Effect of Laser Radiation on Physicochemical and Functional Properties of Human Hemoglobin *In Vitro*

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Exposure to laser radiation increases pH and isoelectric point of human hemoglobin solution, improves the acid-base properties, increases affinity for oxygen, and decreases the Bohr effect in comparison with intact hemoglobin. The mechanisms underlying these changes are discussed.

Key Words: *hemoglobin; laser radiation; acid-base properties; p50*

Irradiation with light from a low-intensity He-Ne laser increases the degree of blood oxygenation [5]. However, it is unclear to what extent this phenomenon depends on physicochemical and functional properties of hemoglobin (Hb). The present study is an attempt to clarify this issue. Our goal was to find out how Hb properties are affected by laser radiation.

MATERIALS AND METHODS

Hemoglobin solution (150 μM) was prepared from fresh donor blood. After separation of plasma by centrifugation, erythrocytes were destroyed with chloroform without washing [2]. Hemoglobin solution was irradiated in a cuvette (layer thickness 2 cm) for 10, 15, or 20 min at ≈20°C with red light from an He-Ne laser at a wavelength of 632 nm and an output power of 25 mW; the distance between the laser and cuvette was 20 cm. Immediately after irradiation, Hb was analyzed for solubility, acid-base properties, pH, and affinity for oxygen. The Hb solubility was estimated by optical density (OD) at 37°C upon titration with 0.1 N NaOH in a KFK-2 photoelectric colorimeter (the initial OD 0.12 unit). Changes in pH were measured in a pH-meter. The acid-base properties were assessed by the ability of Hb to establish ionic equilibrium in distilled water. For this purpose distilled water (8 ml) was added to a thermostated cuvette (37°C), and pH was adjusted to 6.65 or 7.8. Hemoglobin solution (0.02 ml) was then added with constant stirring, and pH was measured again after 3 min. The Hb affinity for oxygen was estimated by the p50 value (mm Hg) measured as described [6]. The data were analyzed using Student's test for small samples.

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RESULTS

The initial pH of control Hb solutions was 6.39±0.07, the corresponding OD being 0.168±0.010. Titration with NaOH increased pH, which was accompanied by OD rise to 0.247±0.010 with subsequent decline (Fig. 1). Judging from these values, Hb solubility was minimal within a narrow pH range, where electrostatic repulsion of Hb molecules is minimal and the distance between oppositely charged molecules is short. These pH values correspond to the isoelectric point in the control samples (6.70±0.03), which agrees with the literature data [4].

Laser radiation rendered Hb more alkaline: pH of Hb samples increased to 6.48±0.05 after 10 min of exposure, 6.59±0.05 after 15 min, and 6.67±0.07 after 20 min. The titration curves shifted to higher

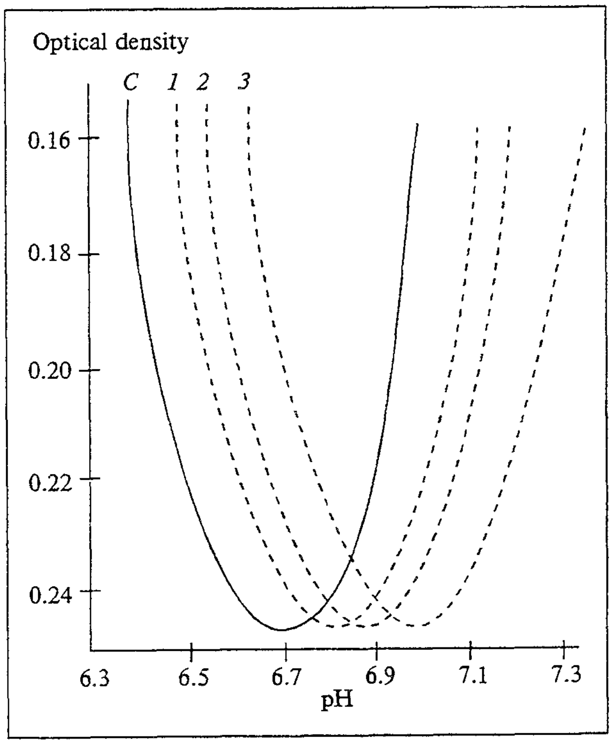

Fig. 1. Hemoglobin solubility as a function of pH of the medium (n=10). Here and in Fig 2: *1*, *2*, and *3* are 10, 15, and 20 min of laser irradiation, respectively. *C*) control samples.

