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Cadmium-Binding Polypeptides in Cadmium-Treated *Zinnia*

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ABSTRACT: Five-week-old seedlings of *Zinnia elegans* were hydroponically cultured in Hoagland's solution containing 45 μM Cd^{2+} with or without one week of prior exposure to 10 μM Cd^{2+}. Profiles of HPLC indicated that plants previously exposed to 10 μM Cd^{2+} developed more sulfurhydryl groups and glutathione than plants not exposed to that prior treatment. Individual peaks of sulfurhydryl groups displayed a prominent increase at 24 h, following by a gradual decrease to initial levels of sulfurhydryl after 96 h. Further isolation and purification of metal-binding complexes was done using DE 52, Sephadex, and HPLC chromatographic separations. Two fractions of the LMW complex contained more than 90% of the histidine-like substance in total amino acids. The HMW complex had five peaks in the HPLC profile. One major peak contained more than 85% of the histidine-like substance, whereas other peaks contained less than 0.1% of the histidine, along with phytochelatins with n number 3 and 4.

KEY WORDS: Cadmium, Glutathione, Phytochelatins, Sulfurhydryl group, *Zinnia elegans*.

INTRODUCTION

Exposure to heavy metals, such as cadmium and copper, may cause plants to induce the synthesis of cysteine-rich polypeptides in cytoplasm. These polypeptides, called phytochelatins, can sequester metals from the cellular milieu and regulate the cellular concentration of metals by accumulation in vacuoles (Steffens, 1990; Rauser, 1990). The structure, organization, and expression of metallothionein genes related to copper and zinc have been reported in *Arabidopsis* (Zhou and Goldgrough, 1994) and other plants (Kawashima *et al*, 1992).

Phytochelatins have been well documented in higher plants, algae, and fungi (Grill *et al*, 1985; Howe and Merchant, 1992). The molecules consist of repeating units of γ-glutamylcysteine followed by a C-terminal glycine, and their structure indicates that plants synthesize them by a post-translationally activated, metal dependent, enzymatic pathway from a precursor, glutathione (Grill *et al*, 1985; Steffen *et al*, 1986). The biosynthetic pathways of phytochelatin through glutathione, by the action of phytochelatin synthase, were reported in fission yeast, *Silence cucubalus*, and in rice (Grill *et al*, 1987; Hayashe *et al*, 1991; Yuan *et al*, 2000).

Although phytochelatin synthesis is activated *in vivo* and *in vitro* by various ions, such as Cd^{2+}, Ag^{+}, Bi^{3+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+}, and Au^{+}, stable metal-phytochelatin complex formation has only been described for Cd^{2+} and Cu^{2+} (Grill *et al*, 1987; Grill *et al*, 1989). *In vivo*, there are two distinct types of metal-phytochelatin complexes: (1) a low molecular

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weight (LMW) complex containing mainly phytochelatin, and Cd^{2+}; and (2) a high molecular weight (HMW) complex consisting of phytochelatin, Cd^{2+}, and acid labile sulfide (Murasugi *et al*, 1983). The HMW metal-phytochelatin complex has a higher Cd^{2+} binding capacity and the bound Cd^{2+} is more resistant to acid displacement (Dameron *et al*, 1989). There are two lines of evidence for heavy metal detoxification by HMW complexes: (1) in cultured cells of *Datura anoxia*, the heavy metal-induced synthesis of phytochelatin peptides was insufficient in itself to confer Cd^{2+} tolerance (Delhaize *et al*, 1989); and (2) in Cd^{2+} sensitive mutants of fission yeast, a deficiency in the HMW complex-fraction was indicated by gel filtration (Mutoh and Hayashi, 1988).

In this present study, the induction of phytochelatins in Cd^{2+} treated plants was examined, along with separation, characterization, and amino acid analysis of both LMW and HMW heavy metal-binding complexes.

MATERIALS AND METHODS

A. Plant materials

Seeds of *Zinnia elegans* were sterilized in 2% hypochlorite containing 0.1% Tween 20 for 20 min. After being thoroughly washed, seeds were placed on mesh plastic containers and immersed in Hoagland's solution for germination and hydroponic culturing. The nutrient solutions were changed every three days. Five-week-old seedlings, exposed to 10 μM $Cd(NO_3)_2$ for one week or not so exposed, were transferred to nutrient solution containing 45 μM $Cd(NO_3)_2$ at different time intervals. Roots of Cd^{2+} treated plants were collected for analysis.

B. Assays of sulfurhydryl groups

The determination of total sulfurhydryl groups in plants followed the method of Ellman (1961). Plant tissues were quenched with liquid nitrogen and stored at –20°C. A 0.3 g aliquot of frozen tissue was ground up with a mill, stirred in 1.2 ml of 6.7% salfosalicylic acid at 4°C for 10 min, and centrifuged at 13,000 x g for 30 min. A 100 ml volume of supernatant was mixed with 1 ml of Ellman's reagent (0.1 mM 5, 5'-dithio-bis(2-nitrobenzoid acid in 50 mM phosphate buffer), gently shaken for 2 min, and its absorbance measured at 412 nm. Glutathione was used as a standard for calibration.

