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Cadmium Infiltration of Detached Pea Leaves: Effect on its Activated Oxygen Metabolism

By

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Summary

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The effect of infiltrating excised pea leaves with 100 µM $CdCl_2$ on the activity of different antioxidative enzymes and the possible involvement of cadmium in the generation of oxidative stress was studied. Excised pea leaves (*Pisum sativum* L., cv Lincoln) from 15-day-old plants were infiltrated with water (controls) or 100 µM $CdCl_2$ and then were incubated with the same solutions for 1, 2, and 6 days, under natural light, at room temperature. In these conditions, Cd increased the lipid peroxidation of leaves, especially after 6 days incubation. Catalase was significantly decreased after 1 day incubation with Cd, while superoxide dismutase (SOD) and guaiacol peroxidase (Gpx) activities were increased, although changes were only significant for SOD activity. Under these conditions, the level of H_2O_2 in pea leaves could be increased as a result of Cd treatment, and it seems very likely that an oxidative stress situation could be induced.

Introduction

Cadmium is a toxic heavy-metal for humans, animals and plants, and is one of the widespread trace pollutants with a long biological half-life (WAGNER 1993). This metal enters the environment mainly from industrial processes and phosphate fertilizers and then is transferred to animals and humans through the food chain (WAGNER 1993).

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The toxic action of cadmium is still not completly understood and apparently is a multifactorial process. Cadmium produces alterations in the functionality of membranes by inducing changes in their lipid composition, and by affecting membrane-associated enzymatic activities, such as the H^+-ATPase (FODOR & al. 1995). Photosynthesis is also sensitive to Cd which can both inhibit chlorophyll biosynthesis (STOBART & al. 1985) and stimulate its degradation (SOMASHEKARAIAH & al. 1992), and Cd can also depress the activity of enzymes involved in CO_2 fixation (DE FILIPPIS & ZIEGLER 1993). Some studies have suggested that oxidative stress could be involved in the toxicity of this metal. In animal tissues it has been demonstrated that cadmium induces changes in the antioxidant status by increasing superoxide radical production and lipid peroxidation and by decreasing enzymatic and nonenzymatic antioxidants (SUGIYAMA 1994). Apparently, cadmium in the presence of ascorbate can produce DNA damage as a result of hydroxyl radicals produced in a Fenton-type reaction (LITTLEFIELD & HASS 1995). However, in plants less information is available on Cd toxicity. In *Phaseolus vulgaris* seedlings, the toxicity of Cd has been related to the increase of lipid peroxides and the decrease in catalase and superoxide dismutase (SOD) activities (SOMASHEKARAIAH & al. 1992). Lipid peroxidation was also enhanced by Cd treatment in *Phaseolus aureus* seedlings (SHAW 1995), but in this case catalase, guaiacol peroxidase, and ascorbate peroxidase activities were also enhanced by Cd. In different yeast strains, Cu,Zn-SOD was also inhibited by this metal while catalase activity remained almost unchanged (ROMANDINI & al. 1992).

In this work, the effect of infiltrating excised leaves with $CdCl_2$ on the activity of different antioxidative enzymes and the possible involvement of Cd in the generation of oxidative stress was studied.

Material and Methods

Pea leaves (*Pisum sativum*, cv. Lincoln) were excised from 15-day-old plants and were subjected to vacuum infiltration with 100 µM $CdCl_2$ at room temperature, with three infiltration cycles of 2 min. Control leaves were infiltrated with distilled water under the same conditions. Then, leaves were incubated with distilled water (controls) and with 100 µM $CdCl_2$ (samples) under natural light for 1, 2 and 6 days, at room temperature. Leaves were washed and homogeneized in 50 mM Tris-HCl buffer (pH 7.5), containing 0.1 mM EDTA, 2 mM 1,4-Dithiothreitol (DTT), 0.2% (v/v) Triton X-100, and 1 mM phenylmethylsulphonyl fluoride (PMSF) (1/4; w/v), in a Sorvall Omnimixer. The homogenates were centrifuged at 27,000 g for 20 min and the supernatants were used for the determinations.

Total SOD activity (EC 1.15.1.1) was assayed according to the ferricytochrome c method of MCCORD & FRIDOVICH 1969, and SOD isoenzymes were individualized by native polyacrilamide gel (PAGE) on 10% acrylamide gels and were localized by the photochemical method of BEAUCHAMP & FRIDOVICH 1971. Catalase activity (EC 1.11.1.6) was determined according to AEBI 1984 and native-PAGE was carried out on 6% acrylamide gels. Catalase activity was localized in the gels as described by WOODBURY & al. 1971. Guaiacol peroxidase activity was determined according to QUESSADA & MACHEIX 1984.

Lipid peroxidation was determined by measuring the concentration of thiobarbituric acid reacting substances (TBARS) as described by BUEGE & AUST 1972. Proteins were determined according to BRADFORD 1976 using bovine serum albumine as standard. Data were subjected to one-way analysis of variance. When the effect was significant ($P<0.01$), differences between means were evaluated for significance by using Duncan's multiple-range test ($P<0.01$).

