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Molecular Docking of *Anopheles gambiae* and *Aedes aegypti* Glutathione S-Transferases Epsilon 2 (GSTE2) Against Usnic Acid: an Evidence of Glutathione Conjugation

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ABSTRACT

The aim of this study was to develop a theoretical model using Anopheles gambiae *GSTE2 structure as template for* Aedes aegypti *GSTE2 by homology modeling Docking simulations were performed for both the enzymes against usnic acid in neutral and anionic forms. Ramachandran plot revealed that 93.9% of the GSTE2 model residues were located on most favored regions. Model evaluation was made by the ANOLEA and GROMOS analysis. Docking results indicated that the enzymes were able to form glutathione-conjugate with usnic acid in both the forms (anionic and neutral).*

Key words: Anopheles gambiae, Aedes aegypti, molecular modeling, insecticide resistance

INTRODUCTION

The Glutathione S-tranferases (GSTs) is a highly promiscuous enzyme super family that plays an essential role in cytoplasm detoxification of a large range of xenobiotic compounds in many organisms (Che-mendoza 2009). The GSTs displays multispecificity for substrate metabolism, involved in the catalysis of endogenous and xenobiotic compounds (Che-mendoza 2009). GSTs also function as non-enzymatic binding proteins (known as ligandins) participating in the intracellular transport (Listowsky et al. 1988) and signaling processes (Adler et al. 1999; Cho et al. 2001). This diversity of enzymatic and non-enzymatic functions is explained by the genetic capacity to encode different GST isoforms by the organisms (Che-mendoza 2009).

The main reaction catalyzed by the GSTs is the conjugation of the tripeptide glutathione (GSH) to a hydrophobic and cytotoxic compound, resulting in a new conjugate that is more soluble. In insects, there are six GSTs class described: Delta, Epsilon, Omega, Sigma, Theta and Zeta (Ding et al. 2003; Tu and Akgul 2005). The epsilon class is arthropod specific and is involved in insecticide metabolism and resistance. This GST class is represented at least by eight members in the mosquitoes: GSTE1, GSTE2, GSTE3, GSTE4, GSTE5, GSTE6, GSTE7 and GSTE8 (Ding et al. 2003).

In this protein superfamily, there is one specific enzyme, the GSTE2 that has been associated with the resistance to chemical insecticides on the mosquitoes *Anopheles gambiae* and *Aedes aegypti*, respectively the main malaria and dengue fever vectors (David et al. 2005; Lumjuan et al. 2007). The GSTE2 aminoacid sequence is available for both the species (Ding et al. 2003; Lumjuan et al. 2007), but only the *An. gambiae* GSTE2

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(AgGSTE2) has its tridimensional structure solved by crystallography (Wang et al. 2008).

The usnic acid (UA) (Fig. 1) is a secondary metabolite found in several species of lichens (Ingo 2002). This compound has received attention due is its wide variety of pharmacological activities, such as antimicrobial, analgesic and anti-inflammatory (Vijayakumar et al. 2000; Luzina et al. 2010). The insecticidal activity of the AU has also been the subject of scientific studies. Cetin et al. (2008) investigated the insecticides effects of AU against *Culex pipiens* mosquito larvae and determined the insecticidal activity by bioassays.

In this study a homology model was built and evaluated for the *A. aegypti* glutathione S-transferase 2 (AaGSTE2). Docking simulations were performed where GSTE2 from *Aedes aegypti* and *An. gambiae* were used as receptors and anionic and neutral usnic acid forms were treated as ligand.

