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Purification and Characterization of a Novel Laccase from the Edible Mushroom *Hericium coralloides*

Ya-Jie Zou[1], He-Xiang Wang[2], Tzi-Bun Ng[3], Chen-Yang Huang[1], and Jin-Xia Zhang[1]*

[1]*Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China*
[2]*State Key Laboratory for Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing 100193, P. R. China*
[3]*Department of Biochemistry, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, P. R. China*

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A novel laccase from the edible mushroom *Hericium coralloides* was purified by ion exchange chromatography on diethylaminoethyl (DEAE) cellulose, carboxymethyl (CM) cellulose, and Q-Sepharose columns followed by fast protein liquid chromatography gel filtration on a Superdex 75 column. Analysis by gel filtration and SDS-PAGE indicated that the protein is a monomer in solution with a molecular mass of 65 kDa. Its N-terminal amino acid sequence was AVGDDTPQLY, which exhibits partial sequence homology to previously isolated laccases. Optimum activity was observed at pH 2.2 and at 40°C. The enzyme showed activity toward a variety of substrates, the most sensitive of which was 2,2′-azinobis [3-ethylbenzothiazolone-6-sulfonic acid] diammonium salt (ABTS). The degradation activity toward substrates was ABTS > N,N-dimethyl-1,4-phenylenediamine > catechol > 2-methylcatechol > pyrogallol. The laccase did not exert any antiproliferative activity against Hep G2 or MCF 7 tumor cell lines at a concentration of 60 μM, unlike some previously reported mushroom proteins, but showed significant activity toward human immunodeficiency virus-1 (HIV-1) reverse transcriptase with an IC_{50} of 0.06 μM.

Keywords: laccase, mushroom, *Hericium coralloides*, purification

Introduction

Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) is a multicopper blue phenoloxidase that catalyzes the oxidation of polyphenols, methoxy-substituted phenols, aromatic diamines, and a range of other compounds by a one-electron transfer mechanism using molecular oxygen as an oxidant (Sakurai, 1992; Xu, 1996; Xu *et al.*, 1996; Piontek *et al.*, 2002). Laccases are widely distributed in higher plants and fungi (Messerschmidt and Huber, 1990) and have also been found in insects and bacteria (Diamantidis *et al.*, 2000; Kramer *et al.*, 2001). Laccases have been the subject of intense research in recent decades because of their broad substrate specificity. Potential uses for laccases include textile-dye bleaching (Kierulff, 1997), pulp bleaching (Palonen and Viikari, 2004), food improvement (Minussi *et al.*, 2002), the bioremediation of soils and water (Li *et al.*, 1999; Wesenberg *et al.*, 2003), polymer synthesis (Marzorati *et al.*, 2005), the development of biosensors and biofuel cells (Trudeau *et al.*, 1997; Tayhas *et al.*, 1999), synthetic dye decolorization (Nagai *et al.*, 2002), bioremediation (Jaouani *et al.*, 2005) and chemical synthesis (Karamyshev *et al.*, 2003). Two of the most intensively studied areas in the potential industrial application of laccases are delignification and pulp bleaching and the bioremediation of environmental pollutants.

Because laccases are versatile biocatalysts in biotechnological processes, a major goal in the field has been the purification and characterization of a novel laccase. To date, more than 100 laccases have been purified from fungi and characterized to various degrees. Many edible mushrooms such as *Cantharellus cibarius* (Ng and Wang, 2004), *Pleurotus eryngii* (Wang and Ng, 2006a), *Hericium erinaceum* (Wang and Ng, 2004a), and *Tricholoma giganteum* (Wang and Ng, 2004b) can also produce laccases. However, the laccase of *Hericium coralloides*, a well-known wild edible mushroom, has not yet been identified. The objectives of this study were (1) to purify laccase from *H. coralloides* and (2) to characterize the enzymatic properties of this laccase.

Materials and Methods

Isolation of laccase

Dried fruiting bodies (100 g) of *H. coralloides* were extracted with distilled water (3 ml/g) using a Waring blender. The homogenate was centrifuged (13,000×g, 20 min), and Tris-HCl (1 M, pH 7.2) was added to the supernatant to a final concentration of 10 mM Tris. The supernatant was then loaded onto a DEAE-cellulose column (2.5×20 cm) that had been pre-equilibrated with 10 mM Tris-HCl buffer (pH 7.2). After elution of the flow through (D1), three adsorbed peaks, D2, D3, and D4, were eluted with step gradients of 50 mM NaCl, 150 mM NaCl, and 500 mM NaCl, respectively. Fraction D4 was subsequently subjected to ion exchange chromatography on a CM-cellulose column (1.0×15 cm, Sigma) that had been pre-equilibrated with 10 mM NH_4OAc buffer (pH 5.2). After elution of the flow through (CM1), the adsorbed materials were eluted with step gradients of 50 mM NaCl, 150 mM NaCl, 500 mM, and 1 M NaCl. Three adsorbed

*For correspondence. E-mail: zhangjx1210@yahoo.com.cn; Tel.: +86-10-8210-6207; Fax: +86-10-8210-6207