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Study of photodissociation parameters of carboxyhemoglobin

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Abstract. **The general properties of photodissociation of carboxyhemoglobin (HbCO) in buffer solutions of whole human blood are studied by the flash photolysis method on a setup with intersecting beams. It is shown that the efficiency of photoinduced dissociation of the HbCO complex virtually linearly depends on the photolytic irradiation intensity for the average power density not exceeding 45 mW cm^{-2}. The general dissociation of the HbCO complex in native conditions occurs in a narrower range of values of the saturation degree than in model experiments with the hemoglobin solution. The dependence of the pulse photolysis efficiency of HbCO on the photolytic radiation wavelength in the range from 550 to 585 nm has a broad bell shape. The efficiency maximum corresponds to the electronic Q transition (porphyrin $\pi - \pi^*$ absorption) in HbCO at a wavelength of 570 nm. No dissociation of the complex was observed under given experimental conditions upon irradiation of solutions by photolytic radiation at wavelengths above 585 nm.**

Keywords: *carboxyhemoglobin, photodissociation, natural photolysis conditions.*

1. Introduction

It is known that the reason for poisoning by carbon monoxide (CO) is the formation of stable carboxyhemoglobin (HbCO) in blood [1, 2]. At present the only efficient method for reducing the HbCO level is hyperbaric oxygenation [3]. The authors of papers [4 – 6] propose the alternative method of detoxification of carbon monoxide poisoning by means of the reversible photolytic dissociation of the heme – CO bond in a carboxyhemoglobin molecule in conjunction with the use of some molecules capturing carbon monoxide. They showed that sodium hypochlorite molecules can be used for this purpose [6]. It is obvious that the development of this method requires the detailed knowledge of the physical foundations of the HbCO photolysis.

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Hemoglobin and its derivatives are most thoroughly studied molecules among all hemoproteins. Let us describe briefly the photodissociation of HbCo. The Fe – CO bond dissociates due to the reconstruction of the molecular orbitals of the complex from binding to antibinding ones [7]. The reconstruction occurs due to the $\pi - \pi^*$ absorption of light by porphyrin at a wavelength of 570 nm [8]. As a result, the Fe – CO bond can be weakened due to a fast (50 fs) reversible charge transfer process from the d_π orbital of iron, which is involved in binding with CO, to the vacant orbital of the porphyrin ring [9]. Due to the weakening of the bond between Fe and C atoms, the crystalline field of the carbon atom decreases so that electrons of the iron atom are redistributed from the t_{2g} orbitals to the e_g orbitals, resulting in the final neutralisation of the Fe – CO bond. In this case, the reverse transition of an electron with the circular orbital of porphyrin occurs to one of the t_{2g} orbitals of iron, which is already not related to the carbon atom in the CO molecule.

The dissociation of the Fe-CO bond occurs at several stages involving the absorption of light by the complex at a wavelength of 570 nm, the transition to the first excited Q state, the 'vibrational' increase in the Fe – CO distance, and intersystem crossing to the dissociative state, in which the CO molecule moves away from the Fe atom. The CO molecule can either again bind to the iron atom or diffuse from the heme pocket [10 – 12]. For the convenience of description, the α spiral of each globule is divided into regions form A to H and all amino acids are successively numerated. The two most probable diffusion paths are considered: the CO molecule can leave the globule either due to rotational vibrations of the His64(E7) globule site or, which is less probable, through the hydrophobic channel between sites B, G, and H [12, 13]. The CO diffusion at room temperature occurs via the so-called holes (density fluctuations) in the globule, i.e. through cavities formed due to microscopic vibrations of globular chains [14].

At the same time, after the dissociation of the Fe – Co bond, the stress of the bond between iron and nitrogen of the imidazole ring of proximal histidine decreases. As a result, proximal histidine is displaced deep in the helix, which leads to allosteric transformations of the entire tetramer. The intersubunit bonds are strengthened and neighbouring subunits in dimers are transformed from the R conformation to the more energetically favourable T conformation, which determines the type of the saturation curve of carboxyhemoglobin [15 – 21]. Due to the successive transformation of subunits from the 'weakened' R conformation to the 'strained' T conformation during

photolysis, the dependence of the number of bound tetramers on the number of ligands has the characteristic s shape.

