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Purification and studies on characteristics of cholinesterases from *Daphnia magna*[*]

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Abstract: Due to their significant value in both economy and ecology, *Daphnia* had long been employed to investigate in vivo response of cholinesterase (ChE) in anticholinesterase exposures, whereas the type constitution and property of the enzyme remained unclear. A type of ChE was purified from *Daphnia magna* using a three-step procedure, i.e., Triton X-100 extraction, ammonium sulfate precipitation, and diethylaminoethyl (DEAE)-Sepharose™-Fast-Flow chromatography. According to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), molecular mass of the purified ChE was estimated to be 84 kDa. Based on substrate studies, the purified enzyme preferred butyrylthiocholine iodide (BTCh) [with maximum velocity (V_{max})/Michaelis constant (K_m)=8.428 L/(min·mg protein)] to acetylthiocholine iodide (ATCh) [with V_{max}/K_m=5.346 L/(min·mg protein)] as its substrate. Activity of the purified enzyme was suppressed by high concentrations of either ATCh or BTCh. Inhibitor studies showed that the purified enzyme was more sensitive towards inhibition by tetraisopropylpyrophosphoramide (*iso*-OMPA) than by 1,5-*bis*(4-allyldimethylammoniumphenyl) pentan-3-one dibromide (BW284C51). Result of the study suggested that the purified ChE was more like a type of pseudocholinesterase, and it also suggested that *Daphnia magna* contained multiple types of ChE in their bodies.

Key words: Crustacea, Pseudocholinesterase, Cholinesterase, Substrate preference, Selective inhibitors
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1 Introduction

Cholinesterases (ChEs) are serine hydrolases. They can further be divided into two molecular types, i.e., acetylcholinesterase (AChE, EC 3.1.1.7) and pseudocholinesterase (PChE, EC 3.1.1.8), based on the substrate preferences and the responses towards selective inhibitors (Villatte and Bachmann, 2002). Regardless the types, ChEs are strongly inhibited by anticholinesterases such as organophosphates (OPs) and cabamates (CBs). This allows them to serve as effective biomarkers in the field (Sanchez-Hernandez *et al*., 1998; Stien *et al*., 1998; Kirby *et al*., 2000; den Besten *et al*., 2001; Forget *et al*., 2003; Quintaneiro *et al*., 2006; Jung *et al*., 2008; Elhalwagy *et al*., 2010; Sáenz *et al*., 2010; van Oosterom *et al*., 2010; Printes *et al*., 2011).

Daphnia is a genus of zooplankton that belongs to the phylum Arthropoda, the order Crustacea, and the family Daphniidae. They feed on phytoplankton and are preyed upon by aquatic vertebrates and macro-invertebrates. The unique responses of ChE levels in *Daphnia* when exposed to anticholinesterases have been observed multiple times (Gälli *et al*., 1994; Guilhermino *et al*., 1996; Sturm and Hansen, 1999; Barata *et al*., 2001; Carvalho *et al*., 2003; Duquesne, 2006; Vesela *et al*., 2006; Dámasio *et al*., 2007; Jemec *et al*., 2007; Printes *et al*., 2008; Duquesne and Küster, 2010; Coelho *et al*., 2011; Li and Tan, 2011). According to Sturm and Hansen (1999), up to 80% ChE inhibition was observed in *Daphnia* after the animals

