UNIVERSITI PUTRA MALAYSIA

ACETYLCHOLINESTERASE FROM *Osteochilus hasselti* FOR THE DETECTION OF INSECTICIDES AND HEAVY METALS

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By

MOHD KHALIZAN BIN SABULLAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Partial Fulfilment of the Requirements for the Degree of Master of Science

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Dedicated to my family and friends.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

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Chairman: Assoc. Prof. Mohd. Yunus Bin Abdul Shukor, PhD

Faculty : Biotechnology and Biomolecular Sciences

The use of acetylcholinesterase to detect insecticides such as carbamate and organophosphate is well known. However, new sources of AChE are constantly being sought since the most crucial preparation of AChE rarely is sensitive to all insecticides. At the same time, literature data shows that certain AChEs are sensitive to heavy metals and by searching new sources, AChE can be applied for the detection of heavy metals as well. In this work, an inhibitive assay for insecticide and heavy metals was developed using partially purified fraction of AChE from *Osteochilus hasselti* using Procainamid Sephacryl S-1000 with a purification fold of 8.1 and a recovery yield of 55.3% were obtained. The partially purified fraction showed optimal activity at pH 7.0 and optimal temperature in the range of 25^{o}C to 30^{o}C. Acetylthiocholine iodide (ATC) was chosen as specific substrate with higher maximal activity (V_{max}) of 742.43 µmole/min and the lowest Michaelis Constant (K_m) at 0.0086 mM followed by Propionylthiocholine Iodide (PTC) and Butyrylthiocholine Iodide (BTC). All of the carbamate groups such as carbaryl, bendiocarb, carbofuran, methomyl and propoxur, and several oxonated OPs such as chlorpyrifos, diazinon,

malathion and parathion showed significant inhibition more than 50% of AChE activity. Partially purified AChE was not inhibited by tricholrfon, dimethoate and acephate. ANOVA analysis showed that all of the inhibition seen was significant compared to control ($p<0.05$) in the absence of insecticide. The carbamates; carbaryl, and the oxonated OPs; diazinon showed significantly lower IC_{50}s in *O. hasselti* compared to *E. electricus* while parathion and diazinon showed overlapped IC_{50}s. Chromium, copper and mercury inhibited 80%, 61.3% and 35% of AChE activity, respectively. The heavy metals exhibited exponential decay type inhibition curves with calculated IC_{50} for copper, and chromium at 0.198 and 0.076 mg l^{-1}, respectively. The effect of sample matrix in detecting insecticides using tap water, sea water and waters from two pristine rivers samples spiked with carbamate showed no significant differences amongst each other ($p>0.05$) indicating no matrix effect. Among all the vegetable samples studied, chili shows the presence of insecticide and validated by HPLC analysis. Sungai Juru exhibited higher inhibition proved by ICP-OES high with amount of chromium and copper while other river samples showed low inhibition except for Endau Rompin national water park sample. It can be summarized that *O. hasselti* AChE is a new alternative source as biosensor to detect contaminant because the sensitivity of this enzyme is almost similar compare to other commercialize AChE. For further studies, it is recommended that full purification should be carried out to understand the fundamental differences between this AChE and other AChEs to further understand the mechanism of heavy metals and insecticides inhibition.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

ASETILKOLINESTERES DARIPADA *Osteochilus hasselti* UNTUK MENGESAN RACUN SERANGGA DAN LOGAM BERAT

OLEH

MOHD KHALIZAN BIN SABULLAH

Ogos 2011

Pengerusi: Prof. Madya Mohd. Yunus Bin Abdul Shukor, PhD

Fakulti : Biotechnologi and Sains Biomolekul

Penggunaan asetilkolinesteres untuk mengesan karbamat dan organofosfat telah diketahui ramai. Walaubagaimanapun, sumber AChE terbaru masih dikaji kerana penyediaan penting AChE adalah amat jarang sekali dapat mengesan kesemua jenis racun serangga. Pada masa yang sama, data literatur menunjukkan AChE tertentu adalah sensitif dalam mengesan logam berat dan dengan mencari sumber terbaru, AChE juga boleh diaplikasikan sebagai pengesan logam berat. Dalam kajian ini, satu asai perencatan untuk racun serangga dan logam berat telah pun dibangunkan menggunakan fraksi penulenan separa AChE dari *Osteochilus hasselti* menggunakan Prosainamida Sephacryl S-1000 dengan penulenan sebanyak 8.1 kali ganda dan hasil perolehan sebanyak 55.3% telah pun diperoleh. Fraksi separa tulen menunjukkan aktiviti AChE adalah optimum pada pH 7 dan suhu optima pada jarak 25^{o}C hingga 30^{o}C. Asetiltiokolin Iodida (ATC) telah dipilih sebagai substrat spesifik dengan kelajuan maksima tertinggi (V_{max}) iaitu 742.43 µmole/min dan pekali Michaelis (K_m) terendah iaitu 0.0086mM diikuti Propioniltiokolin Iodida (PTC) dan Butiriltiokolin Iodida (BTC). Kesemua kumpulan karbamat seperti karbaril, bendiokarb, karbofuran,

