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Ultraviolet-B Effects on *Spirulina platensis* Cells: Modification of Chromophore–Protein Interaction and Energy Transfer Characteristics of Phycobilisomes

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Exposure of ultraviolet-B (280–320 nm, 1.9 mW m^{-2} s^{-1}) radiation of intact *Spirulina platensis* for 9 h caused specific loss of the 85.5 KDa anchor protein of phycobilisomes, the major light-harvesting antenna complex of photosystem II. Associated with the loss of 85.5 KDa protein, the UV-B irradiation also caused photobleaching of phycobilins and alteration in the chromophore protein interactions, as evidenced from the visible circular dichroic measurements, and it also affected the energy transfer process within the phycobilisomes, as inferred from the low-temperature, 77 K, fluorescence spectral analysis. Our results, thus, clearly demonstrate for the first time that the phycobilisomes effectively act as targets for UV-B induced damage of photosynthetic apparatus in cyanobacteria.

Depletion of stratospheric ozone has led to the enhanced levels of ultraviolet (UV) in the solar radiation (1). The deleterious effects of UV radiations on living organisms have been well characterized (2,3). Various types of UV radiation such as UV-A, UV-B and UV-C have specific effects on the oxygenic photosynthetic organisms (4). In recent years, the inhibitory effects of UV-B (280-320 nm) on the photosynthetic organisms particularly on higher plants have been carried out in many laboratories (5-8). It has been shown that UV-B irradiation arrests growth (7), supresses chlorophyll (Chl) biosynthesis (4) inhibits electron transport (9) and net photosynthesis (10). Of the two photosystems, the photosystem (PS)II has been found to be highly susceptible while photosystem (PS)I is some what resistant to UV-B radiation (11).

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UV-B induced loss of water oxidation capacity of PSII, due to loss of mostly D1 and also D2 protein of the PSII reaction centres have been well characterized (12, 13). It has been claimed that the absorption of UV-B by quinones of the photosynthetic electron transport chain enhances the free radical induced damage of PSII reaction centres protein D1/D2 and causes irreversible damage to the photosynthetic apparatus (14,15).

The studies of UV-B effects on cyanobacteria are limited. The cyanobacteria are oxygenic photosynthetic prokayotes and are exposed to various types of environmental stresses which, in turn, have facilitated them to be colonized in a range of habitats. Cyanobacterial phycobilisomes (PBS) serves as the primary light harvesting antenna for PSII, and are attached to the thylakoid membrane (16,17). PBS do absorb in the near UV-B region of the solar radiation and thus becomes a potential target for UV-B attack (18). In earlier studies with UV-B effects on cyanobacteria, high intensity of UV-B exposures were used to monitor the effect of UV-B on photosynthesis. The work of Kulandaivelu *et al.* (4) demonstrated that UV-B exposure of *Synechococcus* cells inhibited O2 evolution and altered the spectral characteristics of PBS due to partial uncoupling of energy transfer. Recently similar results were noted by Rajagopal and Murthy (19). Exposures of high intensity of UV-B to intact cells and isolated *Synechococcus* PBS have been shown to bring about gradual but unspecific damages to linker and anchor polypeptides (20). Recently Lao and Glazer (17) have demonstrated the quantum yield of photodamage of PBS by UV-B is much higher than visible light in *Anabaena sp*, and also UV-B induces photodamage of PBS structure and function. Sah *et al.* (18), however, using a relatively low dose of UV-B exposure reported that UV-B altered the protein-pigment interactions in *Synechococcus*-PBS which was linked to a decrease in the extent of the 75 KDa PBS anchor polypeptide.

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In post of the previous studies, a high intensity of UV-B were used. Therefore, in our present study, we have used moderate intensity of UV-B (1.9 mW m^{-2} S^{-1}) which is comparable to the natural level of UV-B irradiance, to monitor the spectral changes in PBS of Spirulina platensis a filamentous commercially important cyanobacterium (21). Our results, in this *in vivo* study, very clearly demonstrates that the 85.5 KDa anchor polypeptides (22) are the specific and possibly the primary target of UV-B attack. Further, our results also suggest that UV-B alters the energy transfer in PBS possibly due to a) the modification of chromophore-protein interaction and b) to the dissociation of phycocyanin (PC) rods from allophycocyanin (APC) core.

