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MOLECULAR MODELLING OF *ANOPHELES STEPHENSI* - ACETYLCHOLINESTERASE

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ABSTRACT: *Anopheles stephensi* is one of the dominant vector species in India transmitting malaria in human. Vector population control is one of the strategies of integrated vector management. Traditionally pesticides have been in use to control mosquitoes. Acetylcholinesterase (AChE) is the target for these pesticides. Three-dimensional structure of AChE for this prevalent mosquito species in India, is wanting in databases. To overcome the insecticide resistance, novel and safer compounds are of need and structure of target protein as well to employ the principle of drug discovery. In structural Biology, theoretical modelling (Homology/Comparative modelling) of protein is an important approach helps in solving some of the important biological problems viz. site directed mutagenesis, mutation causing disease, molecular function and structure based design of specific ligands. Homology model of AChE with primary sequence has been generated with template protein having 41% sequence identity, refined model has been validated, subjected to stereochemistry check and superimposed.

KEYWORDS: Acetylcholinesterase, Homology modelling, Structure based drug discovery

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1.INTRODUCTION

Mosquitoes are well known vectors which transmit infectious diseases between humans or from animals to humans. *Aedes*, *Anopheles* and *Culex* are the three genera of mosquitoes prevalent and cause diseases like Chikungunya, Dengue fever, Lymphatic filariasis, Rift Valley fever, Yellow fever, Zika (by *Aedes* sp.), Malaria and Lymphatic filariasis (by *Anopheles* sp.), Japanese encephalitis, Lymphatic filariasis and West Nile fever (by *Culex* sp.) (WHO 2017, Web-1). This mosquito species can affect more than half of the world's population as latter live in areas of mosquito presence. The purpose of vector control is to limit contact between humans and vectors, and to reduce vector populations so that they are unable to transmit disease so human suffering and deaths can be prevented. Integrated vector management [1] includes multiple interventions; environmental, biological and chemical (use of insecticides) [2] control measures. Organophosphates and Carbamate (OPs-irreversible and Cs-reversible) block the enzyme Acetylcholinesterase (EC 3.1.1.7) [3], [4] resulting severe disturbance in neurotransmission. Hence, these insecticides are called acetylcholinesterase inhibitors-AChEIs [5]. AChE breaks down the acetylcholine the neurotransmitter into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation [6]. AChEIs inhibit the cholinesterase enzyme from breaking down ACh (acetylcholine) causing accumulation of it at cholinergic synapse [7] leading to uncontrolled, rapid twitching of some muscles, paralyzed breathing, convulsions, and in extreme cases, death. Insecticide resistance has been a problem in all insect groups that serve as vectors of communicable diseases [8], [9], [10]. Hence, acetylcholinesterase inhibitors (AChEIs), interacting with the enzyme as their primary target, are applied as relevant drugs and toxins [5]. Anopheline mosquitoes transmit malarial parasite in human. There are 465 recognized species of genus *Anopheles* [11] of them about 70 species have capacity to serve as vector for malaria [12]. *Anopheles stephensi* (Liston 1901) has been recognized as a dominant vector species (DVS) of malaria in urban areas of the Persian Gulf and India [13[, [14], [15]. There has not been resolved, 3-dimensional structure of AChE present yet in the databases for *Anopheles stephensi*. Comparative modelling (Homology modelling) approach can replace this want and help curb menace of mosquito by subjecting the model in searching new pesticides. Since proteins with similarity in their sequences have shown similar structures, structure of a protein with its primary sequence known can be elucidated. Comparative modelling (Homology modelling) is the computational technique of predicting the structure of proteins where the primary sequence (amino acids) of a protein (target) if shares significant similarity (~30% or more) with sequence that of the protein (template) with resolved 3-dimensional structure. The steps involved in homology modelling are (a) Identification of template (b) Alignment of sequences: target and template (c) Model building (d) Refining and validation of model [16].

