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Phytochelatins in Cadmium-Sensitive and Cadmium-Tolerant *Silene vulgaris*[1]

Chain Length Distribution and Sulfide Incorporation

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In response to a range of Cd concentrations, the root tips of Cd-tolerant plants of *Silene vulgaris* exhibit a lower rate of PC production accompanied by a lower rate of longer chain PC synthesis than those of Cd-sensitive plants. At the same Cd exposure level, stable PC-Cd complexes are more rapidly formed in the roots of Cd-sensitive plants than in those of tolerant plants. At an equal PC concentration in the roots, the PC composition and the amount of sulfide incorporated per unit of PC-thiol is the same in both populations. Although these compounds might play some role in mechanisms that contribute to Cd detoxification, the ability to produce these compounds in greater amounts is not, itself, the mechanism that produces increased Cd tolerance in tolerant *S. vulgaris* plants.

In response to excessive uptake of heavy metals, plants produce metal-binding nonprotein SHs, called PCs, that have the general structure $(\gamma\text{-Glu-Cys})_n\text{Gly}$ (Gekeler et al., 1989). PCs are believed to be involved in the cellular homeostasis of biologically essential metals. Metal-depleted diamine oxidase and carbonic anhydrase can be reactivated in vitro by PC-Cu and PC-Zn complexes, respectively (Thumann et al., 1991). PCs can also effectively detoxify heavy metal ions. Metal-sensitive enzymes are 10- to 1000-fold more sensitive to free Cd than to PC-bound Cd (Kneer and Zenk, 1992). The role for PCs in metal detoxification is substantiated by the fact that inhibition of PC synthesis, either through sulfur starvation or by treatment with buthionine sulfoximine, increases the sensitivity of plants and cell suspensions to heavy metals, regardless of whether biologically essential or nonessential metals are concerned (Steffens et al., 1986; Huang et al., 1987; Reese and Wagner, 1987b; Schultz and Hutchinson, 1988; Salt et al., 1989; de Knecht et al., 1992). PCs also have been considered to play a role in metal tolerance, here defined as a naturally or artificially selected heritable increase in the capacity to tolerate higher concentrations of metal exposure. Increased tolerance has been suggested to result from overproduction of PCs (Bennetzen and Adams, 1984; Steffens et al., 1986) or a faster synthesis of longer chain species of PC (Delhaize et al., 1989), forming more stable complexes than shorter forms (Hayashi and Nakagawa, 1988). A third possibility that has been suggested is that increased incorporation of sulfide into PC-metal complexes increases both the stability and the potential amount of metal bound per unit of PC-SH (Reese et al., 1988; Reese and Winge, 1988). The relevance of the presence of sulfide in PC-metal complexes to metal detoxification is substantiated by the observation that mutants of *Schizosaccharomyces pombe* that produce only PC-Cd complexes without sulfide are hypersensitive to Cd (Mutoh and Hayashi, 1988). Incorporation of sulfide in PC-Cd complexes can result in the formation of CdS crystallites, which might be the storage form of Cd in the vacuole of fission yeast (Ortiz et al., 1992).

In a previous study, we demonstrated that sensitive plants of *Silene vulgaris* produce more PCs than tolerant plants when exposed to the same external Cd concentration (de Knecht et al., 1992). Moreover, L-buthionine sulfoximine treatment increased Cd sensitivity in sensitive plants but not in tolerant plants. These results strongly suggest that differential Cd tolerance in *S. vulgaris* is not affected by differential PC production per se. However, in that study, PC concentrations were calculated as total nonprotein SH minus GSH. The possibility remains that differential tolerance is due to differences in the chain lengths of PCs produced or in the amount of sulfide incorporated into PC-metal complexes, as suggested by results of another study of *S. vulgaris* (Verkleij et al., 1990). In the present study, we compared PC chain length distribution and amount of sulfide incorporation in PC-Cd complexes from Cd-sensitive and tolerant *S. vulgaris*.

MATERIALS AND METHODS

Plant Culture

Cd-sensitive plants from the *Silene vulgaris* population Amsterdam and Cd-tolerant plants from the population Plombières were grown as described by de Knecht et al.

