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Cadmium Tolerance by *Stigeoclonium tenue* Kütz: Partial Characterization of Cadmium-Binding Proteins

M VANAJA, N V N CHARYULU and K V N RAO
Plant Physiology Laboratory, Botany Department, Osmania University, Hyderabad 500 007

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Cadmium toxicity and mechanism of cadmium tolerance were investigated in *Stigeoclonium tenue* Kütz isolated from industrial effluents from Ramagundam (AP). The soluble protein band profile revealed the induction of a new protein band upon exposure to cadmium. The two protein bands (P_2 and P_3) (present in the control), were native, while a third one (P_1, present in the treated ones) was induced upon exposure to cadmium. The protein bands showed different cadmium-binding capacities at increasing concentrations of cadmium—P_1 showed four-fold increase while P_2 and P_3 bands showed three-fold increase. The mechanism of cadmium-tolerance in *Stigeoclonium tenue* involved cadmium-binding by both native and induced proteins.

Key Words: Stigeoclonium, Cadmium tolerance, Cadmium-binding proteins

Introduction

Heavy metals (density $> 5g\ cm^{-3}$) such as Cd, Pb, Hg, Cu and Zn cause serious injury to living organisms. Pollution by industrial effluents containing various heavy metals distrubs the ecology and vegetational pattern of both aquatic and terrestrial habitats (Wolverton & McDonald 1978). Certain organisms have been shown to have the ability to develop tolerance to heavy metal ions (Kagi & Vallee 1961, Noel-Lambot 1975, Webb 1979, in animals; (Rugstad & Norseth 1975), in human cells *in vitro*; Antonovics et al. 1971, Leu-Kim & Rauser 1986, Grill et al. 1987, Salt et al. 1989 in plants; Macara 1978, Grill et al. 1986, in yeast; Chopra 1975, Mitra et al. 1975: in bacteria; and Butler et al. 1980, Bariaud et al. 1985, Reddy & Prasad 1989, in algae).

Tolerance to heavy metals is achieved in different ways by exclusion (Baker 1978), accumulation in vacuoles (Ernst 1982), binding by cell walls (Turner 1970), metal-tolerant enzymes (Wainwright & Woolhouse 1975), induction of metallothioneins (Bartolf et al. 1980, Rauser & Curvetto 1980, Leukim & Rauser 1986), induction of phytochelatins (Grill et al. 1985, 1986, 1987, Salt et al. 1989) and lower accumulation and decreased affinity to heavy metal (Bariaud et al. 1985).

Stigeoclonium tenue Kütz. occurs in diverse habitats including polluted waters. It has been shown to tolerate a high metal pollution (McLean 1974). It has also been suggested as an indicator of chromium and copper pollution (Palmer 1959). In the present investigation, toxicity and mechanism of cadmium tolerance under laboratory culture conditions, as the level of tolerance and mechanism of resistance depend upon both the algal species and the heavy metal involved (Bariaud et al. 1985), was studied in *S. tenue*.

Material and Methods

S. tenue was collected and isolated from the industrial effluents of Ramagundam (A P). After isolation, the alga

was raised in axenic cultures. The alga was grown in modified Bold's medium (Bischoff & Bold 1963) with the addition of $Na_2 SiO_3 . 9H_2O$ (58 mg L^{-1}) and $Na_2 CO_3$ (20 mg L^{-1}) instead of NaCl, at *p*H 6.9. The cultures were maintained with cool continuous white fluorescent illumination at 20 ± 1^0 C.

After obtaining maximum growth in the basal medium, the alga was grown at different concentrations of cadmium, viz. 1, 5 and 10 ppm for one week. *S. tenue* grown in basal medium without cadmium served as the control. After one week the cultures were harvested and the algal material was thoroughly washed repeatedly with deionised double glass distilled water and 2mM cysteine solution to remove adsorbed cadmium as per Bariaud et al. (1985). Pigments and interfering compounds were removed by washing thrice with chilled acetone. The soluble proteins from the resulting algal mat were extracted according to Lue-Kim and Rauser (1986). The amount of protein in different samples was estimated as per Lowry et al. (1951). The separation of soluble proteins by discontinuous non-SDS PAGE was carried out according to the method of Steward and Barber (1964) and Steward et al. (1965). Hundred μg of protein for each sample was loaded on each gel. For all the samples the gels were run in duplicate. One set of the gels was stained with amido black and diffusion-destained and scanned in a gel scanner. The other set was stored in a freezer. By comparing with the stained gels, the areas of unstained gels were sliced—each piece containing single protein band. The protein bands from unstained sliced gel pieces were extracted with 30% H_2O_2 and analysed for Cd^{2+} in atomic absorption spectrophotometer to know the amount of cadmium bound by each protein band. The amount of cadmium present in the industrial effluent from Ramagundam was also analysed by atomic absorption spectrophotometry.

Results

Cadmium level from the industrial effluent from which the alga was isolated varied from 0.01 to 0.10 ppm. However, the alga showed normal growth even at 10 ppm of Cd^{2+} in culture conditions.

