clear that PS-II is the main target for Cu stress. To rule out the non susceptibility nature of PS-I catalyzed electron transport was measured by using reduced DCPIP as donor and MV as an acceptor. These measurements clearly demonstrate that Cu shows partial inhibition in PS-I catalyzed electron transport activity at 40 µM only 10% inhibition was noticed. To clearly identify the target pigment protein, the absorption spectral properties of *Spirulina* cells have been measured. After incubation 60 Cu µM caused the loss in the absorption capacity of cyanobacterium, *Spirulina platensis* (Fig 1) was observed. Since thylakoid membranes are the sites for the performance of PS-II photochemistry an attempt has been made to analyze the changes of thylakoid membrane lipids. For this purpose TBA and TCA reagents are to measure the MDA formation. The UV-B treatment caused an increase in the lipid peroxidation (Table 4). This lipid peroxidation extent has been enhanced in the presence Cu stress are able to specifically affect PS-II catalyzed electron transport most probably at the level of LHC-II or reducing side of PS-II or both. But when heavy metal stress was applied there was damage on PS-II photochemistry. In addition the heavy metal stress causes more lipid peroxidation in thylakoid membrane and effect the functional aspects of PS-II in cyanobacterium, *Spirulina platensis*.

Table 1: Effect of copper on Whole chain electron transport activity in cyanobacterium, *Spirulina platensis*

Concentration of $CuCl_2$ (µMoles)	**Whole chain electron transport activity$H_2O \rightarrow MV$µMoles of $O_2\downarrow$mg $chl^{-1}h^{-1}$**	**Percentage loss**
Control	186±19	0
20	143±10	23
40	102±8	45
60	85±7	55
80	62±4	67

Table 2: Effect of copper on PS-II catalyzed electron transport activity in cyanobacterium, *Spirulina platensis*

Concentration of $CuCl_2$ (µMoles)	PS-II activity $H_2O \rightarrow pBQ$ µMoles of $O_2\uparrow$ mg $chl^{-1}h^{-1}$	Percentage loss
Control	225±19	0
20	165±14	27
40	110±12	31
60	82±8	64
80	72±6	68

Table 3: Effect of Copper on PS-I catalyzed electron transport activity in cyanobacterium, *Spirulina platensis*

Concentration of $CuCl_2$ (µMoles)	PS-I activity $H_2O \rightarrow pBQ$ µMoles of $O_2\downarrow$ mg $chl^{-1}h^{-1}$	Percentage loss
Control	325±27	0
20	305±27	6
40	293±24	10
60	272±21	16
80	252±21	23

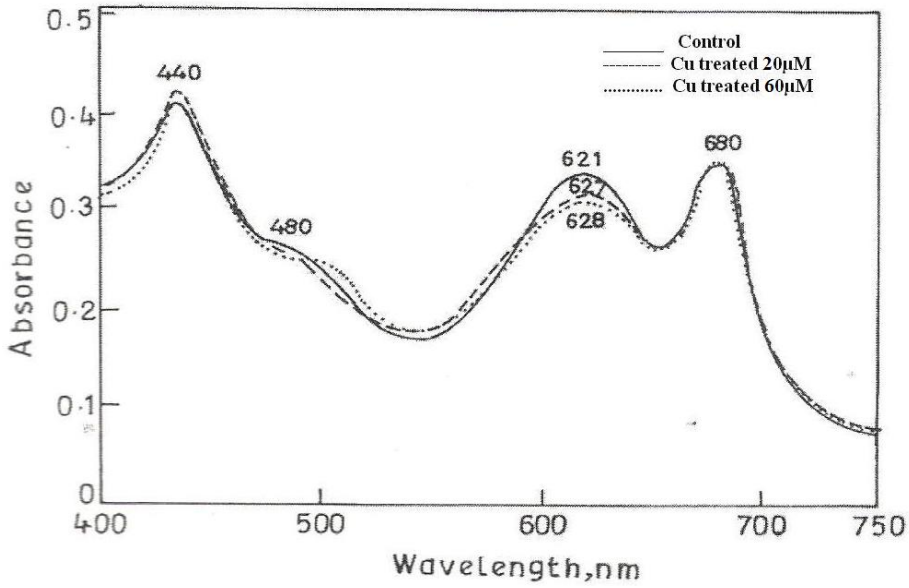

Fig: 1: Effect of copper on the absorption spectrum of intact cells of cyanobacterium, *Spirulina platensis*

Table 4: Effect of Copper on Lipid peroxidation in cyanobacterium, *Spirulina platensis*

Concentration of $CuCl_2$ (µMoles)	**Lipid peroxidation n moles of MDA/g. FW**	**Percentage enhancement**
Control	35±2.3	0
20	49±3.2	40
40	60±4.7	71
60	69±4.7	97

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