2. MATERIALS AND METHODS

2.1. Primary sequence of Target

Primary sequence of AChE for *An. stephensi* [17] was procured form UniprotKB [18] with Entry: P56161. This sequence was also found in NCBI protein database with ACCESSION No. 1808210A.

2.2. Template Identification

The sequence was submitted to NCBI's (Web-2) standard protein BLAST server in fasta format [19]. PSI-BLAST (Position-Specific Iterated BLAST) [20] algorithm with BLOSUM62 (amino acid substitution matrix) as score parameter and the search set of same genus (Taxonomy ID: 7165) were opted to select suitable template from PDB [21] (Web-3). RID-DERNY4B5014 identity number was generated for this BLAST. The program first performed a gapped BLAST database search from which a position-specific score matrix was constructed, which replaced the target sequence for the next round of database searching. PSI-BLAST was iterated till significant alignment of target sequence with database was found.

2.3. Alignment of sequences

Target sequence with 664 residues and template with 553 residues were realigned using Clustal Omega [22] where HMM Iterations: 5, full distance matrix during; initial alignment and alignment iteration had been the parameters for alignment.

2.4. Model Building

For the process of homology or comparative modelling of protein three-dimensional structures, MODELLER was used [23]. Target and template sequences in alignment was provided to MODELLER which calculated a model containing all non-hydrogen atoms by implementing comparative protein structure modelling by satisfaction of spatial restraints [24].

2.5. Refining and validation of Model

The loop in between residues 131-166, was modelled with MODELLER. This model was submitted to GalaxyWeb Refine (Web 4) for protein structure refinement and side chain quality [25]. The method first did rebuild side chains and performed sidechain repacking and subsequent overall structure relaxation by molecular dynamics simulation. Refinement of the model was measured by GDT-HA [26] and MolProbity score [27] where Ramachandran plot was reobtained with UCSF Chimera alpha v 1.13 (buid 41662) [28].

2.6. Superimposing Model:

AChE model for *Anopheles stephensi* was superimposed with that of its template to identify structural similarities specially the folds in structures. The overall root mean square deviation (RMSD) [29] of all corresponding C-alpha atoms gave an idea of the similarity between the two structures. Matchmaker tool [30] in UCSF Chimera [28] was used for superimposition.

3. RESULTS AND DISCUSSION

3.1. Primary sequence of Target

Amino acid sequence of *Anopheles stephensi* AChE with 664 residues and 74,629 Mass (Da) in its canonical form is as under:

```
>sp|P56161|ACES_ANOST Acetylcholinesterase OS=Anopheles stephensi OX=30069 PE=3
SV=1
MFVNQRTRRPYMSVFVLVLGAAVICPAYGIIDRLVVQTSSGPIRGRSTMVQGREVHVFNGVPFAKPPVDSLRFKKPVPA
EPWHGVLDATRLPPSCIQERYEYFPGFAGEEMWNPNTNVSEDCLYLNIWVPTKTRLRHGRGLNFGSNDYFQDDDDFQRQ
HQSKGGLAMLVWIYGGGFMSGTSTLDIYNAEILAAVGNVIVASMQYRVGAFGFLYLAPYINGYEEDAPGNMGMWDQALA
IRWLKENAKAFGGDPDLITLFGESAGGSSVSLHLLSPVTRGLSKRGILQSGTLNAPWSHMTAEKALQIAEGLIDDCNCN
LTMLKESPSTVMQCMRNVDAKTISVQQWNSYSGILGFPSAPTIDGVFMTADPMTMLREANLEGIDILVGSNRDEGTYFL
LYDFIDYFEKDAATSLPRDKFLEIMNTIFNKASEPEREAIIFQYTGWESGNDGYQNQHQVGRAVGDHFFICPTNEFALG
LTERGASVHYYYFTHRTSTSLWGEWMGVLHGDEVEYIFGQPMNASLQYRQRERDLSRRMVLSVSEFARTGNPALEGEHW
PLYTRENPIFFIFNAEGEDDLRGEKYGRGPMATSCAFWNDFLPRLRAWSVPSKSPCNLLEQMSIASVSSTMPIVVMVVL
VLIPLCAWWWAIKKNKTPPHPQVILETRAFMH
```

3.2. Template Identification

PDB ID: 5X61 chain-B [31] with 553 residues, 39% identity with target, 87% query cover and with Evalue 5e -144 is the identification of template in PSI-BLAST (Table 1). The identified template is AChE of *Anopheles gambiae* shown in Figure 1.

