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ULTRAVIOLET– B LIGHT INDUCED CHANGES IN ENERGY TRANSFER OF PHOTOSYSTEM II IN THE INTACT CELLS OF *SPIRULINA PLATENSIS*

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ABSTRACT
In this investigation an attempt has been made to study the effect of Ultraviolet-B (UV-B) radiation (2 Wm^{-2}) on spectral properties of light harvesting complex (LHC) and energy transfer in the cyanobactrium, *Spirulina platensis*. Our results clearly indicated that there are alterations in the spectral properties as evidenced from the shift in both absorption and fluorescence emission peaks of phycocyanin (PC). PAM fluorescence studies clearly indicated that there is a rise in the F_0 value which reflects the alterations of LHC in the above cyanobacterium.

INTRODUCTION

The cyanobacteria photosynthetic apparatus is very similar to those of higher plants, except its antenna pigment complexes. The cyanobacterial photosynthetic apparatus principally consists of three types of macromolecular complexes: photosystem I(PSI), photosystem II (PS II) and phycobilisomes (PBsomes). PSI and PSII are intrinsic complexes where as PBsomes arranged on the outer surface of thylakoid membrane in the form of beads (Gantt, 1981; Liu *et al.,* 2005). The PBsomes, which biochemically consists of water soluble PBPs such as APC, C-PC and PE together with linker polypeptides. PBPs, primarily composed of alpha, beta polypeptides (in some phycoerythrins, there is a special type of subunit, the gamma subunit) are a brilliantly colored group of disc shaped proteins bearing covalently attached open chain tetrapyrrolle known as phycobilins (Liu *et al.,* 2005). Linker polypeptides located between PBsomes and thylakoid membranes can provide structural connection between adjacent PBPs and stabilize the PBsome structures 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 Chl containing thylakoid membrane of the photosynthetic cells. Kulandaivelu *et al.,* (1989) showed that prolonged exposure of UV-B causes destruction of Chl pigments and leads to the inhibition of PS II photochemistry. Fluorescence analysis indicated that Chl *b* is the main target in photo system comparative to the other photosynthetic pigments (Lischanthaler *et al.,* 1992; Lambreva *et al.,* 2005).

In this study an attempt has been made to study the effect of UV-B on spectral properties and energy transfer in the cyanobacterium, *Spirulina platensis*. In addition to that we have made an attempt to study the time dependant effect of UV-B exposure on PS II photochemistry by using Chl fluorescence kinetics. The main objective of the study is to understand the mechanism of UV-B stress induced change in energy transfer in PBSs to PS II in these cyanobacteria.

MATERIALS AND METHODS

Spirulina platensis was grown axenically in Zarrouks medium (Zarrouk, 1966) at $25 \pm 2^{o}C$ under continuous illumination ($20Wm^{-2}$).The culture was agitated by passing filtered air. The log phase cells were harvested in to fresh growth medium in to Petridish and exposed to UV-B radiation at influence rate of $2Wm^{-2}$ (obtained from A Philips TL 20 type 05 type in the spectral range of 280-320 nm and width a peak at 312 nm) for different intervals (20-80 min). Then the cell suspension was taken for scanning the absorption spectra from 400-750 nm by using a Hitachi-557double beam spectrophotometer. Fluorescence emitted by the whole cells was measured at room temperature with excitation at 580 nm in a Perkin Elmer LS-5 spectrofluoremeter.

RESULTS AND DISCUSSION

In this investigation an attempt has been made to know the effect of UV-B radiation on spectral properties using intact

cells for different intervals in *Spirulina*. Fig. 1 shows the absorption spectra of intact *Spirulina* control and UV-B treated cells measured at room temperature. From this, the intact cells exhibited two major peaks and two shoulders in the absorption spectrum. The peak at 680 nm is due to the absorption of Chl *a* and peak at 440 nm is due to Soret band of Chl *a*. The shoulder at 621 nm is due to the residual PC and a hump at 480 nm is mostly due to the carotenoid absorption. Treatment of UV-B radiation showed the peak shift in the PBPs from 621 to 632 when exposed to 40 and 60 min of duration respectively (Fig. 1). In control cells the ratio between absorption of Chl at 440/680 nm was 1.17. UV-B treatment could not cause any change in the above ratio (Table 1). Similarly the ratio between carotenoids (480 nm) and Chl (680 nm) was observed to be 0.59 in control and in treatment the ratio was 0.55. The ratio between PC (621 nm) and Chl (680 nm) was observed to be 0.98 in case of control and 0.85 in the case of UV-B exposed for 80 min. Thus it is clear that among the several pigment proteins, PC absorption gets affected more when compared to that of others. Since the absorption properties are related to light harvesting in PS II, we have measured fluorescence emission spectra of PC. The room temperature fluorescence emission spectra of intact cells exhibit various emission bands at different wavelengths depending on the excitation wavelength. Due to the presence of PXB chromophore in PBsome, we have excited the cells with 545 nm light. When the cells were excited in the PC absorbing region at 540 nm, an emission peak is observed mainly at 652 nm due to PC and also a hump is observed at 684 nm due to Chl *a* emission (Fig. 2). The cells treated with UV-B caused gradual decrease in the PC fluorescence and induced 9 nm peak shifts towards the red region of the spectrum after 40 min of UV-B exposure. After 80 min of UV-B treatment caused almost 40% decreases in the fluorescence intensity (Table 2). The decrease in the fluorescence intensity indicated that energy transfer from PBsomes to the photosystem was suppressed. The other possibility is uncoupling of energy transfer between PC and APC. Thus it is clear that the UV-B affects the spectral properties of PBsomes in short term incubations and also induces uncoupling of energy transfer in PBPs. UV-B radiation also decreases Chl flurorescence with the fast components accelerated and the slow components retarded, suggesting the formation of additional quenchers of excitation energy in reaction centers (Renger *et al.*, 1989). It has been indicated that plastoquinone with its three redox states (quinine, semiquinone anion and the quionl) may act as a primary UV-B photosensitive molecule since all these forms absorb to the same extent in UV-B region (Melis *et al.*, 1992). To identify the real target of UV-B, fluorescence kinetics has been measured using PAM fluorimeter. The fluorescence emission increases from an initial level, F_0 to maximum level F_m. This F_0 can be observed with a very weak modulated light and this light is incapable of carrying out the photochemistry of PS II (Schreiber, 1986). This rise from F_0 to F_m or the difference

