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A Biophysical Study on Hemoglobin Molecule Irradiated by near Ultraviolet Waves

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Abstract: This study was designed to detect the possible changes in some biophysical properties of hemoglobin molecule like relative viscosity, refractive index and the ratio in absorbance at 578 to 542 nm in the spectrum following exposure to near ultraviolet irradiation. Forty eight mature male rats were used and divided into eight groups, according to the dose of exposure; The first group was not subjected to any radiation and was considered as the control group. Groups (2-8) were exposed to near ultraviolet doses ($7.5x10^{-3}$-$67.5x10^{-3}$ J/cm^2) for 15-60 minutes once. Blood samples were collected and some biophysical properties of hemoglobin were measured. Results revealed that, irradiation with near ultraviolet waves induced a slight increase in relative viscosity and refractive index of hemoglobin. The A_{578} /A_{542} ratio slightly decreased as the doses of irradiation increased. It was concluded that exposure to high dose of near ultraviolet radiation induced hazardous changes in the biophysical properties of hemoglobin of exposed rats.

Key words: Near ultraviolet · radiation · biophysics · hemoglobin · viscosity · rats

INTRODUCTION

Functional dynamics and transitions between equilibrium conformations of hemoglobin depend upon environmental factors [1]. Hemoglobin A (Hb A) with α2-β2 tetramer structure exhibited positive cooperativity in oxygen binding. It has been extensively investigated with various methods, since it serves as a basic model for general allosteric proteins. Currently, elucidation of a structural mechanism of cooperativity is a major subject of Hb studies.

X-ray crystallographic studies have demonstrated the presence of two distinct quaternary structures, called T (tense) and R (relaxed) states,

which correspond to the low-and high-affinity states, respectively. Typical structures are practically seen for the deoxy and CO-bound forms of Hb A. The cooperative oxygen binding of Hb has been explained in terms of a reversible transition between the two quaternary structures, switching of which takes place at a certain number of bound ligands [2]. It is known that near ultraviolet (240-420 nm) is absorbed by both protein and heme in the hemoglobin molecule [3].

Changes in the heme-globin contact exert a discernible influence on spectra and cooperative oxygen binding [4].

Amino acid residues in the B10 helix of alpha-and beta-chains can play different roles in regulating the functional properties and stability of the hemoglobin molecule [5].

Photolysis of hemoglobin at 366 nm during 5, 15 and 30 minutes induced the recovery of a part of the enzymatic activity. A variable alteration of hemoglobin after labeling could explain that the complete removal of 1-(2-nitrophenyl) ethyl (NPE) groups did not restore its full oxidative activity [6].

The current study was designed to investigate some biophysical properties of hemoglobin due to the exposure to near ultraviolet irradiation.

MATERIALS AND METHODS

Animals: Forty eight male rats (weighing 250-300 g) obtained from Animal House of the National Research Centre were used. Rats were kept in metal cages under routine light system and freely provided with concentrate ration and fresh water. Rats were divided into eight groups. Each group contained six rats.

Experimental design: The whole body of rats from all groups (except the first group which was saved as the

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control group) was exposed to the near ultraviolet rays for 15-60 minutes as follow:

Groups	1	2	3	4	5	6	7	8
Dose X 10^{-3} (Joul/cm^2)	0	7.5	15	22.5	37.5	45	52.5	67.5

The irradiation was carried by a mercury arc lamps (distance = 22cm), using a specific filters. The temperature was kept at 20±1°C [7]. The intensity of the light was calibrated by using a simple direct reading ultraviolet-sensitive photovoltaic cell (Philips, Netherland)

Blood samples were taken from all rats in sterile test tubes containing heparin, blood was centrifuged at 3000 rpm for 10 min., then the plasma was removed and packed cells were washed with 5 ml, saline and centrifuged, this process was repeated three times. The erythrocytes sediments were treated with 4-folds ice-cold deionized water to obtain hemolysate [8]. The absorbance at the wave lengths 578 and 542 nm was measured by using Shimadzu UV-visible double beam automatic recording spectrophotometer and the ratio between them was calculated.

At constant temperature and concentration of hemoglobin (4.1x10-5 M), the relative viscosity was calculated by measuring of time of flow with constant volume of samples using Oswalt Capilay Viscometer according to the equation

$$\eta_s / \eta_w = t_s d_s / t_w d_w$$

Where as:

η_s is the sample viscosity
η_w is the water viscosity
t_s is the time of flow for sample
t_w is the time of flow for water
d is distance which is constant for the same viscometer.

The refractive indices were measured using an Abbe'refractometer, manufactured by Carl Zeiss Jena / 234174, Germany. Its sensitivity is $1x10^{-4}$ illuminated a monochromatic light source.

Data were computed and illustrated by figures.

RESULTS

Figure 1 shows the changes in the relative viscosity of hemoglobin in rats irradiated by different doses of near ultraviolet waves. Relative viscosity of hemoglobin slightly increased with increasing of exposure dose of near ultraviolet waves.

Figure 2 Shows the changes in the refractive index of hemoglobin in rats irradiated by different doses of near ultraviolet waves, whereas the refractive index is increased as the doses increase, especially before the dose of $60x\ 10^{-3}$ joul/cm^2

Figure 3 Shows the changes in the A_{578} /A_{542} of hemoglobin in rats irradiated by different doses of near ultraviolet waves, whereas the ratio slightly decreased as the doses increase.

DISCUSSION

The elevation in the relative viscosity of hemoglobin in rats exposed to near ultraviolet waves in this study could be attributed to the aggregation and unfolding of proteins in hemoglobin parts, whereas the structural dynamics play a role in the functional processes of metaloproteins [9].

