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Purification and Characterization of Carboxylesterases of a Rice Brown Planthopper, *Nilaparvata lugens* Stål

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More than 10 molecular forms of carboxylesterases were observed with α-naphthyl acetate as substrate in a rice brown planthopper (BPH), *Nilaparvata lugens* Stål, using isoelectric focusing. The three most active ones, E_1, E_2 and E_3, purified with gel permeation/chromatofocusing chromatography were characterized. Their subunit molecular mass varied between 62 and 64 kDa and the pI ranged from *c.* 4.7 to 4.9. They were immunologically related and showed no difference in sensitivity toward the inhibition of paraoxon, methyl paraoxon, and malaoxon. E_1 consistently exhibited much higher activity than the other two isozymes toward some model substrates, i.e. α-naphthyl acetate and butyrate, β-naphthyl acetate, and 4-nitrophenyl acetate as well as some insecticides. While malathion and *trans*-permethrin were readily hydrolyzed by these isozymes, very limited or no degradation of cypermethrin and *cis*-permethrin was detected. The carboxylesterases of BPH, being unable to hydrolyze parathion, could bind strongly the potent anticholinesterase paraoxon and oxons of several organophosphorus insecticides, rendering them nontoxic. Resistant BPH had higher activity and quantity of carboxylesterases (notably E_1) than susceptible BPH. Protein subunits immunologically related to E_1 of BPH were detected in two other rice planthoppers (*Laodelphax striatellus* and *Sogatella furcifera*) and two aphids (*Myzus persicae* and *Aphis gossypii*), but not in the green rice leafhopper (*Nephotettix cincticeps*) and southern house mosquito (*Culex quinquefasciatus*).

Nilaparvata lugens Carboxylesterase isozymes Purification Insecticide resistance

INTRODUCTION

Nilaparvata lugens Stål, a rice brown planthopper (BPH), was the most devastating insect pest on rice in many parts of Asia during the 1970s. Outbreaks of this planthopper could lead to severe hopperburn and total loss of the rice crop if no effective control was achieved (Dyck and Thomas, 1979). As the consequence of the use of intensive chemical sprays, resistance to carbamates and organophosphorus insecticides in this insect was soon reported in Taiwan (Lin *et al.*, 1979), Japan (Nagata *et al.*, 1979) and the Philippines (Heinrichs, 1979). Chung and Sun (1983) observed that carboxylesterase (EC 3.1.1.1) hydrolysis was closely involved in BPH resistance to malathion. Dai and Sun (1984) subsequently proposed that this enhanced esterase activity also confered a major part of BPH resistance to permethrin and other pyrethroids of primary alcohol esters, such as phenothrin. Chang and Whalon (1987) resolved eight esterases with pIs ranging between 4.3 and 5.3 from multiresistant BPH homogenate using isoelectric focusing and all eight forms were able to hydrolyze α- and β-naphthyl acetate, malathion, *cis*- and *trans*-permethrin at different rates. The current study purified the three most active (among a total of >10) molecular forms of carboxylesterases from BPH and determined their biochemical and toxicological properties, as well as their immunological relationship with proteins from some other insects.

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MATERIALS AND METHODS

Insects

Several strains of the brown planthopper were used: a susceptible S strain introduced from Agricultural Research Station, Japan ICI Ltd in March 1993, a susceptible P strain introduced from the International Rice Research Institute, Philippines in July 1992, a field F strain collected in September 1991 from Chiayi Prefecture, a malathion-resistant R-mal strain selected from F strain. In addition, BPH collected from Kwangtung (freeze-dried), Taichung and Nantou were also used. The BPH was reared on rice seedlings.

Additional homopterous insects used include white-backed planthopper (*Sogatella furcifera*), smaller brown planthopper (*Laodelphax striatellus*), corn hopper (*Peregrinus maidis*), green rice leafhopper (*Nephotettix cincticeps*), zigzag leafhopper (*Recilia dorsalis*), green peach aphid (*Myzus persicae*) and cotton aphid (*Aphis gossypii*).

Purification of carboxylesterases

Approximately 2 g of adult BPH was homogenized with a Polytron at 0°C in 10 ml of 0.1 M Tris–HCl buffer (pH 7.2) containing 5 mM EDTA. The supernatant after 1 h of ultracentrifugation at 100,000 *g* at 4°C (Beckman L5-40) was used as the enzyme source. It was subjected to ammonium sulfate fractionation and activity toward α-naphthyl acetate (α-NA) was determined for each fraction. Proteins precipitated between 40 and 60% ammonium sulfate (containing >65% activity) were dissolved in 2–3 vol of 0.02 M Tris–HCl buffer (pH 7.2). After dialysis the enzyme solution was introduced onto a Sepharose 6B column (2.2 × 20 cm) equilibrated with the same buffer used for dissolution. The column was eluted with this Tris–HCl buffer at 14.5 ml/h and fractions of 2.4 ml were collected and tested for activity toward 4-nitrophenyl acetate (NPA). Active fractions were pooled and concentrated with Centricon-10 (Amicon) to *c.* 2 ml; 5 vol of 0.025 M histidine–HCl buffer (pH 6.2) was added and the sample was again concentrated to *c.* 0.5 ml. It was then loaded onto a polybuffer exchanger PBE 94 gel column (1.0 × 20 cm) (Pharmacia) equilibrated with 0.025 M histidine–HCl buffer (pH 6.2). The column was first washed with the equilibrating buffer for 5 min at 14.5 ml/h and then eluted with 10-fold diluted polybuffer 74–HCl (pH 4.0) (Pharmacia) at 6.5 ml/h. Fractions of 1.3 ml were tested for activity toward NPA and those with activity were individually concentrated with Centricon-10 for 30–60 min, depending on activity. Each concentrated fraction was analyzed for α-NA activity on isoelectric focusing and fractions with only one band of the same pI were pooled for purity analysis with SDS–PAGE. Those containing more than one band were collected and concentrated to <0.5 ml for further purification. In the final step, the enzyme solution was introduced onto a Sephadex G-75 column equilibrated as Sepharose 6B column given previously and the column was eluted with 0.02 M Tris–HCl buffer (pH 7.2) at 6.5 ml/h. Fractions (1.1 ml) were tested for NPA activity and active fractions were pooled and analyzed for purity with SDS–PAGE. All purification steps were performed at 4°C.

