UNIVERSITI PUTRA MALAYSIA

THE ASSESSMENT OF CHOLINESTERASE FROM THE MUSCLE OF Anabas testudineus AS DETECTION OF METAL IONS

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FBSB 2015 49

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Dissertation submitted in partial fulfilment for the requirement for the course of
BCH 4999
Project in the Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia.

June 2015

PENGESAHAN

Dengan ini adalah disahkan bahawa tesis projek yang bertajuk "The assessment of cholinesterase from the muscle of *Anabas testudineus* as detection of metal ions" telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh Ain Aqilah binti Basirun (165325) sebagai syarat untuk kursus BCH4999 Projek.

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ABSTRACT

Anabas testudineus, also known as Climbing Perch is a freshwater fish which originated from India and mostly inhabited in rivers and lakes. It came from family 'Anabantidae' and in Malaysia it is called 'ikan puyu'. *A. testudineus* is a very hardy fish and is having high commerial value. Cholinesterase (ChE) was purified from the muscle extracts of *A. testudineus* through ion-exchange chromatography (DEAE-cellulose). In this study, ChE was partially purified with a purification folding and recovery yield of 2.012 and 14.46% respectively. Native-PAGE analysis was done to show the degree of purity. Optimisation study of muscle ChE was carried out shows that for muscle ChE, the specific substrate is propionylthiocholine iodide (PTC) at 2.5 mM, with optimum temperature and pH of 30°C and pH 9.0 respectively using Tris-HCl buffer. Metal ions inhibition study has shown that mercury had inhibited at highest percentage of enzyme activity, at 93.4%. Half-maximal inhibitory concentration by mercury obtained in this study was at 0.9752 mg/L. Hence, the ion-exchange chromatography shows that it is one of the useful purification methods to purify ChE from muscle of *A. testudineus* and ChE purified from this can be a useful biosensor for metal ion pollution monitoring in aquatic system.

ABSTRAK

Anabas testudineus atau lebih kenali sebagai ikan puyu di Malaysia merupakan salah satu ikan air tawar yang berasal dari India dan hidup di habitat seperti sungai dan tasik. Ia dikategorikan dalam kumpulan 'Anabantidae'. Ikan puyu merupakan ikan yang tahan lasak dan mempunyai pasaran ikan yang tinggi. Kolinesteres (ChE) yang diestrak daripada otot *A. testudineus* telah ditulenkan melalui proses kromatografi penukaran ion menggunakan DEAE-selulosa sebagai medium penulenan. Dalam kajian ini, separa penulenan telah dilakukan dan hasil penulenan adalah 2.012 dan pemulihan enzim sebanyak 14.46%. Analisis poliakrilamid gel elektroforesis natif (Native-PAGE) telah dijalankan untuk menguji tahap penulenan. Kajian tentang keadaan optima ChE ini telah dilakukan. Ia merangkumi profil substrat yang spesifik iaitu PTC, 2.5 mM, 30°C sebagai suhu optima, dan pH optima iaitu pH 9 dalam penimbal tris-HCl. Analisis perencatan aktiviti ChE oleh ion logam telah dilakukan. Semua ion logam menunjukkan perencatan terhadap aktiviti enzim ChE dan merkuri telah menunjukan peratusan perencatan tertinggi iaitu 93.4%. Kajian separa perencatan (IC_{50}) oleh merkuri juga dilakukan dengan hasil perencatan sebanyak 0.9752 mg/L. Justeru, penulenan melalui kaedah kromatografi penukaran ion menjanjikan satu kaedah penulenan yang berguna untuk menulenkan ChE daripada estrak otot *A. testudineus* dan ChE daripada estrak ini boleh dijadikan sebagai biopenanda yang berguna untuk mengesan pencemaran ion logam di dalam system pengairan.

ACKNOWLEDGEMENT

Alhamdulillah, praised to Allah for giving me greatest strength and opportunity to commit myself finishing this project.

