Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3 (1): 233-238

Received: 03-12-2014
Accepted: 09-01-2015

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Comparative binding mode of organophosphates, pyrethroids against modelled structures of acetylcholinesterase and alpha amylase in *Blattella germanica*

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Abstract
The majority of the human health sufferings and economical depletion worldwide is caused due to the pests and *Blattella germanica (*German cockroaches) being one among them. These are a reason for causing allergic reactions, including asthma and allergic rhinitis. In the present study, in silico analysis of potential pesticides among the organophosphate–monochrotophos and pyrethroids-fenvalerate, cypermethrin which acts against these pest by inhibiting the metabolic enzymes. In this pursuit, we performed homology modeling and docking studies against validate drug targets, Alpha amylase (α amylase) and acetyl cholinesterase(AChE) that are essential enzymes for the pest metabolism. Docking analysis revealed monochrotophos to be the potential pesticide against acetylcholinesterase with a highest E_score of -14.4365 while it showed a score of -9.9768 with α- amylase. However, fenvalerate showed an E_scores of -10.4656 and -11.2168 with α- amylase and acetylcholinesterase where as cypermethrin did not show any hydrogen bond interactions with both the drug targets.

Keywords: *Blattella germanica,* Docking, Homology Modeling, organophosphate, pesticide, Pyrethroid

1. Introduction
The hypersensitive disorder, allergy is one of the major diseases encountered in children across India over a few years [1], associated with asthma, allergic rhinitis and atopic dermatitis [2]. Delineating on allergic studies focused the view on cockroaches as one of the major sources of allergens [3] across the world. These allergens may be found in house dust, feces, secretions, cast off skin and even the entire body [4] of the cockroach, which can spread through air and manifest the allergic symptoms after inhalation [5] provoking several diseases. Cockroaches are one of the important groups of pests in the urban community existing over 360 million years [6]. Besides spoiling food substances, they are one of the modes of transfer of pathogens for several human diseases including helminths, poliomyelitis, bacteria, fungi and protozoa [7, 8, 9, 10]. Known as potential vectors for nosocomial infections [11], they have been the bed of several antibiotic resistance bacteria [12]. These are recognized by different names at different parts of the globe and among which the German cockroaches and Blattela germanica occur widely in the hotels and food outlets than in household locations [13, 14]. Being one of the popular indoor pests of low economical status, it substantializes as one of the important pests across the world [15]. A cockroach belongs to the order Blattaria with about 5000 species under 398 genera in 28 families [16, 17]. Of theses 35 species are associated with human habitations and eat on human eatables [18].

1.1 Acetylcholinesterase
Acetylcholinesterase (AchE, EC 3.1.1.7) is a potential enzyme present in the German cockroaches, performs certain crucial functions like synapses of cholinergic neuron [19] in central and peripheral nervous systems. AchE is a drug target of interest for a host of diseases associated with nervous system besides acting as an enzyme of interest for palliative Alzheimer's and subsequently portrays itself as a possible insecticide [20]. Elaborate studies on acetylcholinesterase uncover the insect specific cysteine residue which a target site for the novel insecticide development [19, 21]. Researchers worked on two AchE genes, B *gace1* and B *gace2* and the results explained that it was B *gace1* responsible for the hydrolysis of AchE [22] whereas B *gace2* performs the supplementary functions [22]. However, their study noted the presence of both the types of enzymes at the m-RNA level.

1.2 Alpha amylase

These are one of the vital enzymes that degrades the woody cellulose and are present largely in termites, including cockroaches and therefore absorbs the degraded products as a source of nutrients [23, 24, 25]. Degradation occurs by a coupled mechanism in which the salivary enzyme endo-β – 1-4 glucanase cleaves the cellulose and further the subsequent reactions are carried out by cellobiohydrolase and β-glucosidase eventually converting it to glucose [26] which is utilized as an energy yielding source. Three different types of enzymes exists based on the mechanism of cleavage they perform *viz* [27], α amylase [EC 3.2.11], β amylase [EC 3.2.1.2] and γ amylase which aid in the digestive functions.