The dynamics of HbCO dissociation is quite complicated [22–30]. Without going into details, we point out only that relaxation to a free T globule occurs within a microsecond after the absorption of light by heme in a subunit. There exist four schemes of successive transformations from six intermediate states.

Apart from a huge amount of information on the HbCO photolysis available at present, more specific data concerning the proceeding of this reaction under natural conditions are required in our case.

It is shown, for example, that photochemical processes induced by UV radiation in hemoglobin under model and native conditions are different as a whole [31]. Therefore, it is necessary to reveal differences in the general properties of photodissociation under model conditions and conditions close to natural experimental conditions. In our opinion, the most illustrative in this respect is the dose-effect dependence, which gives information on the linearity of the process, and also the dependence of the photolysis efficiency on the complex concentration in the material under study. It was shown earlier that the saturation degree of a solution is a parameter that determines in principle the possibility of observing the reaction [4, 5]. However, the dependence of the photolysis efficiency on the saturation degree under native conditions can differ from this dependence in model experiments. In addition, this dependence should demonstrate generally speaking the limits of applicability of photolysis for detoxification for different degrees of poisoning.

The question of whether the photodissociation efficiency depends on the photolytic radiation wavelength remains open. Also, it is not clear how far in the red spectral region the photolytic radiation can produce dissociation of the complex. Undoubtedly, the irradiation of blood saturated with HbCO at wavelengths above 570 nm will allow one to increase the safe intensity of laser radiation and to minimise the probability of damaging erythrocytes due to the reduction of absorption in oxyhemoglobin. The dependence of the photolysis efficiency for different hemoglobin forms on the photolytic radiation wavelength has not been adequately studied. It is stated usually that this dependence is absent. However, as mentioned above, it is the Q transition that causes the dissociation of the Fe–CO bond. In this case, it is reasonable to assume that the red boundary of the photolysis efficiency is located in the region of the HbCO absorption band (560–600 nm).

In this paper, we studied the photolysis of carboxyhemoglobin caused by pulsed laser irradiation of human blood solutions saturated with carbon monoxide. To investigate the photodissociation of carboxyhemoglobin in these solutions, we solved the following problems:

(i) The experimental setup for studying the photodissociation of the HbCO complex was built and optimal parameters at which the detection of photolysis of the complex is most reliable were determined.

(ii) The dependence of the HbCO photodissociation efficiency on the photolytic radiation intensity was obtained.

(iii) The dependence of the HbCO photodissociation efficiency on the complex concentration in solutions was obtained and compared with model studies.

(iv) The dependence of the HbCO photodissociation efficiency on the photolytic radiation wavelength in the region of the α absorption band of HbCO was plotted.

2. Materials and method

We studied blood samples from one donor – a healthy 23 year old man. The Hb level analysed in blood was 155 g L^{-1} and the HbCO level did not exceed 10 %. The non-hemolytic solution was prepared by diluting the initial blood with a phosphate buffer (0.1 M, Na_2HPO_4+ NaH_2PO_4) maintaining a constant pH level of 7.4 (according to [32]) to obtain the optical density of the solution ~ 1 at the photolytic radiation wavelength (according to [4]). The final dilution corresponded to the protein heme concentration of 0.5×10^{-4} M. Then, sodium dithionite (0.1 g mL^{-1}) was added to the solution, which provided the presence of only HbCO and DoxHb (desoxyhemoglobin) in the solution. The solution saturation with carbon monoxide was achieved by its blowing through the solution. The initial concentration of HbCO in the solution was reduced by blowing argon through the solution (to produce the oxygen-free atmosphere) or by dilution with an unsaturated prepared solution. We used in experiments freshly prepared solutions at room temperature.

The studies were performed by using the experimental setup based on the scheme with intersecting beams [29–31] (Fig. 1).

