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Sensitivity of crude and partially purified acetylcholinesterase from fish to carbamates and organophosphates

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Abstract

In this work, the selective sensitivity of carbamates and organophosphates (OP) towards crude and partially purified fractions of AChE from the brain of *Pangasius pangasius* is reported. ANOVA analysis showed that all carbamates tested whether in crude or partially purified fractions showed significant inhibition to AChE activity as compared to control ($p<0.05$). ANOVA analysis also showed that there was no significant difference between crude and partially purified fraction in terms of inhibition caused by carbamates ($p>0.05$). Although OPs such as acephate and dimethoate did not show significant inhibition compared to control in the crude fraction, they showed significant inhibition of 8.9 and 9.3% as compared to control ($p<0.05$) in the partially purified fraction. In addition, although the rest of the OP showed significant inhibition compared to control in both fractions, OPs such as diazinon and trichlorfon showed significantly higher inhibition in the partially purified fraction compared to crude fraction ($p<0.05$) suggesting that partially purified AChE is more sensitive to OPs compared to the crude fraction.

Key words

Carbamates, Organophosphates, AChE, *Pangasius pangasius*

Introduction

Insecticides are so widely used that their residues are found in fruits, vegetables and other foods. This makes insecticide detection an important global agenda (Ekanayake *et al.*, 2011). Acetylcholinesterase (AChE), the enzyme that degrades the neurotransmitter acetylcholine present at the synapse, is one of the widely used enzymes for the detection of insecticides in various samples especially vegetables and fruits (Perić and Petrović, 2011). *Electrophorus electricus* and *Drosophila melanogaster* are common sources of AChE (Villatte *et al.,* 1998). There has been an attempt to use enzymes from other fish species aside from *E. electricus* (Bocquene *et al.,* 1990). Fish has been traditionally used as one of the test organisms for the bioassay of a variety of toxicants such as insecticides (Khan and Khan, 2012), pesticides (Qamar *et al.,* 2012), textile dyes (Oyewo and Don-Pedro, 2006) and heavy metals (Kousar and Javed, 2012; Javed, 2012). This reflects the sensitivity of fish to toxicants. During preliminary works on using the brains of *P. pangasius* as a source of AChE, it was discovered that for certain insecticides, the sensitivity towards AChE is the same whether crude or affinity-purified fraction was used, and for OPs there is a significant difference in terms of inhibition between crude and partially purified fraction. In this work the selective sensitivity towards carbamates and organophosphates of crude and partially purified fraction of AChE from P. pangasius is reported.

Materials and Methods

Preparation of partially purified AChE from fish: *Pangasius pangasius* was used as a fresh water test organisms. The fish weighing 800-1000 g and ≈ 30 cm in length were obtained from the hatchery at Universiti Putra Malaysia, Serdang, Selangor. The fish were killed by decapitation and the whole brain was dissected out immediately. Preparation of crude brain extract and affinity chromatographic separation of the crude enzyme was performed using procainamide, a ligand specific for the choline-binding site

according to the method outlined by Tham *et al.* (2009).

AChE assay: AChE activity was estimated following the method of Ellman *et al.* (1961) with modification for a 96 well microplate assay. This method employed acetylthiocholine iodide (ATC) as a synthetic substrate for AChE. Acetylthiocholine iodide was broken down to thiocholine and acetate by AChE and thiocholine reacted with 5, 5'-dithio-bis-2-nitrobenzoate (DTNB) to produce a yellow color. The quantity of yellow color which develops over time is a measure of the activity of AChE. AChE activity was expressed as the amount of acethylthiocholine iodide (mmol) broken down by AChE per minute. The specific activities were given as mmole hydrolyzed min-1 mg-1 protein or U mg^{-1} of protein and were calculated on the basis of an extinction coefficient of 13.6 mM^{-1} cm^{-1} (Ellman *et al.,* 1961).

The insecticides to be incorporated in the screening assay were dissolved in either acetonitrile or deionized water to a final concentration of 1 mg l^{-1}. The reaction mixtures contained 150 µl of 0.1 M potassium phosphate buffer pH 8.0, 20 µl DTNB (0.067 mM) followed by 50 µl of the pesticides (final concentration was 1 mg l^{-1}), and subsequently 10 µl of enzyme. The reaction mixture was incubated in dark for 30 min at room temperature. Then, 20 µl of ATC (0.5 mM) was added into the reaction mixture and left to stand for 10 min at room temperature before the absorbance was read at 405 nm. The control was run through the same procedure except substituting pesticides with potassium phosphate buffer pH 8.0. Values are means ± SE. All data were analyzed using Graphpad Prism version 3.0 and Graphpad InStat version 3.05. Comparison between groups was performed using a Student's t-test or a one-way analysis of variance (ANOVA) with post hoc analysis by Tukey's test (Miller and Miller, 2000). $P < 0.05$ was considered statistically significant.

