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cDNA Sequence and mRNA Expression of a Putative Glutathione S-Transferase from the Mole Cricket, *Gryllotalpa orientalis*

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The glutathione S-transferases (GSTs) are enzymes responsible for the protection of cells from chemical toxicants and oxidative stress. We describe here the cDNA sequence and mRNA expression of a putative GST from the mole cricket, *Gryllotalpa orientalis*. The *G. orientalis* GST cDNA sequences comprised of 621 bp encoding 207 amino acid residues. The multiple sequence alignment of *G. orientalis* GST gene with other known insect GSTs showed several conserved residues that may be essential for the enzymatic activity of the protein. Phylogenetic analysis of the deduced amino acid sequences of *G. orientalis* GST gene with other insect GST sequences revealed that the *G. orientalis* GST gene belongs to class I GST, forming a strong monophyletic group (100% bootstrap value) exclusively for class I GSTs from a diverse insect species. Northern blot analysis confirmed midgut-specific expression at transcriptional level, evidencing the midgut as a site for GST synthesis.

Key words: Insect, Mole cricket, *Gryllotalpa orientalis*, Glutathione S-transferase, cDNA sequences, mRNA expression, Phylogeny

Introduction

The glutathione S-transferases (GSTs) (EC 2.5.1.18) are detoxification enzymes and appear to act to protect cells from chemical toxicants and oxidative damages (Daniel, 1993; Salinas and Wong, 1999). GSTs have the capacity to conjugate reduced glutathione on the thiol of cysteine to various electrophiles and to bind to a variety of hydrophobic or amphipathic molecules (Armstrong, 1991; Clark, 1989; Daniel, 1993; Listowsky *et al.*, 1988; Pickett and Lu, 1989; Rushmore and Pickett, 1993). Functional GSTs are either homodimers or heterodimers of subunits. The mammalian GST subunits have been classified into six groups, alpha, mu, pi, sigma, theta, and microsomal subunit.

Insect GSTs have been divided into two major groups, GST1 and GST2 (Grant and Matsumura, 1989; Fournier *et al.*, 1992; Snyder *et al.*, 1995; Franciosa and Berge, 1995). The insect GST1 is serologically distinct from GST2. The insect GSTs were expressed in the midgut (Tate *et al.*, 1982; Snyder *et al.*, 1995), fat body (Chien and Dauterman, 1991), hemolymph cells and other tissues (Franciosa and Berge, 1995). In insects, GSTs play an important role in the resistance and detoxification to insecticides. The insect GSTs are highly induced by plant chemicals, insecticides and other xenobiotics (Motoyama and Dauterman, 1980; Riskallah *et al.*, 1986; Snyder *et al.*, 1995; Yu, 1982, 1989). In insects, isolation of GST genes has been reported from various insect species belonging to the orders Blattodea, Diptera, Lepidoptera and Hemiptera (Arruda *et al.*, 1997; Beall *et al.*, 1992; Feng *et al.*, 1999; Fournier *et al.*, 1992; Huang *et al.*, 1998; Prapanthadara *et al.*, 1998; Snyder *et al.*, 1995; Toung *et al.*, 1990; Toung and Tu, 1992; Vontas *et al.*, 2002; Wang *et al.*, 1991). Such investigation on insect GSTs increases molecular information of the structural characteristics and functional mechanisms. However, much of GST for orthopteran species is still unknown.

The mole cricket, *Gryllotalpa orientalis* (Burmeister), is a singly known species of the family Gryllotalpidae in Korea, and distributed in Asia and many European and African countries (Nevo *et al.*, 2000). In order to obtain molecular information of the mole cricket, therefore, we

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have previously constructed the cDNA library using *G. orientalis* whole bodies (Kim *et al.*, 2002). The Sec61p ã subunit and chemosensory protein homologue genes have been identified by screening EST (expressed sequence tag) of the *G. orientalis* cDNA library (Kim *et al.*, 2002, 2003).

In this study, we report the cDNA sequence of the putative GST gene from *G. orientalis* and compared the amino acid sequences with other insect GSTs. Also, we found a tissue-specific expression of the *G. orientalis* GST in the midgut.

Materials and Methods

Animals

The mole cricket, *Gryllotalpa orientalis* (Burmeister), was collected in Gimhe city, Korea (Kim *et al.*, 2002).

cDNA library screening, nucleotide sequencing and data analysis

G. orientalis larval cDNA library (Kim *et al.*, 2002) was screened to generate the expressed sequence tags (ESTs). Sequencing of randomly selected clones harboring cDNA inserts was performed. For DNA sequencing, plasmid DNA was extracted by Wizard mini-preparation kit (Promega). Sequence of each cDNA clone was determined using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, Foster City, CA). The sequences were compared using the DNASIS and BLAST programs provided by the NCBI. GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program.

