

# **Purification and characterization Of L-Amino acid oxidase from Naja Nigricollis Venom**

## **Thesis**

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**BY**

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*I would like to dedicate this work to my husband, my mother, Shereen and my baby for their great help and support.*

# Contents

	<i>Page</i>
• <b>List of tables</b> .....	I
• <b>List of figures</b> .....	II
• <b>Abbreviations</b> .....	IV
• <b>Aim of the work</b> .....	VII
• <b>Introduction</b>	
○ General introduction .....	1
○ General composition of snake venom .....	6
○ Snake venom biomedical applications .....	13
○ Snake Venom L-Amino Acid Oxidases .....	24
○ Physical Properties of Snake Venom LAAOs .....	28
○ Enzymatic Properties of LAAOs .....	36
○ Immunological Properties of LAAOs .....	40
• <b>Materials and Methods</b>	
○ Crude venom .....	51
○ Protein estimation: Ultraviolet absorption method .....	54
○ Screening of the LAAO activity for protein fractions obtained from various purification steps .....	55
○ Fractionation of crude Naja nigricollis venom and identification of the LAAO activity:	
I) Gel filtration chromatography on sephadex G-75 .....	59

II) Cation exchange chromatography .....	63
○ Determination of the purity of fraction VIIb and its molecular weight by Disc SDS-PAGE .....	67
○ Assessment of carbohydrate content in fractionVIIb ....	72
○ Determination of optimum pH of LAAO of fraction VIIb .....	74
○ Effect of temperature on LAAO activity of fraction VIIb .....	76
○ Effect of some cations on LAAO activity of fraction VIIb .....	77
○ Effect of some inhibitors on LAAO activity of fraction VIIb .....	79
○ Effect of adding CaCl <sub>2</sub> on LAAO activity of fraction VIIb pre-incubated with EDTA .....	81
○ Measurements of LAAO kinetic constant .....	83
● <b>Results .....</b>	<b>84</b>
● <b>Discussion .....</b>	<b>105</b>
● <b>Recommendations .....</b>	<b>115</b>
● <b>Summary .....</b>	<b>116</b>
● <b>References .....</b>	<b>119</b>
● <b>Arabic Summary .....</b>	<b>—</b>

# List of Tables

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
<b>Table (1):</b>	Physiochemical Properties of Some Purified Venom L-Amino Acid Oxidases .....	27
<b>Table (2):</b>	Screening fractions obtained from gel Filtration chromatography for LAAO activity ...	86
<b>Table (3):</b>	Screening fractions obtained from cation exchange chromatography for LAAO activity ...	89
<b>Table (4):</b>	Summary of purification steps of LAAO from Naja Nigricollis venom .....	90
<b>Table (5):</b>	Determination of carbohydrate content in fraction VIIb .....	93
<b>Table (6):</b>	Determination of optimum pH of LAAO activity of fraction VIIb .....	94
<b>Table (7):</b>	Effect of temperature on LAAO activity of fraction VIIb .....	96
<b>Table (8):</b>	Effect of some cations on LAAO activity of fraction VIIb .....	98
<b>Table (9):</b>	Effect of some inhibitors on LAAO activity of fraction VIIb .....	99
<b>Table (10):</b>	Effect of adding metal ions on LAAO activity of fraction VIIb pre-incubated with EDTA .....	101

# List of Figures

<i>Figure No.</i>	<i>Title</i>	<i>Page No.</i>
<b>Figure (1):</b>	Cobra snake .....	2
<b>Figure (2):</b>	Naja Nigricollis .....	4
<b>Figure (3):</b>	The biological effects of snake venoms during the envenomation process .....	12
<b>Figure (4):</b>	N-terminal amino acid sequence of some snake venom L-amino acid oxidases .....	31
<b>Figure (5):</b>	Deduced amino acid sequence of Agkistrodon halys blomhoffii L-amino acid oxidase .....	32
<b>Figure (6):</b>	Reaction mechanism for the oxidation of L- phenylalanine by L-amino acid oxidase .....	40
<b>Figure (7):</b>	Disc gel electrophoresis apparatus .....	71
<b>Figure (8):</b>	Gel filtration chromatography on sephadex G-75 .....	84
<b>Figure (9):</b>	Cation exchange chromatography on CM sephadex C-25 .....	87
<b>Figure (10):</b>	Disc SDS-Page of fraction VIIb obtained from cation exchange chromatography .....	91

