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MODELLING P1-M BINDING TO ENZYMES OF THE CHITIN DEGRADATION PATHWAY IN ASIAN CORN BORER, *Ostrinia furnacalis* (Guenee)

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ABSTRACT

Discovery and development of new mode of action chemical entities is important for increasing the number of effective tools for management of insect resistance to insecticides. Inverse *in silico* molecular docking was utilized to identify possible enzyme targets of P1-M in *O. furnacalis*. Tight binding interaction of P1-M was observed in chitinase (-8.6 kcal/mol) and β-N-Acetyl-D-hexosaminidase (-8.1 kcal/mol), respectively. Hydrogen bonding and hydrophobic interactions contributed by the amphiphilic property of P1-M resulted in tight binding in the active site of the target enzymes. Molecular interaction of P1-M with *O. furnacalis* chitin synthase could not be properly evaluated because of the lack of accurate model of its 3D structure. Taken together, the structure-based *in silico* docking study revealed that P1-M is a possible strong inhibitor of enzymes responsible for chitin degradation pathway in *O. furnacalis*.

Key words: *in silico*, inverse virtual docking, mode of action, P1-M,

INTRODUCTION

In silico approach for solving biological problems is becoming increasingly popular because of improvements in computational power and availability of molecular docking software capable of accurately predicting binding interaction of chemical ligands to their true molecular targets. Recently, *in silico* approaches have entered the lead discovery process of agrochemical industry (Speck-Planche et al., 2012a, 2012b). These methods can discover new bioactive compounds with ideal properties at lower cost in much quicker time. It has been used to investigate the efficacy of insecticides (Elamathi et al. 2014) and identifying new insecticide targets (Adebiyi et al., 2015).

Bioinformatics databases containing three dimentional (3D) structures of proteins from various biological sources, including 3D structure of proteins from insects, are freely available from the internet. The 3D structures of both target protein and insecticidal compound are required for *in silico* analysis. The development of new insecticides through *in silico* method could be a cost-effective way of developing new, reduced risk and effective insecticides.

One important insect pest of corn is the Asian corn borer (ACB), *Ostrinia furnacalis* (Guenee). Several commercial chemical insecticides, including transgenic *Bacillus thuringiensis* (Bt) corn, are available to farmers for managing ACB populations.

However, insecticide resistance could potentially develop against these ACB management tools. The development of insecticide resistance is a dynamic and complex process, depending directly on genetic, physiological, behavioral and ecological factors of the arthropod pests, and depending indirectly on operational factors including categories of insecticides used as well as the application timing, rate, coverage and method (Zhu et al., 2016). In view of the threat of insect resistance development, a regular screening program for new insecticidal compounds is helpful.

The Philippines has a rich actinomycete flora which has not been fully investigated for the presence of novel insecticidal compounds. To date, only one natural insecticidal compound, P1-M derived from a local strain of actinomycete, has been reported (Alcantara, 2015). The objectives of this study are: 1) to simulate by *in silico* method, the binding interaction of P1 to chitinase and β-N-Acetyl-D-hexosaminidase, two key enzymes involved in chitin degradation pathway in ACB, and 2) to predict the possible molecular mode of action of P1-M.

MATERIALS AND METHODS

Ligand 3D Structure. The structural formula of P1-M insecticidal compound was elucidated by proton and carbon NMR as previously reported (Alcantara, 2015). The 3D structure of P1-M was generated using an online cheminformatics tool (www.molinspiration.com.Tanimoto coefficient as a measure of compound structure similarity was determined using ChemMine server (www.chemmine.ucr.edu/similarity).

Target Protein Structure. The 3D structures of selected enzymes of chitin degradation pathway in *O. furnacalis* were retrieved from the Protein Data Bank (www.rcsb.org). These enzymes were chitinase (3W4R) and β-N-Acetyl-D-hexosaminidase (3WMB). Stereochemical stabilities of the protein 3D structures were evaluated using Ramachandran plot analysis (ww.mordred.bioc.cam.ac.uk/~rapper/rampage).