MacVector (ver. 6.5, Oxford Molecular Ltd.) was used to align the amino acid sequences of GSTs. Including the thirteen GenBank-registered GST amino acid sequences, phylogenetic analysis was performed using PAUP* (Phylogenetic Analysis using Parsimony) version 4.0 (Swofford, 2000). The tree was obtained by bootstrap analysis with the option of heuristic search (1,000 replications). The information and accession numbers of the 13 insect GST sequences in the GenBank are described in Table 1. Helical and non-helical regions of *G. orientalis* GST were predicted by the Garnier-Robson method (Garnier *et al.*, 1978).

RNA isolation and Northern blot analysis

Total RNA was isolated from fat body, midgut, and epidermis of the *G. orientalis* using the Total RNA Extraction Kit (Promega, Madison, WI). Total RNA (10 μg/lane) from the *G. orientalis* was separated on glyoxalation gel (McMaster and Carmichael, 1977), transferred onto a

Table 1. Glutathione S-transferases from various insect species

Common name	Species	Sequence name	Length of amino acid	GenBank accession number	References
Orthoptera					
Mole cricket	*Gryllotalpa orientalis*	*Go*GST	207		This study
Blattodea					
German cockroach	*Blattella germanica*	*Bg*GST	204	O18598	Arruda *et al.* (1997)
Lepidoptera					
Silkworm	*Bombyx mori*	*Bm*GST	218	CAA07071	Unpublished
Spruce budworm	*Choristoneura fumiferana*	*Cf*GST	203	AAF23078	Feng *et al.* (1999)
Cotton bollworm	*Helicoverpa armigera*	*Ha*GST	170	AAL23839	Unpublished
Tobacco hornworm	*Manduca sexta*	*Ms*GST	247	AAF16718	Robertson *et al.* (1999)
Tobacco hornworm	*Manduca sexta*	*Ms*GST1	203	AAA92881	Snyder *et al.* (1995)
Tobacco hornworm	*Manduca sexta*	*Ms*GST2	217	AAA92880	Snyder *et al.* (1995)
Diptera					
Yellow fever mosquito	*Aedes aegypti*	*Aa*GST-2	218	AAK64286	Unpublished
African malaria mosquito	*Anopheles gambiae*	*Ag*GST1-3	218	AAC79992	Ranson *et al.* (1998)
African malaria mosquito	*Anopheles gambiae*	*Ag*GST1-6	209	AAC79995	Ranson *et al.* (1998)
Fruit fly	*Drosophila mauritiana*	*Dma*GST1-1	200	P30105	Unpublished
House fly	*Musca domestica*	*Md*GST	241	P46437	Franciosa and Berge (1995)
House fly	*Musca domestica*	*Md*GST-1	208	CAA43599	Unpublished

nylon blotting membrane (Schleicher & Schuell, Dassel, Germany) and hybridized at 42°C with a probe in a buffer containing 2 × PIPES, 50% formamide, 1% sodium dodecyl sulphate (SDS) and blocking reagent (Boehringer Mannheim, Mannheim, Germany). The probe used to detect the GST transcripts was 621 bp for *G. orientalis* GST cDNA cloned in this study and labeled with [α-^{32}P] dCTP (Amersham, Arlington Heights, IL) using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA). After hybridization, the membrane filter was washed three times for 30 min each in 0.1% SDS and 0.2 × SSC at 65°C, and finally exposed to autoradiography film.

Results and Discussion

A cDNA clone showing similarity to the reported other insect GSTs was obtained by screening the cDNA library of the mole cricket, *G. orientalis*. The nucleotide sequence of the cDNA clone was analyzed and its amino acid sequence was deduced. The nucleotide and deduced amino acid sequences of a cDNA encoding a putative member of insect GST are presented in Fig. 1. The sequences of the *G. orientalis* GST cDNA comprised of 621 bp encoding 207 amino acid residues.

