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Heavy metal induced changes in the spectral properties of *Anacystis nidulans*

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Abstract

Copper caused bleaching of phycocyanin changing the pigment-protein interaction. On cadmium treatment the energy was not efficiently transferred to photosystem (PS) 2 and a spillover of energy occurred to PS1. Thallium treatment induced a general decrease in absorption and fluorescence of chlorophyll and phycobilisomes, while *Anacystis* was resistant to lead.

Additional key words: blue green algae, cadmium, carotenoids, chlorophyll, copper, lead, thallium.

Introduction

Heavy metals affect the electron transport activity at multiple sites (Murthy and Mohanty 1991). Photosystem (PS) 2 is more sensitive to heavy metals as compared to PS1. PS2 was affected *e.g.* by Cr and Pb in *Nostoc muscorum* (Dubey *et al.* 1986, Prasad *et al.* 1991), Cd in *Nostoc linkia* (Husaini *et al.* 1991), Cu, Cd, Zn in *Anacystic nidulans* (Singh and Singh 1987), Cu in *Anabaena doliolum* (Rai *et al.* 1991), Cu, Zn and Cd in *Chroococcus paris* (Les and Walker 1984), Hg in *Spirulina platensis* (Murthy and Mohanty 1991). However, the data on the effect of heavy metals on light-harvesting complex, *i.e.* phycobilisomes of cyanobacteria, are scanty (Murthy and Mohanty 1991). In this study we report the effect of Cu, Cd, Pb and Tl on the pigment-protein complexes of phycobilisomes in *Anacystis nidulans.*

Materials and methods

Cultures of the blue green algae *Anacystis nidulans* ARM 336 (obtained from the National Facility for Blue-Green Algae, Indian Agricultural Research Institute, New Delhi) were grown autotrophically in the BG 11 medium (Stanier *et al.* 1971). The cultures were aerated continuosly with aquarium air pumps (model *Troppi-666*, India) at 25 ± 2 °C and irradiated with cool white fluorescent lamps (irradiance of

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20 W m^{-2}). Cells were subjected to different concentrations of heavy metals (5, 10 and 15 μM of Cu, Cd, Tl and 75, 100 and 150 μM of Pb) in the mid-log phase.

Spectra of intact cells treated with different metal concentrations of were taken at room temperature by a *160A Spectrophotometer* (*Shimadzu*, Kyoto, Japan - absorption spectra) and (*Perkin-Elmer, LS-5*, London, England, - fluorescence spectra, 10 nm excitation slit, 5 nm emission slit). Cells were exposed to 440 nm radiation to excite chlorophyll (Chl) a and to 545 nm to excite phycobilisomes. Cells equivalent to 5 g m^{-3} of Chl *a* were used for recording the spectra. The Chl concentration was determined by the formula of Mackinney (1949).

Results

Absorption spectra of the untreated cells showed three peaks. The peaks at 622 and 680 nm corresponded to the absorption of PC and Chl *a*, respectively, while the peak at 440 nm represented the Soret band of Chl *a* (Fork and Mohanty 1986). In copper treated cells the PC peak was more affected than the other peaks. 5 μM of copper had a very small effect on the PC peak (data not shown) but at 15 μM this peak was reduced along with a significant reduction in the Chl peak thereby changing the PC/Chl *a* ratio to 0.85 (Table 1).

Table 1. Effect of heavy metals on the absorption properties of the intact cells of *Anacystis nidulans*. Cells were grown in the presence of 15 μM Cu, Cd, Pb or Tl for 4 d. Car - carotenoids, Chl - chlorophyll, PC - phycocyanin.

Heavy metal	Peak position [nm]			Pigment ratios 440/680	Car/Chl 490/680	PC/Chl 622/680
Control	440	622	680	1.44	1.047	1.095
Cu	440	-	680	1.64	1.176	0.851
Cd	440	622	678	1.51	1.100	1.125
Pb	440	622	680	1.44	1.047	1.095
Tl	440	622	680	1.60	1.175	1.075

In cadmium treated cells (15 μM) a peak shift of 2 nm towards the blue region at 680 nm was observed, whereas 5 μM of Cd caused a peak shift of only 1 nm (not shown). The absorbance at 680 nm was also significantly reduced, thereby increasing the PC/Chl a ratio from 1.095 to 1.125 (Table 1). While lead had no affect on the absorption spectra of the cells, in the thallium treated cells, both Chl and PC were affected.

Fluorescence emission spectra of cells: By excitation at 440 nm the cells showed two emission peaks: 650 nm emanated from phycobilisomes, and 685 nm from Chl. Peak position of Chl *a* emission was shifted by all applied concentrations of Cd (by 2 - 4 nm) and by higher concentrations of Cu and Tl. The PC emission shifts were induced only by Cu (3 - 5 nm to shorter wavelengths) and Cd (6 - 8 nm to

longer wavelengths) (Table 2). The peak ratios (PC/Chl *a*) showed always evident changes, with the exception of the Pb treatment (Table 2).

