

Physicochemical and kinetic properties of purified arginase from Camel and Bovine liver cytosol

A thesis Submitted by

Rasha Elsherif Hassan Ibrahim

M.Sc. in Biochemistry (2005)

Biochemistry Department

Faculty of Science-Ain Shams University

*As a requirement for degree of Doctor of philosophy in
Biochemistry*

Under supervision of

Dr. Mohamed M. Abdel Fattah

Dr. Tahany M. Maharem

Professor of Biochemistry

Biochemistry Dept.

Faculty of Science

Ain Shams University

(God rest his soul)

Professor of Biochemistry

Biochemistry Dept.

Faculty of Science

Ain Shams University

Dr. Walid E. Zahran

Assistant professor of Biochemistry

Biochemistry Dept.

Faculty of Science

Ain Shams University

Biochemistry Department

Faculty of Science

Ain Shams University

2009

*Physicochemical and kinetic properties of
purified arginase from Camel and Bovine
liver cytosol*

Board of Scientific Supervision

Dr. Mohamed M. Abdel Fattah

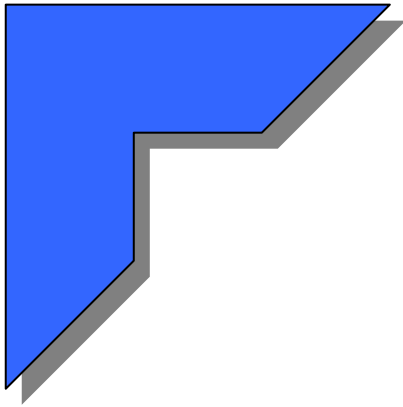
*Professor of Biochemistry
Biochemistry Dept.
Faculty of Science
Ain Shams University
Cairo – Egypt
(God rest his soul)*

Dr. Tahany M. Maharem

*Professor of Biochemistry
Biochemistry Dept.
Faculty of Science
Ain Shams University
Cairo - Egypt*

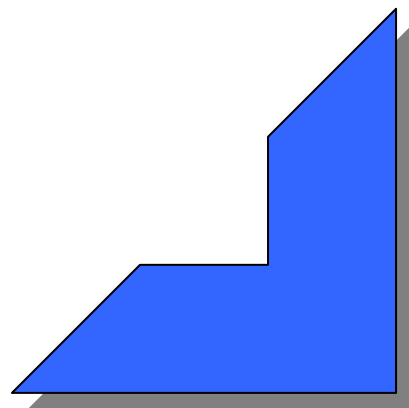
Dr. Walid E. Zahran

*Assistant professor of Biochemistry
Biochemistry Dept.
Faculty of Science
Ain Shams University
Cairo - Egypt*



*This Thesis has not been submitted to this or any
other University.*

Rasha Elsherif Hassan



Dedication

Special thanks and gratitude to my beloved parents, brother and my beloved husband who are behind every successful aspect of my life. If every one is as kind as them, earth would be an utopia. Special thanks, love and care to my lovely son Abdullah. Finally, I want to dedicate this thesis to every member in my family especially to my grandfather and my grandmother (God rest their soul).

CONTENTS

	Page
Abstract	i
List of Figures	iii
List of Tables	xi
List of Abbreviations	xiii
Aim of the Work	xvi
Introduction	1
Materials and Methods	36
Materials	36
1- Enzyme source	36
2- Chemicals	36
3- Buffers	37
Methods	37
- Analyses	37
1- Arginase assay	37
2- Protein determination	40
- Purification of arginase	42
1- Preparation of crude homogenate	42
2- Heat treatment	42
3- Ammonium sulphate fractionation	43
4- DEAE cellulose column chromatography	44

5- SP-Sepharose column chromatography	46
6- Gel filtration chromatography	47
- Sephadex G-100-120 column	47
- Sephacryl HR-300 column	48
- Molecular weight determination	49
1- Gel filtration chromatography	49
2- Native polyacrylamide gel electrophoresis	52
3- SDS-Polyacrylamide gel electrophoresis	56
- Determination of isoelectric point	62
- Determination of carbohydrate content	65
- Determination of manganese content	66
Results	68
Discussion	142
Summary	158
References	165
Arabic Summary	
Arabic Abstact	

ABSTRACT

Rasha Elsherif Hassan Ibrahim, Physicochemical and kinetic properties of purified arginase from camel and bovine liver cytosol.

Ph.D. Thesis, Biochemistry Department, Faculty of Science, Ain Shams University.

In the present study, camel liver was chosen as a source of arginase, since no information is available about this enzyme from this species, so this is the first time to purify arginase from camel liver cytosol. Arginase was also purified from bovine liver cytosol and some of the physicochemical and kinetic properties of the purified arginase from the two sources were studied and compared.

