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Interaction affinity of Delta and Epsilon class glutathione-s-transferases (GSTs) to bind with DDT for detoxification and conferring resistance in *Anopheles gambiae*, a malaria vector

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ABSTRACT

Background & objectives: The enzyme glutathione-s-transferases (GSTs) are associated with detoxification of DDT, as experimentally proved in *Anopheles gambiae*. Insect GSTs are classified into six classes and among them Delta and Epsilon class GSTs have been implicated in detoxification of organochlorine insecticides. Both Delta and Epsilon GSTs produce, in total, 24 transcripts that result in the production of corresponding enzyme proteins. However, the conventional assay estimates the level of total GSTs and relates to development of resistance to DDT. Hence, it would be more reliable to estimate the level of the specific class GSTs that shows higher affinity with DDT. This would also lead to design a specific molecular tool for resistance diagnosis.

Methods: Of the 24 GSTs, computational models for 23 GSTs, which are available in Swiss-Prot database, were retrieved and for the remaining one, D7-2, for which no model is available in the data bank, a structural model was developed using the sequence of *An. dirus* B with a PDB ID of 1R5A as the template. All the models were docked with DDT in the presence of reduced glutathione.

Results: The energy output showed that Delta, D6 has the highest interaction affinity with DDT. Hence, this particular GST (D6) is likely to get elevated on exposure of mosquitoes to DDT.

Interpretation & conclusion: It would be, therefore, possible to design a specific molecular assay to determine the expression level of such high affinity transcript(s) and to use for resistance diagnosis reliably in the vector surveillance programme.

Key words *Anopheles dirus; An. gambiae;* DDT; Delta and Epsilon classes; GSH; glutathione-s-transferases

INTRODUCTION

Insecticide resistance is a growing problem in the control of disease spreading insect vectors. The basic mechanisms underlying resistance to commonly used insecticides such as organochlorines, organophosphates, carbamates and synthetic pyrethroids are well-known. Resistance mechanisms are broadly of two types, target site insensitiveness and enzyme-based detoxification[1]. In mosquitoes, detoxification is performed by a group of enzymes such as esterases, oxidases and glutathione-s-transferases (GSTs). Detoxification of insecticides occurs when enhanced levels of these enzymes prevent the insecticide from reaching its site of action. GSTs are the major family of detoxification enzymes which get activated in the presence of reduced glutathione (GSH) and bind to DDT (Dichlorodiphenyl-trichloroethane) molecules and convert them into a non-toxic lipophilic compound DDE (Dichlorodiphenyl-ethane)[2]. Many resistant insects have elevated level of GSTs and such over expression of this enzyme is considered to be responsible for the development of resistance to organochlorine insecticides including DDT, hexachlorocyclohexane (HCH) and cyclodiene[3]. Recently, elevated level of GST activity for DDT detoxification has been confirmed in *Anopheles culicifacies* and *An. annularis*[4].

Insect GSTs are classified into six classes, viz. Delta, Epsilon, Zeta, Sigma, Omega, and Theta by comparative analysis of *Drosophila melanogaster* and *An. gambiae* genomes[5–8]. The Delta and Epsilon class GSTs, which are encoded by multiple genes, have been implicated in detoxification, particularly in conferring resistance to organochlorine group of insecticides[9–12]. In *An. gambiae*, an important African malarial vector, the increased rate of DDT dehydrochlorination in the resistant vector strain was associated with quantitative increase of GST enzymes that included Delta and Epsilon classes[13]. GSTs consist of a specific-glutathione (GSH)-binding site (G-site) next to a non-specific electrophilic ligand-binding site (H-site). The high level of diversity in this region is responsible

for the differences in substrate specificities[14–15].

