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Effect of high temperature on photosynthetic electron transport activities of the cyanobacterium *Spirulina platensis*

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Abstract

The activities of photosystem 2 (PS2) and whole chain electron transport declined in high temperature treated cells at the room temperature beyond 35 °C, while photosystem 1 (PS1) showed increased activity. Thylakoid membrane studies did not exhibit increase in PS1 activity indicating that the enhancement of PS1 activity is due to permeability change of cell membranes. However, the electron transport activity measured from reduced duroquinone to methylviologen which involves intersystem electron transport was extremely sensitive to high temperature. The activity of PS2 at different irradiance, which was accompanied by alterations in absorption and fluorescence emission properties, indicated changes in the energy transfer processes within phycobilisomes. Thus high temperature has multiple target sites in photosynthetic electron transport system of *Spirulina platensis*.

Additional key words: absorption spectra; energy transfer; fluorescence emission spectra; photosystems 1 and 2.

Introduction

High temperature (HT) affects photosynthetic electron transport in thylakoid membranes at multiple sites (Berry and Björkman 1980, Quinn and Williams 1985, Mohanty *et al*. 1987). In higher plants, HT causes inhibition in PS2 catalysed electron transport whereas it enhances PS1 catalysed electron transport activity (Armond *et al*. 1973, Thomas *et al.* 1984, Mohanty *et al.* 1987). The inhibition of PS2 catalysed electron transport activity by HT is most probably due to either damage to the oxygen evolving complex (OEC) (Katoh and San Pietro 1967, Cramer *et al.* 1981) or due to changes in the organisation of thylakoid membranes (Yamashita and Butler 1968, Hirano *et al.* 1981, Gounaris *et al.* 1983, Wada *et al.* 1984). A variety of reasons for the enhancement of PS1 activity after HT exposure has been proposed, *i.e*., opening a new donor site to PS1 (Thomas *et al.* 1984), enhanced permeability of specific donors to the donation sites (Sabat and Mohanty 1989), spillover of excitation energy or change in the absorption cross section of PS1 (Ivanov *et al.* 1986, Velitchova *et al.* 1989). Studies related to the effect of HT on cyanobacterial photosystems are scanty. Therefore we studied the effect of HT (30 to 60 °C) on photosynthetic electron transport properties of intact cells and thylakoids isolated from the cyanobacterium *Spirulina platensis.*

Materials and methods

Spirulina platensis was grown axenically in the medium of Zarrouk (1966) at 25±2 °C under the irradiance of 40 μmol(photon) m^{-2} s^{-1}. The cells were harvested by centrifuging the culture at 6 000×*g* for 10 min. The collected cells were suspended in 25 mM HEPES-NaOH buffer (pH 7.5) at a chlorophyll (Chl) concentration of 2 kg m^{-3} and exposed to 30 to 60 °C for 30 min in dark. After the HT treatment the samples were cooled to room temperature in the dark. Thylakoid membranes from *Spirulina* cells were isolated according to Shubin *et al.* (1991) with some modifications. *Spirulina* cells equivalent to 1 g fresh mass were suspended in 10 cm^3 of 25 mM HEPES-NaOH containing 20 mM NaCl buffer (pH 7.5) with 1 mM PMSF (α-toluenesulfonyl fluoride). The cells were

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Abbreviations: Chl, chlorophyll; Cyt b_6f, cytochrome b_6f; DCPIP, 2,6-dichlorophenol-indophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; DQ, duroquinone; ETC, whole chain electron transport activity; HT, high temperature; MV, methylviologen; OEC, oxygen evolving complex; PBS, phycobilisomes; pBQ, *p*-benzoquinone; PS, photosystem.
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disrupted by sonication at 4 °C, at an amplitude of 15 μm, with 15-17 bursts of 15 s each, in an *MSE* sonicator (*Usamisonix*, Farmingdale, NY, USA). The thylakoids were separated from the cell debries by centrifugation at 9 000×*g* for 5 min. The supernatant was again centrifuged at 40 000×*g* for 90 min at 4 °C. The pellet was resuspended in 2-3 cm^3 of HEPES-NaOH buffer (pH 7.5) at Chl *a* concentration of 2-3 kg m^{-3}.

Room temperature absorption spectra were measured with *Shimadzu-UV-260* spectrophotometer. Fluorescence emission spectra were recorded at room temperature using *Perkin-Elmer LS-5* spectrofluorometer by the method of Murthy *et al.* (1989). Electron transport activities were analysed using an oxygen electrode at 25 °C and saturating irradiance of 420 μmol(photon) m^{-2} s^{-1}. The PS1 catalysed electron transport activity was measured using reduced DCPIP (2,6-dichlorophenolindophenol) as electron donor and methyl viologen (MV) as acceptor. The PS2 activity was measured by using *p*-benzoquinone (pBQ) as electron acceptor. The whole chain electron transport activity (ETC) assay was done in intact cells using MV as an acceptor by following the procedure of Robinson *et al.* (1982). The intersystem electron transport activity was measured using reduced durohydroquinone (DQH_2) as a donor to plastoquinone pool by following the method of Izawa and Pan (1978).