Electrophoresis

SDS–PAGE was performed according to Laemmli (1970) using 3.75% acrylamide stacking gel and 12.5% acrylamide running gel and Bio-Rad Mini-Protean II electrophoresis cell. The proteins were stained with Coomassie blue (Fairbanks *et al.*, 1971). Isoelectric focusing (IEF) between pH 4.5 and 5.4 was performed on Bio-Rad model 111 mini IEF cell according to the manufacturer's instruction. The gel was stained with Coomassie blue for proteins or for carboxylesterase activity using α-NA as substrate.

Measurement of carboxylesterase activity

Both BPH homogenates and purified carboxylesterases were used for activity measurements. To prepare the homogenate, 40 mg adult BPH was homogenized with a Polytron in 10 ml 0.02 M sodium phosphate buffer (pH 7.8) at 0°C. The homogenate was centrifuged (Sigma 202 MK) at 10,000 *g* for 15 min at 4°C. The supernatant was filtered through cheesecloth and used as the enzyme source. For measurements of subcellular distribution of carboxylesterase activity, the crude homogenate was subject to a series of centrifugation (Sigma 202 MK and Beckman L5-40). At least three samplings of BPH were taken for each determination.

The method of van Asperen (1962) was modified for carboxylesterase activity toward α-NA, β-NA and α-naphthyl butyrate (α-NB). Proper amount of enzyme solution and 40 μl 0.02 M substrate in ethanol were dissolved in 0.02 M sodium phosphate buffer (pH 7.8) to make a final volume of 2.5 ml. The reaction was terminated after 10 min at 28°C with 0.5 ml 0.3% fast blue B salt solution (with 3.5% SDS). Absorbance (600 nm for α-NA and α-NB and 555 nm for β-NA) was read with a Beckman DU-70 spectrophotometer and enzyme activity was determined from standard curves made from α- and β-naphthol. For NPA activity, absorbance change at 400 nm was recorded for 1 ml reaction mixture at 25°C in 0.1 M Tris–HCl buffer (pH 8.2) that contained proper amount of enzyme solution and 10 μl 0.05 M NPA (in acetone). Enzyme activity was calculated using molar extinction coefficient (16.36 mM^{-1} cm^{-1}) of 4-nitrophenol (Ljungquist and Augustinsson, 1971). No enzyme was included for the control. At least three replicates were made. For K_m and V_{max} determinations, purified carboxylesterase isozymes were used and five concentrations (30–100 μM) of NPA were used. Results were analyzed by a double reciprocal plot.

For hydrolysis of insecticides, proper amounts of purified carboxylesterase isozymes and insecticides (in ethylene glycol monomethyl ether) were added to 0.1 M Tris–HCl buffer (pH 8.0) to make a final volume of 1.0 ml. The insecticides used were 10 μl of 10 mg/ml malathion and malaoxon solution, 15 μl of 1 mg/ml parathion solution, 20 μl of 1 mg/ml *trans*/*cis*-permethrin and cypermethrin. After 30–60 min incubation at 37°C, 4 ml *n*-hexane was added to terminate the reaction. The remaining insecticides were quantitated using gas-chromatograph. No enzyme was added for the controls. Two or three replicates were carried out. A Varian 3400 gas–liquid chromatograph equipped with 1.5% OV-17 and 1.95% OV-210 glass column (2 mm × 2 m) was used. For organophosphorus insecticides, a flame photometric detector (P-mode) at 300°C and a column of 240°C were used; carrier gas velocity was 20 ml/min. For pyrethroids, a ^{63}Ni electron capture detector at

320°C and a column of 270°C were used; carrier gas velocity was 40 ml/min.

Western blot analysis

Polyclonal antiserum against purified E_1 was raised in rabbit using routine procedures and serum collected was partially purified by ammonium sulfate fractionation (Harlow and Lane, 1988). After separation by SDS–PAGE, the proteins were transferred to nitrocellulose paper and Western blot was performed (Sambrook *et al.*, 1989). The secondary antibody used was alkaline phosphatase conjugated affinipure goat antirabbit IgG at 1:5000 dilution with bromochloroindolyl phosphate/nitroblue tetrazolium as substrate.

Inhibition and recovery of carboxylesterases

To 0.1 M Tris–HCl buffer (pH 8.2) proper amount of purified carboxylesterases and 10 μl of varied concentrations of malaoxon, paraoxon or methyl paraoxon (dissolved in ethylene glycol monomethyl ether) were added. After 2 min incubation at room temperature, 10 μl of 0.05 M NPA was added. The remaining activity was determined as described before. The control had only the solvent used to dissolve the organophosphates. Two to three replications were made. I_{50} was calculated.

