Proceedings of the 7th Hungarian Congress on Plant Physiology, 2002
S2-P22

Volume 46(3-4):121-122, 2002
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Effect of Cd treatment on phytochelatin synthesis in maize

Gabriella Szalai*, Tibor Janda, Avi Golan-Goldhirsh[1], Emil Páldi

Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary, [1]The Jacob Blaustein Institute for Desert Research, Ben Gurion University of the Negev, Israel

ABSTRACT Phytochelatins are Cys-rich peptides enzymatically synthesized by γ-Glu-Cys dipeptidyl transpeptidase, generally known as phytochelatin synthase. In the present work the enzymes of phytochelatin synthesis and cadmium (Cd) accumulation were investigated during Cd stress. Young maize plants were treated with 0.1 mM Cd for 48 hours. Roots and leaves were used for the experiments. The Cd was accumulated mainly in young leaves during the treatment but there was also accumulation in older leaves. In the roots the amount of Cd increased continuously. The Cd concentration was much higher in the roots than in the leaves. There were no changes in the photosynthetic parameters. The γ-glutamylcysteine synthetase (γ-ECS) activity did not change in the first six hours but there was a great increase in activity after one day. The activity of glutathione synthetase did not change in the leaves but increased to a slight extent in the roots after one hour. The phytochelatin synthase activity did not change after one day, but decreased on the 2nd day in the roots. An increase in the activity could be seen in the leaves during the 1st day, after which it remained constant.
Acta Biol Szeged 46(3-4):121-122 (2002)

KEY WORDS

cadmium
phytochelatin synthase
Zea mays L.

Plants respond to heavy metal toxicity in a variety of different ways. Such responses include immobilization, exclusion, chelation and compartmentalization of the metal ions, and the expression of more general stress response mechanisms such as ethylene and stress proteins. Phytochelatins (PCs) are enzymatically-synthesized Cys-rich peptides. Grill et al. (1989) first identified enzyme activity from cultured cells of *Silene cucubalis* that synthesized PCs from glutathione (GSH) by transferring a γ-Glu-Cys moiety from a donor to an acceptor molecule. The reaction involved the trans-peptidation of the γ-Glu-Cys moiety of GSH onto initially a second GSH molecule to form PC2 or, in later stages of the incubation, onto a PC molecule to produce an *n*+1 oligomer. This γ-Glu-Cys dipeptidyl transpeptidase has been named PC synthase (PCS). *In vitro* the partially purified enzyme was active only in the presence of metal ions. The best activator tested was Cd, followed by the cations Ag, Bi, Pb, Zn, Cu, Hg and Au. These metals also induce PC biosynthesis in *in vivo* plant cell cultures. Similar PC synthase activities have been detected in pea (Klapheck et al. 1995), tomato (Chen et al. 1997) and *Arabidopsis* (Howden et al. 1995). Crude enzyme preparations from the roots of pea, which normally contain both GSH and homo-GSH (γ-Glu-Cys-β-Ala), were able to use GSH efficiently and homo-GSH or γ-Glu-Cys-Ser less efficiently as substrates for PC synthesis. In the presence of both GSH and homo-GSH, the synthesis of homo-PCs was enhanced (Klapheck et al. 1995). Little is known about the tissue specificity of PC synthase expression and/or PC biosynthesis. In the only study on tissue-specific PC synthase expression to date, activity was detected in the roots and stems of tomato plants but not in the leaves or fruit (Chen et al. 1997). The aim of this work was to investigate the activity of the enzymes (γ-Glu-Cys, GSH and PC synthase) involved in PC synthesis in the leaves and roots of maize plants during Cd treatment.

*Corresponding author. E-mail: szalaig@mail.mgki.hu

Materials and Methods

Maize plants (hybrid Norma) were grown in a plant growth chamber (Conviron PGV-36) in hydroponic solution for two weeks, then treated with 0.1 mM Cd for 1, 6, 24 or 48 hours. The Cd contents of the roots and leaves were measured according to Hegedűs et al. (2001).

Roots and 3rd fully developed leaves were used for the further measurements.

The chlorophyll fluorescence induction parameters of the leaves were determined at room temperature using a pulse amplitude modulated (PAM) fluorometer.

γ-glutamylcysteine and glutathione synthetase activities were determined using the method of Hell and Bergmann (1988, 1990).

Phytochelatin synthase activity was measured according to Chen et al. (1997).

Results and Discussion

The Cd was accumulated mainly in young leaves during the treatment, but there was also some accumulation in older leaves. In the roots the amount of Cd increased continuously. The Cd concentration was much higher in the roots than in the leaves.

There were no changes in the photosynthetic parameters (F_v/F_m, quantum yield of PS II) during 0.1 mM Cd treatment. A slight decrease was seen after 48 hours in the F_v/F_m parameter if the concentration of Cd was 0.5 mM or 1 mM. The decrease in the quantum yield parameter was much more pronounced. It can thus be seen that the 0.1 mM Cd treatment did not cause extensive damage to the plants.

