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Resistance pattern and point mutations of insensitive acetylcholinesterase in a carbamate-resistant strain of housefly (*Musca domestica*)

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Abstract

To examine the resistance pattern and point mutations present in the carbamate-resistant strain of housefly (SH-CBR), *Musca domestica*, from Shanghai, China, the fly's resistance spectrum and k_i ratios to organophosphate (OP) and carbamate (CB) insecticides were determined. The cDNA encoding acetylcholinesterase (AChE) from both a susceptible control strain (SH-S) and the SH-CBR strain of housefly were cloned and sequenced using RT-PCR. Based on two major patterns of target site resistance suggested by Russell et al. [R. J. Russell, C. Claudianos, P. M. Campbell, I. Horne, T. D. Sutherland, J. G. Oakeshott, Two major classes of target site insensitivity mutations confer resistance to organophosphate and carbamate, Pestic. Biochem. Physiol. 79 (2004), 84–93.], the present results indicate a Pattern I resistance: resistance to CBs was greater than that to OPs; the SH-S/SH-CBR k_i ratios were also greater for CBs than for OPs. The cDNA was 2079 nucleotides long, encoding a protein of 693 amino acids. The cDNA deduced amino acid sequence of the housefly had a high degree of identity with previously described insect AChEs and exhibited the same structural feature as vertebrate *Tc*AChE. Three mutations in the region of the active site, V261L, G343A, and F408Y, which were identical to those identified in the 77M and YBOL housefly strains, were identified in the AChE from the SH-CBR strain. Additionally, a novel mutation, D422L, was found at the outer surface of the protein where the residue presumably could not interact directly with amino acid residues lining the active site gorge. Homologous modelling of housefly AChE, based on the high resolution crystal structure of fruit fly (*Drosophila melanogaster*) AChE, indicated that D422 may be involved in a salt bridge with H341. The distance between D422 and H341 is predicted to be 3.8 Å, and modification of the charge on the side chain of D422 (D to V) would likely affect the profile of the acyl pocket via the Y339 component. Such a structural change in the acyl pocket in the mutant may underlie the decreased affinity of AChE for CBs.

Keywords: Insensitive acetylcholinesterase; Insecticide resistance; Point mutations; *Musca domestica*

1. Introduction

In insects, acetylcholinesterase (AChE, EC 3.1.1.7) encoded by the *ace* gene, catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh) at cholinergic synapses of the central nervous system. It is the target of the largest group of insecticides, organophosphate (OP), and carbamate (CB) compounds, which phosphorylate or carbamylate the active site serine to block the hydrolysis of ACh. AChE blockade is ultimately fatal for affected insects. Intensive use of these insecticides over the past 50 years has led to the development of resistance in many target species. This resistance originates mainly from AChE modification that renders the enzyme less sensitive to the inhibitors.

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These altered forms of AChE have widely differing spectra insensitivity between species, as well as a marked range of insensitivity to different compounds within a species.

The insensitivity displayed by various-resistant species is very complex. First, distinct genes encode the AChE target: only one *ace* gene (now called *ace*2) exists in *Drosophila melanogaster* [1,2], *Musca domestica* [3,4], *Lucilia cuprina* [5], and *Bactrocera oleae* [6]. But in many other insects and ticks there are at least two genes, termed *ace1* and *ace2*, encoding AChE (e.g. *Anopheles gambiae* [7] *Culex pipiens* [8–10], *Culex tritaeniorhynchus* [11], *Anopheles albimanus* [12] *Boophilus microplus* [13], *Aphis gossypii* [14], *Myzus persicae* [15], and *Schizaphis graminum* [16]). Second, though multiple AChE genes were identified in the above species, only one of them has been demonstrated to be related to resistance. Although *ace2* has a high similarity with the *D. melanogaster* gene [17,9,10], it is *ace*1 in which mutations that correlate with insensitivity to OPs and CBs in *C. pipiens* and *M. persicae* have been found [18,10,11]. Attempts to find mutations in the *ace*2 gene have failed in most insects, however such mutations have been reported in *D. melanogaster*, *M. domestica*, *B. oleae*, and *L. cuprina*. In these four species, the *ace2* gene is the only AChE gene present [1–6] and mutations have been shown to confer resistance. Third, different mutations, either single or in combination, may confer varying spectra of resistance [3]. Based on the similarities and differences in resistance profiles of these cases as assessed using bioassay and biochemical criteria, two major classes of *ace* mutations that confer resistance have been identified [19]. Fourth, though the presence of insensitive AChE has been demonstrated in some species, such as in *B. microplus*, *Nephotettix cincticeps* [20], *Helicoverpa armigera* [21], *Bemisia tabaci*, and *Trialeurodes vaporariorum* [22], no corresponding mutations(s) could be found.

