SeGSTo, a novel glutathione *S*-transferase from the beet armyworm (*Spodoptera exigua*), involved in detoxification and oxidative stress

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Abstract Members of the glutathione *S*-transferase superfamily can protect organisms against oxidative stress. In this study, we characterized an omega glutathione *S*-transferase from *Spodoptera exigua* (SeGSTo). The *SeGSTo* gene contains an open reading frame (ORF) of 744 nucleotides encoding a 248-amino acid polypeptide. The predicted molecular mass and isoelectric point of SeGSTo are 29007 Da and 7.74, respectively. Multiple amino acid sequence alignment analysis shows that the SeGSTo sequence is closely related to the class 4 GSTo of *Bombyx mori* BmGSTo4 (77 % protein sequence similarity). Homologous modeling and molecular docking reveal that Cys35 may play an essential role in the catalytic process. Additionally, the phylogenetic tree indicates that SeGSTo belongs to the omega group of the GST superfamily. During *S. exigua* development, SeGSTo is expressed in the midgut of the fifth instar larval stage, but not in the epidermis or fat body. Identification of recombinant SeGSTo via SDS-PAGE and Western blot shows that its molecular mass is 30 kDa. The recombinant SeGSTo was able to protect super-coiled DNA from damage in a metal-catalyzed oxidation (MCO) system and catalyze the 1-chloro-2,4-dinitrobenzene (CDNB), but not 1,2-dichloro-4-nitrobenzene (DCNB), 4-nitrophenethyl bromide (4-NPB), or 4-nitrobenzyl chloride (4-NBC). The optimal reaction pH and temperature were 8 and 50 °C, respectively, in the catalysis of CDNB by recombinant SeGSTo. The mRNA expression of *SeGSTo* was up-

regulated by various oxidative stresses, such as CdCl₂, CuSO₄, and isoprocab, and the catalytic activity of recombinant SeGSTo was noticeably inhibited by heavy metals (Cu²⁺ and Cd²⁺) and various pesticides. Taken together, these results indicate that SeGSTo plays an important role in the antioxidant and detoxification of pesticides.

Keywords Antioxidant enzyme · Detoxification · Oxidative stress · *Spodoptera exigua* · Beet armyworm

Introduction

Glutathione *S*-transferases (GSTs; EC 2.5.1.18) have been investigated extensively in both prokaryotic and eukaryotic organisms and are able to conjugate with reduced glutathione (GSH) to metabolize xenobiotics (Li et al. 2009). Moreover, glutathione *S*-transferases are also responsible for combating oxidative stress and protecting the genetic material from damage by reactive oxygen species (ROS) including hydrogen peroxide (H₂O₂), superoxide anion (O₂^{•−}) and hydroxyl radical (OH[•]) (Enayati et al. 2005).

Most GSTs are present in homo- as well as heterodimeric forms, and each subunit contains two binding domains, a GSH-binding site (G-site) and a hydrophobic binding site (H-site). The G-site is located in the N-terminal thioredoxin-fold domain that is highly conserved across different GST groups; the H-site is located in the C-terminal alpha helical domain that is highly varied among different groups that endows each GST with distinct characteristics in topology and substrate specificity (Gullipalli et al. 2010). In the G-site, tyrosine (Tyr), serine (Ser), or cysteine (Cys) residues have been demonstrated to be the essential mediator of GSH catalytic activation. Tyr/Ser plays critical roles in promoting the

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formation and stabilization of the highly reactive thiolate anion via providing a hydrogen bond (Saranya Revathy et al. 2012).

The glutathione *S*-transferase family is a multifunctional superfamily that can be categorized based on the sequence similarities, 3D structure, and immunological cross-reactivity into at least 15 groups including alpha, beta, delta, epsilon, kappa, lambda, mu, omega, phi, pi, rho, sigma, tau, theta, and zeta (Hayes et al. 2005). In insects, glutathione *S*-transferases can be classified into six groups including the ubiquitous omega, sigma, theta, and zeta groups, insect-specific delta and epsilon groups, as well as unclassified GSTs (Chelvanayagam et al. 2001; Ranson et al. 2002).

