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INHIBITORY EFFECT OF CADMIUM ON THE GROWTH AND NITRATE UTILIZATION OF *COELASTRUM ASTROIDEUM*[1,2]

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Abstract

The inhibitory effect of Cd on the growth and the ultilization of nitrate in *Coelastrum astroideum* cells was studied. During Cd-treatment, glutamate pool was prominantly decreased in size. The test of the sensitivity of the enzymes involving in nitrate and ammonia assimilations to Cd showed that the lowering in glutamate pool size was not resulted from the inhibition of enzyme activity, but probably due to the decrease in nitrate uptake. A considerable amount of potassium ions were released from cells to medium, when algal cells were treated with high dose of Cd. The release of potassium ions could be lowered by the presence of high concentrations of calcium and magnesium ions. Calcium was very effective in lowering the toxicity of Cd. Up to 60% of Cd-toxicity to cell growth could be relieved by adding a high dose of calcium into culture medium

Key words: *Coelastrum astroideum*; cadmium; cell growth; nitrate uptake; free amino acid; K^+-release; Ca^{2+}-effect.

Introduction

Cadmium is not an essential element for algae, but rather a typical contaminant in waters. Algal cells are able to uptake and accumulate a considerable amount of cadmium (Hart and Scaife, 1977; McLean and Williamson, 1977; Mang and Tromballa, 1978; Soeder *et al.*, 1978; Geisweid and Urbach, 1983). However, cadmium is toxic to algal cells because it may repress the growth of cells at very low concentrations (Klass *et al.*, 1974; Müller and Payer, 1979; De Filippis *et al.*, 1981; Hornung *et al.*, 1981; Irmer *et al.*, 1984). Based on the study with plant

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materials it has been ascertained that cadmium may affect on some enzymes (Vallee and Ulmer, 1972; Lee *et al.*, 1976; Webb, 1979), photosynthesis, especially on photosystem II (Li and Miles, 1975; Van Duijvendijk-Matteoli and Desmet, 1975; Baszynski *et al.*, 1980), respiration (Lee *et al.*, 1976). In addition to the above actions, the toxic effect of cadmium also has been interpreted as the damage of cell membranes (De Filippis, 1979; Strickland *et al.*, 1979). A notable result of membrane damage is the change in permeability and therefore the leakage of electrolytes such as potassium ions. The inhibitory effect of cadmium on cells is therefore manifold.

Nitrate is the predominant nitrogen source for the growth of algal cells. Nitrate is assimilated to ammonium before it is incorporated into keto acids to form various nitrogenous components of the cells (Hewitt, 1975; Miflin and Lea, 1977; Tyler, 1978). The utilization of nitrate by algal cells has been found to be more susceptible to cadmium than photosynthesis, because the former process can be inhibited by cadmium of very low concentrations at which photosynthesis is not yet affected (Hofslagare *et al.*, 1985). The study of the inhibitory action of cadmium on nitrate utilization is therefore of great interest. In this paper the effect of cadmium on the growth and the assimilation of nitrate in the cells of a green alga, *Coelastrum astroideum*, was studied.

Materials and Methods

The green alga *Coelastrum astroideum* De-Not. (Strain 2069) was isolated from Taipei, Taiwan. Algal cells were cultivated at 30±1°C under light-dark (LD) period of 14:10 hours. The illumination of 8-10 Klux was provided by five fluorescent tubes. The cultures were aerated with 1.5% CO_2 in air. Algal cells were grown in inorganic nutrient solution (Kuhl, 1962). A log-phase grown culture was maintained with daily diluting the culture prior to the onset of light.

For the assay of enzyme activities in cell free extract algal cells were comminuted with a vibrogen cell mill (Edmund Bühler Co.). The activities of nitrate (NR) and nitrite reductases (NIR) were assayed as described by Tischner (1976). The activity of glutamine synthetase (GS) was determined according to the transferase activity of the enzyme (Rhodes *et al.*, 1975). The dehydrogenase activities of glutamate (GDH) and alanine (ADH) were measured according to Strickland (1969) and Joy (1969), respectively. Aspartate aminotransferase (AAT) activity was assayed by the coupled reaction with malate dehydrogenase (Bergmeyer, 1974). Protein in the extract was quantified by the modified Lowry method (Schachterle and Pollack, 1973). Chlorophyll content was estimated by the method of Mackinney (1941). Cadmium was given in form of chloride salt.

To analyse the free amino acids, algal cells were comminuted with a cell mill.

