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Cloning and expression of a JHA-inducible glutathione *S*-transferase gene in the common cutworm *Spodoptera litura* (Lepidoptera: Noctuidae)

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ABSTRACT

The juvenile hormone titer during the last instar is a crucial determining switch for metamorphosis in lepidopterans. We previously observed that the induction of glutathione *S*-transferase (GST) activity by a juvenile hormone analog (JHA) is reversely proportional to the occurrence of JHA-induced supernumerary instars following topical application of JHA to *Spodoptera litura* during the last instar period. In this paper, at least five JHA-induced GSTs were purified by glutathione-affinity chromatography, and one was further isolated for determination of the N-terminal sequence. The corresponding cDNA was cloned and named *SlGST1* (GenBank accession no. AY506545). *Sl*GST1 was classified into the epsilon class of GSTs by phylogenetic analysis. Northern blot analyses further showed that the *SlGST1* in fat bodies can be induced by JHA, particularly in 0-day-old sixth-instar larvae, in which induction was much higher than that in 1- and 2-day-old sixth-instar larvae. To our knowledge, this is the first JHA-inducible GST to have been identified, and we believe that it will provide new insight into the role of GST in insect development, particularly during metamorphosis.

Introduction

Glutathione *S*-transferases (GSTs; E.C. 2.5.1.18), a group of multifunctional dimeric enzymes (homo- or heterodimers with subunits ranging from 20 to 30 kDa), catalyze the conjugation of glutathione with a wide spectrum of endogenous and xenobiotic compounds for detoxification, protection from oxidative damage, isomerization, and intracellular transportation (Hayes and Pulford, 1995). In mammals, GSTs comprise at least eight classes: alpha, mu, pi, theta, sigma, omega, zeta, and kappa (Sheehan et al., 2001). With the mammalian GST nomenclature system extended to insect GSTs, six classes of insect cytosolic GSTs have been identified (sigma, zeta, theta, delta, epsilon, and omega), and several remain unclassified (Chelvanayagam et al., 2001; Sheehan et al., 2001; Ding et al., 2003). Among these, delta and epsilon GSTs are unique in insects and are primarily involved in the process of xenobiotic metabolism (Ranson et al., 2001; Ding et al., 2003).

GSTs are also involved in the processes of insect development. In *Choristoneura fumiferana*, GSTs are involved in diapause and are regulated by insect molting hormones (Feng et al., 1999, 2001). GSTs from *Anopheles gambiae* and cutworms *Spodoptera litura* are regulated variously in different developmental stages (Strode et al., 2006; Huang et al., 2011). A delta GST identified from *Corcyra cephalonica* has been proposed to be involved in development, according to its expression profile and hormone-regulated activity (Gullipalli et al., 2010). The expression of the sigma and delta classes of GST in *Bombyx mori* is regulated by a juvenile hormone analog (JHA) and 20-hydroxyecdysone (Zou et al., 2011). These results imply that GSTs may play roles in certain physiological processes of insect development.

Juvenile hormone (JH) is one of the most important hormones in insects and its level determines the switch of metamorphosis. During the larval–pupal transition, the JH level in insect hemolymph drops to a fairly low level, and thereby triggers the pupal development (Riddiford et al., 2003). Application of exogenous JH in insect larval–pupal transition stage results in supernumerary larvae (Hatakoshi et al., 1986; Wu and Lu, 2008). We previously reported that GST activity in *S. litura* fat bodies was induced significantly by JHA, and this induction phenomenon only occurred in 0-day-old final (sixth)-instar larvae, whereas no induction was observed in 1- and 2-day-old sixth-instar larvae (Wu and Lu, 2008). In the present study, the GST most significantly induced by JHA in *S. litura* fat bodies was purified by affinity chromatography and isolated from gels. Following the determination of its N-terminal 16 amino acids, the corresponding cDNA was cloned and its expression in the larval fat bodies of cutworms was determined.

Materials and methods

Experimental insects

S. litura egg masses collected from taro fields were reared on an artificial diet (40% wheat flour, 20% yellow bean powder, 20% white

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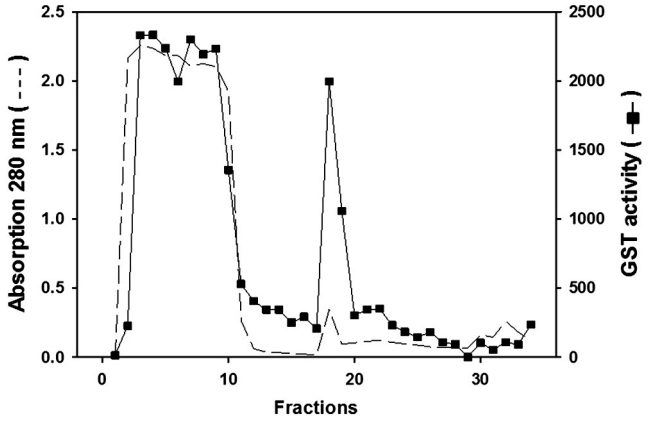

Fig. 1. Elution profile for affinity chromatography of GSTs from *Spodoptera litura* larval fat bodies. Proteins were detected by absorption at 280 nm (——), and GST activity was measured using CDNB as the substrate (—■—).

