Journal of Insect Science OPEN ACCESS

RESEARCH

Purification, Characterization, and Sensitivity to Pesticides of Carboxylesterase From *Dendrolimus superans* (Lepidoptera: Lasiocampidae)

Chuan-shan Zou, Chuan-wang Cao, Guo-cai Zhang, and Zhi-ying Wang[1]

School of Forestry, Northeast Forestry University, Harbin 150040, P.R. China
[1]Corresponding author, e-mail: zhiyingwangnefu@aliyun.com

Subject Editor: Yu-Cheng Zhu

J. Insect Sci. 14(260): 2014; DOI: 10.1093/jisesa/ieu122

ABSTRACT. Through a combination of steps including centrifugation, ammonium sulfate gradient precipitation, sephadex G-25 gel chromatography, diethylaminoethyl cellulose 52 ion-exchange chromatography and hydroxyapatite affinity chromatography, carboxylesterase (CarE, EC3.1.1.1) from sixth instar larch caterpillar moth, *Dendrolimus superans* (Lepidoptera: Lasiocampidae) larvae was purified and its biochemical properties were compared between crude homogenate and purified CarE. The final purified CarE after hydroxyapatite chromatography had a specific activity of 52.019 μmol/(min·mg protein), 138.348-fold of crude homogenate, and the yield of 2.782%. The molecular weight of the purified CarE was approximately 84.78 kDa by SDS-PAGE. Three pesticides (dichlorvos, lambda-cyhalothrin, and avermectins) showed different inhibition to crude CarE and purified CarE, respectively. In vitro median inhibitory concentration indicated that the sensitivity of CarE (both crude homogenate and final purified CarE) to pesticides was in decreasing order of dichlorvos > avermectins > lambda-cyhalothrin. By the kinetic analysis, the substrates alpha-naphthyl acetate (α-NA) and beta-naphthyl acetate (β-NA) showed lesser affinity to crude extract than purified CarE. The results also indicated that both crude homogenate and purified CarE had more affinity to α-NA than to β-NA, and the K_{cat} and V_{max} values of crude extract were lower than purified CarE using α-NA or β-NA as substrate.

Key Words: carboxylesterase, purification, *Dendrolimus superans*, pesticide sensitivity, biochemical characteristic

Carboxylesterase (CarE, EC3.1.1.1) belongs to the class of serine hydrolases hydrolyzing a wide variety of carboxylic acid esters (Shukla 2012). CarEs are a family of enzymes ubiquitously expressed in all living organisms including animals, plants, insects, and microbes (Cashman et al. 1996, Yu et al. 2009). CarEs have a variety of physiological functions, such as detoxification, allelochemical metabolism and tolerance, degradation of neurotransmitters, metabolism of specific hormones and pheromones, defense, and behavior (Vogt et al. 1985, Taylor and Radic 1994, Vontas et al. 2002, Vogt 2005). Previous researches have shown that CarEs played a key role in defense against xenobiotic compounds in insects (Maymó et al. 2002, Sogorb and Vilanova 2002, Field and Blackman 2003). Some researches have also reported that insects CarEs are involved in metabolism of organophosphates (OPs), carbamates, and pyrethroids and resistance to these pesticides. Increased CarEs activity was associated with pesticide resistance of insects, which had been confirmed in *Culex tarsalis*, *Tribolium confusum*, *Habrobracon hebetor*, *Lucilia cuprina*, *Musca domestica*, and *Aphis gossypii* (Whyard et al. 1995, Perez-Mendoza et al. 2000, Smyth et al. 2000, Wool and Front 2003, Pan et al. 2009). Moreover, different CarE from various insects showed different characteristics and properties to detoxify pesticides, even closely related species. Constitutive expression and protein component of CarEs are highly diversified in different pest insects, resulting in a broad hydroxylation range of carboxylic esters to their component alcohols and acids (Durand et al. 2010, Oakeshott et al. 2010, Ramsey et al. 2010). The biochemical characteristics of CarEs from many insects had been studied including *Blattella germanica*, *Bombyx mori*, *Culex quinquefasciatus*, *Tribolium castaneum*, and *Oryzaephilus surinamensis* (Ketterman et al. 1992, Murthy and Veerabhadrappa 1996, Prabhakaran and Kamble 1996, Haubruge et al. 2002, Lee 2011). However, characteristics of many insects CarEs such as the larch caterpillar moth, *Dendrolimus superans* (Lepidoptera: Lasiocampidae), remain unknown.

The *D. superans* (namely larch caterpillar; Lepidoptera: Lasiocampidae), mainly distributed in Russia (Siberia), North Korea, Hokkaido of Japan, Mongolia, Xinjiang, Hebei Province, and inner Mongolia of China, is a key lepidopterious pest species of forests, which caused great harm to pines such as *Pinus koraiensis* Sieb. et Zucc, *Pinus tabulaeformis* Carrière, *Pinus sylvestris var. mongolica* Litv., *Picea asperata* Mast., *Abies fabri* (Mast.) Craib (Li et al. 2002). Many investigations on the occurrence and prediction, physiological ecology, and control of the *D. superans* had been studied in previous researches (Xiao 2003, Dong et al. 2009, Guo et al. 2011). For example, the dichlorvos, lambda-cyhalothrin, and avermectins insecticide are usually used as chemical control strategy (Lasota and Dybas 1991, Wu 2003, Amweg et al. 2005, Lehnert et al. 2011). It also had been confirmed that *D. superans* larvae could be controlled effectively by three pesticides (Xiao 2003, Tang 2012, Zhou et al. 2012, Song et al. 2013). In this study, the authors further investigated the purification and kinetic characterization of CarE from *D. superans* larvae in order to understand the physiological and biochemical functions of CarE from *D. superans* in response to insecticides.