After centrifugation (20,000 xg) the protein in the supernatant was eliminated by precipitation with trichloroacetic acid (2N). The pH of supernatant was adjusted to 2.2 before it was lyophilized. Free amino acids were analysed by a Dionex D-300 amino acid analyser.

The uptake of nitrate by cells was measured with a ion selective nitrate electrode (Dr. W. Ingold AG.) which was connected with an ion analyser and a recorder. Algal cells grown in the nutrient medium in deficiency of nitrate were used as material. The release of potassium ions from cells to medium was detected with a potassium electrode which was also connected with an ion analyser and a recorder.

Results

Cadmium (Cd) is rather toxic to *Coelastrum astroideum* cells. One μM Cd already resulted in a significant repression in the growth of cells (Fig. 1). When Cd in culture medium was higher than 200 μM, there was no cell growth at all.

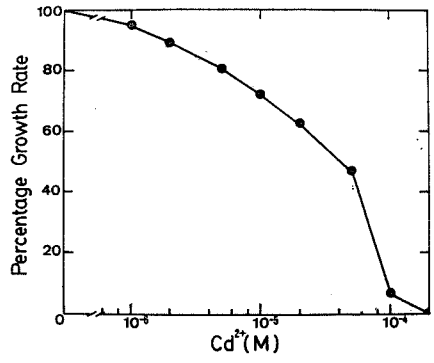

Fig. 1. Decrease in growth rate of *Coelastrum astroideum* cells due to increasing cadmium concentrations in culture medium.

In order to realize the effect of Cd on the utilization of nitrate, the change of free amino acid pool in response to Cd-treatment was studied. 300 μM Cd was added to algal culture grown in the light. The change in free amino acid pool was then analyzed after two-hour incubation in the light. During Cd-treatment, different amino acid decreased in its pool size with different magnitude. As shown in Table 1, those amino acids that markedly decreased in their pool size were glutamate, aspartate, arginine, methionine, alanine, glycine and serine. Among them, the change in pool sizes of aspartate, methionine, alanine, glycine and serine seemed not to be specific to Cd-treatment, because it also could be observed, when algal culture was incubated in darkness instead of being incubated with toxic dose of Cd.

Table 1. *Change of the pool sizes of free amino acids in response to cadmium treatment (300 μM) and dark incubation.*

Amino acid	Control*	+Cd*	+Dark*
Glu	53.86	28.46	47.65
Arg	3.75	1.66	5.09
Asp	19.57	6.45	9.16
Met	0.93	0.25	0.39
Thr	0.85	1.15	1.10
Lys	4.89	4.01	4.45
Ile	2.22	1.82	2.32
Ala	25.48	17.93	20.43
Leu	1.69	2.69	2.10
Val	3.12	3.60	3.78
Gly	5.76	3.90	4.58
Ser	10.37	5.66	5.05
Phe	0.50	0.64	0.56
Tyr	0.37	0.60	0.51
His	0.25	0.28	0.38

* All the data were given in relative unit per mg protein.

The change in pool size of glutamate was specially noteworthy. The assay of the activity of enzymes involving in the biosynthesis of this amino acid, namely nitrate and ammonium assimilations, showed that all of them were inhibited by Cd to different extent (Fig. 2). The enzymes involving in nitrate assimilation, namely NR and NIR, were more sensitive to Cd than those involving in the biosynthesis of glutamate (GS and GDH), the assimilation of ammonium. Also the activity of the key enzymes accounting for the biosynthesis of alanine and aspartate family, ADH and AAT respectively, was little affected by high dose of Cd. Among the enzymes tested, NR was most susceptible to Cd. However, the activity of this enzyme was not seriously inhibited by Cd of the lethal dose. In the presence of 0.2 mM Cd, the growth of cells had been totally inhibited. Nevertheless, as tested *in vitro*, only about 30% of NR activity was inhibited by Cd at this concentration.

The uptake of nitrate by cells would also be affected by Cd. When Cd was added to algal culture grown in the light, the uptake of nitrate was inhibited immediately after the addition. The threshold concentration of Cd resulting in a visible decrease in nitrate uptake was as low as 5 μM (Fig. 3). The extent of inhibition in nitrate uptake increased with increasing Cd concentrations. About 50% of nitrate uptake was inhibited by 0.2 mM Cd during short term measurement (5 min).