In the light of the above, it is very essential to eradicate these pests and further to snub the transmission of the human diseases. The aim of the present investigation is to assess the potential pesticide on the protein drug targets of the cockroaches and culled on two imperative proteins essential for cockroaches, acetylcholinesterase and alpha amylase.

2. Materials and Methods

2.1 Sequence Retrieval and Analysis

The protein drug targets for the present investigation are the α amylase and the acetyl cholinesterase. The protein sequences in their FASTA format were retrieved from Swissprot database with Id Q2KJQ1 and Q2PZG3 respectively. Subsequently its primary structure analysis was performed by Protparam [28], a tool which allows understanding the details of the protein. Further, its secondary structure was studied by SOPMA **(**self-optimized prediction method) [29]. The protein was then subjected to BLAST for identifying the template structure.

2.2 Homology Modelling and Validation

The protein was modelled for attaining its 3D structure using Discovery Studio (Accelrys 2.5) [30]. The obtained/ constructed protein was then validated by RAMPAGE and PROSA. The obtained template was subjected to homology modelling against the query adopting the Discovery Studio (Accelrys 2.5). Validation of the modeled protein was performed using RAMPAGE [31] and PROSA [32, 33].

2.3 Molecular Docking

2.3.1 Docking

The protein ligand docking was performed using the Molecular Operating Environment (MOE) [34], using the London dG scoring function. The ligands were minimized before initiating the docking using CHARM m 99 force field. After all the default parameters were set and to obtain the minimum energy structures, the ligands were allowed to be flexible. The best ligand interaction was analyzed and assessed at the end of the dock results. However, the poses are scored adopting the London dG scoring function which estimates the free energy, ΔG of the binding of the ligand from a given pose for assessing the binding affinity. The binding energy can be analysed either by grid based algorithm or by full force field.

2.3.2 Protein preparation

The modelled protein structure, α amylase and Acetyl cholinesterase in their PDB formats were imported on to the Discovery Studio (Accerlys 2.5). The protein chemistry of the missing hydrogen was corrected after which the heteroatoms and the crystallographic water molecules were removed from the protein [35]. Valence monitor options and alternate conformations were used to connect the crystallographic disorder and the unfilled valence atoms. The proteins were then subjected to energy minimization steps by applying the steepest descent method which was then followed by the conjugate gradient method until the convergence gradient was satisfied [35]. Further, the active sites of the protein were identified by site finder.

2.3.3 Ligand selection and preparation:

The ligands selected for the present investigation are the known Organophosphate- monochrotophos and pyrethroids - cypermethrin and fenvalerate. Their structures are drawn on Chem Sketch (ACDLABS 12.0) [44]. Removal of duplicates was done and bonds were added to it. The CHARMm force field was used to minimize the energy and thereafter using catalyst the 3D structures generated [45].

3. Results and Discussion:

3.1 Sequence Retrieval and Analysis:

Sequence of the protein, α amylase was retrieved from SWISSPROT in FASTA format. Protparam analysis of the primary sequence revealed the total number of amino acids to be 480 with a molecular weight of 53700.9 and a theoretical pI 6.05. Further, the data revealed the total number of negatively charged residues as 49 while that of positively charged residues as 40. However, the estimated half-life is found to be 0.8 hours mammalian reticulocytes, in vitro, 10min (yeast, in vivo and 10 hours (Escherichia coli, in vivo) with the N-terminal of the sequence considered is Q (Gln).Thereafter, the secondary structure of the protein was analysed by SOPMA which revealed the protein alpha helixes (31.04%) and beta bridges (0.00%) and the random coil was 36.46%.

The protein, acetylcholinesterase, was retrieved from the SWISSPROT in its FASTA format. The primary structure analysis performed by Protparam informed the number of amino acids as 692 with a molecular weight of 77569.8 and the theoretical pI was 5.70. The end terminal sequence was M (Met) with the estimated half life of 30 hours mammalian reticulocytes, in vitro, >20 hours (yeast, in vivo and >10 hours (*E. coli*. In vivo). The SOPMA studies showed the alpha helix (31.07%) and beta bridges (0.00%).