The recovery of inhibited carboxylesterase was determined according to Cuany *et al.* (1993). To 2.5 ml purified E_1 was added 25 μl of 0.03 M paraoxon or malaoxon (in ethylene glycol monomethyl ether). After 1 h of incubation at 0°C, the mixture was passed through a Sephadex G-75 column (1 × 20.5 cm) which was subsequently eluted with 0.02 M Tris–HCl buffer (pH 7.2) at 10 ml/h. Fractions of 1 ml were collected and monitored for 280 nm absorbance. Each fraction was tested for antiacetylcholinesterase (AChE) activity. Protein fractions free from unbound malaoxon or paraoxon were pooled and carboxylesterase activity toward NPA was determined after 1, 2 and 3 h. For the control, the same procedures were followed except that the solvent used to dissolve the organophosphates was added. Fractions corresponding to those of insecticide treatments were also tested for antiAChE activity. AChE from bovine erythrocyte was used and AChE activity was determined using the method of Ellman *et al.* (1961).

TABLE 1. Subcellular distribution of carboxylesterase activity in the brown planthopper

Subcellular fraction	Total protein		Total activity[a]		Specific activity
	mg	%	μmol/min	%	μmol/min/mg protein
1000 *g* ppt	1.99 ± 0.10[b]	33	0.33 ± 0.02	4.1	0.17 ± 0.01
10,000 *g* ppt	0.83 ± 0.05	14	0.45 ± 0.01	5.5	0.53 ± 0.04
100,000 *g* ppt	0.90 ± 0.05	15	0.20 ± 0.02	2.4	0.22 ± 0.01
100,000 *g* sup	2.29 ± 0.01	38	7.14 ± 0.06	88	3.12 ± 0.03

[a]α-naphthyl acetate was used as substrate.
[b]Mean ± SE of three replicates.

Labeling of carboxylesterases with [1,3-^{3}H]diisopropyl fluorophosphate (DFP)

A homogenate of BPH in 0.02 M sodium phosphate buffer (pH 7.8) was centrifuged at 10,000 *g* (4°C) for 30 min. 0.5 ml supernatant (equivalent to 50 mg BPH) was added to 20 μCi [^{3}H]DFP (10 Ci/mmol, NEN Inc.) dissolved in propylene glycol. After 1 h incubation at room temperature the mixture was dialyzed overnight. The proteins were separated with SDS–PAGE or IEF and the dried gel was processed for fluorography on Kodak X-ray film.

Protein determination

A bicinchoninic acid protein assay was used according to the manufacturer's (Pierce) instruction whenever sufficient protein was available. Otherwise, absorbance at 280 nm was used for estimation. Bovine serum albumin was used as reference.

RESULTS

Purification of carboxylesterases in BPH

Nearly 90% of the total carboxylesterase activity in BPH was found in the supernatant after the sedimentation of microsomal fraction at 100,000 *g* ultracentrifugation of the homogenate (Table 1). Sepharose 6B chromatography of the fraction precipitated between 40 and 60% ammonium sulfate (containing 65% of the total carboxylesterase activity) resulted in *c.* 6-fold increase of specific activity [Fig. 1(A) and Table 2]. More than 10 molecular forms of carboxylesterase in terms of activity toward α-naphthyl acetate [Fig. 1(B)] were observed on IEF gel. To resolve these forms chromatofocusing chromatography (pH 6.2 to 4.0) was used and the three most active ones, E_1, E_2 and E_3, were collected [Fig. 2(A)]. Further gel filtration on Sephadex G-75 of E_2 fractions removed a smaller contaminating protein [Fig. 2(B)]. Table 2 summarizes the results of a typical purification experiment.

Characterization of carboxylesterase isozymes in BPH

Both the molecular mass and pI of the three purified carboxylesterase isozymes differed slightly (Fig. 3 and Table 3), and they did not appear to vary significantly in terms of the kinetic parameters, K_m and V_{max}, toward NPA (Table 3). Their activities toward several model substrates are given in Table 4. While E_1 had the highest and E_3 had the lowest activity toward all four substrates tested, α-NB was the preferred substrate for all three isozymes.

Among the organophosphorus insecticides tested, only malathion could be hydrolyzed by these three isozymes and no degradation of malaoxon and parathion was detected (Table 5). These isozymes hydrolyzed *trans*-permethrin at considerably higher rates than they could hydrolyze *cis*- permethrin and cypermethrin. E_1 consistently displayed the highest and E_3 the lowest activity toward these insecticides (Table 5).

