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# ACTION OF LIGHT ON THE RELATIVE ACTIVITY OF PEROXIDASE IN THE AEROBIC OXIDATION OF LIGNIN

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It has been established that the activity of peroxidase depends to a considerable degree on the radiation dose and the spectral composition of the light. A special sensitivity of the enzyme to the action of the blue and red regions of the spectrum has been shown. In the investigation it was found that light with a wavelength greater than 520 nm promotes the liberation, and short wave light the consumption, of oxygen during the aerobic oxidation of lignin in the presence of peroxidase.

It has been shown [1] that the cambial tissue and the wood of the new annual layer of the pine contains oxidative enzymes: peroxidase and polyphenol oxidase. The substrate for these enzymes is the coniferin of the cambial tissue and its derivatives which, after the cells have died off, form lignin. Such external factors as light, temperature, soil pH, the presence of trace elements, etc., exert a great influence on the degree of lignification of the plant. According to [2], light can penetrate into wood to a depth sufficient to reach the cambial layer which, undoubtedly, has an influence on the metabolism of phenolic compounds. Ryumina [3] has shown that the formation of the bast fibers of hemp and their lignification is connected with the passage of the plant through a light-controlled stage of development. A number of workers have studied the influence of light on lignification and have obtained results showing that the processes of lignification are completed in the dark in the case of the ash [4] and in the tissues of carrot galls [5] in various young herbs and in coniferous and broad-leaved trees [6]. In a determination of the influence of light of different wavelengths, it was established that yellow-green light stimulates lignification. A number of authors have shown a maximum sensitivity of the biosynthesis of phenols to the red and blue regions of the spectrum [7-9].

We have investigated the influence of light on the catalytic activity of peroxidase and its capacity for the aerobic oxidation of lignin, which is connected with it. The activity of the enzyme is affected both by the dose and by the spectral composition of the light. According to the results obtained, with an increase in the dose of radiation there is a redistribution of the activity of the native peroxidase. Thus, while at a low dose of radiation the long-wave region of the spectrum ($\lambda$ = 540-660 nm) plays the most active role, with an increase in the radiation dose the rise in the activity of the peroxidase is due to the blue region of the spectrum ($\lambda$ = 365-420 nm). A considerable contribution to the activation of the enzyme is made by light with a wavelength of 545-590 nm, where, according to [10], are located the absorption bands of peroxidase ($\lambda_{max}$ = 548 and 583 nm). It is obvious that under the influence of radiation in this region of the spectrum an excitation of the active center of the enzyme takes place which is reflected in its catalytic activity. The action spectrum for peroxidase is shown in Fig. 1.

A somewhat different pattern was observed when lignin is added to the reaction mixture. In this case, it was not a redistribution of the activity of the peroxidase that took place but a fall in it with an increase in the radiation dose. The maximum ratio $A_\lambda/A_T$, where $A_\lambda$ is the activity of the peroxidase on irradiation at different wavelengths and $A_T$ is the

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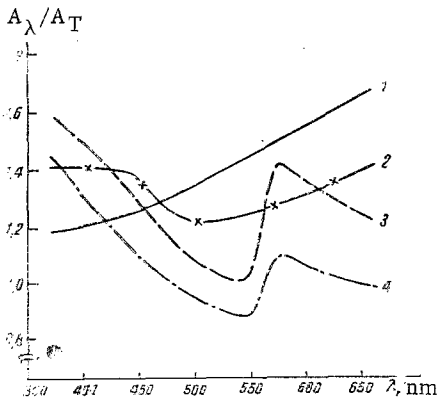



