UV-Induced Transformation of Human Carboxyhemoglobin

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The effect of UV-radiation (240-390 nm, 151-4530 J/m^2) on structural changes in human carboxyhemoglobin is studied. Photodissociation of CO ligand from heme and conversion of hemoprotein into oxyhemoglobin and methemoglobin were accompanied by changes in spectral and electrophoretic characteristics (number, mobility, and content of fractions) of the studied hemoprotein.

Key Words: *radiation dose; carboxyhemoglobin; electron absorbance spectrum; electrophoretic characteristics*

Photochemical transformations of hemoproteins, blood components and active acceptors of UV quanta, have been extensively studied. Among hemoproteins, hemoglobin is of particular importance because of its functions.

Human blood contains various forms of hemoglobin. The effect of UV radiation on physicochemical properties of oxyhemoglobin (HbO_2) was studied in detail, while its effect on carboxyhemoglobin (HbCO) was less explored.

In vivo transition of HbO_2 into HbCO can be induced by a number of factors. Heme degradation is accompanied by carbon monooxide (CO) formation, which can block up to 1% oxygen binding sites in hemoglobin molecules. Moreover, impaired ecological situation, air and pollution, high concentration of CO in the atmosphere stimulate conversion of some hemoglobin molecules into HbCO and its accumulation in the blood. This impairs blood functions, since heme in the hemoglobin molecule is characterized by a 200-fold higher affinity for CO than for O_2. In light of this, new effective and rapid means for converting HbCO into more active hemoprotein forms showed developed. It has been shown that UV radiation induce photodissociation of CO from hemoproteins in human HbCO solution [3, 8,9]. The aim of the present study was to analyze physicochemical properties of photoproducts formed in this reaction.

MATERIALS AND METHODS

Experiments were carried out using aqueous solutions of HbO_2 (10^{-4}-10^{-5} mol/liter) obtained from donor blood as described elsewhere [7] with some modifications [2].

Oxyhemoglobin was converted into HbCO by bubbling with CO released in the reaction between formic and concentrated sulfuric acid. Formation of HbCO was assessed by characteristic UV and visible absorption spectra.

Hemoprotein solution (3 ml) in a thermocontrolled cuvette (20±1°C) was irradiated with a DTR-400 quartz-mercury lamp using a UFS-1 filter (240-390 nm light transmission range). The samples were irradiated for 1, 3, 6, 9, 15, and 30 min at 151 J/m^2/min radiation power. Structural modifications of HbCO were assessed by absorbance spectra within 230-700 nm wavelength range recorded using an SF-46 spectrophotometer and electrophoretic patterns.

HbCO was analyzed by polyacrylamide gel (PAAG) disc electrophoresis using a Reanal apparatus and reagents. Electrophoresis was performed in 7% stack and 5.5% separating PAAG and Tris-glycine

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buffer (pH 8.3). The gels were stained with Amido Black 10B and washed with 7% acetic acid.

Densitograms of control and experimental samples were used for calculation of some electrophoretic parameters. For instance, the width of electrophoretic fraction was measured as the base of a triangle outlined with the corresponding peak on densitogram, while the contents of hemoprotein fractions were calculated as the surface under the corresponding peaks [5]. The electrophoretic mobility (EM) of HbCO fractions was calculated as described previously [10].

The data were processed statistically using Statgraphics software.

RESULTS

Absorbance spectra of intact human HbCO had two maxima in the UV range (270-278 and 342-345 nm) and 3 absorbance bands (Fig. 1) in the visible range (420, 540, and 575 nm), which agrees with published data [1,2,4]. Electrophoresis showed that HbCO molecules were homogenous and form a single electrophoretic band (Fig. 2).

UV-irradiation in a dose of 151 J/m² reduced optical density of HbCO in absorbance maxima at 272, 345, and 540 nm. However, this reduction was significant only for the Soret band (0.950±0.023 *vs.* 1.043±0.019 in the control). Moreover, a new peak at 629 nm appeared. Electrophoretic pattern of modified hemoprotein was also different: apart from the major band typical of native HbCO, two slow fractions with EM 0.33 and 0.25, respectively, appeared, which suggested the formation of new photoproducts.

