Comparison of the Blood Component Optical Transmission Spectra of White Rats with and without Ethanol Intoxication

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Received: 18.09.2003

Abstract

In this report we present spectroscopic transmission studies of hemoglobin extracted from the vein blood of laboratory rats free of and exposed to ethanol intoxication, which are performed in order to analyze the relevant spectral differences. The animals were divided into the intoxicated group (IG) and the control group (CG). The results show a noticeable difference of the absorption/transmission spectra of hemoglobin extracted from the blood of these two groups. The difference in the absorption spectra is probably associated with the fact that the vein blood of ethanol-intoxicated animals includes more RHb than that of the CG. The deoxygenation process rate for the hemoglobin of intoxicated animals reaches a higher value and is quicker than that for the CG. It means that the affinity of the IG hemoglobin to oxygen is smaller than that for the CG. The minimum in the transmission spectra of hemoglobin with the dissolved cibacron blue colouring agent is shifted to a longer wavelength range (620 nm). The transmittance value for the IG is smaller than that for the CG, implying probably that the immunoglobulin G (IgG) in the IG possesses a more essential decomposition of its spatial structure, when compare with the CG. The changes in the transmission spectra of hemoglobin are detected after adding H_2O_2 and bromthymol blue, indicating the protein conformational changes for the IG animals.

Key words: transmission spectra, hemoglobin, ethanol intoxication, white rats.

PACS: 42.66.-p, 33.55.-b

Introduction

Just of the absence of impartial methods for identification of mental disorders, the unexpected loss of the unity of mental functions, the mental and emotional deviations, the behaviour reaction of the mentally diseased persons still remains unforeseen. At present, the etiology of such a mental disease as alcoholism has not yet been specified. Different viewpoints on the causes of this disease exist. The most commonly accepted causes are heredity and social factors. It is known that the alcoholism is connected with alteration of gene-enzyme system, particularly alcohol dehydrogenase and aldehyde dehydrogenase [1]. To determine the etiology of mental diseases, it would be possible to use a direct sequenation of DNA loci responsible for the pathological processes. However, the diseases have a polygenetic etiology and the mentioned method is expensive and laborious. Since the mental diseases are entailed by the development of the oxidative stress, formation of the active forms of oxygen [2,3], that can activate conformation oxydative modification and destruction of blood plasma proteins, would be reflected in spectral optical characteristics. It is also known that the mental

diseases are entailed by alteration of the albumin molecule [4], which may also affect the vibration spectra in the IR region, the Raman spectra, fluorescence spectra and the circular dichroism [5].

A lot of works have been devoted to studies of the ethanol influence on the blood system [6-9]. It has been noted that the rate of protein fractions of blood serum changes its value, when compare the cases of ethanol intoxication and the absence of the latter [8]. Depression of the hemocoagulation [10] and increase in the circulated blood volume [11] have been also observed. A decrease in the erythrocytes and hemoglobin quantity and the fetal hemoglobin level has been found in the study [12]. However, the mechanism of ethanol action on physiochemical properties of the hemoglobin has not been determined for certain.

The aim of this study is to make a first step in the determination of alcoholism etiology with the spectroscopic methods and to clarify the changes in the transmission spectra of hemoglobin under the action of ethanol.

Experimental

The experimental studies were carried on the white rats (females) outbreed with the average weight of 250g. The rats were placed into the individual cages under the conditions of a free access of 15% water solution of ethanol (for the intoxicated group of animals abbreviated hereafter as the IG). The volume of the used liquid and the weight of animals were controlled daily.

After the 10-day test, the rats with a clear alcohol motivation (the average daily usage of ethanol was 7.1g/kg of their weight) were placed into separate cages for further forcible alcohol intoxication. The blood sampling from the tail vein was conducted after 1 and 1.5 months of 15% ethanol usage. Those samples were subjected to the investigation. To have a reference for the obtained results, the blood of a control group (CG) of animals (without the ethanol intoxication) was used. The heparin was used as anticoagulant. After centrifugation of the whole heparinizated blood, the supernatant (plasma) was separated. The red blood cell hemolization was performed with the 30mM phosphate buffer (pH 7.36). The hemolization was used for the study of optical transmission spectra.

The light transmission spectra were measured in the spectral range of 0.4-0.8 μm. To study the conformational changes of IgG and Hb, the cibacron blue (8mg of cibacron blue dissolved in 100ml 0.1M acetate buffer, pH 4.8) and the bromthymol blue dye (10^{-5}M) were used, respectively (the details see, e.g., in [12]).

Results and discussion

As seen from Figure 1, the absorption spectra of hemoglobin for the IG and CG animals exhibit a maximum at 560nm. The transmittance ratios for the hemoglobin of the IG and CG animals are shown in Figure 2. A difference in the light absorption of the IG and CG means that the vein blood of ethanol-intoxicated animals consists of a larger quantity of RHb than the blood of the CG.

