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A STUDY ON THE EFFECT OF HEAVY METALS ON SDS-PAGE PROTEIN PROFILES OF *Spirulina platensis*

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ABSTRACT

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) analyses of the total protein profile of *Spirulina platensis* was studied to know the effect of heavy metals Silver and Chromium. The excitation spectra of heavy metal treated cells showed the changes in chromophore protein interaction of various PBPs due to the shift in peak position. Further studies by SDS-PAGE revealed that the heavy metal treatment with either of Cr and Ag caused the decrease in the B subunit of PC and the loss of 41, 34 kDa linker polypeptides. Between these two heavy metals, Ag is responsible for the loss of above polypeptides in large extent than that of Cr. In addition, an additional polypeptide at 27 kDa was noticed which could be degratory product of high molecular weight containing 41 kDa. However, the isolated PBsomes from Cr and Ag pretreated cells exhibited the characteristic blue shift in the peak position of absorption and emission and the decrease of PC fluorescence emission intensity and loss of 22 kDa as observed in earlier with heavy metal treated intact cells. The study clearly indicated that upon incubation of intact cells with Cr or Ag the PC of PBsomes gets irreversibly altered such that the changes of PBsomes even persist after the isolation of PBsomes from heavy metal treated samples.

KEYWORDS: *Spirulina platensis*, silver, chromium, SDS page.

INTRODUCTION

Cyanobacteria are among the most known widespread, morphologically distinct and abundant prokaryotes. They are oxygenic photosynthetic autotrophs, originally considered as a class of algae, the blue-green algae possessing a unique ability in fixing atmospheric nitrogen. Cyanobacteria are known to adapt to environmental stresses by suitably modifying their proteome (Apte and Bhagwat, 1989). Living organisms, especially micro-organisms, are exposed to various types of natural stresses such as nutrient limitation, pesticides, pollution, drought, salinity, temperature, pH, light intensity and quality etc. Cyanobacteria is a group of Gram-negative bacteria that can survive in a wide variety of extreme environmental conditions. These cyanobacteria are exposed to heavy metals which are indispensable to the modern agricultural practice. The use of these pesticides over the years has resulted in problems caused by their interactions with the biological systems in the environment and has deleterious effects on cyanobacteria (Pankratz, 2003). To survive in the stressful conditions all organisms including bacteria will have to adopt different strategies at ecological, physiological, biochemical and molecular level. A molecular technique that was proved to be useful in typing bacterial strains is sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of whole cell bacterial proteins, wherein differences seen in protein bands in different circumstances could successfully be used to group tolerant bacteria. A Protein in the cyanobacterial thylakoid membranes was identified to be a sensitive protein to environmental stress conditions. Under various unfavorable conditions such as drought, nutrition deficiency, heat, chemical stress, ozone fumigation as well as UV-B and visible light stresses the turnover of this protein gets affected (Giardi, 1997). Rath and Adhikary (2007) demonstrated that the exposure of estuarine cyanobacterium *Lyngbya aestuarii* to UV-B radiation resulted in differential expression of cellular proteins. The aim of the work was to study protein profile changes and differentially expressed proteins in three cyanobacteria under the study.

MATERIAL AND METHODS

Microorganism and culture condition

The experimental organism *S. platensis,* the mother culture was obtained from National Facility for Blue

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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 at Department of Biochemistry, Sri Venkateswara University, Tirupati. Andhra Pradesh. 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 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. Measurement of optical density (O.D.) is particularly suitable for determination of growth of *S. platensis*.

Protein extraction in cyanobacteria

The cyanobacteria cultures were centrifuged at 10 600 g for 5 min and the medium was poured out and cultures were resuspended in 80% acetone solution and sonicated using a sonifier cell disruptor (Branson Digital Sonifier S- 450D, USA) for 20 s each in an ice bath, with 40 s cooling breaks up to one minute at 70% intensity. The sonicated samples were left overnight at 40^0C. The samples were then centrifuged at 10,600 g for 5 min and the pellets obtained were suspended in the solubilization buffer containing 7.5 ml of ultra-pure water, 2.5 ml of 1MTris– HCl pH 6.8, 16 ml of 10% SDS, and 1 ml of 80% glycerol (v/v) (Gentili *et al*., 2005). Finally, sample buffer-mercaptoethanol, Sodium containing deionized water, Dodecyl sulphate (SDS), 1M Tris-Hcl (pH 6.8), glycerol and bromophenol blue at a ratio of 2:1 was added to the samples followed by 3 min boiling.