Irradiation in a dose of 453 J/m² reduced the absorbance in the Soret band (D=0.955) and 540- and 575-nm peaks, indicating changes in the microenvironment of nitrogen atoms in the heme pyrrole rings. Electrophoretic pattern of photomodified protein was also altered: one slow fraction disappeared and a minor fast fraction containing approximately 6.17% protein (EM=0.95) appeared. EM of this fast fraction was similar to that of human HbO_2 fraction [6]. Protein content in the slowest fraction (EM=0.24) decreased 2-fold (from 15.08 to 7.47%).

Irradiation in a dose of 906 J/m² increased the absorbance in the 628-630-nm band, which indicated the accumulation of methemoglobin (MtHb) and induced a blue shift in the Soret band (to 417 nm); optical densities in this band and in the 540-nm peak (0.103±0.006) were considerably lower than in the control. Electrophoresis revealed the following changes: slow fraction disappeared, some molecules from the major fraction passed into fast

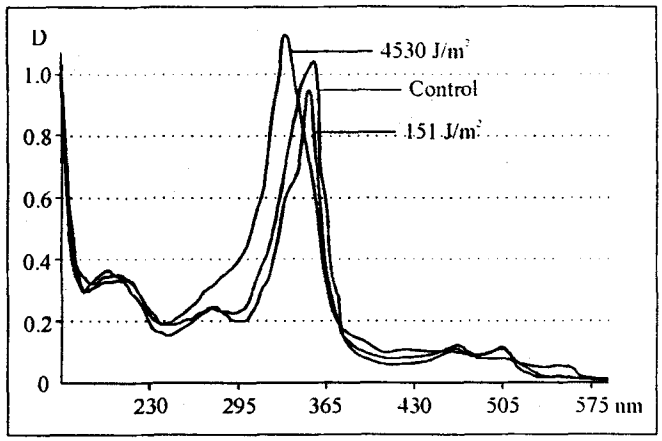

Fig. 1. Absorbance spectra of human carboxyhemoglobin exposed to different doses of ultraviolet radiation.

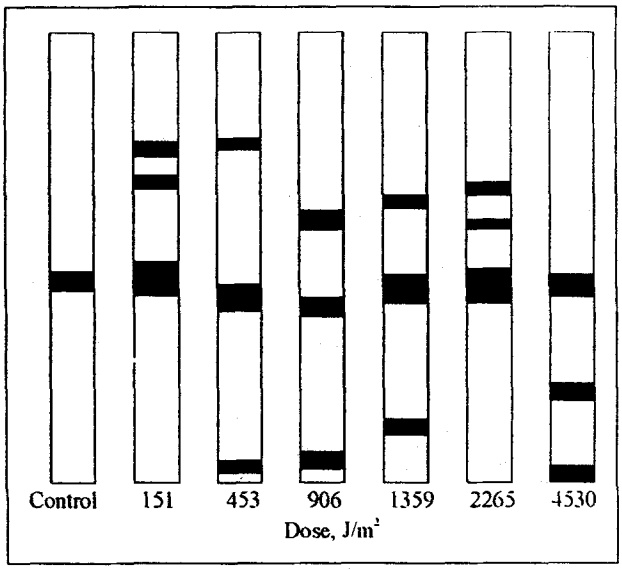

Fig. 2. Electrophoregrams of human carboxyhemoglobin exposed to different doses of ultraviolet radiation.

