BIOLOGIA PLANTARUM 54 (1): 117-120, 2010

BRIEF COMMUNICATION

ABA or cadmium induced phytochelatin synthesis in potato tubers

A. STROIŃSKI*, T. CHADZINIKOLAU, K. GIŻEWSKA and M. ZIELEZIŃSKA

Plant Physiology Department, University of Life Sciences, Wołyńska 35, PL-60637 Poznań, Poland

Abstract

Short-term treatment of potato (*Solanum tuberosum* L.) tuber discs with $CdCl_2$ solution elevated both the *StPCS1* transcript level, phytochelatin synthase (PCS) activity and contents of phytochelatin (PC) and abscisic acid (ABA). Similar effects but less marked were noticed after treatment of tuber tissues with ABA solution. Cd-treatment increased also cysteine (CYS) content but did not change glutathione content. If ABA treatment preceded Cd-treatment, the elevation of CYS and PC contents were limited. The data suggest the participation of ABA in the regulation of PCS.

Additional key words: phytochelatins, phytochelatin synthase, *Solanum tuberosum*

The cadmium treatment may lead to plant growth inhibition, chlorosis and deficiencies of some essential elements (Stiborová *et al.* 1987, Stroiński *et al*. 1990, Karcz and Konopka 2007, Maksimović *et al*. 2007). Cd can intensify some harmful processes in plants, namely oxidative stress (Hendry *et al.* 1992, Stroiński and Kozłowska 1997, Sanita di Toppi and Gabrielli 1999, Skórzyńska-Polit *et al.* 2003) or inactivation of macromolecules and cellular structure (Stroiński *et al.* 1990, Jiang *et al.* 2009). The resistance of plant cells to Cd depends, among other things, on the content of phytochelatins (PC), which chelate cadmium ions. These peptides are synthesized from glutathione and related thiols by PC synthases (PCS; γ-glutamyl-cysteine dipeptidyl transpeptidases; EC 2.3.2.15) in the presence of heavy metal ions (Grill *et al.* 1989, Maitani *et al.* 1996, Clemens 2006). PCS genes were characterized in *Arabidopsis thaliana* (*AtPCS1*, *AtPCS2*), in wheat (*TaPCS1*), in *Brassica juncea* (*BjPCS1*), in rice (*OsPCS1*) (Cobbett and Goldsbrough 2002) and in potato (*StPCS1*) (Nuc *et al.* 2003). AtPCS1 did not exhibit transcriptional regulation by Cd in *Arabidopsis*, although its activity was increased in the presence of Cd (Ha *et al.* 1999, Vatamaniuk *et al.* 2000). In other plants the biosynthesis of some phyto-chelatin synthases showed transcriptional and/or post-transcriptional regulation by Cd (Clemens *et al.* 1999, Stroiński and Zielezińska 2001, Cobbett and Goldsbrough 2002).

Until now there have been scarce reports characterizing hormonal participation in plant response to Cd. Our initial experiment revealed that Cd infiltrating potato tuber tissues significantly changed ABA content in those tissues (Chadzinikolau and Stroiński 2003). Cd treatment resulted in an increase in ABA content in leaves of rice (Hsu and Kao 2003, 2005, Hsu *et al.* 2006) and in roots of *Typha latifolia* and *Phragmites australis* (Fediuc *et al.* 2005). ABA mediates plant responses to environmental stress, such as high salinity, drought, low temperature and mechanical wounding (Giraudat *et al.* 1994, Xiong *et al*. 2002, Shinozaki *et al.* 2003). Usually under these stress conditions ABA accumulation was observed and this effect was associated with growth inhibition (Cheng *et al.* 2002, Eckardt 2002). The present paper describes the effect of cadmium and exogenous ABA on ABA, cysteine (CYS), glutathione (GSH) and PC content and on *PCS1* gene expression and PCS activity in potato tuber tissues.

