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Journal of Molecular Catalysis B: Enzymatic 41 (2006) 81–86
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Enhanced dye decolorization efficiency by citraconic anhydride-modified horseradish peroxidase

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Received 5 February 2006; received in revised form 11 April 2006; accepted 19 April 2006
Available online 5 June 2006

Abstract

Bromophenol blue and methyl orange removal capabilities of citraconic anhydride-modified horseradish peroxidase were compared with those of native horseradish peroxidase. Citraconic anhydride-modified horseradish peroxidase showed higher decolorization efficiencies for both dyes than native horseradish peroxidase. Upon the chemical modification, the decolorization efficiencies were increased by 1.8% and 12.4% for bromophenol blue and methyl orange, respectively. The quantitative relationships between decolorization efficiencies of dyes and reaction conditions were also investigated. Experimental data revealed that aqueous phase pH, reaction time, temperature, enzyme concentration and ratio of dye and H_2O_2 play a significant role on the dye degradation. Lower dose of citraconic anhydride-modified horseradish peroxidase was required than that of native enzyme for the decolorizations of both dyes to obtain the same decolorization efficiencies. Citraconic anhydride-modified HRP exhibited a good decolorization of dye over a wide range of dye concentration from 8 to 24 or 32 $\mu mol\,l^{-1}$ at 300 $\mu mol\,l^{-1}$ H_2O_2, which would match industrial expectations. Kinetic constants for two different dyes were also determined. Citraconic anhydride-modified horseradish peroxidase shows greater affinity and catalytic efficiency than native horseradish peroxidase for both dyes.

Keywords: Horseradish peroxidase; Dye decolorization; Citraconic anhydride modification; Bromophenol blue; Methyl orange

1. Introduction

Approximately 10,000 different dyes and pigments are produced annually worldwide and used extensively in the dye and printing industries [1]. It is estimated that about 10–14% of the total dye used in the dyeing process may be found in wastewater [2]. These dyes are considered to be recalcitrant, and toxic. They resist microbial biodegradation and are, therefore, not easily degraded in wastewater treatment plant [3]. Thus, treatment of dye is yet one of the challenging tasks in environmental field. Currently available methods such as chemical oxidation, reverse osmosis, adsorption, etc., suffer from disadvantages such as high cost, regeneration problem and secondary pollutant/sludge generation [4]. Recently, researchers have been focusing their attention to enzymatic treatment. Many peroxidases such as lignin peroxidase, manganese peroxidase, soybean peroxidase, horseradish peroxidase (HRP) and laccase, etc., were applied to decolorize and degrade dye in industrial effluents [1,4,4–7]. However, due to inactivation, large amounts of enzyme are required to achieve a high degree of decolorization, thus limiting its use in industrial situation. Immobilization of enzyme is one of the methods to overcome these limitations. Mohan et al. reported that acrylamide gel immobilized HRP showed effective performance compared to free HRP and alginate entrapped HRP [7]. Cheng et al. also reported that aluminum-pillared interlayered clay (Al-PILC) immobilized HRP could be applied over a broader range of pH from 4.5 to 9.3 for phenol removal and had better storage stability than free enzyme [8]. Chemical modification of HRP surface has been performed to improve its stability and catalytic efficiency. Our previous papers reported that modification of HRP by phthalic anhydride improved HRP's stability and catalytic activity both in aqueous buffer and some organic solvents [9–11]. O'Brien and Ó'Fágáin demonstrated that phthalic anhydride- and ethylene glycol-bis-(succine acid *N*-hydroxysuccinimide ester)-modified HRP showed slightly greater bleaching ability at 65 °C than native HRP for some of dyes [12]. However, to the best of our knowledge, no report has appeared on systematic evaluation of efficiency of modified

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1381-1177/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2006.04.011

HRP in degradation of dyes. We also reported that HRP-modified by citraconic anhydride (CA) had a greater thermostability both in aqueous buffer and organic solvents than native HRP [13]. Thus, in order to investigate the decolorization efficiencies of dye by CA-modified HRP, effects of parameters such as aqueous phase pH, temperature, H_2O_2, HRP and dye concentration on the dye decolorization efficiencies, were investigated, and then compared with those of native enzyme. The kinetic constants for selected dyes were also determined.

2. Materials and methods

2.1. Chemicals

Horseradish peroxidase was purchased from Shanghai Lizhu Dong Feng Biotechnology Co. Ltd. and had a specific activity of 250 purpurogallin units/mg and $RZ = A_{402}/A_{280} = 3.0$. Citraconic anhydride was purchased from Alfa Aesar. Methyl orange and bromophenol blue (analytical grade, the structure is shown in Scheme 1) were obtained from Shanghai Reagent Company. All other reagents were of analytic grade.

