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Incorporation of Cadmium by *Acetabularia calyculus*

R. Reyes,[1] R. Salazar,[2] E. García[1]

[1] Simón Bolívar Universidade, Departamento de Biologia de Organismos, Apartado Postal 89000, Caracas, Venezuela
[2] Bioanalysis Department, Science School, Sucre, Oriente University, Post Office Box 145, Cumaná, Venezuela

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The toxic effects of high concentrations of lead, mercury, cadmium, zinc, and copper on aquatic organisms have been widely researched (Roesijadi 1992; Prasad et al. 1998; Bonifacio and Montaño 1998; Salazar and Reyes 2000). Algae have long been used as biological indicators of heavy metal contamination in aquatic environments because of their capacity to incorporate these elements from the surrounding medium (Travieso et al. 1999). Cadmium inhibits algal growth and reduces the rates of respiration and photosynthesis (Ilangovan et al. 1998). It is also easily bioaccumulated by both algae and marine invertebrates (Soderlund et al. 1998).

Acetabularia calyculus has been used as an experimental model to study the incorporation of heavy metals and to test cell response to these elements (García 1993; García and Reyes 1996, 1998; Reyes, 1999). The purpose of this research was to study the ability of *A. calyculus* to incorporate cadmium in both short and long terms, as well as the participation of the nucleus in this process.

MATERIALS AND METHODS

Samples of *A. calyculus* were collected at Morrocoy National Park in the western coast of Venezuela. The algae were thoroughly washed to remove microorganisms and sediments and placed in artificial seawater, referred to as MS (Shepard 1970). They were grown for two weeks under sterile conditions at a temperature of 23-25°C for alternate 12-hour periods of light (3-4W/$m^{2)}$ and darkness in a growth chamber 4-cm long. Vegetative cells with caps were selected; the nucleus of some were removed for the incorporation trials.

The kinetics of incorporation was studied in both whole and anucleated cells for short periods of time ranging from 5 minutes to 6 hours under controlled conditions of light, and for longer periods of 1 to 7 days in alternate 12-hour cycles of light and darkness. The experiments were performed three times, using 20 cells for each experiment and each trial time. The algae were placed in PVC receptacles containing 100 ml of MS amended with 1, 10, and 25 mg/L of $CdCl_2$.

Correspondence to: R. Reyes

The total content of cadmium in the cells was determined after rinsing twice with MS and then drying on filter paper. The cadmium, adsorbed into the walls of *Acetabularia,* was removed by rinsing, and analyzed according to EPA procedures (1976). The cells were sumerged in 60° C water for four hours and then diluted with 70% HNO_3. The total content of cadmium, assesed by atomic absorption spectrophotometry using a Perkin-Elmer 2380 spectrophotometer, was expressed in mg of cadmium per gram of dry alga.

The tests with anucleated cells were carried out on rhizoids whose nuclei were cut off and discarded. The anucleated cells were grown in MS for 24 hours prior to incubation in $CdCl_2$. All tests were performed in the presence of penicillin (1 mg/ml) to prevent bacterial contamination. Linear regression analysis based on the east-square method was applied to asses the relevance of the relationship between the concentration of cadmium and the time of exposure in the experiments of short-term kinetics. The relevance of both variables in the long-term kinetics was assesed by means of a two-way variance analysis (Sokal and Rohlf, 1995), by comparing whole and anucleated algae.

RESULTS AND DISCUSSION

The incorporation of cadmium by *Acetabularia* depended on the time of exposure, with a period ranging from 5 to 360 minutes (Figure 1). Regression analysis indicated that the correlation between the metal content and the time of exposure was highly significant for both whole and anucleated algae for the three concentrations tested.

Cadmium concentration inside the cell increased linearly during the 6 hours of exposure without reaching a steady state. These results suggested the existence of rapid mechanism of cadmium absorption. Similar results were obtained for mercury uptake by *A. calyculus* (García 1993).

Several authors have suggested that the main mechanism for heavy metal incorporation in plants operate in the cytoplasmic matrix and that the subsequent accumulation and storage occurs in the vacuole (Vogeli and Wagner 1990). The high uptake of cadmium noted in *Acetabularia* may be related to this mechanism, as its cytoplasm includes a 5 to 10 μm-thick band arranged around a central vacuole which takes up most of the cellular volume (Berger et al. 1987).

In the exposures ranging from 1 to 7 days, the rate of cadmium uptake by the cells increased up to the third day of exposure. The cadmium content in the alga dropped significantly thereafter (Figure 2).