3.3. Alignment of sequences

Target sequence with 664 residues and template with 553 residues were realigned (Figure 2) using Clustal Omega [22] where HMM Iterations: 5, full distance matrix during; initial alignment and alignment iteration had been the parameters for alignment. Percent Identity between two sequences has been 41.7%.

3.4. Model Building

MODELLER generated a reliable homology model (Figure 3) for *Anopheles stephensi*-AChE target sequence.

3.5. Refining and validation of Model

Refined model by GalaxyWeb Refine, produced 5 models. The model with the best validation score is as in Table 2.

GDT-HA measures backbone structure accuracy of the protein model whereas Molprobity measures overall physical corrections in structure of protein and includes Clash Score; Number of atomic clashes per 1000 atoms, Poor Rotamers; rotamers outliers and Ramafavoured. Ramachandran [32] favoured backbone torsion angles for residues are in the model plot (Figure 4).

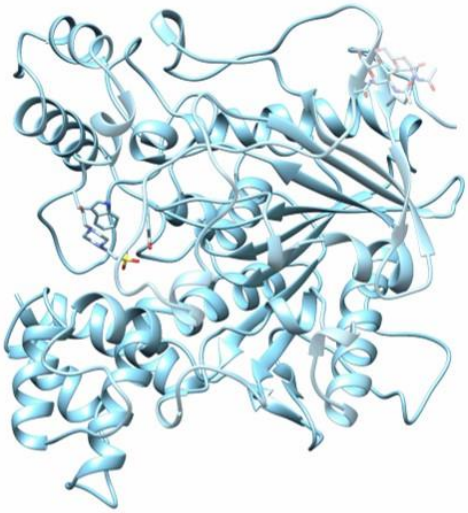

Figure 1. Template PDB ID: 5X61 chain-B.

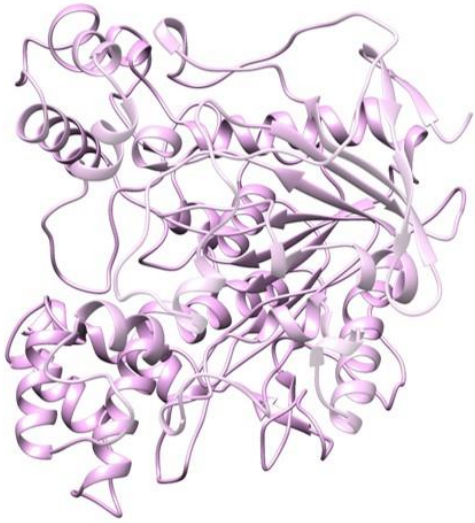

Figure 3. Homology Model based on template PDB ID: 5X61 chain-B.

