Effect of Helium-Neon Laser on Activity and Optical Properties of Catalase

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The effects of laser (632.8 nm) on functional and spectral properties of catalase at pH 6.0-7.4 were studied. Laser irradiation led to photoactivation of the enzyme at pH 7.1-7.4. Changes in the spectral properties of photomodified hemoprotein were found in the absorption spectrum of the protein component: apoenzyme displayed protective effects in relation to ferroporphyrin. Structural modifications of catalase induced by helium-neon laser irradiation correlated with its functional properties. These results can be used in clinical practice to design the individual management program.

Key Words: *helium-neon laser; catalase; absorption spectrum*

Nondrug therapy is now gaining wide acceptance in Russia. Starting from the 1980s, laser irradiation attracts considerable attention. Experimental and clinical studies showed that laser irradiation attenuates inflammatory reactions by shortening the exudative and proliferative phases, inducing vasodilation, improving blood flow in tissues, and stimulating regeneration [11]. It is believed that these effects are related not only to photodynamic effects of the laser beam increasing intracellular Ca^{2+} concentration and activating blood cells, but also to photolysis of NO metallocomplexes, which promotes the formation of endothelium-derived relaxing factor [3].

Identification of the acceptor of light energy is the topical problems. A comparison of the absorption maxima of biologically important compounds with the wavelength of a helium-neon (He-Ne) light suggests that iron- and copper-containing enzymes play the major role in the absorption of radiation. It was reported that low-energy red laser radiation activates some metalloenzymes [3,5,9]. However, the mechanisms of laser effects on biological objects are still unclear and, therefore, the individual patient management programs are based entirely on an empirical approach.

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During the alteration phase of inflammation, catabolic processes in inflamed tissues cause metabolic disturbances in the major component of the connective tissue [6]. Neutrophilic granulocytes (the main source of extracellular H_2O_2) migrate into the inflammatory focus [6,12]. This leads to tissue acidosis, and high H^+ concentrations affect blood cells migrating into the inflammatory focus.

From this point of view, studies of the effects of He-Ne laser on structural and functional properties of catalase at pH 6.0-7.4 are of considerable interest. Since homeostasis is aimed at the maintenance of a dynamic equilibrium by attenuating the effects of various disturbing factors, physicochemical properties of hemoprotein at pH 7.0-7.4 are studied by us in details.

MATERIALS AND METHODS

Catalase (5×10^{-6} mol/l) from bovine liver (Olaine chemical plant) was used. The enzyme was dissolved in 0.01 M phosphate buffer (pH 6.0-7.4). An aliquot of hemoprotein solution (4 ml) was irradiated with He-Ne laser (632.8 nm, 4-6 mW) for 5, 10, and 15 min.

Catalase absorption spectra were recorded on a SF-46 spectrophotometer. Standard 10-mm quartz cuvettes were used. Optical density was recorded with increments of 5 nm or 1 nm (at absorption maxima).

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Catalase activity was measured spectrophotometrically by optical density at 250 nm (absorption maximum of H_2O_2) [15]. The amount of H_2O_2 (in moles) degraded by 1 mg catalase over 1 sec was taken as a unit of activity.

The results were analyzed using Student's *t* test (Statgraphics software); the differences were considered to be significant at $p<0.05$.

RESULTS

The catalytic activity of catalase was maximum at pH 6.2-6.9. At pH 7.1-7.4 catalase activity remained practically unchanged, being 50% of the maximum.

Catalase absorption spectra at pH 6.0-7.4 were characterized by the presence of two peaks corresponding to protein (maximum at 256-280 nm) and hematin components of the enzyme (maximum at 402 nm) and a diffuse band at 618-626 nm (Fig. 1, *a*). The absorption spectrum of catalase at pH 6.6 recorded by us agrees with published data [10]. The absorption spectra at pH 2.8 and 12.0 demonstrate changes in the shape and intensity of both peaks [1]. This indicates structural modifications of catalase molecule in strongly acidic or alkaline medium (compared to neutral medium).

Changes in pH did not affect light absorption by the hematin component of the molecule. The intensity of the Soret band decreased only at pH 6.0 (Fig. 1, *a*). Changes in spectral characteristics of the protein solution were found only in the UV band of the absorption spectrum. Increasing the concentration of H^+ shifted the absorption maximum towards the short-wave region (Fig. 1, *b*). This shift was maximum at pH 6.0 (to 256 nm). These data indicate changes in the microenvironment of chromophores in the protein component of the enzyme. It can be suggested that the contribution of phenylalanine residues into the total spectrum of catalase increases, and light absorption by tyrosine and tryptophan residues decreases (the molecule contains 30 phenylalanine residues [10]).

