Bull. Environ. Contam. Toxicol. (1997) 58:543–549

Environmental Contamination and Toxicology

Effect of Cadmium on the Bioelement Composition of *Nostoc* UAM208: Interaction with Calcium

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Received: 24 July 1996/Accepted: 6 January 1997

Heavy metals may cause effects on the cyanobacterial cell including possible damage to the membranes and leakage from cells resulting in the loss or reduction of essential bioelements. There are many reports in the literature concerning morphological, biochemical and physiological changes caused by cadmium in cyanobacteria, but data on the influence of cadmium on the ion balance of the cell dealing with the interactive effect of cadmium and calcium are limited (Jensen et al. 1982, 1986).

Calcium has been found to exert a protective role against heavy metal toxicity in a variety of organisms (Trevors et al. 1986). We previously reported that calcium is able to counteract the toxic effect of cadmium towards growth, photosynthesis, nitrogenase activity and pigment content of the cyanobacterium *Nostoc* UAM208 (Mateo et al, 1994; Fernandez-Piñas et al. 1995). In the present study, we analyzed the content of essential ions, as affected by cadmium treatment, to search for possible mechanisms of heavy metal damage and toxicity in *Nostoc*. We also studied whether calcium enrichment (1.1 mM final concentration) has any influence on the heavy metal effect on those ionic contents.

MATERIALS AND METHODS

The cyanobacterium *Nostoc* UAM208 was used for this study (Fernandez-Piñas et al. 1991a). Batch cultures were grown in N-free medium as described by Mateo et al. (1986) at 26°C and were aerated under a constant light intensity of 40 $\mu Em^{-2}s^{-1}$.

For toxicity assays, cadmium as $CdCl_2$ was added to fresh medium at a concentration of 0.49 mM (55 $\mu g.mL^{-1}$). This yielded a free Cd^{2+} concentration of 4.89 µM (0.55 $\mu g.mL^{-1}$) which corresponded to the EC_{50} calculated previously for this cyanobacterium (Fernandez-Piñas et al. 1991a). After 30 min (time required for the formation of the complexes between cadmium and medium components), cells in the logarithmic growth phase at an average initial inoculum of 0.3 $\mu g.mL^{-1}$ dry weight were added. Tests were performed in aerated cultures at 26°C and incubated

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under illumination of 40 $\mu Em^{-2}s^{-1}$. Calcium enrichment was achieved by adding $CaCl_2$ to a final concentration of twice (1.1 mM) that in the standard medium (0.55 mM). Cadmium and the excess of calcium were added simultaneously. Addition of calcium did not change the free Cd^{2+} concentration in the culture medium as monitored with a cadmium electrode.

Measurements of free Cd^{2+} were made with a model 901 Orion Ionalyzer. The sensors used were: model 94-48A Orion Solid-state specific-ion electrode and a model 90-02 double-junction reference electrode (with 10% KNO_3 in the outer compartment). Measurements were made under constant light and temperature, Temperature was thermostatically controlled with a circulating bath at 25°C. 5 M $NaNO_3$ was used as the ionic strength adjustor (ISA). Before each measurement experiment, the electrode was polished with Orion polishing strips to give reproducible and rapid response. The concentration range of the cadmium electrode is between 0.1 M and 10^{-7} M (11,200 to 0.01 $\mu g.mL^{-1}$). Test solutions with fresh culture media, media components, cadmium as $CdCl_2$ and ISA were placed in polyethylene vessels and allowed to equilibrate for 30 min; measurements were made and were reproducible to ± 4%. A blank solution was used for low level measurements. The slope (millivolt difference for a ten-fold change in cadmium concentration) was always within the range of a correct electrode operation (27 ± 2 mV for temperature between 10 and 25°C).