Based on the studies carried out so far in *An. gambiae*, Delta class comprises of 12 different genes (D1-D12), out of these D1 gene gives rise to four different transcripts, D7 and D11 genes produce two different transcripts each and each one of the remaining genes (except D9) gives rise to a single transcript and no information is available for D9 gene in the existing databases for *An. gambiae*. Epsilon class encompasses eight different genes (E1-E8); each gives rise to a single transcript. All the 16 transcripts of Delta class GSTs, except D7-2 and eight transcripts of Epsilon class GSTs have modbase structure deposited in the Swiss-Prot database. Further, structure of one of the transcripts of D1 gene, agGST1-6, was proved experimentally[16] and its affinity with S-hexyl glutathione (GTX), a hexyl glutathione was understood from crystallographic studies. Similarly, the structure of E2 GST was confirmed by X-ray crystallographic analysis[17].

Therefore, the Delta and Epsilon class GSTs produce a total of 24 transcripts that result in the production of corresponding enzyme proteins. However, to detect the development of resistance by mosquitoes to DDT, the conventional biochemical assay estimates the elevated level of total GSTs. Hence, it would be more reliable if the specific class of GST enzyme which has the highest molecular interaction affinity with DDT is estimated and used for screening of field mosquito population for development of DDT resistance.

Therefore, a comparative study was undertaken by docking of Delta and Epsilon class GSTs in their bioconfirmative form (GSTs and reduced GSH [glutathione] complex) with DDT using bioinformatics tools. This information would be useful to improve the biochemical analysis of the specific class GST enzyme that would further lead to design of a more specific molecular tool to determine the expression status of the particular type of GST enzyme.

MATERIAL & METHODS

The computational models of eight Epsilon (E1-E8) and 15 Delta class GSTs (D1-D8 and D10-D12) (except D9 and D7-2), that are reported to be responsible for DDT resistance in *An. gambiae* were retrieved from the Swiss-Prot database (*http://www.expasy.ch/sprot/*). The Delta class GST, D9 was not considered in the present study as its corresponding gene has no information in the data bank. But, for D7-2, while there was no structural model deposited in the Swiss-Prot database, its sequence details are available. Hence, the D7-2 GST protein that consists of 191 amino acid residues, with an ID of A0NDD6 was subjected for modeling. The NCBI, BLAST was used to select a template for the D7-2 query sequence based on the highest sequence identity. As a result, the sequence of *An. dirus* B with a PDB ID of 1R5A having an identity of 95% was chosen as the template and a homology model of D7-2 was developed using MODELLER. The stereo chemical quality of the model was determined by the Structure Analysis and Verification Server (*http://nihserver.mbi.ucla.edu/SAVS/*). The other modeled proteins of Epsilon and Delta class GSTs retrieved from the database were also reassessed for their quality using the same server (PROCHECK analysis). The modeled D7-2 was examined by superimposing it on the template 1R5A in Chimera Visualizer (Fig.1a). Subsequently, to establish the correctness of the model, its RMSD details (Fig. 1b) were obtained using the Superpose online server tool (*http:/wishart.biology.ualberta.ca/SuperPose/*)[18].

CASTp (*http://sts-fw.bioengr.uic.edu/castp/*) was used to find out the surface pockets of the retrieved as well as the modeled proteins and Q-site Finder (*http://www.bioinformatics.leeds.ac.uk/qsitefinder/help.html*), an online tool and a new method of ligand binding site prediction, was used to predict the active sites of the proteins. Additionally, Fuzzy-Oil-Drop (*http://www.bioinformatics.cm-uj.krakow.pl/activesite/*) was also used to confirm the active sites predicted by the CASTp and Q-site Finder. The G-site (an active site for reduced GSH) and H-site (an active site for DDT) of GST enzymes were predicted using the three online servers. Since, reduced glutathione is necessary for the activation of GST, its chemical structure was taken from the PUBCHEM database (*http://pubchem.ncbi.nlm.nih.gov/*). Similarly, the chemical structure of DDT was also retrieved from the PUBCHEM database and their corresponding PDB files were obtained by uploading the smiles notations of the compounds to the online molecular format converter (*http://www.molecular-networks.com/online_demos/*