methomil dan propoxur, dan beberapa organofosfat oxon seperti klorpirifos, diazinon, malathion and parathion menunjukkan perencatan ketara melebihi 50% pada aktiviti AChE. AChE separa tulen tidak direncatkan oleh triklorfon, dimethoat dan asefat. Analisis ANOVA menunjukkan kesemua perencatan adalah ketara berbanding dengan kawalan ($p<0.05$) tanpa kehadiran racun serangga. Karbamat; carbaryl dan OP oxon; diazinon menunjukkan IC_{50} rendah yang ketara dalam *O. hasselti* berbanding dengan *E. electricus* manakala parathion dan diazinon menunjukkan pertindihan IC_{50}. Kromium, kuprum dan merkuri telah merencat 80%, 61.3% dan 35% aktiviti AChE, masing-masing. Apabila diuji pada kepekatan yang berbeza, logam berat menunjukkan lekuk kemerosotan eksponan dengan pengiraan IC_{50} untuk kuprum dan kromium pada 0.198 dan 0.076 mg l^{-1}, masing-masing. Kesan sampel matrik untuk mengesan racun serangga menggunakan air paip, laut dan sungai tidak tercemar yang telah ditambah bersama karbamat menunjukan masing-masing tiada perbezaan ketara ($p>0.05$) yang menunjukan tiada kesan matrik. Daripada kajian sampel sayur-sayuran, cili menunjukan kehadiran racun serangga dan dibuktikan melalui analisis HPLC. Sungai Juru menunjukkan perencatan tinggi dimana menunjukkan kepekatan yang tinggi bagi kromium dan kuprum dibuktikan melalui ICP-OES sementara sampel sungai lain menunjukkan perencatan yang rendah kecuali sampel dari Taman Negara Endau Rompin. Ini boleh disimpulkan bahawa AChE *O. hasselti* merupakan alternatif terbaru pengesan biologi untuk pencemaran kerana kesensitifan enzim ini hampir sama berbanding AChE komersil yang lain. Bagi kajian akan datang, adalah disyorkan untuk menjalankan penulenan penuh untuk memahami perbezaan prinsip antara AChE ini dan AChE yang lain bagi kefahaman lebih lanjut tentang mekanisma perencatan logam berat dan racun serangga.

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I certify that an Examination Committee met on **3 August 2011** to conduct the final examination of Mohd Khalizan bin Sabullah on his Master of Science thesis entitled "Acetylcholinesterase from *Osteochilus hasselti* for detection of insecticides and heavy metals" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date :

DECLARATION

I declare that the thesis is my original work except for quotation and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at other institutions.

MOHD KHALIZAN BIN SABULLAH

Date: 3 August 2011

LIST OF TABLES

TABLE OF FIGURES

LIST OF ABBREVIATIONS

Å	Angstrom
≈	Almost equal to
°C	degree Celsius
%	percent
3D	Three Dimensional
AChE	Acetylcholinesterase
ACh	Acetylcholine
ATC	Acetylthiocholine Iodide
BuChE	Butyrylcholinesterase
BTC	Butyrylthiocholine Iodide
ChE	Cholinesterase
ChO	Choline Oxidase
CE	Carbofuran equivalence
DDVP	Dichlorvos
DTNB	5,5'-dithiobis(2-nitrobenzoic acid)
DTT	dichlorodiphenyltrichloroethane
g	gravity (Relative Centrifugal force)
HPLC-MS	High Performance Liquid Chromatography Mass Spectrophotometer
HCL	Hydrochloric acid
IC_{50}	fifty percent inhibition concentration
GC-MS	Gas Chromatography Mass Spectrophotometer
kDa	kilodalton
$K_{m\ (app)}$	Apparent Michaelis-Menten Constant

M	molar
mg	miligram
mM	milimolar
mg/l	milligram per liter
µl	microliter
nm	nanometer
NaOH	Sodium hydroxide
OP	Organophosphate
OD	optical density
pH	-log concentration of H^+ ion (*Puissance hydrogene)*
PTC	Propionylthiocholine Iodide
PMSF	phenylmethylsulfonylfluoride
PAGE	polyacrylamide gel electrophoresis
PrChE	Propionylcholinesterase
SEM	Standard Error Mean
SDS	sodium dodecyl sulphate
TEMED	N,N,N',N'-tetramethyl-ethylenediamine
V_{max} (app)	Apparent Maximum velocity
v/v	volume/ volume
w/v	weight/ volume

TABLE OF CONTENTS