

MINI REVIEW

Insect glutathione transferases and insecticide resistance

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Abstract

Glutathione transferases (GSTs) are a diverse family of enzymes found ubiquitously in aerobic organisms. They play a central role in the detoxification of both endogenous and xenobiotic compounds and are also involved in intracellular transport, biosynthesis of hormones and protection against oxidative stress. Interest in insect GSTs has primarily focused on their role in insecticide resistance. GSTs can metabolize insecticides by facilitating their reductive dehydrochlorination or by conjugation reactions with reduced glutathione, to produce water-soluble metabolites that are more readily excreted. In addition, they contribute to the removal of toxic oxygen free radical species produced through the action of pesticides. Annotation of the *Anopheles gambiae* and *Drosophila melanogaster* genomes has revealed the full extent of this enzyme family in insects. This mini review describes the insect GST enzyme family, focusing specifically on their role in conferring insecticide resistance.

Keywords: insects, insecticide resistance, detoxification, glutathione transferases.

Introduction

The glutathione transferases (GSTs) are a large family of multifunctional enzymes involved in the detoxification of a wide range of xenobiotics including insecticides (Salinas & Wong, 1999). GSTs primarily catalyse the conjugation of electrophilic compounds with the thiol group of reduced glutathione (GSH), generally making the resultant products more water soluble and excretable than the non-GSH

conjugated substrates (Habig *et al.*, 1974). In addition, some GSTs catalyse a dehydrochlorination reaction using reduced glutathione as a cofactor rather than a conjugate (Clark & Shamaan, 1984). GSTs are important in cancer epidemiology and drug resistance (Tew, 1994; Hayes & Pulford, 1995) and hence are well studied in mammals. The majority of studies on insect GSTs have focused on their role in detoxifying foreign compounds, in particular insecticides and plant allelochemicals and, more recently, their role in mediating oxidative stress responses. (Clark *et al.*, 1986; Wang *et al.*, 1991; Fournier *et al.*, 1992; Ranson *et al.*, 2001; Vontas *et al.*, 2001; Sawicki *et al.*, 2003).

Classification and nomenclature

There are at least two ubiquitously distributed distantly related groups of GSTs, classified according to their location within the cell: microsomal and cytosolic. A third group of GSTs, the Kappa class, are located in mammalian mitochondria and peroxisomes (Morel *et al.*, 2004; Lander *et al.*, 2004) and are structurally distinct from the microsomal and cytosolic GSTs (Robinson *et al.*, 2004). To date, genome data mining has failed to detect any members of this GST class in insects.

A single microsomal GST gene is present in the genome of the fruitfly *Drosophila melanogaster* whereas the mosquito *Anopheles gambiae* has three microsomal GST genes (Toba & Aigaki, 2000; Ranson *et al.*, 2002). The microsomal GSTs are trimeric, membrane-bound proteins. Although very different in structure and in origin, the microsomal GSTs catalyse similar reactions to the cytosolic GSTs (Gakuta & Toshiro, 2000; Prabhu *et al.*, 2001). Microsomal GSTs have not been implicated in the metabolism of insecticides and will not be discussed further in this review.

Insect cytosolic GSTs were initially assigned numbers according to their order of elution from the various purification procedures employed or isoelectric points (Clark *et al.*, 1985; Prapanthadara *et al.*, 1993). Later two immunologically distinct classes of GSTs were recognized in houseflies and designated as class I and class II (Fournier *et al.*, 1992). The class II insect GSTs are encoded by a single gene in all species studied to date (Beall *et al.*, 1992; Reiss & James, 1993; Synder *et al.*, 1995) although two distinct transcripts are produced by alternative splicing of the *A. gambiae* class II gene (Ding *et al.*, 2003). The class I

doi: 10.1111/j.1365-2583.2004.00529.x

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insect GSTs, in contrast, are encoded by a multigene family in *Anopheles* mosquitoes, *D. melanogaster* and *Musca domestica* (Toung *et al.*, 1993; Zhou & Syvanen, 1997; Ranson *et al.*, 2002).

