MODELLING THE STRUCTURE OF GLUTAMATE GATED CHLORIDE CHANNEL (GLUCL) IN *ATHENA TUMIDA*MURRAY : AN EFFECTIVE INSECTICIDE TARGET FOR APICULTURE

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ABSTRACT

Beekeeping has been practiced by the human beings since ancient times mainly for the precious honey stored by the bees in the combs. Small hive beetle or Athena tumida Murray is a small, dark-coloured beetle that lives in beehives that can damage the comb, stored honey and pollen. High infestation rates by the beetle may cause bees to abandon their hive it can be a marker for the diagnosis of colony collapse disorder for honey bees. Several pesticides are currently being used against the small hive beetle. The chemical fipronil (Combat Roach Gel) is one such pesticide that affects the nervous system of insects and is applied inside the corrugations of cardboards which are used for making beehives. It is important to know how to control the spread of the infection by knowing the molecular mechanism of interaction of the insecticide with the beetle. Very little is known about the structure, position and molecular interaction of the insecticide fipronil onA.tumida. In this present investigation, we have tried to predict the 3D structure of GluCl channel in A.tumidain order to decipher the position where the insecticide fipronil acts in the nervous system of the beetle, A.tumida.

Keywords: *Insecticide, GluCl-channels, Honey bees, Binding sites, Fipronil.*

Introduction

A small flying insect called honey bee is considered to be one of the most beneficial insects gifted by nature to us. Beekeeping has been practiced since the ancient times mainly for the precious honey stored by the bees in the combs. Honey was the first sweet food to be tasted by the prehistoric humans who lived in forests and rock shelters. India has some of the oldest recorded honey harvesting industry which has been depicted in the form of paintings by prehistoric man in the rock shelters. With the development of civilization, honey acquired a unique status in the lives of the ancient people. Honey was regarded as a magical substance in ancient cultures and was believed to have controlled the fertility of women and cattle, and also of lands and crops (Abrol, 2009).

Small hive beetle (SHB) or *Athena tumida* Murray is a small, dark-coloured beetle that lives in beehives and pest that destroys the honey bee colonies, it can affect all the parts of the bee hives, like the comb, stored honey, and pollen(Hood et al, 2014). The small hive beetle (SHB) (*A.tumida* Murray), family Nitidulidae, is a scavenger and parasite of colonies of honeybee (Evans et al, 2018). Looking at the rate at which SHB is invading new countries and continents, it is important to look for the control a mechanism. It is likely to be transported internationally by accident, because of the invasive character of the species outside its native range (Office International des Épizooties (OIE), 2013, Idrissou et al, 2018, Idrissou et al, 2019, Dongmei et al 2018).

In order to protect the colonies, the beekeepers use the insecticide, Fipronil (Johnson et al, 2010). Fipronil is a broad-spectrum insecticide belonging to the phenylpyrazolechemical family. Fipronilhas been proven to disrupt the insect's central nervous system by blocking GABA-gated chloride channels and glutamate-gated chloride (GluCl) channels (Aufavure et al, 2012; Raymond-Delpech et al 2005). GluCl channels is not found in mammals, so the use of fipronil is less likely to affect the human beings(Bloomquist, 2001).Thus, the beekeepers who are using fipronil to save their bee hives will not have to worry about any adverse impact of honey consumption by human beings. Thus, the bio-safety of

mammals, especially of human beings is not compromised by the use of insecticide, fipronil.

Effective management of the various types of insects requires insight into the physiological and genetic basis of insecticide resistance, which is possible through wet laboratory and dry laboratory approaches.The small hive beetle genome is likely to serve as a resourcefor insecticide susceptible target sites (Rinkevich and Bourgeois, 2020). Bloomquist (2003) demonstrated the usefulness of the chloride channels as a target for developing selective insecticides. GABA-gated chloride channels are responsible for inhibitory currents in the insect central nervous system, while the insectglutamate-gated chloride channels (GluCl) are highly effective targets that can be used against selected insects. We have very limited knowledge about the structure of GluCl in *A. tumida* and the molecular interaction of fipronil with GluCl and its effect on*A.tumida*nervous system (Rinkevich and Bourgeois, 2020). Thus, the present investigation aims to provide the rational structure of the target protein and the interaction of fipronil with the target protein to show the insecticidal effect.

Materials and Methods

Sequence Retrieval

The sequence of glutamate gated chloride channel (GluCl) of *A.tumida* was retrieved from REFSEQ database of NCBI with accession number XP_019865726.

Template selection

A search for a template was performed using BLAST-P (Altschul et al, 1990)against PDB database(Westbrook et al, 2003) from NCBI interface and it was found to be *C.elegans*GluCl (PDB ID: 3RHW, Hibbs and Gouaux, 2011).

