UNIVERSITI PUTRA MALAYSIA

PARTIAL PURIFICATION AND CHARACTERISATION OF ALKALINE PHOSPHATASE FROM HEPATOPANCREAS AND INTERTINE OF RED TILAPIA, (TILAPIA MOSSAMBICA)

VANITHA MARIAPPAN.

FBSB 2005 30

PARTIAL PURIFICATION AND CHARACTERISATION OF ALKALINE PHOSPHATASE FROM HEPATOPANCREAS AND INTESTINE OF RED TILAPIA, (*Tilapia mossambica*)

By

VANITHA MARIAPPAN

Thesis Submitted to the School of Graduate Studies,Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Master of Science

July 2005

DEDICATION

Specially dedicated to the memory of my dear aiyah, late **Mr. MARIAPPAN ARUNASALAM** who left me & my siblings 15 years ago and to my beloved amma **BABY MARIAPPAN** who have been standing strong all her life just for us……

Aiyah even if you are gone for long,

Your presence I feel within me,

But you will always be in my memory,

As sweet as it can be,

All my life forever & ever…

HARD IS LIFE,

FOR HE WHO DESIRE DEATH,

BUT LIVES ON,

FOR THE SAKE OF HIS LOVED ONE!!!

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

PARTIAL PURIFICATION AND CHRACTERISATION OF ALKALINE PHOSPHATASE FROM HEPATOPANCREAS AND INTESTINE OF RED TILAPIA, (*Tilapia mossambica*)

By

VANITHA MARIAPPAN

July 2005

Chairman: **Professor Nor Aripin Shamaan, PhD**

Faculty: **Biotechnology and Biomolecular Sciences**

Alkaline phosphatase (EC 3.1.3.1) is a metalloenzyme, which catalyze nonspecific hydrolysis of phosphate monoesters. Partial purification was conducted on alkaline phosphatase (ALP) extracted from hepatopancreas and intestine of red tilapia, (*Tilapia mossombica*) using two main steps – ammonium sulphate precipitation and ion exchange chromatography on DEAE – 52. Samples from the ion-exchange step were analysed for ALP activities and characterised by SDS-PAGE. SDS-PAGE analysis showed 2 identical bands and was found to have molecular weight of 68, 000 Da (hepatopancreas ALP) and 180, 500 Da (intestinal ALP) subunits. Overall, purification fold obtained from the final step are 1.8 and 21.9 for hepatopancreas and intestinal

respectively, with recovery of only 0.22% from hepatopancreas and 0.01% from intestine. The specific activity of the enzyme was 1.72 X 10^{-2} μmol min^{-1} mg^{-1} and 2.93 X 10^{-1} μmol min^{-1} mg^{-1} from hepatopancreas and intestine respectively. The ALP from hepatopancreas remained stable at temperatures up to 50°C, and ALP from intestine enzyme had an optimum temperature of 60°C. The optimum pH for both hepatopancreas and intestine ALP of *Tilapia mossambica* is pH 10. The positive monovalent alkali metal ions (Li^+, Na^+ and K^+) have no effect on the ALP enzyme activity. However, the positive divalent alkali metal ions (Mg^{2+} and Ca^{2+}) activate the enzyme activities. Heavy metal ions (Zn^{2+}, Cu^{2+}, Cd^{2+} and Hg^{2+}) were found to inhibit the enzyme activity.

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

PENULENAN SEPARA DAN PENCIRIAN ENZIM ALKALINE FOSFATASE DARI HEPATOPANKREAS DAN USUS IKAN TILAPIA MERAH (*Tilapia mossambica*)

Oleh

VANITHA MARIAPPAN

Julai 2005

Pengerusi: **Profesor Nor Aripin Shamaan, PhD**

Fakulti: **Bioteknologi dan Sains Biomolekul**

Alkaline fosfatase (EC 3.1.3.1) adalah metalloenzim yang hidrolisis secara takspesifik fosfat monoester. Penulenan separa telah dijalankan ke atas alkaline fosfatase (ALP) dari hepatopankreas dan usus ikan tilapia merah (*Tilapia mossambica*) dengan menggunakan dua kaedah utama iaitu pemendakan amonium sulfat dan kromatografi penukaran ion DEAE – 52. Sampel dari penukaran ion DEAE yang mengandungi aktiviti ALP dianalisis dan dicirikan melalui kaedah elektroforesis SDS. Analisis SDS-PAGE telah menunjukkan 2 jalur yang serupa dan berat molekulnya adalah 68, 000 Da (ALP hepatopankreas) dan 180, 500 Da (ALP usus) bagi subunitnya. Secara keseluruhannya, faktor penulenan yang diperolehi daripada kaedah terakhir ialah 1.8

(hepatopankreas) dan 21.9 (usus) dengan pemulihan hanya 0.22% dari hepatopankreas dan 0.01% dari usus. Aktiviti spesifik enzim ialah 1.72 X 10^{-2} U mg^{-1} bagi hepatopankreas dan 2.93 X 10^{-1} U mg^{-1} bagi usus. ALP hepatopankreas adalah stabil pada suhu sehingga 50°C dan ALP usus pula mempunyai suhu optimum 60°C. pH optimum bagi kedua-dua hepatopankreas dan usus *Tilapia mossambica* adalah pH 10. Ion monovalen logam alkali positif (Li^+, Na^+ and K^+) tidak memberikan sebarang kesan kepada aktiviti ALP. Bagaimana pun, ion divalen logam alkali positif (Mg^{2+} and Ca^{2+}) mengaktifkan aktiviti enzim. Ion logam berat pula (Zn^{2+}, Cu^{2+}, Cd^{2+} and Hg^{2+}) menghalang tindak balas enzim.

