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A review on the structure and mechanism of cytosolic Glutathione S transferases in mosquitoes

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Abstract
Glutathione S transferases are functionally diverse enzymes that have a consensus structure. These enzymes play a vital role in detoxification, protecting cells from oxidative cells and insecticide resistance in mosquitoes. This review includes the structure and function of various GSTs isolated from *Anopheles gambiae* and *Aedes aegypti*. The mosquito GSTs have a canonical fold that is made of a thioredoxin-like N-terminal domain and an alpha helical C-terminal domain. The enzyme has a conserved glutathione binding domain and a relatively variable substrate binding site that is hydrophobic in nature. Differences within the enzymes reveal adaptation to various ecological and chemical pressures. In addition to their role in developing insecticide resistance- the enzymes can also be used in biosensing. This review highlights the importance of mosquito GSTs both as a tool and target for insect physiology, chemical ecology and public health.

Keywords: *Aedes aegypti*, *Anopheles gambiae*, Glutathione S transferases, cytosolic GSTs

Introduction
Glutathione S transferases (EC 2.5.1.18) are key enzymes that participate in various biotransformation processes [1]. They play a crucial role in cellular detoxification [2]. They also help to protect against damage from various harmful substances like reactive oxygen species, electrophilic species and environmental carcinogens [3]. They also act to protect the cancer cells from various chemotherapeutic medications [4]. These enzymes are found in different kingdoms from prokaryotes to eukaryotes are a part of the detoxification processes that have been around for more than 2 billion years [5]. In both the prokaryotic and eukaryotic systems these enzymes participate as primary Phase II Biotransformation enzymes [6]. They make up a superfamily of versatile enzymes that participate in different functions like nucleophilic coupling of tripeptide glutathione to potentially harmful foreign compounds like cancer causing byproducts formed during Phase I metabolism [7]. The tripeptide is made up of three amino acids glutamic acid (Glu), Cysteine (Cys) and Glycine (Gly) [8]. They also neutralize electrophilic properties making the end products more hydrophilic. This increase in hydrophilic property of the end products aids in the removal of the xenobiotic compound from the cell by Phase III enzymes [9]. The Glutathione S-transferases also function as isomerases, peroxidases in addition to acting as thiol transferases [10]. The Glutathione S transferases make a very ancient superfamily of proteins that have likely evolved from a thioredoxin-like ancestor [11]. All the GSTs share sequential and structural similarities with many stress related proteins. They share the thioredoxin-like fold with other cysteine and GSH binding proteins [12]. It is postulated that GSTs evolved from in response to development of oxidative stress. Multiple GST classes might have arisen by gene amplification, followed by divergence involving mechanisms like DNA shuffling [13].

General structure of GSTs
Generally, GSTs are homo- or hetero-dimeric proteins with each monomer having a weight of about 23-30 kDa [14]. Formation of heterodimers has been proposed to be restricted only when the two dimers belong to the same class of GSTs. This is necessary for compatible interface interactions between the subunits [15]. Evidence from crystallography of the homodimer enzymes have shown that the homodimers are related by a two-fold symmetry axis [16]. Each monomer has two domains [17]. The N-terminal domain (Domain I) is composed of β strands and α helices. This domain has the thioredoxin-like fold motif βαβαββα which is structurally conserved across the GST family [18]. The C terminal (Domain II) is composed entirely of helices. The 4-8 helices in the domain are characteristic of the GST class [19].

The two domains are linked by a linker loop made up of about 10 amino acids [20]. Each subunit of GST has two ligand-binding sites namely the 'G' and 'H' sites. Together they form the catalytic active site [21]. When compared to the H-Site, the G-site is more hydrophilic and structurally conserved within the GST families [22]. The G-site is mainly found in the N-terminal domain. It binds to the GSH group. It also prepares the sulphur containing thiol group for nucleophilic attack on the electrophilic substrates [23]. The relatively hydrophobic H-site adjacent to the G-site is present in the C-terminal domain and binds to the electrophilic substrates [24]. The amino acids present in the H-site participate in the positioning of the electrophilic centres of different substrate compounds of endogenous and exogenous origin for nucleophilic GSH attack. The H-site of the GSTs play a critical role in multiple substrate binding [25]. This helps the insects to tolerate various environmental stresses that it may encounter. Mutations in the amino acid sequences of the H-site alter the enzymatic activities of these transferases. These variations on the catalytic sites are responsible for the variable enzymatic activities to different substrates [26]. When compared to the G-site, H-site has more variations in their primary and secondary structures. The hydrophobicity of the H-sites also vary across GST classes and also within the individual GSTs [27]. The hydrophilic amino acids help in the formation of the hydrophobic pocket of the H-site beside the GSH-binding domain [28]. The H-site of the AgGSTe2 in *Anopheles gambiae* is most likely responsible for the binding of the enzyme to DDT [29]. The diversity of H-sites account for the wide variety of xenobiotic substrates to which the GSTs can bind and catalyse various biotransformation reactions [30].

