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Rapid communication

Efficacy of amphiphile-modified laccase in enzymatic oxidation and removal of phenolics in aqueous solution

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Abstract

This study is mainly related to the characterization of a chemically modified laccase from the *Trametes* sp. for use in the oxidation and the removal of phenolics in aqueous systems. An amphiphilic copolymer, polyalkyleneoxide-*co*-maleic anhydride was used as a modifier. The amphiphile-modified laccase displayed higher catalytic activity along with improved thermal stability in the oxidation reaction of 2,6-dimethoxyphenol. The chemical modification resulted in a 20- to 10-fold increase in thermal stability at 25 and 40 °C, respectively. The modified laccase was applied to remove a range of phenolic compounds including mono-, di-, and tri-chlorophenols, cresol, and xylenol, all of which usually behave as hazardous pollutants of natural environments. The modified laccase exhibited a remarkably improved efficiency over that of the native laccase in terms of removing various phenolic substances. These results revealed that the enzymatic oxidation by the amphiphile-modified laccase is suitable for the efficient removal of phenolics in aqueous systems.

Keywords: Amphiphile; Chemical modification; Laccase; Oxidative removal; Phenolic pollutants; Polyalkyleneoxide-*co*-maleic anhydride; Thermal stability

1. Introduction

Contaminated water containing phenolic compounds is produced by various industrial processes such as oil refining, dying, photo processing, metal plating, coal conversion, and circuit-board manufacturing process [1–3]. As most of the phenolic compounds are toxic and cause coloration of the receiving waters, it is therefore essential to decontaminate effluent containing such compounds. The effluents contaminated with phenolic compounds are conventionally treated by various methods including solvent extraction, distillation, adsorption, and chemical oxidation. Although these methods are useful, they have some drawbacks such as high cost, incomplete purification, formation of other hazardous byproducts, or restricted applicability as regards which pollutants they can effectively remove.

Microbial or enzyme-based treatments for the removal of phenolic compounds have offered some distinct advantages over physical and chemical removal methods [4–8]. Thus, using enzymes as decontaminating agents has received great attention because of their potential to remove pollutants from the environment without creating the harsh side effects associated with other methods. The significant advantages of this enzymatic method include the mild condition of enzymatic treatment, the requirement of only trace amounts of enzymes, the ability to decontaminate low concentrations of contaminants, and the ability to handle large volumes of effluent as well.

Peroxidases, laccases, and tyrosinases catalyze the oxidation of phenolics using either hydrogen peroxide or molecular oxygen, generating phenoxy radicals that react with themselves or other phenolics to form dimers. These re-

Abbreviations: AAP, 4-aminoantipyrine; CP, chlorophenol; DCP, dichlorophenol; DM, degree of modification; DMP, 2,4-dimethoxyphenol; HPLC, high performance liquid chromatography; ME, modified enzyme; NE, native enzyme; PAOMA, polyalkyleneoxide-*co*-maleic anhydride; RA, relative oxidative activity; TCP, trichlorophenol; TNBS, 2,4,6-trinitrobenzenesulfonate; WR, weight ratio of modifier to enzyme

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actions eventually lead to the production of higher oligomers and polymers of low solubility, which then precipitate and can be readily removed by sedimentation or filtration. Laccases, one of the more promising groups of enzymes, have been used to decontaminate phenol-polluted systems. Laccases (EC 1.10.3.2), which are multi-copper oxidases, utilize molecular oxygen for the oxidation of phenolic compounds; in contrast, peroxidases require hydrogen peroxide, which is harmful to the environment. Therefore, laccases are environmental-friendly and potentially attractive catalysts. Since they have a wide range of substrate specificity and can utilize various types of aromatic compounds as substrates, they are very useful in the process of detoxification and decolorization of various phenolic pollutants [9–12].

However, the use of these enzymes for practical applications, mainly as agents for environmental cleanup purposes, is still prevented by several limitations. In general, low stability and the potential for drastic reductions in enzymatic activity have always been considered as hindrances to the practical application of enzymatic systems.

