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Research progress on the mechanism of fungal degradation of dyes

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Abstract

In the early 1980s, Glenn and Gold first discovered and demonstrated that the white rot fungus Phanerochaete Chrysosporium (P.C.) has excellent decolorization and degradation effects on some synthetic dyes. Later, domestic and foreign scholars conducted in-depth research and concluded that the degradation of refractory organic pollutants such as dyes by white rot fungi consists of two processes: intracellular and extracellular. In the cell, it mainly affects the synthesis of enzymes. The enzymes mainly include H2O2 production enzymes composed of glyoxal oxidase, glucose enzyme, etc., and lignin peroxidase, manganese peroxidase, laccase, etc. of lignin-degrading enzymes. But outside the cell, the lignin-degrading enzyme system mainly participates in the reaction as a catalyst, thereby realizing the efficient and broad-spectrum degradation of various refractory organic pollutants. Among the lignin degrading enzymes, laccase (Lac), lignin peroxidase (Lip), and manganese peroxidase (Mnp) are the three most common enzymes produced by fungi in dye degradation. The characteristic of degradation allows the fungus to avoid the accumulation of toxic substances to be toxic to the cells, which makes the application of the fungus more extensive.

Keywords: **White rot fungus, laccase , manganese peroxidase, lignin peroxidase.**

1. Manganese peroxidase

Manganese peroxidase (MnP, EC 1.11.1.13) is produced by a specific class of Basidiomycetes, which are mainly produced by two Basidiomycete lines, White Rot Fungi and Soil Subtilis [1]. MnP consists of a heme group and a Mn^{2+} to form its active center, and is essentially a heme-containing glycosylation peroxidase with a molecular weight of 40-47 KDa and an isoelectric point of 4.2-4.9[2]. The structure is similar to that of two enzymes, lignin peroxidase and polyphenol oxidase, and is special compared to other peroxidases in that its substrate is an organic acid. At present, the crystal structure of MnP has been basically studied. There are two Ca atoms on the proximal and distal sides of heme, which are respectively combined with some amino acids and water molecules. It plays an important role in the thermal stability of the enzyme. Some proteins (such as bovine serum albumin) and Mn^{2+} can improve its stability and also promote the improvement of its enzymatic activity [4]. The catalytic cycle of manganese peroxidase (shown in Figure 1.1) starts with two electrons donated by H_2O_2 to form an intermediate-complex I, which can oxidize Mn^{2+} to Mn^{3+} or oxidize phenolic compounds to their corresponding free radicals, At the same time, the enzyme is reduced to a one-electron oxidation intermediate, that is, complex II. Complex II absolutely requires Mn^{2+} as a reducing agent. While oxidizing Mn^{2+} to Mn^{3+}, it is itself reduced to complex I state. Yang Xiuqing et al. [5] studied the conversion mechanism of manganese peroxidase produced by white rot fungus SQ01 to biphenyl intermediate metabolites and found that the conversion of

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HOPDA by manganese peroxidase is a cleavage between Cα-Cβ and the release of benzyl groups to form aromatic acids. Yang et al.[6] proposed that the manganese peroxidase secreted by Irpex lacteus F17 can decolorize malachite green through N-terminal demethylation and oxidative cleavage of the C=C double bond (as shown in Figure 1.2).