The intensity of absorption peaks of the protein component at pH 6.3-7.3 remained unchanged. The optical density at the absorption maximum at pH 6.0 and 7.4 increased by 26 and 5.7%, respectively, compared to that at pH 6.6 (optimum for catalytic activity of the enzyme, Fig. 1, *a*). This was probably associated with exposure of chromophores (aromatic amino acids) to the solvent.

Light scattering in protein solutions was estimated at 330 nm corresponding to minimum absorption of the hemoprotein [2]. This parameter remained unchanged at pH 6.0-6.6 and decreased with alkalization (by 18% at pH 7.0, 7.1, and 7.4 and by 23% at pH 7.2 and 7.3). Since the absorption spectrum in this pH range characterizes the interaction between the protein and hematin components of the enzyme, our findings suggest that various H^+ concentrations change structural and functional properties of catalase by affecting binding of apoenzyme to hematin.

In further experiments, we analyzed properties of photomodified catalase.

At pH 6.0-7.0, functional properties of catalase significantly changed only after a 15-min He-Ne laser irradiation (Fig. 2). At pH 7.1-7.4, catalase activity increased after irradiation for 5 and 10 min. It should be emphasized that in the studied pH range (except for pH 7.4), prolonged exposure to He-Ne laser (15 min) decreased catalase activity. Hence, low-intensity He-Ne laser irradiation dose-dependently changed functional properties of catalase. Structural and functional

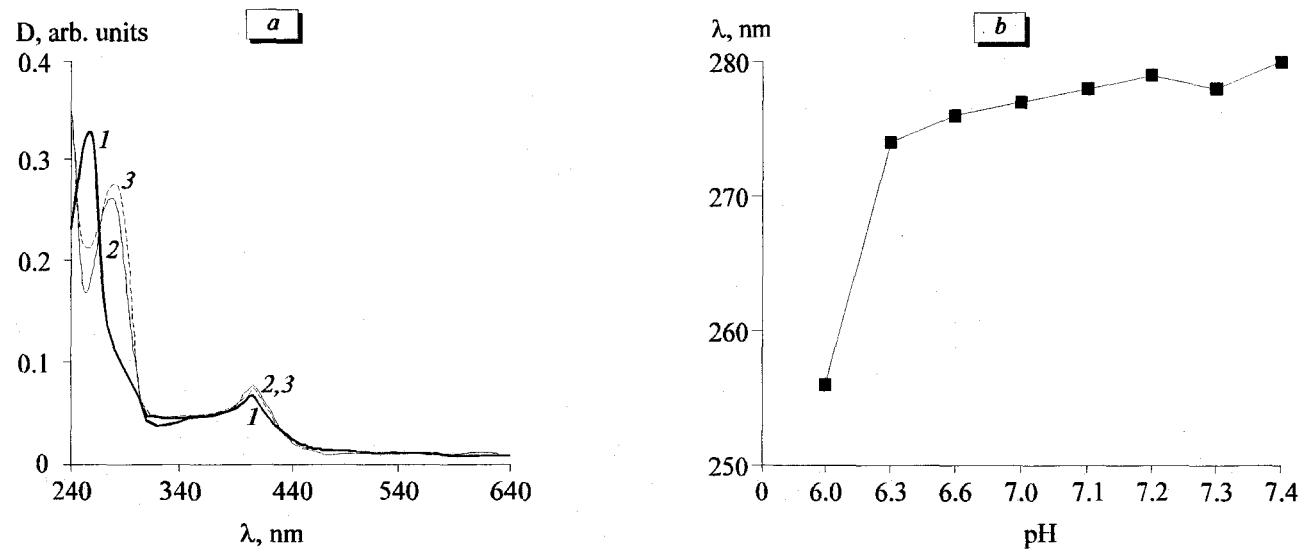

Fig. 1. Absorption spectra of catalase (*a*) and absorption maximum of its protein component (*b*) at pH 6.0 (*1*), 6.6 (*2*), and 7.4 (*3*).