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were exposed for 24 h to dichlorvos, parathion, and aldicarb, respectively, at concentration of 0.25, 1, and 250 μg/L. Meanwhile the authors found 24-h LC_{50} values (lethal concentration that kills 50% of the test animals in a given time) of the three insecticides were 0.233, 2.35, and 227.6 μg/L, respectively. A recent study conducted by Coelho *et al*. (2011) revealed an 88% ChE activity inhibition in *Daphnia* exposed for 48 h to trichlorfon of 0.25 μg/L, while the study revealed a 48-h LC_{50} of 0.29 μg/L. A study conducted by Duquesne and Küster (2010) revealed 80% ChE activity inhibition in *Daphnia* being exposed to 1.5 μg/L parathion-methyl for 24 h while the paper revealed that the insecticide had a 48-h LC_{50} of 0.6–7.0 μg/L. All of the above studies confirmed that, as has been observed in fish and many other aquatic vertebrates, ChE inhibition in *Daphnia* occurs only at near-lethal concentrations. Due to the existence of so-called compensating metabolism, anticholinesterase exposures trigger the onset of accelerated enzyme synthesis to rectify functional disability (Kaufer *et al*., 1999; Khattab and Ali, 2007). However, it should be noted that it is difficult to measure the actual inhibition of the ChEs in concentrations far below the lethal ones. To solve the problem, it is necessary to develop methodology for quantifying of ChE in bio-samples. Enzyme-linked immunosorbent assay (ELISA), a sort of immunoassay which combined sensitivity and specificity, is believed capable to fulfill the task. In order to quantify ChE in *Daphnia*, it is necessary to develop antibodies of the enzyme. One of the several possible ways to do this is to purify ChEs from the animals.

Despite the known importance of ChEs in *Daphnia*, the constitution and properties of those ChEs remain largely unstudied. It is known that acute toxicities of anticholinesterases could be two orders of magnitude higher in *Daphnia* than in aquatic vertebrates such as fish and amphibians. For example, according to Coelho *et al*. (2011), the 48-h LC_{50} of trichlorfon for *Daphnia magna* was 0.29 μg/L, whereas 96-h LC_{50} of the same insecticide for early-life stage and adult zebrafish was 25.4×10^3 and 28.8×10^3 μg/L, respectively. Studies on crude extracts indicated that the ChE from *Daphnia magna* showed characteristics of both AChE and PChE (Diamantino *et al*., 2007; Vesela *et al*., 2008). Before the ChE had been purified, it was difficult to say whether the species contained a single type of ChE with characteristics of both AChE and PChE, or if it contained two or more types of ChE with different characteristics. An understanding of *Daphnia* ChE composition would ultimately aids the sensitive detection of exposure of anticholinesterases in field conditions.

2 Materials and methods

2.1 *Daphnia* rearing

A pure-bred strain of *Daphnia magna* (i.e., 62 *Daphnia magna*), obtained from the Institute of Environmental Health and Related Product Safety, China Centers for Disease Control (CDC), was used as the enzyme source in this study. The *Daphnia* was cultured in dechlorinated tap water with a pH of 7.0 and hardness of 45 mg/L (counted with $CaCO_3$). It was fed daily with the unicellular algae, *Scemedesmus subspicatus*.

2.2 Chemicals

An Amplex® Red Acetylcholine (ACh)/ AChE Assay Kit (A12217), a product of Invitrogen™ (Eugene, Oregon, USA), was used. The DEAE-Sepharose™-Fast-Flow used was bought from GE Healthcare (Little Chalfont, Buckinghamshire, UK). Acetylthiocholine iodide (ATCh), *S*-butyrylthiocholine iodide (BTCh), 1,5-*bis*(4-allyldimethylammoniumphenyl)pentan-3-one dibromide (BW284C51), and tetraisopropylpyrophosphoramide (*iso*-OMPA) were bought from Sigma-Aldrich® (St. Louis, USA). Bovine serum albumin (BSA) and protein markers were bought from Biosharp® (Huaibei, Anhui, China). Coomassie Blue G_{250} and R_{250} were bought from Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The other reagents were domestic products of analytical degree unless specifically mentioned.

2.3 ChE activity determination

A method introduced by Zhou *et al*. (2000) was employed in this study for measuring ChE activity in the process of enzyme purification. The method was more sensitive than that of Ellman *et al*. (1961). To start the reaction, an aliquot of 100 μl diluted sample was mixed with 100 μl 400 μmol/L 10-acetyl-3,7-dihydroxyphenoxazine that contained 2 U/ml

horseradish peroxidase, 0.2 U/ml choline oxidase, and 100 μmol/L ACh. After incubation for 30 min at room temperature (i.e., 25 °C) in the dark, fluorescence of the mixture was measured with a Tecan Genios Microplate Reader (Tecan Austria GmbH, Groedig, Austria) at an excitation wavelength of 530 nm and emission wavelength of 595 nm.

