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High-light–induced Changes on Photosynthesis, Pigments, Sugars, Lipids and Antioxidant Enzymes in Freshwater (*Nostoc spongiaeforme*) and Marine (*Phormidium corium*) Cyanobacteria

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ABSTRACT

We studied the effects of high-light exposure (500 μmol m^{-2} s^{-1} of photosynthetic active radiation) on the cyanobacteria *Nostoc spongiaeforme* Agardh, a fresh-water alga, and *Phormidium corium* Agardh (Gomont), a marine alga, with respect to photosynthesis, pigments, sugar content, lipid peroxidation, fatty acids composition, antioxidant enzymes activity and DNA. It was seen that the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm), which is indicative of photosynthetic efficiency, decreased because of the light treatment. The damage to photosynthesis occurred in the antenna system and the photosynthetic II reaction center. Photobleaching of photosynthetic pigments was also observed. High-light treatment also resulted in decreased sugar content, which was probably due to the effect on photosynthesis. Peroxidation of membrane lipids, indicating oxidative damage to lipids and a high level of unsaturation in the cell membrane, was also observed. The activity of antioxidant enzyme superoxide dismutase and ascorbate peroxidase was increased, probably as a result of oxidative damage observed in the form of lipid peroxidation. Quantitative decreases in phospholipid and glycolipid levels were also observed. The level of unsaturated fatty acids in total lipids and glycolipids remained unchanged in both species; however, the level of saturated fatty acids decreased, which slightly changed the ratio in favor of unsaturated fatty acids. Degradation of DNA was also observed in both species. There was a transient plateau 2–4 h after exposure to high-light treatment in the Fv/Fm ratio and in levels of phycobilisome pigments, sugars and antioxidant enzymes after an initial decrease 1 h after the treatment. These findings may indicate a period of partial adaptation to high light that is due to the efficiency of protective processes operational in the two species, which subsequently failed after a longer exposure duration of 4–6 h.

INTRODUCTION

Solar radiation is the ultimate source of energy on earth and conversion of solar radiation into a chemical form of energy only occurs in photosynthetic organisms. Light is one of the most important factors determining the growth of cyanobacteria in their natural habitat. In aquatic ecosystems, solar radiation affects physical, chemical and biological processes, such as thermal stratification and vertical distribution of nutrients, in photoautotrophs, herbivores and consumers. Although primary producers rely on sunlight for photosynthesis, exposure to higher levels of photosynthetic active radiation (PAR) than those required for growth can lead to the inhibition of photosynthesis in algae and plants, particularly during longer period of exposure. This process is known as photoinhibition and it affects photochemical reactions (1–3) by generating active oxygen species, which can oxidize membrane proteins, lipids and pigments, resulting in membrane unstability and photobleaching of the photosynthetic pigments and affecting growth and survivability of the organism. Algae have the same mechanism of photoinhibition as that found in more-complex plants (4). Cyanobacteria populations, particularly those in rice fields in the tropics, are often exposed to high levels of sunlight and investigators have described the photoinhibitory effects of intense PAR in various types of algae, including decreased growth, increased photobleaching of pigments and tissue damage (5,6). Cyanobacteria in general are adapted to grow in conditions of relatively low levels of light and have developed a number of protective mechanisms that permit the optimal use of absorbed light energy while avoiding oxidative damage induced by excessive excitation. These mechanism include enzymatic (7) and nonenzymatic antioxidant systems (8), production of photoprotective compounds (9) and chromatic adaptation due to changes in the content and ratio of phycobilisomes (10).

Cyanobacteria provide a good model system for studies of response and acclimation to various stresses. In this study we investigated the effect of PAR, which exceeds light conditions ideal for growth of cyanobacteria, on photosynthesis, pigments, lipids, fatty acids, sugars, antioxidant enzymes and DNA in *Nostoc spongiaeforme* Agardh, a fresh-water cyanobacteria present in rice fields in India, and *Phormidium corium* Agardh (Gomont), a cyanobacteria isolated from coral reefs off Lakshadweep Island, India.

MATERIALS AND METHODS

Culture conditions. The cultures were grown under routine conditions in autoclaved liquid culture media (BG 11 for fresh-water algae and ASN III for marine algae) according to the methods of Rippka *et al.* (11). Cultures were maintained in 100 mL conical flasks filled to 40% of their volume and kept on a shaker set to a temperature of 30°C ± 2°C. The flasks were exposed to cool white fluorescent tubes providing approximately 80 μmol

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m^{-2} s^{-1} PAR at the culture level, with a 12 h photoperiod. *Nostoc* and *Phormidium* (kindly provided by Dr. C. Raghukumar from National Institute of Oceanography, Goa) were allowed to grow for 14 days and 30 days to obtain their respective logarithmic phase of growth (balance growth phase) (12). The balance growth phase for both the cyanobacteria was determined on the basis of the Fv/Fm ratio, chlorophyll content and fresh and dry weight measurements.

Exposure to high light. The algal cultures was exposed to high irradiance of approximately 500 μmol m^{-2} s^{-1} PAR (Li-cor, Model Li-189) at the culture level for up to 6 h in a double-walled cuvette at a constant temperature of 30°C maintained by controlled circulation of water. The cavity of the cuvette for treating the culture was 2 cm in diameter. Most of the earlier photoinhibition studies involving algae were conducted using solar radiation, which also includes UV radiation, and most of the reported results also pertain to UV radiation. To study the effect of intense PAR, the cyanobacteria were exposed to artificial light source in this study using two slide projectors with a halogen lamp (25 V and 150 W) placed on opposite sides of the culture. Culture was constantly stirred at a slow speed to avoid shading effects during the treatment and to avoid mechanical damage to the cells.

Photosynthesis measurements. The Fv/Fm ratio, which an indicator of photosynthetic efficiency, was measured using a chlorophyll fluorometer (PAM, Walz Germany) according to the protocol of Sharma *et al.* (13). Culture was dark adapted for 10 min before measurement at room temperature. The dark-adapted culture was exposed to modulated light with an intensity of 3–4 μmol m^{-2} s^{-1} to measure initial fluorescence (Fo). This was followed by an exposure to a saturating pulse of white light of 4000 μmol m^{-2} s^{-1} to provide the maximum fluorescence (Fm). Variable fluorescence (Fv) was determined by subtracting Fo from Fm (Fv = Fm – Fo) and the Fv/Fm ratio was calculated.

