Purification and Characterization of an Esterase Conferring Resistance to Fenitrothion in *Oryzaephilus surinamensis* (L.) (Insecta, Coleoptera, Silvanidae)

Sung-Eun Lee,*,† Edith M. Lees,‡ and Bruce C. Campbell†

Plant Protection Research Unit, Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710, and Department of Agricultural Chemistry and Soil Science, Faculty of Agriculture, The University of Sydney, Sydney, NSW 2006, Australia

Esterases from a fenitrothion-resistant strain (VOSF) of the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), are presumed to play a role in conferring resistance to malathion, fenitrothion, and chlorpyrifos-methyl. Colorimetric assays showed a significant positive correlation between increased resistance to fenitrothion in strains of *O. surinamensis* examined and elevated esterase hydrolytic activity to substrates of *p*-nitrophenyl acetate, α-naphthyl acetate, and *β*-naphthyl acetate. Esterase zymograms showed different banding patterns between VOSF and an insecticide-susceptible strain, VOS48. A major esterase in the VOSF strain, not detected in VOS48, was purified and characterized by chromatographic and electrophoretic techniques. On the basis of SDS−polyacrylamide gel eletrophoresis, the molecular mass of the purified esterase from VOSF was 130 kDa and consisted of two 65 kDa subunits. Additional properties of this enzyme are discussed.

Keywords: *Oryzaephilus surinamensis; esterase; enzyme purification; malathion; fenitrothion; chlorpyrifos-methyl*

INTRODUCTION

Oryzaphilus surinamensis (L.) (Coleoptera: Silvanidae), the saw-toothed grain beetle, is a major worldwide pest of stored foods, particularly grains, milled products, and dried fruits. This insect was not a major pest until routine use of insecticides on storage grains preferentially eliminated natural predators and competing species. Additional reliance on insecticides as grain protectants to control infestation has now led to insecticide resistance in *O. surinamensis*. Development of resistance in *O. surinamensis* has been reported frequently (Collins et al., 1993; Herron et al., 1996; Wallbank, 1996). In the early 1960s malathion was used against stored grain pests, but from 1968 to 1979 widespread resistance to malathion necessitated use of new postharvest protectants in Australia. Since 1977 fenitrothion (12 mg/kg of grain), in a mixture with bioresmethrin (1 mg/kg of grain) plus piperonyl butoxide as a synergist (bioresmethrin: piperonyl butoxide, 1:10), has been used extensively on grain in Australia (Attia and Frecker, 1984). Champ and Dyte (1976) recorded cross-resistance to fenitrothion in Australian malathion-resistant *O. surinamensis*. However, fenitrothion continued to be used because resistance was not sufficient to cause total failure of the insecticide for acceptable levels of control in silos. Although fenitrothion is still in current use, alternative insecticides have become necessary (Kotze and Wallbank, 1996; Milson, 1995). Chlorpyrifos-methyl is now being used in grain storage sites where fenitrothion resistance is sufficient to cause problems. However, resistance to chlorpyrifos-methyl and pirimiphos-methyl has already been detected (Collins et al., 1993; Kotze and Wallbank, 1996; Milson, 1995; Wallbank, 1996). Collins et al. (1992) examined a range of possible resistance mechanisms, including esterase, mixed function oxidase, and glutathion *S*-transferase activity in different strains of *O. surinamensis*. The strains studied included VOS3, a field strain selected when malathion was the sole organophosphate insecticide used by the grain industry before the introduction of fenitrothion and chlorpyrifos-methyl; VOSF, a field strain selected with fenitrothion; and VOSCM, a field strain selected with chlorpyrifos-methyl. Collins et al. (1992) found total esterase activity ratios for VOS3, VOSF, and VOSCM relative to VOS48, an insecticide-susceptible strain of *O. surinamensis*, to be 11.6×, 12.2×, and 25.9×, respectively, suggesting esterases play a role in the detoxification of all of these pesticides, but perhaps more significantly for chlorpyrifos-methyl. A preliminary study conducted in our laboratory showed, additionally, the VOSF strain had an esterase isozyme pattern different from that of VOS48. This paper presents our findings on esterase activity in strains of *O. surinamensis* susceptible and resistant to fenitrothion. We also present results of purification and characterization of the major esterase conferring fenitrothion resistance in the VOSF strain.

EXPERIMENTAL PROCEDURES

Chemicals. *p*-Nitrophenylacetate (PNPA), α-naphthyl acetate (α-NA), *β*-naphthyl acetate (*β*-NA), α-naphthol, *β*-naphthol, *p*-nitrophenol, Fast Blue B salt (*o*-dianisidine, tetrazotized zinc chloride complex), and tris(hydroxymethyl)aminomethane (Tris) were all purchased from Sigma Chemical Co. Fluon (polytetrafluoroethylene) was from ICI. All chemicals used were of the highest grade commercially available.

* Corresponding author [telephone (510) 559-5841; fax (510) 559-5777; e-mail sel@pw.usda.gov].

† U.S. Department of Agriculture.

‡ The University of Sydney.