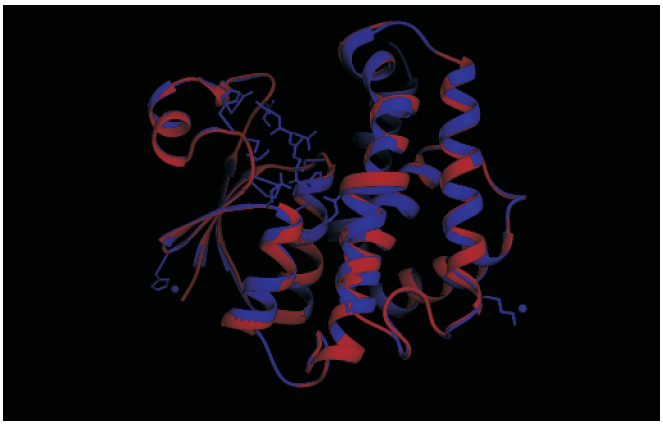


Fig. 1a: Three dimensional homology model of D7-2 superimposed on the template, 1R5A; GST is shown in ribbon form.

SuperPose used the sequence alignment to guide the superposition

Local RMSD

	Alpha carbons	*Back bone*	*Heavy*	*All*
RMSD	0.14	0.19	0.76	0.76
Atoms	190	760	1532	1532
	Structure		*Residues*	
	1R5A chain 'A' PDBB		2–191 2–191	

Global RMSD

	Alpha carbons	*Back bone*	*Heavy*	*All*
RMSD	0.14	0.19	0.76	0.76
Atoms	190	760	1532	1532
	Structure		*Residues*	
	1R5A chain 'A' PDBB		2–191 2–191	

SuperPose output images

WebMol
MolScript superposition image
Difference distance matrix

SuperPose output text files

Sequence alignment
Difference distance matrix
Superposition (PDB)
RMSD report
Back to SuperPose home page

Fig. 1b: RMSD details of the superimposed model D7-2 on the template, 1R5A.

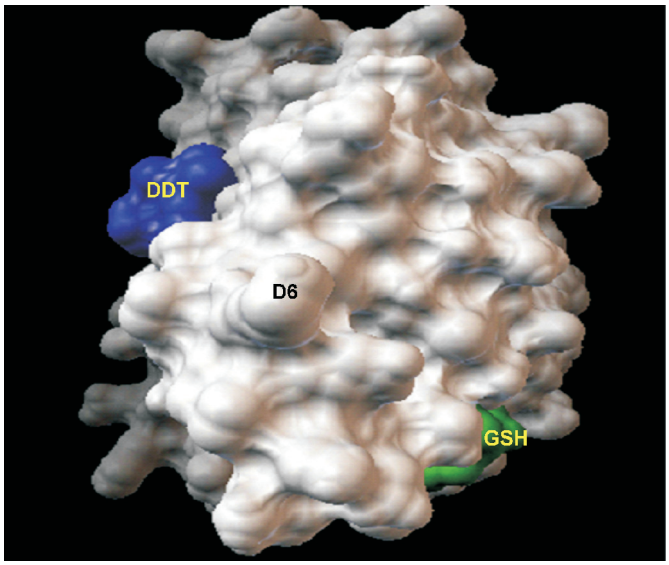


Fig. 2: Docking results of reduced GSH with D6, a Delta class GST.

corina). The transcript models that were validated using PROCHECK analysis were initially docked with GSH (Fig. 2) and the resulted (GSH + GST) (Fig. 3) complex was subsequently docked with the ligand, DDT using AutoDock to rank the transcript showing the highest interaction affinity. Labeled molecular level close up of the hydrogen bonds between GSH and GSTs has been shown in Fig. 4 and hydrophobic patch was established by DDT interaction with GST at position PHE 117 (Fig. 5).