These results indicated that *A. calyculus* was saturated with cadmium after 3 days of exposure. The binding of cadmium during this period suggested the presence of a tolerance mechanism that allows the organism to offset the toxic effects of the metal. Metal-binding proteins, called class III metallothioneins or phythochelatines,

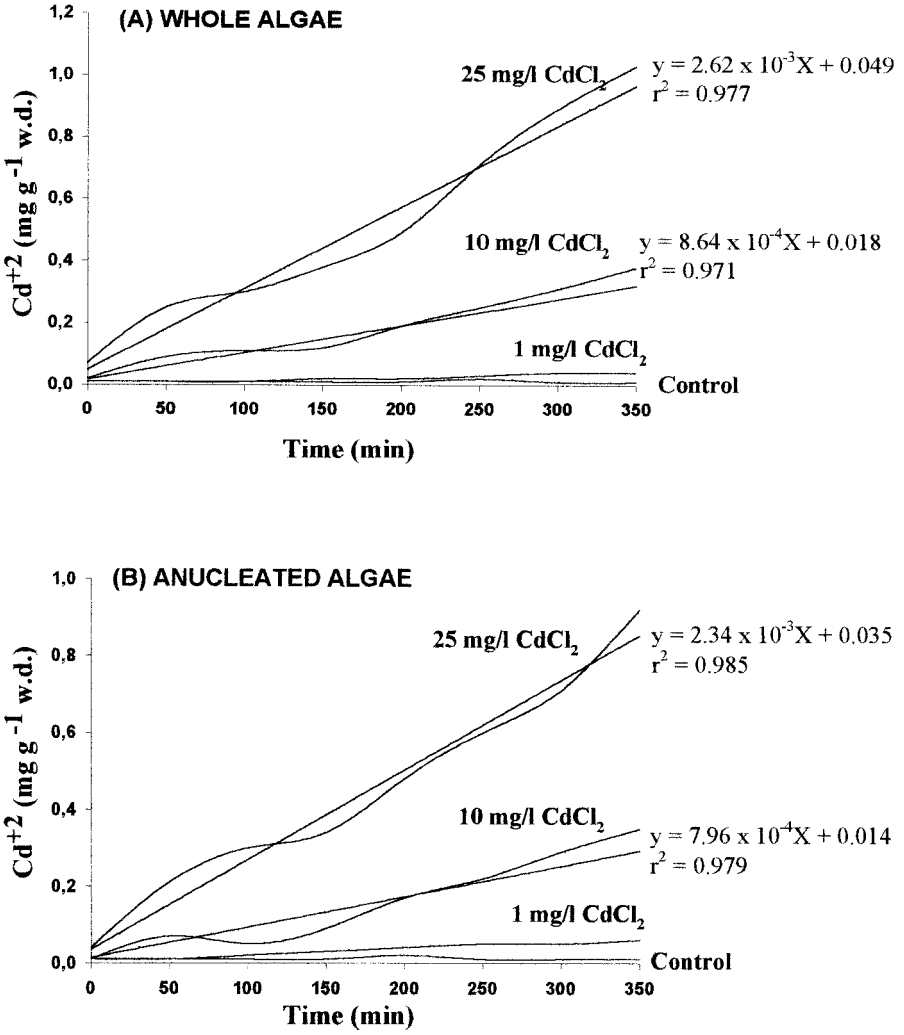

Figure 1. Cadmium uptake in *Acetabularia calyculus*. (A) Whole algae (B) Anucleated algae

have been discovered in autotrophic organisms. These proteins differ from I and II metallothioneins for they are not transcription-induced (Fowler et al. 1987). The algae *Dunaliella* and *Chlorella pyrinoidosa,* produce class III metallothioneins when exposed to a high concentration of cadmium (Gadd 1990) and *A. calyculus* produces mercury-binding proteins (Garcia and Reyes 1996, 1998).