HPLC separation of sulfurhydryl groups followed the method of Vogeli-Lange and Wagner (1990) with minor modifications. A 0.3 g aliquot of frozen tissue powder was put into an Eppendorf tube and immediately stirred with 0.6 ml of 3.5 N HCl for 6 h, followed by centrifuging at 13,000 x g for 30 min. Acid soluble supernatant was applied to a C18 reverse-phase column of a Beckman 126 AA HPLC that was pre-equilibrated with 0.05 % (v/v) phosphoric acid. This was followed by fractionation with a linear gradient of 0-20 % acetonitrile in 0.05 % phosphoric acid at a flow rate of 0.75 ml/min. Collected fractions with a retention time within 20 min were measured at 412 nm. Glutathione was used as a standard for calibration.

C. Quantitation of acid labile sulfur

A 500 ml quantity (0.5 g of plant tissues in 0.75 ml of 50 mM Tries buffer, pH 7.6) of crude plant extract was thoroughly mixed with 1 ml of 1 M lead acetate, and then allowed to react with 100 ml of 6 % NaOH by shaking for 10 sec. An additional 250 ml of 0.1% N,

N-dimethyl-p-henyldiamine dihydrochloride was added to the reaction mixture and stirred until it became clear. The resulting solution was allowed to react with 100 ml of 0.31 % $FeCl_3$ and the stand for 1 h, followed by measurement with a Hitachi 3400 spectrophotometer at 670 nm.

D. Preparation and purification of metal-binding complexes

Harvested roots were washed thoroughly with water, air dried, quenched with liquid nitrogen and ground into a fine frozen powder with a mill. The resulting material was collected in an ice-cooled beaker. Approximately 25 g of tissue powder was mixed with 50 ml of 50 mM Tris (pH 8.0) and homogenized in a Polytron at 12,000 rpm. The crude root extracts were centrifuged at 8,000x g for 20 min. The supernatant was further filtered through nylon cloth and centrifuged at 12,000x g for 30 min.

The purification of metal-binding complexes followed the methods described by Grill *et al* (1985). The clarified supernatant of root extract was loaded onto a Whitman DE 52 column (2.6 x 10 cm), washed with 1.4 l of 50 mM Tris (pH 8.0), and then eluted with 400 ml of 0-0.8 M KCl gradient in 50 mM Tirs (pH 8.0) at a flow rate of 45 ml/h. The Cd^{2+} binding fractions were collected, ultrafiltrationally concentrated using an Amicon YM1 filter, and applied onto a Sephadex G-50 column. Elution was done with 50 mM Tris (pH 8.0) at a flow rate of 30 ml/h. Two fractions were pooled separately, ultrafiltrated with an Amicon YM1, and lyophilized for amino acid analysis and further identification by HPLC.

E. Cd^{2+}-binding polypeptide characterization

The Cd^{2+} binding complexes were further resolved using a C18 reverse-phase column (Waters/Millipore 086344). After being acidified with 0.5 % (v/v) phosphoric acid, both the HMW and LMW Cd-binding complexes were further separated and identified by HPLC.

F. Analysis of amino acid composition

Samples of HMW and LMW Cd^{2+} binding complexes, obtained from gel filtration, or different fractions in HPLC (corresponding to peak 1 and 2 of LMW Cd^{2+} binding complex and peaks 1-5 of HMW Cd^{2+} binding complex) were hydrolyzed by mixture with HCl and trifloroacetic acid (1:1) at 150°C for 3 h. After this they were lyophilized and analyzed for amino acid composition on a Beckman 6300 high performance amino acid analyzer.

RESULTS

A. Changes in sulfhydryl groups in Cd^{2+}-treated plant

Plants growing in the 45 μM Cd^{2+} treatment (no prior exposure) had seven major peaks of sulfhydryl compounds (Figs. 1). Peak 2 in the HPLC profile of sulfurhydryl compounds is glutathione, and there were no significant changes in the different peaks for these plants. However, plants exposed to 10 μM Cd^{2+} and then grown in 45 μM Cd^{2+} medium had seven major sulfhydryl-containing peaks and several other minor peaks (Fig. 2). A time course of sulfhydryl production in these pre-exposed plants indicated that peaks 3 and 4 increased up to the highest level at 24 h, and then decreased gradually to a lower level (similar to that at 0 h) at 96 h. Peaks 3 and 4 had 49% and 46% of the total sulfhydryl groups, respectively, for plants exposed for 24 h to the Cd^{2+} treatment (Fig. 2D, not considering glutathione, in peak 2). Peak 2 decreased gradually to a lower level at 48 h after treatment, while peaks 1, 5, 6, and 7

