ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

High Temperature Induced Alterations in Photosystem II Photochemistry of the Cyanobacterium - *Spirulina Platensis.*

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ABSTRACT

The effect of high temperature (30°C-45°C) was studied on photosystem II photochemistry by incubating the intact cells of *Spirulina platensis* at different temperatures for 15 minutes. Between two photosystems, photosystem II catalysed electron transport is more sensitive to high temperature than that of photosystem I. Fluorescence emission spectral measurements clearly demonstrated that the energy transfer in photosystem II is susceptible to high temperature. It also revealed that uncoupling of energy transfer takes place between phycocyanin and chlorophyll *a* in intact cells of *Spirulina* and there by affects the photosystem II photochemistry.

Keywords : Fluorescence, High temperature, Photochemistry, Photosystem II, *Spirulina platensis.*

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ISSN: 0975-8585

INTRODUCTION

High temperature (HT) affects photosynthetic electron transport in thylakoid membranes at multiple sites [1-3]. In higher plants, HT causes inhibition is photosystem (PS) II catalysed electron transport whereas it enhances PS I catalysed electron transport activity [3-5].The inhibition of PS II catalysed electron transport activity by HT is most probably due to either damage to the oxygen evolving complex (OEC) [6,7] or due to changes in the organization of thylakoid membranes [8-11]. A variety of reasons for the enhancement of PS I activity after HT exposure has been proposed, *i.e.*, opening a new donor site to PS I [5], enhanced permeability of specific donors to the donation sites [12], spillover of excitation energy or change in the absorption cross section of PS I [13,14]. Studies related to the effect of HT on cyanobacterial photosystems are scanty. Therefore we studied the effect of HT (30^{o}C to 50^{o}C) on photosynthetic electron transport properties of intact cells of the cyanobacterium, *Spirulina platensis.*

MATERIALS AND METHODS

Spirulina platensis was grown axenically in the medium of Zarrouk [15] at 25±2^{o}C under the irradiance of 40 µmol (photon) m^{-2} s^{-1}. The cells were harvested by centrifuging the culture at 6000 xg 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^{o}C to 45^{o}C for 15 min in dark. After the HT treatment the samples were cooled to room temperature in dark. Fluorescence emission spectra were recorded at room temperature using Perkin–Elmer LS-5 spectroflurometer by the method of Murthy *et al.* [16]. Electron transport activities were analysed using an oxygen electrode at 25^{o}C and saturating irradiance of 420 µmol (photon) m^{-2} s^{-1}. The PS I catalysed electron transport activity was measured using reduced DCPIP (2,6-dichlorophenolindophenol) as electron donor and methyl viologen (MV) as acceptor. The PS II activity was measured by using *p*-benzoquinine (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* [17].

RESULTS AND DISCUSSION

In this communication an attempt has been made to characterise target site to identify the inhibition in PS II catalysed electron transport in the cyanobacterium *Spirulina platensis* under high temperature stress. To achieve that objective whole chain electron transport activity has been measured using MV as an electron acceptor. Control cell exhibited the whole chain electron transport activity equal to 158 µ moles of O_2 consumed [Table 1]. High temperature treatment (30-45^{o}C) caused inhibition in whole chain electron transport activity in an intensity dependent manner and at 35^{o}C, 47% inhibition was noticed. The possible reason for the inhibition of whole chain electron transport could be either alterations at the level of PS II or PS I or at both. To confirm the target site PS II catalysed electron transport was assayed using pBQ as an Hill acceptor [Table 2]. Control cells exhibited the activity equal to 372 µ moles of O_2 evolved. The treatment of cells with high temperature exhibited loss in Hill activity and at 35^{o}C, 50% inhibition in electron transport was noticed. The possible reason for the loss in PS II catalysed electron transport could be alterations in extrinsic polypeptides of water oxidation complex [18]. To confirm susceptibility difference of PS II with PS I, the PS I catalysed electron transport has been measured using reduced DCPIP as donor. High temperature surprisingly induced enhancement in the PS I catalysed electron transport and at 40^{o}C temperature , 52% increase was noticed [Table 3]. The possible reasons for the increase of PS I activity could be opening of a new site towards DCPIP donation [5]. But in the case of intact cells high temperature causes the changes in the permeability barrier like cell wall and therefore more reduced DCPIP could be entering into the cell. As a result the observed PS I electron transport enhancement could be possible. To identify the target in PS II catalysed electron transport the energy transfer has been measured in terms of phycocyanin fluorescence [Table 4]. Upon excitation with 545 nm light phycocyanin fluorescence light at 652 nm in control cells of *Spirulina platensis.* The treatment of high temperature gradually increased the fluorescence emission intensity and at 40^{o}C, 60% enhancement in phycocyanin fluorescence noticed. In addition the peak position was shifted from 652 nm to 642 nm which could be due to structural alterations in phycocyanin. The increase in the fluorescence emission could be due to uncoupling of energy transfer from phycocyanin to chlorophyll in PS II of the above cyanobacterium as has been suggested earlier by Murthy *et al* [16] in the case of mercury toxicity in *Spirulina platensis.* Thus high temperature mainly effects energy transfer in PS II and there by causes inhibition in PS II catalysed electron transport in the above cyanobacterium.

ISSN: 0975-8585

Table 1: Effect of temperature (30-45°C) on whole chain electron transport activity ($H_2O \rightarrow MV$) in cyanobacterium *Spirulina platensis*:

Temperature (°C)	Whole chain electron transport activity ($H_2O \rightarrow MV$) μ moles of O_2 consumed mg Chl^{-1} h^{-1}	Percentage of loss
25	158 ± 13	0
30	147 ± 11	7
35	84 ± 6	47
40	35 ± 3	78
45	-	-

Table 2: Effect of temperature (30-45°C) on PS II catalyzed electron transport actvity ($H_2O \rightarrow PBQ$) in cyanobacterium *Spirulina platensis*:

Temperature (°C)	PS II catalyzed electron transport ($H_2O \rightarrow PBQ$) μ moles of O_2 evolved mg Chl^{-1} h^{-1}	Percentage of loss
25	372 ± 33	0
30	342 ± 29	8
35	186 ± 15	50
40	67 ± 5	82
45	-	-

Table 3 : Effect of temperature (30-45°C) on PS I catalyzed electron transport activity ($DCPIPH_2 \rightarrow MV$) in cyanobacterium *Spirulina platensis*:

Temperature (°C)	PS I catalyzed electron transport ($DCPIPH_2 \rightarrow MV$) μ moles of O_2 consumed mg Chl^{-1} h^{-1}	Percentage increase
25	105 ± 8	0
30	126 ± 10	20
35	147 ± 12	40
40	159 ± 14	52
45	179 ± 15	71

Table 4: Effect of high temperature (30-45°C) on phycocyanin fluorescence emission intensity of intact cells of cyanobacterium, *Spirulina platensis*.

Temperature (°C)	Phycocyanin fluorescence emission intensity (Rel.units)	Peak Position (nm)	Percentage of enhancement
25	51 ± 4	652	0
30	63 ± 3	650	23
35	75 ± 5	646	47
40	82 ± 6	644	60
45	71 ± 5	642	39

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