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Oxidative stress related enzymes in response to chromium (VI) toxicity in *Oxya chinensis* (Orthoptera: Acridoidae)

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Abstract: The toxic effects of Cr(VI) on antioxidant enzymes of *Oxya chinensis* (Orthoptera: Acridoidae) were determined. 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 Cr(VI). Fifth-nymphs of *O. chinensis* insects were injected with Cr(VI) with different concentrations (0, 75, 150, 225, 300, 375, 450 mg/kg of body weight). The results showed that Cr(VI) led to the change of SOD, CAT, and GPx activities at different concentrations, which revealed that: (1) The oxidative stress of SOD increased with the increase of Cr(VI) concentration. (2) With the increase of Cr(VI) concentrations, CAT activities for females increased at lower concentrations, but decreased at higher concentration range, which indicated that antioxidant system of *O. chinensis* was not influenced by the presence of Cr (VI). A very similar response to Cr(VI) effect for males indicated that Cr(VI) concentrations were not high enough to damage *O. chinensis* in terms of CAT. (3) The GPx activity for females increased in all treatments, which revealed that the damage power of Cr(VI) was increased with the increase of Cr(VI) concentrations in terms of GPx, but the effect was not so remarkable. There was not a consistent trend of GPx activities for males in all treatments of Cr(VI). Cr(VI)-induced changes in antioxidant enzymes were different for SOD, CAT and GPx, of which the tendency was that activities generally changed with increase of concentrations of Cr(VI) suggesting SOD, CAT, and GPx could serve as indices of oxidative stress to some extent.

Keywords: Cr(VI); *Oxya chinensis*; oxidative stress; superoxide dismutase(SOD); catalase (CAT); guaiacol peroxidase(GPx)

Introduction

Chromium(Cr) is one of the heavy metals that cause serious environmental contamination in soil, sediments, and ground waters(Bartlett, 1991; Witmer, 1991; Mei, 2002). It is found Cr-contaminated soils and ground waters at some production sites are formed through anthropogenic activity including stainless steel production, corrosion inhibition, and wood preservation. Cr toxicity and mobility are strictly dependent on its speciation. Cr in nature exists in the form of Cr(Ⅵ) and Cr(Ⅲ). Cr(Ⅵ) compounds have been demonstrated to be approximately 1000 fold more cytotoxic and mutagenic than Cr(Ⅲ) compounds (Debasis, 2001; Valérie, 2003). Both Cr(Ⅵ) and Cr(Ⅲ) are biologically active and oxidative states of Cr. They are involved in redox cycling with the production of reactive oxygen species(ROS) (Klein, 1991; Stohs, 1995; Xie, 2001). A series of *in vitro* and *in vivo* studies have demonstrated that Cr(Ⅵ) induces an oxidative stress through enhanced production of ROS toxicity affecting mainly lipids, proteins, carbohydrates and nucleic acids (Snow, 1991; Debasis, 2001). ROS induces superoxide radical ($\cdot O^{2-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical($\cdot OH$), some of these species are generated by phagocytes and plays an active role in immunological host defense in Cr(Ⅵ) induced cell injuries (Dinauer, 1992; Dawes, 2000; Wang, 2004). After entering the cell through an anion transport system, Cr(Ⅵ) is able to generate a whole spectrum of ROS(Debasis, 2001; Xie, 2001; Wang, 2004). However, living organisms use various means, such as enzyme mechanisms including superoxide dismutase(SOD), catalase(CAT), and guaiacol peroxidase(GPx) to control the noxious effects of ROS(Lee, 2003; Damien, 2004). SOD, which dismutases O_2^- radical to generate H_2O_2, abolished both $\cdot OH$ radical and Cr(Ⅴ) signal generated by Cr(Ⅵ) stimulation, suggesting that O_2^- radical acts as a source for the generation of Cr(Ⅴ) and $\cdot OH$ radical through Haber-Weiss (Eq. (1)—(3)) as follows:

