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Cadmium Toxicity to Photosynthesis and Associated Electron Transport System of *Nostoc linckia*

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The significance of cadmium as an aquatic contaminant has long been realized. It is during the last few years that much attention has been focussed on growth, O_2 evolution, carbon fixation, nitrogenase activity, ultrastructural changes and localization of Cd in cell cytoplasm (see Rai *et al.* 1981; Vymazal 1987; Rai *et al.* 1989). Notwithstanding, the impact of Cd on structural and functional characteristics of phytoplanktons in natural systems has also been studied (Wong 1987).

It is worth mentioning that despite much emphasis already given to understand the toxicity of Cd nothing is known about the site of its action in cyanobacteria and algae. Therefore the present work has been undertaken not only to find out the site of action of Cd in cyanobacteria but also to know the mechanism of inhibition of photosynthetic electron transport, a process responsible for the generation of ATP and NADPH, which are essential for carbon fixation. The present study compares the sensitivities of photosystem I (PS I), photosystem II (PS II) and redox coupling between the two photosystems of *Nostoc linckia* exposed to different concentrations of cadmium.

MATERIALS AND METHODS

Nostoc linckia was grown axenically in modified Chu 10 medium (Gerloff *et al.* 1950) buffered with 4 mM Tris/Hcl buffer (pH 8) under 14.4 Wm^{-2} light intensity and a 14:10 h photoperiod at 26±2°C. Stock solution of $CdCl_2$ was filter sterilized by passing through Millipore membrane filters (0.45 µM) before adding to the culture medium. Three concentrations of test metals viz. (i) effective concentration (EC_{50}), (ii) one concentration above and (iii) one concentration below EC_{50} were taken to study their effects.

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The protein content of the cells was determined by the method of Lowry et al. (1951). Photosynthetic O_2 evolution of exponentially growing Nostoc cells (300 µg protein ml^{-1}) was determined in terms of µmole O_2 mg^{-1} protein h^{-1} by a calibrated Clark type O_2 electrode, enclosed in a 10 ml airtight reaction vessel illuminated with 12.0 Wm^{-2} light at 25±2°C. This was connected to an oxygen analyzer (Universal Biochem. Model M76T, India). Cell free thylakoid membrane of Nostoc linckia was prepared following the method of Lien (1976).

Hill reaction assays were carried out within three hours of membrane preparation by polarographic method (Lien 1976) and expressed in terms of either evolution or consumption of O_2.

Reaction 1. H_2O ---> p-benzoquinone (PBQ) (assayed as O_2 evolution).

Reaction 2. H_2O ---> Ferricyanide (assayed as O_2 evolution).

Reaction 3. Ascorbate - 2,6-dichlorophenol-indophenol sodium salt (DCPIP) ---> methyl viologen (MV) (assayed as O_2 consumption).

The addition of 3-(3,4 dichlorophenyl)-1,-1 dimethylurea (DCMU) (0.01 ml, 5mM) blocked the PS II electron transport.

Carbon fixation was determined by measuring the uptake of 14_C from $NaH^{14}CO_3$ by the method of Rai and Raizada (1986) and expressed as dpm (disintegration per minute).

The total ATP content was measured by luciferin-luciferase assay using LKB-1250 Luminometer. The ATP was extracted by using 4% TCA supplemented with 2 mM EDTA.

RESULTS AND DISCUSSION

The effect of different concentrations of cadmium on O_2 evolution, 14_C incorporation and ATP content of Nostoc linckia is given in Table 1. Cadmium inhibited the electron transport chain, 14_C uptake and ATP content in a concentration dependent manner. The test cyanobacterium pre-exposed to different doses of cadmium (0.01 to 0.05 µg ml^{-1}) and incubated for 1 h under normal growth conditions, showed higher inhibition of carbon assimilation as compared to O_2 evolution. These results are in essential agreement with those of Rai (1989), where 14_C incorporation has been suggested to be the most sensitive parameter in metal toxicity evaluations. The inhibitory action of cadmium on photosynthesis as observed in the present study is due to the inhibition of phytosystem I and II (see Table 2).