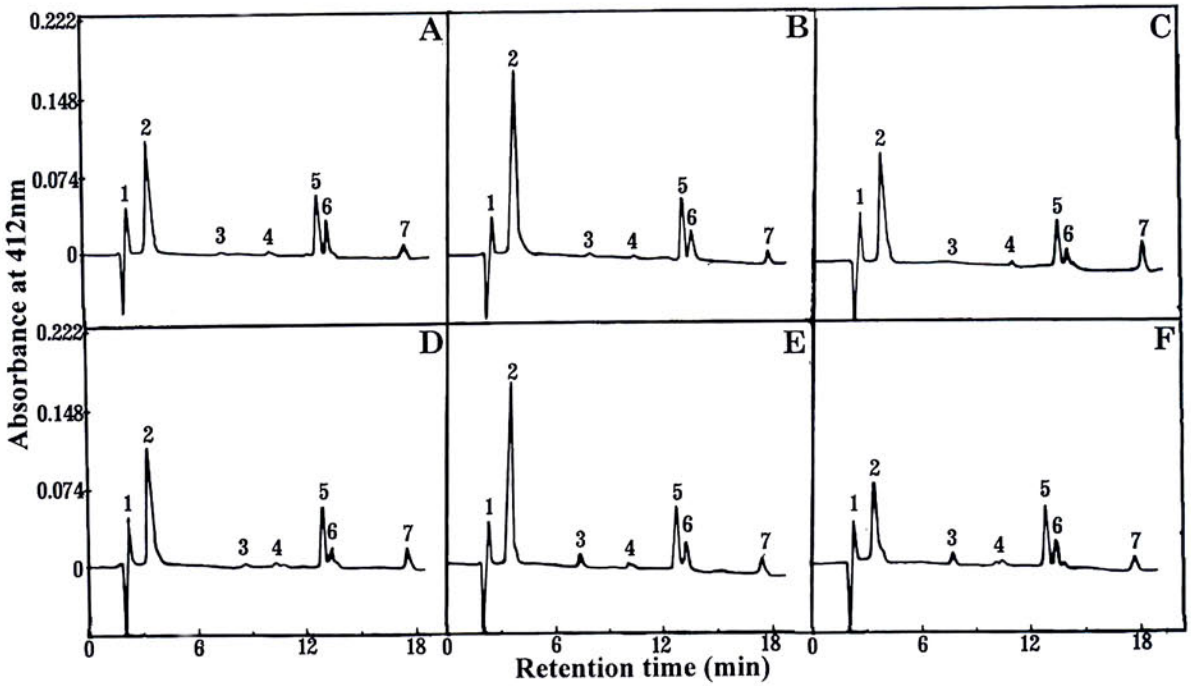

Fig. 1. HPLC profile of sulfurhydryl groups in plain 45 μM Cd^{2+} plants. The durations of Cd^{2+} incubation in A, B, C, D, E and F are 0, 3, 12, 24, 48 and 96 h respectively.

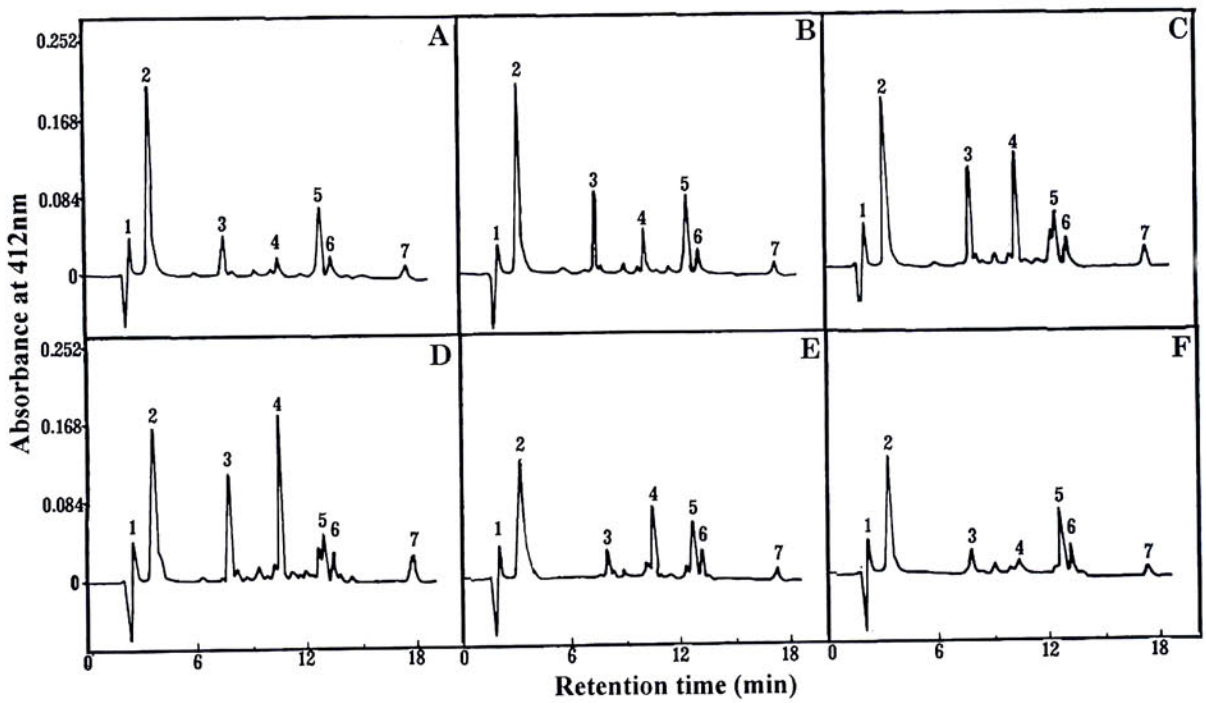

Fig. 2. HPLC profile of sulfurhydryl groups in five-week-old plants pretreated with 10 μM Cd^{2+} for a week and then treated in 45 μM Cd^{2+} for different time intervals. The durations of Cd^{2+} incubation in A, B, C, D, E and F are 0, 3, 12, 24, 48 and 96 h, respectively.

did not have significant changes within 96 h. Plants previously exposed to 10 μM Cd^{2+} not only induced more than one type of sulfurhydryl group but also displayed different responses of individual sulfurhydryl groups (Figs. 1 and 2). These results indicate prior exposure to Cd^{2+} at a sub-lethal concentration can induce greater production of sulfurhydryl compounds in plants when they are subsequently transferred to a medium containing a higher Cd^{2+} concentration.

