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# Purification and Characterization of Acetylcholinesterase from Oriental Fruit Fly [*Bactrocera dorsalis* (Hendel)] (Diptera: Tephritidae)

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An acetylcholinesterase (AChE, EC 3.1.1.7) was purified from the head of the insecticide susceptible oriental fruit fly, *Bactrocera dorsalis* (Hendel), by affinity chromatography of Triton X-100 extract. The degree of purification was about 8183-fold with recoveries of 52%. The molecular mass of purified AChE was 116 kDa for its native protein (nonreduced form) and 61 kDa for its subunits (reduced form) as revealed on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE), suggesting that the homodimer of AChE linked with disulfide bonds. Nondenaturing PAGE of the purified AChE revealed only one molecular form. The maximum velocities ($V_{max}$) for hydrolyzing acetylthiocholine (ATC), propionylthiocholine, and S-butyrylthiocholine were 833.3, 222.2, and 57.5 $\mu$mol/min/mg, and the Michaelis constants ($K_m$) were 87.9, 26.9, and 195.3 $\mu$M, respectively. More than 97% of AChE activity was inhibited by 10 $\mu$M eserine or BW284C51, but only 53% of the activity was inhibited by ethopropazine at the same concentration. On the basis of the substrate and inhibitor specificities, the purified enzyme appeared to be a true AChE. Nevertheless, the purified AChE exhibited some distinctive characteristics including (i) a lack of the substrate inhibition phenomenon when using ATC as the hydrolyzing substrate and (ii) a higher $V_m$ value for ATC than AChE from other insect species. These biochemical properties may show that AChE purified from the oriental fruit fly may have structural differences from those of other insect species.

**KEYWORDS: Acetylcholinesterase; kinetics; purification**

## INTRODUCTION

Acetylcholinesterase (AChE, EC 3.1.1.7) plays an important role in neurotransmission at cholinergic synapses by rapidly hydrolyzing the excitatory neurotransmitter acetylcholine into choline and acetic acid (*1*−*4*). Because it is the key enzyme in an insect's central nervous system, several inhibitors of this enzyme have been developed (*5*, *6*). Two classes of compounds, organophosphorus and carbamate insecticides, are commonly used to quasi-irreversibly inhibit AChE. However, more than 30 years of use of these insecticides has resulted in several resistant insect species whose altered AChEs are less sensitive to insecticide with these compounds (*7*, *8*).

Because it is biologically significant to insect neurophysiology and pest resistance, AChE has attracted a great deal of attention. Much research has explored how AChE alteration may relate to insect resistance. However, most of these studies have used unpurified AChE, the homogenates of body parts, or the whole body (*9*−*15*). Crude AChE extracts, containing contaminating non-AChE factors, such as carboxylesterases, can affect the measurement of AChE and produce misleading results.

AChE has been purified from only a few insect species, including *Musca domestica* (*14*), *Lygus hesperus* Knight (*16*), *Leptinotarsa decemlineata* (*17*), *Helicoverpa armigera* (*18*), *Diabrotica virgifera virgifera* (*6*), *Galleria mellonella* L. (*19*), *Schizaphis graminum* (*20*), and the cotton aphid, *Aphis gossypii* Glover (*21*).

In Taiwan, the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is the most serious fruit pest, damaging up to 180 000 ha of fruit trees annually. Organophosphorus and carbamate insecticides are commonly used to control this pest. Field populations have developed resistance progressively to the organophosphorus and carbamate insecticides as revealed from recent resistance monitoring (*22*). Altered AChE is considered a common cause of resistance in the fruit fly toward organophosphorus insecticides (*23*, *24*). However, the biochemical characteristics of AChE in the oriental fruit fly have not been done.

We present here a simple, effective, two-step method for the purification of oriental fruit fly AChE and have characterized

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the biochemical and molecular properties of the purified enzyme. The knowledge obtained from this study should facilitate further investigations on this important enzyme in the oriental fruit fly and be helpful in the development of strategies for the resistance management of this pest.

## MATERIALS AND METHODS

**Insects.** The oriental fruit fly used in this study came from an insecticide susceptible laboratory strain. Three to five days old adults were either used immediately or frozen under −80 °C for further purification of AChE.

