Spectral Characteristics of Hemoglobin Taken from the Blood of Rats Subjected to Durable Ethanol Intoxication

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Abstract

We have studied the conformation state of the hemoglobin taken from the blood of laboratory rats of four generations that suffered durable ethanol intoxication. The rats have been intoxicated with adding 15% aqueous ethanol solution to their ration, instead of water. The colouring agents, Coomassi G-250 and Cibacron Blue F3G-A, have been used as probes. The obtained results show definite differences in the hemoglobin absorption spectral maxima for the control and intoxicated groups of animals. It has been found that the absorption band centred at the wavelength 566,8nm is available for the complexes Coomassi G-250–CNMetHb of the control group of rats. In case of the same complexes for the intoxicated rats, the maximum corresponds to the wavelength in range of 571.4–580.7nm. The Cibacron-MetHb complex is characterized with an intensive band at 624-628nm. It has been shown that intensification of hypo-chromic effect depends on the distance of ethanol-intoxicated (EI) posterity from their parents. The mechanisms for the appearance of new hemoglobin fraction in the EI rats' generation have been discussed.

Key words: optical spectroscopy, alcohol intoxication, hemoglobin, methemoglobin, cyan-methemoglobin

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Introduction

Alcoholism as one of pathological states of organism is accompanied by hystoxical hypoxia. Such the hypoxia appears as a result of disturbance of respiratory function, oxygen transportation and its assimilation with the tissues.

In case of hypoxia with different etiology, disturbance of the dynamic equilibrium balance between some of the oxygen-dependent processes in organism manifests itself in the activation of free-radical reactions. These reactions are accompanied with heaping of the active metabolites of oxygen (AMO): $O_2^{\cdot-}$, H_2O_2 and OH [1]. Heaping of the AMO in the cells and tissues can lead to development of oncological, cardiovascular and nervous-mental diseases. This is verified by founding of metabolites, which are typical for such the reactions [2, 3, 5-8]. It is not exception that the mentioned reactions take place at the hypoxia induced with the alcohol intoxication.

In the healthy organism, there exists a balance among the transport of oxygen, its using and deposition. It is conditioned by a matched interaction of oxygen transportation with the

blood stream, reverse coupling of oxygen with the hemoglobin, as well as by its diffusion through the barrier system. It is known that the hemoglobin represents a principal component of the oxygen transportation system. Oxygenation and deoxygenation of the hemoglobin is connected with the dynamic equilibrium balance of the structural states of the molecule ($T \Leftrightarrow R$) – strained and relaxation. Thus, the unite act of oxygenation and deoxygenation is accompanied with the changes in conformation of the hemoglobin molecule, which is conditioned by the peculiarities of spatial structure of this protein [9].

It has been shown by *Bozhko G.Kh.* and *Voloshin P.V.* [4] and *Ngnuyen L.B.* and *Peterson C.M.* [10] that, under the alcohol intoxication, the hemoglobin can be modified due to interaction with acetaldehyde, a principal ethanol metabolite. Concerning oxygenation and deoxygenation of hemoglobin at the alcohol intoxication, the literature data are quite pure. As far as we know, the information about the structural-functional state of hemoglobin for the posterity of ethanol-intoxicated parents is absent. The methodical approaches for studies of the modified hemoglobin have not been well grounded, too. Therefore, the aim of the present paper is to make a comparative study of the conformation state of hemoglobin withdrawal from the blood of ethanol-intoxicated (EI) rats and their posterity, using the technique of probing optical spectroscopy.

Materials and Methods of Study

We used in our experiments the white rats (both males and females) with the weight of 250–300g, and their first, second and third breeds. Each next rat breed was born from the EI parents with the age of 3-6 months. The weight of the new-generation EI animals was 150-200g. The rats were held on the basis of vivarium ration. The alcohol intoxication was carried out daily, with supplying 15% aqueous ethanol solution, instead of water. Before the experiment beginning, the rats were tested for inclination to the ethanol with the so-called "two-bottle method" suggested by *Burov Yu.V.* and *Vedernikova A.N.* [11]. They were separated into the following groups: the control group (0) – daily usage of water; the investigated group (1) – rats (parents) that used 15% ethanol during 10 months; the investigated group (2) – the first breed that was EI during 6 months (their parents used ethanol during 3 months); the investigated group (3) – the second breed, EI during 3 months; and, finally, the investigated group (4) – the third breed, EI during 3 months.

The blood was withdrawn with decapitating. The hemoglobin was separated from the blood with *D.L.Drabkin* technique [12]. The separated hemoglobin was changed to the cyanmet-type and the concentration was determined with the aid of formula

$$C_{mg/ml} = 1.45 D_{540} n, \qquad (1)$$

where D denotes the optical density of the solution at the light wavelength of 540nm, n the concentration of the starting solution, and 1.45 means the millimolar absorption coefficient of hemoglobin recalculated on tetramer.