Materials and Methods

Insects. The *D. superans* larvae were collected from Kecskes Teng Qi, Chifeng city, Inner Mongolia, China, and fed by *Larix gmelinii* leaves at 25 ± 1°C, photoperiod 14:10 (L:D) h and 70 ± 5% relative humidity. The sixth instar larvae were used in this study.

Chemicals. All the pesticides used were technical grade and their purity in this study was as follows: 93.50% dichlorvos (Zhengzhou Sanonda Weixin Pesticides Ltd., Co., China); 95.00% lambda-cyhalothrin (Heilongjiang Pingshan Forestry Chemical Company, China); 93.80% avermectins (Jilin Tonghua Pesticides Chemical Ltd., Co., China). Alpha-naphthyl acetate (α-NA), beta-naphthyl acetate eserine (β-NA), 2-dichloro-4-nitrobenzene, Coomassie brilliant blue (G-250), bovine serum albumin, Fast Blue B salt, sodium dodecyl sulfate (SDS), 2-ethoxy-1-ethoxycarbonyl-12-dihydroquinoline, Sephadex G-25, ECH Sepharose 4B, poly ethylene glycol 20000 (PEG20000), procainamide, N,N,N′,N′-tetramethylethylenediamine, TEMED, were

© The Author 2014. Published by Oxford University Press on behalf of the Entomological Society of America.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

purchased from Sigma Chemical CO., USA. Bio-Gel HTP Hydroxyapatite was purchased from Bio-Rad Laboratories, Inc., USA. Acrylamide, ammonium persulphate (APS), methylene bis acrylamide, *Tris* (Hydroxymethyl) aminomethane; glycine were purchased from Shenzhen Hua Xinrui Biological Technology Ltd., Co., China. All chemicals and reagents were analytical grade or better.

Homogenate Preparation. Ten sixth instar *D. superans* larvae were dissected, and the digesta in the intestine was removed, then the epidermis and other tissues were rinsed using 10 mM cold phosphate buffer (pH 6.8). All tissues were homogenized with 10 mM cold phosphate buffer (pH 6.8), and the supernatant was centrifuged at 4°C, 8,000 × *g* for 15 min. The final supernatant was centrifuged at 4°C, 12,000 × *g* for 10 min as crude enzyme and stored into −20°C.

CarE Activity Test. CarE activity was determined according to the method of Van Asperen with modification (Asperen 1962). Briefly, 0.05 ml crude enzyme extract and 2.00 ml 3×10^{-4} mol/liter α-NA containing eserine (1:1) were mixed and then were incubated at 35°C for 10 min. The catalytic reaction was terminated using 0.90 ml chromogenic reagent (5% SDS:1% Fast Blue B salt = 5:2, v/v). The specific activity was determined at 600 nm by spectrophotometer assay. CarE activity was calculated as micrometer α-naphthol produced per minute per milligram of protein. The specific activity unit of CarE was defined as μmol/(min·mg protein). Each assay was repeated three times. Protein concentration was estimated by the method of Bradford (1976), using bovine serum albumin as the standard protein (Laemmli 1970, Bradford 1976).

Purification of the CarE. CarE from sixth instar *D. superans* larvae has been purified by a combination of ammonium sulfate gradient precipitation, sephadex G-25 gel chromatography, diethylaminoethyl cellulose 52 ion-exchange chromatography, and hydroxyapatite affinity chromatography. All purification steps were carried out at 4°C unless otherwise stated.

Ammonium Sulfate Precipitation. Ammonium sulfate gradient precipitation (saturation was from 20 to 90%) was carried out at 4°C, and the obtained precipitate was centrifuged at 8,000 × *g* for 20 min at 4°C. Each precipitate was dissolved in 5.0 ml 10-mM cold sodium phosphate buffer (pH 6.8), and specific activity and protein content of each precipitate were determined. All assays were repeated three times. The optimal saturation for precipitating objective protein was chosen by the specific activity account.

Sephadex G-25 Gel Filtration. After ammonium sulfate gradient precipitation, the precipitate were resuspended and dialyzed for 24 h against three changes of 10-mM sodium phosphate buffer at 4°C. The dialyzed solution was filtrated on Sephadex G-25 gel column (15 by 300 mm) equilibrated with 10-mM cold phosphate buffer (pH 6.8). An aliquot of 12 ml sample was added into the column using 10-mM cold phosphate buffer (pH 6.8) as eluent with a flow rate of 30 ml/h, then the elution and fraction (3.0 ml) were begun and collected. CarE activity and protein content from each fraction were assayed, and five fractions containing higher CarE activity were combined and concentrated to 6.0 ml by PEG20000 for next column.

DEAE-52 Ion-Exchange Chromatography. The sample from Sephadex G-25 gel filtration was applied into DEAE-52 ion-exchange chromatography column (16 by 300 mm), equilibrated with 50-mM cold phosphate buffer (starting buffer, pH 6.8). The column was washed with 120 ml of starting buffer (containing 0.6 M sodium chloride) and 5.0 ml of fractions were collected at a flow rate of 24 ml/h. The CarE activity and protein content from each fraction were assayed; five fractions with higher CarE activity were combined and then dialyzed for 24 h against three changes of 50 mM sodium phosphate buffer at 4°C. The sample solution was concentrated to 10.0 ml by PEG20000 for hydroxyapatite affinity chromatography.