Extraction of photosynthetic pigments. Cyanobacterial cells were collected after centrifugation of culture at 8000 ***g*** for 15 min. The supernatant was discarded and the pellet of algal cells was dried in lyophilizer (Snijders, Holland). A total of 0.1 g of lyophilized algal cells were extracted in 1 mL 80% vol/vol methanol in a homogenizer at 4°C under dim light, followed by centrifugation at 6000 ***g*** for 10 min at 4°C. The samples were filtered through a 0.2 μm filter before HPLC analysis.

Analysis of photosynthetic pigment. The pigments were separated by HPLC with a reverse-phase column (Waters Spherisorb ODS, 25 μm × 4.6 mm × 250 mm) and a PDA detector (Waters 2996) according to the method described by Sharma and Hall (14). A total of 20 μL of filtered sample was injected into the HPLC. The gradient for separation was 0–100% ethyl acetate in acetonitrile-water (9:1 vol/vol) over 25 min with flow rate of 1.2 mL/min. The quantity of pigments was calculated from peak area value using β-carotene as an external standard. Identification of pigments was performed by comparison of the retention time against standard values and analysis of the spectral profile of individual peaks with a PDA detector in the range of 400–700 nm.

Harvesting the algal tissue for various analysis. Algal culture (control or treated) was harvested by centrifuging at 6000 ***g***. The algal pellet was homogenized in a tissue homogenizer and redissolved in fresh culture medium with a ratio of 1:5 (wt/vol). The resuspended pellet was involved in the various analyses discussed in this article.

Estimation of phycobilisomes. Cell samples were concentrated by centrifugation for 15 min at 6000 ***g***, 0.1 g pellet was resuspended in 5 mL of 20 m*M* sodium acetate buffer (pH 5.5) and cells were broken using sonicator (Bandelin UW 2200, Germany) at 50% power with 9 cycles for 1 min. Phycobilisomes were precipitated by incubation with 1% streptomycin sulphate (wt/vol) for 30 min at 4°C and were collected by centrifugation at 8000 ***g*** for 30 min at 4°C. The amount of phycocyanin, allophycocyanin and phycoerythrin were calculated according to the methods of Liotenberg *et al.* (15).

Estimation of chlorophyll a. Cell suspension (1 mL) was centrifuged at 10 000 ***g*** for 10 min at 4°C and the pellet was extracted in 5 mL of methanol (90% vol/vol) followed by centrifugation at 10 000 ***g*** for 10 min at 4°C. The chlorophyll *a* content was calculated from the absorbance of the methanolic extract at 665 nm according to the method described by Tandeau de Marsac and Houmard (16).

Estimation of total sugar content. Total sugar content in resuspended algal suspension (0.5 mL) was determined using anthrone reagent according to the method described by Plummer (17). A total of 4 mL of anthrone reagent (2% wt/vol) in concentrated sulphuric acid was added to the cell suspension and incubated at 100°C in a water bath for 10 min. The mixture was cooled and the absorbance was read at 620 nm by means of a double-beam UV-Visible spectrophotometer (Schimadzu, 2450). Total sugar content was calculated using a standard curve established with a glucose solution.

Estimation of reducing sugar content. The amount of reducing sugars present in the sample was determined using 2-4,dinitrosalicylic acid according to the method of Plummer (17). Resuspended algal suspension (1 mL) was mixed with 2 mL of distilled water and 1 mL of dinitrosalicylic acid reagent (4% dissolved in alkaline sodium potassium tartarate) and boiled for 5 min in a water bath. The mixture was cooled at room temperature and the level of extinction was measured at 540 nm. The total sugar content was calculated using a standard curve established with a glucose solution.

Determination of glycogen content. Glycogen content was determined according to the protocol of Liotenberg *et al.* (15). Resuspended algal suspension (1 mL) was hydrolyzed by boiling for 20 min with 50 μL of 50% sulphuric acid to which 5 mL of O-Toluidine reagent was added. Samples were boiled for 10 min and cooled on ice. The concentration of glucose released by the acid hydrolysis was calculated from the absorbance at 635 nm using a standard graph established with a glucose solution.

Peroxidation of cell membrane lipids. Lipid peroxidation was determined by measuring the production of TBA-MDA (malondialdehyde) adduct formation, which signifies the formation of polyunsaturated fatty acid peroxides in biological systems, according to method described by Sharma *et al.* (13). A total of 5 mL of resuspended algal culture was again centrifuged and the algal pellet was homogenized in 0.5% trichloroacetic acid (TCA). The homogenate was made up to 5 mL of final volume with TCA and centrifuged at 6000 ***g*** for 15 min. The supernatant was collected and used for measuring the peroxidation of membrane lipids. A total of 1 mL of supernatant was added to a test tube containing 2.5 mL of freshly prepared 0.5% thiobarbaturic acid (TBA) in 20% TCA and allowed to incubate for 30 min at 90°C in a water bath. After incubation, the supernatant was allowed to cool at room temperature and was centrifuged for 2 min at 1000 ***g*** to settle the debris and nonspecific precipitate. The optical density was measured at 532–600 nm with a UV-visual spectrophotometer (Schimadzu UV-2450). Peroxidation of lipids was measured using an extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Extraction of total lipids. Total lipids were extracted according to the methods of Turnham and Northcote (18). Freshly harvested algal pellets (8 g) were boiled in 5 mL of isopropanol for 2 min to inhibit the lipase activity and were then dried under nitrogen gas. The dried pellet was homogenized in chloroform-methanol (1:2 vol/vol) to achieve a final volume of 15 mL with 0.01% BHT added as an antioxidant in the lipid-extraction solvent system. Lipid extract was centrifuged for 5 min at 2000 ***g*** to remove cell debris. A total of 0.8 mL of distilled water was added the to supernatant, followed by 5 mL of chloroform and 5 mL of 0.88% potassium chloride in a separating funnel to achieve a chloroform-methanol-water ratio of 1:1:0.9. The mixture was shaken vigorously for 5 min and allowed to separate for 30 min. The solvent phase was collected and concentrated under nitrogen gas. The dried lipid extract was redissolved in 5.0 mL of chloroform and used for quantitative determination of different class of lipids.

Estimation of glycolipid level. The level of glycolipids was estimated by determining the sugar content in total lipid extract according to the method of Kushawa and Kates (19). The absorbance of the orange color was read at 490 nm against a reagent blank. The amount of sugars in the unknown sample was calculated using a standard curve established with glucose solution.

Estimation of phospholipids level. The level of phospholipids was estimated by determining the amount of phosphorus in total lipid extract according to the Bartlett method described by Christie (20). A series of standard samples using sodium biphosphate was analyzed at 830 nm and used as a standard curve for the quantitation.

