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ROLE OF NON-CYCLIC PHOTOPHOSPHORYLATION IN NITROGEN FIXATION IN *NOSTOC MUSCORUM*

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Nostoc muscorum is a diazotrophic and photoautotrophic cyanobacterium. The membrane-bound PS II complex of cyanobacteria is involved in the process of O_2 evolution and non-cyclic photophosphorylation. The herbicide DCMU an inhibitor of non-cyclic photophosphorylation, was supplied to the organism to estimate the contribution of this metabolic pathway towards nitrogen fixation. It was found that DCMU induced a 40% decrease in growth rate and 98% decrease in amount of N_2 fixed. The N_2 fixing capacity could be almost restored by supplying ATP to the organism. Therefore, DCMU mediated inhibition of nitrogen fixation was due to non-availability of ATP generated by non-cyclic photophosphorylation.

Keywords : Cyanobacteria; DCMU; Nitrogen fixation.

Introduction

Cyanobacteria are photosynthetic prokaryotes that evolve oxygen from water. Similar to eukaryotic algae and green plants, the membrane-bound PS II complex of cyanobacteria is involved in the process of O_2 evolution and non-cyclic photophosphorylation. The principle light harvesting structures for the PS II in cyanobacteria are phycobilisomes, pigment-protein complexes that are located on the surface of the thylakoid membranes. Phycobiliproteins are known to be concentrated in PS II in cyanobacteria.

Photosystem II produces 'reductant' and ATP, both of which are required by nitrogenase for nitrogen fixation. There are observations which suggest that PS II products are not utilized by the nitrogenase [1-4]. However, few workers are of the opinion that PS II reactions do affect nitrogen fixation [5-7].

In the present investigation an attempt has been to determine the role of non-cyclic photophosphorylation in nitrogen fixation in Nostoc *muscorum*. Using DCMU it is possible to prevent photolysis of water and consequently block non-cyclic photophosohorylation. If non-cyclic photophosphorylation has a role in nitrogen fixation then DCMU mediated inhibition of non-cyclic photophosphorylation is expected to inhibit growth rate as well as nitrogen fixation by cutting down the supply of ATP.

Materials and Methods

Nostoc muscorum was grown from our laboratory cultures in BG II medium[8]. The pH of the medium was adjusted to 7.8 before autoclaving. Phosphate was autoclaved separately and mixed after cooling the medium to avoid precipitation. DCMU was dissolved in distilled water and matured for 7 days to complete dissolution. The organism was grown in 100 ml flasks containing 50 ml of medium (MM). The cultures were grown at room temperature with a light

Table 1. Effect of DCMU and ATP on the growth rate

S. No.	Composition of medium	Rate of Growth (Day^{-1})	% decrease in growth rate*
1.	MM	0.50	-
2.	MM + DCMU (50 µg)	0.47	6.00
3.	MM + DCMU (100 µg)	0.45	10.00
4.	MM + DCMU (150 µg)	0.40	20.00
5.	MM + DCMU (200 µg)	0.30	40.00
6.	MM + DCMU (200 µg) + ATP (10^{-5} M)	0.30	16.66
7.	MM + DCMU (200 µg) + ATP (10^{-4} M)	0.33	13.33
8.	MM + DCMU (200 µg) + ATP (10^{-3} M)	0.40	1.10
9.	MM + DCMU (200 µg) + ATP (10^{-2} M)	0.50	0.00