**Chemicals.** Acetylthiocholine iodide (ATC), 1,5-bis(4-allyldimethylammonium phenyl)pentan-3-one dibromide (BW284C51), S-butyrylthiocholine iodide (BTC), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), eserine hemisulfate, ethopropazine hydrochloride, $\beta$-mercaptoethanol, propionylthiocholine iodide (PTC), and Triton X-100 were purchased from the Sigma Chemical Company (St. Louis, MO). The ECH Sepharose 4B molecular markers (High Molecular Weight-SDS Calibration Kit for Electrophoresis and High Molecular Weight Calibration Kit for Native Electrophoresis) were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Tetraethylammonium iodide ($Net_4I$), procainamide, and *N*-ethoxycarbonyl-2-ethoxy-1, 2-dihydroquinoline (EEDQ) were purchased from ACROS Organics (New Jersey, United States). The bovine serum albumin (BSA) protein assay standard II was purchased from Bio-Rad Laboratories (Hercules, CA).

**Optimization of Conditions for AChE Extraction.** To determine the optimal pH for the AChE extraction, 0.1 g of frozen oriental fruit fly heads was homogenized in 5 mL of ice-cold 0.1 M phosphate buffer containing 0.5% (v/v) Triton X-100 at each of seven pH conditions ranging from 5.8 to 8.0. The homogenates were centrifuged for 15 min at 13000*g* at 4 °C, and the supernatants were used as the enzyme sources for the AChE assay according to the spectrophotometric method of Ellman et al. (*25*) using ATC as the substrate. One unit of AChE activity was defined as the amount of enzyme needed to hydrolyze 1 $\mu$mol of ATC per min at 37 °C under the assay condition.

To determine the optimal concentration of Triton X-100 to extract AChE from the oriental fruit fly, six batches of 0.1 g of frozen oriental fruit fly heads were homogenized in 5 mL of ice-cold 0.1 M phosphate buffer (pH 7.4). Each batch contained a different concentration of Triton X-100, ranging from 0 to 1.0% (v/v). The homogenates were centrifuged, and the supernatants were used for the AChE assay as described above.

**Purification of AChE.** After extraction by Triton X-100, AChE was purified from the heads of adult fruit fly by affinity chromatography using procainamide, a ligand specific for the choline binding site. The procainamide affinity column was prepared according to manufacturer's instructions and stored at 4 °C using ECH Sepharose 4B as the coupling gel and EEDQ for ligand immobilization (*20*, *26*, *27*). Briefly, procainamide and EEDQ solutions were prepared by dissolving 0.68 g of procainamide in 5 mL of water and 0.062 g of EEDQ in 5 mL of ethanol. The two solutions were mixed and then added to 10 mL of ECH Sepharose 4B. The procainamide−ECH Sepharose 4B coupling reaction was carried out by gently mixing the solution with a rotator at 4 °C overnight. The procainamide-coupled Sepharose 4B was poured into an 1 cm × 7.5 cm glass column and washed successively with 50% ethanol, 20 mM Tris-HCl buffer (pH 7.0), and 1 M NaCl solution. The column then was equilibrated with 0.1 M phosphate buffer (pH 7.4) containing 0.5% (v/v) Triton X-100.

To extract AChE from the fly head, 8 g of frozen heads was homogenized in 80 mL of ice-cold 0.1 M phosphate buffer (pH 7.4) containing 0.5% (v/v) Triton X-100 and centrifuged at 13000*g* for 15 min at 4 °C. The supernatant was first filtered through two layers of cheesecloth and then through a Whatman no. 1 filter to remove the lipids. The filtrate, containing AChE, was applied to the procainamide-based Sepharose 4B affinity column equilibrated with the same extraction buffer. The column was washed extensively with 50 mM phosphate buffer, 0.05% (v/v) Triton X-100 (pH 7.4), and 50 mM NaCl (PTS) buffer until the absorbance at 280 nm fell below 0.01. The AChE was then eluted with 30 mM $Net_4I$ in PTS buffer. The constant flow rate was maintained at 30 mL/h with a peristaltic pump, and fractions of 1.0 mL were collected after the elution solution was applied to the column. The purity of AChE from oriental fruit fly was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE). The fractions containing purified AChE were pooled, dialyzed against PTS buffer to remove the $Net_4I$, and concentrated using a concentrator (Amicon, model 8050) at 4 °C.

**Determination of Protein Concentration.** The protein content was determined by the Bradford method using BSA as the standard (*28*). The spectrophotometric assays were carried out with a spectrophotometer at a wavelength of 595 nm.