Homology modelling

A three-dimensionalhomology model of the GluCl was developed using the tool called Modeller v9.10 program(Sali and Blundell, 1993),using standardized protocol described in detailed elsewhere (Meshram et al, 2020a; Meshram et al, 2020b). Briefly, the homology model was built on the concept of "satisfaction of spatial restraints methodology" employed in Modeller v9.10 An inbuilt script inside Modeller known as Align2Dwas used to align the glutamate-gated chloride (GluCl) channels of *A.tumida*, against the template structure. Align2D command is a modification of the classical end-to-end alignment strategy using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970).

The sequence of glutamate gated chloride channel (GluCl) of *A.tumida*(Accession number XP_019865726) was retrieved from REFSEQ database of NCBI. BLAST-P (Altschul et al, 1990) search was carried out against the PDB database to identify suitable homologous structure of the protein. From the hits obtained, the best one was selected and used as template for homology modelling.The final models were build using the automodel class of Modeller and further energy minimized using Swiss PDB viewer 3.7 (SPDBV3.7) (Guex and Peitsch, 1997).

The structure of singlechain of (GluCl) of *A.tumida* was developed using homology modelling using Modeller v9.10 programusing the template from *C elegans*. The model of GluClfrom*C. elegans* was already co-crystallized with its inhibitor Ivermectin. Subsequently, the oligomerization of GluCl was performed by applying crystallographic symmetriesto yield the pentameric, functionally active,quaternary state.The binding site was inferred from the template structure that was co-crystallized with an inhibitor. Well-known and standard tools were used for the evaluation of the models such as Ramachandran plot (Willard et al, 2003) and WhatCheck/WhatIf (Hooft et al, 1996).

The molecular docking was performed as per an established protocol published elsewhere (Meshram et al, 2020a). The model was visualised in SPDBV software and docking was done using PyRx 0.8program(Dallakyan and Olson, 2015) to locate the binding and orientation of fipronil on the predicted model of glutamate-gated chloride (GluCl) channel of *A.tumida.*AutoDock 4.2 was used in backendfor performing molecular docking (Huey et al, 2007: Morris et al, 2009)while

LigPlot+ (Laskowski&Swindells, 2011; Wallace et al, 1995) program was used to understand the molecular interaction i.e. hydrophobic reaction, hydrogen bonding etc.

Results

Homology modelling

As no experimental structure was available for glutamate-gated chloride (GluCl) channels of *A.tumida*, a homology model was built. For this, first a template search was conducted and this search yielded seven hits with comparable e-values and bit score. The most significant hit was the structure of *C. elegans* glutamate-gated chloride channel (GluCl)(PDBID 3RHW) having 76% query coverage and sharing 55.21% sequence identity with the query sequence.This structure also had the bound substrate Ivermectin init.The alignment of GluCl of *A. tumida* with the structure of GluCl in *C. elegans* using Align2D program is shown in Fig. 1. The homology model generation was performed using Modeller.

```
 _aln.pos          10        20        30        40        50        60
template1A  SDSKILAHLFTSG-YDFRVRPPTDNG--GPVVVSVNMLLRTISKIDVVNMEYSAQLTLRESWIDKR
query       KEKEVLDQILGPGRYDARIRPSGVNGTDGPAIVRVNIFVRSISKIDDVTMEYSVQLTFREQWLDER
 _consrvd         *      * ** * **   **  **  * **   * ***** * **** *** ** * * *
 _helix      999999999999
 _beta                                 999999999999999999 999999999999999999

 _aln.pos     70        80        90       100       110       120       130
template1A  LSYGVKGDGQPDFVILTVGHQIWMPDTFFPNEKQAYKHTIDKPNVLIRIHNDGTVLYSVRISLVLS
query       LKFNDFG-GRLKYLTLTEANRVWMPDLFFSNEKEGHFHNIIMPNVYIRIFPYGSVLYSIRISLTLS
 _consrvd    *     * *      **     **** ** ***    * *  *** ***   * **** **** **
 _helix
 _beta                99999         999999   99999   999999999 99999999999999

 _aln.pos       140       150       160       170       180       190
template1A  CPMYLQYYPMDVQQCSIDLASYAYTTKDIEYLWKEHSPLQLKVGLSSSLPSFQLTNTSTTYCTSVT
query       CPMNLKLYPLDRQICSLRMASYGWTTDDLVFLWKEGDPVQV-V-KNLHLPRFTLEKFLTDYCNSRT
 _consrvd    *** *  ** * * **   ***  ** *   ****  * *  *     ** * *    * ** * *
 _helix                                              9999999
 _beta       999       99999999999      9999999   99999        9999999999999999

 _aln.pos  200       210       220       230       240       250       260
template1A  NTGIYSCLRTTIQLKREFSFYLLQLYIPSCMLVIVSWVSFWFDRTAIPARVTLGVTTLLTMTAQSA
query       NTGEYSCLKVDLLFKREFSYYLIQIYIPCCMLVIVSWVSFWLDQGAVPARVSLGVTTLLTMATQTS
 _consrvd    *** ****      ***** ** * *** ********** *  * **** ********  *
 _helix                  99999999999999999999999999   99999999999999999999
 _beta         999999999999999

 _aln.pos      270       280       290       300       310       320       330
template1A  GINSQLPPVSYIKAIDVWIGACMTFIFCALLEFALVNHIANAG-TTEWNDISKRVDLISRALFPVL
query       GINASLPPVSYTKAIDVWTGVCLTFVFGALLEFALVNYASRSDMHRENMKKQRRQCELEHA-----
 _consrvd    ***  ****** ****** * * ** * *********          *      *      *
 _helix      999999      99999999999999999999999999999  99999999999999999999
 _beta

 _aln.pos          340
template1A  FFVFNILYWSRFGH
query       --------------
 _consrvd
 _helix      9999999999
 _beta
```