RESULTS & DISCUSSION

This current *in-silico* analysis was carried out based on the observation made in a wet lab study that confirmed

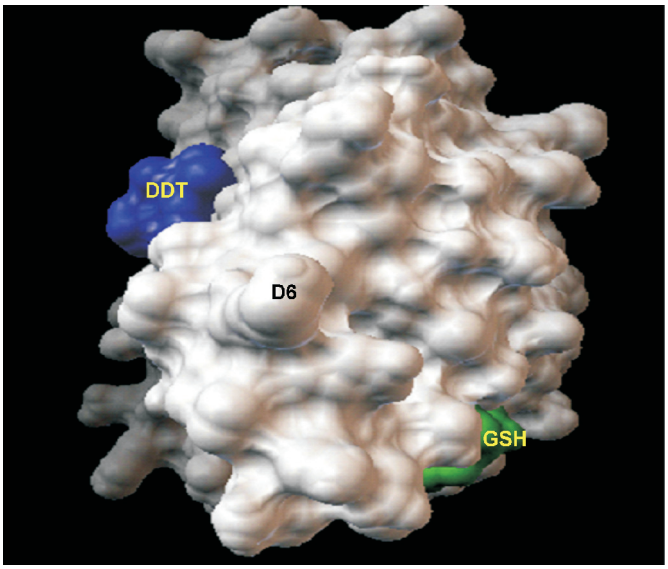


Fig. 3: Docking results of DDT with D6 GST in the presence of reduced GSH.

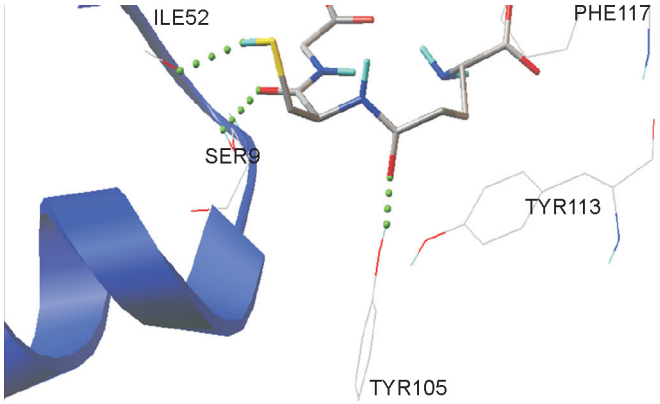


Fig. 4: Molecular interaction of GSH and GST.

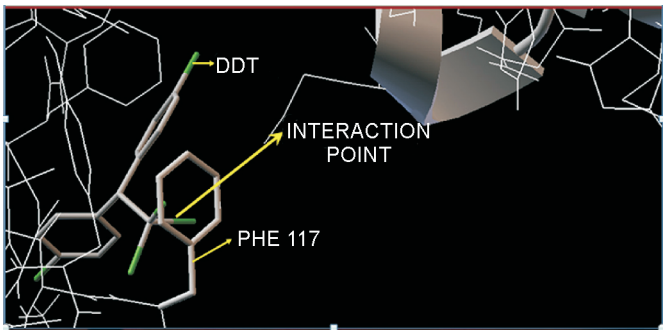
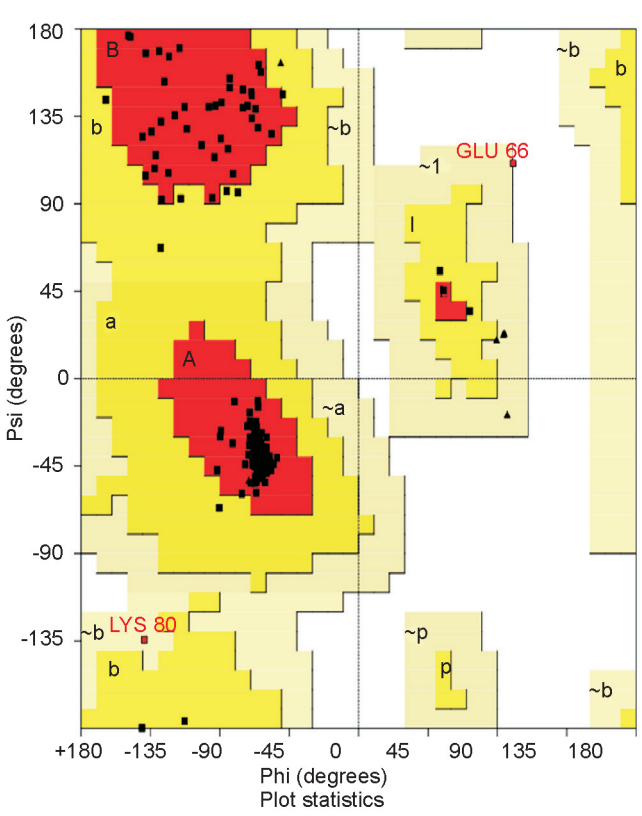