**Electrophoretic Analysis of Purified AChE.** Electrophoresis was carried out with Hoefer Mighty Small II SE 250 and SE 260 Mini-Vertical Gel Electrophpresis Units (Piscataway, NJ). The SDS−PAGE (*29*) was used to estimate the molecular masses of the nonreduced and reduced AChE. The AChE samples were treated by incubation in 2% (w/v) SDS solution with or without 1.25% (v/v) $\beta$-mercaptoethanol in a dry bath at 100 °C for 5 min, and they were loaded onto an 10% SDS polyacrylamide gel. The gel was run for 1.5 h at 100 V at room temperature. AChE in the gels was localized by silver staining (*30*).

The procedure of nondenaturing PAGE was similar to the SDS−PAGE except that no SDS was used and it was performed in a 4 °C cold room during electrophoresis. For the analysis of the interaction between AChE and Triton X-100, both the gels and the electrode buffer were incorporated with or without 0.1% (v/v) Triton X-100 (*16*). The purified AChE was loaded (in duplicate) onto each lane of a 3−12% gradient polyacrylamide gel. After the gel was run for 2 h at 8 mA, it was cut into two halves. One half of the gel was used to stain the AChE activity with ATC as a substrate for 1 h at 37 °C by the method of Karnovsky and Roots (*31*) based on hydrolysis of a thiocholine ester and formation of an insoluble copper complex, whereas the other half was used to stain total proteins using the silver stain method (*30*).

**Effect of pH and Temperature.** The optimum pH for AChE activity was determined at 37 °C by varying the pH of the reaction from 5.0 to 8.8. The optimum temperature for AChE activity was determined by assaying the enzyme at various temperatures ranging from 15 to 45 °C.

**Thermal Stability.** Thermal inactivation of the AChE was determined by incubating the purified enzyme in a water bath for 10 min at various temperatures and then cooling it to room temperature for 5 min. This procedure was repeated at seven different temperatures ranging from 25 to 60 °C each in triplicate.

**Substrate Specificity and Kinetics of Purified AChE.** The substrate specificity of purified AChE was studied with three thiocholine esters (ATC, PTC, and BTC) according to the method of Ellman et al. (*25*) as modified by Zhu and Clark (*17*). We studied the AChE activities at 12 different concentrations (3−30000 $\mu$M) of each substrate. Controls in the absence of AChE were included in the assays to correct nonenzymatic hydrolysis of each substrate at high concentrations. The maximum velocities ($V_{max}$) and the Michaelis constants ($K_m$) for each substrate were determined according to a Lineweaver−Burk plot (*32*). The turnover number ($k_{cat}$) was calculated according to the molecular mass of purified AChE and $V_{max}$. The substrate specificity constant ($k_{cat}/K_m$) was determined based on $k_{cat}$ and $K_m$ (*33*).

**Inhibition of Purified AChE.** The inhibitor specificity of purified AChE was studied according to the method of Zhu and Brindley (*34*). Purified AChE was preincubated with nine different concentrations of each of three inhibitors (eserine, BW284C51, and ethopropazine) at 25 °C for 5 min. The remaining activity of AChE was determined. The median inhibition concentration ($IC_{50}$) for each inhibitor was determined based on log-concentration vs probit (% inhibition) regression analysis.

## RESULTS

**Optimization of Conditions for AChE Extraction.** The optimal pH value for oriental fruit fly AChE extraction was indicated by an increased enzyme activity from pH 5.8 to 6.8 and reached its peak at pH 7.4 (**Figure 1A**). No significant difference in the enzyme activity was noted between the ranges of pH 6.8 and 7.8. At pH 8.0, however, the enzyme activity

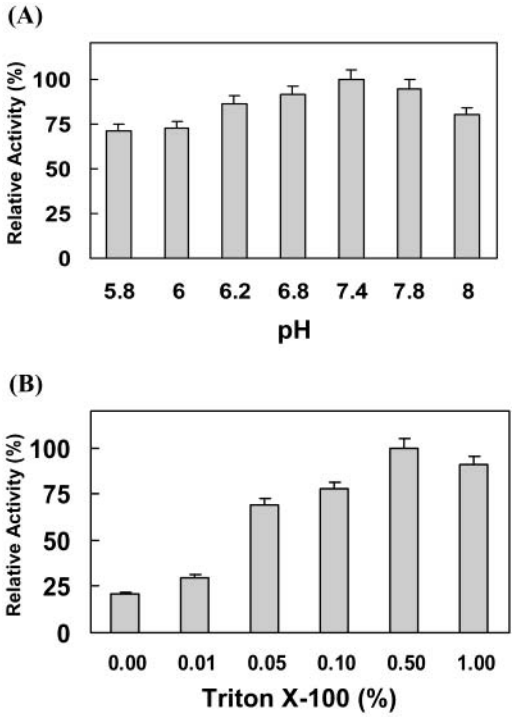


**Figure 1.** Optimal pH condition and Triton X-100 concentration for AChE extraction. (**A**) Effects of pH on AChE extraction. The percentage of AChE activity was calculated based on the highest AChE activity at pH 7.4. The results are the means of three replications ($n = 3$). (**B**) Effects of Triton X-100 on AChE extraction. The percentage of AChE activity was calculated based on the highest activity at 0.5% Triton X-100. The results are the means of three replications ($n = 3$).