Fig 1: Alignment by Align2D.

Structure validation

The evaluation and validation of the structure was done by using WhatCheck/WhatIf(Hooft et al, 1996), ProCheck (Laskowski et al, 1993) and Ramachandran plot statistics (Willard et al, 2003).Generally, models with the majority of residues falling in the allowed region of the Ramachandran plot are considered correctly folded structures (Willard et al, 2003).

In order to judge the efficacy of the prediction method, the model was tested for 47 structural tests implemented in the WhatCheck/WhatIfprogram. This program essentially checks for proteingeometry as well as nomenclature to determine the correctness of the given model. The results of these 47 checks are classified into three categories depending on the severity of the problem identified. For instance, the observations flagged as "warning" signifies observation with trivial severity, while those marked with "errors" flag demands immediate attention. The third category "notes" might indicate some statistical figure or some other comment on the test conducted in WhatIf. The generated model for GluCl channel from *A.tumida*passed the majority of tests; some warnings were there in the template itself. All these results clearly indicate that the proposed model has sound stereochemicaland geometric properties as well. This model validation was done for the monomeric chain of glutamate channel for *A.tumida*. Then oligomerization was done for the formation of the complete structure which was a pentamer.

Table 1: Ramachandran Plot statistics for the residues in the predictedGluCl structure.

	No. of residues	**% of residues**
Most favoured regions [A,B,L]	262	90.7%
Additional allowed regions [a,b,l,p]	24	8.3%
Generously allowed regions [~a,~b,~l,~p]	3	1.0%
Disallowed regions [XX]	0	0.0%
Non-glycine and non-proline residues	289	100.0%
End-residues (excl. Gly and Pro)	5	
Glycine residues	17	
Proline residues	14	
Total number of residues	325	

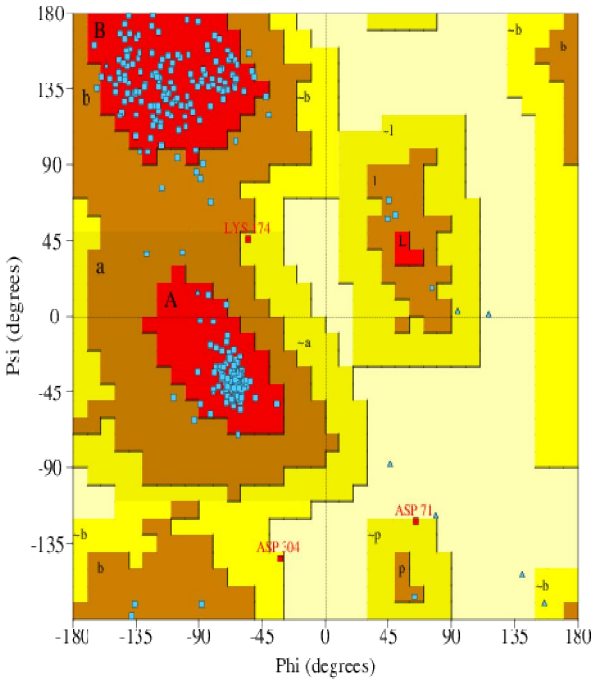

Fig 2.Ramachandran plot of modelled GluCl from *A.tumida*

Ramachandran plot shows that, no residue is present in the disallowed region; the monomer was present in core region which means the model which is developed is perfect stereochemicallyaccording to Ramachandran Plot statistics. Two hundred and sixty two (262) amino acids are in most favoured region, while three amino acid residueswere in generously allowed region and core region of Ramachandran plot. Thus, with 90% data in most favoured region and 8% data in additionally allowed region and 1% in generously allowed region, with none (0%) of the residues is found in the disallowed region. Thus, stereo-chemically the model is perfect.