As the volume of insect sequence data increased, additional GSTs were identified that did not clearly fit within class I or II and the mammalian system of GST nomenclature was adopted whereby classes are designated using Greek letters (Chelvanayagam *et al.*, 2001). Phylogenetic comparison of insect and mammalian GST genes showed that the insect class II GSTs are orthologous to the Sigma GST class found in a diverse range of species from nematodes to mammals (Agianian *et al.*, 2003). In contrast, the class I GSTs are unique to insects and were re-named Delta GSTs. A second large class of GSTs, the Epsilon class, is also restricted to insects (Ranson *et al.*, 2001). The Delta and Epsilon GST classes have expanded independently in *D. melanogaster* and *A. gambiae*, suggesting that these enzymes play important roles in the adaptation of these species to their specific environments (Ranson *et al.*, 2002).

The majority of the remaining cytosolic insect GSTs are members of the Zeta, Theta and Omega classes (Board *et al.*, 1997, 2000; Ranson *et al.*, 2002). The relatively high degree of conservation of these GST genes across taxa suggests that they play essential steps in conserved physiological pathways. The complete inventory of *A. gambiae* cytosolic GSTs includes three genes, *GSTu1*, *GSTu2* and *GSTu3*, that cannot be readily assigned to the existing classes. They share less than 40% amino acid identity with other insect GSTs and are physically separate from other members of the GST gene family on the mosquito chromosomes (Ding *et al.*, 2003). These may represent novel classes of insect GSTs but have been temporarily designated unclassified (u). The phylogenetic relationship between the different classes of GSTs found in insects is shown in Fig. 1.

By extending the mammalian GST classification system to encompass insect GSTs the nomenclature of individual insect GST genes and proteins has been clarified (Chelvanayagam *et al.*, 2001). Individual GST subunits are now assigned names indicating the species from which they were isolated and the GST class. They are also given a number that may reflect the order of discovery or the genome organizations. For example, *AgGSTd12* is the twelfth member of the *A. gambiae* Delta class of GST subunits to be identified. The distribution of different classes of GSTs in insects and other animals is shown in Table 1.

Enzyme structure

Cytosolic GSTs are hetero- or homo-dimeric proteins approximately 25 kDa in size. The polypeptide chain of each monomer folds into two domains joined by a variable linker region. The N-terminal domain (residues 1–80) consists of four beta sheets and three flanking alpha helices