Fig. 5: Molecular interaction of GST and DDT.

the involvement of GSTs in conferring DDT resistance in the malaria vectors, *An. culicifacies* and *An. annularis*[4]. There was a 3-fold increase in GST activity in DDT-resistant *An. annularis* compared with its susceptible population. In *An. culicifacies*, the median GST activity (71.8 µmol/min/mg) was almost the same as estimated in the DDT-resistant *An. annularis* (74.6 µmol/min/mg), suggesting that the GST activity estimated in *An. culicifacies*



Residues in most favoured regions [A, B, L]	157	(92.4)
Residues in additional allowed regions [a, b, l, p]	11	(6.5)
Residues in generously allowed regions [~a, ~b, ~l, ~p]	1	(0.6)
Residues in disallowed regions	1	(0.6)
Number of non-glycine and non-proline residues	170	(100.0)
Number of end-residues (excl.Gly and Pro)	2	
Number of glycine residues (shown as triangles)	7	
Number of proline residues	12	
Total number of residues	191	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor not greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Figures in parentheses indicate percentages.

Fig. 6: Ramachandran plot of the D7-2 GST model. The plot calculations on the 3D homology model of D7-2 GST were computed with the PROCHECK programme. The plot statistics are: residues in most favoured regions [A, B, L] : 157 (92.4%); residues in additional allowed regions [a, b, l, p] : 11 (6.5%); residues in generously allowed regions [-a, -b, -l, -p] : 1 (0.6%); residues in disallowed regions : 1 (0.6%); number of non-glycine and non-proline residues : 170 (100%); number of end residues (excluding glycine and proline) : 2; number of glycine residues (shown as triangles) : 7; number of proline residues : 12; and total number of residues : 191.

could be an elevated level for detoxification of DDT. Furthermore, the GST activity in DDT-resistant *An. culicifacies* and *An. annularis* was significantly higher than that in the DDT-susceptible *An. fluviatilis*, which had a GST activity of 20 µmol/min/mg[4].

In total, structural models are available for 24 GSTs, eight of Epsilon class and 16 of Delta class. Among these, 23 were the retrieved ones and the remaining one was developed during the current study. The overall quality factor of the model developed for D7-2 was 92.4% as obtained from the Ramachandran plot (Fig. 6). The quality factor for all the retrieved models was around 90% as revalidated using PROCHECK analysis (Table 1).

Finding out the active site residues of GST showing the highest affinity for GSH interaction and subsequently of GST + GSH complex for DDT interaction is an important step to carry out the docking process. In an earlier crystallographic study, one of the transcripts of Delta class D1 gene (D1-1) was examined in relation to GSH binding with GST and, based on formation of more number of hydrogen bonds, identified ILE 52 as the active site residue showing the highest affinity for GSH binding[16]. This residue is present in the G-site of the D1-1 transcript. The G-site consists of 19 amino acid residues (LEU 6, SER 9, ALA 10, PRO 11, LEU 33, MET 34, HIS 38, HIS 50, ILE 52, GLU 64, SER 65, ARG 66, TYR 105, PHE 108, TYR 113, ILE 116, PHE 117, PHE 203, and PHE 207) and out of these, ILE 52 showed the highest interaction affinity with GSH[15]. Hence, ILE 52 was considered in the current study as the suitable active site for carrying out the docking of GSH with the GSTs of both the Epsilon and Delta classes.

The crystallographic study with *An. dirus* confirmed that either PHE (117 and 203) or TYR (105 and 113) residues of the bioactively conformed GST form the hydrophobic pathway for binding with DDT[19], and out of these four residues, according to the docking results obtained in the current study, PHE 117 was found to show a higher interaction with DDT. Further, the studies with *An. gambiae* have illustrated that the H-site, which is meant for DDT (ligand) binding, composed of 12 amino acid residues, including those four residues that were related to DDT binding in the study with *An. dirus*[19]. So PHE and TYR could be the residues involved in DDT binding in the case of *An. gambiae* also. Thus, involvement of these two residues, with PHE showing a higher interaction, in DDT binding with GSH + GST complex is clearly confirmed. Based on this information, PHE 117 could be considered as the suitable active site residue for DDT interaction. However, literature evidence did not explain about the position of ILE 52 (G-site residue) and PHE 117 (H-site residue), i.e. whether both are located in the same pocket or not. Our results from CASTp ,Q-SITE FINDER and Fuzzy Oil Drop analysis confirmed that both the G-site residue (ILE 52) (Fig. 7a) and the H-site residue (PHE 117) (Fig. 7b) are present in the same pocket,

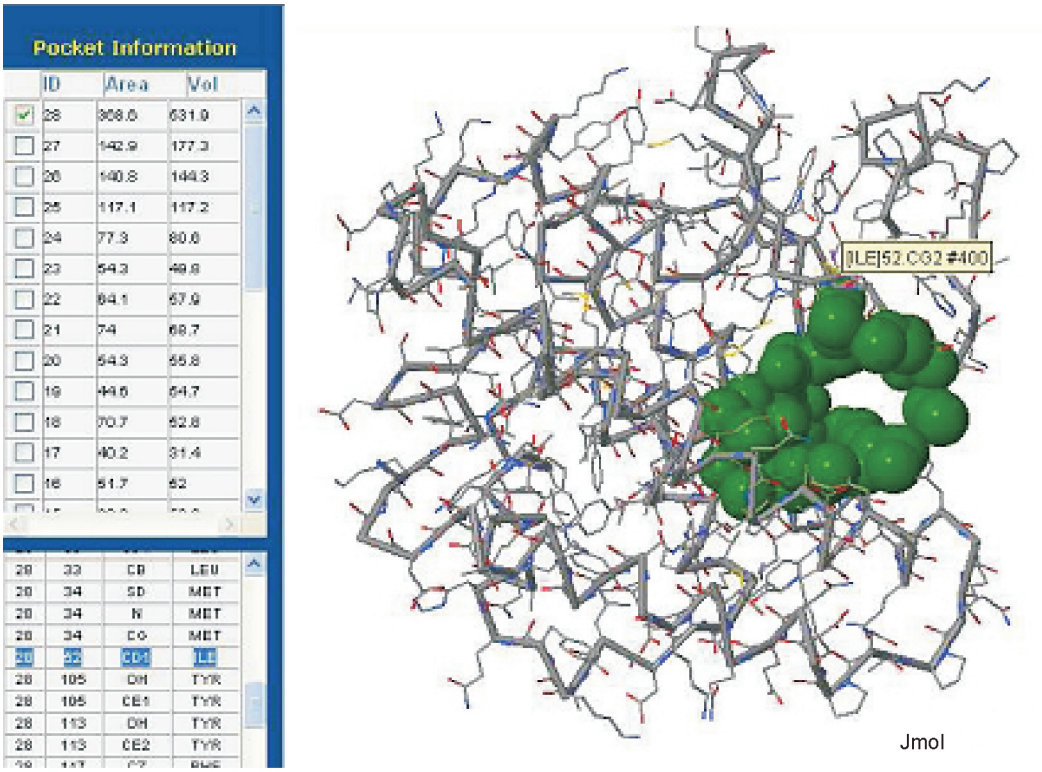


Fig. 7a: CASTp results showing the G-site residue (ILE 52) present in the pocket ID 28 at different sites.

