UNIVERSITI PUTRA MALAYSIA

PURIFICATIONS AND CHARACTERIZATIONS OF CHOLINESTERASE FROM DIFFERENT ORGANS OF LATES CALCARIFER BLOCH

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PURIFICATIONS AND CHARACTERIZATIONS OF CHOLINESTERASE FROM DIFFERENT ORGANS OF *LATES CALCARIFER* BLOCH

By

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In Malaysia, many rivers have been greatly polluted by industrial effluents. Fish are ubiquitous organisms that have many features with a potential as a biomarker of heavy metals pollution. Recently, cholinesterase (ChE) from inhibition studies on fish has emerged to be one of great potential biomarkers for heavy metals monitoring. This study was aimed to assess the capability of ChE from selected *Lates calcarifer* organs namely brain, gill, liver, muscle, and kidney to detect metal ions because they tend to bioaccumulate and will give a great threat towards living organism. ChE was purified through ammonium sulphate precipitation and ion exchange chromatography. The optimum ChE activity for all organs determined to be at 25°C and in 0.1 M Tris-HCl buffer, pH 8.0. Each organ was able to hydrolyse different synthetic substrates. Brain, gill, and kidney showed a strong affinity towards acetylthiocholine iodide (ATC). Liver ChE hydrolysed butyrylthiocholine iodide (BTC) at a faster rate than other organs, while muscle ChE showed an optimum enzyme activity when propionylthiocholine iodide (PTC) was used as the substrate. Ten heavy metals namely argentum (Ag), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), cobalt (Co), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn) were chosen for the inhibition study. When tested with a specific substrate for each organ, the results showed that the brain ChE was inhibited by Ag, As, Cd, Cr, and Hg. Different results were obtained for gill ChE, which was inhibited by Cu, Hg, and Pb only, while liver ChE was inhibited by almost all of the heavy metals used, but only Cd and Co did not show an inhibition of >50%. Muscle ChE was inhibited by Pb, while kidney ChE was very sensitive towards Pb. The results can be further used in biomarker studies for addressing heavy metals pollution in water bodies.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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September 2015

Chairperson : Siti Aqlima Ahmad, PhD

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In Malaysia, many rivers such as Sungai Juru and Sungai Merbok have been greatly polluted by industrial effluents from electronics, basic and fabricated metal products, chemical plants, and transport equipment industries. Fish are ubiquitous organisms that have many features with a potential as a biomarker of heavy metals pollution. Recently, cholinesterase (ChE) from inhibition studies on fish has emerged to be one of the great potential biomarkers for heavy metals monitoring. The aim of this study was to assess the capability of ChE from selected *Lates calcarifer* organs namely brain, gill, liver, muscle, and kidney to detect metal ions because they tend to bioaccumulate and will give a great threat towards living organism. The ChE was purified through ammonium sulphate precipitation and ion exchange chromatography. The optimum ChE activity for all organs was determined to be at 25°C and in 0.1 M Tris-HCl buffer, pH 8.0. Each organ was able to hydrolyse different synthetic substrates. Brain, gill, and kidney showed a strong affinity towards acetylthiocholine iodide (ATC). Liver ChE hydrolysed butyrylthiocholine iodide (BTC) at a faster rate than other organs, while muscle ChE showed an optimum enzyme activity when propionylthiocholine iodide (PTC) was used as the substrate. Ten heavy metals namely argentum (Ag), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), cobalt (Co), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn) were chosen for the inhibition study. When tested with a specific substrate for each organ, the results showed that the brain ChE was inhibited by Ag, As, Cd, Cr, and Hg. Different results were obtained for gill ChE, which was inhibited by Cu, Hg, and Pb only, while liver ChE was inhibited by almost all of the heavy metals used, but only Cd and Co did not show an inhibition of >50%. Muscle ChE was inhibited by Pb, while kidney ChE was very sensitive

towards Pb. The results showed that different substrates gave different inhibition effects towards the heavy metals. The results from this study can be further used in biomarker studies for addressing heavy metals pollution in water bodies.