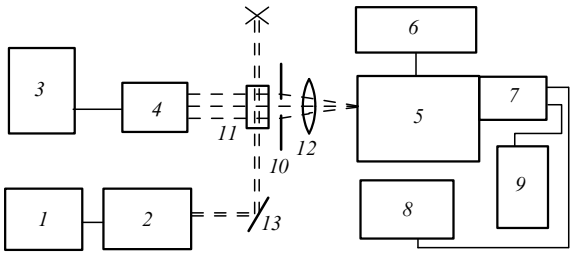

Figure 1. Scheme of the experimental setup: (*1*) power supply and laser controller; (*2*) laser; (*3*) probe laser power supply; (*4*) probe laser; (*5*) monochromator; (*6*) power supply and monochromator controller; (*7*) photomultiplier; (*8*) photomultiplier power supply; (*9*) digital multimeter; (*10*) rectangular aperture; (*11*) quartz cell with solution; (*12*) focusing lens; (*13*) steering mirror.

Two photolytic radiation sources were used. The dependences of the photolysis efficiency on the radiation intensity and carboxyhemoglobin concentration were studied by using the 532-nm second harmonic of a *Q*-switched LTI-404 Nd : YAG laser emitting 25-ns, 60-kW pulses. The laser beam cross section area was 0.5 cm^2. By varying the pulse repetition rate from 1 to 50 Hz and, correspondingly, the average power from 1.5 to 42.5 mW, we obtained the average intensity of photolytic radiation from 3 to 85 mW cm^{-2}.

The dependence of the photolysis efficiency on the photolytic radiation wavelength was studied by using a tunable pulsed solid-state LKI-301 laser pumped by a pulsed LTI-404 Nd : YAG laser. The active media of the LKI-301 laser were polymethyl methacrylate matrices of two types doped either with the unsubstituted rhodamine dye or rhodamine 6G. Stable lasing was achieved in the

wavelength range from 544 to 600 nm. The maximum pulsed power at the maximum of luminescence of unsubstituted rhodamine was 4 kW.

Integrated variations in the optical density of solutions caused by photolytic radiation were detected with a F-4800 digital multimeter by measuring the voltage across the load resistance of a PEU-30 photomultiplier detecting probe radiation. The probe radiation wavelength was selected with a MCD-2 monochromator scanning automatically the spectrum. The accuracy of measuring the optical density ΔD was 0.001 for the photomultiplier signal level $U_0 = 1$ V.

The relative concentration of HbCO in the solution was measured with a spectrophotometer by the expression [33]

$$C_{\mathrm{HbCO}} = \frac{D_{534} - 0.72D_{563}}{0.43D_{563}},$$

where D_{534} and D_{563} are the optical densities of the solution at wavelengths 534 and 563 nm, respectively.

By measuring the dependence of the HbCO photolysis efficiency on the photolytic radiation wavelength, it is necessary to take into account the wavelength dependence of the laser radiation. In addition, the absorption in the solution in the wavelength range under study decreases with increasing wavelength. It is clear that the photolysis efficiency, which is directly related to the absorbed energy, also depends on the wavelength. For this reason, this dependence was measured in the general form by normalising the output voltage of the photomultiplier to the absorption in the solution at the appropriate wavelength and to the spectral dependence of the tunable laser intensity:

$$I(\lambda) = \frac{I_{\mathrm{exp}}^{\mathrm{rel}}(\lambda)}{I_{\mathrm{sol}}^{\mathrm{rel}}(\lambda) I_{\mathrm{las}}^{\mathrm{rel}}(\lambda)},$$

where $I(\lambda)$ is the photolysis efficiency in relative units; $I_{\mathrm{exp}}^{\mathrm{rel}}(\lambda)$ is the experimental dependence of the output photomultiplier voltage on the photolytic radiation wavelength normalised to its maximum; $I_{\mathrm{sol}}^{\mathrm{rel}}(\lambda)$ is the absorption spectrum of the solution normalised to its maximum; and $I_{\mathrm{las}}^{\mathrm{rel}}(\lambda)$ is the wavelength dependence of laser radiation power normalised to its maximum. No normalisation to the absorption spectrum of the cell was performed because absorption in the cell was independent of the wavelength in the spectral range under study.