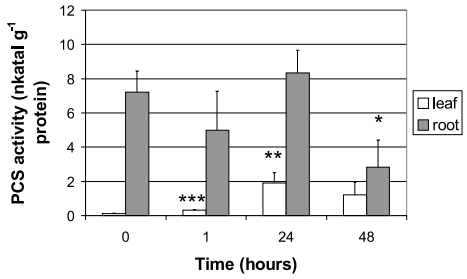

Figure 1. Changes in the phytochelatin synthase activity during 48 h Cd treatment in young maize plants. (*, **, ***: significant at p<0.05, p<0.01, p<0.001 levels, respectively).

The γ-glutamylcysteine synthetase (γ–ECS) activity did not change in the first six hours, but there was a considerable increase in the activity after one day. This decreased again on the second day, but this decreased activity was still higher than the initial level. The activity of glutathione synthetase (GS) did not change in the leaves, but increased to a slight extent in the roots after one hour. Earlier studies showed that γ–ECS is rate-limiting for GSH synthesis in plants (Noctor et al. 1997). Other results support this view, because the overexpression of γ-ECS in unstressed Indian mustard plants led to increased GSH levels (Zhu et al. 1999b). Since the overexpression of γ-ECS increased the γ-EC, GSH and PC levels in transgenic seedlings, it would appear that γ-ECS is rate-limiting for both GSH and PC production in Cd-stressed plants. Under Cd stress conditions, however, earlier research showed that the overexpression of GS led to an increase in GSH and PC levels, suggesting that GS limited GSH production under these conditions. This has been explained by suggesting that in Cd-treated plants Cd activated γ-ECS, so that γ–EC accumulated and GS became rate-limiting (Zhu et al. 1999a). On the other hand, the observations that GSH and PC levels were increased by the overexpression of γ-ECS and that Cys levels were increased by Cd suggest that γ-ECS is rate-limiting as well. Thus, it appears that the two enzymes co-limit GSH production under Cd stress (Zhu et al. 1999b).

PC synthase (PCS) activity was measured in the roots and leaves in young maize plants. The activity did not change after one day but decreased on the 2nd day in the roots. An increase in the activity could be seen in the leaves during the 1st day, after which it remained constant (Fig. 1).

Since plants assimilate various metal ions from the soil, the first organ exposed to these ions in the root. The localization of PCS in the roots and stems could provide an effective means of restricting Cd to these organs by chelation in the form of Cd-PC complexes. This enzyme has been studied mainly in cell cultures, but there are a few data about whole plants. Activity was detected in the roots and stems of tomato plants, but not in the leaves or fruit (Chen et al. 1997). However, PCs can accumulate in tomato leaves after the exposure of plants to Cd^{2+} (Reese et al. 1992), suggesting that PCS may be regulated in this organ, the enzyme being synthesized only in response to metal exposure. The results also show that the activity in the roots was constitutive, while in the leaves it was induced by Cd ions.

Acknowledgments

The authors are gratefully indebted to Edit Kövesdi and Zsuzsa Kóti for their technical assistance. This work was supported by the Hungarian National Science Foundation (OTKA T 037195) and a scholarship from the Jacob Blaustein Foundation to G. Szalai.

References

Chen J, Zhou J, Goldsbrough PB (1997) Characterization of phytochelatin synthase from tomato Physiol Plant 101:165-172.

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

Hegedűs A, Erdei S, Horváth G (2001) Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. Plant Sci 160:1085-1093.

Hell R, Bergmann L (1988) Glutathione synthetase in tobacco suspension cultures: catalytic properties and localisation. Physiol Plant 72:70-76.

Hell R, Bergmann L (1990) γ–Glutamylcysteine synthetase in higher plants: catalytic properties and subcellular localisation. Planta 180:603-612.

Howden R, Goldsbrough PB, Andersen CR, Cobbett CS (1995) Cadmium-sensitive, *cad1*, mutants of *Arabidopsis thaliana* are phytochelatin deficient. Plant Physiol 107:1059-1066.

Klapheck S, Schlunz S, Bergmann L (1995) Synthesis of phytochelatins and homo-phytochelatins in *Pisum sativum* L. Plant Physiol 107:515-521.

Noctor G, Strohm M, Jouanin L, Kunert K-J, Foyer CH, Rennenberg H (1997) Synthesis of glutathione in leaves of trangenic poplar over-expressing γ–glutamylcysteine synthetase. Plant Physiol 112: 1071-1078.

Reese RN, Wagner GJ (1987) Effects of buthionine sulfoximine on Cd-binding peptide levels in suspension-cultured tobacco cells treated with Cd, Zn, or Cu. Plant Physiol 84:574-577.

Zhu YL, Pilon-Smits EAH, Jouanin L, Terry T (1999a) Overexpression of glutathione synthetase in *Brassica juncea* enhances cadmium accumulation and tolerance. Plant Physiol 119:73-79.

Zhu YL, Pilon-Smits EAH, Tarun AS, Weber SU, Jouanin L, Terry N (1999b) Cadmium tolerance and accumulation in Indian mustard is enhanced by overexpressing γ–glutamylcysteine synthetase. Plant Physiol. 121:1169-1177.