Partial characterization of glutathione S-transferases from different field populations of *Liposcelis bostrychophila*

Dou, W., Niu, J.-Z., Xiao, L.-S., Deng, Y.-X., Wang, J.-J.*#
Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection, Southwest University, Chongqing 400716, P. R. China, E-mail: jjwang7008@yahoo.com

* Corresponding author
# Presenting author

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Abstract

Glutathione S-transferases (GSTs) from different field populations of *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) were purified by glutathione-agarose affinity chromatography and characterized subsequently by their Michaelis-Menten kinetics toward the artificial substrates 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH). The specific activity of the affinity of purified GST toward CDNB was highest in lab population, 2.7-fold higher than that of Guanghan population with the lowest value observed. GSTs of lab population exhibited higher apparent Michaelis-Menten constants (Km) and higher maximal velocity (Vmax) values than those of Jianyang and Guanghan populations, revealing that the latter two populations exhibited significantly higher affinities to the test substrates. Inhibition kinetics showed that all test compounds (ethacrynic acid, curcumin, diethyl maleate, bromosulfalein, and carbosulfan) possessed significant inhibitory effects on GSTs. Curcumin appeared to be the most effective inhibitor. Compared to the other compounds, diethyl maleate and carbosulfan exhibited their I50s (the concentration required to inhibit 50% of GSTs activity) at higher concentrations.

Keywords: GSTs, purification, Psocids, Xenobiotic compounds, Field populations

1. Introduction

Liposcelis bostrychophila Badonnel is a prevalent insect pest in large grain depots in China. Routine fumigations of warehouses and storage facilities with methyl bromide have failed to control the pest (Ho and Winks, 1995). Moreover, the serious damage by psocids in Australia has increased in recent years, mostly due to the failure of almost all currently registered grain protectants against these pests (Nayak and Daglish, 2006, 2007).

Glutathione S-transferases (GSTs; E.C. 2.5.1.18) are a large family of multifunctional enzymes found ubiquitously in aerobic organisms (Clark et al., 1984; Ranson et al., 2001). The majority of studies on insect GSTs have focused on their role in detoxifying xenobiotic compounds, in particular insecticides and plant allelochemicals and, more recently, their role in mediating oxidative stress responses (Fournier et al., 1992; Ranson et al., 2001; Vontas et al., 2001, 2002; Sawicki et al., 2003). Increased GST activity has been detected in strains of insects resistant to organophosphates and organochlorines (Fournier et al., 1992), and this enzyme family has recently been implicated in resistance to pyrethroid insecticides (Kostaropoulos et al., 2001; Vontas et al., 2001). What's more, GST-based resistance to insecticides was described to be facilitated by the increase in the level of expression of one or more GSTs.

Currently, research on the psocid pest genus *Liposcelis continues* to increase, but knowledge of the pest remains limited compared to other stored-products insect pests. Very recently, GSTs from *L. bostrychophila* and *L. paeta* were purified and their partial characterizations analyzed from the perspective of resistance of different strains (Dou et al., 2009; Wu et al., 2009). In this paper, the partial characterization of purified GSTs were investigated and further comparatively analyzed from different field populations of *L. bostrychophila*.

2. Materials and methods

2.1. Chemicals and insecticides

Reduced glutathione (GSH, Sigma), 1-chloro-2, 4-dinitrobenzene (CDNB, Shanghai Chemical Ltd.), and other biochemical reagents were of analytical grade. The xenobiotic compounds used for the inhibition bioassays were: ethacrynic acid, 94% curcumin, 97% diethyl maleate (all above from Sigma, St. Louis, MO, USA), bromosulfalein (Dow AgroSciences LLC, USA), and 86% carbosulfan (Zhejiang Chemical Co. Ltd., China).

2.2. Test insects

Three field populations of L. paeta were collected from wheat warehouses in Jianyang and Guanghan of Sichuan Province, China in 2004, and Beibei of Chongqing Municipality, China in 1990. The insects were reared on an artificial diet consisting of whole wheat flour, skimmed milk, and yeast powder (10:1:1) in a temperature controlled room at 27 ± 1 ºC, r.h. 75%-80% and a scotoperiod of 24 h. Stock colonies were never exposed to blended gas or insecticides. All experiments were conducted under the conditions described above with three to five day-old adult females of all populations.

2.3. Purification of enzyme

GSTs from different field populations of *L. bostrychophila* were purified employing glutathione-agarose affinity chromatography as described by Fournier et al. (1992).

