In Silico Comparision of the Ligand Binding Domain of Ecdysone Receptor of Two Lepidopterous Insect Pests, Helicoverpa Armigera and Spodoptera Litura

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Abstract

The physiological process of molting in insects is governed by hormones. The ecdysteroid hormones coordinate the major stages of insect development by binding to the ecdysone receptor (EcR). Attempts to control insects through targeting the EcR are now gaining importance. In this study the protein sequence of ecdysone receptor of Helicoverpa armigera (Hub) and Spodoptera litura (Fab) were modeled specifically by extracting geometric restraints for corresponding atoms using the Homology modeling approach. The 3D structural comparison of the targets shows that the structures are closely related to each other with the low RMS value of 0.70Å. We analyzed the similarity in the ligand binding domain (LBD) of Ecdysone receptor of the lepidopteran pests by fixing the non-steroidal, synthetic agonist BYI06830 used in agrochemical pest control as the ligand. The comparison of EcR in complex with non-steroidal synthetic agonist results in partially overlapping residues in the LBD and the residue involved in the H-bond formation was found to be identical. We suggest that these findings of ligand-dependent binding pocket similarities have potential applications for developing a potent solution against the challenging pests.

Keywords: Ecdysone receptor, Lepidopterans, Homology modeling, Synthetic agonist, Molting, Dibenzoylhydrazines, Molecular docking, Ligand binding domain.

Introduction
Protection of crops from insect pest is vital in order to meet the food requirements of the burgeoning human population. Lepidopteran insects are among the major pests of

several economically important crops and their control requires a multi-pronged intervention. While insecticides have been used in several IPM (Insect pest management) programmes, pheromone traps have been frequently used to capture and kill insect pests in the field. The capacity of novel chemicals to disturb the mating and molting processes of insects has been capitalized for pest control. The molting hormone, 20-Hydroxyecdysone (ecdysterone or 20E), is a naturally occurring ecdysteroid hormone that controls the molting of arthropods (Thummel, 1995, 1996). During insect development, it binds to the ecdysone receptor, a ligand-activated transcription factor found in the nuclei of insect cells (Riddiford, 2000). This in turn leads to the activation of many other genes, as evidenced by chromosomal puffing at over a hundred sites. Ultimately the activation cascade causes physiological changes that result in molting (Henrich, 2005). In recent years research is focused on targeting the ecdysone receptor with the aim to disrupt the molting process of insects and facilitate insect control. **Insect growth regulators control insect population, by primarily** regulating molting, metamorphosis and many other physiological and developmental processes (Williams, 1956; Fox, 1990; Mondal et.al., 2000). Non-steroidal dibenzoylhydrazines such as RH5849 and RH5992 exert their insecticidal effect by binding to the 20-hydroxyecdysone binding site and activating the ecdysteroid receptors permanently (Wing et al., 1988, Smagghe et al., 1994; Wurtz et al., 2000). Their comprehensive effects and high selectivity as well as lower toxicity to non-target animals and the environment provide new tools for integrated pest management

EcR is the target of the environmentally safe bisacylhydrazine insecticides used against pests which cause severe damage to agriculture. N-tert-butyl-N,N'-dibenzoylhydrazines (DBHs) were discovered as molting hormonal agonists, and causes incomplete molting in insects leading to death (Hsu, 1991; Wing et.al., 1998). A number of DBH analogs with various substitutes at benzene rings were synthesized and the structure-activity relationship (SAR) studies performed (Takehiko Ogura et.al., 2005). Recently, four DBH compounds including tebufenozide (Hsu et.al., 1997), methoxyfenozide (Carlson et.al., 2001), **chromafenozide (**Sawada et.al., 2003) and halofenozide (Dhadialla et.al., 1998) have been commercialized. Chromafenozide is found to be significantly potent against various lepidopterous insects, but at the same time almost non-toxic to non-lepidopterous species, including pollinators, predators and parasitoids. As chromafenozide has a low toxicity profile in mammals and non-target organisms, and has minimum impact on the environment, it would be an ideal agent for integrated pest management (IPM) (Horowits et.al., 2004; Mikio Yanagi et.al., 2006). Even though 20E is commonly used as molting hormone in most of insects and has similar potency among insects, SARs of non-steroidal ecdysone agonists varied among insect species. The reason for the difference of SARs between ecdysteroids and non-steroidal compounds is disclosed by the three dimensional structure analysis of ligand-bound EcR, showing that ponasterone A (PonA), one of the most potent ecdysteroids, does not necessarily overlap with a **chromafenozide** analog (**BYI06830**) in the binding pocket, and therefore, the interaction between EcR and DBHs can be species-dependent (Billas et.al., 2009; Holmwood et.al., 2009)

In this study we compare the structural level relationship between the EcR of Spodoptera litura and Helicoverpa armegira and analyze the LBD with respect to the lepidopteran specific bisacylhydrazine BYI06830. The LBD was compared to understand the adoptability of same kind of ligand into the binding pocket of two different pests. Identifying the location of ligand binding sites on a protein is of fundamental importance for a range of applications including molecular docking, de novo drug design and structural identification and comparison of functional sites.

