Changes of the Photosynthetic Apparatus in *Spirulina* Cyanobacterium by Sodium Stress

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- Z. Naturforsch. 55c, 16-22 (2000); received July 2/September 10, 1999

Thylakoid Membrane, Photosystem I and II, Spirulina, Sodium Stress, Phycobilisomes

Spirulina platensis trichomes grown in Zarrouks medium having total Na^+ concentration as 0.14 M when transfered to fresh Zarrouks medium containing enhanced level of Na^+ ions equal to 0.86 M showed 30% more accumulation of Na^+ intracellularly as compared to the control. An inhibition of photosystem II activity to almost 66% was observed. Also due to this exposure to high Na^+ , the room temperature absorption characteristics of *Spirulina* trichomes and the thylakoid membrane preparations were altered indicating changes in the chromophore protein interactions and alterations in the phycocyanin/allophycocyanin ratio; there by affecting the energy harvest and energy transfer processes. An increase in the carotenoid absorption was two fold over the control in the treated sample. Similarly, room temperature and low temperature (77 K) fluorescence emission spectra collectively suggested alterations in the chlorophyll a emissions, F 726 of photosystem I reflecting changes in the lipid protein environment of the thylakoid. Our results indicate that in *Spirulina* the enhanced Na^+ level alters the energy harvest and transfer processes. It also affected the emission characteristics of chlorophyll a of photosystem I.

Introduction

The ubiquitous distribution of cyanobacteria expose these organisms to a variety of environmental conditions such as high or low temperature, high irradiance, drought and salinity (Schubert and Hagemann, 1990 and Tandeau de Marsac and Houmard, 1993). These organisms are able to survive in extreme conditions by adopting specific adaptive strategies to the stress factors (Joset et al., 1996). For instance, exposure of cells to salt concentrations that are physiologically above those present intracellularly, affect the cells due to increase of the osmotic pressure and the ionic concentration. Under osmotic stress synthesis of osmoprotective compounds such as sucrose, trehalose, glucosylglycerol and glycine betaine takes place as protective measure (Reed et al., 1986). For ionic stress specific mechanism of transporters are known to be expressed (Gorham et al., 1985). Rate of respiration is known to increase under ionic stress (Molitor et al., 1986). It has been also shown that ionic stress is met through enhancement of AT-Pase activity, modification of membrane lipid composition (Joset *et al.*, 1996) and increased cyclic electron transport around PSI (Canaani, 1990; Endo *et al.*, 1995; Hibbino *et al.*, 1996).

At physiological concentration the involvement

the critical demands of cyanobacteria exposed to

At physiological concentration the involvement of Na+ has been found in the uptake system, photosynthetic water oxidation, nitrogenase activation and internal pH regulation (Zeng and Vonshak, 1998 and Congming and Vonshak, 1999). Cations Like Mn²⁺, Ca²⁺ and Mg²⁺ and anions like Cl⁻, HCO₃⁻ are known to regulate photosynthetic electron transport (Debus, 1992). In cyanobacteria, the depletion of both Ca²⁺ and Na⁺ has been shown to alter PS II function (Brand et al., 1983). However the effect of excess sodium ions on the phycobilisome and thylakoid structure is not known. Spirulina is a thermophilic and alkalophilic, non heterocystous filamentous cyanobacterium. Owing to the specific growth conditions (35-37 °C, pH 10) required for this algae its outdoor mass culture is easier. It is an important source for various chemicals. Moreover, synthesis of the important chemicals have been found to be pronounced in the salt-exposed cells. Most of studies

Abbreviations: Chl a, Chlorophyll a; PS II, photosystem II; PS I, photosystem I; PC, phycocyanin; APC, allophycocyanin; PBS phycobilisome.



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carried out on stress effects on *Spirulina* photosynthesis were related to temperature stress, metal ions and UV-B radiation. Accordingly the present investigation was carried out to study the effect of enhanced level of sodium ions on the photosynthetic apparatus of this cyanobacterium.

Our results suggest a modification in the organisation of the thylakoid membrane and the spectral profile of the photosynthetic pigments in response to the enhanced internal sodium level due to exposure of *Spirulina* trichomes to high Na⁺ ions in the culture medium.

