INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
VOLUME-2, ISSUE-2 APRIL-JUNE-2012 ISSN 2231-4490
Coden : IJPAES www.ijpaes.com

Received: 01st April-2012 *Revised: 04th April-2012* *Accepted: 07th April-2012*
Research article

ALTERED SPECTRAL PROPERTIES OF PHOTOSYNTHETIC APPARATUS IN THE CYANOBACTERIUM, *SYNECHOCOCCUS* 6301 UNDER INFLUENCE OF PHOTOINHIBITION

Chandra Sekhar, Y.S.S[1]., Brahmaji Rao P[1], Prasanna Reddy.B[3] and Murthy.S.D.S[2]

[1]Department of Environmental Sciences, Acharya Nagarjuna University, Guntur, A.P-522 510.

[2]Department of Biochemistry, S.V.University, Tirupati, Andhra Pradesh - 517 502.

[3]Department of Quality Control, Nosch labs Pvt Ltd, Hyderabad, A.P. India

Email: *sdsmurthy@rediffmail.com*

ABSTRACT: In this investigation an attempt has been made to develop spectral properties of cyanobacteria as an indicator to identify the high light stress (105-450 Wm^{-2}). High light caused alterations in the absorption as well as fluorescence emission properties of phycocyanin and stopped the energy transfer to photosystem II in cyanobacterium, *Synechococcus* 6301.Chlorophyll fluorescence measurements proved that the existence of inhibitor site near reducing of photosystem II.

Key words: Electron transport, Photosystem, Photoinhibition, Synechococcus 6301.

Abbreviations: LHC - Light harvesting complex; PS - Photosystem; PBPs: Phycobiliproteins; PBsomes-Phycobilisomes;

INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes whose light harvesting complex of PS II is made up of PBPs. These complexes are arranged on the outer surface of thylakoid membrane in the form of beads [1 2]. The light is harvested by the PBS and transferred to the RC in the following sequence: PC → APC → APCB → Chl *a* [3,4]. Several environmental factors are known to affect the energy transfer process from PC to Chl *a* i.e. low temperature [5], high temperature [6, 7], heavy metals [Hg: 8], nitrogen stress [9], Salt stress [10]. Murthy (1991) [8] showed that mercury at low concentrations acts as a potent inhibitor of energy transfer between PC and Chl *a* in *Spirulina platensis*. High light intensity caused changes in the ratio of LHC pigments, Chl *a/b* in the green algae *Dunaliella salina* [11]. Prolonged illumination of isolated PS II RC under aerobic condition causes photo destruction of Chl [12]. Lichtenthalar's group used fluorescence parameters to analyze the effect of high light on photosynthetic pigments and concluded that Chl *b* is the main target for photoinhibition [13]. There is an evidence in the literature suggesting that variations in the Chl *a/b* ratio occur naturally as a response to the changes in the irradiance [13, 14]. Studies related to the effect of high light on cyanobacterial light harvesting system are scanty. Hence in this chapter an attempt has been made to study the effect of high light on spectral properties and energy transfer in the cyanobacterium, *Synechococcus* 6301.

MATERIALS AND METHODS

Synechococcus 6301 was grown axenically in BG -11 medium [15] at 25±2^{0}C under continuous illumination (≈ 15 Wm^{-2}). Throughout the growth period the culture was agitated by the passage of filtered air. After five days of growth (late log phase) cells were harvested by centrifuging at 9,000g for 5 min, washed twice with 20 mM Tricine- KOH buffer (pH 7.5) that contained 400 mM sucrose, 10 mM KCL and 10 mM EDTA (disodium salt) and centrifuged as above. Intact cells were exposed to different intensities of light (105-450 Wm^{-2}).

The absorption spectra of a suspension of spheroplasts with or without Hg^{2+} were recorded using Shimadzu UV-3000 double beam fluorescence emitted by spheroplasts was measured at room temperature with excitation at 545 nm in a Perkin Elmer (LS-5) spectrofluoremeter [16,17]. Fluorescence spectra were not corrected for the spectral sensitivity of the photomultiplier. Cells equivalent to 5 µg of Chl were used for all fluorometric assays. The concentration of Chl was determined by the method of MacKinney [18].