The conformation state of hemoglobin was studied using the probing spectroscopy. The organic dyes Coomassi G-250 and Cibacron Blue F3G-A produced by "Fluca" played a role of the probe. The structure of these compounds consists of the benzoine- and quinoid-type chromophores, as well as the active groups that can be coupled with the respective groups on the surface of protein molecule. The samples for the analysis were prepared in the following manner: to the 2ml of cyan-methemoglobin (CNMetHb) - 50μM the 2ml of aqueous solution was added (100 μM) Coomassi G-250. The optical spectra were measured after 10min of incubation. To 2ml of the methemoglobin solution (MetHb) - 50μM, 2ml of Cibacron Blue F3G-A (100 μM) was added, prepared on the basis of 0.1M acetate buffer (pH 4.8). This time the optical spectra were measured after 5 min of incubation.

Finally, the optical spectra were measured with the aid of Specord M-40 spectrometer in the 450-750nm spectral range.

Results and Discussion

It is known that hemoglobin molecule can create complexes with the other compounds by means of cation and anion groups that exist on the globule surface. The number of active groups increases with uncoiling the protein globule. Such uncoiling may be induced with the action of exogenous or endogenous factors. Action of both inorganic and organic compounds belongs to those factors. In the conditions of ethanol intoxication, the acetaldehyde and the AMO are expected to be the compounds that could modify the hemoglobin. In our previous papers [13-15], we have presented the optical spectra in the UV and visible ranges for the hemoglobin taken from the blood of EI rats. The particular differences in the optical spectra observed in the UV range make a background for deeper studies of possible structural changes in this gem-protein in the rats' posterity. In this connection, we have studied the hemoglobin of EI rats and their posterity, using the probing optical spectroscopy.

The experimental data (see Table 1 and Figure 1) evidence that the spectral characteristics of CNMetHb–Coomassi G-250

Table 1.

	Solution	Duration of the ethanol intoxication of the parents, month				Absorption maximum wavelength, nm
		(1)	(2)	(3)	(4)	
1	Coomassi G-250	-	-	-	-	585.4
2	CNMetHb	-	-	-	-	544.2
	HbO_2	-	-	-	-	541.7 (β); 576.6 (α)
A. CNMetHb + Coomassi G-250 complexes						
3	Control group (0)	-	-	-	-	566.8 – 568.8
4	EI group, 10 months – group (1) **P**	-	-	-	-	572.0
5	EI group, 6 months – group (2) $\mathbf{F_1}$	3	6	-	-	571.4 – 578.0
6	EI group, 3 months – group (3) $\mathbf{F_2}$	1	3	3	-	573.3 – 580.7
7	EI group, 3 months – group (4) $\mathbf{F_3}$	1	3	3	3	575.5; 576.0; 577.3 – 580.0
B. MetHb + Cibacron blue complexes						
1	MetHb	-	-	-	-	630
2	Cibacron blue	-	-	-	-	615 – 617
3	Control group (0)	-	-	-	-	624.3 – 626.5
4	EI group, 6 months – group (2) $\mathbf{F_1}$	3	6		-	626.5; 624.9
5	EI group, 3 months – group (3) $\mathbf{F_2}$	1	3	3	-	628.1; 625.7
6	EI group, 3 months – group (4) $\mathbf{F_3}$	1	3	3	3	624.9 – 628.1

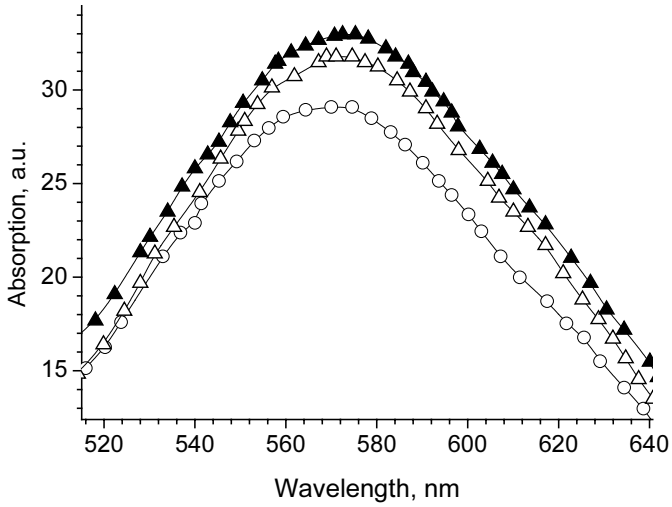

Fig. 1. Absorption spectra of the CNMetHb solution with the Coomassi G-250 dye: open circles – group (0), λ_{max}=566.8 nm; full triangles – group (1); open triangles – group (2), λ_{max}=571.4 nm.

complexes of the control and investigated groups are different. So, the hemoglobin complexes of the control group of rats manifest the absorption maximum at the wavelength of 566.8nm, while the spectra for the rats that were intoxicated during 10 months are characterized with the maximum at 572nm.

