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Activity of the enzymes of the antioxidative system in cadmium-treated *Oxya chinensis* (Orthoptera Acridoidae)

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Abstract

One purpose in this research was to determine the toxic effects of Cd on antioxidant enzymes of *Oxya chinensis* (Orthoptera: Acridoidae). Changes in the activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (GPx) were measured in *O. chinensis* insects injected with Cd^{2+}. Fifth-nymphs of *O. chinensis* insects were injected with Cd^{2+} at different concentrations (0, 0.55×10^{-4}, 1.10×10^{-4}, 1.65×10^{-4}, 2.20×10^{-4}, and 2.75×10^{-4} g g^{-1}). An increase in SOD activity in *O. chinensis* was observed at 1.10×10^{-4} to 2.75×10^{-4} g g^{-1} Cd^{2+}. The SOD activity was lower at $2.20 \times 10^{-4}$and 2.75×10^{-4} g g^{-1} than that at 1.10×10^{-4} and 1.65×10^{-4} g g^{-1}. It appears that SOD had a positive protective effect at low Cd^{2+} concentrations, and that this effect disappeared at high Cd^{2+} concentrations. CAT activity was accelerated to varying degrees at 1.10×10^{-4} to 2.75×10^{-4} g g^{-1} for males and at 1.10×10^{-4}, 2.20×10^{-4}, and 2.75×10^{-4}g g^{-1} for females. CAT showed a strong detoxification effect with all treatments. GPx activity decreased with increasing Cd^{2+} concentration with all treatments for males and at 2.20×10^{-4} and 2.65×10^{-4}g g^{-1} for females. We showed that GPx activity had a weak detoxification function with all treatments for males and at high Cd^{2+} for females. Thus, CAT had a strong detoxification effect, whereas SOD had a medium and GPx had a weak detoxification effect. Among the three enzymes, CAT played an important role in the damaging mechanisms of reactive oxygen species in *O. chinensis* insects. Alterations of the antioxidant enzyme level under environmental stresses are suggested as indicators of biotic and abiotic stress.

Keywords: Cadmium (Cd); Oxidative stress; Superoxide dismutase (SOD); Catalase (CAT); Guaiacol peroxidase (GPx); Insect; *Oxya chinensis*

1. Introduction

Cadmium (Cd) is a non-essential heavy metal that is toxic to living organisms. In the periodic table of elements, it belongs to GroupII B (Harrison and Hoare, 1980). It is a trace element that is ubiquitous in soil. It has anthropogenic effects in activities such as the non-ferrous metal industry, mining, the production, use, and disposal of batteries, and disposal of metal-contaminated waste and sludge. Application of pesticides and phosphate fertilizers leads to dispersion of Cd (Alloway, 1995). This situation has been found in Taiyuan, Shanxi Province, China, because it is a city with heavy industry in which there are many industrial groups or companies. Owing to accumulation effect, heavy-metal pollution in the soil subsystem of the city is becoming more and more serious. The present and potential harm of heavy-metals have been widely noted (Wang et al., 2002b).

Cd is a highly toxic environmental contaminant. It presents a serious hazard to public health and is a threat to most life forms (Breakman et al., 1997). Metal trace elements, including Cd, cause severe damage at each level in living organisms, from populations and communities to cell elements (Schützendübel et al., 2001). Several studies have shown that metal trace elements are, at the cellular level, often involved in oxidative stress, which results from the production of reactive

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oxygen species (ROS). ROS includes the superoxide radical ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\bullet}OH$), all of which affect mainly lipids, proteins, carbohydrates, and nucleic acids (Damien et al., 2004). The importance of antioxidant enzymes is generally emphasized in the prevention of oxidative stresses by scavenging of ROS. The antioxidant system comprises several enzymes such as superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (GPx). Superoxide radicals that are generated are converted to H_2O_2 by the action of SOD, and the accumulation of H_2O_2 is prevented in the cell by CAT and GPx. It has been demonstrated that the activities of SOD, CAT, and GPx are induced both in plant species (Fornazier et al., 2002a; Bhattacharjee, 1997/98; Lee and Shin, 2003; Skórzyńska-polit et al., 2003–2004; Pereira et al., 2002; Gallego et al., 1999) and in animal species (Francisco et al., 2000; Sarkar et al., 1998; Rashed, 2001) by some external factors, but less is known about the activity of antioxidant enzymes in insects, especially *Oxya chinensis* insects, in response to Cd stress. One aim in the present study was to investigate the effect of Cd stress in terms of the activities of SOD, CAT, and GPx in *O. chinensis* insects.

