In-Silico Docking Studies on Insecticide Resistance Acetylcholinesterase (Ache) Gene in Aphis Gossypii and Bemisia Tabaci

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Abstract

An insect may employ behavioral strategies or have particular physiological characteristics or modified biochemical mechanisms that enable it to survive in the environment which would be toxic to the normal population, which is otherwise called insecticide resistance phenomenon. Mechanisms of insecticide resistance found in insects may include three general categories viz, modified behavioral mechanisms, physiological mechanisms and biochemical mechanisms. In order to understand the insecticide resistance mechanism specially using in silico bioinformatics tools and techniques, we focused towards the third category biochemical mechanism of insecticide resistance. Ache (Acetylcholinesterase) is one among the enzymes/proteins targeted by most of insecticides, as a result reduced sensitivity of AChE to organophosphates and carbamates which act by inhibiting AChE, a most common type of alteration of target site which lead to resistance. Here this work considered Aphis gossypii and Bemisia tabaci insect's AChE genes and performed the in silico docking with the eight insecticide compounds and tracked two most efficiently docked compounds i.e., tetrachlorvinphos and dicrotophos based on binding affinity to the receptor.

Keywords: Acetylcholinesterase gene, Aphis gossypii, Bemisia tabaci, organophosphate insecticides, in-silico docking

I. INTRODUCTION

Humans have been controlling or attempting to control insect and other arthropod pests, plant pathogens, weeds, rodents, and other vertebrate pests for thousands of years. Insect pests have developed resistance to insecticides faster than beneficial organisms, limiting the integration of biological and chemical controls. The evolution of resistance to insecticides has become a serious problem world-wide.

Aphis gossypii, whitefly Bemisia tabaci and others developed resistance to the major groups of chemical pesticides like organophosphorus, synthetic pyrethroids, organochlorinates and other new groups of pesticides. *Aphis gossypii* is a tiny insect or greenfly in the superfamily Aphidoidea in the order Hemiptera. It is a true bug and sucks sap from plants. *Bemisia tabaci* belonging to the order Hemiptera and superfamily Aleyrodidae is one of the most destructive pests of mainly vegetables and ornamental crops around the world.

Bemisia tabaci is a cryptic species complex with at least 32 species having been discovered so far based on the 3.5 % divergence limit of the partial mitochondrial cytochrome oxidase subunit 1 (mtCO1) sequence.[1,2,3,4,5]. In insects, AChE is a target for organophosphorus and carbamate compounds, which remain widely used pesticides around the world. Modification of AChE to an insensitive form can be related to the increased AChE activity and has been demonstrated as the most important mechanism providing resistance to the organophosphates and/or carbamates in some pests. [6, 7, 8]

II. MATERIALS AND METHODS

A. Acetylcholinestrase (AChE) gene sequence retrieval (Protein):

The retrieval of AChE gene sequences of *Aphis gossypii* and *Bemisia tabaci* were carried out from NCBI GenBank. The gene ID are as *Aphis gossypii*- 22212715 and *Bemisia tabaci*- 15736683. BLAST is performed to obtain the similar sequence. Later Multiple Sequence Alignment is carried out in ClustalW2 to obtain the best similar sequence. The tertiary structure of the retrieved gene sequence is obtained from the PDB database [*Aphis gossypii*-1GQR and *Bemisia tabaci*- 4FNM].

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B. Retrieval of organophosphate compounds from ZINC database (Ligand):

The collection of the ligands for organophosphate compounds (monochrotophos, acephate, mevinphos, chlorfenvinphos, dicrotophos, crotoxyphos, dichlorvos, heptenophos) is done by ZINC data base and retrieved the information in SDF file format. The AChE gene shows insecticide resistance for above said organophosphate compounds.

