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

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﴿قالوا سبحانك لا علم لنا إلا ما علمتنا

إنك أنت العليم الحكيم﴾

صدق الله العظيم

سورة البقرة الآية: 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 carica* latex using chromatographic separation on CM-Sepharose and DEAE-Sepharose columns 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 to be 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, acetone, 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 NaN_3). 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 dye.

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