Bioscience Research Communications
Vol. 15, No. 4, August 31, 2003
Printed in Nigeria

0795-8072/2003 $12.00 + 0.00

http://www.klobexpublishers.com

BRC 2003035/15401

The Impact of Cadmium on Some Mitochondrial Antioxidative Enzymes and Lipid Peroxidation in Maize Root

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(Received July 18, 2003)

ABSTRACT: The study is an investigation into the role of mitochondrial Mn-sensitive superoxide dismutase (MnSOD) and peroxidase (POX) enzymes in Cd toxicity in maize. Soil treated with Cd at concentrations of 10 and 20 ppm significantly reduced seedling % emergence and fresh weight of root of 7, 14 and 28-day old maize. These Cd concentrations also inhibited shoot height and leaf area of maize seedling. The accumulation of Cd in the root of the plant for up to 28 days was about 100-fold. Cd at the three concentrations studied increased significantly the activities of mitochondrial MnSOD and POX isolated from 7, 14 and 28-day maize root. Soil treatment with increasing concentration of Cd results in increased generation of TBARS in maize root. The effect of Cd on all the parameters studied was concentration dependent. These results are discussed in relation to the likely role of antioxidant enzymes in ROS detoxification generated by Cd contamination in maize.

Keywords: Cadmium, Maize, Mitochondria, Antioxidative, Enzymes, Vegetative

Introduction

Cadmium (Cd) is a non-essential heavy metal that is toxic to both plant and animal. It accumulates in different tissues and organs (Thevenod and Friedmann,1999) as well as in several tissues of higher plants including maize (Kovacevic *et al*., 1999). Cd can cause the production of reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl ion ($OH^{\cdot}$) in plants (Foyer *et al*., 1997). Phytochelatins are known to bind Cd and other heavy metals , thereby reducing the concentration of free Cd that could cause direct damage to plant tissues as well as the production of ROS (Grill *et al*., 1987; Rauser, 2000).

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In the plant cell, ROS are generated in chloroplast, mitochondria, peroxisomes, cell wall and nitrogen-fixing nodules as unwanted byproducts (Casolo *et al*., 2000; Becana *et al.*, 2000; Foyer *et al*., 1994). The production and removal of these ROS have to be adequately regulated since their accumulation in high concentrations causes damage to proteins, lipids and DNA. There is the need therefore for cell organelles to evolve strategies for dealing with its generation, accumulation, detoxification and damage to macromolecules. In plant mitochondria, about 1% of total oxygen consumption goes into the production of ROS and the major sites are complex I and the ubisemiquinone in complex III (Møller, 2001; Puntarulo *et al*., 1988).

ROS formation arises from the interaction of reduced form of some electron transport chain (ETC) components such as flavins and ubiquinone. ETC is usually kept sufficiently oxidized in order to prevent or limit ROS generation. In the event of its production, it is detoxified by Mn-sensitive superoxide dismutase (MnSOD), peroxidases, catalase, ascorbate/glutathione cycle and the thioredoxin system. MnSOD is known to catalyse the conversion of O_2^- to H_2O_2 in mitochondria and peroxisomes while the peroxidase system (POX) is one of the enzymes that breakdown H_2O_2 to H_2O and O_2 (Bowler *et al*., 1991; Møller, 2001; Eshdat *et al*., 1997; Foyer *et al*., 1994).

In this present study, we report the effect of Cd on some mitochondrial antioxidative enzymes and lipid peroxidation in maize roots. Also, growth of the maize seedlings as affected by Cd is discussed.

Materials and Methods

Plant material and growing conditions:

Seeds of maize (*Zea mays* L), presoaked in distilled water were germinated and grown in 3Kg soil (acid washed sand) mixed with 450ml Hoagland's Solution. It was supplemented with different concentrations of Cd (0, 5, 10, and 20ppm) as $CdCl_2$. The soil was put in bags and each contained five seeds. The seedlings were grown in a greenhouse with temperature of between 25 – 36 ^oC. The seedlings were constantly watered in order to maintain the initial weight of the bag. The roots were harvested at 7, 14, and 28 day intervals and washed in distilled water. The samples were stored in a freezer at -20 ^oC and used for mitochondrial isolation within three days.