Psocid samples of 50 mg were homogenized manually on ice in 1.5 m Lsodium phosphate buffer (20 mM, pH 7.2). The homogenate was centrifuged for 5 min at 5000 g and 4ºC. The pellet was discarded and the supernatant was again centrifuged for 15 min at 17,500 g and 4ºC. Finally, the supernatant was filtered through a 0.45 µm Millex-HV filter (Millipore, USA) and loaded on a GSH-reduced agarose gel column (GE Healthcare, USA) with a bed volume of 2 mL. The column was equilibrated with 20 mM sodium phosphate buffer, pH 7.2 and washed with the same buffer. The bound GST enzyme was eluted with 50 mM Tris-HCl buffer, pH 8.0, containing 5 mM reduced GSH. The collected fractions (200 µLeach) containing GST activity were pooled for further analysis.

2.4. Enzyme activity and kinetics

GSTs activities were determined using 1-chloro-2, 4-dinitrobenzene (CDNB) and reduced GSH as substrates according to Habig et al. (1974) with slight modifications. The total reaction volume was 250 µL, consisting of 50 µLeach enzyme solution, CDNB (1% ethanol (v/v) included) and GSH in Tris-HCl buffer, giving final concentrations of 0.24 mM and 2.4 mM of CDNB and GSH, respectively. The non-enzymatic reaction of CDNB with GSH measured without enzyme solution served as control. The change in absorbance was measured continuously for 5 min at 340 nm and 37°C in a Thermomax kinetic microplate reader. Changes in absorbance per minute were converted into nmol CDNB conjugated/min/mg protein using the extinction coefficient of the resulting 2, 4-dinitrophenyl-glutathione: ϵ340nm = 9.6 mM /cm (Habig et al., 1974). Protein concentrations of the enzyme samples were determined at 595 nm and bovine serum albumin (BSA) as standard (Bradford 1976).

Values of the apparent Michaelis-Menten constant (Km) and maximal velocity (Vmax) of purified GSTs from L. bostrychophila were determined for CDNB and GSH, respectively. The activity was recorded toward a range of concentrations of CDNB (0.08-0.96 mM) or GSH (0.8-9.6 mM), while the concentration of the other substrate was kept constant at 2.4 mM or 0.24 mM of GSH or CDNB, respectively. Km and Vmax values were calculated by SPSS 10.0 (SPSS, Inc., USA) using the Michanelis-Menten equation.

2.5. Inhibition studies

The inhibition studies were performed using the standard GST assay conditions but in the absence and presence of various concentrations of inhibitors. Stock solutions of the inhibitors ethacrynic acid, bromosulfalein, curcumin, diethyl maleate, and carbosulfan were prepared in ethanol and diluted with Tris-HCl (50 mM, pH 7.5), thus the highest ethanol concentration was 1% in the test solutions. Twenty five µLof the enzyme source and 25 µL inhibitor solutions with appropriate concentrations were firstly incubated for 5 min at 25oC and then added to the substrate mixture as described above.

3. Results

3.1. Purification of GSTs from different field populations of L. bostrychophila

The chromatography elution profile from GSH-affinity chromatography column was shown in Figure 1. Totally, first 8 tubes (1 mL per tube) were collected and assayed. Both the fourth tubes exhibited highest specific activity of GSTs in Jianyang and Guanghan populations. For lab population, the highest activity of GSTs was recorded in fifth tube. Table 1 presents the results of the purified GSTs activities from different populations. The specific activity of GSTs in lab population was 1.21 μmol min/mg, 2.7-fold higher than that of Guanghan population (lowest value was recorded, 0.47 μmol/min/mg).

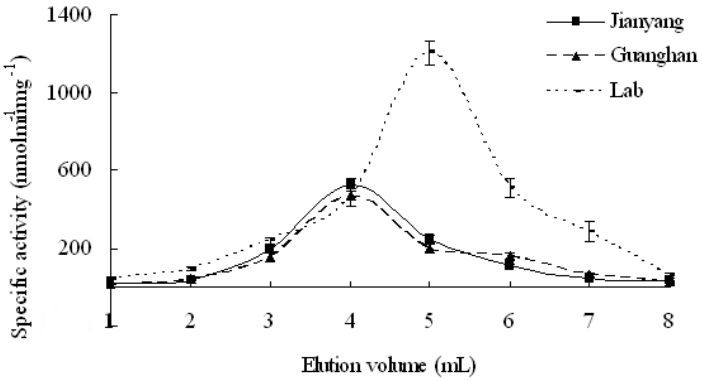

Figure. 1 The elution profile for GSTs activities from different field populations of *Liposcelis bostrychophila*. Each value represents the mean ± SE of three independent experiments.

Table 1 Activity comparison of GSTs purified from different field populations of Liposcelis Bostrychophila.

Population	Total protein (μg)	Total activity (nmol/min)	Specific activity (μmol/min/mg)
Jianyang	6.81 ± 0.37 a	3.57 ± 0.12 a	0.53 ± 0.04 a
Guanghan	7.08 ± 0.37 a	3.33 ± 0.13 a	0.47 ± 0.02 a
Lab	7.94 ± 0.30 b	9.59 ± 0.20 b	1.21 ± 0.06 b

Note: Each value represents the mean (M ± SE) of three replications. Means within the same column followed by different letters are significantly different at $P < 0.05$.