C. Protein- Ligand docking:

This involves the steps that include loading the protein receptor which is already downloaded (1GQR) by starting Discovery Studio. In the Files Explorer, right-click a directory to which you would prefer to save protocol data. Click Set Default from the context menu. Choose File, Open from the menu bar, open the file pdb1GQR. This opens the 3D structure view and now we have to prepare the protein by adding hydrogen and by deleting the water molecules. The protein preparation is done and then click on the success to view the results. Later, we have to definine the receptor and start searching for binding sites. In the 3D Structure View, select any atom of the protein receptor by clicking it. The selected atom is highlighted with a yellow square. In the Binding Site tools, click "Define" selected molecule as Receptor, which will this define the protein molecule as the receptor. Find "Sites" from Receptor Cavities which will employ a cavity detection algorithm that identifies binding site cavities inside the protein receptor, the sites are sorted by size, and the largest site is displayed. Later, we have to define the ligand molecules by selecting the file and selecting the ligands and adding them by choosing the insert option. Finally, run the Docking protocol .
Then select on the dock option.After the docking procedure we will obtain the docked result. Click on result to view and calculate the Energy Binding value.

III. RESULTS

A. Ache protein as a receptor

The obtained protein (AChE) belongs to "Esterase lipase" domain super family which confirms that the obtained protein sequence and structure is valid insecticide resistance responsible protein. Since the selected sequence is reported with its valid protein structure in considering insects (PDB ID of *Aphis gossypii* 1GQR and PDB ID of *Bemisia tabaci* 4FNM), which helps to directly consider the protein structure as a receptor molecule for this study.

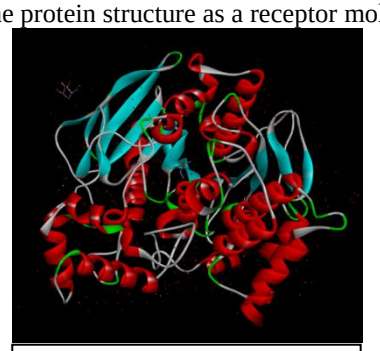


Fig.3.1.a. Protein Structure of 1GQR
(Aphis gossypii) as a receptor



Fig.3.1.b. Protein Structure of 4FNM (Bemisia tabaci) as a receptor

B. Retrieval of ligand molecules:

In the present study we have retrieved set of eight organophosphate chemical molecules (insecticide compounds) which are pre-reported showing resistance by above said insect pests. These compound structures are retrieved from the ZINC small molecule database http:\\zinc.docking.org\.The 8 set of organophosphate compounds from ZINC database are Mevinphos- 387426, Monocrotophos- 13827879**,** Tetrachlorvinphos-13783166, Acephate-2034488, Chlorfenvinphos-2040511**,** Dichlorvos-1853865**,** Dicrotophos-1587404 and Heptenophos-1849749

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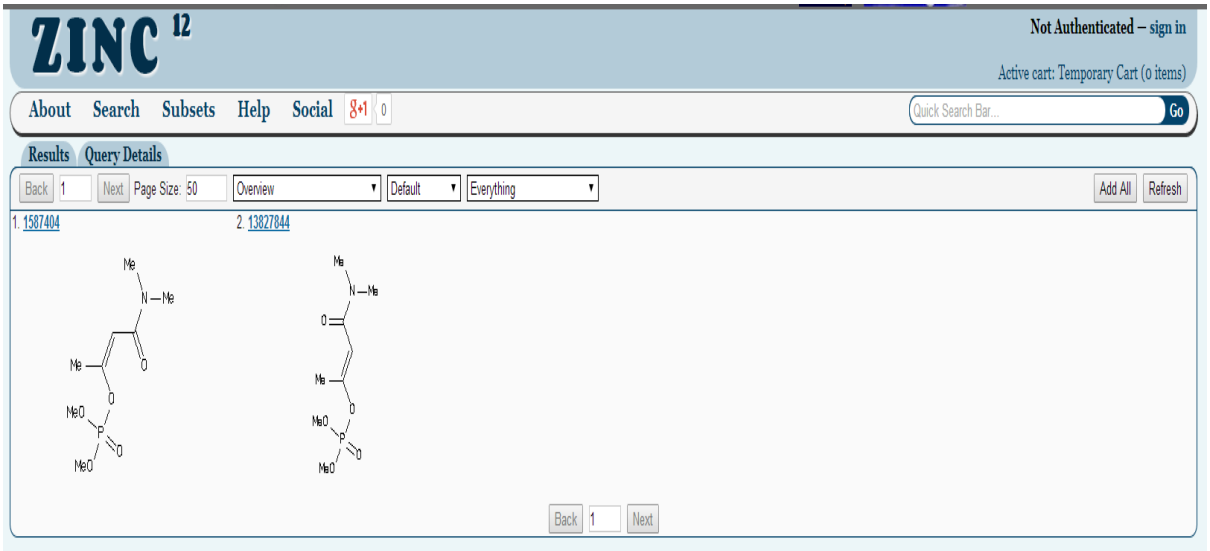