3.2 Homology Modeling and Validation:

The sequence retrieved for α-amylase from the Swissprot was loaded onto the BLAST to obtain the template against all the proteins in the database. Based upon the highest identity 58.5%, the template 1VIW was selected. Likewise, the sequence retrieved for Acetylcholinesterase was loaded onto the BLAST to obtain the template. Based on the highest identity, 47.0% the chain A of 4QWW was preferred as the best template. Further, the selected proteins were modelled and its 3D structure was predicted on Discovery Studio (Accerlys 2.5) shown in figure 1 and 2. The structures were later viewed with Rasmol and SPDBV.

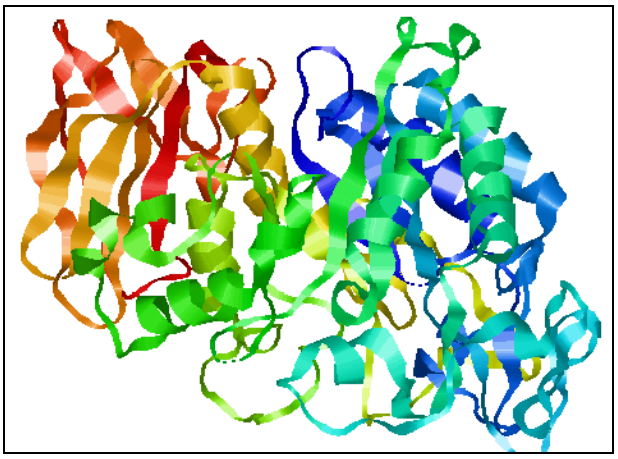

Fig 1: Structure of homology modelled Alpha amylase

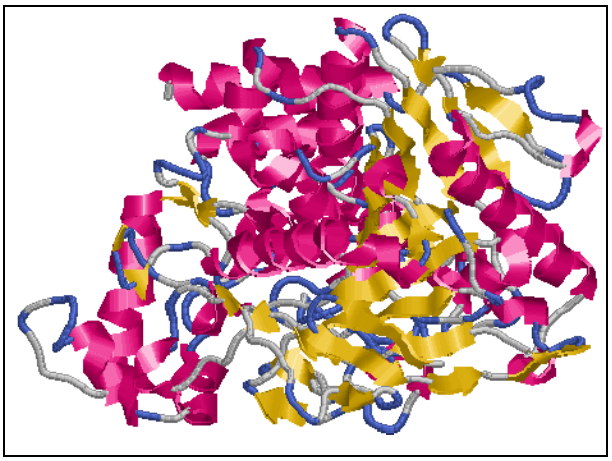

Fig 2: Structure of homology modelled Acetylcholinesterase

3.3 Verification of the modelled proteins:

The modeled proteins α –amylase and Acetylcholinesterase were verified for quality using the available software RAMPAGE and PROSA. RAMPAGE Server was used for validation of protein structure which revealed that the modeled target α –amylase has significant stereochemical quality in the Ramachandran plot with favorable region (90.4%), allowed region (7.1%), disallowed region (2.5%) shown in figure 3 and that of the modeled target structure of acetylcholinesterace has significant stereochemical quality in the Ramachandran plot with favorable region 90.6%), allowed region (7.9%), disallowed region (1.5%) shown in figure 4.

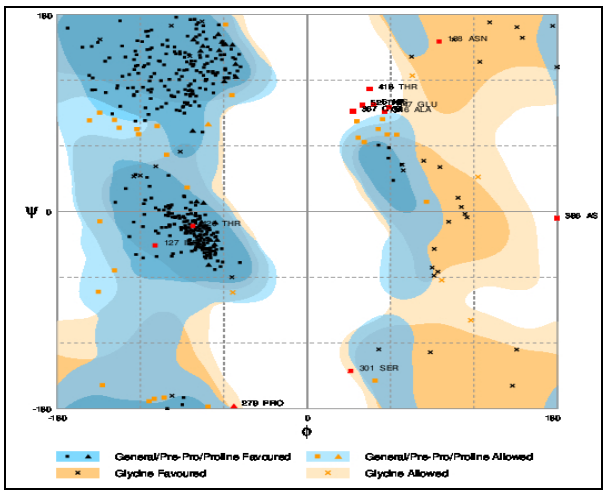

Fig 3: RAMPAGE analysis showing 90.4% residue favorable regions.