**Table 1.** Summary of the Purification Steps for Oriental Fruit Fly AChE by Triton X-100 Extraction and Procainamide-Based Affinity Chromatography

| steps | total activity ($\mu$mol/min) | total protein (mg) | specific activity ($\mu$mol/min/mg) | yield (%) | purification factor (fold) |
|---|---|---|---|---|---|
| Triton X-100 extraction | 35.095 | 413.369 | 0.085 | 100 | 1 |
| affinity column | 18.237 | 0.026 | 690.931 | 51.966 | 8183 |

decreased. The condition for AChE extraction was further optimized by the addition of 0.5% (v/v) Triton X-100 (**Figure 1B**). The enzyme activity increased with increases in the Triton X-100 concentration and reached a maximum at 0.5% (v/v). The AChE activity was slightly inhibited at 1.0%. In summary, the optimal extraction condition for AChE purification would be 0.1 M phosphate buffer, pH 7.4, containing 0.5% (v/v) Triton X-100.

**Purification of AChE from the Oriental Fruit Fly.** The AChE from the oriental fruit fly was successfully purified by procainamide-based affinity chromatography following Triton X-100 extraction. Typically, the overall purification factor and yield were 8183-fold and 52%, respectively (**Table 1**). This yield was considerably higher than those found in previous purifications of other AChEs (*6*, *19*−*21*, *35*−*38*). Approximately 26 $\mu$g of purified AChE with a specific activity of about 690 $\mu$mol/min/mg protein was obtained from 8 g of the oriental fruit fly heads. These results were reproducible over several purification attempts during the course of the study. The degree of purification was further confirmed by SDS−PAGE using the silver staining method (see below).

**Molecular Properties of Purified AChE.** The SDS−PAGE analysis showed one major protein band for AChE samples

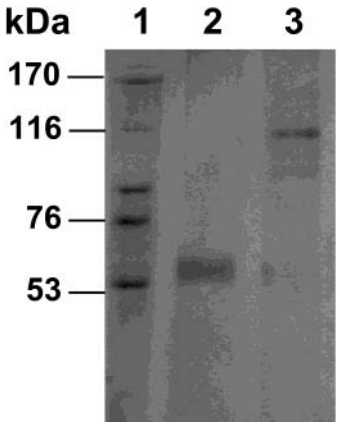


**Figure 2.** SDS–PAGE analysis of purified AChE. The purified AChE samples were mixed with the sample buffer with or without reducing agent and heated at 100 °C for 5 min in a dry bath. The treated samples were loaded onto a 10% SDS–polyacrylamide gel. Electrophoresis was run for 1.5 h at 100 V at room temperature. The protein bands were visualized by silver staining. Lane 1, molecular weight markers (High Molecular Marker-SDS Calibration Kit for Electrophoresis, Amersham Pharmacia Biotech); lane 2, AChE treated with the reducing agent $\beta$-mercaptoethanol; and lane 3, AChE not treated with reducing agent.

either treated or not treated with the reducing agent $\beta$-mercaptoethanol after the gel was stained by silver nitrate (**Figure 2**). The molecular mass of the purified AChE was 116 kDa for nonreduced (native) and 61 kDa for reduced AChE (subunit). These results indicated that each molecule of AChE consisted of two very similar or identical subunits connected with a disulfide bond.

Affinity chromatographically purified AChE was also analyzed by nondenaturing PAGE in the absence or presence of Triton X-100 in both the gels and the electrode buffer system. There was only one band in both electrophoretic conditions (**Figure 3**). The band migrated much more slowly in the presence of Triton X-100, which indicated that the purified AChE and Triton X-100 may interact.

**Characteristics of AChE.** The AChE was shown to have a pH optimum of about pH 7.4 (**Figure 4A**) and a temperature optimum of about 37 °C (**Figure 4B**). Irreversible denaturing of purified AChE mainly occurred above 45 °C (**Figure 5**), which made it seem as though AChE decreased sharply after exposition at 40 °C, after already being partially inactivated at 45 °C.