Esterification of fatty acids. Fatty acid methyl esters were prepared for gas chromatograph (GC) analysis according to the protocol of Christie (20). The internal standard (1 m*M* heptadeconoic acid) was added to the lipid sample and was subjected to methanolysis in the presence of methanoic-HCl at 68–70°C for 2 h. The methyl esters were extracted with three successive portions of hexane and treated with 5 mL of saturated solution of sodium bicarbonate and washed with 5 mL of distilled water. The upper (hexane) solution was evaporated to dryness in a water bath at 35–40°C with the help of nitrogen gas. The methyl esters were placed in a small volume of fresh hexane and 2 μL of sample was injected into the injector port of the gas chromatograph (GC-Nucon).

Assay of superoxide dismutase (SOD) level. The SOD level was assayed according to the method of Boveris (21). A wet pellet of alga (0.5 g) was

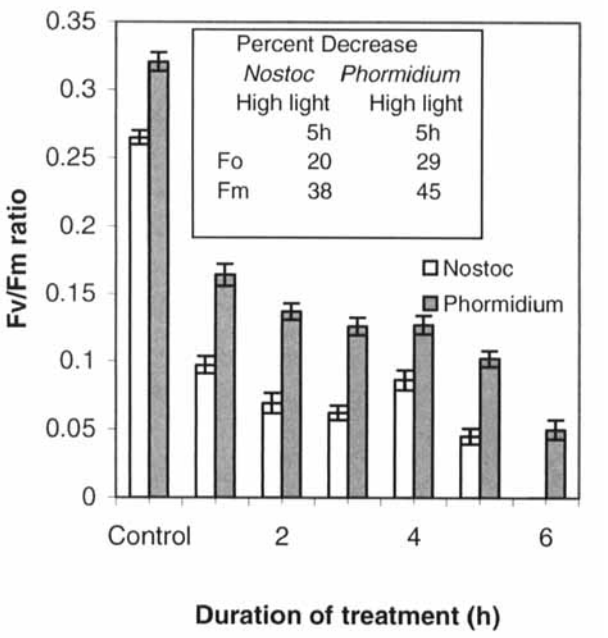

Figure 1. Effect of high light administered for up to 6 h on the Fv/Fm ratio in *Nostoc spongiaeforme* and *Phormidium corium*. Each bar represents mean ± SD ($n = 6$). Inset show the percent decrease observed in both Fo and Fm, compared with control.

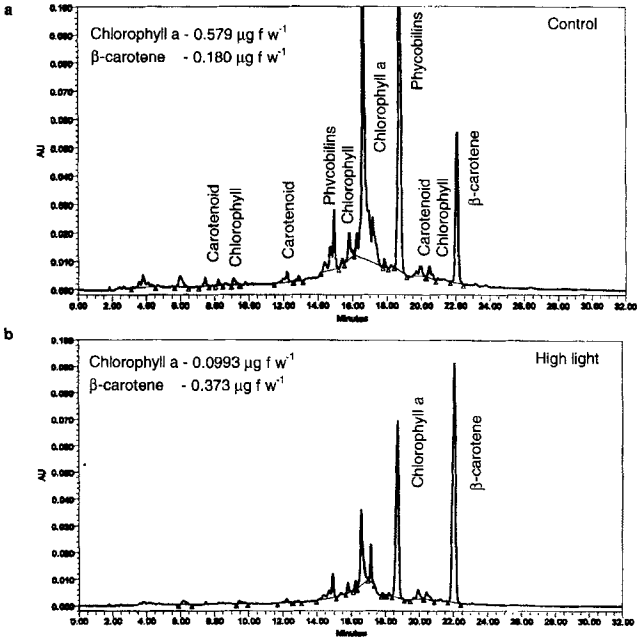

Figure 2. HPLC profile of photosynthetic pigments at 445 nm in control (a) and after exposure to high light for 6 h (b) in *Nostoc spongiaeforme*. Numbers show the quantity of the respective molecules calculated using β-carotene as external standard on the peak area basis.

extracted in 5 mL of 50 m*M* sodium dihydrogen phosphate buffer by means of a tissue homogenizer. The extract was centrifuged for 10 min at 6000 ***g*** and supernatant was used for SOD assay. A total of 100 µL of tissue extract was added to 2.6 mL of assay buffer containing 6 m*M* ethylenediaminetetraacetic acid (EDTA) in 10 m*M* sodium carbonate buffer (pH 10.2) and 300 µL of 4.5 m*M* epinephrine. Absorbance was recorded at 480 nm. A set of standard with epinephrine but without tissue extract was also assayed separately to calculate the activity. The protein concentration of the supernatant was determined using the method of Lowry *et al.* (22).

Assay of ascorbate peroxidase (APX). Peroxidase activity was assayed according to the protocol of Sankhalkar and Sharma (23). A wet pellet of alga (0.5 g) was extracted in 5 mL of 100 m*M* sodium dihydrogen phosphate buffer (pH 7.5), 1 m*M* EDTA and 5 m*M* ascorbate by means of a tissue homogenizer. The extract was centrifuged for 15 min at 6000 ***g*** at 4°C and the supernatant was used for peroxidase assay. Activity was assayed in a reaction mixture containing potassium hydrogen phosphate (100 m*M*), EDTA (1 m*M*), sodium ascorbate (1.5 m*M*), hydrogen peroxide (10 m*M*) and tissue extract. The decrease in absorbance was recorded for 3 min at 290 nm. Protein concentration of the supernatant was determined according to the method of Lowry *et al.* (22).

DNA isolation and separation. DNA was isolated using hexadecyltrimethyl ammonium bromide (CTAB) according to the protocol of Kaufmann *et al.* (24).

RESULTS

Effect on chlorophyll fluorescence

Figure 1 shows the effects of high light on chlorophyll fluorescence (*i.e.* the Fv/Fm ratio) in *N. spongiaeforme* and *P. corium*. There was an initial decrease in the Fv/Fm ratio during the first 2 h after the onset of light treatment, followed by either a slight increase, as in the case of *Nostoc* organisms, or a plateau, as in the case of *Phormidium* organisms. A subsequent longer duration treatment resulted in a much greater decrease in the Fv/Fm ratio for *N. spongiaeforme* and *P. corium*. High-light treatment for 6 h resulted in complete inhibition of the Fv/Fm ratio in *Nostoc spongiaeforme*, whereas an 84% decrease was observed in *P. corium*.