A multiple sequence alignment of the deduced amino acid sequences of *G. orientalis* GST cDNA with other

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1   ATGGCTCTGAAGTACAAATTGACATATTTTGATTGTAAGGCTTTGGGTGAACCTATTCGT
1   M  A  L  K  Y  K  L  T  Y  F  D  C  K  A  L  G  E  P  I  R
61  TTTCTTTTCGTTATGGTGGAATTGAATTTGAAGATGATCGTTTTGAGAGTGAGATGTGG
21  F  L  F  R  Y  G  G  I  E  F  E  D  D  R  F  E  S  E  M  W
121 CCTCAACTAAAATCAAAAATGCCATTTGGACAAGCTCCTGTTTTGGAGTATGATGGAAAA
41  P  Q  L  K  S  K  M  P  F  G  Q  A  P  V  L  E  Y  D  G  K
181 GTCATTAATCAATCTGTGGCTATTTCTCGCTATGCAGCAAAGAAAGTTGGATTAGCAGGT
61  V  I  N  Q  S  V  A  I  S  R  Y  A  A  K  K  V  G  L  A  G
241 GCTGATGATTGGGAAGCATTACAGATAGATGCTACTGTCGATACAATTACTGATATGCGT
81  A  D  D  W  E  A  L  Q  I  D  A  T  V  D  T  I  T  D  M  R
301 TTGAAAATTGCTAATTATCATTATGATAATGATGCTGCATCTAAGGAGAAGAAAAAAGAA
101 L  K  I  A  N  Y  H  Y  D  N  D  A  A  S  K  E  K  K  K  E
361 CCTTTAATGAAAGAAACAATTCCTTACTACATGCCAAAATTTGAAGAACAAGTAAAAAGA
121 P  L  M  K  E  T  I  P  Y  Y  M  P  K  F  E  E  Q  V  K  R
421 AATGGTGGTTACTTTGTGAATAATAAGTTAACCTGGGCTGATCTTTATTTTGTGGCTGTA
141 N  G  G  Y  F  V  N  N  K  L  T  W  A  D  L  Y  F  V  A  V
481 TTGGACTACCTAAATTGGATGGTGGGAGAAAAGATCCTTCTTGAAGGTGGTCCTACTCTG
161 L  D  Y  L  N  W  M  V  G  E  K  I  L  L  E  G  G  P  T  L
541 AATAAACCTACCGCGAAACTGGTGTTGGAAAATTACCACCTATTTAAAGCTTGGATTGCC
181 N  K  P  T  A  K  L  V  L  E  N  Y  H  L  F  K  A  W  I  A
601 AAGCGCCCTGACACAACATACTGA
201 K  R  P  D  T  T  Y  *
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Fig. 1. The nucleotide and deduced amino acid sequences of *G. orientalis* GST. The start codon ATG is boxed and the termination codon is shown by asterisk. A putative N-glycosylation site is underlined.

insect GST sequences is shown in Fig. 2. Alignment of the *G. orientalis* GST sequences with those of GSTs from several other species indicates the extent of the identity that exists. Particularly, the presumed glutathione-binding sites, Tyr-9, Gln-64, and Asp-99, in mammals (Rushmore *et al.*, 1993; Reinemer *et al.*, 1991; Ji *et al.*, 1992, 1995) were well conserved in some insect species, such as *Blat-*

