Computational study of interaction between *CYP319A1* and Organophosphates in the Cattle Tick, *Rhipicephalus Microplus*

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Abstract

Molecular docking is a key tool to determine the interactions between proteins and ligand molecules. Here we report the interaction between organophosphates, organic esters of phosphoric acid and CYP319A1 cytochrome P450, CYP319A1 present in Rhipicephalus microplus. CYP319A1 sequence analysis was performed with the help of different computational tools and then modelled using Phyre² web portal. Protein modelling involved the construction of 3D structure of the protein and further validation using SAVES sever. The modelled structure was docked with different acaricides such as coumaphos, cyclophos and diazonin using Glide software in Schrodinger Software package. The results obtained from this in silico studies will lead to a better understanding of the binding efficacy of the protein towards ligand molecules.

Keywords: *Rhipicephalus microplus, Organophosphates, Cytochrome P450, Homology modeling, docking*

1. Introduction

The cattle tick, *Rhipicephalus (Boophilus) microplus*, is an most important vector of diseases affecting livestock, humans and companion animals. Control of tick infestation is very much needed for the development of agriculture and livestock industries. Acaricide dipping and spraying are the most common methods used to control tick population. Chemicals, including organophosphates, synthetic pyrethroids, amitraz and organochlorines play important role in controlling *Rhipicephalus* species [1]. Among these acaricides, the most commonly used are the organophosphates, a diverse group of chemicals which have been widely used in both domestic and industrial settings. The long term use of these chemicals to control ticks could promote resistance and also hinders the control of diseases caused by *R. (B) microplus*. Molecular studies related to OP compounds such as coumaphos, cyclophos, diazonin and chlorphenvinphos has been well studied in ticks, especially in *R.(B) microplus* [2,3,4]. The mechanisms of resistance have been attributed to be enhanced metabolism, reduced absorption of the chemical and target site mutations [5, 6].

In the last decade various research groups have identified several target genes that involved in acaricide resistance in *Rhipicephalus (Boophilus) microplus*. However, the exact mechanism behind this resistance still remains elusive[7]. A coumaphos-resistant Mexican San Roman strain *R. microplus*, when exposed to coumphas, showed an increased expression of Cytochrome P450, which is involved in the detoxification of many classes of chemicals [8]. P450s play a major role in metabolic resistance to arthropods to a range of different insecticide or acaricide classes including organophosphates, pyrethroids, organochlorines etc [9]. Cytochrome P450 enzyme systems are involved in both bioactivation and detoxification of acaricides and are capable of metabolizing the acaricides rapidly. Therefore P450-insecticide interaction studies can definitely aid in the development of novel and unique tools for controlling resistant populations. To date, three *CYP* genes have been identified in *R. (B) microplus*, of these *CYP319A1* is the protein selected for the current study [10, 11, and 12].

In silico analyses of cytochrome P450 proteins helps to understand the properties and specifications of the proteins thereby improving our knowledge base on the protein molecule. These analyses also comprises of protein modelling and docking. Molecular docking is referred as an optimization problem that describes the exact fit of the ligand molecule in the active site of the receptor. The binding affinity to the target molecule can be predicted by the orientation of the ligand molecule [13]. Elevated Cytochrome P450 activity is well documented in many OP resistant studies [14]. Therefore, interaction studies between Cytochrome P450 protein and acaricides can serve as an appropriate tool for the

identification and analysis of protein - ligand binding sites to increase binding efficacies. In this work, the 3D model of R. *microplus* Cytochrome P450 protein CYP319A1 was predicted and interaction with commonly used organophosphates- coumaphos, cyclophos and diazonin was done with a goal to understand the CYP protein – acaricide affinity.

2. Materials and Methods

2.1 Sequence retrieval

Cytochrome P450 *CYP319A1* sequence of *R. (B) microplus and its* and functional information was obtained from Uniprot, a database of protein sequence (http://www.uniprot.org). The accession id of the protein is Q9NB96. The protein sequence was retrieved in the FASTA format.