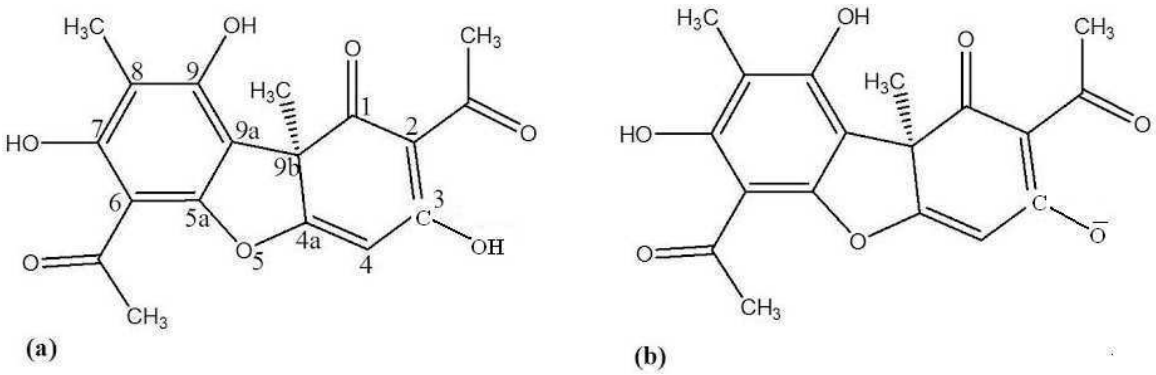

Figure 1 - Usnic acid in its (a) neutral and (b) anionic form.

MATERIALS AND METHODS

Homology modelling of the AaGSTE2

The AaGSTE2 and *An. gambiae* GSTE2 (AgGSTE2) amino acid sequences were obtained from Vectorbase data bank (AaGSTE2 ID number: AAEL007951-PA; AgGSTE2 ID number: AGAP009194-PA) and submitted to BLAST2p sequence (Altschul et al. 1997) for seeking the homology between them. The AgGSTE2 structure was obtained on PDB database (PDB ID: 2imi; Resolution: 1.4 Å) and was selected for template to build the AaGSTE2 model. The SWISS-MODEL workspace (http://swissmodel.expasy.org/) was used to construct the structural model for the target sequence (Arnold et al. 2006). For structural optimization, the final model was submitted to an energy minimization on the Chiron web Server-http://troll.med.unc.edu/chiron/index.php (Ramachandran et al. 2011).

Model evaluation and database submission

The AaGSTE2 was analyzed by PROCHECK (Laskowski et al. 1993) for the structure quality evaluation. Ramachandran diagram was requested to access the stereochemical quality. The ANOLEA mean force potential (Melo and Feytmans 1998) and the GROMOS force field (Scott et al. 1999) were used to evaluate the local quality of the structural model. After validation, the theoretical model was submitted to a public online repository, the PMDB – Protein Model Data base (its structure is available for public access under the ID code PM0079164).

Preparation of receptor and ligand structures

The initial coordinates of the AU were developed with the Gaussian 03 program (Frisch et al. 2004). The method B3LYP and 6-31+G (d,p) functions basis set was applied to calculate geometry of lower energy, which was submitted to the docking. The ligands were assigned with Gasteiger charge parameters (Gasteiger and Marsili 1980) and all polar hydrogen were removed. The AgGSTE2 and AaGSTE2 PDB files were prepared as receptors by adding hydrogen, assigning Kollman charges (Weiner et al. 1984) and converting to pdbqt files. The glutathione tripeptide was treated as co-factor.

Molecular docking simulations

The docking experiment was performed on the Autodock 4.3.2 software (Morris et al. 2010). Docking simulations were run using Lamarckian Genetic algorithm (LGA). The grid points for Autogrid calculations were set to be 52 × 52 × 52 Å with the active site residues at the center of the grid box. The docking parameters were set to a LGA calculation of 10,000 runs. The energy evaluations were set to 1,500,000 and 27,000 generations. The Population size was set to 150 and the rate of gene mutation and the rate of gene crossover were set to 0.02 and 0.8, respectively. The obtained conformations were then summarized, collected and extracted by using Autodock Tools. The first and the last conformation was analyzed from a 10-ranked set of each complex using the VMD-Visual Molecular Dynamics (Humpfrey et al. 1996).

RESULTS AND DISCUSSION

The protein Blast output revealed an excellent score (353 bits) and no gaps were found. The entire alignment is shown in Figure 2. The stereochemical quality of the predicted model was confirmed by the Ramachandran plot results (Fig. 3), whose 93.9% of residues were within the most favored regions. The local energy evaluation of the model by ANOLEA and GROMOS showed low energy values in most residues. Both ANOLEA and GROMOS energies showed negative values for the majority of protein residues. It indicated that they were located on favorable energy environment. These results suggested that the AaGSTE2 model displayed structural quality and reliability. It is the first structural model available for the AaGSTE2 enzyme, and the information about its structure could be very useful for further studies.