B. Effect of Cd^{2+} on the content of glutathione in plants

Glutathione is an indicator of plant defensive response to external stresses, such as water stress, salinity, radiation, extreme high and low temperatures, heavy metals, and various air pollutants (Smith *et al,* 1990). In the present study, glutathione production was enhanced by prior exposure of plants to a sublethal concentration of Cd^{2+}.

The quantitative study indicated that the production of glutathione in plants can be enhanced by the presence of Cd^{2+} in culture medium (Fig. 3). Plants pretreated with 10 μM Cd^{2+} for one week and then treated with 45 μM Cd^{2+} accumulated a higher quantity of initial glulathione, reaching 161 mole/g. f.w. at 3 h after treatment and then decreasing to 100 mole/g. f.w. Plants with no prior exposure to Cd prior to treatment with 45 μM Cd^{2+} had a lower initial quantity of glutathione (80 mole/g. f.w.), although the quantity increased by 70% at 3 h and at 48 h after treatment. They also contained a lower quantity of glutathione at the end of the testing period (60 mole/g. f.w. at 96 h). These results indicate that prior exposure to Cd^{2+} at a lower concentration could enhance the production of glutathione at initial and final stages.

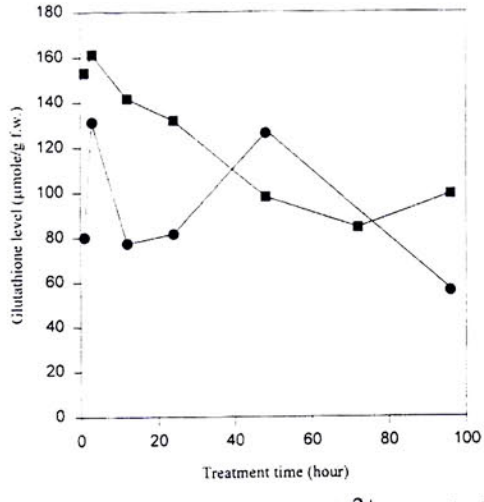

Fig. 3. Comparison on the production of glutathione in 45 μM Cd^{2+}-treated plants with (-■-■-) or without (-●-●-) the pretreatment of 10 μM Cd^{2+} for one week. Active fractions of glutathione obtained from HPLC separation as shown in Figures 1 and 2 were compared with glutathione standard used as marker of retention time and quantitative calibration.

C. Isolation and purification of LMW and HMW Cd^{2+}-binding complexes

Two major fractions of peptides (I and II, Fig. 4) had overlapping Cd^{2+} peaks in the profile of the ion exchange column. Approximately 60% of total Cd^{2+} was associated with peptide peak II. Fractions I (tubes 13-21) and II (tubes 25-33) were collected. Two fractions containing Cd^{2+} and peptides were pooled for further separation on a Sephadex G-50 column.

Fractions containg the HMW Cd^{2+} binding complex (tubes 7-9) and the LMW Cd^{2+} binding complex (tubes 12-19) were collected from gel filtration (Fig. 5), and lyophilized to determine amino acid composition and individual phytochelatin in the HPLC profiles.

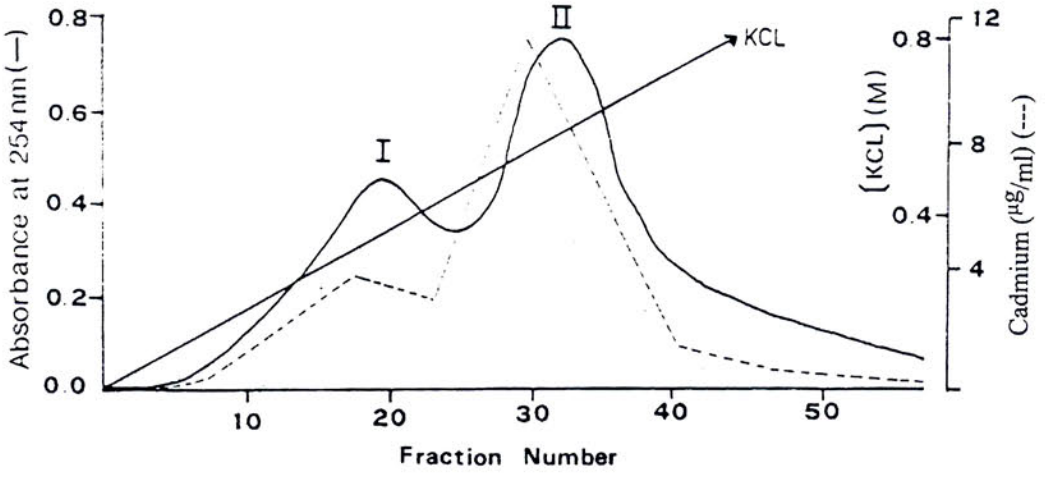

Fig. 4. DE52 profile of containing cadmium-binding complex in plants. The five-week-old plants were pretreated with 10 μM Cd^{2+} for one week and then with 45 Cd^{2+} μM for 5 days.

