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Characterization of UV- B radiation induced alterations in energy transfer in photosystem II of the cyanobacterium, *Spirulina platensis* using *in vivo* and *in vitro* studies.

Praveena B, and Murthy SDS*.

Department of Biochemistry, Sri Venkateswara University, Tirupathi-517502.India.

ABSTRACT

In the present study an attempt has been to study the effect of Ultraviolet-B (UV-B) radiation (0.7 Wm^{-2} – 2.8 Wm^{-2} for about 30 min) on the spectral properties of intact cells and light harvesting complex in cyanobacterium, *Spirulina platensis.* To know the alterations in light harvesting complex, isolation of phycobilisomes is done and their spectral properties both absorption and fluorescence are studied in which altered spectra is observed indicating that phycobilisomes are the target for UV-B radiation. Our results clearly demonstrated that there are alterations in the spectral properties in both absorption and fluorescence emission peaks of phycocyanin (PC).

Keywords: Phycobilisomes, *Spirulina platensis*, UV-B radiation.

Corresponding author

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INTRODUCTION

UV radiation is one of the serious issues since past few decades due to industrialization. Increase in the industrialization results in the increase in atmospheric pollutants. These pollutants are being responsible for the depletion of ozone layer in the stratosphere [1]. Prolonged exposure of UV-B radiation leads to the loss of functional ability of photosynthetic apparatus of both plants and cyanobacteria [2]. Cyanobacterial photosynthetic apparatus principally consists of three types of macromolecular complexes : Photosystem I (PS I) Photosystem II (PS II) and Phycobiliproteins (PBPs) constitute the major light harvesting pigments which are attached to the outer surface of the thylakoid membranes in aggregated complex called phycobilisomes (PBsomes) [3]. The PBsomes, which biochemically consists of water soluble PBPs such as allophycocyanin (APC), phycocyanin (C-PC), phycoerythrin (PE).The light energy absorbed by PBsomes is transferred to the reaction centre of photosystem II through the antenna chlorophylls. PBPs , primarily composed of polypepetides like alpha and beta which are brilliantly coloured group of disc shaped proteins bears covalently attached open chain tetrapyrrole known as phycobilins [4]. Linker polypeptides located between PBsomes and thylakoid membranes can provide structural connection between adjacent PBPs and stabilise the PBsome structure and also they can modulate the absorption and fluorescence properties to facilitate or directly participate in energy transfer from the rod to the core and eventually to the chlorophyll (Chl) containing thylakoid membrane of the photosynthetic cells. Kulandaivelu *et al.,* [5] showed that prolonged exposure of UV-B causes destruction of chlorophyll pigments and leads to the inhibition of PS II photochemistry. Absorbance and fluorscence analysis indicated that PBsomes are the main target in photosystem comparative to the other photosynthetic pigments. In this the study an attempt has been made to study the effect of UV-B on the spectral properties of intact cells and isolated PBsomes in the cyanobacterium, *Spirulina platensis.*

MATERIALS AND METHODS

Spirulina platensis was grown axenically in Zarrouks medium [6] at 25 ± 2^{o}C under continuous illumination (20 Wm^{-2}). The cells agitated by passing filtered air. The log phase cells were harvested in to fresh growth medium in to petri dish and expressed to UV-B radiation (obtained from A Philips TL 20 type 05 type in the spectral range of 280-320 nm and width a peak at 312 nm) of different intensities (0.7 Wm^{-2} – 2.8 Wm^{-2}) for about 30 min. Then the cell suspension is taken for scanning the absorption spectra from 400-750 nm by using Hitachi-557 double beam spectrophotometer and also fluorescence emitted by the whole cells was measured at room temperature with excitation at 545 nm in a Perkin Elmer LS-5 Spectrofluorimeter.

PBsomes were isolated from the cell suspension according to the method of Gantt *et al.,* [7]. These isolated PBsomes are used for the analysis of spectral measurements (both absorption and fluorescence). Circular dichroism (CD) was performed on a JASCO J-720 Spectro-polarimeter calibrated for a single height with d-10, amino-camphor sulfonic acid (Kattayama chemical Co., Japan) and a neodymium glass filter for wavelength within 0.2 nm. CD spectra were recorded between 300-750 nm at room temperature by loading the samples in 1 mm path length cylindrical quartz cuvette.