Materials and Methods

Target and Ligand Serach

The protein sequences of Ecdysone receptor of the targets (H. armigera and S. litura) were collected from the sequence database NCBI (http://www.ncbi.nlm.nih.gov/). The chemical 3D structure of the ligand (HWG-synthetic agonist BYI06830) was collected from PUBCHEM through NCBI search.

Homology Modelling

The EcR sequences of S. litura and H. armegira (ABX79143 and ABN11286) was modeled with the homology modeling server GENO3D (http://geno3d-pbil.ibcp.fr). The target sequences were submitted to the GENO3D tool to find the template using PSI-BLAST method against Protein Data Bank (PDB). From the PSI-BLAST results the correct template were selected based on the sequence similarity and submitted again for modeling the structure. The structures were modeled by extracting geometrical restraints (dihedral angles and distances) for corresponding atoms between the query and the template and the 3D construction of the protein by using a distance geometry approach (Combet et.al., 2002).

The geometric parameters were evaluated using Ramachandran plot produced by the PROCHECK analysis (Luthy et al., 1992). ProSA (https://prosa.services.came.sbg.ac.at/prosa.php) was used to check 3D models of protein structures for potential errors (Sippl., 1993).

Structure Alignment

The secondary structures of the targets were analysed using the tool SOPMA (Geourjon and Deleage., 1995). The 3D structure comparison was implemented using the Swiss-PDBviewer3.7.

Docking

The intermolecular complex of ligand and the targets were formed using the tool PATCH DOCK (http://bioinfo3d.cs.tau.ac.il/PatchDock/). Patch dock is a web server works based on the geometry-based molecular docking algorithm (Duhovny, 2002; Schneidman-Duhovny, 2003 & 2005). The intermolecular complexes were analysed for tracing the LBD using the ARGUS LAB (Thompson, 2004). ArgusLab is a molecular modeling program that runs on Windows 98, NT, and 2000.

Results

Homology Modelling

The PSI-Blast results were short listed carefully based on the alignment file provided by the tool. A sequence identity of 97% and 88.6% was observed for the EcR of H. armigera and S. litura respectively when matched with the D chain of Hormone/Growth Factor Receptor of Helicoverpa virescens (PDB code: 1R1K). The selected templates were submitted to the GENO3D tool for modeling. Three models were generated for each target. Among the three models, the top was filtered based on the Energy score provided by the tool. The top model of the targets has the energy value of -10902.90 kcal /mol and -10634.20 kcal /mol respectively.

