Optical Spectra of Hemoglobin Taken from Alcohol Dependent Humans

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Abstract

Optical spectra of CNMetHb and CNMetHb-Coomassi G-250, taken from the blood of humans with alcohol dependence, are studied in the spectral range of 450–750nm. The shifts in the spectral absorption maxima of CNMetHb-Coomassi G-250 complexes are observed for the diseased persons with alcohol dependence. The obtained results show that the hemoglobin structure of alcohol dependent humans is changed.

Keywords: absorption spectra, hemoglobin, alcoholism

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Introduction

It is well established that reactive oxygen species are involved in acute and chronic alcohol intoxication (see, e.g., [1]). Biochemical signs of the oxidation damage could be detected using the experiments with animals exposed to ethanol, as well as alcoholic patients. For example, *Hazelett S.E. et al.* [2] have shown that hemoglobin-acetaldehyde (HbA1-AcH) may serve a useful marker enabling to detect alcohol abuse, especially in the populations where the other markers have been shown to fail. The acetaldehyde reacts quite easy with the compounds that include ethanol groups, primary amino-groups, sulfhydryl and carbonyl groups. By means of the mass spectrometry technique, nuclear magnetic carbon-13 resonance method and the Raman spectroscopy, it has been shown in earlier works that, at sufficient amount of acetaldehyde, five of the six possible sites of peptide pentalysine are modified [3,4]. The authors [5] have ascertained sensitivity of hemoglobin molecules to alcohol intake, while measuring and comparing the fractions of hemoglobin A1 for 43 normoglycemic men and women placed in alcohol treatment centre and 41 healthy volunteers. According to [5], this fraction is significantly larger for the alcoholic patients, if compare with the healthy subjects. It is worthwhile to stress that the process of hemoglobin conformation changes may be experimentally detected with the optical spectroscopy methods [6,7]. In our recent papers [8,9] we have reported of sufficient sensitivity of the optical spectra of hemoglobin coupled with the colouring CNMetHb-Coomassi agent and taken from ethanol intoxicated white rats. A so-called batochromic shift has been observed for the absorption band of CNMetHb, when compare with that of CNMetHb-Coomassi

Table 1. Characteristic parameters of the optical spectra of hemoglobin CNMetHb-Coomassi G-250 complexes, measured for the alcohol dependent and healthy persons.

	Group of persons	Wavelength of the absorption maximum, nm	Batochromic shift with respect to CNMetHb solution, nm	Hypsochromic shift with respect to Coomassi G-250 solution, nm	Shift of the absorption maximum wavelength with respect to that of the control group, nm
1	Control (n=10)	*566.1	22.7	–17.3	–
2	Alcoholism, treated (n=13)	*555.9	12.5	–27.5	–10.2
3	Alcoholism, non-treated (n=6)	*570.6	27.2	–12.8	+4.5
4	Psychic disorders due to alcohol intake, treated (n=4)	*561.1	17.7	–22.3	–5

Here n means a number of persons under test, "*" the absorption maximum wavelength, and the signs "+" and "–" correspond to shifts in the absorption maximum respectively towards the long- and short-wavelength regions, when compare to the control-group spectral maxima.

complexes for the control group. In the present paper we would like to make sure that the same is true for humans with alcohol dependence.

Materials and methods

In our spectroscopic studies, we used the blood of a control group of volunteers (the number of volunteers n=10), a group of persons diseased by alcoholism (treated disease, n=13), another group of the diseased persons (non-treated alcoholism, n=6) and, finally, a group characterized by psychic disorders acquired due to alcohol intake (treated, n=4). The diseased persons had previously undergone a course of medical treatment at the Lviv Regional Psychiatric Hospital.

Heparinized blood was used for the analysis. The blood was taken from the elbow vein. The erythrocytes were detached from plasma with the aid of centrifuge operating at 500g. The hemoglobin was extracted with the *Drabkin* technique [10] and its concentration was determined using the *Kaushakovskiy* method [11]. The conformation changes in the hemoglobin were determined by means of optical probe spectroscopy method, with Coomassi G-250 (produced by Fluca company, Switzerland) chosen as a probe colouring agent. The samples were prepared for the analysis according to the scheme that provided for adding of water solution (2ml) of Coomasi G-250 (50 μM) to the water solution (2ml) of cianmethhemoglobin (CNMetHb, 50 μM). The optical spectra were studied in the spectral range of 450–750 nm, using Specord M-40 spectrophotometer.

Results and discussion

The results of the spectroscopic studies of CNMetHb-Coomassi G-250 complexes are shown in Fig. 1 and Table 1. One can see that the hemoglobin of persons with the alcohol dependence, which were medically treated, can form the complexes with Coomassi G-250. The structure of Coomassi G-250 includes chromatic groups of benzoic and quinoid type, together with sulfhydryl and carbonyl groups. These groups can interact with the corresponding groups on the surface of protein molecule, creating stable coloured complexes in that way.