ACKNOWLEGEDEMENTS

First of all, I would like to thank GOD for his blessing.

I wish to convey my deepest gratitude and greatest appreciation to my supervisor Assoc. Prof. Dr. Nor Aripin Shamaan for his guidance, advice, encouragement, keen interest, and support throughout my whole project and course of the study. I am thankful to Dr. Nor Aripin for being very understanding when I was going through tough times especially when I was ill and hospitalised.

Words cannot express my heartfelt thanks to my co-supervisors; Dr. Yunus Abd Shukor and Prof. Dr. Mohd Arif Syed for their supervision, providing useful information, constructive suggestions, invaluable advice, and for extending their time discussing about my research work.

Furthermore, I am also indebted to Mr. Jasni from the Hatchery Unit, Universiti Putra Malaysia for providing fresh live tilapia fishes used for the experiments without any charge.

Thanks are also extended to the member of Toxicology Lab especially to Noor Azlina Masdor and Suhaidah Ahmat @ Amirrudin for their invaluable help, assistance and co-operation and the fun we had during work for the past two years. Thanks to my fellow graduate students especially Yap Wai Sum, Lailatul Jumaiyyah, Anthony Chin Chee Meng, Palaniammal Krishnan and Putri Noor Faizah, for being such a wonderful friends.

A very special thanks goes to my dearest friends; Mr. Sunil Bhalla and Dr. Sreeramanan Subramaniam for their moral support, sincere advice, and encouragement throughout the completion of this study. I really appreciate it. Thank you friends!!!

Last but definitely not least, I am deeply grateful and thankful to my beloved mother who have helping me financially, and for the love she showed me all my live; also to my sisters, brother, brothers in-law, my first ever niece and my boyfriend for their support and encouragements. Without the understanding and love of these people everything would have been impossible. GOD BLESS YOU ALL AND THANK YOU!

I certify that an Examination Committee met on 20th July 2005 to conduct the final examination of Vanitha Mariappan on her Master of Science thesis entitled "Partial Purification and Characterisation of Alkaline Phosphatase from Hepatopancreas and Intestine of Red Tilapia (*Tilapia mossambica*)" 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:

Radzali Muse, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Johari Ramli, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Muhajir Hamid, PhD
Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Yasmin Anum Mohd Yusof, PhD
Associate Professor
Faculty of Medicine
Universiti Kebangsaan Malaysia
(External Examiner)

GULAM RUSUL RAHMAT ALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 22 AUG 2005

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory committee are as follows:

Nor Aripin Shamaan, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohd Arif Syed, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Mohd Yunus Abdul Shukor, PhD
Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 08 SEP 2005

DECLARATION

I hereby declare that the thesis in based on my original work except for quotations and citations that have been duly acknowledged. I also declare that it has been not been previously or concurrently submitted for any degree at Universiti Putra Malaysia or other institutions.

VANITHA MARIAPPAN

Date: 12/8/05

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

α	- alpha
β	- beta
° C	- degree Celsius
%	- percentage
Abs	- absorbance
ALP	- alkaline phosphatase
APS	- ammonium persulphate
BSA	- bovine serum albumin
cm	- centimetre
Da	- Dalton
DEAE	- diethylaminoethylcellulose
IEF	- isoelectric focusing
kD/kDa	- kilodalton
kg	- kilogram
mA	- mili Amp
mg	- miligram
ml	- mililiter
min	- minute

mins	- minutes
MW	- molecular weight
NaOH	- sodium hydroxide
$(NH_4)_2SO_4$	- ammonium sulfate
nm	- nanometer
pH	- (-) log concentration of H^+
p-NPP	- para nitrophenyl phosphate
p-NP	- para nitrophenol
SDS	- sodium dodecyl sulphate
U	- unit
µmol	- micromole
µg	- microgram
X g	- gravity
w/v	- weight/volume
w/w	- weight/weight

CHAPTER 1

INTRODUCTION

The existence of enzymes has been known for well over a century. Some of the earliest studies were performed in 1853 by the Swedish chemist Jon Jacob Berzelius who termed their chemical action catalytic. It was not ever since James B. Sumner of Cornell University purified and crystallizes the enzyme urease from the jack bean (EC 3.5.1.5) for the first time in the year 1926 as mentioned in Dixon and Webb, (1979); biochemists have successfully purified perhaps, thousands of enzymes thus far.

Alkaline phosphatase, ALP, (EC 3.1.3.1) is found in abundance in nature. ALP is a hydrolase and catalyzes the hydrolysis of various bonds. It has found in wide application especially in molecular biology, medical, and industries. It is a hydrolytic enzyme which catalyses the cleavage of a chemical bond with the addition of water (McComb *et al.*, 1979). ALP is in a group of enzyme acting on ester bonds and these esterases are subdivided into those acting on phosphoric monoester hydrolases, the phosphatases under an alkaline condition (McComb *et al.*, 1979).