ENZYMATIC OXIDATION OF LIGNIN AND COMPOUNDS MODELING IT.

III. FORMATION OF SINGLET OXYGEN IN THE PEROXIDASE OXIDATION OF LIGNIN

V. A. Strel'skii and É. I. Chupka

UDC 535.379.5:577.158

The main emitters of radiation in the aerobic oxidation of lignin are the carbonyl groups in an excited state and singlet oxygen. It has been shown that the main source of $O_2(^1\Delta)$ may be the radical anion $O^{\bullet}_2$. Singlet oxygen and the radical anion are by-products of the radical oxidation of lignin.

We have previously shown that the peroxidase oxidation of lignin and of a number of compounds modelling its structural units takes place through a stage of the formation of electronically excited states and is accompanied by chemiluminescence (CL) [1, 2].

In the present paper we present results obtained in a study of the spectral composition of the radiation arising on the aerobic oxidation of lignin in the presence of peroxidase.

From the CL spectrum with the aid of limiting glass filter we isolated a number of characteristic bands with maxima at 490, 560, 630, and 750 nm (Fig. 1). In addition to these bands, we also detected the emission of radiation in the far red region of the spectrum (above 715 nm) the individual components of which could not be isolated because of the low sensitivity of FÉU-84-2 photomultiplier to this region of the spectrum.

The appearance in the CL spectrum of the band with λ_{max} 560 is due, according to [3], to the processes involved in the deactivation of excited carbonyl groups of the substrate. The concentration of carbonyl groups in lignin is 1.0-1.26 meq/g [4] and depends on the method of isolating the lignin from wood.

The radiation with wavelengths of 634 and 703 nm relates to the deactivation of dimeric forms of singlet oxygen $O_2(^1\Delta)$ [5]:

$$2O_2(^1\Delta) \longrightarrow 2O_2(^3\Sigma) + h\nu_{703}^{634}.$$

The radiation at 634 nm corresponds to the transition of both molecules of product into the ground vibrational state; if one of the molecules of the product proves to be in the first vibrational level, radiation will be observed at 703 nm. The band at 634 nm is convenient for monitoring, since its intensity is proportional to the square of the concentration of singlet oxygen.

To confirm the hypothesis concerning the formation of $O_2(^1\Delta)$ in the system, we investigated the quenching of the radiation in the 630 and 705 nm regions by 1,3-diphenylisobenzofuran (DPBF), which is one of the most reactive acceptors of singlet oxygen. The results show that the addition of the acceptor led to a fall in the intensity of CL due to the quenching of the $O_2(^1\Delta)$ both in the 630 nm region and in the 705 nm region (Fig. 2). The change in the intensity of the radiation on the addition of DPBF obeyed the Volmer–Stern equation*:

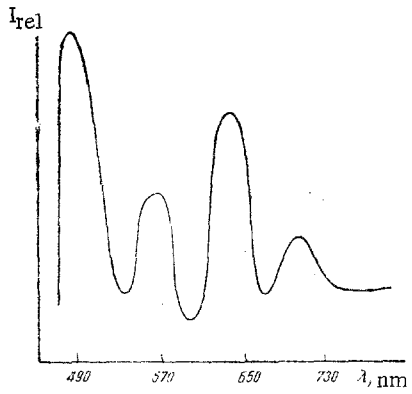
$$\sqrt{\frac{I_0}{I}} = 1 + K[A],$$

where I_0 is the intensity of the CL without the acceptor;

*In view of the fact that the intensity of the radiation was proportional to the square of the concentration of $O_2(^1\Delta)$, in place of the ratio I_0/I we used the expression $\sqrt{I_0/I}$.

Siberian Scientific-Research Institute of Cellulose and Board, Bratsk. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 510-514, July-August, 1984. Original article submitted June 28, 1983.

 0009-3130/84/2004-0482$08.50



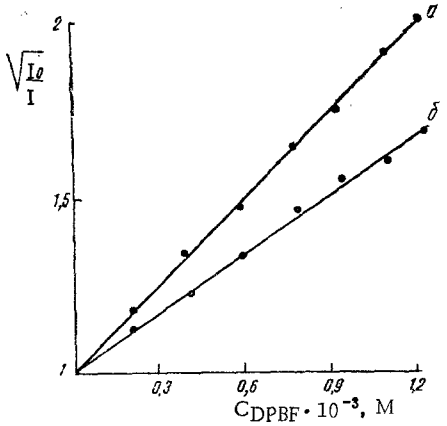


Fig. 1 Fig. 2

Fig. 1. CL spectrum arising in the peroxidase-catalyzed aerobic oxidation of lignin. The solvent was dioxane. The concentration of enzyme was 10^{-6} M and that of lignin 10^{-3} nominal moles/liter. Temperature 30°C.