Sodium dodecyl poly acrylamide gel electrophoresis (SDS-PAGE) assay

The extracted whole cell proteins from the isolates together with higher and lower range of protein molecular weight marker were mixed with SDS PAGE sample buffer in a 2: 1 ratio and the mixtures were heated in a heater block for 3 min at 100^0C. After cooling the samples at room temperature, the insoluble materials were removed by centrifugation. The supernatants thus obtained were submitted to SDSPAGE followed by electrophoresis at 70 V until the bromophenol blue dye front reaches the bottom of the gel. Following electrophoresis, the gel was stained overnight with Coomassie Blue R-250 and then destained in the same solution. Finally, the whole cell protein profiles of the samples were visualized under Trans white light and captured using Alpha innotech. All the experiments were performed in three independent replicates and only those spots present in at least two gels of the independent set were taken for analysis.

RESULT AND DISCUSSION

SDS-PAGE polypeptide profile of total soluble protein of control and heavy metal treated cells were depicted in Fig 1 and Fig 2 and the SDS-PAGE of total protein of control cells of *Spirulina* resolved its components in the range of 97.4 kDa to 14.4 kDa (Fig 1). The PBPs of intact cells were in the range of 16-22 kDa. Since these proteins are chromopore linked, they can be seen on the gel prior to the staining with comassive brilliant blue. Figure 1 shows the polypeptide profile of *Spirulin*a which were treated with 50 to 100 µM of Cr. The treatment caused gradual disappearance of 22 kDa. In addition it causes a decrease in 32 and 34 kDa poplypeptides which are related to reaction center of PS II. Cr treatment, in addition exhibited the appearance of 27 kDa polypeptide which could be most probably degradative product of higher molecular weight containing polypeptide (41 kDa). Figure 2 shows the effect of Ag on the polypeptide profile of total protein of control and Ag treated *Spirulina* cells. In the case of Ag the effect is more pronounced and it caused the maximum disappearance of 34 kDa, 41 kDa and 22 kDa. The 22 kDa polypeptide is known to be related to the PC β subunit of PBsomes. Thus the heavy metal treatment might be inducing some of the proteases which can specifically degrade the polypeptides related to PS II reaction center and LHC of PS II (β subunit of PC).

To support the above results the experiments were planned in two ways a) *in vivo* studies, where the PBsomes were be isolated from the heavy metal treated cells b) *in vitro* studies, where the treatment given to the PBsomes after their isolation from control cells. To achieve this an attempt was made to isolate the PBsomes from control and heavy metal treated samples by using sucrose density gradient. After their isolation sucrose was removed by dialysis and samples were used for spectral and electrophoretic measurements. Figure 3 shows the spectral characteristics of isolated PBsomes. The absorption spectra of PBsomes exhibited main peak at 615 nm due to the PC (phycocyanin) and a shoulder at 650 nm is due to APC (Allo Phycocyanin). The PBsomes excited with 545 nm light beam showed characteristic emission at 670 nm emanating from the longer wave length absorption

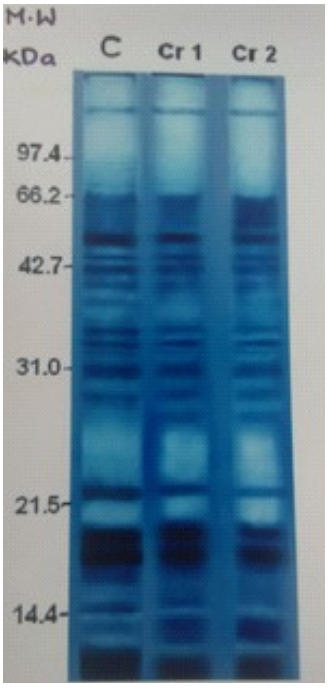

Fig 1. SDS-PAGE polypeptide profile of total soluble proteins of control *Spirulina* cells and different concentrations (50, 100 µM) of chromium treated cells. (C-control; Cr1- 50 µM; Cr2-100 µM)