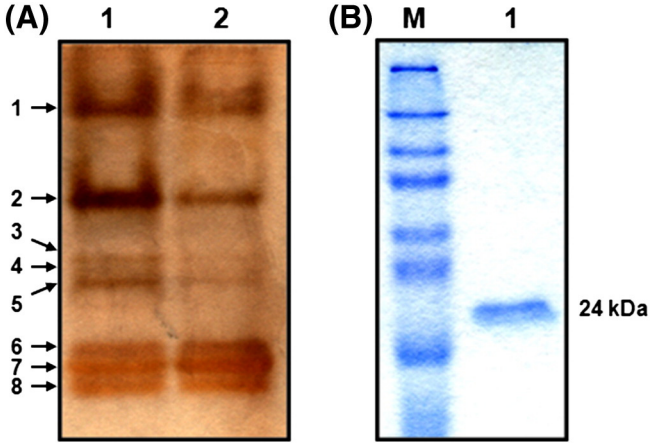

Fig. 2. Gel electrophoresis profiles of the bound GST fraction. (A) Native PAGE analysis of the bound fraction of *Spodoptera litura* larval fat bodies from GSH–agarose affinity columns. Lanes 1 and 2 represent JHA-treated and control larvae, respectively. (B) SDS-PAGE analysis of band 2 in panel A. M: molecular marker.

Dutch runner bean powder, and 18% yeast powder in 2% agarose) in an environmental chamber at 25 °C with a 12:12-h L:D photoperiod (Ou-Yang and Chu, 1988). Newly hatched larvae were reared together in plastic cups (9.5 cm diameter × 5.5 cm height) until pre-ecdysis of the fifth instar. The pre-ecdysed larvae were randomly chosen and maintained individually in a 30-well rearing plate without any diet for molting. After ecdysis, at the beginning of day 0, a sufficient amount of diet was added to each well to feed the larvae.

Chemicals

1-Chloro-2,4-dinitrobenzene (CDNB), glutathione (GSH; reduced form) and GSH–agarose affinity gel were purchased from Sigma Chemical Company (St Louis, MO). Pyriproxyfen, a JHA, was a gift from Sumitomo Chemical Taiwan Co., Ltd (Taipei, Taiwan). All chemicals were of analytic grade.

Isolation of JHA-induced GST

Our previous results showed that 100 μg JHA/larva had the most pronounced effect on the metamorphosis of sixth-instar larvae and that maximal GST induction activity can be achieved at 24 h after treatment of a 0-day-old sixth-instar larva with 100 μg JHA/larva (Wu and Lu, 2008). Therefore, these dosage and timing were applied to isolate JHA-induced GSTs.

Newly ecdysed (0-day-old) sixth-instar larvae were topically treated with 100 μg pyriproxyfen in 1 μL acetone on the dorsal cuticle of the abdomen using a microliter syringe (Hamilton Co., Reno, NV); the control group was treated with 1 μL acetone. Unless otherwise stated, all procedures in the purification scheme were performed at 4 °C. Working with groups of 30 larvae, 200 fat bodies were collected from treated and control female larvae 24 h after treatments, rinsed in 1.15% KCl, and homogenized in 75 mL of ice-cold 100 mM Tris–Cl buffer (pH 8.0, with 1 mM EDTA and 1 mM DTT) by a motor-driven tissue grinder for 2 min. The crude homogenate was centrifuged (20,000 ×*g*, 20 min, 4 °C), and the supernatant was filtered sequentially through 5- and 0.45-μm MF™ membrane filters (Millipore, Bedford, MA) to remove cell debris and floating lipids. The filtrate was then applied to a GSH–agarose affinity column (1 × 10 cm), eluted with 100 mM Tris-buffer (pH 8.0) at 9 mL/h, and collected at 20 min/fraction. The eluate was monitored for absorption at 280 nm, and each fraction was analyzed for GST activity toward CDNB. The bound GSTs were eluted with 10 mM GSH and concentrated using an Amicon Centricon-10 (Millipore) to remove GSH. The protein concentration was estimated using a BCA protein assay reagent kit (Pierce, Rockford, IL), using bovine serum albumin as the quantitative standard. All samples were stored at −80 °C for further analysis.

Polyacrylamide gel electrophoresis (PAGE)

The eluate from the GSH affinity agarose gel was analyzed via native PAGE and SDS-PAGE. SDS-PAGE was performed according to the method of Laemmli (1970) using 4.0% stacking gels and 12.5% running gels (0.75 mm thick) in a Bio-Rad Mini Protean II Cell (Bio-Rad Laboratories, Inc. Hercules, CA). Protein samples for SDS-PAGE were prepared in a 5× sample buffer (0.312 M Tris–Cl, pH 6.8, 0.05% bromophenol blue, 50% glycerol, 10% SDS, and 25% β-mercaptoethanol) and heated in boiling water for 5 min. They were then applied to the SDS-PAGE, stacked at 80 V, and resolved at 120 V. After electrophoresis, the protein bands were visualized with Coomassie Brilliant Blue R-250.

For native PAGE, all procedures followed those of the SDS-PAGE but without the addition of SDS. After electrophoresis, the gel was subjected to silver staining.

Table 1
GST purification steps

Step	Total volume (mL)	Total protein (mg)	Total activity (nmol/min)	Specific activity (nmol/min/mg protein)	Yield (%)	Purification ratio
1. Centrifugation (20,000 ×*g*)	69.80	706.4	1126.0	1.6	100.1	1.0
2. Filtration (5.0 μm)	68.00	671.8	1047.9	1.6	93.1	1.0
3. Filtration (0.45 μm)	68.00	548.1	1040.5	1.9	92.4	1.2
4. Bound fraction	0.75	0.4	11.8	32.2	1.1	20.2
5. Unbound fraction	8.00	78.6	156.4	2.0	13.9	