Zinc Detoxification by a Cyanobacterium from a Metal Contaminated Bay in Brazil

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ABSTRACT

We describe here the trapping of zinc in polyphosphate granules of the cyanobacterium \textit{Synechocystis} \textit{aquatilis} NPBS-3. Cells were cultured in 25 $\mu$M of zinc chloride and prepared for electron microscopy and energy-dispersive X-ray analysis. Some ultrastructural features were changed by zinc exposure, the increase of glycogen granules number being the main change. The polyphosphate granules contained phosphorus, sulphur, calcium, iron and zinc. The trapping of zinc in polyphosphate granules seemed to be an effective way of detoxifying the metal and surviving in the bay. As a non-specific mechanism, these polyphosphate granules could also be effective in trapping other metals in excess.

Keywords: Heavy metals, zinc, Sepetiba Bay, polyphosphate granules, glycogen granules, detoxification

INTRODUCTION

Sepetiba Bay, located southwest of Rio de Janeiro city, Brazil, is a well-known metal-contaminated environment. Zinc, cadmium and chromium have been pointed as critical metals (Penna Franca et al., 1984). The cyanobacterium \textit{Synechocystis} \textit{aquatilis} NPBS-3 was isolated from phytoplankton samples collected in Sepetiba Bay. The cells have been maintained in laboratory (Universidade Federal do Rio de Janeiro - NPPN culture collection) since then. Investigations of physiological changes due to zinc have been done and differences in growth and photosynthetic rates relatively to control samples have been pointed (Andrade et al., 1993, 1994). More recently, zinc retention by these cells was confirmed by atomic absorption spectrophotometry as 20-fold the control when they grew under 25 $\mu$M of zinc, the EC$_{50}$ (effective concentration which causes the inhibition of 50% of cell growth relatively to control samples) found for the metal (Andrade and Pfeiffer, 1999). These studies have confirmed the cyanobacterium tolerance to zinc.

As a trace element, zinc only becomes toxic to organisms above certain concentrations, in the range of micro or millimolar. This is the case in waters of many metal-contaminated environments. As a consequence, metal-tolerant organisms develop as selection takes place (Robinson, 1989). The selection of such organisms is explained by the many metal-detoxification or metal-controlling mechanisms found.

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According to Simkiss (1993), one of the abilities of living systems is to have cells capable of regulate and compartmentalise ions from their surroundings, and this would lead to biomineralization towards the production of amorphous minerals. Among them there are granules containing phosphorus, calcium and magnesium. Their amorphous structure is important for both presumable functions of stocking and detoxifying ions (Taylor and Simkiss, 1993). These minerals have been found in invertebrates and their use as environmental monitors of metal pollution has been suggested (Simmons et al., 1994).

Polyphosphate granules are a common structure in bacteria. They are the bacterial counterparts for the phosphorus granules, and probably these two structures have some similar functions in cells. The elemental composition of the polyphosphate granules of cyanobacteria is, usually, phosphorus, magnesium, potassium and calcium (Baxter and Jensen, 1980a); sometimes, sulphur is also present (Rai et al., 1990).

Here we show the presence of zinc in the polyphosphate granules of the cyanobacterium *Synechocystis aquatilis* NPBS-3 cultured in 25 μM of zinc chloride. Besides, we also show the increase in the number of glycogen granules in these cells.

**MATERIALS AND METHODS**

**Growth of *Synechocystis aquatilis* NPBS-3**

*Synechocystis aquatilis* NPBS-3 cells were grown photoautotrophically and axenically in 1 L of ASM-1 medium (Gorham et al., 1964). Cultures were maintained at 28°C and continuous illumination provided by 2 cool white fluorescent tubes, giving a surface irradiance of 17 μEm²·s⁻¹. Flasks were sparked with sterile air throughout the growth period. ZnCl₂ concentrations provided were 3.2 μM (control) and 25μM (EC₅₀). An inoculum of 1-2 x 10⁶ cells ml⁻¹ was used to start cultures.