The measurement error was determined as a sum of the experimental error and the scatter in the set of measurements. The experimental error was determined by the scale factor of the digital multimetre equal to 1 mV.

3. Results and discussion

Upon exposing solutions not saturated with carbon monoxide to photolytic radiation, no changes in the photomultiplier output signal were detected, whereas in solutions saturated with CO, we observed variations in transmission. Therefore, variations in the output signal upon irradiation of solutions saturated with CO are directly related to the presence of carboxyhemoglobin in the solution.

After photolytic irradiation, the optical density of the solution returned to its initial value and did not change in the absence of irradiation, which suggests that dissociation is reversible and protein is stable to irradiation (for the intensities of photolytic and probe radiations used in experiments).

We found from the transmission spectra of solutions exposed to photolytic radiation that strongest changes in transmission at the specified average irradiation intensity occurred at wavelengths 540, 570, 555, and 435 nm. These wavelengths correspond to strongest changes in the optical density of blood solutions, in accordance with data presented in [34].

The optical density at 540 nm deceased during irradiation, demonstrating the dissociation of the HbCO complex. The optical density at 570 nm decreased similarly, which was also caused by the photodissociation of carboxyhemoglobin. Absorption at a wavelength of 555 nm slightly increased, which was explained by the appearance of desoxyhemoglobin during photolytic irradiation. Finally, the increase in absorption at 435 nm (the Soret band of desoxyhemoglobin) was strongest, an order of magnitude larger than absolute changes at the wavelengths mentioned above.

Figure 2 shows the dependence of the amplitude of a change (decrease) in the output signal at a wavelength of 435 nm on the average intensity of photolytic radiation during irradiation of blood solutions at HbCO concentrations 57 % and 88 %. Measurements were performed at laser pulse repetition rates of 5, 10 and 25 Hz. An increase in the pulse repetition rate resulted in the proportional increase in the average radiation intensity. The errors presented in Fig. 2 are the scatter of the measurements summed up with the instrumental error ($\Delta I = 1$ mV). Points correspond to the root-mean-square measurement results. One can see that these dependences can be quite accurately approximated by straight lines intersecting the coordinate origin; therefore, even the maximum intensity of photolytic radiation does not cause saturation in the change of the HbCO concentration in the solution. This means that the maximum dissociation efficiency, i.e. the 100 % dissociation of HbCO in the solution can be achieved at a higher average intensity of photolytic radiation and also when the volume in which the reaction proceeds is isolated from the solution to exclude the mixing of free globules with globules bound with the ligand. One can also see from Fig. 2 that as the degree of solution saturation by the carboxyhemoglobin is increased from

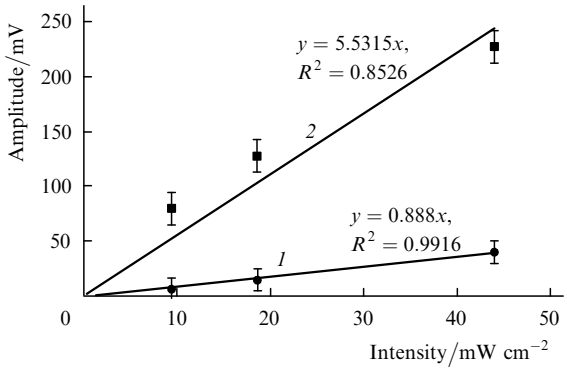

Figure 2. Dependences of the amplitude of a change (decrease) in the output photomultiplier signal at a wavelength of 435 nm on the average intensity of photolytic radiation at 532 nm upon irradiation of the whole blood solution at the HbCO concentrations 88 % (*1*) and 57 % (*2*). The experimental dependences are fitted by the straight lines described by the corresponding equations with the confidence level presented.

57 % to 88 %, a change in the output signal decreases approximately by a factor of six. It is clear from these dependences that photolytic radiation with a pulse repetition rate of 25 Hz provides the maximum effect.