10.1021/jf991258f CCC: $19.00
Published on Web 08/25/2000

Insects and Culture Method. The two strains of *O. surinamensis* used in this study were supplied by Dr. H. A. Rose, Department of Crop Science, The University of Sydney, Australia. VOS48, the fenitrothion-susceptible reference strain, has been in continuous laboratory culture since 1973. VOSF is a composite field strain that was maintained in the laboratory in Sydney under fenitrothion selection. The strains were maintained in our laboratory without further insecticide selection, in 750 mL glass jars. Prior to inoculation, nutrient mix [200 g mixture of cracked wheat, rolled oats, and brewer's yeast (7:7:1, w/w)] was placed in the jars, which were then heated at 110 °C for 1 h to kill any potentially contaminating mites, insects, or fungal spores. Jars were then inoculated with 20 mg (≈50 individuals) of insects and incubated at 28 °C for 8 weeks, by which time a large population (>2000 individuals) of insects had developed. Insects were collected by shaking open jars over a stainless steel tray with Fluon coated on the sides.

Determination of the Resistance of Two Strains of *O. surinamensis* to Organophosphorus Insecticides. Insecticide resistance was assessed according to the standard FAO impregnated filter paper assay method (Champ and Dyte, 1976) to determine LC_{50} values for the VOSF and VOS48 strains with malathion and fenitrothion. The insecticides were dissolved in a mixture of ondina oil/acetone/petroleum ether (1:1:3, v/v). Insecticide solutions (0.5 mL) of different concentrations were applied to (7 cm diameter) Whatman No. 1 filter papers, and the acetone and petroleum ether were allowed to evaporate. The filter papers were placed in aluminum foil lined PVC trays (80 × 330 × 420 mm), and an aluminum ring (4.3 cm inner diameter and 2.9 cm high) coated inside with Fluon was placed on each filter paper. Two batches of ~40 adult beetles each were exposed to a graded series of four, five, or six concentrations of malathion and fenitrothion for 24 h at 28 °C. For determination of resistance to malathion in VOSF, one batch of insects was exposed to six concentrations of malathion. The insects were then transferred to small vials, containing 2 g of rolled oats, for 24 h, after which time survivors were counted. The LC_{50} values were calculated by Probit analysis (Finney, 1971). Control mortality was accounted for by Abbott's formula (Abbott, 1925). Resistance factors were calculated as the ratio of the LC_{50} for a resistant strain, VOSF, to the LC_{50} for the susceptible strain, VOS48. In this study all insecticide concentrations used or LC_{50} values determined are reported as percent (w/v) insecticide in ondina oil.

Preparation of Extracts of Esterases. Enzyme preparation was carried out at 4 °C. Five grams of adults from each of the two strains was homogenized with a pestle and mortar in 20 mL of 10 mM Tris-HCl buffer, pH 7.5, containing 0.5% (w/v) Triton X-100. The resultant homogenates were filtered through Miracloth to remove exoskeletal and tissue debris and centrifuged at 12100*g* for 20 min using a Sorvall Superspeed RC-2B centrifuge. The supernatants were reserved as crude enzyme extracts.

Enzyme Assays. The esterase assay was carried out according to the method of Mackness et al. (1983). The reaction mixture contained 50 μL of crude enzyme, 50 μL of 150 mM *p*-nitrophenyl acetate (PNPA) in ethanol, and 9.2 mM Tris-HCl buffer, pH 7.5, to give a final concentration of 2.5 mM PNPA in a total volume of 3 mL. The reaction was initiated by the addition of enzyme extract and incubated at 25 °C for 4 min. Absorbance was determined at 405 nm. A standard curve was prepared in the range of 0−220 nmol of *p*-nitrophenol (PNP). The hydrolysis of the naphthyl acetates (α-NA and β-NA) was determined as described by Devonshire (1977). The reaction mixture contained 50 μL of crude enzyme, 25 μL of 30 mM α-NA or β-NA in acetone to give a final concentration of 0.25 mM of α-NA or β-NA, and 9.75 mM Tris-HCl buffer, pH 7.5, in a total volume of 3 mL. The reaction was initiated by the addition of enzyme extract and incubated at 25 °C for 60 min, and the reaction was stopped by adding 0.5 mL of 0.1% Fast Blue B salt in 3.5% (w/v) sodium dodecyl sulfate. After the reaction mixture had been maintained for 15 min at room temperature, absorbance values at 605 nm, for α-NA, and at 550 nm, for β-NA, were determined. Standard curves for both α-naphthol (α-N) and β-naphthol (β-N) were prepared with a range of 0−50 nmol. All enzyme assays were replicated six times, and data were subjected to a one-way ANOVA. Means were compared and tested for significant difference by the Scheffe test at the $P = 0.05$ level (SAS, 1995).