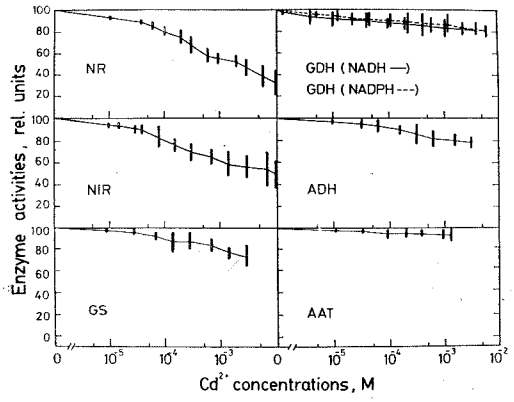

Fig. 2. Inhibition of the activities of nitrate reductase (NR), nitrite reductase (NIR), glutamine synthetase (GS), glutamate dehydrogenase (GDH), alanine dehydrogenase (ADH) and aspartate aminotransferase (AAT) of *Coelastrum astroideum* cells by cadmium of different concentrations.

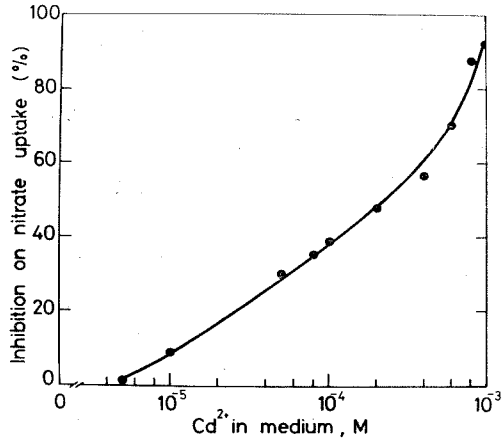

Fig. 3. Short term inhibition of nitrate uptake in *Coelastrum astroideum* cells in the light in exposing to various cadmium concentrations. The percentage inhibition was estimated at 5th min after cadmium addition to a culture with density of 33.67 μg chlorophyll/ml.

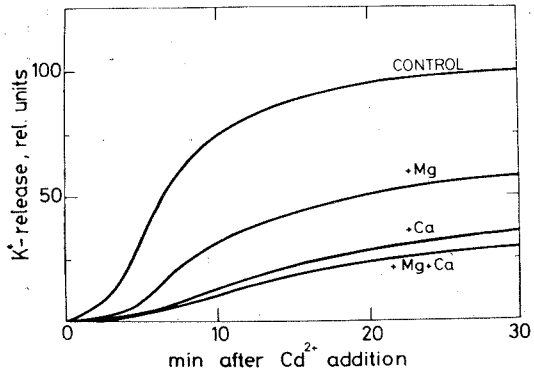

Fig. 4. Effectiveness of calcium (6 mM) and magnesium (25 mM) on repressing the potassium ion release from *Coelastrum astroideum* cells due to cadmium-treatment (200 μM). Culture density: 38.05 μg chlorophyll/ml.

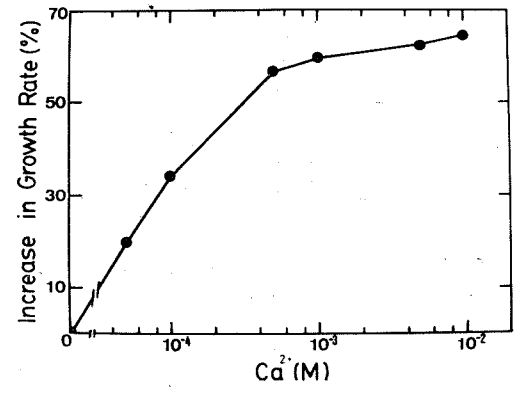

Fig. 5. Effect of calcium concentrations on lowering the cadmium toxicity to the growth of *Coelastrum astroideum* cells. The measurement of growth rate was based on the chlorophyll increase in a light-dark growth cycle in the presence of 100 μM cadmium.

Like many other heavy metals, Cd may also affect on cell membranes and result in the change in the permeability of membranes and therefore the release of potassium ions from cells to medium. The release of potassium ions could be decreased to some extent, when the medium contained a proper amount of calcium and/or magnesium ions. As shown in Fig. 4, calcium was more effective than magnesium in decreasing the release of potassium ions from cells. A better result was obtained, when calcium was added in combination with magnesium.