RESULTS AND DISCUSSION

In this investigation an attempt has been made to know the effect of UV-B radiation (0.7 Wm^{-2} – 2.8 Wm^{-2} for 30 min) on the spectral properties using intact cells of *Spirulina platensis.* Fig 1 shows the absorption spectra of intact cells of *Spirulina* control and UV-B treated (2.1 Wm^{-2}) cells measured at room temperature. The peak at 680 nm is due to absorption of Chl *a* , peak at 623 nm is due to the absorption of PC of PBS , a hump at 490 nm is due to the absorption of carotenoids and a peak at 435 nm is due to presence of Chl *a* [8]. After treatment, there was a gradual decrease in PC absorption intensity. UV- treatment of 2.1 Wm^{-2} caused maximum decrease with peak shift from 623 nm to 626 nm in the PC absorption with minor changes in the Chl and carotenoid spectral properties. This indicates that there could be alterations in PBPs due to UV-B radiation in the above cyanobacteria. To confirm this PC fluorescence emission spectra at room temperature of intact cells in *Spirulina* are measured (Fig 2). In control cells upon excitation with 545 nm light beam an emission peak at 655 nm emanating from PC was prominent in this spectra. Treatment of cells with UV-B caused gradual decrease in the PC fluorescence and induced the 1 nm peak shift towards the red region of the spectrum. The decrease in the fluorescence intensity indicates the alteration in the energy transfer of light within the PBsomes. Thus alteration in electron transport could arise due to dissociation of PBsomes.

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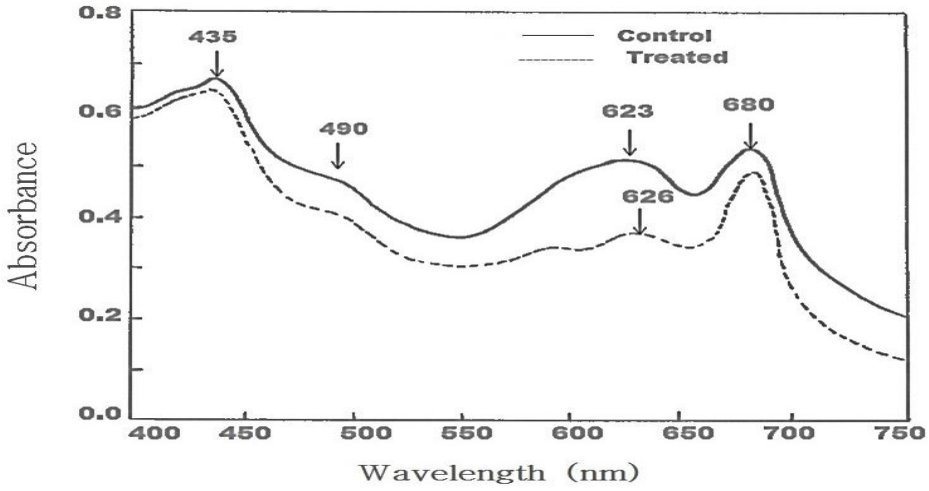

Fig 1: Effect of UV-B (2.1 Wm^{-2}) on the absorption spectra of the intact cells of the cyanobacterium, *Spirulina platensis.*