Results and discussion

The increase in temperature from 25 to 35 °C caused 49 % inhibition in the electron transport activity. Further rise in temperature to 45 °C caused 83 % loss in ETC (Table 1). The reason for the inhibition of ETC could be due to alteration at the level of OEC polypeptides or to the loss of Mn ions as has been suggested by Nash *et al.* (1985). A similar extent of inhibition was observed in PS2 catalysed electron transport assay indicating that the PS2 alterations are responsible for the observed inhibition in ETC. The PS2 activity declined with increasing temperature and at 45 °C it became negligible (Table 1). This loss in PS2 activity could be due to changes in organisation of thylakoid membrane structure (Satoh and Murata 1980, Wada *et al.* 1984): OEC is the part of PS2 most sensitive to HT stress. In contrast to PS2, PS1 catalysed electron transport activities were enhanced upon HT exposure. The increase in temperature beyond 45 °C further enhanced the PS1 activity by 80 % (Table 2). The enhancement in PS1 activity could be due to the enhanced donation of reduced DCPIP as reported earlier for thylakoids of higher plant cells (Thomas *et al.* 1984).

Table 1. Effect of high temperature (HT) on whole chain and PS2 catalysed electron transport activities of cells of *Spirulina platensis*. For whole chain electron transport 3 cm^3 of reaction mixture contained reaction buffer (25 mM HEPES-NaOH) (pH, 7.5) with 20 mM NaCl, 0.5 mM MV, 1 mM sodium azide, and cells equivalent to 15 μg Chl. For PS2 electron transport, 3 cm^3 of reaction mixture contained reaction buffer, 0.5 mM pBQ, and intact cells equivalent to 15 μg of Chl. The SD was not more than 10 %.

Temperature	Electron transport activity [nmol(O_2) kg^{-1}(Chl) s^{-1}]			
[°C]	$H_2O \rightarrow MV$	% loss	$H_2O \rightarrow pBQ$	% loss
25	42.0 ± 4.0	0	85.0 ± 8.0	0
35	21.0 ± 2.0	49	51.0 ± 4.9	40
40	7.1 ± 1.0	83	12.7 ± 1.3	85
45	0	100	0	100

Table 2. Effect of high temperature (HT) on PS1 catalysed electron transport activity [nmol(O_2) kg^{-1}(Chl) s^{-1}] ($DCPIPH_2 \rightarrow$ MV) in intact cells and thylakoids of *Spirulina platensis.* Three cm^3 of reaction mixture contained reaction buffer, 5 mM ascorbate, 0.1 mM DCPIP, 10 μmol DCMU, 0.5 mM MV, 1 mM sodium azide, and thylakoids equivalent to 15 μg of Chl. The SD was not more than 10 %.

Temperature	PS1 catalysed electron transport activity			
[°C]	Cells	% increase	Thylakoids	% increase
25	91 ± 8	0	135 ± 12	0
35	113 ± 10	25	139 ± 12	3
45	136 ± 12	50	148 ± 13	10
55	163 ± 15	80	162 ± 12	12

To find whether the HT mediated enhancement was present in thylakoids, effect of HT was studied in thylakoid membranes (Table 2). The increase in temperature from 35 to 55 °C caused marginal increase in PS1 catalysed electron transport activity indicating that the observed enhancement in PS1 activity of intact cells was related to enhanced permeability of reduced DCPIP only. The enhancement in PS1 activity in higher plant thylakoid membranes might be due to opening of new site for DCPIP near to Cyt b_6f. Sundby *et al.* (1996) suggest that migration of LHC2 from PS2 to PS1 is responsible for the enhancement of PS1 activity. Unlike higher plant thylakoids, cyanobacterial thylakoids are insensitive to HT and are not able to show PS1 mediated stimulation due to HT treatment (Table 2).

To find out the target site of HT in intersystem electron transport before DCPIP donation, an electron transport assay has been measured using DQH_2 as donor to plastoquinone (Table 3). HT treatment (40 °C) caused 55 % loss in intersystem electron transport activity suggesting the existence of sensitive site between plastoquinone and plastocyanin.