Analyses for calcium, magnesium, iron, zinc, manganese and potassium concentrations were performed using a Perkin-Elmer 4000 Atomic Absorption/Emission Spectrophotometer operated in the acetylene-air flame mode with hollow cathode lamps. Analyses for each element were determined against the appropriate standard solution from Carlo Erba Chemicals. The spectrophotometer operated at a wavelength of 422.7 nm for calcium, 285.2 nm for magnesium, 248.3 for iron, 213.9 for zinc and 279.5 for manganese (detection limits- 20 μgL^{-1} calcium, 1 μgL^{-1} magnesium, 2 μgL^{-1} iron, 0.4 μgL^{-1} zinc and 1 μgL^{-1} manganese). Potassium was measured by atomic emission. For the analyses, samples of cells that had been exposed to cadmium were taken at 4, 24 and 96 hr after inoculation. Cells were collected by centrifugation (23,000 x g, 10 min.), washed three times with 10 mM EDTA, pH 7.5 and dried. Wet digestions of the dried samples with a mixture of nitric-sulfuric-perchloric acids in the proportion 5:1:2 were performed. The digestion was left at 4°C for 12 hr and later boiled slowly in a sand bath until only perchloric acid remained, as evidenced by the appearance of dense, white fumes. The clear solutions resulting from this oxidation process were then diluted with double-distilled water. To assure the accuracy of the digestion and atomic absorption method, several samples were run after atomic absorption standards additions, since methodology was only tested previously with leaf standard materials, Percentages of recovery were above 90%.

The intracellular contents of the divalent cations calcium, magnesium, iron, zinc, manganese, and the monovalent cation potassium were determined in control, cadmium-treated cells, and cadmium-treated cells supplemented with 1.1 mM calcium.

Data in the figures are the means and standard deviations of at least three independent experiments with duplicate cultures and samples within each experiment. Student's *t* tests were performed to determine the statistical significance of differences between control and cadmium-treated cultures.

RESULTS AND DISCUSSION

The effect of cadmium on calcium content is shown in Figure 1a. Short-term exposure (4 hr) resulted in an increase in calcium content as compared to the control, this was clearly significant in cadmium-treated cells supplemented with extra calcium. However, no significant differences on the effect of cadmium on calcium content could be detected at longer times of exposure (24-96 hr). There was also a substantial increase in the levels of calcium in those cultures treated with cadmium and supplemented with 1.1 mM calcium after 24 hr of treatment.

Cadmium caused a significant increase in zinc and manganese contents after short-term exposure (Fig. 1b and c, respectively). The extra calcium supplement also resulted in an increase in the content of both elements in cadmium-treated cultures after 4 and 24 hr exposure.

The most important feature of the effect of cadmium on iron content was the drastic decrease (40%) in this element content after 24 hr (Fig, 2a). Calcium enrichment protected against that loss of iron.

The analysis of magnesium (Fig. 2b) demonstrated that, apart from the observed significant (Student's *t* test, $P < 0.05$) increase after 4 hr of treatment, a significant decrease in magnesium content in the presence of cadmium after 24 hr (43% decrease) and 96 hr (65% decrease) occurred. This reduction in magnesium content could be overcome by increasing the concentration of calcium in the culture medium as Figure 2b shows.

There are reports in the literature (Gadd & Mown 1983; Jensen et al. 1982, 1986) relating heavy metal toxicity to a significant loss of intracellular potassium. We examined the influence of cadmium on the potassium content of *Nostoc* UAM208 and the interaction with increased calcium. The content of potassium was affected significantly (Student's *t* test, $P < 0.05$) by cadmium, there was a 60% reduction of this ion after 96 hr of exposure (Fig. 2c). Addition of 1.1 mM calcium reduced the Cd^{2+}- induced potassium loss by 50%.