Fig. 1

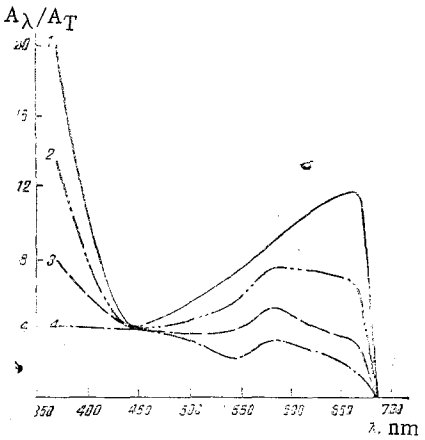



Fig. 2

Fig. 1. Change in the relative activity of peroxidase as a function of the wavelengths of the acting light. Concentration of peroxidase $10^{-6}$ M; pH 5.7; T = 295°K. Radiation doses: 1) $6 \cdot 10^4$ kJ·sec/mole; 2) $12 \cdot 10^4$ kJ·sec/mole; 3) $18 \cdot 10^4$ kJ·sec/mole; 4) $24 \cdot 10^4$ kJ·sec/mole.

Fig. 2. Change in the relative activity of peroxidase on the introduction of lignin as a function of the wavelength of the acting light. Concentration of lignin 200 mg/liter. For the experimental conditions, see Fig. 1.

activity of the enzyme in the absence of radiation, was observed at a dose of $6 \cdot 10^4$ kJ·sec/mole both in the blue and in the red regions of the spectrum (Fig. 2). The peroxidase activity was inversely proportional to the radiation dose at wavelengths close to the absorption maxima of the native enzyme. It must be mentioned that on radiation with light having a wavelength of 440-540 nm the peroxidase activity did not depend on the radiation dose, and on irradiation with light having $\lambda > 680$ nm there was a fall to the level of activity in the absence of radiation.

As has been shown [11], the lignin groupings most readily passing into the excited state are the carbonyl structures and structures with conjugated double C—C bonds. It has been established that during the photooxidation of polymers hydroperoxide groups are formed. On irradiation with light the polymeric hydroperoxides decompose in accordance with the following equations:

$$ROOH \dashrightarrow R^{\cdot} + {}^{\cdot}OOH, \quad (1)$$

$$ROOH \dashrightarrow RO^{\cdot} + {}^{\cdot}OH. \quad (2)$$

The energy of quanta with a wavelength greater than 300 nm is sufficient to cleave RO—OH and R—OOH bonds, the energies of dissociation of which are 176 and 284 kJ/mole, respectively [12]. In addition, a hydroperoxide can accept an electron from the $Fe^{2+}$ cation that is present in the active center of peroxidase and acts as a reducing agent.

Photodecomposition can take place in accordance with the scheme

$$Fe^{2+} + RO{-}OH \dashrightarrow Fe^{3+} + RO^{\cdot} + OH^{-}. \quad (3)$$

The occurrence of photodecomposition by scheme (3) was detected with the aid of benzidine, which acts as an organic indicator and gives a characteristic coloration in the presence of oxidizing agents — in this case, $RO^{\cdot}$ and $Fe^{3+}$ [13]. The rate of oxidation of benzidine will be made up of the oxidative actions of $Fe^{3+}$ — the active center of the enzyme — and of the radicals $RO^{\cdot}$ formed in the photooxidation of lignin. The reaction shows a dark after-effect of considerable length (more than 30 min after irradiation for 5 min with full-spectrum light). The maximum rate of oxidation is achieved after 10 min of this dark stage.

Aerobic oxidation catalyzed by peroxidase under the action of light causes a change in the UV absorption spectra of lignin. Thus, irradiation of the peroxidase—lignin system with light having a wavelength of 313 nm led to the appearance in the absorption spectrum of the

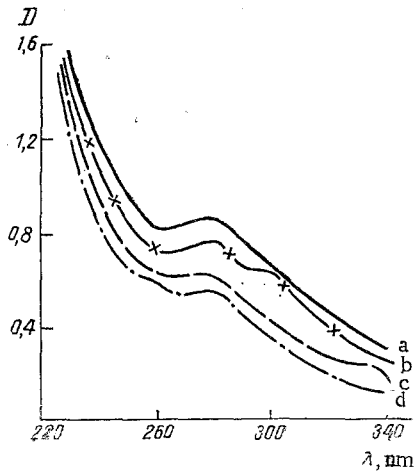
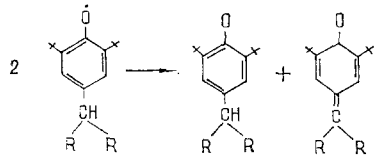



