SPECTROPHOTOMETRIC INVESTIGATION OF THE KINETICS OF THE OXIDATION OF MODEL COMPOUNDS AND LIGNIN BY A PEROXIDASE–H_2O_2 COMPLEX

V. A. Strel'skii, A. V. Beigel'man, and É. I. Chupka

UDC 634.0.813.1:541.41

It has been established that in relation to the increase in the maximum initial rate of oxidation of model compounds and of lignin by a peroxidase–H_2O_2 complex, the substances studied form the following sequence: guaiacol, α-guaiacylpropanol, ferulic acid, α-guaiacylpropanone, and α-lignin. On the basis of the results of the investigation it is suggested that the oxidation of lignin is carried out by a peroxidase–O_2 complex. With a rise in the concentration of hydrogen peroxide the oxidation of model compounds is inhibited.

One of the most important questions of the biosynthesis of lignin is the system of dehydrogenating enzymes causing the polymerization of p-hydroxycinnamyl alcohols into lignin macromolecules. Mason and Cronyn [1] have shown that such enzymes may be a peroxidase and a laccase. In tissues undergoing lignification both enzymes are usually present, but it has been reported that in bamboo shoots [2] the activity of the peroxidase is higher. S. M. Manskaya [3], investigating the peroxidase and phenol oxidase of the cambium in the formation of the wood of the pine, also showed an action of these enzymes on coniferin, which may be a precursor of lignin. Model experiments with a whole series of phenolic derivatives and pure enzymes have shown the basic role of peroxidase in the lignification of the plant cell [4]. The work of Steelink [5, 6] has shown that the oxidation of model compounds and of lignin by a peroxidase–H_2O_2 complex takes place by a radical mechanism. It has been shown by the ESR method that the maximum concentration of radicals is proportional to the concentrations of peroxide and enzyme. When the oxidant was only O_2 or H_2O_2 without the enzyme, no formation of radicals was observed. In the same investigation it was shown that the aerobic oxidation of the model compounds is catalyzed by peroxidase at very low concentrations of hydrogen peroxide. In a study of the enzymatic oxidation of phenols of the type of α-methylsyringyl alcohol, it was established that ultraviolet irradiation catalyzes this process.

Spectrophotometric [7] and potentiometric [8] studies of the oxidation of substrates by the peroxidase–H_2O_2 complex have been performed. But, unfortunately, they mainly have a descriptive nature and do not report concrete results on the kinetics of the oxidation of different substrates. Our aim was a quantitative evaluation of the processes of oxidation of model compounds and of lignin by the peroxidase–H_2O_2 complex.

We used the spectrophotometric method of following the oxidative activity of the peroxidase–H_2O_2 complex with respect to the substrates selected. The change in optical density for model compounds and lignin was measured at the absorption maxima of the oxidation products in 0.1 M phosphate buffer, pH 5.7.

As model compounds we used α-guaiacylpropanol $\lambda_{max}^{C_2H_5OH}$ 275 log nm; log ε 3.47, α-guaiacylpropanone $\lambda_{max}^{C_2H_5OH}$ 275,305 nm; log ε 3.5; 3.40, ferulic acid ($\lambda_{max}^{C_2H_5OH}$ 315 nm, log ε 3.17), guaiacol ($\lambda_{max}^{C_2H_5OH}$ 270 nm, log ε 3.27), and ethanol ligin ($\lambda_{max}^{C_2H_5OH}$ 280 nm, log ε 4.78). The time from the mixing of the solution to the beginning of recording was not more than 5 sec.

Under the action of the peroxidase–H_2O_2 complex the UV spectra of the model compound and lignin changed. Thus, in the UV spectrum of the products of the oxidation of ferulic acid a maximum appeared at 285 nm, and in the absorption spectrum of α-guaiacylpropanol a shift in the absorption maximum in the long wave direction (λ_{max} 290-295 nm) was observed. The oxidation of guaiacol and of α-guaiacylpropanone formed colored products. On the oxidation of lignin by the peroxidase–H_2O_2 complex no characteristic change in the spectrum took place. In

Siberian Scientific-Research Pulp and Board Institute, Bratsk. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 109-112, January-February, 1982. Original article submitted June 12, 1981.

