The Bioscan 7(1) : 23-25, 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EXPLOITATION OF PHYCOBILIPROTEINS SPECTRAL PROPERTIES AS AN INDICATOR OF HEAVY METAL TOXICITY

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KEY WORDS
Absorption
Allophycocyanin
Cyanobacteria
Fluorescence
Phycocyanin

Received on :
08.11.2011

Accepted on :
19.12.2011

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ABSTRACT
Exposure of intact cells of *Spirulina* to silver ions (5-15 μM) caused decrease in the phycocyanin absorption and loss in the phycocyanin fluorescence emission indicating the altered energy transfer from phycocyanin to chlorophyll *a* under *in vivo* conditions. The *in vitro* measurements of spectral properties of phycobilisomes isolated from silver treated cells, clearly demonstrated that phycobilisome energy transfer is the major target for silver action. Thus phycocyanin is the major susceptible pigment protein for silver ion action which is evidenced from the decrease of fluorescence emission of phycocyanin.

INTRODUCTION

Excess use of fungicides and pesticides and release of industrial effluents is responsible for the accumulation of heavy metals in the aquatic environment. The increase in of heavy metal toxicity in water bodies' results in impairment of plant growth and loss of biomass production (Prasad, 2004). Therefore there is a need for the indicator protein to identify the presence of heavy metal toxicity. Cyanobacteria are photosynthetic prokaryotes which respond to the variety environmental stresses (Foy *et al.*, 1978; Clijsters and Van Assche, 1985; Murthy, 1991). These organisms contain specialized pigment protein called phycobiliproteins, as light harvesting complexes (Bryant and Stirewalt, 1990).They are phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC). Murthy *et al.* (1989) showed that the above pigment proteins are sensitive and exhibit spectral alterations in *Spirulina* under mercury stress. Therefore in this investigation an attempt has been made to analyze the effect of silver ions (5-15 μM) on spectral properties of phycobiliproteins in *Spirulina platensis* both and under *in vivo* and *in vitro* conditions.

MATERIALS AND METHODS

Spirulina platensis trichomes were grown in Zarrouk's medium (Zarrouk, 1966) at 25 $\pm$ 2ºC under continuous illumination (15 Wm^{-2}). *In vivo* experiments were conducted by incubating the cells with silver (Ag) (5-15 μM) for 10 min under continuous stirring. For *in vitro* studies the cells were treated with Ag ions (3 μM) for 12 h and the phycobilisomes (PBsomes) have been isolated. The PBsomes isolated were according to the method of Gantt *et al.* (1979) with slight modifications. The PBsomes were recovered from the 1.0 M region as an intense blue band. Sucrose was removed from the isolated PBsomes by using dialysis, with against 0.75 K. PO_4 (pH 7.0) PBsomes and intact cells both were used for spectral measurements. The absorption spectra of intact cell suspension and PBsomes were taken by using a Hitachi - 557 double beam, spectrophotometer as described by Murthy *et al.* (1989). The emission spectral of cell samples and PBsomes were measured by using Perkin-Elmer spectrofluoremeter. Cells equivalent to 15 μg of Chl were used for spectral measurements, where as PBsomes equivalent to 30 μg of protein was used for both absorption as well as fluorescence emission measurements.

RESULTS AND DISCUSSION

Initially after giving the silver ions (5-15 μg) treatment for 10 min, the absorption characteristics of different pigment proteins present in the control cells of *Spirulina platensis* was measured. The peak at 679 nm is due to the absorption of Chl *a*; at 621 nm is due to the absorption of PC and 480 nm and carotenoids is due to a peak at 438 nm is due to the soret band of Chl *a* (Fork and Mohanty, 1986). The treatment of intact cells of *Spirulina* with different concentrations silver (5-15 μg) caused drastic decrease in phycocyanin absorption by marginally affecting the Chl a and carotenoid absorption. In addition there is a 7 nm blue shift of PC indicating the structural alterations in PBsomes (Fig. 1). Similar observations were made by Murthy *et al.* (1989) in *Spirulina* under mercury stress. Since silver affected the phycocyanin absorption quite extensively further studies were made by measuring room temperature phycocyanin fluorescence. The control

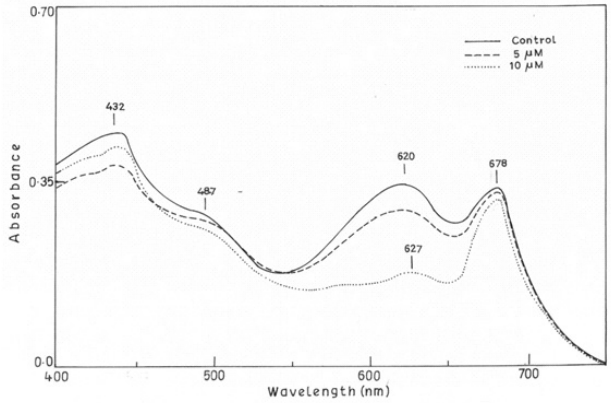

Figure 1: Effect of Ag ions on the absorption spectra of intact cells of *Spirulina platensis*

