UNIVERSITI PUTRA MALAYSIA

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

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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

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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

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

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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.

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TABLE OF CONTENT

		Page
DEDICATION		**iii**
ABSTRACT		**iv**
ABSTRAK		**vi**
ACKNOWLEDGEMENTS		**viii**
APPROVAL		**x**
DECLARATION		**xii**
LIST OF TABLES		**xvi**
LIST OF FIGURES		**xviii**
LIST OF ABBREVIATIONS		**xxii**
CHAPTER		
1.0	**INTRODUCTION**	**1**
2.0	**LITERATURE REVIEW**	**5**
2.1	Development of fishing industry in Malaysia	5
2.2	Culture of hybrid of tilapia	8
2.2.1	Taxonomy and distribution	8
2.2.2	Life history characteristics	11
2.2.3	Hepatopancreas and digestive system	14
2.3	Phosphatase	17
2.3.1	Role of phosphatase	19
2.4	Specificity of Acid and Alkaline Phosphatase	28
2.5	Definition of Alkaline Phosphatase	31
2.5.1	Characteristics of ALP	35
2.5.2	ALP as a hydrolase	41
2.5.3	Molecular and physical properties of ALP	46
2.5.4	Active site of ALP	48
2.5.5	Functions of ALP	52
2.6	Diseases related to significant of ALP	54
2.6.1	Diseases of bone hyperparathyroidism	54
2.6.2	Paget's disease	55
2.6.3	Osteomalacia	55

2.6.4 Bone tumors 56
2.6.5 Disease of the liver 56
2.6.6 Infectious hepatitis 57
2.6.7 Bone fractures 57
2.6.8 Other diseases if greater or lower than normal ALP level 57
2.6.9 Effects of ALP in fish 58
2.7 Theory of ammonium sulphate precipitation 58
2.8 Theory of ion exchange chromatography 59
2.9 Theory of isoelectric focusing 59

3.0 MATERIALS AND METHODS 60
3.1 Materials 60
3.1.1 Sources of fish 60
3.1.2 Chemical and biochemical reagents 61
3.1.3 Apparatus 62
3.2 Methods 63
3.2.1 Determination of protein content 63
3.2.2 Assay of enzyme activity 66
3.2.3 Extraction of crude enzyme 68
3.2.4 Purification of ALP enzyme 69
3.2.5 Ion-exchange chromatography on DEAE 72
3.2.6 Relative molecular weight determination 76
3.2.7 Isoelectric focusing 82
3.2.8 Characterisation on ALP enzyme 83
3.2.9 Experimental design and statistical analysis 85
3.2.10 Flow chart of the research project 86

4.0 RESULT AND DISCUSSION 87
4.1 Assay of the enzyme activity 87
4.2 Extraction of crude enzyme 88
4.3 Purification of ALP enzyme 90
4.3.1 Ammonium sulphate precipitation $(NH_4)_2 SO_4$ 90
4.3.2 Dialysis 97
4.4 Ion-exchange chromatography 104
4.4.1 Sample binding 104
4.4.2 DEAE-52 chromatography 106
4.5 Relative molecular weight determination 112
4.5.1 SDS-polyacrylamide gel electrophoresis 112

4.5.2 Determination of gel percentage 113
4.5.3 Molecular weight determination 118
4.6 Isoelectric focusing 121
4.7 Characterisations of the ALP enzyme 125
4.7.1 Determination of protein content and enzyme 125
4.7.2 Effect of incubation time on ALP 126
4.7.3 Effect of temperature on ALP 127
4.7.4 Effect of pH on ALP 130
4.7.5 Effect of metal ions on ALP 132
4.7.6 Effect of heavy metals on ALP 137
4.7.7 Assay of the Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) 143

5.0 CONCLUSION 145

REFERENCES 148
APPENDICES 156
BIODATA OF THE AUTHOR 171

LIST OF TABLES

Table		Page
1.	Hydrolysis of various O- and S-substituted monoesters of phosporothioic acid by alkaline and acid phosphatases.	29
2.	Molecular weights of ALP in different sources	36
3.	Types of glycoprotein found in different sources	38
4.	Physical properties of ALP	47
5.	Total protein and activities of the ALP enzyme from the organs of *Tilapia mosssambica*.	89
6.	Summary of the purification for alkaline phosphatase from *Tilapia mossambica* hepatopancreas.	110
7.	Summary of the purification for alkaline phosphatase from *Tilapia mossambica* intestine.	110
8.	Protein content and enzyme activities of the ALP enzyme from the purified hepatopancreas and intestine of *Tilapia mosssambica*.	125
9.	Comparison of characterizations of hepatopancreas and intestine of *Tilapia mossambica*.	147