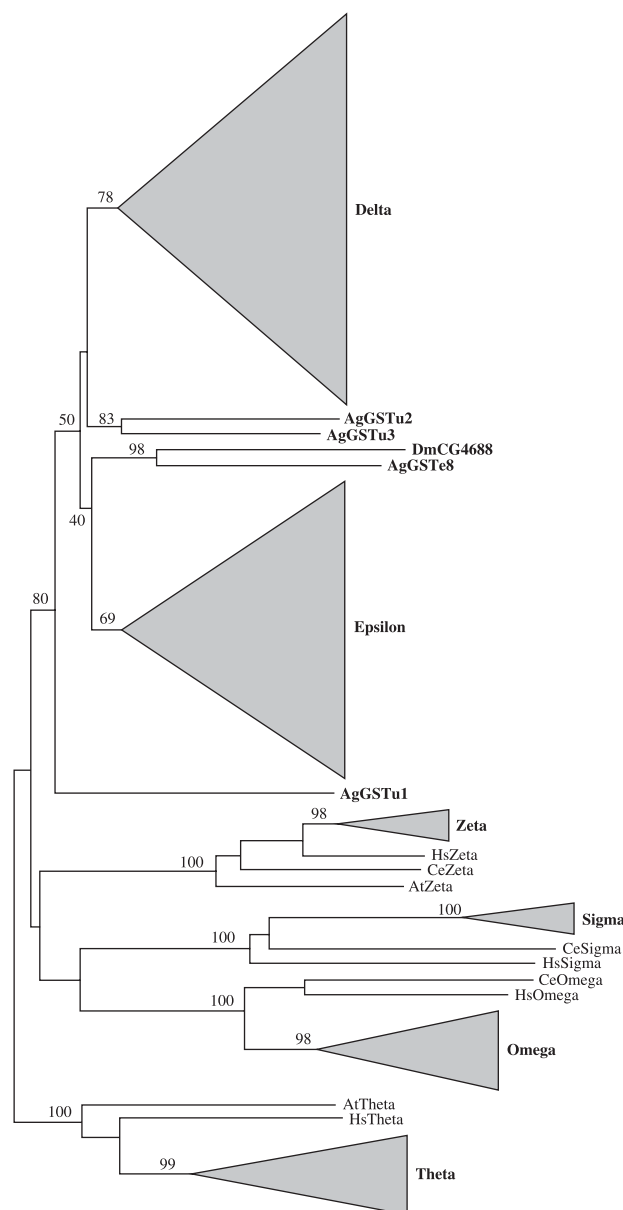


Figure 1. Neighbor joining tree illustrating the relationship between the classes of GSTs found in insects. The amino acid sequence of all cytosolic GSTs from *Anopheles gambiae* and *Drosophila melanogaster* were aligned with GSTs from mammals, plants and nematodes from the Zeta, Sigma, Omega and Theta classes using CLUSTALW. For clarity, a single GST peptide was selected for each class from the non-insect phyla (accession numbers and wormbase protein IDs: HsZeta, O43708; CeZeta, WP:Ce25559; AtZeta, AF288182; CeSigma, WP:Ce04834; HsSigma, O60760; CeOmega, WP:Ce15815; HsOmega, P78417; AtTheta, AJ131580; HsTheta, S44358). The tree was constructed by the neighbor-joining method from a similarity matrix of pair-wise comparisons made using the Jukes–Cantor algorithm. Bootstrap values (500 replicates) are shown at selected nodes. The nodes of highly supported insect GST classes have been collapsed. Those insect GSTs whose classification is unresolved are shown in bold. Ag = *Anopheles gambiae*; At = *Arabidopsis thaliana*; Ce = *Caenorhabditis elegans*; Dm = *Drosophila melanogaster*; Hs = *Homo sapiens*. See text for further details.

Table 1. Summary of insect GST classes

GST class	Distribution	Putative transcript no. in <i>A. gambiae</i>	Putative transcript no. in <i>D. melanogaster</i>	Examples in other insect species
Delta	Insects only	15	11	<i>L. cuprina</i> , <i>M. domestica</i> , <i>N. lugens</i> , <i>C. variipennis</i> <i>P. xylostella</i> , <i>M. sexta</i> , <i>M. domestica</i>
Epsilon	Insects only	8	14	
Omega	Nematodes, insects, mammals	1	5	<i>M. domestica</i> , <i>B. germanica</i> , <i>C. fumiferana</i> , <i>M. sexta</i>
Theta	Mammals, insects and plants	2	4	
Sigma	Molluscs, helminths, nematodes, insects, mammals	1	1	
Zeta	Plants, nematodes. Insects, mammals	1	2	

and adopts a conformation similar to the thioredoxin domain found in many proteins that bind GSH or cysteine (see reviews by Dirr *et al.*, 1994; Wilce & Parker, 1994; Sheehan *et al.*, 2001). This domain contains the majority of residues involved in the binding of glutathione (the G-site). The larger C-terminal domain consists of a variable number of alpha helices. The variable hydrophobic H-site, which interacts with the electrophilic substrates, is largely formed from residues in the C-terminal domain. Although each monomeric active site functions independently, their quaternary structure is essential for their activity (Danielson & Mannervik, 1985).

Mode of action

In a GST-catalysed conjugation reaction, one molecule of reduced GSH and one molecule of a second substrate are combined to form a thioester. The reaction proceeds via substrate binding, the activation of the thiol group of GSH and subsequent nucleophilic attack by the anionic GSH on the bound hydrophobic compound (Atkins *et al.*, 1993). This conjugation neutralizes the electrophilic sites of the lipophilic substrate and protects the cellular components, especially the nucleophilic oxygen and nitrogen of DNA from electrophilic attack of nucleophiles. Conjugation also renders the product more water soluble and therefore more readily excretable from the cell.

GSTs have a high affinity towards GSH and because this tripeptide is present at high intracellular concentrations the GSH binding site of GST may always be occupied. The 'active site residue' in the N-terminal domain interacts with and activates the sulphhydryl group of glutathione. In most mammalian GSTs the active site residue is a tyrosine (Karshikoff *et al.*, 1993; Wilce & Parker, 1994) but in the Delta and Epsilon insect GST classes this role is performed by a serine residue (Board *et al.*, 1995).