Deoxygenation kinetics of hemoglobin was studied with using the red blood cells of the CG and IG rats. The hemolizate deoxygenation was carried on with the depression method, using the modified saturator [13]. The temporal dependences of the Hb deoxygenation rate were

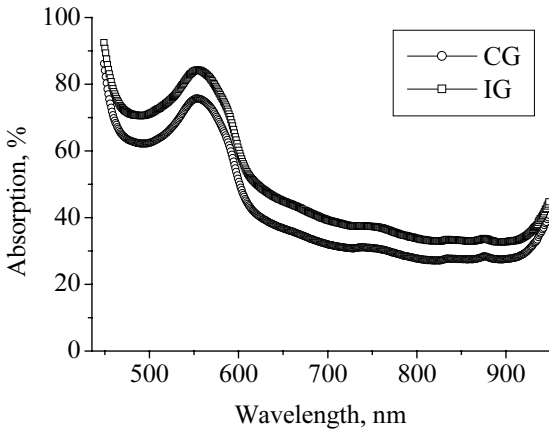

Fig. 1. Absorption spectra of the white rat hemoglobin.

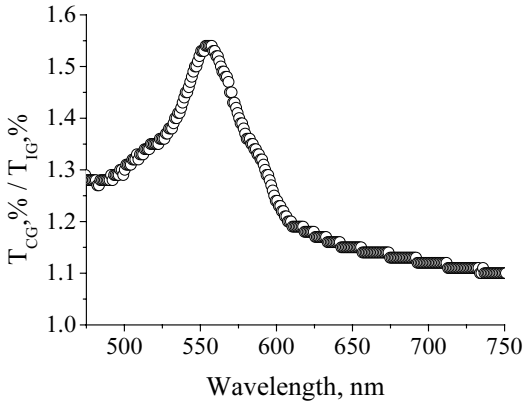

Fig. 2. Transmission ratio for the hemoglobin of the IG and CG animals.

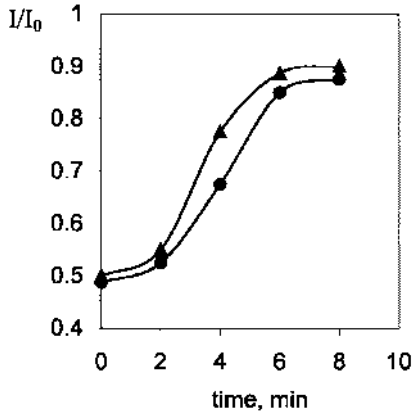

Fig. 3. Temporal dependences for the white rat hemoglobin deoxygenating process (circles correspond to the CG and triangles to the IG).

studied with the spectroscopic absorption method at the wavelength of 560nm (i.e., the wavelength of the maximum light absorption in RHb). The results are presented in Figure 3. It is clearly visible that the deoxygenation process is quicker just for the hemoglobin of the IG animals.

The hemoglobin deoxygenation velocity for the IG reaches its maximum value in 4 minutes, while the same for the CG hemoglobin corresponds to 6 minutes (Figure 4).

The studies of the transmission spectra of hemoglobin were also performed under the conditions of adding different dyes and chemical agents. For example, a 60nm-shift of the transmittance minimum (i.e., up to 620nm) was observed after adding the cibacron blue dye (see Figure 5). The transmittance values for the IG

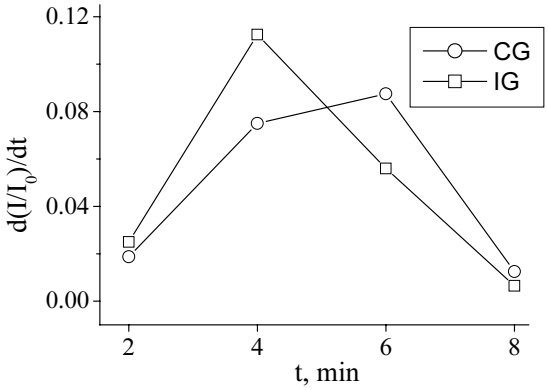

Fig. 4. Temporal dependences of the deoxygenation velocity.

are smaller than those characteristic for the CG. Probably, it means that the IgG manifests a more essential conformational change in the IG, when compare with the CG. After adding 0.3% hydrogen peroxide (H_2O_2), the additional trans-

