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PHOTOINHIBITION INDUCED ALTERATIONS IN PHOTOSYNTHETIC ELECTRON TRANSPORT ACTIVITIES OF THE CYANOBACTERIUM *SYNECHOCOCCUS* 6301

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ABSTRACT
In this investigation an attempt has been made to study the highlight (Photoinhibition) induced alterations in the cyanobacterium *Synechococcus* 6301. The electron transport activities have been measured using oxygen electrode with spheroplasts of the intact cells using artificial electron donors, accepters and inhibitors. They indicate that whole chain and photosystem II catalyzed electron transport activities are more sensitive to high light (410 - 620 Wm^{-2}) when compare to that of photosystem I. Light intensity measurements (420 - 15 Wm^{-2}) gave clear indications that the energy transfer from phycobilisomes to photosystem II reaction centre is major target since the inhibition at light saturating conditions than at light limiting conditions.

INTRODUCTION

The source of energy, which helps in the maintenance of biosphere on the earth, is sunlight. This light is converted to chemical energy by the process of photosynthesis. Therefore light can also be bad when plants are exposed to high intensities of light under adverse environmental conditions (Kyle *et al*., 1987). There is an evidence that too much of light can affect plant growth leading to the reduction of plant productivity and crop yield. This stress condition is known as photoinhibition, which was reported as long ago (Chow and Andersson, 1987; Barber and Andersson, 1992). Strong illuminations of light to oxygenic photosynthetic organisms results in decreased CO_2 fixation, inhibition of photosynthetic electron transport and oxygen evolution (Powles,1984) Biochemical protection from potentially damaging excess irradiance has been demonstrated by oxygen uptake reactions (Osmond and Grace, 1995). Photoinhibition causes mainly photo inactivation of PS II catalyzed electron transport and irreversible damage to the reaction centers (RCs) (Aro *et al*., 1993; Kruse *et al*., 1997). The 32 kDa, Q_B binding protein of the PS II RC is known as D_1 protein. It is involved in the early stages of damage caused by photoinhibition. The damage to the 32 kDa was thought to be the primary cause of photoinhibition. The possible reason for the damage of D_1 protein is the production of oxyradicals from molecular oxygen (Trebst *et al*., 1988; Andersson and Aro, 2001). There is general argument that light induced impairment of electron transport is not a consequence but rather the cause of protein damage and transfer of D_1 protein (Arntz and Trebst, 1986). Several *in vitro* studies suggested that PS I photochemical activity could be inhibited by strong light (Hui *et al*., 2000; Rajagopal *et al*., 2003). In the initial experiments PS I inactivation was observed only in the presence oxygen. Later the inactivation of PS I was observed under strongly reducing oxidants in *Bryopsis* chloroplast (Satoh and Fork, 1982). Particularly photo inhibitory effect on the energy transfer in PS II and electron transport activities of the above cyanobacterium has been studied both under *in vivo* and *in vitro* conditions to identify the site (s) of action.

MATERIALS AND METHODS

Synechococcus 6301 was grown axenically in BG-11 medium (20) at 25 ± 2ºC under continuous illumination (15 Wm^{-2}). Throughout the growth period the culture was agitated by the passage of filtered air after 5 days of growth (late log phase) cells were harvested by centrifuging at 9000 g for 5 mins, 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. Spheroplasts were prepared by incubating the intact cells at 37ºC in the presence of lysozyme (1mg/mL) for 3 h according to Newman and Sherman (1978).The photochemical activities were assayed polarographically with a Clark-type oxygen electrode according to Murthy *et al*. (1989). The 3 mL of reaction mixture used for the assay of whole chain electron transport contained suspension buffer, 0.5 mM Methyl viologen (MV) and 1 mM

Sodium azide.The three mL of reaction mixture used for the assay of the PS II catalyzed electron transport contained suspension buffer and 0.5 mM 2,6-dichlrophenol indophenol (DCPIP). The reaction mixture (3 mL) used for assaying the PS I catalyzed electron transport consisted suspension buffer, 0.1 mM DCPIP, 5 mM ascorbate, 1mM azide, 5 μM DCMU and 0.5 mM MV. In the entire assays spheroplasts equivalent to 15 μg chlorophyll was used. Spheroplasts were incubated with or without Hg^{2+} for 5 min in the dark before measurements were carried out at 25ºC under saturating illumination by white light (intensity (420 Wm^{-2}).