Materials and Methods

Pure culture of Spirulina platensis was obtained from Central Food Technological Research Institute, Mysore, India. The trichomes were grown at 26 ± 2 °C in Zarrouk's medium (Zarrouk, 1966) under continuous white light illumination (30 µmol m⁻² sec⁻¹) as described elsewhere (Kolli et al., 1998). The culture medium was continuously bubbled with filtered air. The mid-log phase culture was harvested by centrifugation at 12000 xg for 15 min and was washed twice with fresh growth medium. The washed trichomes were resuspended at a final chlorophyll a concentration of 3 µg ml⁻ ¹ in fresh medium containing excess total sodium ions (0.86 M). This medium was prepared by increasing the amount of NaHCO3, NaCl and NaNO3 in the Zarrouk's medium (initial concentration of Na⁺ is 0.14 m) so that final concentration of total Na⁺ ions is 0.86 M in the medium. Increase in any specific anion content due to additions of the above salts in the medium was considered negligible as compared to Na+ ions. Trichomes suspended in the medium having an enhanced Na+ level were incubated in the dark for 24 h. A parallel set of control cells were also kept. The control and treated trichomes were washed twice in 50 mm Hepes (N-[2-hydroxyethyl] piperazine -N'-[2ethanesulfonic acid]) buffer pH (7.0) for further studies.

In order to determine a suitable concentration of sodium ions which could affect the *Spirulina* trichomes but, is non lethal i.e., a concentration of sodium ions from which the cyanobacterial culture would recover and grown upon transfer to fresh medium lacking enhanced sodium ion level. The treated samples (*Spirulina* trichomes) suspended

in Zarrouk's medium containing excess sodium (0.86 M for 24 h) for sodium stress and the control samples were resuspneded in fresh Zarrouk's medium without excess sodium ion at 0.5 μ g ml⁻¹ chlorophyll *a* concentration and allowed to grow under continuous white light illumination (30 μ Em⁻² sec⁻¹) with constant bubbling of filtered air. The growth of the above two sets were monitored at interval of 48 h in terms of increase in chlorophyll *a*.

Measurement of internal Na+ concentration

The cellular Na⁺ concentration in control and high Na⁺-treated *Spirulina* trichomes were estimated by atomic absorption spectrometry at 589 nm using Philips PU 9200 atomic absorption spectrophotometer. The control and treated cells were washed with 1 mm EDTA and dried at 80 °C followed by digestion with 50% nitric acid for the measurements.

Measurement of pigments and protein

Phycocyanin and allophycocyanin were estimated by the method of Bennet and Bogorad (1980). Pigment and protein content were measured before and after exposure of the cells to the excess Na⁺ ions. Chl a was extracted in methanol and estimated according to Kolli et al. (1998). The total carotenoids were extracted in acetone according to Lichtenthaler (1987). Protein was estimated according to Lowry et al. (1951), using bovine serum albumin (fraction 5) as standard. Intensity of light during growth curve was measured using Li-cor radiometer model (LI–189, Li-cor, USA).

Spectral measurements

Room temperature absorption spectra was measured in the intact trichomes and in the thylakoid membrane preparation at Chl *a* concentration of 5 µg ml⁻¹. The absorption spectra were recorded using Hitachi U-2000 spectrophotometer. Room temperature and 77 K fluorescence emmision spectra were measured in LS-5 Perkin Elmer spectrofluorimeter as described by Sah *et al.* (1998). Chl *a* concentration was 6 µg ml⁻¹ in case of room temperature fluorescence measurements and 3 µg ml⁻¹ in case of 77 K fluorescence measurements.

Room temperature fluorescence excitation spectra were recorded in Shimadzu RF-540 at a Chl a concentration of 6 µg ml⁻¹.

PS II (photosystem II) activity was measured polarographically at 20 °C under saturating light intensity with *p*-benzoquinone as electron acceptor according to Kolli *et al.* (1998).

Results and Discussion

Our preliminary investigations showed that dark incubation of Spirulina trichomes in the normal fresh medium did not alter the physiological functions. We incubated the Spirulina trichomes in medium containing more than the recommended levels of Na+ ions in the medium in dark for 12 h to 24 h. No significant spectral alterations were seen up to an excess Na+ ions of 0.86 m. However, 0.86 м Na⁺ imposed changes in the Spirulina structure. To ascertain if dark incubation of Spirulina in medium containing excess Na+ ion did increase the internal cellular Na+ level, we estimated internal Na⁺ concentration using atomic absorption spectrometry. Intracellular accumulation of Na+ was found to be 30% more in the treated as compared to the control trichomes (0.9 ppm Na⁺ in the control and 1.3 ppm in the treated). It was also found that the treated trichomes took a little longer time to adapt when transferred to the fresh medium as compared to the control set. However they were able to recover at a normal rate of growth (data not shown).