MATERIALS AND METHODS

Pure culture of *Spirulina platensis* was obtained from Central Food Technology Research Institute, Mysore, India. The cells were grown at 26 ± 2 °C in Zarrouk's medium under continuous white light illumination (30 μ moles m^{-2} sec^{-1}) as described elsewhere (23). The culture medium was continuously bubbled with filtered air. The midlog phase culture was harvested by centrifugation at 12,000×g for 15 min and washed with fresh growth medium and finally 1 gm fresh weight of cells (trichomes) were suspended in 70 ml of fresh growth medium. The cells were exposed to UV-B using photodyne UV-B tube lights (Photodyne, USA) for 9 h with continuous stirring and control sample was incubated in dark for 9 h. UV-B intensity was measured using VLX-312 nm UV meter from Vilber-Lourmat, France. Both control and UV-B exposed samples were maintained at 26 ± 2 °C.

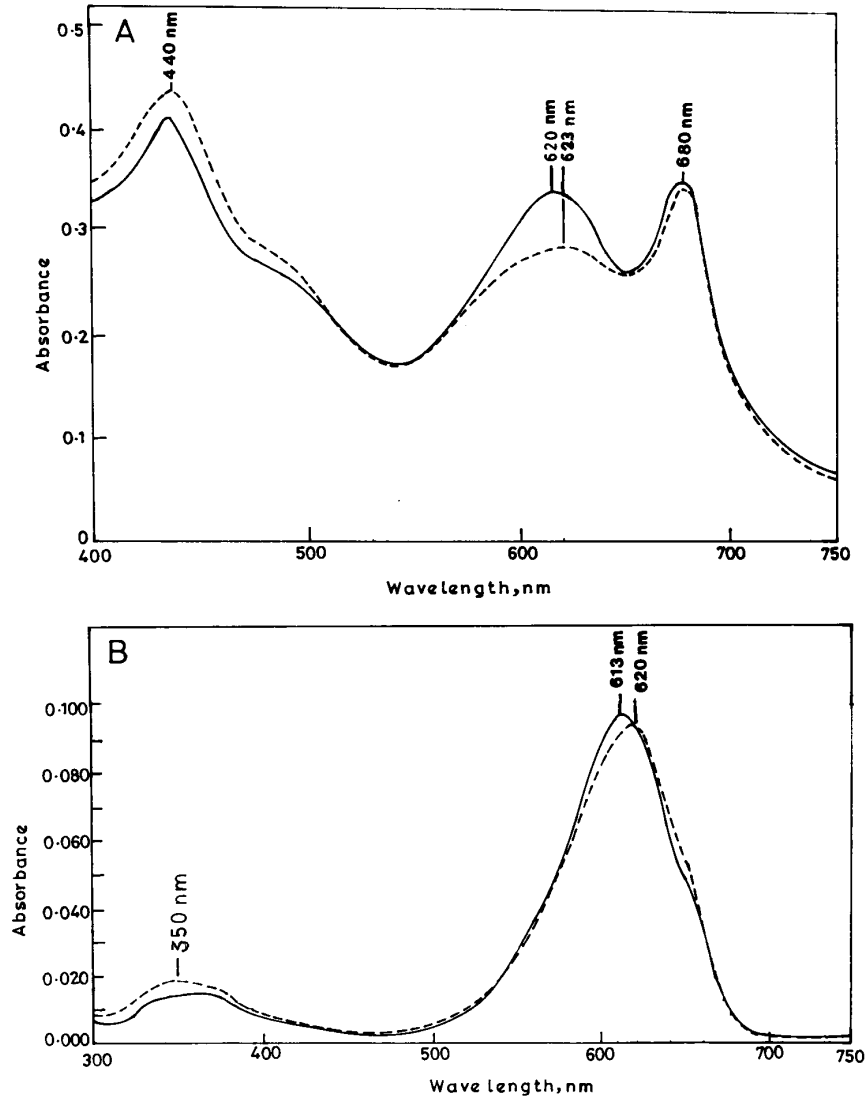

FIG. 1. (A) The room temperature absorption spectra of intact *Spirulina platensis* cells, (—) represents the spectrum for cell in dark for 9 h, and (----) represents the spectrum for cells exposed to UV-B (1.9 mW m^{-2} s^{-1}) for 9 h. For other details see materials and methods. (B) The room temperature absorption spectra of PBS isolated from dark incubated control (—) for 9 hrs and UV-B exposed (----) *Spirulina* cells for 9 h. The absorption spectra were recorded on equal protein basis.