Fig. 2. Volmer–Stern relation in the coordinates $\sqrt{I_0/I} = f[A]$ on the quenching of singlet oxygen by DPBF. For the experimental conditions, see Fig. 1: a) λ_{max} = 630 nm; b) λ_{max} = 700–705 nm.

I is the intensity of the radiation on the addition of DPBF;

[A] is the concentration of acceptor (DPBF);

K is the quenching constant, equal to the product of the actual lifetime of the excited state in the absence of a quenching agent, τ_0, to the quenching rate constant k_q.

The experimental value of K was determined from the tangent of the angle of slope of the straight line in the coordinates $\sqrt{I_0/I} = f[A]$. For λ_{max} 630 nm, $K = 2.7 \cdot 10^3$ liter/mole. The lifetime of singlet oxygen in dioxane is $3 \cdot 10^{-5}$ sec [6]. Substituting the values obtained for K and τ_0 in the formula

$$k_q = \frac{K}{\tau_0},$$

we determined the value of the quenching rate constant k_q for singlet oxygen. The constant for the rate of quenching for $^1O_2$ for radiation with λ_{max} 630 nm was $0.9 \cdot 10^8$ liter/mole sec, and for the 700–705 nm region $0.36 \cdot 10^8$ liter/mole·sec.

It was established by a control experiment that DPBF does not interact with peroxides. The rate constant of quenching k_q agrees well with the analogous values obtained by the methods of pulsed photolysis [6] and of chemiluminescence [7] for DPBF.

The addition to the reaction mixture of known CL activators (eosin, dibromoanthracene, etc.) in concentration of 10^{-5}–10^{-3} M led to an approximately 1.5-fold increase in the concentration of singlet oxygen.

Peroxidase is an enzyme liberated by a wood-destroying fungus in the plant cell. The influence of other structural elements of the cell wall (cellulose, hemicelluloses) has been shown on the natural process of oxidation of lignin, and we therefore investigated the influence of a number of carbohydrates on the formation of singlet oxygen. The use of pentosans as additives showed that arabinose and xylose in concentrations below 10^{-4} M intensified the radiation in the 630 nm region. A further increase in the concentration of the additive led to a fall in the CL in the red region of the spectrum. The addition of galactose led to a rise in the intensity of the radiation in this region of the spectrum (Fig. 3).

The formation of singlet oxygen in the oxidation of lignin may be the result of several processes taking place in parallel in the system. $O_2(^1\Delta)$ can arise in the transfer of energy from an excited carbonyl group to molecular oxygen in the ground state [8], and also in the

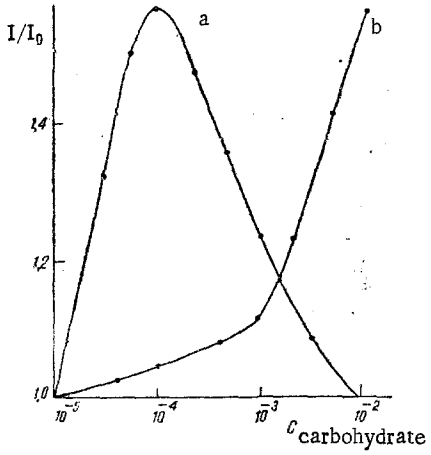


Fig. 3

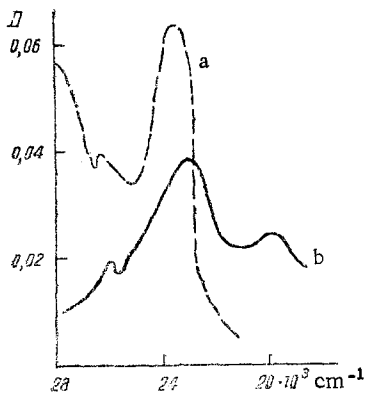


Fig. 4

Fig. 3. Influence of carbohydrates on the intensity of CL in the 630 nm region: a) arabinose; b) galactose.

Fig. 4. Spectrum of compound III of peroxidase: a) native peroxidase + O_2; b) peroxidase + lignin + O_2.

decomposition of dioxetanes [9]. The transfer of energy to molecular oxygen in this system is unlikely because of the influence of various factors (the large amount of carbonyl groups in the lignin, their small lifetime in the excited state, the presence of impurities, etc.).