**Electron Microscopy**

Cells were collected by centrifugation at the end of cultivation period. They were washed 3 times in a small volume of PBS (pH 7.4) and centrifuged. Fixation was carried out in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. Post-fixation was done with 1% osmium tetroxide for one hour. Cells were washed, dehydrated in acetone series and embedded in Polybed 812. Sections were cut with a diamond knife and collected on copper grids. They were then stained in uranyl acetate and lead citrate. Sections were observed under the transmission electron microscopes Zeiss CEM 902 and Zeiss 900.

**Energy-Dispersive X-Ray Analysis (EDXA)**

A special specimen preparation was done for Energy-Dispersive X-ray Analysis (EDXA). Small volumes of a very dense culture were collected by centrifugation in a microcentrifuge. ASM-1 medium was taken out and cells were washed with distilled water two times. They were embedded in the hydrophilic resin Nanoplast (Ted Pella, Inc.) for 48h at 40°C and polymerised for 48 h at 60°C. Ultrathin sections were cut with a diamond knife and placed on nylon grids. Analysis was carried out in a Jeol 1200 EX electron microscope operating at 80kV with a Noran detector system, for 200 seconds. A background spectrum was obtained from a cytoplasm region of the same cell, in the same conditions, and subtracted from the polyphosphate body spectrum.

**RESULTS AND DISCUSSION**

Cells were collected at the late exponential period (after 25 days of the inoculum); at this stage a reasonable number of cells could be obtained for electron microscopy and analytical studies. Growth curves for control and zinc-treated cells are presented on Fig. 1. Micrographs of a control cell and a zinc-treated cell are respectively seen in Figs. 2 and 3. Glycogen and polyphosphate granules are pointed. Looking at the micrographs, one could see that the amount of glycogen granules increased in the zinc-treated samples, and the size of each granule seemed smaller than that of control cells. In stressed photosynthetic organisms, it is common an increase in energy and carbon storage structures, such as glycogen.
Figure 1 - Growth curves of *Synechocystis aquatilis* NPBS-3 grown on ASM-1 medium under 3.2 μM (control) and 25 μM of ZnCl₂.

Figure 2 - Transmission electron micrograph of *Synechocystis aquatilis* NPBS-3 cells in control growth conditions. (P) Hole in the section occupied previously by a polyphosphate body and (G) glycogen granules are shown. Bar = 0.5μm.

Figure 3 - (a) Transmission electron micrograph of *Synechocystis aquatilis* NPBS-3 cells grown on 25 μM Zn. (T) Thylakoid membranes, (G) glycogen granules and (PI) pili. Bar = 0.5μm. (b) Insert shows an ultrathin section containing one polyphosphate granule embedded in the hydrophilic resin nanoplast. Bar = 0.38μm.
This result is in agreement with those of Andrade et al. (1993) who found an increase in photosynthetic activity for *Synechocystis* cells treated with zinc. Although not clearly visible in the electronmicrograph on Fig. 3a, polyphosphate granules were found in cells grown on zinc chloride (e.g. fig. 3b). Based on the findings of other authors, we decided to check for the presence of zinc in the cytoplasm and in the polyphosphate granules. Energy-dispersive X-ray analysis (EDXA) showed the presence of zinc in the polyphosphate bodies of some zinc-treated cells. Fig. 4 shows the net spectra of a polyphosphate body, after subtracting the background. A discrete zinc peak was present, and also the peaks of phosphorus, sulphur, calcium, iron, sodium, magnesium, and aluminium. Zinc was not found in other regions of the cell. The presence of zinc in this structure could represent a way of detoxifying the excess metal. EDXA of granules of control cells showed the same elements, with the exception of zinc. As previously stated, these cells could accumulate about 20 times the amount of zinc of the control cells (Andrade and Pfeiffer, 1999). As the control cells had no detectable zinc when examined by EDXA, it was expected small zinc peaks in zinc-treated cells.

