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Effect of cadmium on hydrolytic enzymes in maize root and coleoptile

Beáta Široká, Jana Huttová, Ladislav Tamás, Marta Šimonovičová, & Igor Mistrík

Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 14, SK–84523 Bratislava, Slovakia
tel.: ++421-2-59426116, e-mail: Ladislav.Tamas@savba.sk

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Impact of cadmium on activity of some hydrolytic enzymes was studied in maize seedlings exposed to different concentration of Cd (1–1000 μM) for 24 h. Our results confirmed high sensitivity of maize seedlings to Cd as even the lowest 1μM Cd was able to induce 30% reduction of root growth. The inhibition of root growth was dose dependent and positively correlated up to 50 μM Cd with the loss of root cell viability of detected by Evans blue uptake. At higher Cd concentrations (250–1000 μM) no further increase in Evans blue uptake was observed in spite of nearly total root growth inhibition. With the exception of phosphatase activity at low Cd concentration, the activity of hydrolytic enzymes determined in 1 cm long apical part of primary root decreased with increasing Cd concentration. Glucosidase and esterase were found to be the most sensitive to Cd. In comparison with enzymes activity in apical part of primary root the activity of enzymes in basal part and in coleoptile showed significantly lower sensitivity to Cd and also a stimulation of enzyme activity was observed. In contrast to a significant inhibition of hydrolases observed at 1 mM Cd in the apical part of root in *in vivo* experiments we did not confirm their inhibition by the same Cd concentration *in vitro*. In addition to Cd toxicity to maize seedlings possible role of the studied enzymes in root growth inhibition are discussed.

Key words: cadmium, cell viability, esterase, glucosidase, maize, phosphatase, root growth inhibition.

Introduction

Cadmium as a non-essential metal is strongly toxic for many plant species. It inhibits root growth and biomass accumulation by disturbing basic physiological processes. Interaction of Cd with –SH groups of biomolecules is proposed to be the general mechanism of Cd toxicity (Padmaja et al., 1990). General symptom of Cd-treated plants is the induction of oxidative stress, which can cause oxidative modifications of proteins (Romero-Puertas et al., 2002). Several Cd-detoxification mechanisms are described, e.g. chelating of Cd by Cd-binding peptides, compartmentation of Cd into the vacuoles or its immobilization by mucilage (Sanita di Toppi & Gabrielli, 1999). Cd rapidly and strongly binds to cell wall material thus affecting cell wall metabolism. Rapid removal of Cd from the cell wall and its subsequent immobilization in the cytoplasm is related to the higher

Cd-tolerance of roots (INOUHE et al., 1991). Several hydrolytic enzymes including glucosidases, esterases, acid and alkaline phosphatases, were proposed to be involved in plant morphogenesis, especially in cell wall metabolism that is necessary for its turnover and plasticity (BALEN et al., 2003; BORDENAVE et al., 1995; HARVEY et al., 2001; SANO et al., 2003).

The aim of the present study was to characterize the impact of low and high Cd concentrations *in vivo* and *in vitro* on acid and alkaline phosphatases, non-specific esterase, and β-glucosidase in maize roots and coleoptiles.

Material and methods

Plant growth and stress conditions

Following surface sterilization with 12% H_2O_2 the maize seeds (*Zea mays* L. cv. Tina) were placed in filter paper rolls soaked with distilled water and germinated in the dark at 25 ℃ and 98% RH. Three-day-old seedlings with 5–6 cm long primary seminal root were transferred into hydroponics containing 0.2 mM $CaCl_2$ solution (pH 4.5). After 24 h of incubation the solution was changed for a fresh 0.2 mM $CaCl_2$ solution (control) or in case of Cd-treated seedlings for 0.2 mM $CaCl_2$ solution containing different concentrations of $CdCl_2$ (1, 10, 50, 250, 1000 μM) and the seedlings were exposed for the next 24 h. Then the root length was measured by a ruler and the roots and coleoptiles were used for cell viability analysis or stored at −70 ℃ until analyzed for enzyme activity.

Determination of cell viability

The loss of cell viability was evaluated using Evans blue staining method (BAKER & MOCK, 1994). Freshly harvested roots were stained with 0.25% (v/v) aqueous solution of Evans blue for 15 min. After washing with distilled water for 30 min, 10 root tips (10 mm long) were excised and soaked with 500 μL of N, N-dimethylformamide for 1 h at room temperature. Optical density of the released Evans blue was measured spectrophotometrically at 600 nm.