3.2. Kinetics of GSTs

GSTs difference among the different populations was also observed for kinetic parameters (Table 2). The statistical analyses revealed that GSTs in lab population showed a lower affinity to the substrate CDNB or GSH (i.e. higher Km values, 0.33 mM or 6.53 mM, respectively). Meanwhile, GSTs in lab population have higher catalytic activities for CDNB and GSH (i.e. higher Vmax values, 2.37 nmol/min and 3.21 nmol/ min).

Table 2 Kinetic properties of GSTs from different field populations of *Liposcelis bostrychophila*.

	GSH		CDNB	
Population	Km (mM)	Vmax (μmol/ min/mg pro)	Km (μM)	Vmax (μmol/min/ mg pro)
Jianyang	3.4 ± 0.1 a	2.17 ± 0.24 b	289 ± 21 b	1.19 ± 0.12 a
Guanghan	3.5 ± 0.2 a	1.30 ± 0.12 a	138 ± 19 a	1.15 ± 0.13 a
Lab	6.5 ± 0.2 b	3.21 ± 0.29 c	331 ± 13 c	2.37 ± 0.14 b

Note: Each value represents the mean (M ± SE) of three replications. Means within the same column followed by different letters are significantly different at $P < 0.05$.

3.3. In vitro inhibition of GSTs

Based on their I50s (the concentration required to inhibit 50% of GSTs activity), the efficiencies of the tested inhibitors were compared and the corresponding I50s of the tested compounds were listed in Table 3. All compounds exhibited good inhibition effects on GSTs in vitro, and curcumin was considered to be most effective inhibitor. Among the different field populations, the lab population seemed least sensitive to all the test compounds (highest I50s were observed). For Jianyang and Guanghan populations, no significant differences were seen.

Table 3 The I50 inhibition constants of xenobiotic compounds on purified GSTs from different field populations of Liposcelis Bostrychophila.

	I50 (μM)		
Inhibitor	**Jianyang**	**Guanghan**	**Lab**
Ethacrynic acid	0.324 ± 0.057 a	0.278 ± 0.064 a	0.696 ± 0.131 b
Curcumin	0.0080 ± 0.0013 a	0.0073 ± 0.0011 a	0.0096 ± 0.0013 a
Diethyl maleate	2.28 ± 0.34 a	2.13 ± 0.31 a	3.33 ± 0.46 b
Bromosulfalein	0.225 ± 0.046 ab	0.167 ± 0.043 a	0.326 ± 0.057 b
Carbosulfan	6.32 ± 0.73 a	6.19 ± 0.34 a	9.05 ± 1.02 b

Note: Each value represented the mean (M ± SE) of three replications. Means within the same row followed by different letters are significantly different ($P < 0.05$).

4. Discussion

In the current study, the partial characterization of purified GSTs from different field populations of *L. bostrychophila* was compared. Important differences were observed when considering the kinetics assays. For both the tested substrates, the Lab population expressed higher Kms suggesting lower affinities of GSTs toward the substrates in this population.

For *Liposcelis bostrychophila* , a previous study on crude preparations of GSTs has revealed that there was some correlation between resistance development and the quantity of GSTs (Dou et al., 2006). Following GSTs purification and partial characterization analysis for L. bostrychophila and L. paeta, the relationship between GSTs level and resistance of different strains or field populations were further clarified (Dou et al., 2009; Wu et al., 2009). The current results demonstrate that the two populations from Sichuan province possess less specific activities while kinetics analysis reveales that these two populations exhibited significantly higher affinities to the test substrates. GSTs acitivity difference between the two populations may result from local differences insecticide practice.

The CDNB conjugating activity of psocids GSTs was shown to be inhibited by several xenobiotic compounds tested. Among the compounds, curcumin expressed the most effective inhibitory effects with its I50 values at sub-micromolar concentrations. In Anopheles dirus, bromosulfophthalein exhibited its I50 values of 0.805 μM (Prapanthadara et al., 1996). Similarly, some insect GSTs exhibited I50 values for ethacrynic acid at sub-micromolar concentrations, i.e., *Nilaparvata lugens* (40 nM), *Blattella germanica* (350 nM), and *Spodoptera frugiperda* (150nM) (Yu and Huang, 2000; Vontas et al., 2002; Yu, 2002).

Glutathione S-transferases are a large family of multifunctional enzymes involved in the detoxification of a wide range of xenobiotics including insecticides (Enayati et al. 2005). In insect species, many GST enzymes are differentially regulated in response to various inducers or environmental signals or in a tissue- or developmental-specific manner. The current study presented some basic biochemical information of GSTs from different field populations of the psocid, and surely will help in understanding the exact role in detoxification of the various GST isoenzymes and to understand the evolutionary aspects of detoxification related to each insect species.

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