Inverse In silico Docking. The software Autodock Vina (Trott and Olson, 2010) as implemented in PyRx 0.9.4 (http://pyrx.sorceforge.net) was used to generate an ensemble of docked conformations of the P1-M ligand in the target receptor macromolecules. Autodock Vina utilizes a Lamarckian Genetic Algorithm (LGA) for conformational sampling (Morris et al., 1998). Inverse virtual screening was conducted with a rigid receptor and flexible ligand conformation. Ligand binding site in each target macromolecule and receptor grid parameters (i.e., search space in the receptor) were determined by using the SiteHound server (Hernandez et al., 2009). The P1-M ligand was energy minimized using default energy minimization parameters in PyRx. All heteroatoms from protein 3D structures were deleted, followed by structure refinement and energy minimization with $3D^{refine}$ server (Bhattacharya et al., 2016). All docking computations were performed with a MacIntosh computer with quadcore Intel Core i5 CPU running at 2.7 Ghz.

Visualization. The 3D visualizations of docked conformations of P1-M bound to target macromolecules were performed using the program, Chimera Ver. 1.80 (http://www.rbvi.ucsf.edu/chimra/). 2D plots of P1-M-macromolecule interactions were prepared using LigPlot V1.4.5 (Wallace et al., 1996).

RESULTS AND DISCUSSION

A modern concept for the development of new insecticides is the target-based approach. This method consists of five major steps: target protein identification and subsequent validation, identification and optimization of chemical lead structures, and lastly, *in vitro* testing of the optimized lead structures. The first step, target identification, very often involves bioinformatics searches for proteins that can serve as molecular points of attack for insecticidal compounds (Krasky et al., 2007). To support the lead identification and lead optimization process (steps 3 and 4 of the target-based approach), cheminformatics methods like homology modeling and ligand docking can be used (Gasteiger, 2007). A combined bioinformatics and cheminformatics approach was used in this study to investigate the possible mode of action of P1-M insecticide.

Accuracy of docking protocol in predicting bound conformations of the ligand to a target macromolecule using PyRx Autodock Vina was demonstrated by re-docking of the native ligand (i.e., TMX) to the active site in *O. furnacalis* β-N-Acetyl-D-hexosaminidase (Figure 1). Docking poses that reproduced experimental geometry at < 3Å RMSD are considered good (Bordogna et al., 2011). Results of inverse docking showed that P1-M preferentially binds (-8.60 kcal/mol) to chitinase (Fig. 2A). Ramachandran plot (Fig. 3A) analysis showed that residues in the active site of the enzyme were not outlier residues. About 98% of all residues were in the favourable conformation within the 3D structure of chitinase (Table 1). P1-M formed one hydrogen bonding (Trp372) and fourteen hydrophobic (Trp34, Trp107, Glu148, Tyr149, Ala192, Phe194, Arg195, Asp218, Leu219, Trp223, Tyr243, Leu246, Arg274, and Phe309) interactions with amino acid residues in the active site of ACB chitinase (Fig. 4A). The amino acid residue, Phe194 in the active site of ACB chitinase, was previously reported to be crucial for chitin hydrolysis (Chen et al., 2014). The highly hydrophobic nature of the active site might have contributed to the high binding affinity (i.e, -8.6 kcal/mol) of P1-M to ACB chitinase. Chitinase was reported to be essential to moulting in ACB (Chen et al., 2014). Chitinase has been previously proposed as an attractive target for the development of insecticides (Asano, 2003).