To date, GSTs from different invertebrate and vertebrate groups have been demonstrated to play key roles in relieving oxidative stress and in the detoxification of foreign chemicals. However, only a few of the GST families have been systematically investigated in insect species including *Anopheles gambiae*, *Apis mellifera*, *Bombyx mori*, *Drosophila melanogaster*, and *Tribolium castaneum* (Ding et al. 2003; Corona and Robinson 2006; Yu et al. 2008; Ranson et al. 2002; Shi et al. 2012). In *Apis cerana cerana*, AccGSTO2 and AccGSTS1 play a crucial role in cellular antioxidant defenses (Yan et al. 2013; Zhang et al. 2013). Moreover, many studies focused on the insect-specific GST groups (delta and epsilon) that are important in the metabolism of pesticides and development of resistance (Enayati et al. 2005; Lumjuan et al. 2011; Low et al. 2010). Indeed, evidence for the involvement of GSTo in the detoxification of organophosphorus insecticides and antioxidative processes was observed in *B. mori* (Yamamoto et al. 2009).

The beet armyworm *Spodoptera exigua* is a serious pest that causes massive agricultural losses worldwide, especially in developing countries. Data from the Arthropod Pesticide Resistance Database indicate that *S. exigua* has developed insecticide resistance to 38 active ingredients (APRD 2016). Therefore, understanding the mechanisms of insecticide resistance would help in pest management. In this study, we cloned SeGSTo from the cDNA library of *S. exigua*. Bioinformatic analysis at different levels and perspectives was performed to deduce the functions of SeGSTo. We measured the expression profile of *SeGSTo* in different tissues and developmental stages. Furthermore, we investigated the enzyme activity and interaction between recombinant SeGSTo and pesticides.

Materials and methods

Animals

Beet armyworm (*S. exigua*) was reared in an incubator under optimal culture conditions (temperature: 25 ± 1 °C, relative

humidity: 60 ± 5 %, photoperiod: 16-h light/8-h dark) with an artificial diet, according to the method of Wan et al. 2012.

cDNA library screening and bioinformatic analysis

Total RNAs were isolated from all the developmental stages of *S. exigua* larvae using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and the target clone encoding *SeGSTo* was identified by library sequencing using an ABI 310 automated DNA sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). To confirm the sequence of the *SeGSTo*, plasmid DNA from the clone was extracted using the Wizard Mini-Prep Kit (Promega) and sequenced. Subsequently, the sequence was validated using BLAST software from NCBI (<http://www.ncbi.nlm.nih.gov/BLAST>). Simultaneously, the functional domains were identified using the Conserved Domains tool (<http://www.ncbi.nlm.nih.gov/cdd/>).

Based on the BLAST results, the top ranked amino acid sequences with higher similarity were downloaded and aligned with the SeGSTo sequence using MacVector (ver. 6.5, Oxford Molecular Ltd.). Meanwhile, the protein parameters (molecular mass and isoelectric point) of SeGSTo were predicted using the Compute pI/Mw tool (http://web.expasy.org/compute_pi/). For the investigation of the tertiary structure and the interaction between SeGSTo and GSH, SWISS-MODEL (<http://swissmodel.expasy.org/>) was employed to build the 3-D structure (Arnold et al. 2006), and AutoDock (The Scripps Research Institute) was used to mimic the reaction. The phylogenetic tree was constructed with MEGA 6 (Tamura et al. 2013) to assess the relationship with other GST sequences submitted to NCBI.

Recombinant SeGSTo expression and purification

The genetically engineered *Autographa californica* nucleopolyhedrovirus (AcNPV) expressing recombinant SeGSTo was produced using the Bac-to-Bac® Baculovirus Expression System (Invitrogen) and its host cell line, *Spodoptera frugiperda* (Sf9). The *SeGSTo* sequence was PCR-amplified from *pBluescript-SeGSTo* using the forward primer 5'-GAATTCATGGTTGGAACAAACAAGGGA\AAC-3' and the reverse primer 5'-CTCGAGATGATGAT\GATGATGATGCTTCTGGATCAGGAAATCGTAAAC-3'. The reverse primer of *SeGSTo* contained a His-tag to facilitate the purification of the recombinant protein. The PCR cycling conditions were as follows: 94 °C for 3 min, 30 cycles of amplification (94 °C for 30 s, 58 °C for 60 s, and 72 °C for 1 min), and 72 °C for 5 min. The PCR products were purified and ligated into the transfer vector, the pGEM-T vector (Promega), before being inserted into the expression vector pFastBac1. Subsequently, the engineered plasmids were extracted using the Wizard mini kit (Promega, Madison, WI,