The PROCHECK result of the top model shows that most of the residues fall on the allowed region of the Ramachandran plot. The percentage of residues in the "core" region were found to be 87.0%, 86.9% respectively (Fig: 1 a, b) for H. armigera and S. litura. The stereochemical quality of the models was found to be satisfactory. The ProSA shows that the top model energy plot is reliable to the deposited NMR and X-ray structures in PDB database (Fig: 2 a, b).
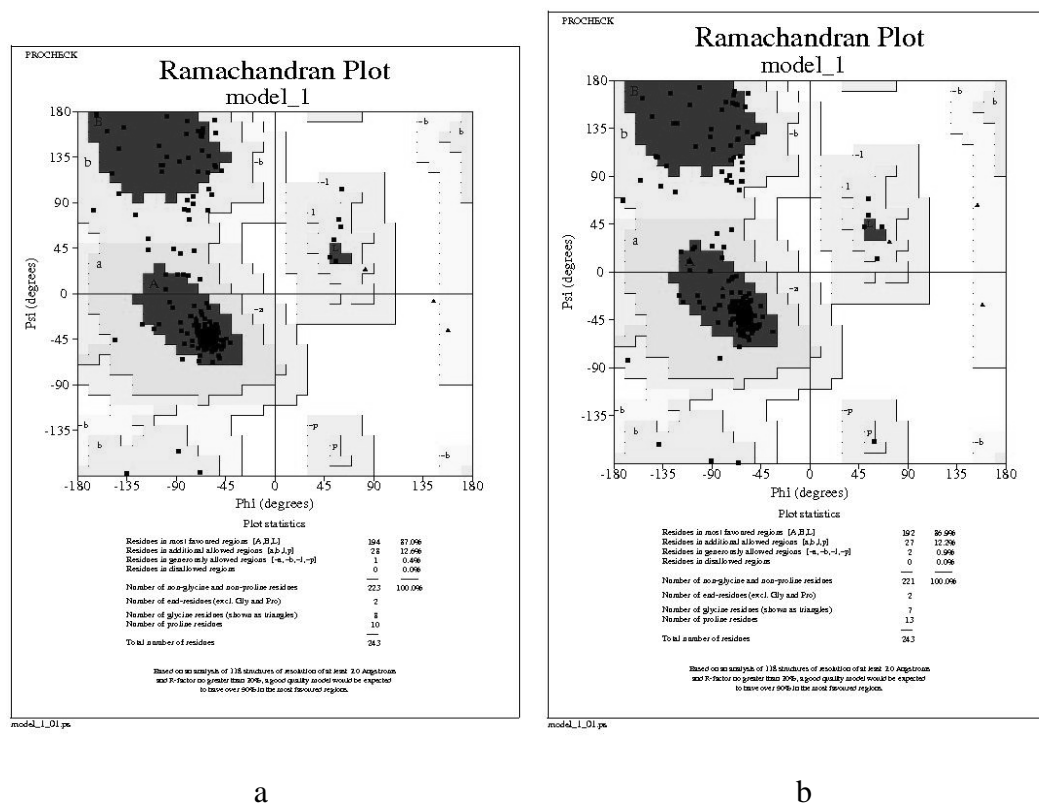

a   b

Figure 1: Ramchandran plot of the EcR of a) H. armigera and b) S. litura. (The most favored regions are coloured red, additional allowed, generously allowed and disallowed regions are indicated as yellow, light yellow and white fields, respectively)
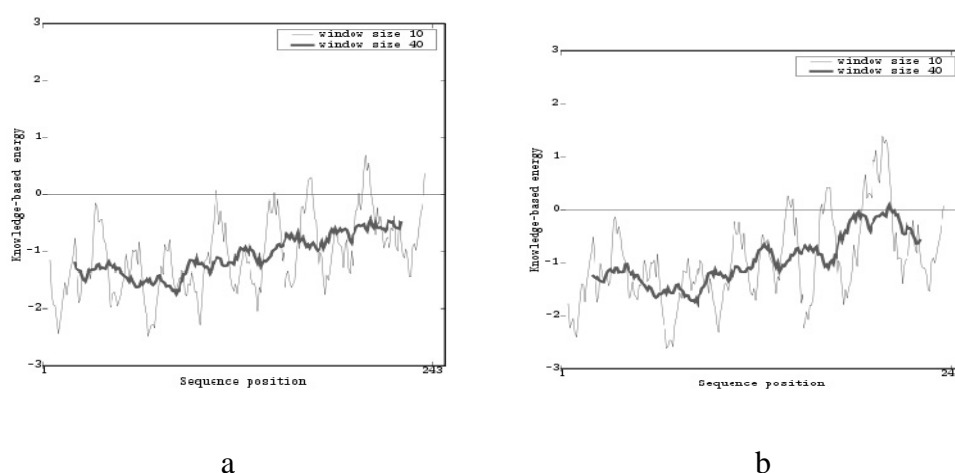
a   b

Figure 2: Energy plot of EcR of a) H. armigera and b) S. litura (solid line) with the template (Dotted lines) produced by ProSA.