2.4 Protein content determination

Protein concentration in samples was determined by method of Bradford (1976) using BSA as standard.

2.5 ChE extraction and purification

2.5.1 Preparation of crude extract

Daphnia larger than 0.42 mm was used as a source of enzyme. After being washed at least three times with ultra-pure water, the collections (3.7437 g) were homogenized for 3 min in 20 ml 50 mmol/L Tris-HCl (pH 7.5) containing 0.25% Triton X-100, 0.05 mol/L NaCl, and 1 mmol/L ethylenediaminetetraacetic acid (EDTA) (i.e., Buffer A). After that, a JY 92-II Ultrasonic Cell Disruption System (Ningbo Scientz Bio-Tech Co., Ltd., China) was employed at a power of 200 W for 99 repetitions of 5-s use followed by 5 s of rest. After 1-h centrifugation at 12857×*g*, the supernatant was collected for precipitation with ammonium sulfate. All of above operations were conducted at 4 °C.

2.5.2 Ammonium sulfate precipitation

Solid ammonium sulfate was added to the crude extract derived from above, step by step, to obtain saturation levels of 0%–45%, 45%–60%, and 60%–100%, respectively. Within each step, the mixture underwent a stirring for 10 min, a stay for 1 h on ice, and a centrifugation for 1 h at 12857×*g*. After the centrifugation, the pellet was collected for measuring ChE activity and protein content, while the supernatant was taken for precipitation at the next higher saturation. Before the measurement, the pellet was re-suspended in Buffer A to go through a centrifugation for 5 min at 12857×*g*. All above operations were conducted at 4 °C.

2.5.3 DEAE-Sepharose™-Fast-Flow chromatography

The pellet precipitated by ammonium sulfate of 60%–100% saturation was re-suspended in 5 ml of 50 mmol/L Tris-HCl (pH 8.1) containing 1 mmol/L EDTA (i.e., Buffer B). After a centrifugation for 5 min at 12857×*g*, the supernatant was dialyzed for 24 h against Buffer B. The buffer was changed at least three times during the dialysis in order to completely remove the salt.

The dialyzed sample was loaded on a glass column (with inner diameter of 1.0 cm) filled with 10 cm DEAE-Sepharose™-Fast-Flow that was previously equilibrated with Buffer B. After being washed with Buffer B of 60 ml (corresponding to 20 tubes), the sample was eluted in sequence with 50 mmol/L Tris-HCl buffer (pH 8.1) spiked with NaCl of 0.1, 0.2, 0.4, 0.6, and 0.8 mmol/L, respectively, at a flow rate of 36 ml/h. Effluents were collected every 3 ml with BSZ-100 (an automatic collector manufactured by Shanghai Jingke Industrial Co., Ltd., China). ChE activity and protein content in each effluent were measured by the method described above. Effluents with considerable activity (i.e., greater than 0.1 U/ml) of ChE were pooled and then dialyzed against Buffer B to remove NaCl. All above operations were conducted at 4 °C.

2.6 SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the procedure described by Laemmli (1970). Samples to be measured involved the crude extract, the pellet precipitated by ammonium sulfate of 60%–100% saturation, and the pooled effluents with ChE activity >0.1 U/ml. After being diluted with sample buffer (i.e., 1 mol/L Tris-HCl buffer of pH 6.7 that contained 2.5% (25 g/L) SDS, 10% (100 g/L) sucrose, and 0.05% (500 mg/L) bromophenol blue), the samples were put in boiling water for 5 min and then on ice for 10 min. Thereafter they were put on top of the gel together with their markers. Sample of 10 μl was spiked with the marker of 5 μl. "Low" marker was employed together with the separating gel of 8.5%, and "high" marker was employed together with the separating gel of 12.5%. The stacking gel concentration was 5% (50 g/L).

The "high" marker consisted of chicken egg white ovalbumin (44.3 kDa), BSA (66.4 kDa), rabbit muscle phosphorylase B (97.2 kDa), *Escherichia coli* β-galactosidase (116.0 kDa), and pig skeletal muscle

myosin (200.0 kDa). The "low" marker consisted of lysozyme (14.3 kDa), soybean trypsin inhibitor (20.1 kDa), bovine carbonic anhydrase (29.0 kDa), chicken egg white ovalbumin (44.3 kDa), BSA (66.4 kDa), and rabbit muscle phosphorylase B (97.2 kDa).

