Effects of chlorpyrifos on glutathione S-transferase in migratory locust, Locusta migratoria

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\textbf{A R T I C L E I N F O}

\textbf{A B S T R A C T}

Chlorpyrifos is a typical organophosphate pesticide and is among the most widely used worldwide. The objective of the present investigation was to assess the effect of chlorpyrifos exposure on glutathione S-transferase in Locusta migratoria. In the present study, chlorpyrifos (0.1, 0.2, and 0.4 mg g\textsuperscript{-1} body weight) was topically applied in the abdomen of locusts. The GST activity, mRNA levels of ten L. migratoria GSTs and protein levels of four representative GSTs were detected. The results showed that chlorpyrifos treatment caused significant decrease of 1,2-dichloro-4-nitrobenzene (DCNB) and p-nitro-benzyl chloride (p-NBC) activities, whereas 1-chloro-2,4-dinitrobenzene (CDNB) activity was not altered in locusts. The mRNA levels of seven L. migratoria GSTs, including LmGST\textsubscript{3}1, LmGST\textsubscript{3}2, LmGST\textsubscript{3}4, LmGST\textsubscript{3}5, LmGST\textsubscript{3}6, LmGST\textsubscript{1}1, and LmGST\textsubscript{1}2, were decreased after chlorpyrifos exposure. The protein levels of LmGST\textsubscript{3}5, LmGST\textsubscript{1}1 and LmGST\textsubscript{1}2 were significantly decreased at higher doses of chlorpyrifos. However, chlorpyrifos elevated the mRNA and protein expression of LmGST\textsubscript{1}1. It indicated that LmGST\textsubscript{1}1 might contribute to the resistance of locust to organophosphate pesticides such as chlorpyrifos, whereas the decrease in other GSTs might be an economic compensation by the insect to differentially regulate the expression of enzymes involved in the detoxification of insecticides on the expense of those that are not.

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1. Introduction

Pesticides remain for various reasons the main tool to control crop pests and combat disease vectors in agricultural and public health operations [1]. Chlorpyrifos is among the most widely used organophosphate pesticides (OPs) worldwide, which has been used in homes and on farms and detected in agricultural and fishery operations [2]. Chlorpyrifos exerts its toxic effects mainly by the oxon to inhibit the activity of acetylcholinesterase [4]. Detoxification of the oxon occurs via hydrolysis by esterases or by conjugation with glutathione (GSH), which is catalyzed by glutathione S-transferase (GST) [5]. In addition, it has been postulated to have multiple effects on the target cells including generation of reactive oxygen species and induction of intracellular oxidative stress thereby disrupting normal cellular development and differentiation [6].

GSTs are multifunctional enzymes involved in detoxification of xenobiotics and can protect insects against various plant allelochemicals and chemical insecticides. The insect cytosolic GSTs can be classified into six major classes along with several unclassified genes [7]. Among them, sigma, omega, zeta, and theta have representatives across Metazoa whereas delta and epsilon are specific to Insecta and Holometabola, respectively [8].

The migratory locust, Locusta migratoria, is the most widespread locust species. Frequent applications of insecticides have inevitably resulted in development of resistance in some natural populations of the locust [9–12]. GSTs involved in OPs detoxification play an important role in developing resistance to insecticides [13]. Moreover, GSTs could be inducible by certain insecticides and other chemicals [7]. In our previous study, we have identified 10 putative cytosolic GSTs, fall into four classes (delta, sigma, theta, and unclassified) [14]. Transcriptional activation of GST by various xenobiotics remains an active area of research due to their important role in detoxification reactions [15].

Recent studies revealed that chlorpyrifos is able to regulate the transcription of some classes of GSTs in mammalian and fish [4,16]. However, the transcription regulation of insect GST genes by chlorpyrifos has not yet been investigated. To improve our understanding of the toxicity mechanisms and responses of different GST classes to chlorpyrifos, a better understanding of GSTs is required. The evaluation of the effects of chlorpyrifos on insect GSTs transcription is of relevance. In this study, we examined the changes in GSTs activity, transcription of ten locust GST isoforms, and...
protein levels of four kinds of locust GSTs (GSTs5, GSTd1, GSTt1, and LmGSTu1) in locust. We evaluated the dose-dependent responses of different locust GST isoforms, and examined possible mechanisms underlying these changes.

2. Material and method

2.1. Insect and treatment

*L. migratoria* were purchased from the Insect Protein Co., Ltd. of Cangzhou City in China and reared in the laboratory with wheat sprouts in 22 × 22 × 22 plastic cages at 28 °C under 14:10 h light:dark photoperiod.

Four different concentrations (0, 10, 20, and 40 μg/mL) of chlorpyrifos (Sigma, St. Louis, MO, USA) solution were prepared in acetone as a solvent. Each of 16–20 third-instar nymphs in one replicate was topically applied with 3 μL of each chlorpyrifos solution or acetone (control) in the abdomen between the second and third sterna. Each dose (0.1, 0.2, and 0.4 mg g⁻¹ body weight) or control was repeated three times. The mortality rates at each dose were 10%, 30%, and 50%, respectively. All surviving locusts from each group were collected and immediately stored at −80 °C for biochemical analysis after 24 h treatment.