Fig 3.2 ZINC Database result for the ligand Dicrotophos

C. Receptor-ligand docking

In silico docking has been carried out in Discovery studio tool by using Ache protein (Aphis gossypii*)* 1GQR and (Bemisia tabaci*)* 4FNM as a receptor with 8 organophosphate compounds as a ligand molecules.

D. Active Pocket Prediction:

Followed by the preparation of the receptor we have subjected the processed receptors for the active pocket (binding sites) prediction and obtained the following binding site in the receptors respectively.

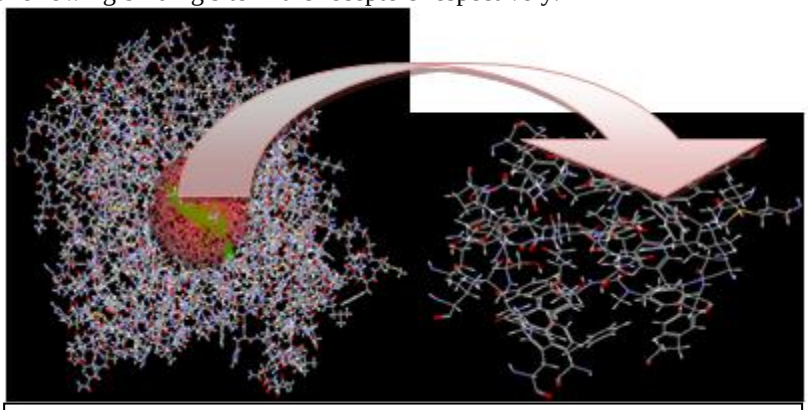


Fig.3.4.a. Prepared protein along with predicted binding site for 1GQR for Aphis gossypii

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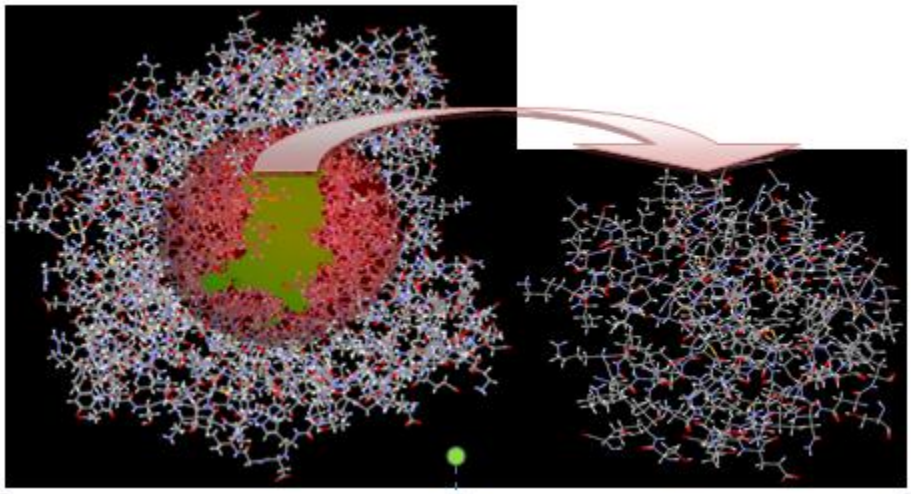