Numerous studies have been conducted in order to overcome these limitations. Several methods based on protein engineering [13], immobilization in solid supports [14], isolation from thermophilic organisms [15], the use of additives [16], and chemical modification with polymeric [17–19] and low-molecular weight compounds [20,21] have been reported to be successful for preparing stable enzymes. In particular, an amphiphilic polymer, polyalkyleneoxide-*co*-maleic anhydride (PAOMA), have been widely used as enzyme modifiers in order to overcome the limitations associated with unmodified enzymes [22,23].

The aim of the present study was to prepare a chemically modified laccase using an amphiphilic polymer, PAOMA, and to characterize the modified enzyme in terms of its oxidative activity, thermal stability, and the removal efficiency of various phenolics.

2. Materials and methods

2.1. Materials

Laccase obtained from the *Trametes* sp. was a donated sample from Daiwa Kasei K.K., Osaka, Japan. The molecular weight and isoelectric point of the laccase were 6.2×10^4 and 3.0, respectively. Polyalkyleneoxide-*co*-maleic anhidride, SUNBRIGHT AGM-0530®, was kindly supplied from Nippon Oil & Fat Co., Japan, and this copolymer was used as the modifier. The chemical structure of the copolymer are shown in Fig. 1. All phenolic substrates used in this study, i.e., 2,6-dimethoxyphenol (DMP), *o*-, *m*-, *p*- chlorophenols (CPs), 2,4- and 2,6-dichlorophenols (DCPs), 2,4,6-trichlorophenol (TCP), cresol, and xylenol, were obtained from Wako Pure Chemical Industries, Ltd., Japan. All chemicals were used directly without further purification.

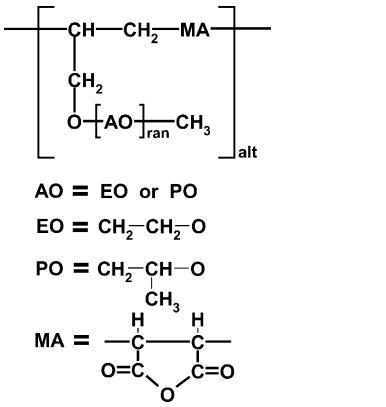

Fig. 1. Structure of the polyalkyleneoxide-*co*-maleic anhydride (PAOMA). The random copolymerization degree (ran) and the alternative copolymerization degree (alt) are 32 and 30, respectively. The EO/AO is 0.6.

2.2. Chemical modification of laccase

The chemical modification of laccase with the PAOMA copolymer was based on the condensation reaction between a free amino group of the enzyme surface and a maleic-anhydride group of the copolymer (Fig. 2).

The chemical modification of laccase with the PAOMA copolymer was performed according to a previously described method [22,23]. The native laccase was incubated with different amounts of PAOMA copolymer in a solution of 0.15 M borate buffer, pH 8.5, at 2 °C with gentle stirring. The laccase concentration in the mixture was 2 wt.%, and that of the modifier was 2–18 wt.%. The resulting solution was diluted at the required concentration with the appropriate buffer and then was used for the following experiments (the modified laccase obtained by this method is referred to hereafter as "ME" and the native laccase is referred to as "NE").

2.3. Degree of modification

The degree of modification (DM) was defined as a percentage of the number of modified amino groups in the modified protein relative to the number of free amino groups in the native protein. The number of free amino groups in the protein molecule was reported to be proportional to the spectrophotometric absorbance at 420 nm obtained from 2,4,6-trinitrobenzenesulfonate (TNBS) method [24]. The measurement of absorbance of the TNBS reaction mixture was carried out using a UV-3210 spectrophotometer (HITACHI,

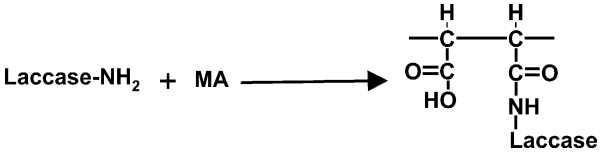

Fig. 2. Chemical modification of laccase using PAOMA as a modifier. MA represents the maleic anhydride group in PAOMA molecule. Reaction was carried out at pH 8.5, 2 °C.