Arginase was isolated and purified from camel and bovine liver using heat denaturation followed by ammonium sulphate fractionation, DEAE-cellulose column chromatography, SP-Sephacryl column chromatography and gel filtration using Sephadex G-100-120 and Sephacryl HR-300 columns.

It was observed from the present investigation that there is a similarity between the purified camel and bovine liver arginases in some properties including the non-adsorption on anion exchanger column, the oligomeric structure of the

enzyme, the non-cooperativity of subunits, the efficiency constant, the susceptibility to the inhibition by some amino acids, the requirement for metal ions as cofactor, the alkaline optimum pH, the stability at 4°C, the cold lability through freezing and thawing cycle and storage at -20°C. On the other hand, purified camel liver arginase differs from the purified bovine liver enzyme in its chromatographic behaviour on the cation exchanger column, higher molecular weight (174-180 kDa) versus (133-140 kDa), slightly alkaline *pI* value (7.7) versus the slightly acidic to neutral *pI* values (5.9, 7), higher carbohydrate content 2.7% versus 0.6%, affinity towards arginine with K_m value 7.1mM versus 14.3mM of bovine liver enzyme, its inhibition by some metal ions as Sr^{+3} and Zn^{+2} . Also, it was found that camel liver arginase is more thermostable with an optimum temperature of 70°C and E_a of about 11,752cal/mole versus an optimum temperature of 55-60°C and E_a of about 10,420cal/mole for bovine liver arginase which demonstrates that camel liver enzyme has a relative structure rigidity. Finally, although purified camel liver arginase was affected by freezing at -20°C and by repeated freezing and thawing it proved to be more stable than the purified bovine liver enzyme.

Key words: *Camel, bovine, liver, arginase, purification, physicochemical and kinetic properties.*

LIST OF FIGURES

Figure no.	Legand	Page
Fig. (1)	Urea cycle	2
Fig. (2)	a) Ribbon plot of rat liver arginase structure. b) Binuclear Mn^{+2} center of rat liver arginase and its coordination interactions.	7
Fig. (3)	Proposed arginase mechanism.	10
Fig. (4)	Diagram of the major metabolic fates of arginine.	11
Fig. (5)	Polyamine synthesis from arginine in mammalian cells.	13
Fig. (6)	Postulated pathways of L-arginine metabolism and their relationship to airway responsiveness, inflammation and remodeling.	22
Fig. (7)	Standard curve of urea covering the range (55-500 μ M).	39
Fig. (8)	Standard curve of BSA covering the range (20-200 μ g/ml).	41
Fig. (9)	A plot of log molecular weight of standard proteins versus V_e/V_o using Sephadex G-100-120 gel filtration chromatography.	50
Fig. (10)	A plot of log molecular weight of standard proteins versus V_e/V_o using Sephacryl HR-300	51

	gel filtration chromatography	
Fig. (11)	A plot relating relative mobility (R_f) and molecular weight of standard proteins using 10% native polyacrylamide gel electrophoresis.	60
Fig. (12)	A plot relating relative mobility (R_f) and molecular weight of standard proteins using 15% SDS-polyacrylamide gel electrophoresis.	61
Fig. (13)	A plot relating isoelectric point (pI) and relative mobility of standard proteins using native isoelectric focusing.	66
Fig. (14)	Standard curve of glucose covering the range (2-20 μ g/0.5ml).	67
Fig. (15)	DEAE-cellulose column chromatography of camel liver ammonium sulphate precipitation protein solution.	74
Fig. (16)	DEAE-cellulose column chromatography of bovine liver ammonium sulphate precipitation protein solution.	75
Fig. (17)	SP-Sepharose column chromatography of camel concentrated dialyzed DEAE-cellulose enzyme solution.	79
Fig. (18)	SP-Sepharose column chromatography of bovine concentrated dialyzed DEAE-cellulose enzyme solution.	80

Fig. (19)	Gel filtration of camel dialyzed pooled SP-Sephrose enzyme solution on Sephadex G-100-120 column at a flow rate 20ml/hour and 2ml/fraction.	81
Fig. (20)	Gel filtration of bovine dialyzed pooled SP-Sephrose enzyme solution on Sephacryl HR-300 column at a flow rate 20ml/hour and 2ml/fraction.	82
Fig. (21)	SDS-polyacrylamide gel electrophoresis pattern of camel liver arginase samples from different purification steps.	84
Fig. (22)	SDS-polyacrylamide gel electrophoresis pattern of bovine liver arginase samples from different purification steps.	85
Fig. (23)	Native polyacrylamide gel electrophoresis of purified camel and bovine liver arginases referring to individual standard molecular weight markers.	91
Fig. (24)	Native molecular weight determination of purified camel and bovine liver arginases using 10% native polyacrylamide gel electrophoresis.	92
Fig. (25)	Molecular weight determination of purified camel liver arginase from calibration curve of protein markers with known molecular weight using Sephadex G-100-120 gel filtration.	93