**Substrate and Inhibitor Specificities and Kinetic Analysis of Purified AChE.** Three substrates, including ATC, PTC, and BTC, were used to study the effect of substrate concentration on the activity of purified AChE from the oriental fruit fly (**Figure 6**). The optimal substrate concentrations were rather broad, and the substrate inhibition phenomenon at higher concentrations was not seen. Kinetic studies determined the affinity of AChE to its different substrates. The $K_m \pm$ SD values were 87.9 ± 9.5 $\mu$M for ATC, 26.9 ± 1.4 for PTC, and 195.3 ± 22.0 for BTC (**Table 2**). The hydrolyzing efficiencies of AChE for the three substrates, as indicated by their $V_{max}$ values, were 833.3 ± 19.3 $\mu$mol/min/mg protein for ATC, 222.2 ± 11.0 for PTC, and 57.5 ± 3.6 for BTC. These results indicated that the affinities of purified AChE to ATC and BTC were 3.3- and 7.3-fold, respectively, lower than that to PTC, as indicated by their $K_m$ values. In addition, the hydrolyzing efficiencies of purified AChE for ATC and PTC were 14.5- and 3.9-fold higher than that for BTC, as indicated by their $V_{max}$ values. The turnover numbers ($k_{cat}$) of AChE from oriental

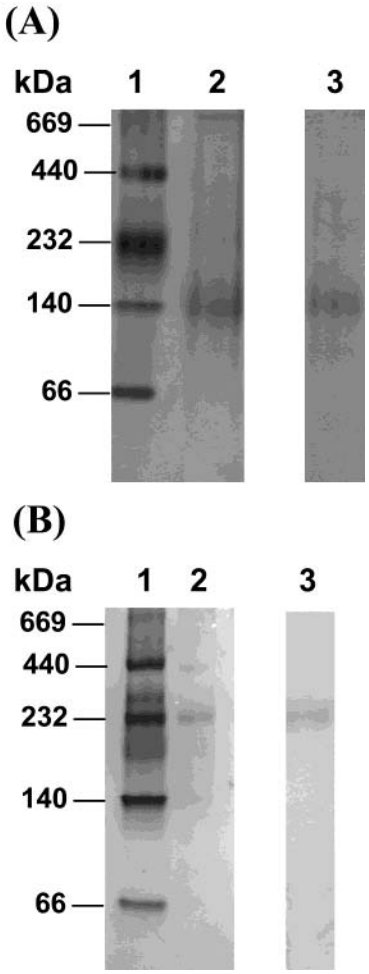


**Figure 3.** Nondenaturing PAGE analysis of the purified AChE in the absence (**A**) or presence (**B**) of 0.1% Triton X-100. The purified AChE was loaded to each lane of a 3–12% gradient polyacrylamide gel. The gel was run for 2 h at 8 mA in a 4 °C cold room. After electrophoresis, the gel was cut into two halves. One half was stained for AChE activity (lane 3) using ATC as a substrate. The color was developed for 1 h at 37 °C. The other half was stained for total protein with silver (lanes 1 and 2). Lane 1 was a molecular weight marker (High Molecular Marker Calibration Kit for Native Electrophoresis, Amersham Pharmacia Biotech).

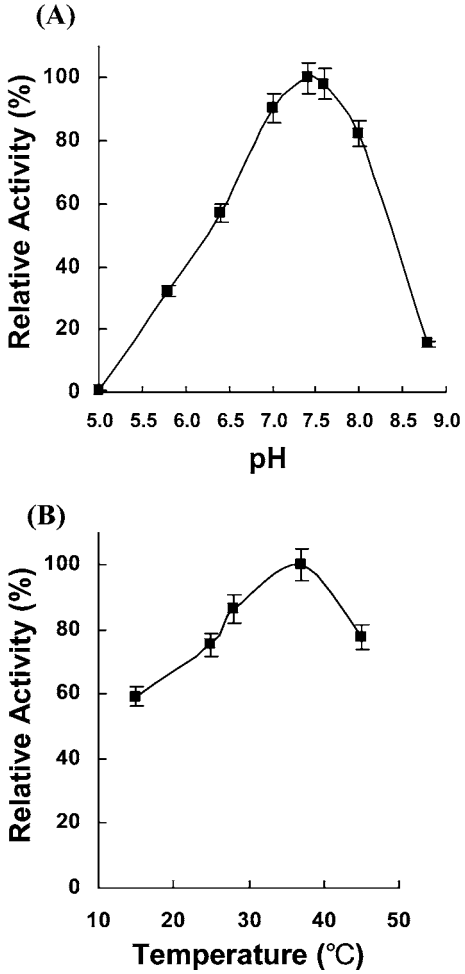


**Figure 4.** pH (**A**) and temperature (**B**) activity profiles of oriental fruit fly AChE. The activities are expressed as a percentage of the maximal activity, and the results are the means of three replications ($n = 3$). The purified enzyme was used.

