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Short communication

Glutathione *S*-transferase in the midgut tissue of gypsy moth (*Lymantria dispar*) caterpillars exposed to dietary cadmium

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ABSTRACT

Activity of glutathione S-transferase (GST) in midgut of gypsy moth caterpillars exposed to 10 and 30 μg Cd/g dry food was examined. Based on the enzyme reaction through conjugation with glutathione, overall activity remained unaltered after acute and chronic treatment. No-observed-effect-concentration (10 μg Cd/g dry food) significantly increased activity only after 3-day recovery following cadmium administration. Almost all comparisons of the indices of phenotypic plasticity revealed statistically significant differences. Despite the facts that GST has important role in xenobiotic biotransformation, our results indicate that this enzyme in insect midgut does not represent the key factor in cadmium detoxification.

1. Introduction

Living organisms are exposed to multiple environmental stressors and have evolved to cope with them. The gypsy moth (*Lymantria dispar*, Lepidoptera) is a wide spread phytophagous insect in Europe, Asia and North America, with a great economic impact on forests.

The cadmium pool in a forest ecosystem mainly originates from anthropogenic activities. It is highly toxic metal without any physiological function in the organism. Phytophagous pest insects are ravenous consumers, so the alimentary tract, especially the midgut, as the major segment, is a possible point where cadmium has harmful effects (Beaty et al., 2002). Cadmium can influence cellular iron levels, decrease glutathione content and inhibit glutathione-related enzymes (Peakall et al., 1999), while lipid peroxidation is considered to be the primary mechanism of cadmium toxicity. Most insects lack Se-dependent glutathione peroxidase. Glutathione S-transferase isozymes possess selenium independent peroxidase activity and, together with independent mechanisms ascorbate peroxidase (APOX), metabolize peroxides in insect midgut tissues (Yepiskoposyan et al., 2006).

Glutathione S-transferase acts in multiple isoenzymatic forms as part of important intracellular mechanisms of detoxification present in almost all animals. Glutathione conjugation is considered to be one of the major modalities for xenobiotic detoxification for maintenance of homeostasis. So far, the major role of GSTs in insects is considered to be resistance to various xenobiotics through their selenium-independent peroxidase activity. By catalyzing conjugation of lipid peroxidation final products with glutathione, these enzymes metabolize hydroperoxides (Krishnan and Kodrik, 2006). For heavy metal detoxification, GST probably binds metal in the GSH binding domain at the N-terminal end (Salazar-Medina et al., 2010).

The main questions addressed in this study are the modality of changes in GST peroxidase activity during chronic and three-day acute cadmium dietary treatments, the ability of GST to return to control levels after 3 days recovery, plasticity of the enzyme during stress and genetic (egg-masses) differences in response to cadmium ingestion. Detecting alterations in biochemical parameters (biomarkers), may allow us to detect alterations in the environment and thereby monitor long-term changes in biota induced by different stressors.

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2. Material and methods

2.1. Insects

The experiment was performed on twenty randomly chosen egg-masses collected in Opovo poplar forest, 30 km north of Belgrade, Serbia. Larvae were reared and distributed into seven experimental groups (acute, chronic and recovery) as described earlier (Vlahović et al., 2013; Vlahović, 2009).

Two cadmium levels were tested: 10 and 30 μg Cd/g dry food (NEOC and LOEC, respectively) (Scheme 1). Cadmium concentrations in the diet were calculated in relation to the relative amount in $Cd(NO_3)_2 \times 4H_2O$.

2.2. Homogenate preparation and enzyme analysis

Individual larvae were anesthesized and decapitated on ice. The whole gut was excised, midgut tissue extracted (peritrophic membrane with content was removed) and washed with ice-cold insect physiological iosotonic saline, containing 7.1 mM $CaCl_2$, 22.0 mM Na_2 β-glycerophosphate, 13.5 mM $MgSO_4$, 26.9 mM $MgCl_2$, 29.5 mM KCl, and 23.9 mM glucose. The pH was adjusted to 6.8 by titration with KH_2PO_4 (Davenport and Wright, 1985). Midguts were pooled by weight, homogenized in 0.25 M sucrose, sonicated and centrifuged at 10500*g*/90 min. Midgut tissue pools were formed for each experimental treatment (7 groups). For every egg-mass (20), homogenate was made from five midguts.

GST activity was determined through conjugation reaction of 1-chloro-2,4-dinitrobenzene (CDNB) with the SH groups of glutathione (Habig et al., 1974). Enzyme activity was expressed as Units per mg of protein.

The results were evaluated in the STATISTICA 4.5 program. The influence of cadmium treatments was examined by one-way analysis of variance. The probability (P) value was set at 0.05.