Hydroxyapatite Affinity Chromatography. Hydroxyapatite chromatography column was prepared according to the method of Urist et al. (1984). The combined fractions with higher CarE activity through DEAE-52 ion-exchange chromatography were applied to a hydroxyapatite column (15 by 150 mm, Bio-Gel HTP Gel, Bio-Rad Laboratory) equilibrated with 100 ml 50-mM phosphate buffer (pH 6.8). CarE activity fractions were eluted with 400-mM phosphate buffer (pH 6.8) with a flow rate of 36 ml/h. Five fractions with higher CarE activity were pooled and concentrated to 10 ml using PEG20000. An aliquot of 10 ml purified sample was dialyzed for 24 h against three changes of 50-mM sodium phosphate buffer at 4°C. Through above steps, purified CarE was eventually obtained.

SDS-PAGE. The molecular weight of CarE was determined by polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was carried out on 10% gels according to the method of Laemmli (1970) using standard proteins (M_r 10,000–170,000 Da) (Laemmli 1970). The molecular weight of CarE was derived from the plot of log molecular weight versus relative mobility.

Kinetic Analysis. Enzyme kinetics of crude CarE homogenate and purified CarE were compared by recording activity toward five concentrations (3.0×10^{-4}, 1.5×10^{-4}, 0.75×10^{-4}, 0.375×10^{-4}, and 0.1875×10^{-4} mol/liter) of α-NA and β-NA. The maximal velocity (V_{max}) and Michaelis–Menten (K_m) values to the both substrates (α-NA and β-NA) were determined from Lineweaver–Burk plots, and calculated by using Enzfit software (Elsevier) (Pan et al. 2009). The turnover numbers (K_{cat}) were calculated according to the molecular weights of the enzymes (crude homogenate and purified CarE) and each V_{max}. Substrate specificity constant (K_{cat}/K_m) was calculated according to K_{cat} and K_m.

In Vitro Inhibition Assay. In vitro inhibition of CarE in sixth instar *D. superans* larvae by dichlorvos, avermectins, and lambda-cyhalothrin were determined according to the method of Young et al. (2005). The crude and purified CarE were preincubated with 0.0001, 0.0010, 0.0100, 0.1000, and 1.0000 mg/ml of dichlorvos, avermectins, and lambda-cyhalothrin for 10 min at 35°C, respectively. The remaining specific activities of the CarE were assayed using α-NA as substrate, and IC_{50} values of CarE to each pesticide were calculated by the linear equations. At the same time, crude and purified CarE dealt with distilled water as control, each assay was repeated three times.

Results

Purification. The result of ammonium sulfate gradient precipitation was shown in Fig. 1A. The CarE showed the maximum specific activities at 60% saturated ammonium sulfate. The CarE precipitated by 60% saturated ammonium sulfate was pooled into the sephadex G25 gel chromatography column. The CarE activity and protein content from each fraction were shown in Fig. 1B. The peak of CarE activity and protein content appeared at the 14th fraction. The CarE from sephadex G25 gel chromatography was pooled into diethylaminoethyl cellulose 52 ion-exchange chromatography column for further purification, 24 fractions had been collected by elution. Two peak values of specific CarE activity appeared at the 7th and 20th fractions while three peak values of protein content appeared at the 7th, 17th, and 19th fractions, respectively (Fig. 1C). The CarE purified by 52 ion-exchange chromatography column was collected and then poured into the hydroxyapatite affinity chromatography column for final purification. Among all 23 fractions collected by elution, both the maximum specific activity and protein content values of CarE appeared at the 19th fraction (Fig. 1D).

CarE from sixth instar *D. superans* larvae was sequentially purified by 60% sulfate ammonium precipitation, G25 Sephadex, DEAE-52 ion exchange chromatography, and hydroxyapatite chromatography. By using α-NA as substrate, the specific activity and total activity of CarE crude homogenate were 0.376 μmol/(min·mg protein) and 287.284 μmol/min, respectively. After the CarE was purified with 60% sulfate ammonium precipitation, the specific activity was 0.571 μmol/(min·mg protein) and 1.528-fold higher than crude homogenate. As the CarE being purified by G25 Sephadex, 9.486-fold higher activity than crude homogenate and 3.567 μmol/(min·mg protein) of specific activity were obtained. However, the specific activity of CarE reached 6.979 μmol/(min·mg protein) through the third step purification with DEAE-52 ion exchange chromatography and 18.561-fold of the crude