Experiments were carried out using tubers of potato (*Solanum tuberosum* L.) cv. Bzura (cultivar tolerant to cadmium; Stroiński *et al.* 1990). Discs (18 mm in diameter. 4 mm thick) were infiltrated with H_2O or with ABA solution (0.1 mM) under two vacuum impulses for 5 min, washed with distilled water and incubated for 24 h in a moist chamber at 20 °C in the dark. Next day, they were infiltrated in similar manner with $CdCl_2$ solution (1 mM), washed with distilled water, and finally incubated in a moist chamber at 20 °C in the dark for 24 h. Then the materials were immediately frozen in liquid nitrogen.

Received 11 June 2007, *accepted* 21 September 2008.
Abbreviations: ABA - abscisic acid; CYS - cysteine; GSH - glutathione; PC - phytochelatin; PCS - phytochelatin synthase.
Acknowledgements: This study was supported by KBN grant No. 6 P06A 014 20.
* Corresponding author; fax: (+48) 61 8487179, e-mail: astroins@o2.pl

Thiols (cysteine, glutathione and PC) were isolated and separated by a reverse-phase HPLC with post column derivatization as described earlier (Tukendorf and Rauser 1990, Stroiński and Zielezińska 1997). PCS activity was determined according to Grill *et al.* (1989). The amount of PC was calculated from a comparison to GSH standard. Total protein contents were measured according to Bradford (1976).

Total RNA was extracted from tuber tissues using the phenol-chloroform method (Branch *et al.* 1989). Poly (A) RNA was isolated from the total RNA with a *Dynabeads* mRNA isolation kit (*Dynal*, Hamburg, Germany) and used to prepare the cDNA. The first-strand cDNAs were synthesized by reverse transcription from 0.1 - 0.5 μg of mRNA isolated from *Solanum tuberosum* by using AMV reverse transcriptase (*Promega*, Madison, USA) and an oligo dT primer according to the manufacturer's instructions. Amounts of synthesized cDNA for the PCR reaction were measured spectrofluorometrically by using the OilGreen dye with oligonucleotide standard M13 (*Molecular Probes*, Carlsbad, USA). The PCR reaction was carried out by using 2× PCR *Master Mix* (*Fermentas*, Vilnius, Latvia), which contained a concentrated solution of 0.05 U mm^{-3} *Taq* DNA polymerase (recombinant). Specific primers were used for the phytochelatin synthase gene *(StPCS1)*: 5'AAATGGAAAGGGCCTTGGAG 3' and 5' GACCCAGTGAGGGGGATA 3' (Nuc *et al.* 2003). PCR products were separated electrophoretically on a 1.1 % agarose gel with the addition of ethidium bromide.

The isolation and estimation of ABA by high-performance liquid chromatography (HPLC) were done according to Moore (1990) with ABA-methyl ester as an internal standard for estimating extraction efficiency. Amounts of ABA were calculated from its peak area, and peak area of internal standard using Chromatography Station program (*Data Apex*, Prague, Czech Republic).

To determine the soluble (Cd_S) and bound (Cd_B) cadmium, the potato tissues were homogenized in 200 mM phosphate buffer at pH 7.8 and the resultant homogenates were centrifuged at 20 000 *g* for 20 min. Supernatants were collected to estimate Cd_S concentrations. On the other hand, to determine Cd_B concentration, the precipitates were wet mineralized to ash by the use of acid mixture (36 % HCl + 20 % $HClO_4$ + H_2O, 2:1:1, v/v). Cadmium was determined by means of flame atomic absorption spectrometry.

The data presented in the figures are means of three or more independent replications. The significance of differences between means was calculated using unpaired *t*-test.

Our previous studies indicated that a short-term treatment of potato (*Solanum tuberosum* L.) tuber discs with $CdCl_2$ induced an increase of phytochelatin synthase (PCS) activity in concentration and time dependent manner (Stroiński and Zielezińska 2001).