Results and Discussion

Cadmium treatment produced a significant increase in the lipid peroxidation of pea leaves after 6 days incubation with this metal (Table 1). This increase in lipid peroxidation was linked to the bleaching of the tissue. Lipid peroxidation was also increased in control leaves with incubation time, but this change was not statistically significant.

Table 1. Effect of Cd infiltration on lipid peroxidation and antioxidative enzymes in pea leaves. Values represent mean ± SEM of three replicates. LSD = least significant difference by Duncan's multiple range test ($P<0.01$)

Treatment	Lipid peroxidation nmol MDA mg^{-1} prot	catalase μmol H_2O_2 min^{-1} mg^{-1} prot	SOD U mg^{-1} prot	GPx U mg^{-1} prot
Control				
1d	0.64±0.01	264.4±12.1	6.0±0.2	44.3±1.5
2d	0.69±0.07	167.5±18.8	9.4±0.9	69.4±1.5
6d	0.89±0.05	89.9±5.10	8.7±0.5	74.5±3.5
100 μM Cd				
1d	0.77±0.09	99.6±13.1	7.8±0.9	44.3±2.9
2d	0.79±0.04	174.1± 3.3	12.0±0.4	77.7±2.2
6d	1.48±0.08	132.5±4.8	21.0±1.1	83.3±0.7
LSD ($P<0.01$)	0.19	32.53	2.15	n.s.

Catalase activity was strongly depressed after 1 day incubation with Cd (Table 1). The activity in control leaves experimented a gradual decrease as a result of incubation time (Table 1). Native-PAGE analysis of catalase showed a single widespread band of activity which experimented a slight increase in its Rf value after 6 days incubation, in both control and Cd-treated leaves (Fig. 1). This change in mobility of catalase could be due to a modification in the isoelectric point of the protein and was dependent exclusively on the incubation time and not on the Cd treatment.

Total SOD activity was increased during the incubation period, in both control and Cd-treated leaves, but the increase was only significant with Cd (Table 1). The enhancement in SOD activity was due to a rise in the activities of all the isoenzymes present in the extracts, especially the Mn-SOD and Cu,Zn-SOD II (Fig. 2). In relation to guaiacol peroxidase activity, this followed a similar pattern to SOD, experimenting a slight increase during the treatment, but the differences with respect to the control leaves were not statistically significant (Table 1).

The response of catalase and SOD activities of pea leaves to Cd infiltration is different to that reported for *Phaseolus vulgaris* seedlings which was characterized by a significant reduction of these two antioxidative activities (SOMASHEKARAIAH & al. 1992), and is similar to that of *Phaseolus aureus* seedlings where the treatment with Cd produced an enhancement of catalase and guaiacol peroxidase activities (SHAW 1995).

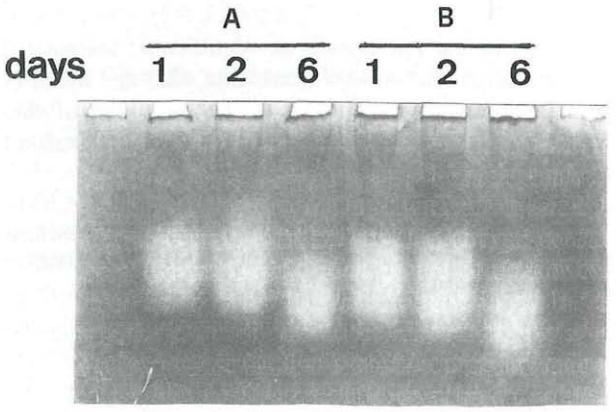

Fig. 1. Catalase isoenzyme activity. Samples (20 µg protein) from control (A) and Cd-treated leaves (B) were subjected to native-PAGE and isoenzymes were localized as described in Material and Methods.

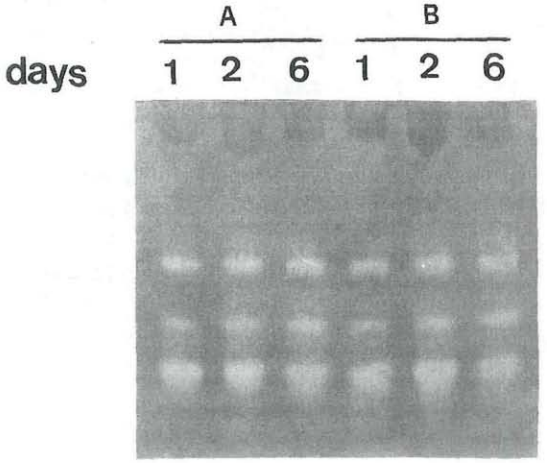

Fig. 2. SOD isoenzyme activity. Samples (90 µg protein) from control and Cd-treated leaves (B) were subjected to native-PAGE and SOD isozymes were visualized in the gels as described in Material and Methods.

Cadmium infiltration of pea leaves produced very similar symptoms to those of leaf senescence, characterized by an increase in lipid peroxidation and tissue bleaching, an enhancement in SOD activity and a decrease in catalase activity (PASTORI & DEL RÍO 1994). SOD and catalase work in conjunction to provide an efficient defence against free radicals produced during oxidative cell metabolism. The uncoupling of both activities as a result of Cd treatment could give rise to an increase in the level of H_2O_2 and, under these conditions, it seems very likely that an oxidative stress situation could be induced.

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