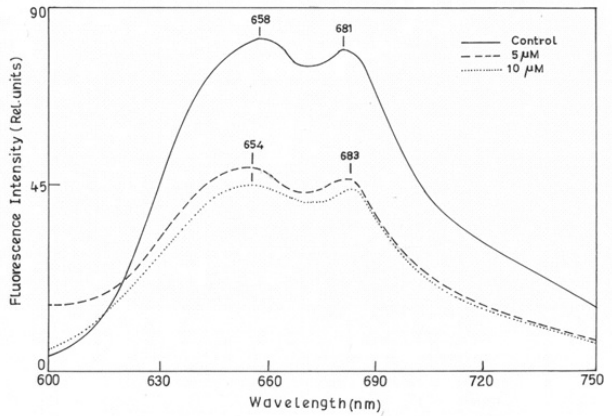

Figure 2: Effect of Ag on phycocyanin fluorescence emission spectra of the intact cells

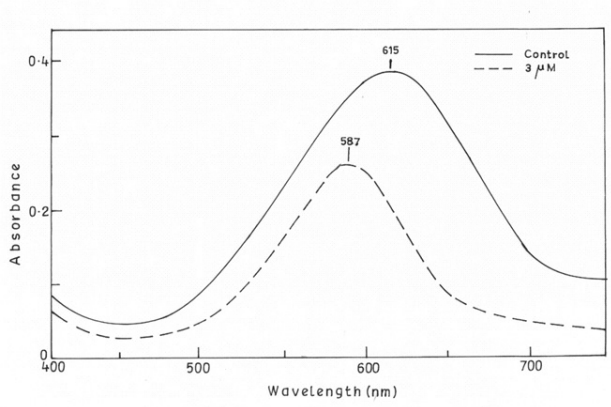

Figure 3: Absorption spectra of isolated PBsomes from control and Ag treated cells

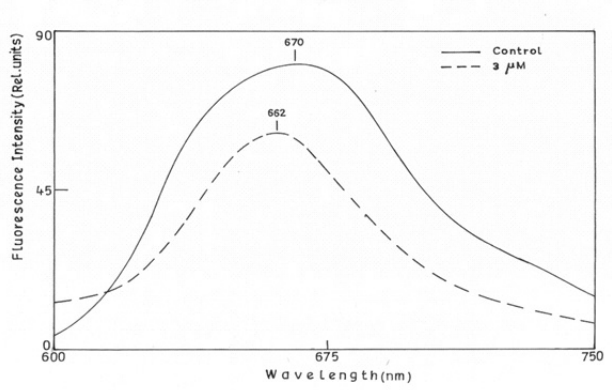

Figure 4: Fluorescence emission spectra of isolated PBsomes from control and Ag treated cells

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* (Singhal *et al.,* 1981; Fork and Mohanty, 1986). Fig. 2 shows the phycocyanin fluorescence emission spectra of silver treated *Spirulina* intact cells. With the treatment of Ag ions drastic decrease was noticed in the fluorescence emission intensities and with 10 μM of silver ions almost 50 % of loss in the fluorescence intensity of phycocyanin was observed. It clearly demonstrates that selected heavy metal (Ag) 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 Ag treated cells by using sucrose density gradient. After removal sucrose the spectral properties have been measured (Fig. 3 and 4). The absorption spectra of PBsomes exhibit a main peak at 615 nm. The PBsomes isolated from 3 μM of Ag 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, PBsome samples which were isolated from silver exposed (3 μM) *Spirulina* cells were used for the measurement of phycocyanin fluorescence emission. Silver is able to cause 42% decrease in the fluorescence intensity and blue shift in the emission peak from 670 nm to 662 nm. The decrease in the fluorescence intensity indicates the change in the energy transfer and blue shift gives information about structural changes in the PBsomes (Fig. 4). Similar reports were made by Murthy *et al.* (1989) during the toxic effect of mercuric chloride ($HgCl_2$) on the spectral properties of phycobiliproteins in the same organism. Thus silver 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 PBsomes.

ACKNOWLEDGEMENT

S.D.S.Murthy is thankful to University Grants Commission for providing Major Research Project (2011-2014) F. No. 39-244/2010(SR)

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