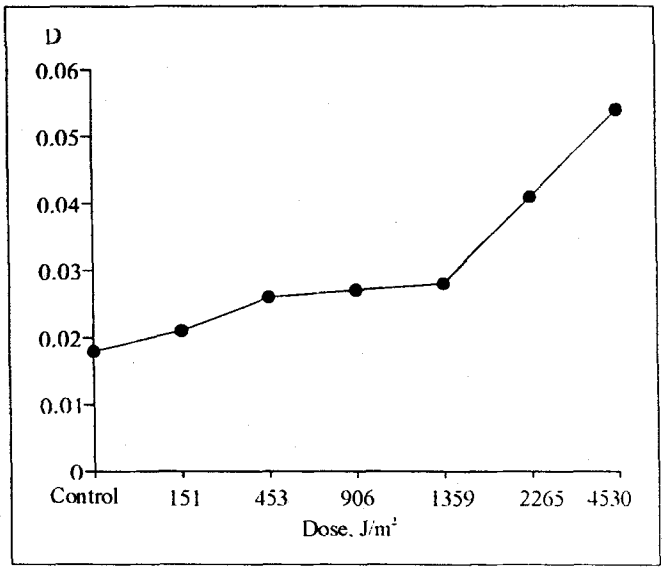

Fig. 3. Optical density (628-630 nm) of human carboxyhemoglobin solutions exposed to different doses of ultraviolet radiation.

fraction (EM=0.93), so that protein content in this fraction increased to 62%, and a new slow component (EM=0.40) with high protein content (24.98%) segregated from the major HbCO fraction.

We have previously demonstrated that under these conditions human MtHb migrated in PAAG as a single fraction with EM=0.42. It can be assumed that slow HbCO fraction is presented by oxidized hemoglobin (MtHb). Thus, HbCO exposed to UV irradiation in a dose of 906 J/m² is a mixture of various hemoprotein isoforms: apart from HbCO, it contains HbO_2 and MtHb isoforms.

In the spectra of HbCO exposed to UV irradiation in a dose of 1359 J/m², the Soret band shifted to 409 nm and overlapped with the 342-345-nm band, while the corresponding absorbance surpassed the control level (1.125±0.011). The content of MtHb (optical density at 628-630 nm attained 0.028± 0.004) increased. The fastest electrophoretic fraction in this sample migrated more slowly (EM=0.86), while its protein content decreased to 14.37%. The content of protein in the middle fraction (EM=0.54) increased to 76.23%.

UV-irradiation in a dose of 2265 J/m² shifted aromatic absorbance band downward (λ=269 nm) probably due to photooxidation of UV-quantum acceptors. The Soret band shifted to 406 nm and its optical density increased; β-band at 540 nm was smoothened; the absorbance at 628-630 nm became more intensive. Electrophoregrams of these samples were characterized by the presence of two slow fractions with EM 0.42 and 0.34, respectively, while the major hemoprotein fraction became diffuse (0.72-cm width), which reflected its heterogeneity.

The maximum dose of UV irradiation (4530 J/m²) shifted the Soret and aromatic absorbance bands to 404 and 268 nm, respectively; β-band disappeared and a new peak at 495-500 nm appeared (D=0.105); optical density in the 628-630-nm band increased. This dose induced the formation of two fast fractions with EM 0.96 and 0.78, respectively, while the major HbCO fraction became narrower (0.48-cm width).

Electrophoresis of UV-irradiated HbCO revealed fractions with characteristics similar to thouse of HbO_2 and MtHb.

It can be hypothesized that the appearance of slow (EM=0.24-0.42) and fast (EM=0.78-0.42) fractions on electrophoregrams is associated with UV-induced dissociation of CO from HbCO molecules, followed by their oxygenation and photooxidation yielding HbO_2, MtHb, and their photomodified derivatives. This assumption is confirmed by changes in absorbance spectra and electrophoretic patterns: formation of HbO_2 is evidenced from the shift of the Soret band from 420 to 417 nm and the appearance of fast electrophoretic fractions with EM= 0.96-0.93. Further shift in the Soret band to 404 nm and the appearance of 495-500- and 628-630-nm bands as well as the downward shift in the protein band and the shift of the 342-345-nm band to the shoulder reflect the formation of MtHb,

