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STUDIES ON TOXIC EFFECT OF CHROMIUM AND SILVER HEAVY METALS ON PHOTOSYSTEM II IN CYANOBACTERIA

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ABSTRACT

In the present study, the chromium and silver ions have been used to identify the alterations in PBPs and analyzed their effect on the energy transfer process of PS II in the above cyanobacterium. By choosing the heavy metals Cr and Ag, attempts were made by incubating the cells and isolated pigment proteins for 24 and 72 h in light with different concentration revealed that the treatment of Cr showed a concentration dependent effect on whole chain electron transport activity and 49 per cent inhibition was noticed with 50 µM of Cr. Similarly Ag is able to cause 51 per cent inhibition with 10 µM concentration. Also, the exploitation of cyanobacteria as spectral probes, related to the Hill activity by exposing the cells to different illumination intensity of white light (105 to 300 µmoles) using neutral density filters suggest that the reason for the inhibition of PS II activity at low light intensity (105 µmoles) could be alterations at the light harvesting complex.

KEYWORDS:

INTRODUCTION

The cyanobacteria (Blue-green algae) are the most ancient, filamentous, photosynthetic bacteria that use water as electron donor in photosynthesis giving out oxygen. There are two important species, *Spirulina maxima* and *Spirulina platensis*. These are multicellular organisms, which are multiplied by binary fission. Depending upon the presence of chlorophyll a, botanists included it as micro algae in the class *Cyanophyceae*; but depending on its structural characteristics shown as a prokaryotic bacterium considered by the bacteriologist (Nagendra Babu *et al.,* 2008). *Spirulina platensis* has been commercially used in several countries as health foods, feed, bio-fertilizers and applications in biotechnology because of its valuable constituents such as proteins, vitamins, minerals, carbohydrates, lipids and polyunsaturated fatty acids. They have anti-cancer properties and immune promoting effects. *S. platensis* is an attractive source of various bioactive substances such as sterols function as antimicrobial agents, polysulfated polysaccharides as antiviral agents, phycobilliproteins and carotenoids as antioxidants, mycosporine-like amino acids (MAAs) and scytonemin as photoprotectants, polyunsaturated fatty acid (PUFA) as serum lipids levels reduction and HDL-cholesterol increasing, Gamma-linolenic acid (GLA) as rheumatoid arthritis, eczema, diabetes, multiple trauma, and premenstrual syndrome. Among the phycobiliproteins derived from *S. platensis*, the most abundant is phycocyanin (PC), a brilliant blue colour pigment have greater importance because of its various biological and pharmacological properties e.g. antioxidant, antiviral, anti-cancer, neuro-protective, hepatoprotective, antitumor, radical scavenging, radioprotection and anti-inflammatory properties (Li *et al*., 2005; Bhat and Madyastha, 2000; Ivanova *et al*., 2010 and Roamy *et al*., 1999). The present study was conducted to investigate to know the deep insights of Electron transport measurements, whole chain electron transport and photosystem II through standard assays.

MATERIAL AND METHODS

Microorganism and culture condition

The experimental organism *S. platensis,* the mother culture was obtained from National Facility for Blue Green Algal collection, New Delhi, India and cultured autotropically. The cells were transferred from the agar slants to liquid medium i.e. cultivated in Zarrouk's medium (1966). Experiments to evaluate the effect of different stress conditions were carried out in departmental laboratory. Conical flasks of 100 ml capacity were prepared containing 50 ml *S. platensis* culture with initial optical density 0.1 for all treatment groups. The cultures

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placed at west facing window receiving natural day light at temperature 30 ± 2°C and shaken gently thrice a day to avoid clumping and enhance the growth.

Growth measurements

Measurement of optical density (O.D.) is particularly suitable for determination of growth of *S. platensis*. The basic advantage of turbidity technique in growth rate measurements is the possibility of taking repeated readings on increase in turbidity of the same batch of the suspension of cells.

Extraction and estimation of photosynthetic pigments

Cultures were taken by thoroughly shaking the flask and cells are harvested by centrifuging at 9000 xg for 5 min. The pellets were washed twice with reaction buffer containing (25 mM HEPES-NaOH buffer (pH 7.5) containing 20 mM Nacl) and suspended in the same buffer.