Purification of Esterase from VOSF Strain. Beetles of VOSF (10 g) were homogenized in 50 mL of 10 mM Tris-HCl buffer, pH 7.5. The crude extract was applied to a Sepharose CL-6B gel filtration column (85 cm × 2.6 cm) equilibrated with 10 mM Tris-HCl buffer, pH 7.5, and eluted with the same buffer. Fractions (5 mL) were collected, and 50 μL of each was assayed for esterase activity using PNPA as substrate. Protein was determined according to the method of Bradford (1976) using 50 μL of sample solution and 2.5 mL of diluted (1 to 5) Bio-Rad Coomassie Blue concentrate. Active fractions were pooled, rechromatographed on a DEAE-Sephacel column (69 cm × 1.6 cm), and eluted with a linear 0−0.5 M NaCl gradient in 300 mL of 10 mM Tri-HCl buffer, pH 7.5, at 1.0 mL min^{-1}. Fractions (2 mL) were collected and assayed for esterase using PNPA. Conductivity of the NaCl gradient was determined using a Radiometer CDM 83 conductivity meter. Active fractions were combined and concentrated to a volume of 2 mL using an Amicon Concentration YM 10 membrane. The concentrated eluate was applied to a Sephacryl S-300 column (90 cm × 1.6 cm) equilibrated with 10 mM Tris-HCl buffer, pH 7.5, and eluted with the same buffer. Fractions (1 mL) were collected and assayed for esterase using PNPA. Fractions containing esterase activity were pooled and concentrated to a final volume of 1 mL and stored at −80 °C until required.

Electrophoresis (PAGE) of Purified Esterase. Electrophoresis was carried out on 7.5% (w/v) polyacrylamide gels at pH 8.5 using a Bio-Rad mini-protein II dual vertical gel slab cell system. Samples of esterase in a solution of 20% (w/v) sucrose containing bromothymol blue were loaded onto stacking gels (3%, w/v). Electrophoresis was carried out at 100 V for 10 min and then at 200 V (maximum current of 2.5 mA) for ~2 h until the buffer front (dye) ran to the bottom of the gel. The gels were stained for esterases with a solution of 30 mM α-NA in acetone (1 mL) and 0.2% (w/v) Fast Blue RR salt in 0.2 M phosphate buffer, pH 6.0 (50 mL) (Denvonshire, 1977). Gels were stained for protein using Coomassie Blue (Fairbanks et al., 1971).

Determination of Molecular Mass and Subunit Size of Purified Esterase. The molecular weight of the purified esterase was determined by gel chromatography using the method of Andrews (1965). A Fractogel TSK HW-55(F) column (100 cm × 2.2 cm) equilibrated with 50 mM Hepes−KOH buffer, pH 7.1, containing 100 mM KCl and 20% (v/v) glycerol was calibrated using 1 mL aliquots of blue dextran containing 20% (w/v) sucrose to determine void volume (V_0) and six proteins, thyroglobulin, ferritin, catalase, BSA, chymotrypsinogen A, and cytochrome *c*, of known molecular mass as standards. The elution volumes (V_e) for the standard proteins were determined by measuring the absorbance at 280 nm of 1 mL aliquots of 2 mL fractions using a Hitachi 100-10 spectrophotometer. Next, aliquots of the purified esterase (1 mL) were applied to the column, and the activity of 2 mL fractions was determined by assaying PNPA hydrolysis.

The purified esterase from VOSF was examined by SDS−PAGE. Proteins included as electrophoretic size standards were thyroglobulin, ferritin, catalase, BSA, ovalbumin, lactate dehydrogenase, and lactalbumin. The gels were stained with Coomassie Blue as described.

Determination of Inhibition Kinetic Constants by Fenitrothion and Fenitrooxon on the Purified Esterase. For the inhibition kinetics, stopped-time inhibition assays were conducted using PNPA as substrate. The purified enzyme was incubated with a series of concentrations of the respective insecticide, and then at various times aliquots were withdrawn and residual activity was determined by measuring the rate of substrate hydrolysis. The activities were divided by those measured in the absence of insecticide as control. The bimolecular rate constants (k_a) for the formation of acylated enzyme were derived as described previously (Main and Dauterman,

Table 1. LC_{50} Values for Two Strains of *O. surinamensis* in Response to Malathion, Fenitrothion, and Chlorpyrifos-methyl[a]

strain	*n*	LC_{50}(95% FL), g L^{-1}	RF
		Malathion	
VOS48	540	0.0238 (0.0191−0.0285)	1
VOSF	629	0.232 (0.173−0.310)	9.7
		Fenitrothion	
VOS48	781	0.0014 (0.0013−0.0015)	1
VOSF	420	0.136 (0.116−0.161)	97
		Chlorpyrifos-methyl	
VOS48	440	0.0251 (0.0181−0.0327)	1
VOSF	543	0.123 (0.106−0.145)	4.9

[a] Resistance factors (RF) for the VOSF strain have been calculated relative to the susceptible strain, VOS48. LC_{50} values are in g L^{-1} of ondina oil.

Table 2. Esterase Activities of Two Strains of *O. surinamensis*[a]

	esterase activity substrate		
strain	PNPA	α-NA	β-NA
VOS48	5.98 ± 0.04 (1)a	4.45 ± 0.12 (1)a	3.44 ± 0.12 (1)a
VOSF	17.6 ± 0.23 (2.9)b	9.86 ± 0.19 (2.2)b	7.24 ± 0.04 (2.1)b

[a] Esterase activities are expressed as μmol of substrate (*p*-nitrophenyl acetate, PNPA; α-naphthyl acetate, α-NA; β-naphthyl acetate, β-Na) hydrolyzed min^{-1} g of $beetles^{-1}$. Figures in parentheses indicate the ratio of activity in the resistant strains in comparison with the susceptible strain, VOS48. Data (mean ± SE) were determined from six replicates. Means within a column followed by a different letter are significantly different [$P = 0.05$; Scheffe test (SAS, 1995)].

