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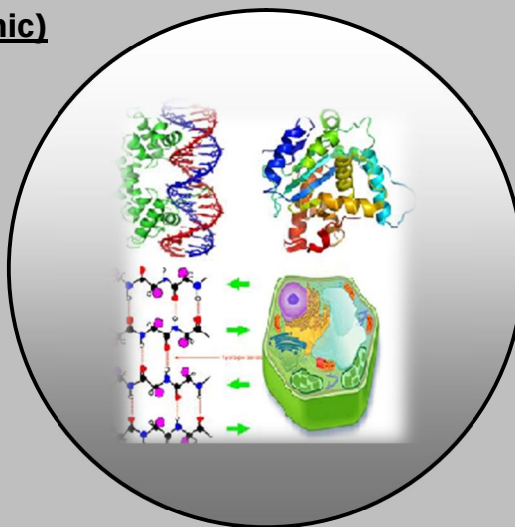
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RESEARCH PAPER

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Cadmium Induced Photoinhibition of Photosynthesis in Cyanobacterium *Aphanothece stagnina* Grown Under Various Concentrations of Nitrogen and Phosphorus

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ABSTRACT

The present communication is an attempt to study the response of photoinhibitory light and cadmium stress singly and in combination to each other, in presence of various concentrations of nitrate and phosphate on cyanobacterium Aphanothece stagnina. Results suggest that both the nutrients regulate the photoinhibition of photosynthesis under cadmium stress in a concentration dependent manner. A marked reduction in photosynthetic oxygen evolution due to photoinhibitory light and cadmium stress was observed. High levels of nitrate and phosphate were found to decrease the effect of photoinhibitory light and cadmium by enhancing the photosynthesis capacity for the assimilation of organic compounds resulting in decreased cadmium toxicity.

Key Words: Cadmium, Photoinhibitory light, Photosynthetic oxygen evolution and Nutrient levels.

INTRODUCTION

In natural field conditions, photosynthesis organisms are exposed to a wide range of light regimes, ranging from growth limiting to growth inhibiting irradiance. Excess light is the most whelming and omnipresent stress which decreases the intrinsic efficiency of organism termed photoinhibition (Ogren, 1988).

When plants are exposed to additional environmental stress co-occurring in nature, their interactions are complex and result in intensification and overlapping of stress through organism responses (Ogren and Oquist, 1984). An understanding of toxicologic and physiologic responses of algae and cyanobacteria on heavy metals alone and in combination with high light intensity is of particular relevance when attempting to predict the impact of cations on aquatic environment. The interaction of light with heavy metal is of common occurrence in water bodies and most favored habitat of cyanobacteria due to relative abundance of trace metals and their chemical speciation in the Archean and Proterozoic oceans lead to the conclusion that marine cyanobacteria have trace metal sensitivities (Sanito et al., 2003). Cyanobacteria interactions with heavy metals such as Hg, Pb, Cr, have been frequently reported (Bapatista and Vasconcelos, 2006). Whereas cyanobacterial interactions with cadmium are still undetermined. Cadmium is one of the most hazardous trace elements in the environment because of its high toxicity to all components of aquatic community (Vymazal, 1987). Cadmium has received widespread attention because of its accelerated release into the environment as the result of industrial pollution. In early ocean, it present at very low concentration but today the concentrations are much higher leads to toxicity to the cyanobacteria. Although, there are several reports about regulation of the susceptibility of photoinhibition in cyanobacteria by nutrient elements, but none of the studies have been conducted earlier in comprehensive manner to understand the problem. Thus, the present study aims to look into the effect of nitrogen and phosphorus concentration on the photoinhibitory effect in cyanobacterium *Aphanothece stagnina* in presence of photoinhibitory light alone and in combination with cadmium.

MATERIAL AND METHODS

Experimental organism and culture conditions

Cyanobacteria *Aphanothece stagnina* was used in the present study. Axenic culture of *A. stagnina* was well grown and maintained in modified nitrogen containing BG-11 medium (Huges et al., 1958), under $75 \mu \text{mol photon m}^{-2} \text{s}^{-1}$ light intensity at $27 \pm 1^\circ \text{C}$ and photoperiods of 14:10 h light and dark cycle controlled by electronic automatic timer (MDS Switch Gear Ltd. India). The exponentially grown cyanobacterial cells were used throughout the experiment, conducted in replicates of three.

Photoinhibitory light treatment

Photoinhibitory light was provided by metal halogen lamp (Osram HQI-TS-500 W) mounted in reflector. the desired photon flux densities was obtained by varying the distance between the source and the cyanobacterial sample glass tubes (1.5 cm diameter) immersed in a temperature controlled water bath $27 \pm 1^\circ \text{C}$ provided by gentle stirring. Photon flux density (PFD) was measured with the help of power meter (Spectra Physics USA mode 407, A-2). For photoinhibitory treatment, cell suspension was exposed to $1000 \mu \text{mol photon m}^{-2} \text{s}^{-1}$ light intensity for 30 minutes alone and in the presence of different concentration of cadmium.