Fig.3.4.b Prepared protein along with predicted binding site for 4FNM for Bemisia tabaci

E. Ligand Preparation

After the detection of the binding site the next step we carried was to prepare the ligand molecule which is an important step towards the docking.

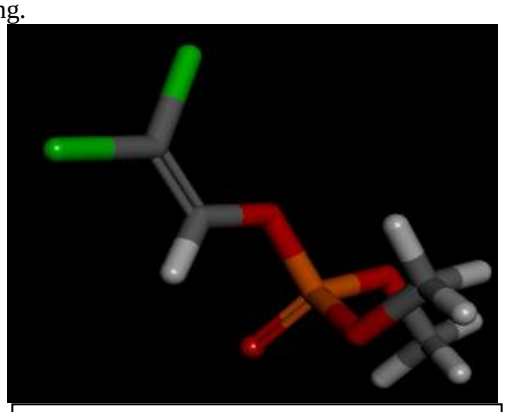


Fig 3.5.a. Prepared ligand structure 1GQR for Aphis gossypii

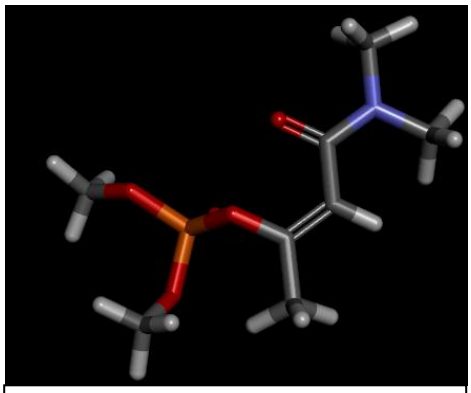


Fig.3.5.b. Prepared ligand structure for 4FNM for Bemisia tabaci

F. Receptor- Ligand docking

The following figure showing the actual step of Receptor- Ligand interaction and also showing the labeled amino acids bonding with the Ligand molecule.

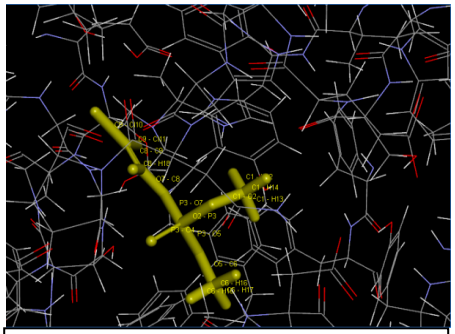


Fig.3.6.a Receptor Ligand interaction between 1GQR and Tetrachlorvinphos (Ligand) for *Aphis gossypii*

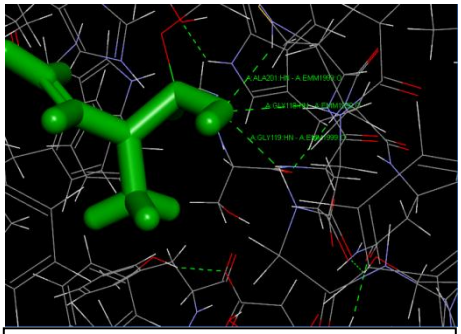


Fig.3.6.b Receptor Ligand interaction between 4FNM and Dicrotophos (Ligand) for *Bemisia tabaci*

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Based on the binding energy of the considering ligand molecules we have identified two best Ligand molecules like Tetrachlorvinphos and Dicrotophos which has interacted with the minimal binding energy for *Aphis gossypii* and *Bemisia tabaci* respectively.