In control materials used in the present experiments, we found small amounts of Cd, and low levels of the *StPCS1* transcript and PCS activity. Cadmium treatment of tuber tissues caused a considerable accumulation of Cd about thirty times and simultaneous increase in *StPCS1* transcript level and PCS activity. Cd stress also increased the contents of CYS and PC (Table 1, Fig. 1). Similar CYS changes caused by Cd treatment were observed in soybean seedlings (El-Shintinawy and El-Ansary 2000) and in maize roots (Nocito *et al.* 2002). At the same time, the GSH content did not change in the tuber tissues (Table 1). Previously Cd-induced decrease in GSH content was observed in roots of maize seedlings (Tukendorf and Rauser 1990) and in spinach roots (Tukendorf 1993).

Until now there have been no reports which would characterize the hormonal participation in the regulation of cadmium detoxification, particularly in the regulation of PCS activity. The ABA content increased approximately four times after Cd application. Similar results were found by Hsu and Kao (2003, 2005), Hsu *et al.* (2006) and Fediuc *et al.* (2005). The exogenous ABA treatment of tuber tissues increased the endogenous

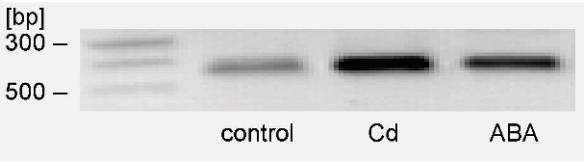

Fig. 1. Influence of exogenous ABA and Cd on expression of *StPCS1*. RT-PCR were conducted using 40 ng of total mRNA extracted from tissues exposed or not exposed to $CdCl_2$ (0.1 mM) for 48 h, or with ABA (0.1 mM) for 24 h. Specific primers to *StPCS1* cDNA for 352 bp product were used.

Table 1. Effect of exogenous ABA and Cd on Cd, ABA, CYS, GSH and PC content and on PCS activity in potato tuber tissues. Values are the means of three replicates ± SE. The values marked with different letters are significantly different at $P = 0.05$.

	Control	ABA	ABA + Cd	Cd
Soluble Cd content [μg g^{-1}(f.m.)]	0.90 ± 0.1 [a]	-	22.60 ± 1.5 [b]	21.30 ± 1.4 [b]
Bound Cd content [μg g^{-1}(f.m.)]	2.20 ± 0.2 [a]	-	30.60 ± 4.0 [b]	55.20 ± 0.6 [c]
ABA content [μg g^{-1}(f.m.)]	0.44 ± 0.0 [a]	0.88 ± 0.0 [b]	1.56 ± 0.1 [c]	1.72 ± 0.1 [c]
CYS content [nmol g^{-1}(f.m.)]	7.40 ± 1.0 [a]	5.00 ± 0.5 [b]	8.00 ± 1.4 [a]	12.30 ± 1.0 [c]
GSH content [nmol g^{-1}(f.m.)]	15.30 ± 1.1 [a]	15.70 ± 1.3 [a]	16.60 ± 1.0 [a]	19.40 ± 1.4 [a]
PC content [nmol g^{-1}(f.m.)]	6.70 ± 0.7 [a]	11.90 ± 1.5 [b]	26.00 ± 2.6 [c]	36.30 ± 3.5 [c]
PCS activity [nmol(PC) mg^{-1}(protein) h^{-1}]	4.40 ± 1.1[a]	8.70 ± 1.6[b]	13.60 ± 3.6[c]	16.20 ± 2.1 [c]

ABA content (Table 1) and it confirmed earlier observations of the positive feedback in ABA biosynthesis by ABA (Audran *et al.* 2001, Xiong and Zhu 2003). However in this experiment, ABA can be accumulated nonspecifically in plant tissue by passive transport.

Simultaneously, this ABA treatment induced significant increase in the *PCS1* transcript level, PCS activity and PC content (Table 1, Fig. 1) similarly as Cd treatment. Additionally, the experiments when ABA treatment preceded Cd stress indicated that these effects were not additive (Table 1, Fig. 1). It seems that both factors can modulate PCS activity differently using signaling pathways possessing some common elements (Knight and Knight 2001).

These observations, for the first time, can suggest the participation of ABA in the regulation of phytochelatin synthase, the main step of the cadmium detoxification process.