$$O_2^- + Cr(Ⅵ) \rightarrow Cr(Ⅴ) + O_2, \quad (1)$$

$$Cr(Ⅴ) + H_2O_2 \rightarrow Cr(Ⅵ) + \cdot OH + OH^-, \quad (2)$$

overall,

$$O_2^- + H_2O_2 \rightarrow O_2 + \cdot OH + OH^-, \quad (3)$$

this enzyme may enhanced the dismutation of O_2^- to generate H_2O_2 and $\cdot OH$ radical, resulting in an enhancement of apoptosis. As shown in Eq. (1)—(3), O_2^- is required for both Cr(Ⅴ) and $\cdot OH$ generation. SOD may also reduce Cr (Ⅴ) generation from Cr(Ⅵ) due to dismutation of O_2^- and reduced $\cdot OH$ generation, resulting in a decrease in apoptosis. Depending on these two factors, SOD may either enhance or decrease apoptosis. CAT and GPx, which are specific scavengers for H_2O_2, decreased $\cdot OH$ radicals signal but did not abolish it. H_2O_2 can cause apoptosis indirectly through its ability to generate $\cdot OH$ radical upon reaction with Cr(Ⅴ). The accumulation of H_2O_2 is prevented in the cell by CAT and GPx, and they are specific scavenger for H_2O_2, decreased $\cdot OH$ radical signal bit did not abolish it(Donahue, 1997; Lee, 2003; Wang, 2004).

Oxya chinensis (Orthoptera: Acridoidae) have long been known highly harmful pest to crops and forage plants. Therefore, the insects have been the subjects of numerous studies throughout the world. *O. chinensis* is one of the most common and widespread insect in Asia and is abundant in rice paddies, in sugar cane and other gramineous plants. Because this organism causes extensive damage in an agriculturally important sector, this species has received much more attention at different levels. In recent years, grasshoppers continue to be a major critical pest of break crops in China(Han, 2002; Ren, 2002). Several authors have demonstrated that Cr(Ⅵ) could affect the SOD, POD, and GPx activities in plant species and in animal species(Wang, 2004), but less is known about the activity of antioxidant enzymes in *O. chinensis* in response to Cr(Ⅵ) stress.

The aim of the present study was to investigate the

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effects of Cr(Ⅵ) stress in terms of activities of SOD, CAT, and GPx in *O. chinensis*. Specially, the present work attempted to clarify whether non-specific enzymatic indices are of any diagnostic value in respect to environmental metal pollution and to provide a basis for studying mechanism of insect adaptation to complex environmental stress. The information of insectival toxicology can also be enriched.

1 Materials and methods

1.1 Insect models

Fifth-instar nymphs of *O. chinensis* 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 seven days in the laboratory.

1.2 Acute toxicity experiment

$K_2Cr_2O_7$ was dissolved in triple-distilled water, and the insects were injected (4 μl, ip) with different doses of Cr(Ⅵ) (0, 75, 150, 225, 300, 375, 450 mg/kg of body weight) at 2 to 3 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: 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℃. The number of dead insects was determined and used for the calculation of LD_{50} with probit analysis (Finney, 1970). The LD_{50} was 291.0 mg/kg.

Based on the pervious experiment, the activities of SOD, CAT, and GPx in head, thorax, abdomen, and hind femur of *O. chinensis* were detected. All of them were highest in thorax, so the activities of SOD, CAT, and GPx were measured with the thorax in present study.

1.3 Enzyme extraction

For enzyme extraction, the method from the kit of the Nanjing Jiancheng Bioengineering Institute was as follows: thoraxes were homogenized in the Co for 1.5 min in buffer (pH 7.4) containing 0.01 mol/L Tris-HCl, 0.0001 mol/L EDTA-Na_2, 0.01 mol/L sucrose and 0.8% sodium chloride. The homogenate (1∶10 w/v) was centrifuged at 15000 g (4℃) 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 (Smith, 1985).

1.4 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 the system of xanthine and xanthine oxidase. One unit of SOD activity was defined as the amount of SOD required for 50% inhibition of the system of xanthine and xanthine oxidase reaction in 1 ml enzyme extraction of per milligram of protein.