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Abbreviations: FPLC, fast-performance liquid chromatography; PC, phytochelatin; SH, thiol; SSA, 5-sulfosalicylic acid.

(1992). After a preculture of 14 d, the plants were transferred to a fresh nutrient solution buffered with 2 mM Mes/KOH (pH 5.2). Cd was added as $CdSO_4$ at the appropriate concentrations. Control plants were placed in solution without Cd. At the time of harvest, Cd was removed from the root surface by placing the roots in a solution of 10 mM $CaCl_2$ for 30 min at 0°C. Root tips (10-mm apical segments) of six plants per Cd concentration were clipped, pooled, immediately frozen in liquid nitrogen, lyophilized, and stored under vacuum for future analysis.

Cd Tolerance

The Cd tolerance of both populations was inferred from the dose-response curves for the effect of Cd on the increase in length of the longest root throughout a 3-d exposure. To facilitate the measurement, roots were stained black with active carbon before the addition of Cd (Schat and ten Bookum, 1992).

Assay of GSH and PCs

PCs and GSH were extracted by homogenizing 10 mg of lyophilized root tip tissue in 1 mL of a 5% (w/v) SSA solution with 6.3 mM diethylenetriaminepentaacetic acid, using a mortar, pestle, and quartz sand. After the material was centrifuged at 10,000*g* for 10 min, the supernatants were filtered over 0.45-μm organic filters (FHUP 04700; Millipore) and immediately assayed.

GSH and PCs were separated by HPLC on a Nova-Pak C_{18} column (60 Å, 4 μm, 3.9 × 150 mm, i.d.; Waters catalog number 36975), with a Nova-Pak C_{18} precolumn (Waters catalog number 15220), using the method of Tukendorf and Rauser (1990) with the following modifications. The flow rate through the column was 0.5 mL min^{-1}. The sample volume was 50 μL. The gradient program was 0.1% (v/v) TFA for 2 min, followed by a gradient of 0 to 20% (v/v) acetonitrile in 0.1% TFA for 16 min. The column was then regenerated by washing with 50% acetonitrile in 0.1% TFA and equilibrated in water with 0.1% TFA for 10 min. The eluent was derivatized with 1.8 mM 5,5′-dithiobis(2-nitrobenzoic acid) in 0.3 M potassium phosphate buffer and 15 mM Na_2EDTA (pH 7.8), which was added at a flow rate of 0.25 mL min^{-1} by an Eldex postcolumn pump (model A-30-sw-2). The mixture passed through a RXN 1000 coil (volume 1 mL) with a residence time of 80 s. The *A* of the derivatized material was measured at 412 nm by a 991-photodiode array detector (PDA-991, Waters). Retention times and peak areas were determined with a computerized integration program (Waters Maxima). The SH concentrations are expressed as GSH equivalents, based on peak areas of GSH standards.

Identification of PCs

To identify the peaks obtained by HPLC, supernatants (0.5 mL) of root extracts were loaded on a FPLC-PEPRPC column (HR 5/5, Pharmacia) and eluted with a linear gradient of 0 to 25% acetonitrile in 0.1% TFA for 12.25 min (flow rate 0.7 mL min^{-1}). The column was equilibrated with 0.1% TFA in water. Fractions (0.5 mL) were collected and rechromatographed by HPLC as described above, both with and without addition of the crude supernatant. The fractions that produced a single peak that co-eluted with one of the supernatant peaks were analyzed for amino acid composition. The SH concentration in the fractions was determined by mixing the fractions with an equal volume of the 5,5′-dithiobis(2-nitrobenzoic acid) solution described above and measuring the A_{412}.

The amino acid analysis was performed as described by Bank et al. (1988). The dried fractions were successively oxidized with performic acid for 4 h at 0°C, dried, and hydrolyzed in 6 M HCl + 0.1% (v/v) phenol for 1 h at 150°C in evacuated sealed tubes in a Waters Pico-Tag Workstation. Amino acids were derivatized with phenylisothiocyanate and separated by HPLC on a reversed-phase Pico-Tag column (Waters, catalog No. 88131).