 0009-3130/82/1801-0102$07.50

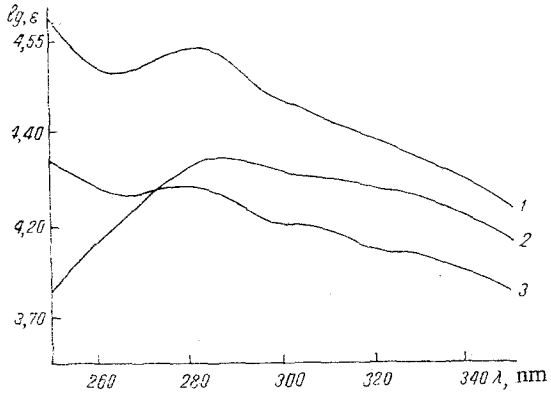

Fig. 1. UV spectra of ethanol lignin and the products of its oxidation: 1) initial spectrum of ethanol lignin (C_{lignin} = 0.18 g/liter); 2) spectrum of the products of the oxidation of the lignin by peroxidase ($C_{peroxidase} = 10^{-4}$ M; C_{lignin} = 0.18 g/liter; $C_{H_2O_2} = 0$); 3) spectrum of the products of the oxidation of the lignin by the peroxidase—H_2O_2 complex ($C_{peroxidase} = 10^{-4}$ M; C_{lignin} = 0.18 g/liter; $C_{H_2O_2}$ = 0.09 M).

the reaction of peroxidase with lignin (in the absence of H_2O_2) a fall in absorption in the 250-265 nm region was observed (Fig. 1). Thus, the conversion of lignin and its model compounds can be monitored from the change in the optical density in the ultraviolet and visible regions of the spectrum.

The kinetics of the enzymatic oxidation of lignin and its model compounds are considered in accordance with the Scheme [9]:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES; \quad ES + T \xrightarrow{k_3} E + P, \tag{1}$$

where E represents the enzyme; S represents a substrate (in this case, hydrogen peroxide); T represents a substrate (model compound or lignin); and P represents the product.

The constants and maximum rates of the oxidation reaction were calculated in the manner described by Ashmore [9]. For a bimolecular reaction between substrates, the initial rate of oxidation is determined from the equation

$$V_0 = \frac{k_1 k_3 [S]_0 [T]_0 [E]_0}{k_2 + k_3 [T]_0 + k_1 [S]_0}. \tag{2}$$

This relation shows that the maximum rate $k_1[S]_0[E]_0$ is reached when $[S]_0$ is kept constant and $[T]_0$ rises, and another maximum rate $k_3[T]_0[E]_0$ is obtained with a constant $[T]_0$ and a rise in $[S]_0$. From this it is possible to determine k_1 and k_3, and then k_2 from the equation

$$K = \frac{k_2 + k_3 [T]_0}{k_1}, \tag{3}$$

where K is a constant.

Hence, k_2 depends on the magnitude selected for $[T]_0$. The dependence of the rate of the reaction on the initial concentrations of hydrogen peroxide and substrate (guaiacol) is shown in Fig. 2a, b. The model compounds of the structural units of lignin form the following sequence in relation to the maximum rate of oxidation: guaiacol > α-guaiacylpropanol > ferulic acid > α-guaiacylpropanone > lignin. The constants and maximum rates of the oxidation reactions are given in Table 1.

The rate of oxidation of the model compounds by hydrogen peroxide did not exceed $0.5 \cdot 10^{-10}$ M·sec^{-1}. The initial rate of oxidation of lignin by peroxidase in the absence of hydrogen peroxide amounted to $0.17 \cdot 10^{-7}$ M·sec^{-1}.

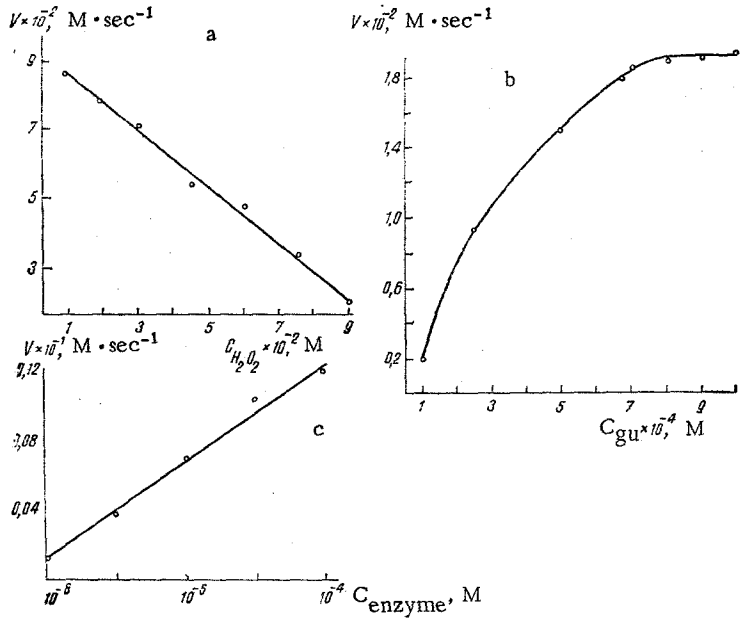

Fig. 2. Dependence of the initial rate of oxidation of guaiacol on the concentrations: a) of hydrogen peroxide ($C_{peroxidase}$ = 10^{-4} M; $C_{guaiacol}$ = 10^{-3} M; pH 5.7); b) of the substrate ($C_{peroxidase} = 10^{-4}$ M; $C_{H_2O_2}$ = 0.09 M); c) of the enzyme ($C_{guaiacol}$ = 10^{-3} M; $C_{H_2O_2}$ = $0.9 \cdot 10^{-3}$ M).

