Purification and Characterization of Peroxidase from *Euphorbia tirucalli* Latex

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تنقية ودراسة خواص البيروكسيديز من العصارة اللبنية لنبات *الإيوفوربيا تريكالي*

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List of Abbreviations

AAP	Aminoantipyrene
ABTS	2,2'–Azino–di[3-ethyl-benzthiazoline-6-sulphonic acid]
APS	Ammonium persulfate
APx	Ascorbate peroxidase
A. sativum	*Allium sativum*
BSA	Bovine serum albumin
CBB G-250	Coomassie brilliant blue G-250
CBB R-250	Coomassie brilliant blue R-250
CcP	Cytochrome c peroxidase
cDNAs	Complementary deoxyribonucleic acid
C. jambhiri	*Citrus jambhiri*
CM	Carboxymethyl
Da	Dalton
DEAE	Diethylaminoethyl
DOPA	3,4-Dihydroxyphenylalanine
DTNB	5,5'-Dithiobis-(2-nitrobenzoic acid)
DTT	Dithiothreitol
E.C.	A classification of enzymes according to the Enzyme Commission of the International Union of Biochemistry and Molecular Biology
E. characias	*Euphorbia characias*
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay

List of Abbreviations (Cont.)

ELP	*Euphorbia* latex peroxidase
E. nyikae	*Euphorbia nyikae*
E. tirucalli	*Euphorbia tirucalli*
FPLC	Fast protein liquid chromatography
HIV	Human immunodeficiency virus
HRP	Horseradish peroxidase
IAA	Iodoacetic acid
i.d.	Inner diameter
IgG	Immunoglobulin G
KDa	Killodalton
K_m	Michaelis constant
Mb	Megabases
NADPH	Nicotinamide adenine dinucleotide phosphate
OPD	O-Phenylene diamine
PMSF	Phenylmethylsulfonyl fluoride
PNP	Peanut peroxidase
POD	Peroxidase
Rf	Electrophoretic mobilities
S. melongena	*Solanum melongena*
SDS-PAGE	Sodium dodecyl sulfate - polyacrylamide gel electrophoresis
Spp.	Species

List of Abbreviations (Cont.)

TEMED	N, N, Ń, Ń-Tetramethethylenediamine
TMB	3,3',5,5' Tetramethylbenzidine
TP	Turnip peroxidase

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Purification and Characterization of Peroxidase from *Euphorbia tirucalli* Latex

Submitted by: Rasha Azouz Mohamed Azouz

Abstract

A cationic peroxidase from *Euphorbia tirucalli* latex (ELP) has been purified to homogeneity using chromatography on a Sephacryl S-200 and carboxymethyl-Sepharose columns. The Cationic peroxidase ELP is proved to be pure on SDS - PAGE, and its molecular weight was 44 kDa. A study of substrate specificity revealed the affinity of ELP to oxidize some phenolic substrates in the order of ABTS > guaiacol > *o*-phenylenediamine > aminoantipyrene, while ELP had no affinity towards ascorbic acid and *o*-dianisidine. The K_m of ELP for hydrolysis of H_2O_2 was 15 mM. The K_m values of electron donor substrates were also determined. The enzyme had pH and temperature optima at pH 7.0 and a temperature of 40 °C, respectively. ELP was stable at 10 - 60 °C and unstable above 60 °C. The thermal inactivation of ELP was characterized by a rapid decline followed by a relative stability in activity on exposure to heat. However, the thermal inactivation of ELP was almost changed in the presence of Ca^{2+} ions. Most of the different examined compounds and

metal ions had partial inhibitory effects on ELP except for Ca^{2+} which had a vital role in activation of the enzyme and Mg^{2+} which had a slight activation effect.

Keywords: Calcium ions; *Euphorbia tirucalli*; Latex; Peroxidase; Thermal inactivation.

Introduction

Euphorbia tirucalli is a species of the plant kingdom belonging to the Euphorbiaceae family. The Euphorbiaceae is among the larger families of flowering plants with 300 genera and 8000 species. This family is one of the most diverse families that range from herb, shrub and tall tree. The succulent species is characterized by the presence of irritant white milky latex. The great majority of this family is tropical or subtropical, but even in temperate North America there are 27 genera with over 100 native or naturalized species. **(Willis, 1973; Grady and Webster, 1986; Mabberley, 1987; Webster, 1994; Radcliffe-Smith and Esser, 2001).**

Peroxidases (E.C. 1.11.1.X; donor: hydrogen peroxide oxidoreductase) are enzymes that utilize hydrogen peroxide or other peroxides to oxidize a second reducing substrate, which can be a wide variety of organic and inorganic compounds. Peroxidases act as antioxidant enzymes by protecting cells and tissues against the toxic effects of peroxides. They are also involved in a variety of defense mechanisms toward pathogens based on the so called oxidative burst, in which the levels of H_2O_2 and other reactive oxygen species (mainly superoxide) rapidly increase **(Moerschbacher, 1992; Lamb and Dixon,**

1997; Blee *et al.*, 2001). In addition, these enzymes are directly involved in the synthesis of important metabolites in plants **(Passardi *et al.*, 2007)**.

Peroxidases can be divided into three classes, on the basis of amino acid sequence: class I includes bacterial, fungal and plant intracellular enzymes in the mitochondria and chloroplasts; class II consists of secretory fungal peroxidases such as manganese peroxidase and lignin-degrading peroxidases; class III consists of secretory plant peroxidases. Best examples of class III peroxidases are the enzymes extracted from horseradish, peanut, barley and the latex *Euphorbia* **(Medda *et al.*, 2003).**

Typically, class III peroxidases may exist under an extremely high number of isoforms within the same species, potentially implicated in different functions **(Veitch, 2004).** Plant peroxidases are receiving increasing attention due to their extensive potential in clinical, biochemical, biotechnological as well as industrial applications **(Ryu *et al.*, 1993; Kim and Moon, 2005).**

The only source of commercial peroxidase is from horseradish roots **(Kamal and Behere, 2008),** which are cultivated in cool climates but not in Egypt. Economic sources

of the enzyme include a limited number of plants such as turnip **(Hamed *et al.,* 1998)**, soybean **(Sessa and Anderson, 1981)**, peanut **(Hu and Van Huystee, 1989)**, animals and a few species of microorganisms **(Kanayama *et al.,* 2002)**.

Currently, peroxidases are used in organic synthesis for the production of polymers and for the biotransformation of various drugs and chemicals **(Colonna *et al.,* 1999; Mohamed *et al.,* 2008[a])**. These enzymes could also be exploited for the detoxification and remediation of various aromatic pollutants such as phenols, aromatic amines, 2,4,6-trinitrotoluene and dyes which are present in waste water/industrial effluents coming out from several industries such as textile, dyes, printing, paper and pulp **(McEldon and Dordick, 1996; Akhtar *et al.,* 2005[a]).**

Peroxidase can promote a large variety of reactions; therefore it can exhibit a degree of versatility unsurpassed by any other enzyme **(Clemente, 1998).** A class III cationic peroxidase was isolated from the latex of the Mediterranean shrub *Euphorbia characias* and its main biochemical features were characterized **(Medda *et al.,* 2003).**