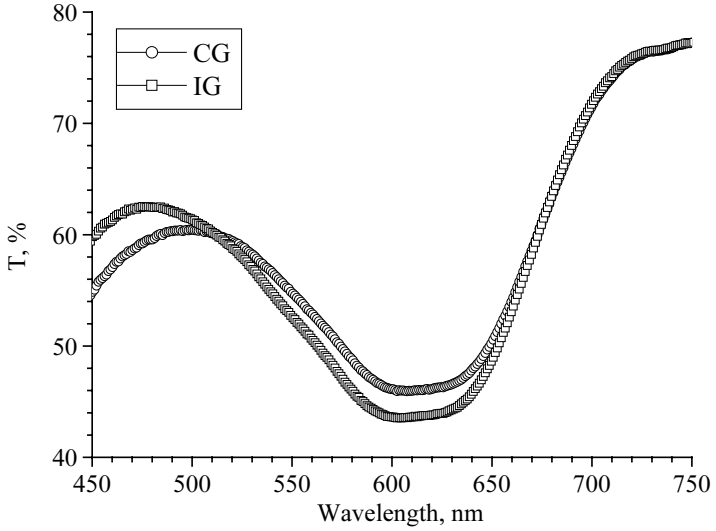

Fig. 5. Transmission spectra of the white rat hemoglobin with the cibacron blue dye added.

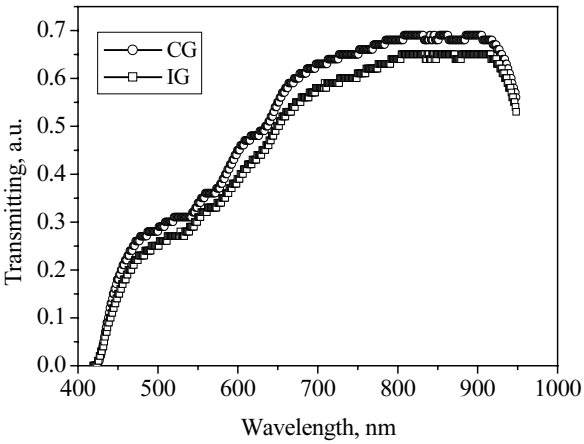

Fig. 6. Transmission spectra of the white rat hemoglobin with H_2O_2 added.

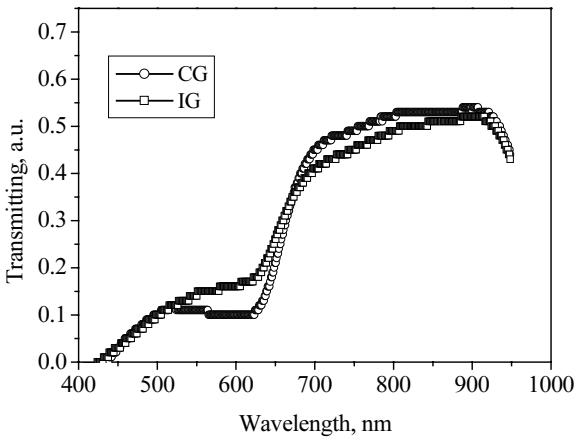

Fig. 7. Transmission spectra of the white rat hemoglobin with both H_2O_2 and bromthymol blue dye added.

mittance minimum at the wavelength of 630nm was observed in the spectra (Figure 6). The existence of this minimum can be related to the appearance of MetHb. It is interesting to note that this minimum is more clearly distinguished in the CG than in the IG. Adding subsequently the bromthymol blue dye leads to keeping the anomalous spectral behaviour only in the range of 600-630nm (see Figure 7). The MetHb is more clearly distinguished in the IG spectrum. The changes detected in the transmittance spectra of hemoglobin after adding H_2O_2 and bromthymol blue indicate the protein conformational changes for the IG animals. Most probably, this is connected with the fact that the other centres of colouring agent bounding with protein appear in the IG hemoglobin.

Conclusions

Basing on the spectroscopic transmission studies of hemoglobin extracted from the vein blood of laboratory rats exposed to the ethanol intoxication and the CG animals, the following results are revealed:

1. The IG hemoglobin affinity to the oxygen is smaller than that of the CG, as shown with the spectroscopic study of hemoglobin solutions. The difference in the absorption spectra of hemoglobin in the IG and CG is probably associated with the fact that the vein blood of ethanol-intoxicated animals consist of a larger quantity of deoxy-hemoglobin than the blood of the CG rats.
2. The minimum in the transmission spectra of hemoglobin with the dissolved cibacron blue colouring agents is shifted towards the long-wavelength region (620nm). The transmittance value in the IG is smaller than in the CG, implying probably that the immunoglobulin G (IgG) for the IG manifests a more essential decomposition of its spatial structure, in comparison with the CG.
3. The changes in the transmission spectra of hemoglobin were detected after adding H_2O_2 and bromthymol blue, thus indicating the protein conformational changes in the IG animals.

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