It is my greatest privilege to express my deepest gratitude to all people who contributes directly or indirectly in conducting this research project work successfully. I want to express my greatest thanks to my beloved supervisor Professor Dr. Mohd Arif Syed for giving me motivational support in finishing this project and also as a coordinator for this BCH 4999 course. I would also like to express my deepest gratitude to Dr. Siti Aqlima Ahmad for helping and guiding me to improve my knowledge and skills doing the experiment while working on this project.

Besides, I would also give the warmest gratitude to all seniors and members of the Bioremediation and Enzymology Labs for treating me well since I have been in these labs. Special thanks to Mohd Khalizan and Nursabrina Hayat for teaching and guiding me to conduct this project. Furthermore, I would like to thank to my labmates, Shakirah Abdul Wahab Sha'arani, Izzuanuddin Mohammed Iqbal, Nur Muhammad Syahir, Wong Yoong Fei, and Low Weini for the greatest cooperation and being supportive while working on this project.

Finally yet importantly, this research would not be done successfully without the greatest support from my beloved parent, Basirun Razak and Norleha Bakar as well as my siblings, Amirah Asyikin, Anis Hidayah, Muhamad Adib, and also not to be forgotten my best friend, Rudy Fadhlee Mohd Dollah. Special thanks to them for supporting me until with the end of this project. Thank you very much.

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LIST OF ABBREVIATIONS

%	Percent
°C	Degree Celsius
µl	Microlitre
ACh	Acetylcholine
AChE	Acetylcholinesterase
ATC	Acetylthiocholine iodide
BCh	Butyrylcholine
BChE	Butyrylcholinesterase
BSA	Bovine serum albumin
BTC	Butyrylthiocholine iodide
ChE	Cholinesterase
DTNB	5,5-dithio-bis-2-nitrobenzoate
DEAE	Diethylaminoethyl
et al.,	And friends
HCl	Hydrochloric acid
L	Litre
M	Molar
mg	Milligram
Min	Minute
ml	Millilitre
mM	Millimolar
PAGE	Polyacrylamide gel electrophoresis
PMSF	Phenylmethylsulfonyl flouride
PrCh	Propionylcholine
PrChE	Propionylcholinesterase
PTC	Propionylthiocholine iodide
U	Unit of activity

CHAPTER 1

INTRODUCTION

In recent years, there has been an increase of awareness on the wide occurrence of heavy metals pollution in the environment especially towards the aquatic system. Heavy metals are able to transform into persistent metallic compound in which it can accumulate in organisms' body system, disturbing the food chain and eventually threaten human life (Zhou *et al*., 2007). In trace amounts, metal ions are actually help to maintain the homeostasis as well as being important for cellular growth (Sabullah *et al*., 2014). The presence of metal ions normally facilitates the formation of enzyme – substrate complex. However, some metal ions that have similarities with substrates will form stable conjugates with the active site of cholinesterase (ChE). Therefore, the alteration of the active site conformation fails the binding of substrate to the enzyme ChE (Glusker *et al.*, 1999).

The uses of ChE extracted from the aquatic organisms such as fish as a biomarker for the detection of the effect of anticholinesterase has been developed based on the study of biological responses of organisms to pollutants especially in aquatic system (Monteiro *et al*., 2005). This is because ChE itself is the crucial enzyme for signal termination at cholinergic synapse by rapid hydrolysis of the neuron acetylcholine in the brain. The interruption of anticholinesterase by pollutants such as pesticides, metal ions at nerve system will cause the accumulation of acetylcholine at synaptic cleft and eventually causing the organism to face paralysis and death. In liver, ChE also acts as detoxifier (Sabullah *et al*., 2014).

From the early studies, the uses of inhibitive enzyme-based assay of heavy metals using ChE enzyme as detector is significant due to low – cost, fast and need no tedious technique to be done (Sabullah *et al*., 2014). Therefore, the objectives of this study are:

1. To extract and partially purify cholinesterase (ChE) from the muscle of *Anabas testudineus.*
2. To characterise ChE that isolated from the muscle of *A. testudineus*.
3. To investigate the potential of ChE isolated from *A. testudineus* as a monitoring tool to the exposure of heavy metals pollution.

