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Subcellular Distribution and Chemical Form of Cadmium in Bean Plants

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ABSTRACT

The subcellular distribution and chemical form of Cd in bean plants grown in nutrient solutions containing Cd were investigated. Cd was accumulated mainly in roots and to a minor extent in leaves. Subcellular fractionation of Cd-containing tissues (pH 7.5) showed that more than 70% of the element was localized in the cytoplasmic fraction in roots as well as in leaves. Little Cd (8 to 14%) was bound either to the cell wall fraction or to the organelles. Gel filtration of the soluble fraction showed Cd to be associated mainly with 5,000 to 10,000 molecular weight components in roots, and 700 to 5,000 molecular weight components in leaves. Small amounts of Cd were found in the high molecular weight proteins (molecular weight 150,000). Only traces of Cd could be detected as a free ion. Chemical characterization of the low molecular weight components resulted in the identification of nine amino acids which were identical in roots and leaves. Cd in bean plants is assumed to be bound to peptides and/or proteins of low molecular weight.

Cd is a major environmental contaminant (8, 22). The essentiality of this element for either plants or animals has not yet been demonstrated, whereas its toxicity in higher concentrations is well established (8, 16). The molecular basis for this toxicity remains largely unknown. Interest has focussed primarily on the relationship between substrate parameters and plant concentration (12, 20), the general toxicity symptoms (10, 26), and specific effects on transpiration and photosynthesis (1, 2). Numerous *in vitro* and *in vivo* studies on the effects of Cd revealed that it is either an inhibitor or activator of enzymes, such as nitrate reductase, malate dehydrogenase, and peroxidase (16, 17). However, little is yet known about transport, intracellular localization, and binding of Cd *in situ* (3, 5, 18). Knowledge of its intracellular distribution pattern should help, therefore, in assigning its effects on cellular activities and should also be valuable in defining its possible biochemical roles.

The present paper reports the fractionation of Cd-treated bean plants into subcellular components and the characterization of the chemical form and binding of this element.

MATERIALS AND METHODS

Plant Culture. Seeds of *Phaseolus vulgaris* L. cv. Sankt Andreas were germinated on moist Vermiculite, and individual seedlings were transferred to an aerated nutrient solution (15) 5 days after germination. The pH was adjusted to 5.5. After 3 days, Cd was supplied to the nutrient solution in a final concentration of 0.45 mg $CdCl_2$/l. Plants were grown in a greenhouse under the following conditions: 50% RH, 16/8-h light cycle (2,800 ft-c), and a day/night temperature of 25/20 C. At the time of the harvest, the plants had developed primary and first trifoliate leaves. Roots and leaves were analyzed separately. Roots were washed several times with deionized H_2O and blotted between paper towels prior to weighing. The experiments were repeated three times.

Fractionation of Leaves and Roots. Leaves and roots were homogenized using a mortar and pestle in a medium containing 0.25 M sucrose, 50 mM Tris-HCl (pH 7.5), and 1 mM dithioerythritol. All steps were performed at 4 C. The resulting brei was strained through eight layers of cheesecloth, and liquid was expressed from the residue. The residue was washed twice with the grinding medium. The pooled washes, together with the first filtrate, were centrifuged at 300*g* for 30 s. The resulting pellet combined with the residue of the cheesecloth filtration contained mainly cell walls and cell wall debris and was designated as cell wall fraction (I). The supernatant of the first centrifugation step was then centrifuged at 20,000*g* for 45 min to sediment cell organelles. The pellet was taken as organelle fraction (II). The resultant supernatant solution referred to as soluble fraction (III) was employed in subsequent characterization studies as described. Fractions I and II were analyzed for their Cd content. The supernatant, fraction III, was further fractionated by gel filtration using Sephadex G-100, G-25, and G-10 (Pharmacia Fine Chemicals, Uppsala, Sweden). An aliquot of fraction III was applied to a column (100 × 2.6 cm) of G-100 using 50 mM Tris-HCl (pH 7.5) and 0.1 mM dithioerythritol as eluting buffer. Fractions containing Cd were pooled and chromatographed on calibrated G-25 (roots) or G-10 (leaves) columns (70 × 2.3 cm). Fractions were collected on an LKB Ultrarac fraction collector. The effluents were monitored at 280 nm with a Gilford spectrophotometer 2400 S. The activities of the glutamate dehydrogenase and malate dehydrogenase were determined according to references 9 and 23.