Vegetative Parameters:

Emergence (%) records were taken for a period of 7 days and seeds that failed to sprout after that period were regarded as having not germinated.

Seedling growth rate was measured using shoot height and leaf area. The height of the plant was measured from the soil level to the terminal bud using a transparent metre rule. Leaf area was measured by comparing the weight with that of a cut-out, traced on standard paper of known weight to area ratio (Eze, 1965).

Mitochondrial Isolation and Enzyme Assays:

About 50g of the maize root was used for isolation of mitochondria by the method of Douce *et al*. (1987) employing the technique of density gradient centrifugation.

MnSOD assay was by the procedure of McCord and Fridovich (1969). One unit of activity was defined as the amount of enzyme required to inhibit the ferrocytochrome C reduction by 50% in the assay.

POX activity was assayed spectrophotometrically at 470nm in a reaction mixture containing 100mM Citric acid/K_2PO_4 buffer (pH 5.0), 33mM guaiacol and 0.3mM H_2O_2 at 25 ^oC (Lagrimini, 1991). Horse radish POX (Sigma) was used as standard.

Protein was estimated by Lowry *et al*. (1951) method with BSA as standard protein.

Determination of Lipid Peroxidation:

Assay of lipid peroxidation as estimated by the reaction of lipid peroxides with thiobarbituric acid was carried out by the method of Shaw (1995). 1,1,3,3-tetraethoxypropane was used as a standard of lipid peroxide.

Cadmium Analysis:

A quantity of the root was dried overnight in an oven at 105 ^{o}C. This portion was ground in a mortar. Ground dry root samples (about 200g) were digested with 2ml Conc HNO_3 in a pressurized Microwave (Kurner, Rosenheim, Germany). Cd in solution was determined with AAS (Carl Zeiss, Jena, Germany).

Statistics:

All statistical analyses were done by ANOVA to test the significant difference among all the groups and the Duncan's Multiple Range test used to compare the means. A p value less than 0.05 was considered to be statistically significant.

Results

Table 1 show that Cd adversely affected the emergence of maize seedlings and fresh weight of the roots. Cd at a concentration of 5 ppm did not inhibit percentage emergence. However, 10 and 20 ppm concentrations inhibited seedling emergence by 11 and 20% respectively. These same concentrations also caused a significant reduction in the fresh weight of maize root of the 7, 14 and 28-day old plants. Again, 5 ppm had no significant effect on fresh weight; though small decrease was observed. The decrease in weight of root by Cd was concentration dependent.

Table 1: Effect of Cd on emergence and fresh weight of maize root.

Cd Concentration (ppm)	Emergence %	Fresh wt of root (g)		
		7 days	14 days	28 days
0 (Control)	95.0±3.8^{a}	0.80±0.03^{a}	2.29±0.3^{a}	5.61±0.2^{a}
5	95.1±4.6^{a}	0.76±0.03^{a}	1.83±0.2^{a}	4.90±0.3^{b}
10	84.3±3.7^{b}	0.68±0.04^{b}	1.33±0.2^{b}	4.54±0.3^{b}
20	75.1±3.1^{c}	0.65±0.02^{b}	0.84±0.1^{c}	3.02±0.3^{c}

Results are expressed as mean ± S.E.M of three determinations.

Values on the same column with superscripts of the same letter are not significantly different from each other.

In Table 2, growth of maize seedlings was remarkably inhibited by Cd at 10 and 20 ppm. Shoot height and leaf area were reduced by the two concentrations for 7, 14 and 28-day old seedlings. 5 ppm of Cd decreased significantly the shoot height of the 28-day old seedling only. The effect of Cd was concentration dependent.

Table 3 show that Cd at the three concentrations studied increased the activities of mitochondrial MnSOD and POX isolated from 7, 14 and 28-day maize root. The stimulation of the MnSOD activity was greater in the 14-day old than the 7-day old maize root for all the concentrations studied. The increase in the 7-day old root was comparable to the 28-day old. The magnitude of stimulation of the POX activity in the 14-day old root was greater than the 7- and 28-day old roots. The stimulation of both MnSOD and POX activities by Cd was dose-dependent.

