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Heavy metal induced physiological changes in the antioxidative response system

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Heavy metal stress induced alterations in the activities of several representatives of the enzymatic antioxidant defense system such as guiacol peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) were comparatively studied in barley seedlings treated by Zn and Cd. Although roots were the main sites of metal accumulation, the oxidative damage was detrimental mainly in the leaves. Our experiments clearly show that the investigated heavy metals have quite different effects on plant metabolism and induce the protective enzymatic system in different ways.

It is inevitable that we should cope with the environmental damages induced by increased concentration of heavy metals. One of the common characteristics of these events is the oxidative stress generating active oxygen species (AOS) (Salin 1987).

Plants have evolved various protective mechanisms to eliminate or reduce AOS species. One of them is the enzymatic antioxidant system, including SOD, POD, CAT and APX. Each of these enzymes has physiological function under non-stressed conditions, but their activity/or quantity is increased under oxidative stress.

The comparison of the the antioxidative enzymatic system in seedlings exposed to two essentially different heavy metals, namely Zn and Cd should reveal essential elements of this defense system. While Zn is an essential microelement that is indispensable for normal plant growth at low concentration and is toxic only at high concentration, Cd has no vital function in plants developing under "natural" conditions.

Materials and Methods

Plant material

Barley seedlings (*Hordeum vulgare* L. cv. Triangle) were grown in hydroponics of a half strength Hoagland solution in a growth chamber as described (Hegedüs et al. 2001). The seven-day-old seedlings were transferred into fresh medium supplemented with different concentrations of $CdCl_2$ or $ZnSO_4$.

Determination of Cd and Zn content content

The metal content was determined by atomic absorption spectroscopy (Horváth et al. 1996).

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Chlorophyll determination

The chlorophyll content was determined in 80 % acetone extract of 0.1 g leaf as described by Arnon (1949).

Preparation for enzyme activities

One g of plant tissue was homogenized with three fold excess of buffer containing 0.05 M Tris (pH 8.0), 0.004 M citric acid, 0.008 M cysteine, 0.005 M ascorbic acid, 0.01 M $MgCl_2$ and sucrose (Arulsekar and Parfitt 1986). The tissue extract was centrifuged at 10000xg for 30 min. The supernatant was used for further analyses.

POD and CAT activities were determined using guaiacol and H_2O_2 substrates, respectively as described by Chance and Maehly (1955), while the APX activity was measured according to Nakano and Asada (1981). The activities were expressed in mkat/g.fr.w.

Results and Discussion

Both heavy metals have been accumulated in the plants proportionally with the progress of incubation time and metal concentration. The primary site of the metal accumulation was the root.

The increasing Cd accumulation resulted in the reduction of the chlorophyll content of leaves but there was no substantial, visible sign of the Zn treatment. The highest (1mM) Zn concentration and the longest (7day) treatment had no effect on the chlorophyll content of leaves, while 0,3-1mM Cd concentrations resulted in 60 % reduction after a 4-day treatment. The latter observation is not surprising, as Cd has been proved to be an aggressive, oxidative damage inducing agent and an effective competitor for essential metal cofactors participating in the chlorophyll biosynthesis (Sanitá di Toppi and Gabbrielli 1999). Zn-ions at high concentration also induce oxidative damage (Chaoui et al. 1997), but at the same time it is an essential cofactor of chlorophyll-synthetizing d-aminolevulinic acid dehidratase. Our observation can be explained at least in two different ways. For one the intracellular localisation of Zn is different from that of Cd, for the other the antioxidative protective system can overcome the detoriate effect of Zn.

To answer these questions we have determined the activity of characteristic antioxidative enzymes.

The POD activity was determined in Zn- and Cd-treated seedlings, and the data are presented in Figure 1. The POD activity did not change in roots after heavy metal treatment.

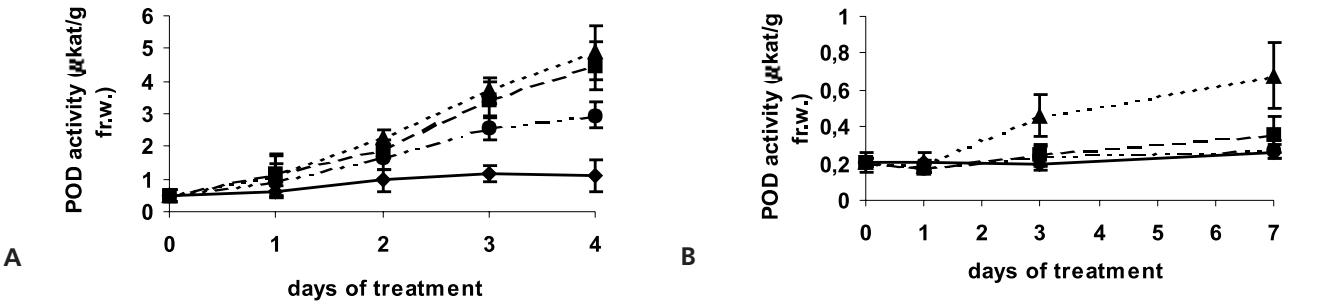

Figure 1. POD activity after Cd (A) and Zn (B) treatment in the leaves of barley seedlings. (®) control; (l) 0,1 mM; (n) 0,3 mM; (s) 1 mM Zn and Cd, respectively.

On the contrary, enhanced POD activity was detected in leaves, although the extent of increase was different in Zn and Cd treated seedlings. Cd-treatment even at the lowest concentration resulted in a substantial increase of enzyme activity, while Zn provoked considerable enzyme activity increase only at the highest concentration and aft er longer incubation time (7 day).

Catalase which is located mostly in peroxysomes and participates in the breakdown of the photorespiratory H_2O_2 (Foyer et al. 1994) could not be detected in roots and showed no significant alterations in neither experimental system.The APX is one of the most important enzymes playing crucial role in eliminating poisonous H_2O_2 from plant cells (Foyer et al. 1994). In Cd-treated seedlings the APX activity of roots was proportionally increased with higher Cd concentration and further enhanced during further incubation. It should be emphasized that at all Cd concentrations the APX activity was dramatically reduced after the third day of treatment. In leaves the APX activity exhibited similar changes as those found in roots but no inhibition could be detected at the highest, 1 mM Cd concentration. Zn-treatment did not casued significant changes in APX activity at any concentrations and after any days of treatment neither in roots nor in leaves. This fact is an additional proof emphasizing the essentially different effects of the heavy metal Zn and Cd on plant metabolism.

We conclude, that there is a fundamental difference in the metabolic changes induced by the heavy metal Zn and Cd. Cadmium induces drastic changes that are reflected in the decrease of chlorophyll content and the radical changes in the activity of the antioxidative enzyme system. Another heavy metal, zinc induces much milder changes in the plant metabolism that is reflected in the constant chlorophyll content, the CAT and APX activity. The only parameter indicating the toxicity of Zn ions at high concentration was the increase of POD activity in leaves. We conclude, that the reason of less toxicity of Zn should be either in the different localization of Zn or the most effective phytochelation of the heavy metal.

On the basis of our experiments we suggest a simple, cheap method for the early detection of heavy metal damage on plants; namely the determination of POD activity of different plant tissues. By means of this test we can make a distinction between the consequences of a detrimental (*i.e.* Cd) and a less harmful (*i.e.* Zn) heavy metal exposure of the plants.

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