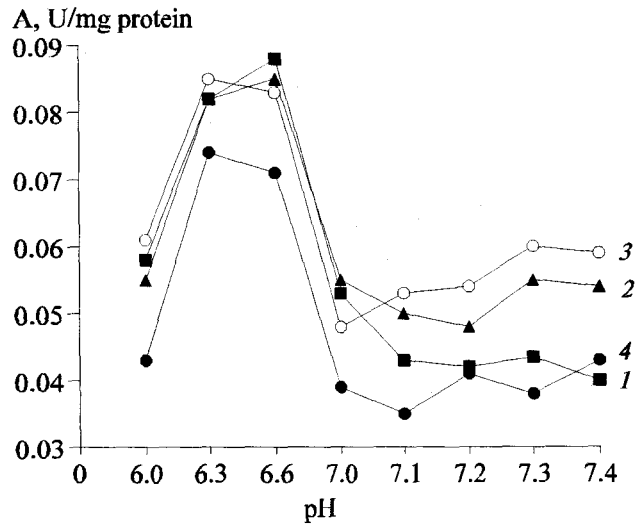

Fig. 2. Catalase activity (A) as a function of pH after exposure to He-Ne laser: native enzyme (*1*); irradiation for 5 (*2*), 10 (*3*), and 15 min (*4*).

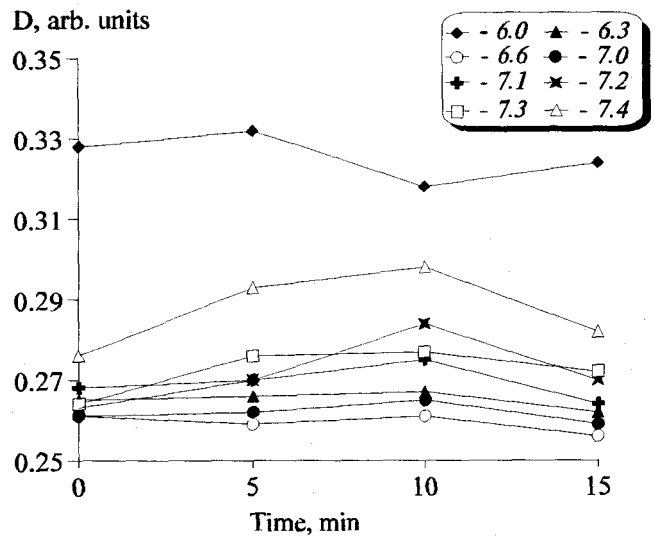

Fig. 3. Optical density of catalase at the absorption maximum of its protein component as a function of the time of He-Ne laser irradiation at pH 6.0-7.4.

properties of catalase at H^+ concentrations corresponding to its maximum catalytic activity were practically insensitive to monochromatic coherent light with a wavelength of 632.8 nm.

Spectral characteristics of catalase modified by He-Ne laser irradiation were studied to evaluate possible structural modifications, which determine changes in activity of this protein macromolecule. The analysis of absorption spectra showed that these changes involved the protein component of the molecule, rather than ferroporphyrin chromophores. The energy absorbed by hematin is probably transferred to apoprotein, which indicated protective effects of the protein globule on hematin. Similar effect was determined previously for catalase irradiated with UV light at 250-400 nm [10]. Protective effects of the polypeptide chain on the hematin component of catalase were revealed after exposure of its water solutions to γ-irradiation [12].

The intensity of the absorption peak in the UV band decreased after a 15-min irradiation of the enzyme at pH 6.3-6.6 (Fig. 3). At pH 7.0, light absorption by protein chromophore remained unchanged. At pH 7.1-7.4, He-Ne laser irradiation for 5 and 10 min increased optical density in the absorption maximum of the protein component; after a 15-min irradiation, the intensity of the absorption band decreased. Light scattering was unchanged under these experimental conditions.

L. I. Irzhak *et al.* [7] analyzed physicochemical and functional properties of human hemoglobin exposed to *in vitro* laser irradiation. It was shown that irradiation of hemoglobin (as well as catalase) affects mainly chromophores of aromatic amino acids in polypeptide chains.

These data indicate that structural modification of catalase correlate with its functional activity. High optical density in the UV band after exposure to laser irradiation for 5 and 10 min at pH 7.1-7.4 corresponds to increased enzyme activity. At the same time, the decrease in light absorption by the catalase protein component after a 15-min exposure to He-Ne laser irradiation in the studied pH range is accompanied by reduction of catalase activity.

These data contribute to understanding of the molecular mechanisms of He-Ne laser effects on complex proteins and explain the opposite changes in catalase activity during laser therapy at various stages of inflammation [4,5,8,9]. Our results can be used in clinical practice to design the individual patient management program.

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