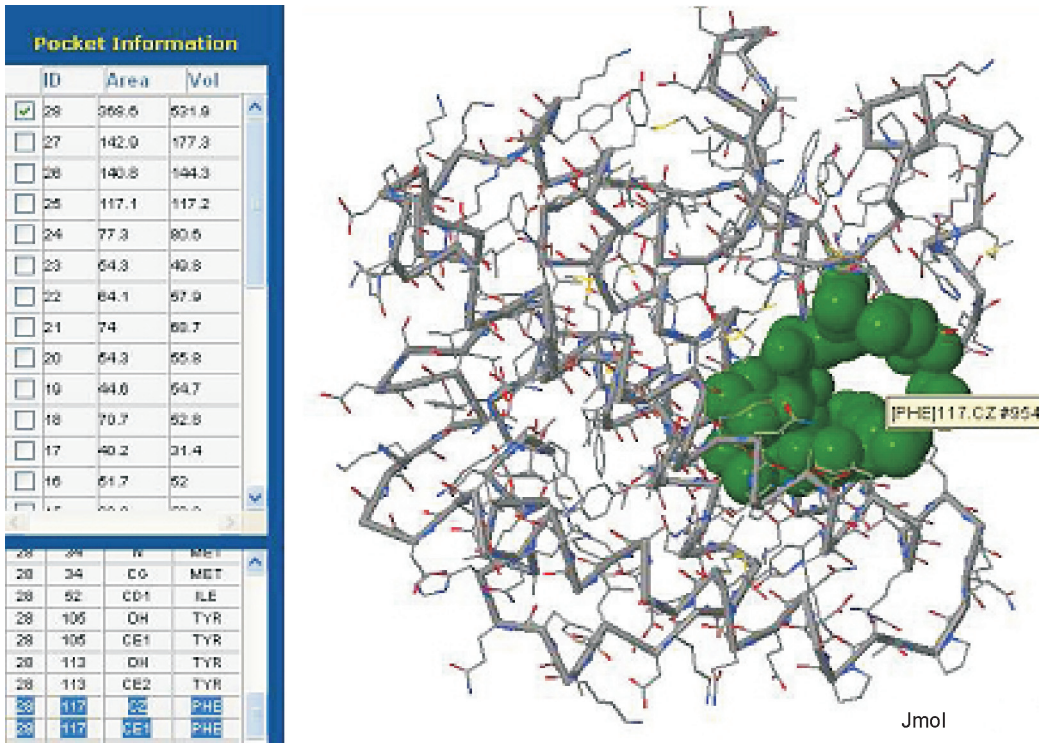


Fig. 7b: CASTp results showing the H-site residue (PHE 117) present in the pocket ID 28 at different sites.

but at different sites. The residues PHE and ILE are, respectively at position 117 and 52 in both D1-1 and D1-3. Apart from these two transcripts, in all others, PHE and ILE are present at different positions not at 52 and 117; these two positions are occupied by other amino acid residues. It has been reported that all the 24 Epsilon and Delta

Table 1. Quality factor for Delta and Epsilon classes GSTs as validated by PROCHECK analysis

