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Characterisation of an anionic peroxidase from horseradish cv. Balady

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ABSTRACT

An anionic peroxidase POIII, molecular weight 56 kDa, was purified from the roots of horseradish cv. Balady. The enzyme exhibited high activity towards *o*-phenylenediamine and guaiacol, while *o*-dianisidine had moderate peroxidase activity. Pyrogallol and *p*-aminoantipyrine had low affinity toward POIII. POIII was found to have a temperature optimum at 40 °C; the enzyme activity remained stable up to 40 °C and retained 87%, 51% and 29% of its activity at 50, 60 and 70 °C, respectively. The enzyme exhibited more than 50% of activity in the pH range between 4.0 and 8.0 with its pH optimum at 5.5. Several metal cations had partial inhibitory effects toward POIII. Fe^{3+} enhanced the activity of the enzyme by 160% at 5 mM. All the metal chelators caused partial inhibitory effects toward POIII, except for EDTA at 1 mM, which had no effect on the enzyme.

1. Introduction

Peroxidases can be considered as bifunctional enzymes that can oxidise various substrates in the presence of H_2O_2 but also produce reactive oxygen species. Plant peroxidases participate in a broad range of physiological processes including the removal of hydrogen peroxide, the oxidation of toxic compounds, the biosynthesis of cell walls (lignin and suberin), indole-3-acetic acid regulation, ethylene biosynthesis, defence responses towards wounding, and other stresses (Blee et al., 2003; Passardi, Penel, & Dunand, 2004). Peroxidases can also catalyse the formation of diferuloyl and isodityrosine linkages in both primary and secondary cell walls (Almagro et al., 2009; Kawamura, Wakabayasihi, Hoson, Yamamoto, & Kamisaka, 2000; MacAdam & Grabber, 2002). The specific functions of individual peroxidases are often difficult to understand because of their low substrate specificity and the existence of many isoenzymes (Tognolli, Penel, Greppin, & Simon, 2002).

Among all peroxidases, Japanese horseradish peroxidase has received special attention owing to its potential applications. Oxidative polymerisation of phenols and aromatic amines, conducted by horseradish peroxidase in water and water-miscible organic solvents, may lead to new types of aromatic polymers. Peroxidase has a potential for soil detoxification, while horseradish peroxidase, as well as soybean and turnip peroxidases have been applied to the bioremediation of waste waters contaminated with phenols, cresols and chlorinated phenols. Peroxidase has also been used for practical analytical applications in diagnostic kits, such as quantification of uric acid, glucose, cholesterol, lactose, etc. Enzyme-linked immunosorbent assay (ELISA) tests, in which peroxidase enzyme is the most common enzyme used for labelling an antibody, are a simple and reliable way of detecting toxins, pathogens, cancer risk in bladder and prostate, and many other analytes (Hamid & Khalil-ur-Rehman, 2009). Therefore, the aim of this study is to purify peroxidase from the roots of horseradish cv. Balady and study the physical and chemical characterisation of the enzyme.

2. Materials and methods

2.1. Plant material

Roots of horseradish cv. "Balady" were obtained from a local market in Jeddah, Saudi Arabia.

2.2. Chemicals

Hydrogen peroxide, guaiacol, *o*-phenylenediamine, *o*-dianisidine, pyrogallol, *p*-aminoantipyrine, sodium dodecyl sulphate (SDS), molecular weight markers for gel filtration and all resins and reagents for electrophoresis were obtained from Sigma Chemical Co. (St. Louis, MO). Sephacryl S-200, diethylaminoethyl (DEAE)-Sepharose and molecular weight markers for SDS–polyacrylamide gel electrophoresis were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Other chemicals were of analytical grade.

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