The position and chemical properties of the amino acid in the active site of GSTs are very important for the enzyme's substrate binding affinity and catalytic functions. In delta and epsilon GSTs, His-38 is kept as a polar or charged residue [31]. In these classes His-50 is part of a conserved motif NPQHTVPTL. Both these residues are within the distance of polar interaction with the glycyl carboxylate moiety of GSH [13]. Ser-9 residues are conserved across epsilon and theta families of GSTs. This residue forms a hydrogen bond with GSH thiolate and stabilizes it [32]. The active site of a number of enzymes have been elucidated using X-ray crystallography and site-directed mutagenesis.

Classes of GSTs

In mosquitoes like most insects at least six classes (Epsilon, Delta, Zeta, Omega, Theta and Sigma) of cytosolic GSTs have been identified [20]. The enzymes are classified based on amino acid sequences, immunological and chromatographic properties, and phylogenetic relationships [33]. In spite of having very less similar sequences, the enzyme GSTE8 has been placed in Epsilon Class because of the position of the gene encoding the enzyme adjacent to the other Epsilon GSTs [34]. A few of the GSTs cannot be assigned to any class due to conflicting signals from different techniques that are used. Three cytosolic GST genes found in *An. gambiae* have not been assigned to any particular class with certainty [35]. Orthologs of these three enzymes are found in *Ae. aegypti* but not in *Drosophila melanogaster* [36].

Delta GSTs

The Delta class of GSTs is the largest class of all the glutathione S transferases [37]. These enzymes have high levels of expressions in the preimaginal stages [38]. The enzymes have been extensively studied partly due to the ease with which they can be purified using glutathione based affinity chromatographic columns. In *An. gambiae* there are about 12 Delta GST genes [35]. Among them, the GSTd1 gene is alternatively spliced expressing four biochemically distinct subunits [38]. In *Aedes aegypti* eight delta genes have been identified till date while in *Culex quinquefasciatus* there are 14 delta GSTs discovered till date [13]. Evolutionarily, the Delta GSTs diversified after the split of the nematoceran and Cyclorrhapha diptera lineages about 250 million years ago. This diversification of Delta GSTs in different dipteran families suggests the crucial role of these enzymes in different adaptive mechanisms insects have evolved to survive in their environment [20].

Using X-ray crystallography and site-directed mutagenesis to study the active site of delta GST GSTD3-3 in *Anopheles dirus*, the G-site was found to have residues Ser-9, Pro-11, Leu-33, His-38, Cys-51, Ile-52, Pro-53, Glu-64, Ser-65, Arg-66, and Met-101 [13]. The residues were within a 4.0 Å distance cutoff of GSH. The residue Ser-65 was found to be most conserved of all the residues across all the GST classes. This residue participates in hydrogen bond formation with GSH γ-glutamyl carboxylate [40]. The residues Ile-52 and Glu-64 have hydrophobic or polar properties across all the classes. The amide group of Ile-52 forms a hydrogen bond with the backbone carbonyl group of GSH cysteinyl group. Glu-64 participates in the formation of a salt bridge with the amino group of γ-glutamyl moiety of GSH [13].