In prokaryotic organisms, metal detoxification occurs by (i) enzymatic reduction or oxidation of the metal, which is converted to a less toxic form; (ii) methylation and diffusion of the methyl-metal through the membrane; (iii) active efflux systems; and (iv) complexation (Nies, 1992; Wood and Wang, 1983). Metals can be complexed by proteins like metallothioneins (Robinson et al., 1990), polymers of the cell wall and capsule (Beveridge, 1981; 1989) or by polyphosphate granules (Baxter and Jensen, 1980b; Jensen et al., 1982a; Rai et al., 1990; Keasling and Hupf, 1996; Gonzalez and Jensen, 1998).

The cyanobacterium *Synechocystis aquatilis* NPBS-3 is no doubt tolerant to zinc (Andrade and Pfeiffer, 1999). This tolerance could be a result of natural selection in the bay where it comes from. This microorganism had been extensively investigated before for a metallothionein gene similar to the *smtA* of *Synechococcus* cells (Robinson et al., 1990). No conclusions came up for the existence of a similar gene (Andrade, 1997). Surprisingly, *Synechocystis* from Sepetiba Bay tolerates a higher concentration of zinc (it survives up to 32 µM of ZnCl₂, data not shown) than *Synechococcus* for which this highly specialized metal detoxification mechanism, an specific metallothionein, has been reported (Turner et al, 1993). The higher zinc concentration that *Synechococcus* can tolerate is 14 µM.

Besides the presence of zinc, the polyphosphate granules of *Synechocystis aquatilis* NPBS-1 presented phosphorus, sulphur, magnesium, sodium and calcium, common components of polyphosphate granules (Webster et al., 1984), and also aluminium and iron, less common components of these structures (Spring et al., 1998; Lins and Farina, 1999). Such granules have been proposed to have a role in chelating metals such as calcium, potassium and magnesium (Wood and Clark, 1988). However, some authors have related them to the detoxification of heavy metals in cells of prokaryotic and eukaryotic organisms. The cyanobacterium *Plectonema boryanum* exposed to 20 ppm of zinc, aluminium, manganese or lead for 1 h showed high amounts of these metals inside polyphosphate bodies (Torres et al., 1998). Studies in *Anabaena cylindrica* have shown the accumulation of aluminium in polyphosphate granules and claimed the participation of these structures as a detoxification mechanism (Petterson et al., 1985). Stereologic studies of zinc-exposed *Anabaena variabilis* showed that high concentrations of zinc caused an increase in the relative volume of these structures (Rachlin et al., 1985). The same was found for cadmium-exposed *Plectonema boryanum* (Rachlin et al., 1982). Zinc was detected in the polyphosphate...
granules of zinc-exposed *Chlorella saccharophila*, but not in these structures of *Navicula incerta* and *Nitzschia closterium*; in contrast, lead was found in the polyphosphate granules of these three algae species (Jensen et al., 1982b). Besides, Hashemi et al. (1994) found that *Anabaena variabilis* cells with large pools of polyphosphate were more resistant to metal even though the metals were not localized in the polyphosphate granules.

There are no doubts that polyphosphate granules are certainly involved in metal detoxification by *Synechocystis aquatilis* NPBS-3. If the polyphosphate granules sequestrate zinc and these structures are not membrane-bounded in bacteria, the free Zn\(^{2+}\) in the cytoplasm will decrease. This will alleviate the cell of metal contamination.

**CONCLUSIONS**

Metal detoxification mechanisms as polyphosphate granules, although less sophisticated, seem to be more effective than a metallothionein, which is sometimes very specific for binding only one metal. The non-specificity of the granules gives them the ability of binding many different metals. Although we investigated zinc only, it is known that the bay where *Synechocystis* was isolated is also contaminated with cadmium, lead and chromium (Penna Franca et al., 1984). The mechanism proposed here seems to be effective for surviving in a metal polluted environment and could be the reason for selection of metal-tolerant species such as *Synechocystis aquatilis* NPBS-3. Hence, we suggest further investigations on the role of polyphosphate granules of this cyanobacterium in the presence of these other pollutants of the bay.

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