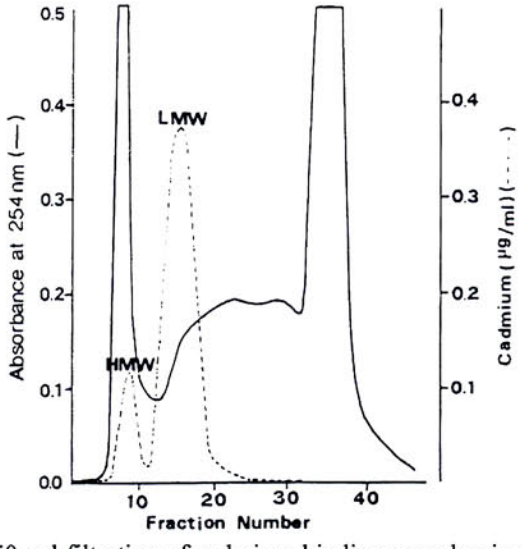

Fig. 5. Profile of Sephadex G-50 gel filtration of cadmium-binding complex in 45 μM Cd^{2+}-treated plants with pretreatment of 10 μM Cd^{2+} for one week.

D. Identification of LMW and HMW Cd^{2+}-binding complexes

The composition of Cd^{2+} binding complexes were quite different in LMW vs. HMW (Table 1). The LMW Cd^{2+} binding complex did not contain acid labile sulfurs, whereas the HMW complex had an extremely high level of them. Moreover, the HMW Cd^{2+} binding complex had a much higher content of sulfurhydryl groups and Cd^{2+} than the LMW complex. This indicates that the HMW complex has a higher capacity for chelating Cd^{2+}. Analysis of amino acid composition of both LMW and HMW complexes indicated a high content of

glutamic acid, cysteine, and glycine (Table 2). The percentages were higher in the HMW complex. Both complexes contained a high concentration of histidine-like substance (60.7% and 50.7%, respectively).

Table 1. Components of cadmium -binding complexes.

	HMW	LMW
	n mol	
Sulfurhydryl groups	700.48	129.58
Cadmium	41.00	2.00
Acid labile sulfur	1506.00	—

Table 2. Amino acid composition of purified HMW and LMW Cd-binding Complexes obtained from Sephadex 50 gel filtration.

Amino acid	Percentage of contents	
	HMW	LMW
Asp	3.3	3.7
Thr	0.1	0.5
Ser	1.9	1.4
Glu	20.2	14.0
Pro	1.0	0.5
Gly	7.6	6.3
Ala	1.7	0.6
1/2Cys	6.8	2.5
Val	1.5	0.6
Met	0.1	0.1
Ile	0.8	0.3
Leu	1.4	0.7
Tyr	0.3	0.7
Phe	4.8	0.6
His	50.8	67.7
Lys	1.0	0.3
Arg	0.6	0.4

There were two peaks on the HPLC profile of the LMW Cd^{2+} binding complex (Fig. 6). The amino acid composition of peaks 1 and 2 obtained from this complex held a large quantity of histidine-like substance (99.3% and 91.3%, respectively) and their retention times were shorter than that of glutathione. The ratios of glutamic acid to glycine in peaks 1 and 2 were almost equal to one (Table 3).

Five major peaks and some minor peaks were identified in the HPLC profile of the HMW Cd^{2+} binding complex (Fig. 7). Peak 1 (Table 4) contained a high percentage of histidine-like substance (85.2%) and its ratio of glutamic acid to glycine was approximately 2. In peaks 2 to 5, the histidine-like substance content was just 0.1%, and glutamic acid, cysteine, and glycine were predominant. The summed percentage of three major amino acids in peaks 2, 3, 4 and 5 were 93.5, 93.7, 95.0 and 95.2%, respectively. The ratios of glutamic acid to glycine in peaks 2, 3, 4, and 5 were 3, 3, 4, and 4, respectively. The mechanism for contaminated amino acid mobility of phytochelains needs to be resolved.

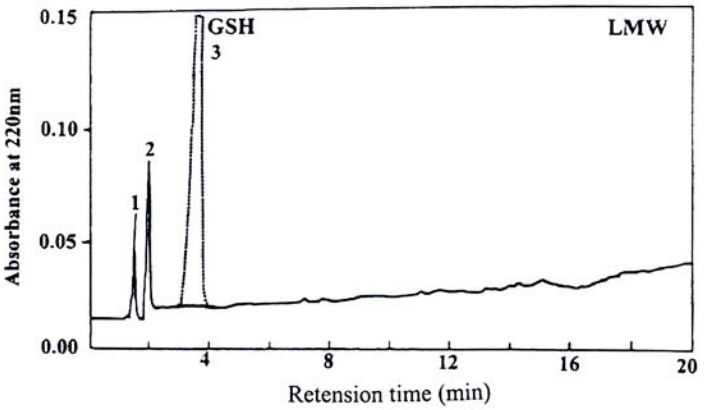

Fig. 6. HPLC profile of LMW Cd-binding complex in 45 μM Cd^{2+}-treated plants with pretreatment of 10 μM Cd^{2+} for one week.