The analysis of the experimental data testifies that the hemoglobins under study manifest different abilities to create the complexes with the colouring agent. The shifts

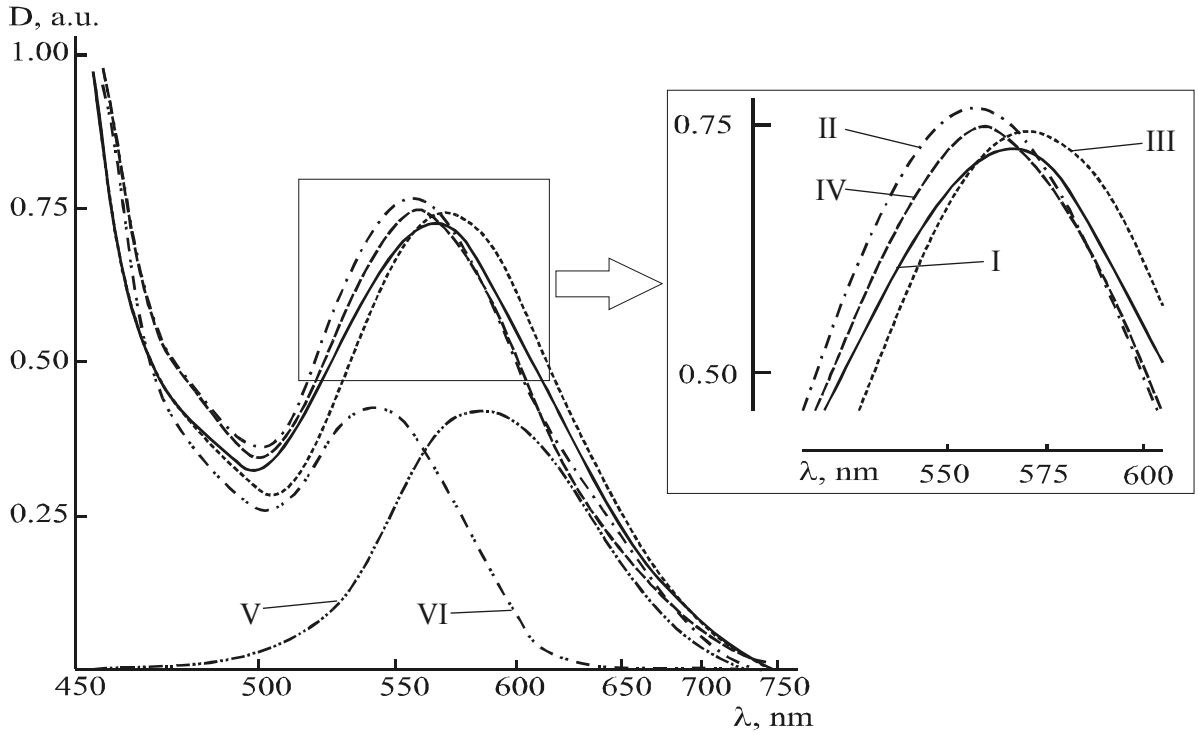

Fig. 1. Averaged absorption spectra of the hemoglobin complexes of alcohol dependent persons and healthy donors: I – control group, II – alcoholism (treated), III – alcoholism (non-treated) and IV – persons with psychic disorders as a result of alcohol intake (treated). The curves V and VI correspond to the absorption spectra of Coomassi G-250 and CNMetHb solutions, respectively. The insert shows enlarged part of the absorption spectra in the regions close to the maxima.

of the absorption maxima of hemoglobin complexes towards the long-wavelength region (from 566.1nm to 570.6nm), which are peculiar for the non-treated persons with alcoholism disease, suggest the conclusion mentioned above. As seen from Table 1, the hemoglobin of the not-treated alcohol dependent persons exhibits better affinity to the colouring agent, when compare with that of the healthy persons. When compare to the absorption band of CNMetHb and CNMetHb-Coomassi G-250 complexes of the control group, the batochromic shift has been observed. We have earlier noticed similar behaviour of the optical spectra of hemoglobin of the laboratory rats that suffered from alcohol intoxication [8,9]. The difference in the hemoglobins of these two groups might cause further differences in the process of heme transition from the relaxed state to the tensed one. Such the conformation transitions are related to the differences in conformation of hemoglobin subunits and a quaternary structure, in general. On the other side, the change in the oxygen affinity of hemoglobin is associated with the change in the quaternary structure. The characteristic absorption maximum for the treated persons with the alcohol dependence is shifted towards the position of the absorption maximum typical for the control group, irrespective of the presence or absence of psychic disorders. Probably, the mentioned behaviours of the hemoglobin taken from the different groups of peoples suggest that the hemoglobin becomes partially uncoiled under the alcohol influence, thus producing more free remains for the reaction with the colouring agent. We have already observed the same behaviour when studying the hemoglobin of ethanol intoxicated laboratory rats [12].

Conclusions

Basing on the results obtained in this work, one can conclude the following:

- the shifts in the characteristic absorption maxima observed in the optical spectra of hemoglobin for the alcohol dependent

persons suggest the conformation changes in the hemoglobin;

- there exists a difference in the optical spectra of CNMetHb-Coomassi G-250 complexes characteristic of the treated and non-treated persons with the alcohol dependence;
- under the conditions of alcohol dependence, the change in the human hemoglobin is similar to that of the alcohol intoxicated white rats;
- the presented method of absorption spectroscopy could be used in clinical practice for revealing alcoholism.

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