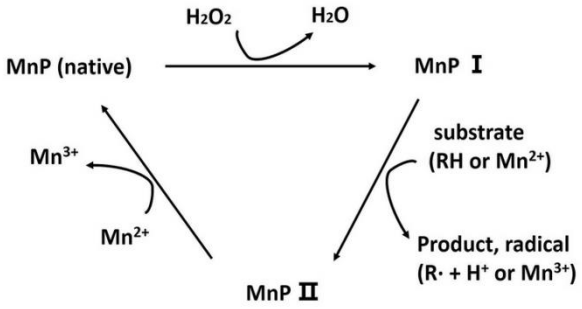

Fig. 1.1 The catalytic mechanism of manganese peroxidase

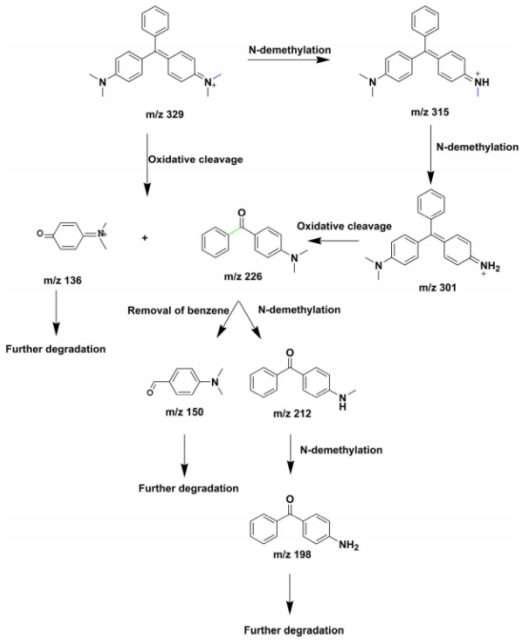

Fig. 1.2 The degradation pathway of Malachite green by manganese peroxidase

2. Lignin peroxidase

Lignin peroxidase (LiP, EC1.11.1.14) is composed of multiple complex forms of glycosylated heme proteases, and its partial amino acid sequence is the same as that of MnP [7], which is produced during the secondary metabolism of cells [8], the molecular mass is generally 37-47kDa, the isoelectric point is about 3.5, the optimum enzyme activity temperature is 35-55°C, and the optimum pH is 2-5.

Factors affecting the enzymatic activity of lignin peroxidase include temperature, pH, metal ions, inhibitors, activators, etc.[9]. Veratrol is a common substrate for lignin peroxidase, and its presence can Keep the enzyme stable, and then promote the increase of enzyme activity; EDTA, azide, cyanide, 3-amino-1, 2, 4-tripyrrole are common inhibitors of lignin peroxidase, they The existence of LPS will seriously affect the activity of the enzyme, and then affect the enzymatic reaction in the reaction system; the participation of lignin peroxidase in the enzymatic catalytic reaction requires the addition of H2O2 to start, but when there are no other reducing substrates in the reaction system , H2O2 will cause partial irreversible inactivation of the enzyme[10]. The catalytic degradation mechanism of LiP is: LiP can oxidize electron-rich aromatic compounds. When attacking the substrate through an electron transfer body, it can take an electron from the benzene ring of the aromatic compound, oxidize it into

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free radicals, and then chain The reaction generates many different free radicals, leading to the cleavage of major bonds in the substrate molecule, followed by a series of cleavage reactions.

3. Laccase

Laccase (LiP, EC1.10.3.2) was originally isolated from the sap of Japanese sumac. It is a copper-containing polyphenol oxidase and a glycoprotein. The molecule contains 4 copper ions. These 4 copper The ion plays a decisive role in the catalytic oxidation reaction. Its molecular weight generally ranges from 59 KDa to 390 KDa, the isoelectric point is between 3.0 and 6.0, the optimum enzyme activity temperature is 25 to 50 °C, and the pH is between 4.0 and 6.0. Laccase comes from a wide range of sources. For example, plants and fungi contain a certain amount of laccase. The molecular weight of laccase from different sources will be very different. Even the same source of laccase will have different molecular weights. There are many factors affecting laccase, such as temperature, pH, metal ions, ionic strength, inhibitors, etc. Generally speaking, different sources of laccase have different optimal pH ranges; different concentrations of ionic strength will also produce different enzyme activities; because halide, cysteine, EDTA and SDS are copper chelators, So they are also common laccase inhibitors.

The functions of laccase are very powerful, such as the synthesis of lignin, plant defense and other biological functions. In addition to these functions, in order to cope with the serious environmental pollution problems faced by human society today, laccase has been widely used in industries such as decolorization of printing, dyeing, and papermaking wastewater. The catalytic mechanism of laccase on different types of substrates is manifested in: the formation of substrate free radicals, the laccase catalyzes the oxidation of substrates, the synergistic effect of the four copper ions in the laccase molecule to transfer electrons and the change of valence to realize the oxidation of O_2. reduction. It is precisely because of this catalytic mechanism that laccase acts on a wide range of substrates and can catalyze the oxidation of many compounds [11].

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