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Research article

ACTION OF SELECTED HEAVY METAL IONS ON THE SPECTRAL PROPERTIES OF ISOLATED PHYCOBILIPROTEINS FROM THE CYANOBACTERIUM, *SYNECHOCOCCUS*-6301

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ABSTRACT: Phycobiliproteins were separated by using hydroxyapatite column chromatography from phycobilisomes of *Synechococcus* 6301. Thick blue fractions have been identified by spectroscopically as phycocyanin (PC) and light blue fraction has been identified as allophycocyanin (APC). Between PC and APC, PC seems to be more sensitive to action of Mercury (Hg^{+2}) or Copper (Cu^{+2}) as evidenced from the spectral alterations of PC in the above organism.

Keywords: heavy metals; *synechococcus*-6301; spectral properties

INTRODUCTION

Phycobilisomes are light harvesting protein pigment complexes attached to the thylakoid membranes of cyanobacteria [1 and 2). The major components of phycobilisomes (PBsomes) are bilin containing proteins namely phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC) [3]. In cyanobacteria another pigment called phycoerythrocyanin (PEC) replaces PE whose synthesis is regulated by light quantity not by light quality [4]. The structure intactness of PBsome will be maintained by certain colourless polypeptides which are known as linker polypeptides in addition to the α and β of phycobiliproteins [5]. The molecular architecture of PBsome is such that the excitation of energy absorbed by then is transferred to the reaction centre of photosystem II (PS II) which will be approximately 80-90% [6]. A variety of environmental factors are known to affect the efficiency of energy transfer from PC to Chl *a* by affecting the pigment protein interaction heat treatment [7], nitrogen stress [8]. In this investigation an attempt has been made to study the effect of several metal ions on phycobiliprotein spectral properties from the cyanobacterium, *Synechococcus* 6301.

MATERIALS AND METHODS

Synechococcus 6301 cells were cultured in BG-11 medium in glass flasks at temperature of 25 ± 2^0C under continues illumination (15 Wm^{-2}) as described earlier by others [9 and 10]. PBsomes were isolated from *Synechococcus* 6301 cells by using the method of Gantt *et al* [3]. Briefly the cells were disrupted by sonication. After incubation in the presence of Triton-X100 for 40 min large fractions were removed by centrifugation at 30,000xg for 30 min. The supernatant was layered as on a sucrose density gradient (2.0, 1.0, 0.5 and 0.25 M). The PBsomes were concentrated in the 1.0 M region after spinning the gradients at 1,40,000xg for 5 h at 20^0C. The PBsomes were pooled after removal from 1 M and dialyzed over night in 0.75 M phosphate buffer; P^H 7.0. PC and APC were separated by loading dissociated PBsomes on hydroxyapatite column [3].

PC and APC were incubated in the presence and absence of heavy metals ($HgCl_2$/$CuSO_4$) for 5 min in phosphate buffer before measuring spectral properties. Absorption spectra were recorded using Shimadzu UV -3000 Spectrophotometer, Japan. Room temperature fluorescence emission spectra were measured in a Perkin Elmer LS-5 spectrofluorometer. Protein content was measured by following the procedure of Lowry *et al* [11].

RESULTS AND DISCUSSION

To characterize the effect of selected heavy metal ions ($HgCl_2$/$CuSO_4$) phycobiliproteins have been separated using dissociated PBsomes through hydroxy apatite column. After development of column with 5 mM phosphate buffer thick blue fractions were collected and used for spectral characterization. Similarly after complete elution of the above fractions, the column was developed with 0.3 M phosphate buffer and light blue fractions were collected and analyzed by using both visible spectrometry and fluorimetry. The thick blue fractions exhibited a single peak at 617 nm with out any absorption at 650 nm (Fig 1a and b). When the sample was excited with 545 nm light, an emission peak at 646 nm was noticed. This fraction is identified as phycocyanin. When this fraction is exposed to different concentrations of $HgCl_2$ and $CuSO_4$ individually there is a decrease in magnitude of absorption peak and as I case of well as in fluorescence emission properties (Table 1 and 2). The reasons for the decrease could be due to alterations of pigment protein interaction in PC due to the exposure to selected heavy metals. Therefore these alterations could be responsible for the change of energy transfer in PBsome.

To verify whether the other light blue fraction pertaining to (APC) is susceptible to heavy metal action and hence their spectral properties are analyzed. The light blue fraction was eluted with 0.3 M of phosphate buffer. Fig.2 (a and b) shows that spectral properties that of APC. The absorption maxima of light blue fraction was 651 nm with a shoulder at 625 nm. When this fraction was excited with 580 nm light an emission peak was observed at 663 nm. This clearly indicates the presence of APC. When this fractions were treated with $HgCl_2$ or $CuSO_4$ (1 - 4 µM) neither change in peak position and absorption capacity nor fluorescence emission properties were observed indicating that non susceptibility of APC to heavy metal action, (Table 3 and 4). Thus based on the results it can be inferred that out of the two pigment fractions (PC and APC) PC is more susceptible for the action of heavy metals than APC.