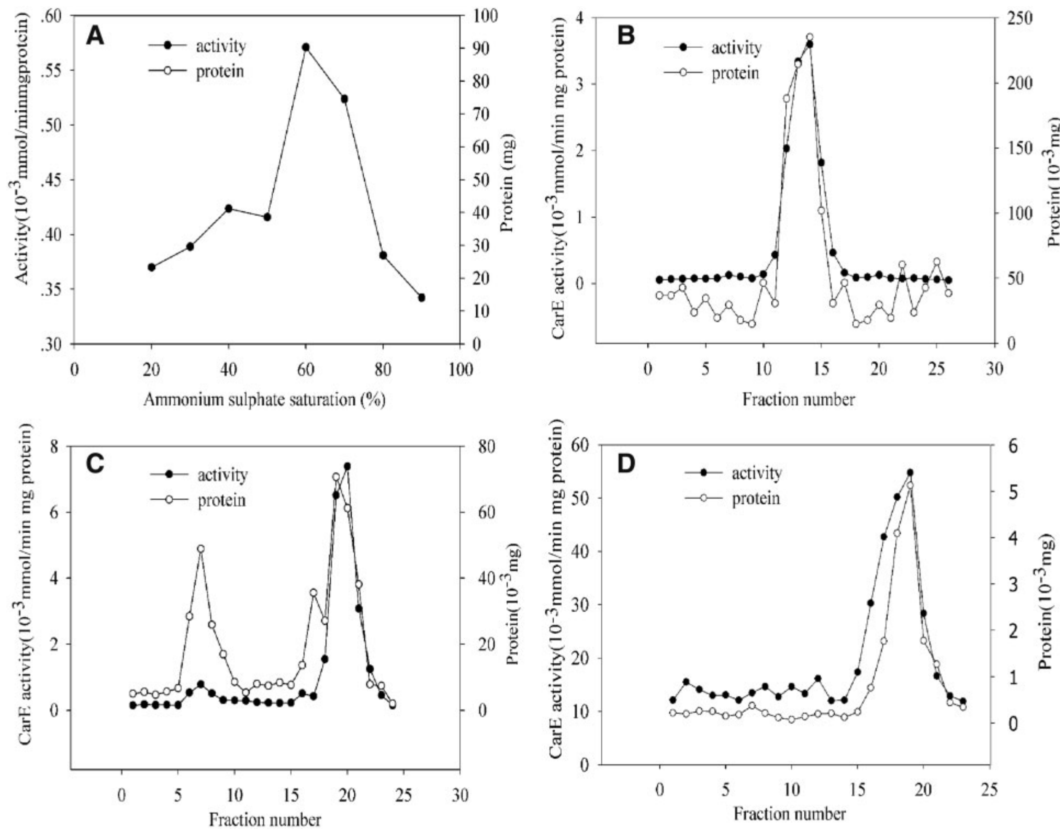

Fig. 1. Purification of CarE from sixth instar *D. superans* larvae by combinatorial steps of ammonium sulfate gradient precipitation, sephadex G-25 gel chromatography, diethylaminoethyl cellulose 52 ion-exchange chromatography, and hydroxyapatite affinity chromatography. (A) Activity and protein content after being precipitated by various saturation (from 20 to 90%) of sulfate ammonium. (B) Elution profile of sephadex G-25 gel chromatography. (C) Elution profile of diethylaminoethyl cellulose 52 ion-exchange chromatography. (D) Elution profile of hydroxyapatite affinity chromatography.

Table 1. The properties of CarE at every purification stage

Step	Total protein (mg)	Specific activity (μmol/min·mg protein)	Total activity (μmol/min)	Purification (-fold)	Yield (%)
Crude homogenate	764.052 ± 58.266	0.376 ± 0.008	287.284	1	100
60 % Sulfate ammonium precipitation	394.896 ± 73.564	0.571 ± 0.004	225.407	1.518	78.461
Gel filtration (G25 Sephadex)	34.912 ± 3.869	3.567 ± 0.057	124.521	9.486	43.344
DEAE-52 ion exchange chromatography	10.669 ± 0.661	6.979 ± 0.581	74.456	18.561	25.917
Hydroxyapatite chromatography	0.154 ± 0.019	52.019 ± 5.898	7.991	138.348	2.782

The values represented the means and standard errors from three replicates.

homogenate after the purification. After CarE was purified by final hydroxyapatite chromatography, the special activity reached 52.019 μmol/(min·mg protein), which was 138.348-fold of the crude homogenate (Table 1).

SDS-PAGE and Molecular Weight Estimation. SDS-PAGE of crude homogenate and final purified CarE was showed in Fig. 2. For purified CarE, one clear protein band appeared in the lane3 which molecular weight was between 70 and 100 kDa. The molecular weight of the purified CarE from sixth instar *D. superans* larvae was estimated about 84.78 kDa by the rate-flow value method. Analysis of crude homogenate by SDS-PAGE revealed several vague protein bands in the lane 1. The results showed that CarE in crude homogenate from sixth instar *D. superans* larvae had been purified using SDS-PAGE analysis.

Kinetic Analysis. Three kinetic parameters including the maximum reaction rate (V_{max}), Michaelis–Menten constant (K_m) and turnover number (K_{cat}) for the sixth instar *D. superans* larvae were listed in Table 2.

Using α-NA and β-NA as substrates, V_{max} values of final purified CarE were $5.041 \times 10^1 \pm 3.508$ and $1.756 \times 10^2 \pm 1.819 \times 10^1$ μmol/(min·mg protein), which were 205.503- and 143.113-fold compared with the crude homogenate, respectively. K_m values of final purified CarE were $4.325 \times 10^{-5} \pm 1.310 \times 10^{-5}$ and $1.615 \times 10^{-4} \pm 2.315 \times 10^{-5}$ mol/liter, which were only 0.503- and 0.428-fold of crude homogenate. The K_{cat} values of final purified CarE were 106.084- and 135.012-fold compared with the crude homogenate, respectively. Two substrates of both α-NA and β-NA showed lesser affinity to crude extract than purified CarE. Three parameters determined by using α-NA as substrate were lower than using β-NA as substrate. The results demonstrated that crude homogenate and purified CarE had more affinity to α-NA than to β-NA.

Inhibition Sensitivity of CarE to Pesticide. The inhibitions of pesticides, dichlorvos, avermectins, and lambda-cyhalothrin, showed distinct dose–effect relationships between CarE activity and pesticide. The CarE activity decreased with the concentration increase of pesticides.