We studied the dependence of the HbCO photodissociation efficiency on the complex concentration by using whole blood solutions with different concentrations of carboxyhemoglobin. The maximum average intensity of photolytic radiation determined by a pulse repetition rate of 25 Hz was 45 mW cm^{-2}. The amplitude of changes in the output signal was detected at wavelengths 570, 540, 555, and 435 nm. Preliminary experiments showed that at all wavelengths, except 435 nm, dissociation was not detected when the HbCO concentration in solutions was lower than 40 % and higher than 80 %. We prepared six groups of solution samples with HbCO concentrations from 40 % to 89 %. Measurements were performed for each of the samples at all four wavelengths. The absolute values of signal variations were averaged in each group of samples (at the given concentration). Figure 3 presents the results obtained. The points show the average values of the scatter in experimental data. Hereafter, errors are the dispersion of measured values summed up with the instrumental error.

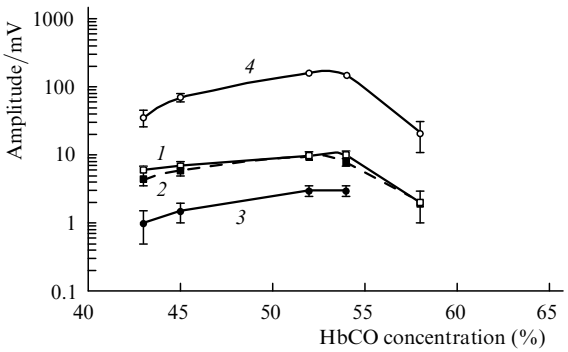

Figure 3. Dependences of the amplitude of a change in the output signal on the HbCO concentration at wavelengths 570 (*1*), 540 (*2*), 555 (*3*), and 435 nm (*4*) upon exposing whole blood solutions to photolytic radiation with the average intensity 45 mW cm^{-2}.

Figure 4 shows the dependences of the relative (normalised to the maximum) output signal amplitude variations on the HbCO concentration measured at different wavelengths. One can see that these dependences are virtually the same within the relative error for all probe radiation wavelengths. This fact suggests that the HbCO photodissociation efficiency is directly proportional to the efficiency of desoxyhemoglobin production and these processes depend on the degree of solution saturation in the same way. The maximum of the signal amplitude change for all the four dependences is observed at the HbCO concentration close to 50 %. The nature of this dependence was discussed in [35]. In our opinion, the dependence of the HbCO photolysis efficiency on the HbCO concentration is determined by the interpulse secondary binding of CO and Hb (for CO molecules outside the globule) and rapid (heminal) binding of CO and Hb (for CO molecules located in the heme pocket of the globule). The competition between these processes depends on the amount of bound HbCO tetramers and free CO molecules in the solvent.

Thus, the HbCO dissociation can be detected most accurately by the change in the output signal level at a

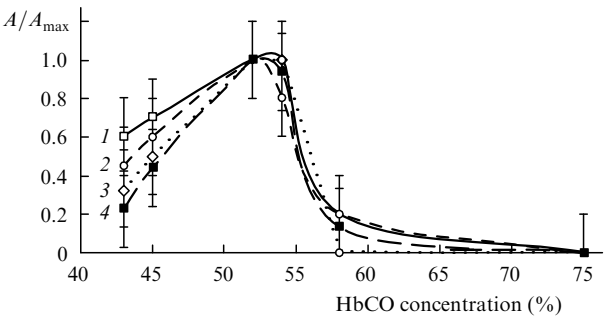

Figure 4. Dependences of the relative change in the output signal on the HbCO concentration at probe radiation wavelengths 570 (*1*), 540 (*2*), 555 (*3*), and 435 nm (*4*) upon exposing whole blood solutions to photolytic radiation of intensity 45 mW cm^{-2}.

wavelength of 435 nm by using the whole blood solution with the initial relative concentration of HbCO close to 50 %. The dissociation efficiency increases with increasing irradiation intensity at least up to 45 mW cm^{-2}. Let us compare the dependence of the photolysis efficiency on the HbCO concentration obtained under our experimental conditions with this dependence in model experiments.