Isolation of PBsomes

The PBsomes were isolated according to the method of Gantt *et al*. (1979) with slight modifications. Cells were grown up to mid-log phase in the medium and harvested by filtration on a whatsman no.1 filter paper using a Millipore filter assembly. Harvested cells were washed twice and suspended in 0.75 M K-phosphate buffer (pH 7.0) in the cell suspension 1 mM phenylmethylsulphonyl floride (PMSF), 2 mM EDTA and 1 mM sodium azide were incubated. The cells were disrupted by ultrasonification at amplitude of 15 microns in MSE ultrasonic disintegrator. The unbroken cells were removed by centrifugation a 6000 xg for 10 min by using Hitachi SCR2OBA (Japan). The obtained supernatant was incubated with 1.2 per cent Triton X-100 for 40 min at 20ºC. Then the PBsomes were separated from the thylakoids by centrifuging it at 30,000 xg for 30 min at 20ºC. The supernatant was then layered on a buffered sucrose density gradients (pH 7.0) containing 1 mM azide. The PB somes were concentrated in the 1.0 M region after spinning the gradients at 1,40,000 xg for 5 hrs at 20ºC in a preparative ultracentrifuge (L870M Beckman). The PBsomes were removed from the 1.0 M region as an intense blue band. Sucrose was removed from the isolated PSsomes by using dialysis with 0.75 K-PO4 (pH 7.0) and PBsomes were sued for both spectral and electrophoretic measurements.

Electron transport measurements

Electron transport activities were measured with Clark-type oxygen electrode (Hansatech, UK). The electron assembly consists of a platinum cathode and silver anode, they were saturated with KCL. An electrical measurement of oxygen is directly proportional to the current flow between the cathode and anode. These electron transport measurements were done under saturating light intensity (4000 µ mol photons $m^{-2}s^{-1}$) at 25ºC. The assay mixture (25 mM HEPS-NaoH (pH 7.5) 20 mM NaCl an intact cells equivalent to 12 to 15 mg Chl *a*) was continuously stirred during measurement of electron transport activity. For measurement of Electron transport activity at different light intensities, neutral density filters were used.

Whole chain electron transport assay

Whole chain electron transport assay (H_2O'! MV) was measured in intact cells in terms of O_2 consumption using MV as electron acceptor (Kok *et al*., 1965). The reaction mixture contained reaction buffer (25 mM HEPES-NaoH (pH 7.5), 20 mM NaCl, 0.5 mM MV, 1 mM sodium azide and the intact cells equivalent to 12 to 15 µg Chl *a*.

Photosystem II assay

Para benzoquinone (pBQ) was used to measure the PS II catalyzed electron transport (H_2O'! pBQ) in the intact cells. Being a lipophilic compound pBQ enters in to the intact cells and accepts electrons at PQ pool in electron transport chain (Warburg and LUthgens, 1944; Trebst, 1974). The reaction mixture contained reaction buffer (same as used in cell havesting), 0.5 mM freshly prepared pBQ and the intact cells equivalent to 12 to 15 µg Chl *a*.

RESULT AND DISCUSSION

Phycobilisomes are the light harvesting pigment protein complexes of PS II in the cyanobacterium *Spirulina platensis*. Any alterations in the spectral properties lead to the inhibition of PS II catalyzed electron transport activity. Therefore after creating the heavy metal ions, a comparative study has been made among electron transport activities by using various donors, acceptors and inhibitors. The artificial electron acceptor, MV which accepts electrons at the reducing side of PS II has free access to the thylakoid membrane, even in the case of intact cells of *Spirulina platensis* (Robinson *et al*., 1982).

Table 1. Effect of various concentrations of chromium and silver on whole chain electron transport assay in cyanobacterium *Spirulina platensis*

Concentration (µM)		whole chain electron transport activity (µ moles O_2 consumed mg ChI^{-1} h^{-1}) $H_2O \rightarrow MV$		Per cent Inhibition	
Chromium	**Silver**	**Chromium**	**Silver**	**Chromium**	**Silver**
Control	Control	253 ± 21	251 ± 20	0	0
25	5	211 ± 20	203 ± 17	17	19
50	10	128 ± 10	123 ± 11	49	51
100	15	95 ± 8	85 ± 6	62	66

Table 2. Effect of various concentrations of chromium and silver on photosystem II catalyzed electron transport activity ($H_2O \rightarrow MV$) in cyanobacterium

Concentration (µM)		PS II catalyzed electron transport activity $H_2O \rightarrow$ Pbq (µ moles O_2 consumed mg ChI^{-1} h^{-1})		Percentage loss	
Chromium	**Silver**	**Chromium**	**Silver**	**Chromium**	**Silver**
Control	Control	369 ± 28	376 ± 29	0	0
25	5	325 ± 26	310 ± 27	12	18
50	10	201 ± 24	191 ± 21	46	49
100	15	143 ± 17	171 ± 18	61	55

Table 3. Effect of chromium and silver on PS II catalyzed electron transport assay ($H_2O \rightarrow$ pBQ) in cyanobacterium *Spirulina platensis*