Index of phenotypic plasticity was calculated according to Cheplik (1995), using the following equation:

$$PP_{Ch} = \frac{\overline{X}_C - \overline{X}_T}{\overline{X}_C} * 100$$

$\overline{X}_C$, $\overline{X}_T$ Mean value of GST activity of the pooled homogenate of a single egg-mass in favorable conditions and under cadmium treatments, respectively.

Wilcoxon's test was employed for comparison of indices of phenotypic plasticity for cadmium effects. We used the *F* test to evaluate comparisons of the variances and plasticities of GST between different metal treatments.

3. Results

Mean values for GST specific activities showed no alterations during acute and chronic treatments at either cadmium concentration when compared to control values (Fig. 1). Norms of reaction showed heterogeneous variations of GST in different egg-masses under the same cadmium treatments. During recovery from both chronic treatments we detected increases of GST specific activity, but this was statistically significant only for the lower cadmium concentration compared to control. During recovery from long-term ingestion at 10 μg Cd/g dry food, larvae from 15 egg-masses showed increased activity, while those from 14 egg-masses enhanced GST activity after three days recovery from 30 μg Cd/g dry food.

One-way ANOVA established that only during recovery from 10 μg Cd/g dry food cadmium significantly affected change of GST activity ($F = 4.116$; $P = 0.021$).

Almost all Z-values, obtained by comparison of indices of plasticities, were statistically significant (Table 1). Mean values of indices of phenotypic plasticity were negative for most treatments. Only the indices of phenotypic plasticity during chronic cadmium effect at 30 and acute effect at 10 μg Cd/g dry food had a positive value. Plasticity was significantly higher during the chronic effect at 30 μg Cd/g dry food than that after recovery from this cadmium concentration as well as chronic treatment at 10 μg Cd/g dry food. Moreover, the index of plasticity was higher after recovery from 10 μg Cd/g dry food than after recovery from 30 μg Cd/g dry food and long-term treatment at 10 μg Cd/g dry food. Egg-masses subjected to short-term treatment at the higher cadmium concentration showed greater plasticity than during acute treatment at the lower amount as well as after long-term effect of the high dietary cadmium concentration. There were no statistically significant F values for comparisons of standard deviations.

4. Discussion

The role of glutathione S-transferases in detoxification of heavy metals is still unclear. It is obvious that neither cadmium concentration examined in our experiment was sufficient to alter GST activity. Essential processes and proteins involved in detoxification of peroxides induced by cadmium, as well as cadmium itself, are sequestration, metallothionein and ascorbate peroxidase—APOX (Hopkin, 1989; Mathews et al., 1997). In gypsy moth larvae, a large number of negative correlations between GST activity and fitness components (larval mass, development duration, mortality and relative growth rate) were found during exposure to dietary cadmium (Vlahović, 2009). A possible reason is energy allocation towards processes enabling survival in stressful environmental conditions. Amounts of cadmium used in our research were probably adequate for *de novo* synthesis of metallothionein. There are two possible explanations for unchanged GST activity. First, cadmium may form a complex with glutathione and modulate the enzyme activity (Iscan et al., 1995). The second mechanism is direct metal binding to GST protein (Xu et al., 2015).

Absence of enzyme changes implies that even low cadmium concentrations and short-term impacts activate different defense mechanisms (APOX, metallothionein) or indirectly attenuate GST activity by binding to GSH. Mirčić et al. (2013) established that activity of APOX increased and glutathione concentration decreased in 4th instar *Lymantria distar* larvae exposed to 50 μg Cd/g dry food. Long lasting cadmium treatment was previously found to cause much structural and functional reorganization in midgut tissue. A three-day of recovery period from 10 μg Cd/g dry food provides an opportunity for GST in tissue repair and re-establishment of homeostasis. After chronic treatment at 30 μg Cd/g dry food inhibition and/or alteration in the GSH pool was stronger than after application of the lower cadmium concentration. The GSH pool was probably redirected towards detoxification via ascorbate peroxidation as a more efficient and cheaper process for peroxide elimination. Direct GST inhibition provoked by cadmium binding to the enzyme or to glutathione was more expressed.

Phenotypic plasticity is one of the major modalities by which different organisms cope with environmental changes. In novel conditions, species with greater adaptive plasticity have a better chance of surviving. Invasive species have greater plasticity than noninvasive ones (Wilson et al., 2009). Our conclusion is that the plasticity response of GST activity in the twenty families examined during cadmium treatments is generally uniform. Negative values for indices of phenotypic plasticity point to the direction of plasticity under the effect of cadmium when compared with controls. Our results for the calculated index of plasticity support previous statements explaining inhibition of enzymes for