Effect on photosynthetic pigments

The effect of high light on photosynthetic pigments in *N. spongiaeforme* and *P. corium* was studied using HPLC (Figs. 2 and 3). The different types of photosynthetic pigments present were chlorophyll *a*, β-carotene, phycobilins and xanthophylls. As a result of the high-light treatment, in *Nostoc* organisms chlorophyll *a* content decreased considerably, the phycobilin level decreased and β-carotene content increased considerably, whereas in *Phormidium* species chlorophyll *a* content decreased to lesser extent but the phycobilin level and β-carotene content decreased considerably.

Effect on phycobilins

The effect of high light on phycobilins was studied in *N. spongiaeforme* and *P. corium* (Fig. 4). High-light treatment of *N. spongiaeforme* showed a maximum decrease of 93% in phycocyanin level, 75% in allophycocyanin level and 69% in phycoerythrin level after 6 h of treatment, as compared with controls (Fig. 4a). The decrease was not linear with respect to the duration of treatment. Phycoerythrin content remained similar for the first 3 h of treatment and then decreased slight during the next 2 h, after which a rapid decrease was seen in *Nostoc* organisms. Similarly, the decrease in allophycocyanin level during the first 3 h after treatment was only 10%; a slightly greater decrease was observed during the next 2 h, after which the decrease was very rapid. The phycocyanin level decreased by 60% during the first hour of treatment, after which it was stable until the fourth hour of treatment and subsequently decreased rapidly. In *Phormidium* organisms, more or less similar results were observed except that

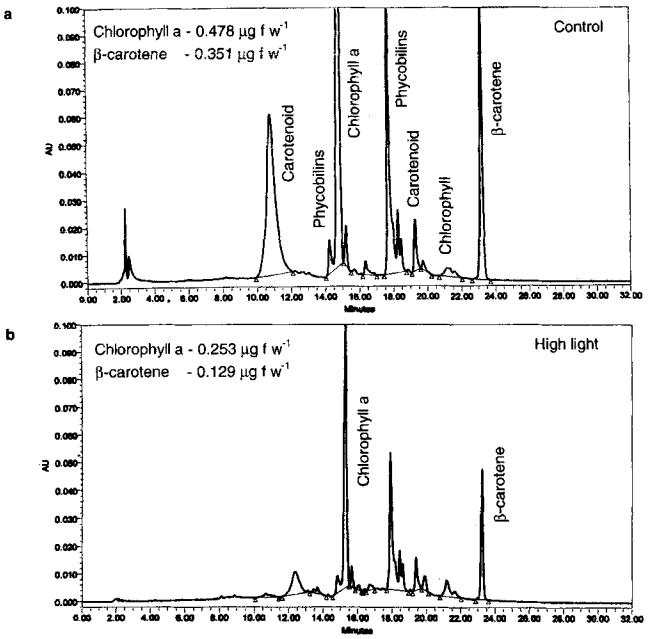

Figure 3. HPLC profile of photosynthetic pigments at 445 nm in control (a) and after exposure to high light for 6 h (b) in *Phormidium corium*. Numbers show the quantity of the respective molecules calculated using β-carotene as external standard on the peak area basis.

the phycocyanin content in *Phormidium* organisms was much greater than that observed in *Nostoc* organisms (Fig. 4b).

Figure 4c shows the effect of high light on chlorophyll *a* content. Chlorophyll *a* content decreased in both species of cyanobacteria as a result of high-light treatment.

Effect on sugars

High light intensity resulted in decreased sugar content in both *N. spongiaeforme* and *P. corium* (Fig. 5). Six hours of high-light treatment resulted in a 66% decrease in the total sugar content in *N. spongiaeforme*, compared with a 42% decrease in *P. corium*, compared with their respective controls (Fig. 5a). High-light treatment decreased reducing sugar content in both species (Fig. 5b). Reducing sugar content during 6 h of treatment was less affected in *N. spongiaeforme* (55%) than in *P. corium* (74%), compared with their respective controls. High-light treatment resulted in similar decreases (60%) in the glycogen content in both species (Fig. 5c). The transient increase or stability was also observed in reducing sugar and glycogen contents within 2–3 h after initiation of treatment in both the species studied in a generally decreasing trend.

Effect on peroxidation of lipids

Peroxidation of membrane lipids (measured with on the basis of MDA formation) was observed in both *P. corium* and *N. spongiaeforme* as a result of the high-light treatment (Fig. 6). High-light treatment administered for 6 h resulted in a 40% increase in the peroxidation level in membrane lipids in *N. spongiaeforme* and a 52% increase in *P. corium*, compared with their respective controls.

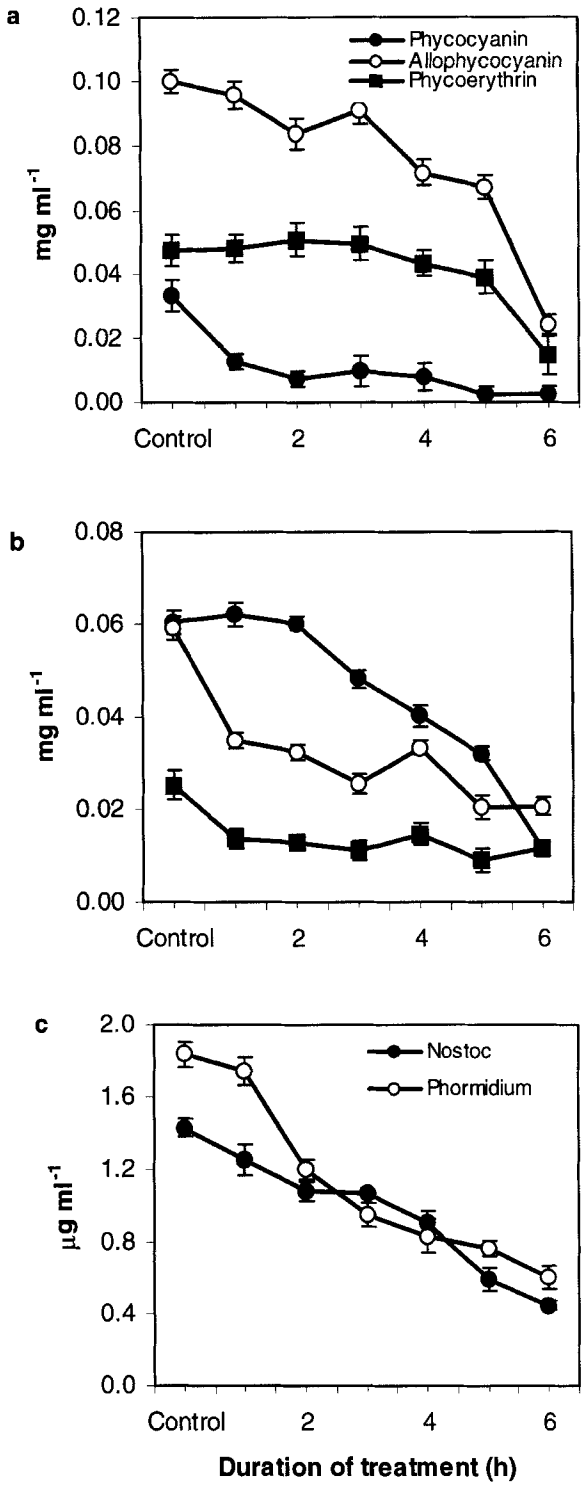

Figure 4. Effect of high light administered for up to 6 h on phycobilins in *Nostoc spongiaeforme* (a) and *Phormidium corium* (b) and chlorophyll *a* concentration in both species (c). Each bar represents mean ± SD (n = 3–6).