Calcium can not only repress the release of potassium ions from cells, but also enhance the growth rate of cells in the presence of high dose of Cd. The presence of 1 mM calcium in culture medium might result in a decrease in the toxicity of 100 μM Cd to cell growth up to 70% (Fig. 5). The effectiveness of calcium no lowering the Cd toxicity was related with its concentration, when it was lower than 1 mM. When calcium concentration was higher than 1 mM, there was no further enhancement in growth rate of cells treated with Cd.

Discussion

The pool size of some free amino acids, as shown above, would be affected by Cd-treatment. A notably specific change to Cd-treatment was the lowering in the pool size of glutamate. Such a change could be resulted from inhibition of any process accounting for the biosynthesis of this amino acid. Glutamate has been found to be synthesized in algal cells via the pathway catalyzed either by GS/GOGAT or by GDH (Tyler, 1978; Guerrero *et al.*, 1981). The test of the sensitivity of these enzymes to Cd showed that they were little affected. Also the assay of the enzymes involving in nitrate assimilation, NR and NIR, indicated that they were only partially inhibited by lethal dose of Cd. It is therefore concluded that the inhibition of enzyme activity by Cd may not be the main reason for the lowering in glutamate pool size during Cd-treatment. Apart from the inhibition of enzyme activity, the decrease in glutamate pool size may also be resulted from the lowering in the supply of reductant needed for the assimilation of nitrate as well as of ammonium. Lee *et al.* (1976) have found that Cd may affect the respiration rate and some enzymes involving in reductant generation. Nevertheless, the study of nitrate uptake indicates the inhibition of this process by Cd is more noteworthy. At low concentration Cd may result in a pronounced inhibition of nitrate uptake. Moreover, the inhibition can be detected immediately after the addition of Cd. This implicates that Cd acts directly on plasmalemma where nitrate uptake is taken place. It was reported that the visible inhibition of heavy metals on cell growth would be due to the action on disturbing the nutrient uptake of cells (Pitman, 1964; Oberländer and Roth, 1975). This may also be the case for Cd to *Coelastrum astroideum* cells.

Like many other heavy metals, Cd also induced the release of potassium ions from cells to medium. The release of potassium ions is considered to be a sympton of membrane damage and to be predominantly due to the change in membrane permeability, rather than due to the exchange with heavy metal (Hassall, 1963; De Filippis, 1979). The addition of high dose of calcium to culture medium, as shown above, was very effective in repressing the release of potassium ions. Furthermore, calcium was able to lower the toxicity of Cd to the growth of algal cells. The lowering of heavy metal toxicity by the presence of other divalent metal ions such as magnesium, manganese and iron has been reported in many organisms (Habermann, 1969; Steemann Nielsen and Kamp-Nielsen, 1970; Kinkade and Erdman, 1975; Foster and Morel, 1982; Stauber and Florence, 1985). The action of these divalent metals has been interpreted as an effect either of inhibiting the uptake of heavy metals or of compensating the inhibitory effect of heavy metals. Our results (data not shown) indicated that the uptake of Cd by cells was not significantly decreased by the presence of high does calcium and magnesium. It is therefore presumed that the major role played by these divalent ions in lowering the toxicity of Cd is to protect the plasmalemma from being affected by Cd, just like the case in *Chlorella* cells to copper (Wu and Lorenzen, in preparation).

In the inhibition of the utilization of nitrate, Cd may act on enzymes involving in the assimilation of nitrate and on the uptake of nitrate. However, the action on the latter seems to be more important, because the latter is more susceptible to Cd. The repression of nitrate uptake by Cd is probably related with the damage of plasmalemma due to Cd-treatment because nitrate uptake is taken place at there. The inhibitory effect of Cd on the function of plasmalemma of the algal cell is still less known. A further study is needed.

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鎘對空球藻生長及硝酸離子代謝之抑制

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本實驗以由本地分離之空球藻 (*Coelastrum astroideum* De-Not.) 爲材料，研究重金屬鎘對其生長及硝酸離子代謝之影響。當以鎘處理時，細胞內之穀氨酸含量會顯著下降，檢驗穀氨酸之合成酵素及硝酸離子同化酵素對鎘之敏感度發現鎘對這些酵素會有抑制作用，但抑制量不大。鎘對硝酸離子吸收之抑制，似較爲重要。鎘在低濃度即會造成硝酸離子吸收之大量抑制。硝酸離子吸收之受抑制似與細胞膜之受傷害有關。鎘會造成細胞質膜之傷害及鉀離子之釋放。鈣和鎂離子會明顯抑制鉀離子流失。鈣離子並可減低鎘離子所造成之傷害而提高細胞生長率，其作用可能在於保護細胞質膜。