Reaction 1. $H_2O \rightarrow$ PBQ is dependent on PS II and does not have an absolute requirement for PS I for an efficient coupling between the two photosystems (assayed in terms of O_2 evolution). This reaction was found sensitive to both low and high concentrations of Cd. A four fold decrease in PS II activity was observed with increase in Cd concentration from 0.01 to 0.05 μg ml^{-1}. PS I activity (Reaction 3) which was assayed in terms of O_2 consumption showed less inhibition at a low concentration (0.01 μg ml^{-1}). However, the toxicity increased by approximately eight fold when Cd concentration was raised from 0.01 to 0.05 μg ml^{-1}.

Table 1. Effect of different concentrations of cadmium on photosynthetic O_2 evolution, ^{14}C incorporation and ATP content of Nostoc linckia

Cd concentration (μg ml^{-1})	O_2 evolution (μM O_2 mg^{-1} protein h^{-1})	^{14}C uptake (dpm mg^{-1} protein h^{-1})	ATP content (ng ATP mg^{-1} protein h^{-1})
Control	48.2±1.8*	656137±32885	1941±91.6
0.01	44.2±2.2[a] (8.2%)**	544453±32666[a] (17.0%)	1771±27.5[a] (8.7%)
0.03	39.0±1.5[b] (19.0%)	417831±16704[b] (36.3%)	1299±61.6[b] (33.0%)
0.05	31.5±1.7[b] (34.6%)	275475±18670[b] (41.9%)	1014±47.0[b] (47.7%)

* Mean ± S.D.
** Per cent inhibition

Values significantly different from control have been marked: [a]$P<0.05$, [b]$P<0.01$ (student's 't' test).

Reaction 2. $H_2O \rightarrow$ Ferricyanide (assayed as O_2 evolution which is absolutely dependent on a functional PS, II, PS I and redox coupling between the two photosystems, was slightly inhibited (9.6%) at 0.01 μg ml^{-1} Cd and approximately 50% at high concentration (0.05 μg ml^{-1}). Since ATP and NADPH are the primary requirements for CO_2 fixation, one can reasonably infer that inhibition of PS II activity both at low as well as high concentrations, and PS I activity at high concentration of Cd was responsible for unavailability of ATP and NADPH and hence for the inhibition of photosynthetic carbon fixation and O_2 evolution. It is evident from Table 1 that ATP content of cyanobacterium was strongly and equally inhibited by Cd as ^{14}C incorporation. Therefore it becomes clear that reduction in ATP synthesis was responsible for inhibition of ^{14}C incorporation.

Table 2. Effect of cadmium on Hill activity by membrane preparation from *Nostoc linckia* (µmole O_2 evolved or consumed mg^{-1} protein h^{-1})

Cd concentration (µg ml^{-1})	Reaction 1 $H_2O \rightarrow PBQ$	Reaction 2 $H_2O \rightarrow$ Ferricyanide	Reaction 3 Ascorbate-DCPIP $\rightarrow$ MV
Control	29.85±1.5*	38.29±2.3	45.34±1.8
0.01	26.02±1.6[a] (12.8%)**	34.60±1.9[a] (9.6%)	43.50±1.9 (4.0%)
0.03	20.40±1.0[b] (31.6%)	23.50±1.3[b] (38.5%)	37.99±1.9[a] (16.2%)
0.05	15.46±0.8[b] (48.2%)	17.30±1.0[b] (54.8%)	29.56±1.8[b] (34.8%)

* Mean ± S.D.
** Per cent inhibition
Values significantly different from control have been marked: [a]$P<0.05$, [b]$P<0.01$ (student's 't' test).

These results clearly indicate that PS II is the primary site of action of Cd for photosynthetic electron transport as was observed by Vierke and Struckmeier (1977) in Spinach chloroplast and by Samson *et al*. (1988) for *Dunaliella tertiolecta* for copper. Since the chloroplast of eukaryotic algae and cyanobacteria are phylogenetically related (Lang 1968) we believe that PS II may be the primary site of action of Cd in cyanobacteria and algae.

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