Effect on glycolipids and phospholipids

P. corium had greater quantitative decreases in glycolipid and phospholipid content than those observed in *N. spongiaeforme* (Fig. 7). High-light treatment for 6 h resulted in a 55% decrease in the glycolipid content in *Phormidium* organisms, whereas the

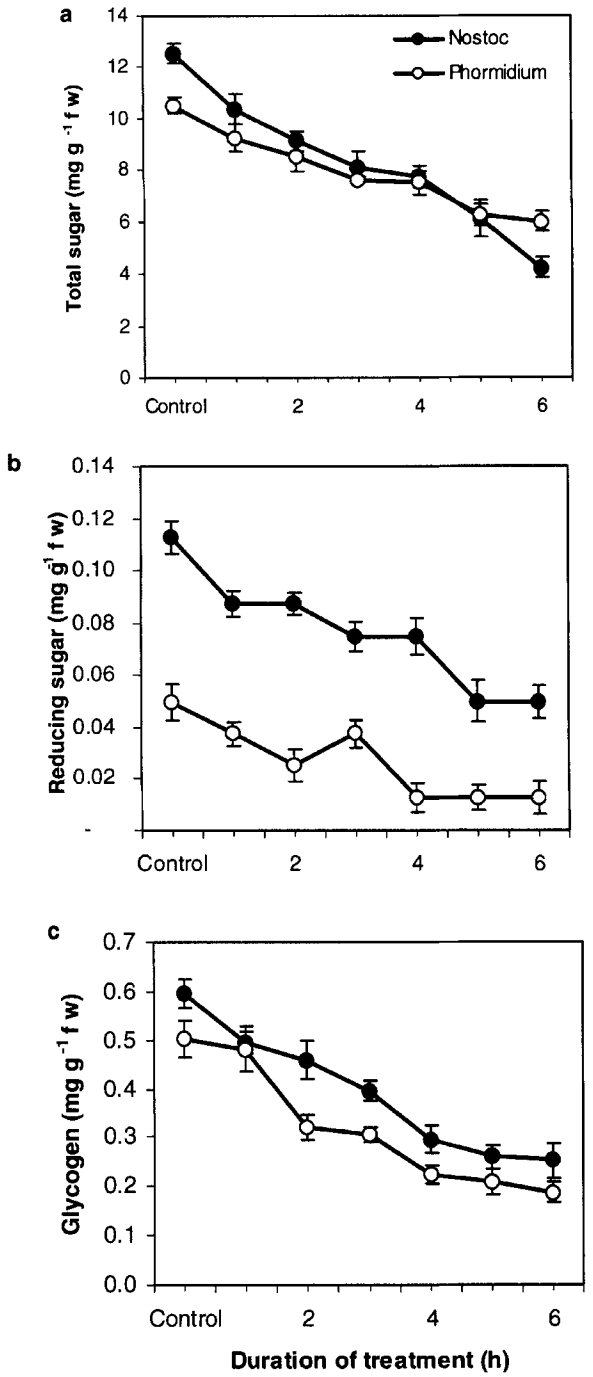

Figure 5. Effect of high light administered for up to 6 h on levels of total sugar (a), reducing sugar (b) and glycogen (c) in *Nostoc spongiaeforme* and *Phormidium corium*. Each bar represents mean ± SD (n = 4).

decrease in *Nostoc* organisms was 35% during the same duration of treatment (Fig. 7a). Similarly, the phospholipid content decreased by 46% in *Phormidium* organisms, compared with only a 17% decrease in *Nostoc* organisms (Fig. 7b).

Effect on fatty acid

In both species, the fatty acid groups present were lauric acid C_{12}, myristic acid C_{14}, palmitic acid C_{16}, stearic acid C_{18}, oleic acid $C_{18:1}$, linoleic acid $C_{18:2}$ and linolenic acid $C_{18:3}$. In

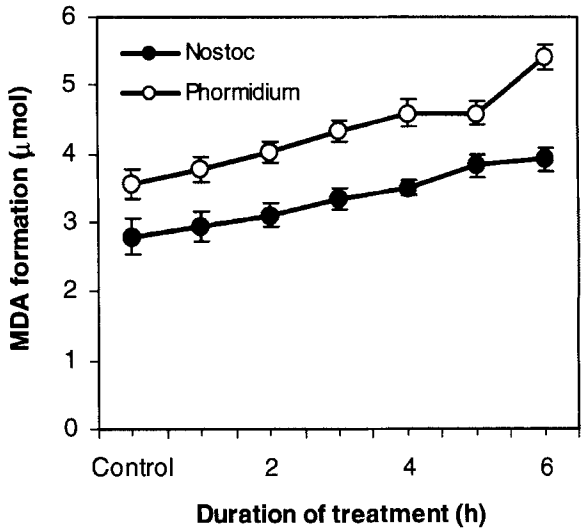

Figure 6. Effect of high light administered for up to 6 h on lipid peroxidation in *Nostoc spongiaeforme* and *Phormidium corium*. Each bar represents mean ± SD (n = 5).

N. spongiaeforme high-light resulted in a slight decrease in levels of saturated fatty acids and no appreciable change in levels of unsaturated fatty acids, whereas levels of both saturated and unsaturated fatty acids remained virtually unchanged in *P. corium* (Tables 1 and 2).

Effect on SOD and APX

An increase in the activity of SOD and APX was observed in both species as a result of high-light treatment (Fig. 8). SOD activity was relatively higher in *Phormidium* organisms, whereas APX activity was relatively higher in *Nostoc* organisms. Six hours of treatment resulted in 28% and 48% increases in the activity of SOD in *N. spongiaeforme* and *P. corium*, respectively, compared with their respective controls (Fig. 8a).