Changes in pigment and protein content

Table I lists the changes in fresh weight, dry weight and PC/APC ratio of control and Na⁺ treated *Spirulina* trichomes. Almost no change in fresh weight and chl *a* contents in the *Spirulina*

filaments were observed. However, a marginal increase in dry weight was seen. The PC/APC ratio which controls energy transfer from phycobilisomes to chlorophyll a (Fork and Mohanty, 1986) decreased to 65% in the treated cells thereby indicating drastic change in the light harvesting antenna of the Spirulina. The total protein increased to almost two fold while the Chl a in the intact trichomes remained unaltered after the treatmnent. However, in thylakoids isolated from control and treated Spirulina trichomes increase in Chl a content was ~20%, but the thylakoid protein increased by 25%, thus changing the pigment protein ratio. The PS II activity (H₂O→p-benzoquinone) in the treated sample was reduced to 60% when compared with the control (see Table I).

Scanning electron micrograph of intact trichomes revealed loss in spirality and the disappearance of transverse cross walls in the treated (data not shown). Similar reports in relation to the variations in the growth conditions have been found (Jeeji Bai and Seshadri, 1980, and Jeeji Bai, 1985).

Absorption spectra

The room temperature absorption spectra of the Na⁺-treated intact trichomes showed higher absorption in the visible region of the spectrum when compared with the control set (data not shown). This could be due to the scattering and internal sieve effect. The absorption spectra of thylakoid membrane prepared from the control and treated cells exhibited peak absorption at 680 nm (Fig. 1 A) due to Chl a along with its soret band at 440 nm. A hump at 490 nm due to carotenoid absorption (Shubin et al., 1991) was more pronounced in the Na⁺-treated thylakoid. Presence of residual

Table I. Changes in mass, pigment protein composition and PS II activity of Na⁺ treated Spirulina.

	Intact trichomesThylakoid membrane preparations										
	Fresh weight [g]	Dry weight[g]	Total carotenoids [µg ml ⁻¹]	PC/ APC	Chl <i>a</i> [μg ml ⁻¹] (A)	Protein [µg ml ⁻¹] (B)	Ratio A/B	PS II activity (µmol O ₂ evolved mg ⁻¹ protein min ⁻¹)	Chl <i>a</i> [μg ml ⁻¹]	Protein [μg ml ⁻¹] (D)	Ratio C/D
Control	4.52	0.08	2.5	2.8	0.2	5.89	0.03	400.0	2.8	200.0	14.4
Treated	4.56	0.10	2.9	1.8	0.2	10.20	0.02	266.0	2.9	250.0	11.1

PS II activity was assayed as $H_2O \rightarrow p$ -benzoquinone as given in Materials and Methods. All the experiments were carried out four times and the deviation did not exceed more than 5%.

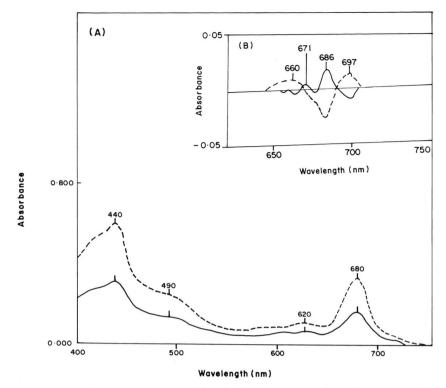


Fig. 1 A. Room temperature absorption spectra of thylakoid membrane isolated from control (——) and treated (——) Spirulina platensis B. Second order derivative spectra of (A). The absorption spectra were recorded an equal Chl a basis, 5 μ g ml⁻¹.

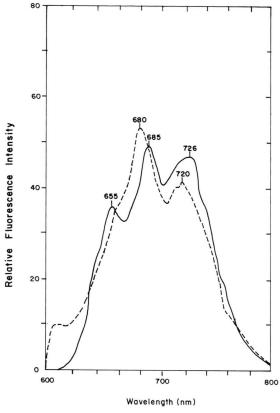
phycobilisomes contributed to the 620 nm absorption. Second order derivative spectra showed two peaks one at 671 nm and the other at 686 nm in case of control (see inset Fig. 1) while the treated thylakoids showed absorption maxima at 660 and 697 nm. This shift of 10 nm in Chl *a* absorption could be due to the disorganization of the thylakoid membrane. Presence of enhanced Na⁺ ion in the medium seems to have altered the lipid protein environment. Similar results have been found in case of the thylakoid membrane under Hg²⁺ ion stress in *Spirulina* by Murthy and Mohanty, (1995) and on temperature stress by Gounaris *et al.* (1984).