Figure 5 presents data obtained in [4] for solution of horse hemoglobin and the curve obtained in our paper for photolysis at a wavelength of 435 nm (as the most illustrative).

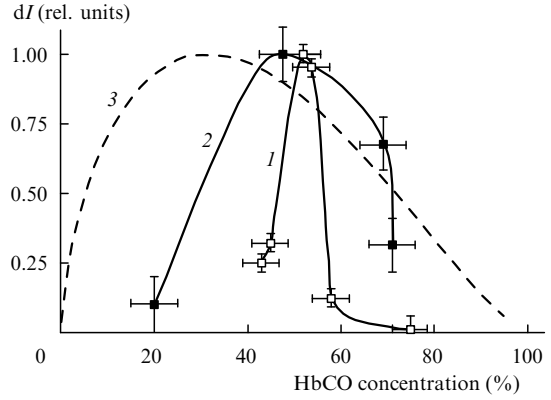

Figure 5. Experimental dependences of the relative dissociation efficiency on the initial HbCO concentration in the buffer whole blood solution (*1*) and horse haemoglobin solution (*2*), and the model dependence for oxyhemoglobin (*3*).

It was pointed out in [4] and is demonstrated in Fig. 5 that experimental points in the region of high saturation degrees (at the HbCO concentration above 45 %) can be approximated by the theoretical curve

$$dI = \frac{nC_{HbCO}^{n-1}}{(1 + C_{HbCO}^{n})^2}$$

for oxyhemoglobin. Here, dI is the relative yield of the HbCO dissociation; C_{HbCO} is the relative concentration of carboxyhemoglobin in solution; n is the index of cooperation set equal to 2.7 (physiological limits are 2.3 – 3). Recall that the index of cooperation (Hill index) is a parameter determining the slope of the saturation curve of hemoglobin and the degree of its affinity to the ligand.

In the region of low saturation degrees [the point of curve (*2*) corresponding to the concentration 20 %], the unambiguous approximation for oxyhemoglobin is impossible even for Hill indices exceeding 2.7. One can see that the experimental dependence of the dissociation efficiency on the HbCO concentration obtained in our paper has a narrower peak of the increase in the signal amplitude compared to the theoretical curve and the curve from [4].

As a whole we can say that the dependence of the HbCO photodissociation efficiency on the complex concentration in solution cannot be unambiguously described by the theoretical dependence for oxyhemoglobin because, unlike curve (*2*) in Fig. 5, even the points corresponding to concentrations above 45 % cannot be fitted within the measurement error by curve (*3*) in Fig. 5.

The integration of these curves should give the s-like dependence of the degree of hemoglobin saturation by carbon monoxide. A narrower peak of the increase in a change in the signal amplitude in whole blood solutions suggests that in this case the curve of HbCO saturation by carbon monoxide is steeper than this curve for free hemoglobin and than the theoretical curve corresponding to oxyhemoglobin. However, this does not mean that the degree of cooperation for the whole blood solution is higher than that for pure hemoglobin solution. This is explained by the fact that the expression obtained in [4] does not reflect the conditions under which the reaction proceeds. The important factors in our experiments are the laser pulse duration and repetition rate. These parameters determine the efficiency of the repeated binding of Fe and CO of two types – the rapid heminal binding in the case when the ligand did not leave yet the heme pocket, and the interpulse binding of a free CO molecule with heme. In addition, it is clear that the density of tetramers, which can be neglected in solutions of free globules, makes a considerable contribution to dissociation as a whole. Therefore, generally speaking, the dependence obtained by using the integral from the experimental curve is no the real curve of hemoglobin saturation by carbon monoxide.

The 'narrower' dependence of the dissociation efficiency in blood solutions compared to this dependence for whole hemoglobin solutions means that the integrated photodissociation efficiency under native conditions is lower than in model conditions. Photolysis in an erythrocyte is a more 'rigorous' process than photodissociation in a free tetramer. This result is consistent with the earlier data obtained in [36]. The authors of this paper showed that the HbCO dissociation in erythrocytes occurs at two stages and is a whole hampered compared to the dissociation of HbCO in the free form. The first stage is the so-called normal phase, which is identical to the HbCO photolysis in the solution of free tetramers. The second phase is the so-called slow phase. The authors of [36] explain it by the fact that the escape of CO molecules from cells is hindered due to decelerated diffusion through the erythrocyte membrane, dense packing of tetramers and an increase in the probability of recombination with neighbours. In this case, the ratio of the integrated photodissociation rates in solution and erythrocyte is 5 : 4.