The following table shows the Ligand binding energy of all the ligands at its different poses but the least energy binded is distinguished by red color.

Table - 1
The binding energy of 1GQR and Tetrachlorvinphos (Ligand) for *Aphis gossypii*

Ligand Name	*Binding Energy (kcal/mol)*	*Ligand Energy (kcal/mol)*	*Protein Energy (kcal/mol)*	*Complex Energy (kcal/mol)*	*Entropic Energy (kcal/mol)*
ZINC02040511	*150.37332*	*72.71515*	*-21695*	*-21471.80997*	*19.64470*
ZINC03874261	*12722.79802*	*609.97028*	*-21695*	*-2362.13014*	*18.63180*
ZINC13783166	*-8.34895*	*-16.94189*	*-21695*	*-21720.18928*	*19.69900*
ZINC13827879	*144.33430*	*6.18363*	*-21695*	*-21544.38051*	*18.42480*
ZINC2034488	*216.56896*	*37.99557*	*-21695*	*-24734.94524*	*18.65090*
ZINC1853865	*748.20414*	*38.83584*	*-21695*	*-24202.46979*	*18.56390*
ZINC1587404	*801.46802*	*39.75350*	*-21695*	*-24948.28825*	*18.67130*
ZINC1849749	*64.79333*	*38.51582*	*-21695*	*-24886.20062*	*18.63500*

Table - 2
The binding energy of 4FNM and Dicrotophos (ligand) for *Bemisia tabaci*

Ligand Name	*Binding Energy (kcal/mol)*	*Ligand Energy (kcal/mol)*	*Protein Energy (kcal/mol)*	*Complex Energy (kcal/mol)*	*Entropic Energy (kcal/mol)*
ZINC02040511	*-4.93623*	*4.98551*	*-24990*	*-24989.46049*	*18.51570*
ZINC03874261	*41.51525*	*15.39496*	*-24990*	*-24932.59956*	*18.59610*
ZINC13783166	*-4.39589*	*5.93329*	*-24990*	*-24987.97237*	*18.50680*
ZINC13827879	*4626.92788*	*195.38992*	*-24990*	*-20167.19197*	*18.60250*
ZINC2034488	*65.15366*	*2.62669*	*-24990*	*-24921.72942*	*18.52410*
ZINC1853865	*493.09859*	*7.35926*	*-24990*	*-24489.05192*	*18.59960*
ZINC1587404	*-104.74495*	*8.37259*	*-24990*	*-25085.88213*	*18.59930*
ZINC1849749	*37.88898*	*281.47329*	*-24990*	*-24670.14750*	*18.63300*

It is very clear from the above results that the ache protein (receptor) showing a strong interaction with the ligands like Tetracholrvinphos (ZINC13783166) and Dicrotophos (ZINC01587404) with the minimal binding energy value of -8.34895 (kcal/mol) and 104.74495 (kcal/mol) respectively. But the featured result generated by docking tool it is very much necessary to take into consideration of the "Complex Energy". The complex energy will gives an qualitative approach, which indicates how the free energy of the interacted complex reflects on the dissociation constant of the Ligand which in-turn the intensity of binding. So based on the complex energy parameter of the docked result it is clear that Tetrachlorvinphos (ZINC13783166) and Dicrotophos (ZINC01587404) has interacted with the efficient complex energy.