2.2 Primary sequence and Secondary Sequence Analysis of CYP319A1

The physical and chemical parameters of the selected sequence was analyzed using ProtParam tool (http://web.expasy.org/protparam/). The computed parameters take account of the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, instability index, aliphatic index and grand average of hydropathcity (GRAVY). The transmembrane region of the protein was predicted by TMHMM (http://www.cbs.dtu.dk/services /TMHMM/). SOPMA server was used to predict secondary structure of the protein [15] (https://npsa-prabi.ibcp.fr/).

2.3 Three dimensional structure generation and validation

Phyre[2] (Protein Homology/analogy Recognition Engine V 2.0) is one of the most reliable tools used in the current study for the prediction of three dimensional structure of the selected protein [16]. It is an user friendly interface used for protein modelling. The generated PDB format of the structure from Phyre2 sever was viewed by using RASMOL visualization tool. The secondary structures are shown different types of interaction between the atoms. Secondary structures like alpha helix, beta sheets, random coils and extended strands were analysed using RasMol. The structure validations of the selected proteins were identified by Ramachandran plot and stereochemical analysis of the generated structure was performed using SAVES server (The Structure Analysis and Verification Server version 4). The quality of the refined structure was validated by using SAVES server (http://nihserver.mbi.ucla.edu/saves).

2.4 Ligands

Most commonly used organophosphates - coumaphos, cyclophos, and diazonin were selected as ligand molecules. These structures of the ligand molecules were obtained from Pubchem database (http://pubchem.ncbi.nlm.nih.gov/). The pubchem IDs of the chemical analogues are CID: 2871, CID: 3071448 and CID: 11970448. The structures of these compounds, saved in sdf format, were used further for docking studies. The properties of the selected ligands were also analysed. The ligand preparation was done by using Ligprep maestro in Schrodinger software package.

2.5 Docking studies

Interaction studies of the selected or modelled protein and the selected ligand compounds were done by Schrodinger molecular modelling package. It is a computational simulation method that is used to find out the best fit and orientation of ligand molecule in to the site of the receptor. The software used for the docking study was 'GLIDE', which is a ligand binding program in Schrodinger package. It provides complete spectrum of speed and accuracy for the high throughput virtual screening of large number of chemical compound to extremely accurate binding mode of prediction. The selected protein was prepared by Protein Preparation Wizard in Schrodinger maestro. The active sites were identified by Sitemap. Glide High throughput Virtual Screening (HTVS) docking was performed to find out the best binders and the results were analysed by Glide score and Glide docking energy. Affinity of the ligand molecule to the target protein can be calculated by analysing interaction energy scores, which is often expressed in kcal/mol.

3. Results and Analysis

3.1 Primary and secondary sequence analysis

Protparam tool was used for the analysis of *CYP319A1* protein. The retrieved FASTA sequence of the CYP319A1 protein sequence was subjected to ProtParam analysis. The amino acid sequence length and molecular weight of the sequence was 531 and 60900.8 respectively. The number of positively and negatively charged residues in the given sequence was found to be 60 and 69 respectively. Total number of atoms is 8614. Aliphatic index of the protein was 94.01, which is defined as the relative volume occupied by aliphatic side chains and is regarded as the thermal stability of globular protein. Grand Average of Hydropathy (GRAVY) of the protein was - 0.116. Atomic composition of the protein is given below.

Atomic composition:

Carbon	C	2735
Hydrogen	H	4334
Nitrogen	N	754
Oxygen	O	762
Sulfur	S	29

SOPMA secondary structure of prediction tool predicted 47.27 % alpha helix, 15.82% extended strands, 5.27% beta

turns and 31.64% random coils in *CYP319A1*. The transmembrane structure of *CYP319A1* was predicted by using TMHMM server. The target protein constitutes two transmembrane helixes Figure 1. Sequence positions of the predicted helixes ranges between 4-26 and 56-78.
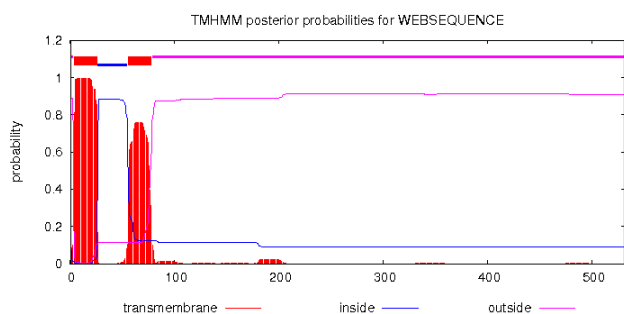

Figure1: TMHMM prediction of *CYP319A1*

3.2 3D Structure generation and validation

Phyre[2]-webportal was used to generate the three dimensional structure of *CYP319A1*. The predicted structure is shown in Figure 2. The structure constitutes 4280 atoms, 4383 bonds and 368 hydrogen bonds. It also possesses 19 helices, 50 turns and 17 strands.
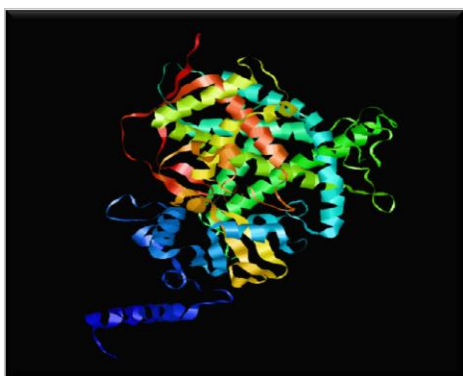

Figure: 2 CYP319A1 structure predicted in Rasmol viewer

The predicted model of the protein has been validated by SAVES server which showed the quality of the protein. After analysis of SAVES result we observed that the overall quality of the predicted structure was 57.170. The results were analysed using Ramachandran plot showed that 85.4 % residues fell in the allowed region. The results are shown in Table 1.

Table: 1 Structure details and statistical evaluation

Plot statistics & RASMOL Structure Details	*CYP319A1*
Residue in most favoured region (%)	85.4 %
Residues in additional allowed regions (%)	8.3 %
Residues in disallowed regions (%)	6.2 %
Number of helices	19
Number of Strands	17
Number of Turns	50

3.3 Docking studies

Organophosphates group of acaricides were selected as ligands for *in silico* docking studies. The properties of the ligands are shown in Table 2. The docking of the selected ligands and *CYP319A1* protein was done using Glide Docking Software and the results are presented in the Table 3 and Figure 3. Coumaphos showed -37.788 glide energy and good glide score -7.559 towards target protein-hydrogen bond interaction were seen between leucine residue (LEU123) in protein and oxygen in ligand with a bond length of $2.36A^0$. Cyclophos showed -26.709 glide energy and glide score -6.864. Hydrogen bond formation was observed between glycine residue (LEU123) and oxygen atom in ligand molecule. Diazonin had the least glide energy compared to the selected compounds, viz., -22.881 glide energy ,and -5.188 glide score. The hydrogen bond interaction was seen between Leucine residue (LEU123) and oxygen atom in the ligand at a distance of $2.04 A^0$.