It is clear as a whole that in solution only a globule prevents the escape of a dissociated CO molecule to solvent. However, in an erythrocyte a CO molecule located in a heme pocket after the rapture of the bond with iron will again bind with iron with a greater probability than a free tetramer. This occurs because the dense packing of a tetramer in a cell should considerably reduce the conformation mobility of a globular chain and, hence, reduce the probability of diffusion of CO from the heme pocket. In addition, even CO molecules that escaped from the globule can again bind with neighbouring free tetramers. Finally, the last obstacle for the escape of CO from erythrocytes is the erythrocyte lipid membrane, which also reduces the efficiency of CO diffusion to solvent.

We studied the dependence of the HbCO photodissociation efficiency on the photolytic radiation wavelength by irradiating solutions with relative HbCO concentrations 40 % and 60 % by pulses from a tunable LKI-301 laser operating at a pulse repetition rate of 25 Hz. The results are presented in Fig. 6. We took arithmetic mean values from a set of detected changes $I_{exp}(\lambda)$ in the output signal for each laser wavelength. To reduce the probability of error in the measurements of $I_{exp}(\lambda)$, the laser wavelength was monotonically increased or decreased or was fixed. As a result, a set of values $I_{exp}(\lambda)$ corresponded to each laser wavelength, which were obtained for different samples and were then averaged.

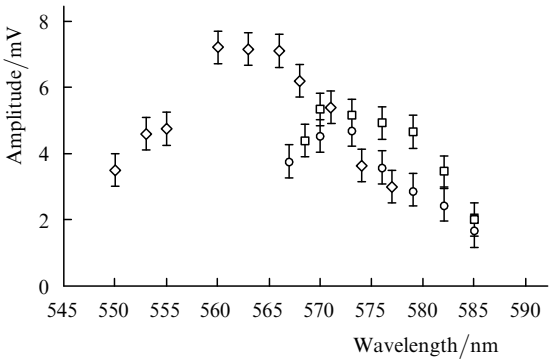

Figure 6. Dependences of the amplitude of a change (decrease) in the output signal at a wavelength of 435 nm on the photolytic radiation wavelength during irradiation of solution containing 60 % of HbCO by a unsubstituted rhodamine laser (◇) and rhodamine 6G laser (□) and also during irradiation of solution containing 40 % of HbCO by a rhodamine 6G laser (○).

Variations in the output signal measured in solutions depending on the photolytic radiation wavelength were normalised to the relative absorption spectrum of solutions and the relative power spectrum of the LKI-301 laser. The dependence of the relative HbCO photodissociation efficiency on the photolytic radiation wavelength obtained in this way is presented in Fig. 7.

The maximum photodissociation efficiency corresponds to the maximum of the α absorption band of HbCO. This fact is consistent with results obtained in [6] and [7]. According to these works, the absorption of light at 570 nm is a 'trigger mechanism' of HbCO photolysis.

Figure 8 presents the energy level diagram for the atomic Fe – CO group involved in photolysis, indicating the nature of electronic bonds [8]. It is obvious that the dissociation probability (when the Fe – CO system is in RS state) is directly related to the probability of population of the Q state, i.e. to the Q transition probability. In turn the probability of this transition is determined by the population of the lower vibronic state of the system.

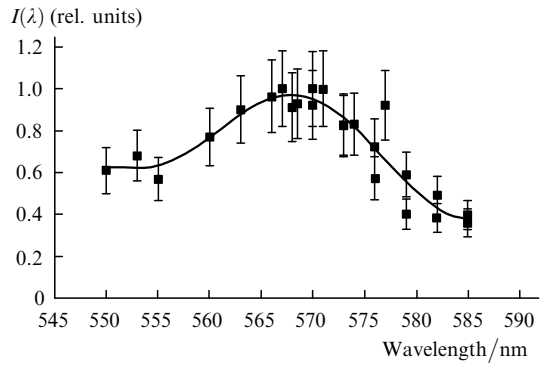

Figure 7. Dependence of the relative photodissociation efficiency of the HbCO complex in the buffer human blood solution on the photolytic radiation wavelength.