Our results showed a significant (Student's *t* test, $P < 0.05$) increase in the cellular content of nearly all of the elements studied after 4 hr exposure to cadmium. This may have been correlated with an enhanced permeability caused by direct binding of cadmium to specific sites in the membrane such as hydroxyl, carboxyl, phosphoryl, amino and sulphydryl groups. This binding by changing the membrane structure may modulate the permeability of the membrane to specific ions, resulting in an increased permeability to ions, culminating in an unbalanced flux of vital ions

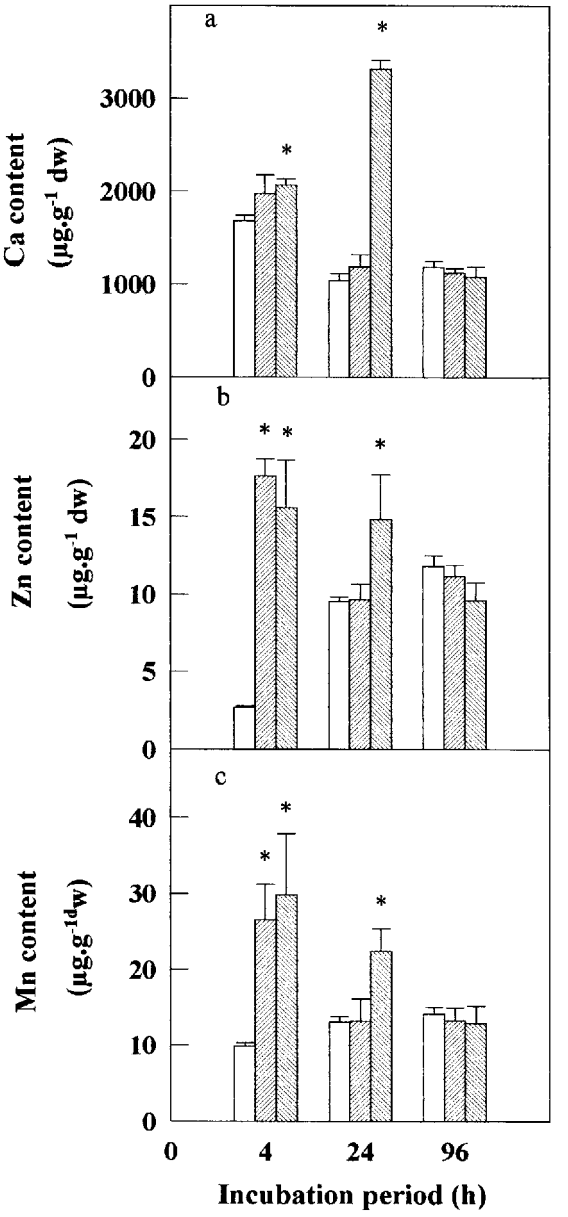

Figure 1. Effect of cadmium and increased calcium on (a) calcium, (b) zinc and (c) manganese content of *Nostoc* UAM208.

control

4.89 µM Cd^{2+} (0.55 mM Ca^{2+})

4.89 µM Cd^{2+} (1.1 mM Ca^{2+})

* indicate statistically significant differences (Student's *t* test, $P < 0.05$) with respect to the control.

into the cells. These results could indicate that the cell membrane is probably the primary site of toxicity of cadmium.

The effect of cadmium on essential elements at longer exposure times was characterized by significant decreases in Mg^{2+}, Fe^{2+} and K^{1+} contents, whereas the contents of Mn^{2+}, Zn^{2+} and Ca^{2+} remained close to control values.

In cyanobacteria the unbalanced content of iron could be related to the inhibitory effects of cadmium on photosynthesis and nitrogen fixation observed in this strain (Fernandez-Piñas et al. 1995), since iron as a cofactor of many enzymes is involved in photosynthesis, chlorophyll reduction, dinitrogen fixation and other biological oxidations.

The important decrease in magnesium content recorded after 24 and 96 hr of exposure could explain the negative effect of cadmium on the chlorophyll content of this cyanobacterium (Fernandez-Piñas et al. 1991b). Jensen et al. (1982, 1986) also reported loss of magnesium from polyphosphate bodies of *Plectonema boryana* and *Anabaena variabilis* treated with cadmium.

Cadmium caused loss of cellular potassium in *Nostoc* UAM208. Enhanced potassium release from the cell seems to be a typical phenomenon upon addition of various kinds of toxic agents; heavy metals like Cd, Cu, Pb and Hg have been shown to cause potassium loss from yeast cells (Gadd & Mowll 1983), algae (Overnell 1975), cyanobacteria (Jensen et al, 1982, 1986) and *Azolla* (Sela et al. 1988). Heavy metals may cause a breakdown in the permeability barrier to potassium, causing a large efflux of potassium from the cell.