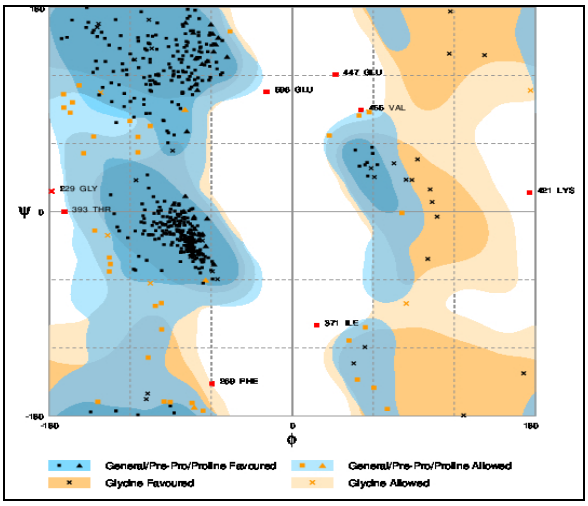

Fig 4: RAMPAGE analysis showing 90.6% residue favorable regions.

ProSa results revealed that the protein model α amylase matched with NMR region of the plot with Z- score (-8.98) and that of AchE matched NMR region of the plot with Z-score (10.03).

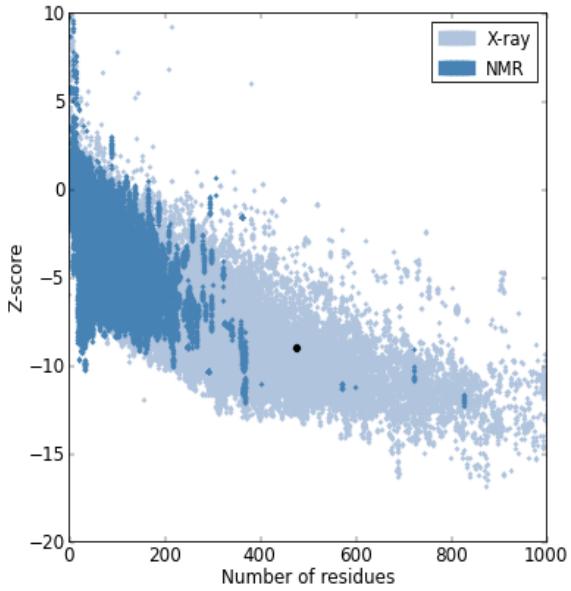

Fig 5: PROSA analysis of α amylase showing residues at NMR region

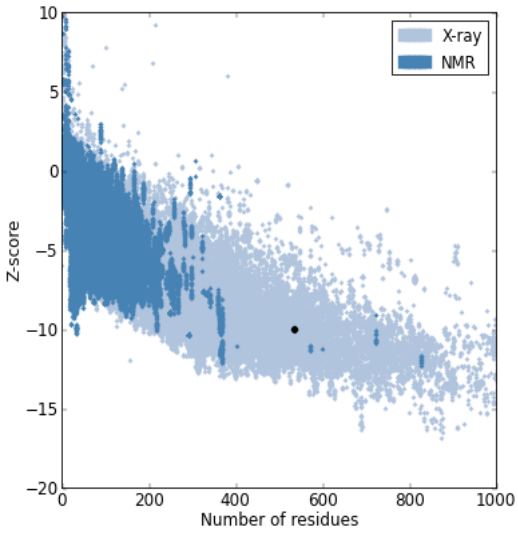

Fig 6: PROSA analysis of AchE showing residues at NMR region

3.4 Molecular Docking
Molecular docking was performed adopting the MOE software using the London dG algorithm. The prepared protein and the ligands were loaded on to the MOE software and were allowed to dock after the active site prediction of the proteins by Site finder tool of MOE

3.5 Dock results
The selected ligands were allowed to dock with the homology modeled proteins. For each ligand 10, 3D structures were generated. The dock results were analyzed using software package MOE program (Molecular Operating Environment) designed by the Chemical Computing Group. The parameters used for the docking were, Total Runs = 50, Cycle/Runs = 15, Iteration Limit=10 000, Potential Energy Grid: ON, Annealing Algorithm: Simulated Annealing. For its accomplishment the PDB files of both the ligand and the protein were loaded into the MOE and then results were read based on ligand binding orientation, binding affinity, and binding-free energies.

