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ALTERED ENERGY TRANSFER IN PHYCOBILISOMES OF THE CYANOBACTERIUM, *SPIRULINA PLATENSIS* UNDER THE INFLUENCE OF CHROMIUM (III)

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ABSTRACT

Phycobilisomes act as major light harvesting complex in photosystem II of cyanobacteria. In this investigation an attempt has been made to study the effect of chromium (Cr) in the energy transfer of phycobilisomes. Our results indicate that chromium (III) (50 and 100 µM) is able to cause alterations in absorption and energy transfer with intact cells at 100 µM concentration during short term incubation. The phycobilisomes isolated from chromium (50uM) treated cells also exhibited changes in absorption as well as fluoroscence emission properties in the red shift in the peak position. Thus chromium acts as an energy transfer inhibitor both under *in vitro* and *in vivo* conditions.

Key worlds : *Absorption, Allophycocyanin, Cyanobacteria, Fluorescence, Phycocyanin.*

INTRODUCTION

Phycobiliproteins (PBPs) are unique light harvesting pigment proteins present in cyanobacteria, red algae and cryptomonads, but not in higher plants. Unlike plant light harvesting chlorophyll (Chl) proteins, these PBPs are arrayed in subcellular structures which are known as phycobilisomes (PBSs). These structures allow the pigments to get arrayed geometrically in a manner which help to optimize the capture light and transfer of energy [1-4]. The major components of PBSs are the bilin containing proteins: Phycoerythryin (PE), Phycocyanin (PC) and Allophycocyanin (APC). The last two pigment protens PC and APC are present in cells of cyanobacteria and red algae [5 – 6], while PE is available component and its presence is resulted by the available quality of light [7-9].

The energy transfer in the PBSs (PE → Chl *a*) can be influenced by several environmental factors such as Hg [10], Cu [11]. Studies related to the effect of Cr (III) on energy transfer studies are scanty. Hence in this investigation an attempt has been made to study the effect of Cr both short term (10 min) and long term (12 h) using intact cells of *Spirulina platensis* as well as isolated PBSs.

MATERIALS AND METHODS:

Spirulina platensis trichomes were grown in Zarrouk's medium [12] at 25 ± 2° C under continuous illumination (15 Wm^{-2}). *In vivo* experiments were conducted by incubating the cells with Cr (100 µM) for 10 min under continuous stirring. For *in vitro* studies the cells were treated with Cr ions (50 µM) for 12 h and the PBSs have been isolated. The PBSs isolated were according to the method of Gantt *et al* [13] with slight modifications. The PBSs were recovered from the 1.0 M region as an intense blue band. Sucrose was removed from the isolated PBSs by using dialysis, with against 0.75 K. PO_4 (pH 7.0) buffer. PBSs and intact cells both were used for spectral measurements. The absorption spectra of intact cell suspension and PBSs were taken by using a Hitachi - 557 double beam, spectrophotometer as described by Murthy *et al* [14]. The emission spectral of cell samples and PBSs were measured by using Perkin-Elmer spectrofluoremeter [14]. Cells equivalent to 15 µg of Chl were used for spectral measurements, where as PBSs equivalent to 30 µg of protein was used for both absorption as well as fluorescence emission measurements.

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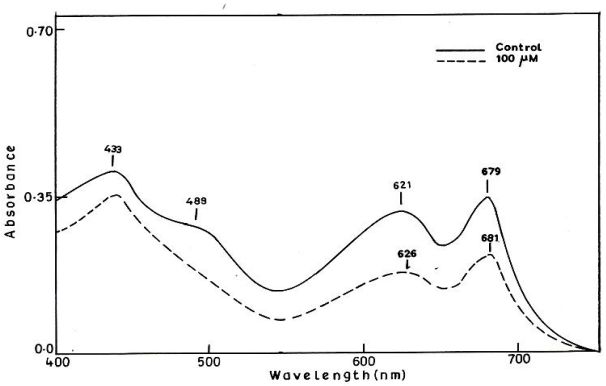

Fig. 1 Effect of 'Cr' ions on the absorption spectra of intact cells of *Spirulina platensis.*

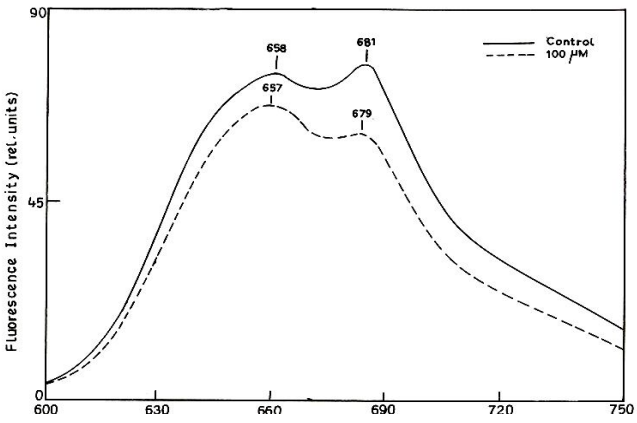

Fig. 2 Effect of 'Cr' on phycocyanin fluorescence emission spectra of the intact cells.