Protein extraction and enzyme assays

Plant tissues (1 cm long apical and 3 cm long basal part of primary root, and coleoptile) were ground to a fine powder in a cold mortar in liquid nitrogen and the resulting powder was rehomogenised in 40 mM succinate/NaOH buffer, pH 4.0 with a homogeniser (Heidolph DIAX 900). After filtration the homogenate was centrifuged at 1 500 *g* for 5 min, then at 12 000 *g* for 15 min and finally at 150 000 *g* for 30 min (Beckman L8-M). After passing through Sephadex G-25 the resulting supernatant (soluble fraction) was used for analysis.

Enzyme activities were determined photometrically using microplate reader (SLT-Laborinstruments, Austria). Each experiment was repeated at least three times. Total protein content in the extracts was determined with Bradford method using Bovine serum albumin as standard (BRADFORD, 1976). Changes in enzyme activities were expressed as a percentage of control.

The reaction mixture for acid phosphatase (EC 3.1.3.2) contained 100 μL of Na-acetate buffer (0.1 M, pH 5.2), 50 μL of 4-nitrophenylphosphate (2mg/mL) and appropriate volume of sample. The reaction was stopped after 30 min incubation at 37 C by adding 50 μL of Na-phosphate buffer (0.4 M, pH 7.2) and absorbance was measured at 405 nm against the control reaction without sample. For alkaline phosphatase (EC 3.1.3.1) the glycine/NaOH (0.025 M, pH 10) buffer was used and 1 mM $MgCl_2$ was added. Substrate was the same as for acid phosphatase and reaction was measured at 405 nm (THAKER et al., 1996).

Assay for non-specific estearse (EC 3.1.1.3) contained 100 μL of Tris/HCl buffer (0.1 M, pH 7.2), 50 μL 4-nitrophenylacetate (2mg/mL solubilized in 20% acetone), and the sample according to WARD & BAMFORTH (2002).

Na-acetate buffer (0,1M, pH 5.2) was used for β-glucosidase (EC 3.2.1.21) activity analysis with 0.5 mg/0.1mL of *p*-nitrophenyl-D-glucopyranoside as a substrate. The reaction was stopped by adding 0.5 volume of 10% Na_2CO_3 per reaction. The released nitrophenol was measured at 405 nm (PISLEWSKA et al., 2002).

For *in vitro* analysis Cd (up to 1 mM) was added to the protein extract from root tips and incubated at 4 ℃ for 24 h.

Results and discussion

In the present work the broad range of Cd concentrations was used to detect the toxic effect of Cd on some hydrolytic enzymes in different parts of maize root and coleoptile. The lowest applied concentration (1 μM) represents the naturally occurring concentration in Cd-polluted soils, whereas 1 mM Cd causes acute stress, which is lethal for many plant species (SANITA DI TOPPI & GABRIELLI, 1999). Root growth inhibition as a general symptom of Cd toxicity was observed already at 1 μM Cd concentration and increased with elevated Cd concentration up to 50 μM (Fig. 1A). At this (50 μM) as well as at higher Cd concentrations nearly total inhibition of root growth was detected. Positive correlation between root growth inhibition and loss of cell viability was also detected (Fig. 1B). Elevated Evans blue uptake started to increase at 1 μM and raised up to 50 μM Cd concentration. At higher Cd concentrations no further increase in Evans blue uptake was observed.

Similarly as with almost all heavy metals, the first target of Cd toxicity is the cell wall of the root cells. Cd binds rapidly to the cell wall components disturbing its basic physiological processes.

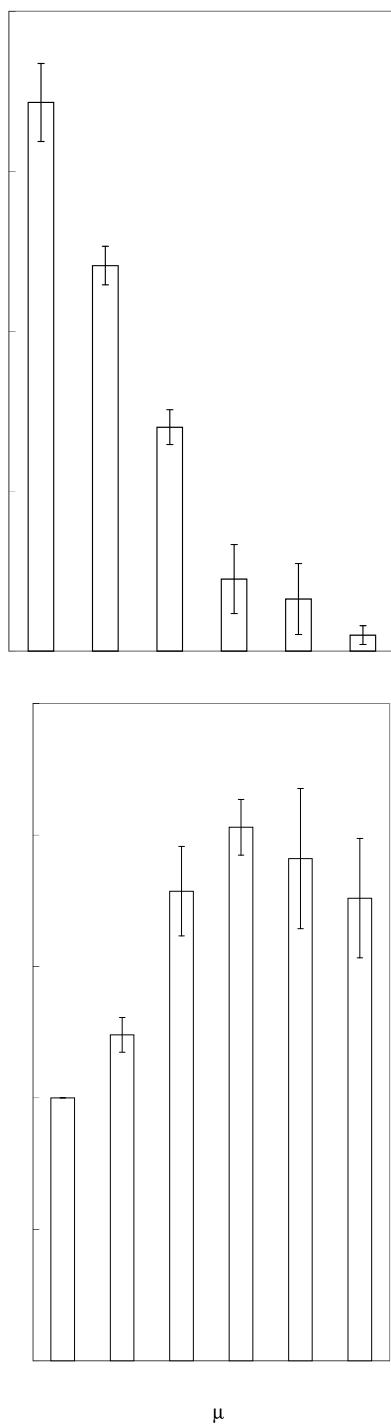


Fig. 1. Root length (A) and Evans blue uptake (B) after 24 h exposure of maize seedlings to 0, 1, 10, 50, 250 and 1000 μM Cd. Means ± SD of five experiments.