In biological materials the formation of singlet oxygen is usually connected with the appearance of a O_2^- radical anion in the system [10], this being the product of the one-electron reduction of oxygen. The O_2^- radical anion has been detected in many oxidative processes catalyzed by various enzymes [11]. Singlet oxygen can be formed by the following scheme

$$\dot{O}_2 + e \dashrightarrow O_2^-, \quad (1)$$

$$2O_2^- \dashrightarrow H_2O_2 + O_2 + O_2(^1\Delta), \quad (2)$$

$$HO_2^{\cdot} + O_2^- \dashrightarrow HO_2^- + O_2(^1\Delta). \quad (3)$$

Singlet oxygen may also appear in the system as the result of the recombination of $HO_2^{\cdot}$ radical formed in the stage of initiation of a chain process:

$$HO_2^{\cdot} + HO_2^{\cdot} \dashrightarrow H_2O_2 + O_2(^1\Delta). \quad (4)$$

Since the peroxidase activity of the enzyme is connected with the presence of hydrogen peroxide in the system, routes (2) and (4) for the formation of singlet oxygen $O_2(^1\Delta)$ appear to be the most probable.

According to Odajima and Yamazaki [12], compound (III) of peroxidase is a $Fe^{3+} \rightarrow O_2^-$ complex and is detectable from the nature of the absorption in 418-425 nm region. The complex may subsequently dissociate with the release of the O_2^-, which takes part in oxidative processes and in the formation of singlet oxygen. We recorded characteristic absorption at $\lambda = 425$ nm which may serve as an indirect confirmation of the formation of compound (III) of peroxidase and, consequently, also of the radical anion O_2^- (Fig. 4). Since peroxidase also takes a direct part in the processes involved in the biosynthesis of lignin, the appearance of these forms of oxygen in the oxidation of the phenylpropane structures that are precursors of lignin in the plant cell must be expected.

EXPERIMENTAL

The apparatus for recording superweak radiations has been described previously [1]. The isolation of the bands of the radiation was carried out with a set of limiting glass filters taking into account their transmission factors and the sensitivity of the FEU-84-2 photomultiplier for each region of the spectrum. The absorption spectrum of compound (III) of peroxidase was recorded on a Specord UV-VIS spectrophotometer in the range of 75-125% transmission. The solutions of dioxane lignin isolated from pine wood by Pepper's method [13] and the DPBF were prepared in the necessary proportions and mixed before the addition

of the enzyme. The reactions were initiated by the addition of peroxidase (Reanal, RZ 0.6, activity by the o-dianisidine method 350-500 units/mg) to the system. As CL activators we used aqueous solutions of commercial preparations of arabinose, xylose, galactose, eosin, and Methylene Green, and dioxane solutions of dibromoanthracene and o-phenanthroline.

SUMMARY

It has been established that the main emitters of the radiation arising in the aerobic oxidation of lignin in the presence of peroxidase are carbonyl groups in the excited state and singlet oxygen. The rate constants of the quenching of $O_2(^1\Delta)$ by 1,3-diphenylisobenzofuran in the regions of radiation of 630 and 700-750 nm has been determined. It has been shown that the main source of singlet oxygen may be the radical anion $O_2^{\cdot-}$. Singlet oxygen and the $O_2^{\cdot-}$ radical anion are by-products of the radical oxidation of lignin. It is quite likely that both these forms of oxygen may also be produced in the biosynthesis of lignin.

LITERATURE CITED

1. V. A. Strel'skii, and É. I. Chupka, Khim. Prir. Soedin., 762 (1982).
2. V. A. Strel'skii and É. I. Chupka, Khim. Prir. Soedin., 765 (1982).
3. D. Slawinska, I. Slavinski, K. Polewski, and W. Pukacki, Photochem. Photobiol., 30, 71 (1979).
4. B. D. Bogomov, The Chemistry of Wood and the Principles of the Chemistry of High-Molecular-Weight Compounds [in Russian], Moscow (1973), p. 389.
5. N. V. Shinkarenko and V. B. Aleskovskii, Usp. Khim., 50, No. 3, 406 (1981).
6. V. Ya. Shlyapintokh and V. B. Ivanov, Usp. Khim., 45, No. 2, 202 (1976).
7. I. Kruk, K. Lichszteld, and T. Michalska, Z. Phys. Chem., 260, No. 2, 371 (1979).
8. N. M. Émanuél' and A. L. Buchachenko, The Chemical Physics of the Aging and Stabilization of Polymers [in Russian], Moscow (1982), p. 359.
9. I. Stauff, H. Schmidkunz, and G. Hartmann, Nature (London), 198, No. 4877, 281 (1963).
10. I. B. Afanas'ev, Usp. Khim., 47, No. 6, 977 (1979).
11. D. I. Metelitsa, The Activation of Oxygen by Enzyme Systems [in Russian], Moscow (1982), p. 255.
12. T. Odajima and I. Yamazaki, Biochim. Biophys. Acta, 206, 71 (1970).
13. I. M. Pepper, P. E. Baylis, and E. Adler, Can. J. Chem., 39, No. 8, 1241 (1959).