

## TABLE OF CONTENTS

	<b>Page</b>
<b>ACKNOWLEDGEMENT</b>	iii
<b>ABSTRACT</b>	iv
<b>TABLE OF CONTENTS</b>	x
<b>LIST OF TABLES</b>	xiv
<b>LIST OF ILLUSTRATIONC</b>	xv
<b>ABBREVIATIONS</b>	xviii
<b>CHAPTER I: INTRODUCTION</b>	1
<b>I. INTRODUCTION</b>	1
The cancer cells	1
Biochemistry of the malignant cells	3
Tumor marker	3
Colon Cancer	4
<b>II. LITERATURE REVIEW</b>	6
<b>I. Molecular characterization and isoenzymes of ALP</b>	6
Genetic and Expression	6
Structural of ALP	8
Anchoring of alkaline phosphatase to cell membrane	10
Glycosylation of ALP	10
Isoenzymes and isoforms of ALP presented in serum	11
<b>II. Physiological function of alkaline phosphatase</b>	12
Role of intestinal ALP in lipid transport	14

	<b>Page</b>
III. Age and sex distribution of Intestinal ALP in serum	14
IV. Intestinal ALP in normal and diseases	14
V. Intestinal ALP in carcinoma	16
<b>III. OBJECTIVES</b>	<b>18</b>
<b>CHAPTER II: MATERIAL AND METHODS</b>	<b>19</b>
<b>A. Material</b>	<b>19</b>
1. Specimens	19
2. Instruments	20
3. Chemical and Reagents	21
<b>B. Methods</b>	<b>23</b>
I. Method for determination of total alkaline phosphatase activity	22
1. Total ALP activity measured by Double-beam UV-Visible Spectrometer (Shimudzu UV 160A)	23
2. Total ALP activity measured by a microplate reader (Model EL 340)	24
3. Quality control of total ALP activity determination	24
II. Liver function test for screening patient conditions	24
III. Preparation ALP isoenzyme from tissues	25
1. Preparation of Liver ALP from bovine liver tissue	25
2. Preparation of ALP isoenzyme from patient specimens	25
IV. Partial purification of ALP isoenzymes from serum or enzyme solution by anion exchange chromatography	26
Precision of partial purification	26
Total protein determination	26
V. Identification of Chromatographic Fractions for IAP isoenzyme.	27
1. Chemical inhibition test.	27
2. Identification of anion chromatographic fractions for IAP isoenzyme by electrophoretic method (standard method)	28
2.1 Agarose gel electrophoresis	28

	<b>Page</b>
VI. Study of IAP properties	30
1. Heat inactivation	30
2. Amino acid inhibition test	30
3. Reactivity with IAP monoclonal antibody	31
VII. Biochemical characterizations of The IAP isoforms	31
1. Sialylation (Neuraminidase treatment)	31
2. Lectin precipitation of sugar moieties of the ALP isoenzymes	32
3. Treatment with Phosphatidylinositol phospholipase C (PI-PLC)	32
4. Affinity chromatography: Concanavalin A chromatography	32
VIII. Molecular weight determination : Western blot analysis	33
1. Separation of protein by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)	33
2. Electrotransfer of separated ALP isoenzyme from the gel into the blotting paper	34
3. Labeling of the transferred ALP isoenzyme by antibodies conjugated with the enzyme ( Immunodetection )	35
4. Detection of the labeling ALP isoenzyme signal	35
IX. Statistic Evaluation	36
 <b>CHAPTER III: RESULTS</b>	 37
I. Method for determination of total alkaline phosphatase activity	37
1. Quality control of total ALP activity determination	37
2. Total ALP activity measured in serum of normal and colon cancer patients	40
II. Liver function test for screening patient conditions	40
III. Preparation ALP isoenzyme from tissues	41
1. Preparation of Liver ALP from bovine liver tissue	41
2. Preparation of ALP isoenzyme from patient specimans	41

	<b>Page</b>
IV. Partial purification of ALP isoenzymes from serum of normal and colon cancer by anion exchange chromatography	44
V. Identification of Chromatographic Fractions for IAP isoenzymes.	47
1.. Chemical inhibition tests	47
2. Identification of anion chromatographic fractions for IAP isoenzyme by electrophoretic method	49
VI. Study of IAP properties	52
1. Heat inactivation of the IAP isoenzyme	52
2. Amino acid inhibition test	55
3. Reactivity with IAP monoclonal antibody	56
VII. Biochemical characterizations of The IAP isoforms	59
1. Sialylation (Neuraminidase treatment)	59
2. Lectin precipitation of sugar moieties of the ALP isoenzymes.	63
3. Affinity chromatography: Concanavalin A chromatography	66
4. Molecular weight determination : Western blot analysis	70
 <b>CHAPTER IV: DISCUSSIONS AND SUMMERY</b>	
I. Discussion	75
II. Summary	82
 <b>REFERENCES</b>	 83
 <b>APPENDIX</b>	 102
[A] Reagent preparations	102
[B] Chromatography data	109
[C] Surgical pathology report	111
 <b>CURRICULUM VITAE</b>	 118

## LIST OF TABLES

<b>Table</b>	<b>Page</b>
1     Quality control of ALP activity determination in serum and partial purified ALP fractions	38
2     LFT determined in serum of colon cancer patients	40
3     ALP activities, protein concentrations and specific activities in tumor and corresponding normal tissues of patients with colon cancer	42
4     The precision of protein partial purification on DEAE -Sephacel column chromatography	44
5.    Total activity of ALP in normal and colon cancer sera and pooled fraction, 1-4 in the first protein peak.	45
6.    Chemical inhibition tests on ALP fractions of normal and patients with colon cancer and liver disease.	48
7.    Effect of Amino acid on ALP activity in fractionated sera and tissues	55
8.    Lectin precipitation of carbohydrate side chain of ALP isoenzymes in normal and colon cancer sera comparison with those of liver disease sera and standards.	65