On the other hand, β-N-Acetyl-D-hexosaminidase, considered a novel enzyme to *O. furnacalis*, was revealed to be essential for normal pupation process in *O. furnacalis* (Liu et al., 2011). Ramachandran plot (Fig. 3B) analysis showed that residues in the active site of the enzyme were not outlier residues. About 98% of all residues were in the favourable conformation within the 3D structure of the enzyme (Table 1). The amino acid residues of β-N-Acetyl-D-hexosaminidase interacting with P1-M are shown in Figure 3B. Two amino acid residues (Val327 and Trp483) formed hydrogen bonds with P1-M. Nine amino acid residues (Trp 322, Glu 328, Asp 367, Trp 448, Tyr 475, Val 484, Trp490, Trp 524, and Glu 526) were in hydrophobic contact with P1-M. These two types of molecular contacts might explain the tight binding (-8.10 kcal/mol) of P1-M to the active site in ACB β-N-Acetyl-D-hexosaminidase.

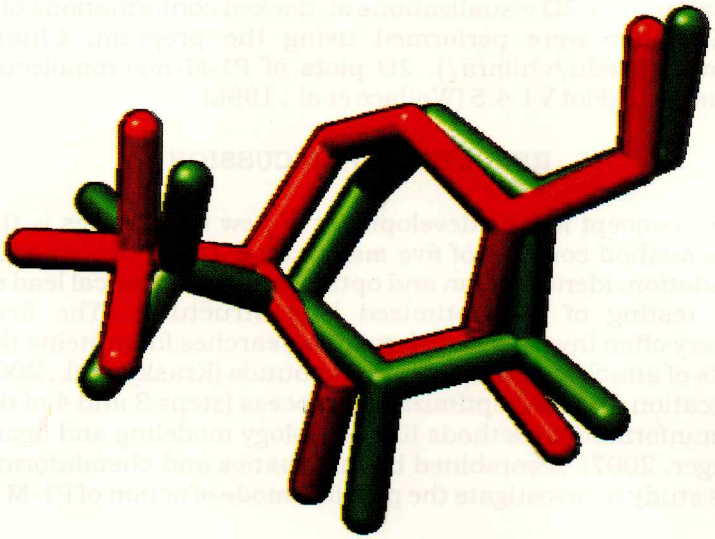

Figure 1. Binding pose of TMX in *Ostrinia furnacalis* β-N-acetyl-D-hexosaminidase (Protein Data Bank ID: 3NSN). Green sticks represent bound conformation of TMX in crystal structure of β-N-acetyl-D-hexosaminidase. Red sticks represent re-docked conformation of TMX in crystal structure of β-N-acetyl-D-hexosaminidase. Re-docking was performed with Autodock Vina as implemented in PyRx 0.9.4. Root mean square deviation = 2.92 Å.

Table 1. Results of *in silico* protein structure modeling and molecular docking of protein targets in *Ostrinia furnacalis*.

Target	Source of 3D structure	% residues in favoured region [a]	% residues in allowed region[b]	% residues in outlier region[c]	Binding affinity to P1-M [d] (kcal/mol)
Chitinase	PDB 3W4R	98.20	1.30	0.50	-8.60
β-N-acetyl-D-hexosaminidase	PDB 3NSN	97.90	1.90	0.20	-8.10

a, b, c values were determined from Ramachandran plots shown in Figure 3.
d, Values were determined by inverse molecular docking using Autodock Vina.