References

Audran, C., Liotenberg, S., Gonneau, M., North, H., Frey, A., Tap-Waksman, K., Vartanian, N., Marion-Poll, A.: Localisation and expression of zeaxanthin epoxidase mRNA in *Arabidopsis* in response to drought stress and during seed development. - Aust. J. Plant. Physiol. **28**: 1161-1173, 2001.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-252, 1976.

Branch, A.D., Benenfeld, B.J., Robertson, H.D.: RNA fingerprinting. - Methods Enzymol. **180**: 130-154, 1989.

Chadzinikolau, T., Stroiński, A.: Influence of cadmium-abscisic acid interaction on phytochelatin synthase activity in potato tuber. - Polish J. nat. Sci. 2003 (Suppl. 1): 105, 2003.

Cheng, W.H., Endo, A., Zhou, L., Penney, J., Chen, H.C., Arroyo, A., Leon, P., Nambara, E., Asami T., Seo, M., Koshiba, T., Shenn, J.: A unique short-chain dehydrogenase/reductase in *Arabidopsis* glucose signaling and abscisic acid biosynthesis and functions. - Plant Cell **14**: 2723-2743, 2002.

Clemens, C.: Evolution and function of phytochelatin synthases. - J. Plant Physiol. **163**: 319-332, 2006.

Clemens S., Kim E.J., Neumann, D., Schroeder, J.I.: Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. - EMBO J. **18**: 3325-3333, 1999.

Cobbett, C., Goldsbrough, P.: Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. - Annu. Rev. Plant Biol. **53**: 159-182, 2002.

Eckardt, N.A.: Abscisic acid biosynthesis gene underscores the complexity of sugar, stress, and hormone interactions. - Plant Cell **14**: 2645-2649, 2002.

El-Shintinawy, F., El-Ansary, A.: Differential effect of Cd^{2+} and Ni^{2+} on amino acid metabolism in soybean seedlings. - Biol. Plant. **43**: 79-84, 2000.

Fediuc, E., Lips, H., Erdei, L.: *O*-acetylserine (thiol) lyase activity in *Pragmites* and *Typha* plants under cadmium and NaCl stress conditions and the involvement of ABA in the stress response. - J Plant Physiol. **162**: 865-872, 2005.

Giraudat, J., Parcy, F., Bertauche, N., Gosti, F., Leung, J., Morris, P.C., Bouvier-Durand, M., Vartanian, N.: Current advances in abscisic acid action and signaling. - Plant mol. Biol. **26**: 1557-1577, 1994.

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

Ha, S.B., Smith, A.P., Howden, R., Dietrich, W.M., Bugg, S., O'Connell, M.J., Goldsbrough, P.B., Cobbett, C.S.: Phytochelatin synthase genes from *Arabidopsis* and the yeast, *Schizosaccharomyces pombe*. - Plant Cell **11**: 1153-1164, 1999.

Hendry, G.A.F., Baker, A.J.M., Ewart, C.F., Cadmium tolerance and toxicity, oxygen radical processes and molecular damage in cadmium-tolerant and cadmium-sensitive clones of *Holcus lanatus*. - Acta bot. neerl. **41**: 271-281, 1992.

Hsu, Y.T., Kao, C.H.: Role of abscisic acid in cadmium tolerance in rice (*Oryza sativa* L.) seedlings. - Plant Cell Environ. **26**: 867-874, 2003.

Hsu, Y.T., Kao, C.H.: Abscisic acid accumulation and cadmium tolerance in rice seedlings. - Physiol. Plant. **124**: 71-80, 2005.

Hsu, Y.T., Kuo, M.C., Kao, C.H.: Cadmium-induced ammonium ion accumulation of rice seedlings at high temperature is mediated through abscisic acid. - Plant Soil **287**: 267-277, 2006.

Jiang, W.S., Liu, D.H., Xu, P.: Cd-induced system of defence in the garlic root meristematic cells. - Biol. Plant. **53**: 369-372, 2009.

Karcz, W., Kurtyka R. Effect of cadmium on growth, proton extrusion and membrane potential in maize coleoptile segments. - Biol. Plant. **51**: 713-719, 2007.

