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Modeling analysis of GST (glutathione-S-transferases) from *Wuchereria bancrofti* and *Brugia malayi*

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Abstract:

GST (glutathione S-transferases) are a family of detoxification enzymes that catalyze the conjugation of reduced GSH (glutathione) to xenobiotic (endogenous electrophilic) compounds. GST from Wb (*Wuchereria bancrofti*) and Bm (*Brugia malayi*) are significantly different from human GST in sequence and structure. Thus, Wb-GST and Bm-GST are potential chemotherapeutic targets for anti-filarial treatment. Comparison of modeled Wb and Bm GST with human GST show structural difference between them. Analysis of the active site residues for the binding of electrophilic co-substrates provides insight towards the design of parasite specific GST inhibitors.

Keywords: Filarial parasites; GST (glutathione-S-transferases) homology modeling; structural deviations; active site residues

Background:

Lymphatic Filariais is an infectious disease that causes serious social and economic burden. **[1, 2]** Filariasis is caused by worms, *Wuchereria bancrofti (Wb*), *Brugia malayi (Bm) and Brugia timori* belonging to the order "Filariidae". The worm is transmitted to man by different mosquito species. *Wb* is responsible for 90% of the cases worldwide followed by *Bm* which is confined to some regions of Southeast and Eastern Asia. **[3]** Filarial disease management requires effective anti-filarial inhibitors. **[4]** Current anti-filarial drug discovery aims towards the development of safe and effective macrofilaricide (a drug targeting adult filarial worms). GST (glutathione S-transferases) are a family of detoxification enzymes that catalyze the conjugation of reduced GSH (glutathione) to xenobiotic (endogenous electrophilic) compounds. They protect tissues against oxidative damage and are involved in the intracellular transport of hydrophobic substrates such as non-catalytic carrier proteins. **[5]** The worm and human GST are structurally different. **[6]** Hence, the worm GST is a promising chemotherapeutic target. Previous studies show defense mechanisms evolved by the worm against the host immune system. **[7]** The worm develops the capacity to neutralize host-derived reactive oxygen species (H_2O_2, super oxide radicals, hydroxyl ions, and nitric oxide). **[8]** The worm GST provides the defense against electrophilic and oxidative damage. **[9]** Therefore, it is our interest to study the structural features of GST from human and worm using homology modeling techniques. Here, we describe the structural differences between human and worm GST towards the design of potential inhibitors as anti-filarial drugs.

Methodology:

The protein sequences (208 residues long) for *Wb*-GST (Q86LL8) and *Bm*-GST (O02636) were obtained from the Swiss-Prot Database. The protein databank (PDB) contains several GST structures from different species ((*Schistosoma japonicum* (PDBID: IM9A), *Fasciola hepatica* (PDBID: IFHE), *Sus scrofa* (PDBID: 2GSR), *Homo sapiens* (PDBID: 19GS)). However, structures for *Wb* GST and *Bm* GST were not available. Sequence analysis using PSI-BLAST show *Wb* GST and *Bm* GST having 42% and 41% sequence identity (highest homology compared to other known structures) with *Sus scrofa,* respectively. Therefore, we used the structure of *Sus scrofa* GST (PDB: 2GSR) as template for building homology models for *Wb* GST and *Bm* GST using MOE (molecular operating environment), an automated molecular modeling tool. **[10]** The predicted models were evaluated for geometry, stereo-chemistry and energy distributions. The models were systematically analyzed using WHATIF **[11]** for various structural properties. The model was also evaluated using the model assessment procedure described elsewhere by Luthy, Bowie and Eisenberg. **[12]** The *Bm-GST* predicted model contains 96.6% residues in the favored regions and 99.5% residues in the allowed regions of the Ramachandran Plot. Similarly, *Wb-GST* predicted model contains 97.1% residues in the favored regions and 99.0% residues in the allowed regions of the Ramachandran Plot. We then superimposed the predicted models of *Wb* GST and *Bm* GST with the crystal structure of human GST for the calculation of RMSD (root mean square deviation) of the Cα backbone atoms of all residues in GST. **[13]**

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Results and Discussion:

An active GST is a homodimer of a 208 residue long monomer consisting of two domains (smaller α/β domain and larger α domain). The N-terminal small domain (residues 1 to 74) is an α/β structure **[14]** with the folding topology βαβαββα arranged in the order β_2, β_1,β_3 and β_4 with β_3 anti-parallel to the others, forming a regular β-sheet with a right-handed twist surrounded by three α-helices. The C terminal, large domain 2 (82-208 residues) is α-helical. GST does not contain the typical α-class α-9 helix which distinguishes between α and π-class enzymes. The residues that interface the two βαβ and ββα motifs are Trp 38, Phe 8, Val 33, Cys 47, Leu 52 and Leu 43 in human π GST. In *Wb*-GST and *Bm*-GST the residues Val 33, Cys 47, and Leu 43 are replaced by Ile 38, Phe 47 and Met 43. The human π-class GST recognizes GSH by an induced-fit mechanism **[15]** and the apo-enzyme helix α-2 is flexible. **[16]** The active site residues (Tyr 49 and Cys 47) binding to GSH are not conserved in *Wb*-GST and *Bm*-GST and they are replaced by Phe 49 and Phe 47. The human GST forms a disulphide bond between Cys 47 and Cys 101 under oxidized conditions and thus making the enzyme inactive. **[17, 18, 19]** In *Wb*-GST and *Bm*-GST the Cys residues (Cys47 and Cys101) are replaced by Phe and Thr, respectively. The effect of this mutation in *Wb*-GST and *Bm*-GST is not known. A previous study shows the inactivation of GST and not able to bind GSH. **[20]** Therefore, it is important to document the residue level mutations between human, *Bm* and *Wb* GST sequences and their significance in 3D structures.

Residue Positions	Human	*Wb*	*Bm*
008	Y	Y	Y
010	V	I	I
013	R	L	L
035	V	A	A
101	C	T	T
104	I	A	T
108	Y	Y	Y
205	G	G	G

Table 1: Residue changes between human, *Bm* and *Wb* GST is shown. These residues are involved in the formation of H-site. The sequence residue positions are with respect to the human GST sequence.

Residue Positions	Human	*Wb*	*Bm*
07	Y	Y	Y
12	G	G	G
13	R	L	L
38	W	W	W
44	K	K	K
49	Y	F	F
51	Q	Q	Q
52	L	L	L
53	P	P	P
64	Q	Q	Q
65	S	S	S
71	H	H	H
97	E	R	R
98	D	D	D

Table 2: Residue changes between human, *Bm* and *Wb* GST is shown. These residues are involved in the formation of G-site. The sequence residue positions are with respect to the human GST sequence.

The residues involved in the formation of H-site (Xeno-biotic binding site) binding pocket are shown in the Table 1 and the residues involved in the formation of G-site (GSH binding site) binding pocket are given in Table 2. A further understanding of residue changes in H and G-site between human, *Bm* and *Wb* GST is critical. Tyr 108 in H site is known to enhance GSH binding **[19]** and this residue is conserved in all π-class GSTs. The hydrogen bonding interaction between the hydroxyl group of Tyr 108 and the amide nitrogen of Gly 204 is also been observed in mouse, pig and human π-class structures. **[14]** A comprehensive understanding of residue mutation at the H and G sites in human, *Bm* and *Wb* will provide insight towards the design of an GST inhibitor specifically for *Bm* and *Wb*.

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Conclusion:
DEC **(**Diethyl carbamazine**)** is the only drug that is commonly used for Filariasis control**.** Therefore, it is important to design effective anti-filarial drugs. The comparison of modeled *Wb* and *Bm* GST structures with human GST structure provide insights towards the design of GST inhibitors. This study also demonstrates the effect of mutations towards function among homologous sequences.

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