Fig. (26)	Molecular weight determination of purified bovine liver arginase from calibration curve of protein markers with known molecular weight using Sephacryl HR-300 gel filtration.	94
Fig. (27)	SDS-polyacrylamide gel electrophoresis of purified camel and bovine liver arginases referring to standard molecular weight proteins.	95
Fig. (28)	Molecular weight determination of purified camel and bovine liver arginases using 15% SDS-polyacrylamide gel electrophoresis.	96
Fig. (29)	Native isoelectric focusing of purified camel and bovine liver arginases with respect to standard <i>pI</i> markers.	97
Fig. (30)	Isoelectric point (<i>pI</i>) determination of purified camel and bovine liver arginases using 5% native polyacrylamide gel electrophoresis.	98
Fig. (31)	Effect of enzyme concentration on the velocity of purified liver arginase catalyzed reaction. (a) Camel liver arginase (9-64μg protein/assay). (b) Bovine liver arginase (6.5-46μg protein/assay).	101
Fig. (32)	(a) Michaelis Menten plot of purified camel liver arginase as a function of arginine concentration. (b) Lineweaver-Burk plot relating purified camel liver arginase activity to arginine concentration.	102

Fig. (33)	(a) Michaelis Menten plot of purified bovine liver arginase as a function of arginine concentration. (b) Lineweaver-Burk plot relating purified bovine liver arginase activity to arginine concentration.	103
Fig. (34)	Hill plot relating $\text{Log } (v/V_{\text{max}}-v)$ and $\text{Log } [S]$ of purified liver arginase catalyzed reaction. (a) the substrate concentration was varied (10-50mM) for camel liver arginase reaction. (b) the substrate concentration was varied (20-60mM) for bovine liver arginase reaction.	108
Fig. (35)	Effect of pH on the activity of purified (a) camel liver arginase, (b) bovine liver arginase, using 50mM sodium acetate buffer pH (3.6-5.6), 50mM potassium phosphate buffer pH (6-8), 50mM Tris-HCl buffer (7.2-9) and 50mM carbonate-bicarbonate buffer pH (9.3-10.7).	110
Fig. (36)	Effect of temperature on the purified (a) camel liver arginase. (b) bovine liver arginase.	111
Fig. (37)	Arrhenius plot relating $\text{Log } v_o$ and $1/T$ to determine the E_a for purified liver arginase catalyzed reaction. (a) the incubation temperature was varied (288-333K) for camel liver arginase reaction. (b) the incubation temperature was varied (283-343K) for bovine liver arginase reaction.	112

Fig. (38)	Thermostability of purified camel liver arginase. (a) The enzyme sample was preincubated alone at (25°C, 37°C and 55°C). (b) The enzyme sample was preincubated with Mn ⁺² at the same temperatures.	117
Fig. (39)	Thermostability of purified bovine liver arginase. (a) The enzyme sample was preincubated alone at (25°C, 37°C and 55°C) (b) The enzyme sample was preincubated with Mn ⁺² at the same temperatures.	118
Fig.(40)	Lineweaver-Burk plot relating purified liver arginase reaction velocity to arginine concentration in absence and presence of different L-ornithine concentrations with constant concentration of (a) camel liver arginase (0.036 mg protein/assay). (b) bovine liver arginase (0.02 mg protein/assay).	129
Fig. (41)	Lineweaver-Burk plot relating liver arginase reaction velocity to arginine concentration in absence and presence of different L-lysine concentrations with constant concentration of (a) camel liver arginase (0.036 mg protein/assay). (b) bovine liver arginase (0.02 mg protein/assay).	130
Fig.(42)	Lineweaver-Burk plot relating purified liver arginase reaction velocity to arginine	131

	concentration in absence and presence of different L-valine concentrations with constant concentration of (a) camel liver arginase (0.036 mg protein/assay). (b) bovine liver arginase (0.02 mg protein/assay).	
Fig. (43)	Lineweaver-Burk plot relating purified liver arginase reaction velocity to arginine concentration in absence and presence of different L-leucine concentrations with constant concentration of (a) camel liver arginase (0.036 mg protein/assay). (b) bovine liver arginase (0.02 mg protein/assay).	132
Fig. (44)	Lineweaver-Burk plot relating purified liver arginase reaction velocity to arginine concentration in absence and presence of different DL-isoleucine concentrations with constant concentration of (a) camel liver arginase (0.036 mg protein/assay). (b) bovine liver arginase (0.02 mg protein/assay).	133
Fig. (45)	Effect of L-ornithine on purified liver arginase activity. (a) L-ornithine concentration was varied (5-50mM) for camel liver arginase reaction. (b) L-ornithine concentration was varied (2-40mM) for bovine liver arginase reaction.	134