Table 1: E-Scores

S. No	Name of the Ligand	Alpha Amylase	Acetylcholinesterase
1.	Monochrotophos	-9.9768	-14.4365
2.	Cypermethrin	-10.6974	-11.7572
3.	Fenvalerate	-10.4656	-11.2168

4. Interpretation of the interactions
4.1 Monochrotophos
The E-Score of monochrotophos was found to be -9.9768 when docked with α amylase. It was observed that the docking was established with one hydrogen bond between the protein and ligand. OG of SER 155amino acid residue participated in the formation H-bond with N atom of the ligand as H acceptor with the distance of 2.82 and score of 33.5%.

On the other hand, the monochrotophos interacted strongly with the protein AchE with five hydrogen bonds with an E_ score of -14.4365. The ligand map elucidated that 3 TYP, 1SER and 1 HIS residues were involved. Further, TYR 232 of AchE established bonds with their OH atom of the ligand. However, it was observed that O atom of ligand was crucial in forming the strongest bond *viz* 1st O atom of the ligand interacted with TYR residue with a score of 99.1% and with a distance of 2.32 while 2nd O produced a much lesser score of 63.3% with a bond distance of 2.29. On the other hand, OG atom of SER 233 interacted with O of the ligand with a distance of 2.60 and a score of 82.9%. The OH group of TYR 439 formed a bond with a score of 66.4% and a distance of 2.50 upon its interaction with O atoms of the ligand. The NE atom of HIS 550 joined with O of the ligand with a score and distance of 22.5% and 2.59 respectively. Herein all the residue acts as H acceptors. The result acknowledges the effective work of monochrotophos on AchE with five hydrogen bonds on contrary, with α amylase with only H hydrogen bond.

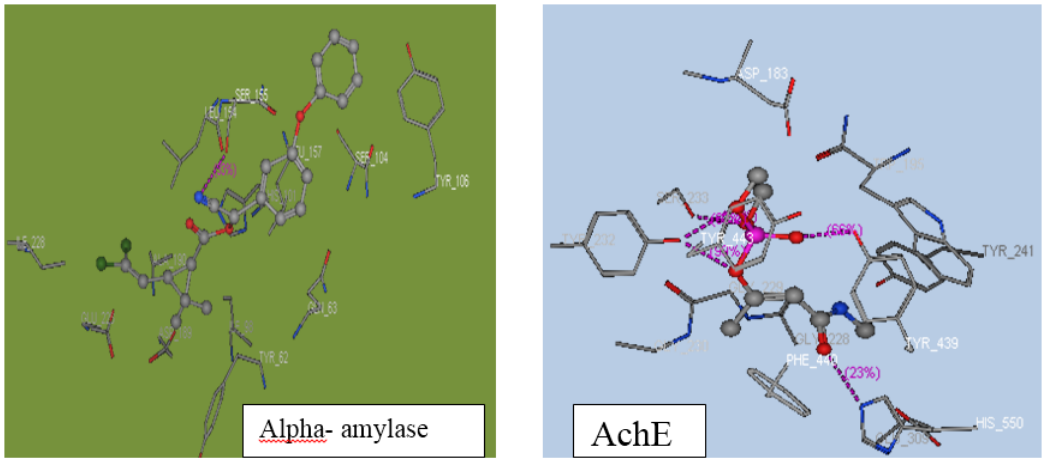

Fig 7: Receptor-Ligand interactions (pink) of Alpha- amylase and AchE with monochrotophos

4.2 Cypermethrin
When the pesticide cypermethrin was docked with the protein α amylase, no H bond interactions were observed. However, the dock database showed E-score to be -10.6974. Similar results were noted with AchE, with the E score of -11.7572. While no H bond interactions were noticed.