The results for spectroscopic studies of the CNMetHb−Coomassi G-250 complexes of the EI rats posterity (see Figures 2 and 3) are of particular interest. The hemoglobin of the second (3) and third (4) breeds is characterized with different possibilities for creation of complexes with the probe, when compare to the case of the control group and even the hemoglobin of the intoxicated parents (Table 1).

In order to continue our previous studies for the Cibacron Blue F3G-A dye, we have now measured the absorption spectra of MetHb−Cibacron complexes. The optical spectra of this dye mixed with the methemoglobin of rats of the second and third breeds are presented in Figures 4.

A comparison of the spectra for the hemoglobin of the rats taken from the control and intoxicated groups testify a different

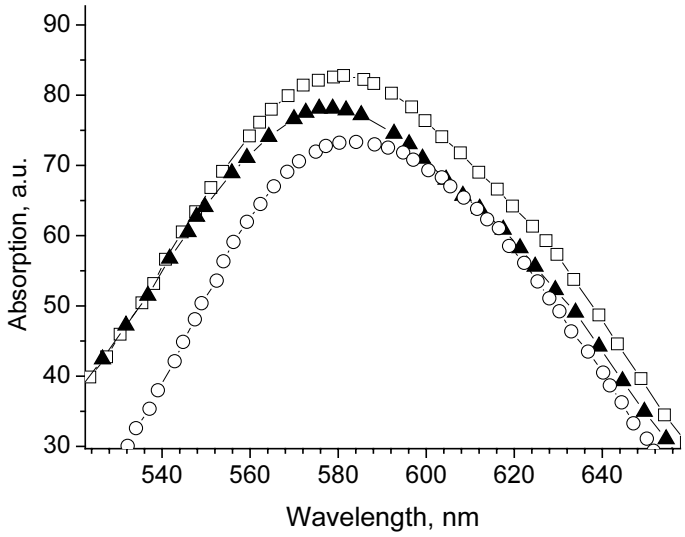

Fig. 2. Absorption spectra of the CNMetHb complexes with Coomassi G-250 dye for the first (2) and second (3) breeds of EI rats: full triangles – first breed (2), EI during 6 months, λ_{max}=578.0 nm; open squares – second breed (3), EI during 3 months, λ_{max}=580.7 nm; open circles - Coomassi G-250, λ_{max}=584.7 nm.

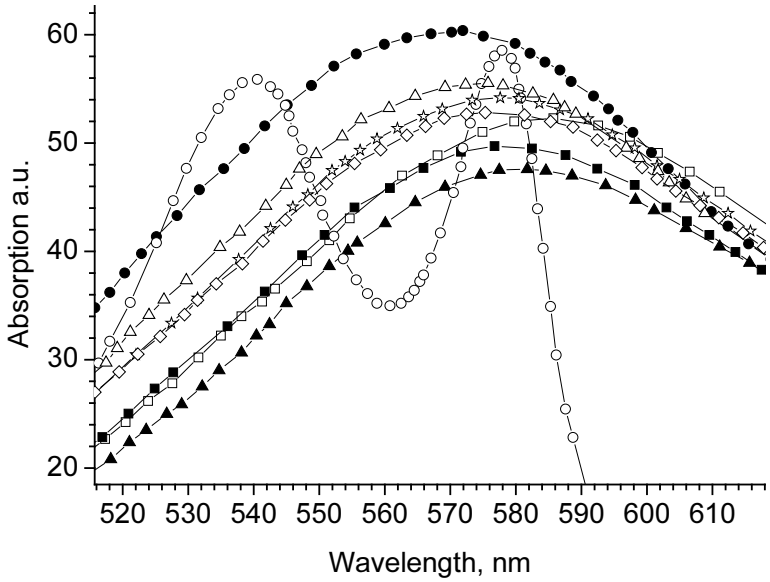

Fig. 3. Absorption spectra of the CNMetHb complexes with Coomassi G-250 dye for the second (3) and third (4) breeds of EI animals: open squares – CNMetHb+Coomassi (3), λ_{max}=576.0 nm; full triangles - CNMetHb+Coomassi (4), λ_{max}=580.0 nm; full squares – CNMetHb+Coomassi (4), λ_{max}=577.3 nm; full circles – control group (0) CNMetHb + Coomassi, λ_{max}=568.8 nm; open circles - HbO_2 β-band λ_{max}=541.7, α-band λ_{max}=576.6 nm; stars – CNMetHb + Coomassi (3), λ_{max}=573.3 nm; open triangles – CNMetHb + Coomassi (3), λ_{max}=571.4 nm; rhombuses – CNMetHb + Coomassi (4), λ_{max}=575.5 nm.

possibility for creation of the complexes with the dyes (see Figures 1 – 4 and Table 1). At the same time, the affinity of the protein and the ligand, whose role is played by the dye, is related to different numbers of the active coupling centres on the surface of the protein molecule.