Knight H., Knight M.R.: Abiotic stress signalling pathways: specificity and cross-talk. - Trends Plant Sci. **6**: 262-267, 2001.

Maksimović, I., Kastori, R., Krstić, L., Luković, J.: Steady presence of cadmium and nickel affects root anatomy, accumulation and distribution of essential ions in maize seedlings. - Biol. Plant. **51**: 589-592, 2007.

Maitani, T., Kubota, H., Sato, K., Yamada, T.: The composition of metals bound to class III metallothionein (phytochelatin and its desglycyl peptide) induced by various metals in root cultures of *Rubia tinctorum.* - Plant Physiol. **110**: 1145-1150, 1996.

Moore, R. Abscisic acid is not necessary for gravitropism in primary roots of *Zea mays.* - Ann. Bot. **66**: 281-283, 1990.

Nocito, F.F., Pirovano, L., Cocucci, M., Sacchi, G.A.: Cadmium-induced sulfate uptake in maize roots. - Plant Physiol. **129**: 1872-1879, 2002.

Nuc P.W., Chadzinikoalu, T., Nuc, K., Stroiński, A.: *Solanum tuberosum* mRNA for phytochelatin synthase (pcs1 gene). Accession: AJ548472. - EMBL/GenBank/DDBJ databases, 2003.

Sanita di Toppi, L., Gabrielli, R.: Response to cadmium in higher plants. - Environ. exp. Bot. **41**: 105-130, 1999.

Shinozaki, K., Yamagucchi, K., Seki, M.: Regulatory network of gene expression in drought and cold stress responses. Curr. Opin. Plant Biol. **6**: 410-417, 2003.

Skórzyńska-Polit, E., Drążkiewicz, M., Krupa, Z.: The activity of the antioxidative system in cadmium-treated *Arabidopsis thaliana*. - Biol. Plant. **47**: 71-78, 2003.

Stiborová, M., Ditrichová, M., Březinová, A.: Effects of heavy metal ions on growth and biochemical characteristic of photosynthesis of barley and maize seedling. - Biol. Plant. **29**: 453-467, 1987.

Stroiński, A., Floryszak-Wieczorek, J, Woźny, A.: Effects of cadmium on the host-pathogen system. I. Alteration of potato leaves and *Phytophthora infestans* relations. - Biochem. Physiol. Pflanz. **186**: 43-54, 1990.

Stroiński, A., Kozłowska, M. Cadmium-induced oxidative stress in potato tuber. - Acta Soc. Bot. Polon. **66**: 189-195, 1997.

Stroiński, A., Zielezińska, M.: Cadmium effect on hydrogen peroxide, glutathione and phytochelatins levels in potato tuber. - Acta Physiol. Plant. **19**: 127-136, 1997.

Stroiński, A., Zielezińska, M.: Cadmium and oxidative stress influence on phytochelatin synthase activity in potato tuber. - Acta Physiol. Plant. **23**: 157-160, 2001.

Tukendorf, A.: Changes in glutathione in detoxification of cadmium and excess of copper in spinach plants. - Acta Physiol. Plant. **15**: 175-183, 1993.

Tukendorf, A., Rauser, W.E.: Changes in glutathione and phytochelatins in roots of maize seedlings exposed to cadmium. - Plant Sci. **70**: 155-166, 1990.

Vatamaniuk, O.K., Mar, S., Lu, Y.P., Rea, P.A.: Mechanism of heavy metal ion activation of phytochelatin (PC) synthase: blocked thiols are sufficient for PC synthase-catalyzed transpeptidation of glutathione and related thiol peptides. - J. biol. Chem. **275**: 31451-31459, 2000.

Xiong, L., Schumaker, K.S., Zhu, J.-K.: Cell signaling during cold, drought and salt stress. - Plant Cell Suppl.: S165-S183, 2002.

Xiong, L., Zhu, J.-K.: Regulation of abscisic acid biosynthesis. - Plant Physiol. **133**: 29-36, 2003.