Table 2: Effect of Cd on growth of maize seedlings.

Cd Conc. (ppm)	Shoot height (cm)			Leaf Area (cm^2)		
	7 days	14 days	28 days	7 days	14 days	28 days
0 (Control)	6.4±0.4 [a]	13.0±0.5 [a]	21.1±1.1 [a]	6.6±0.2 [a]	28.9±2.4 [a]	102.0±8.1 [a]
5	6.1±0.3 [a]	12.1±0.4 [a]	18.8±0.6 [b]	6.7±0.4 [a]	26.4±3.8 [a b]	88.5±8.6 [a]
10	5.4±0.2 [b]	9.8±0.3 [b]	14.7±1.8 [c]	5.6±0.4 [b]	24.3±1.3 [b c]	66.3±5.6 [b]
20	4.7±0.1 [c]	8.2±0.2 [c]	11.6±1.2 [c]	5.8±0.3 [b]	16.3±0.8 [d]	52.7±6.1 [c]

Results are expressed as mean ± S.E.M of three determinations.
Values on the same column with superscripts of the same letter are not significantly different from each other.

Table 3: Mitochondrial MnSOD and POX activities of maize root as affected by Cd.

Cd Conc. (ppm)	MnSOD activity			POX activity		
	7 days	14 days	28 days	7 days	14 days	28 days
0 (Control)	35.08±0.9 [a]	33.95±1.0 [a]	36.55±1.1 [a]	26.13±0.7 [a]	24.91±2.4 [a]	21.82±0.8 [a]
5	40.57±1.3 [b]	43.40±0.8 [b]	39.58±0.7 [b]	30.21±0.8 [b]	30.41±1.0 [b]	28.23±1.9 [b]
10	46.35±1.1 [c]	50.11±1.2 [c]	45.36±0.8 [c]	36.76±1.0 [c]	38.11±1.2 [c]	33.62±1.5 [c]
20	53.70±1.5 [d]	58.52±1.6 [d]	51.34±1.3 [d]	37.55±0.7 [c]	46.02±0.8 [d]	38.78±0.9 [d]

Results are given as mean ± S.E.M of three determinations. Enzyme activity is expressed as units/mg protein.
Values on the same column with superscripts of the same letter are not significantly different from each other.

Table 4: Cd levels in maize root and TBARS concentrations in mitochondria after soil treatment with Cd.

Cd Conc. (ppm)	Cd levels in root (mg/kg dry wt)			TBARS conc. (nmol TBARS/mg protein)		
	7 days	14 days	28 days	7 days	14 days	28 days
0 (Control)	0.012	0.022	0.027	2.65±0.1 [a]	2.76±0.2 [a]	2.68±0.2 [a]
5	104.7	163.2	170.4	2.54±0.1 [a]	2.61±0.1 [a]	3.43±0.2 [b]
10	235.7	250.1	265.7	3.46±0.1 [b]	3.91±0.3 [b]	4.95±0.1 [c]
20	333.2	350.4	352.3	4.34±0.3 [c]	4.90±0.2 [c]	5.89±0.3 [d]

Results are given as mean ± S.E.M of three determinations.

Values on the same column with superscripts of the same letter are not significantly different from each other.

This study shows that soil treatment with increasing concentration of Cd results in increased accumulation and generation of Cd and TBARS in maize root (Table 4). As expected, the amount of Cd accumulated in the root of plant grown in soil treated with 5 ppm was less than that grown in 10 and 20 ppm treated soil. The rate of uptake/accumulation of Cd by the root decreased with duration of growth. 5 ppm of Cd had no significant effect on mitochondrial TBARS except for the 28-day old maize root where an increase was observed. Higher concentrations of Cd caused an increase in the level of TBARS which was greatest in the 28-day old plant.

