International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 04 Issue: 07 | July -2017 www.irjet.net p-ISSN: 2395-0072

Whole chain electron transport under nitrogen stress in Spirulina platensis

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Abstract-*Sensitivity of photosystems, partial electron transport reactions of photosystem-II are measured Nitrogen stress brings 50% loss of whole chain electron transport activity. The increase in the incubation period from 24 hours to 48 hours brings an appreciable loss of whole chain electron transport activity. A decrease in the nitrogen concentration in the growth medium brings loss in Photosystem-II activity. Photosystem-I mediated electron transport is less sensitive to nitrogen stress compared to that of Photosystem-II.*

Key Words: stress, electron transport, photosystems, nitrogen spirulina

INTRODUCTION

To induce the nitrogen stress, Spirulina platensis intact cells were suspended in 40 µM, 60 µM and 80 µM concentration containing nitrate rich 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. For studying electron transport, an oxygen electrode was used and the activities of photo systems in terms of oxygen evolution / consumption were studied. Nitrogen stress induced time dependent inhibition in MV photoreduction mediated by both PS II and PS I.

MATERIALS AND METHODS

Estimation of protein content:

The total cell protein or pigment protein content was determined with Folin- Ciocalteau reagent according to Lowry *et al.* [1]. The reagents used were as follows:

Reagent 1: 2% of sodium carbonate in 0.1N NaOH.
Reagent 2: 0.5% $CuSO_4$ in 1 % sodium-potassium tartarate.
Reagent 3: Mixture of 50 ml of reagent 1 + 1 ml of reagent 2.
Reagent 4: Diluted Folin-Ciocalteau reagent (1: 1 v/v).

After harvesting and washing the cells, the proteins of the cells were precipitated by the addition of 10% TCA. The precipitated protein was collected by centrifugation. The pellets were suspended in 1N NaOH and thoroughly mixed. To the 1 ml of diluted protein solution whose content has to be determined, 5 ml of reagent 3 was added. After thoroughly mixing, it was allowed to stand for 10 min at room temperature. To this solution 0.5 ml of reagent 4 was added rapidly after thoroughly mixing and it was allowed to stay for 30 min. After developing the colour, the optical density was measured at 600 nm in a Shimadzu UV 150 spectrophotometer against a reagent blank which did not have any protein solution.

A set of BSA solutions (Sigma fraction IV) (10- 100 µg) were taken for preparing standard curve for protein estimation.

Preparation of the thylakoid membrane fragments:

The cells were harvested as mentioned above and suspended in the reaction buffer. The cells were then broken by passing the above cell suspension twice through a prechilled French Press (Aminco, SLM Instuments, USA), at 20,000 psi. 30 ml of fragmented cell suspension was centrifuged at 9,000 xg for 5 min at 4°C to remove unbroken cells. The resulting supernatant was then centrifuged at 30,000 xg for 45 min at 4°C. The pellet was suspended in the minimal volume (3 ml) of reaction buffer. All the centrifugation steps were done in Hitachi centrifuge. On Chl basis, it was estimated that the yield of thylakoid membrane fragments was about 10%.

RESULTS

Time dependent effect of nitrogen depletion at 60 µM of nitrate on whole chain electron transport was studied by using oxygen electrode. Under the concentration of 33% nitrate in the growth medium 44% loss was noticed after 24 h of incubation.

The increase in the incubation period to 48 h brought 54% loss in the whole chain electron transport. Further reduction of nitrate concentration to 40 µM brought significant inhibition in whole chain electron transport activity and 52% loss in the whole chain electron

transport activity was noticed after 24 h of incubation. The increase in the incubation period from 24 h to 48 h brought appreciable loss of whole chain electron transport to 68%. From the above study it is clear that 40-80 µM nitrate concentration is effective in the induction of nitrogen starvation. Time dependent studies clearly demonstrate that 24 h of incubation under nitrogen stress is sufficient to bring 50% loss of whole chain electron transport.

From the literature it is clear that the reason for the inhibition of whole chain electron transport could be due to two reasons i.e., either in the alteration at the level of PS II catalyzed electron transport or the changes at PS I catalyzed electron transport.

To distinguish the sensitivity of photosystems, the partial electron transport reactions of PS II and PS I are measured independently. Since the nitrogen depletion inhibited the whole chain electron transport, its effect on PS II catalyzed pBQ Hill reaction in intact cells is investigated. pBQ is an artificial electron acceptor and it accepts electron from PQ pool [2],[3]. pBQ being lipophilic in nature, it enters easily in to intact cells of *Spirulina.* Control cells exhibited a high rate of PS II dependent O_2 evolution activity (375 µmoles of O_2 evolved mg Chl^{-1} h^{-1}).