The electrophoresis was carried out at 60 V and the gel was stained with Coomassie Blue R_{250}. To plot mobility factor against molecular mass of the markers, a linear equation was established. The equation was employed for estimating molecular mass of unknown bands.

2.7 Kinetic studies

2.7.1 Substrate preference

Catalytic activities of the purified ChE corresponding to different concentrations of substrates were measured in this study. The method employed for enzyme activity determination was basically the same as that described in Section 2.3 except that ATCh and BTCh were used instead of ACh. To take ChE activity as the dependent variable and substrate concentration as the independent variable, Michaelis-Menten equations were established using data processing system (DPS)©. The values of the Michaelis constant (K_m) and maximum velocity (V_{max}) that related the two substrates were estimated from their respective equation.

2.7.2 Sensitivity to inhibitors

Inhibitor studies were carried out with two selective inhibitors of ChE, i.e., BW284C51 and *iso*-OMPA. Enzyme sources involved were the crude extract, the pellet precipitated by ammonium sulfate of 0%–45% saturation, and the pooled effluents of DEAE-Sepharose™-Fast-Flow chromatography with ChE activity greater than 0.1 U/ml. The method for ChE activity determination was basically the same as that described in Section 2.3 except that ATCh was used instead of ACh.

3 Results

3.1 Purification of ChE

About 400 μg of ChE was purified from nearly 4 g of *Daphnia* by the four-step procedure, i.e., ultrasonic cell disruption, Triton X-100 extraction, ammonium sulfate precipitation, and DEAE-Sepharose™-Fast-Flow chromatography. Nearly 19% recovery was achieved at the end of the procedure. The purified ChE had specific activity of 330.8 U/mg of protein (Table 1).

Fig. 1 shows the precipitation profile of ChE and that of total protein at three ammonium sulfate saturation levels (i.e., 0%–45%, 45%–60%, and 60%–100%). The residual amounts of ChE and total protein in the supernatant of 100% saturation of ammonium sulfate are also displayed in Fig. 1. One can see from Fig. 1 that a low proportion (i.e., <5% and close to 10%) of ChE was precipitated at saturation levels of 0%–45% and 45%–60%, respectively. The supernatant of 100% saturation contained no ChE. More than 80% of the ChE was precipitated at the saturation level of 60%–100%. This fraction was

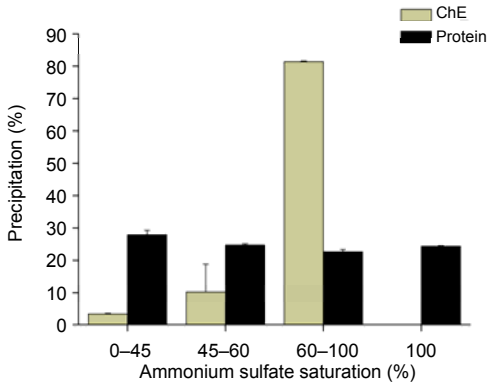

Fig. 1 Precipitation of ChE and total protein at different saturation levels of ammonium sulfate

Percentages of ChE and total protein precipitated at each saturation level were calculated based on total ChE activity and total protein content of the crude extract. Each column is a mean of three measurements. Vertical bars indicate SD

Table 1 Purification procedure with respect to ChE from *Daphnia magna*

Procedure	Volume (ml)	Protein content (mg/ml)	Total protein (mg)	Total activity (U)	Specific activity (U/mg protein)	Recovery[a] (%)	Purification factor[b]
Triton X-100 extraction	20	6.5780	132.000	701	5.332	100.0	1.0
Ammonium sulfate precipitation	10	0.6453	6.500	571	87.870	81.5	16.5
DEAE-Sepharose™-Fast-Flow chromatography	1.2	0.3333	0.399	132	330.800	18.8	62.0

[a] Recovery (%): total activity in each step/total activity in the first step; [b] Purification factor: specific activity in each step/specific activity in the first step

therefore used for further purification with DEAE-Sepharose™-Fast-Flow column.