The linear regression equation was derived from Log_{10} (pesticide concentration) versus the inhibition rate (%), then the median inhibition concentration (IC_{50}) was calculated by the linear equations. Results of IC_{50} values of dichlorvos, avermectins, and lambda-cyhalothrin to crude homogenate and purified CarE were shown in Table 3. Among three pesticides, the IC_{50} values of dichlorvos were $3.309 \times 10^{-3} \pm 2.946 \times 10^{-4}$ and $1.105 \times 10^{-1} \pm 7.344 \times 10^{-2}$ µg/ml, respectively, showing that CarE from crude homogenate or final purified CarE was the most sensitive to dichlorvos. Sensitivity of CarE to pesticides was in decreasing order of dichlorvos > aver mectins > lambda-cyhalothrin.

Discussion

Many esterases such as CarE from *B. mori*, *T. castaneum*, *O. surinamensis*, and *C. quinquefasciatus* had been purified by combinatorial methods (Ketterman et al. 1992, Murthy and Veerabhadrappa 1996, Haubruge et al. 2002, Lee 2011). There were dissimilar results for purified CarEs from different insect species by different methods (Murthy and Veerabhadrappa 1996, Haubruge et al. 2002, Lee 2011). In this study, CarE from sixth instar *D. superans* larvae was purified by a series of techniques, with 138.348-fold and 2.782% yield compared with the crude homogenate. The molecular weight of the purified CarE was approximately 84.78 kDa. There was much more impurities in the Lepidoptera insect larvae than in Coleopteran larvae body, which caused lower purification efficiency of CarE from sixth instar *D. superans* larvae.

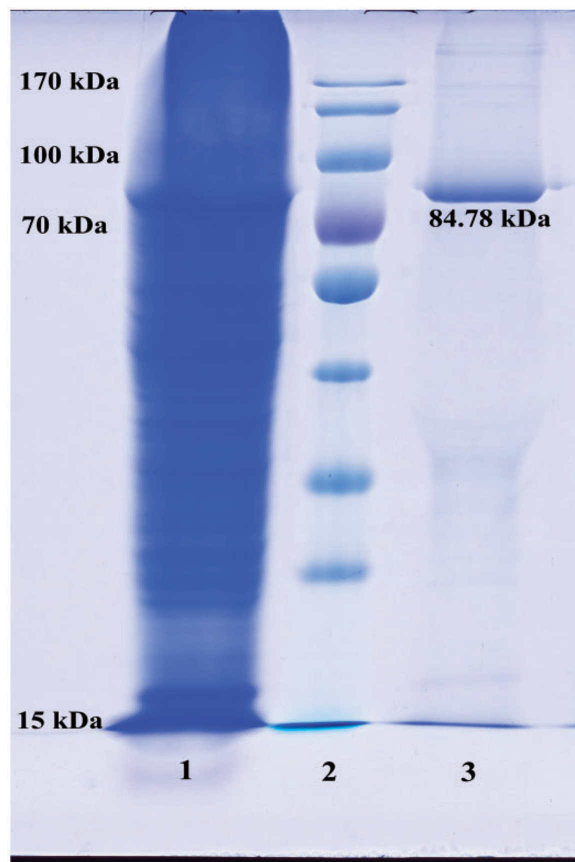

Fig. 2. SDS-PAGE of crude homogenate and final purified CarE. Lane 1: crude homogenate; Lane 2: molecular-mass markers; and Lane 3: final purified CarE.

The kinetic parameters of crude homogenate and final purified CarE were further studied in this study. The crude CarE showed lesser affinity to tested substrates both α-NA and β-NA than purified CarE. Both crude homogenate and purified CarE showed more affinity to β-NA than to α-NA; this result was dissimilar with K_m values of esterase substrates determined on crude supernatants of adult females from susceptible and resistant strains of *Tetranychus urticae* (Van Leeuwen and Tirry 2007). The distinct result was because of enzymes from different organisms which showed different characteristics. As we know, turnover number (K_{cat}) indicates catalytic efficiency of the enzyme; the larger K_{cat} indicated more rapid the catalytic events at the enzyme's active site. In crude extract, non-target protein reduced the catalytic efficiency of CarE to substrates which caused that the K_{cat} of crude extract was lower than purified CarE, whether using α-NA or β-NA as substrate. Moreover, the V_{max} values of crude extract were smaller than purified CarE because the crude extract was with lower catalytic efficiency.

CarEs are hydrolases that involved in the detoxification of many insecticides such as pyrethroid, carbamate, and organophosphorus. Inhibition of CarE activity by organophosphorus and carbamate insecticides has been used as a biomarker of pesticide exposure in many organisms (Wheelock et al. 2008). CarE could be inhibited by many specific pesticides. CarE activities present in *Biomphalaria glabrata, Lumbriculus variegates*, and *Lumbricus terrestris* were inhibited significantly by carbaryl and chlorpyrifos-oxon (Sanchez-Hernandez and Wheelock 2009, Vejares et al. 2010). In *Chilina gibbosa*, CarE activity was also inhibited by azinphos-methyl with 1,000 µg/liter of 50% inhibition concentration (Bianco et al. 2013). CarE activity in deltamethrin resistance strain of *A. gossypii* could be inhibited by *S,S,S*-tributyl phosphorotrithioate (Chang et al. 2010). The dichlorvos also has significant inhibition to CarEs in *Liposcelis bostrychophila* and *Liposcelis entomophila* (Wang et al. 2004), *Dysdercus koenigii* (Singh and Singh 1990), and *Spodoptera litura* (Muthusamy and Karthi 2011). However, the similar results were also obtained in this study. The CarE from sixth instar larvae of *D. superans* was highly inhibited in vitro by dichlorvos. Except for OP pesticides, the pyrethroids and microbial pesticides also affected CarE activity (Sogorb and Vilanova 2002, Heidari et al. 2005). Our results show that the sensitivity of CarE (crude extract and purified CarE) to three pesticides was in decreasing order of dichlorvos > avermectins > lambda-cyhalothrin. The avermectins and lambda-cyhalothrin showed lesser inhibition than dichlorvos. The pesticides with different mechanism and chemical structure showed distinct inhibition to CarE. In addition, CarEs from different source were all inhibited by the same pesticide (Fridovich 1969, Gao 1998,

