African Journal of Basic & Applied Sciences 2 (3-4): 60-63, 2010
ISSN 2079-2034

Nitrogen Chlorosis Induced Alterations in the Photosynthetic Electron Transport Activities of the Cyanobacterium, *Spirulina platensis*

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Abstract: Among the tested concentrations of nitrate, 200 μM is essential for normal growth of the cyanobacterium, *Spirulina platensis* in the medium. Nitrogen starvation can be induced by the maintenance of nitrate concentrations below 60 μM in the medium. Wholechain electron transport measurements showed alterations both nitrate concentrations and incubation time dependent. Appreciable effects were observed after 24 h of incubation under low nitrogen concentrations. Between two photosystems, PS II catalyzed electron transport is more susceptible than PS I for nitrogen starvation. Inhibition in PS II clearly demonstrates the existence of alterations at LHC, phycobilisomes of the cyanobacterium.

Key words: Cyanobacteria · Nitrogen starvation · Electron transport · Photosystem · Spirulina platensis

INTRODUCTION

Nitrogen is a qualitatively important bioelement which is incorporated into the biosphere through assimilatory process carried out by the cyanobacteria. Numerous nitrogen containing compounds can be used by different organisms as source of nitrogen. Many of them are capable of fixing nitrogen. The cyanobacteria were found to be either nitrogen fixersor non-nitrogen fixers. In the absence of combined nitrogen sources, nitrogen fixing cyanobacteria avoid nitrogen deficiency by fixing molecular nitrogen [1-3]. Whereas non-nitrogen fixers respond to nitrogen deprivation by degrading their photosynthetic pigments resulting change in the colour of cultures from blue green to yellow, a process called chlorosis [1, 4-7]. Chlorosis also may occur upon starvation for other essential nutrients [8]. Of the other chloretic reactions, nitrogen starvation has studied extensively [9, 10]. Most of the studies have focused on the degradation of the major light-harvesting complexes the phycobilisomes, which proceeds in an ordered mannerG23 [11]. Researches were conducted to know the mechanisms in the degradation of the light harvesting complexes in the cyanobacteria, i.e., phycobilisomes [8, 12]. But, studies related to the alterations of thylakoid membrane and membrane functions regarding photosynthetic light reactions are scanty. Therefore, in this investigation, an attempt has been made to study the effect of nitrogen deprivation on the electron transport activities in the cyanobacterium *Spirulina platensis*.

MATERIALS AND METHODS

Spirulina platensis is a prokaryotic photosynthetic non-nitrogen fixing cyanobacterium, which is spherical in nature. The mother culture was obtained from National Facility for Blue green algal Collection, New Delhi, India and cultured autotropically in Zarrouk's medium [13] at 25 ± 2°C under continuous illumination (20 Wm^{-2}). Log phase cells were collected by centrifugation at 5000 xg for 10 minutes, then they were suspended in the different low concentrations of nitrate containing medium. After the incubation for different intervals, the cells were suspended in the HEPES-NaOH buffer (pH 7.5) and electron transport activities are measured at a concentration of 15 μg Chl/ 3ml. Photochemical activities were measured polorographically with Clark type oxygen electrode. The reaction mixture suitable for assaying the whole chain electron transport contained in addition to suspension buffer, 0.5 mM methyl viologen (MV) and 1 mM sodium azide was done according to the method of Kok *et al.* [14]. The assay mixture of the PS II catalyzed electron transport contained suspension buffer and freshly prepared solution of 0.5 mM p-benzo quinine (pBQ) [15]. For assay of PS I catalyzed electron transport the reaction mixture contained reaction buffer as stated before, 5 mM ascorbate, 1 mM TMPD/0.1 mM DCPIP/ 1 mM DAD, 10 μM DCMU, 0.5 mM MV, 1mM sodium azide and thylakoid membrane fragments equivalent to 12 to 15 μg Chl *a* [16]. Cells equivalent to 15 μg chlorophyll (Chl) was used in all electron transport assays.