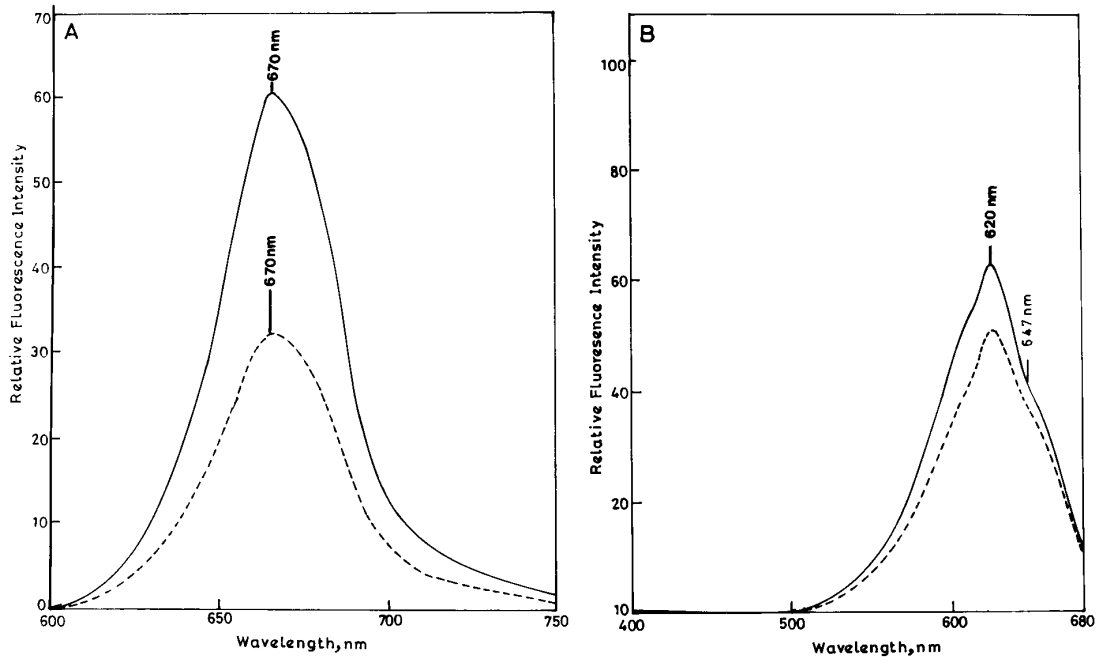

FIG. 2. (A) Room temperature fluorescence emission spectra of PBS isolated from dark incubated control (—) and UV-B exposed (----) *Spirulina* cells. Samples were excited at 585 nm. Excitation and emission slit width were 5 and 10 nm, respectively. (B) Room temperature fluorescence excitation spectra of PBS isolated from dark incubated control (—) and UV-B exposed (----). *Spirulina* cells were exposed to UV-B for 9 h and control cells were incubated in dark for 9 h. Excitation spectra were recorded for 695 nm emission. The slit width for both excitation and emission were set at 5 nm and 10 nm respectively.

The PBS were isolated according to Gantt *et al.* (16), albeit with some modifications. 1 gm fresh weight of *Spirulina* cells were suspended in 10 ml of 1M K-phosphate buffer at pH 7.0 containing 1mM PMSF, 1mM sodium azide, 2mM EDTA, 2% 2-mercaptoethanol. The cells were disrupted by sonication at an amplitude of 15 μm with 7-8 bursts of 15 sec each in MSE sonicator, USA. Now, the sonicated cells were incubated with 2% Triton-X 100 for 35 min and then the crude extract of PBS was separated from the cell debris by centrifugation at 35,000 $\times$ g for 40 min. The supernatant was collected and layered on sucrose density step gradient (2.0M, 1.0M, 0.5M, 0.25M). The PBS were obtained at 1.0M sucrose region after spinning the gradient using swing-out rotors at 1,50,000 $\times$ g for 6 h in an ultracentrifuge.

Protein of PBS was estimated according to Lowry *et al.* (24). The amounts of PC and APC in the PBS were estimated as in reference (25).

Absorption spectra were recorded by Hitachi U-2000 spectrophotometer. Room temperature and 77K fluorescence emission spectra were obtained on model LS-5 Perkin Elmer spectrofluorimeter as described earlier (18). Fluorescence excitation spectra were recorded on Shimadzu RF-540 spectrofluorimeter as described else where (18).

Whole cell absorption spectral analysis were done on equal chlorophyll (Chl) basis (6 μg/ml). Room temperature spectral analysis of PBS were made on equal protein concentration basis (30 μg/ml), and for 77K fluorescence a protein concentration of 10 μg/ml was used in 1.0 M K-phosphate buffer containing 30% glycerol, pH 7.0.