2.2. GST activity assays

Locusts were homogenized in buffer (pH 7.4) containing 0.01 M Tris–HCl, 0.1 mM EDTA-2Na, 0.01 M sucrose, and 0.8% sodium chloride. The homogenate (1:10 w/v) was centrifuged at 15,000 × g for determination of enzyme activity. Ten microliters of supernatant was used in a total volume of 200 μL of a reaction mixture or acetone (control) in the abdomen between the second and third sterna. Each dose (0.1, 0.2, and 0.4 mg g⁻¹ body weight) or control was repeated three times. The mortality rates at each dose were 10%, 30%, and 50%, respectively. All surviving locusts from each group were collected and immediately stored at −80 °C for biochemical analysis after 24 h treatment.

2.3. qPCR analysis

The total RNA was isolated from 80 mg whole bodies of locusts using 1 ml TRIzol reagent (TaKaRa, China). Total RNA was then treated with DNase I (TaKaRa, China) and cDNA was synthesized using the First Strand cDNA Synthesis kit (Fermentas, Glen Burnie, MD).

The mRNA levels were detected by quantitative reverse transcription PCR (qRT-PCR) using specific primers (Table 1). The qPCR was performed using ABI 7300 real-time PCR detection system (ABI, USA) and Maxima SYBR Green qPCR Master Mix kit (Takara, China). A melting curve was established for each sample. The relative expression of each GST gene was determined using cycling parameters of an initial denaturation at 95 °C for 3 min followed by 40 cycles of 95 °C for 20 s, 55 °C for 20 s and 72 °C for 20 s. The threshold cycle (Ct) value for each dilution was then plotted against the log of its concentration, and Ct values for the experimental samples were plotted onto this dilution series standard curve. Target quantities were calculated from separate standard curves generated for each experiment. Relative expression values (REVs) were then determined by dividing the quantities of the target sequence of interest with the quantity obtained for *β-actin* as an internal reference gene. The qPCR was repeated three times for each gene. Each replication was performed based on an independent RNA sample preparation and consisted of two technical replications.

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2.4. Western blot analysis

Total protein was extracted from 100 mg third-instar nymphs in 1 ml ice-cold lysis buffer (1% Nonidet P40, 1 mM EDTA, 125 mM sodium fluoride, 0.5 mM sodium vanadate, 2.5 μg/mL of aprotinin, 50 μg/mL of leupeptin, 25 μM PMSF, and 25 μg/mL of Trypsin inhibitor). Protein concentration was determined according to the method of Bradford using bovine serum albumin as a standard [17]. The lysates were centrifuged at 13,000 rpm for 15 min and supernatants were collected. SDS–PAGE was performed using 150 μg protein samples and precast 12% resolving and 4% stacking Tris–HCl gels (Bio-Rad). Separated proteins were then transferred to a nitrocellulose membrane (Millipore, Billerica, MA). After blocking (blocking solution: 5% non-fat milk dissolved in PBS + 0.1% Tween 20, pH 7.4) proteins were incubated overnight at 4°C with anti-GST antibodies, the process of obtaining were described previously [18], at a concentration of 1:200 (for GSTd1), 1:500 (for GSTt1), or 1:5000 (for GSTs5 and GSTu1). Exposure to fluorescently labeled secondary antibody (1:3000) [IRDye 680CW goat anti-rabbit IgG (H + L), LI-COR] was followed by scanning and detecting with LI-COR Odyssey Infrared Fluorescent System.

2.5. Statistical analysis

Results were expressed as mean ± standard error and the data were analyzed using one way ANOVA for significant differences between chlorpyrifos exposure groups and the control groups (SPSS, Version 17.0). A level of \( p < 0.05 \) was accepted as statistically significant.

3. Results

3.1. Effect of chlorpyrifos on \( L. \) migratoria GST activity

The GST activities were assessed with three kinds of substrates in locusts (Fig. 1). Chlorpyrifos treatment caused statistically significant decrease of DCNB and pNBC activities in a dose-dependent manner, whereas CDNB activity was not significantly altered in locusts treated with all tested concentration of chlorpyrifos. A decrease down to 0.67-, 0.53-, and 0.49-fold in the DCNB activity was observed at various doses of chlorpyrifos. pNBC activity was reduced to 0.53-, 0.47-, and 0.41-fold, respectively.
3.2. Effect of chlorpyrifos on mRNA levels of L. migratoria GSTs

We tested ten L. migratoria GSTs mRNA levels by real time PCR. The mRNA levels of seven L. migratoria GSTs, including LmGSTs2, LmGSTs3, LmGSTs4, LmGSTs5, LmGSTs6, LmGSTt1, and LmGSTu1, were decreased significantly at different tested doses of chlorpyrifos compared with the control (Fig. 2A and B). Among them, LmGSTs2 mRNA levels decreased at all tested concentrations. The decrease of LmGSTs6 mRNA levels was observed only in 40 μg/mL chlorpyrifos group. The other five L. migratoria GSTs mRNA levels decreased after both 20 and 40 μg/mL chlorpyrifos treatment. The mRNA levels of LmGSTs1 and LmGSTs7 were unaltered in locusts treated with all tested concentrations of chlorpyrifos (data not shown). Whereas chlorpyrifos induced significantly the expression of LmGSTt1 mRNA at all tested concentrations (Fig. 2B).