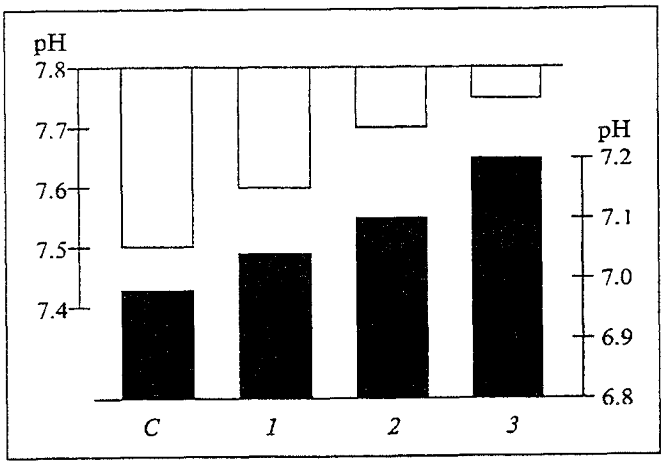

Fig. 2. pH values of the medium after ionic equilibrium was reached. *C*) control Hb solutions.

pH, so that after 10 min of irradiation the isoelectric point increased by 17% compared with that in the control samples, after 15 min of irradiation it increased by 28%, and after 20 min by 41%.

In the control samples, ion equilibrium between Hb and medium was reached after 3 min, when the medium pH was 0.33 unit higher than the initial value of 6.65 and 0.29 unit lower than the initial value of 7.8 (Fig. 2). After 20 min of irradiation, the ability of Hb to increase the medium pH was almost 60% higher than in the control, while its ability to acidify the medium decreased to the same extent.

The p50 values in the control samples did not differ from the normal [1]. Laser irradiation for 10 min had no appreciable effect on the functional properties of Hb. After longer exposure, particularly after 20 min, Hb affinity for oxygen increased considerably (Table 1).

Doubling the exposure time from 10 to 20 min increased Hb affinity for oxygen by 12%. This is much lower compared with the shift in physicochemical properties (Fig. 3), which showed almost linear dependence on the exposure time (radiation dose). These properties are related to each other and reflect specific features of ionization of Hb molecules induced by laser radiation. A question arises: why changes in Hb affinity for oxygen are smaller than those in its physicochemical properties? This discrepancy can be at least partially explained by the fact that Hb binds oxygen when the samples are stirred in the air, and oxyHb is then irradiated. Thus, Hb isolated from venous blood is "arterialized" and acquires the R conformation with specific molecular organization and functional properties, for example, high affinity for oxygen [1]. This accounts for the statistically significant increase in Hb affinity for oxygen observed only after a 20-min exposure. In addition, the discrepancy in the dynamics of physicochemical properties and oxygen affinity of Hb may be associated with determination of these parameters [6].

In irradiated samples, the relationship between p50 and pH was much weaker than in the control. In fact, the Bohr effect calculated from the formula: $H^{+}=\Delta \lg p50/\Delta pH$ was equal to 0.28, when the data obtained after 10-min and 20-min exposure were compared. This value is almost two times lower than that for native human Hb [1]. Consequently, changes in Hb affinity for oxygen observed in this study were determined not only by pH of the medium. To a certain extent it was influenced by metHb, whose generation, if any, in response to laser radiation so far has not been studied. A reduction in the relative proportion of metHb was observed in whole blood after laser irradiation [5]. The mechanism by which hemic iron is oxidized does not operate in Hb solu-

TABLE 1. p50 Values (mm Hg) in Control and Irradiated Hb Samples ($\bar{X}\pm m$; n=10)