Our results showed that calcium is able to protect the cells against potassium, magnesium and iron loss caused by cadmium. It has been shown in *Saccharomyces cerevisiae* that the Cd^{2+}-induced K^{+} release was reduced by calcium (Kessels et al. 1985). A similar effect of calcium in this cyanobacterium could explain the recovery in the element content found by increasing the concentration of calcium in the culture medium. The ameliorative behavior of calcium on the Cd^{2+}-induced K^{+} release from *Saccharomyces cerevisiae* was attributed to their homologous chemistry rather than the cation size.

Another possible explanation for the protective role of calcium on the element content could be based in an involvement of the macroelement in the formation of coprecipitates with cadmium and phosphate resulting in the immobilization of soluble cadmium into the cells with a subsequent detoxifying effect as has been proposed by several authors (Jensen et al. 1986; Sela et al. 1990). Thus, the antagonistic function of calcium on cadmium toxicity may account for a calcium-mediated recovery in this cyanobacterium.

Acknowledgement. This work was supported by Comision Interministerial de Ciencia y Tecnologia (N° PB92-1097 and PB95-0217-l).

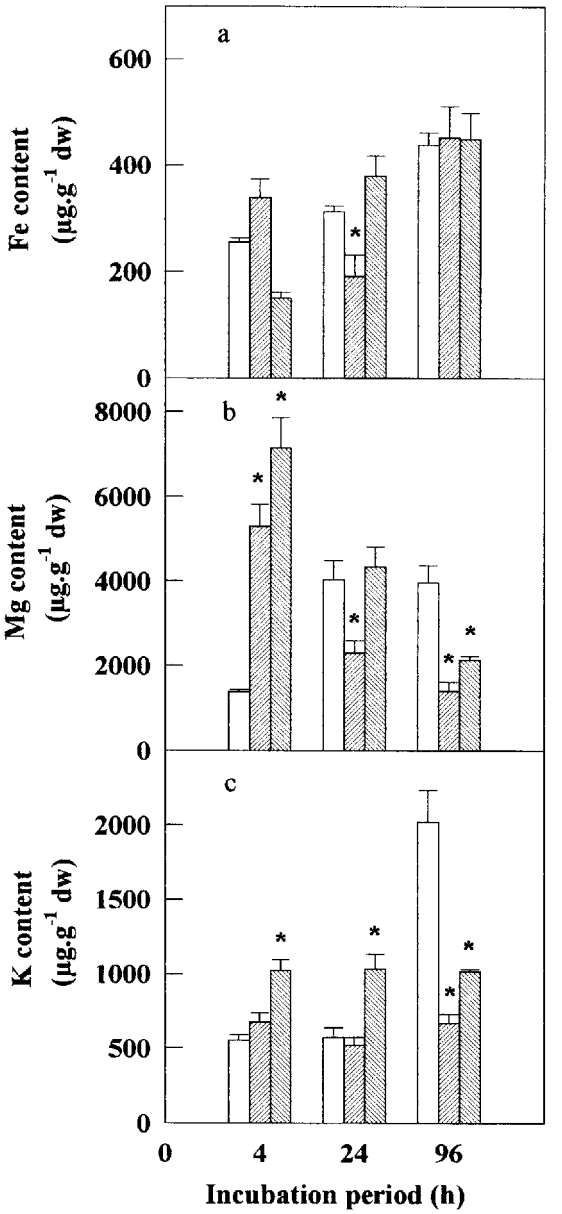

Figure 2. Effect of cadmium and increased calcium on (a) iron, (b) magnesium and (c) potassium content of *Nostoc* UAM208.

control

4.89 µM Cd^{2+} (0.55 mM Ca^{2+})

4.89 µM Cd^{2+} (1.1 mM Ca^{2+})

* indicate statistically significant differences (Student's *t* test, $P < 0.05$) with respect to the control.

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