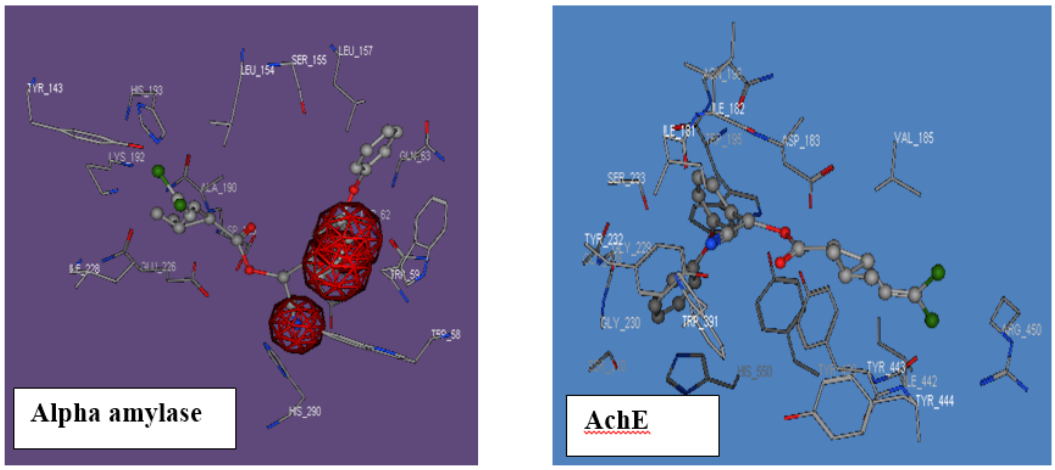

Fig 8: Receptor-Ligand interactions (pink) of Alpha- amylase and AchE with cypermethrin

4.3 Fenvalerate

Fenvalerate interacted with α amylase with the E_ Score of -10.4656. Further, it was noted that OG atom of SER 155 residue shared the H bond with N of the ligand with 69.3% score and the distance of 2.32. When docked with AchE it produced a score of -11.2168.

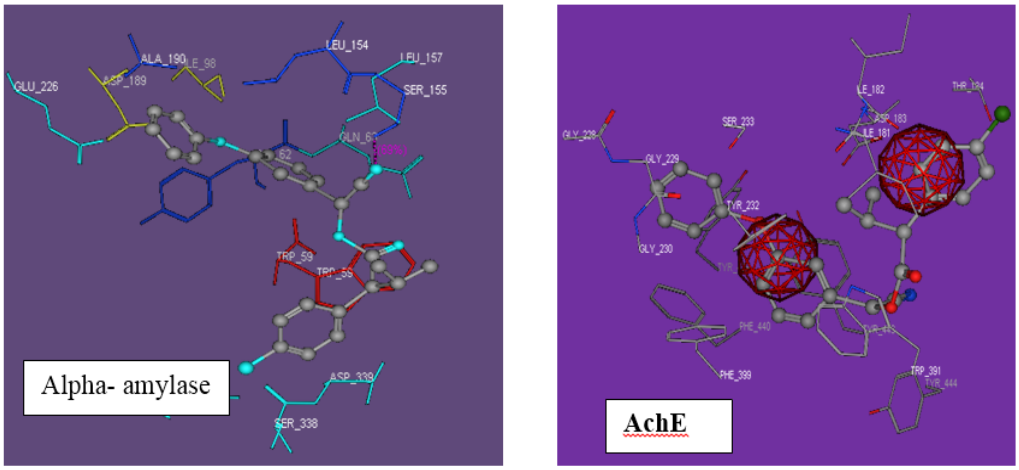

Fig 9: Receptor-Ligand interactions (pink) of Alpha- amylase and AchE with Fenvalerate

5. Conclusion

German cockroaches are the most significant potential vectors which transmit the diseases across human and give rise to several allergic reactions. Reports stating the insecticide [46, 47] resistance in them due to several mechanisms are available [48, 49]. Our results successfully evaluated the potential organophosphate keeping at bay all the confusion and elevate acetyl cholinesterase as best drug target above the α-amylase. Our results favour monochrotophos to be the potential pesticides; however, pest biochemical studies are essential to further enhancing their efficacy.

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