The elution profile of ChE and that of total protein are diagrammed in Fig. 2. Two peaks of ChE appeared with increasing NaCl concentration. One occurred at a concentration of 0.1 mol/L (corresponding to tubes 20th–28th), and the other came forth at 0.2 mol/L (corresponding to tubes 50th–55th). The two peaks are referred to as Fraction A and Fraction B, respectively.

Fig. 3 diagrams the results of the SDS-PAGE for different extractions and fractions. As indicated in Fig. 3a, a large portion of proteins of low molecular mass was removed from the crude extract by ammonium sulfate precipitation with a <60% saturation. According to the result of SDS-PAGE as indicated in Fig. 3b, a main band with molecular mass close to that of rabbit muscle phosphorylase B and a faint band with molecular mass between soybean trypsin inhibitor and bovine carbonic anhydrase were detected in Fraction A. As for Fraction B, no remarkable band was detected except the main band with molecular mass close to that in Fraction A. This suggested that Fraction B was more pure than Fraction A. The molecular mass of the main band in Fraction B was estimated to be 84 kDa.

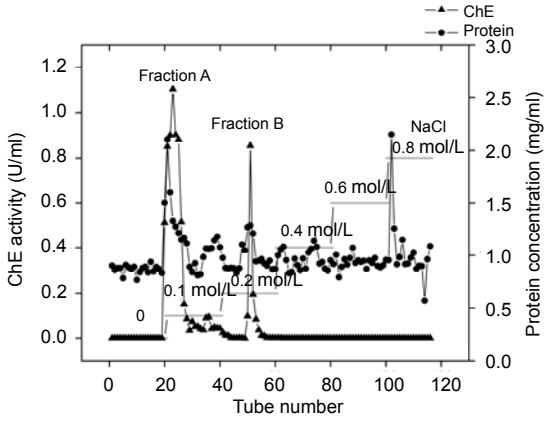

Fig. 2 Effluent pattern of ChE and total protein from DEAE-Sepharose™-Fast-Flow ion-exchange chromatography with increasing NaCl concentration

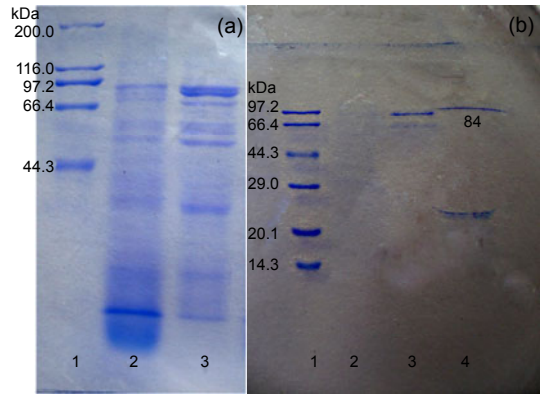

Fig. 3 Diagram of SDS-PAGE for ChE of different sources
(a) Crude extract and some pellet precipitated by ammonium sulfate precipitation. Lanes listed from left to right are: (1) markers of high molecular mass, (2) crude extract, and (3) pellet precipitated by ammonium sulfate of 60%–100% saturation. (b) Crude extract and fractions from DEAE-Sepharose™-Fast-Flow chromatography. Lanes listed from left to right are: (1) markers of low molecular mass, (2) crude extract, (3) purified ChE (i.e., Fraction B), and (4) partially purified ChE (i.e., Fraction A)

3.2 Substrate preference of the purified ChE

Activities of the purified ChE (i.e., Fraction B) at different concentrations of ATCh and BTCh are shown in Fig. 4. Both ATCh and BTCh can be hydrolyzed at a comparable rate by the purified enzyme. The values of K_m and V_{max} were estimated to be 0.035732 mmol/L and 301.1458 U/mg protein, respectively, for BTCh, 0.056151 mmol/L and 300.1667 U/mg protein, respectively, for ATCh. Based on these estimations, the value of V_{max}/K_m was 8.428 L/(min·mg protein) for BTCh, and it was 5.346 L/(min·mg protein) for ATCh. Activity of the purified enzyme was inhibited by high concentrations of either ATCh or BTCh.