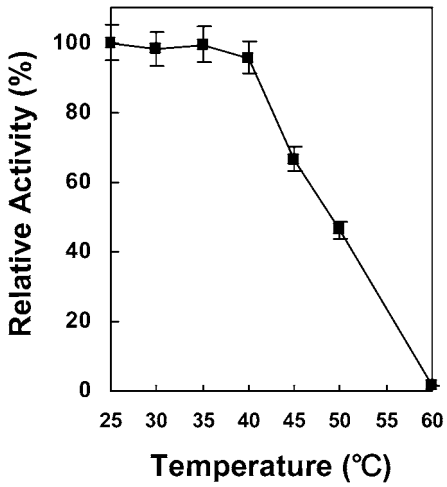


**Figure 5.** Thermal stability of oriental fruit fly AChE. The activities are expressed as a percentage of the maximal activity, and the results are the means of three replications ($n = 3$). The purified enzyme was used.

fruit fly for ATC, PTC, and BTC were 97000, 26000, and 6700, respectively. The substrate specificity constants ($k_{cat}/K_m$) of AChE from oriental fruit fly for ATC, PTC, and BTC were 1103, 968, and 34, respectively. The substrate specificity constant for ATC was 32-fold higher than that for BTC, indicating that AChE had a much higher specificity to ATC than to BTC.

The purified AChE was highly sensitive to inhibition by eserine, a general inhibitor for both AChE and butyrylcholinesterase (BChE, EC 3.1.1.8) and BW284C51, a relatively specific inhibitor for AChE, but was much less sensitive to inhibition by ethopropazine, a relatively specific inhibitor for BChE (**Figure 7**). At a concentration of 10 $\mu$M, both eserine and BW284C51 inhibited 99 and 97% of the enzyme activity, respectively. However, only 53% of the enzyme activity was inhibited by ethopropazine at the same concentration. The median inhibition concentrations ($IC_{50}$, mean ± SD) were $(2.80 \pm 0.57) \times 10^{-8}$ M for eserine, $(8.59 \pm 1.67) \times 10^{-8}$ M for BW284C51, and $(1.17 \pm 0.65) \times 10^{-5}$ M for ethopropazine.

## DISCUSSION

The two-step fly head AChE purification procedure that we developed, Triton X-100 extraction and procainamide-based affinity chromatography, afforded a 8183-fold purification factor with a yield of 52%. The purified enzyme was shown to be

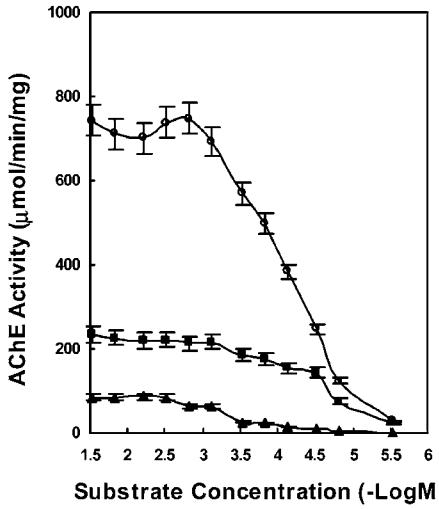


**Figure 6.** Effect of substrate concentrations on AChE activity. The effect of substrate concentration was investigated using the model substrates ATC (○), PTC (■), and BTC (▲). The results are the means of five determinations ($n = 5$).

**Table 2.** Kinetic Parameters of AChE Purified from Oriental Fruit Fly in Hydrolyzing Three Model Substrates[a]

| substrates | $K_m$ ($\mu$M) | $V_{max}$ ($\mu$mol/min/mg) | $V_{max}/K_m$ (ratio) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_m$ ($\mu M^{-1}$ $min^{-1}$) |
|---|---|---|---|---|---|
| ATC | 87.92 ± 9.50 | 833.33 ± 19.34 | 9.48 | 97000 | 1103 |
| PTC | 26.87 ± 1.42 | 222.22 ± 11.02 | 8.27 | 26000 | 968 |
| BTC | 195.29 ± 21.96 | 57.47 ± 3.56 | 0.29 | 6700 | 34 |

[a] The results are presented as the means ± SD ($n = 5$).

electrophoretically homogeneous with a single band resolved on SDS−PAGE using silver staining.

