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Photosynthetic Pigments under nitrogen stress in Spirulina platensis

Udaya Bhaskara Rao .P

Research Scholar, Department of Biotechnology,Rayalaseema University, Kurnool-518007 ,Andhra Pradesh,India

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Abstract-Incubation of cells with 60 µM Nitrogen containing growth medium causes a decrease in Phycocyanin emission and induces a shift in the peak position. Nitrogen stress induces alteration in energy transfer and structural changes in the phycobiliprotiens.Nitrogen stress causes 50% decrease in Phycocyanin emission and 40% decrease in Chl a emission. Phycobiliprotiens are the major targets among the other pigment proteins. The stress is induced after 24 hours in 60 µM nitrate containing growth medium.

Key Words:stress, phycobiliprotiens, structural changes , nitrogen, spirulina platensis

INTRODUCTION

In cyanobacteria, the phycobiliproteins constitute the major light harvesting pigment protein complexes, which are attached to the outer surface of the thylakoid membranes [1],[2]. The light energy absorbed by PBsome is transferred to the PS II reaction center (RC) in the following sequence: PC→ APC →Chl a [3],[4].

A variety of environmental factors are known to affect the efficiency of energy transfer from PC to Chl a i.e., high temperature [5], low temperature [6] From the in vivo results, it is clear that nitrogen stress can influence the energy transfer from PC to Chl a in intact cells of Spirulina.

Micro and macro nutrients are required to perform physiological activities in cyanobacteria. 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. In cyanobacteria, two types of categories are observed. They are diazotrophic cyanobacteria and non-diazotrophic ones.

In the absence of combined nitrogen sources, diazotrophic cyanobacteria avoid nitrogen deficiency by fixing molecular nitrogen. Non-diazotrophic strains respond to nitrogen deprivation by degrading their photosynthetic pigments resulting change in the colour of cultures from blue green to yellow, a process is called chlorosis [7], [8].

This chlorosis also can occur upon starvation for other essential nutrients although the chlorotic response shows subtle differences at cellular level depending on the nature of limiting nutrient factor [9],[10].

MATERIALS AND METHODS

Extraction and estimation of photosynthetic pigments

Cell suspension was drawn after thoroughly shaking the flasks and cells were collected by centrifuging at 9,000 xg for 5 min. The pellets were washed twice with reaction buffer (25 mM HEPES- NaOH buffer (pH 7.5) containing 20 mM NaCl) and suspended in the same buffer.

Chlorophyll a and carotenoids:

The washed pellets were resuspended in methanol or 80% acetone and kept at 5°C for 10 min. the extract was taken and centrifuged by using table top Remi centrifuge for 10 min at 6,000 xg. The supernatant was taken for Chl a estimation and optical density was measured at 665 nm (for methanol) or 663 nm (for 80% acetone) in a Shimadzu UV150 spectrophotometer.

Chl a amount was calculated using the extinction coefficient, E_{663}=82.04 mg ml^{-1} cm^{-1} (for methanol extracted samples) according to Mackinney [11].

Carotenoids were measured in 80% acetone by measuring the optical density at 480 nm. Carotenoids amount was calculated by using the extinction coefficient. E_{480} /cm 1%= 2,500 by following the method of Jensen ([12].

RESULTS

In absorption spectra, the peak at 681 nm is due to the absorption of Chl a peak; at 621 nm is due to the absorption of PC of PBsomes; a hump at 480 nm is due to the absorption of carotenoids; and a peak at 437 nm is due to soret band of Chl a [4].

Nitrogen depletion to 60 µM caused huge decrease in PC absorption by marginally effecting in the chlorophyll and carotenoid absorption. In addition, nitrogen stress caused a red shift in the peak position by 3 nm in PC absorption. This decrease and alterations in PC absorption could be due to changes in the apoprotein and chromophore interaction. The effect was further characterized by calculating the absorption ratios of different pigment proteins such asA_{440}/ A_{680}, A_{470}/A_{680} and A_{621}/ A_{680} (Table 1).