REFERENCES

Askar, A.K., Kudi, A.C. and Moody, A.J. 2011. Purification of soluble acetylcholinesterase from sheep liver by affinity chromatography. *Application of Biochemistry and Biotechnology,* 15: 336-346.

Assis, C.R.D., Bezerra, R.S. and Carvalho, L.B. 2011. Fish cholinesterase as a biomarker of organophosphate and carbamate pesticide. *Pests Control and Pesticides Exposure and Toxicity Assessment,* 13: 253-278.

Alves, L.M., Lemos, M.F., Correia, J. and Novais, C.S. 2014. Characterization of cholinesterases present in brain and muscle tissues of juvenile blue shark (*Prionace glauca*). *Journal of Frontier in Marine Medicine*, 7: 1-17.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry*, 72(1-2): 248–254.

Castro, J.A. 1967. Effect of alkylating agents on human plasma cholinesterase; the role of sulfhydryl group in its active center. *Biochemical Pharmacology,* 17: 295-303.

Cokugras, A.N. 2003. Butyryl; structure and physiological importance. *Turkish Journal of Biochemistry*, 28: 54-61.

Colovic, M.B., Kristic, D.Z., Lazarevic, T.D., Bondzic, A.M. and Vasic, V.M. 2013. Acetylcholinesterase inhibitors; pharmacology and toxicology. *Current Neuropharmacology,* 11: 315-335.

Devi, M. and Fingerman, M. 1995. Inhibition of acetylcholinesterase activity in the central nervous system of the red swamp crayfish, *Procaambarus clarkia* by mercury, cadmium, and lead. *Bulletin of Environment Contamination and Toxicology*, 55: 746-750.

Ding, Y.H., Wu, X.M. and Fang, J.B. 2011. Purification and characterization of acetylcholinesterase from brain tissues of *Oreochromis aurea* and its application in environmental pesticide monitoring. *Sciences in Cold and Arid Regions*, 3(4): 339-343.

El-Demerdash, F.M. and Elagamy, E.I. 1999. Biological effects in *Tilapia nilotica* fish as indicators of pollution by cadmium and mercury. *International Journal of Environmental Health Research*, 9(3): 173-186.

Ellman, G.L., Courtney, K.D., Andres, J.V. and Featherstone, R.M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology,* 7: 88-95.

Falugi, C. and Aluigi, M.G. 2012. Early appearance and possible function of non-neuromuscular cholinesterase activities. *Frontier in Molecular Science*, 54: 1-12.

Frasco, G.R., Colletier, J.P., Weik, M., Carvalho, F., Guilhermino, L., Stroja, J. and Fournier, D. 2007. Mechanism of cholinesterase inhibition by inorganic mercury. *Federation of European Biochemical Societies Journal,* 7: 88-95.

Fuentes, G.R. and Buchott, G.G. 2004. Characterization of cholinesterase activity from different tissues of Nile tilapia. *Marine Environmental Research,* 58: 505-509.

Glusker, J.P., Katz, A.K. and Bock, C.W. 1999. Metal ions in biological systems. *The Rigaku Journal,* 16(2): 8-19.

Gomes, I.D.L., Lemos, M.F.L., Soares, A.M.V.M., Barata, C. and Faria, M. 2014. The uses of cholinesterase as potential biomarker; in vitro characterization in the polychaete, *Capitella teleta*. *Marine Pollution Buletin,* 85: 179-185.

Green, A. A., and Hughes, W. L. 1955. Protein fractionation on the basis of solubility in aqueous solutions of salts and organic solvents. *Methods Enzymology,* 1: 67-90.

