ACETYLCHOLINESTERASE ACTIVITIES IN LABORATORY-REARED AND FIELD-COLLECTED STRAINS OF TOBACCO BUDWORM, *HELIOTHIS VIRESCENS* (F.)

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Abstract

Adult-head acetylcholinesterase (AChE) activity from a methyl parathion-resistant strain (**OPR**) of tobacco budworm, *Heliothis virescens* (F.), was 27-fold less sensitive to inhibition by methyl paraoxon when compared to AChE activity from a methyl parathion-susceptible strain (**OPS**). Acetylcholine-sterase activities from adult heads of resistant *H. virescens* were less sensitive to the N-methyl carbamate propoxur and inhibited by monocrotophos. Acetylcholinesterase activities from a laboratory-maintained, multi-resistant strain (**PYR**), a standard laboratory-maintained susceptible strain (**HRV**), and two Georgia field strains (collected from **Bainbridge** and **Tifton**) were also assessed. The multi-resistant strain was 27-fold less sensitive to inhibition by methyl paraoxon; AChE activity from the lab-susceptible, HRV strain was comparable to OPS AChE activity; and the field strains were ca. 2-fold less sensitive to inhibition by methyl paraoxon. Responses of AChE to inhibition by monocrotophos ($7x10^{-5}$ M) and propoxur ($7x10^{-4}$ M) indicated that the OPS and OPR strains were homozygous for the S and R enzyme, respectively. A scatterplot of AChE activities from the PYR, HRV, and the two field strains compared to the OPS and OPR genotypes indicated that the Bainbridge strain was comparable to the OPS strain and possibly homozygous for the S enzyme; whereas, the PYR, HRV, and Tifton strains were not completely homozygous.

Introduction

Resistance to insecticides has been documented in more than 430 species of arthropods (1), and is often associated with increased enzymatic detoxication of an insecticide or reduced sensitivity of a target protein to inhibition or binding by the insecticide (2,3). Decreased sensitivity of acetylcholinesterase (AChE) to inhibition by organophosphate and carbamate insecticides has been implicated as a mechanism of resistance to those chemicals in a variety of insect species (4).

Withincreasing demands for the use of insecticides and the implementation of effective resistance management strategies, biochemical and genetic methods have been developed to detect and to monitor the resistance in insect field populations (5). One such method that has been adopted is the use of a microtiter plate assay to estimate the activity of AChE in response to inhibition by various organophosphate and carbamate insecticides in tissue preparations from single insects (6,7).

Herein, we report the results from a survey of AChE activities of adult heads from several laboratory-maintained and field-collected strains of tobacco budworm using a microtiter plate assay.

Materials and Methods

Strains:

OPR--a methyl parathion-resistant strain obtained from Dr. T.M. Brown (Clemson University)

OPS--an organophosphate-susceptible strain obtained from Dr. T.M. Bown (Clemson University)

PYR--a multi-resistant strain; a pyrethroid-resistant strain that has been crossed and selected into a pyrethroid-susceptible, organophosphate-resistant genetic background (8,9). This strain was obtained from Dr. T.M. Brown (Clemson University).

HRV--a laboratory-maintained insecticide susceptible strain.

Bainbridge--a lab strain established from a field sample collected from a cotton field near Bainbridge, GA in August 1995.

Tifton--a lab strain established from a field sample collected from a cotton field near Tifton, GA in August 1995.

Topical median lethal doses for methyl parathion against the OPR and PYR strains were estimated to be >600 ug/g larva. Topical median lethal doses for the OPS and HRV strains were estimated to be <15 ug/g larva. Susceptibilities of the Bainbridge and Tifton strains were not assessed.

Inhibitors:

The organophosphates (methyl paraoxon, methyl parathion, and mono-crotophos) and N-methyl carbamate (propoxur) used in this study were supplied by Dr. T.M. Brown (Clemson University).

Inhibition of Adult-Head AChE (10, 11):

o Individual heads were homogenized in 0.5 ml MOPS buffer (0.1M, pH 7.5) using 5 strokes of a hand-held ground glass tissue homogenizer (Kontes, Model 20).

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o Each homogenate was transferred to a 1.5 ml microcentrifuge tube and centrifuged for 2.0 min in Beckman benchtop microcentrifuge.

o Supernatants were transferred to clean 1.5 ml microcentrifuge tubes and used as the enzyme source.

o Reaction mixtures (with and without inhibitor) were prepared as follows:
-the inhibitor (in 100 ul of acetone) or 100 ul of acetone alone was mixed into 15 ml of MOPS buffer (0.1 M, pH 7.5) containing 0.6 mM acetylthiocholine iodide and 2.4 mM 5,5'-dithiobis-2-nitrobenzoic acid

o 20 ul of enzyme were added to a well of a 96-well microtiter plate

o 100 ul of the appropriate reaction mixture was added to the microplate well to initiate the reaction

o AChE Activity (mOD/min) was determined using a microtiter plate reader by measuring absorbance (405 nm) at 5, 10, 15, 30, and 60 min time periods

o Rate values for inhibited enzyme were determined from tangents drawn to inhibition curves (12) and divided by appropriate uninhibited control rates, the ln percentage activity remaining was plotted against time and the slope of the best-fit regression line was divided by the inhibitor concentration to obtain the bimolecular reaction constant