RESULTS AND DISCUSSION:

Initially after giving the Cr ions (100 µM) treatment for 10 min, the absorption characteristics of different pigment proteins present in the control cells of *Spirulina platensis* was measured. The peak at 433 nm is due to the soret band of Chl *a;* at 489nm the peak is due to carotenoids, at 621 nm is due to the absorption of PC and peak at 679 nm is due to the absorption of Chl *a* [15]. The

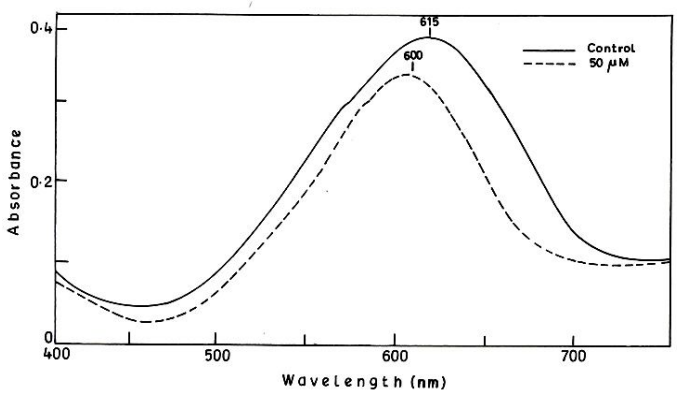

Fig. 3 Absorption spectra of isolated PBSs from control and 'Cr' treated cells.

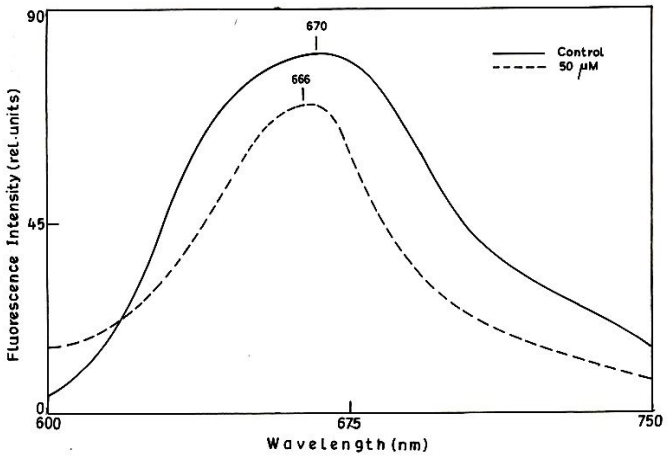

Fig. 4 Fluorescence emission spectra of isolated PBSs from control and 'Cr' treated cells.

treatment of intact cells of *Spirulina* with different concentrations chromium (100 µM) caused drastic decrease in phycocyanin absorption by marginally affecting the Chl *a* and carotenoid absorption. In addition there is a 5 nm red shift of PC indicating the structural alterations in PBSs regarding the chromophore attachment with apoprotein (Fig. 1). Similar observations were made by Murthy *et al* [14] in *Spirulina* under mercury stress. Since chromium affected the phycocyanin absorption quite extensively further studies were made by measuring room temperature phycocyanin fluorescence. The control cells excited with 545 nm light beam exhibited an emission peak at 658 nm which indicates that the energy is transferred from PC to Chl *a* [14; 16]. Figure 2 shows the phycocyanin fluorescence emission spectra of chromium treated *Spirulina* intact cells. With the treatment of Cr ions drastic decrease was noticed in the fluorescence emission intensities and with 100 µM of chromium ions almost 50 % of loss in the fluorescence intensity of phycocyanin was observed. It clearly demonstrates that selected heavy metal (Cr) induced alterations in the energy transfer from PC to Chl *a* by inducing the structural changes in the phycobiliproteins.

To correlate the results of *in vivo* experiments with *in vitro* experiments, phycobilisomes have been isolated from control as well as Cr (50 µM)treated cells (12 h) by using sucrose density gradient. After removal sucrose the spectral properties have been measured (Fig 3 and 4). The absorption spectra of PBSs exhibit a main peak at 615 nm. The PBSs isolated from 50 µM of Cr treated cells, caused a decrease in the absorption of PC by 40% and shifted the peak position from 615 nm to 600 nm. Since the absorption properties are related to the fluorescence emission of PC, PBSs samples which were isolated from chromium exposed (50 µM) *Spirulina* cells were used for the measurement of phycocyanin fluorescence emission. Chromium is able to cause 42% decrease in the fluorescence intensity and blue shift in the emission peak from 670 nm to 666 nm. The decrease in the fluorescence intensity indicates the change in the energy transfer and blue shift gives information about structural changes in the PBSs (Fig 4).

Similar reports were made by Murthy *et al* [14] during the toxic effect of mercuric chloride ($HgCl_2$) on the spectral properties of phycobiliproteins in the same organism. Thus chromium is able to cause alterations in the energy transfer from PC to Chl *a* in the *Spirulina* both under *in vivo* as well as *in vitro* conditions by inducing changes in PBSs.

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