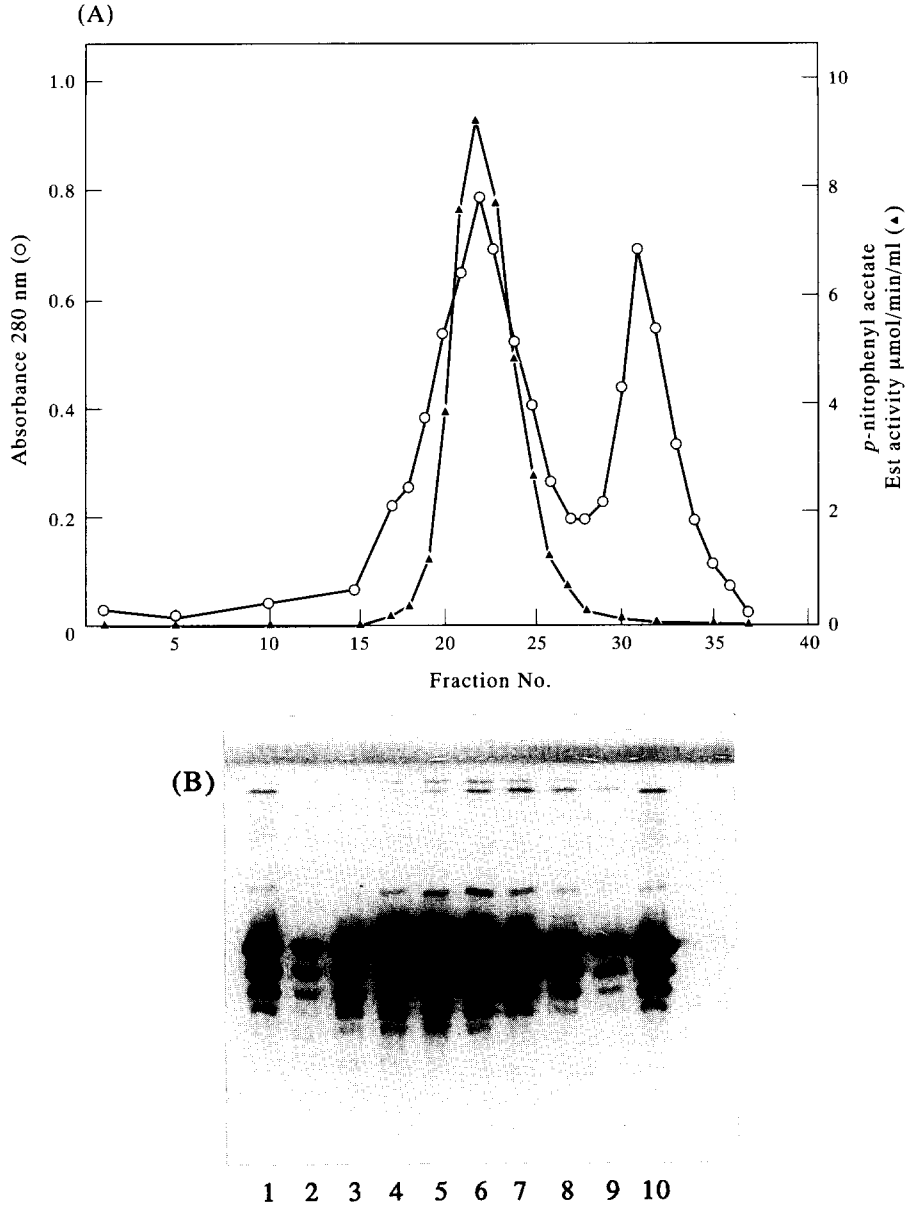

FIGURE 1. (A) Sepharose 6B chromatography and (B) subsequent IEF of carboxylesterases from the brown planthopper. The gel was stained for activity toward α-naphthyl acetate. Lanes 1 and 10, homogenate; lanes 2–9, fraction Nos 19–26.

Paraoxon proved to be a very potent inhibitor of the carboxylesterases of BPH while methyl paraoxon and malaoxon were also strong inhibitors (Table 6). There was no obvious difference in sensitivity to these organophosphates among the three isozymes (Table 6). To study the recovery of inhibited carboxylesterases, the incubation mixture of purified E_1 and paraoxon or malaoxon was passed through Sephadex G-75 column.

TABLE 2. Purification of carboxylesterase isozymes (E_1, E_2 and E_3) of a field (F) strain of the brown planthopper

Step	Total protein (mg)	Total activity[a] (μmol/min)	Specific activity (μmol/min/mg protein)
100,000 *g* sup	105	303	2.89
40–60% $(NH_4)_2SO_4$ ppt	14.4	196	13.6
Sepharose 6B	9.7	165	17.0
Chromatofocusing			
E_1	1.13	78.6	69.3
E_3	0.34	5.2	15.3
Sephadex G-75			
E_2	0.26	8.2	31.5

[a]α-naphthyl acetate was used as substrate.

The first peak (monitored at 280 nm) was tested for possible presence of free insecticide by measuring its inhibition of bovine erythrocyte acetylcholinesterase (AChE). Up to 99% of the AChE activity of the control was detected when the eluate of the first peak was added (data not shown), indicating the paraoxon– or malaoxon–E_1 complex was free from unbound insecticides. (The second peak which was identified as free insecticide caused 97% reduction of AChE activity under similar conditions. Data not shown.) Practically no recovery of the inhibited E_1 was observed in 3 h, suggesting insignificant dephosphorylation of the inhibited E_1 (data not shown).

The significant cross-reactivity between E_1 and E_2/E_3 in Western blot analysis implies that these three carboxylesterase isozymes of BPH were immunologically related [Fig. 4(A)].

Carboxylesterase isozymes in different strains of BPH

Compared with the susceptible strain, the carboxylesterase activity toward α-NA of two resistant BPH strains increased with their resistance levels to malathion