Table 2. Kinetic parameters of CarE in sixth instar *D. superans* larvae

Substrates	V_{max} (µmol/mg·min protein)	K_m (mol/liter)	K_{cat} (s)
Crude homogenate			
α-NA	$4.253 \times 10^{-1} \pm 1.914 \times 10^{-2}$	$8.601 \times 10^{-5} \pm 1.939 \times 10^{-5}$	$5.490 \times 10^{-1} \pm 4.917 \times 10^{-2}$
β-NA	$1.227 \pm 2.057 \times 10^{-1}$	$3.775 \times 10^{-4} \pm 4.166 \times 10^{-5}$	$1.708 \pm 1.213 \times 10^{-1}$
Final purified CarE			
α-NA	$5.041 \times 10^{1} \pm 3.508$	$4.325 \times 10^{-5} \pm 1.310 \times 10^{-5}$	$5.824 \times 10^{1} \pm 5.901$
β-NA	$1.756 \times 10^{2} \pm 1.819 \times 10^{1}$	$1.615 \times 10^{-4} \pm 2.315 \times 10^{-5}$	$2.306 \times 10^{2} \pm 1.624 \times 10^{1}$

The parameters (V_{max}, K_m, and K_{cat}) were estimated from Lineweaver–Burk plots and used to calculate the specificity constant of each substrate. The values represented the means ± standard errors from three replicates.

Table 3. Median inhibition concentration (IC_{50}) of pesticides to CarE-specific activity in sixth instar *D. superans* larvae

Pesticides	IC_{50} (μg/ml)	
	Crude homogenate	Final purified CarE
Dichlorvos	$3.309 \times 10^{-3} \pm 2.946 \times 10^{-4a}$	$1.105 \times 10^{-1} \pm 7.344 \times 10^{-2a**}$
Avermectins	$1.259 \times 10^{1} \pm 4.780 \times 10^{-1b}$	$1.025 \times 10^{1} \pm 4.780 \times 10^{-1b**}$
Lambda-cyhalothrin	$4.529 \times 10^{2} \pm 1.672 \times 10^{2c}$	$1.951 \times 10^{1} \pm 1.973^{c**}$

IC_{50} values were calculated by the linear equations, the values represented the means and standard errors from three replicates; Means followed by a different letter in same column are significantly different by the Duncan test ($P < 0.05$); Means followed by ** in same row are extremely significant difference by the *t*-test ($P < 0.01$).

Denholm et al. 1999). The IC_{50} values of crude homogenate and purified CarE from sixth instar *D. superans* larvae to three pesticides were different because there was more target protein CarE in purified CarE than crude extract in per unit protein. Non-target enzymes significantly impacted the biochemical characteristics of target enzymes as inhibitors (Mann and Keilin 1938, Vontas et al. 2002, Ma et al. 2011). The IC_{50} value of dichlorvos to the final purified CarE was 33.39-fold of crude homogenate. IC_{50} values of avermectins and lambda-cyhalothrin to the purified CarE showed slightly smaller than the crude homogenate. In insect organisms, other various non-CarE enzymes in crude homogenate such as acetylcholinesterase, multi-function oxidase, cytochrome P450 enzymes, and glutathione S-transferase significantly impacted on the biochemical properties of CarE. The competition inhibition mechanism of pesticides to various enzymes needed to be further studied.

In summary, these results will provide some basic information for understanding the physiological and biochemical properties of the CarE in *D. superans*. We will further study the CarE involved in toxicology mechanism in *D. superans*.

Acknowledgments

This work was supported by the Fundamental Research Funds for the Central Universities (2572014CA15), 'Twelfth Five Year' National Science and Technology Project in Rural Areas (2011BAD37B01-4), the S&T Plan Projects of Heilongjiang Province (GA09B203-3), and Academic Teacher Support Project of NEFU.

References Cited

Amweg, E. L., D. P. Weston, and N. M. Ureda. 2005. Use and toxicity of pyrethroid pesticides in the Central Valley, California, USA. Environ. Toxicol. Chem. 24: 966–972.

Asperen, K. V. 1962. A study of housefly esterases by means of a sensitive colorimetric method. J. Insect Physiol. 8: 401–416.

Bianco, K., M. S. Yusseppone, S. Otero, C. Luquet, C. Ríos de Molina Mdel, and G. Kristoff. 2013. Cholinesterases and neurotoxicity as highly sensitive biomarkers for an organophosphate insecticide in a freshwater gastropod (*Chilina gibbosa*) with low sensitivity carboxylesterases. Aquat. Toxicol. 144: 26–35.

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

Cashman, J. R., B. Y. Perotti, C. E. Berkman, and J. Lin. 1996. Pharmacokinetics and molecular detoxication. Environ. Health Perspect. 104(Suppl 1): 23.

Chang, J., C. W. Cao, and X. W. Gao. 2010. The effect of pretreatment with S,S,S-tributyl phosphorotrithioate on deltamethrin resistance and carboxylesterase activity in *Aphis gossypii* (Glover) (Homoptera: Aphididae). Pestic. Biochem. Physiol. 98: 296–299.