Discussion

The present research was intended towards elucidating the roles of MnSOD and POX in the toxicity of Cd as represented by lipid peroxidation and plant growth. Cd was observed to accumulate in the roots by about 100-fold. The rate of accumulation declined remarkably with time of growth. Cd retention in root might be due to cross-linking of Cd to carboxyl group of the cell wall and/or to an interaction with thiol residues of soluble proteins (Barcelo and Poschenrieder, 1990; Leita *et al*., 1993; Lozano-Rodriguez *et al*., 1997).

Cd was observed to reduce seedling emergence, growth and fresh weight of maize root in this study. Other researchers had reported similar effects of Cd in maize (Lozano-Rodriguez *et al*., 1997; El-Enany, 1995; Khan *et al*., 1984; Florijin and van Bensichem, 1993) and other crops (Osubor and Anoliefo, 1999; Leita *et al*., 1993; Landberg and Greger, 1994). These observed adverse effects of Cd on the above parameters are attributable to the generation of ROS which cause oxidative damage to lipids and proteins and inhibit cellular energy production. (Foyer *et al*., 1997; Gallego *et al*., 1999).

Treatment of the soil with 10 ppm and above of Cd resulted in increased TBARS concentrations in the root mitochondria. The levels of these reactive materials is an index of lipid peroxidation and therefore of oxidative stress. The induction of oxidative stress by Cd is mediated by the formation and accumulation of ROS such as O_2^-, H_2O_2, $OH^\cdot$ (Foyer *et al*., 1994; 1997). In plant mitochondria, SOD catalyses the dismutation of O_2^- to H_2O_2. However it is not too clear how the H_2O_2 formed in mitochondria is metabolized to H_2O and O_2. H_2O_2 is capable of diffusing out of the mitochondria into the extracellular space (MØller, 2001). The observation in this research that the activities of MnSOD and POX were elevated by Cd indicates that both enzymes may have a role in the removal of ROS in the mitochondria. To date, different responses depending on the plant species and the length of Cd treatment have been reported by investigators in the activities of MnSOD and POX. The increase in the activities of the enzymes have been observed in studies involving other crops (Dalurzo *et al*., 1997; Schickler and Caspi, 1999; Vitoria *et al*., 2001; Shaw, 1995; Hendry *et al*., 1992). However, others have reported no changes or decrease in their activities as a result of Cd contamination (Fornazier *et al*., 2002; Bhattacharjee, 1998; Gallego *et al*., 1996; Williamson and Scandalios, 1992). It is postulated that the decrease in these activities results in the increase of ROS which normally would have been detoxified.

In this experiment, we reported that Cd stimulated the MnSOD and POX activities probably due to increased levels of ROS. The increased activities of these enzymes will tend to reduce the ROS concentrations. This could explain the ability of the plant to resist Cd treatment at 5 ppm. However, increase in the concentration of Cd may give rise to increased levels of ROS which might have overwhelmed the ability of the two enzymes and other antioxidant mechanisms to deal with it. This may probably explain ineffectiveness of the increased enzyme activities to sufficiently deal with Cd toxicity at higher concentrations. The sustained increase in the activities of the enzymes irrespective of the enhanced toxicity/damage as reflected by increased levels of TBARS is feasible because H_2O_2 does not inactivate MnSOD but rather a signaling molecule to POX activity (Boeler *et al*., 1994).

Schützendübel *et al*. (2001) had reported elevated ‘unspecified POXs (ie enzymes oxidizing phenolic substrates such as guaiacol) activity in pine roots by exposure to Cd. Th increased enzyme activity resulted in increased concentration of phenolics and lignification in response to Cd. The Cd induced cell wall rigidification was consistent with the observation that root growth is significantly inhibited after Cd exposure (Punz and Sieghardt, 1993; Kahle, 1993). It was then postulated that the lignified root tips may have lost their capacity for nutrient uptake and thus their ability to sustain plant growth (Schützendübel and Polle, 2002). The reduction in fresh weight of root and growth retardation observed in this study may have been due to increased lignification of the root tips as a result of increased POX activity.

In conclusion, Cd has been shown to induce lipid peroxidation and stimulate MnSOD and POX activities in mitochondria of maize root. This implies that These enzymes may play some role in the molecular processes of the organelle occasioned by Cd toxicity.

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