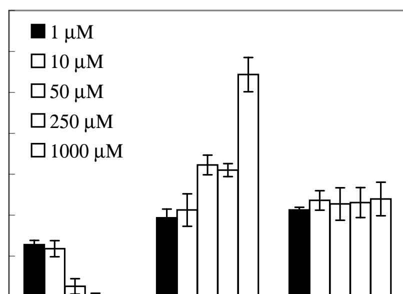
Rapid removal of Cd from the cell wall is probably responsible for higher Cd-tolerance of mono- comparing to dicotyledonous plants (INOUHE et al., 1994). In addition to other factors influencing the composition and function of cell walls an important role of hydrolytic enzymes in cell wall metabolism is well documented in plant kingdom (COSGROVE, 2001).

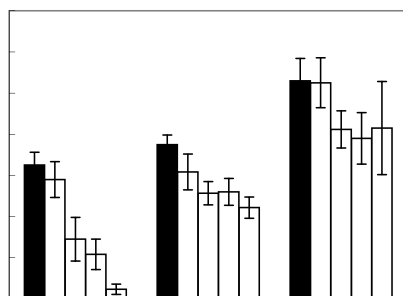
As shown in Fig. 2, significant inhibition of glucosidase and esterase activity was exhibited in the apical part of roots already at 1 μM Cd concentration and this inhibition increased with elevated Cd concentrations (up to 1 mM). Similar course of esterase activity inhibition was observed also in the basal part of roots, whereas in coleoptile it was only slightly inhibited at higher Cd concentrations. In contrast to esterase, glucosidase activity markedly increased with Cd concentration in the basal part of roots, but no significant changes were detected in glucosidase activity in coleoptile.

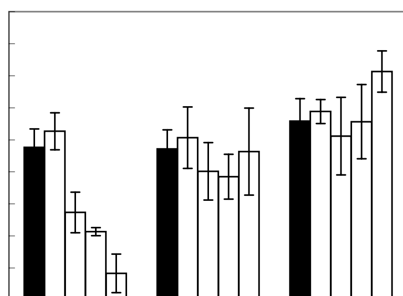
The inhibition of acid and alkaline phosphatase was detected only at higher Cd concentrations (50, 250 and 1000 μM) in the apical part of roots (Fig. 2). Slight increase in acid phosphatase activity was detected in coleoptiles especially at the highest 1000 μM Cd concentration.

Cell wall phosphatases play an important role in cell wall modifications required during cell wall regeneration and extension (PFEIFFER, 1996; SANO et al., 2003). According to our results it seems that the Cd-induced inhibition of phosphatase activity is not responsible for the observed root growth inhibition, because these two parameters were not correlated; inhibition of phosphatase activity was observed only at Cd concentrations that caused complete cessation of root growth.

In contrast, strong correlation was observed between the Cd-induced root growth inhibition and inhibition of glucosidase and esterase activities in the apical part of root. Glucan-hydrolases are expressed mainly in elongating cells and in young tissues suggesting their role in rapid turnover and modification of the cell wall (HARVEY et al., 2001). Similarly to glucosidase, the esterase activity is necessary for the cell wall metabolism in removing methyl groups and cleaving ester linkages mainly in rapidly growing young tissues (BORDENAVE et al., 1995; COSGROVE, 2001). Our results suggest that one of the possible toxic effects of Cd, which is probably involved in Cd-induced root growth inhibition, is a decrease of esterase and glucosidase activities in the maize root tip. In contrast to significant inhibition of hydrolases observed with 1 mM Cd in the apical part of roots, in *in vivo* experiments (Fig. 2) we did not







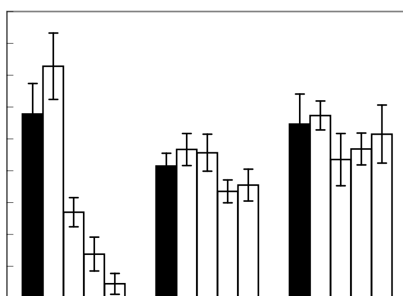


Fig. 2. Glucosidase (Glu), esterase (Est), acid phosphatase (AcPh) and alkaline phosphatase (AlPh) activity in apical part of root (A), basal part of root (B) and coleoptile (C) after 24 h exposure of maize seedlings to 1, 10, 50, 250, and 1000 μM Cd. Means $\pm$ SD of five experiments.