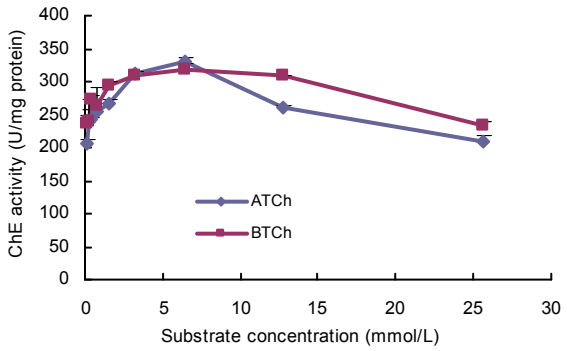

Fig. 4 Effect of substrate (either ATCh or BTCh) concentrations on activity of purified ChE
Each data point is a mean of three replications (n=3). Vertical bars indicate SD. Values of Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) were calculated based on Michaelis-Menten equations, i.e., V=301.1458S/(0.035732+S) for BTCh, and V=300.1667S/(0.056151+S) for ATCh, where V is enzyme activity, and S is substrate concentration

3.3 Sensitivity of ChE to selective inhibitors

Sensitivities of ChE towards two selective inhibitors, i.e., BW284C51 and *iso*-OMPA, were illustrated in Fig. 5. The crude extract and the pellet precipitated by ammonium sulfate of 0%–45% saturation were more sensitive to BW284C51 than to *iso*-OMPA (Figs. 5a and 5d). Conversely, Fraction A and Fraction B were more sensitive to *iso*-OMPA than to BW284C51 (Figs. 5b and 5c).

4 Discussion

4.1 Characteristics of the purified ChE

The common features between Fraction A and Fraction B were that the two fractions had a main band at a molecular mass of about 90 kDa and that they were more sensitive to *iso*-OMPA than to BW284C51. Published data on molecular mass of subunit of ChE from various species indicated the values ranging from 65 to 94 kDa (Table 2). Based on the data, the faint band in Fraction A, which showed a molecular mass between 20.1 to 29.0 kDa, was not likely to be a type of ChE. One could, therefore, infer that the activity of ChE in Fraction A is derived from the main band. Since the main band in Fraction B was similar in terms of mobility to the main band in Fraction A, and both Fraction A and Fraction B responded similarly to the selective inhibitors, it is rational to infer that the main band in Fraction B was a type of ChE.

The value of V_{max}/K_m is considered to be an indicator of substrate affinity (Principato *et al*., 1988). The higher the value, the more intense the affinity is. The results of the substrate study as shown in Fig. 4 indicate that the value of V_{max}/K_m for the purified ChE (i.e., Fraction B) was 58% higher in cases where BTCh was employed instead of ATCh. This indicates that the purified ChE has a higher affinity towards