Analysis of PC-Cd Complexes

Root material (100 mg dry weight) was homogenized in 10 mL of a solution containing 20 mM Tris-HCl, 1 mM PMSF, 1 g of PVP (Polyclar, pH 7.2) at 4°C, using a mortar, pestle, and quartz sand. The homogenate was centrifuged at 10,000*g* for 10 min to remove PVP and quartz sand and then at 100,000*g* for 90 min at 4°C. Supernatant was applied to an FPLC-Mono Q anion-exchange column (HR 5/5, Pharmacia) and eluted with a linear salt gradient (0–1 M NaCl) in 20 mM Tris-HCl (pH 8.0). Fractions (1 mL) were collected and assayed for SH, Cd, and sulfide. SH and Cd concentration were determined as described by de Knecht et al. (1992). Sulfide concentrations were quantified as described by King and Morris (1967). The amount of Cd present in the fractions did not interfere with this assay. The sulfide concentration in a standard solution was completely recovered up to 5 mM Cd (data not shown).

To study the formation of the PC-Cd complexes, these complexes were extracted from lyophilized root systems of three plants as above in the presence of 5 mM β-mercaptoethanol. Supernatants were applied to an FPLC-Superose 12 gel filtration column (HR 10/30) and eluted in 100 mM NH_4HCO_3 (pH 8.0). Fractions (1.5 mL) were collected and assayed for Cd and sulfide concentration.

Determination of Cd Concentrations

The Cd concentration in the roots was calculated from the Cd concentration in the supernatant of the SSA extracts that was measured using atomic absorption spectrophotometry. The Cd concentrations calculated in this way were similar to those obtained after wet ashing in $HClO_4$-HNO_3, as described by Verkleij and Prast (1989).

RESULTS

Effect of Cd on Root Growth

The effect of Cd on root growth in Cd-sensitive and Cd-tolerant plants is shown in Figure 1. In sensitive plants, the highest Cd concentration that did not affect root growth was approximately 1 μM. Fifty percent inhibition occurred at 45 μM and 100% inhibition occurred at 180 μM Cd. In tolerant

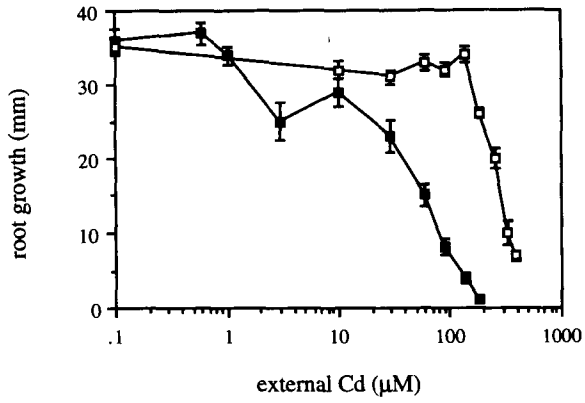

Figure 1. Effect of Cd on root growth of Cd-sensitive (■) and tolerant (□) *S. vulgaris*. Plants were exposed to Cd for 3 d. Values are means ± SE of nine plants.

plants these concentrations were 135, 250, and 490 μM, respectively.

PC Concentrations and Cd in Root Tips

Plants of both populations were exposed to increasing Cd concentrations for 3 d. The HPLC chromatogram (Fig. 2) of