Results and Discussion

Bimolecular rate constants of AChE from each of the laboratory-maintained and field-collected strains tobacco budworm were determined (Table 1). The AChE activities of the HRV and OPS strains were most rapidly inhibited by methyl paraoxon; whereas the AChE activities of the PYR and OPR strains were least inhibited by methyl paraoxon. The inhibition of the AChE activities of the field strains, Bainbridge and Tifton, were intermediate. Both the PYR and OPR AChE activities were ca. 27-fold less sensitive to inhibition by methyl paraoxon when compared to AChE activity of the OPS strain. The field strain AChE activities were ca. 2-fold less sensitive to inhibition by methyl paraoxon. These data confirm previous work (11) and suggest that decreased sensitivity of AChE is a factor contributing to organophosphate resistance in the PYR and OPR strains.

Acetylcholinesterase activities were assessed from each of the strains fol-lowing inhibition by propoxur (an N-methyl carbamate) and monocrotophos (an organo-phosphate). Propoxur was a selective inhibitor of OPS AChE activity whereas monocrotophos was shown to be a selective inhibitor of OPR AChE activity. These data confirmed earlier observations by Brown and Bryson (11). The responses of preparations from these strains and the HRV, PYR, and field-collected strains to inhibition by propoxur and monocrotophos were compared (Figure 1). All of the OPS responses were in the lower right cluster of the scatterplot; whereas, all of the OPR responses were in the upper left cluster of the scatterplot. Eighty-six percent of the HRV sample responses were in the lower right cluster. PYR responses were equally distributed between the upper left cluster and an intermediate cluster. Most of the Tifton responses were associated with the lower right cluster (although some responses were more intermediate), while all of the Bainbridge responses were associated with the lower right cluster. Genetic linkage analysis indicated that AChE inhibition characteristics were controlled by a single gene, *Aceln*, and that the SS genotypes were fully susceptible to propoxur. Furthermore, the RR genotypes were fully susceptible to monocrotophos, and the RS genotypes were partially inhibited by either compound (Heckel and Brown, unpublished; 11). Our data indicated that the Bainbridge AChE was comparable to the OPS AChE in its response to inhibition by propoxur and monocrotophos and suggested that the Bainbridge strain may be homozygous for the susceptible enzyme. Based on these data, the genotypes of the PYR, HRV, and Tifton strains were more varied.

Conclusions

o OPR and PYR AChE activities were less sensitive to inhibition by methyl paraoxon as compared to OPS AChE activity

o AChE activities of the Bainbridge and Tifton field strains were intermediate

o Based on responses of AChE activities following inhibition by propoxur and monocrotophos, the OPS and Bainbridge strain would be assigned the SS genotype, the OPR strain would be assigned the RR genotype, and the YR, HRV, and Tifton strains would not be completely homozygous

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Table 1. Inhibition constants for acetylcholinesterase from tobacco budworm, *Heliothis virescens*, adults following exposure to methyl paraoxon.[a]

Strain	Ki x 10-3 (SE)[b]
OPS	47.4 (0.32)
OPR	1.75 (0.31); 27.0[c]
HRV	54.0 (4.57); 0.88
PYR	1.73 (0.20); 27.3
Bainbridge	23.0 (2.70); 2.06
Tifton	32.3 (3.75); 1.47

[a] Pooled activities from individual heads; inhibited in the presence of substrate
[b] Bimolecular reaction constants (M^{-1} min^{-1})
[c] Quotient of K*i* OPS divided by K*i*.

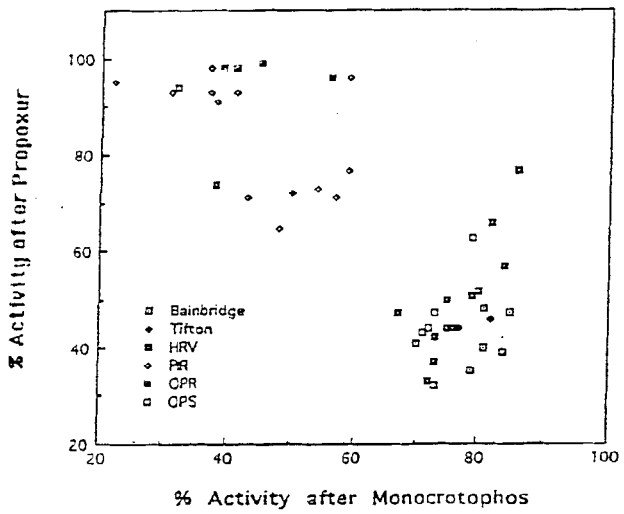

Figure 1. Acetylcholinesterase activities in tobacco budworm head preparations in resonse to inhibition by propoxur (7×10^{-4}) and monocrotophos (7×10^{-5}).