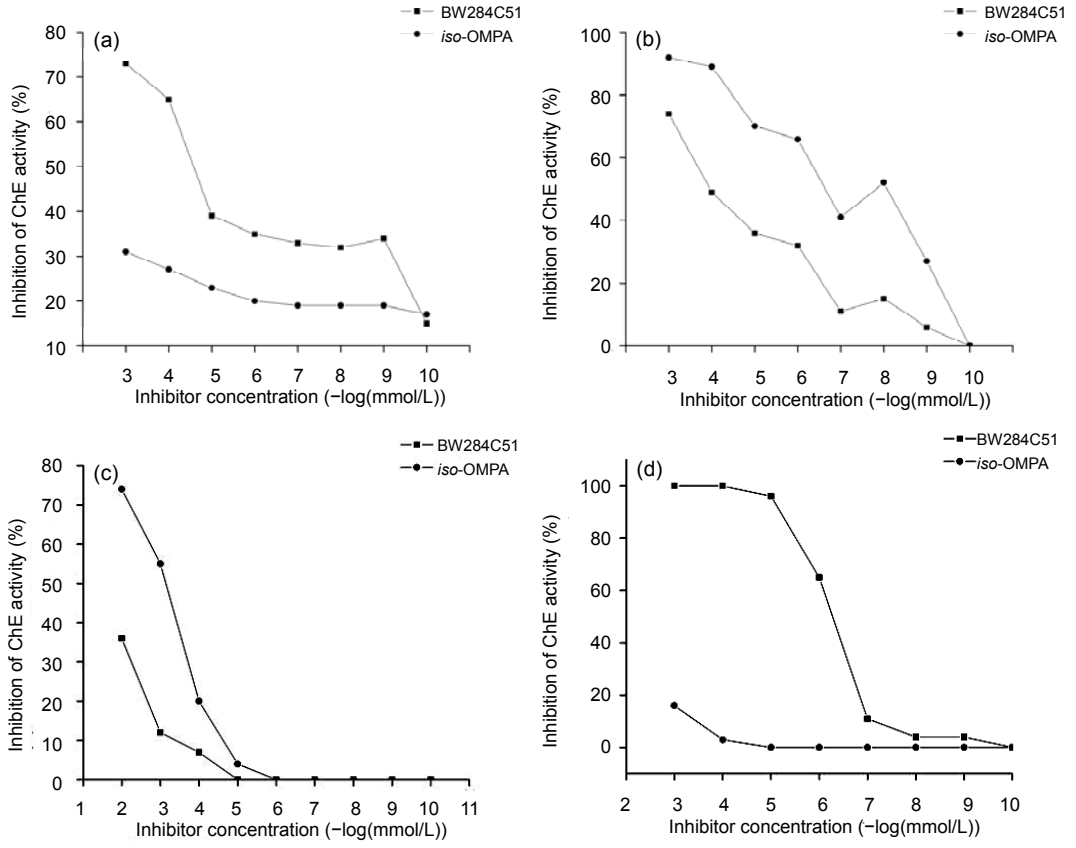

Fig. 5 Inhibition of *Daphnia* ChE activity by selective inhibitors

(a) Crude extract; (b) Partially purified ChE (i.e., Fraction A); (c) Purified ChE (i.e., Fraction B); (d) Pellet precipitated by ammonium sulfate of 0%–45% saturation

Table 2 Molecular mass of subunit(s) of ChE from different sources

Source of the enzyme		Molecular mass of the subunit(s) (kDa)	Source of the data
Specie	Tissue		
Silkworm *Bombyx mori* L.	Head	77.8	Peng *et al*., 2008
Fish *Scomberomorus niphonius*	Brain	85	Zhu *et al*., 2006
Horse	Serum	72	Li *et al*., 2006
Nematode *Nippostrongylus brasiliensis*	Whole bodies	66	Zhang and Yang, 2006
Leafhopper *Nephotettix cincticeps* Uhler	Whole bodies	75	Kato *et al*., 2004
Human	Cerehellum	67	Zhang *et al*., 1999
Colorado potato beetle *Leptinotarsa decemlineata*	Whole bodies	65	Zhu and Clark, 1994
Pigeon *Columba livia*	Serum	84	Khattab *et al*., 1993
Legume bug *Lygus hesperus* Knight	Whole bodies	79–94	Zhu and Brindley, 1992

BTCh. In spite of that, the enzyme was inhibited by excess substrates. This information implies that the purified ChE was a type of PChE with some properties of AChE.

The results of inhibitor studies are shown in Figs. 5b and 5c and indicate that both Fraction A and Fraction B were more sensitive to *iso*-OMPA than to BW284C51. BW284C51 and *iso*-OMPA are known selective inhibitors for AChE and PChE, respectively (Chuiko, 2000; Kousba *et al*., 2003). This supports the idea that Fraction A and Fraction B are more like types of PChE.