Hayat, N.M., Sabullah, M.K., Shukor, M.Y., Syed, M.A., Dahalan, F.A., Khalil, K.A and Ahmad, S.A. 2014. The effect of pesticides on cholinesterase activity by using fish as a biomarker. *Nanobio Bionano,* 1: 1-8.

Jamsari, A.F.J., Muchlisin, Z.A., Musri, M. and Azizah, M.N.S. 2010. Remarkably low genetic variation but high population differentiation in the climbing perch, *Anabas testudineus* based on the mtDNA control region. *Genetic Molecular Research,* 9: 1836-1843.

Kim, J.H., Stevens, R.C., MacCoss, M.J., Goodlett, D.R., Scheirl, A., Richter, R.J., Suzuki, S.M. and Furlong, C.E. 2010. Identification and characterization of biomarkers of organophosphorus exposures in humans. *Advance Experimental Medicinal Biology*, 660: 61-71.

Koenig, M. and Solé, M. 2014. Muscular cholinesterase and lactate dehydrogenase activities in deep sea fish from the NW Mediterranean. *Marine Environmental Research,* 94: 16-23.

Lang, G.J., Shang, J.Y., Chen, Y.X., Chui, Y.J, Wang, Q., Tang, Z.H. and Zhang, C.X. 2010. Expression on the housefly acetylcholinesterase in a bioreactor and its potential application in a detection of pesticide residues. *World Journal of Microbiology Biotechmolecular,* 26: 1795-1801.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature,* 227: 680-685.

Leibel, W.S. 1988. Characterization of a pseudocholinesterase purified from surgeonfish tissues confirms the atypical nature of this enzyme. *Journal of Experimental Zoology,* 247(3): 198-208.

Leticia, A.G. and Gerardo, G.B. 2008. Determination of esterase activity and characterization of cholinesterases in the reef fish *Haemulon plumier*. *Ecotoxicology and Environmental Safety*, 71: 787-797.

Lopes, R. M., Filho, M.V.S., Salles, J.V., Bastos, Z.F.C. and Bastosk, J.C. 2014. Cholinesterase activity of muscle tissue from freshwater fishes: Characterization and sensitivity analysis to the organophosphate Methyl-paraoxon. *Environmental Toxicology and Chemistry*, 33(6): 1331-336.

Masson, P., Schopfer, L.M., Bartels, C.F., Frometit, M.T., Ribes, F., Nachon, F. and Lockridge, O. 2001. Substrate activation in acetylcholinesterase induced by low pH or mutation in the π-cation subsite. *Biochimica et Biophysica Acta*, 1594: 313-324.

Monteiro, M., Quintaneiro, C., Morgado, F., Soares, A.M.V.M. and Guilhermino, L. 2005. Characterization of the cholinesterase present in head tissues of the estuarine fish *Pomotoschistus microps;* application to biomonitoring. *Ecotoxicology and Environmental Safety*, 62: 341-347.

Miao, Y., He, N. and Zhu, J.J. 2010. History and new developments of assays for cholinesterase activity and inhibition. *Chemical Reviews*, 110(9): 5216-5234.

Mirica, K.A., Lockett, M.R., Snyder, P.Y., Shapiro, N.D., Mack, E.T., Nam, S. and Whitesides, G.M. 2012. Selective precipitation and purification of monovalent proteins using oligovalent ligands and ammonium sulfate. *Bioconjugate Chemistry*, 23: 293-299.

Morel, N., Bon, S., Greenblatt, H.M., Belle, D.V., Wodak, S.J., Sussman, J.L., Massoulie, J. and Silman, I. 1999. Effect of mutations within the peripheral anionic site on the stability of acetylcholinesterase. *The American Society for Pharmacology and Experimental Therapeutics*, 55: 982-992.

Rang, H.P., Dale, M.M. and Ritter, J.M. 2001. *Cholinergic transmission. In: Pharmacology*. 4th Edition. Harcourt Publisher Ltd. United Kingdom. pp. 110-138.