RESULTS AND DISCUSSION

In our earlier communication an attempt has been made to identify the targeted photosystem to high light stress and our results indicated that PS II seems to be altered in the cyanobacterium, *Synechococcus* 6301. To confirm our preposition the effect of high light has been studied on the spectral properties of cells after exposing them for 30 min to different intensities of light (105- 450 Wm^{-2}). Table 1 shows the absorption characteristics of control cells of *Synechococcus* 6301. The peak of 680 nm is due to absorption of Chl *a*, peak at 620 nm in due to absorption of PC of phycobilisomes, a hump at 490 nm in due to absorption of carotenoids and a peak at 435 nm is due to the presence of Chl *a* [19]. After treatment, there was a decrease in PC/Chl ratio regarding absorption capacity. This indicates that there could be alterations in phycobiliproteins of PBsomes.

Table 1: Effect of high light intensity on absorption spectral properties (ratios) of intact cells of the cyanobacterium, *Synechococcus* 6301.

High light treated sample	Absorption Ratio		
	PC/Chl	Chl/Chl	Caro/Chl
Control	1.1	1.3	0.85
105 Wm^{-2}	0.9	1.2	0.80
215 Wm^{-2}	0.7	1.1	0.78
450 Wm^{-2}	0.6	1.1	0.76

As high light altered specifically PC absorption in a significant manner, an attempt has been made to analyze the effect of high light on PC fluorescence emission spectra on intact cells of *Synechococcus* 6301 (Table 2). Control sample upon excitation with 545 nm light exhibited an emission peak at 655 nm for PC. Treatment cells with high light intensity (105-450Wm^{-2}) caused gradual decrease in the PC fluorescence emission and induced 4nm red shift in the peak position. At 450 Wm^{-2} of high light induced 45% loss in the fluorescence intensity. The decrease in fluorescence intensity indicates the alterations in energy transfer of light within the PBsomes. This shift in the peak position indicates the bleaching of chromophore of PC. Similar observations regarding the PC alterations have been studied with Cu toxicity and Hg toxicity in the cyanobacterium, [20 and 8]. In higher plant system similar alterations have been reported under photoinhibition as Chl protein spectral alterations [13].

Table 2: High light induced alterations in the phycocyanin fluorescence emission properties in intact cells of the cyanobacterium, *Synechococcus* 6301.

High light treated sample	Phycocyanin fluorescence	
	Intensity (rel. units)	Peak position, nm
Control	89	655
105 Wm^{-2}	73	654
215 Wm^{-2}	62	657
450 Wm^{-2}	51	659

To ascertain the existence of the inhibition site in PS II, Chl *a* fluorescence properties have been used as a tool to identify the target. The effect of high light was studied on Chl *a* fluorescence in the presence and absence of diuron, our inhibition of electron transport at Q_B site of D_1 protein. Table 3 clearly demonstrated that in control sample the ratio of Chl *a* fluorescence in the presence and absence of diuron was 1.84 indicating the 100% effecting of PS II. But the high light treatment caused the ratio to drop from 1.84 to 1.15 indicating the damage of PS II at reducing side in intact cells of *Synechococcus* 6301. Our results are in against that the reason for the inhibition by high light could be the alterations at D_1 protein of PS II. Thus high light induces alterations in the energy transfer from PC to Chl *a* in PS II as well as affects the electron transport by targeting D_1 protein of PS II in the cyanobacterium, *Synechococcus* 6301.

Table 3: Effect of high light intensity on chlorophyll *a* fluorescence in the presence and absence of DCMU (herbicide) in the cyanobacterium, *Synechococcus* 6301.

High light treated sample	*Chl* a fluorescence		Ratio
	+DCMU	-DCMU	
Control	52	96	1.84
105 Wm^{-2}	47	75	1.59
215 Wm^{-2}	41	54	1.31
450 Wm^{-2}	39	46	1.15

REFERENCES

[1] Gantt, 1981. Phycobilisomes. Annu. Rev. Plant Physiol. 32:327-347.

[2] Liu Z, Yan H, Wang K, Kuang T, Zhang J, Gui L. and Chang W, 2004. Crystal structure of spinach major light harvesting complex at 2.72 Å resolution. Nature. 428: 287-292.