Table 1: Effect of UV-B radiation (2Wm^{-2}) for different intervals (20-80mins) on absorption properties of the intact cells of *Spirulina platensis*

UV-B radiation min	Absorption ratio		
	440/680	480/680	621/680
Control	1.17	0.59	0.98
20	1.16	0.57	0.97
40	1.15	0.56	0.91
60	1.14	0.57	0.89
80	1.14	0.55	0.85

Table 2: Effect of UV-B radiation (2Wm^{-2}) on the PC fluorescence emission properties of the intact cells of *Spirulina platensis*

UV-B exposure, min	Phycocyanin fluorescence emission Intensity(relative units)		Percent decrease Peak position (nm)
Control	75 ± 4	652	0
20	65 ± 4	655	14
40	58 ± 3	651	27
60	50 ± 2	665	33
80	45 ± 2	665	40

Table 3: Effect of UV-B radiation (2Wm^{-2}) on fluorescence kinetics of thylakoids membrane isolated from intact cells of *Spirulina platensis*

UV-Bexposure min	Fluorescence parameter(in terms of distance,cm)			
	F_0	Fv	Fm	Fv/Fm
Control	2.1	5.1	702	0.71
20	203	4.5	6.8	0.66
40	204	4.1	6.5	0.63
60	2.6	3.8	6.4	0.59
80	2.8	3.5	6.3	0.55

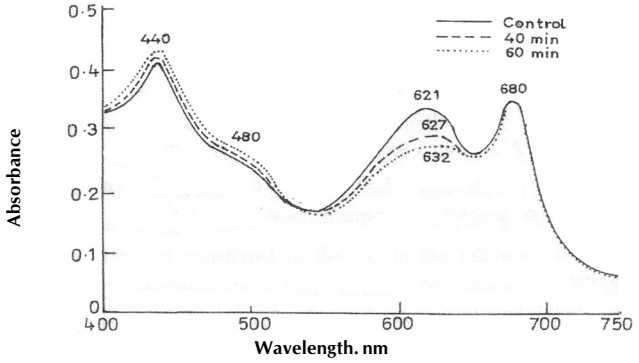

Figure 1: Effect of UV-B radiation (2Wm^{-2}) on the absorption spectra of the Intact cells of *Spirulina platensis*

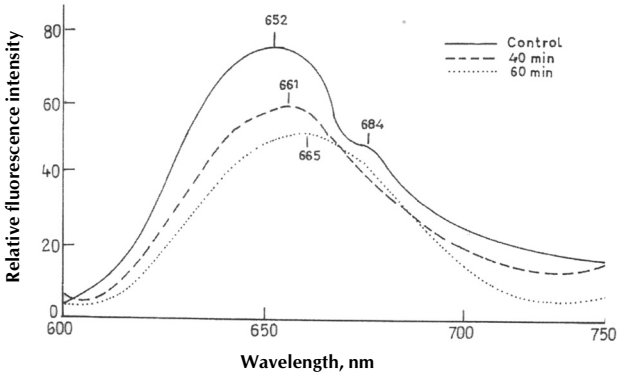

Figure 2: Effect of UV-B radiation (2Wm^{-2}) for different intervals (40and 60min) on the fluorescence emission spectra of intact cells of *Spirulina platensis*

is called variable fluorescence, F_v. F_0 gives information about the status of LHC. In this investigation, fluorescence intensity measurements were made by using PAM kinetic spectroflourimeter. To the control cells, weak modulated light caused a rise upon excitation which is nothing but F_0. Further illumination with strong light caused enhancement in the signal to Fm. This UV-B treatment caused changes in the F_0, F_v and F_m of thylakoids isolated from intact cells. Table 3 shows the change in the Fv/Fm ratio which is nothing but the photochemistry of PS II. The treatment of UV-B caused rise in the F_0 from 2.2 to 2.8 cm where as a drop was noticed in the case of F_v from 5.1 to 3.5 cm (Table 3). Similarly F_m value was changed from 7.2 to 6.3 cm due to the increase in the UV-B exposure from 20 to 80 min. The loss in the F_V and F_m indicates the PS II activity due to the damage of LHC at the level of PS II (Campbell *et al*., 1998). Similar observations have been made in *Spirulina* under the influence of mercury (Murthy *et al*., 1991). Thus UV-B radiation mainly affects energy transfer by acting at the level of light harvesting complex, phycobilisomes and causes loss in PS II photochemistry.

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