USA). The identity of the inserted sequence was confirmed using the ABI 310 automated DNA sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). According to the Bac-to-Bac Baculovirus Expression System protocol (Invitrogen), the recombinant bacmid DNA containing the *SeGSTo* sequence was obtained via homologous recombination in MAX efficiency DH10Bac cells (containing bacmid and helper). The Cellfectin II reagent (Invitrogen) was used to increase the efficiency of recombinant bacmid DNA transfection into the Sf9 cells. TC100 medium (Gibco BRL) supplemented with 10 % fetal bovine serum (FBS, Gibco BRL) was used to culture the transfected cells at 27 °C. After 7 days, the Sf9 cells were further passaged in TC100 medium at 27 °C. After 3 days, the amplified recombinant baculoviruses were harvested and the recombinant proteins were purified using the MagneHisTM Protein Purification System (Promega). The quality and concentration of the recombinant protein was determined using the Bio-Rad Protein Assay Kit, and the molecular weight was measured using 12 % SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

In vitro DNA protection assay

The DNA protection capability of SeGSTo was measured using metal-catalyzed oxidation (MCO) DNA cleavage protection assays as described previously (Hu et al. 2010). Different concentrations of purified recombinant SeGSTo protein (20 to 80 ng/mL) were added into the MCO system (16.5 μ M FeCl₃, 3.3 mM DTT) to detoxify the oxidative damage of DNA. The reaction mixtures were incubated in a total volume of 50 μ L at 37 °C for 2.5 h. Subsequently, pUC18 super-coiled plasmid DNA (1000 ng) was added to each reaction mixture and incubated at 37 °C for 2.5 h. DNA degradation was visualized by electrophoresis through a 1.0 % agarose gel containing ethidium bromide.

Collection of tissues, RNA isolation, and Northern blot

Different tissues including the epidermis, fat body, and midgut were collected from healthy *S. exigua* worms by dissection under a stereomicroscope (Zeiss, Jena, Germany) with a cooled platform. The tissues were washed with phosphate-buffered saline (PBS; 140 mM NaCl, 27 mM KCl, 8 mM Na₂HPO₄, and 1.5 mM KH₂PO₄, pH 7.4) and fast frozen in liquid nitrogen before storage at -80 °C for RNA extraction. According to the manufacturer's protocol for the TRIzol reagent (Invitrogen, Carlsbad, CA, USA), no more than 0.2 g tissue was homogenized with 1 mL TRIzol for obtaining high-quality RNA from the epidermis, fat body, and midgut respectively. Total RNAs (5 μ g/lane) from different tissues were loaded on a 1.0 % formaldehyde/agarose gel and separated through electrophoresis. Subsequently, the RNAs were transferred onto a nylon blotting membrane (Schleicher &

Schuell). The *SeGSTo* cDNA was labeled with [α -³²P] dATP (Amersham Biosciences) to produce the radioactive probe using the Prime-It II Random Primer Labeling kit (Stratagene, La Jolla, CA, USA). As previously described (Hu et al. 2010), the radioactive cDNA probe was diluted in hybridization buffer, which consisted of the following: 5 $\times$ SSC (1 $\times$ SSC consisting of 0.15 M sodium chloride and 0.15 M sodium citrate), 5 $\times$ Denhardt's solution (0.1 % each of BSA, Ficoll, and polyvinylpyrrolidone), 0.5 % SDS, and 100 mg/ml denatured salmon sperm DNA. The nylon blotting membrane was incubated with the radioactive cDNA probe at 42 °C for hybridization. The membrane was washed with 0.1 % SDS and 0.2 $\times$ SSC at 65 °C for 30 min (3 repeats) before exposure to autoradiography film at -80 °C.