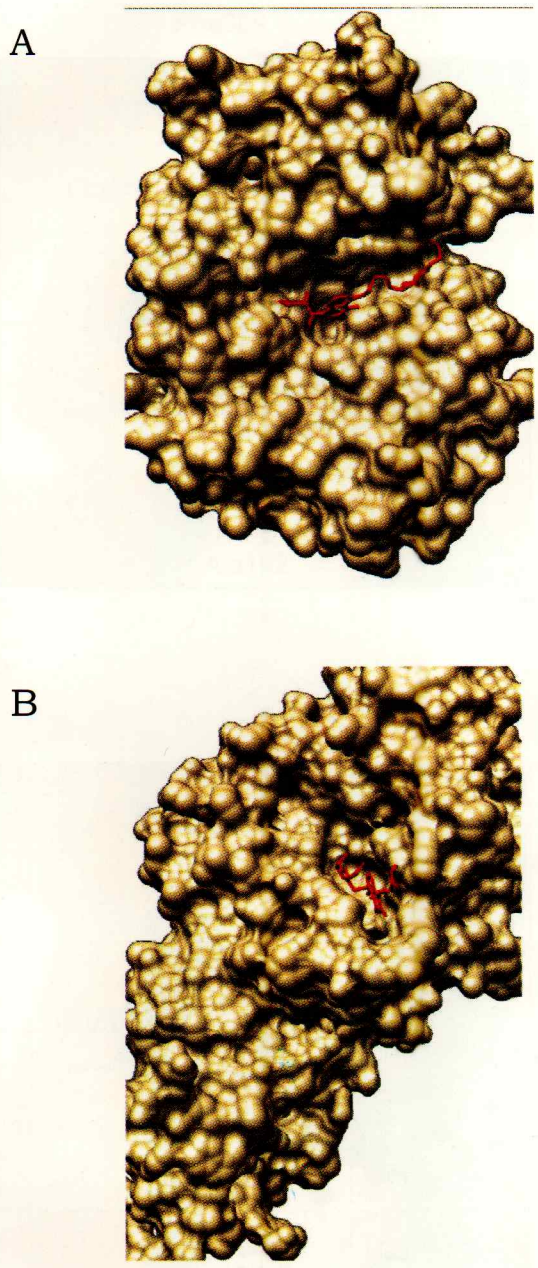

Figure 2. Bound conformation of P1-M (red stick) in *Ostrinia furnacalis* **(A.)** chitinase, and **(B.)** β-N-acetyl-D hexosaminidase. Binding poses of P1-M were estimated with inverse molecular docking with Autodock Vina in PyRx 0.9.4.