In a study by Chen et al. 2003 [41], the structure of agGSTd1-6, a delta class Glutathione S-transferase from a DDT-resistant strain of *Anopheles gambiae* was elucidated. This enzyme converts DDT to a harmless compound by the process of dehydrochlorination. This reaction leads to the formation of 1,1-dichloro-2,2-bis-(p-chlorophenyl)ethane (DDE), a non-toxic compound through an in glutathione conjugate 1-(S-glutathionyl)-1,1-dichloro-2,2-bis-(p-chlorophenyl)- ethane (GS-DDE) intermediate. The authors used a molecular replacement method to determine the structure of the enzyme. Molecular replacement method is used to determine the 3-D structure of a protein when the structure of similar proteins are known. The enzyme was first purified and crystallized giving five different crystal forms of the enzyme. A primitive orthorhombic form of the enzyme was used for determining the structure. The refined structure of the enzyme when complexed with S-hexylglutathione (GTX) had bond lengths with root mean square deviation of 0.006 Å. The bond angles had a rms deviation of 1.2°. Both these values indicate that the model used by the authors to propose the possible structure of the enzyme is reliable. The Ramchandran plot is used to find how realistic the protein conformation is based on the steric constraints. It evaluates the backbone dihedral angles (ϕ and Ψ) of amino acid residues. The plot showed that most of the residues (90.4%) had ideal and stable conformations indicating a well-refined model of the enzyme. Some of the residues (8.0%) had acceptable conformations while even a fewer percentage of residues (1.6%) were in permissible regions due to local strains. The absence of non-glycine residues in disallowed regions further renders support to the model used. Like all GSTs, agGSTd1-6 is also a dimer. The distance between the 209 α carbons of the two residues is 0.70 Å. The enzymes adopt a conserved structure common to most GSTs. It has eight alpha helices (H1-H8) and four beta strands (B1-B4). The structure can be divided into two distinct domains: a N-terminal domain with 78 residues (G-site) and a C-terminal domain with 123 residues (H-site). The two domains are

linked by a short hinge loop with 6 residues. The N-terminal domain consists of a four-stranded mixed beta-sheet at its centre with H1 (residues 9-22) and H3 (residues 64-76) helices on one side and H2 (residue 40-47) helix on the other side. The secondary structures of the enzyme in the N-terminal domain are arranged in a ßαßαßßα motif. In this motif the B3 (residues 53-57) beta strand is antiparallel to the other beta strands namely: B1 (residues 1-5), B2 (residues 26-30) and B4 (residues 60-63). This mixed beta-sheet adopts a topology of '-1+2-1'. A proline residue in its cis-conformation ar 53rd position appears to be critical for the formation of the active site. Similar cis-residues are found in all GST structures till date [41]. The C-terminal domain has 5 alpha helices namely H4 (residues 86-115), which is slightly bent due to the presence of Glycine at 102 position; H5 (residues 123-142); H6 (residues 154-169); H7 (residues 177-189); H8 (residues 193-209). The active site of the enzyme is formed by the residues Leu6, Ser9, Ala10, Pro11, Leu33, Met34, His38, His50, Ile52, Glu64, Ser65, Arg66, Tyr105, Phe108, Tyr113, Ile116, Phe117, Phe203 and Phe207 and can be divided into two subsites, a glutathione (GSH) binding site and a hydrophobic binding site. The G-site is hydrophilic and polar while the H-site is hydrophobic. A GSH-analogue, GTX, was used to determine the positions of the hydrogen bonds formed by the active sites of the enzyme with its substrate. The gamma-glutamyl moiety of GTX formed hydrogen bonds with side chains of Glu64, main chain and hydroxyl group of Ser65 and side chains of Arg66. The cysteinyl moiety of the analogue also formed hydrogen bonds with the main chain of Ile52 and amide N atom of Ile52. The glycyl group bonds with the carbonyl group and side chain of His50. It also forms a hydrogen bond with His38 bridged by a water molecule. The sulphur atom of the analogue forms another hydrogen bond with the hydroxyl group of Ser9. These hydrogen bonds and interactions help position the GSH moiety properly within the a G-site of the active site of the enzyme. The S-hexyl moiety of GTX covers only a small portion of the large and open H-site of the catalytic site of the enzyme. This H-site is composed of amino acid residues that are hydrophobic like Leu6, Ala10, Pro11, Leu33, Met34, Tyr105, Phe108, Tyr113, Ile116, Phe117, Phe203 and Phe207.