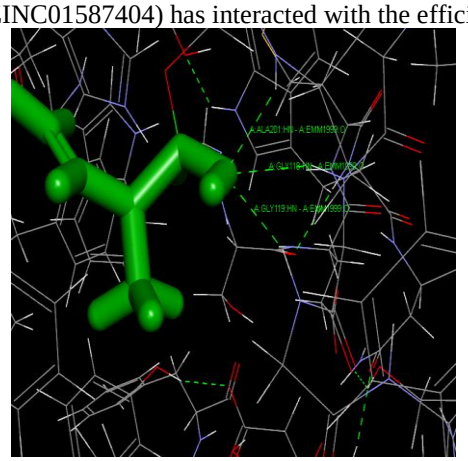


Fig.3.6.c Receptor ligand docking of 4FNM and Dicrotophos

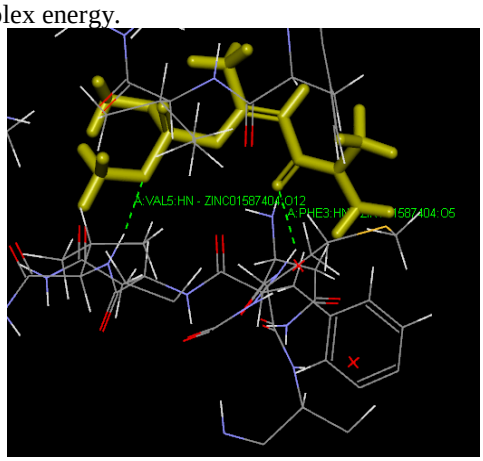


Fig.3.6.d Receptor ligand docking of 1GQR and Tetrachlorvinphos

Also considering the number of bonds established in due course of interaction conveys about the strength of its binding for the receptor. Here in the present study 1GQR receptor of the *Aphis gossypii* and Tetrachlorvinphos (Ligand) established a three bonds at the Alanine 201 position and two Glycine at 118 and 119th position of the receptor, where as 4FNM receptor of *Bemisia tabaci* and Dicrotophos (ligand) interacted with the two bonds at 5th position of the Valine and 3rd position of Phenylealanine of the receptor, which is considerable efficient bonding pattern.

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IV. DISCUSSION

The current study started with intention to reveal the structural level understanding of the Ache protein. Researchers have reported that ache gene/protein plays a vital role in expression of the insecticide resistance mechanism in insects. In order to know the fact how AChE gene responsible for resistance mechanism, this current study has selected to approach using Structural Bioinformatics (*In-silico* Docking). Understanding of the Ache protein by *in-silico* docking with class of organophosphates(monochrotophos, acephate, mevinphos, chlorfenvinphos, dicrotophos, crotoxyphos, dichlorvos, heptenophos) came up with significant single and most efficient Ligand molecule for each receptor molecule (1GQR of Aphis gossypii and 4FNM of Bemisia tabaci). Further this each Ligand molecule were analyzed for the reason it stood efficient ligand among set of four, it was very much clear from the results part of this work that each Ligand molecule (ZINC13783166- Tetrachlorvinphos of *Aphis gossypii and* ZINC01587404- Dicrotophos of *Bemisia tabaci*) has established a strong binding with the receptor.

V. CONCLUSION

In this study "Complex Energy" parameter played an explicable role in identifying how the binding energy and intensity of Ligand binding reflects in deciding which insecticide (ligand) shows high resistance. Finally this study came up logical strategy that, high affinity ligand binding to the receptor will perform well and brings the expected bio-activity, on the other hand if ligand binds very loosely,with high complex energy value to the receptor it may experience easy disassociation with the receptor which in turn cannot brings the expected result. Applying this strategy to the present study it is clear that among the final two insecticides Dicrotophos for *Bemisia tabaci* will shows the possibility of the less resistance when compared to the Tetrachlorvinphos for *Aphis gossypii* because Dicrotophos is having lower complex energy value (-25085.88213 Kcal/Mol) where as for Tetrachlorvinphos energy value is -21720.18928 kcal/mol. This comparison clearly tells that more negative the complex energy value, the higher the rate of dissociation, in turn cannot attain the expected bioactivity of killing insect pest with the particular chemical compound. And this will be the converse for another Ligand if it is having lesser negative complex energy value, which in turn chemical compound (Ligand) binds firmly to the receptor and significantly brings the expected result of killing the insect.

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