[3] Fork, D.C. and Mohanty, P, 1986. Fluorescence and other characteristics of blue-green algae (Cyanobacteria), red algae and cryptomonads. In: Light Emission by Plants and Bacteria (Govindjee, Amsez, 1. and Fork, D.C., eds.) pp. 451-496, Academic Press, New York.

[4] Li DH, Xie J, Zhao JQ, Xia A, Li D. and Gong Y, 2004. Light – induced excitation energy transfer in the photosynthetic reaction center of Rhodopseudomonas. Biophys. J.70:2469-2492

[5] Schreiber, U, 1979. Cold-induced uncoupling of energy transfer between phycobilins and chlorophyll in Anacystis nidulans. Antagonistic effects of monovalent and divalent cations and of high and low pH. FEBS Lett. 107: 4-9.

[6] Singhal, G.S., Mohanty, P. and Govindjee, 1981. Effect of preheating intact cells on pigments revealed by absorption and fluorescence spectra. Z. Pflanzen-physiol. 103: 217228.

[7] Murthy S.D.S, Ramanaiah V.V. and Sudhir P, 2004. High temperature induced alterations in energy transfer in phycobilisomes of the cyanobacterium, Spirulina platensis. Photosynthetica.42:615-617.

[8] Murthy, S. D. S, 1991. Studies on bioenergetic processes of cyanobacteria: Analysis of the effect of selected heavy metal ions on energy linked process. Ph. D. thesis, Jawaharlal Nehru University, New Delhi.

[9] Yamanaka G., Glazer A.N, Williams R.C, 1980. Molecular architecture of a light-harvesting antenna. Comparison of wild type and mutant Synechococcus 6301 phycobilisomes. J. Biol. Chem. 255: 11004-11010.

[10] Sudhir P, Pogoryelov D, Kovacs L, Garab, G. and Murthy, S. D. S, 2005. The effects of salt stress on photosynthetic electron transport and thylakoid membrane proteins in the cyanobacterium, Spirulina platensis. J.Biochem. and Molecular Biol. 38:481-485.

[11] Smith BM, Morrissey PJ, Guenther JE, Nemson JA, Harrison MA, Allen JF, and Melis A, 1990. Response of the photosynthetic apparatus in Dunaliella salina (green algae) to retardation stress. Plant. Physiol. 93:1433-1440.
[12] Telfer A, Las D, Rivas J. and Barber J, 1990. Biochim. Biophys. Acta. 1060:106-114.
[13] Lischanthaler H K, Burkart S, Schindler C. and Stober F, 1992. Changes in photosynthetic pigment and in vivo chlorophyll fluorescence parameters under photoinhibitory growth conditions. Photosynthetica. 27: 343-353.
[14] Lambreva M, Stoyanova-Koleva D, Baldjiev G. and Tsonev T, 2005. Early acclimation changes in the photosynthetic apparatus of bean plants during short term exposure to elevated CO2 concentration under high temperature and light intensity. Agriculture, Ecosystems Environment. 106: 29-232.
[15] Stanier,R.Y, Kunisawa.R., Mandai,M. and Cohen-Bazire,G, 1971. Purification and properties of unicellular blue green algae (order chrococcales); Bacteriol. Rev. 35: 171–205.
[16] Murthy S D S, Sabat S C. and Mohanty P, 1989. Mercury induced inhibition of photosystem II activity and changes in the emission of fluorescence from phycobilisome in intact cells of the cyanobacterium Spirulina platensis. Plant Cell Physiol. 30 1153–1157.
[17] Murthy, S.D.S. and Mohanty, P, 1991a. Mercury induces alteration of energy transfer in phycobilisomes by selectively affecting the pigment protein, phycocyanin in the cyanobacterium Spirulina platensis. Plant Cell Physiol. 32 231-237
[18] MacKinney, G, 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem. 140: 315-322.
[19] Fork, D.C. and Mohanty, P, 1986. Fluorescence and other characteristics of blue-green algae (Cyanobacteria), red algae and cryptomonads. In: Light Emission by Plants and Bacteria (Govindjee, Amsez, 1. and Fork, D.C., eds.) pp. 451-496, Academic Press, New York.
[20] Park J W and Sauer K, 1991. Subunit selective bleaching of C-phycocyanin from Synechococcus PCC 6301 strain AN 112 by Cu 2+ and sodium perchlorate. Biochim. biophys. Acta 1056: 181-189.