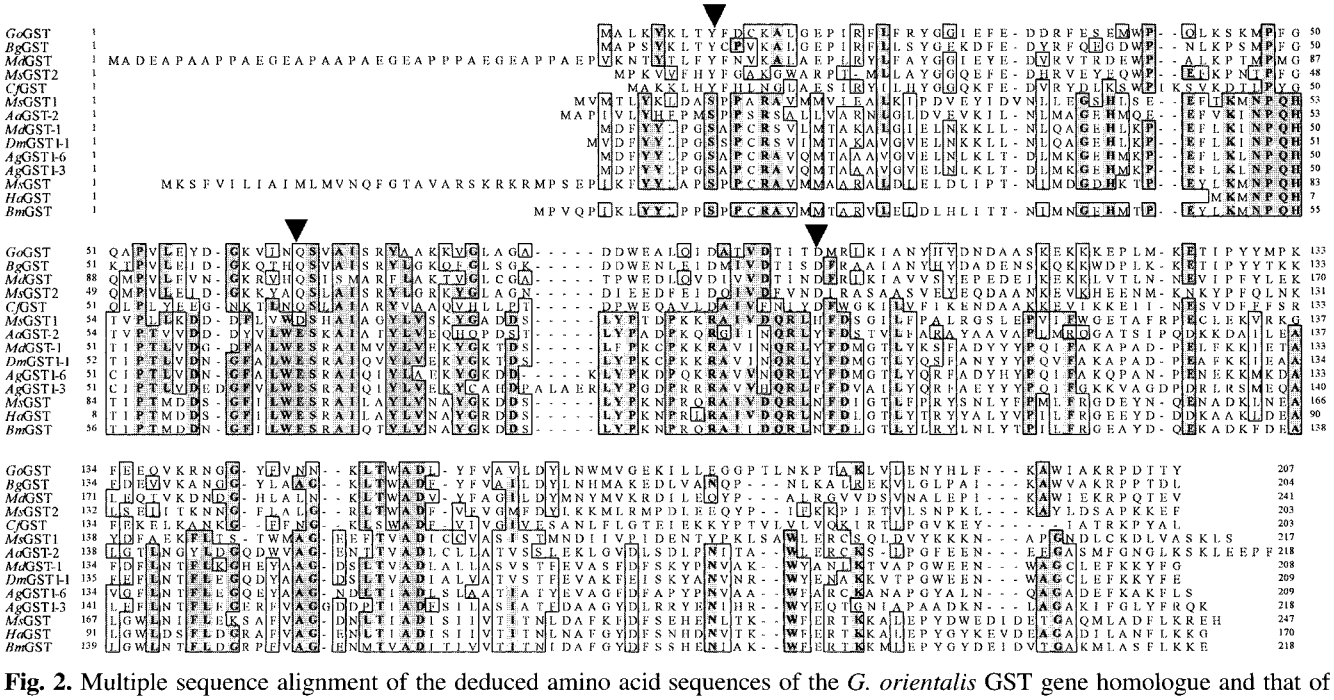
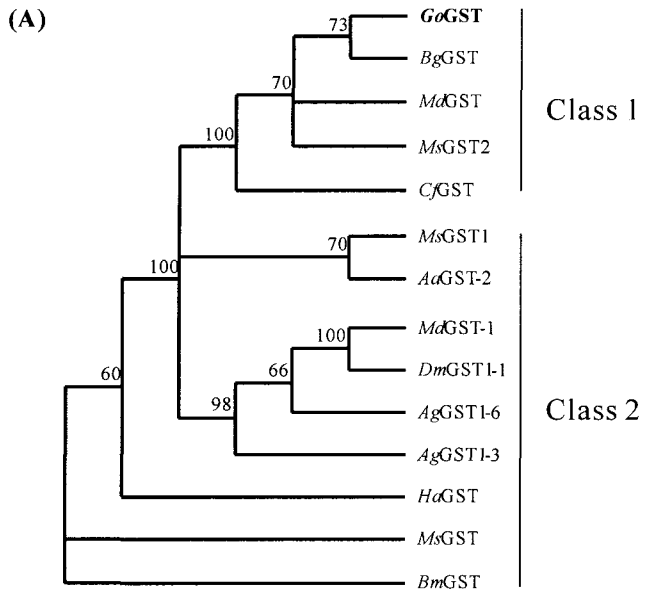


Fig. 2. Multiple sequence alignment of the deduced amino acid sequences of the *G. orientalis* GST gene homologue and that of other insects. The solid boxes are the residues that are identical to those of *G. orientalis*. The residues involved in glutathione binding in the *B. germanica* GST, *BgGST* (Arruda *et al.*, 1997) are indicated with vertical arrows.



(B)

Percent similarity

Sequence	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *Go*GST		67	51	51	53	25	23	25	27	26	23	21	20	26
2. *Bg*GST	57		55	55	50	22	21	24	25	24	21	19	19	23
3. *Md*GST	41	39		47	44	20	20	22	22	22	21	25	17	24
4. *Ms*GST2	40	41	32		42	20	22	23	23	25	23	19	20	23
5. *Cf*GST	35	31	27	29		23	23	25	26	23	21	18	19	22
6. *Ms*GST1	12	14	11	12	11		54	46	49	46	49	45	39	50
7. *Aa*GST-2	11	11	9	11	13	35		57	57	58	59	51	46	60
8. *Md*GST-1	13	15	9	12	13	36	41		93	79	70	52	46	59
9. *Dm*GST1-1	14	14	10	11	12	35	40	84		78	72	54	48	62
10. *Ag*GST1-6	16	16	12	14	12	35	38	68	67		73	53	48	61
11. *Ag*GST1-3	13	13	10	12	13	33	36	54	55	63		53	45	61
12. *Ha*GST	12	12	12	10	10	30	34	35	37	40	38		58	76
13. *Ms*GST	13	13	8	13	11	27	33	31	33	35	33	52		67
14. *Bm*GST	15	14	12	12	11	34	43	41	44	45	44	66	60	