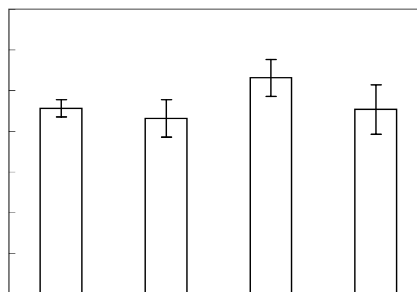


Fig. 3. *In vitro* activity of glucosidase (Glu), esterase (Est), acid phosphatase (AcPh) and alkaline phosphatase (AlPh) in protein extract isolated from 1 cm long apical part of maize root, to which 1 mM Cd was externally added and incubated at 4 ℃ for 24 h. Means $\pm$ SD of three experiments.

confirm similar inhibition with the same Cd concentration *in vitro* (Fig. 3). Probably Cd inhibits these hydrolases *in vivo* indirectly, for example by Cd-induced oxidative modification of proteins (ROMERO-PUERTAS et al., 2002). This assumption can be supported by our observations with Cd-induced loss of cell viability, which positively correlated with inhibition of both root growth and esterase and glucosidase activities.

Acknowledgements

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References

BAKER, C. J. & MOCK, N. M. 1994. An improved method for monitoring cell death in cell suspension and leaf disc assays using Evans blue. Plant Cell Tissue Organ Cult. **39:** 7–12.

BALEN, B., KRSNIK-RASOL, M. & SIMEON-RUDOLF, V. 2003. Isoenzymes of peroxidases and esterases related to morphogenesis in *Mammillaria gracillis* Pfeiff. tissue culture. J. Plant Physiol. **160:** 1401–1406.

BORDENAVE, M., GOLDBERG, R., HUET, J. C. & PERNOLLET, J. C. 1995. A novel protein from mung bean hypocotyl cell wall with acetyl esterase activity. Phytochem. **38:** 315–319.

BRADFORD, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:** 248–254.

COSGROVE, D. J. 2001. Wall structure and wall loosening. A look backwards and forwards. Plant Physiol. **125:** 131–134.

INOUHE, M., MITSUMUNE, M., TOHOYAMA, H., JOHO, M. & MURAYAMA, T. 1991. Contribution of cell wall and metal-binding peptide to Cd- and Cu-tolerances in suspension-cultured cells of tomato. Bot. Mag. Tokyo **104:** 217–229.

INOUHE, M., NINOMIYA, S., TOHOYAMA, H., JOHO, M. & MURAYAMA, T. 1994. Different characteristics of roots in the cadmium-tolerance and Cd-binding complex formation between mono- and dicotyledonous plants. J. Plant Res. **107:** 201–207.

HARVEY, A. J., HRMOVA, M. & FINCHER, B. 2001. Regulation of genes encoding β-D-glucan glucohydrolases in barley (*Hordeum vulgare*). Physiol. Plant. **113:** 108–120.

PADMAJA, K., PRASAD, D. D. K. & PRASAD, A. R. K. 1990. Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* L. seedlings by cadmium acetate. Photosynthetica **24:** 399–405.

PFEIFFER, W. 1996. Auxin induces exocytosis of acid phosphatase in coleoptile from *Zea mays*. Physiol. Plant. **98:** 773–779.

PIŚLEWSKA, M., BEDNAREK, P., STOBIECKI, M., ZIELIŃSKA, M. & WOJTASZEK, P. 2002. Cell wall-associated isoflavonoids and β-glucosidase activity in *Lupinus albus* plants responding to environmental stimuli. Plant Cell Environ. **25:** 29–40.

ROMERO-PUERTAS, M. C., PALMA, J. M., GÓMEZ, M., DEL RÍO, L. A. & SANDALLIO, L. M. 2002. Cadmium causes the oxidative modification of proteins in pea plants. Plant Cell Environ. **25:** 677–686.

SANITA DI TOPPI, L. & GABRIELLI, R. 1999. Response to cadmium in higher plants. Env. Exp. Bot. **41:** 105–130.

SANO, A., KIADA, R., MAKI, H. & KANEKO, T. S. 2003. Involvement of an acid phosphatase on cell wall regeneration of tobacco protoplasts. Physiol. Plant. **119:** 121–125.

THAKER, V. S., SAROOP, S., VAISHNAV, P. P. & SINGH Y. D. 1996. Physiological and biochemical changes associated with cotton fibre development. A. Physiol. Plantarum, **18:** 111–116.

WARD, R. E. & BAMFORTH C. W. 2002. Esterases in barley and malt. Cereal Chem. **79:** 681–686.

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