1963). The reactivation experiments were performed by incubating the purified enzyme with the respective insecticide for 30 min so that the enzyme was >90% inhibited. Aliquots were withdrawn to measure the esterase activity of the reactivating enzyme, as well as a control, over time. The pseudo-first-order inhibition rate constants were calculated and used to determine the various inhibition constants (Aldridge and Reiner, 1972). Organophosphorus insecticides inhibit esterases by rapid esterification of a serine residue in the active site. This reaction is followed by a rate-determining slow deacylation. The generally accepted reaction mechanisms is:

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EI \xrightarrow{k_2} EI' + P_1 \xrightarrow[H_2O]{k_3} E + P_2$$

where EI is the Michaelis complex and EI′ is the acylated enzyme.

RESULTS

The LC_{50} values determined for the three insecticides, malathion, M(S), fenitrothion, F(S), and chlorpyrifos-methyl, CM(S), for the two strains of *O. surinamensis* and the resistance factor (RF) for VOSF relative to VOS48 are shown in Table 1. The VOSF strain showed cross-resistance to the three insecticides when compared to VOS48. Adults of the VOSF strain of *O. surinamensis* had resistance factors to M(S), F(S), and CM(S) of 9.7, 97, and 4.9, respectively.

The levels of esterase in adult insects of the two strains of *O. surinamensis* are shown in Table 2. The fenitrothion-resistant strain showed 2.9-, 2.2-, and 2.1-fold elevated esterase activity in comparison with VOS48 for PNPA, α-NA, and β-NA, respectively.

The resulting PAGE of protein aliquots of two strains of *O. surinamensis* stained for esterase activity is shown in Figure 1. At least three different esterases in the VOSF strain were identified by α-NA staining. The

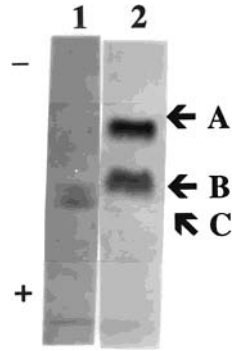

Figure 1. Native PAGE of crude extracts of adults of two strains of *O. surinamensis* stained for esterase activity with α-naphthyl acetate and 0.1% Fast Blue RR salt: (lane 1) VOS48; (lane 2) VOSF. Standard 15 μL aliquots (equivalent to 0.5 μg) were applied to the gels.

Table 3. Purification Table of the Major Esterase, Designated A, from VOSF Strain of *O. surinamensis*[a]

stage	total act. (units)	total protein (mg)	sp act. (units/mg of protein)	purifn (*x*- fold)	yield (%)
crude extract	47.5	329	0.144	1.0	100
Sepharose CL-6B concentrate	36.3	100.2	0.362	2.52	76.6
DEAE-Sephacel concentrate	16.3	8.21	1.98	6.94	34.3
Sephacryl S-300 concentrate	0.34	0.02	17.0	118.0	0.72

[a] Enzyme was assayed with PNPA as substrate and unit of activity is μmol of *p*-nitrophenol released min^{-1}.

VOSF pattern shown in Figure 1 had a major strongly stained band, designated A, not detected in VOS48.

The major esterase, A, of VOSF as shown by PAGE (Figure 1) was purified. A Sepharose CL-6B elution profile of esterase from the VOSF strain is shown in Figure 2a. A small proportion of the esterase activity was eluted with the void volume, but most activity was represented by a single large peak. A DEAE-Sephacel elution profile of the pooled fractions containing the majority of the VOSF esterase activity from the Sepharose CL-6B column is shown in Figure 2b. A large proportion of the esterase activity eluted in a peak at a conductivity of ∼12 mS cm^{-1}. A Sephacryl S-300 elution profile of the pooled and concentrated fractions containing VOSF esterase activity from the DEAE-Sephacel column is shown in Figure 2c, and only one peak was detected. Purification factors at each stage of the process are shown in Table 3, showing that the VOSF esterase was eventually purified 118-fold. The results of PAGE of the Sephacryl S-300 concentrate containing the purified esterase from VOSF are shown in Figure 3. Only one esterase band on the gel appeared. The molecular mass of the purified esterase from the VOSF was found to be 130 kDa as shown in Figure 4. The subunit size for the VOSF enzyme was shown to be 65 kDa using SDS−PAGE as shown in Figure 5.