Electrophoresis was performed on SDS-PAGE according to Laemmli (26) with 12.5% polyacrylamide gel and were run at a constant voltage of 200V. Samples on equal protein basis (20μg) were loaded on each lane. For determination of molecular mass weight, Pharmacia molecular markers were used. Densitometric analysis were done on an ultrascan (Pharmacia, Sweden) using a 633 nm He-Ne laser.

Circular dichroism (CD) spectra were measured on JASCO model J-720 spectropolarimeter coupled with a data processor. Samples of PBS at concentration 60 μg/ml were prepared in 1.0 M K-phosphate buffer, pH 7.0. CD spectra were recorded between 750-300 nm at room temperature by loading the samples in 1 mm path length cylindrical quartz cuvette. Spectra were signal averaged and base line of the solvent was subtracted. The CD data were expressed as ellipticity in millidegree (18).

RESULTS AND DISCUSSION

Studies with isolated PBS clearly show that the UV-B (280-320 nm) radiations even at low doses get absorbed by the cyanobacterial PBS and induce alterations in the energy transfer (4,18) and dissociation of PBS (18). At high enough UV-B intensity, photodamage to PBS occur (17).

We have, therefore, investigated the effect of moderate intensity ($\sim$1.9mW m^{-2} s^{-1}) of UV-B on the cyanobacterium. Prolonged (9 h) exposure of UV-B to the *Spirulina platensis* cells brought about a loss in absorption intensity by phycobilins (Fig.1A) together with a shift in the peak absorption by PBS from 620 nm to 623 nm. The soret peak at 440 nm due to Chl a absorption showed a small increase while no significant alteration in the red absorption band of Chl a could be observed (Fig.1A). Figure 1B depicts the absorption spectra of intact PBS isolated from UV-B exposed and dark

adapted control *Spirulina* cells. The room temperature absorption spectrum of control (untreated) PBS exhibited a typical profile with a major peak at 613 nm and another small peak at 350 nm as reported earlier (25,27). The PBS from UV-B exposed cells exhibited a red shift in the absorption peak by 7 nm and a small increase in absorption intensity at 350 nm (Fig.1B). The shift in PBS absorption band was found to be dose dependent as the extent of red shift was observed to 3 to 3.5 nm after 6 h as against 7 nm after 9 h exposure. The PBS isolated from *Spirulina* cells kept for 9 h in light or dark yielded same spectral characteristics and the cells showed no loss of photosynthetic activity as monitored by PBQ Hill reaction (data not shown).

After 9 h of UV-B radiation exposure, the phycocyanin (PC) to APC ratio decreased from 2.46 to 1.95 suggesting that UV-B induced a preferential bleaching of PC. Figure 2A shows the room temperature emission spectra of PBS from control and UV-B exposed *Spirulina* cells excited at wavelength 585 nm which is absorbed mainly by PC. The room temperature emission spectrum of control PBS exhibited a typical emission peak at 670 nm as reported earlier for *Spirulina* (21,27). The PBS of UV-B exposed cells, on equal protein basis, showed a loss of emission intensity by 33% and 46% after 6h and 9h exposure,

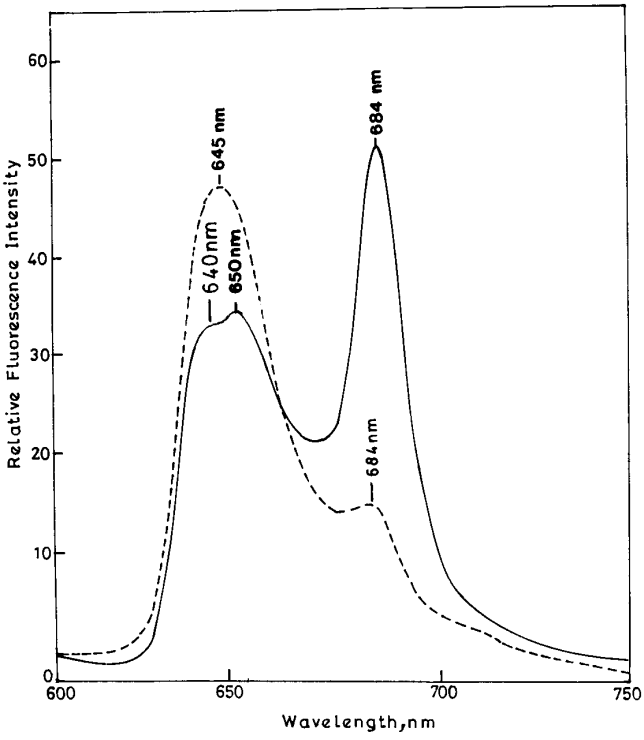

FIG. 3. Low temperature (77K) fluorescence emission spectra of PBS isolated from 9 h dark incubated control (—) and 9 h UV-B exposed (----) *Spirulina* cells. Samples were excited at 585 nm. The excitation and emission slit width were 5 and 10 nm, respectively. Other details are as in material and methods.