Given the complications described above, the aim of the present study was to classify the resistance pattern and point mutations of the carbamate-resistant (SH-CBR) strain of the housefly to identify the precise genetic adaptation that plays a role in insecticide resistance. We determined the SH-CBR housefly's resistance spectrum and k_i ratios to OPs and CBs, and cloned and sequenced the genes encoding AChE from the sensitive (SH-S) and the SH-CBR strains of housefly using RT-PCR. Here, we observed a Pattern I resistance as described by Russell et al. [19]. These findings suggest that, of the six mutations identified in AChE of the SH-CBR strain, four may be associated with resistance.

2. Materials and methods

2.1. Housefly strains

The SH-CBR housefly strain from a suburb of Shanghai has been described [23]. This strain had been selected with propoxur before 2003, and then has been altered to treat with methomyl every five generations at LC_{70}–LC_{80} since that year. Methomyl has been used because it was more effective than propoxur. The SH-S housefly was collected from Jiangshu Province in 1976. Since then, the SH-S flies have been raised in the laboratory and have never been exposed to any insecticides.

2.2. Insecticide Bioassay

All insecticides were obtained from Chem Service (West Chester, PA). Insecticide resistance bioassays were performed with topical application of the compounds as previously described by Shi et al. [23].

2.3. AChE Preparation and Determination of Bimolecular Rate Constants (k_i)

AChE preparation and determination of bimolecular rate constants (k_i) were carried out as previously described by Shi et al. [23]. All tests were carried out in triplicate in the presence or absence of the AChE inhibitor, all inhibitors tested were also from Chem Service (West Chester, PA), bimolecular rate constants (k_i) were analyzed and fitted using DynaFit 3.17 software (BioKin, WI).

2.4. RT-PCR and molecular cloning of AChE gene

Total RNA was extracted from adult housefly heads of the SH-CBR and the SH-S strains by Trizol® reagent (Life Technologies) following the manufacturer's instructions. First strand cDNA was synthesized from the total RNA (25 μg) with reverse transcriptase (Takara) with 200 ng oligo (dT) as a primer. The AChE coding region was amplified by PCR based on nucleotide sequence (deposited in the GenBank Accession No. as AF 281161) using two gene specific primers: AAGGATCCATGGCTCGGTCTGTAAG (sense) and CGGAATTC TTATTGGAAAATGCTATTG (anti-sense). PCR was carried out in 35 cycles of 94 °C for 30 s, 52 °C for 60 s, and 72 °C for 90 s. The amplified fragments of the predicted size were purified by low melting point agarose gel (Life Technologies) and subcloned into the pBluescript II SK (+) plasmid cloning vector (Strategene) for DNA sequencing analysis. The selected positive clones were sequenced with universal M13 and specific primers following the dideoxynucleotide chain terminating method [24].

2.5. Analysis of housefly AChE structure

Sequences were analyzed with the aid of BioEdit software (Version 5.0). DNA and deduced amino acids sequences were compared with the updated data in GenBank/EMBL. The structure of *Dm*AChE was obtained from the Protein Data Base (http://www.rcsb.org/pdb; ID ref 1Q09) and homology modeling of the housefly amino acid sequence was conducted with Rasmol and Swiss PDB viewer software based on the crystal structure of *Dm*AChE.