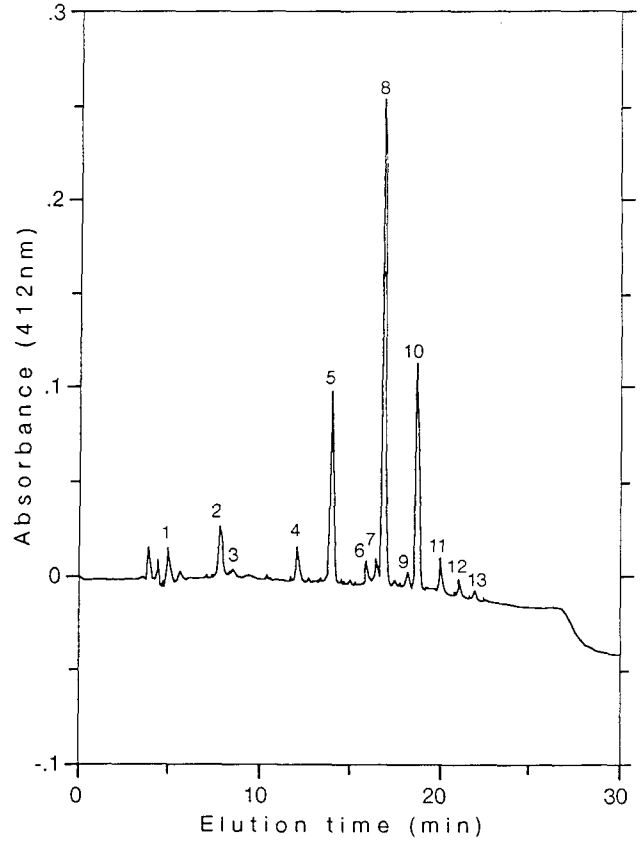

Figure 2. HPLC profile of SSA extract from root tips of Cd-exposed sensitive *S. vulgaris*. The identified peaks are: 1, Cys, 2, GSH; 3, γ-GluCys; 5, PC_2; 8, PC_3; 10, PC_4; 4, 6, 7, 9, 11, and 12, unidentified SHs.

Table I. *Amino acid composition of purified PCs*

PCs in roots of *S. vulgaris* were purified with FPLC-PEPRPC and analyzed for Gly, Cys, and Glx (Glu/Gln) by Pico-Tag HPLC after oxidation, hydrolyzation, and derivatization. The values of the amino acids in PCs were adjusted for those found in a GSH standard.

Compound	Gly	Cys	Glx	Gly:Cys:Glx
	nmol/sample			
PC_2	220	430	446	1:1.96:2.03
PC_3	66	196	217	1:2.97:3.28
PC_4	33	130	137	1:3.89:4.12

acid root-tip extracts revealed three predominant peaks (5, 8, and 10), that contained only Glu, Cys, and Gly. Based on the molar ratio of Gly to Glu and Cys, they contained PC_2, PC_3, and PC_4, respectively (Table I). Peaks 1, 2, and 3 corresponded to Cys, GSH, and γ-GluCys, respectively. Upon exposure to high Cd concentrations new peaks with different retention times than those of the SHs mentioned above appeared (peaks 4, 6, 7, 9, 11, and 12). However, the total amount of SH units in these peaks was always less than 15% of the total SH present in the samples. More than 90% of the SH groups directly measured in the extract were recovered following HPLC separation.

The concentrations of SHs (GSH, PC_2, PC_3, and PC_4), total PCs, and internal Cd in the apical root segments are presented

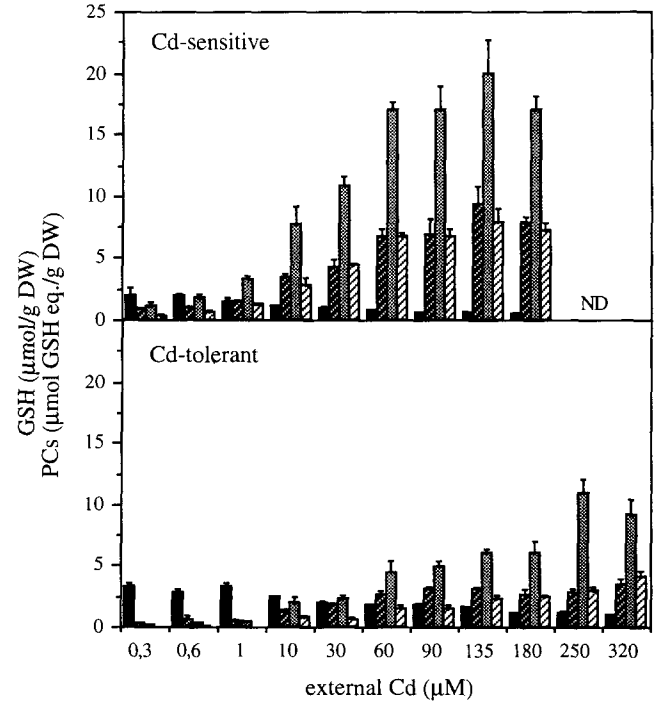

Figure 3. Concentrations of GSH (■), PC_2 (▨), PC_3 (▩), and PC_4 (▧) in root tips of Cd-sensitive (top) and Cd-tolerant (bottom) *S. vulgaris* exposed to increasing Cd concentrations for 72 h. Values are means ± SE of three extracts of six plants each. ND, Not determined; DW, dry weight.