2. Materials and methods

2.1. Insects

Fifth-instar nymphs of *O. chinensis* (Orthoptera: Acridoidea) were collected with insect nets from Yuanping (113°4′E, 38°40′N), Shanxi Province, China, in August 2003. The samples were caught directly from a field, transferred to iron-wire cages (620 mm × 510 mm × 400 mm), and brought alive to the laboratory. The habitat of *O. chinensis* insects is a field of bulrushes on the banks of a river. *O. chinensis* insects were acclimatized for 7 days in the laboratory.

2.2. Acute experiment

$CdCl_2{\cdot}2.5H_2O$ was dissolved in triple-distilled water, and the insects were injected (4 μL, i.p.) with different doses of Cd^{2+} (0, 0.55×10^{-4}, 1.10×10^{-4}, 1.65×10^{-4}, 2.20×10^{-4}, and 2.75×10^{-4} g g^{-1} of body weight) at two to three abdominal segments. Control insects received an injection with an equal volume of triple-distilled water. Each dose was repeated three times and was injected into 20–22 male and female individuals. Twenty-four hours after being injected, the insects were separated into two groups (Table 1): one consisted of live insects and was used for the analysis of SOD, CAT and GPx, and those in the other group were dead. After the insects had been separated, they were immediately stored at −80 °C. The number of dead insects was determined and used for the calculation of LD_{50} with probit analysis (Finney, 1970). The LD_{50} was 110.21×10^{-4} g g^{-1}.

In previous experiments (Li and Gong, 1998), the activities of SOD, CAT, and GPx in insects were found to be high in metabolizing organs. The CAT and GPx activity of *O. chinensis* insects in the thorax and abdomen was detected. As shown in Table 2, the CAT activity in the abdomen was a little higher than that in the thorax, and the GPx activity in the thorax was higher than that in the abdomen for females. In view of the effect of food in the alimentary canal on the result and the high GPx activity of females in the thorax, the thorax was chosen for comparing the different activities of SOD, CAT, and GPx in the same body part of all *O. chinensis* insects tested.

Table 1
Number of *O. chinensis* live, dead, and total expose to Cd

	Cd ($\times 10^4$ g g^{-1})						
	0	0.55	1.10	1.65	2.20	2.75	3.30
Group 1							
Alive (numbers)	20	14	10	9	5	5	3
Dead (numbers)	0	6	12	12	16	15	17
Total (numbers)	20	20	22	21	21	20	20
Group 2							
Alive (numbers)	21	16	12	6	5	5	2
Dead (numbers)	0	5	9	16	17	16	19
Total (numbers)	21	21	21	22	22	21	21
Group 3							
Alive (numbers)	20	15	10	7	8	5	2
Dead (numbers)	0	5	12	15	14	16	17
Total (numbers)	20	20	22	22	22	21	19

2.3. Enzyme extraction

For enzyme extraction, the method included in the kit of the Nanjing Jiancheng Bioengineering Institute was followed: thoraxes were homogenized in the Co for 1.5 min in buffer (pH 7.4) containing 0.01 mol L^{-1} Tris–HCl, 0.0001 mol L^{-1} EDTA-2Na, 0.01 mol L^{-1} sucrose, and 0.8% sodium chloride. The homogenate (1:10 w/v) was centrifuged at 15,000 × *g* (4 °C) for 20 min, and the supernatant was stored on ice for determination of enzyme activity.

To determine the protein concentration of all samples, we followed the method of Smith et al. (1985).

2.4. Activity assay

The activity of SOD, CAT, and GPx was determined spectrophotometrically according to the method of the Nanjing Jiancheng Bioengineering Institute with a Microplate reader (Spectra MAX 190).

SOD activity was assayed spectrophotometrically at 550 nm by use of a xanthine and xanthine oxidase system. One unit of SOD activity was defined as the amount of SOD

Table 2
GPx and CAT activity in the thorax and abdomen (GPx: U (mg prot)$^{-1}$, CAT: U (mg prot)$^{-1}$)

	GPx (female)	GPx (male)	CAT (female)	CAT (male)
Thorax	5.46	3.89	4.07	3.86
Abdomen	3.95	5.31	5.46	4.65