Enzymatic activity assays

The enzymatic activity of recombinant SeGSTo was measured according to a previously published method (Habig et al. 1974) with slight modifications. Each reaction mixture contained 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), 10 mM reduced GSH, an appropriate amount of recombinant SeGSTo, and 100 mM sodium phosphate buffer (pH 6.5), which was used to control the pH value and supplement the reaction mixture up to 100 μ L. The reaction mixtures were incubated in a water bath at 25 °C for 5 min before measuring in an ultraviolet spectrophotometer (SHIMADZU UV-1800). A blank reaction control without the recombinant protein was monitored in parallel to normalize the absorbance. Meanwhile, other potential substrates of recombinant SeGSTo including 1,2-dichloro-4-nitrobenzene (DCNB), 4-nitrobenzyl chloride (4-NBC), and 4-nitrophenethyl bromide (4-NPB) were also analyzed as described above. The optimal temperature and pH required for recombinant SeGSTo were measured using CDNB as the substrate. Furthermore, the ability of heavy metals to inhibit recombinant SeGSTo was investigated in vitro by adding CuSO₄ (0.5, 1, 2, 4, 7, or 10 μ M) or CdCl₂ (5, 10, 20, 40, 70, or 100 μ M) into the reaction mixtures, as previously described (Qin et al. 2012). Meanwhile, the influence of time was determined by incubating the reaction mixtures for different time points ranging from 5 to 30 min in the presence of CuSO₄ or CdCl₂ at a concentration of 0.5 or 5 μ M, respectively.

Enzyme-pesticide interaction analysis

The interaction between recombinant SeGSTo and different pesticides, including insecticides (imidacloprid, clothianidin, indoxacarb, cyhalothrin, dinotefuran, and isoprocarb), herbicides (cyhalofop and bispyribac), and fungicide (carbendazim and dimetachlone), was measured as described previously using CDNB as the substrate (Lai et al. 2011; Pavlidi et al. 2015). For each reaction, 50 μ L of a solution containing 1 μ M purified

recombinant SeGSTo and 100 μ M each pesticide in 100 mM sodium phosphate buffer (pH 6.5) was incubated at 25 °C for 30 min. Subsequently, the residual activity of SeGSTo was assessed according to the method described above.

Feeding experiment

S. exigua larvae were fed the cabbage with CdCl₂, CuSO₄, or isoprocarb on the first day of the fifth instar stage using leaf dipping method. Double-distilled water was served as control. For each treatment, CdCl₂, CuSO₄, or isoprocarb was diluted to 1 mg/mL, and the midgut was individually collected at 3, 6, 9, and 12 h post-treatment. RNA was isolated as described above, and genomic DNA was removed via mixing DNase-I (ThermoFisher, Waltham, MA, USA) with 2 μ g total RNA. Then first-strand cDNA was synthesized according to the protocol of First Strand cDNA Synthesis Kit (ThermoFisher). Triplicate first-strand cDNA for each sample served as templates for qRT-PCR using the SoFast™ EvaGreen® Supermix (Bio-Rad, Hercules, CA, USA) with an iQ2 Optical System (Bio-Rad). The PCR procedure conditions included an initial denaturation at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s, and 60 °C for 10 s. After all reactions, a melting curve analysis from 55 to 95 °C was applied to ensure consistency and specificity of the amplified product (Lu et al. 2013). The relative expression was calculated using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen. 2001), and *S. exigua* Ribosomal protein L10 (*SeRPL10*) was used as a reference gene for normalization (Zhu et al. 2014). The primers for *SeGSTo* were: forward primer 5'-CCTCAACGACCTCTACTC-3' and the reverse primer 5'-TCTGTAACCTTGCTGGAT-3'.

Statistical analysis

The data are expressed as the mean $\pm$ SE values from triplicate experiments and are analyzed for statistical significance using a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using the SPSS statistical software package (version 11.5; SPSS, Inc.). Differences with *P* values less than 0.05 were considered to be statistically significant for all treatments.

Results

Cloning and bioinformatic analysis

In the present study, the full-length sequence of *SeGSTo* obtained from the cDNA library of *S. exigua* was 981 bp and contains an open reading frame (ORF) of 744 nucleotides encoding a 248-amino acid polypeptide (Fig. 1a). The results from the Compute pI/Mw tool showed that the theoretical molecular mass is 29,007 Da, and the predicted isoelectric

point is 7.74. Two functional domains were identified via conserved domains: a putative glutathione binding domain (G-site) of 88 amino acids (10–97) and a putative electrophilic substrate binding domain (H-site) of 123 amino acids (112–234) (Fig. 1b). The nucleotide sequence of *SeGSTo* has been submitted to GenBank (GenBank accession number: KF595079).