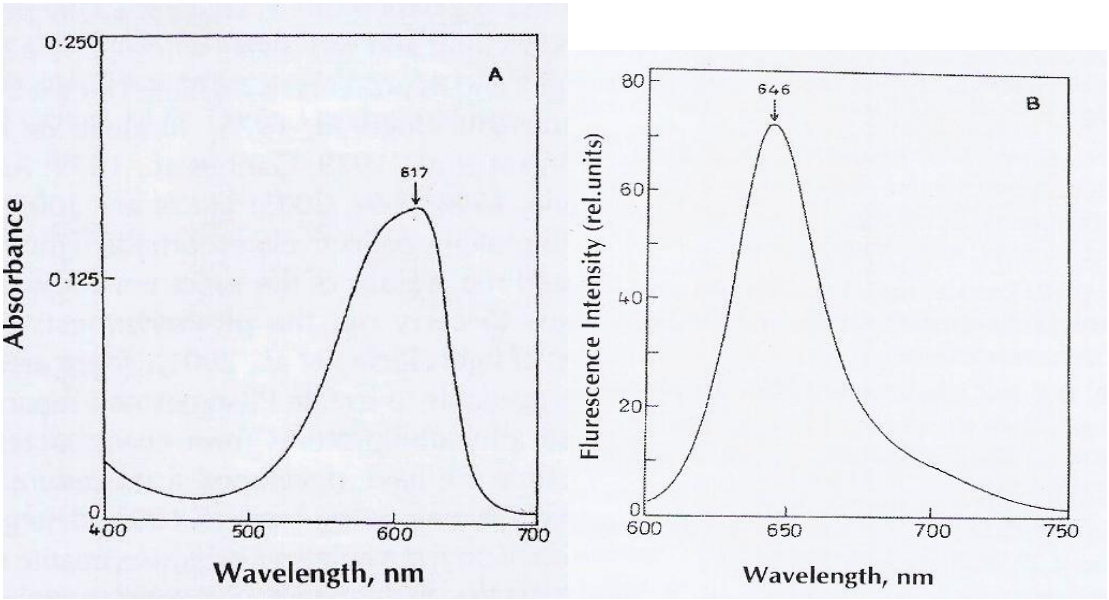

Fig 1: Spectral properties of purified phycocyanin of the cyanobacterium, *Synechococcus*-6301

a: Heavy metals impact on absorption spectra of phycocyanin
b: Heavy metals impact on fluorescence emission spectra of phycocyanin

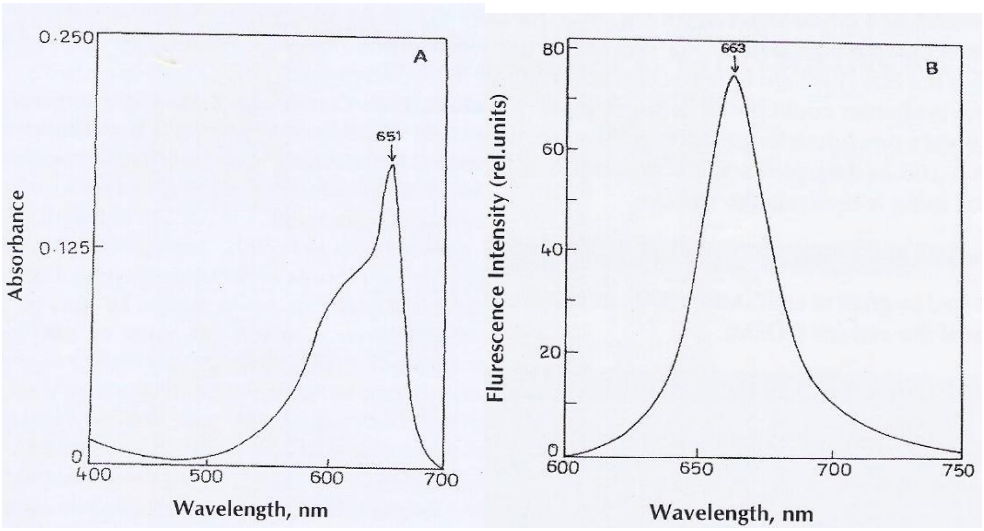

Fig 2: Spectral properties of purified allophycocyanin of the cyanobacterium, *Synechococcus*-6301

a: Heavy metals impact on absorption spectra of allophycocyanin
b: Heavy metals impact on fluorescence emission spectra of allophycocyanin

Table 1:Effect of heavy metals on PC absorption spectral properties of *Synechococcus*-6301

Heavy metal	Concentration (µM)	Absorption (OD)	Peak position (nm)
Control	**0**	**0.145**	**617**
$HgCl_2$	**1.5**	**0.119**	**611**
	3.0	**0.102**	**609**
$CuSo_4$	**2.0**	**0.127**	**615**
	4.0	**0.110**	**611**

Table 2: Effect of heavy metals on PC fluorescence emission properties of *Synechococcus*-6301

Heavy metal	Concentration (µM)	Fluorescence (rel. units)	Peak position (nm)
Control	**0**	**85**	**646**
$HgCl_2$	**1.5**	**62**	**644**
	3.0	**41**	**642**
$CuSo_4$	**2.0**	**71**	**645**
	4.0	**57**	**644**

Table 3: Effect of heavy metals on APC absorption spectral properties of *Synechococcus*-6301

Heavy metal	Concentration (µM)	Absorption (OD)	Peak position (nm)
Control	**0**	**0.156**	**651**
$HgCl_2$	**1.5**	**0.154**	**650**
	3.0	**0.153**	**650**
$CuSo_4$	**2.0**	**0.153**	**651**
	4.0	**0.152**	**650**

Table 4: Effect of heavy metal ions on APC fluorescence emission properties of *Synechococcus*-6301

Heavy metal	Concentration (µM)	APC Fluorescence (rel. units)	Peak position (nm)
Control	**0**	**72**	**663**
$HgCl_2$	**1.5**	**70**	**662**
	3.0	**68**	**662**
$CuSo_4$	**2.0**	**69**	**663**
	4.0	**67**	**662**

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