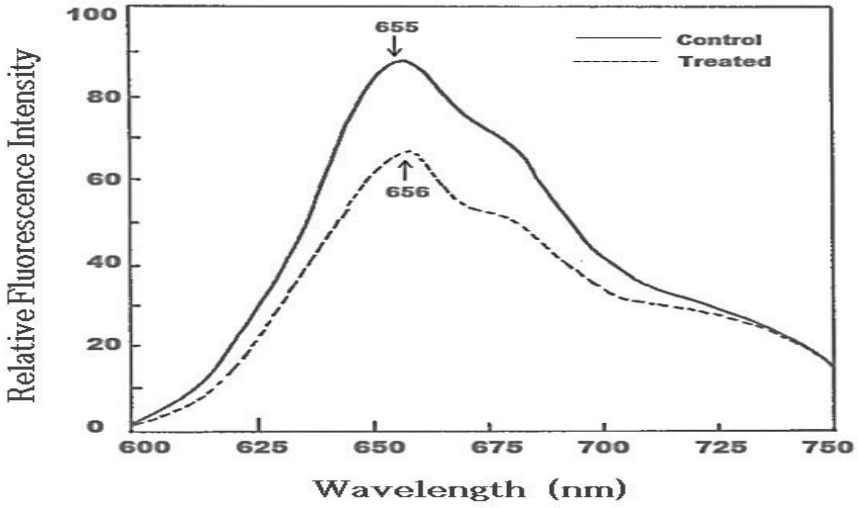

Fig 2: Effect of UV-B (2.1 Wm^{-2}) on the PC fluorescence emission spectra of the intact cells of the cyanobacterium, *Spirulina platensis.*

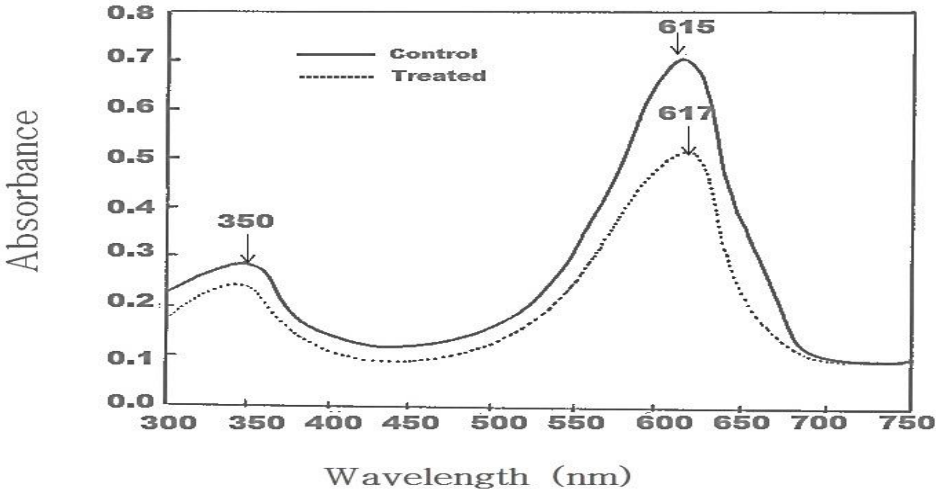

Fig 3: Absorption spectra of PBsomes, isolated from UV-B treated *Spirulina* cells . PBS equivalent to 15 µg of protein were suspended in 0.75 K-PO_4 buffer (pH 7.0)

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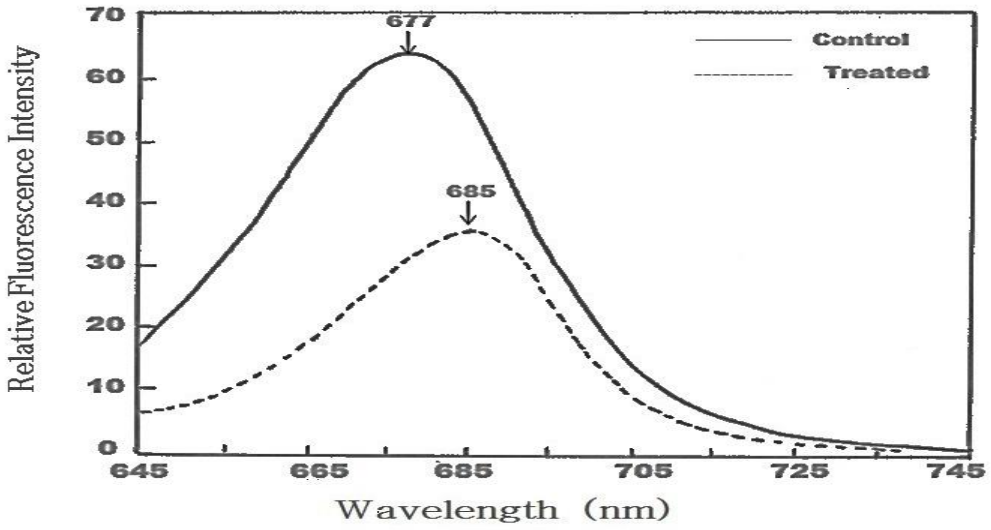

Fig 4: Effect of UV-B radiation on fluorescence emission spectra of PBsomes isolated from *Spirulina* cells. PBS were excited with 545 nm. PBS equivalent to 15 µg of protein were suspended in 0.75 K-PO_4 buffer (pH 7.0).