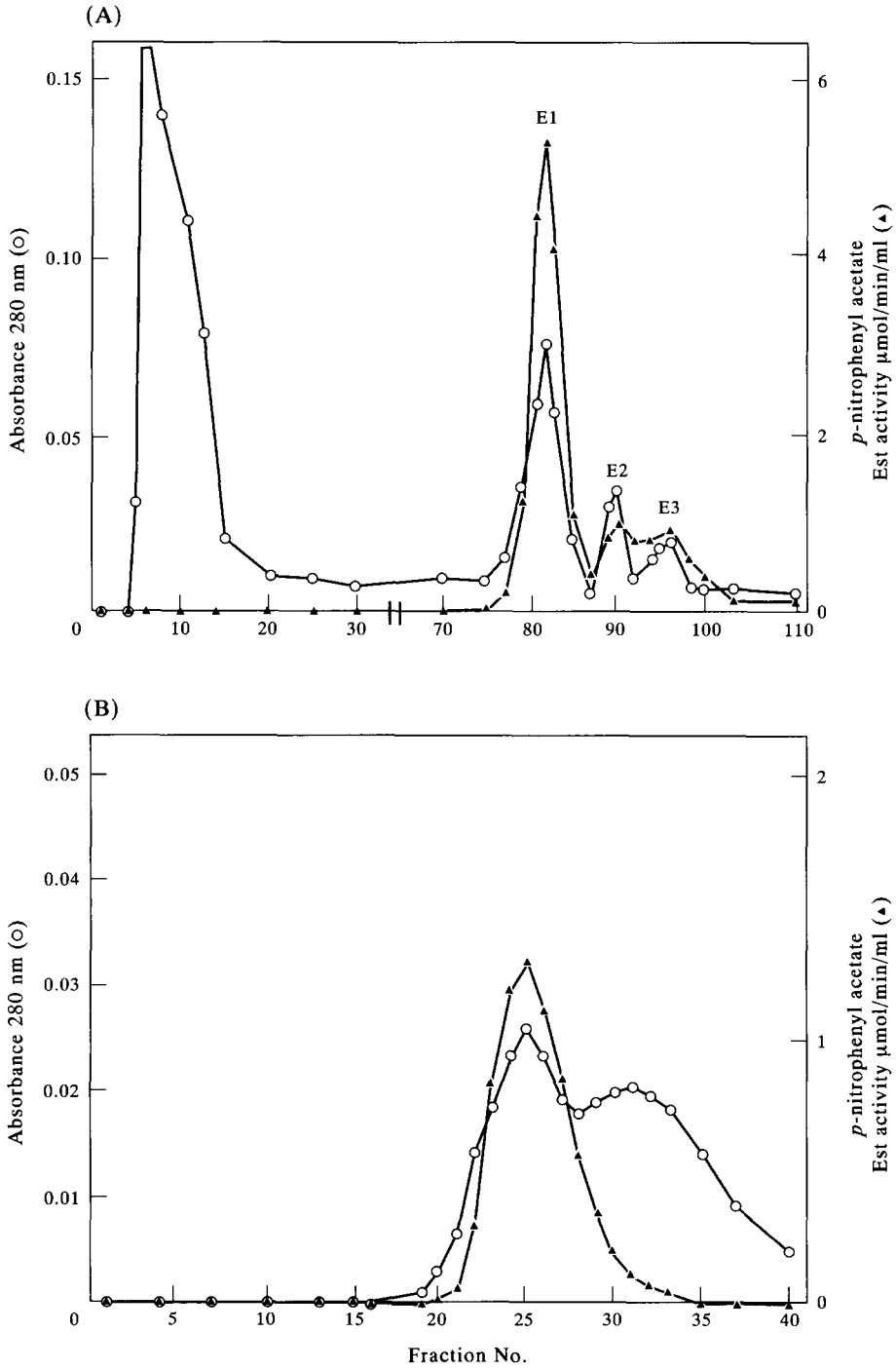

FIGURE 2. (A) Chromatofocusing chromatography of isozymes E_1, E_2 and E_3 in fraction Nos 19–26 of Sepharose 6B chromatography of carboxylesterases from the brown planthopper, and (B) subsequent Sephadex G-75 chromatography of E_2 fractions.

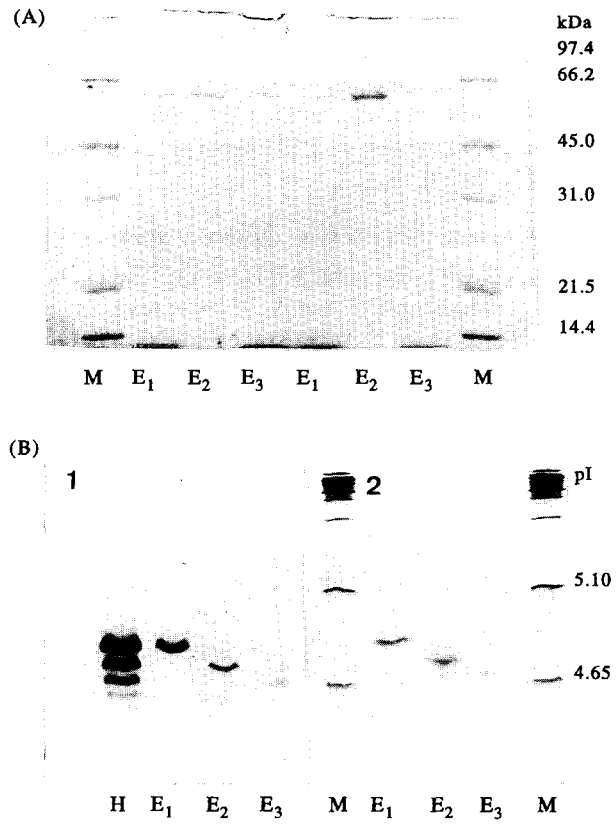

FIGURE 3. (A) SDS–PAGE and (B) IEF of three purified carboxylesterases E_1, E_2 and E_3 of the brown planthopper. M, protein markers; and H, homogenate. B_1 was stained for activity toward α-naphthyl acetate, and B_2 was stained with Coomassie blue.

and permethrin (Table 7). The same profiles of carboxylesterase isozymes were found in all strains tested (Fig. 5). Western blotting of homogenate supernatants from different strains probed with polyclonal antiserum against E_1 shows that malathion-resistant strain had considerably higher amounts of E_1 and immuno-related proteins [Fig. 4(B)]. In order to further quantify carboxylesterase in these strains, proteins were labeled with [^{3}H]DFP following the separation of homogenates by SDS–PAGE and IEF. The fluorograph clearly indicates that the resistant field F strain had higher quantity (*c.* 4-fold) of DFP-binding proteins than the susceptible P strain [Fig. 6(A)]. While the quantities of all three carboxylesterase isozymes were higher in resistant strains, E_1 was overproduced to the greatest extent [Fig. 6(B)].