Transcripts of GST genes (*An. gambiae*)	Query sequence length (residues)	Template sequence		Sequence identity (%)	Swiss-Prot Model ID	Residues in most favoured region (quality factor) (%)
		Length/ PDB ID	Organism			
Delta class GSTs						
D1-1	209	209/1pn9	*An. gambiae*	100	Q93113	96.2
D1-2	216	218/1r5a	*An. dirus* B	43	Q7PH26	92.4
D1-3	209	209/1pn9	*An. gambiae*	84	Q93112	95.1
D1-4	219	219/1jlw	*An. dirus* B	84	O77462	93
D2	209	209/1pn9	*An. gambiae*	52	Q94999	94
D3	210	210/1v2a	*An. dirus* B	86	Q7PQ95	94.1
D4	212	210/1v2a	*An. dirus* B	62	Q5TT03	94.7
D5	216	210/1v2a	*An. dirus* B	60	Q7QB59	92.1
D6	222	210/1v2a	*An. dirus* B	42	Q7PQB4	92.2
D7-1	218	218/1r5a	*An. dirus* B	95	O76483*	93.1
D7-2	191	218/1r5a	*An. dirus* B	95	A0NDD6	92.4
D8	223	209/1pn9	*An. gambiae*	46	Q5TTE5	92.5
D10	211	210/1v2a	*An. dirus* B	56	Q7QA79	91.9
D11-1	214	209/1jlv	*An. dirus* B	56	Q7PQ57	94.7
D11-2	217	209/1jlv	*An. dirus* B	47	Q5TT04	89.5
D12	211	209/1v2a	*An. dirus* B	41	Q7QA80	94.1
Epsilon class GSTs						
E1	224	218/1r5a	*An. dirus* B	32	Q7PVS5	90.6
E2	221	209/1pn9	*An. gambiae*	41	Q9GPL8	91.8
E3	223	209/1pn9	*An. gambiae*	36	Q7PVS2	93.5
E4	225	209/1pn9	*An. gambiae*	39	Q7PVS7	95.7
E5	230	209/1pn9	*An. gambiae*	41	Q7PVS8	92.8
E6	227	218/1r5a	*An. dirus* B	27	A0NG89	89.1
E7	225	209/1pn9	*An. gambiae*	36	Q7PVS4	96.2
E8	217	218/1r5a	*An. dirus* B	27	Q8WQJ5	93.1

*The primary accession code: AF 251478.

class GSTs have been implicated in the process of detoxification of DDT[8]. The pocket prediction analysis done in the current study also confirmed the presence of PHE and ILE in the same pocket in all the 24 computational GST models. Therefore, though present at different positions in different transcripts, considering their presence within the same pocket, the two residues, PHE and ILE should be considered as the key residues involved in DDT cleavage and hence, significance was given to the residues and not to the position they occupy. Further, the pocket having both PHE and ILE residues was taken as the active site for the interaction of GSH + GST with DDT.

The energy budget obtained as the output from the docking of the 24 models of Epsilon and Delta class GSTs with DDT in the presence of reduced GSH is given in (Table 2). Out of the 24 modeled GSTs, Delta 6 had shown the lowest binding energy followed by Delta 4 and Epsilon 2, indicating their highest degree of affinity with DDT in order. Therefore, these three proteins could be considered to be the major factor for the detoxification of DDT.

CONCLUSION

Based on the degree of interaction affinities, the modeled GST from D6 transcript, which showed the highest affinity with DDT, is likely to get over-expressed on mosquito exposure to the insecticide. Following further confirmation through wet lab studies, it would be possible to design a specific molecular assay to

Table 2. Energy budget output from the docking of GST with DDT bound with reduced GSH

GST class/ transcripts	Binding energy (kcal/mol)	DDT interactive residue
Delta class		
D1-1	–9.19	PHE 117
D1-2	–6.42	PHE 210
D1-3	–7.37	PHE 117
D1-4	–7.82	PHE 123
D2	–6.38	PHE119
D3	–6.60	PHE194
D4	**–32.60**	PHE97
D5	–9.00	PHE157
D6	**–34.47**	PHE105
D7-1	–8.02	PHE115
D7-2	–8.17	PHE115
D8	–8.78	PHE3
D10	–7.68	PHE196
D11-1	+1.44e+05	PHE108
D11-2	+3.04e+05	PHE211
D12	–7.61	PHE181
Epsilon class		
E1	–8.18	PHE 111
E2	**–10.35**	PHE 108
E3	–6.31	PHE 117
E4	–10.07	PHE 113
E5	–7.52	PHE 112
E6	–7.94	PHE 109
E7	–7.02	PHE 110
E8	–7.72	PHE 119

determine the expression level of such high affinity transcript(s) and this could be used for detection/monitoring of resistance more reliably among the field vector populations.

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