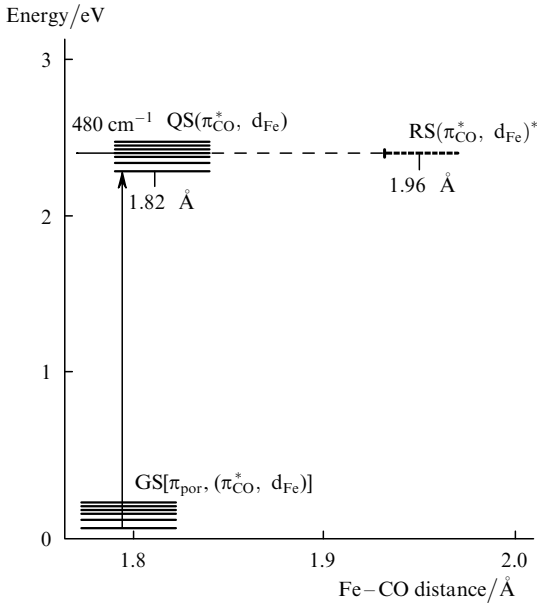

Figure 8. Energy level diagram for the initial dissociation stage of the heme–CO complex [8]; GS, QS, and RS are the ground state, the first excited (quintet state), and unstable (repulsive state), respectively. The equilibrium Fe–CO distances for QS and RS states are 1.82 and 1.96 Å; the difference of energies of these states is 480 cm^{-1}.

It is known that the absorption spectrum of a heme has no rotational structure. Vibrational motions in a heme are so complicated that vibrational bands are not resolved in the electronic absorption spectra (see, for example, [37]). However, the characteristic motions of individual groups in the complex, in particular, of the atomic Fe–CO can be distinguished [38]. We will assume that the energy level diagram for the Fe–CO group in the heme Hb is similar to this for a free diatomic molecule at least for several first vibronic states. Such an approximation is reasonable because the potential curves of the stable states of the atomic Fe–CO group presented in [7, 8] are close to the Morse potential shape. Distances between the vibrational sublevels of the Fe–CO group are small, but the vibrational spectrum of Fe–CO is resolved. It is clear that to the left of the maximum of the dissociation efficiency curve (at lower wavelengths) photodissociation is possible because transitions from any populated vibrational sublevel of the ground state to the vibrational–rotational levels of the Q state are possible. At lower wavelengths (for example, for the second harmonic of the Nd : YAG laser), dissociation is possible because the system obviously can efficiently relax from higher-lying excited states to the Q state. To the right of the maximum of the dissociation efficiency curve, i.e. when the absorbed energy is lower than the purely electronic transition energy, the reaction occurs in fact similarly to the appearance of anti-Stokes regions in luminescence and Raman spectra and is determined by the presence of populated nonzero vibrational sublevels at temperatures above absolute zero. Finally, as expected, the maximum of the dependence of the dissociation efficiency on the photolytic radiation wavelength corresponds to the Q transition energy, i.e. the purely electronic transition provides the maximum dissociation (obviously due to the high population, at least, the lower vibrational sublevels of the ground electronic state of the Fe–CO system).

4. Conclusions

We have studied the photodissociation of the carboxyhemoglobin complex in buffer solutions of whole human blood. The dependences of the photolysis efficiency on the average photolytic radiation intensity, the degree of saturation of solutions by carboxyhemoglobin, and the photolytic radiation wavelength have been obtained. The results obtained in the paper have shown that the use of photolysis for reducing the amount of carboxyhemoglobin in blood should be more efficient for the saturation degree of HbCO 40 % – 60 % when photolytic radiation at a wavelength of 570 nm is used.

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