Denholm, I., J. A. Pickett, and A. L. Devonshire. 1999. Insecticide resistance: from mechanisms to management. CAB International, Wallingford, United Kingdom.

Dong, S. B., B. Z. Zhu, and H. B. Liu. 2009. Occurrence and control techniques of *Dendrolimus superans* in Boli County. Protect. For. Sci. Technol. 6: 13.

Durand, N., G. Carot-Sans, T. Chertemps, F. Bozzolan, V. Party, M. Renou, S. Debernard, G. Rosell, and M. Maïbèche-Coisne. 2010. Characterization of an antennal carboxylesterase from the pest moth *Spodoptera littoralis* degrading a host plant odorant. PLos One 5: e15026.

Field, L. M., and R. L. Blackman. 2003. Insecticide resistance in the aphid *Myzus persicae* (Sulzer): chromosome location and epigenetic effects on esterase gene expression in clonal lineages. Biol. J. Linnean Soc. 79: 107–113.

Fridovich, I. 1969. Superoxide dismutase: an enzymatic function for erythrocuprein. J. Biol. Chem. 244: 6049–6055.

Gao, X. W., Y. Zhao, X. Wang, X. L. Dong, and B. Z. Xu. 1998. Induction of carboxylesterase on *Helicoverpa arogera* by insecticides and pant allelochemicals. Acta Emyomologica Sinica 41(S1): 5–11.

Guo, H. M., W. Z. Tu, J. D. Li, and W. Han. 2011. Method of meteorological forecast of *Dendrolimus superans* in Hohhot. J. Inner Mongolia For. 4: 015.

Haubruge, E., M. Amichot, A. Cuany, J. B. Berge, and L. Arnaud. 2002. Purification and characterization of a carboxylesterase involved in malathion-specific resistance from *Tribolium castaneum* (Coleoptera: Tenebrionidae). Insect Biochem. Mol. Biol. 32: 1181–1190.

Heidari, R., A. L. Devonshire, B. E. Campbell, S. J. Dorrian, J. G. Oakeshott, and R. J. Russell. 2005. Hydrolysis of pyrethroids by carboxylesterases from *Lucilia cuprina* and *Drosophila melanogaster* with active sites modified by *in vitro* mutagenesis. Insect Biochem. Mol. Biol. 35: 597–609.

Ketterman, A. J., K. G. Jayawardena-Indrananda, and J. Hemingway. 1992. Purification and characterization of a carboxylesterase involved in insecticide resistance from the mosquito *Culex quinquefasciatus*. Biochem. J. 287(Pt 2): 355.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685.

Lasota, J. A., and R. A. Dybas. 1991. Avermectins, a novel class of compounds: implications for use in arthropod pest control. Annu. Rev. Entomol. 36: 91–117.

Lee, S. E. 2011. Purification and characterisation of a carboxylesterase from a chlorpyrifos-methyl-resistant strain of *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). Aust. J. Entomol. 50: 187–194.

Lehnert, M. P., R. M. Pereira, P. G. Koehler, W. Walker, and M. S. Lehnert. 2011. Control of *Cimex lectularius* using heat combined with dichlorvos resin strips. Med. Vet. Entomol. 25: 460–464.

Li, L., H. W. Meng, and X. Sun. 2002. Bionomics of *Dendrolimus superans* (Butler). Neimenggu Nongye Daxue Xuebao 23: 101–103.

Ma, M., B. Zhang, and S. C. Li. 2011. Purification and partial characterization of acetlcholinesterase from *Pardosa astrigera* L. Koch. J. Cell Anim. Biol. 5: 11–16.

Mann, T., and D. Keilin. 1938. Haemocuprein and hepatocuprein, copper–protein compounds of blood and liver in mammals. Proc. R. Soc. Lond. Ser. B: Biol. Sci. 126: 303–315.

Maymó, A. C., A. Cervera, R. Sarabia, R. Martínez-Pardo, and M. D. Garcerá. 2002. Evaluation of metabolic detoxifying enzyme activities and insecticide resistance in *Frankliniella occidentalis*. Pest Manag. Sci. 58: 928–934.

Murthy, K., and P. S. Veerabhadrappa. 1996. Purification, characterization and properties of carboxylesterase from the midgut of the silkworm, *Bombyx mori* L. Insect Biochem. Mol. Biol. 26: 287–296.

Muthusamy, S., and R. Karthi. 2011. Pesticide detoxifying mechanism in field population of *Spodoptera litura* (Lepidoptera: noctuidae) from South India. Egypt. Acad. J. Biol. Sci. 3: 51–57.

Oakeshott, J. G., C. Claudianos, P. M. Campbell, R. D. Newcomb, and R. J. Russell. 2010. Metabolic enzymes associated with xenobiotic and chemosensory responses in *Nasonia vitripennis*. Insect. Mol. Biol. 19(Suppl. 1): 147–163.

Pan, Y., H. L. Guo, and X. W. Gao. 2009. Carboxylesterase activity, cDNA sequence, and gene expression in malathion susceptible and resistant strains of the cotton aphid, *Aphis gossypii*. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 152: 266–270.

Perez-Mendoza, J., J. A. Fabrick, K. Y. Zhu, and J. E. Baker. 2000. Alterations in esterases are associated with malathion resistance in *Habrobracon hebetor* (Hymenoptera: Braconidae). J. Econ. Entomol. 93: 31–37.

Prabhakaran, S. K., and S. T. Kamble. 1996. Biochemical characterization and purification of esterases from three strains of German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae). Arch. Insect Biochem. Physiol. 31: 73–86.