The degree of purification is not readily comparable between species as it obviously reflects the initial amount of extraneous tissue. However, in this study, extracts were made from only the heads of the insects. Differences caused by insect materials and assay conditions usually preclude direct comparisons of studies by different investigators. The increase of specific activity from 0.085 units/mg protein in the initial extract from heads to 691 units/mg protein in the purified AChE preparation indicated that AChE in the initial extract accounted for only 0.0123% of the total proteins. This amount is approximately half of the 0.02% value reported in the initial homogenate of *Drosophila* heads (*39*). The purification factor was larger than AChE purified from heads of *G. mellonella* (283-fold) (*19*) but smaller than that from heads of *L. decemlineata* (39000-fold) (*17*, *35*) and *D. virgifera virgifera* (20000-fold) (*6*). However, the activity yield of 52% was comparable to the published yields using the same method for other species, e.g., 54% for *Rhyzopertha dominica* (*5*) and 51% for *L. decemlineata* (*17*).

Using ATC as a substrate, the specific activity of purified AChE has been reported to be 1350 units/mg protein for *Drosophila* heads (*39*), 385 for horn fly (*40*), 50 for Western

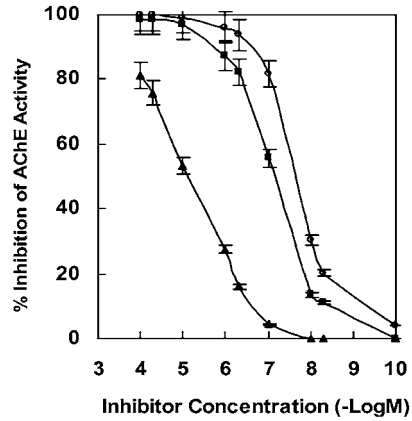


**Figure 7.** Effect of inhibitor concentrations on AChE activity. The inhibitor with a different concentration was mixed with diluted AChE. The remaining AChE activity was determined after the mixture was incubated for 5 min at 25 °C. Results are the means of five determinations ($n = 5$). Eserine (○), BW284C51 (■), and ethopropazine (▲).

tarnished plant bug (*36*), 128 for Colorado potato beetle (*17*), 5 for lesser grain borer (*5*), 1456 for cotton aphid (*21*), and 691 for oriental fruit fly (this study). The variation in the specific activity of the purified AChE among different insect species may be partially due to the degree of the purity of AChE but might also be due to variation in the insect species. Our results are also consistent with the notion that insects in Hemiptera, Coleoptera, and Lepidoptera exhibit lower levels of AChE activity than in Diptera (*41*).

The SDS−PAGE analysis revealed a rather distinctive 116 kDa protein for the AChE sample untreated with the reducing agent and a single 61 kDa band for the treated sample. The result indicates that AChE purified from oriental fruit fly was dimeric, as has been found in the AChEs of other insects (*41*). The molecular mass of the AChE subunit of oriental fruit fly was virtually the same as those purified from *Drosophila* (56 kDa) (*39*), horn fly (54 kDa) (*42*), Colorado potato beetle (65 kDa) (*17*), lesser grain borer (56 kDa) (*5*), waxmoth (60 kDa) (*19*), greenbug (72 kDa) (*20*), and cotton aphid (63.5 kDa) (*21*). As 60−65 kDa is almost universally reported as the subunit size from invertebrate and vertebrate sources (*19*), this value is consistent with previously reported results.