The alignment of multiple amino acid sequences showed that the *SeGSTo* sequence is much more closely related to the type 4 GSTo of *B. mori* BmGSTo4 (77 % protein sequence similarity), compared with the three type 1 GSTos and two type 3 GSTos (Fig. 1b). The tertiary structure of *SeGSTo* was constructed based on the deduced protein sequence by the SWISS-MODEL program using a *B. mori* glutathione *S*-transferase omega (PDB: 3WD6) as the template (Yamamoto et al. 2013). The structure of *SeGSTo* obtained from homologous modeling includes 12 helices and 5 beta strands (Fig. 1c). Meanwhile, the molecular docking analysis using AutoDock revealed that Cys35 may play an essential role in the catalytic process. Furthermore, the helix 1 and beta strand 2 (belonging to the G-Site) and helices 5 and 12 (belonging to the H-Site) collectively forms the catalytic site (Fig. 1d).

The phylogenetic tree was constructed based on the known protein sequences of GSTs from different insect species via MEGA 6.0. The GSTs were classified to six groups: omega, zeta, sigma, theta, delta, and epsilon; the phylogenetic tree indicated that the GST from *S. exigua* in the present study belongs to the Omega group (Fig. 2).

Fig. 1 Bioinformatic analysis of *SeGSTo*. **a** The cDNA and amino acid sequence analysis of the *SeGSTo* (GenBank accession no. KF595079). The initial codon (ATG) is highlighted with a box, and the termination codon is marked with an asterisk. The putative glutathione binding region (G-site) is underlined, and the electrophilic substrate binding region (H-site) is double-underlined. The conserved G-site residues are marked with squares, and the substrate binding pockets (H-site) are indicated with circles. **b** Amino acid sequence alignment of *SeGSTo* with other known GSTs. Identical residues are shown in solid boxes. The putative G-site and H-site are indicated by solid and dashed lines, respectively. The conserved G-site residues are indicated with asterisks, and the substrate binding pockets (H-site) are marked with circles. The sources for the sequences used in the alignment are as follows: *SeGSTo* (this study, GenBank accession no. KF595079), *Bombyx mori* GSTo4 (BmGSTo4; GenBank accession no. NP_001108461.1), *Danaus plexippus* GSTo1 (DpGSTo1; GenBank accession no. EHJ65988.1), *Papilio xuthus* GSTo1 (PxGSTo1; GenBank accession no. KPI97298.1), *Spodoptera litura* GSTo1 (SIGSTo1; GenBank accession no. AEG75845.1), *B. mori* GSTo3 (BmGSTo3; GenBank accession no. NP_001040435.1), and *S. litura* GSTo3 (SIGSTo3; GenBank accession no. AIH07602.1). **c** The homologous modeling structure of *SeGSTo*. The structure was constructed using SWISS-MODEL. The α -helices are highlighted in red, and the β -strands are highlighted in blue. **d** The predicted interaction between *SeGSTo* and GSH. The GSH analogue (*S*-hexyl-GSH) is indicated using sticks and colored according to atom type (for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

Fig. 2 Phylogenetic analysis of SeGSTo with known GSTs. This tree was constructed using MEGA 6 with the algorithm of Neighbor-Joining Tree, and the numbers on the branches represent the bootstrap values for 1000 replicates

Expression of SeGSTo

To depict the expression profile of *SeGSTo*, total RNAs were isolated from three tissues (epidermis, fat body, and midgut) to conduct northern blot analysis. The results of a northern blot analysis demonstrate that *SeGSTo* is only expressed during the fifth instar in the midgut (Fig. 3a). The expression of *SeGSTo* was not detected in the epidermis or fat body (data not shown).

To further investigate the function of the *SeGSTo* gene, the baculovirus transfer vector pFastBac1 was engineered to express the ORF of *SeGSTo* under the control of the AcNPV polyhedrin promoter. The recombinant baculovirus was generated in Sf9 cells by transfection with bacmid DNA. The recombinant SeGSTo purified from the recombinant baculovirus-infected insect cells was analyzed via SDS-PAGE and confirmed by Western blot, which indicated that the approximate molecular mass of recombinant SeGSTo was 30 kDa (Fig. 3b).