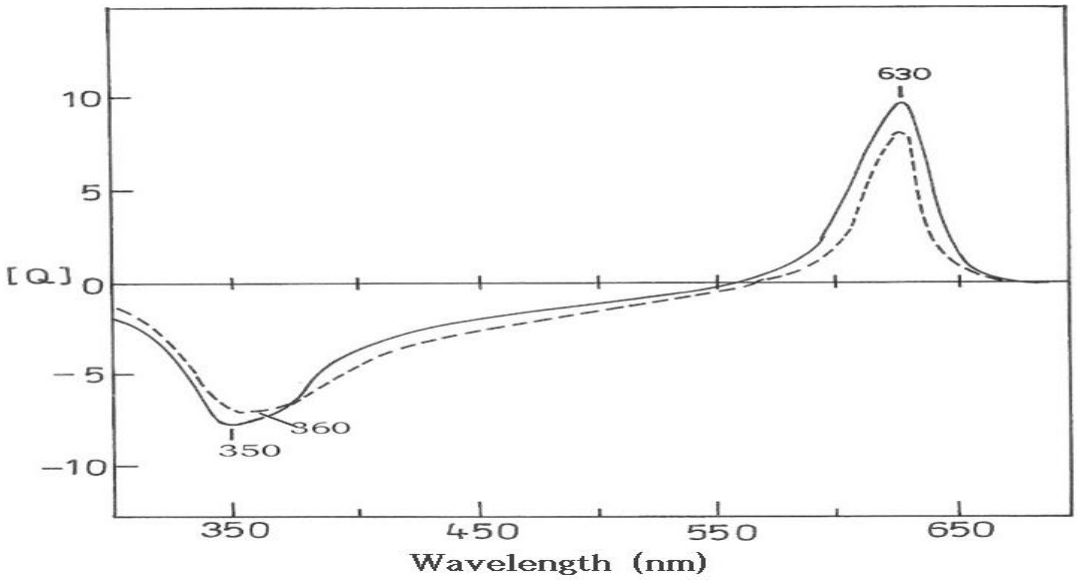

Fig 5: CD spectra of isolated phycobilisomes from control and UV-B treated intact cells.

To further examine the possibility PBsomes are isolated from *Spirulina*, control and treated samples are used for the characterizing the spectral properties. The absorption spectrum of PBPs exhibited maximum absorbance at 615 nm due to PC and a shoulder at 350 nm due to the presence of APC (Fig 3). The PBsomes isolated from the UV-B treated cells showed the decrease in the absorption intensity with a 2 nm peak shift towards red region of the spectrum. This decrease in the absorption capacity could be due to the alteration in the PBPs in treated samples. The PBPs upon excitation showed the emission peak at 677 nm emanating from the large wavelength from PBPs, APCB (Fig 4). From this it is clear that the energy transfer in isolated PBsomes is intact and it occurs form PC to APCB. UV-B treatment causes drastic decrease in the fluorescence intensity by 70% and shifted the peak towards the blue region of the spectrum by 8 nm indicating the alterations in the pigment protein interaction of PBPs. Our spectral measurements are in agreement with the observation of Gantt *et al*. [7]. To identify the specific alterations in PBsomes, polypeptide and chromophore protein interaction, the CD spectra of control and UV-B treated samples was measured. As shown in Fig 5 the control phycobilisomes CD spectrum exhibited a trough at 350 nm and a positive band in the visible region of the CD spectrum. UV-B treated conditions cause shift in the peak and the suppression of trough observed at 350 nm almost by 100%. These drastic changes in the CD spectrum seem to rise because of the dissociation of

phycobilisomes and also possible due to change in the chromophore protein interaction. Spectral measurements of isolated PBsomes clearly demonstrated that the energy transfer from PC to APC B is getting affected under *in vitro* conditions.

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