Concentration (µM)		Duration time (h)		PS II catalyzed electron transport activity $H_2O \rightarrow$ pBQ (µ moles O_2 consumed mg ChI^{-1} h^{-1})		Per cent inhibition	
Chromium	**Silver**	**Chromium**	**Silver**	**Chromium**	**Silver**	**Chromium**	**Silver**
Control	Control	0	0	385 ± 30	383 ± 31	0	0
50	10	24	12	301 ± 29	293 ± 28	22	24
		48	24	206 ± 24	201 ± 22	46	48
		72	36	125 ± 15	131 ± 11	67	65

Table 4. Effect of illuminated light intensity on chromium and silver induced inhibition of photosystem II catalyzed electron transport activity ($H_2O \rightarrow pBQ$)

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

Therefore the effect of selected heavy metal ions (Cr, Ag) on the whole chain electron transport activity (H_2O'! MV) in the presence and absence opf heavy metal ions was studied (Table 1). Control cells showed high rate of oxygen consumption involving whole chain electron transport activity (253 μ O2 consumed mg ChI^{-1} h^{-1}). The treatment of Cr showed a concentration dependent inhibition in whole chain electron transport activity. 25 μM of Cr caused 17 per cent inhibition in whole chain electron transport activity. Further increase in the concentration to 50 μM and 100 μM brought 49 per cent and 62 per cent inhibition respectively. Also, Table 1 shows concentration dependent effect of Ag on whole chain catalyzed electron transport activity. The 51 per cent inhibition was noticed with 10 μM of Ag and further rise in the concentration caused 66 per cent inhibition with 15 μM of Ag. From the data, it is clear that the reason for the inhibition of whole chain electron transport activity could be due to two possibilities, a) either the alteration at the level of PS II catalyzed reaction center or at the level of LHC b) both.

To verify the above preposition the partial electron transport reactions of PS II have measured by using pBQ as Hill acceptor. PBQ is an artificial electron acceptor which accepts electrons from PQ pool (Warburg and Luthgens, 1944; Trebst, 1974). pBQ is a lipophilic acceptor which enters easily into intact cells of *Spirulina* and reaches the PQ pool. Control cells exhibited a high rate of PS II dependent oxygen evolving catalyzed electron transport activity (369 μ mol of O_2 evolved mg^{-1} chI h^{-1}). The treatment of Cr as expected showed concentration dependent inhibition in PS II activity was noticed with 50 μM of Cr (Table 2). Further rise in the concentration to 100 μM induced 61 per cent loss in the PS II activity with 10 μM of Ag. Further rise to 15 μM brought 55 per cent loss in PS II activity (Table 2). To compare whether time dependent effect is associated with the above two mentioned heavy metals, a time dependent study was made by choosing the 50 μM of Cr and 10 μM of Ag. To achieve this, the cells were incubated at different intervals from 12 to 72 h and the PS II time dependent effect of Cr on PS II catalyzed electron transport activity. 46 per cent inhibition of PS II activity was noticed with 50 μM of Cr after 48 h of incubation. Further rise in the incubation period could bring 67 per cent inhibition in the Hill activity.

The data in the Table 3 shows the time dependent effect of Ag on pBQ mediated Hill reaction. In this case of Ag, 48 per cent of inhibition was noticed after 24 h of incubation unlike Cr. The loss in the PS II catalyzed electron transport activity under heavy metal stress could be due to three reasons: a) the alteration at the level of WOC b) changes at the PS II reaction center level or LHC or c) modification at the level of reducing side of PS II. Similar reports were made in the photosynthetic electron transport activity of PS II in the same cyanobacterium under heavy metal stress (Murthy, 1991; Ranjani, 2003). From the results it is quite clear that the PS II catalyzed electron transport is main target for heavy metal stress (Cr and Ag) in the cyanobacteirum *Spirulina platensis*. Since the main objective of this study is exploitation of the cyanobacteria as spectral probes, PS

II catalyzed electron transport activity at different light intensities was measured. For this study, a variable light intensity form 3000 µ moles to 105 µ moles were used with special filters, neutral density filters. These were helpful in reducing the light intensity as per the convenience. Form this study it was clear that the inhibition was more at light saturating conditions than that of light limiting conditions. The difference of inhibition between light saturating and light limiting conditions in case of Cr was 13 per cent. Similar results are also observed in the case of Ag stressed cyanobacterial cells. In the case of Ag the difference of inhibition between light saturating conditions and light limiting conditions was 12 per cent (Table 4). The main reason for the inhibition of PS II activity at light limiting conditions in both the cases was due to alterations in the LHC of PS II. In cyanobacteria, PBsomes can lead to the inhibition of its function i.e. PS II catalyzed electron transport activity.

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