The enzyme of PChE-type was reported to be separated from species of Crustacea such as spiny lobster *Palinurus vulgaris* (Talesa *et al*., 1992), mantis shrimp *Squilla mantis* (Principato *et al*., 1988), and brine shrimp *Artemia salina* (Acey *et al*., 2002). According to Mehrani (2004), the K_m of human butyerylcholinesterase was estimated to be (0.018±5) and (0.083±12) mmol/L in case that BTCh and ATCh were respectively used as substrates. According to Guo *et al*. (1993), the K_m of duck serum cholinesterase was estimated to be 0.098 mmol/L when BTCh was used as a substrate. This indicated that PChEs might differ in terms of their kinetic properties when collected from different animals or tested with different substrates. The results of the substrate study, as shown in Fig. 4, indicate that the K_m of Fraction B was estimated to be 0.035732 and 0.056151 mmol/L when BTCh and ATCh were used as substrates, respectively. It was very possible that the K_m of the purified ChE was between the human butyerylcholinesterase and the duck serum cholinesterase.

4.2 Type constitution of ChE in *Daphnia*

The results of the inhibitor study, as shown in Fig. 5a, indicate that the crude extract was more sensitive to BW284C51 than to *iso*-OMPA. This result was verified by a former study conducted by Diamantino *et al*. (2007). The study showed that the no observed effect concentration (NOEC) towards the crude extract was <0.0078 mmol/L for BW284C51 and it was 0.5 mmol/L for *iso*-OMPA. Also, the study indicated that the apparent K_m of the crude extract was estimated to be 4.28 and 83.13 μmol/L, respectively, for ATCh and BTCh. All these studies suggested that *Daphnia* contained enzyme of AChE-type in the bodies.

According to Figs. 5b–5d, the pellet precipitated by ammonium sulfate of 0%–45% saturation contained ChE that was more sensitive to BW284C51, whereas the pellet precipitated by ammonium sulfate of 60%–100% saturation (from which Fraction B and Fraction A derived) contained ChE that were more sensitive to *iso*-OMPA. This suggested that AChE-type enzyme might have resided in the pellet precipitated by ammonium sulfate of 0%–45% saturation, and it was removed from the crude extract in process of ammonium sulfate precipitation.

It is known that AChE and PChE coexist in mammals. AChE is responsible for terminating nervous action at postsynaptic membrane by hydrolyzing neurotransmitter ACh, whereas PChE is more like a scavenger to prevent anticholinesterases such as OPs and CBs from reaching their targets (Doctor *et al*., 1993; Raveh *et al*., 1993; Maxwell *et al*., 1999; Wang *et al*., 2004; Lenz *et al*., 2005). Their roles in Arthropoda are not completely clear. It is believed

that most species of insect have single AChE which can hydrolyse both ACh and butyrylcholine. In spite of that, multi-types of ChE were found in species including ticks *Boophilus microplus* (Nolan *et al*., 1972), house flies *Musca domestica* (Tripathi and O′Brien, 1972), spring grain aphids *Schizaphis gramina* (Brestkin *et al*., 1985), honey bees *Apis melifera* (Belzunces and Colin, 1991), Colorado potato beetles *Leptinotarsa decemlineata* (Ioannidis *et al*., 1992; Wierenga and Hollingworth, 1993), mosquitos *Culex pipiens* (Bourguet *et al*., 1996), and common oystesr *Crassostrea gigas* (Bocquené *et al*., 1997). Two hypotheses could be employed to explain that: the first involves the existences of two ChE-encoding genes and the second comes down to the mechanism of posttranslational modification (Bocquené *et al*., 1997; Villatte and Bachmann, 2002).

Considering that different types of ChE might differ in their sensitivity towards inhibition and this might be decisive with regard to survival of *Daphnia* exposed to anticholinesterases, the type of ChE in 0%–45% ammonium sulfate precipitation should be separated and its properties should be investigated in further studies.

5 Conclusions

A type of ChE with molecular mass estimated to be 84 kDa was purified from bodies of *Daphnia magna*. The purified enzyme could be regard as an untypical PChE based on the fact that: (a) It hydrolyzed both ATCh and BTCh with comparable rate and showed slight preference to BTCh; (b) It could be inhibited by high concentration of either ATCh or BTCh; (c) It was more sensitivity to *iso*-OMPA than to BW284C51.

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Compliance with ethics guidelines

Yan-xia YANG, Li-zhi NIU, and Shao-nan LI declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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