Table 1

Concentration of $NaNO_3$ (µM)	Absorption ratio		
	440/680	470/680	621/680
Control	1.14	0.88	0.97
80	1.11	0.86	0.86
60	1.12	0.86	0.82
40	1.14	0.87	0.79

The ratio of absorption at soret band region to the absorption at red region of chlorophyll in control cells was 1.14. Nitrogen stress from 80µM to 40µM did not bring any change in the ratio of chlorophyll absorption. The ratio of absorption at carotenoid region to the absorption at the red region of the chlorophyll was 0.88 in control cells (Table 1).
The increase of nitrogen stress could not influence the ratio of carotenoids to chlorophyll absorption. This shows that nitrogen stress could affect neither chlorophyll nor carotenoids. The ratio of absorption at PC region to the absorption at red region of the chlorophyll was 0.97 in control cells.
Nitrogen stress brought gradual decrease in the ratio from 0.87 to 0.82 in 20 µM nitrate medium containing grown cells. This clearly demonstrates that phycobiliproteins are major targets among other pigment proteins.

As PC absorption was quite extensively affected by nitrogen stress, The room temperature PC fluorescence emission spectra of Spirulina cells which were grown with less nitrogen containing medium was measured.

In control cells excited at 545 nm, an emission peak at 647 nm emanating from PC was prominent in the spectrum [4],[5].

Incubation of cells with 60 µM and 40 µM nitrogen containing medium exhibited decrease in the PC fluorescence emission and induced blue shift in the peak position by 2 nm. Control cells exhibited 75 relative units of PC fluorescence with an emission peak at 647 nm.

Nitrogen stress induced by the depletion of the nitrogen from 80 µM to 40 µM gradually brought the decrease from 14% to 39%. In addition, 5 nm blue shift was noticed in the nitrogen stressed samples (Table 2).

Table 2

Concentration of $NaNO_3$(µM)	Phycocyanin fluorescence emission		Percent decrease
	Intensity (Relative units)	Peak position	
Control	75	647	0
80	65	646	14
60	54	642	38
40	46	642	39

This clearly demonstrates that the nitrogen stress induced alteration in energy of energy transfer from PC to Chl a and structural changes of phycobiliproteins.

To confirm the alteration in energy transfer from PC to Chl a, the cells were excited with 440 nm light specially targeting Chl a present in the PS II. In control cells two peaks at 654 and 680 nm were prominent in the spectrum. 654 nm peak is contributed by PC where as 680 nm is contributed by PS II Chl a.

Nitrogen stress (60 µM grown cells) caused 50% decrease in PC emission and 40% in Chl emission with a blue shift by 4 nm. This clearly confirms the structural alterations of Chl proteins. From these spectra it is clear that the energy is not flowing with 100% efficiency from PC to Chl a. Table 14 shows the ratio of Chl a to PC emission of control cells was 0.87. In treated sample, the ratio has enhanced to 1.21 indicating the improper energy transfer in nitrogen depleted cells (60 µM).

In cyanobacteria, the phycobiliproteins constitute the major light harvesting pigment protein complexes, which are attached to the outer surface of the thylakoid membranes [1],[2]. The light energy absorbed by PBsome is transferred to the PS II reaction center (RC) in the following sequence: PC→ APC →Chl a [3],[4].

A variety of environmental factors are known to affect the efficiency of energy transfer from PC to Chl a i.e., high temperature [5], low temperature [6], From the in vivo results, it is clear that nitrogen stress can influence the energy transfer from PC to Chl a in intact cells of Spirulina.

DISCUSSION

Several workers have made studies related to the nitrogen starvation in cyanobacteria. Most of the investigations have focused on the degradation of the major light-harvesting complexes, the phycobilisomes which proceed in an ordered manner[13].

The studies related to the alterations of thylakoid membrane and membrane functions regarding photosynthetic light reactions are scanty. Therefore the effect of nitrogen deprivation on the organization of thylakoid membranes, PBsomes, electron transport and energy transfer process in the cyanobacterium Spirulina platensis were studied.

To induce the nitrogen stress Spirulina platensis intact cells were suspended in 40 µM, 60 µM and 80 µM concentration nitrate containing medium. The cells which were grown in 220 µM nitrate containing medium exhibited no changes in the growth characteristics.

The stress is induced after incubation of cells for 24 h in 60 µM nitrate containing medium. Therefore 40 µM, 60 µM and 80 µM nitrate containing media as stress inducers were chosen and the effect of nitrogen limitation on electron transport and energy transfer processes of this cyanobacterium is studied.

CONLUSIONS

The stress due to nitrogen depletion affects phycobiliprotiens and induces changes in energy transfer from phycocyanin to chlorophyll a. The nitrogen stress at different concentrations of nitrate in the growth medium shows its effect on photosynthetic pigments.

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