Refractive index of hemoglobin in the current exposed rats increased with increased dose of near ultra violet irradiation. This finding agrees with the previous results [10], as a biophysical indicator which showing the effect of near ultraviolet irradiation on the salt bridge of tetramer hemoglobin and may lead to different possibilities of trimer, dimmer and monomers. The condition is as a result of the intermolecular interaction which refer to the stabilization of hemoglobin as a macromolecule [7].

The changes in A_{578}/A_{542} ratio confirmed the conversion of oxyhemoglobin to methemoglobin, the degree of conversion increased with the elevation of the dose. Proteins are dynamic system wriggle and breath and their motion is essential to their function. The intensity of abnormal motion seems to be related to the high doses of the near ultraviolet and is reversible in case of low doses below $60x10^{-3}$ joul/cm^2. So, decrease of this ratio showing an imbalance of the two bands at 542 and 578 nm which indicate a defect in bonds between heme iron and nitrogen in porphyrin ring and heme-heme interaction bands, respectively [10].

From this study, it was concluded that exposure to near ultraviolet radiation has some hazardous effects on the biophysical properties of hemoglobin such as, a defect in bonds between heme iron and nitrogen in porphyrin ring, heme-heme interaction band, unfolding of proteins, intermolecular interaction and a disturbance in, the stabilization of hemoglobin molecule and as a result in the function of hemoglobin.

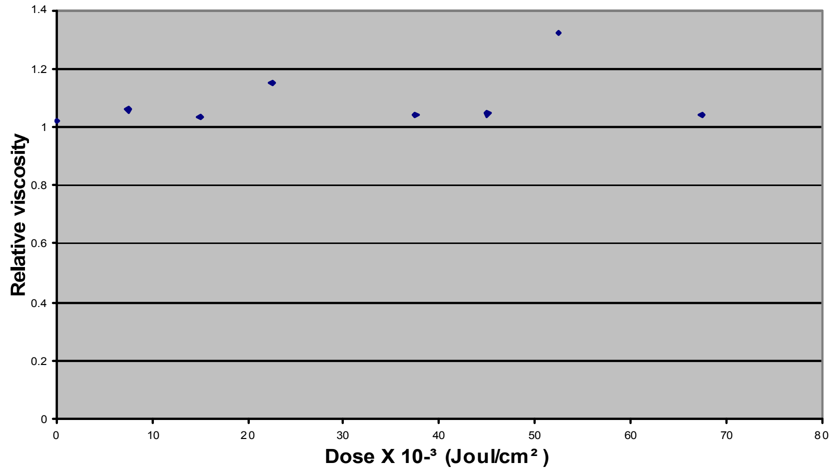

Fig. 1: Effect of different doses of near ultraviolet waves on the relative viscosity of hemoglobin in exposed rats

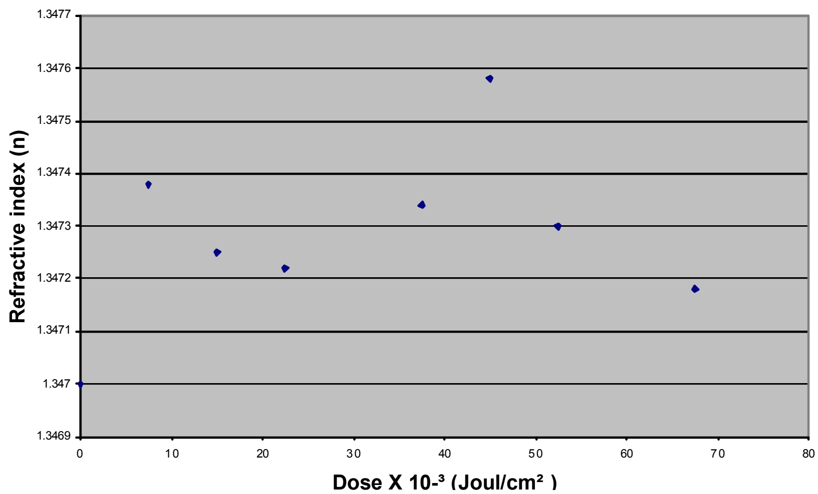

Fig. 2: Effect of different doses of near ultraviolet waves on the Refractive index of hemoglobin in exposed rats

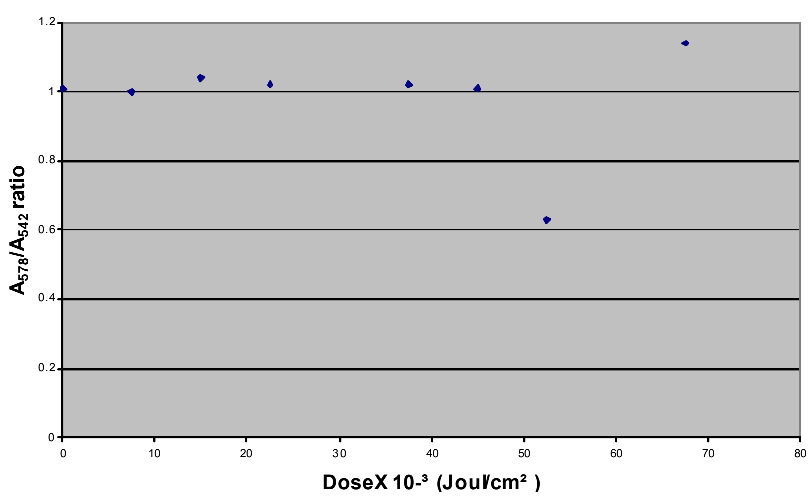

Fig. 3: The changes in A_{578} /A_{542} ratio of hemoglobin in rats exposed to near ultraviolet waves

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