APX activity increased by 46% in *N. spongiaeforme* and by 35% in *P. corium* after 3 h of high-light treatment. However, additional treatment resulted in a decrease in APX activity in both cyanobacteria (Fig. 8b).

Effect on DNA

Degradation of DNA after 6 h of high-light treatment was observed in both species. Some of the effect we observed (largely in *Nostoc* organisms) might also have been due to storage of the isolated DNA overnight (Fig. 9).

DISCUSSION

Both cyanobacteria differed in their fluorescence parameters and their sensitivity to high-light exposure. High-light treatment resulted in a decrease in photosynthetic efficiency, which was due to a decrease in Fo and Fm. A decrease in Fo is an indicator of decreased excitation energy reaching the photosynthetic II (PS II) reaction center, whereas a decrease in Fm is an indicator of damage to the PS II reaction center itself. The Fv/Fm ratio shows that the cells suffered significant photoinhibition, apparently in two phases: a big decrease occurred during the first hour of treatment and an additional decrease occurred 4–6 h after treatment initiation. Similar decreases in the Fv/Fm ratio in other cyanobacteria

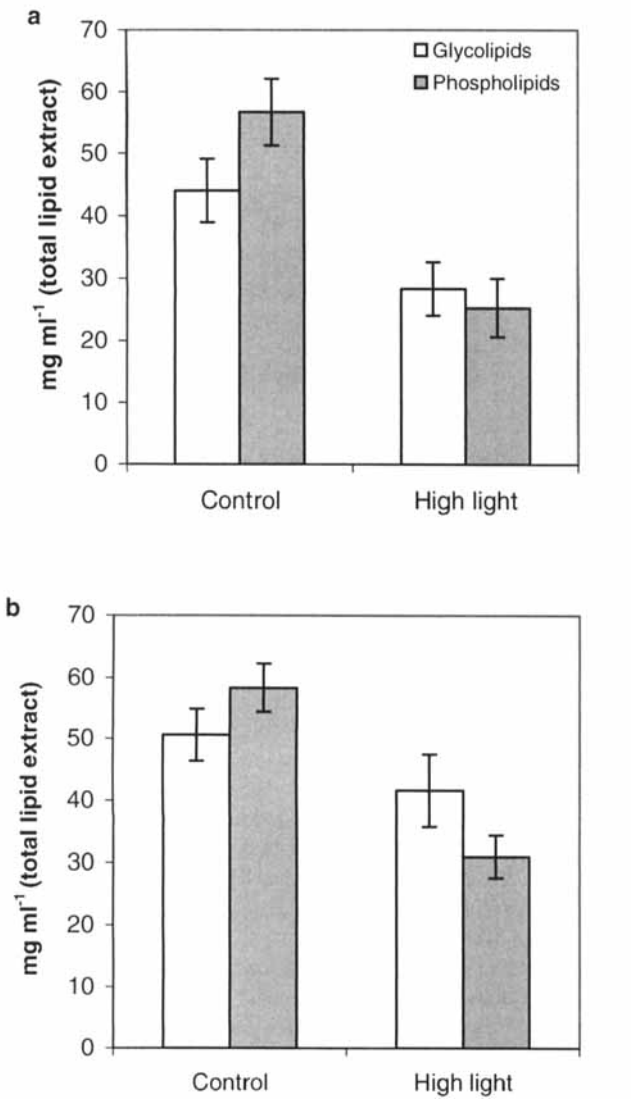

Figure 7. Effect of high light up to 6 h on glycolipid and phospholipid content in *Nostoc spongiaeforme* (a) and *Phormidium corium* (b). Each bar represents mean ± SD ($n = 3$).

correlate with the accumulation of reactive radicals (25) and result from photoinhibition of the PS II repair cycle and of PS II function itself (26).

Herrman *et al.* (27) reported that solar radiation caused strong photoinhibition even after a short period of exposure. A reduction in the photosynthetic activity of algae near midday was also observed under field and laboratory conditions (28). Decreases in photosynthetic efficiency and changes in the pigments due to white light in green alga *Spongiochloris* species (29), *Chlorella* and *Scenedesmus* species (30) and the red alga *Dunaliella salina* (31) were also reported. All of these effects on photosynthesis affect the productivity of this process.

The decreases in almost all photosynthetic pigments except for β-carotene in *Nostoc* organisms observed in this study may well be due to oxidation of pigments under conditions of excess light. The greater decrease in chlorophyll *a* content due to high light in *Nostoc* organisms in our study may also be related to the greater decrease in the Fv/Fm ratio in this species. The observed increase in the β-carotene content in *Nostoc* organisms may be related to

Table 1. Effect of high light on total and glycolipid fatty acids in *Nostoc spongiaeforme*

	Mean total lipid level (% mole), by study group		Glycolipid level (% mole), by study group	
Fatty acid	Control	High light	Control	High light
Lauric acid (C_{12})	11.4 ± 0.5	10.3 ± 0.4	17.9 ± 0.8	16.7 ± 0.6
Myristic acid (C_{14})	18.2 ± 0.8	17.6 ± 0.7	20.6 ± 0.6	19.4 ± 0.8
Palmatic acid (C_{16})	15.4 ± 0.7	14.8 ± 0.7	15.1 ± 0.6	14.1 ± 0.7
Stearic acid (C_{18})	9.5 ± 0.4	9.1 ± 0.6	25.6 ± 0.9	25.3 ± 0.8
Oleic acid ($C_{18:1}$)	3.4 ± 0.3	3.4 ± 0.3	8.8 ± 0.5	8.8 ± 0.3
Linoleic acid ($C_{18:2}$)	20.9 ± 0.9	20.7 ± 0.8	37.7 ± 0.7	37.7 ± 0.8
Linolenic acid ($C_{18:3}$)	3.5 ± 0.2	3.5 ± 0.3	9.2 ± 0.4	9.2 ± 0.3

Data are mean ± SD.

protection and/or adaptation against high light during the initial stages of photoinhibition, resulting in a nonlinear decrease in the Fv/Fm ratio (transient plateau), because β-carotene can act as a nonenzymatic antioxidant that can quench reactive oxygen species. *Phormidium* organisms had a relatively high phycocyanin content, which made them more susceptible to high-light damage than *Nostoc* organisms, which have a far lower phycocyanin content. The dramatic decrease in phycocyanin content after 1 h of high-light treatment may also play a role in the subsequent transient increase in the Fv/Fm ratio. A slight transient increase in the allophycocyanin level and a plateau in the phycoerythrin level during the early stages of photoinhibition in both *Nostoc* and *Phormidium* could also be important with respect to transient increase and plateau observed in *Nostoc* and *Phormidium*.