Structure Alignment of ECR of H. Armigera and S. Litura

The SOPMA result shows that of EcR of H. armigera mainly comprises of 48.22% of Alpha helix, 40.24% of Random coil, 7.69% of extended strand, 3.85% of Beta turn. The EcR of S. litura comprises of 41.33% of Alpha helix, 44.56% of Random coil, 8.67% of extended strand and 5.44% of Beta turn. This indicates that both the structures consist of more number of Alpha helix and Random coils. The 3D structural comparison of the targets shows that the structures are closely related to each other with the low RMS value of 0.70Å (Fig: 3).
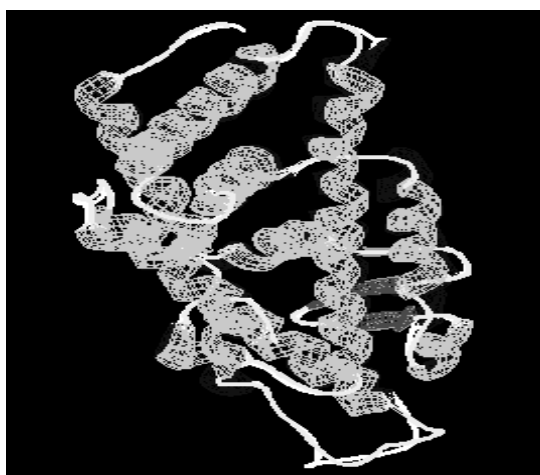

Figure 3: 3D Structure comparison of targets-EcR of H. armigera (Helix-light blue, coils-yellow, Strands- pink) with EcR of S.litura (Helix-dark blue, coils-white, Strands- orange)

Docking

The synthetic agonist BYI06830 (C23H28N2O4) has almost similar molecular formula to Chromafenozide (C24H30N2O3). BYI06830 has the molecular weight of 396.47942 [g/mol], 1 Hydrogen bond donor, 4 Hydrogen bond acceptor and 3 Rota table bonds. Thus these molecules can bind tightly with the target by the hydrogen bonds.

The patch dock server has produced 20 solutions for each docking process. Among the solutions, the best solution was picked out based on the ACE (Atomic Contact Energy) and the Geometric shape complementarily score. The best solution of EcR of H. armigera, S. litura with HWG-synthetic agonist BYI06830 has the ACE value of -377.48, -298.87 and the score of 4708, 5492 respectively.

The best intermolecular complex obtained from patch dock server was analysed using Argus lab and the results showed a group of amino acids present in the ligand (synthetic agonist) binding domain (Table-1)**.** The amino acids found in the LBD of S. litura and H. armigera are comparatively similar with overlapping residues such as "218ASN, 222CYS, 53ILE, 134, 225, 236LEU, 56, 94, 95, 127, 221MET, 50,111PHE, 54, 57, 60THR, 240TRP, 122TYR, 98,109,130VAL". Although conserved residues were present at the LBD, some of the residues vary such as 101ARG, 91SER and 215GLY in the LBD of S. litura and 112ALA, 233PRO in LBD of H. armigera (Fig 4a,b). The H-bond analysis revealed the side chain atom of the polar residue 210ASN forms a C-H...O type H-bond with the ligand in S. litura and H. armegira.
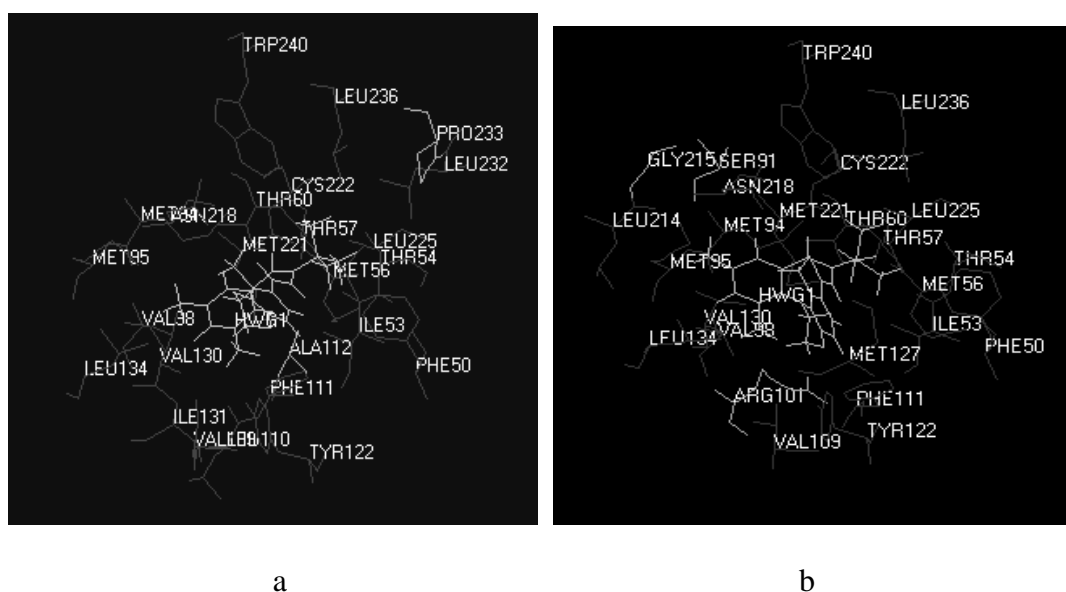

a   b

Figure 4 : LBD of EcR in a) H. armegira and b) S. litura.(Ligand denoted in Green colour, Amino acids indicated in red colour are conserved overlapping residues and the amino acids indicated in orange colour are varying amino acids).