Results and Discussion

The AChE enzyme was partially purified from the brain of *P. pangasius* using procainamide-Sephacryl S-1000-based affinity chromatography. Table 1 shows that the partial purification of the enzyme was successful with 6-fold purification and a good yield of 50%.

ANOVA analysis showed that all carbamates tested whether in crude or partially purified fractions showed significant strong inhibition as compared to control ($p<0.05$). ANOVA analysis also showed that there was no significant difference between crude and partially purified fraction in terms of amount of inhibition caused by carbamates ($p>0.05$). Although OPs such as acephate and dimethoate did not show significant inhibition compared to control in the crude fraction, they showed significant inhibition of 8.9 and 9.3 % as compared to control ($p<0.05$) in the partially purified fraction. In addition, although the rest of the OP showed significant inhibition compared to control in both fractions, OPs such as diazinon and trichlorfon showed significantly higher inhibition in the partially purified fraction compared to crude fraction ($p<0.05$) suggesting that partially purified AChE is more sensitive to OPs compared to the crude fraction.

Table 1 : Purification table for *C. batrachus* brain AChE

Fraction	Total protein (mg)	Total activity (U)	Specific activity (U mg^{-1})	Purification fold	Yield (%)
Crude	1200	100,050,112.1	83,375.1	1.0	100.0
Partially purified	100	50,146,635.10	501,466.4	6.0	50.1

Similar works in the studies of the effect of heavy metals on the proteases bromelain (Masdor and Said, 2011) and papain (Masdor and Said, 2012) showed marked increase in inhibition once the commercial crude preparation is further purified. One of the probable reasons for the improvement in sensitivity of AChE to insecticides is during purification, possible insecticide-binding proteins other than the enzyme being studied is removed. This makes the bioavailability of the target toxicant to acetylcholinesterase to increase and hence the lower IC_{50} values observed. This removal of insecticide-binding protein after purification is dependent upon the type of insecticides being studied with OPs more affected than carbamates. More studies are needed to identify the noncholinesterase OPs-binding protein in crude enzyme preparation of *P. pangasius*. Recently, OPs have been discovered to form covalent bonds with tyrosine and lysine residues instead of the usual serine found in AChE. The binding of OPs to noncholinesterases has been suggested as a possible mechanism seen in low dose OPs toxicity (Duysen *et al.*, 2001).

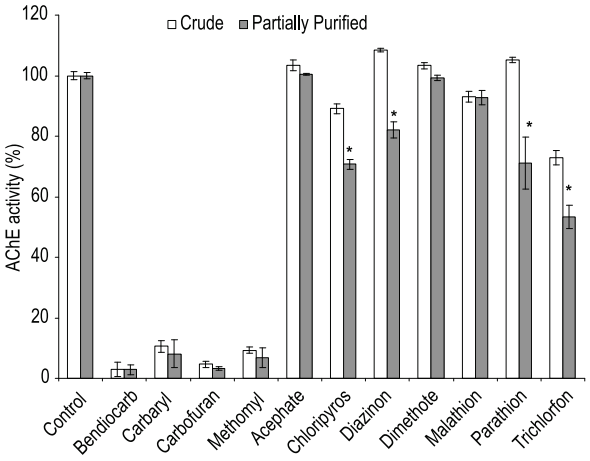

Fig. 1 : Effect of various pesticides on the enzymatic inhibition of crude (□) and partially purified (■) AChE from *P. pangasius*. Mean values significantly different from those obtained from crude enzyme are marked by stars ($p<0.05$). Values are mean of 3 replicates ± SD

In conclusion, the overall results suggest that partially purified fraction was less sensitive to carbamates and more sensitive to OPs as compared to crude fraction. Further studies on greater inhibition by OPs compared to carbamate in the partially purified fraction needs to be investigated. In general, purification of enzymes removes unwanted inhibitors and other isoenzymes in the preparation that might reduce enzyme activity through unspecific binding of substrates to isoenzymes or depletion of available inhibitors that bind to other proteins through weak forces (Scopes, 1994).

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