Protection of super-coiled DNA by recombinant SeGSTo

To confirm the DNA protection capacity of recombinant SeGSTo, a nicked DNA assay was performed in a metal-catalyzed oxidation (MCO) system in the presence or absence of recombinant SeGSTo. The super-coiled plasmid DNA pUC18 was employed as an index to determine the efficiency of DNA protection. The results based on gel mobility

Fig. 3 Expression of SeGSTo. **a** *SeGSTo* expression during *S. exigua* development. Total RNA was isolated from the midgut of *S. exigua*. RNA was separated using 1.2 % formaldehyde agarose gel electrophoresis, transferred onto a nylon membrane, and hybridized with a radiolabeled *SeGSTo* cDNA probe, (a–d) represent the first, second, third, and three and half day of the fifth-instar larvae,

Fig. 4 In vitro DNA protection assay of super-coiled pUC18 plasmid DNA by recombinant SeGSTo in a metal-catalyzed oxidation (MCO) system. *Lane 1*: pUC18 incubated with water; *Lane 2*: pUC18 only with 1.65 mM DTT; *Lane 3*: pUC18 only with 16.5 mM FeCl₃; *Lane 4*: pUC18 with MCO system; *Lanes 5–7*: pUC18 with MCO system and a concentration gradient (20, 40, and 80 ng/mL) of purified SeGSTo. The bands corresponding to the nicked form (NF) and the super-coiled form (SF) of pUC18 plasmid DNA are indicated on the right side

indicated that the metal-catalyzed oxidation (MCO) system was able to nick super-coiled plasmid DNA through hydroxyl radicals (OH·) in the absence of recombinant SeGSTo, while DNA was protected from nicks in the presence of recombinant SeGSTo at all concentrations (Fig. 4).

Biochemical characterization of recombinant SeGSTo

To demonstrate the enzymatic characteristics of recombinant SeGSTo, the enzyme kinetic parameters of K_m and V_{max} toward four substrates in the presence of GSH were analyzed. As shown in Table 1, the results indicated that recombinant SeGSTo exhibits low catalytic activity toward CDNB alone (21.64 ± 0.94 nmol/min/mg).

For further investigation, the optimal reaction temperature and pH of recombinant SeGSTo were determined using CDNB as the substrate. The results showed that the catalytic activity reached the highest point as the temperature increased to 50 °C, and the optimal pH was 8 (Fig. 5a, b). The inhibition of catalytic activity by heavy metals was determined using

respectively; (e) represents the first day of prepupa. **(b)** SDS-PAGE (left) and Western blot analysis (right) of recombinant SeGSTo expressed in baculovirus-infected Sf9 insect cells and purified using the MagneHis™ Protein Purification System. Recombinant SeGSTo was identified using a His-tag antibody. Molecular weight standards are used as size markers

Table 1 Determination of the specific activities of recombinant SeGSTo toward different substrates

Substrate	[Substrate] (mM)	[GSH] (mM)	Temperature (°C)	pH	OD λ_{max} (nm)	K_m (mM)	V_{max} (nmol/min/mg)
CDNB	1.0	10.0	25.0	6.5	340	0.65 ± 0.023	21.64 ± 0.94
DCNB	1.0	10.0	25.0	6.5	345	n.d	n.d
4-NPB	0.5	1.0	25.0	6.5	310	n.d	n.d
4-NBC	1.0	1.0	25.0	6.5	310	n.d	n.d

The values of specific activity presented in the table are the means of three independent experiments $\pm$ SE

n.d. not detectable

CDNB as the substrate. The data revealed that Cu^{2+} was able to reduce the catalytic capability of recombinant SeGSTo to approximately 5 % in the dosage of 10 μM or exposure recombinant SeGSTo in Cu^{2+} for 30 min (Fig. 6). Cd^{2+} was able to reduce the catalytic capability of recombinant SeGSTo to approximately 10 % in the dosage of 100 μM . The recombinant SeGSTo maintained the 40 % capability of catalyzing the CDNB after exposure in Cd^{2+} for 30 min (Fig. 7). Comparing Cu^{2+} with Cd^{2+} , Cu^{2+} was more efficient than Cd^{2+} in reducing the catalytic activity of recombinant SeGSTo in a dose- and exposure time-dependent manner.

Recombinant SeGSTo interactions with pesticides

To investigate the effect of pesticides on the catalytic activity of recombinant SeGSTo, different pesticides including insecticides (imidacloprid, clothianidin, indoxacarb, cyhalothrin, dinotefuran, and isoprocarb), herbicides (cyhalofop and bispyribac) and fungicides (carbendazim and dimetachlone) were added into the reaction mixtures with CDNB as the substrate to determine the inhibition of pesticides toward recombinant SeGSTo. The data demonstrated that dimetachlone reduced the catalytic activity of recombinant SeGSTo dramatically and imidacloprid interfered with the catalytic process marginally (Fig. 8).