Metal treatment

Stock solution of CdCl_2 was prepared in glass sterilized double distilled water and freshly prepared solution was filter sterilized ($0.45 \mu\text{m}$) using Millipore membrane filter (USA) before the preparation of working concentrations in culture medium. Before being exposed to high light treatment, cyanobacterium was incubated to different concentrations of cadmium with $50 \mu\text{mol photon m}^{-2}\text{s}^{-1}$ at $27 \pm 1^\circ\text{C}$.

Nitrate and phosphate treatment

Exponentially growing cells were harvested from normal culture suspension by centrifugation at 5000 rpm for 5 min, washed twice with sterile distilled water and suspended in nitrate and phosphate deficient (N- free) medium for 48 h with $75 \mu\text{mol photon m}^{-2}\text{s}^{-1}$ light intensity. Cells were again harvested, washed twice with double distilled water to remove adhered substance. Resultant cyanobacterium were allowed to grow separately with different concentrations (0.015, 1.5 and 3.0 mg/100 ml) of nitrate and phosphate (0.5, 50 and 150 $\mu\text{g}/100\text{ml}$) for 8 h under $75 \mu\text{mol photon m}^{-2}\text{s}^{-1}$ PAR light intensity at $27 \pm 1^\circ\text{C}$ temperature. These cells were again divided to incubate with different concentrations of cadmium (50, 100, 150 $\mu\text{M CdCl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$) before 1 h to photoinhibitory light treatment. Photosynthetic activity was assayed by measuring photosynthetic oxygen evolution.

Measurement of photosynthetic oxygen evolution

Photosynthetic oxygen evolution was measured in a water jacketed electrode cell of 5 ml capacity by using a Clark-type O_2 electrode (Digital Oxygen System Model 10, Rank Brothers U.K.) in temperature controlled air tight reaction vessel at $27 \pm 1^\circ\text{C}$. For oxygen evolution measurement, 5 ml of treated or untreated cell sample was withdrawn in electrode cell. Actinic light was provided by a slide projector fitted with a 250 W halogen lamp which was fed by a constant current power supply. The light beam was passed through water jacket and the light intensity ($360 \mu\text{mol photon m}^{-2}\text{s}^{-1}$) was set as desired at the centre of the electrode cell.

RESULTS AND DISCUSSION

A maximum photosynthetic oxygen evolution was recorded in control for cultures supplemented with various concentrations of nitrate. On exposure to photoinhibitory light, photosynthetic O_2 evolution declined significantly. Photoinhibitory light in combination with various concentrations of cadmium (Cd) decreases the photosynthetic oxygen evolutions further. *A. stagnina* cells exposed to minimum Cd (50 μM) concentration and a combination of photoinhibitory light supplemented with maximum nitrate 03.0 mg/100 ml showed minimum decline of 44% in photosynthetic oxygen evolution over control. Whereas cells exposed to photoinhibitory light treatment in presence of maximum Cd (150 μM) with minimum nitrate supplementation of 0.015 mg/100ml showed maximum inhibition of 81% in photosynthetic oxygen evolution. Similar trend was observed in *A. stagnina* cells exposed to photoinhibitory light alone and in combination with Cd in presence of different concentrations of phosphate.