The structure of another delta class of GST, adGSTD5-5 was proposed by Udomsinprasert et al. 2005 [37]. The enzyme was isolated from *Anopheles dirus*. The results from the molecular replacement model confirmed that the final refined model had amino acid residues 2-215, one glutathione sulfonic acid molecule bound to the G-site and 44 water molecules that are often associated with protein crystals. The enzyme has a conformational structure that corresponds to the canonical structure of GST enzymes. Like all GSTs, it is present as a dimer in the physiologically active state. The G-site was mostly formed by residues from the N-terminal thioredoxin domain. The author proposed that a Ser11 residue appeared to be responsible for the activation of GSH thiol residue during catalysis. However they also opined that without direct evidence like site directed mutagenesis, there is a possibility that a serine, cysteine or tyrosine residue nearby can be a major player in the catalytic reactions. This enzyme has a His119 residue that is rare in the H-site of delta GSTs. The H-site of delta GSTs have hydrophobic amino acids. However in adGSTD5-5 a water molecule is present in the H-site that has a hydrogen bond linkage with the His119 residue. The helix alpha8 of the enzyme has much less distance to its C-terminal domain compared to other insect GSTs. This might be due to the presence of smaller amino acid residues in the enzyme when compared to other similar enzymes. The active site of the enzyme is also more elongated and has more polar residues when compared to other isoenzymes. The increase in polarity and positive and negative charges lend specificity to GSH and substrate binding. The uniqueness of this enzyme suggests that the structural plasticity of the Delta GSTs are not restricted to their H-sites only. In addition to the biotransformation of xenobiotics, this enzyme can also be involved in oogenesis and embryogenesis in *An. dirus*.

Epsilon GSTs

Another large class of insect specific GSTs are the epsilon GSTs. Till date eight epsilon GSTs have been isolated from *An. gambiae* and three have been discovered from *Ae. aegypti* till date [36]. The epsilon GSTs are characterised by low activity with 1 chloro 2,4, dinitrobenzene [38]. This class of GSTs are also not efficiently retained by glutathione based affinity chromatographic columns [43]. The epsilon GSTs have evolved independently in the nematoceran and cyclorrhaphan dipteran lines [44]. Like other GSTs, these enzymes are involved in the detoxification of xenobiotics. A few epsilon GSTs in members of the Family Culicidae have peroxidase properties. So they might also be involved in protection from oxidative stress [45].

A study was done by Wang et al. 2008 [29] to deduce the structure of agGSTe2. agGSTe2 is an epsilon GST isolated from *Anopheles gambiae*. It contains 221 residues. The enzyme is divided into two distinct domains and a linker amino acid segment (residues 80-89). The N-terminal domain is smaller (residues 1-79) while the C-terminal domain is larger (residues 90-221) composed entirely of alpha helices. The N-terminal domain has a bababba pattern. The mixed Beta strands occupy the central core of the protein and are flanked on one side by helices H1 (residues 13-25), H3 (residues 68-79) and on another side by H2 (residues 44-47). The B3 Beta strand (residues 57-60) is anti-parallel when compared to the other beta strands B1 (residues 4-8), B2 (residues 29-33) and B4 (residues 63-66). The C-terminal domain also known as the H-site is a right handed bundle made up of five alpha helices: H4 (residues 90-121), H5 (residues 128-145), H6 (residues 157-169), H7 (residues 181-191) and H8 (residues 196-221). Among them H4 is antiparallel to H5. It is interrupted by a kink (Val106 and Leu107) which is a distinguishing feature of this enzyme. The authors also crystallized the binary complex of the enzyme with its coenzyme, tripeptide glutathione (GSH) to study the enzyme's cofactor binding site. Each enzyme was found to have one GSH molecule bound to it. In the Ramachandran plot all the amino residues were found to be in the most favoured zone of the plot. The results obtained by the authors did not find any significant difference between the structure of the unbound enzyme and the enzyme bound to its cofactor GSH. Differences were only observed in the N- and C-terminal where variations are expected. The cofactor binding site is a narrow elongated cleft found at the interface of the N and C domains. This cofactor binding site or the active site can be divided into a cofactor binding site (G-site) and a substrate binding site that is made up mostly of hydrophobic amino acids (H-site). In this enzyme the G-site is made up of five amino acid residues (His53, Ile55, Glu67, Ser68, and Arg112). With the exception of Ile55, all the other amino acids are hydrophilic and polar in nature. The ɣ-glutamyl moiety of the GSH cofactor forms a hydrogen bond with the side chain of Glu67, hydroxyl group and amide group of

