PURIFICATION AND CHARACTERIZATION OF HYALURONIDASE FROM CERASTES CERASTES VENOM

THESIS

Submitted in Partial Fulfilment for the Master Degree in Biochemistry



 \mathbf{p}

HAB MOHAMED HELMY MAHMOUD

M. B.; B. Ch.

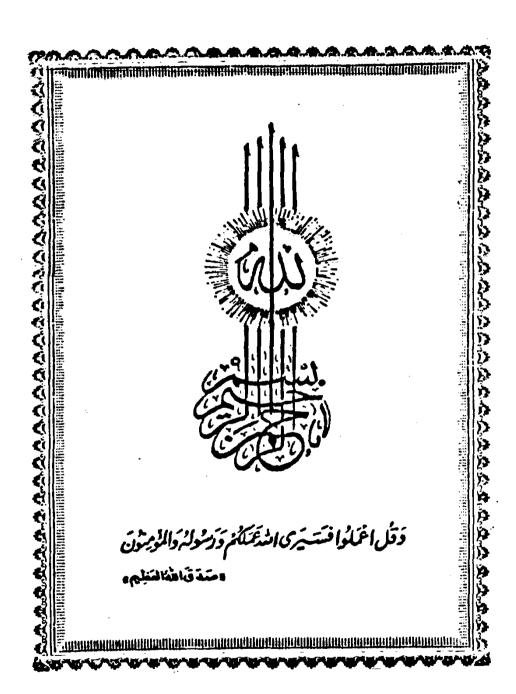
Supervisor

Prof. Dr. FATHY MOHAMED TASH

Professor of Biochemistry
Faculty of Medicine, Ain Shams University

Faculty of Medicine
Ain Shams University

1985





DÉDICÁTED TO MY FATHER & MOTHER

This research project is carried out under grant No. 840505 by the Foreign Relation Coordination unit of the Supreme Council of Universities.

ACKNOWLEDGMENT

I would like to express may deepest gratitude to my advisor Dr. Mohamed Farid El-Asmar, Professor of Biochemistry, Faculty of medicine, Ain Shams University, for his technical assistance, guidance and generous help.

I want to express my gratitude and thanks to Dr. Fathy Tash, Prof. of Biochemistry, for his supervision, guidance, and true cooperation, and to Dr. Emtiaz Shaaban, and Dr. Rashwan Farag for their supervision and actual readiness to help.

My sincere gratitude and thanks are also extended to my professor, Dr. Salah El-Din Zaky Eid, Head of Biochemistry department, Faculty of medicine, Ain Shams University, for his encouragement and support.

Finally thanks to all my colleagues in Biochemistry department for being cooperative and helpful.

	CONTENTS	Page
ι.	AIM OF THE WORK	1
2.		3
	* Classification and distribution of venomous snakes	
	in the world	6
3	* General composition of snake venoms	10
	* The protein component in snake venoms	14
•	* Distribution of venoms in the body of snakebite	
	victims	15
	* Enzymes in snake venoms	18
	* Biological value of enzymes in snake venoms in	
	biochemical research	19
	* Hyaluronidases	22
	* Mammalian sources of hyaluronidase enzyme	23
	* Hyaluronidases in venoms	24
	* Classification of hyaluronidases	27
	* Purification and characterization of testicular	
	hyaluronidase	30
	* Lysosomal hyaluronidases	31
	* Properties of different hyaluronidases	35
	* Kinetics of hyaluronidases	37
	* Biological significance of hyaluronidases	38

		Page			
	* Different hyaluronidase substrates	40			
	* Different methods for assay of hyaluronidase				
	activity	44			
	* Gel Filteration chromatography	47			
з.	MATERIALS & METHODS	53			
	* The crude venom	53			
	* Purification procedure for hyaluronidase	54			
	* Protein determination	57			
•	* Preparation of standard curve for protein				
	estimation	59			
	* Estimation of the protein content in the crude				
	venom	60			
	* Assay of hyaluronidase activity	61			
	* Assay of hyaluronidase activity in the crude ven				
	* Effect of enzyme concentration on enzyme activity	66			
	* Effect of pH on hyaluronidase activity	67			
	* Effect of temperature on enzyme activity	70			
	* Effect of substrate conc. on enzyme activity	71			
	* Effect of some cations on enzyme activity	72			
	* Effect of different anions on enzyme activity	73			
	* Effect of EDTA, DME and Iodoacetamide on enzyme				
	activity	74			
4.	RESULTS	75			
5.		105			
		116			
7.	REFERENCES	118			
	ARABIC SUMMARY.				