Han *et al.* (5) reported a significant percent decrease in levels of chlorophyll *a* and phycocyanin in *Anabaena* species after exposure to intense PAR and suggested that it might be an adaptation strategy to prevent absorption of excess energy by the organism. Xenopoulos *et al.* (32) reported that the chlorophyll *a* concentration in phytoplankton decreased rapidly because of exposure to natural light and a short-lived ozone-thinning period. The accessory light-harvesting pigment phycocyanin was bleached more rapidly and drastically than were phycoerythrin and chlorophyll *a* during exposure to solar radiation (33). Neidhardt *et al.* (31) also observed that high-light resulted in lower pigment content, a highly truncated chlorophyll antenna size and accumulation of photo-damaged PS II centers in the chloroplast thylakoids in *Dunaliella salina* cells. They also suggested the possibility of maximizing photosynthetic productivity and light use by minimizing the light-

Table 2. Effect of high light on total and glycolipid fatty acids in *Phormidium corium*

	Total lipid level (% mole), by study group		Glycolipid level (% mole), by study group	
Fatty acid	Control	High light	Control	High light
Lauric acid (C_{12})	11.3 ± 0.4	11.3 ± 0.6	11.6 ± 0.5	11.6 ± 0.5
Myristic acid (C_{14})	17.6 ± 0.7	17.5 ± 0.6	13.4 ± 0.5	13.8 ± 0.5
Palmatic acid (C_{16})	14.8 ± 0.8	14.7 ± 0.7	9.8 ± 0.3	9.8 ± 0.4
Stearic acid (C_{18})	9.1 ± 0.4	9.0 ± 0.5	16.6 ± 0.7	16.6 ± 0.6
Oleic acid ($C_{18:1}$)	3.2 ± 0.1	3.2 ± 0.2	5.7 ± 0.4	5.7 ± 0.3
Linoleic acid ($C_{18:2}$)	19.9 ± 0.5	19.9 ± 0.4	35.0 ± 0.8	35.0 ± 0.9
Linolenic acid ($C_{18:3}$)	3.3 ± 0.2	3.3 ± 0.4	5.9 ± 0.4	5.9 ± 0.3

Data are mean ± SD.

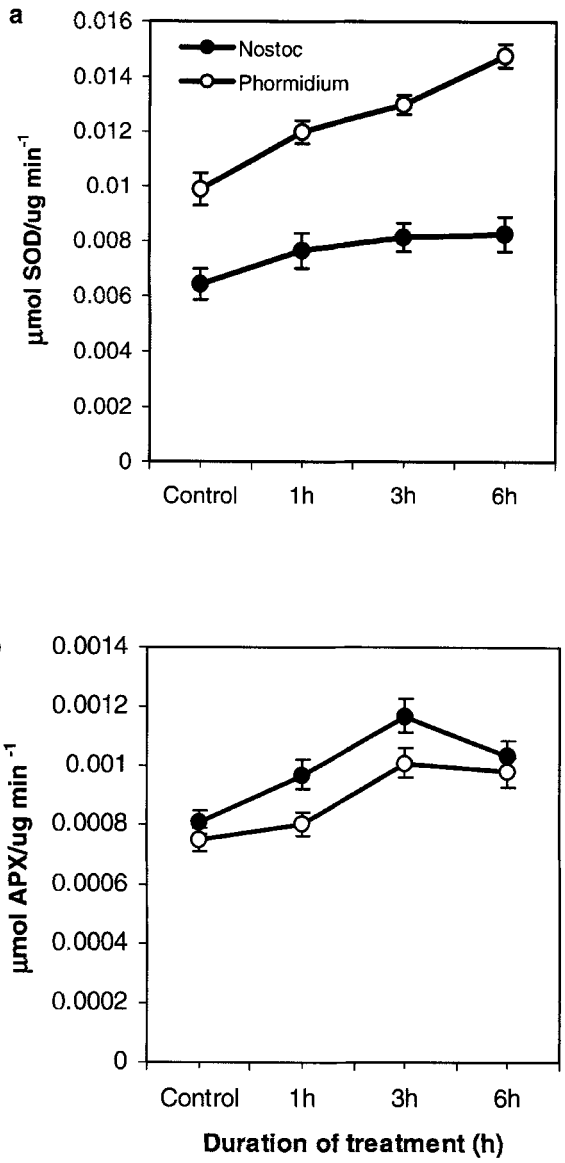

Figure 8. Effect of high light administered for up to 6 h on superoxide dismutase (a) and ascorbate peroxidase (b) activity in *Nostoc spongiaeforme* and *Phormidium corium*. Each bar represents mean ± SD ($n = 5$).

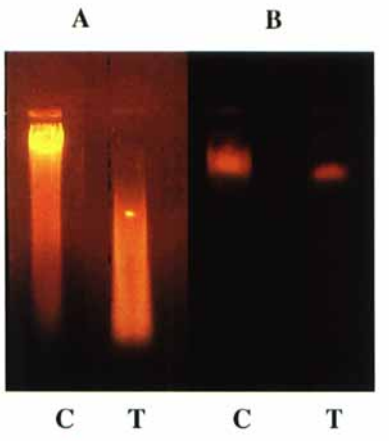

Figure 9. Effect of high light administered for up to 6 h on DNA in *Nostoc spongiaeforme* (a) and *Phormidium corium* (b). C represents the control and T represent 6 h of high light treatment.

harvesting chlorophyll antenna size, thereby indicating that, if light absorption can be reduced by pigments (chlorophyll *a* and phycobilins) at the antenna level, than the extent of photoinhibition can be decreased. Nomsawai *et al.* (34) also reported a decrease in the number of phycobilisomes (phycocyanin and allophycocyanin) when cells of *Spirulina platensis* were shifted from low light to high light. They also observed the disappearance of large phycocyanin transcripts and 33 kDa linker polypeptides of phycobilisomes. Schwarz and Grossman (35) reported that *Synechococcus* nbIR mutant cells were unable to properly modulate the phycobilisome level and died rapidly because of the inability to degrade its light-harvesting complex when exposed to high light. The nbIR mutant cells, which also had more phycobilisomes than wild-type cells, were unable to properly modulate the phycobilisome level during exposure to high light, which compromised their survival. Enlargement of chlorophyll antenna size and an increase in the chlorophyll *a* level after shifting *D. salina* cells from high light to low light was reported by Masuda *et al.* (36), which suggests that these pigments might protect against photoinhibition by limiting the overload of excitation energy and reverting back during exposure to decreased or normal levels of light.

