Note

The Role of Laccase in the Enzymic Oxidation of α,β -Unsaturated Aromatic Alcohols[†]

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In our previous papers,¹⁻³⁾ it was reported that *Fusarium* species excreted into culture filtrates an enzyme which oxidizes α,β -unsaturated alcohols in the side chains of lignin-related aromatic compounds and milled wood lignins to their corresponding aldehydes. During the course of our study on the properties of the enzyme, we have found that the alcohol oxidase activity is greatly accelerated by guaiacol in the presence of laccase. This paper describes this phenomenon.

In this experiment, partially purified alcohol oxidase obtained by the method reported previously²⁾ was used. Laccase was obtained as a homogeneous protein from a cell-free extract of *Fusarium* sp. A13 according to the method reported previously.⁴⁾ To avoid the oxidation of substrate by the laccase, 3,4-dimethoxycinnamylalcohol was used as a substrate for the alcohol oxidase. The alcohol oxidase activity was determined as follows: A reaction

TABLE I. EFFECT OF PHENOLS AND LACCASE ON ALCOHOL OXIDASE ACTIVITY

The reaction mixture, containing 50 μ g of alcohol oxidase, 0.5 μ mol of 3,4-dimethoxycinnamylalcohol, and if added, one μ mol of each phenol and 20 μ g of laccase in one ml of 0.1 M phosphate buffer, pH 6.0, was incubated for 2 hr at 30°C with shaking.

Addition	Relative activity
None	100
Guaiacol	105
Catechol	91
Pyrogallol	94
Laccase	103
Laccase + guaiacol	200
Laccase + catechol	94
Laccase + pyrogallol	118

FIG. 1. Effects of Laccase and Guaiacol on the Alcohol Oxidase Activity.

The reaction mixture, containing 25 μ g of the alcohol oxidase and 0.25 μ mol of 3,4-dimethoxycinnamylalcohol, and if added, 20 μ g of laccase, 0.5 μ mol of guaiacol in 0.5 ml of 0.1 M phosphate buffer, pH 6.0, was incubated for the indicated time at 30°C with shaking. —●—, substrate + (laccase and guaiacol) without addition of the alcohol oxidase; —●—, substrate + the alcohol oxidase; ---●---, substrate + the alcohol oxidase + laccase or guaiacol; —○—, substrate + the alcohol oxidase + (laccase and guaiacol).

FIG. 2. Effect of Guaiacol Concentration on the Alcohol Oxidase Activity.

The reaction mixture, containing 100 μ g of the alcohol oxidase, 0.25 μ mol of 3,4-dimethoxycinnamylalcohol, 20 μ g of laccase and the indicated amount of guaiacol, was incubated for 1.5 hr at 30°C with shaking. —●—, without addition of laccase; —○—, with addition of laccase.

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Fig. 3. Effect of Laccase Concentration on Alcohol Oxidase Activity.

The reaction mixture, containing 100 μ g of the alcohol oxidase, 0.5 μ mol of 3,4-dimethoxycinnamylalcohol, 1 μ mol of guaiacol and the indicated amount of laccase, was incubated for 1 hr at 30°C with shaking.

—●—, without addition of guaiacol; —○—, with addition of guaiacol.

mixture containing the substrate, the enzyme, and other additions in 0.5 ml of 0.1 M phosphate buffer, pH 6.0 was incubated at 30°C with shaking for the period indicated. After incubation 2.0 ml of 0.1 N NaOH was added to the reaction mixture and the 3,4-dimethoxycinnamaldehyde produced was determined by measuring the absorbance at 340 nm. The enzyme activity was expressed as absorbance at 340 nm.

As shown in Table I, the alcohol oxidase activity was greatly accelerated by guaiacol in the presence of laccase. Other phenols such as catechol and pyrogallol were not effective as stimulants, even in the presence of laccase. Figure 1 shows that the substrate was not oxidized by laccase alone or a combination of laccase and guaiacol in the absence of the alcohol oxidase during long incubations. As shown in Figs. 2 and 3, the acceleration rate was proportional to the concentrations of laccase or guaiacol in the presence of the other. The data shown in Table II indicate that the oxidation of guaiacol by laccase was reduced by the oxidation of 3,4-dimethoxycinnamylalcohol by the alcohol oxidase. These data suggest that the acceleration rate seemed to be dependent on the rate of oxidation of guaiacol by laccase. A laccase-glucose: quinone oxidoreductase cycle⁵⁾ and a laccase-cellobiose: quinone oxidoreductase cycle⁶⁾ have been postulated in white-rot fungi in relation to lignin biodegradation. The results reported here suggest that laccase may function as a link in an electron-transport chain which participates in the oxidation of lignins. A proposed mechanism for an alcohol oxidase-laccase cycle is given in Scheme 1. The oxidation reaction catalyzed by the alcohol oxidase also proceeded in the presence of oxygen, even in the absence

TABLE II. EFFECT OF ALCOHOL OXIDASE ON THE OXIDATION OF GUAIACOL BY LACCASE

The reaction mixture, containing 30 μ g of laccase and 3 μ mol of guaiacol, and if added, 3,4-dimethoxycinnamylalcohol 3 μ mol, alcohol oxidase 50 μ g in 0.1 M phosphate buffer, pH 6.0, was incubated for 30 min at 30°C with shaking.

Addition	Guaiacol oxidation (O.D. at 470 nm)
None	0.28
3,4-Dimethoxycinnamylalcohol	0.27
Alcohol oxidase	0.24
Alcohol oxidase + 3,4-dimethoxycinnamylalcohol	0.05

SCHEME 1.

of guaiacol and laccase. The alcohol oxidase preparation used in this experiment was not a homogeneous protein, so it is possible that the enzyme preparation consisted of two types of enzymes, an alcohol: oxygen oxidoreductase and an alcohol: quinone oxidoreductase. The detailed mechanism and physiological function of the proposed oxidation-reduction system are under investigation in relation to lignin biodegradation.

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