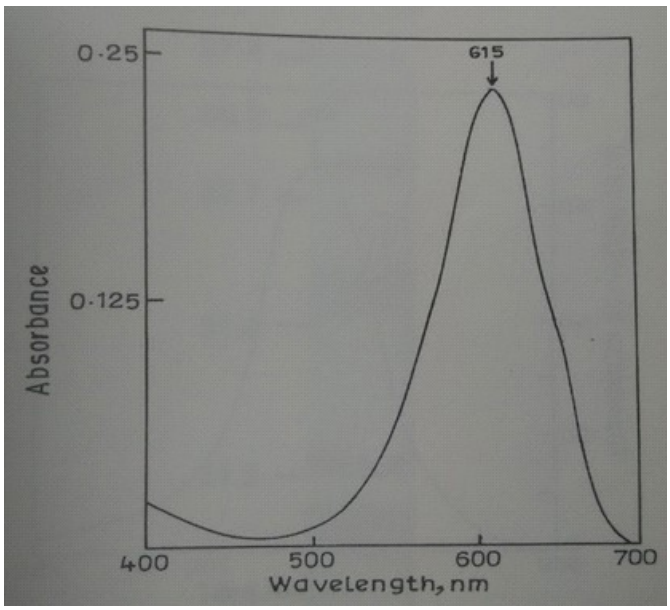

Fig 3. Absorption spectra of isolated phycobilisomes from *Spirulina platensis*

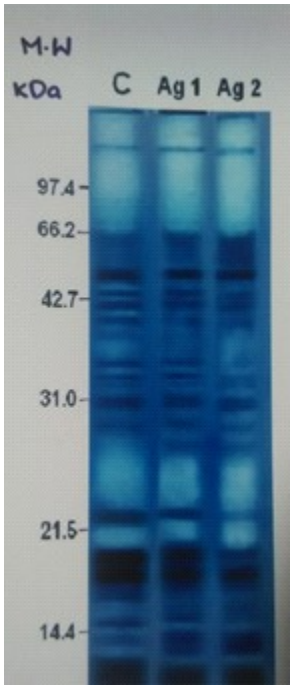

Fig 2. SDS-PAGE polypeptide profile of total soluble proteins of control *Spirulina* cells and different concentrations (10, 15 µM) of silver treated cells. (C-control; Ag1- 10 µM; Ag2 - 15 µM)

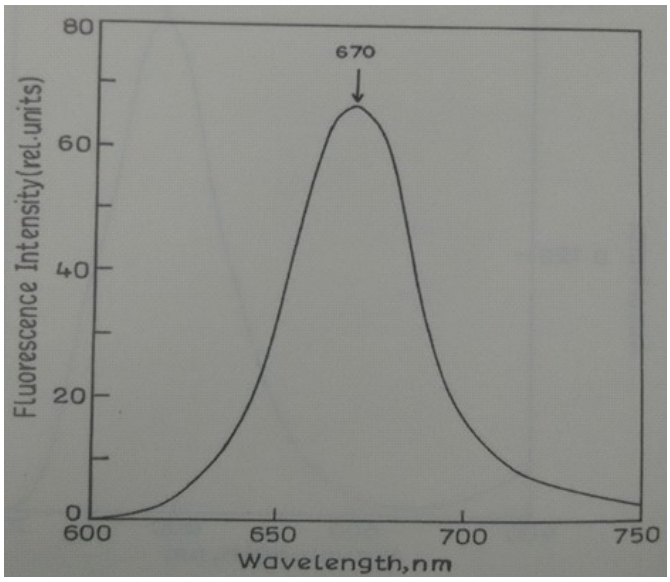

Fig 4. Flurescence emission spectrumof isolated phycobilisomes from *Spirulina platensis*

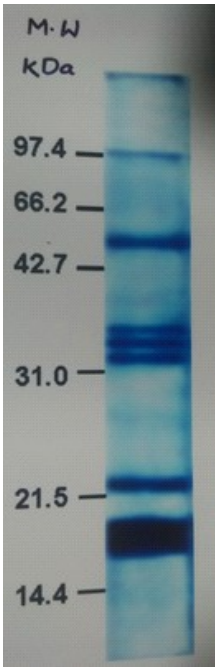

Fig 5. SDS-PAGE polypeptide gel profile of control phycobilisomes of *Spirulina platensis.* Protein equivalent to 30 µg was used in the electrophoretic studies