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RESULTS AND DISCUSSION

Cells grown in 200 μM nitrate containing medium exhibited no changes in growth characteristics. The stress being induced after incubation of cells under 60, 40 and 20 μM nitrate containing media. Transfer of cells to nitrogen depleted medium (60-20 μM) showed time dependent inhibition in whole chain electron transport ($H_2O \rightarrow MV$) activity (Table 1). With 60 μM nitrate concentration containing medium incubated cells showed 46% loss after 48 h with 40 μM nitrate containing cells showed 43% loss after 48 h and 20 μM nitrate containing cells showed 53% loss after 48 h. Further incubation for 72 h caused 70 % loss. Time dependent studies clearly demonstrated that 24 h of incubation under nitrogen stress is sufficient to bring 50 % loss of whole chain electron transport (Table 1). The reason for the loss of whole chain electron transport could be either due to alterations at PS II or PS I catalyzed electron transport. To conform weather the target is PS II or PS I we have measured the PS II/ PS I catalyzed electron transport independently. Since the nitrogen depletion inhibited the whole chain electron transport, we have investigated its effect on PS II catalyzed pBQ Hill reaction in intact cells (Table 2). After 24 h of incubation under 60 μM nitrate concentration the electron transport activity exhibited 17 % loss in treated sample. Further decrease in nitrate concentration exhibited 33 and 53 % loss for 40μM and 20 μM nitrate concentrations respectively. The reasons for the loss of PS II catalyzed, electron transport could be changes at three levels: a) alterations at water oxidation complex, b) changes at LHC II or c) modification at reducing side of PS II [6, 17].

When compare to whole chain and PS II catalyzed electron transport, PS I catalyzed reactions could not be assayed in intact cells of *Spirulina* as reduced DCPIP/TMPD/DAD did not readily enter in to the intact cells. Therefore we have isolated thylakoid membranes by following the procedure of Murthy *et al.* [18, 19] and studied the status of PS I under nitrogen deprivation. The PS I activity of thylakoid membranes isolated from the 60 μM containing media exhibited 17 % loss after incubation for 24 h (Table 2). The inhibition has enhanced from 17 % to 25 % in the 40 μM nitrogen stress sample after 24 h of duration. Further decrease in the concentration to 20 μM could not cause much inhibition and only 38% loss was observed. The reason for the loss of PS I activity under nitrogen stress could be alterations at the level of reaction centre (P_{700}) or changes at oxidizing side or at the reducing side of PS I. Thus nitrogen stress affects the PS II and PS I in a preferential manner. Thus when compare to PS II catalyzed electron transport, PS I catalyzed electron transport seems to be resistant to nitrogen stress induced alterations.

To probe the exact site of nitrogen stress, we have measured the PS II activity at different light intensities (Table 3). For this study we have varied the light intensity from 105 to 3000 μ moles by using neutral density filters. The inhibition observed under light saturating conditions (3000 μmoles) was 53 % in nitrogen stressed samples. The inhibition noticed under light limiting conditions (105 μmoles) was 40 % (Table 3). Thus, the inhibition under nitrogen stress was 13 % more at light saturating conditions when compare to that of light limiting conditions. The inhibition at light saturating conditions could be due to alterations in the electron transport chain

Table 1: Effect of nitrogen stress (60, 40 and 20 μM $NaNO_3$) on whole chain electron transport assay ($H_2O \rightarrow MV$) for different intervals (24-72 h). The values are average of 3 separate experiments. The SD is not more than 10%.

Concentration $NaNO_3$ (μM)	Duration Time (h)	Whole chain electron transport activity(μ moles O_2 consumed mg Chl^{-1} h^{-1})$H_2O \rightarrow MV$
Control	0	242 ± 25 (0)
60	24	212 ± 25 (12)
	48	129± 13 (46)
	72	114 ±10 (52)
40	24	165 ± 18 (32)
	48	139± 15 (43)
	72	113 ± 14 (53)
20	24	130 ± 15 (46)
	48	113± 13 (53)
	72	73 ± 10 (70)

Table 2: Effect of nitrogen stress (60, 40 and 20 μM $NaNO_3$) on PS II and PS I catalyzed chain electron transport activities. The values are average of 3 separate experiments. The SD is not more than 10%.

		Electeron transport	
Concentration $NaNO_3$ (μM)	Duration Time (h)	PS II	PS I
Control	24	363 ± 25 (0)	415 ± 49 (0)
60		299 ± 15 (17)	346 ± 32 (17)
40		242 ± 15 (33)	312 ± 30 (25)
20		170 ± 10 (53)	260 ± 27 (38)

Table 3: Effect of nitrogen stress (20 μM $NaNO_3$) on photosystem II catalyzed electron transport activities under different light intensities.

	PSII catalyzed electron transport activity(μ ?moles O_2 evolved mg Chl^{-1} h^{-1})	
Light intensity μ moles	Control	Nitrogen stress (20μM)
105	42 ± 4	25 ± 5 (40)
1100	92 ± 11	50 ± 12 (46)
2050	192 ± 21	92 ± 16 (52)
3000	368 ± 32	176 ± 18 (53)

of PS II in nitrogen stressed samples, where as inhibition at light limiting conditions is due to the changes in energy transfer and alterations in the light harvesting complex (phycobilisomes) of cyanobacteria under nitrogen stress. Similar observations had made by Murthy [20] in the same organism under heavymetal stress.

Thus, from the our study it is clear that depending on the availability of nitrogen concentration, it shows multiple effects on photosynthetic electron transport in the cyanobacterium, *Spirulina platensis*.

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