Insect AChE exists mostly in different native forms. For example, *M. domestica* and *L. hesperus* Knight AChE have been reported to have three forms (*14*, *16*), and in the Colorado potato beetle, *L.. decemlineata* (Say), the purified native AChE had two molecular forms (*17*). In contrast, results with the purified AChE from the oriental fruit fly obtained from nondenaturing PAGE, whether in the presence or absence of Triton X-100, showed only one major band, suggesting a single molecular form. This band corresponded to the silver-stained protein band, which suggests that we had only one major molecular form of AChE in our purified AChE samples. Because there was a striking difference in migration during nondenaturing electrophoresis with or without Triton X-100, the major molecular form

**Table 3.** Kinetic Parameters Using ATC as Substrate and $IC_{50}$ Values of Different Inhibitors of AChE Purified from Different Insect Species

| species | $K_m$ ($\mu$M) | $V_{max}$ ($\mu$mol/min/mg) | $IC_{50}$ (M) for different inhibitor | | |
|---|---|---|---|---|---|
| | | | eserine | BW284C51 | ethopropazine |
| Colorado potato beetle | 14.9 | 177.7 | $2.3 \times 10^{-8}$ | $5.4 \times 10^{-9}$ | $8.7 \times 10^{-6}$ |
| lesser grain borer | 24.2 | 18.7 | $0.9 \times 10^{-9}$ | $8.0 \times 10^{-8}$ | $1.1 \times 10^{-6}$ |
| Western corn rootworm | 19.7 | 184.8 | $2.6 \times 10^{-8}$ | $1.9 \times 10^{-8}$ | $1.1 \times 10^{-5}$ |
| greenbug | 57.6 | 78.0 | $4.8 \times 10^{-7}$ | $1.5 \times 10^{-7}$ | $1.0 \times 10^{-4}$ |

of AChE purified here could be an amphiphilic form (*43*). In other words, the AChE purified from oriental fruit fly was affected by the nature of the detergent, and the slower migration may have been due to binding between the detergent and the hydrophobic domain of the amphiphilic AChE molecule, which then retarded the migration of the protein (*43*).

Substrate inhibition of enzyme activity at high concentrations is a typical characteristic for AChE but not for BChE and, therefore, has been accepted widely as one of the criteria used to distinguish between AChE and BChE (*41*). However, our study has not indicated any significant substrate inhibition with AChE purified from oriental fruit fly. Similar results have been reported previously in studies of AChE from Western flower thrip (*44*) and altered AChE from organophosphorus and/or carbamate insecticide resistant tobacco budworm (*45*) and Colorado potato beetle (*35*), none of which exhibited excess substrate inhibition. Several researchers have hypothesized that substrate inhibition is due to the binding of excess substrate to peripheral anionic sites, leading to an inactive enzyme−substrate−substrate complex (*46*−*48*). This hypothesis suggests that peripheral anionic sites of oriental fruit fly AChE might be structurally different from those on AChE of other insect species.

From the kinetic point of view, the relative efficiency of BTC vs ATC hydrolysis by purified AChE, as determined by the $V_{max}$ (BTC):$V_{max}$ (ATC) ratio, was 0.069 for the oriental fruit fly. The ratio is smaller than that obtained from Western tarnished plant bug (0.098) (*34*) and only 10% of the AChE purified from *Drosophila* (0.6) (*39*). Although smaller ratios have also been found to be 0.032 for lesser grain borer (*5*) and Colorado potato beetle (*17*) AChEs, these ratios are still larger than those found in vertebrates such as electric eel (0.005) and human erythrocytes (0.01) (*39*). Apparently, the substrate specificity of AChE from insects is lower than that of AChE from vertebrates as judged by theses two substrates.

The $K_m$ and $V_m$ values for Colorado potato beetle, lesser grain borer, Western corn rootworm, and greenbug AChE using ATC as the substrate are summarized in **Table 3** (*5*, *6*, *17*, *20*). In the present study, we found the oriental fruit fly AChE to have an unusually high $V_m$ (833.3) value than the AChE from any insect species that have been reported previously. The $IC_{50}$ values of different inhibitors for the above species AChE are also shown in **Table 3** (*5*, *6*, *17*, *20*). These inhibitory features have been used as one way of distinguishing AChE from BChE (*49*). On the basis of the enzyme's substrate and inhibitor specificities, we concluded that the enzyme purified from oriental fruit fly is a true cholinesterase (i.e., AChE).

In summary, our current study has found AChE purified from the oriental fruit fly to be a true AChE with a higher efficiency in hydrolyzing ATC than BTC and a high sensitivity to inhibition by eserine and BW284C51 but much less sensitivity to ethopropazine. However, the purified AChE was significantly different from AChE purified from many other insects in several aspects in its lack of substrate inhibition phenomenon when using ATC as a hydrolyzing substrate, which has long been considered as a typical AChE characteristic from many other insects, and its unusually higher $V_m$ value for ATC than AChE from other insect species. These biochemical differences may reflect structural differences of AChE purified from the oriental fruit fly as compared with AChE from other insect species and affect the effectiveness of AChE inhibiting insecticides used for fruit fly control.

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