The soluble protein band profile showed differences in protein band pattern between control without cadmium and the treated ones having cadmium. The protein bands were designated as P_1, P_2 and P_3 based on

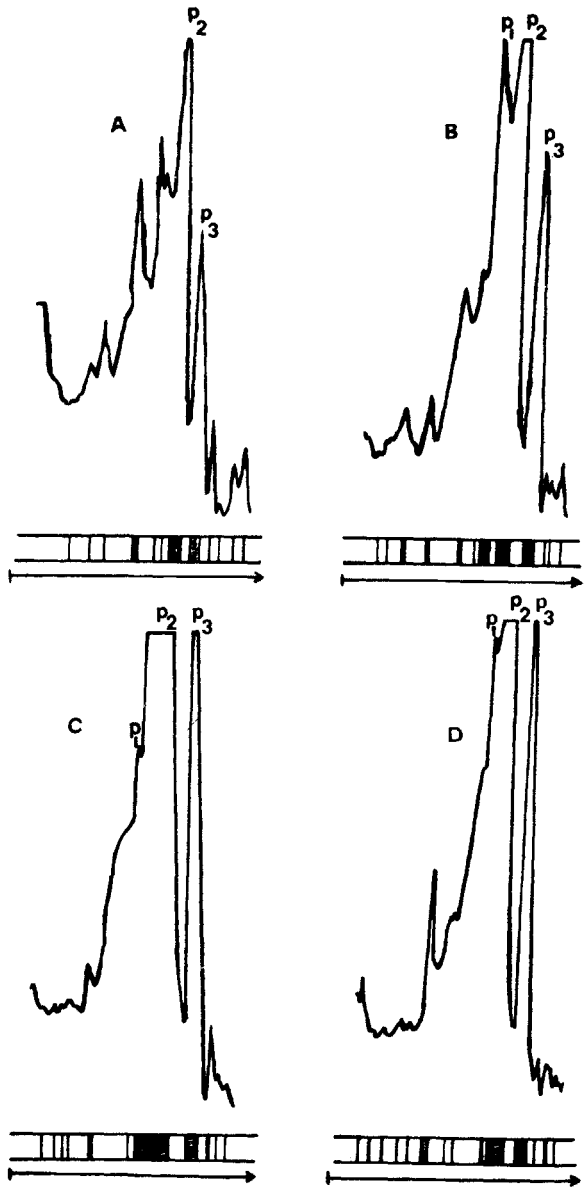

Figure 1 Gel scan showing changes in the protein band pattern upon exposure to increasing concentration of Cd^{2+} **A**, Control; **B**, 1 ppm; **C**, 5 ppm and **D**, 10 ppm of Cd^{2+}. P_1, P_2, and P_3 are Cd^{2+}-binding proteins. Arrow below each gel indicates the direction of electrophoretic run from origin to the tracking dye front.

relative mobility (R_m) values (figure 1). The concentration of protein bands P_2 and P_3 increased with the increasing concentration of cadmium. The protein band P_1 was found only in Cd^{2+} treated samples, while P_2 and P_3 bands were present in all, including control. The amount of Cd^{2+} bound by the protein bands also increased with the increasing concentrations of Cd^{2+} (table 1). Maximum amount of Cd^{2+} was bound by P_3 band (a three-fold increase in binding capacity) followed by P_2 band (also three-fold increase) and P_1 band (four-fold increase) (table 1).

Table 1 *Amount of cadmium bound by different protein bands in* ***S. tenue*** *upon exposure to increasing concentration of cadmium in the medium*

Concentration of Cd^{2+} in the medium	Amount of Cd^{2+} bound (μg)		
	P_1	P_2	P_3
0 (Control)	Nil	Nil	Nil
1 ppm	20 μg	40 μg	50 μg
5 ppm	50 μg	70 μg	80 μg
10 ppm	80 μg	110 μg	160 μg

Discussion

Cadmium as an environmental pollutant and its responses in plants stimulated research in recent years on its response in plants. (Verma & Katz 1978, Page et al. 1972). The level of toxicity and the mechanism of tolerance varies from species to species and from metal to metal. In the present investigation *S. tenue* grew well even at 10 ppm of Cd^{2+}, though a concentration of 0.005 to 2.0 ppm cadmium was reported to be toxic in *Spirodela polyrrhiza* (Jaiswal & Srivastava 1985). However, *S. tenue* has been reported to tolerate high levels of metal pollution (Mc Lean 1974).

The mechanism of tolerance to cadmium toxicity has been shown to mainly consist of (a) the production of cysteine rich low molecular weight metallothioneins or metallothionein-like heat-stable proteins (Robinson & Jackson 1986, Bartolf et al. 1980, Leu-Kim & Rauser 1986, Fujita & Kawanishi 1986), (b) phytochelatins, which are sulfur-rich, low molecular weight polypeptides containing only three amino acids L-cysteine, L-glutamic acid and glycine (Grill et al. 1985, 1986, 1987) and (c) lower accumulation and decreased affinity for cadmium (Bariaud et al. 1985). Metallothioneins and/or phytochelatins are generally induced upon exposure to cadmium. In the present investigation, cadmium was bound by three protein bands P_1, P_2 and P_3, of which only the protein band P_1 was induced by exposure to cadmium. The protein bands P_2 and P_3 which were native, i.e. present even in the absence of cadmium (control) showed an increase in their concentration with increasing concentration of Cd^{2+}. The three different bands have also shown different degrees of cadmium-binding capacities. In *Scenedesmus quadricauda*, only one protein band of 8Kd was induced by cadmium (Reddy & Prasad 1989).

In addition to the induction of a new protein upon exposure to cadmium, the present study shows that certain proteins which were present prior to cadmium exposure also bind cadmium. Thus, it is suggested that the mechanism of cadmium tolerance in *S. tenue* involves both native and induced proteins in detoxification and homeostasis of cadmium toxicity. Further work on molecular weight determination and estimation of sulfhydryl groups of Cd^{2+}-binding proteins is in progress.

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