2.2. Chemical modification

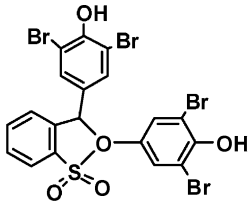
Chemical modification was based on our previous method [10,14]. Twenty-five microlitres 50% (v/v) citraconic anhydride in 0.1 mol l^{-1} phosphate buffer (pH 7.4) and 2 ml 1 mg ml^{-1} HRP in 0.1 mol l^{-1} phosphate buffer (pH 7.4) were mixed. The reaction proceeded at 4 °C for 1 h and was then dialyzed against 0.1 mol l^{-1} phosphate buffer (pH 7.4) at 4 °C to removal excess reagent.

The degree of modification was about 50% estimated by the method of Snyder and Sobocinski [14].

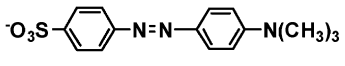
2.3. Peroxidase activity assay

The enzyme activity was assayed by colorimetric method [11]. Reaction mixture containing 10 mmol l^{-1} phenol, 0.2 mmol l^{-1} hydrogen peroxide and 2.4 mmol l^{-1} 4-aminoantipyrin (4-AAP) in a total volume of 3.0 ml was incubated at 30 °C. All reagents were dissolved in 0.05 mol l^{-1} phosphate buffer (pH 7.0). The reaction was then started by adding 0.1 ml of diluted enzyme solution, and the initial increase in absorbance was monitored at 510 nm during 1 min. Under such conditions, the rate of formation of colored product which absorbs light at a peak wavelength of 510 nm was calculated using a molar extinction coefficient of 7100 l mol^{-1} cm^{-1}. One unit of peroxidase activity was defined as the amount of the enzyme consuming 1 μmol of hydrogen peroxide per minute under the assay conditions.

HRP concentration was estimated from its Soret absorbance (molar extinction coefficient at 402 nm = 102 l mol^{-1} cm^{-1}) [11].



Bromophenol blue



Methyl orange

Scheme 1. The structure of bromophenol blue and methyl orange.

2.4. Dye assay

Quantitative estimation of the dye in the aqueous phase was carried out by colorimetry. A solution of 0.24 mol l^{-1} of the dye was scanned over a wavelength range of 200–700 nm by using Shimadzu UV2450. λ_{max} and absorbance at λ_{max} were determined. λ_{max} for bromophenol blue and methyl orange is 592 and 462 nm, respectively. And then the molar extinction coefficient was calculated (31.09 and 26.81 l $mmol^{-1}$ cm^{-1} for bromophenol blue and methyl orange, respectively).

2.5. Dye degradation

The dye was first dissolved in 50 mmol l^{-1} citrate buffer (pH 5.0). The assay medium was 50 mmol l^{-1} citrate buffer. Typically, 0.1 ml of diluted enzyme solution and 30 μl of 30 mmol l^{-1} H_2O_2 were used in 3 ml of reaction mixture for the assay. The decrease in absorbance of the dye solution at the respective λ_{max} was monitored. Series of experiments were performed by varying the process parameters such as aqueous phase pH, reaction temperature, reaction time, enzyme concentration and dye concentration. The conditions are detailed in the legends to figures.

2.6. Kinetic studies

The kinetic experiments were performed using constant enzyme and H_2O_2 concentration as the dye degradation and varying the concentration of substrate under the optimum conditions for dye degradation.

The values given in the paper represent the mean of three independent sets of experiments with S.D. of less than 5%.

3. Results and discussion

Enzyme has an optimum pH range at which its activity is maximum. The efficiency of dye removal by CA-modified HRP (CA-HRP) at various pH values of the reaction mixture is depicted in Fig. 1. The similar result was obtained in the dye degradation using native HRP (data not shown). From Fig. 1, we can find that the decolorization efficiency of bromophenol blue was highest at pH 4.0–5.0. The bell-shaped curve with a defined pH optimum was also obtained in HRP and phthalic anhydride-modified HRP catalyzed removal of phenol [8,9]. The reason is that bromophenol blue and phenol are also phenolic compound. However, the optimum pH for bromophenol blue was different from that for phenol.

However, the decolorization efficiency of methyl orange decreased with the increase of pH value of the reaction mix-