Ser68. While the cofactor's cysteinyl group forms two hydrogen bonds with the main chain carbonyl group and the nitrogen atom present in the amide group of Ile55. The glycyl moiety forms only one hydrogen bond with His53. Arg112 participates in three hydrogen bonds with the oxygen atoms present in the glycyl group and the gamma-glutamyl region of GSH. These hydrogen bonds help in the formation of an electron-sharing network and stabilization of the thiolate anion. The thiolate ion is formed from the thiol group of GSH. The formation of the thiolate anion is crucial for the nucleophilic attack by the enzyme to be successful. Residues Pro13, Pro14, Leu36, His41, Gln52, Thr54, Pro56, His69, and Phe108 might be involved in the proper positioning and orientation of the GSH molecule in its binding site. The authors found the Ser12 residue to be conserved in this enzyme playing a role in the stabilization in the thiolate anion by forming a hydrogen bond through its hydroxyl group. The active site of the enzyme has a well-defined V-shaped pocket where the substrate binds located adjacent to the GSH-binding site. The substrate binding site is made up of amino acids (Leu9, Leu11, Ser12, Pro13, Pro14, Leu36, Leu37, His41, Ile55, Phe108, Met111, Phe115, Leu119, Phe120, Leu207, and Phe210) most of which are hydrophobic in nature. The side chains of the amino acids Arg112, Glu116, and Phe120 form a cap-like pocket. Several attempts to crystallize DDT-apo-agGSTe2 complex were unsuccessful. This result indicated that the binding of the cofactor GSH is necessary for the substrate molecule to bind to the apoenzyme.

The authors also used computational modelling to mimic the binding of a DDT molecule to the substrate binding site of the enzyme by assuming minimum collision with surrounding residues. They found that the planar p-chlorophenyl ring of DDT fits quite well to a subpocket of the binding site while spatial hindrance is met by the other ring from the side chains of Met111 and Phe115. The authors proposed a possible elimination reaction mechanism for the enzymatic reaction catalysed by the GST enzyme based on the little distance of about 2.0 Å between the beta hydrogen of DDT and S atom of GSH in the proposed computational model. The binding of a DDT molecule to the H-site, the activated thiolate group of the GSH cofactor initiates a nucleophilic attack on the positively charged β hydrogen of DDT. In this process a HCl molecule is eliminated converting the DDT to DDE. This type of elimination reaction has been reported by Ranson et al. 2001 [47] in the enzyme agGSTe2.

In a study by Pontes et al. 2016 [46], the binding and affinity of DDT with AgGSTE2 and AgGSTE5 were studied. The authors used variants of AgGSTE2 enzymes having Phe or Leu at 120 position to study the AgGSTE2 enzyme. DDT binds to the enzyme in a well-defined conformation such that the beta hydrogen of the molecule is within 2 Å of the thiolate group of GSH. This conformation allows the accepted elimination reaction mechanism for conversion of DDT to DDe. This conformation of DDT with AgGSTE2 enzyme was the same irrespective of the presence of residues Phe120 or Leu120. When molecular docking simulations were done it was found that along with Phe120, Phe115, Phe121 and Phe210 restricts the G-site. The four aromatic amino acids help DDT to bind to the G-site through hydrophobic interactions. The simulations show that Phe120 is the main anchorage point for DDT acting as the facilitator for binding of DDT to AgGSTE2 enzyme.