Sabullah, M.K., Sulaiman, M.R., Shukor, M.Y.A., Syed, M.A., Shamaan N.A., Khalid, A. and Ahmad, S.A. 2014. The assessment of cholinesterase from the liver of *puntius javanicus* as detection of metal ions. *The Scientific World Journal*, 2014: 1-9.

Scopes, R.K. 1988. *Protein purification, principles and practice.* 2nd Edition. Springer-Verlag, New York. pp. 267-269.

Sussman, J.L. and Granberry, A. Acetylcholinesterase: A story of substrate traffic and inhibition by green mamba snake toxin http://proteopedia.org/index.php [accessed on 12 February 2015].

Shulze, H., Varlova, S., Villatte, F., Bachmann, T.T. and Schmid, R.D. 2003. Design of acetylcholinesterases for biosensor applications. *Biosensors and Bioelectronics*, 18: 201-209.

Sole, M., Loberaa, G., Aljinovica, B., J. Ríosb, L.M. Parrab, F. and Maynoua, J.E. 2008. Cholinesterases activities and lipid peroxidation levels in muscle from shelf and slope dwelling fish from the NWMediterranean: Its potential use in pollution monitoring. *Scientific of the Total Environment,* 402: 306-317.

Soorya, S.R., Devi, C.A., Binitha, R.N., Amrutha, B.V., Jayalekshmy, G. and Sunny, F. 2012. Oxidative stress experienced by freshwater fish *Anabas testudineus* exposed to sewage effluents of Parvathyputhenar, Kerala. *Biodiversity Utilization and Threats,* 85: 769-780.

Srivastava, A.K. and Singh, V.K. 2015. Influence of temperature on the fecundity and acetylcholinesterase activity of the fresh water snail *Lymnaea acuminata*. *Journal of Biology and Earth Sciences,* 5(1): 34-39.

Srinivas K., Sridhar, G.R. and Allam, A.R. 2012. Secondary Structure of Butyrylcholinesterase. *Journal of Diabetes Metabolism,* 3(5): 1-6.

Tham, L.G., Perumal, N., Syed, M.A., Shamaan, N.A. and Shukor, M.Y. 2009. Assessment of Clarias batrachus as a source of acetylcholinesterase (AChE) for the detection of insecticides. *Journal of Environmental Biology,* 30(1): 135-138.

Teng, T.L., Harpst, J.A., Lee, J.C., Zinn, A. and Carlson, D.M. 1976. Composition and molecular weights of butyrylcholinesterase from horse serum. *Archives of Biochemistry And Biophysics,* 176: 71-81.

Whitehead, A., Anderson, S.L., Ramirez, A. and Wilson, B.W. 2005. Cholinesterases in aquatic biomonitoring: Assay optimization and species-specific Characterization for a California native fish. *Ecotoxicology,* 14: 597-606.

Xuereb, B., Noury, P., Felten, V., Garric, J. and Geffard, O. 2007. Cholinesterase activity in *Gammarus pulex* (Crustacea Amphipoda): Characterization and effects of chlorpyrifos. *Toxicology,* 236: 178- 189.

Yang, Y.X., Niu, L.Z. and Li, S.N. 2013. Purification and studies on characteristics of cholinesterases from *Daphnia magna. Journal of Biomedicine and Biotechnology*, 14(4): 325-335.

Zhan, C.G. and Gao, D. 2005. Catalytic mechanism and energy barriers for butyrylcholinesterase-catalysed hydrolysis of cocaine. *Biophysical Journal*, 89: 3863-3872.

Zhong, F., Lisi, G.P., Collins, D.P., Dawson, J.H. and Pletneva, E.V. 2013. Redox-dependent stability, protonation, and reactivity of cysteine-bound heme proteins. *Proceeding of The National Academy of Sciences of the United States of America,* 1: 306-315.

Zhou, Q., Zhang, J., Fu, J., Shi, J. and Jiang G. 2008. Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. *Analytica Chimica Acta*, 606: 135-150.