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Species marker for developing novel and safe pesticides

Yuan-Ping Pang*

Computer-Aided Molecular Design Laboratory, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905, USA

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Abstract—Current anticholinesterase pesticides developed during World War II are toxic to mammals because they target a catalytic serine residue of acetylcholinesterases (AChEs) in insects and in mammals. A sequence analysis of AChEs from 68 species and three-dimensional models of the greenbug and English grain aphid AChEs reported herein reveal that a cysteine residue is present at the active sites of greenbug and aphid AChEs but absent at those of mammalian AChEs. This discovery enables the design of novel and safe pesticides that target the cysteine residue rather than the ubiquitous serine residue.

The greenbug (*Schizaphis graminum*) and English grain aphid (*Sitobion avenae*) are devastating grain pests whose feeding activities damage wheat, sorghum, and barley. According to U.S. Department of Agriculture's 2005 annual report, estimated economic losses caused by these pests totaled $100 million in six U.S. states. Greenbugs are also found in North, Central, and South America, Europe, Africa, the Middle East, and Asia, and aphids have been present since 1912 in southern Europe, central Asia, the Middle East, and Africa.

Acetylcholinesterase (AChE), a serine hydrolase that is vital for regulating the neurotransmitter acetylcholine in mammals and in insects, has long been used as a target for pesticides. This enzyme has a deep and narrow active site, the bottom and opening regions of which are known as catalytic and peripheral sites, respectively.[1,2] Current anticholinesterase pesticides (organophosphates and carbamates developed during the World War II era) for controlling insects react with a serine residue at the catalytic site, thus disabling the function of AChE. Because the serine residue is also present in AChEs of other species, the use of these pesticides has been limited by their toxicity to mammals. Although it has long been assumed that humans are not harmed by low applications of the anticholinesterases as insects are more sensitive to the chemicals than humans, a recent report by the U.S. Environmental Protection Agency's Office of Inspector General indicates that some anticholinesterases can enter the brain of fetuses and young children and may destroy cells in the developing nervous system.[3]

One safe way to control these pests without harm to mammals is to develop new pesticides that target a conserved and pest-specific region of AChE. This type of pesticides would be less toxic to mammals, because, unlike current pesticides, they would not react with the catalytic serine residue of mammalian AChEs. Identification of this AChE region relies on three-dimensional (3D) models of grain pest AChEs. However, the 3D models of grain pest AChEs have been unavailable until now.

I have computationally determined the 3D models of *Schizaphis graminum* and *Sitobion avenae* AChEs using homology modeling and refinement with multiple molecular dynamics simulations (MMDSs).[4] The protein sequences of these AChEs were obtained from GenBank (AF321574 for *Schizaphis graminum*[5] and AY819704 for *Sitobion avenae*[6]). The homology-MMDS protocol was validated through successful identification of small-molecule inhibitors of a homology-MMDS-derived 3D model of a protease.[7] Recently, this MMDS refinement protocol has proven successful in refining a homology model nearly identical to a crystal protein structure [Protein Data Bank (PDB) ID: 1XE1] that was provided by the Protein Structure Prediction Centre for critical assessment of techniques for protein structure prediction and refinement. Relative to the 1XE1 crystal structure, the alpha carbon root mean square

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* Tel.: +1 507 284 7868; fax: +1 507 284 9111; e-mail: pang@mayo.edu

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deviation (CRMSD) of the MMDS-refined model was 1.7 Å, whereas the CRMSD of the initial homology model was 4.6 Å (see Supplementary Fig. S1 online).

A homology model of *Schizaphis graminum* AChE (*Sg*AChE) was first generated with the SWISS-MODEL program[8] according to the sequence alignment using five AChE crystal structures as templates (PDB IDs: 1QTI, 1AMN, 1VOT, 2ACK, and 1EEA).[9,10] These crystal structures all have 41% sequence identity to *Sg*AChE. There were five regions of insertion and two regions of deletion in the *Sg*AChE sequence that aligned with those of the crystal structures (see Supplementary Fig. S2 online). The substrate-bound *Sg*AChE model was then built by manually docking acetylcholine into the active site of the homology model. The docking was guided by the substrate-bound *Torpedo* AChE (PDB ID: 2ACE).[1] The resulting complex was refined using 295 different molecular dynamics simulations (2.0 ns for each simulation with a 1.0-fs time step and with a different seed for initial velocity) performed on 590 Apple G5 processors. An average of 147,500 trajectories of the complex obtained at 1.0-ps intervals during the last 0.5-ns period of these simulations was subjected to a second-round refinement with a new set of 295 different molecular dynamics simulations (0.5 ns for each simulation with a 1.0-fs time step and with a different seed for initial velocity). A new average of 147,500 trajectories of the complex obtained at 1.0-ps intervals during the second set of the simulations was used as a refined 3D model of *Sg*AChE. The refined model differed from the homology model and the X-ray structure of human AChE (hAChE) primarily in three adjacent loops of residues 73–82 (loop 1), 283–293 (loop 2), and 335–352 (loop 3) that compose part of the peripheral site of AChE (Fig. 1).

The 3D model of *Sitobion avenae* AChE (*Sa*AChE) was then obtained from the refined 3D model of *Sg*AChE by mutating 17 residues of *Sg*AChE to the corresponding residues of *Sa*AChE as the two enzymes share 96% sequence identity. The resulting *Sa*AChE model was refined with the same approach used for the *Sg*AChE model. The two refined models are nearly identical; the CRMSD between the two is 0.87 Å. Both were deposited to the PDB on June 17, 2006 (PDB IDs for *Sg*AChE and *Sa*AChE are 2HCP and 2HCQ, respectively).