CAT activity was determined spectrophotometrically by measuring the decrease of absorbance at 240 nm due to H_2O_2 decomposition only for the thorax. One unit of CAT activity was defined as the amount of enzyme required for 0.50—0.55 H_2O_2 absorbance of substrate of one gram protein per second.

GPx was assayed spectrophotometrically by use of glutathione (GSH) as substrate by measuring the decrease of enzymatic reaction of GSH (except the effect of non-enzymatic reaction) at 412 nm. One unit of GPx activity was defined as the decrease amount of 1 nmol/L GSH (except the effect of non-enzymatic reaction) in system of enzymatic reaction of one milligram protein per minute.

O. chinensis insects were taken from the refrigerator and separated with a pair of ophthalmologic scissors into four parts (head, thorax, abdomen, and hind femur). The thorax was prepared for assays of SOD, CAT, and GPx activity.

1.5 Statistical analysis

The obtained data of oxidative stress were analyzed with Duncan's multiple-rang test ($P < 0.05$) by using ANOVA of SPSS 10.0 statistical software. The values of SOD, CAT, and GPx activities were expressed as mean ± *SE* from four independent replicate measurements.

2 Results and discussion

2.1 SOD activity

2.1.1 Variation in SOD activity

Information on SOD activity is shown in Table 1. The tendency was that SOD activity increased at lower concentrations and could not be induced at higher concentrations for females. The activities of SOD were about 130.9%, 258.7%, 175.1%, 177.7%, and 162.9% compared with the control values at 75, 150, 225, 300, 375 mg/kg Cr(Ⅵ), respectively. It was found that the maximum of activity SOD was 111.94 U/mg prot at 150 mg/kg Cr(Ⅵ) and the minimum of activity SOD was 56.64 U/mg prot at 75 mg/kg Cr(Ⅵ) with respect to the control. For males, the trend of SOD activity for females was similar to that for females, but they were higher than that of the control except for at 75 mg/kg. The SOD activity of 75, 150, 225, 300, 375 mg/kg Cr(Ⅵ) were about 92.9%, 139.6%, 171.2%, 129.9%, 147.5% of the control. The maximum of SOD activity was 88.48 U/mg prot at 225 mg/kg Cr(Ⅵ), and the minimum of that was 48.05 U/mg prot at 75 mg/kg Cr(Ⅵ) with respect to the control.

Table 1 Effect of Cr(Ⅵ) concentration on the activity of SOD

Cr(Ⅵ), mg/kg	SOD activity of female, U/mg prot	Treatment/CK, %	SOD activity of male, U/mg prot	Treatment/CK, %
0	43.28 ± 9.82		51.68 ± 5.05	
75	56.64 ± 5.60	130.9	48.05 ± 4.78	92.9
150	111.94 ± 28.36	258.7	72.13 ± 2.64	139.6
225	75.78 ± 6.87	175.1	88.48 ± 10.40	171.2
300	76.92 ± 7.66	177.7	67.14 ± 7.87	129.9
375	70.51 ± 24.25	162.9	76.24 ± 14.58	147.5

Notes: Values of SOD activity are expressed as mean of four replicate ± *SE*

2.1.2 ANOVA analysis for the SOD activity

As shown in Table 2, there was no significant difference ($P > 0.05$) in SOD activity influenced by Cr(Ⅵ) treatments. Duncan's multiple range test demonstrated that the significant differences ($P < 0.05$) for the activities of SOD were found between 0 and 150 mg/kg, and between 75 and 150 mg/kg, respectively. There were no significant differences ($P > 0.05$) for the activities of SOD among 225, 300 and 375 mg/kg Cr(Ⅵ).

For male, the activities of SOD were significant different

($P < 0.05$) in all treatments of Cr(Ⅵ) from Table 3. Duncan's multiple range test demonstrated that when the concentrations of Cr(Ⅵ) were 0, 75, 150 and 300 mg/kg; 0, 150, 300 and 375 mg/kg; and 150, 225, 300 and 375 mg/kg, the differences for the activities of SOD were not found ($P > 0.05$) among them. And the significant differences($P < 0.05$) for the activities of SOD were found between 0 and 225 mg/kg, between 75 and 225 mg/kg, and between 75 and 375 mg/kg, respectively.

Table 2 Results of ANOVA analysis for the activities of SOD in *O. chinenesis* (female fifth instar nymphs)

Sources	*SS*	*df*	*MS*	*F*	*P*
Between group	10780.27	5	2156.26	1.99	> 0.05
Within group	19507.61	18	1083.76		
Total	30288.88	23			

Notes: *SS*. sum of squares; *df*. degree of freedom; *MS*. mean square; *F*. *F* = mean square of between treatments/mean square of within treatments; *P*. significance level

Table 3 Results of ANOVA analysis for the activities of SOD in *O. chinenesis* (Male fifth-instar nymphs)

Sources	*SS*	*df*	*MS*	*F*	*P*
Between group	4664.08	5	932.82	3.20	< 0.05
Within group	5255.04	18	291.95		
Total	9919.12	23			

Notes: The same as Table 2

For SOD, the activity increased at lower concentrations and then decreased a at higher concentrations, which suggested that the oxidative stress increased with increasing Cr(Ⅵ) concentration. At lower Cr(Ⅵ) concentration, *O. chinensis* could convert the O_2^- to H_2O_2 to induce the damage on them. At higher Cr(Ⅵ) concentration, they were not able to do so immediately, so they could be damaged. This demonstrated that Cr(Ⅵ) generated more and more serious damage on *O. chinensis* with the increase of Cr(Ⅵ) concentration.

2.2 CAT activity

2.2.1 Variation in CAT activity

Information on CAT activity is given in Table 4. With increasing Cr(Ⅵ) concentration, CAT activity increased at lower Cr concentrations and kept about a constant value at higher concentrations for female, and they were higher than that of the control. The activities of CAT at 75, 150, 225, 300, 375 mg/kg Cr(Ⅵ) were 1.0-, 1.8-, 1.0-, 1.3- and 1.2-fold of the control. The maximum of CAT activity was 101.80 U/g prot at 150 mg/kg Cr(Ⅵ) and the minimum of that was 55.59 U/g prot at 225 mg/kg Cr(Ⅵ) with respect to the control. The CAT activity of 225 mg/kg Cr(Ⅵ) was lower than that of both 150 and 330 mg/kg Cr(Ⅵ). For male, CAT activity was about a constant value at lower concentrations except for that at 150 mg/kg Cr(Ⅵ) and decreased at 375 mg/kg Cr(Ⅵ), that is needed to be further studied, but they were lower than the control in all treatment of Cr(Ⅵ). They were 0.97-, 0.76-, 0.99-, 0.92- and 0.79-fold of the control at 75, 150, 225, 300, 375 mg/kg, respectively. The maximum of CAT activity was 77.81 U/g prot at 225 mg/kg Cr(Ⅵ) and the minimum of that was 59.63 U/g prot at 150 mg/kg Cr(Ⅵ) with respect to the control.

2.2.2 ANOVA for the CAT activity

Table 5 shows that the ANOVA did not reveal any significant differences ($P > 0.05$) on CAT activity in all treatments of Cr(Ⅵ). Duncan's multiple range test showed that significant differences ($P < 0.05$) for the activities of CAT were found between 0 and 150 mg/kg Cr(Ⅵ), between 75 and 150 mg/kg, and between 150 and 225 mg/kg, respectively. There were no significant differences ($P > 0.05$) for the activities of CAT among 0, 75, 225, 300 and 375 mg/kg, and among 150, 300 and 375 mg/kg, respectively.

Table 4 Effect of Cr(Ⅵ) concentration on the activity of CAT

Cr(Ⅵ), mg/kg	CAT activity of female, U/g prot	Treatment/CK, %	CAT activity of male, U/g prot	Treatment/CK, %
0	55.44 ± 4.68		78.89 ± 12.56	
75	57.34 ± 7.43	103.4	76.6693 ± 5.82	97.2
150	101.80 ± 20.44	183.6	59.63 ± 5.99	75.6
225	55.59 ± 7.72	100.3	77.81 ± 7.89	98.6
300	72.15 ± 14.36	130.1	72.36 ± 10.85	91.2
375	67.99 ± 12.25	122.7	62.65 ± 7.95	79.4

Notes: Values of CAT activity are expressed as mean of four replicate ± *SE*

Table 5 Results of ANOVA analysis for the activities of CAT in *O. chinenesis* (female fifth-instar nymphs)

Sources	*SS*	*df*	*MS*	*F*	*P*
Between group	6337.38	5	126.49	2.09	> 0.05
Within group	10927.47	18	607.08		
Total	17264.91	23			

Notes: The same as Table 2

Table 6 shows that the ANOVA did not reveal any significant differences ($P > 0.05$) on CAT activity in all treatment of Cr(Ⅵ). Duncan's multiple range test showed that the differences for the activities of CAT were not found ($P > 0.05$) among all treatments of Cr(Ⅵ).

Table 6 Results of ANOVA analysis for the activities of CAT in *O. chinenesis* (male fifth-instar nymphs)

Sources	*SS*	*df*	*MS*	*F*	*P*
Between group	1364.14	5	272.83	0.87	> 0.05
Within group	5646.02	18	313.67		
Total	7010.16	23			

Notes: The Same as Table 2

For CAT, Cr(Ⅵ) could induce the change of CAT activities at different Cr(Ⅵ) concentrations. With the increase Cr(Ⅵ) concentrations, CAT activities were increased for female at lower concentrations and then decreased at higher concentrations, and they were higher than that of the control. This showed that antioxidant system of *O. chinensis* could work properly at all treatments of Cr(Ⅵ). A very similar response to Cr(Ⅵ) effect for male was observed in changes of CAT activity except for that at 150 mg/kg Cr(Ⅵ), that is needed to be further studied, but they were lower than the control in all treatments of Cr(Ⅵ). This showed that Cr(Ⅵ) concentrations of all treatments were not enough to damage *O. chinensis*.

2.3 GPx activity

2.3.1 Variation in GPx activity

GPx activities of different Cr(Ⅵ) concentrations had been presented in Table 7. It showed that Cr(Ⅵ) could induce the change of GPx activity. The GPx activity increased in all experiments for males, but it was lower than that of the control value. The GPx activities at 75, 150, 225, 300, 375 and 450 mg/kg Cr(Ⅵ) were 41.4%, 52.5%, 57.9%, 101.0%, 103.8% and 135.8% of the control, respectively. It was found that the maximum of activity GPx was 10.11 U/mg prot at 450 mg/kg Cr(Ⅵ) and the minimum of activity GPx was 3.08 U/mg prot at 75 mg/kg Cr(Ⅵ) with respect to the control. For males, there was not a distinct trend of change of the GPx activities in all treatments of Cr(Ⅵ). The GPx activities at 75, 150, 225, 300, 375 and

450 mg/kg Cr (Ⅵ) were 43.3%, 40.6%, 18.9%, 305.8%, 283.0% and 256.4% of the control, respectively. The highest GPx activity was 21.78 U/mg prot at 300 mg/kg Cr(Ⅵ) and the minimum of activity GPx was 1.35 U/mg prot at 225 mg/kg Cr(Ⅵ) with respect to the control.

Table 7 Effect of Cr(Ⅵ) concentration on the GPx activity

Cr(Ⅵ), mg/kg	GPx activity of female, U/mg prot	Treatment/CK, %	GPx activity of male, U/mg prot	Treatment/CK, %
0	7.44 ± 1.26		7.12 ± 0.74	
75	3.08 ± 0.11	41.4	3.08 ± 0.24	43.3
150	3.90 ± 0.54	52.5	2.89 ± 0.65	40.6
225	4.31 ± 0.58	57.9	1.35 ± 0.29	18.9
300	7.52 ± 2.39	101.0	21.78 ± 3.22	305.8
375	7.72 ± 2.59	103.7	20.16 ± 4.63	283.0
450	10.11 ± 2.68	135.8	18.27 ± 2.68	256.4

Notes: Values of GPx activity are expressed as mean of four replicate ± *SE*

2.3.2 ANOVA for the GPx activity

It did not reveal any significant differences ($P > 0.05$) on GPx activity in all treatments of Cr(Ⅵ) in Table 8. Duncan's multiple range test showed that the significant differences ($P < 0.05$) for the activities of GPx were found between 75 and 450 mg/kg, 150 and 450 mg/kg, and 225 and 450 mg/kg, respectively. There were no differences ($P > 0.05$) for the activities of GPx among 0, 75, 150, 225, 300 and 375 mg/kg, and among 0, 300, 375 and 450 mg/kg, respectively.

Table 8 Results of ANOVA analysis for the activities of GPX in *O. chinenesis* (Fifth-instar femal nymphs)

Sources	*SS*	*df*	*MS*	*F*	*P*
Between group	157.55	6	26.26	2.10	> 0.05
Within group	262.43	21	12.50		
Total	419.98	27			

Notes: The same as Table 2

It revealed significant differences ($P < 0.01$) on GPx activity in all treatments of Cr(Ⅵ) in Table 9. Duncan's multiple range test showed that the significant differences ($P < 0.05$) for the activities of GPx were found between 0 and 300 mg/kg Cr(Ⅵ), between 75 and 300 mg/kg, and 150 and 300 mg/kg, 250 and 300 mg/kg, 0 and 375 mg/kg Cr, 75 and 375 mg/kg, 150 and 375 mg/kg, 225 and 375 mg/kg, 0 and 450 mg/kg, 75 and 450 mg/kg, 150 and 450 mg/kg, and 225 and 450 mg/kg, respectively. There were no significant differences ($P > 0.05$) for the activities of GPx among 0, 75, 150 and 225 mg/kg, and among 300, 375 and 450 mg/kg, respectively.

Table 9 Results of ANOVA analysis for the activities of GPX in *O. chinenesis* (Fifth-instar male nymphs)

Sources	*SS*	*df*	*MS*	*F*	*P*
Between group	1955.64	6	325.94	14.23	< 0.01
Within group	481.16	21	22.91		
Total	2436.79	27			

Notes: The same as Table 2

For GPx, for females, the GPx activity increased in all treatments of Cr(Ⅵ) compared to the control. This revealed that the damage of Cr(Ⅵ) was increased with increasing Cr(Ⅵ) concentrations at all treatments, but it did not affect them seriously. For males, there was not a clear law of change of the GPx activities in all treatments of Cr(Ⅵ), and this needed to be further studied.

In summary, Cr(Ⅵ) could induce the change of SOD, CAT, and GPx activities. Cr(Ⅵ)-induced changes in antioxidant enzymes were different for SOD, CAT, and GPx with increasing Cr(Ⅵ) concentrations at all treatments.

3 Conclusions

In order to understand the biochemical basis of metal tolerance, biochemical stress-related parameters in *O. chinensis* were studied in the present study. The toxicity of Cr(Ⅵ) was observed in *O. chinensis* at different Cr(Ⅵ) concentrations (Table 1, 4, 7). The results indicate that, Cr(Ⅵ)-induced changes in antioxidant enzymes were different for SOD, CAT, and GPx, of which the tendency was that activities generally increase with concentrations of Cr(Ⅵ), suggesting SOD, CAT, and GPx could serve as indices of oxidative stress to some extent. We demonstrated that multiple mechanisms rather than a single mechanism may be responsible for the capacity of *O. chinensis* insects to resist Cr(Ⅵ). Alterations of the antioxidant enzyme level under environmental stresses are suggested to serve as indicators of biotic and abiotic stress.

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