Score	Expect	Method	Identities	Positives	Gaps
353 bits(907)	1e-128	Compositional matrix adjust.	157/220(71%)	195/220(88%)	0/220(0%)

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Query  1    MTKLILYTLHVSPPCRAVELCAKALGLELEQKTVNLLTKEHLTPEFMKMNPQHTVPVLDD  60
            M+ L+LYTLH+SPPCRAVEL AKALGLELEQKT+NLLT +HL PEF+K+NPQHT+PVLDD
Sbjct  1    MSNLVLYTLHLSPPCRAVELTAKALGLELEQKTINLLTGDHLKPEFVKLNPQHTIPVLDD  60

Query  61   NGTIVCESHAIMIYLVSKYGKDDSLYSKELVKQAKLNAALHFESGVLFARLRFVCEPILF  120
            NGTI+ ESHAIMIYLV+KYGKDDSLY K+ VKQA++N+ALHFESGVLFAR+RF+ E ILF
Sbjct  61   NGTIITESHAIMIYLVTKYGKDDSLYPKDPVKQARVNSALHFESGVLFARMRFIFERILF  120

Query  121  AGGSEIPADRAEYVQKAYQLLEDTLVDDYIVGNSLTIADFSCVSSVSSIMGVIPMDKEKF  180
             G S+IP DR EYVQK+Y+LLEDTLVDD++ G ++TIADFSC+S++SSIMGV+P+++ K
Sbjct  121  FGKSDIPEDRVEYVQKSYELLEDTLVDDFVAGPTMTIADFSCISTISSIMGVVPLEQSKH  180

Query  181  PKIYGWLDRLKALPYYEAANGSGAEQVAQFVLSQKEKNAQ  220
            P+IY W+DRLK LPYYE ANG G   + +FVL++KE+NA+
Sbjct  181  PRIYAWIDRLKQLPYYEEANGGGGTDLGKFVLAKKEENAK  220
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Figure 2 - BLAST 2p sequence output for AaGSTE2 (Query) and AgGSTE2 (Sbjct).

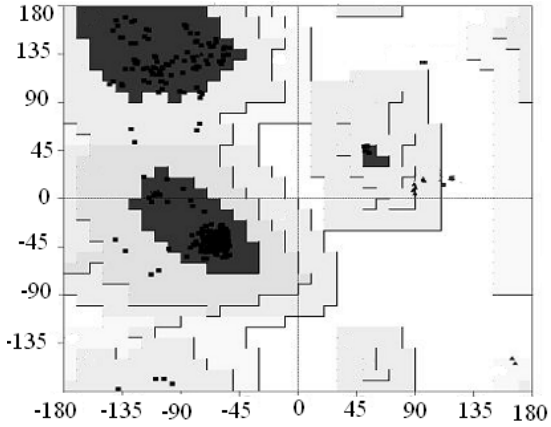


Figure 3 - Ramachandran plot for the AaGSTE2 predicted model.

Due the fact that X-rays structures were not available for AaGSTE2, a homology model was generated to perform the docking simulations. According to Laskowski et al (1993), predicted models are supposed to be reliable if over 90% of residues are situated in the core regions (Ramachandran plot). By this criterion, one could consider the stereochemical quality of the AaGSTE2 model (93.9%) quite satisfactory.

The binding energies for all the complexes displayed negative values. The ANOVA test did not find significant difference ($p > 0.05$) among the docking energies from the neutral and anionic forms as well as it did not point statistical significance between the enzymes. The lowest

energy value obtained was for the first conformation of *AaGSTE2-UA deprotonated* complex (Table 1). The highest energy was observed on the last conformation of the *AaGSTE2-UA neutral* complex (Table 1). Visual analysis showed the usnic acid involved in the G-site pocket in all the conformations.

The distances between usnic acid and GSH are showed at Table 2. In all the complexes, the oxygen from UA was the atom that interacted with the glutathione, which interacted with both hydrogen and sulfur atoms (Fig. 4). The Pearson´s correlation coefficient was negative and significant (r = -0.786; $p < 0.05$), which showed that when AU oxygen-GSH hydrogen distances decreased, the AU-oxygen-GSH sulfur distances increased (Fig. 4).

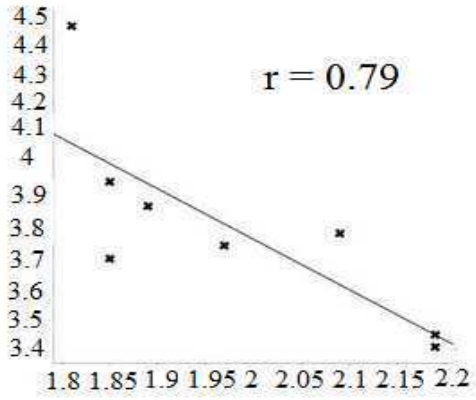


Figure 4 - Plot of AU oxygen-GSH hydrogen distances (X-axis) versus AU-oxygen-GSH sulfur distances (Y-axis). Pearson´s coefficient (r value) are shown.

Table 1 - Docking binding energies (Kcal/mol) of *Anopheles gambie* and *Aedes aegypti* GSTE2 against neutral and deprotonated usnic acid forms.

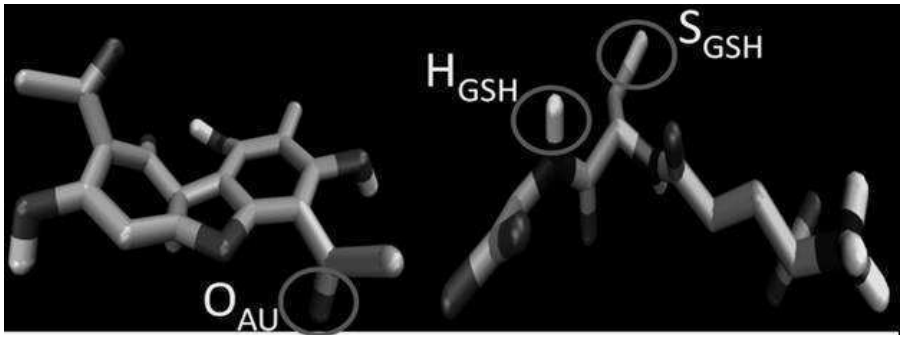
Enzime	Usnic acid (neutral)	Usnic acid (anion)
AaGSTE2-conformation 1	-7.11	-7.63
AgGSTE2-conformation 1	-5.78	-6.37
AaGSTE2-conformation 10	-4.95	-7.37
AgGSTE2-conformation 10	-6.95	-5.51

Table 2 - Distances between UA oxygen and GSH hydrogen and sulfur atoms. The parenthesis shows distances in the last conformation, while other values refer to the first conformation of each complex in the docking energy ranking.

Binding Complexes	Distance Å O-H-GSH	Distance Å O-S-GSH
AgGSTE2-AU neutral	2.19 (1.85)	3.40 (3.69)
AgGSTE2-AU deprotonated	2.19 (1.81)	3.44 (4.45)
AaGSTE2-AU neutral	1.89 (1.85)	3.86 (3.94)
AaGSTE2-AU deprotonated	1.97 (2.09)	3.73 (3.77)

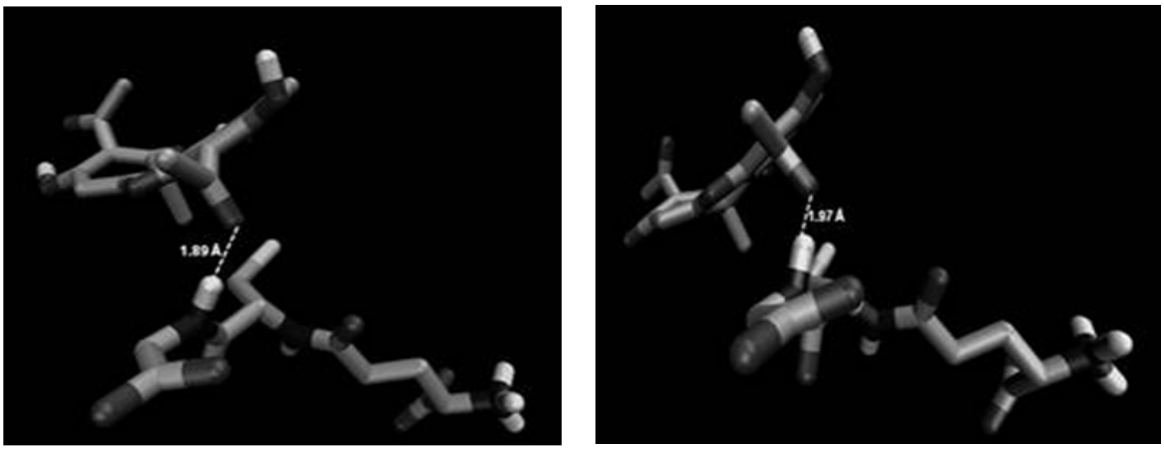
Results suggested that the UA-GSH conjugate was formed by *An. gambiae* and *A. aegypti* GSTE2 activity (Fig. 5). These enzymes should also be able to metabolize the both usnic acid forms: the neutral and anionic (Fig. 6 and 7). These results were the first evidence of usnic acid conjugation by an insect glutathione S-transferase and the first *in-silico* docking study with this compound.