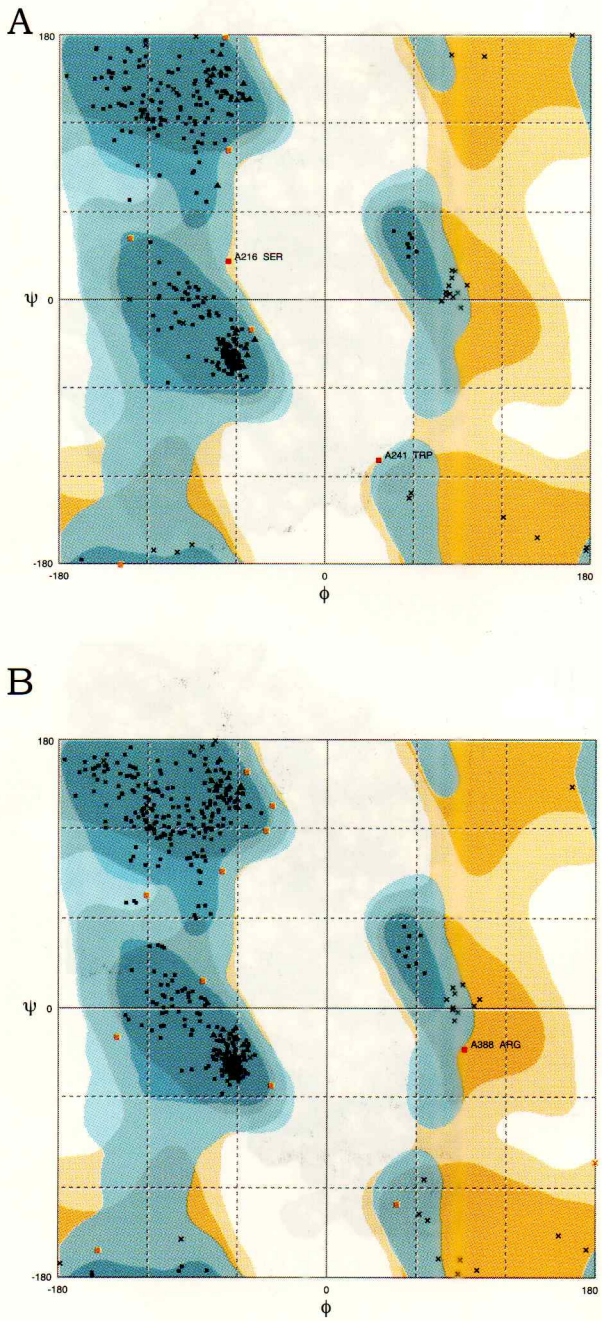

Figure 3. Ramachandran plot of *Ostrinia furnacalis* **(A.)** chitinase, and **(B.)** β-N-acetyl-D hexosaminidase. Plots were created by uploading each protein 3D structure into RAMPAGE server.

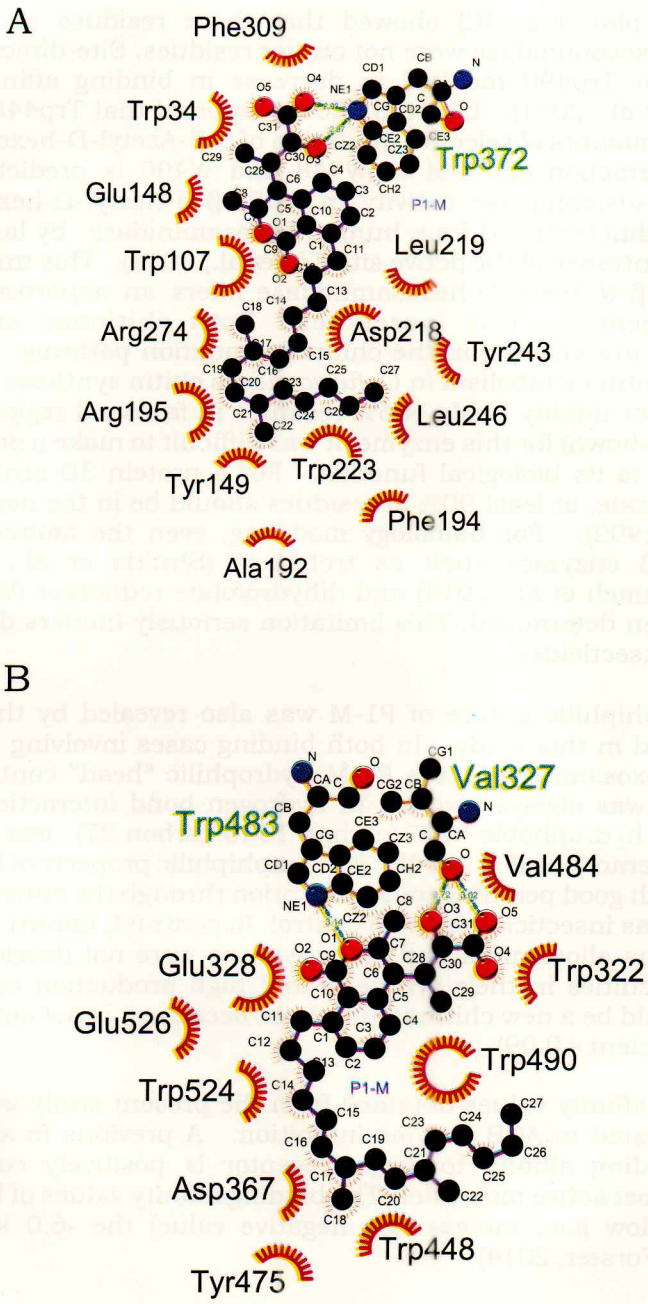

Figure 4. Intermolecular interaction in the protein active site between P1-M and **(A.)** chitinase, **(B.)** β-N-acetyl-D-hexosaminidase. Hydrogen bond interactions are depicted as green dashlines. Hydrophobic contacts are represented by an arc with spokes radiating toward the ligand atoms they contact. Figures were generated with LigPlot.