The change in the optical spectra of hemoglobin in the visible range (450-750nm) is caused by redistribution of electronic density in the system of bonds conjugating the proto-porthyrin ring and the iron atom (Fe^{2+}). In the oxygenation-deoxygenation process, the gem iron exists in the two equilibrium states: low spin ⇔ high spin. The transition of Fe^{2+} from one conformation state to the other leads to breaking gradually the salt bonds between the α-subunits of hemoglobin molecule along the

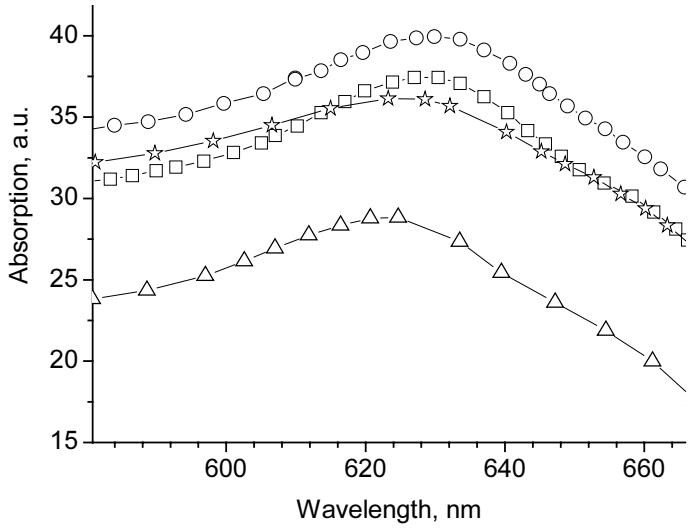

Fig. 4. Absorption spectra of the MetHb complexes with Cibacron dye for the control group (0) and the EI group of the second (3) and third (4) breeds: group (0) – λ_{max}= 627.0 nm; group (2) – λ_{max}=626.5nm; group (3) –λ_{max}=628.1nm; group (4) – λ_{max}= 624.9 nm.

contacts $\alpha_1-\beta_1$ і $\alpha_2-\beta_2$ [9]. As shown in our previous study [13], the change in the optical spectra within the range of 250-400 nm is stipulated by modification of the protein component of the hemoglobin molecule.

Conclusions

Having analysed the literature data [2–10] and our above results, one can come to the conclusion that ethanol intoxication is accompanied by modification of the protein and non-protein components of the hemoglobin. Nevertheless, it is necessary to note that the appearance of the modified hemoglobin cannot be always associated with the reaction with acetaldehyde. It is quite possible that the ethanol in blood can lead to particular uncoiling of the hemoglobin molecule. As a result, the native conformation of the molecule can be changed. Then the conformation changes would lead to disturbance of oxygen access to the gem iron. This is quite logically related to the results of investigation for affinity of hemoglobin to the oxygen obtained with the method of oxygen equilibrium balance curves, where it has been shown that the oxygen affinity to hemoglobin decreases under intoxication with the ethanol [16]. One cannot exclude the fact that, in case of the ethanol intoxication, the active metabolites play a role of inductors of the expression of respective globular genes and synthesis of *de novo* polypeptide chains of the hemoglobin. Such ethanol-induced hemoglobin may be characterized with the changed conformation and it manifests an increased affinity to the modifications. One can assume also that coupling of such the acetaldehyde metabolite should be a protective reaction of organism against the toxic metabolites. We have shown in the present paper that the modified hemoglobin can be detected with the method of probing optical spectroscopy.

Hence, the obtained results testify the essential changes in the conformation of the oxygen-transporting protein-to-hemoglobin for the rats induced by ethanol intoxication. The functional changes represent a sequence of the conformational ones.

The detected changes for the case of the posterity of intoxicated animals may be explained as a post-translation modification, as well as a disturbance of the structure and function of tissue cellular gene mechanism for the blood creation. The ethanol-induced expression of the respective α- or β-globular genes can provoke a creation of tetramers with the different structural-functional properties.

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