Characterization of Cd Form. Plant fractions of either the G-25 or G-10 column containing Cd were pooled and treated with 6 N HCl for 20 h at 100 C (protein hydrolysis). The determination of the resulting amino acids was carried out by means of an automated amino acid analyzer 3201 (LKB).

Determination of Cd. Cd was determined by atomic absorption spectroscopy either in the column effluents or after ashing the Cd-containing tissues at 450 C.

RESULTS AND DISCUSSION

The Cd content of the leaves and roots of bean plants grown in solution culture containing 0.45 mg $CdCl_2$/l was determined at various times after Cd application. As shown in Table I, the Cd content of both leaves and roots increased with time. At 18 days, roots contained 66.0 μg Cd/g and leaves 8.0 μg Cd/g on a fresh weight basis. These Cd contents in the plant tissues caused growth reductions of roots and shoots as well as foliar toxicity symptoms (reddish brown color of the veins and beginning chlorosis). Similarly, the Cd content of the different subcellular fractions increased with time (Table I). However, the proportion of Cd bound to cell

Table I. *Cd Distribution in Subcellular Fractions from Leaves and Roots of Bean Plants at Various Days after Cd Application in Vivo*

Fractions I, II, and III: see "Materials and Methods." Figures are average of three determinations ±SE.

Days after Cd Application		Cd Content				
		2	4	6	8	12
		μg per g dry matter				
Fraction I	Leaves	0.80 ± 0.04	1.60 ± 0.08	1.00 ± 0.12	1.80 ± 0.09	16.0 ± 2.40
	Roots	13.9 ± 2.40	16.5 ± 1.80	25.5 ± 2.00	62.7 ± 7.50	97.5 ± 10.7
Fraction II	Leaves	1.40 ± 0.07	1.80 ± 0.09	1.80 ± 0.09	2.00 ± 0.07	8.00 ± 0.05
	Roots	9.90 ± 0.94	20.4 ± 1.56	42.0 ± 3.90	66.7 ± 8.40	112.5 ± 7.50
Fraction III	Leaves	7.20 ± 0.72	10.4 ± 0.78	13.0 ± 1.32	14.0 ± 1.12	50.0 ± 8.30
	Roots	75.0 ± 8.40	141.0 ± 12.3	232.0 ± 16.9	345.6 ± 39.0	720.5 ± 62.5
Total	Leaves	10.0 ± 2.30	15.0 ± 2.20	16.0 ± 3.20	17.7 ± 3.20	80.0 ± 10.2
	Roots	98.4 ± 8.40	178.0 ± 11.2	322.5 ± 23.0	514.0 ± 62.5	990.0 ± 98.0
Recovery %	Leaves	94.0	92.0	98.8	100.0	92.2
	Roots	100.0	99.3	99.3	91.9	93.5