A. stagnina cells after exposed to photoinhibitory light singly decline in photosynthetic oxygen evolution is due to decreased supply of ATP and NADPH₂ resulting from deleterious effect on photosynthetic apparatus (Sinha et al., 1996). On exposure to a combination of photoinhibitory light with different concentration of cadmium, the effect gets synergistically enhanced resulting in further decrease in photosynthetic oxygen evolution. Since, cadmium is responsible for deterioration of thylakoid membranes which implicates a loss of pigments and photosynthetic activity (Surosz and Palinska, 2004). Besides this, PS II disfunctioning and decline in xanthophylls cycle – a mechanism which protects photosynthetic apparatus (Wisniewski and Dickinson, 2003), adversely affects the photosynthetic potential thus lowering photosynthetic oxygen evolution further. Minimum photoinhibitory effect was observed in cyanobacterial cells with maximum nitrate concentration (3.0 mg/100 ml) because light captured, appeared to be utilized in assimilation of nitrate to ammonia. Whereas, Cyanobacterial cells with minimum concentration of nitrate (0.015 mg/100ml) in culture medium exhibited greater loss in photosynthetic oxygen evolution on exposure to photoinhibitory light in combination with cadmium. Nitrate uptake in cyanobacteria is enhanced by light (Gotham and Rhie, 1981). Exogenous nitrate is transported into the cells where it is reduced to ammonia by the action of nitrate reductase (NR) and nitrite reductase (NIR) enzyme systems. Such nitrate reduction in cyanobacteria is linked to photosynthesis. The ability to fix nitrogen in diazotrophic cyanobacteria depends upon supply of ATP and reductant which are provided either directly by photosynthesis or indirectly from the oxidation of carbohydrate. Assimilation of amino acids depends on photosynthesis and is stimulated by increasing irradiance (Lee-Kadam and Simonis 1982). Nitrogen limitation causes a reduction in thylakoid stacking and light absorptivity (Rhiel et al., 1985, 1986, Plumbey and Schmidt, 1989) by affecting the apparent absorption cross section of PS II and the efficiency of energy transfer by decreasing the size of the antenna (Turpin et al. 1990). It may also affect the enzyme of photosynthetic carbon metabolism, where decline in ribulose 1-5 biphosphate carboxylase per cell is well established (Plumbey and Schmidt, 1989, Beardall, 1991). This corresponds to a large decline in cellular photosynthetic capacity. This decreased ability to dissipate light energy in the reduction of CO₂ can be related to the increased susceptibility of nitrogen-deficient algae to photoinhibition (Prezelin et al, 1986, Rhiel et al., 1986, Kolber et al. 1988). Plants grown in high light along with high nitrogen has the highest tolerance to photoinhibition and highest photosynthetic capacity with fast recovery of photoinactivated PS II (Kato et al., 2002). In present study, cyanobacterial cells treated with maximum nitrate showed minimum photo inhibition on exposure to photoinhibitory light and different cadmium concentrations. It can be attributed to low uptake of cadmium by the cells with high nitrate content or partial utilization of excess light energy in nitrate assimilation or to a combination of both.

Phosphorus is also an important regulatory and constructive component of living organism as a constituent of nucleic acids, phospholipids, ATP and other phosphorus containing components, responsible for oxidation-reduction reaction.

A marked effect of phosphorus content on photosynthetic activity of the *A. stagnina* cells was observed after exposure to photo inhibitory light alone and together, with cadmium. Increased phosphate level reduced the photosynthetic O₂ evolution loss caused by the photo inhibitory light, alone and together, with cadmium. The role of phosphate in antagonizing heavy metal toxicity has been studied (Patterson et al. 1985, Greger et al., 1992, Singh et al., 1993). The interaction between heavy metals and phosphorus is thought to be primarily based on adsorption-precipitation reactions occurring either at the cell surface or outside the cell. Phosphate gets accumulated inside cells as polyphosphate bodies. These polyphosphate granules, because of negative charge, are known to bind a good amount of toxic metals (Singh et al., 1993). Thus, in present study high phosphate supplemented cultures showed less inhibitory effect as the toxic metal cadmium might have bound with polyphosphate bodies and thus cadmium was not found in free state. Thereby, the extent of interruption of photosynthetic electron transport chain and damage to photosynthetic machinery would have been reduced, considerably.

Taking into account these results, we propose the functional role of nitrate and phosphate in the alleviation of the stress caused by photo inhibitory light alone and in combination with cadmium.

Table – Photo inhibition of photosynthesis in cadmium treated *Aphanothece stagnina* cells in presence of different levels of nitrate and phosphate.

Treatment	Photosynthetic oxygen evolution [μ mol O ₂ evolution (mg Chl a) ⁻¹ h ⁻¹]					
	Nitrate concentration (mg/100 ml)			Phosphate concentration (μ g/100 ml)		
	0.015	0.15	3.0	0.5	50	100
Normal light (Control)	140 $\pm$ 3	180 $\pm$ 6	230 $\pm$ 5	175 $\pm$ 4	210 $\pm$ 2	240 $\pm$ 5
Photo inhibitory light	73 $\pm$ 2 (48)	99 $\pm$ 4 (45)	151 $\pm$ 4 (34)	77 $\pm$ 3 (56)	118 $\pm$ 3 (44)	149 $\pm$ 4 (38)
+ 50 μ M Cd	53 $\pm$ 2 (62)	77 $\pm$ 3 (57)	129 $\pm$ 3 (44)	70 $\pm$ 2 (60)	97 $\pm$ 2 (54)	139 $\pm$ 3 (42)
+100 μ M Cd	43 $\pm$ 3 (69)	63 $\pm$ 2 (65)	120 $\pm$ 3 (48)	39 $\pm$ 2 (78)	78 $\pm$ 2 (63)	127 $\pm$ 3 (47)
+ 150 μ M Cd	27 $\pm$ 1 (81)	43 $\pm$ 2 (76)	110 $\pm$ 2 (52)	28 $\pm$ 1 (84)	59 $\pm$ 3 (72)	106 $\pm$ 2 (56)

All the values are means $\pm$ SE.

Data in parenthesis denote percent inhibition over control.

All treatments are significantly different ($p < 0.01$) from control (student 't' test)

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