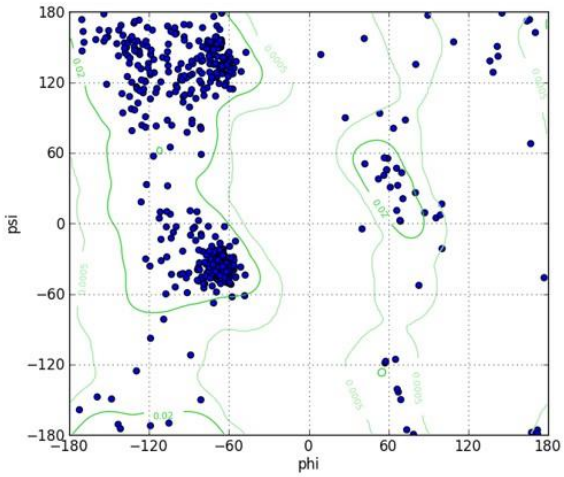

Figure 4. Ramachandran Plot of the refined model

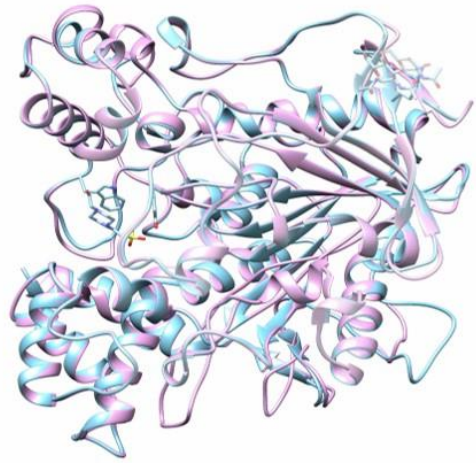

Figure 5. Superimposition of Homology model-AChE (Blue) and template PDB ID: 5X61, chain-B (Pink)

Table 1. Parameters of PSI-BLAST for Template search-Result

Alignment Score	Expect Value	Identities	Positives	Gaps
423bits (1087)	5e-144	231/590(39%)	331/590(56%)	55/590(9%)

Table 2. Model Refine Score by GalaxyWeb Refine

Model	GDT-HA	MolProbity	Clash score	Poor rotamers	Rama favored
Initial	1.0000	3.741	173.9	4.4	89.8
Later	0.9505	2.739	41.5	1.9	93.3

3.6. Superimposing Model

Pairing used both sequence and secondary structure, allowing similar regions of the structures to be superimposed even when their sequence similarity has been low ~41%. Needleman-Wunsch algorithm [33] using BLOSUM-62 [34] substitution matrix superimposes two structures (Target model and

Template) where RMSD between 518 pruned atom pairs is 0.292 Å (Figure 5) and sequence alignment score = 1437.9.