## LIST OF ILLUSTRATIONS

<b>Figure</b>	<b>Page</b>
1. Microscopic appearance of cancer cells	2
2. Evolution of ALP gene family.	7
3. Structural of ALP	9
4. The distribution of ALP activity in Level I control serum in shimudzu UV- Spectrophotometer	39
5. The distribution of ALP activity in Level I control serum in a microplate reader	39
6. Comparison of ALP activity (upper figure) and specific activity (lower figure) in normal and tumor tissues of patients with colon cancer	43
7. Optimal condition variance of partial purification of protein on DEAE-Sephacel column chromatography	44
8. Pattern of partial purification of ALP in normal serum by DEAE-Sephacel column chromatography.	46
9. Pattern of partial purification of ALP in patient serum by DEAE-Sephacel column chromatography	46
10. The inhibition effect of L-phenylalanine and levamisole on fractionated normal serum ALP in the first protein peak. L-phenylalanine more positive (left hand) L-phenylalanine less positive (right hand)	48
11. Identification of ALP fractions by agarose gel electrophoresis	50
12. Agarose gel electrophoresis of ALPs isoenzyme in normal and patient serum samples: comparison of IAP separation with tissue IAP and LAP standards	51
13. Heat inactivation of ALP isoenzymes activities. Percentage of remaining ALP activity were shown as a function of time(min) At 52 °C and 65 °C. [A] : LAP, [B] : IAP and [C] : LAP+IAP	53

<b>Figure</b>	<b>Page</b>
14 Heat inactivation of fractionated ALP in normal serum I (n=7), normal serum II (n=6), Liver disease serum (n=2), colon cancer serum (n=3) compared with IAP and LAP standard	54
15 Heat inactivation of intact and fractionated ALP in normal and paired tumor tissue from the same patient. (n=2 for each)	54
16 Agarose gel electrophoresis demonstrated colon cancer serum treated with various concentration of monoclonal anti IAP.	57
17 Polyacrylamide gel electrophoresis demonstrated normal serum and colon cancer serum treated with various concentration of monoclonal anti IAP	58
18 Agarose gel electrophoresis of ALPs isoenzymes in normal sera untreated and treated with Neuraminidase (Cn and Vn), demonstrating the patterns of migration of ALPs isoenzyme in serum compared with standard ALP isoenzyme.	60
19 Agarose gel electrophoresis of ALPs isoenzymes in sera of normal, colon cancer, and liver disease untreated and treated with neuraminidase (Cn and Vn), demonstrating the patterns of migration of ALPs isoenzyme in serum compared with standard ALP isoenzyme	61
20 Effect of neuraminidase (Cn) on IAP isoforms detected by polyacrylamide gel electrophoresis.	62
21. Lectins precipitation of ALP isoenzymes in normal, patient sera and tissues of patients with colon cancer comparison with the partial purified standards.	65
22. The elution profile of sugar containing in ALP protein ; Peak 1 : The unbound fraction (the molecule contains GPI or Lipid residues), Peak 2 : Weakly bound fraction Peak 3 : Strongly bound fraction	66

<b>Figure</b>	<b>Page</b>
23     The % relative ALP activity of IAP ( Figure 23 - A), LAP standard ( Figure 23 - B) and normal sera blood group A ( Figure 23 - C) and normal sera blood group B (Figure 23 - D) respectively : comparison between before and after treating with PL-C before separation on ConA column.	68
24     % Relative activity of ALP in serum, partial purified normal and corresponding tumor tissues of patient with colon cancer. The same samples, untreated and pre-treated with PI-PLC were separated on ConA column and the patterns of elution were compared. {A} ;serum {B} ; normal tissue and {C}: tumor tissue of the same patient.	69
25     The molecular weight of IAP isoforms in 3 patient sera with that in normal serum.	71
26     The comparison of IAP isoforms in normal, colon cancer and liver disease serum patient with colon cancer and liver	72
27     The comparison of IAP isoforms in serum , normal tissue and tumor tissue obtained from the same patient.	73
28     The IAP isoforms observed in tumor tissue extract with that in normal tissue extract of the same patient.	74
were high molecular mass IAP	
29     Pattern of partial purification of IAP standard on Sepharose 4B (gel filtration) column chromatography.	109
30     Pattern of partial purification of IAP standard on anion exchange chromatography, demonstrated correlation between protein and ALP activity in each fractions.	110



## ABBREVIATIONS

ABS	absorbance
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BBM	brush border membrane
CEA	carcinoembryonic antigen
Con A	Concanavalin A
CV	coefficient of variation
g.	gram
GPI	glycan-phoshatidylinositol
h	hour
IAP	Intestinal alkaline phosphatase
HCl	hydrochloride
kDa	kilodalton
L	litre
LAP	liver alkaline phosphatase
M	Molar
Min	minute
mL	milliliter
mM	millimolar
Mw	molecular weight
mU	milliunit
N	normal
NaOH	sodium hydroxide
nm.	nanometer

OD	optical density
PAGE	polyacrylamide gel electrophoresis
Pea	<i>Pisum sativum</i> agglutinin
PI-PLC	phosphatidylinositol phospholipase-C
PLP	placental alkaline phosphatase
SD	standard deviation
SDS	sodium dodecyl sulfate
TEMED	N,N,N',N'-tetramethyl-ethylene diamine
U	unit
WGA	wheat germ agglutinin
%	percent
$\alpha$	alpha
$^{\circ}\text{C}$	degree Celsius

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved