

Altered photo system ii photochemistry under the influence of cold stress in the cyanobacteria; *Spirulina platensis*

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ABSTRACT

The treatment of intact cells of Spirulina platensis with low temperature (10-25°C) for 45 min caused inhibition in PS II catalysed electron transport activity. The light intensity measurements clearly gave an indication that light harvesting complex (Phycobilisomes) are targets for the cold stress. Low temperature effects the pigment-protein interaction and finally altered the energy transfer from phycocyanin to Chl a in the intact cells of Spirulina platensis which is evident from the both absorption and fluorescence emission measurements.

Key words: Absorption, energy transfer, fluorescence, phycocyanin, *Spirulina platensis*.

INTRODUCTION

Photosynthesis represents an integration of photochemical as well as biochemical processes. Thus, ambient temperature fluctuations will have a direct impact on photosynthesis through its effects on the thermally sensitive biochemical and physiological processes. Cyanobacteria can be found all over the world and in environments from Antarctica, where temperatures never exceed -20 °C [1], to hot springs, where temperatures reach 70 °C [2]. Cyanobacteria found in water pockets of Antarctic lake ice, where temperatures are always below 0 °C, are metabolically active and capable of performing oxygenic photosynthesis [3]. However, the photochemical events of light absorption, energy transfer and charge separation associated with PS II and PS I are insensitive to temperature in the biologically relevant range of 0°C to 50°C [4]. Thus very little information is available on the main target in PS II complex during chilling induced damage. Kee *et al* (1986) [5] showed that chilling induced inhibition in whole chain electron transport as well as PS II catalyzed photochemistry by effect at the level of O₂ evolving complex. The studies related to the effect of low temperature on PS II photochemistry in relation to spectral alterations in cyanobacteria are scanty. Therefore in this investigation an attempt has

been made to study the effect of low temperature on PS II catalyzed electron transport and light harvesting complex of PS II in the cyanobacteria, *Spirulina platensis*.

MATERIALS AND METHODS

Spirulina platensis was grown axenically in the medium of Zarrouk (1966) [6] at 25 ± 2 °C. Under continuous irradiance of $40 \mu \text{mol (photon) m}^{-2} \text{S}^{-1}$. Cells from the late log grown cultures were harvested by centrifuging at 6,000 Xg for 10 min. The collected cells were suspended in 25 mM HEPES-NaOH buffer (pH 7.5) at a Chl concentration of $200 \mu\text{g ml}^{-1}$. Samples were incubated at low temperature ($10\text{-}25$ °C) for 45 min in the Petri dishes under constant stirring. The reaction mixture for PS II catalysed electron transfer ($\text{H}_2\text{O} \rightarrow p\text{-benzoquinone(pBQ)}$) contained the reaction buffer in 25 mM HEPES-NaOH buffer (pH 7.5) and 0.5 mM pBQ[7,8]. The measurements were carried out at 25°C under saturating illumination by white light ($3000\mu\text{moles (photon) density}$) low intensity of light was provided by passing the light through calibrated neutral density filters. The absorption spectra of a suspension of intact cells were recorded using a shimadzu UV-3,000 double beam spectrophotometer operated in the split beam mode. Fluorescence emitted by whole cells was measured at room temperature with excitation at 545 nm in perkin Elmer LS-5 spectrofluorometer. Emission spectra at 685 nm of intact cells at room temperature were measured in the same fluorometer. Emission spectra were not correct for spectral sensitivity of the photomultiplier. Cells equivalent to $50\mu\text{g}$ of Chl were used for all fluorometric assays. In low temperature measurements the samples were diluted with 60% glycerol (v/v) and slowly cooled to liquid nitrogen temperature in dark.

RESULTS AND DISCUSSION

In this study an attempt has been made to study the alterations in the PS II photochemistry of the cyanobacteria *Spirulina platensis*. The fore initially PS II catalysed electron transport has been measured by using pBQ as an electron acceptor. The exposure of cells to different temperature ($10\text{-}25$ °C) caused the inhibition in PS II catalysed electron transport (Table-1) .65% inhibition in PS II catalysed electron transport was noticed with the treatment of intact cells at 10°C for 45 min. These results are in agreement with the observations of Kaniluuga *et al* (1978 b) [9]. To identify the target in PS II catalysed electron transport, a study has been made by exposing the control and treated cells (10°C) to different illumination intensities of light ($12\text{-}446 \text{ Wm}^{-2}$) (Table 2). The inhibition in PS II catalysed electron transport was more at high light intensities (446 Wm^{-2}) than at light limiting conditions (12 Wm^{-2})(Table 2). The reason for the loss at light limiting conditions could be the alteration at light harvesting complex of PS II. Thus low temperature shows multiple effects on photosynthetic electron transport depending on the extent of temperature for which the cells are exposed. The absorption spectra of intact cells showed that low temperature treatment (10°C) caused the decrease in the absorption of light by Phycocyanin at 630 nm (Fig-1) and showed a slight red shift by 2 nm. These results indicate that there is an alteration in the chromophore protein interaction in Phycocyanin. To verify the above preposition cells were excited with 440 nm light to measure the fluorescence emission spectra (Fig-2). In control spectrum an emission peak at 677 nm was prominent. Low temperature treatment caused the decrease in the fluroscence intensity by 40% and shifted the peak by 16 nm .This clearly demonstrates the alterations in the energy transfer from Phycocyanin to Chl *a* . The similar observations were made in this cyanobacteria by murthy (1991) [8]. To clearly pin point

the target low temperature (77K) fluorescence emission spectra of intact cells was measured (Fig 3). This results also confirmed the alterations in the energy transfer as evidence from the decrease of F-695 peak thus the altered energy transfer is responsible for the loss of PS II photochemistry in this cyanobacteria under the influence of low temperature.

Table 1: Low temperature induced alteration in PS II catalysed electron transport of the intact cells of the cyanobacterium *Spirulina platensis*

Temperature ⁰ C	PS II electron transport activity (H ₂ O → <i>p</i> -BQ) μ moles of O ₂ ↑ mg ⁻¹ Chl ⁻¹ h ⁻¹	Percentage loss
25	361 ± 33	0
20	282 ± 26	22
15	209 ± 19	42
10	126 ± 11	65

Table 2: Illuminated light intensity dependent effect on the low temperature induced inhibition of PS II catalysed electron transport activity (H₂O→*p*BQ) in the cyanobacterium, *Spirulina platensis*.

Light intensity(Wm ⁻²)	PS II catalysed electron transport activity μ moles of O ₂ ↑ mg Chl ⁻¹ h ⁻¹		Percentage inhibition
	Control (25 ⁰ C)	Treated (10 ⁰ C)	
12	44 ± 3	30 ± 3	31
125	125 ± 11	81 ± 7	35
262	256 ± 24	154 ± 14	40
446	358 ± 33	201 ± 19	44

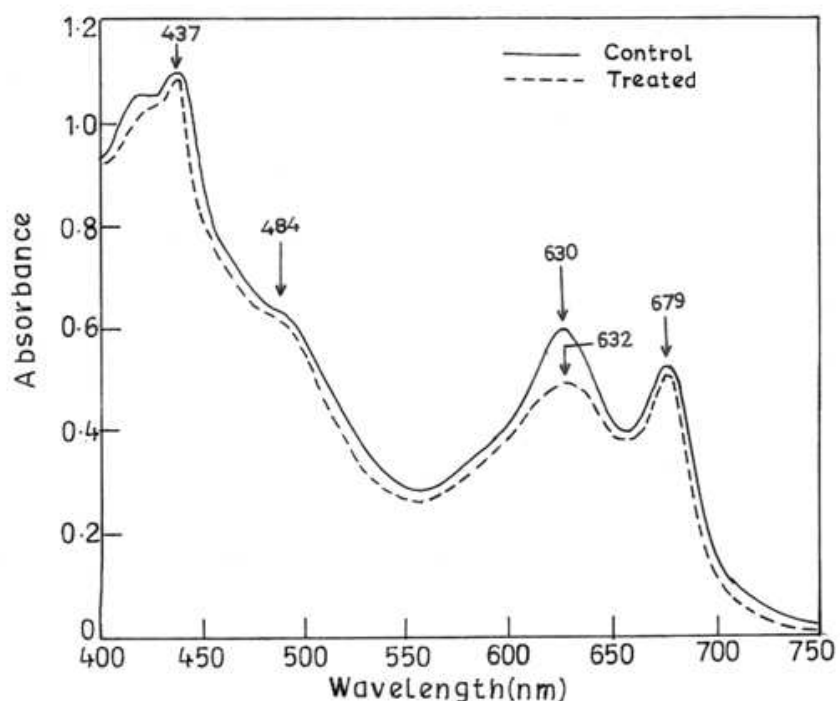


Fig 1: Absorption spectrum of the treated intact cells of the cyanobacterium, *Spirulina platensis*. The cells were treated at 10°C for 45 min before measurements were made.

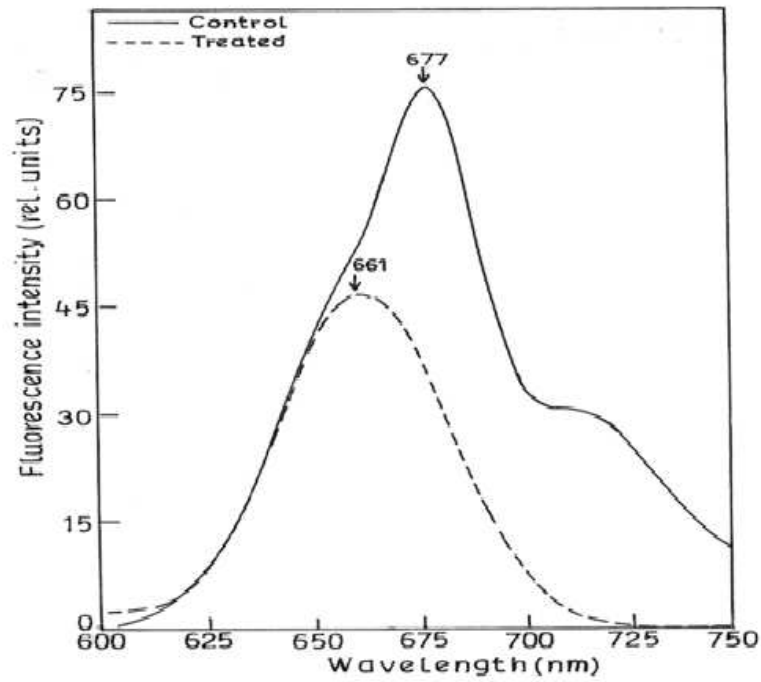


Fig2: Fluorescence emission spectra

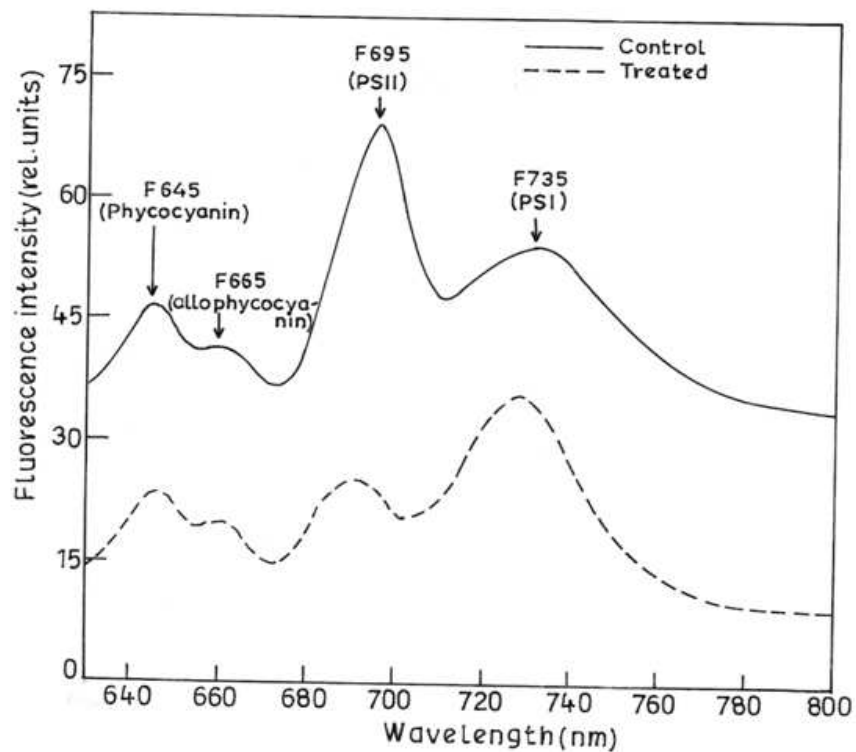


Fig 3: Effect of cold stress (10°C) on 77 K fluorescence emission spectra of intact cells of *Spirulina*

CONCLUSION

Low temperature is one of the most important abiotic factors which limit the growth, productivity and distribution of plants. The low temperature (10°C) is able to cause alterations in the PS II catalysed electron transport activity in the thylakoid membranes of intact cells of *Spirulina*. Cold stress causes alterations in the energy transfer of Phycobilisomes under *in vivo* and *in vitro* conditions as evident from spectral alterations in absorptions as well as fluorescence emission.

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