Omega GSTs

The omega GST was first identified in *Homo sapiens sapiens* [48]. Since this class of GSTs has been found in different organisms. In mosquitoes omega GSTs appear to be encoded by only one gene [39]. However on other dipteran lineages like in *Drosophila melanogaster*, five genes encoding this class of GSTs have been discovered till date [47]. These GSTs appear to protect cells from oxidative stress by removing the S-thiol adducts from various proteins playing a key cellular housekeeping role in the organism [50]. In a study by Board et al. 2000 [51], the authors characterized and studied the structure of GSTO1-1. The authors determined the structure of the enzyme by multiwavelength anomalous dispersion giving a resolution of 2.0 Å. The enzyme had a structure similar to that of other GST enzymes having a canonical GST fold. The enzyme had two domains. A thioredoxin-like N-terminal domain and a C-terminal domain composed entirely of alpha-helical structures. A central four stranded beta-sheet is present in the N-terminal domain that consists of two alpha helices (α1 residues 32-45; α3 residues 85-97) on one side and a 3_{10} helix also known as α2 (residues 60-66). The N-terminal domain of the enzyme is unique. The residues 5-22 make an extended structure that leads into the β1 strand (residue 23-28). In the C-terminal domain there are seven alpha helices. Among them five helices (α4, 5, 6, 7, 8) are found in most of the GSTs. However the last two (α9 residues 219-230 and α10 residues 235-239 fold back and extend on top of the N-terminal domain. This extension of the C-terminal makes several H-bonds with N-terminal domain. This forms a continuous extension with the extension of the N-terminal. The cofactor binding of the enzyme GSTO1-1 distinguishes it from other GSTs. The Cys32 residue forms a disulphide bond with GSTO1-1. This feature is also found in a bacterial GST PmGSTB1-1. The thiol group of Cys32 is located precisely over the helical axis of α1. Similar placement is also seen in the thiol of the N-terminal cysteine present in the Cys-Xaa-Xaa-Cys motif of glutaredoxin and thioredoxin. The structure creates a proximity of Cys side chain to the positive end of the helix dipole. This forms a nucleophilic property in the cysteine residue of thioredoxin and other similar chemicals. Pro33 helps in the proper positioning of the thiol group of Cys32 thereby stabilizing the thiolate form of GSH. Another unique feature of the omega GST is the absence of interaction between the cofactor that is bound to one peptide and groups that are bound to another peptide. Unlike the other peptides, the GSTO1-1 dimer adopts an open V-shaped configuration. The two subunits contact only through the side chains of β4, α3 and α4. The interactions are non-polar with only two salt bridges (Lys114 to Glu91). All other characters of the enzyme at its G-site are similar to that in other enzymes. At the H-site, like other GSTs GSTO1-1 has a definite binding site for the xenobiotic compound adjacent to the GSH binding site. One side of the binding side is formed by Phe-31 and Pro-33 with a reactive Cys32 in between them. Another unique feature of the H-site is the Trp222 residue. The residue has its indole nitrogen pointing into the pocket. This indole nitrogen contributes to the formation of an additional hydrogen bond, making the binding pocket less hydrophobic. The side chain of Arg183 contributes to another polar atom (N-ϵ) which forms the bottom part of the binding cavity. The relatively polar nature of the binding site and its open configuration suggests that the substrate for the enzyme might be a large molecule that is not entirely hydrophobic.

Function of mosquito GSTs

One of the most common reactions carried out by the GST enzymes is conjugation of glutathione. This helps in the

conversion of lipophilic chemicals to hydrophilic ones. This conversion helps in easy removal of the chemicals from the cells [52]. GSTs, besides playing an important role in detoxifying insecticides play a key role in the metabolism of other biomolecules. Glutathione S transferases have catalytic functions to detoxify various organochlorines and organophosphates [9]. Additionally, some of the GSTs might have peroxidase activities that protect the cell from harmful effects of oxidative stress [53].

Discussion

Glutathione S-transferases are a part of a large family of enzymes that involve detoxification of xenobiotic substances [54]. They also play an important role in developing resistance to different chemical insecticides namely organochlorines and organophosphates in mosquitoes [55]. Various works have been done on the structure of GST enzymes. The high resolution structure of the enzymes revealed the presence of a conserved canonical fold in both the apo and ligand bound forms. This structure common to all dipteran GSTs indicates that the Glutathione S-transferase enzymes have diverged while at the same time retaining a highly conserved structure particularly at the G-site [56]. The studies showed that the binding of GSH to the apoenzyme did not induce any major conformational changes in the enzyme. This was supported by very little root mean square deviations between the two forms. The presence of this conserved structure suggests an evolutionary pressure to maintain an efficient glutathione binding environment at the same time allowing structural diversity at the H-site. This diversity helps the H-site to bind to a wide variety of hydrophobic substrates [46]. As a result there are isoform specific regulatory functions and subcellular localization among the various GSTs [57]. The GST family has given rise to different classes in insects by gene duplication and functional diversification [13]. Class-specific adaptation to bind to the various compounds in the substrate binding H-site has risen as a response to various stresses caused due to natural and artificial chemicals. These GSTs can be used as biosensors for environmental monitoring because of their ability to metabolize various organophosphates, organochlorines and pyrethroids. Understanding the structural diversity of various GST enzymes in mosquitoes can help to understand metabolic resistance against various insecticides that are used to control mosquito borne diseases like malaria and dengue [58].

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