

Ain-Shams University

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Purification and characterization of *Ficus carica* latex peroxidase with potential applications for decolorization of the dyes A THESIS

Submitted for the award of the Ph.D. degree of science in

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Date of Examination 28/11/2018



سورة البقرة الأية: ٣٢

Abstract

Alshaimaa Mohamed Elsayed, Purification and characterization of *Ficus carica* latex peroxidase with potential applications for decolorization of the dyes

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

In the present study, three peroxidase isoenzymes were isolated from Ficus latex using chromatographic carica CM-Sepharose DEAE-Sepharose separation and on followed by Sephacryl S-200 column gel filtration. The complete purification was carried out for FP1 (0.1M NaCl / CM-Sepharose fraction of Ficus carica latex peroxidase) only due to the low level of activity and protein content of FP2 (0.1M NaCl / DEAE-Sepharose fraction of Ficus carica latex peroxidase) and FP3 (0.2M NaCl / DEAE-Sepharose fraction of Ficus carica latex peroxidase). The purified isoenzyme FP1 was found monomeric with a molecular weight of 30 kDa by electrophoresis on SDS-PAGE. FP1 and FP3 isoenzymes have the same pH and temperature optima at pH 5.5 and 40°C, respectively, while FP2 has optimum pH at 7.0 and optimum temperature at 30 °C. On the other hand, FP1 and FP2 were thermostable up to 40 and 50°C. respectively, whereas FP3 had a low thermostability at 30°C. FP1 isoenzyme was found to be stable between pH 5.0 to 7.5, and FP2 was stable between pH 4.5 and 7.5, while FP3 was found to be stable in acidic range between pH 4.5 and 5.5. The activity of FP1, FP2 and FP3 was decreased gradually by increasing the concentration of tested organic solvents (ethanol. methanol and DMSO). It was found that the activity of FP1, FP2 and FP3 was increased by increasing the concentrations of some metal cations and decreased by increasing the concentrations of other metal cations and some compounds (EDTA and NaN₃). The three peroxidase isoenzymes have a broad specificity towards some phenolic substrates and o-phenylenediamine showed higher affinity towards the three peroxidase isoenzymes, with K_m values of 3.87, 3.64 and 3.33 mM for FP1, FP2 and FP3, respectively. F. carica latex peroxidase isoenzymes and commercial horseradish peroxidase were able to decolorize many tested synthetic dyes and the extent of decolorization achieved with different dye classes were varied according to different chemical structure of each dve.

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ACKNOWLEDGEMENT

First and foremost, my great praise and sincere thanks should be submitted to **ALLAH**, the kindest and the most merciful, for the kind and continuous support to me.

I wish to express my sincere appreciation to **Prof. Dr. Ahmed Mohamed Salem**, Professor of Biochemistry,

Biochemistry Department, Faculty of Science, Ain-Shams

University, for his valuable guidance, continuous encouragement,

supervision and unlimited support during this study.

I would like to express my sense of gratitude to **Prof. Dr. Afaf Saad Eldin Fahmy,** Professor of Biochemistry, Molecular Biology Department, National Research Centre, for suggesting the theme of this study, her valuable guidance, continuous encouragement, and support she provided throughout my research study and thesis preparation.

I'm also indebted and truly thankful for **Dr. Marwa Galal Eldeen Abdo**, Assistant Professor of Biochemistry, Biochemistry Department, Faculty of Science, Ain-Shams University, for her encouragement, kind advices throughout the study and supervision.

I'm also grateful to **Dr. Somia Shaker Abdel-Ghany,** Assistant Professor of Biochemistry, Molecular Biology