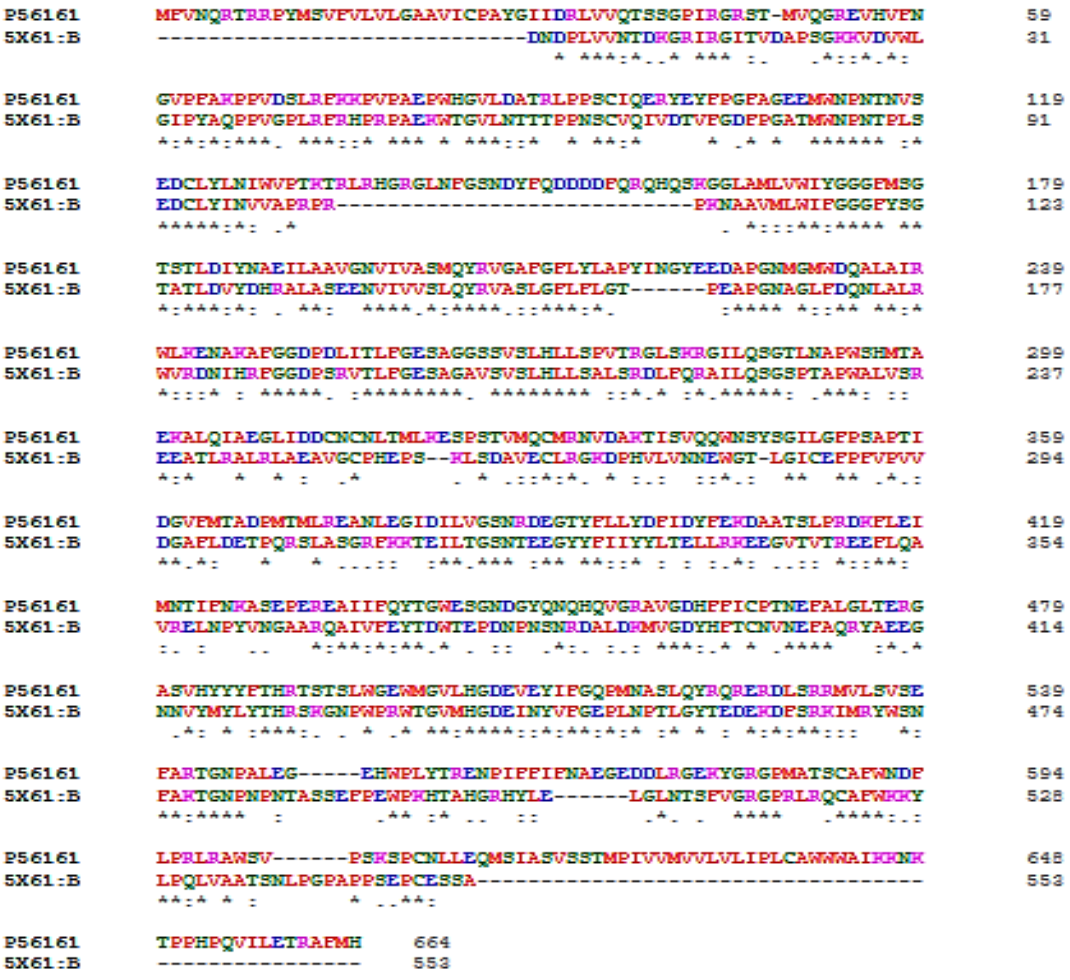

Figure 2. Target and Template Sequence alignment

4. CONCLUSION

Acetylcholinesterase is an important target enzyme in terms of controlling pests including insect-flies-vectors. More than 60 Anopheline mosquito species have been responsible for harbouring malaria across the globe but only one of these species has got AChE 3D structure resolved. *Anopheles stephensi* is an important vector species transmitting malaria in urban India causing human suffering and deaths. This homology model of AChE would be the key in structure based ligand design to inhibit the enzyme in turn curbing the population of the major vector for malaria.

5. ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. Beier JC, Keating J, Githure JI, Macdonald MB, Impoinvil DE, Novak RJ. Integrated vector management for malaria control. Malaria journal. 2008 Dec;7(1):S4.
2. Lacey LA, Lacey CM. The medical importance of riceland mosquitoes and their control using alternatives to chemical insecticides. Journal of the American Mosquito Control Association. Supplement. 1990 Jun;2:1-93.
3. O'Brien RD. Acetylcholinesterase and its inhibition. In Insecticide biochemistry and physiology 1976 (pp. 271-296). Springer, Boston, MA.
4. Fukuto TR. Mechanism of action of organophosphorus and carbamate insecticides. Environmental health perspectives. 1990 Jul;87:245.
5. Colovic MB, Krstic DZ, Lazarevic-Pasti TD, Bondzic AM, Vasic VM. Acetylcholinesterase inhibitors: pharmacology and toxicology. Current neuropharmacology. 2013 May 1;11(3):315-35.
6. Toutant JP. Insect acetylcholinesterase: catalytic properties, tissue distribution and molecular forms. Progress in Neurobiology. 1989 Jan 1;32(5):423-46.
7. Lauder JM, Schambra UB. Morphogenetic roles of acetylcholine. Environmental health perspectives. 1999 Feb;107(Suppl 1):65.
8. Brogdon WG, McAllister JC. Insecticide resistance and vector control. Emerging infectious diseases. 1998 Oct;4(4):605.
9. Ranson H, Lissenden N. Special Issue: Vectors. Mortality. 2015;50(40):30.
10. Liu N. Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. Annual review of entomology. 2015 Jan 7;60:537-59.
11. Harbach RE. Genus ANOPHELES Meigen, 1818. Mosquito Taxonomic Inventory. In Book Genus ANOPHELES Meigen, 1818 Mosquito Taxonomic Inventory City 2011.
12. Service MW, Townson H. The Anopheles vector, pp. 59–84. Essential malariology. 2002.
13. Davidson G. Studies on insecticide resistance in anopheline mosquitos. Bulletin of the World Health Organization. 1958;18(4):579.
14. Singh OP. Bionomics of malaria vectors in India. Malaria Research Centre. 2002:19-31.
15. Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Chareonviriyaphap T, Coetzee M, Mbogo CM, Hemingway J, Patil AP, Temperley WH, Gething PW. A global map of dominant malaria

vectors. Parasites & vectors. 2012 Dec;5(1):69.