Docking studies have already been done for the AgGSTE2 against DDT and DDE (Setzer 2011). Others detoxification enzyme families had also been docked against the insecticide in *Anopheles* mosquito (Chiu et al. 2008). The information about how detox enzymes binds to insecticidal compounds could be extremely useful for specific inhibitors development. The present results showed that both usnic acid forms bound to the proteins, which meant there was a real possibility that AaGSTE2 and AgGSTE2 could metabolize this natural insecticide. These enzymes are promising targets for design new technologies tools as biosensors for the direct monitoring of environmental pollutants, such as insecticides (Chronopoulou et al. 2009). Despite the proven larvicidal activity of usnic acid, this *in silico* study showed that AaGSTE2 and AgGSTE2 enzymes could be involved in usnic acid detoxification.



A B

Figure 5 - The binding atoms for the ligands. O_{AU} = usnic acid oxygen; H_{GSH} = glutathione hydrogen; S_{GSH} = glutathione sulfur.



A B

Figure 6 - Best energy ranked conformations for AaGSTE2-AU netral (A) and AaGSTE2-AU anionic (B) complexes.

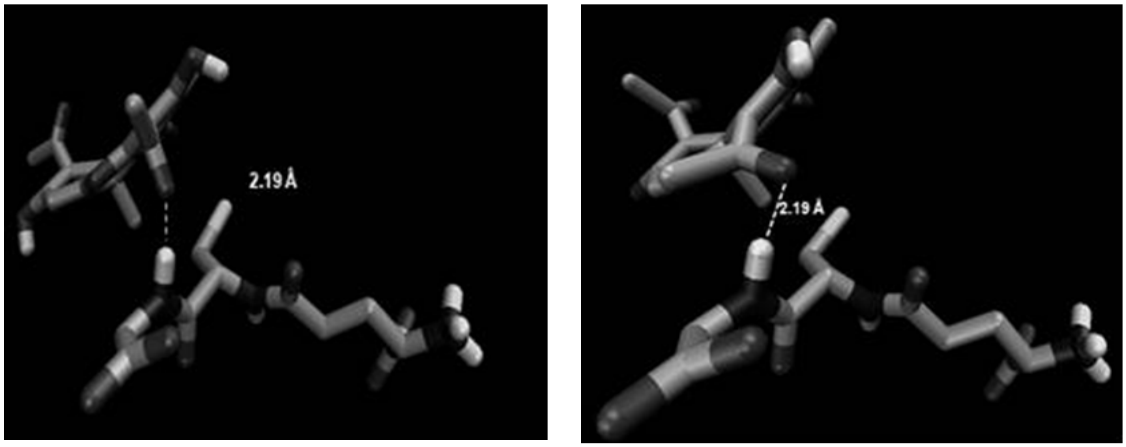


Figure 7 - Best energy ranked conformations for AaGSTE2-AU netral (A) and AaGSTE2-AU anionic (B) complexes.

CONCLUSIONS

The modeling results led to conclude that the predicted model was representative for AaGSTE2, and that it was closely related to the homologous AgGSTE2. This homology relationship was supported by their sequence identity and similarity, structural features and affinity to the substrates. The docking results showed that both

the enzymes (AaGSTE2 and AgGSTE2) displayed a relevant role on enantiomeric usnic acid forms metabolism. Development of a resistance way to usnic acid could results the use of this compound as an insecticide as a safe and efficient for mosquito control in future.

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