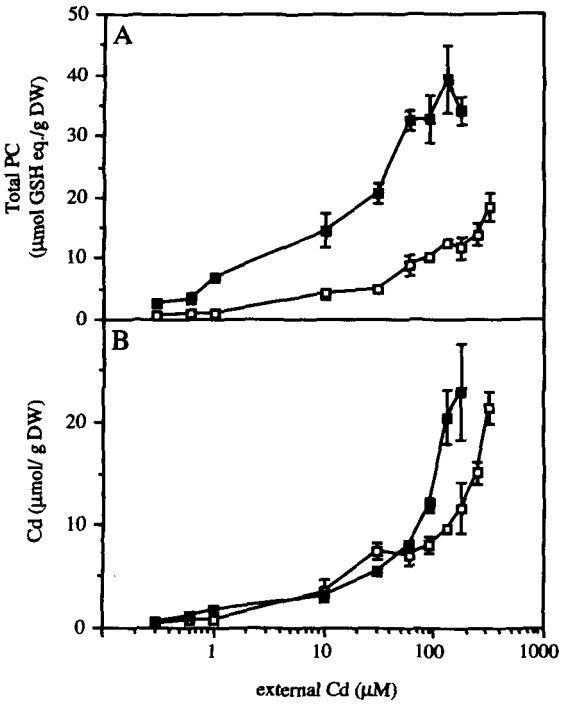

Figure 4. Total PC (A) and Cd concentrations (B) in root tips of Cd-sensitive (■) and tolerant (□) *S. vulgaris* exposed to increasing Cd concentrations for 72 h. Values are means ± SE of three extracts of six plants each. DW, Dry weight.

in Figures 3 and 4, A and B, respectively. At the lowest Cd concentration in the nutrient solution (i.e. 0.3 μM), sensitive and tolerant plants produced PCs. In the sensitive population, the total PC concentration (PC_2, PC_3, and PC_4) in the apical root segments was highest at 135 μM Cd, although there was little difference in PC concentration of roots of plants exposed to higher than 60 μM Cd (Fig. 3). Below this Cd concentration, PC concentrations in sensitive plants were approximately 4 times higher than in tolerant plants (Fig. 4). Between 60 and 320 μM Cd the PC concentration of the tolerant plants continued to increase. In sensitive plants PC_3 was the most abundant at all Cd concentrations, whereas in tolerant plants, PC_2 was predominant, up to 10 μM Cd. At all Cd concentrations, the concentrations of each of the three PCs were always higher in sensitive plants than in tolerant plants. The higher PC concentrations in root tips of sensitive plants were accompanied by lower GSH concentrations.

The internal Cd concentration in the apical root segments of both populations increased with increasing external Cd concentrations (Fig. 4B). The internal Cd concentration was the same in both populations, up to 60 μM external Cd. At higher external Cd concentrations, the concentrations in sensitive plants were higher than in tolerant plants.

Incorporation of Sulfide into PC-Cd Complexes

Extracts of whole root systems of sensitive plants exposed to 10 and 30 μM Cd and of tolerant plants exposed to 30 and 180 μM Cd for both 3 and 7 d were applied to an FPLC anion-exchange column. Tolerant plants were exposed to higher Cd concentrations to obtain equal amounts of PC and to generate a comparable toxic effect. The chromatograms of all extracts contained the Cd-induced PC peak at 0.6 M NaCl, as previously shown by Verkleij et al. (1990) and de Knecht et al. (1992). At the same Cd exposure level, the amounts of SH, Cd, and sulfide in this peak were always higher in sensitive plants. However, the relative amounts of these components did not differ between the two populations (Table II). The SH concentration increased directly in proportion with the Cd concentrations in the same manner in both populations. This was also true for the relation between SH and sulfide. The SH:Cd ratio was approximately 2:1. The sulfide:SH ratio was approximately 1:9. The sulfide:Cd ratio varied between 1:10 and 3:10 in both populations (Table II).