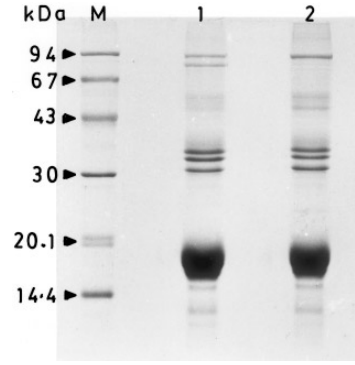

FIG. 4. SDS-PAGE polypeptide profile of PBS isolated from 9 hrs dark incubated control and 9 hrs UV-B exposed *Spirulina* cells. Lane M represents molecular weight markers, Lane 1 dark incubated control, Lane 2 UV-B exposure PBS. A 12.5% gel was used for SDS-PAGE analysis. Molecular masses of PBS polypeptides are estimated using Pharmacia markers.

respectively. The results, there by, suggest that UV-B radiation causes dose dependent photobleaching of phycobilins (17,18). No shift in peak emission upon 585 nm wave length excitation was, however, observed in PBS from UV-B exposed cells. The UV-B exposure induced an apparant shift in peak absorption but not in the emission spectrum. This absorption peak shift may have been due to change in PC to APC ratio in the PBS. The excitation spectra of dark control and UV-B exposed *Spirulina* cell-PBS measured, at 695 nm (F695) both showed excitation maxima at 620 nm due to PC (Fig.2B). In the excitation spectrum of the PBS from UV-B treated cells the hump at 647 nm was less pronounced than the dark control PBS excitation spectrum. Besides, the loss in excitation intensity, the alteration in the excitation spectral profile by UV-B radiation indicated that not only photodamage of phycobilin proteins but also possible alterations in energy transfer process within the PBS by UV-B exposure occurred. In order to ascertein the nature of UV-B induced alterations in emission characteristics of various phycobilins in the PBS, we monitored the emission spectra of PBS of control and UV-B exposed-cells at 77K. The 77K emission spectrum of control PBS, excited at wavelength 585 nm exhibited emission maxima at 640 nm and 650 nm due to PC and another peak at 684 nm due to APC core (28). The 77K emission spectrum of UV-B exposed all PBS showed a decrease in emission intensity. Notably, the 684 nm emission (F684) was drastically reduced (Fig.3). However, the emission by PC at 650 nm was enhanced (Fig.3). The ratio of F684 to F645 in control PBS was to 1.2 while in PBS of UV-B treated cells it was 0.32. These dramatic changes in low temperature emission characteristics by the UV-B exposure of *Spirulina* cells suggest that UV-B induced not only change in PBS chromoprotein ratio, but also induced suppression of energy transfer process from rod PC to core APC within

TABLE 1

Densitometric Analysis of Dark Incubated Control PBS and UV-B Exposed PBS of SDS-PAGE

	Relative area (%) of total sample										
PBS*	94 kDa	85.5 kDa	58 kDa	56.5 kDa	36 kDa	34.5 kDa	31.5 kDa	19.5 kDa	18 kDa	13 kDa	12.5 kDa
Control	5.36	3.77	5.35	—	3.46	2.93	5.07	54.57	8.15	7.81	2.02
UV-B exposed	8.69	—	4.51	2.19	4.06	2.35	5.09	55.08	7.81	7.93	2.11

Note. The total integrated area of the lanes differed by less than 2%. All values were normalized to 100% peak area. The step resolution down the length of the lane is 20 μm.