TABLE 3. Biochemical characteristics of three purified carboxylesterase isozymes of the brown planthopper

Isozyme	Subunit kDa	pI	K_m^a (mM)	V_{max} (μmol/min/mg protein)
E_1	*c.* 62.0	*c.* 4.87	0.198 ± 0.025[b]	90.65 ± 8.57
E_2	*c.* 63.2	*c.* 4.76	0.246 ± 0.033	54.97 ± 5.45
E_3	*c.* 64.0	*c.* 4.68	0.159 ± 0.019	19.69 ± 1.36

[a]4-nitrophenyl acetate was used as substrate.
[b]Mean ± SE of three replicates.

TABLE 4. Substrate specificity of three purified carboxylesterase isozymes of the brown planthopper

Isozyme	Specific activity (μmol/min/mg protein) α-NA[a]	α-NB	β-NA	NPA
E_1	69.30 ± 0.96[b]	108.5 ± 2.8	72.63 ± 1.21	55.37 ± 1.83
E_2	31.50 ± 0.44	46.08 ± 0.65	33.93 ± 0.27	27.59 ± 0.38
E_3	15.32 ± 0.09	24.25 ± 0.22	17.11 ± 0.20	14.03 ± 0.07

[a]α-NA, α-naphthyl acetate; α-NB, α-naphthyl butyrate; β-NA, β-naphthyl acetate; and NPA, 4-nitrophenyl acetate.
[b]Mean ± SE of three replicates.

Immunological cross-reactivity with proteins of other insects

Proteins strongly reacted with E_1 antiserum were present in two homopterous insect pests of rice, i.e. the whitebacked planthopper (*S. furcifera*) and the smaller brown planthopper (*L. striatellus*) (Fig. 7). In addition, *M. persicae* and *A. gossypii* had proteins that reacted less strongly with E_1 antiserum. While *C. quinquefasciatus* and several other dipterous insects had proteins that barely reacted with E_1 antiserum, none was recognized in the lepidopterous insects tested, e.g. *Plutella xylostella*, *Trichoplusia ni*, etc. (data not shown).

DISCUSSION

Hydrolases have long been recognized as one of the major detoxifying enzymes involved in insect metabolism of xenobiotics (Dauterman, 1985). The enzymes detoxify insecticides by hydrolysis and sequestration (Devonshire and Moores, 1982; Cuany *et al.*, 1993). The three purified carboxylesterase isozymes of BPH, which constituted 1–2% of total proteins (Table 2), readily hydrolyzed malathion and *trans*-permethrin; cypermethrin and, especially, *cis*-permethrin were degraded only to a very limited extent. While these isozymes showed no hydrolytic activity toward parathion and malaoxon, they were strongly inhibited by the oxons of these organophosphorus (OP) insecticides. Somewhat less inhibition of these isozymes by carbaryl and *cis*-permethrin (I_{50}s in the range of 10^{-6} M) was also observed (data not shown). No recovery of activity of malaoxon- or

TABLE 5. Degradation of some insecticides by three purified carboxylesterase isozymes of the brown planthopper

Insecticide	μmol degraded/h/mg protein E_1	E_2	E_3
Malathion	16.08 ± 0.39[a]	10.56 ± 0.17	3.20 ± 0.07
Malaoxon	ND[b]	ND	ND
Parathion	ND	ND	ND
Trans-permethrin	9.06 ± 0.19	4.21 ± 0.15	2.35 ± 0.01
Cis-permethrin	0.94 ± 0.07	ND	ND
Cypermethrin	1.84 ± 0.14	0.79 ± 0.02	0.36 ± 0.04

[a]Mean ± SE of three replicates.
[b]ND: not detected. Detection limit: 3.0 nmol/h/mg protein for *cis*-permethrin; 0.64 and 0.36 μmol/h/mg protein for malaoxon and parathion, respectively.

TABLE 6. Inhibition of three purified carboxylesterase isozymes of the brown planthopper

Isozyme	I_{50} M		
	Paraoxon	Methyl paraoxon	Malaoxon
E_1	5.6×10^{-9}	3.7×10^{-7}	2.0×10^{-7}
E_2	4.9×10^{-9}	4.5×10^{-7}	1.6×10^{-7}
E_3	4.4×10^{-9}	3.4×10^{-7}	2.9×10^{-7}

paraoxon-inhibited E_1 was found within 3 h after the removal of unbound oxons. Thus, carboxylesterase of BPH appeared to exert dual actions in the detoxication of insecticides, i.e. a catalytic protein for the hydrolysis of some insecticides, i.e. malathion and *trans*-permethrin, and a binding protein for the oxons of some OPs, i.e. paraoxon, methyl paraoxon and malaoxon, and possibly some carbamates and pyrethroids. Motoyama *et al.* (1984) reported similar findings in the green rice leafhopper, *N. cincticeps*.