Table II. *Relationships between acid-soluble SHs, sulfide, and Cd in PC-Cd complexes isolated from Cd-sensitive and Cd-tolerant S. vulgaris, using FPLC-Mono Q anion-exchange chromatography*

Plants were exposed to various Cd concentrations for 3 and 7 d. Ratios were calculated from the concentrations found in fractions of 1 mL. Results are means ± SE.

Population	SH:Cd	S:Cd	S:SH
Cd sensitive	1.81 ± 0.60	0.20 ± 0.09	0.11 ± 0.05
Cd tolerant	1.95 ± 0.53	0.21 ± 0.10	0.11 ± 0.06

In a separate experiment, SH and Cd concentrations in Tris and SSA extracts were compared (Table III). As mentioned by de Knecht et al. (1992), extraction in Tris buffer yielded lower amounts of SHs than extraction in SSA, approximately to the same extent in both populations. In the case of Cd, however, Tris and SSA extracted equal amounts in sensitive plants, whereas SSA extracted more Cd than did Tris in tolerant plants.

Formation of PC-Cd Complexes

Extracts of whole root systems of sensitive and tolerant plants exposed to 10 and 30 μM Cd for 3 and 7 d were analyzed by gel filtration through FPLC-Superose 12. Assays of the eluents revealed two Cd-containing components, which accounted for 95 to 100% of the soluble Cd present. The first compound was induced by Cd and had an apparent molecular mass of 14.5 kD. As shown previously (Verkleij et al., 1990), this peak consists of PCs. The second component corresponded to a β-mercaptoethanol-Cd complex. In the

Table III. *SH and Cd concentrations in Tris and SSA extracts of roots of Cd-sensitive and Cd-tolerant S. vulgaris exposed to various Cd concentrations*

Population	Treatment	Tris Extracts		SSA Extracts	
		SH	Cd	SH	Cd
	μM Cd	μmol g^{-1} dry wt			
Cd sensitive	10	19.2 ± 3.2	8.9 ± 0.6	25.5 ± 4.5	7.8 ± 0.6
	30	22.5 ± 3.1	8.6 ± 1.1	34.6 ± 5.9	9.9 ± 2.5
Cd tolerant	30	7.5 ± 1.2	6.2 ± 1.7	11.8 ± 1.2	9.4 ± 1.3
	180	20.6 ± 4.3	16.4 ± 0.8	24.3 ± 3.2	20.6 ± 2.0

absence of β-mercaptoethanol, most of the Cd eluted in the PC peak (data not shown).

In roots of both populations, the amount of Cd co-eluting with PCs increased with time and the external Cd concentration at the expense of the amount co-eluting with β-mercaptoethanol. This, however, occurred much faster in sensitive than in tolerant plants (Fig. 5). Seven days after exposure to 30 μM Cd, almost all Cd in the supernatant of sensitive plants was bound to PCs, whereas in tolerant plants approximately 50% of the Cd eluted coincided with β-mercaptoethanol. Sulfide was only detectable in PC-Cd complexes formed in sensitive plants after 7 d at both Cd concentrations (data not shown).

DISCUSSION

In a previous study of PC biosynthesis in *S. vulgaris* (de Knecht et al., 1992), the PC concentration was assessed by subtracting GSH from total acid-soluble nonprotein SH. This presupposes that acid-soluble SH compounds other than PCs and GSH are not present in appreciable amounts. This assumption is confirmed by the present data. In Cd-exposed root tips of sensitive and tolerant populations, GSH and identified PCs together account for at least 85% of the total SHs accumulated. At all Cd concentrations tested, the PCs in root tips are mainly PC_2, PC_3, and PC_4 in both populations. Cys and γ-glutamylcysteine account for 1% or less and are independent of the external Cd concentration (data not shown). These results are consistent with those obtained using root tips of maize (Tukendorf and Rauser, 1990). The amount of unidentified SHs varies in proportion with the PC concentration. Several have a higher retention time than PC_4 and may thus represent longer PCs. Others elute between GSH and the other identified PCs. These components are more abundant in older root parts and increase during breakdown of PCs after arresting the Cd exposure (J. de Knecht, N. van Baren, W.M. ten Bookum, H.W. Wong Fong Sang, P.L.M. Koevoets, H. Schat, and J.A.C. Verkleij, unpublished data). They may be breakdown products of PCs.