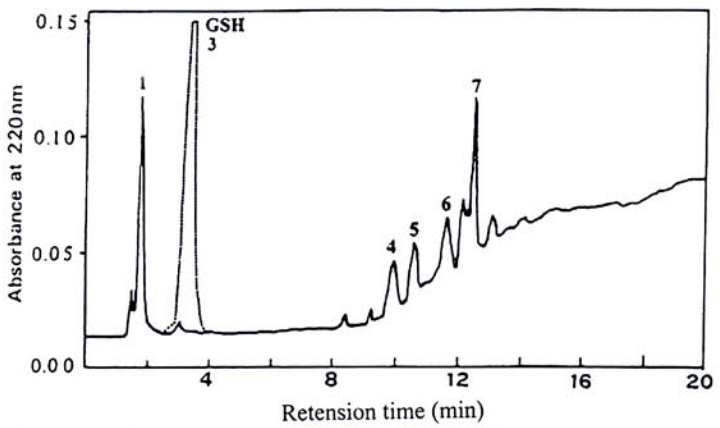

Fig. 7. HPLC profile of HMW Cd-binding complex in 45 μM Cd^{2+}-treated plants with pretreatment of 10 μM Cd^{2+} for one week.

Zinnia elegans plants treated with Cd^{2+} were induced to produce both LMW and HMW Cd-binding complexes containing sulfurhydryl groups. Phytochelatins occurred only in the HMW Cd^{2+} binding complex, with an n number ranging from 3 to 4.

DISCUSSION

Plant production of glutathione, in response to various external and internal stresses, is well documented in the literature (e.g., Smith *et al,* 1990). However, the internal level of glutathione varies with plant species and the experimental conditions. In this study, *Zinnia* pretreated with Cd^{2+} at a sublethal concentration for a period of one week were able to increase their internal concentration of glutathione. In plants not previously exposed to Cd^{2+}, glutathione was decreased on exposure to a high concentration of Cd^{2+}. Glutathione occurred only in the HPLC profile of the HMW complex, in small amounts. Similar results for the LMW complex, without glutathione, were reported in Cd^{2+}-treated fission yeast (Mehar and Mulchandani, 1995).

Table 3. Amino acid composition of purified HMW Cd-binding Complexes obtained from HPLC separation.

Amino acids	Percentage of contents	
	Peak 1	Peak 2
Asp	0.2	0.5
Thr	0.1	0.3
Ser	0.1	0.4
Glu	0.3	1.0
Gly	0.3	1.0
Ala	0.0	1.2
1/2Cys	0.0	0.0
Val	0.0	0.0
Met	0.0	0.0
Ile	0.0	0.5
Leu	0.0	1.0
Tyr	0.0	0.0
Phe	0.0	0.0
His	99.0	92.3
Lys	0.0	0.4
Arg	0.0	1.1
n (Glu/Gly)	1	1

Table 4. Amino acid composition of purified HMW Cd-binding complexes Obtained from HPLC separation.

Amino acid	Percentage of contents					
	Peak 2	Peak 3 GSH	Peak 4	Peak 5	Peak 6	Peak7
Asp	1.4		0.3	1.0	0.7	0.6
Thr	0.2		0.5	0.6	0.3	0.4
Ser	0.3		1.0	1.1	0.8	0.7
Glu	7.2		52.0	52.3	57.4	57.0
Pro	0.0		1.7	0.0	0.3	0.1
Gly	3.4		18.8	17.0	14.9	15.1
Ala	0.3		0.5	0.6	0.2	0.3
1/2Cys	2.8		22.7	24.4	23.7	23.3
Val	0.0		0.0	0.0	0.0	0.0
Met	0.0		0.0	0.0	0.0	0.0
Ile	0.0		0.3	0.3	0.2	0.1
Leu	0.0		0.8	0.7	0.4	0.5
Tyr	0.0		0.0	0.0	0.0	0.0
Phe	0.0		0.0	0.0	0.0	0.0
His	85.2		0.1	0.1	0.1	0.1
Lys	0.0		0.6	0.6	0.3	0.5
Arg	0.0		1.1	1.3	0.8	0.9
n (Glu/Gly)	2		3	3	4	4

Because they contain γ-glutamic acid and are structurally similar, phytochelatins are common with glutathione. Phytochelatins are biosynthesized by phytochelatin synthase with glutathione as a precursor (Grill *et al*, 1989). The Cd^{2+}-induced synthesis of phytochelatins is

accompanied by a reduction of the glutathione pool in roots (Rauser *et al*, 1991) and in cultured cells (Grill *et al*, 1989). Cd^{2+} is the most potent mineral activator for phytochelatin synthase *in vitro* (Yuan *et al*, 1993; Klapheck *et al*, 1995).

The characterization and composition of Cd^{2+} binding complexes varies greatly among organisms, concentration of free ionic Cd^{2+}, exposure time, and nutrient medium (Rauser, 1995). Both LMW and HMW Cd^{2+} binding complexes found in yeast, algae, and higher plants are well documented (Steffens, 1990). The HMW complex contains acid sulfide and is essential for growth of yeasts and plants exposed to a high concentration of Cd^{2+} (Mutoh and Hayashi, 1988; Speiser *et al*, 1992). Acid labile sulfide was found in the HMW complex, but it was absent from the LMW complex of *Zinnia elegans* treated with a lethal concentration of Cd^{2+}. How sulfide, Cd^{2+} and phytochelatins interact is still unknown (Rauser, 1995).