Table 2: Properties of the selected ligands

Sl. No.	Ligand	Molecular formula	Molecular weight	Hydrogen Bond Donors	Hydrogen Bond Acceptors
1	2871 Coumaphos	$C_{14}H_{16}ClO_5PS$	362.765602 g/mol	0	6
2	3071448 Cyclophos	$C_{16}H_{34}O_6P_2S_2$	448.515084 g/mol	0	8
3	11970448 Diazonin	$C_7H_{14}N_2O$	142.19886 g/mol	2	1

Table 3: Docking results of *CYP319A1* and selected ligands

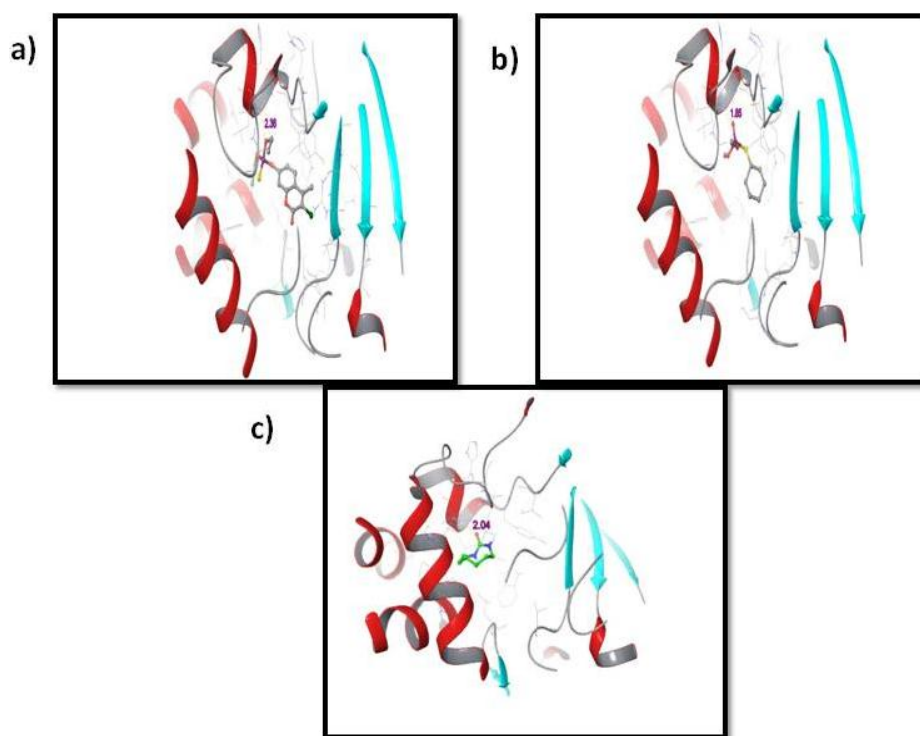| Sl. No,	Ligand	XPG score	Glide energy	H Bond Nos.	H Bond Protein	H Bond Ligand	H bond Length (Å)
1	2871 Coumaphos	-7.559	-37.788	1	LEU 123 (H)	O	2.36
2	3071448 Cyclophos	-6.864	-26.709	1	LEU 123 (H)	O	1.85
3	11970448 Diazonin	-5.188	-22.881	1	LEU 123 (H)	O	2.04

Figure: 3 (a), (b) and (c) shows interaction of *CYP319A1* with coumaphos, cyclophos, and diazonin   compounds respectively

Conclusion

Ticks and tick-borne diseases affect 80 per cent of the world cattle population and are widely distributed throughout the world. At present, control of tick and tick-borne diseases is mainly achieved by the wide spread use of chemical acaricides and the development of resistance by ticks against these chemical has been a major area of concern. Many cases of tick resistance against organophosphates have been reported in many parts of the world. There were several reported studies on the role of Cytochrome P450 in conferring resistance to organophosphates, therefore understanding of acaricides and their target proteins helps to control the infestation caused by tick species. In the present work, *CYP319A1* sequence was analysed and the three dimensional structure was predicted using different computational methods. Phyre[2] predicted 3D structure of the selected protein and was validated. Coumaphos, a fat soluble organophosphate compound showed highest docking score and energy toward target protein sequence and Diazonin showed least binding energy and score. Understanding the interaction mechanism between protein and ligand molecules helps to identifying novel gene targets and may help in designing more efficient chemicals for tick control and management.

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