3.3. Effect of chlorpyrifos on protein expression of L. migratoria GSTs

Four representative L. migratoria GSTs proteins were detected by Western blot (Fig. 3A and B). Chlorpyrifos at 40 μg/mL caused statistically significant decreases of LmGSTs5 protein levels in locusts. The protein levels of LmGSTt1 and LmGSTu1 were significantly decreased at higher doses of chlorpyrifos (20 and 40 μg/mL). On the opposite, chlorpyrifos caused the increases of LmGSTd1 protein levels in a dose-dependent manner.

4. Discussion

Chlorpyrifos, an organophosphate, is one of the widely used insecticides for pest control in various agricultural and animal husbandry operations. When chlorpyrifos enters the body of locust, it moves to all parts of the body. Chlorpyrifos works by blocking acetylcholinesterase which hydrolyzes the neurotransmitter acetylcholine. We focus on the effects of chlorpyrifos on locust GSTs in this study.

GSTs are useful biomarkers for metals and organic pollutants yielding oxidative stress and have also been useful as an indicator of pesticide exposures [19,20]. Insect GSTs could be inhibited by organophosphates, organochlorines, and pyrethroids [21]. In the present study, decreases of DCNB and pNBC activities were observed after chlorpyrifos exposure, which might be a comprehensive effect of different GST subfamilies. Decreases of GST activity were also observed in Oreochromis niloticus, Carassius auratus, and Cyprinus carpio L. after exposed to chlorpyrifos [2,16,22]. However, the GSTs activity decrease is accompanied with not only the decrease of most GSTs transcription but also the increase of some GSTs transcription in kidney and gill after C. carpio exposed to chlorpyrifos [16]. Similar results were observed in the present study. Among the ten locust GSTs, the mRNA levels of five sigma GSTs, LmGSTt1, and LmGSTu1 were decreased while those of LmGSTs1 and LmGSTs7 were unchanged. In accordance with their mRNA levels, the protein levels of LmGSTs5, LmGSTt1 and LmGSTu1 were decreased, too. However, the mRNA levels of LmGSTd1 were significant increased after chlorpyrifos exposure. In accordance with its mRNA levels, the protein levels of LmGSTd1 were increased, though the increased ratio of protein was less than that of mRNA in the highest dose. It might be a delayed effect of protein. In the present study, no GST activities increase was observed after chlorpyrifos exposure, which might be a comprehensive effect of different GST subfamilies. The opposite change of different locust GSTs might due to the different physiological function of different GST families.

The direct participation of GSTs in the mechanism of insecticide detoxification as well as the overexpression of the GST in pesticide-resistant strains of insects has been reported [23,24]. Some GSTs might involve in the detoxification of insecticides [5,25]. We have proved that LmGSTu1 played an important role in chlorpyrifos and carbaryl detoxification [18]. But the expressions of LmGSTu1 mRNA and protein were decreased. It indicated that the chlorpyrifos could not induce but inhibit the expression of LmGSTu1. They are separated events between chlorpyrifos detoxification and inhibition of LmGSTu1. LmGSTs3 might involve in carbaryl detoxification [26]. LmGSTs5 plays significant roles in both carbaryl and malathion detoxification [18]. The sigma and theta classes have a much wider taxonomic distribution and likely play essential housekeeping roles [27,28]. The significant inhibition in GST activity has been noticed after chlorpyrifos exposure animal, which usually is the case under oxidative stress conditions [29,30]. As noted earlier, the unclassified locust GST is mostly closely related to the delta class. The amino acid identity between LmGSTu1 and LmGSTd1 is 38.8% [14]. However, the function differs greatly between them. The unclassified LmGST played significant roles in chlorpyrifos detoxification [18]. Delta GSTs are believed playing certain roles in insect resistance [31]. Previous studies indicate that GST and esterase probable contributions in some cases, is the major mechanism imparting resistance to different insecticides in Choristoneura rosacea [32]. It has been validated that GSTs also have a significant role in insect resistance to OP insecticides [33]. Delta and epsilon family GSTs usually participate in the resistance to insecticides. It has been reported that exposure to sublethal concentrations of chlorpyrifos induced up-expression of LsGSTe1 and LsGSTs2 Laodelphax striatellus [34]. The elevated expression of GST genes induced by insecticides might be related to the enhanced tolerance of this insect to insecticides and xenobiotics. However, the elevation of GST activity was not always observed in resistant strain insects. There was no significant change in the activities of GST between chlorpyrifos-resistant strain and...