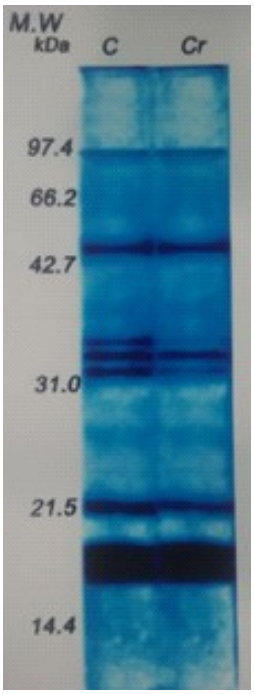

Fig 6. SDS-PAGE polypeptide gel profile of phycobilisomes isolated from control and chromium treated cells of *Spirulina platensis.* Lane 1- control PBsomes, Lane 2- chromium (100 µM) treated PBsomes

species (Fig 4). This indicates that the energy transfer in isolated PB somes in intact and the energy is getting transferred from PC to APC B. These spectral characteristics are in agreement with the observation of earlier workers (Murthy, 1991). Figure 5 shows the polypeptide profile of PBsomes isolated form *Spirulina* cells. Besides bilin carrying subunits of PBps (16 to 22 kDa), five non pigmented polypeptides were clearly associated with PBsomes. The polypeptide at 97 kDa is the anchor polypeptide which links the PBsomes to the thylakoid membrane. The polypeptide between 32 and 37 kDa are the linker polypeptides which helps in the attachment of PC to PC rods. The intense bands between 16 to 22 kDa are the subunits of PBPs (both PC and APC). In this range there are total four polypeptides which belongs to PC (α and β) an APC (α and β). The anchor polypeptide has been demonstrated to be susceptible for proteolytic degradation and hence care should be taken to prevent the degradation during isolation procedure.

To correlate the functional aspects of PBsomes with structural aspects an attempt has been made to resolve the changes in the polypeptide profile of PBsomes which were isolated from both Cr and Ag treated *Spirulina* cells (Fig 6 and 7). SDS-polypeptide profile of PBsomes of control *Spirulin*a cells were resolved in the range of 14.4 kDa to 97.4 kDa. In the electrophoretogram besides what bilin carrying PBPs (16 to 22 kDa), five non pigmented polypeptides were clearly seen (lane 1), associated with PBsomes. The intense bands of the subunits of PBPs both PC (α and β) and APC (α and β) (Fig 6) was clearly seen. The Cr treatment caused loss in the content of 22 kDa polypeptide by 50% without affecting other PBPs. In addition it caused the degradation of 32 and 37 kDa polypeptides without affecting much 35 kDa. In the case of Ag almost 22 kDa was degraded by almost 75% and only faint bands were observed in the case of linker polypeptides (Fig 7). From the data, it is clear that Ag is able to cause more loss in the linker polypeptides and PC β subunit when compared to that of Cr treated samples. This loss in polypeptides could be due to induction of metal ion specific proteases which could degrade b subunit as well as linker polypeptide preferentially.

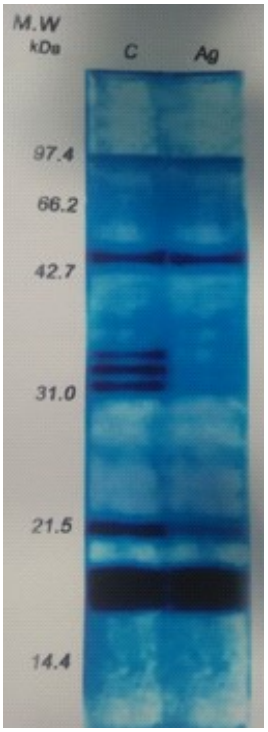

Fig 7. SDS-PAGE polypeptide gel profile of phycobilisomes isolated from control and silver treated cells of *Spirulina platensis.*
Lane 1- control PBsomes, Lane 2- silver (15 µM) treated PBsomes.

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