* LIST OF TABLES *

			Page
Table	(1):	Protein content in the crude venom	77
Table	(2):	Hyaluronidase activity in the crude	
		venom	80
Table	(3):	Hyaluronidase activity in the pooled	
		active fraction	84
Table	(4):	PUrification profile for hyaluronidase	
		enzyme	85
Table	(5):	Effect of metal ions on enzyme activity	9 8
Table	(6):	Effect of some anions on enzyme activity.	101
Table	(7):	Effect of EDTA, iodoacetamide and	
		dimercaptoethanol on enzyme activity	104

* LIST OF FIGURES*

			Page
Fig.	(1):	A standard curve for protein estimation by	
		lowry's method	76
Fig.	(2):	Standard curve for the turbidity of sodium	
		hyaluronate after adding cetyltrimethyl	
		ammonium bromide	79
Fig.	(3):	Elution pattern of crude Cerastes cerastes	
		venom on sephadex G-100(Fine) column	83
Fig.	(4):	Effect of enzyme concentration on enzyme	
		activity	8 7
Fig.	(5):	Effect pH on enzyme activity	-89
Fig.	(6):	Effect of temperature on enzyme activity	91
Fig.	(7):	Effect of substrate concentration on enzyme	
		activity	93
Fig.	(8):	Double reciprocal Lineweaver-Burk plot of	
		1/v versus 1/ \$	95
Fig.	(9):	Effect of some cations on the enzyme acti-	
		vity	97
Fig.	(10):	Effect of some anions on enzyme activity	100
Fig.	(11):	Effect of NaEDTA, dimercaptoethanol and	
		Iodoacetamide on enzyme activity	103

AIM OF THE WORK

Aim of the work

Snake venoms are complex and concentrated mixtures of many enzymes and substances of marked biological concern

The understanding of the net effect of envenomation on the body of bittin victim requires a complete idea about the effect and role of each component separetly. And hence the need for fractionation, purification and characterization of the different venom constituents.

The hyaluronidase enzyme was well known to occur in the majority of snake venom families. It has been implicated in the diffusion of the injected venom into and through tissues, but it has never been shown to definitely participate in this action.

Our task in such a study is to purify the hyaluronidase enzyme from one of Egyptian vipers venom, which is the <u>Cerastes</u> cerastes snake venom.

Characterization of both physical and kinetic behaviour of the enzyme will be explored.

Effect of temperature, pH, metal and non metal ions on it's activity will be attempted.

Calculation of its Michaelis constant (K_m) . for its preferable substrate, hyaluronic acid, is of great value for its characterization.

INTRODUCTION

GENERAL INTRODUCTION

In ancient civilization, the snake embodied the spirit of the earth. At this time, the snake was the attribute of all cosmogonies.

Snakes represented the spirit of air and earth and was the symbol of health, Knowledge, life, and fecundity.

Aaron, the brother of Moses, turned rods into snakes, and † placed a bronze snake on a staff, anyone who was the victim of snake poisoning was saved when he saw this symbol.

Jung (1964) told the curious story of the chemist

Kekule who, in the 19th century, came to define the molecular structure of benzene, influenced by the memory of
ancient symbols, he was dreaming one night of a snake
holding it's tail in it's mouth and on awakening from his
dream, related the circular shape of the snake to the cyclic
structure of benzene.

The "ouroboros" of Africa is a snake which bites its own tail off since it fertilizes itself it is considered a "Source of life"Being venomous, it is also a "Source of death" so the "Ouroboros" is an expression of the idea of life and death at the same time (CHEVALIER, 1973).

In the 16th century, Van Helmont proposed his phlogistic theory, that snake venoms are "irritated spirits" which were "so cold" that they coagulated the blood in the veins and arrested the circulation.