Sucking insects such as BPH, *N. cincticeps* and the smaller brown planthopper, *L. striatellus*, typically had low P450-dependent monooxygenase and high carboxylesterase activities as compared with some chewing insects. The metabolic mechanisms of insecticide resistance observed in these homopterous species (Ozaki and Kassai, 1970; Miyata and Saito, 1976; Dai and Sun, 1984) were suggested to reflect this fundamental difference in the makeup of detoxifying enzymes (Hung *et al.*, 1990). Resistant BPH strains had considerably higher

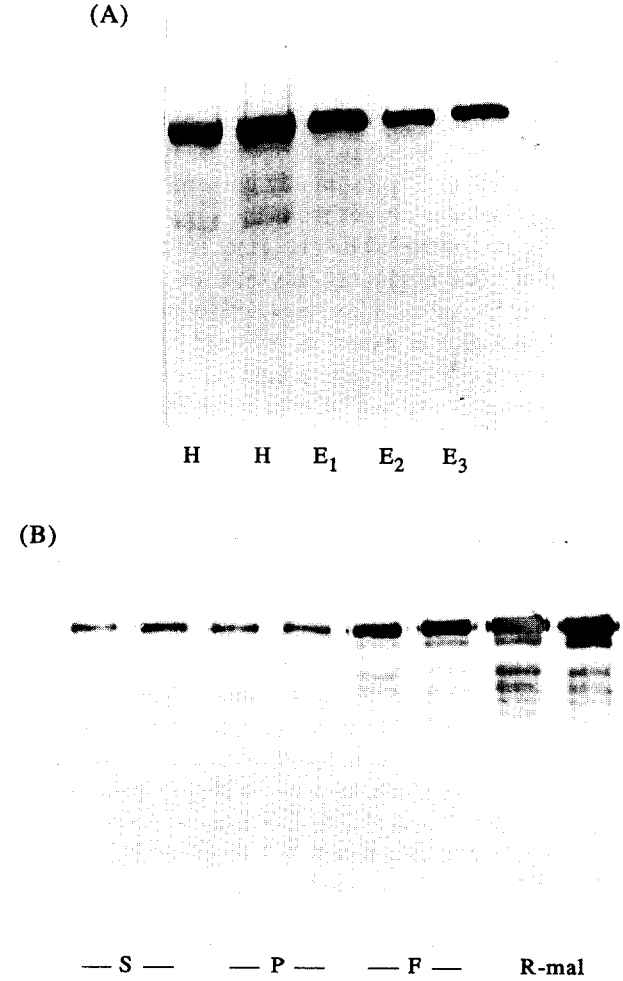

FIGURE 4. Western blot analysis with polyclonal antibody against E_1 after SDS–PAGE of (A) three purified carboxylesterases E_1, E_2 and E_3 (0.45 μg each) and (B) homogenates (same amount of proteins) of several strains of the brown planthopper. H, homogenate; S and P, susceptible strains; F, field strain; and R-mal, malathion-resistant strain.

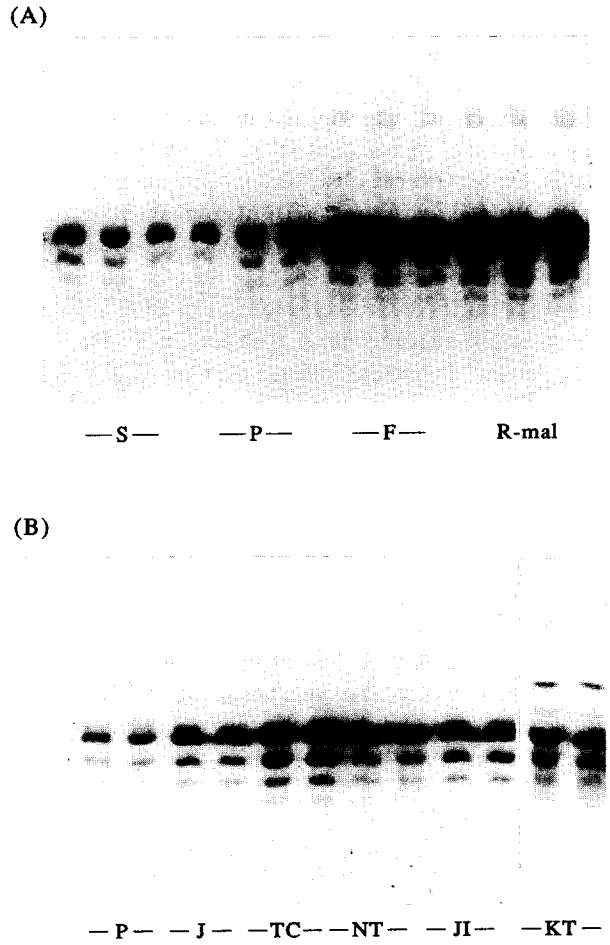

FIGURE 5. IEF analysis of carboxylesterases of several strains of the brown planthopper. (A) S and P, susceptible strains; F, field strain; and R-mal, malathion-resistant strain. (B) P, susceptible strain; J, Japan strain; TC, Taichung strain; NT, Nantou strain; JI, Chiayi strain; and KT, Kwangtung strain. The gel was stained for activity toward α-naphthyl acetate. 5.6 μg of proteins was loaded in each lane.

TABLE 7. Carboxylesterase activity of three strains of the brown planthopper

Strain[a]	Carboxylesterase activity[b]	
	μmol/min/mg adult	Ratio
P	38.9 ± 1.6[c]	1.0
F	128 ± 5	3.3
R-mal	286 ± 8	7.4

[a]P, susceptible strain; F, field strain (resistance ratios to malathion and permethrin 12× and 4×, respectively); and R-mal, malathion-resistant strain (RR to malathion and permethrin 46× and 15×, respectively).

[b]α-naphthyl acetate was used as substrate.

[c]Mean ± SE of three replicates.

carboxylesterase activity (Table 7, Chung and Sun, 1983) than the susceptible strain. Both Western blot [Fig. 4(B)] and [^{3}H]DFP labeling (Fig. 6) show clearly that this increase of activity was due to an overproduction of carboxylesterases in resistant BPH. In view of the consistent isozyme patterns observed in both susceptible and resistant BPH [Figs 5 and 6(B)], it appears that E_1, E_2 and E_3 (and possibly other carboxylesterase isozymes) were all overproduced.