Gene organization

The diversity of reactions catalysed by GSTs is a function of the broad substrate specificities of many individual GST enzymes and the extensive nature of the GST supergene family. In insects, local gene duplications, particularly within

the insect-specific Delta and Epsilon classes, have resulted in expansions of the GST family. Because substitution of a small number of amino acids can have dramatic effects on the substrate specificity of the enzymes (Ortelli *et al.*, 2003), the process of gene duplication, diversification and selection can tailor the repertoire of reactions catalysed by GSTs to suit the particular ecological niche occupied by a species (Ranson *et al.*, 2002).

Further GST diversity is generated by alternative splicing in *Anopheles* mosquitoes and genetic rearrangements leading to gene fusions in *Musca domestica*. Two GST genes are alternatively spliced in *A. gambiae*: four distinct peptides with differing catalytic properties are generated from the Delta class *AgGSTD1* gene (Ranson *et al.*, 1998) and two transcripts, sharing two common 5' exons but differing in their use of 3' exons, are generated from the single Sigma GST gene in this species (Ding *et al.*, 2003). The genome of *M. domestica* contains multiple intronless loci encoding Delta GSTs, some of which appear to have resulted from the fusion between the 5' and 3' ends of different Delta GST genes. It is not known whether all of these housefly GST genes encode functional proteins but it is possible that a rare recombination mechanism may be contributing to GST diversity in this species (Zhou & Syvanen, 1997).

Regulation of GST expression

In non-insect species, many GST enzymes are differentially regulated in response to various inducers or environmental signals or in a tissue- or developmental-specific manner. A similarly complex pattern of regulation is expected for insect GSTs. Two review articles have described the effect of various dietary compounds, insecticides and laboratory inducers on general GST expression (Clark, 1989; Yu, 1996). Now that the full extent of the GST family is known for two insect species, more specific studies can be conducted to determine the factors regulating expression of individual GST genes.

Levels of GST activity vary throughout the life stage of insects. For example, in *Aedes aegypti*, total GST activity measured with 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloronitrobenzene (DCNB) increases during larval

development, reaching its peak in the pupal stage and declining in adults as they age (Hazelton & Laing, 1983). In a preliminary investigation of the expression profiles of *A. gambiae* GSTs, transcripts were detectable for all but one of the genes in 1-day-old adults (Ding *et al.*, 2003). No attempt was made to quantify the expression level in different developmental stages in *A. gambiae* but it is apparent from studies in other insects that the levels of individual enzymes can fluctuate widely during the lifespan of an insect. For example, a Sigma GST from the spruce budworm, *Choristoneura fumiferana*, is expressed at very low levels in feeding larvae but high levels in diapausing larvae (Feng *et al.*, 1999).

Variations in the level of GST activity in different insect tissues have been reported in several species. In cases where the variation in activity is attributed to individual enzymes, such studies can provide valuable insights into the functions of different GSTs. Thus the finding that Sigma GSTs from housefly and *Drosophila* were predominately located in the indirect flight muscles, in association with troponin H, suggested that the role of this GST class was structural rather than catalytic (subsequently, however, these GSTs have been found to play a very important role in protection against oxidative stress) (Franciosa & Berge, 1995; Singh *et al.*, 2001). Very high levels of GST activity have been reported in the fat body and midguts of insects. Both these tissues are important sites for the detoxification of xenobiotics.

GSTs and insecticide resistance

Elevated GST activity has been associated with resistance to all the major classes of insecticides (Prapanthadara *et al.*, 1993; Huang *et al.*, 1998; Vontas *et al.*, 2001). In many cases, the individual GST enzyme(s) involved in resistance have not been identified and GSTs have been implicated by association only (i.e. an increase in GST activity, detected using model substrates, in insecticide-resistant strains of insects vs. their susceptible counterparts). In cases that have been studied in more detail, resistance has been attributed to increases in the amount of one or more GST enzymes, either as a result of gene amplification or more commonly through increases in transcriptional rate, rather than qualitative changes in individual enzymes (Grant & Hammock, 1992; Ranson *et al.*, 2001).