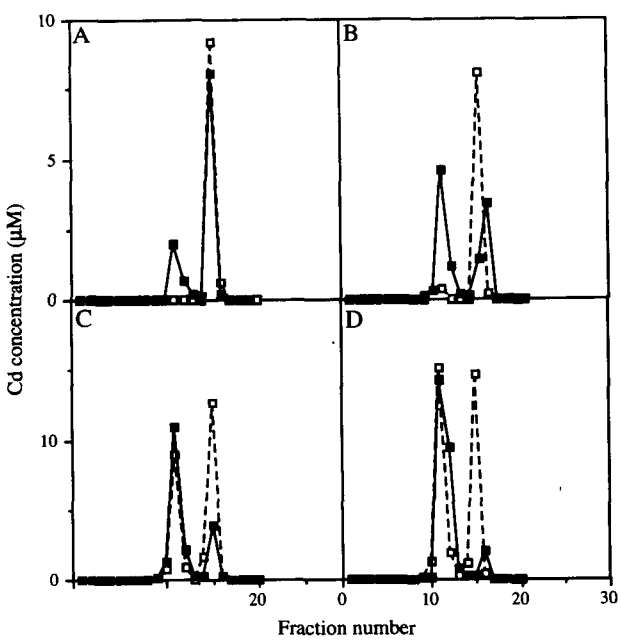

Figure 5. Distribution of Cd between PCs and β-mercaptoethanol in extracts of Cd sensitive (■) and tolerant (□) *S. vulgaris* exposed to 10 (A and C) and 30 μM Cd (B and D) for 3 (A and B) and 7 d (C and D). Extracts were separated by FPLC-Superose 12 gel filtration. Elution rate, 0.4 mL min^{-1}; fraction volume, 1.5 mL. Complexes eluting at fractions 11 and 12 are designated PC-Cd complexes; those eluting at fraction 15 are designated β-mercaptoethanol-Cd complexes.

At the same PC concentration, the PC composition (Fig. 3) and the amount of sulfide incorporated per unit of PC-SH is the same in both populations (Table II). However, tolerant plants reach the same PC concentration as sensitive plants only after exposure to higher Cd concentrations (Fig. 4A). Because longer PCs possess a higher binding affinity for Cd than shorter forms (Hayashi and Nakagawa, 1988), the differences in PC concentration and composition at equal Cd exposure levels probably explain why larger amounts of Cd are retained in PC complexes in sensitive plants when β-mercaptoethanol is present in the extract (Fig. 5). Because the amount of sulfide per SH unit is equal (Table II), the faster formation of stable PC-Cd complexes cannot be explained by an increased amount of sulfide in the PC-Cd complexes. Sulfide-containing PC-Cd complexes isolated from *S. pombe* (Ortiz et al., 1992) and *Brassica juncea* (Speiser et al., 1992) elute in gel filtration separately from those without sulfide. However, only one peak is detectable extracts from *S. vulgaris* (de Knecht et al., 1992). The former two species were exposed to higher Cd concentrations and contain PC-Cd complexes with a much higher S:Cd ratio than *S. vulgaris* (Table II). Reese et al. (1992) demonstrated that the S:Cd ratio in PC-Cd complexes in roots of tomato depends on the Cd concentrations to which the plants are exposed. Below 50 μM Cd the ratios were less than 0.1, which are close to the ratios reported in this paper.

