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

Thesis

Submitted for the Partial Fulfillment of the Master Degree
in Basic Science (Biochemistry)

BY

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2015

Acknowledgements

I would like to express my sincere gratitude to **Prof. Dr. Mohamed Farid El Asmer**, Professor of Medical Biochemistry Department, Faculty of Medicine, Ain Shams University, for suggesting this point of research and for his willing assistance and guidance, I also thank him for providing the equipment and the chemicals necessary to accomplish this work.

My deep appreciation goes to **Prof. Dr. Hanaa El Tayeb Nasser,** Head and Professor of Medical Biochemistry Department, Faculty of Medicine, Ain Shams University, for her continuous support, assistance and guidance throughout the course of the study.

I am also very grateful to **Dr. Ayman Ragaa Basheer**, Lecturer of Medical Biochemistry Department, faculty of Medicine, Ain Shams University, for his continuous supervision and fruitful teaching throughout the work.

Finally, I offer my gratitude to all staff members and colleagues in biochemistry department, faculty of Medicine, Ain Shams University.

DEDICATION

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

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Abbreviations

A.h blomhoffii .....Agkistrodon halys blomhoffii

A˚280 Absorbance at 280 nm.

ADP .....Adenosine diphosphate

B. moojeni .....Bothrops moojeni

B. piraja .....Bothrops piraja

BM .....Bone marrow

C. adamanteus .....Calloselasma adamanteus

C. atrox .....Crotalus atrox

C. dirissus cascavella …Crotalus dirissus cascavella

C. rhodostoma .....Calloselasma rhodostoma

CCL_4 .....Carbon tetrachloride

cDNA .....Complementary deoxyribonucleic acid

CHO .....Carbohydrate

CM-Sephadex .....Carboxy methyl- sephadex

DEAE-Sephadex.....Diethyl amino ethyl-sephadex

DNA .....Deoxyribonucleic acid

DW Distilled water

E.C. No .....Enzyme classification number

EDTA Ethylene diamine tetraacetic acid

ELISA .....Enzyme linked immunosorbent assay

FAD .....Flavin adenine dinucleotide

FMN .....Flavin mononucleotide

g gram

KDa Kilo Dalton

M Molar

mA Milliamber

mM Millimolar

Mw Molecular weight

N. kaouthia .....Naja *kaouthia*

nm Nanometer

O. hannah .....Ophiophagus hannah

OD Optical density

PAGE Polyacrylamide gel electrophoresis

pI .....Isoelecteric point

PLA_2 Phospholipase A_2

PMSF .....Phenylmethanesulfonylfluoride or phenylmethylsulfonyl fluoride

RVV Russelli viper venom

SDS Sodium dodecyl sulphate

SVMPs Snake venom metalloproteases

SVTLEs Snake venom thrombin-like enzymes

TEMED Tetra methyl-ethylene diamine

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

Abbreviations of Amino Acids

C.....Cystiene (Cys)

D.....Aspartic acid (Asp)

E .....Glutamic acid (Glu)

G.....Glycine (Gly)

H.....Histidine (His)

I .....Isoleucine (Ile)

K.....Lysine (lys)

M.....Methionine (Met)

N.....Aspargine (Asn)

P .....Proline (Pro)

R.....Arginine (Arg)

S .....Serine (Ser)

T.....Threonine (Thr)

V.....Valine (Val)

X.....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, Sistrurrus, 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 α-neurotoxin in their venoms **(Meenatchisundarama and Michael, 2010)**. The other two highly toxic components of cobra venoms are cytotoxins and phospholipases A_2, 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).**