VOL. 4, NO. 3, MAY 2009 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

MERCURY INDUCED ALTERATION IN THE PHYCOBILIPROTEINS OF CYANOBACTERIUM, *CYLINDROSPERMUM STAGNALE* (kütz.) ADAPTED UNDER DIFFERENT LIGHT

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ABSTRACT

Phycobilisomes extracted from the cyanobacterium, *Cylindrospermum stagnale* grown under different light conditions (white, red, blue and green) were subjected to graded concentration of mercuric chloride 6 µM, 9 µM and 12 µM. At all concentrations of $Hgcl_2$, partial inhibition in the absorption of the phycobiliproteins was observed, contrarily in 6 µM concentration of red-light, no influence in phycocyanin absorbance was occurred. In comparison phycocyanin and allophycocyanin inhibition was less as 26.2% and 35.7% at higher concentration (12 µM) in red light. Major effect was occurred even at low concentration (6 µM) in phycoerythrin and phycocyanin of blue light and green light. Whereas higher concentrations of $Hgcl_2$ produced dramatic changes at all other light conditions. Significantly inhibition in green light 56% and blue light allophycocyanin was 53.1%, likely 40.4% in white light phycocyanin. The differential percentage of $Hgcl_2$ inhibition towards the bilipigments at different light conditions shows their influence in the protein configuration of the phycobilisomes.

Keywords: c*ylindrospermum stagnales*, light, mercuric chloride, phycobiliproteins.

INTRODUCTION

In cyanobacteria the phycobiliproteins constitute the major light harvesting, which are attached to the outer surface of the thylakoid membrane in aggregated complex called the phycobilisomes [4,5]. A variety of environmental factors are known to affect the efficiency of energy transfer from PBSs to chlorophyll [17]. Heavy metals are known to interfere with a variety of photosynthetic functions [2]. Pioneer studies on the effect of mercurial compounds *p*-chloromercuribenzoate have been made by Pecci and Fujimori group [3,13] While, mercury has been shown to interrupt the flow of electrons at multiple sites, such as plastocyanin [9] and activity of enzymes such as ferredoxin-NADP-oxidoreductase [8]. Although mercuric chloride has been extensively used as an inhibitor of electron transport in chloroplasts [6]. Mercury induced inhibition of photosystem II activity and changes the fluorescence emission of phycobilisome in intact cells of the cyanobacterium *Spirulina platensis* [10] because; in cyanobacteria the phycobilisomes are the likely targets for the heavymetal toxicity. Selectively in *Spirulina platensis* Hg_2^+ affected the pigment protein, phycocyanin [11]. The effect of mercurial compound on the spectral properties of isolated phycoerythrin of *Hydrocoleum* genus has been studied by [14]. In this paper we report the effect of mercury ions on the phycobilisome extract from cultures grown under different filters of cyanobacterium *Cylindrospermum stagnales*, which is not yet studied.

MATERIALS AND METHODS

The cyanobacteria *Cylindrospermum stagnale* (Kütz) was obtained from the culture collection of Center for Advance Studies in Botany, University of Madras. The culture was grown in BG-ll medium amended with NO_3 (15) at 26±1ºC under illumination of 40 µEm-²s-¹ by cool white fluorescent tubes (Philips) in 12:8 h light dark regime. For different light quality studies, flask was covered with red, blue and green filters, (spectral transmittance as shown in Figure-1. The flasks were stirred daily to allow uniform light penetration and circulation of air and nutrients.

Log phase cultures were harvested and given cold acetone treatment with 80%acetone v/v over night to remove chlorophyll. After the removal of chlorophyll cells were washed twice with extraction buffer (phosphatebuffer-0.1M, pH 6.7) and subsequently disrupted using Lab Sonic 2000 sonicator with equal volume of extraction buffer. To separate the PBS from the membrane complex, disturbed cells was incubated in the presence of 2% Triton X-100 for 30 min in the dark by continuous stirring. The homogenized suspension was centrifuged at 12,096xg for 15 min Tandeau de Marsac [16]. 500 µl of the PBS extract was taken from all light conditions as control; for inhibition study, 50 µl of different concentrations of $HgCl_2$ (6 µM, 9 µM and 12 µM) was added to 450 µl of PBS (phycobilisomes) extracts and incubated in dark for 5min. The absorption spectrum was scanned from 400 nm to 750 nm using Beckmann DU40 spectrophotometer and percentage of $HgCl_2$ inhibition was calculated form the spectra.

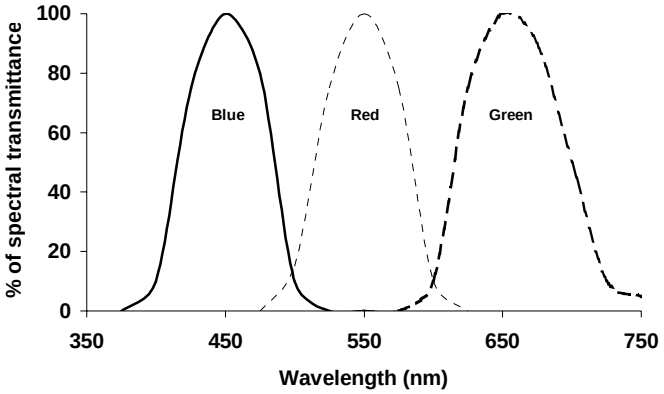

Figure-1. Spectral distribution of color filters.

RESULTS AND DISCUSSIONS

The PBS extracted of the cultures grown under white light and different filters (red, blue and green) have shown difference in their percentage of inhibition $Hgcl_2$ (6 µM -12 µM) in biliprotein PE (phycoerythrin), PC (phycocyanin), and APC (allophycocyanin) content.

Phycoerythrin

Although no distinct PE absorbance peak was found, nevertheless absorbance about 565 nm was focused in the spectra. In Figure-2, under white light condition the spectrum showed obvious changes in the percentage of inhibition at different concentration of mercuric chloride. A low concentration of 6 µM of Hg^+ caused a partial inhibition of 12.4%, nevertheless at higher concentration of 12 µM it was doubled to 25.5%. Likewise, in red light also the absorbance showed partial inhibition of 13.9% in 6 µM Hg^+ concentration, which was increased to 30.3% in 9 µM and finally it, reached 33.03% at 12 µM Figure-3. Whereas in blue filter, relatively high percentage of inhibition was observed at 6 µM (27.25%) and the trend remain almost similar as other higher concentration Figure-4. In green light beside gradual increase in inhibition at 6 and 9 µM, a sharp decrease in inhibition at 12 µM (16.6%) was notable, because of spectral rise and shift towards shorter wavelength Figure-5. Such spectral rise was also observed as other light conditions at higher percentage of inhibition.

Phycocyanin

The absorbance spectra show that *C. stagnales* is a PC producer; thus higher PC synthesis was found in red, blue and white light conditions. Under white light a major shift in the absorbance from 615 to 620 nm and a four-fold increase in inhibition at 12 µM concentrations (40%) could be noticed Figure-2. In red filter there was no inhibition in the absorbance at 6 µM, but a slight contraction in the broadness of the spectra from 540-610 nm was occurred Figure-3. But a huge rise in the inhibition was observed in 9 µM (22.6%) followed by 26.2% inhibition at 12 µM. In Figures, 4 and 5, PC of blue and green light showed higher inhibition from 6-12 µM, significantly the absorbance peak was shifted from 610 to 630 nm in the red region.

Allophycocyanin

Figures, 2-5 shows, the APC absorbance had a high percentage of inhibition at 12 µM Hg^+. In white light at 6 µM, inhibition was 6.7%, which was increased to 6 fold (37.8%) at 12 µM it showed a spectral peak shift from 665 to 668 nm (Figure-2). Under red light (Figure-3), very less inhibition was noticed at less concentration of ions, but it was increased to 4-fold at 12 µM concentration (35.7%) with a spectral shift as white light [11]. Similarly at higher concentration, as high as in blue light 53.12% and in green light 56% was observed, at this time the APC peak in the spectra was completely distorted Figures, 4 and 5.

Pigment synthesis was relatively high in red filter followed by blue, white and green light. PBSomes extracts treated with different concentration of mercury chloride exhibited dramatic changes in their spectral properties. In our study highest percentage of inhibition was observed in APC especially in green and blue light, may be due to changes in quality as well as its sensitivity to high concentration of Hg^+ Figures, 4 and 5. Contrarily [11] no allophycocyanin inhibition was observed at low concentration of Hg^+. In red light no PC inhibition was observed at 6 µM Hg^+, and a less inhibition was found at 12 µM Hg^+. Which shows the site of inhibition was dependent on the concentration of ion used [12] and the synthesis of phycobiliproteins to different light conditions [1,7]. Especially phycoerythrin and allophycocyanin under all conditions are highly affected; hence it is clear that in the phycobilisomes the terminal rods and photosyntem II (680nm) is more sensitive site to heavy metals. Further, differential effect of mercury towards biliprotein could possibly be due to the difference in their protein

conformation Murthy and Mohanty [11] and relative exposure of chromophore groups to the external environment. Perhaps less percentage of inhibition may be resistance of these biliprotein towards mercuric chloride. Our results demonstrated that phycobiliprotein analysis could be useful as a biochemical tool to assess change in light harvesting photosynthetic pigment of algae in response to this common environment pollutant.