In the preparation of heavy metal-phytochelatin complexes in Cd^{2+}- and Cu^{2+}-treated algae, yeasts, and high plants, values of cysteine, glulamic acid and glycine ranging from 45 to 97% of the total amino acid have been reported (Robinson *et al*., 1993). In fission yeast (Kondo *et al*, 1984) and *Rauvolfia serpentina* (Grill *et al*, 1985), the primary structure of Cd-binding peptides, the phytochelatins, were (γ-Glu-Cys)n-Gly, where n = 2 to 7. In the present study, only peaks 4, 5, 6, and 7 of the HMW Cd^{2+} binding complex in the HPLC profile contained a high percentage (more than 93%) of glutamic acid, cysteine, and glycine. The n numbers of (Glu-Cys)n-Gly ranged from 3 to 4 in *Zinnia elegans*, fitting in the category of phytochelatins.

Both HMW and LMW complexes contained a high quantity of sulfurhydryl groups. In fission yeast and other metal-tolerant plants, the LMW complex always chelated with phytochelatins without acid labile sulfur (Ortiz *et al*, 1992; Salt *et al*, 1993). However, the LMW complex in Cd^{2+}-treated *Zinnia* contained more than 90% of the histidine-like substance in total amino acids.

The cellular compartment for the HMW complex in yeast was suggested to be the vacuole, and the LMW complex was found in both the vacuole and cytoplasm (Ortiz *et al*, 1995). Phytochelatins, Cd-phytochelatin complex, and free Cd^{2+} could permeate the tonoplast by the processes of proton/Cd^{2+} antiport or ATP-binding cassette (ABC) Mg-ATPase (Ortiz *et al*, 1992; Salt and Wagner, 1993).

In Cd^{2+}-sensitive *Silene vulgaris,* free Cd^{2+} transported from the root system was indicated (Verkleij *et al*, 1995). By using X-ray absorption spectroscopy of Cd^{2+} treated plants, Salt *et al* (1995) documented that the majority of Cd^{2+} was conjugated with sulfur ligands in roots and coordinated with oxygen or nitrogen ligands in xylem sap. Histidine contains both oxygen and nitrogen. A histidine-nickel complex ligand had previously been found in nickel hyperaccumulation plants of *Alyssum spp* (Kramer *et al*, 1996). In the present study, high histidine-like peaks found in both LMM and HMW is cause for further study because artificial peaks occurred at similar retention times of histidine-like substance in the HPLC profiles.

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LITERATURE CITED

Dameron, C. T., B. R. Smith and D. R. Winge. 1991. Glutathione-coated cadmium-sulfide crystallites in *Candida glabrata*. J. Biol. Chem. **264**: 17355-17360.

Delhaize, E., P. J. Jackson, L. d. Lujan, and N. J. Robinson. 1989. Poly (gamma-glutanylcysteinyl) glycine synthesis in *Datura innoxia* and binding with cadmium. Plant Physiol. **89**: 700-706.

Ellman, G. L., K. D. Courtney, V. Andres and R. M. Featherstone. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. **7**: 88-95.

Grille, E., E. L. Winnacker and M. H. Zenk. 1985. Phytochelatins: the principal heavy metal-complexing peptides of higher plants. Science **230**: 674-676.

Grill, E., E. L. Winnacker and M. H. Zenk. 1987. Phytochelatins, a celss of heavy-metal-binding peptides from plants, are functionally analogous to metallothioneins. Proc. Natl. Acad. Sci. (USA) **84**: 439-443.

Grill, E., E. L. Winnacker and M. H. Zenk. 1989. Phytochelatins, the heavy-metal-binding peptides of plants, are synthesized from glutathione by a specific glutamylcysteine-dipeptidyl transpeptidase (phytochelatin synthase). Proc. Natl. Acad. Sci. (USA) **86**: 6838-6842.

Hayashi, Y, C. W. Nakagawa, N. Mutoh, M Isobe and T. Goto. 1991. Two pathways in the bioxynthesis of cadystins (gEC)nG in the cell-free system of the fission yeast. Biochem. Cell Biol. **69**: 115-121.

Howe, G. and S. Merchant. 1991. Heavy metal-activated synthesis of peptides in *Chlamydomonas reinhardtii*. Plant Physiol. **98**: 127-136.

Kawashima, I., T. D. Kennedy, M. Chino and B. G. Lane. 1992. Wheat E_c metallothionein genes: like mammalian Zn^{2+} metallothionein genes, wheat Zn^{2+} metallothioneinare conspicouosly expressed during embryogenesis. Eur. J. Biochem. **209**: 971-976.

Klapheck, S, S. Schlunz, and L. Bergman. Synthesis of phytochelatins and homo-phytochelatins in *Pisum sativum*. Plant Physiol. **107**: 515-521.

Kondo, N., K. Imai, M. Isobe, T. Goto, A. Murasugi, C. Wada-Nakagawa and Y. Hayashi. 1984. Cadystin A and B, major subunitpeptides comprising cadmium binding peptides induced in fussion yeast-separation, revision of structures and synthesis. Tetrahedron Lett. **25**: 3869-3872.

Kramer, U., J. D. Cotter-Howells, J. M. Charmock, A. J. M. Baker and J. A. C. Smith. 1996. Free histidine as a metal chelator in plants that accumulate nicker. Nature **379**: 635k-638

Mehra, R. K. and P. Mulchandani. 1995. Glutathione-mediated transfer of Cu(I) into phytochelatins. Biochem. J. **307**: 697-705.

Murasugi, A., C. Wada and Y. Hayashi. 2983. Occurrence of acid-labile sulfide in cadmium-binding peptide 1from fission yeast. J. Biol. Chem. **93**: 661-664.

Mutoh, N. and Y. Hayashi. 1988. Isolation of mutants of *Schizosaccharomyces pombe* unable to synthesize cadystin, small cadmium-binding peptides. Biochem. Biophys Res. Commun. **151**: 32-39.

Ortiz, D. F., L. Kreppel, D. M. Speiser, G. Schell, G. McDonald and D. W. Ow. 1992. Heavy metal tolerance in the fission yeast requires an ATP-binding cassette-type vacuolar membrane transporter. EMBO J. **11**: 3491-3499.

Ortiz,D. F., T. Ruscitti, K. F. McCue and D. W. Ow. 1995. Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacuolar membrane protein. J. Biol. Chem. **270**: 4721-4728.

Rauser, W. E. 1990. Phytochelatins. Annu. Rev. Biochem. **59**: 61-86.

Rauser, W. E. 1995. Phytochelatins and related peptides: structure, biosynthesis and function. Plant Physiol. **109**: 1141-1149.

Rauser, W. E., R. Schupp, and H. Rennenberg. 1991. Cysteine, gamma-glutamylcysteine and glutathione levels in maize seedlings. Plant Physiol. **97**: 128-138.

Robinson, N. J., A. M. Tommey, C. Kuske, and P. J. Jackson. 1993. Plant metallothioneins. Biochem. J. **295**: 1-10.

Salt, D. E., R. C. Prince, I. J. Pickering, and I. Raskin. 1995. Mechanisms of cadmium motility and accumulation in Indian mustars. Plant Physiol. **109**: 1427-1433.

Salt, D. E. and G. J. Wagner. 1993. Cadmium transport across tonoplast of vesicles from oat roots. J. Biol. Chem. **268**: 12297-12302.

Smith, I. K., A. Polle, and H. Rennenberg. 1990. Glutathione. In "Stress Responses in Plants: Adaptation and Acclimation Mechanisms (Alscher, R. G. and J. R. Cumming ed)". Wiley-Liss, New York.

Smith, J. A. C., U. Kramer and A. J. M. Baker. 1995. Role of metal transport and chelatin in nickel hyperaccumulation in the genus *Alyssium* In 14th Annu. Symp. Curr. Top. Plant Biochem. Physiol. Mol. Biol: Will Plant Have a Role in Bioremediation? University of Missouri, Columbia

Steffens, J. C. 1990. The heavy metal-binding peptides of plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **41**: 553-575.

Steffens, J. C., D. F. Huant and B. G. Williams. 1986. Accumulation of non-protein metal-binding polypeptides (gamma-glutanyl-cysteinyl)n-glycine in selected cadmium-resistant tomato cells. J. Biol. Chem. **261**: 13879-13882.

Voegeli-Lange R. and G. J. Wagner. 1990. Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves. Plant Physiol. **92**: 1086-1093.

Verkleif, J. A. C., J. A. DeKnecht, H. Harmens and H. Schat. Physiological studies on Zn and Cd tolerance in high plants. In "14th Annu. Symp. Curr. Top. Plant Biochem. Physiol. Mol Biol.:Will Plant Have a Role in Bioremediation?" University of Missouri, Columbia.

Yan, S.-L. C.-C. Tsay and Y.-R. Chen. 2000. Isolation and characterization of phytochelatin synthase in rice seedlings. Proc. Nat'l Sci. Coun. Part B **24**: 202-207

Zhou, J. and P. B. Goldbrough. 1994. Functional homologs of animal and fungal metallothioneins gene from *Arabidopsis* . Plant Cell **6**: 875-884.

鎘處理百日草內鎘結合多胜肽

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摘　　要

五週大的百日草幼苗，以水耕法施以 10 μM 鎘濃度的先處理一週後，移植於含 45 μM 鎘的營養液下處理。在不同的處理時間下，分別收集經前處理及單一的 45 μM 處理的植株進行測試。在高效能液態色層分析圖譜的比較下，有經先處理的植株含較多的氫硫化合物及麩胱甘太，而且在七個波峰的氫硫化合物中的第三和第四波峰在處理 24 小時內遞增至最高，隨後而遞減，在 96 小時時恢復原來的濃度。進一步分離與鑑定低分子與高分子複合物所用的材料都用先處理者。植物的萃取液經 DE 52 的離子交換和 Sephadex-50 膠體過濾柱型色層分析，取得的兩個層析分別是高分子與低分子鎘結合復合物。低分子鎘結合復合物在高效能液態色層分析圖譜中顯示兩個波峰，氨基酸分析儀鑑定兩著所占有的組氨酸量高達 90 % 以上，且其滯留時間比麩胱甘太短。高分子鎘結合複合物則顯示有七個波峰，除第一波峰含有的組氨酸高達 85 % 外，其餘波峰所含的組氨酸都在 0.1 % 以下，植物螯合物中麩氨酸/甘氨酸的比例分別為 3 和 4。

關鍵詞：鎘，麩胱甘太，植物螯合物，硫氫基，百日草。

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