Experiments with split roots indicate that inhibition of root growth is the result of a direct effect of Cd on root cells, both in sensitive and tolerant plants (J. de Knecht, N. van Baren, W.M. ten Bookum, H.W. Wong Fong Sang, P.L.M. Koevoets, H. Schat, and J.A.C. Verkleij, unpublished data), which implies that the tolerance mechanism must operate in the root cells themselves. Therefore, the present results imply that in *S. vulgaris* increased Cd tolerance does not result from an increased accumulation of PCs, from a faster synthesis of longer PCs, or from an increased incorporation of sulfide into PC-Cd complexes in the roots. Cd tolerance in cell lines of *Lycopersicon esculentum* and *Datura innoxia* were also not associated with an overproduction of PCs (Huang et al., 1987; Delhaize et al., 1989); however, Cd-sensitive and Cd-tolerant cells accumulated approximately the same amount of PCs. In contrast to the present results, Verkleij et al. (1990) reported a higher S:Cd ratio in Cd-tolerant *S. vulgaris*. However, this result was obtained after a long-term exposure (3 weeks), which almost killed the root systems of sensitive plants. Therefore, it is possible that the difference in the S:Cd ratio found by these authors is due to a toxic effect rather than to differential tolerance. Upon exposure to Zn and Cu, sensitive *S. vulgaris* plants also produce more PCs than Zn- or Cu-

tolerant plants. This indicates that the amounts of PCs are also not responsible for Cu or Zn tolerance in *S. vulgaris* (de Vos et al., 1992; Schat and Kalff, 1992; Harmens, 1993). A similar result was obtained for Cu-tolerant *Deschampsia cespitosa* (Schultz and Hutchinson, 1988).

It is unlikely that the lower PC concentrations in tolerant plants are due to a reduced uptake of Cd (de Knecht et al., 1992) because the Cd concentrations in root tips of tolerant plants are similar to those in sensitive root tips (Fig. 4B). In spite of the initial SSA-extractable Cd concentrations and the much lower PC concentrations in tolerant plants, the PC-SH:Cd ratio in Tris extracts is the same. This apparent discrepancy seems to be due to the fact that Tris extracts less Cd than SSA from tolerant material, whereas Tris and SSA extracts Cd equally effectively from sensitive material (Table III), at least at the Cd concentrations tested. Apparently, tolerant root material contains a higher proportion of exclusively acid-soluble Cd, which is not bound to PCs. The PC-SH:Cd ratios in the PC-Cd complexes are close to 2:1, both in sensitive and tolerant plants (Table II), which is similar to that found in *Nicotiana tabacum* and *Agrostis gigantea* (Rauser, 1984; Reese and Wagner, 1987a) but does not correspond with the $Cd(SCys)_4$ centers found in PC-Cd complexes of *Rauvolfia serpentina* deduced from Cd-EXAFS (Strasdeit et al., 1991). The lower SH concentrations in Tris extracts, compared to SSA extracts, may be due to oxidation. The loss of SH in Tris is approximately the same for tolerant and sensitive material (Table III).

The difference between the amount of PCs induced by Cd in roots of both populations might be caused by a difference in (a) the specific PC synthase activity, (b) the rate of PC breakdown, or (c) the rate of transport of PC-Cd complexes across the tonoplast. The presence of Cd and PCs in vacuoles of tobacco leaves has been demonstrated following a short exposure to Cd. Because of the low pH, PC-Cd complexes are believed to dissociate in the vacuole, possibly followed by reshuttling of PCs into the cytoplasm or a PC degradation (Vögeli-Lange and Wagner, 1989). In this way, the rate of transport across the tonoplast could influence the rate of PC breakdown and, consequently, the PC concentration. Recently, Ortiz et al. (1992) isolated a gene in *S. pombe*, encoding a vacuolar membrane transporter, that might be responsible for transport of PC-Cd complexes across the tonoplast. It is also possible that the Cd ion itself or another complex in addition to PC-Cd is transported into the vacuole. It is conceivable that differential Cd tolerance in *S. vulgaris* could be based on a difference in a carrier system in the tonoplast. The observed lower PC concentrations in Cd-tolerant plants might then result from a lower Cd concentration in the cytosol caused by a faster transport into the vacuole. This is currently being investigated.

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