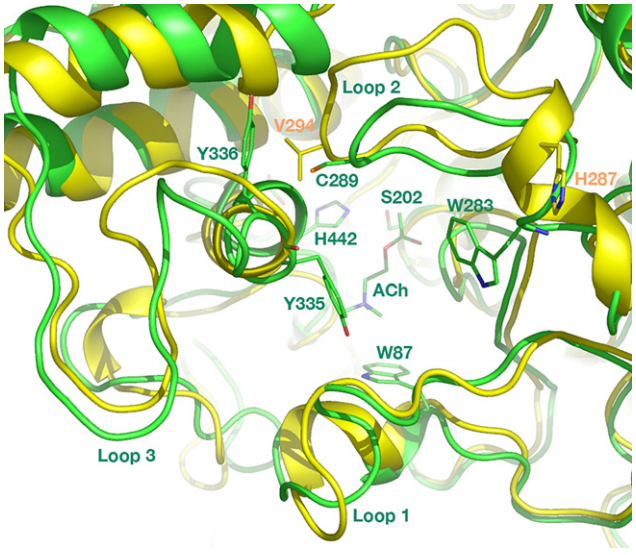

Figure 1. Overlay of *Schizaphis graminum* (green) and human (yellow) acetylcholinesterases from a perspective looking down onto substrate acetylcholine at the catalytic site.

At the peripheral site of the refined models of *Sg*AChE and *Sa*AChE is a cysteine residue, C289, that has favorable sulfur-aromatic interaction[11] with Y336 and is accessible for covalent bonding to small molecules that bind at the active site (Figs. 1 and 2). In hAChE the residue corresponding to C289 of *Sg*AChE is V294; there is no cysteine at the entire peripheral site of hAChE (Fig. 1). A sequence analysis of AChEs from 68 species using CLUSTALW[12] (see Supplementary Fig. S3 online) showed that C289 is present only in AChEs of the greenbug, English grain aphid, oat and wheat aphids, melon and cotton aphids, green peach aphid, German cockroach, lancelet, rice leaf beetle, domestic silkworm, honey bee, African bollworm, beet armyworm, codling moth, and diamondback moth.

It has been reported that a native or engineered cysteine residue near the active site of an enzyme can hook a small molecule that fits, even loosely, the active site as long as the thiol group of the cysteine residue is reacted with a functional group of the molecule[13] It has also been reported that reactive chemicals—which are covalently bonded to an engineered cysteine (H287C in mammalian AChEs) which is also located at the peripheral site of mammalian AChEs (Fig. 1)—are able to interfere with the substrate binding and subsequently inhibit the catalysis of the enzymes,[14,15] and that a relatively less reactive chemical is able to bond covalently to a native cysteine residue at the active site of a cysteine protease after the chemical binds favorably in the proximity of the cysteine residue.[16] Based on these reports and on the proximity of C289 to its active site revealed by the 3D models of *Sg*AChE and *Sa*AChE, it is conceivable that a chemically stable molecule can react with C289 and irreversibly inhibit the two AChEs upon binding to the active site (Fig. 2).

Because of the species specificity of C289 demonstrated by the sequence analysis, C289 can be used as a species marker for developing a new generation of safe pesticides that can covalently bond to C289 in greenbug and aphid AChEs. The absence of a cysteine residue in the peripheral site of mammalian AChEs means that the C289-targeted irreversible inhibitors would have less toxicity to mammals than current pesticides that target the catalytic serine reside present in both mammals and insects. The 3D models of *Sg*AChE and *Sa*AChE are now available at the PDB, and they are useful to the pursuit of structure-based designs for conceptually new and superior pesticides and for ending the use of current toxic anticholinesterase pesticides.

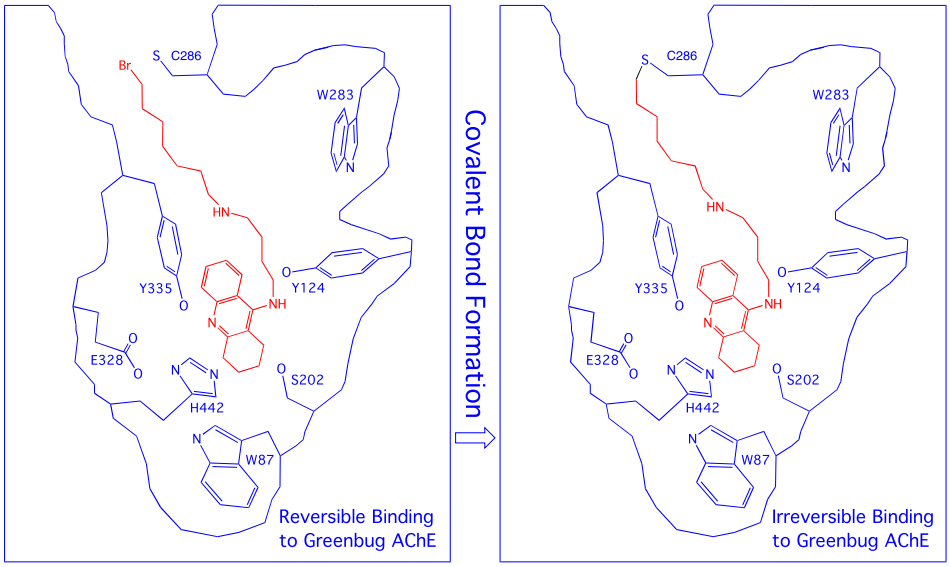

Figure 2. Cartoon representation of a *Schizaphis graminum* acetylcholinesterase that is covalently bonded to an inhibitor upon binding to the active site.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.09.073.

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