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Effect of Cationic Surfactant on the Inhibition of Ligninase by Hydrogen Peroxide

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Abstract: The inhibition of ligninase by hydrogen peroxide in the presence of cationic surfactant CTAB was studied by kinetic spectrophotometric technique. Results showed that addition of CTAB enhanced the inhibition by H_2O_2, but it did not alter the inhibition pattern and the inhibition constant changed little with the concentration of CTAB. Modification of the enzymic protein by the surfactant monomer may be responsible for the above mentioned results.

Keywords: Ligninase, hydrogen peroxide, cationic surfactant, inhibitory kinetics, veratryl alcohol.

Studies of biodegradation of lignin model compounds by enzyme are of great significance to make full use of natural resources, protect ecological environment, and achieve sustainable development. Lignin-degrading white-rot basidiomycetes have been known to produce extracellular heme peroxidases such as ligninase (LiP) and manganese peroxidase (MnP) under ligninolytic conditions[1]. These enzymes have been believed to play a key role in lignin biodegradation[2,3]. Owing to the poor solubility of lignin model compounds in aqueous media, effective degradation of these compounds by hydrophilic LiP and MnP is retarded[4]. Surfactant can help hydrophobic compounds to dissolve in aqueous solutions[5]. Therefore, studies on the catalytic properties of LiP and MnP and their related degradation mechanism in the presence of surfactant are of great importance[6]. These studies are informative for people to increase understanding of the function of surfactant in the biodegradation of lignin model compounds, and to find out a proper medium in which not only the water solubility of these hydrophobic substrates can increase but also the high activity of LiP and MnP can retain.

LiP and MnP are H_2O_2-dependent heme peroxidases; however, excess H_2O_2 (>2mmol·L^{-1}) has an obvious inhibitory effect on the enzymes[7,8]. In order to investigate the effect of surfactants on their catalytic performance and reaction mechanism, the inhibition of LiP by H_2O_2 in the presence of cationic surfactant cetyltrimethylammonium bromide (CTAB) was studied by kinetic spectrophotometric technique. The indicator

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reaction was the ligninase-catalyzed oxidation of veratryl alcohol (VA). To our knowledge, such report has not been documented in literature.

Experimental

The ligninase (LiP) from wood-decaying fungus *Phanerochaete chrysosporium* burds was isolated and purified according to the procedure described in our previous papers[9].

VA is the optimum substrate of LiP, which has no absorbance at 310 nm, but its oxidized product veratraldehyde absorbs strongly at 310 nm. The initial velocity of the indicator reaction was measured as follows: At 30°C, 150 μL VA stock solution(150 mmol·L^{-1}), 2.5 mL citrate buffer (0.1mol·L^{-1}, pH=3.5) or CTAB solution prepared with the buffer, 120 μL H_2O_2 solution were mixed in a cuvette , then 40 μL LiP solution was added to initiate the reaction. After quick mixing, a plot of absorbance (A) at 310 nm *versus* the reaction time (t) was recorded promptly (on Shimadzu UV-240 spectrophotometer), using the corresponding blank without LiP as reference. The initial velocity can be calculated from the linear portion of the A~t curve.

Results and Discussion

Kinetic model of reversible inhibition

Based on the kinetic model of competitive inhibition, the following equation is derived:

$$1/v_0 = y/[S] + 1/v_{max} \quad \text{where} \quad y = (1 + [I]/K_i)\, K_m / v_{max}$$

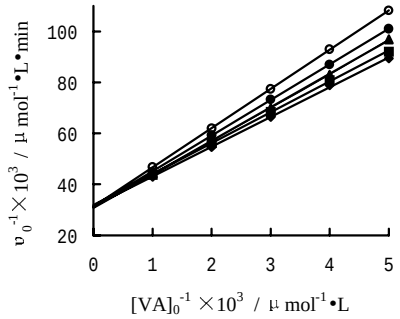
S denotes substrate, *I* denotes inhibitor and K_i, K_m and v_{max} are inhibition constant, apparent Michaelis constant and maximum velocity, respectively. For a given inhibitor concentration, the double reciprocal plot of $1/v_0$ *versus* 1/[S] is linear and, moreover, the lines obtained at different inhibitor concentrations intersect at one point of the $1/v_0$ axis. Ki can be obtained accordingly from the secondary replot of *y* versus [*I*].

Inhibition pattern in the presence of CTAB

Figure 1 is the double reciprocal plot of the initial velocity of the indicator reaction *versus* the concentration of VA at several inhibitory concentrations of H_2O_2 in an aqueous solution of CTAB. At each given concentration of H_2O_2 , the data of υ_0^{-1} and $[VA]_0^{-1}$ were best fitted to a linear line; moreover, these linear lines drawn at different fixed concentrations of H_2O_2 intersected almost at one point at the ordinate, suggesting a reversible competitive pattern; *i.e.*, presence of CTAB did not alter the inhibition pattern.

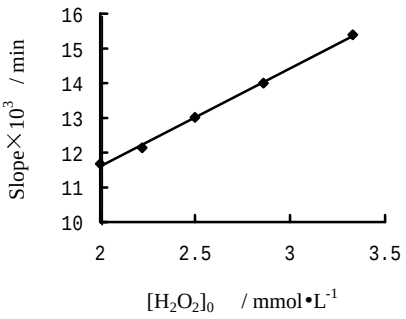
Figure 2 is the secondary replot of the slopes of the double reciprocal lines in **Figure 1** *versus* the corresponding inhibitory concentration of H_2O_2. A linear line was obtained. From its intercept and slope, K_i was calculated to be 2.14 mmol·L^{-1}.

Figure 1 Double reciprocal plot of the initial rate of the indicator reaction versus the concentration of VA at different concentrations of H_2O_2 in an aqueous solution containing CTAB



[CTAB]=1.92×10^{-4} mol•L^{-1}; $[H_2O_2]^{-1}\times10^3$=0.30(o), 0.35(•), 0.40(ω), 0.45(υ), 0.50(σ) μmol^{-1}•L

Figure 2 Secondary replot of the slopes of the double reciprocal lines in **Fig.1** versus the corresponding inhibitory concentration of H_2O_2



Effect of concentration of CTAB on Ki

According to the method described above, the inhibition constants at several selected concentrations of CTAB (lower than, near to and larger than its critical micelle concentration (CMC=9.2×10^{-4} mol·L^{-1})[5]) were obtained, these results, together with Ki in the absence of CTAB, are listed in **Table 1**.

Table 1 Inhibition constants at different CTAB concentrations

[CTAB] mmol•L^{-1}	*Ki* mmol•L^{-1}
0	2.80
0.192	2.14
0.962	2.21
5.78	2.16

As shown in **Table 2**, addition of CTAB made Ki decrease obviously, but K_i only had little change with the increase of the concentration of CTAB. This result indicated that CTAB affected K_i and, moreover, the monomer of CTAB should be responsible for the effect. Based on the fact that CTAB monomer and its micelle have little effect on the reactivity of VA (VA has moderate water-solubility) and on the spectral characteristic of veratraldehyde (measurement of v_0 is based on formation of veratraldehyde), we speculated that modification of the enzymic protein by the monomer of CTAB is responsible for the above mentioned result. Verification of this deduction using other spectroscopic techniques is under way.

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