Table 2. Effect of different concentrations of heavy metals on the fluorescence emission spectra of pigments at room temperature. The pigments were excited at 440 nm.

Heavy metal	Concentration [μM]	Peak position PC	Chl	Peak ratio 650/684 nm
Control	-	650	684	0.557
Cu	5	647	684	0.428
	10	645	684	0.375
	15	-	680	0.260
Cd	5	656	682	0.625
	10	658	680	0.690
	15	658	680	0.759
Pb	75	650	684	0.557
	100	650	684	0.557
	150	650	684	0.557
Tl	5	650	684	0.675
	10	650	682	0.680
	15	650	682	0.685

By exciting at 545 nm, the spectra showed a prominent peak at 650 nm emanating from PC and a shoulder at 685 nm (Fig. 1). 15 μM of Cu caused an almost 90 % decrease in fluorescence emission intensity and the position of the peak shifted from 650 to 645 nm. The largest increase in fluorescence emission was caused by Cd; it was accompanied by a peak shift to 654 nm. In Tl treated cells the decrease in emission intensity was 20 % with no peak shift (Fig. 1).

Fluorescence excitation spectra of control cells showed a peak at 662 nm and a shoulder at 650 nm similar by to those reported by Goedheer (1968) or Fork and Mohanty (1986). In 15 μM Cd treated cells an increase in the intensity of peak at 662 nm was observed, by both the 685 and 715 wavelengths.

Discussion

Copper completely quenched the fluorescence of PC. A blue shift of 5 nm at 650 nm indicates that copper binds to the β-chain of PC and brings a change in the conformation of the protein, which causes a decrease in the absorption as well as fluorescence of PC (Murthy and Mohanty 1991, Park and Sauer 1991). The decrease in fluorescence of Chl and a shift of 3 nm indicated that Cu caused structural alterations in the PS2 as well.

The red shift in peak at 650 nm in cadmium treated cells showed that the major emission was coming from allophycocyanin (APC). This increase in the intensity of APC over PC could be due to changes in aggregation state of the phycobilisomes so that energy is not efficiently transferred to PS2. Schreiber *et al.* (1979) also showed a

cold induced decrease in the energy transfer from phycobilisomes to Chl *a* in *A. nidulans* due to enhancement of APC fluorescence. A red shift in the peak also indicates that energy transfer occurs through a spectrally distinct PC within the rods of phycobilisomes (Yamazaki *et al.* 1984, Bruce *et al.* 1985). The peak at 685 nm also showed an increase in fluorescence intensity. Since the 685 nm peak is

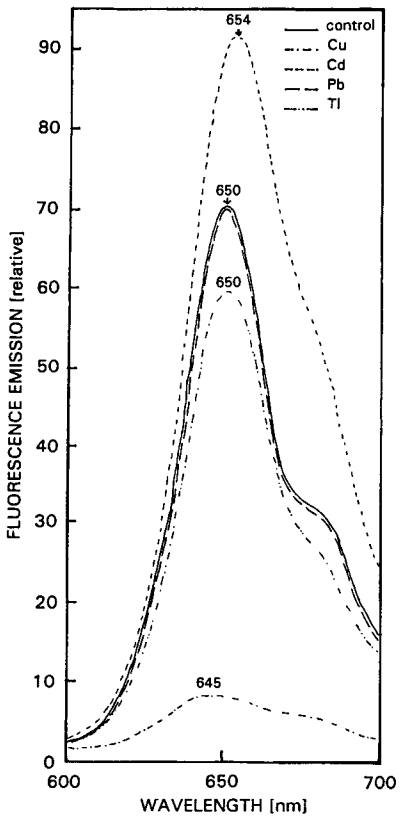

Fig. 1. Fluorescence emission spectra of *Anacystis* cells treated with heavy metals. The cells were exposed to 545 nm to excite phycobilisomes (slit width 10 nm for excitation and 5 nm for emission). Cells equivalent to 5 μg Chl *a* were used.

contributed by long wavelength form of APC and PS2 at room temperature (Gantt 1981), the increase in the intensity of fluorescence at 685 nm could be due to (*a*) alteration in structure of APC so that less energy is transferred to PS2; (*b*) alteration in the structure of PS2 as indicated by the blue shift in the peak, resulting in its inability to accept the energy from phycobilisomes (Murthy 1991); or (*c*) back transfer of energy from Chl to APC (Mohanty *et al.* 1985). The excitation spectra at 685 nm show that PC, APC and APC-β transfer energy to Chl *a* in PS1 (Cho and Govindjee 1970, Mohanty *et al.* 1985) and a back transfer of energy also occurs from PS1 to APC-B.

Lead did not affect the spectral properties of cell showing that *Anacystis* is resistant to lead. According to Lee *et al.* (1992) lead affects *Anacystis* only at concentrations higher than 10^{-3} M. The decrease in absorption as well as fluorescence in Tl treated cells is due to general disintegration of thylakoids.

In summary, our results show that the effect of copper is due to change in pigment-protein interactions while cadmium affects aggregation status of phycobilisomes thereby altering the energy transfer mechanism.

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