* Data taken from Fig. 4, for control lane 1, for UV-B exposure lane 2.

the PBS (18). The SDS-PAGE profile of PBS isolated from dark control and 9h UV-B exposed cells of *Spirulina* is shown in Fig.4. It is quite clear that upon UV-B exposure, the 85.5KDa polypeptide, representing an anchor polypeptide was totally absent in the PBS of UV-B exposed cells. The intensity of 94 KDa polypeptide however, was intensified. The relative extents of the various PBS polypeptides on equal protein basis in the gel were estimated both for control and UV-B exposed PBS (Table 1). The major change in the PBS polypeptide profile is the loss of 85.5 KDa polypeptide by UV-B exposure. The polypeptide with molecular mass 94 KDa increased approximately by ~50%, while there was a considerable increase in intensity of a polypeptide of molecular mass 56.5 KDa. The exact nature of enhancements in these protein bands in SDS-PAGE is not known. However, it is very clear that UV-B exposure did induce specific degradation of 85.5 KDa anchor polypeptide of PBS. While the nature of intensification of 94 KDa band by UV-B (Fig.4) is not totally clear, it is possible that this increase may be due to some enhanced synthesis of this protein. The total disappearace of the 85.5 KDa polypeptide signifies the specific UV-B induced changes in the *Spirulina* PBS. Besides these three, no other polypeptides seemed to be affected by UV-B. Thus, our result show for the first time that the in vivo UV-B radiation of cells causes a specific degradation of 85.5 KDa anchor polypeptide in *Spirulina* PBS. In view of the specific alteration in the PBS polypeptides and changes in absorption and emission characteristics, the CD spectra of dark control and UV-B exposed PBS were, monitored. As shown in Fig.5, the control PBS visible CD spectrum exhibited a trough at 345 nm and a positive band in the visible region of the CD spectrum. The sharp positive CD band due to strong rotational strength at 598 nm and a weak rotational strength represented by a shoulder at 650 nm are conspicuous in the control PBS spectrum. The UV-B exposed cell PBS exhibited a red shift from the 598 nm to 608 nm in the main peak and a 10 nm blue shift from 650 nm to 640 nm at the shoulder. This dramatic change in the CD spectrum seems to arise because of the dissociation of the PBS by UV-B and also possibly of altered protein chromophore interaction of the PC (18).

Recently, Sah *et al.* (18) have shown that exposure of isolated *Synechococcus* PBS to low dose of UV-B of, decreased the α-helicity of PBS apoprotein and induced a drastic change of PBS apoprotein secondary structure. It, thus, appears that exposure of UV-B to *Spirulina* cells also induces similar alterations in the PBS. In summary, we report here, for the first time, that exposure of intact *Spirulina* cells to moderate intensity of UV-B, induces specific loss of a PBS polypeptide of 85.5 KDa which seems to be the anchor polypeptide of PBS (22). UV-B also alters the PC to APC ratio and affects chromophore protein interactions, and alters energy transfer the processes within the PBS as re-

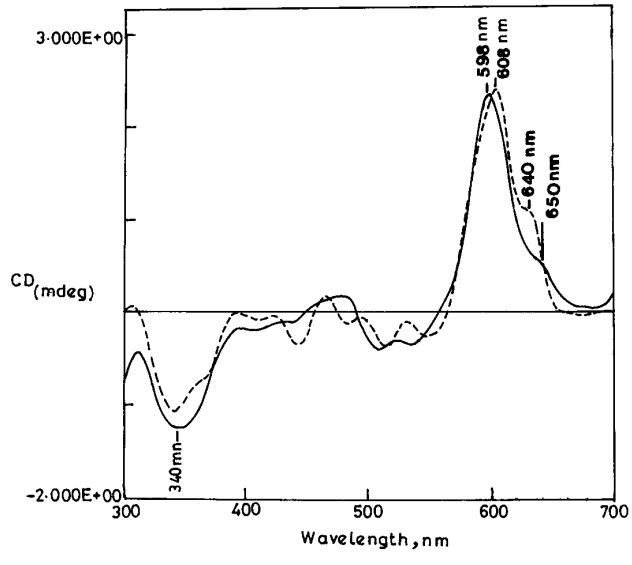

FIG. 5. Room temperature visible CD spectra of PBS of dark incubated control (—) and UV-B exposed (-----) *Spirulina* cells for 9 h. Experimental conditions were as follows: path length 1mm, response 1.0 sec, step size 2.0 nm, scan speed 50 nm/min, ordinate sensitivity 20 mdeg. PBS CD spectra were recorded on equal protein basis.

vealed by the analysis of CD and 77K fluorescence spectra. Thus our results demonstrate that the PBS serve as a specific target for UV-B in some cyanobacterium like *Spirulina*. Thus, enhanced levels of UV-B radiation likely to affect mass culture of *Spirulina* by bringing direct photodamage to the PBS antenna of this organisms.

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