16. Marti-Renom MA, Stuart AC, Fiser A, Sánchez R, Melo F, Šali A. Comparative protein structure modeling of genes and genomes. Annual review of biophysics and biomolecular structure. 2000 Jun;29(1):291-325.
17. Hall LM, Malcolm CA. The acetylcholinesterase gene of Anopheles stephensi. Cellular and molecular neurobiology. 1991 Feb 1;11(1):131-41.
18. UniProt Consortium. UniProt: the universal protein knowledgebase. Nucleic acids research. 2016 Nov 28;45(D1):D158-69.
19. Lipman DJ, Pearson WR. Rapid and sensitive protein similarity searches. Science. 1985 Mar 22;227(4693):1435-41.
20. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research. 1997 Sep 1;25(17):3389-402.
21. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank Nucleic Acids Research. 2000; 28, 235-242.
22. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD. Fast, scalable generation of high- quality protein multiple sequence alignments using Clustal Omega. Molecular systems biology. 2011 Jan 1;7(1):539.
23. Webb B and Sali A. Comparative Protein Structure Modeling Using Modeller. Current Protocols in Bioinformatics, John Wiley & Sons, Inc., 2014; 5.6.1-5.6.32.
24. Šali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. Journal of molecular biology. 1993 Dec 5;234(3):779-815.
25. Lee GR, Heo L, Seok C. Effective protein model structure refinement by loop modeling and overall relaxation. Proteins: Structure, Function, and Bioinformatics. 2016 Sep 1;84(S1):293-301.
26. Kopp J, Bordoli L, Battey JN, Kiefer F, Schwede T. Assessment of CASP7 predictions for template- based modeling targets. Proteins: Structure, Function, and Bioinformatics. 2007 Jan 1;69(S8):38-56.
27. Chen VB, Arendall WB, Headd JJ, Keedy DA, Immormino RM, Kapral GJ, Murray LW, Richardson JS, Richardson DC. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallographica Section D: Biological Crystallography. 2010 Jan 1;66(1):12-21.
28. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera—a visualization system for exploratory research and analysis. Journal of computational chemistry. 2004 Oct 1;25(13):1605-12.

29. Damm KL, Carlson HA. Gaussian-weighted RMSD superposition of proteins: a structural comparison for flexible proteins and predicted protein structures. Biophysical journal. 2006 Jun 15;90(12):4558-73.
30. Meng EC, Pettersen EF, Couch GS, Huang CC, Ferrin TE. Tools for integrated sequence-structure analysis with UCSF Chimera. BMC bioinformatics. 2006 Dec;7(1):339.
31. Han Q, Wong DM, Robinson H, Ding H, Lam PC, Totrov MM, Carlier PR, Li J. Crystal structure of acetylcholinesterase catalytic subunits of the malaria vector Anopheles gambiae. Insect science. 2017 Mar 1.
32. Ramachandran GN, Ramakrishnan C, Sasisekharan V. Stereochemistry of polypeptide chain configurations. Journal of molecular biology. 1963 Jul 1;7(1):95-9.
33. Needleman SB, Wunsch CD. A general method applicable to the search for similarities in the amino acid sequence of two proteins. Journal of molecular biology. 1970 Mar 28;48(3):443-53.
34. Henikoff S, Henikoff JG. Amino acid substitution matrices from protein blocks. Proceedings of the National Academy of Sciences. 1992 Nov 15;89(22):10915-9.

Web Sites:

Web-1: http://www.who.int/mediacentre/factsheets/fs387/en/

Web-2: https://www.ncbi.nlm.nih.gov/

Web-3: www.rcsb.org

Web-4: http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE