UNIVERSITI PUTRA MALAYSIA

THE ASSESSMENT OF CHOLINESTERASE FROM GILLS OF ANABUS TESTUDINEUS AS DETECTION OF METAL IONS

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PENGESAHAN

Dengan ini adalah disahkan bahawa tesis projek yang bertajuk "The Assessment of Cholinesterase from Gills of *Anabus testudineus* as Detection of Metal Ions" telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh Low Weini (165125) sebagai syarat untuk kursus BCH4999 Projek.

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ABSTRACT

Cholinesterase from the gill of *Anabus testudineus* contains mostly AChE. It was partially purified by ammonium sulphate precipitation and DEAE cellulose using ion exchange chromatography with 4.890U of specific activity, 11.7 of purification fold and 3.0% yield. Optimum pH for AChE in gill of *A. testudineus* is 8 using Tris-HCl buffer at temperature 25°C. The optimum acetylthiocholine iodide concentration is 2.5 mM with V_{max} 2.533 and K_m 0.8802 in which the catalytic efficiency of 2.88. For metal ion inhibition, 10 metal ions were tested and AChE in gill of *Anabus testudineus* showed a critically low enzyme activity towards mercury in which the activity is inhibited by 99.05%. However, cobalt and silver had no inhibitory effect on AChE. From IC_{50}, only 0.0123 ppm of Hg was required to reduce the enzyme activity by half.

ABSTRAK

Kolinesteres dari insang Anabus testudineus mengandungi asetilkolinesteres (AChE). Ia ditulenkan secara separa melalui aplikasi kerpasan ammonium sulfat dan kromatografi pertukaran ion dengan menggunakan selulosa DEAE sebagai matriks dengan aktiviti spesifik bernilai 4.89U, faktor penulenan bernilai 11.7 dan hasil perolehan sebanyak 3.0%. pH optima untuk AChE dari insang Anabus testudineus ialah 8 dengan menggunakan penimbal Tris-HCl pada suhu 25°C. Optima kepekatan substrat asetilkolinesteres iodide ialah 2.5 mM dengan nilai V_{max} sebanyak 2.533 dan nilai K_m sebanyak 0.8802 yang menunjukkan kecekapan pemangkin sebanyak 2.88. Sebayak 10 jenis ion logam berat telah diuji dengan AChE dari insang *Anabus testudineus* dan ia menunjukkan aktiviti enzim yang rendah apabila diuji dengan mekuri di mana aktiviti enzim telah direncat 99.05%. Walau bagaimanapun, kobalt dan perak tidak memberi kesan negatif kepada aktiviti enzim. Dari IC50, hanya 0.0123 ppm merkuri diperlukan untuk menurunkan aktiviti enzim ke 50%.

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"There's nothing you can't do, it's all about the willingness."

Low Weini, 2015

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LIST OF ABBREVIATIONS

^{o}C	Degree Celsius
%	Percent
AChE	Acetylcholinesterase
ACh	Acetylcholine
ATC	Acetylthiocholine iodide
BuChE	Butyrylcholinesterase
BTC	Butyrylthiocholine iodide
ChE	Cholinesterase
DTNB	5,5'-dithiobis(2-nitrobenzoic acid)
DDT	Dichlorodiphenyltrichloroethane
HCl	Hydrochloric acid
IC_{50}	Half maximal inhibitory concentration
kDa	Kilo Dalton
Km	Michaelis-Menten constant
M	Molar
mg	Milligram
mM	Millimolar
µl	Microlitre
nm	Nanometre
NaOH	Sodium hydroxide
pH	-log concentration of H+ ion
PTC	Propionylthiocholine iodide
PAGE	Polyacrylamide gel electrophoresis
SDS	Sodium dodecyl sulphate
TEMED	N,N,N',N'-tetramethyl ethylenediamine
Vmax	Maximum velocity
Zn	Zinc
Pb	Lead
Ni	Nickel
Hg	Mercury
Co	Cobalt

Cu	Copper
Cr	Chromium
Cd	Cadmium
As	Arsenic
Ag	Silver

CHAPTER 1

INTRODUCTION

Nowadays, metal pollution of aquatic environment is a rising issue in Malaysia. There are numbers of contributors that account for metal pollution, for example, oil and gas industries, shipping and cargo, and agricultural activities. However, the major cause of pollution is due to the manufacturing sectors, for instance, metal manufacturer such as electroplating, etching and etc which contains a high level of Cadmium, Copper, and Zinc, which is harmful to human (Shazili *et al*., 2007).

In the context of aquatic environment pollution, fish are the most affected and can be considered as the biomarker to measure the level of water pollution (Law and Singh, 1991). Other marine organisms such as microflora and algae have the ability to accumulate metal ions in their cells and as a result, the small fish that these microflora and algae will be enriched with these accumulated metal substances and consequently, human will be affected through the bioaccumulation occurs in food web (Jaffar *et al*., 1998; Ashraf *et al*., 2012). Fish is known as a good biomarker to determine the level of water pollution because it is easily reachable, able to corporate metals and has an ideal size to be sampled (Batvari *et al*, 2007).

Anabas testudineus, commonly known as climbing perch, is a kind of freshwater fish that can survive in the water with very low oxygen level and polluted water. It can also survive without water for 6 to 10 hours (Hughes *et al*., 1970). Due to its ability to survive in polluted aquatic environment, it is suitable to be used as a biomarker to indicate the status of pollution in aquatic environment.

In the context of cholinesterase, it is commonly used in biomarker for determination of contamination due to its effectiveness, especially by pesticides and insecticides. Cholinesterase functions as detoxifying enzyme in human. Thus, the inhibition of cholinesterase is very crucial to be studied.

Therefore, the objectives of this project are:

1. To extract and purify cholinesterase from the gill of *Anabas Testudineus*.
2. To identify the optimum temperature and pH for cholinesterase activity.
3. To determine the in-vitro effect of cholinesterase activity towards heavy metals exposure.

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