Fig. 3. UV spectra of lignin after irradiation with a dose of $24 \cdot 10^4$ kJ·sec/mole: a) initial spectrum of the lignin; b, c) $\lambda_{irr}$ = 313 nm; d) $\lambda_{irr}$ = 620 nm.

lignin of a shoulder at 305-310 nm (Fig. 3, curve b). The irradiation of a solution containing peroxidase, $H_2O_2$, and lignin with the same wavelength caused the formation of a shoulder in the region of absorption at 325-335 nm (Fig. 3, curve c). According to Adler [14] as also to Cook and Norcross [15] quinone methides are responsible for absorption in this region. It is likely that the phenoxy radicals from spatially hindered phenols disproportionate, forming the initial phenol and a quinone methide:



It is possible that this mechanism was also observed in the present case. The irradiation of a reaction mixture containing peroxidase, lignin, and an oxidizing agent ($O_2$ or $H_2O_2$) with red light having a wavelength of 620 nm led to the appearance of an absorption band in the 260-265 nm region (Fig. 3, curve d), which is probably connected with the accumulation of peroxide compounds in the lignin macromolecule.

The aerobic oxidation of lignin depends directly on the amount of oxygen in the reaction mixture. Consequently, considerable interest is presented by the question of the change in the amount of oxygen during the peroxidase oxidation of lignin under the action of radiation. It has been established that light with a wavelength greater than 520 nm and also the dark stage of the process after irradiation promote the formation of oxygen in a peroxidase–lignin mixture. Short-wave radiation causes a consumption of oxygen in the reaction medium. The relative rate of the absorption of $O_2$ is the higher the shorter the wavelength of the irradiating light.

## EXPERIMENTAL

The sources of light were DRSh-500 and DRSh-1000 mercury lamps. To isolate various regions of the spectrum we used a set of glass limiting filters and filters for isolating the lines of a mercury lamp. Irradiation was performed in a glass cell with a column of 8 $cm^3$ having a quartz window with a diameter of 1 cm. The relative change in the amount of oxygen in the solution was measured with the aid of an enclosed Clark electrode at the oxygen reduction potential (–0.5 V). A preparation of horseradish peroxidase (Reanal) was dissolved in 0.1 M phosphate buffer, pH 5.7. A solution containing lignin (200 mg/liter) isolated by Pepper's method [16] and peroxidase ($10^{-6}$ M) was irradiated. In the experiments using $H_2O_2$ as oxidizing agent, the concentration of hydrogen peroxide was 0.03 M. Benzidine of ch. ["pure"] grade in dioxane at a concentration of $10^{-3}$ M was used as an indicator of the activity of the enzyme. The benzidine was added to the reaction mixture after irradia-

tion. The kinetics of the oxidation of the benzidine (and, correspondingly, the change in the activity of the enzyme) was observed from the change in the optical density at $\lambda$ = 410 nm ($\varepsilon^{410}_{\text{dioxane}} = 0.82 \cdot 10^2$ liter/mole·cm). The absorption spectra of the lignin were measured in the UV region on a Spektromom-195 spectrophotometer.

## CONCLUSION

It has been established that the activity of peroxidase depends to a considerable degree on the radiation dose and the spectral composition of the light. A particular sensitivity of the enzyme to the action of the blue and red regions of the spectrum has been shown. The catalytic activity of peroxidase in the presence of lignin is inversely proportional to the radiation dose at wavelengths coinciding with the absorption bands of native peroxidase. It has been established that light with a wavelength greater than 520 nm permits the liberation, and short-wave light promotes the absorption, of oxygen in the peroxidase oxidation of lignin.

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