Table 1: Comparison of amino acids present in the LBD of EcR of S. litura, H. armigera.

S. litura	H. armigera
101ARG	112ALA
218ASN	218ASN
222CYS	222CYS
215GLY	53, 131ILE
53ILE	134, 225, 232, 236LEU
134, 214, 225, 236 LEU	56, 94, 95, 127, 221MET
56, 94, 95, 127,221MET	50, 111PHE
50, 111PHE	233PRO
91SER	54, 57, 60 THR
54, 57,60THR	240 TRP
240TRP	122 TYR
122TYR	98, 109, 130 VAL
98, 109, 130VAL	

Discussion

The homology modeling done based on the structural studies across the Nuclear receptor family have shown that its members share a common modular structure (Krust, 1986; Evans, 1988; Renaud, 2000). The sequence analysis indicated a higher percentage of identity of H. armigera to H. virecence than S. litura to H. virescence. This is quite natural since members of species belonging to the same genera would be more closely related. The modeled structures have the Φ/Ψ dihedral angles in the most favored regions of the Ramachandran plot. The energy plot indicates the local model quality by plotting energies as a function of amino acid sequence position. In general, positive values correspond to problematic or erroneous parts of a model. Markus et.al. (2007) has reported that the PDB structure of 2HYD (multi-drug ABC transporter Sav1866 from Staphylococcus aureus) is found to be correct and close to the data base average with the negative value in the energy plot. Thus, we can infer that the targets structure modeled in this study are reliable to the template structures since they have negative value in the energy plot (Fig 2a,b).

The crystal structures of the LBDs of many nuclear receptors including those complexed with agonists, partial agonists or antagonists have been determined (Egner et.al., 2001). These structures have provided important information on the recognition of the ligands and the mechanism of activation of nuclear receptors, which is useful for designing ligands with the desired modulation activity (Egner et.al., 2001, Schapira et.al., 2000). Recently, crystal structures of the LBD of ultraspiracle (USP), which forms a heterodimer with the EcR, have been determined (Clayton et.al., 2001, Billas et.al., 2003).

Several amino acid substitutions were found in the residues of the binding pocket when the sequence of the HvEcR LBD (H. virescens - a lepidopteran) was compared to that of a dipteran, the fruit fly Drosophila melanogaster **(Talbot et.al.,**

1993). These included Pro353Ser, Met360Ile, Val402Met, Val413Ile, Val434Asn and Ile527Phe. Among these substitutions, the replacement of Val402 of the HvEcR with Met508 of the Drosophila **was the only one in which the size of the side chain in contact with the ligand changed significantly. Kumar** et.al. (2004) **has reported that a single amino acid change leads to discrimination between the ecdysone Non-steroidal agonists.** Recently it has been reported that EcR from H. virescens crystal structures showed that ECD (ecdysteroid) and DAH ligands occupy distinct but overlapping binding cavities and that Val-128 (Val-133 in EcR from H. virescens) is a proximal residue to both PonA and the DAH BYI06830 (Billas et.al., 2003). Further, mutation in the VAL residue to PHE decreased ECD and DAH sensitivity (Kumar et.al., 2004). **The EcR of** S. litura **and** H. armigera **(lepidopteran) are closely related to each other in structure level with overlapping amino acids in the LBD which contains the amino acids such as ILE, MET, THR, ASN and VAL. Also, VAL residues were present in both the LBD of** S. litura **and** H. armigera **which is sensitive and specific for Non-steroidal agonists. These overlapping amino acids could be responsible for the recognition of the** synthetic agonist BYI06830 in the LBD. Some of the insecticides show effect on S. litura but not on H. armigera; for example emamectin benzoate is more effective for Spodoptera litura when compared to H. armigera (Munir et.al., 2005). The similarity in the EcR and the LBD reveals that the same kind of agonist (BYI06830) can be adopted in the binding pocket of EcR for the control of H. armigera and S. litura. The comparative distinction of LBD at the structural level and the interaction with EcR may further aid to discover a novel insecticide against the pests with broad spectrum of activity.

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