10. Differences of freshwater aquaculture production (tan metric) for year 2002 and 2003 170

11. Freshwater fish production values for year 2002 and 2003 170

12. Changes in numbers of freshwater fish aquaculture between year 2002 and 2003 171

13. Differences in (hectares) of freshwater fish aquaculture between year 2002 and 2003 171

LIST OF FIGURES

Figure		Page
1.	The enzyme nomenclature of alkaline phosphatase.	2
2.	Schematic diagram of red tilapia (*Tilapia mossambica*).	8
3.	Schematic diagram of fish anatomy.	13
4.	Schematic diagram of the alkaline phosphatase hydrolyric reaction	31
5.	Molecular model of alkaline phosphatase structure.	33
6.	Two-steps process hydrolysis by ALP.	41
7.	Hydrolysis and phosphotransferase mechanism phosphate monoesters by ALP.	43
8.	The two-step mechanism of alkaline phosphatase.	45
9.	Molecular states of ALP under various conditions.	46
10.	Active site of ALP.	49
11.	Red tilapia (*Tilapia mossambica*).	60

12. Hepatopancreas of fish. 61

13. Electrophoresis apparatus. 62

14. Phosphate hydrolysis of *p* -NPP to *p* –NP. 66

15. Schematic diagram of ion exchange chromatography method. 75

16. Schematic diagram of the SDS-PAGE electrophoresis gel. 78

17. Over all flow chart of the research project. 86

18. (a) Graph of amount of protein (mg) and total enzyme activity of ALP supernatant (µmole min^{-1}) versus percentage of ammonium sulphate precipitation (%) from hepatopancreas supernatant. 91

18. (b) Graph of amount of protein (mg) and total enzyme activity of ALP supernatant (µmole min^{-1}) versus percentage of ammonium sulphate precipitation (%) from intestine supernatant. 92

19. (a) Graph of amount of protein (mg) and total enzyme activity of ALP supernatant (µmole min^{-1}) versus percentage of ammonium sulphate precipitation (%) from hepatopancreas supernatant. 95

19. (b) Graph of amount of protein (mg) and total enzyme activity of ALP supernatant (µmole min^{-1}) versus

percentage of ammonium sulphate precipitation (%) from intestine supernatant. 96

20. (a) Graph of amount of protein (mg) and total enzyme activity of ALP pellet (µmole min^{-1}) versus percentage of ammonium sulphate precipitation (%) from hepatopancreas pellet. 99

20. (b) Graph of amount of protein (mg) and total enzyme activity of ALP pellet (µmole min^{-1}) versus percentage of ammonium sulphate precipitation (%) from intestine pellet. 100

21. (a) Graph of amount of protein (mg) and total enzyme activity of ALP pellet (µmole min^{-1}) versus percentage of ammonium sulphate precipitation (%) from hepatopancreas pellet. 102

21. (b) Graph of amount of protein (mg) and total enzyme activity of ALP pellet (µmole min^{-1}) versus percentage of ammonium sulphate precipitation (%) from intestine pellet. 103

22. pH of DEAE binding versus absorbance at 410nm (ALP enzyme activity). 105

23. (a) Chromatography on DEAE-52 with hepatopancreas from the $(NH_4)SO_4$ step. 108

23. (b) Chromatography on DEAE-52 with intestine from the $(NH_4)SO_4$ step. 109

24. (a) SDS-polyacrylamide gel electrophoresis of under reduced conditions of hepatopancreas alkaline phosphatase of tilapia using a 15% gel. 114

24. (b) SDS-polyacrylamide gel electrophoresis of under reduced conditions of intestine alkaline phosphatase of tilapia using a 15% gel. 115

25. (a) SDS-polyacrylamide gel electrophoresis of under reduced conditions of hepatopancreas alkaline phosphatase of tilapia using a 12% gel. 116

25. (b) SDS-polyacrylamide gel electrophoresis of under reduced conditions of intestine alkaline phosphatase of tilapia using a 12% gel. 117

26. (a) Log standard molecular weight plot against R_f value of hepatopancreas to determine the molecular weight of alkaline phosphatase enzyme. 119

26. (b) Log standard molecular weight plot against R_f value of intestine to determine the molecular weight of alkaline phosphatase enzyme. 120

27. (a) Isoelectric focusing on hepatopancreas 122

27. (b) Isoelectric focusing on intestine 123

28. Influence of incubation time (minutes) on hepatopancreas and intestinal ALP activity. 126

29. Effect of temperature on hepatopancreas and intestinal ALP activity. 129

30. Effect of pH values on hepatopancreas and intestinal ALP activity. 132
31. (a) Activation effects of metal ions on hepatopancreas and intestinal ALP activity Mg^{2+} 135
31. (b) Activation effects of metal ions on hepatopancreas and intestinal ALP activity Ca^{2+}. 136
32. (a) Inhibition effects of heavy metals on hepatopancreas and intestinal ALP activity Zn^{2+} 139
32. (b) Inhibition effects of heavy metals on hepatopancreas and intestinal ALP activity Cu^{2} 140
32. (c) Inhibition effects of heavy metals on hepatopancreas and intestinal ALP activity Cd^{2+}. 141
32. (d) Inhibition effects of heavy metals on hepatopancreas and intestinal ALP activity Hg^{2+}. 142
33. Saturation curves on *p*-nitrophenyl phosphate substrate of both hepatopancreas and intestinal ALP activity. 144

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).