* % decrease is calculated with reference to growth rate in MM

Table 2. Effect of DCMU and ATP on nitrogen fixation

S. No.	Composition of Medium	48 h		72 h		96 h	
		N fixn. (mgl^{-1})	% ** decrease	N fixn. (mgl^{-1})	% ** decrease	N fixn. (mgl^{-1})	% ** decrease
1.	MM	1.10	-	1.40	-	1.29	-
2.	MM + DCMU (50 µg)	0.28	74.55	0.67	52.14	0.61	52.80
3.	MM + DCMU (100 µg)	0.17	84.56	0.43	69.29	0.52	59.69
4.	MM + DCMU (150 µg)	0.09	91.82	0.30	78.57	0.33	74.42
5.	MM + DCMU (200 µg)	0.02	98.19	0.11	92.17	0.20	84.50
6.	MM + DCMU (200 µg) + ATP (10^{-5}M)	0.21	80.01	0.09	93.57	0.06	95.32
7.	MM + DCMU (200 µg) + ATP (10^{-4}M)	0.24	78.79	0.20	85.72	0.18	86.05
8.	MM + DCMU (200 µg) + ATP (10^{-3}M)	0.40	63.64	0.35	75.00	0.30	76.75
9.	MM + DCMU (200 µg) + ATP (10^{-2}M)	0.80	27.27	1.29	7.86	0.94	27.14

** % decrease ic calculated with reference to nitrogen fixed in MM.

intensity of 1800 lux from incandescent bulbs at the culture surface.

Growth was monitored in terms of increase in synthesis of chlorophyll-a (calculated in terms of absorbance values at 665λ).

Readings were taken with the help of Baush & Lamb Spectronic - 20 (1 cm pathlength). Total nitrogen was estimated by modified Microkjeldahl method[9]. The N content was computed as follows:

1ml of 1 N acid = 14 mg nitrogen

1ml of N/25 acid = 0.56 mg nitrogen

$$\text{Amount of nitrogen (mg/l)} = \frac{0.56 \times \text{Vol. of acid} \times 20}{\text{wt. of sample}}$$

$$\text{Amount of nitrogen in the filterate} = \frac{0.56 \times \text{vol. of acid} \times 20}{\text{wt. of filterate}}$$

Results and Discussion

The present investigation has shown some interesting results with regard to the role of PS II (and therefore non-cyclic photophosphorylation) in nitrogen fixation in this organism.

The herbicide DCMU inhibited growth rate ranging from 6 to 40% depending on the concentration of DCMU (Table 1). Stewart and Pearson[6] evidenced that DCMU (3×10^5M) completely inhibits oxygen evolution in aerobically grown *Anabaena* cultures. Padan *et. al*[7] reported that in light, 1μ M DCMU completely inhibited Co_2 fixation in Plecto*nema* for atleast 13h. Growth rate of DCMU (200 μg) treated cells increased in response to variable concentrations of ATP suppli to the medium, and could be restored to normal growth rate i.e. 0.5 doublings per day at higher concentrations of ATP (10^{-2}M).

DCMU treated cells also showed a decline in total nitrogen fixed depending on age of the culture and the concentration of DCMU employed. In 48 h old cultures maximum decrease in nitrogen fixed at saturating DCMU concentration was 98%, however, in aging cultures (72-96 h old) nitrogen fixation increased between 6-14% as compared to that in 48 h old cultures. Weare and Benemann[10] have reported that DCMU strongly inhibited the nitrogenase activity of aging cultures and that PS II may donate electrons to most of the ferredoxin used in nitrogenase reaction in *Anabaena* cultures. Singh *et al*[11] while working on rice field isolate of *Gloeocapsa* sp. have reported that nitrogen fixation diminishes with increasing concentrations of DCMU.

Total inhibition of nitrogen fixation, however, was not observed at higher concentrations of DCMU, indicating thereby that a small percentage of nitrogen fixation is independent of DCMU mediated inhibition. That the inhibition of nitrogen fixation was due to non-availability of ATP generated through non-cyclic photophosphorylation, was indicated when cells treated with DCMU were simultaneously supplied with ATP. Cells of different age groups ranging between 48-96 h observed differential increase in the rate of nitrogen fixed in response to variable

ATP concentraton as shown in Table 2. ATP has a marked effect on the overall increase in the rate of nitrogen fixation. As a matter of fact enhancement of nitrogen fixation was observed several fold in the presence of ATP in contrast to the cells grown in MM only. This observation may have wider implications with regard to energetics of the nitrogen fixation process as a whole.

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