The PS2 activity was measured at both irradiance-saturating and -limiting conditions (Table 4). The inhibi-

Table 3. Effect of high temperature on intersystem electron transport system activity ($DQH_2 \rightarrow MV$) [nmol(O_2) kg^{-1}(Chl) s^{-1}] in intact cells of *Spirulina platensis*. Three cm^3 of reaction mixture contained reaction buffer, 5 mM ascorbate, 0.5 mM DQH_2, 10 μM DCMU, 0.5 mM MV, 1 mM sodium azide, and cells equivalent to 15 μg Chl. The SD was not more than 10 %.

Temperature [°C]	PS1 activity	% inhibition
25	48.0 ± 4.0	0
30	45.0 ± 4.0	6
35	31.0 ± 3.0	35
40	21.0 ± 2.0	55
45	17.0 ± 1.6	65

tion of PS2 catalysed activity was higher at saturating irradiance [420 μmol(photon) m^{-2} s^{-1}] compared to irradiance-limiting conditions [8 μmol(photon) m^{-2} s^{-1}]. This inhibition at irradiance-limiting conditions indicates the alteration of energy transfer from phycobilisomes, the major light-harvesting antennae to PS2. The absorption (Fig. 1*A*) and fluorescence emission (Fig. 1*B*) of phycocyanin (PC) were drastically decreased with the increase in temperature. A 3 nm shift in the fluorescence emission maximum of PC (Fig. 1*B*) was observed in the cells treated with 50 °C for 30 min. At 60 °C, a complete loss of absorption and emission of PC was observed. These spectral alterations suggest the changes in energy transfer. Similar findings were made by Murthy *et al.* (1989) under mercury ion stress in the same cyanobacterium species. According to Li *et al*. (2001) closure of PS2 did not influence fluorescence yields of PS1 in *S. platensis*.

Table 4. The effect of different irradiance [μmol(photon) m^{-2} s^{-1}] on PS2 catalysed electron transport activity ($H_2O \rightarrow pBQ$) [nmol(O_2) kg^{-1}(Chl) s^{-1}] of control (25 °C) and 35 °C treated cells. For other details see Materials and methods.

Irradiance	PS2 activity		% inhibition
	Control	Treated	
420	87.0 ± 8.0	54.0 ± 5.0	38
160	53.0 ± 5.0	34.0 ± 3.0	36
40	26.0 ± 2.0	18.0 ± 1.7	30
8	5.4 ± 0.4	3.9 ± 0.3	27

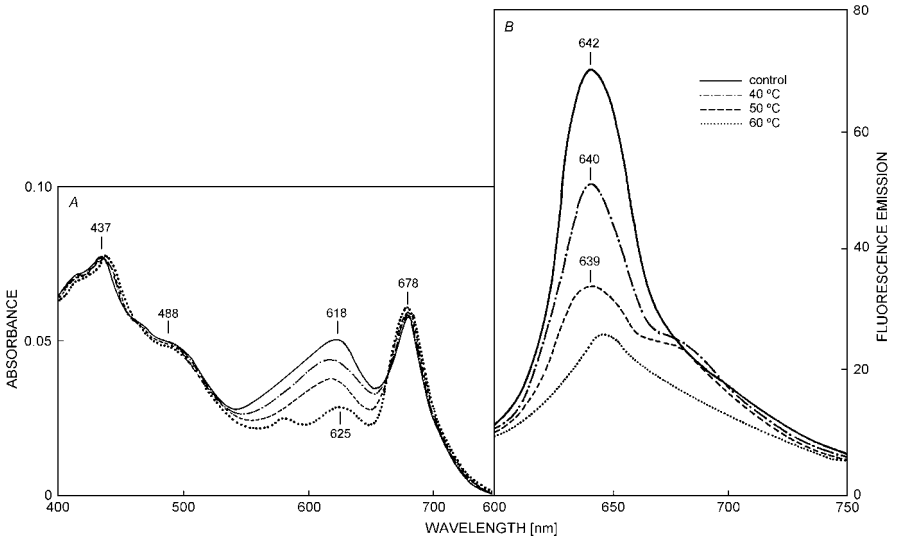

Fig. 1. Effect of high temperature (HT) on (*A*) absorption and (*B*) fluorescence emission spectra of intact cells of *Spirulina platensis*. Cells were given 30 min HT treatment and then were kept in dark for 5 min before the measurements. Intact cells equivalent to 6 μg chlorophyll *a* were suspended in 3 cm^3 of reaction buffer. In *B*, cells were excited at 545 nm to specifically excite phycobilisomes; slide width for measurements of both excitation and emission was 5 nm.

Thus HT treatment causes inhibition of photosynthetic electron transport at multiple sites besides the alterations in energy transfer processes. PS1 mediated enhancement was not observed in cyanobacterial thylakoids unlike in thylakoids of higher plant chloroplasts.

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