Percent identity

Fig. 3. Relationships among amino acid sequences of the *G. orientalis* GST and the known GSTs. (A) Phylogenetic tree of aligned amino acid sequences of the *G. orientalis* GST and other known GSTs. The information of GST sequences extracted from GenBank is described in Table 1. The tree was obtained by bootstrap analysis with the option of heuristic search and the numbers on the branches represent bootstrap values for 1,000 replicates. (B) Pairwise similarities and identities of the deduced amino acid sequences among insect GST sequences including *G. orientalis* GST. The abbreviation is represented in Table 1.

tella germanica GST, *Musca domestica* GST, *Manduca sexta* GST2, and *Choristoneura fumiferana* GST (Table 1).

A phylogenetic analysis using deduced amino acid sequences of known insect GST genes revealed that the *G. orientalis* GST formed a strong subgroup with *B. germanica* GST, *M. domestica* GST, *M. sexta* GST2, and *C. fumiferana* GST (Fig. 3A). The *G. orientalis* GST showed

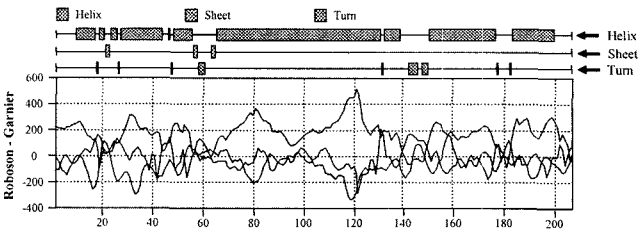


Fig. 4. Predicted helical regions of *G. orientalis* GST.

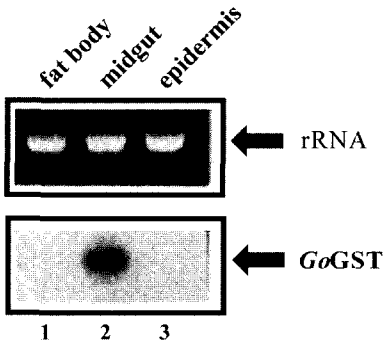


Fig. 5. Northern blot analysis of the *G. orientalis* GST messages. Total RNA was isolated from the fat body (lane 1), midgut (lane 2), and epidermis (lane 3). The RNA was separated by 1.0% formaldehyde agarose gel electrophoresis (upper panel), transferred onto a nylon membrane, and hybridized with the appropriate radiolabelled probe (lower panel). *G. orientalis* GST message is indicated by arrow on the right side of the panel.

57% protein sequence identity to the *B. germanica* GST, 41% to the *M. domestica* GST, 40% to the *M. sexta* GST2, and 35% to *C. fumiferana* GST, respectively (Fig. 3B). In fact, these GSTs are the member of class I GST gene among two well differentiated types of GST genes in insects, and the *G. orientalis* GST was well grouped together with the class I of all other insect GSTs (Feng *et al.*, 1999).

Helical and non-helical regions of *G. orientalis* GST were predicted as shown Fig. 4. The *G. orientalis* GST was predicted to be structure with a helix-turn-helix motif. To confirm the expression of the *G. orientalis* GST gene at transcriptional level, Northern blot analysis was performed using total RNA prepared from the fat body, midgut, and epidermis of *G. orientalis* (Fig. 5). Hybridization signal was detected only at the midgut, indicating that the midgut is a main site for *G. orientalis* GST synthesis. In previous reports, the insect GSTs were expressed in the midgut (Tate *et al.*, 1982; Snyder *et al.*, 1995), fat body (Chien and Dauterman, 1991), hemolymph cells and other tissues (Franciosa and Berge, 1995). In insects, GSTs play an important role in the resistance and/or detoxification to insecticides and other xenobiotics (Motoyama and Dauterman, 1980; Riskallah *et al.*, 1986; Snyder *et al.*, 1995;

Yu, 1982, 1989). In this study, the *G. orientalis* GST is expressed only in the midgut. It is likely that the midgut is a site where large quantities of GST are synthesized for the detoxification and/or resistance of the deteriorated chemicals absorbed from the diet.

In conclusion, we have cloned a novel cDNA encoding putative GST from the mole cricket *G. orientalis*. The gene was belonged to class I of the insect GST and showed a midgut-specific expression at the transcriptional level. We hope that the molecular characterization of the GST in *G. orientalis* in this study will expand the understanding of the insect GSTs and accumulate genetic information of *G. orientalis*, which is largely unknown to us.

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