TABLE 1. Kinetic Characteristics for the Oxidation of Model Compounds and of Lignin in the Peroxidase—H_2O_2 System

Substrate	V_{max} $[H_2O_2]$ – const	V_{max} $[T]_0$ – const	k_1	k_2	k_3
	M × sec^{-1}	M × sec^{-1}	liter × M^{-1} × sec^{-1}	liter × M^{-1} × sec^{-1}	liter × M^{-1} × sec^{-1}
Guaiacol	$2,0\times10^{-2}$	$9,4\times10^{-2}$	$0,2\times10^{5}$	$2,0\times10^{2}$	$9,4\times10^{5}$
α-Guaiacylpropanol	$4,0\times10^{-3}$	$6,5\times10^{-3}$	$1,10\times10^{3}$	$1,65\times10^{2}$	$4,0\times10^{4}$
Ferulic acid	$1,5\times10^{-5}$	$1,3\times10^{-5}$	$2,05\times10^{3}$	$1,0\times10^{2}$	$6,5\times10^{4}$
α-Guaiacylpropanone	$0,25\times10^{-5}$	$0,4\times10^{-5}$	$1,25\times10^{2}$	$0,20\times10^{2}$	$2,0\times10^{4}$
Lignin	$3,3\times10^{-7}$	$0,4\times10^{-7}$	0.20	$1,0\times10^{-3}$	$0,1\times10^{2}$

In the first stage of the oxidation of lignin by the peroxidase—H_2O_2 complex, hydrogen peroxide decomposes actively with the formation of a large amount of oxygen, which is consumed in the oxidation process. In view of this, the rate of oxidation of lignin rises with an increase in the concentration of hydrogen peroxide. In the oxidation of the model compounds, the opposite situation was observed, i.e., the initial rate of oxidation fell with an increase in the concentration of hydrogen peroxide. This can be explained by the competitive inhibition arising between the hydrogen peroxide and the substrate for the active centers of the enzyme. In a study of the kinetics of the oxidation of the model compounds and lignin a linear relationship was found between the concentration of enzyme and the initial rate of oxidation of the substrate (Fig. 2c).

EXPERIMENTAL

A preparation of horseradish peroxidase (Reanal RZ 0.6, activity by the o-dianisidine method 350-500 units/mg) which was presented by the Institute of Biophysics of the Academy of Sciences of the USSR was used. The model compounds and the ethanol lignin was obtained by methods described in the literature [10]. As model compounds we used guaiacol, ferulic

acid, α-guaiacylpropanol, and α-guaiacylpropanone. These models of the structural units of lignin were dissolved in water–ethanol (2:3) and the solutions were brought to the required concentration with distilled water. The peroxidase was dissolved in 0.1 M phosphate buffer, pH 5.7. The spectrophotometric investigations were performed on a Spectromom-195 spectrophotometer with recording on a Karl Zeiss potentiometer. The experiments were performed in a mixture of peroxidase, hydrogen peroxide, and substrate in a volume ratio of 1:2:2 at room temperature.

CONCLUSION

It has been established that with respect to the maximum initial rate of oxidation of model compounds by the peroxidase–H_2O_2 complex the substances studied form the following sequence: guaiacol, α-guaiacylpropanol, ferulic acid, α-guaiacylpropanol, and lignin.

With a rise in the concentration of hydrogen peroxide the initial rate of oxidation of the model compounds falls, which can be explained by an inhibition arising with an increase in the concentration of oxidant.

With a rise in the concentration of hydrogen peroxide oxygen is actively liberated and the initial rate of oxidation of lignin rises although this is not observed in the oxidation of the model compounds. Furthermore, the difference in the initial rate of oxidation of lignin both in the presence of H_2O_2 and without it are inconsiderable. This makes it possible to assume that the oxidation of lignin takes place by a peroxidase–O_2 complex.

LITERATURE CITED

1. H. S. Mason and M. Cronyn, J. Am. Chem. Soc., 77, 491 (1955).
2. T. Higuchi, Physiol. Plantarum, 10, 356 (1957).
3. C. M. Manskaya, Dokl. Akad. Nauk SSSR, 62, 369 (1948).
4. T. Higuchi, Proceedings of the IVth International Congress of Biochemistry (1958).
5. E. S. Cardwell and C. Steelink, Biochim. Biophys. Acta, 184 (1969).
6. M. Young and C. Steelink, Phytochemistry, 12, 2851 (1973).
7. B. Chance, in: Techniques of Organic Chemistry (ed. A. Weissberger), Interscience, New York, Vol. VIII (1953), p. 690.
8. P. George, J. Biochem., 54, No. 2, 267 (1953).
9. P. Ashmore, Catalysis and Inhibition of Chemical Reactions, Butterworth, London (1963).
10. G. F. Zakis, The Synthesis of Model Compounds [in Russian], Riga (1980).