The decrease in photosynthetic pigments could be due to the generation of active oxygen species, as observed as increased MDA formation in this study, because pigments are highly sensitive to oxidation and peroxidation reactions (2). The production of ROS in algae under high-light conditions has been reported (25). This research suggests that changes in the accessory light harvesting pigments, such as phycobilisomes, in order to limit absorption of excess light may prevent photodamage against excess high light by reducing the antenna size, as well as light absorption.

The decrease in the sugar content due to high light in both *N. spongiaeforme* and *P. corium* is largely the result of damage caused to photosynthesis by the treatments, because the decrease in the level of reducing sugar was much greater than that of total sugar (Fig. 5). We have reported that high-light treatment resulted in a decreased Fv/Fm ratio and damage to the lipid membrane and both of these effects (one resulted in decreased photosynthesis and the resulted in a greater maintenance cost) collectively could result in decreased levels of reducing and total sugar. However, studies are lacking on the impact of high light on changes in the carbohydrate status. Work by De Philippis *et al.* (37) showed that increases in glycogen content occurred in *Spirulina maxima* after shifting the culture from low light to high light for a duration of 200 h.

High light resulted in an increased level of oxidative damage to the cell membrane, which may have affected the fluidity of membrane and, in turn, various other biochemical processes, including photosynthesis. Lipids are one of the most sensitive targets for oxidation by elevated reactive oxygen species, particularly at sites where polyunsaturated fatty acids occur in high concentrations. Increased formation of reactive oxygen species by stress factors, such as high light, UV radiation and temperature, is a common process in living organisms, including algae. Although formation of these compounds is a normal part of the metabolism, excessive production can cause damage to DNA, proteins and lipids (38). UV-B radiation is known to cause oxidation of membrane lipids (39). He and Häder (40) observed induction of reactive oxygen species under *in vivo* condition due to UV-B stress in the cyanobacterium *Anabaena*, which was further

enhanced when photochemical reactions of PS II were impaired. The quantitative decrease in phospholipids and glycolipids observed with high light treatment in our study might largely be due to such oxidative damage (Fig. 7). Part of the damage to phospholipids and glycolipids could also be due to impairment of enzymes involved in lipid synthesis during stress conditions (41). They attributed the survival of algae under higher irradiance conditions to membrane fluidity due to a higher level of fatty acid unsaturation. Tedesco and Duerr (42) also shown that high light slightly increased total lipids and percent composition of poly-unsaturated fatty acids, such as γ-linolenic acid, in *S. platensis*. Decreases in the levels of phospholipids and glycolipids, changes in fatty acid composition and changes in the unsaturation level after high-light exposure have been reported in microalgae (43,44). Khotimchenko and Yakovleva (45) reported variations in the lipid composition of the marine green alga *Ulva fenestrata* under different irradiance conditions, which suggests that changes in lipid composition can be considered as a mechanism of adaptation and survival of thalli subjected to variations in solar irradiance. Dawes *et al.* (46) observed that light intensity primarily influenced the content of saturated and tetraunsaturated fatty acids in *Ulva pertusa*. Walsh *et al.* (47) reported that the changes in the fatty acid composition due to high levels of light intensity occurred as a cellular response to reduce the susceptibility of the cyanobacterial membranes to photo-oxidation. Slight decreases observed in saturated fatty acids in *Nostoc* organisms in our study would change the ratio of fatty acids in membranes toward the unsaturation level, leading to increased membrane fluidity and adaptation to high-light conditions. However, changes in fatty acid composition in response to stress conditions still remain a less studied process.

There are a number of defense mechanism against reactive oxygen species, all of which can be categorized into two major groups: enzymatic and nonenzymatic antioxidant systems. This study involved the enzymatic antioxidant response and the increase in the SOD and the APX activity in response to the high-light treatment indicates that it plays an important role in an organism's defense mechanism, because these enzymes remove active oxygen species generated due to excess reduction of the photosynthetic electron transport under excess light conditions. Increased SOD activity has been shown to confer increased protection from oxidative damage. Krause (1) and Foyer *et al.* (48) observed that antioxidant enzymes play an important role in detoxifying ROS and regenerating the reduced forms of the antioxidant. Jansen *et al.* (49) observed higher tolerance against high-light stress in plants by overproducing SOD. Mackerness *et al.* (50) observed that activities of APX, glutathione reductase and SOD were increased in response to UVB irradiation and were higher under high light in pea buds in response to the increased ROS levels. Also, Sen Gupta *et al.* (51) observed higher SOD activity due to environmental stress. It has been reported that even a moderate increase in SOD activity led to improved tolerance to oxidative stress. Similar results have also been reported in algae. Lornmann *et al.* (52) have shown in red algae that increase in tolerance against environmental stresses was attributed to greater content or activities of antioxidant enzymes SOD, APX and GR. Barros *et al.* (53) reported greater increase in SOD, which prevented peroxidation of lipids under higher ROS conditions but no increase in CAT and APX activity, indicating that a particular antioxidant enzyme may be specific to a particular algae. Our data indicate partial adaptation to high light over a short period in both *Nostoc* and *Phormidium* organisms. This adaptation may be result of protective/recovery processes. Our results with antioxidant enzymes indicate that APX activity increased initially for up to 3 h after initiation of treatment and decreased subsequently, which may help in protecting against oxidative damage during the short duration of treatment. We also observed a slight increase in the SOD in *Nostoc* organisms, compared with greater increase in *Phormidium* organisms. A greater decrease in the photosynthetic efficiency (*i.e.* the Fv/Fm ratio) in *Nostoc versus Phormidium* orgnanisms could also be due to the difference in the SOD level between the two species.

High light intensity resulted in damage to DNA, maybe because of the formation of dimers between adjacent pyrimidine bases or perhaps because of radical damage to the DNA. However, further work is needed to identify the actual cause and mechanism of degradation.

Work presented here shows that high light caused oxidative damages affecting photosynthesis, probably through an effect on photosynthetic pigments in the antenna complex and on membrane integrity. The observed effect may be a consequence of the generation of reactive oxygen species as a result of the high-light treatment. Our work also showed that, in response to oxidative damage, the activity of SOD and APX increased to provide protection against the damage. The transient plateau in the Fv/Fm ratio and levels of phycobilisome pigments, sugars and antioxidant enzymes in the cells of these two species after initial decreases due to treatment may indicate a period of partial adaptation to high light that is due to the combined efficiency of protective processes occurring, which subsequently failed after longer exposure. A role for the fatty acid composition of membranes during photoinhibition is also suggested.

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