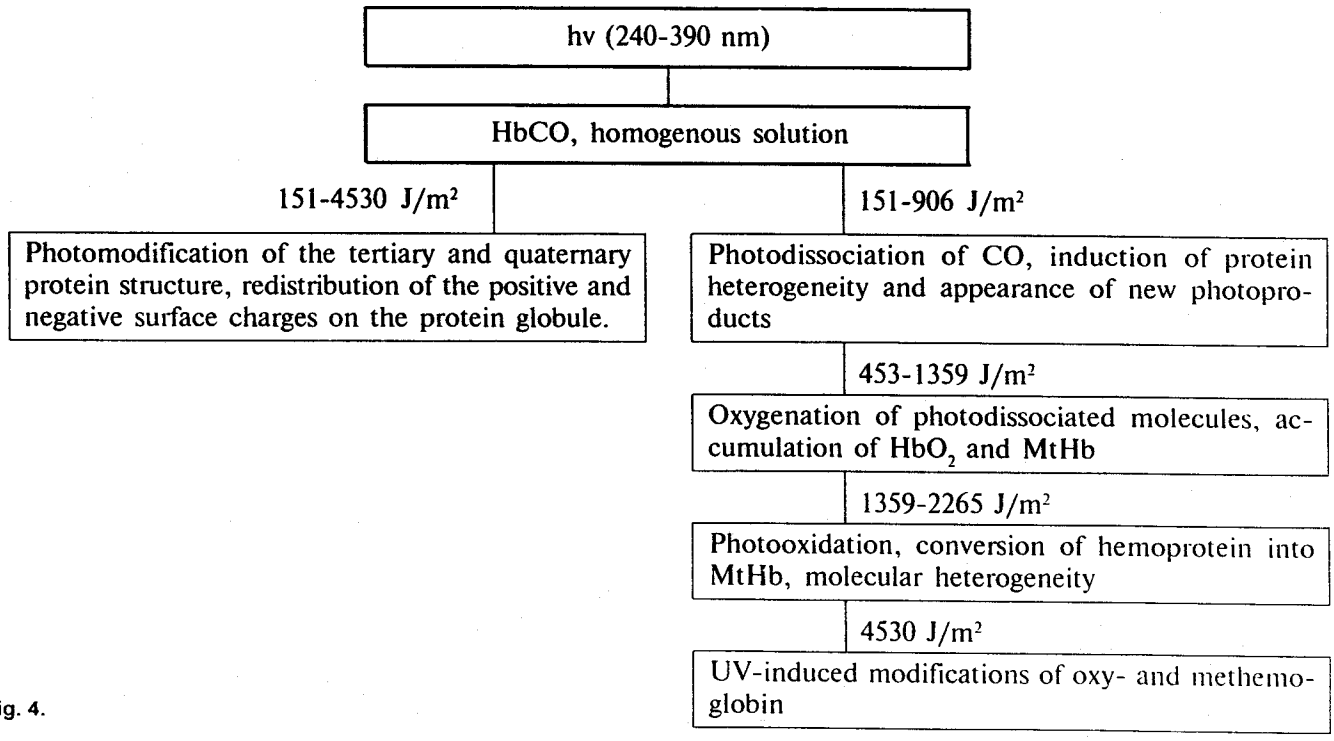

Fig. 4.

which directly depends on the dose of UV-radiation (Fig. 3).

Apart from the appearance of HbO_2 and MtHb, UV-radiation modified the distribution of positive and negative charges on the protein globule and altered its tertiary and quaternary structure, which was accompanied by the appearance of additional electrophoretic fractions with varying mobility. It should be noted that aromatic amino acid residues made no substantial contribution to modifications of hemoprotein structure: optical density of UV-irradiated HbCO at 270-278 nm practically did not differ from the control.

On the basis of these findings and published data [1] we propose the following scheme of UV-induced processes in HbCO molecules (Fig. 4).

Our findings suggest that UV irradiation (240-390 nm) of the blood in doses below 453 J/m^2 can be used as an urgent resuscitation measure in CO intoxication. This procedure can be performed using autotransfusion and UV-irradiation equipment currently used in quantum hemotherapy.

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