Ramsey, J. S., D. S. Rider, T. K. Walsh, M. De Vos, K. H. Gordon, L. Ponnala, S. L. Macmil, B. A. Roe, and G. Jander. 2010. Comparative analysis of detoxification enzymes in *Acyrthosiphon pisum* and *Myzus persicae*. Insect Mol. Biol. 19(s2): 155–164.

Sanchez-Hernandez, J. C., and C. E. Wheelock. 2009. Tissue distribution, isozyme abundance and sensitivity to chlorpyrifos-oxon of carboxylesterases in the earthworm *Lumbricus terrestris*. Environ. Pollut. 157: 264–272.

Shukla, A. 2012. Characterization of mycobacterial estrases/lipases using combined biochemical and computational enzymology. Master Thesis of SRM University, India.

Singh, H. K., and H. N. Singh. 1990. Acetylcholinesterase and carboxylesterase sensitivity to organophosphate poisoning during different developmental stages of *Dysdercus koenigii* (F.). Indian J. Entomol. 52: 50–56.

Smyth, K. A., T. M. Boyce, R. J. Russell, and J. G. Oakeshott. 2000. MCE activities and malathion resistances in field populations of the Australian sheep blowfly (*Lucilia cuprina*). Heredity 84: 63–72.

Sogorb, M. A., and E. Vilanova. 2002. Enzymes involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis. Toxicol. Lett. 128: 215–228.

Song, F. S., X. H. Wang, and W. Zhou. 2013. Investigations on the effect of ultra-low volume aerial spray using 2% avermectin finish and control of *Dendrolimua superans* J. Jilin For. Sci. Technol. 42: 36–39.

Tang, L. Z. 2012. Effect analysis of biological pesticides to control *Dendrolimus superans*. J. Hebei For. Sci. Technol. 3: 7.

Taylor, P., and Z. Radic. 1994. The cholinesterases: from genes to proteins. Annu. Rev. Pharmacol. Toxicol. 34: 281–320.

Urist, M. R., Y. K. Huo, A. G. Brownell, W. M. Hohl, J. Buyske, A. Lietze, P. Tempst, M. Hunkapiller, and R. J. DeLange. 1984. Purification of bovine bone morphogenetic protein by hydroxyapatite chromatography. Proc. Natl Acad. Sci. 81: 371–375.

Van Leeuwen, T., and L. Tirry. 2007. Esterase-mediated bifenthrin resistance in a multiresistant strain of the two-spotted spider mite, *Tetranychus urticae*. Pest Manag. Sci. 63: 150–156.

Vejares, S. G., P. Sabat, and J. C. Sanchez-Hernandez. 2010. Tissue-specific inhibition and recovery of esterase activities in *Lumbricus terrestris* experimentally exposed to chlorpyrifos. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 51: 351–359.

Vogt, R. G. 2005. Molecular basis of pheromone detection in insects. Compr. Insect Physiol. Biochem. Pharmacol. Mol. Biol. 3: 753–804.

Vogt, R. G., L. M. Riddiford, and G. D. Prestwich. 1985. Kinetic properties of a sex pheromone-degrading enzyme: the sensillar esterase of *Antheraea polyphemus*. Proc. Natl Acad. Sci. 82: 8827–8831.

Vontas, J. G., G. J. Small, D. C. Nikou, H. Ranson, and J. Hemingway. 2002. Purification, molecular cloning and heterologous expression of a glutathione S-transferase involved in insecticide resistance from the rice brown planthopper, *Nilaparvata lugens*. Biochem. J. 362(Pt 2): 329.

Wang, J. J., W. X. Cheng, W. Ding, and Z. M. Zhao. 2004. The effect of the insecticide dichlorvos on esterase activity extracted from the psocids, *Liposcelis bostrychophila* and L. entomophila. J. Insect Sci. 4: 1–5.

Wheelock, C. E., B. M. Phillips, B. S. Anderson, J. L. Miller, M. J. Miller, and B. D. Hammock. 2008. Applications of carboxylesterase activity in environmental monitoring and toxicity identification evaluations (TIEs). Rev. Environ. Contam. Toxicol. 195: 117–178.

Whyard, S., A. E. Downe, and V. K. Walker. 1995. Characterization of a novel esterase conferring insecticide resistance in the mosquito *Culex tarsalis*. Arch. Insect Biochem. Physiol. 29: 329–342.

Wool, D., and L. Front. 2003. Esterase variation in *Tribolium confusum* (Coleoptera: Tenebrionidae): genetic analysis of interstrain crosses in relation to malathion resistance. J. Stored Products Res. 39: 237–249.

Wu, G. Q. 2003. Biological pesticide: avermectins. Fine Specialty Chem. 14: 002.

Xiao, P. 2003. Aerial control against *dendrolimus superans* and its influence on natural enemies. For. Pest Dis. 23: 18–20.

Young, S. J., R. V. Gunning, and G. D. Moores. 2005. The effect of piperonyl butoxide on pyrethroid-resistance-associated esterases in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Pest Manag. Sci. 61: 397–401.

Yu, Q. Y., C. Lu, W. L. Li, Z. H. Xiang, and Z. Zhang. 2009. Annotation and expression of carboxylesterases in the silkworm, *Bombyx mori*. BMC Genom. 10: 553.

Zhou, C. M., W. X. Zhao, S. S. Li, D. Nan, and Y. F. Liu. 2012. Control experiments of *Dendrolimus superans* by different concentrations of pesticides. Pract. For. Technol. 12: 52.

Received 2 September 2013; accepted 20 January 2014.