RESULTS AND DISCUSSION

In the present investigation an attempt has been made to study the effect of highlight (HL) on photochemical activities of the spheroplasts of cyanobacterium *Synechococcus* 6301 by exposing the intact cells for 30 min to white light.To achieve this initially whole chain electron transport (WCE) activities have been measured using MV as terminal electron acceptor.The treatment of cells with white light (105-450 Wm^{-2}) caused intensity dependent inhibition after 30 min of exposure and 52 % loss was noticed at 360 Wm^{-2} of light (Table 1). The inhibition in WCE could be due to alterations at the level of PS II (Choquet and Vallon, 2000) or changes at the level of PS I as suggested by others (Rajagopal *et al*., 2003). To probe the above argument PS II catalyzed electron transport has been measured using pBQ as Hill acceptor (Table 2). There is 55% loss in Hill activity after 30 min of exposure to white light

Table 1: Effect of different high light intensities on whole chain electron transport assay ($H_2O \rightarrow MV$) in the spheroplasts of *Synechococcus* 6301. Three mL of reaction mixture contains reaction buffer (25 mM HEPES-NaOH) (pH 7.5) contains 20 mM NaCl, 0.5 mM MV, 1mM sodium azide and thylakoid cells equivalent to 15 μg of Chl. The values are average of three separate experiments and SD is not more than 10%.

Light intensity Wm^{-2}	Whole chain electron transport activity ($H_2O \rightarrow MV$) μmoles O_2 consumed mg Chl^{-1} h^{-1}	Percent loss
Control	175 ± 15	0
105	133 ± 11	24
210	110 ± 9	37
360	84 ± 7	52
450	51 ± 4	71

Table 2: Effect of different high light intensities on PS II catalyzed electron transport assay ($H_2O \rightarrow BQ$) in the spheroplasts of *Synechococcus* 6301. Three ml of reaction mixture contains reaction buffer (25 mM HEPES-NaOH) (pH 7.5) contains 20 mM NaCl, 0.5 mM MV, 1mM sodium azide and thylakoid cells equivalent to 15 μg of Chl. The values are average of three separate experiments and SD is not more than 10%

Intensity of light Wm^{-2}	PS II electron transport activity $H_2O \rightarrow BQ$ μmoles O_2 evolved mg Chl^{-1} h^{-1}	Percent loss
Control	262 ± 21	0
105	193 ± 17	26
210	162 ± 13	38
360	118 ± 9	55
450	68 ± 4	74

Table 3: Effect of different high light intensities on PS I catalyzed electron transport assay ($DCPIPH_2 \rightarrow MV$). Spheroplasts were incubated in the presence of high light. The reaction mixture of PS I catalyzed electron transport assay contained reaction buffer and other ingredients as mentioned in the Materials and methods. The values are average of three separate experiments and SD is not more than 10%

Light intensity (Wm^{-2})	Photosystem I catalyzed electron transport activity ($DCPIPH_2 \rightarrow MV$) μ moles O_2 consumed mg Chl^{-1} h^{-1}	Percent loss
Control	358 ± 31	0
105	318 ± 27	11
210	279 ± 24	22
360	236 ± 19	34
450	211 ± 16	41

(360 Wm^{-2}). The observed inhibition in PS II catalyzed electron transport could be due to free radical mediated damage of D_1 protein of PS II or due to inactivation of WOC as suggested by Ohad *et al*. (1994). Between two photo systems PS II seems to be more sensitive to white light of high intensity. Contrary to PS II, high light treatment caused marginal (34%) inhibition with the similar intensity of light (360 Wm^{-2}). The possible reason for the loss of PS I inhibition could be changes at the level of reaction centre, P700 of PS I or at the level of iron sulphur centers as earlier repeated by others (Sonoike *et al*., 1997) (Table 3). Thus high light intensity exerts multiple effects on the thylakoid membrane photo functions, electron transport in the chloroplast of *Synechococcus* 6301.

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