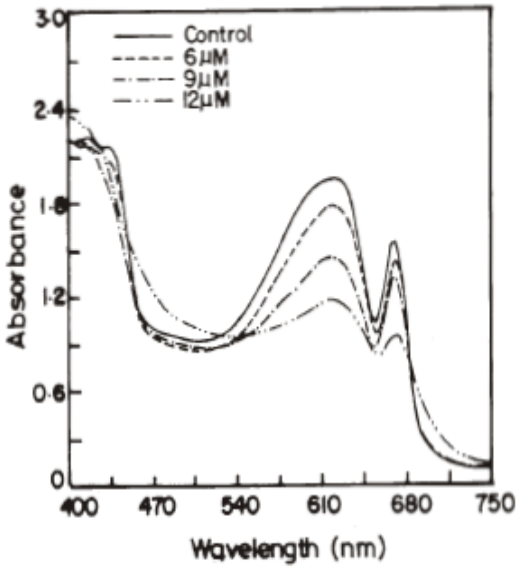

Fig. 2

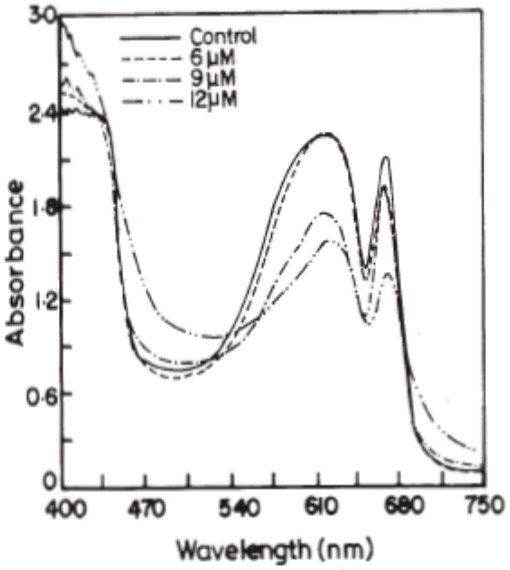

Fig. 3

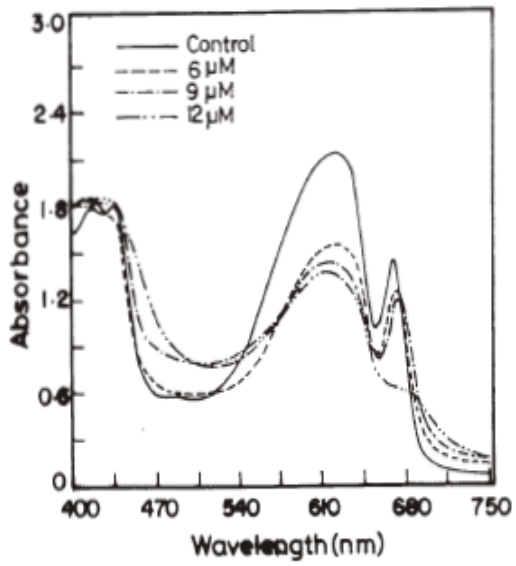

Fig. 4.

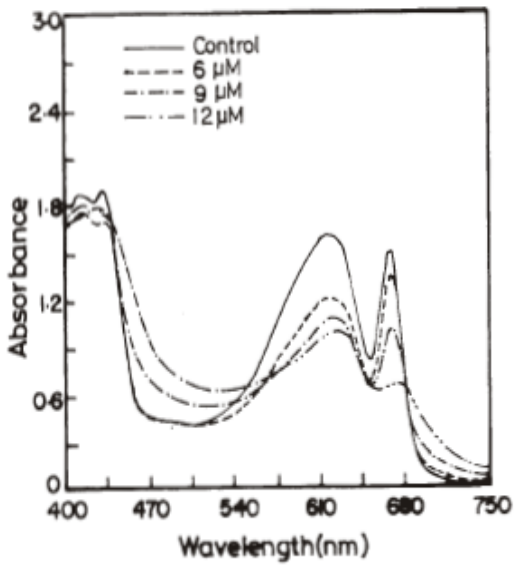

Fig. 5

Figures 2,3,4 and 5. Effect of mercury ions (6-12 µM) on the absorption of phycobilisome extracts adapted to different light conditions **2.** white **3.** red **4.** blue and **5.** green. PBS extract were incubated in $HgCl_2$ for 5 min in dark prior to spectral measurement.

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