Ramachandran plot (Fig. 3C) showed that these residues in the active site of β-N-Acetyl-D-hexosaminidase were not outlier residues. Site-directed mutagenesis of either Trp448 or Trp490 resulted in decrease in binding affinity to the enzyme substrate (Liu et al., 2011). Liu et al. (2011) reported that Trp448 and Trp490 were important determinants of selective inhibition of β-N-Acetyl-D-hexosaminidase. Thus, the binding interaction of P1-M to W448 and W490 is predicted also to inhibit β-N-Acetyl-D-hexosaminidase activity in ACB. β-N-Acetyl-D-hexosaminidase from *O. furnacalis* is differentiated from human hexosaminidase by large conformational changes at the entrance of the active site (Liu et al., 2011). This unique characteristic of *O. furnacalis* β-N-Acetyl-D-hexosaminidase offers an opportunity to develop an insecticide specific to this insect pest. Both chitinase and β-N-Acetyl-D-hexosaminidase are involved in the chitin degradation pathway. Another important enzyme of the chitin metabolism in *O. furnacalis* is chitin synthase. However, because of the overall poor quality (only ~86% residues in favoured region) of the homology model (data not shown) for this enzyme, it was difficult to make a predicted correlation of tight binding to its biological function. For a protein 3D structure model to be considered accurate, at least 90% of residues should be in the most favorable region (Morris et al., 1992). For homology modeling, even the amino acid sequence of interesting ACB enzymes such as trehalase (Shukla et al., 2015), β-carbonic anhydrase (Emameh et al., 2014) and dihydrofolate reductase (Walker et al., 2000) have not yet been determined. This limitation seriously hinders development of new mode of action insecticides.

The amphiphilic nature of P1-M was also revealed by the *in silico* docking results described in this study. In both binding cases involving ACB chitinase and β-N-Acetyl-D-hexosaminidase, the P1-M hydrophilic "head" containing oxygen and nitrogen atoms was always involved in hydrogen bond interactions. On the other hand, the P1-M hydrophobic "tail" (carbon 13 to carbon 27) was always involved in hydrophobic interactions (Fig 4A, 4B). The amphiphilic property of P1-M might provide the molecule with good percutaneous absorption through the cuticle which might lead to practical use as insecticide for ACB control. In contrast, known potent inhibitors of chitinase such as allosamidin and its derivatives were not developed as insecticide because of difficulties in their synthesis and high production costs (Saguez et al., 2008). P1-M could be a new chitinase inhibitor because it is not similar to allosamidin (Tanimoto coefficient = 0.09).

Binding affinity values obtained from the present study were predicted to be positively correlated to ACB enzyme inhibition. A previous *in silico* docking study found that binding affinity to target receptor is positively correlated to strong bioactivity by most active molecules. The binding affinity values of bioactive molecules were largely below (i.e., increasingly negative value) the -6.0 kcal/mol threshold (Shityakov and Forster, 2014).

SUMMARY AND CONCLUSION

The mode of action of P1-M, a natural insecticide isolated from *Streptomyces* sp. strain PCS3-D2, was investigated by *in silico* method. *Ostrinia furnacalis* enzymes (chitinase and β-N-acetyl-D-hexosaminidase) involved in chitin metabolism were selected as possible targets of P1-M. The X-ray-determined 3D structures of chitinase and β-N-acetyl-d-hexosaminidase were retrieved from the Protein Data Bank. Binding of P1-M to both enzymes was modeled using *in silico* molecular docking approach. Binding affinities of P1-M to chitinase and β-N-acetyl-D-hexosaminidase were -8.60 kcal/mol and -8.10 kcal/mol, respectively. P1-M is predicted to attack the chitin degradation pathway by inhibiting the activity of chitinase and β-N-acetyl-D-hexosaminidase in *O. furnacalis*.

In silico molecular docking is a powerful tool in predicting insecticidal activity of compounds to molecular targets in ACB. Future efforts in determining the 3D structure of other key enzymes in different metabolic pathways will increase the chance of success of rational insecticide design intended to provide new tools to manage *O. furnacalis* resistance to insecticides.

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