Fluorescence spectra

Room tenperature fluorescence emission spectra of thylakoid membrane preparation from control trichomes when excited at 440 nm (Chl *a* excitation) showed two peaks, (Fig. 2) one at 655

nm mostly due to APC, a major peak at 685 nm due to Chl a of PS II and APC and a broad emission band at 726 nm due to Chl a of PS I. Thylakoids from the treated Spirulina trichomes showed reduction of 655 nm peak to a hump, F 685 peak shifted to 680 nm and the Chl a emission was found as doublet at 720 nm. A cross over of the fluorescence emission spectra of control and treated appeared at 685 nm suggesting that sodium stress has altered the chlorophyll a emission characteristics of PS I. Salt-induced decrease in the chlorophyll a fluorescence yield has been earlier shown by Mohanty et al. (1974). Moreover, alteration in the Chl a of PS I as indicated by crossover of fluorescence emission spectra due to shift in peak emission has been earlier shown by Rajagopal et al. (1998) under UV-B stress.

Remarkable differences were observed between the 77 K fluorescence emission spectra of treated and control intact trichome of *Spirulina* excited at 440 nm (Fig. 3A). The control spectrum showed



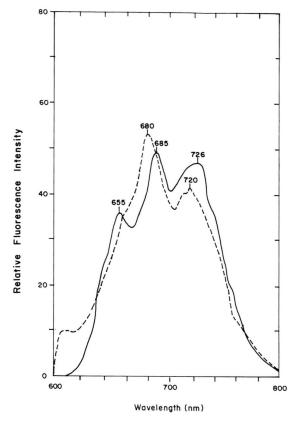


Fig. 2. Room, temperature fluorescence emission spectra of thylakoid membrane isolated from control (——) and treated (——) *Spirulina* trichomes. Samples were excited at 440 nm. The slit. width for both excitation and emission were set at 5 nm and 10 nm respectively. The fluorescence spectra were recorded on equal Chl a basis, 6 μg ml $^{-1}$.

Fig. 3 Low temperature, 77 K fluorescence emission spectra of (A) intact trichomes (B) thylakoid membrane isolated from control (——) and treated (——) *Spirulina platensis*. Samples were excited at 440 nm. The slit width for both excitation and emission were set at 5 nm and 10 nm respectively. The fluorescence spectra were recorded on equal Chl a basis, 3 μ g ml⁻¹.

emission bands at 642 nm (due to PC), 661 nm (due to APC) 689 and 695 nm due to Chl a of PS II and 730 nm due to Chl a of PS I (Goedheer, 1968, and Mohanty et al., 1997). A spectral crossover at 707 nm is again indicative of alteration in PS I chlorophyll a emission characteristics. The 661 nm peak which is due to allophycocyanin (Mohanty et al., 1985) was more pronounced in treated as compared to control trichomes. Schubert and Hagemann (1990) reported similar findings in their work with high salt adapted Synechocystis cells. It is also seen that the peaks at 689 and 695 nm are not well resolved in treated trichomes which could be due to the loss in PS II organisation (Fork and Mohanty, 1986) by sodium stress.

However reduction in fluorescence emission intensity at 730 nm is suggestive of changes in Chl *a* of PS I. Fig. 3B shows the 77 K fluorescence emission spectra of the thylakoid membranes of control and treated *Spirulina* trichomes. Control thylakoid when excited at 440 nm showed 3 distinct peaks at 649, 682 and 726 nm due to PC, Chl *a* of PS II and Chl *a* of PS I respectively while treated thylakoid showed a small shift to the red region there by shifting peak positions to 650, 680 and 728 nm. Similar to the Fig. 3 A a spectral cross over of PS I emission band reflects a change in the Chl *a* of PS I due to Na⁺.

In summary, our results show that exposure of *Spirulina* trichomes to enhanced level of Na⁺ re-

sulted in accumulation of Na⁺ ions which was 30% more in the treated cells as compared to the control. This enhanced level of sodium, induced changes in the absorption characteristics of chlorophyll *a* as evidenced from the derivative spectral analysis. Both room and low temperature chlorohyll *a* fluorescence measurements suggested that the Na⁺ ions possibly effected the Chla belonging to PS I. These changes together with alternation in PC/APC content altered the energy transfer processes.

Acknowledgements

One of us (KV) is grateful to the Council of Scientific and Industrial Research, New Delhi for the financial support in the form of Research Associateship. PM thanks DST B 6.9 for financial support. We also thank the Dean, School of Life Sciences, Jawaharlal Nehru University, New Delhi for providing the laboratory facilities. Assistance of Mr. B. D. Sharma atomic absorption spectrophotometry is gratefully acknowledged.

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