The second-order rate constants for the formation of the acylated enzyme (biomolecular rate constant), k_a, and k_3 values obtained from reactivation kinetic experiments are shown in Table 4. There is no significant interaction of the purified esterase with fenitrothion. However, the purified esterase had a significantly higher rate of binding for fenitrooxon, an oxon derivative of fenitrothion produced by cytochrome P450-dependent monooxygenase. Slow deacylation rates (k_3) with feni-

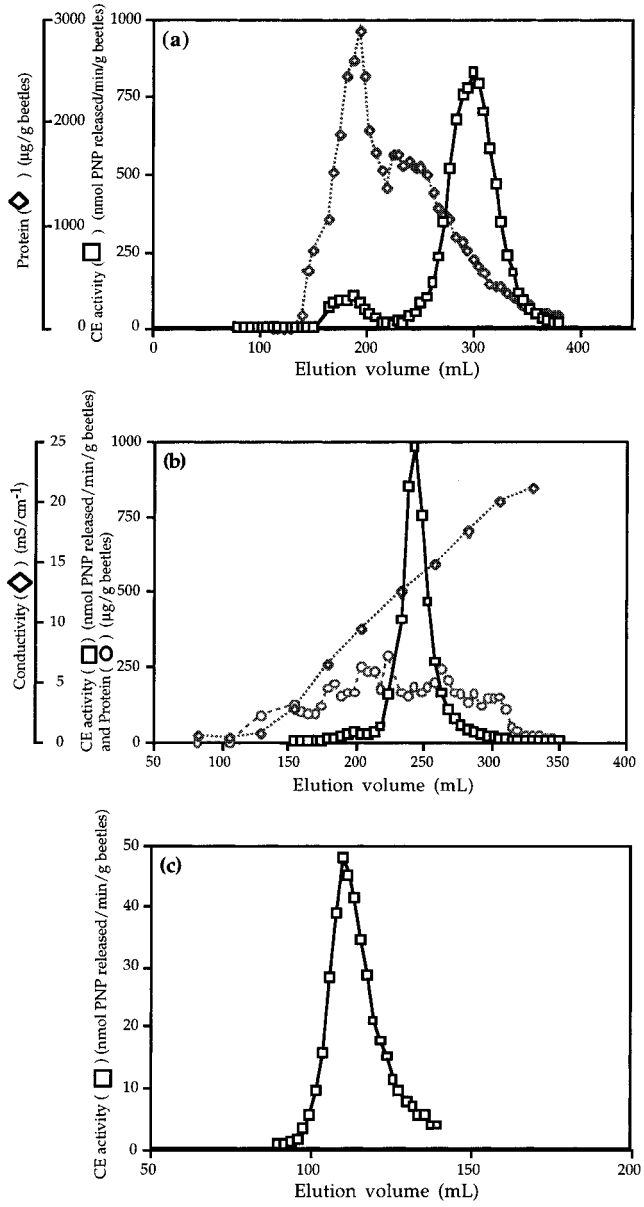

Figure 2. Pufication of the major esterase, designated A, from *O. surinamensis* VOSF strain: (a) Sepharose CL-6B elution profile of a crude extract (12100 g of supernatant); (b) DEAE-Sephacel elution profile of the Sepharose CL-6B concentrate containing esterase activity from *O. surinamensis* VOSF; (c) Sephacryl S-300 elution profile of the DEAE-Sephacel concentrate of the major peak of esterase activity from *O. surinamensis* VOSF. Esterase activity was determined by hydrolysis of PNPA. Protein was determined according to the Bradford (1976) method.

trothion and fenitrooxon indicate that the principal role of the purified enzymes in detoxication may be sequestration as shown in Table 4.

DISCUSSION

In this study, there is a positive relationship between the degree of insecticide resistance and the total esterase activity of *O. surinamensis* as shown in Tables 1 and 2. Resistance factors for these three insecticides reported for VOSF to VOS48 by Collins et al. (1992) were 120 for F(S), 21 for M(S), and 5 for CM(S). Thus, the resistance factors to F(S) and M(S) for VOSF in our study show only a slight decline for F(S) and a >2-fold decline for M(S) over a two year period of culturing in

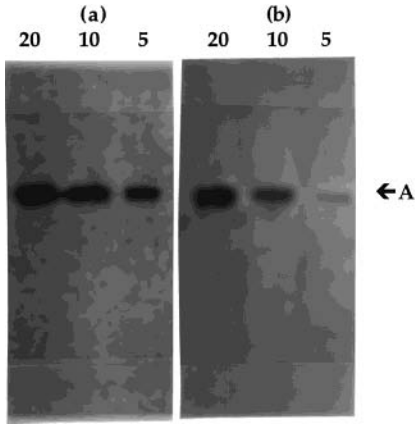

Figure 3. Native PAGE of purified esterase from VOSF strain of *O. surinamensis*. Aliquots (5, 10, and 20 μL) of the concentrated Sephacryl S-300 eluates were applied to the gels. The gels were stained with α-naphthyl acetate and 0.1% Fast Blue RR salt for esterase activity (a) and with Coomassie Blue for protein (b).

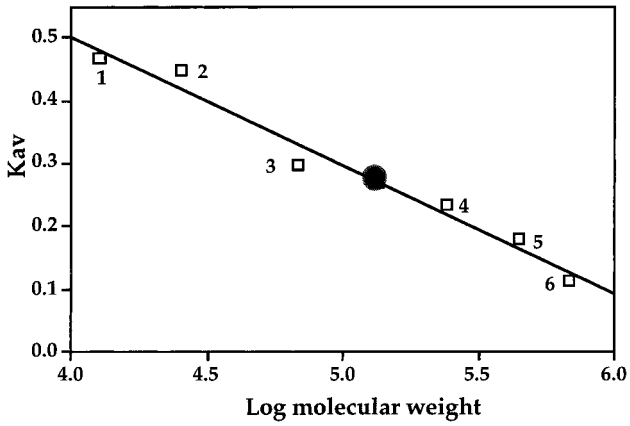

Figure 4. Calibration of the fractogel TSK HW-55(F) column used for estimation of the molecular weight of the esterase purified from VOSF. Standards were (1) cytochrome *c* 12500, (2) chymotrypsinogen A 25000, (3) BSA 68000, (4) catalase 240000, (5) ferritin 450000, and (6) thyroglobulin 669000. The K_{av} value for the VOSF carboxylesterase (●) was 2.71.

the absence of F(S). However, the similar resistance factors to chlorpyrifos-methyl in the VOSF strain reported by Collins et al. (1992) and our study suggest resistance to this pesticide has not declined. Collins (1986) examined the genetic basis of F(S) resistance in *O. surinamensis* and suggested resistance is controlled by several genetic traits, which are possibly not entirely dominant genes. On the other hand, resistance to M(S) in *O. surinamensis* was reported to be controlled by only a single dominant gene (Anonymous, 1981). These differing mechanisms of genetic control may explain the apparent relative heritable stability of F(S) resistance and variability of resistance to M(S) in *O. surinamensis* after multitudinous generations.

The relationship between resistance, the level of esterase activity in insects, and insecticide metabolism is not always clear. Esterases catalyze hydrolysis of several different types of esters, and many insecticides such as organophosphates, carbamates, and pyrethroids contain ester groups. Thus, these types of pesticides could be detoxified by hydrolysis of the ester bond. Figure 6 shows structures of malathion, fenitrothion, and chlorpyrifos-methyl with potential sites of esterase activity. In vitro esterase activity is frequently determined by hydrolysis of simple carboxylesters such as

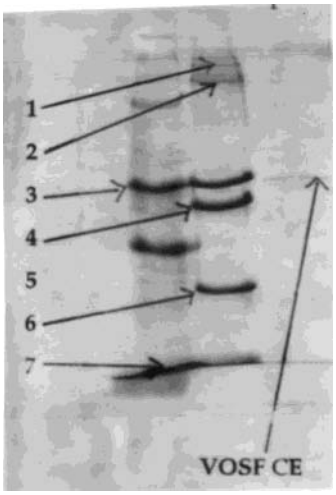

Figure 5. SDS−PAGE of the purified esterase from VOSF strain. The gels were stained for protein with Coomassie Blue. Standards were (1) thyroglobulin 330000, (2) ferritin 220000, (3) BSA 66000, (4) catalase 60000, (5) ovalbumin 45000, (6) lactic dehydrogenase 36500, and (7) ferritin 18500.

Table 4. Bimolecular Rate Constant (k_a) and k_3 Values for Insecticide Interaction with the Purified Esterase from a Fenitrothion-Resistant Strain of *O. surinamensis*[a]

insecticide	k_a (M^{-1} min^{-1})	$10^4 \times k_3$ (min^{-1})
fenitrothion	15.9 ± 2.60 (10^{-2})a	2.1 ± 0.27a
fenitrooxon	0.72 ± 0.06 (10^{-5})b	1.5 ± 0.13b

[a] Means within a column followed by a different letter are significantly different [$P = 0.05$; Scheffe test (SAS, 1995)].

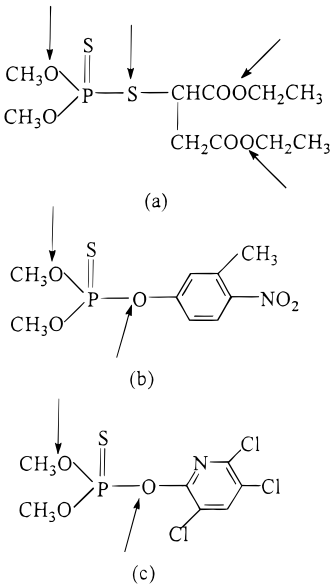

Figure 6. Structures of three insecticides: (a) malathion; (b) fenitrotion; (c) chlorpyrifos-methyl. Arrows indicate possible sites of esterase activity.