Dehydrochlorination is an important mechanism for DDT detoxification. This reaction is catalysed by GSTs (Clark & Shamaan, 1984). The proposed mechanism is shown in Fig. 2. A GSH conjugate of DDT has never been identified, but this tripeptide is an essential cofactor in the reaction. The thiolate anion generated in the active site of the GST acts as a general base and abstracts a hydrogen atom from DDT resulting in the elimination of chlorine to generate DDE. Other organochlorine insecticides, e.g. lindane, are detoxified by conjugation to glutathione.

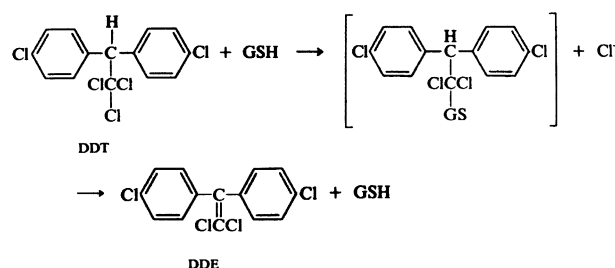


Figure 2. Model of DDT dehydrochlorinase activity of GSTs (after Matsumura, 1985).

Increased rates of DDT dehydrochlorination confer resistance to DDT in many insect species including houseflies and the mosquitoes *Ae. aegypti*, *A. gambiae* and *A. dirus* (Prapanthadara *et al.*, 1993, 1996; Grant *et al.*, 1991). In *Ae. aegypti*, two immunologically distinct forms of GST are overproduced in a DDT-resistant strain (Grant *et al.*, 1991). Overexpression of at least one of these is thought to be controlled by a mutation in a *trans*-acting repressor in the DDT-resistant strain (Grant & Hammock, 1992) but the nature of this repressor is not known. Multiple Epsilon class GSTs from *A. gambiae* are overexpressed in a DDT-resistant strain and one of these, *GSTe2*, encodes an enzyme that has the highest levels of DDT dehydrochlorinase activity reported for any GST (Ortelli *et al.*, 2003). Genetic mapping of the major loci conferring DDT resistance in *A. gambiae* implicate both *cis*- and *trans*-acting factors in the overexpression of the Epsilon class GSTs (Ranson *et al.*, 2000).

GSTs are responsible for many cases of organophosphate resistance (Hayes & Wolf, 1988). The conjugation of glutathione to organophosphate insecticides results in their detoxification via two distinct pathways. In O-dealkylation, glutathione is conjugated with the alkyl portion of the insecticide, e.g. the demethylation of tetrachlorvinphos in resistant houseflies (Oppenoorth *et al.*, 1979). In the second mechanism, O-dearylation, the glutathione reacts with the leaving group, e.g. the detoxification of parathion and methyl parathion in the diamondback moth *Plutella xylostella* (Chiang & Sun, 1993). Recombinant GST enzymes from the diamondback moth and housefly have verified the role of these enzymes in the metabolism of organophosphate insecticides (Huang *et al.*, 1998; Wei *et al.*, 2001).

GSTs have not yet been implicated in the direct metabolism of pyrethroid insecticides. Nevertheless, they may play an important role in conferring resistance to this insecticide class by detoxifying lipid peroxidation products induced by pyrethroids (Vontas *et al.*, 2001). A Delta class GST has been cloned from a pyrethroid resistant strain of *Nilaparvata lugens*. The recombinant protein produced from this gene has high peroxidase activity and it has been proposed that this enzyme is involved in the prevention or repair of oxidative damage induced by insecticide exposure (Vontas *et al.*, 2002). Elevated GSTs in other resistant insects may play a

similar role. GSTs may also protect against pyrethroid toxicity in insects by sequestering the insecticide (Kostaropoulos *et al.*, 2001).

Summary

Advances in genetics and biochemistry have revealed the complexity of the insect GST family. Specific functions of some individual GSTs, particularly their role in detoxifying xenobiotics, have been identified but much remains to be learnt about the endogenous substrates of insect GSTs. Deciphering the precise expression profile of each GST gene may provide clues to function that can subsequently be elucidated in *in vitro* systems.

Acknowledgements

This work was funded in part by a Royal Society Dorothy Hodgkin Fellowship (to H.R.) and a Wellcome Trust Project Grant (to J.H. and H.R.).

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