<b>Figure (11):</b>	Standard curve for sucrose .....	92
<b>Figure (12):</b>	Optimum pH of LAAO activity of fraction VIIb .....	95
<b>Figure (13):</b>	Effect of temperature on LAAO activity of fraction VIIb .....	97
<b>Figure (14):</b>	LAAO Michaelis–Menten kinetics curve .....	103
<b>Figure (15):</b>	LAAO Lineweaver-Burk plot curve .....	104
<b>Figure (16):</b>	Mechanism of chemical reaction catalysed by LAAO .....	105



# Abbreviations

<b>A.h blomhoffii</b>	.....Agkistrodon halys blomhoffii
<b>A°280</b>	Absorbance at 280 nm.
<b>ADP</b>	.....Adenosine diphosphate
<b>B. moojeni</b>	.....Bothrops moojeni
<b>B. piraja</b>	.....Bothrops piraja
<b>BM</b>	.....Bone marrow
<b>C. adamanteus</b>	.....Calloselasma adamanteus
<b>C. atrox</b>	.....Crotalus atrox
<b>C. dirissus cascavella</b>	...Crotalus dirissus cascavella
<b>C. rhodostoma</b>	.....Calloselasma rhodostoma
<b>CCL<sub>4</sub></b>	.....Carbon tetrachloride
<b>cDNA</b>	.....Complementary deoxyribonucleic acid
<b>CHO</b>	.....Carbohydrate
<b>CM-Sephadex</b>	.....Carboxy methyl- sephadex
<b>DEAE-Sephadex</b>	.....Diethyl amino ethyl-sephadex
<b>DNA</b>	.....Deoxyribonucleic acid
<b>DW</b>	Distilled water
<b>E.C. No</b>	.....Enzyme classification number
<b>EDTA</b>	Ethylene diamine tetraacetic acid
<b>ELISA</b>	.....Enzyme linked immunosorbent assay

<b>FAD</b> .....	Flavin adenine dinucleotide	
<b>FMN</b> .....	Flavin mononucleotide	
<b>g</b>	gram	
<b>KDa</b>	Kilo Dalton	
<b>M</b>	Molar	
<b>mA</b>	Milliamper	
<b>mM</b>	Millimolar	
<b>Mw</b>	Molecular weight	
<b>N. kaouthia</b> .....	<i>Naja kaouthia</i>	
<b>nm</b>	Nanometer	
<b>O. hannah</b> .....	Ophiophagus hannah	
<b>OD</b>	Optical density	
<b>PAGE</b>	Polyacrylamide gel electrophoresis	
<b>pI</b> .....	Isoelectric point	
<b>PLA<sub>2</sub></b>	Phospholipase A <sub>2</sub>	
<b>PMSF</b> .....	Phenylmethanesulfonylfluoride	or
	phenylmethylsulfonyl fluoride	
<b>RVV</b>	Russelli viper venom	
<b>SDS</b>	Sodium dodecyl sulphate	
<b>SVMPs</b>	Snake venom metalloproteases	
<b>SVTLEs</b>	Snake venom thrombin-like enzymes	
<b>TEMED</b>	Tetra methyl-ethylene diamine	

<b>U/mg</b>	Unit/ milligram
<b>UV</b>	Ultra violet
<b>µg</b>	Micro gram
<b>FIX</b>	Factor 9

### **Abbreviations of Amino Acids**

<b>C</b> .....	Cystiene (Cys)
<b>D</b> .....	Aspartic acid (Asp)
<b>E</b> .....	Glutamic acid (Glu)
<b>G</b> .....	Glycine (Gly)
<b>H</b> .....	Histidine (His)
<b>I</b> .....	Isoleucine (Ile)
<b>K</b> .....	Lysine (lys)
<b>M</b> .....	Methionine (Met)
<b>N</b> .....	Asparagine (Asn)
<b>P</b> .....	Proline (Pro)
<b>R</b> .....	Arginine (Arg)
<b>S</b> .....	Serine (Ser)
<b>T</b> .....	Threonine (Thr)
<b>V</b> .....	Valine (Val)
<b>X</b> .....	Stands for any amino acid

## **AIM OF THE WORK**

This work aims to:

- 1- Purification of L-Amino acid oxidase enzyme from the venom of *Naja Nigricollis nigricollis* (spitting cobra) from Egypt.
- 2- Determination of the purity and molecular weight of the enzyme by sodium dodecyl sulfate-polyacrelamide gel electrophoresis.
- 3- Studying the biochemical characters of the enzyme e.g effect of changes in pH, temperature, metal ions concentration, different inhibitors and studying kinetic parameters of the enzyme.