SeGSTo in response to various stresses

To investigate the expression levels of *SeGSTo* following the heavy metals and pesticide treatment, *S. exigua* larvae were fed the cabbage with CdCl_2 , CuSO_4 , or isoprocarb on the first day of the fifth-instar stage using leaf dipping method. Subsequently, the expression levels induced by these substances were analyzed by qRT-PCR and the results exhibited that *SeGSTo* could be dramatically induced in the first 6 h and regressed to the normal levels in the following 6 h (Fig. 9). These phenomena indicated that *SeGSTo* might play essential roles in detoxication of heavy metals and pesticides.

Discussion

GSTs comprise a multifunctional family that participates in the detoxification of hydrophobic and electrophilic toxicants including insecticides, fungicides, and herbicides (Qin et al. 2013). However, pesticide exposure in agricultural fields may result in the depletion of reduced glutathione in insects, which requires GSTs to refresh the glutathione. This is the essential mechanism that powers metabolic resistance adaptation and evolution (Zhu et al. 2015). In this study, we cloned and characterized a novel omega class GST gene from the beet

Fig. 5 Effects of temperature and pH on the catalytic activity of recombinant SeGSTo on CDNB. **a** The optimal temperature for activity was determined in the range of 20–70 °C. **b** The optimal pH for activity

was measured using citrate–phosphate–borate buffer with various pH values in the range 2–10. The error bars represent the mean $\pm$ SE values ($n = 3$)

Fig. 6 Effect of CuSO₄ on the activity of recombinant SeGSTo. **a** The enzymatic activity of recombinant SeGSTo toward CDNB was measured in the presence of various concentrations of CuSO₄. **b** The enzymatic

activity of recombinant SeGSTo toward CDNB was measured using different incubation times in the presence of 0.5 μM CuSO₄. The error bars represent the mean ± SE values ($n=3$)

armyworm *S. exigua*. The full length of the *SeGSTo* gene is 981 bp and contains an open reading frame (ORF) of 744 nucleotides encoding a 248-amino acid polypeptide. Protein sequence alignment analysis indicated that the SeGSTo comprised two conserved GST signature motifs: a glutathione binding region (G-site) and an electrophilic substrate binding region (H-site), which play essential roles in detoxification (Deng et al. 2009). Meanwhile, the results showed that SeGSTo is closer to the type 4 GSTo from *B. mori* than other types of GSTos. Moreover, the phylogenetic genetic tree showed that SeGSTo belongs to the omega group (Hu et al. 2014). Furthermore, the 3D structure analysis and molecular docking revealed that Cys35 may play an essential role in the catalytic process and the helix 1 and beta strand 2 (belonging to the G-Site) and helices 5 and 12 (belonging to the H-Site) collectively forms the catalytic site. Thus, the present study identified the first member of the GSTo family in the beet armyworm species and performed bioinformatic analysis from various perspectives and levels.

The GST superfamily has been well explored and illustrated in many insect species. To date, the entire GST families

from various insects have been identified based on the genome sequence; 35, 13, 23, 40, and 41 GSTs have been identified and categorized in *A. gambiae*, *A. mellifera*, *B. mori*, *D. melanogaster*, and *T. castaneum*, respectively (Ding et al. 2003; Corona and Robinson 2006; Yu et al. 2008; Ranson et al. 2002; Shi et al. 2012). In the present study, the expression of *SeGSTo* was detected only in the midgut of the fifth instar larval stage, but not in the epidermis and fat body. This phenomenon may be associated with the antioxidative metabolism induced after ecdysis (Yamamoto, et al. 2011).

In a previous study, the functions of partial GST classes including omega, theta, and zeta have been well defined; these classes play basic roles associated with metabolic activity (Hu et al. 2014). However, the functions of SeGSTo from the essential pest *S. exigua* have not yet been comprehensively described. Herein, the molecular mass of recombinant SeGSTo, was determined using the purified protein expressed in baculovirus-infected insect cells, and the results indicated that the molecular mass of the protein was 30 kDa, which is consistent with the size of known cytosolic GSTs (Mannervik et al. 1998). During advanced investigation of the antioxidation

Fig. 7 Effect of CdCl₂ on the activity of recombinant SeGSTo. **a** The enzymatic activity of recombinant SeGSTo toward CDNB was measured in the presence of various concentrations of CdCl₂. **b** The enzymatic

activity of recombinant SeGSTo toward CDNB was measured using different incubation times in the presence of 5 μM CdCl₂. The error bars represent the mean ± SE values ($n=3$)

Fig. 8 Effect of pesticides on the activity of recombinant SeGSTo toward CDNB. Inhibition of recombinant SeGSTo by insecticides (imidacloprid, clothianidin, indoxacarb, cyhalothrin, dinotefuran, and isoprocarb), herbicides (cyhalofop and bispyribac), and fungicides (carbendazim and dimetachlone) at a concentration of 100 μ M was measured. The error bars represent the mean $\pm$ SE values ($n=3$)

function of recombinant SeGSTo, a DNA protection assay revealed that the recombinant SeGSTo was able to protect super-coiled plasmid DNA from damage in the MCO system, and this result was consistent with previous investigations that maintain that GSTo may play an essential role against oxidative stress (Zhang et al. 2013).

Moreover, we observed the ability of SeGSTo to catalyze different substrates in the presence of reduced GSH. In a previous study, weak conjugation of GSH by GSTo indicated that the enzyme kinetic parameters may be noticeably lower in contrast to the other GSTs (Whitbread et al. 2005). Our data indicated that the $V_{\max}$ of recombinant SeGSTo was far lower than that of recombinant SeGSTe when CDNB was used as the substrate (Wan et al. 2015). In addition, the optimal reaction pH and temperature varied when compared with other types of GSTs (Qin et al. 2013). Therefore, the optimal reaction pH and temperature were determined with CDNB as the substrate, and the results showed that the optimal reaction pH and temperature were 8.0 and 50 $^{\circ}$ C, respectively. These results are similar to the kinetic parameters of other known cytosolic GSTs (Yamamoto et al. 2005; Gullipalli et al. 2010). Furthermore, heavy metals from the environment, especially Cu^{2+} and Cd^{2+} , may dramatically decrease the ability of GSTs to detoxify foreign chemicals and their antioxidative ability (Serafini and Romeu 1991). Additionally, the inhibition patterns of Cu^{2+} and Cd^{2+} in different classes of GSTs were determined in *Locusta migratoria*, and the sigma GST family was the most sensitive group in *L. migratoria* (Qin et al. 2013). In this study, similar results were observed that indicated that Cu^{2+} was able to profoundly weaken the activity of

Fig. 9 *SeGSTo* expression profiles induced by different stressors. Total RNA from *S. exigua* larvae fed with CdCl_2 , CuSO_4 , or isoprocarb isolated from the midgut and double-distilled water served as injection control. Quantitative real-time PCR (qRT-PCR) was performed to analyze the

level of *SeGSTo* mRNA in 3, 6, 9, and 12 h, respectively. The error bars represent the mean $\pm$ SE values ($n=3$). Significant differences are indicated with two asterisks at $P<0.01$

recombinant SeGSTo at lower concentrations than those of Cd²⁺. Previous studies demonstrated that insect-derived GSTs of the delta or epsilon groups might play an essential role in the evolution of resistance to pesticides (Qin et al. 2012; Zhou et al. 2015). Our results indicate that insecticides as well as herbicides and fungicides may reduce the activity of recombinant SeGSTo. In other words, recombinant SeGSTo may conjugate with pesticides in order to protect the insect (Wan et al. 2015; Zhou et al. 2015). The prior investigations illustrated that pesticide not only can up-regulate the expression levels of epsilon class glutathione S-transferases but also can up-regulate the expression levels of omega class (Huang et al. 2011). In this research, heavy metals and pesticide were able to induce the expression of *SeGSTo*, and isoprocarb was the most effective exogenous substance to raise the transcript level of *SeGSTo*. This is a key evidence to support that *SeGSTo* may serve as an essential gene to impair the toxicity toward to themselves and participate in resistance development.

In summary, this study, for the first time, cloned and bioinformatically characterized an omega GST from *S. exigua*. This study also illustrated the enzymatic characteristics of recombinant SeGSTo and revealed the basic function of the protein in the antioxidative process and against pesticides, which offers a molecular basis for characterizing insecticide resistance in *S. exigua*.

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Compliance with ethical standards

Conflict of interests The authors have declared that no competing interests exist.

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