Exposure time, min	Control samples	Irradiated samples
10	26.4±0.7	25.8±0.8
15	26.3±0.6	24.3±0.7
20	26.3±0.6	22.5±0.9

tion. Previously, we showed that metHb formed in Hb solutions irradiated with ultraviolet light contributes to an increase in the Hb affinity for oxygen and to reduction in the Bohr effect [3]. However, the main mechanism underlying alterations of Hb function involves molecular reconformations, when the chromophor groups of aromatic amino acids utilize the energy of short waves. Although the waves of red light from a He-Ne laser are twice as long as those of ultraviolet light, the alterations they cause in Hb properties are virtually the same. Increased affinity for oxygen and decreased Bohr effect have been shown for a number of abnormal hemoglobins (Hb Iwata, Hb Boston, Hb St. Etienne, Hb Zurich, and several others) in which aromatic amino acids (His, Tyr, Phe) have been substituted [1]. Similarities between functional disorders caused by such substitutions, ultraviolet light, and laser radiation suggest that these disorders may have a common origin; thus, the irradiation of Hb solutions with red light from a He-Ne laser affects, directly or indirectly, the chromophores of aromatic amino acids in the polypeptide chains of Hb molecule. A decrease in the Bohr effect may result from the damage to His-121α and His-146β, on which this effect depends by 50% [7-9]. Changes in prosthetic groups of hemes with their system of conjugated bonds caused by laser radiation may also contribute to this decrease.

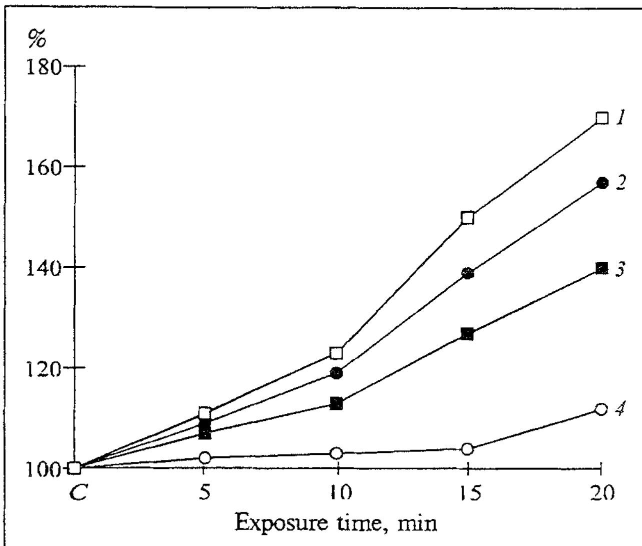

Fig. 3. Changes in Hb parameters as a function of exposure time. The corresponding values in the control samples (*C*) are taken as 100%. *1*) pH; *2*) acid-base properties; *3*) isoelectric point; *4*) hemoglobin affinity for oxygen (p50).

REFERENCES

1. L. I. Irzhak, *Hemoglobins and Their Properties* [in Russian], Moscow (1975).
2. L. I. Irzhak and Yu. V. Loshchinskii, *Byull. Eksp. Biol. Med.*, **115**, No. 6, 565-566 (1993).
3. L. I. Irzhak and V. I. Tayurskii, *Zh. Evolyuts. Biokhim.*, **22**, 507-508 (1986).
4. V. V. Men'shikov, *Lectures on Clinical Biochemistry* [in Russian], Pt. 2, Moscow (1976).
5. A. V. Shaposhnikov and G. A. Linnik, *Izv. Severo-Kavkaz. Nauch. Tsentra Vyssh. Shkoly* (*Journal of the North-Caucasian Center for Higher Education*), No. 3-4, 111-113 (1992).
6. M. Edwards and R. Martin, *J. Appl. Physiol.*, No. 21, 1898-1902 (1968).
7. J. Kilmarin, J. Breen, G. Roberts, and C. Ho, *Proc. Natl. Acad. Sci. USA*, **70**, 1246-1249 (1973).
8. J. Kilmartin and J. Wooton, *Nature*, **228**, 766-767 (1970).
9. K. Nishikura, *Biochem. J.*, **173**, 651-657 (1978).