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The Effect of Malathion on the Activity of Cholinesterase from the Freshwater Shrimp *Caridina* sp.

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ABSTRACT

Acetylcholinesterase (AChE) from *Caridina* sp. was tested for its sensitivity towards the anticholinesterase malathion. Prior to this study, optimal assay conditions were determined to be 25 °C, pH 8.0 and using phosphate buffer. At 10^{-8} M, malathion was able to inhibit AChE activity at 1.75% and this increased to 15.79% with the increasing of malathion concentration to 10^{-4} M. It is proven through this study that the crude extract of AChE is sensitive toward malathion exposure and provide an alternative source for biosensor development.

INTRODUCTION

Organophosphate (OP) insecticides such as malathion is an effective application in the protection of the crops and the control of mosquito and flies [1-3]. Malathion is capable to kill mosquito *Aedes aegypti* even at the larvae stage [4]. The main function of this compound is as a nervous system inhibitor by binding to the acetylcholinesterase (AChE) enzyme [5]. AChE plays a major role in termination the effect of neurotransmitter acetylcholine (ACh) by breaking it down to choline and acetate, acting like an off switch [6].

AChE is well known as the main target of anticholinesterase such as OP and carbamate by inhibiting the metabolisation of ACh through the binding mechanism called phosphorylation and carbamylation at the active site of AChE, respectively [7-9]. Unlike carbamylation process, phosphorylation need the activated form of OP, also called oxon to form a bind at the esteristic site. In the biological system, oxonation was performed through the mechanism of oxidative desulfuration by flavin-containing monooxygenases and cytochrome P450-mediated monooxygenases which can oxidase a wide variety of xenobiotics [10-12].

This bioactivation facillitate the phosphorylation process in which the oxon-OP react the serine hydroxyl group within the AChE esteristic site, phosphorylating this hydroxyl group and yielding a hydroxylated "leaving group". This blockage causes malfunction of AChE and depolarization as a consequence of ACh accumulation in cholinergic synapses [13].

The bioactivation of malathion to malaoxon enhance the binding to AChE. Previous studies have reported on the sensitivity of malathion to various AChE species such as fish [14, 15], rodent [16,17] and bird [18]. *Caridina* sp. is a freshwater prawn found in Malaysia. The use of this organism as a cheap and readily found source of acetylcholinesterase for the detection of malathion has never been carried out.

Therefore, this study was carried out to determine the optimum AChE assay parameter and the effect of malathion at different concentrations on the activity of AChE from crude *Caridina sp.* preparation.

MATERIALS AND METHODS

Sample preparation

The shrimps were killed by freezing in a box of ice for 5 minutes. The shrimp heads were weighted and subsequently homogenized using an electrical homogenizer with the ratio of 1:5 volume of 0.1 M phosphate buffer, pH 8.0. The extractant was centrifuged at 5,000 xg for 5 min at 4 ^{o}C. The supernatant was recovered and stored at -25 ^{o}C, or in ice bath for further analyses.

Enzyme activity and protein content determination

AChE activity from the extract was determined as according to the method of Ellman *et al*. [19]. The assays were done in triplicates in a test tube before the extract was transferred to cuvette for measurement. The mixture of 3 ml of 0.1 M phosphate buffer, pH 8.0, 200 µl of 0.01 M 5,5'-dithiobis-(2-nitrobenzoic acid); DTNB and sample was incubated for 5 minutes. Then, 200 µl of 0.075 M acetylthiocholine iodide; ATCi was mixed and measured spectrophotometrically at 412 nm after incubate for 3 minutes. The protein content was determined based on Bradford method [20] using a series concentration of bovine serum albumin as the standard.

Assay parameters

pH profile

AChE was test separately by replacing the previous buffer with different pH of three buffer namely acetate buffer (pH 3.0, 4.0, 5.0 and 5.5), phosphate buffer (pH 5.0, 6.0, 7.2 and 8.0) and Tris-HCl buffer (pH 7.0, 8.0, 9.0 and 10.0).

Temperature profile

The mixture was separately incubated priorly in different temperature of 15, 20, 25, 30, 40, 50, 60 and 70 ^{o}C for 5 minutes without ATCi added. ATCi mixed throughly and the absorbance was measured after 3 minutes incubation.

In vitro effect of Malathion

The differential concentration of malathion was prepared at the range of 10^{-4} to 10^{-10} M. the assay routinely contained 2.7 ml of selected optimum buffer for assay, 200 µl of 0.01 M DTNB and 300 µl AChE sample. The mixture was incubate at the optimum temperature for 30 minutes (according to Bastos et al. [21]) prior to the addition of ATCi. The absorbance was read at 412 nm.