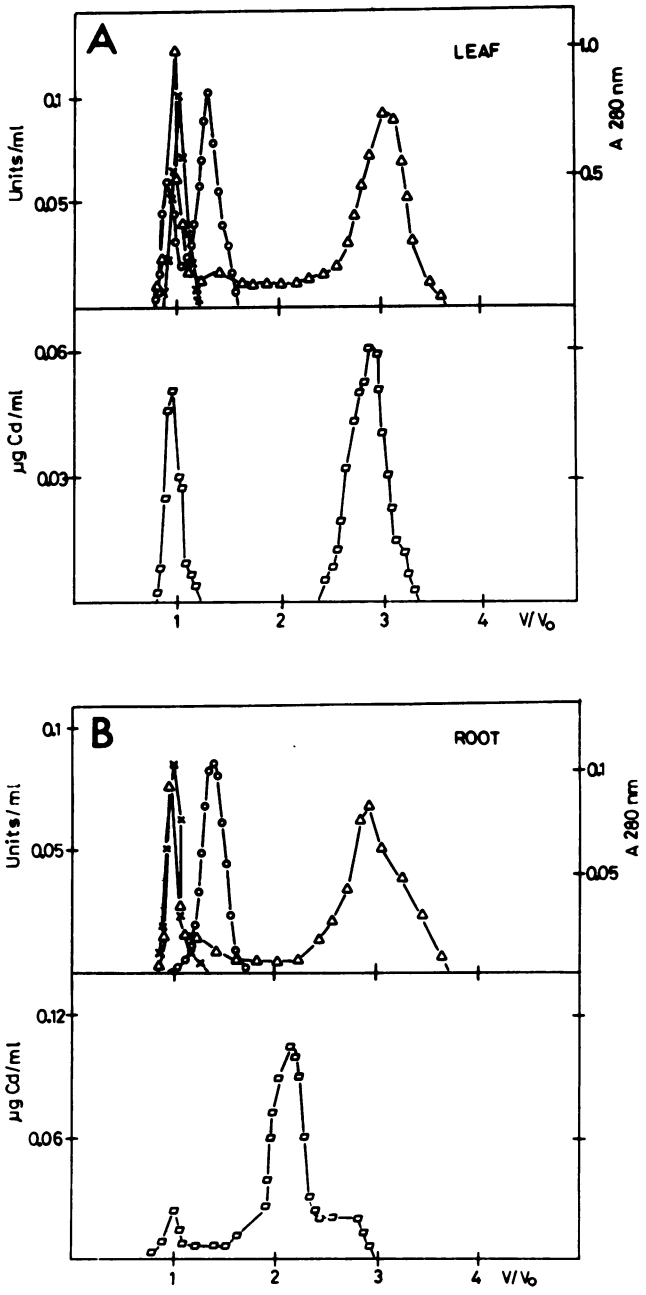

FIG. 1. Sephadex G-100 gel filtration profile of Cd-containing supernatant fractions of bean plants after Cd application *in vivo*. (□——□): Cd; (△——△): *A* at 280 nm; (×——×): glutamate dehydrogenase (GDH); (○——○): malate dehydrogenase (MDH).

walls, organelles, or the supernatant fraction, relative to the total amount of Cd in the tissues, did not change. Furthermore, differential centrifugaton of Cd-containing homogenates (pH 7.5) showed that only a minor part of the element was associated with either the cell walls or the organelle fraction. For example, Cd showed a distribution pattern similar to zinc whose concentration in zinc-treated bean plants was highest in the soluble fraction and low in nuclei, chloroplasts, and cell walls (24, 25). Foliar-applied Cd has been found to be associated mainly with the cell wall fraction and is thought not to be in a physiologically active form (7). Very small amounts of the element were found in nuclear, mitochondrial, and microsomal fractions of soybean plants grown on a Cd-containing sewage sludge (3). Gel filtration of the supernatant containing Cd on Sephadex G-100, performed only with plants, which had been in Cd solution for 18 days, resulted in two main Cd peaks (Fig. 1, A and B). Part of the element eluted with the void volume (V/V_0 1.00) indicating that Cd was bound to proteins with a mol wt >150,000. The peak of this high mol wt protein fraction corresponded to the peak of the activity of glutamate dehydrogenase in leaves and roots, whereas in leaf supernatant a high mol wt polymer of the malate dehydrogenase was found. The binding of Cd to a special protein is of interest because Cd has been shown to stimulate the activity of the glutamate dehydrogenase, but not of the malate dehydrogenase *in vivo* (26). The binding of Cd to macromolecular proteins (mol wt >70,000) has been found in soybean, corn, and yeast (3, 6, 18).

Most of the supernatant Cd was eluted in a second peak from gel filtration on Sephadex G-100 (Fig. 1, A and B). This peak contained nearly all of the total Cd, especially in the root supernatant. The mol wt of the peaks differed between leaves and roots, the root fraction having the higher mol wt. Further separation of each of these Cd-binding fractions resulted in a single Cd peak eluting with the void volume of the columns G-25 (root) and G-10 (leaves), as shown in Figure 2. The mol wt of these fractions, therefore, were >700 in the leaves and >5,000 in the roots. Only traces of Cd could be measured as a free ion in the column effluents corresponding to inorganic salts or ions. According to the elution pattern on the column G-100, the mol wt of the Cd-binding fractions in bean plants should range between 700 and 5,000 in the leaves, and 5,000 and 10,000 in the roots. Similar Cd-binding components of low mol wt have been found in maize and yeast, however, the chemical nature has not been characterized (6, 18). Ni showing the same distribution pattern as Cd in the present case, has also been described to bind to 500–10,000 mol wt components of both roots and shoots in soybean plants (4). An attempt was made to characterize the Cd-binding components