The three purified carboxylesterases of BPH displayed more common features than distinct ones. They had similar subunit molecular masses and pIs (Table 3 and Fig. 3); and they were immunologically related [Fig. 4(A)]. They could bind with and be inhibited by oxons and some other insecticides that were not their preferred substrates (Table 6). However, E_1 consistently exhibited the highest and E_3 the lowest activity toward all substrates tested, with a 4–5-fold difference (Tables 4 and 5). Inclusion of protease inhibitors, e.g. phenylmethanesulfonyl fluoride or trypsin inhibitor, in the buffer used for the homogenization of BPH did not change the electrophoretic pattern of carboxylesterase isozymes. Purified isozymes did not exhibit any changes with time in catalytic activity or electrophoretic behavior. Thus, the possibility of these isozymes being the products of partial proteolysis was excluded. Chung and Sun (1983), using starch gel electrophoresis, studied the carboxylesterase patterns of a susceptible and a malathion-resistant strains, F_1, F_2 and backcross progeny of BPH. The one strongly stained spot (presumably corresponding to E_1, E_2 and E_3) that was absent from the susceptible strain, appeared to be controlled by one gene. If this suggestion should stand, one might say this gene encoding E_1, E_2 and E_3 (and possibly other less active isozymes) was expressed to a greater extent in resistant strains than in susceptible ones. The isozymes might represent the products of different posttranslational modifications of the nascent protein.

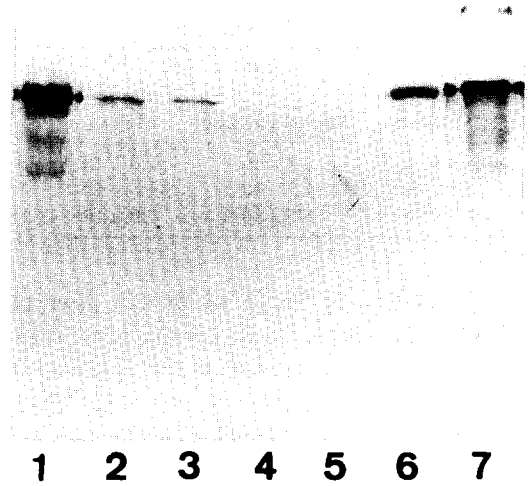

FIGURE 7. Western blot analysis with polyclonal antibody against E_1 after SDS–PAGE of homogenates of some homopterous insects. Lane 1, brown planthopper; lane 2, *M. persicae*; lane 3, *A. gossypii*; lane 4, *N. cincticeps*; lane 5, *R. dorsalis*; lane 6, *S. furcifera*; lane 7, *L. striatellus*; and lane 8, *P. maidis*. 6.25 μg of proteins was loaded in each lane.

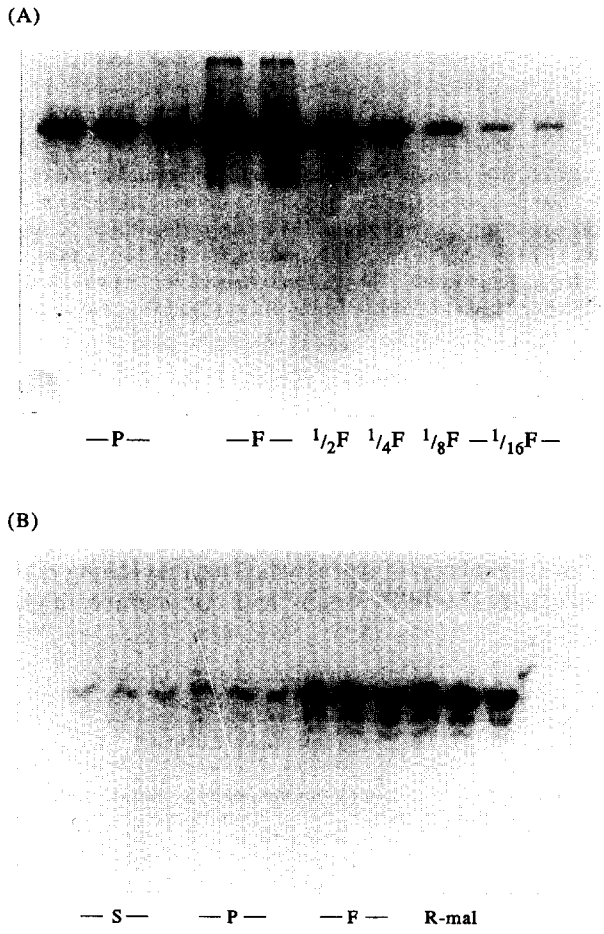

FIGURE 6. Fluorographic analysis of [^{3}H]DFP-labeled carboxylesterases following (A) SDS–PAGE (25 μg of proteins was loaded in each lane) and (B) IEF (10 μg of proteins was loaded in each lane) of homogenates of several strains of the brown planthopper. S and P, susceptible strains; F, field strain; and R-mal, malathion-resistant strain.

Ozaki (1969) first reported high carboxylesterase activity in OP resistant *L. striatellus* and Miyata *et al.* (1976) detected in agar gel electrophoresis only one carboxylesterase band that hydrolyzed malathion. This planthopper and the whitebacked planthopper, *S. furcifera*, had proteins with molecular mass and immunological features similar to carboxylesterases of BPH (Fig. 7). A comparison of detoxifying enzymes of these three planthoppers will be interesting in terms of toxicological significance. A weak immunological cross reactivity between carboxylesterases of BPH and proteins of similar molecular mass (62–64 kDa) in *M. persicae* was observed. Whether the protein(s) recognized by E_1 antiserum of BPH was E_4, the carboxylesterase isozyme well studied in this aphid (Devonshire and Field, 1992), is uncertain.

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