RESULTS AND DISCUSSIONS

The enzyme assay parameter was determined. pH profile of crude homogenate AChE shrimp was from 0.082 to 4.461 µmol/min for all test buffer between pH 3.0 and 10.0 (**Fig.** 1). There were slight differences between phosphate with Tris-HCl buffer, and phosphate with acetate buffer about 0.123 and 0.074 µmol/min at pH 8.0 and 5.0, respectively. The result obtained showed that the maximum activity was reached at pH 8.0 and was dramatically reduced at other pH values. Optimum temperature conditions was evident at 25 ^{o}C with the AChE activity value of 4.191 µmol/min. This also indicated that the assay is suitable to be performed at room temperature (**Fig.** 2).

At low temperature, the enzyme activity is retarded but not denatured. At higher temperatures the thermal effect caused molecular vibrations that lead to the alteration of AChE structure or being denatured associate to substrate failed to hydrolyse. This study has a same optimum temperature value with Ellman et al. [19] and Ribeiro et al. [22]. Although those studies used a similar type of buffer, Ribeiro et al. [22] reported different optimum pH which is at 7.2.

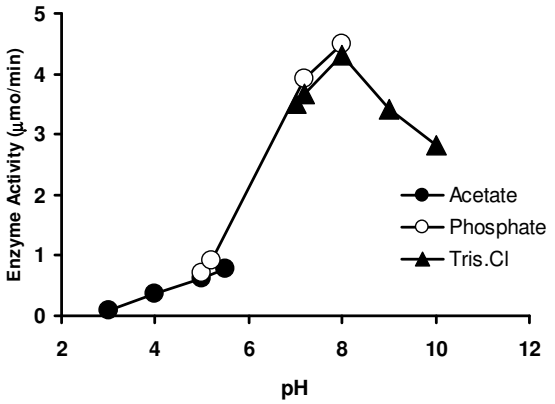

Fig. 1. The effect of AChE activity in different pH of different buffering system. Coefficient of variation for values of each mean were lower than 5%.

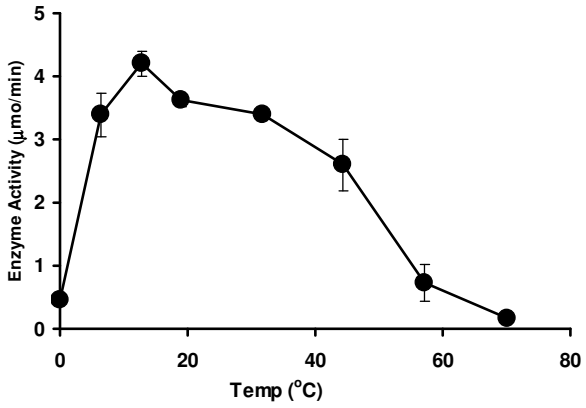

Fig. 2. Shrimp AChE was incubated at different temperature. Error bars represents standard deviation of means (n=3).

Percentage of inhibitions is shown in **Fig.** 4. At concentrations below 10^{-9} M, no inhibition was observed on shrimp AchE activity, while the initial inhibition was determined at 10^{-8} M with a percentage of 1.75% but showed no significant diffferent to control ($p>0.05$). The increasing concentration of malathion was associated with less production of yellow color meaning that the enzyme activitiy was decreased.

The results proved that shrimp AChE was significantly affected by malathion. Studies concerning inhibition of AChE are well documented [21,23] but rarely in prawn. Therefore, the sensitivity obtain has a potential fto develop a biosensor tool to monitor the presene of malathion in the environment [24].

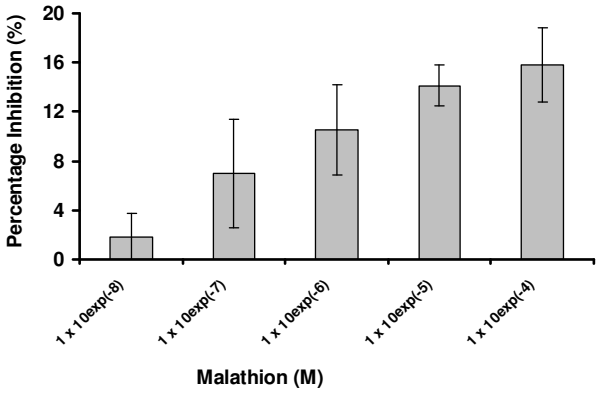

Fig. 3. Shrimp AChE was incubated at various concentrations of malathion. The error bar represent standard deviation of means.

CONCLUSION

The sensitivity of the shrimp *Caridina* sp AChE towards malathion is proven in this study and this study provided a fundamental data to develop a biosensor kit using AChE source from this species. However, further study is needed such as the sensitivity of malathion on purified enzyme, the inhibition constants and field trial studies to asses the efficacy of AchE from this source as a sensitive test for the presence of malathion.

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