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RED LIGHT OF A HELIUM-NEON LASER REACTIVATES SUPEROXIDE DISMUTASE

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Irradiation with light from a helium-neon laser (HNL) is being used on an increasingly wide scale in the treatment of diseases connected with ischemia, hypoxia, and various inflammatory processes [3]. The mechanism of this broad action of HNL is not yet understood. It is evident that the radiation can act only on those components of the living cell which absorb light. The wavelength of HNL radiation is 632.8 nm. The most important oxidoreductases absorb in this region: catalase, superoxide dismutase (SOD), cytochrome C oxidase, ceruloplasmin, etc. [4]. Some workers have recorded activation of certain metalloenzymes by HNL light [1, 2, 6], but no action of laser radiation has hitherto been found on native SOD and ceruloplasmin [9, 13].

To understand the mechanism of action of laser light on biological objects, one fact appears to be important, namely that HNL radiation does not affect normally functioning cells, but has a marked action in cases associated with a pathological process. Damage to the body cells under these circumstances may arise under the influence of active forms of oxygen [14]. In these cases, administration of protective enzymes, notably SOD, has a marked therapeutic effect [15]. This effect may perhaps be connected with the fact that the intrinsic SOD of the cells may be partially inactivated in a pathological focus, where the pH is lowered [5]. In experiments with isolated SOD, disturbance of the structure of the enzyme was observed in an acid medium as shown by changes in optical and paramagnetic properties [12]. The question arises whether the therapeutic action of laser radiation may be connected with photoreactivation of enzymes reversibly inhibited in the pathological focus and, in particular, SOD.

The investigation described below showed that reversible inhibition of the enzyme takes place in an acid medium, and that HNL radiation can reactivate SOD. To shed light on the possible mechanism of this process, it was decided to study the activity and absorption and EPR spectra of SOD and of complexes of copper with histidine, which simulate the structure of the active center of the enzyme.

EXPERIMENTAL METHOD

SOD from bovine erythrocytes (from "Boehringer") was dissolved in 10 mM Tris-HCl buffer, pH 5.9, 7.4, and 8.2. Complexes of copper and histidine were prepared by dissolving 1 mM $CuCl_2$

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TABLE 1. Changes in SOD Activity at pH 5.9 and Irradiation by HNL (M ± m)

pH 5.9, incubation in darkness		Illumination by light from LG-78	
duration of incubation, min	activity, % of control	duration of irradiation, sec	activity, % of control
30	95±2	30	82±2
60	73±1	45	94±1
90	43±4	60	107±2
120	2±2	90	107±3

TABLE 2. Changes in Optical Properties of SOD (0.2 mg/ml) in Acid Medium and after Laser Irradiation

Object	Optical density	
	at 450 nm	at 680 nm
SOD, pH 8.2	0,021	0,011
SOD, pH 5.9 + incubation for 2 h	0,009	0,010
The same + irradiation for 60 sec	0,023	0,012

and 4 mM histidine in 10 mM Tris-HCl buffer. The pH of the samples was adjusted to 5.9 and 8.2, and they were kept at room temperature for 4-6 h.

SOD activity was determined by a modified method [11], activity of the enzyme incubated at pH 7.4 being taken as 100%. Absorption spectra were recorded in the visible region on a "Beckman-7" spectrophotometer. EPR spectra were recorded on a "Variam E-4" radiospectrometer at 77°K.

The source of irradiation was an LG-78 apparatus, generating light with a wavelength of 632.8 nm and with output power of 2 mW, in a beam 3 mm in diameter.

EXPERIMENTAL RESULTS

Chagne in pH toward the acid side may be one condition for inactivation of SOD in a pathological focus [5]. We studied the action of an acid medium on SOD activity. Incubation of SOD in solution at pH 5.9 for 2 h caused virtually complete loss of activity of the enzyme (Table 1). Irradiation of the sample with HNL light caused reactivation of SOD (Table 1). An acid medium thus leads to reversible inhibition of SOD, which is abolished by HNL light:

$$\text{SOD} \underset{\text{light } 632{,}8\text{ nm}}{\overset{\text{pH 5,9, 2 h}}{\rightleftharpoons}} \text{inactive enzyme},$$

To discover the mechanism of the observed effect, absorption spectra of the enzyme were studied. Incubation at pH 5.9 in these experiments caused a change in the optical properties of SOD; absorption was reduced in the region of 450 and 680 nm (Table 2). These results agree with data in the literature [7, 12] and can be explained by rupture of the bond between the copper-histidine complex and zinc in the active center of the enzyme, as a result of protonation of histidine-61 (His-61) [8].

Irradiation with HNL light, and also alkalification of the medium containing the SOD sample, inactivated in an acid medium, to pH 8.2 led to restoration of the optical properties of the enzyme (Table 2).

Additional information on the nature of the changes in the active center of SOD was given by the study of EPR spectra. EPR spectra of SOD in buffer at pH 8.2 (1) and after incubation for 2 h in medium at pH 5.9 (2) are given in Fig. 1. Clearly in an acid medium an increase in A_{II} was observed from $150 \cdot 10^{-2}$ T1 (the value of A_{II} at pH 8.2) to $1.75 \cdot 10^{-2}$ T1, and a decrease in the value of GM were observed. These differences are in agreement with those obtained previously [7, 8, 12] and are evidence of a change in the ligand sphere of copper as a result of protonation of His-61 and rupture of the bond of the organocopper complex with zinc. Irradiation of SOD solution at pH 5.9 for 1 min led to restoration of the parameters of EPR spectra