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

PENULENAN DAN PENCIRIAN ENZIM KOLINESTERES DARIPADA BERLAINAN ORGAN IKAN SIAKAP

Oleh

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Di Malaysia, banyak sungai seperti Sungai Juru dan Sungai Merbok yang teruk dicemari oleh efluen perindustrian daripada industri elektronik, produk asas dan fabrikasi logam, loji kimia dan peralatan pengangkutan. Ikan merupakan organisma yang boleh didapati di mana-mana dan mempunyai banyak ciri yang boleh dijadikan sebagai biopenanda bagi pencemaran logam berat. Sejak akhir-akhir ini, kolinesterase (ChE) daripada kajian perencatan terhadap ikan telah diselidik dan berkembang sebagai salah satu biopenanda yang berpotensi bagi memantau logam berat kerana mereka berkemampuan untuk termendap dan menyebabkan ancaman yang besar terhadap organisma hidup. Tujuan kajian ini adalah untuk menilai kemampuan ChE daripada beberapa organ *Lates calcarifer* (Siakap), iaitu otak, insang, hati, otot, dan buah pinggang bagi mengesan ion logam. ChE ditulenkan dengan kaedah pemendakan amonium sulfat dan kromatografi pertukaran ion. Aktiviti ChE optimum bagi semua organ tersebut ditentukan pada suhu 25°C dan dengan menggunakan 0.1 M penimbal Tris-HCl pada pH 8.0. Setiap organ mampu menghidrolisis substrat sintetik yang berbeza. Otak, insang, dan buah pinggang menunjukkan keafinan yang kuat terhadap asetiltiokolin iodida (ATC). Selain itu, ChE hati menghidrolisis butiriltiokolin iodida (BTC) pada kadar yang lebih pantas, manakala ChE otot menunjukkan aktiviti enzim yang optimum apabila propioniltiokolin iodida (PTC) digunakan sebagai substrat. Sepuluh jenis logam berat, iaitu perak (Ag), arsenik (As), kadmium (Cd), kromium (Cr), kuprum (Cu), cobalt (Co), merkuri (Hg), nikel (Ni), plumbum (Pb), dan zink (Zn) dipilih dalam kajian perencatan. Apabila ujian substrat khusus bagi setiap organ dijalankan, keputusan awal menunjukkan bahawa ChE otak direncat oleh Ag, As, Cd, Cr, dan Hg. Organ-organ lain pula menunjukkan hasil yang berbeza. ChE insang direncat

oleh Cu, Hg, dan Pb. ChE hati pula direncat oleh hampir semua logam berat berkenaan, tetapi hanya Ag, As, Cr, Cu, Hg, Ni, Pb, dan Zn menunjukkan perencatan >50%. Aktiviti ChE otot direncat oleh Pb, sementara ChE buah pinggang amat sensitif terhadap Pb. Ringkasnya, substrat berbeza memberikan kesan perencatan yang berbeza terhadap logam berat. Oleh itu, keputusan daripada kajian ini boleh digunakan dengan lebih lanjut sebagai biopenanda bagi pencemaran air yang disebabkan oleh logam berat.

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TABLE OF CONTENTS

LIST OF EQUATIONS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celcius
µg	Microgram
µL	Microlitre
Abs	Absorbance
Ag	Silver
As	Arsenic
ATC	Acetylcholine iodide
BSA	Bovine serum albumin
BTC	Butyrylthiocholine iodide
Cd	Cadmium
Cr	Chromium
Cu	Copper
DEAE	Diethylaminoethyl
DTNB	5, 5-dithio-bis-2-nitrobenzoate
et al.,	And others
g	Gram
HCl	Hydrochloric acid
Hg	Mercury
HPLC	High Performance Liquid Chromatography
kDa	Kilo Dalton
K_m	Michaelis Menten constant
L	Litre
M	Molar
mg	Miligram
mg L^{-1}	Miligram per litre
min	Minute
mins	Minutes
mL	Millilitre
mM	Milimolar
MW	Molecular weight
PAGE	Polyacrylamide gel electrophoresis
Pb	Lead
PMSF	Phenylmethylsufonyl fluoride

ppm	Part per million
PTC	Propionylthiocholine iodide
TEMED	Tetramethyl-ethylene diamine
U	Unit
WHO	World Health Organization
Zn	Zinc

CHAPTER 1

INTRODUCTION

In 2014, 56.5 kg of fish was consumed per person by Malaysians each year, making Malaysia among the world's top fish consumers (The Star, 2014). Asian sea bass is one of the most popular fish among Malaysians, followed by mackerel, squid, grouper and shrimp. Recently, the local fishery sector was seriously threatened by water pollution caused by heavy metals (The Star, 2014). For example, due to a 40-year accumulated pollution Juru River is dying mainly caused by industrial toxic wastes disposal. Juru River heavily polluted by heavy metal effluents from present industries such as electronics, basic and fabricated metal products, chemical plants, and transport equipment (Alkarkhi *et al*., 2008). They also stated that Juru River was highly polluted with Arsenic (As) and mercury (Hg) with 2.67 and 1.33 ppm respectively.

Fish is very sensitive to temperature changes, natural surroundings and water quality deterioration which made them into a favorite subject biomarker research (Skouras *et al*., 2003). Cellular responses considered as a suitable tool for the early and sensitive detection of chemicals exposure for the assessment of chemicals toxicity at cellular level (Monserrat *et al*., 2007). The use of biomarker for monitoring environmental quality in aquatic ecosystem had raised a great deal because of its economical method, early warning signal and give precise measurement (Paustenbach and Galbraith, 2006; Sarkar, 2006). Biomarkers represent changes that may arise due to the toxic effects of exposure to chemical pollutants from the molecular to the organism level. The response of biomarker occur prior to changes at the population and community levels, thus it has the ability to diagnose causes and act as early warning signals of ecosystem-level damage (Tsangaris *et al*. 2006).

The simplest estimation for toxicant existence was displayed by enzyme-based biomarker in which this method gave multiple advantages such as rapid determination, sensitive even exposed in low concentration of toxicant and low technical application needed (Sabullah *et al*., 2015). Cholinesterase (ChE) was used as a biomarker for metal and organochlorine compound in Kootenai River (Kruse and Scarnecchia, 2002). There are two basic types of ChEs that are the best known and characterized which are acetylcholinesterases (AChE) and butyrylcholinesterase (BChE) (Pezzementi *et al*., 2011). AChE hydrolyzes acetylcholine (Ach) at the neuromuscular junction of vertebrates while in higher vertebrates an evolutionarily related ChE, pseudocholinesterase (BChE and propionylcholinesterase (PChE)) also exist. The function of BChE is unknown but is suggested to play a role in growth and development and sometimes act as a scavenger of cholinergic toxins (Masson and Lockridge, 2010). Various sources of

ChE from aquatic organisms such as *Tilapia mossambica* (Al-Ghais, 2013), *Osteochillus hasselti* (Sabullah *et al*., 2013) and *Periophtalmodon schlosseri* (Sabullah *et al*., 2014) was reported to be a sensitive biomarker with toxicant especially heavy metals. Fish considered as one of a biomarker tool and a highly sensitive enzyme as sentinel species allows the lower contamination levels of pollution detection (Sabullah *et al*., 2015).

The removal of xenobiotic compounds is needed to ensure a safer environment. The *in vitro* inhibition study of fish ChE activity by heavy metals gave multiple information aid standardization of environmental management and treatment to minimize and eliminate the toxicant (Sabullah *et al*., 2015) which *Lates calcarifer* was chosen as the sample because there is lack study of this species for biomonitoring pollution. Furthermore, *L. calcarifer* is important as a commercial and subsistence food fish in Malaysia.

1.1 Aim and Objectives

The main objective of this work is to purify cholinesterase enzyme from different parts of *Lates calcarifer* (Siakap) organ that will be later used as an alternative biosensor for bioremediation of aquatic environment. In this research, purification of the enzyme was performed to obtain a purified enzyme at the end of study. This study embarks the following objectives:

1. To purify cholinesterase from different parts of *Lates calcarifer* (Siakap) organs (brain, gill, kidney, liver and muscle).

2. To determine the optimum assay condition and substrate specificity of purified cholinesterase activity.

3. To characterise the cholinesterase activity by chemical approach using *in vitro* effects of metal ions on purified cholinesterase.

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