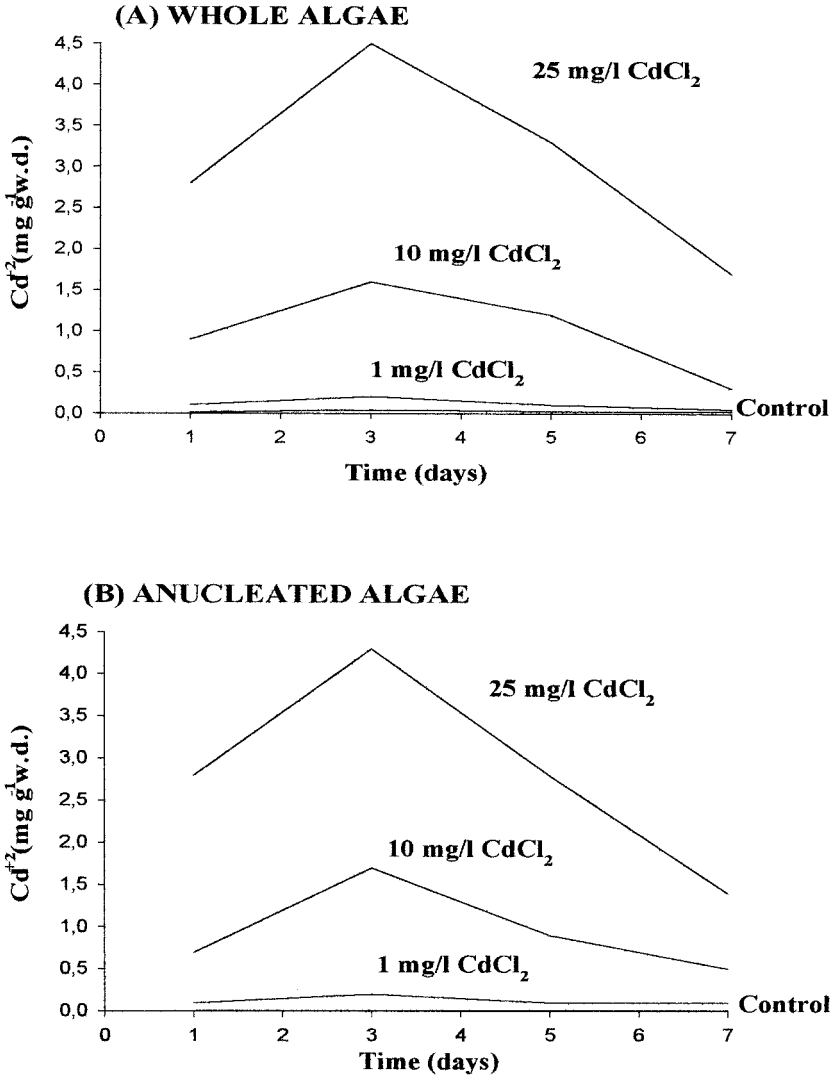

Figure 2. Cadmium Uptake in *Acetabularia calyculus*. (A) Whole Algae (B) Anucleated Algae

In other autotrophic organisms, such algae and plants, cadmium has been found associated with compounds of molecular weights ranging from 5 to 10 kDa, and to proteins of a high molecular weight (Rauser 1995; Carr et al. 1998; Morelly and Pratesi 1997; Leopold et al 1999). It is likely that this induction of metallothioneins may have an effect on tolerance to cadmium toxicity in this alga. Yet, the decrease in cadmium concentration observed after 5 or 7 days of exposure suggested that

other biochemical mechanisms, besides induction, may be involved. In fact, the accumulation of metals may decrease as a result of a change in cell permeability due to either active accumulation or adsorption onto the surface or both (Albergoni et al. 1989). The active excretion of the metal may also be of importance. Proteins in *Saccharomyces cerevisae* and *Schizosaccharomyces pombe* act as pumping devices that store the metal in cell organelles or expel it into the surrounding medium (Li et al. 1997; Perego & Howell 1997). Heavy metals in humans can be excreted by means of conjugation with glutathione. These metal-glutathione conjugates can be directly pumped out to the surrounding medium or excreted by a protein associated to the mechanism of resistance to multiple drugs (Cole et al. 1994). An equivalent cadmium-resistant mechanism has been identified in *S. cerevisae* (Wemmie et al. 1994).

Statistical analysis of the data did not show significant differences in the kinetics of cadmium incorporation between whole and anucleated algae. Similar results have been obtained in algae contaminated with mercury and treated with cycloheximide (Garcia 1993). These results suggest that the process of incorporation and accumulation of the metal was not directly controlled by the nucleus. It seems reasonable to propose that *Acetabularia* reacts to heavy metal stress through mechanisms not directly dependent upon the translation of mRNAs that code polypeptides capable of binding heavy metals onto their molecular structure. The synthesis of phytochelatines may well explain our observations regarding cadmium accumulation by the alga.

Our laboratory is currently performing experiments to test the hypothesis about the presence of cadmium-binding proteins of the type of phytochelatines in *Acetabularia calyculus*.

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