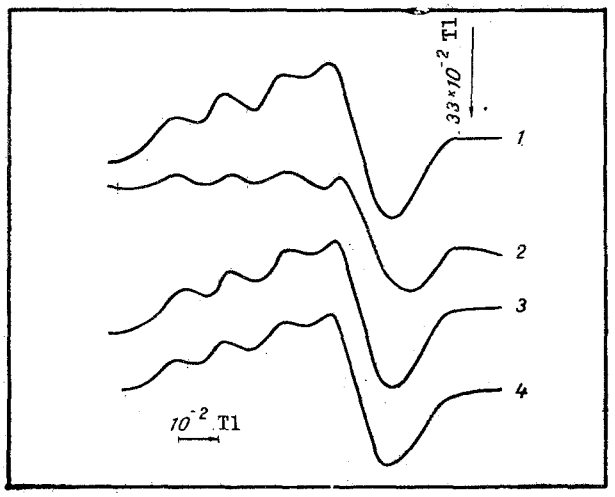


Fig. 1

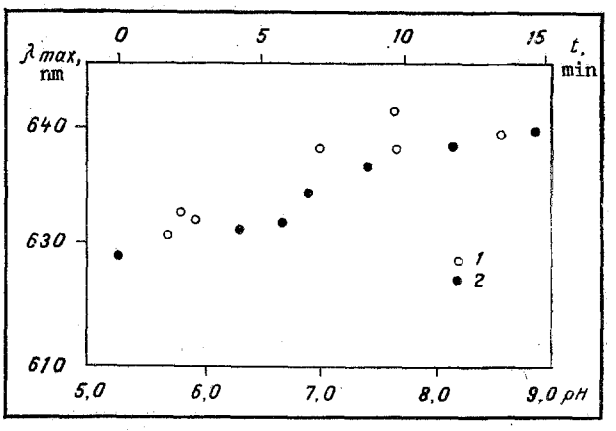


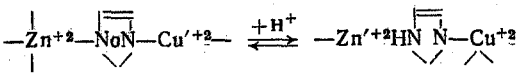
Fig. 2

Fig. 1. EPR spectra of SOD at different pH values before and after action of laser radiation: 1) at pH 8.2; 2) at pH 5.9; 3) the same as 2, after irradiation for 60 sec; 4) the same as 2, immediately after adjustment of pH to 8.2.

Fig. 2. Dependence of position of absorbance maximum of model copper-histidine complexes on pH (1) and on duration of laser irradiation (2).

characteristic of the intact enzyme in medium at pH 8.2. Adjustment of the pH of the solution with alkali to the same value led to similar changes in spectral parameters, i.e., the EPR spectra of SOD, both after irradiation with HNL light in an acid medium and after adjustment of the pH to the control value (8.2) are in full agreement with the EPR spectrum of the intact enzyme at pH 8.2 (Fig. 1).

It was suggested previously [7, 12] on the basis of investigations of absorption, circular dichroism and EPR spectra, and this was subsequently confirmed by an investigation by the spin echo method [8], that protonation of His-61, existing in the form of the imidazolate anion, linking copper and zinc in the active center of SOD, takes place in an acid medium. As a result of attachment of the proton to His-61, the bond between zinc and the copper-histidine complex is ruptured and the enzyme loses its activity:



According to our data, this process at pH 5.9 took 2 h and was accompanied by loss of activity and a change of the optical and paramagnetic properties of SOD. Light of HNL evidently induces deprotonation of the copper-histidine complex in the active center: this is indicated by restoration of the EPR spectrum and optical and enzymic properties characteristic of native SOD in a neutral or weakly alkaline medium. Elementary physicochemical processes taking place in the active center of the enzyme during inactivation in an acid medium and subsequent photoreactivaiton by HNL light can be simulated by using complexes of copper with histidine. The absorption spectrum of such complexes in the long-wave region depend on pH. At pH 5.8-6.0 these complexes were found to have a maximum at 699 nm, which coincides with the wavelength of the light emitted by HNL. Dependence of wavelength of the maximum in the absorption spectrum of the model complexes on pH and on the duration of laser irradiation is shown in Fig. 2. The change in pH of the solution of the complexes from 5.9 to 9.0 leads to a shift of the absorption maximum into the longer-wave region.

Irradiation by HNL light leds to similar shifts in the position of the absorbance maximum for complexes in medium at pH 5.8.

The results of this investigation are evidence that the mechanism of photoreactivation of SOD, when inhibited in an acid medium, consists of deprotonation of His-61, and that the principal acceptor of light is a complex of copper with nitrogenous ligands from histidines −61, −44, −46, and −118 in the active center of SOD.

The action of laser radiation on SOD in an acid medium is reversible. A few minutes after incubation in darkness of the enzyme reactivated by laser light, its optical, paramagnetic, and catalytic properties become the same as before irradiation. In an excited state, the dissociation properties of compounds are known to change [10]. Absorption of a quantum of laser light by a complex of copper with histidine ligands, including in its composition a protonated histidine residue, leads to its conversion into the excited state. This causes a fall of pK to values below the pH of the medium (5.8-5.9) and leads to dissociation of the proton from His-61 and to restoration of the bond between copper and zinc through the imidazole anion thus formed.

The wide distribution of organocopper complexes, similar to that present in the active center of SOD, in the structure of many enzymes of both animals and plants [4] suggests a similar mechanism of photoregulation of their function by red light.

Under pathological conditions at the stage of reversible changes, a most important factor is evidently a change toward acid pH [5]. An increase in the hydrogen ion concentration may lead to the appearance of protonated forms of enzymes, possessing modified catalytic activity [5, 7]. Red light can evidently interact with these forms of enzymes by inducing deprotonation of complexes which accept radiation. Considerations such as these explain the absence of effect of low-energy laser radiation when applied to normally functioning cells and tissues (there is no object with which it can interact) and the marked stimulating effect in the case of irradiation of objects under extremal or pathological conditions.

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