PNPA and α- and β-NA. Generally, enhanced carboxylesterase activity levels are correlated with resistance (Devonshire, 1977; Sigfried and Zera, 1994; Zhu and Brindley, 1990), but several reports have demonstrated a negative correlation between enzyme activity to noninsecticidal substrates and insecticide resistance. Early studies on organophosphate-susceptible and -resistant strains of *Musca domestica* (van Asperen and Oppenoorth, 1959; van Asperen, 1962) showed lower esterase activity in the resistant houseflies in comparison with susceptible strains. Activity assayed with β-NA was similar in susceptible and resistant strains, whereas hydrolytic activity in resistant strains using α-NA was only 5% of activity in a susceptible strain (van Asperen, 1962). It was suggested by Oppenoorth and van Asperen (1960) that the negative correlation between esterase activity and organophosphate resistance was due to an altered esterase enzyme, which could hydrolyze organophosphates but had reduced activity against α-NA. Bush et al. (1993) detected a lower esterase activity to α-NA in a parathion-resistant strain than in a susceptible strain of the codling moth *Cydia pomonella* (L.). They concluded that the reduced esterase activity might result from a modified esterase with much lower specificity to the noninsecticidal α-NA substrate. On the other hand, a positive correlation between esterase activity and insecticide resistance has been demonstrated in the peach-potato aphid, *Myzus persicae* Sulzer. Devonshire (1977) showed that increased esterase activity in this aphid was caused by increased levels of a single isoenzyme, designated E4 on the basis of PAGE analysis. This enzyme hydrolyzed both α-NA and paraoxon, although the rate of hydrolysis of α-NA was 2×10^6 faster than that of paraoxon. The E4 enzymes from resistant and susceptible strains had identical kinetic characteristics, indicating that insecticide resistance was caused by amplification of the E4 esterase and not by a modified protein. Devonshire and Moores (1982) examined the interaction of the E4 isozyme with several organophosphates, carbamates, and pyrethroids and found that the very slow rates of hydrolysis of these compounds were related to the resistance of the insect to the insecticides. Our study shows a clear positive relationship between the degree of insecticide resistance (Table 1) and esterase activity to three noninsecticidal substrates (Table 2) for *O. surinamensis*. Thus, the elevated esterase activity may contribute to the increased resistance in VOSF.

For characterization of insect esterases, Sivakumaran and Mayo (1991) identified nine esterases in the greenbug, *Schizaphis graminum* (Rondani), by PAGE and α-NA staining. Incubation with 10 μM eserine prior to staining with α-NA inhibited the slowest migrating band, P1, indicating that this was a cholinesterase. Incubation with 10 μM paraoxon and 10^{-2} or 10^{-1} mM disulfoton during staining with α-NA indicated that five bands, P2, P3, P4, P5, and P6, were carboxylesterases. Chen and Sun (1994) found >10 isoenzymes of carboxylesterases from a rice planthopper, *Nilaparvata lugens* Stål, using isoelectric focusing. The three most active forms, designated E1, E2, and E3, were purified by Sepharose 6B chromatography and chromatofocusing and were found to have similar subunit molecular masses (62−64 kDa) and p*I* values (4.7−4.9). These three proteins were immunologically cross-reactive to each other using E1 antiserum and showed no difference in sensitivity to organophosphate oxons such as paraoxon, methylparaoxon, and malaoxon. Currently, an esterase involved in resistance to organophosphate insecticides in *O. surinamensis* has been partially purified from a multi-organophosphorus (OP)-resistant strain (7012/lmalRR) (Conyers et al., 1998). The molecular mass of this partially purified esterase was 94 kDa determined by native PAGE. This enzyme was competitively inhibited by OPs, but the enzyme had a 3-fold lower affinity for malaoxon compared to that from the susceptible strain.

CONCLUSION

From our inhibition study, the activity of purified esterase from VOSF was >90% inhibited by 10^{-1} mM F(S) and by 10^{-4} mM F(O), its oxon derivative, fenitrooxon. If the toxicity of fenitrothion is mainly due to oxon metabolites formed in vivo by cytochrome P450-dependent monooxygenases, the resistance is due to the interaction of its oxon derivatives with esterases. In addition, the purified esterase of VOSF did not show hydrolytic activity toward F(S) and F(O). However, the slow deacylation rates to F(S) and F(O) indicate that this purified enzyme may play an important role in fenitrothion detoxification by sequestration properties. Therefore, our purified esterase from the VOSF strain of *O. surinamensis* originating in Australia is different from that of the 7012/lmalRR strain originating in England on the basis of their different biochemical characteristics.

Further molecular biological studies of the purified esterase from the VOSF strain of *O. surinamensis* will contribute information toward a better understanding of resistance mechanisms to organophosphorus insecticides in *O. surinamensis*.

LITERATURE CITED

Abbott, W. S. A method for computing the effectiveness of an insecticide. *J. Econ. Entomol.* **1925**, *18*, 265−267.

Aldridge, W. N.; Reiner, E. In *Enzyme Inhibitors as Substrates: Frontiers of Biology*; Neuberger, A., Tatum, E. L., Eds.; North-Holland: Amsterdam, The Netherlands, 1972; Vol. 26.

Andrews, P. The gel filtration behaviour of proteins related to their molecular weights over a wide range. *Biochem. J.* **1965**, *96*, 595−606.

Anonymous. *Pest Infestation Global Laboratory Report 1977−1979*; Ministry of Agriculture Fisheries and Food Reference Book 343; Stationey Office: London, U.K., 1981; pp 76−79.

Attia, F. I.; Frecker, T. Cross-resistance spectrum and synergism studies in organophosphorus-resistant strains of *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae) in Australia. *J. Econ. Entomol.* **1984**, *77*, 1367−1370.

Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

Bush, M. R.; Abdel-All, Y. A. I.; Rock, G. C. Parathion resistance and esterase activity in codling moth (Lepidoptera: Tortricidae) from North Carolina. *J. Econ. Entomol.* **1993**, *86*, 660−666.

Champ, B. R.; Dyte, C. E. *FAO Global Survey of Pesticide Susceptibility of Stored Grain Pests*; FAO Plant Production and Protection Series 5; FAO: Rome, Italy, 1976.

Chen, W. L.; Sun, C. N. Purification and characterisation of carboxylesterase of a rice brown planthopper, *Nilaparvata lugens* Stål. *Insect Biochem. Mol. Biol.* **1994**, *24*, 347−355.

Collins, P. J. Genetic analysis of fenitrothion resistance in the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae). *J. Econ. Entomol.* **1986**, *79*, 1196−1199.

Collins, P. J.; Rose, H. A.; Wegecsanyi, M. Enzyme activity in strains of the sawtoothed grain beetle (Coleoptera: Cucujidae) differentially resistant to fenitrothion, malathion and chlorpyrifos-methyl. *J. Econ. Entomol.* **1992**, *85*, 1571−1575.

Collins, P. J.; Lambkin, T. M.; Brideman, B. W.; Pulvirenti, C. Resistance to grain-protectant insecticides in coleopterous pests of stored cereals in Queensland, Australia. *J. Econ. Entomol.* **1993**, *86*, 239−245.

Conyers, C. M.; MacNicoll, A. D.; Price, N. R. Purification and characterisation of an esterase involved in resistance to organophosphorus insecticides in the saw-toothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). *Insect Biochem. Mol. Biol.* **1998**, *28*, 435−448.

Devonshire, A. L. The properties of a carboxylesterase from the peach-potato aphid *Myzus persicae* (Sulz.) and its role in conferring insecticide resistance. *Biochem. J.* **1977**, *167*, 675−683.

Devonshire, A. L.; Moores, G. D. A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach-potato aphids (*Myzus persicae*). *Pestic. Biochem. Physiol.* **1982**, *18*, 235−246.

Fairbanks, G.; Steck, T. L.; Wallbank, D. F. Electrophoretic analysis of major polypeptides of human erythrocyte membrane. *Biochemistry* **1971**, *10*, 2606−2617.

Finney, D. J. *Probit Analysis*, 3rd ed.; Cambridge University Press: London, U.K., 1971.

Herron, G. A.; Clift, A. D.; White, G. G.; Greening, H. G. Relationships between insecticide use, grain hygiene and insecticide resistance in *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) on grain-producing farms. *J. Stored Prod. Res.* **1996**, *32*, 131−136.

Kotze, A. C.; Wallbank, B. E. Esterase and monooxygenase activities in organophosphate-resistant strains of *Oryzaephilus surinamensis* (Coleoptera: Cucujidae). *J. Econ. Entomol.* **1996**, *89*, 571−576.

Mackness, M. I.; Walker, C. H.; Rowlands, D. C.; Price, N. R. Esterase activity in homogenates of three strains of the rust red flour beetle *Tribolium castaneum* (Herbst). *Comp. Biochem. Physiol.* **1983**, *74C*, 65−68.

Main, A. R.; Dauterman, W. C. Determination of the bimolecular rate constant for the reaction between organophosphorus inhibitors and esterases in the presence of substrate. *Nature* **1963**, *198*, 551−553.

Milson, J. D. Esterase activity and resistance to organophosphates in *Oryzaephilus surinamensis*. M.Sc.Agr. Thesis, The University of Sydney, 1995.

Oppenoorth, F. J.; van Asperen, K. Allelic genes in the housefly producing modified enzymes that cause organophosphate resistance. *Science* **1960**, *132*, 298−299.

SAS. *SAS User's Guide: Statistics*; SAS Institute: Cary, NC, 1995.

Sigfried, B. D.; Zera, A. J. Partial purification and charaterization of a greenbug (Homoptera: Aphididae) esterases associated with resistance to parathion. *Pestic. Biochem. Physiol.* **1994**, *49*, 132−137.

Sivakumaran, S.; Mayo, Z. B. Electrophoretic characterization of esterase in the greenbug, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae). *J. Kansas Entomol. Soc.* **1991**, *64*, 357−362.

van Asperen, K. A study of housefly esterases by means of a sensitive colorimetric method. *J. Insect Physiol.* **1962**, *8*, 401−406.

van Asperen, K. A.; Oppenoorth, F. J. Organophosphate resistance and esterase activity in houseflies. *Entomol. Exp. Appl.* **1959**, *2*, 48−57.

Wallbank, B. E. Resistance to organophosphorus grain protectants in *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) from off-farm grain storages in New South Wales. *Aust. J. Entomol.* **1996**, *35*, 193−195.

Zhu, K. Y.; Brindley, W. A. Properties of esterases from *Lygus hesperus* Knight (Hemiptera: Miridae) and the roles of the esterases in insecticide resistance. *J. Econ. Entomol.* **1990**, *83*, 725−732.

Received for review November 22, 1999. Accepted July 17, 2000.

JF991258F