Oligomerisation of the monomer was carried out to produce the functionally active model. The model was also checked using 47 WhatCheck/WhatIfwhich was also found to be acceptable. The warnings at some sites were due to the errors in template structure. Except for certain warning there were no serious issues with the structure.We generated 5 models for the five chainsfrom each of the five monomer templates Chain A, B, C, D and E and the final pentamer obtained after applying symmetries can be visualized as shown in Fig 3a.

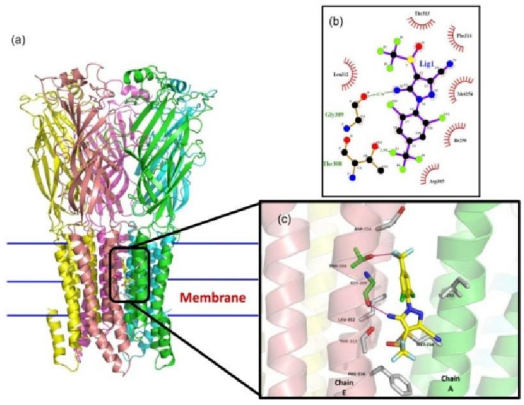

Fig3. (a) Three dimensionalstructure of glutamate chloride channel in A. tumida. (b) Molecular interactions between fipronil and the residues in GluCl (c)Interaction between fipronil and chainA(green)and chainE (purple).

TargetingFipronilagainst GluCl channel from A.tumida

*C. elegans*GluClhas the inhibitor Ivermectin in its structure of glutamate chloride channel. Thus, for molecular docking, the *C. elegans*GluCl inhibitor wasused as a reference for docking fipronil. PyRx 0.8 programthus enabled us to predict how the fipronil might attach at the binding sites of glutamate chloride channel by molecular docking.

Identification of target region in the GluCl in A.tumida for action of fipronil

Modelling and simulation studies provided proactive insights in the structure and function of GluCl channel from *A.tumida*. However, it is also important to identify a set of putative interactions that can be used as a starting point in finding effective insecticidal agents based on the structure of GluCl channel. The predicted free energy of binding of Fipronil was recorded as –5.28 kcal mol^{-1}. Fipronil might be stabilized by the hydrophobic interaction that are provided by the isoleucine 250, methionine 254 from chain A, while aspartate 305, leucine 312, threonine 313 and phenylalanine 316 from chain-E (Fig 3 b & c).

Inhibitor is stabilized by polar interaction in forms of hydrogen bond and our inhibitor forms hydrogen bonds with the residues glycine 309 and threonine 308of chain E. Nitrogen from fipronil might form hydrogen bond with backbone of oxygen of glycine 309. Similarly, the fluorine from the terminal portion of our ligand forms an effective hydrogen bond with the side chain of threonine 308 from chain E and thus this hydrophobic interaction collectively with polar interaction of glycine 309 and threonine 308, stabilise this inhibitor protein complex(Fig 3 b & c). Thus, due to stable interaction and stability imparted due to these hydrophobic interactions and hydrogen bonds, fipronil might inhibit the channel.

Discussion

The collection of honey using apiculture is an extremely important industry in India and over the world. One of the main challenges affecting this industry is the presence of a highly invasive species of beetle known as *A. tumida* which causes severe damage to bees and the honeycombs, Beekeepers have been using Fipronil, a broad-spectrum insecticide which affects GABA-gated chloride channels and glutamate-gated chloride (GluCl) channels, to curb *A. tumida*infestation.But there isvery little understanding of how this insecticide affects *A. tumida*. In this paper we are proposing a possible target, its structure and the molecular interactions that are responsible for pesticidal property of firpronil against *A. tumida* using *in-silico* methods.

We had identified the GluClin *A. tumida*as the potential target for fipronil based on the general property of the insecticide. Since there were no three dimensional structures available for GluCl in *A. tumida*, we modelled the structure of it for the first time. We were able to predict the monomer structure using well established methodologies.The predicted structure was found to be stereo-chemically and energetically stable. Furthermore, we were able to predict the oligomerization state of the protein which was found to be a pentamer (Chains A to E).This oligomer was further used in docking studies to understand how fipronil binds to the biological unit of GluCl. We were able understand that Fipronil interacts with multiple monomers in the channel at the same time to provide a stable complex that might inhibit the GluCl channel. It interacts with Chain A and E using hydrophobic interactions(Chain A: I250, M254, Chain E: D305, L312, T313 and W316)and hydrogen

bonding (Chain E: G309 and T308). Our work will be able to help in understanding the structure of GluCl and the interactions between Fipronil and GluCl. Though further wet lab research needs to be done to validate these results, this work might help in future research in more effective pest control strategies in apiculture.

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