## GENERAL INTRODUCTION

At present, there are more than 600 known species of venomous snakes classified into several families:

- (1) Hydrophidae,
- (2) Elapidae,
- (3) Viperidae,
- (4) Crotalidae,
- (5) Colubridae.

(Panfoli et al., 2010)

**Hydrophidae**; represent sea snakes.

**Elapidae**; comprises 50 genera, including the well known Cobras, the African Mambas, the Kraits.

**Viperidae**; this family comprises the genera Viper, Atractapis, Bitis, Causus, Cerastes, Echis, Adenorhinos, Atheris, Eristicophis, Pseudocerastes and Azempios.

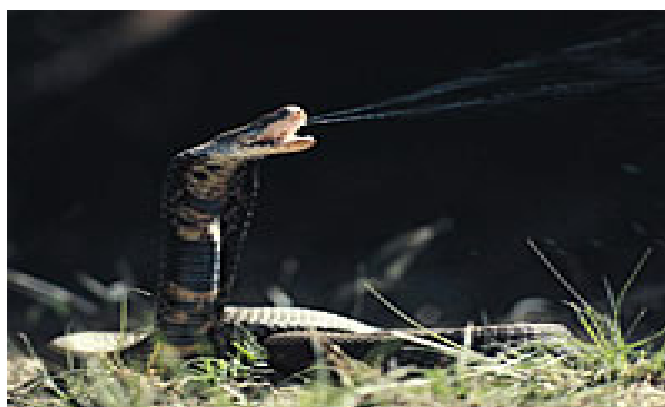
**Crotalidae**; comprises 6 genera; Crotalus, Sistrurus, Agkistrodon, Bothrops, Lachesis and Trimersurus.

**Colubridae**; It is the principle snake family which comprises an enormous group of about 1400 species including roughly 2/3 of world's snakes (**Haddad and Podgorny, 1990**).

## Cobra snake

Most cobra are large snakes, 1.2 - 2.5 m in length. The king cobra *Ophiophagus hannah* which may reach 5.5 m is the largest venomous snake in the world (Veto et al., 2007).

Spitting cobra expulse venom toward the face and/or eyes of potential predators as part of their self defensive. Spit venom does not land as a point but rather is distributed (Young et al., 2009).



**Figure (1):** Cobra snake, (<http://www.google.com>)

The lethal effect of cobra bites is mainly neurotoxic. This is explained by the presence of highly potent  $\alpha$ -neurotoxin in their venoms (Meenatchisundarama and Michael, 2010). The other two highly toxic components of cobra venoms are cytotoxins and phospholipases A<sub>2</sub>, these three types of toxins constitute a major part of cobra venom. However cobra venoms contain also many other less abundant components which

possess very low toxicity or even are not toxic at all. These components, mostly proteins, belong to different structural and functional types, and the reason for their presence in the venom is not always evident.

Some of them are known for many years (e.g., nerve growth factor and cobra venom factor); others (e.g., cysteine rich secretory proteins, CRISPs) were discovered. There are non-lethal proteins with unique biological activities that can be used as biochemical tools, while others may be regarded as potential leads for drug design (Utkin et al., 2007).

### **Naja nigricollis**

Commonly known as the **black-necked spitting cobra**, is a species of spitting cobra found mostly in sub-Saharan Africa. They are moderately sized snakes that can grow to a length of 1.2 to 2.2 m (3.9 to 7.2 ft) in length. Their coloration and markings can vary considerably. Also known as typical cobra (with the characteristic ability to raise the front quarters of their bodies off the ground and flatten their necks in a threatening display) (Spawls and Branch, 1995), with the ability to eject venom from its fangs in self-defense (Hayes et al., 2008).