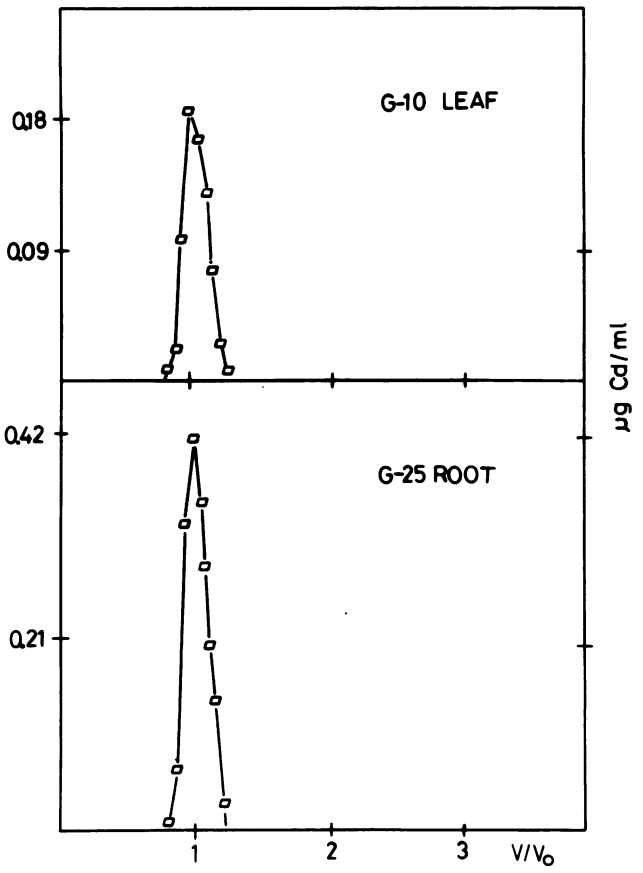

FIG. 2. Sephadex G-25 and G-10 gel filtration profile of Cd-containing supernatant fractions of bean plants. Fractions of the G-100 gel filtration containing Cd (Fig. 1) were pooled and chromatographed again.

described. However, various chemical tests for nucleic acids, ketoacids, carbohydrates, and amino acids failed. Only treatment of the Cd fractions under conditions of protein hydrolyzation and subsequent separation of the products on an automated amino acid analyzer resulted in the detection of Asp, Thr, Ser, Glu, Val, Leu, Ile, Ala, and Lys. These amino acids were the same in both roots and leaves.

The present paper provides evidence that Cd in bean plants is accumulated in the cytosol of the cell and is bound to peptides and/or low mol wt proteins. Compared to this bound fraction of Cd, free Cd ions are of minor importance in the distribution pattern of this element. A specific Cd-binding protein of low mol wt, metallothionein (mol wt 10,000), has been found only in mammalian tissues (13). The synthesis of this protein is induced after Cd application and it is responsible for the adaptation of some organisms to otherwise toxic concentrations of Cd (14). It is possible that a similar mechanism of Cd-binding could also exist in higher plants. However, in the present case the high Cd concentrations in the tissues (Table I) are obviously not detoxified. The toxicity symptoms described earlier clearly indicate an interference of Cd with the metabolism of bean plants. Binding of Cd to metabolites of the plant cell could account for the phytotoxicity of this nonessential element.

Roots of several plant species have been shown to act as a barrier, restricting the transport of Cd to shoots (11). The higher mol wt of the Cd-binding components in the roots of bean plants (Figs. 1 and 2) could be responsible for the different accumulation of Cd in roots and leaves (Table I), as Cd-organo complexes of higher mol wt should only partly be translocated within the plants. The quantitative role of these components in Cd-binding as well as their properties should be elucidated by further investigations.

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