From the above studied it is clear that 24 h of incubation under nitrogen stress was optimum for the induction of nitrogen stress and the effect of extent of nitrogen depletion (80-40 µM) was studied on PS II catalyzed electron transport. After 24 h of incubation under 80 µM nitrate concentration the electron transport activity exhibited 20% loss in treated sample. Further decrease of nitrate concentration in the growth medium to 60 µM brought 34% loss in the PS II activity.

In the sample which was incubated under 40 µM of nitrate for 24 h, exhibited more than 53% loss in pBQ supported Hill reaction. The loss in the PS II catalyzed electron transport under stress could be due to three reasons i) the alterations at the level of WOC, ii) changes at the reaction center or LHC-II and iii) modifications at the reducing side of PS II. Similar reports were made in the photosynthetic electron transport activities of PS II in *Spirulina* under heavy metal stress [4].

DISCUSSION

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 confirm this, the PS II / PS I catalyzed electron transport was measured independently.

pBQ is an artificial electron acceptor which can enter easily into intact cells due to its lipophylic nature (Trebst, 1974). By selecting the 24 h as incubation period, alterations induced by the nitrogen depletion in the intact cells were studied.

There is a concentration dependent increase in the inhibition of PS II catalyzed electron transport activity and at 40 µM of nitrate concentration 53% inhibition was noticed. This inhibition is the PS II catalyzed electron transport activity and could be due to changes at three levels. 1) Alterations at WOC 2). Changes at LHC II 3). Modification at reducing side of PS II.

There was a loss of PS II activity due to shifting of cells from nitrate containing medium to nitrogen depleted medium after 48 h of incubation[5]. PS I mediated electron transport is less sensitive when compared to that of PS II (Table 1).

Since some of the donors and acceptors are unable to enter inside the cells, the thylakoid membranes were prepared and studied the nitrogen stress induced alterations in PS I catalyzed electron transport.

Table 1

Concentration $NaNO_3$(µM)	Duration Time (h)	PS-I catalyzed electron transport activity µ moles of O_2 consumed mg Chl^{-1} h^{-1}	% Inhibition
Control	0	422± 49	0
80		352± 32	17
60	24	317± 30	25
40		268± 27	37

The PS I fragments are free from PC and they exhibited maximum activity (415 µmoles of oxygen consumed). Nitrogen stress gradually caused the inhibition in PS I activity and only 38% inhibition was noticed with severe nitrogen (40 µM) stress. This loss in PS I activity could be due to sensitivity of carotenoids to nitrogen stress [6].To correlate the involvement of LHC II in PS II catalysed electron transport studies were made at different illuminating conditions(Table 2).

Table 2

Light intensity (Wm^{-2})	PS-II catalyzed electron transport activity (µ moles o_2 evolved mg Chl^{-1} h^{-1})		% Inhibition
	Control	Nitrogen stress (60µM)	
470	372	181	52
230	197	94	52
105	96	48	50
12	46	27	42

The nitrogen stress induced inhibition was made at light saturating conditions than at light limiting conditions. The inhibition at light saturating conditions could be due to alterations at electron transport components where as at light limiting conditions is due to changes of energy transfer and alterations in LHC II, phycobilisomes of cyanobacterium Spirulina platensis.

CONCLUSIONS

Nitrogen depletion plays an important role in loss of whole chain electron transport system. The sensitivity of photosystems I and II to the stress due to nitrogen depletion in the growth medium, incubation time and illuminating conditions show their effects whole chain electron transport activity.

REFERENCES

[1] Lowry, a.H., Rosenbrough, J.H., Farr, A.L. and Randall, RJ. (1951) Protein measurement with the Folin-phenol reagent. J. Biol. Chern. 193: 265-275.

[2] Warburg, O. and Luthgens, W. (1944) Naturwissenscheften, 32: 161.

[3] Trebst, A. (1974) Energy conservation in photosynthetic electron transport of chloroplasts. Ann. Rev. Plant Physiol. 25: 423-458.

[4] Murthy, S.D.S. and Mohanty, P. (1991) Mercury induced alteration of energy transfer in phycobilisome by selectively affecting the pigment protein, phycocyanin in the cyanobacterium, Spirulina platensis. Plant Cell Physiol. 32: 231-237.

[5] Allen, M. M., Law, A. and Evans, E. H. (1990). Control of photosynthesis during nitrogen depletion and recovery in a non-nitrogen- fixing cyanobacterium. Arch Microbioll53: 428-431.

[6] Biswal, B., Smith, AJ. and Rogers, L.J. (1994) Changes in carotenoids but not in D1 protein in response to nitrogen depletion and recovery in a cyanobacterium.FEMS microbiology Lett. 116: 341-347.