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Intrinsic Viscosity and Refractive Index of Hemoglobin Molecule Before and After Irradiation by Near Ultraviolet Waves in Rats

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Abstract: This study was carried out to detect the possible changes in some biophysical properties of hemoglobin molecule like intrinsic viscosity, refractive index, buffer capacity and the ratio in absorbance at 578 to 542 nm (NUV) 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 15-105 minutes once. Blood samples were collected and some biophysical properties of hemoglobin molecule as intrinsic viscosity, refractive index, buffer capacity and the ratio in absorbance at 578 to 542 nm were measured. Results revealed that near ultraviolet waves induced a slight increase in intrinsic viscosity and refractive index of hemoglobin. Base buffer capacity increased with doses of radiation, while a decrease of acid buffer capacity was observed as compared to control. Also, a change of H^+ and OH^- receptors on the hemoglobin molecular surface was observed. 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 hemoglobin molecule of exposed rats.

Key words: Ultraviolet-radiation · Biophysics · Hemoglobin-viscosity-rats

INTRODUCTION

Since the energy of an electromagnetic photon increases with decreasing wavelength, the biological effectiveness-defined as the capacity of radiation to produce a specific biological effect-also increases with decreasing wavelength. The largest biological effects are therefore generated in the shortest wavelength range (280-400 nm) of the incident solar radiation spectrum, which is referred to as ultraviolet (UV) radiation. Living organisms (i.e., living systems such as animals, plants and microorganisms) are influenced by solar radiation and especially also by UV radiation [1].

Functional dynamics and transitions between equilibrium conformations of hemoglobin depend upon environmental factors [2]. Hemoglobin A (Hb A) with α2-β2 tetramer structure exhibited positive cooperatively 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 cooperatively is a major subject of Hb studies [3].

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 [4]. It is known that near ultraviolet (240-420 nm) is absorbed by both protein and heme in the hemoglobin molecule [5].

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Changes in the heme-globin contact exert a discernible influence on spectra and cooperative oxygen binding [6].

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 [7].

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 [8, 9].

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.

Experimental Design: Rats were divided into eight groups. Each group contained six rats.

The whole body of rats from all groups (except the first group which was saved as the control group) was exposed to the near ultraviolet rays for 15- 105 minutes as follow:

Groups	1	2	3	4	5	6	7	8
Dose X 10-³ (Joul/cm²)	0	7.5	15	22.5	37.5	45	52.5	67.5

The irradiation was carried by a mercury arc lamps which calibrated to give the previous doses at a certain 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 (4.1 x 10-5 M), the intrinsic viscosity was calculated by measuring of time of flow with constant volume of samples using Oswalt Capilay Viscometer according to the equation (Huggins equation).

$\eta_{sp/c} = [\eta] + K'[\eta]^{2}C$, where K'is the Huggins coefficient and related to solute-solute interaction [10].

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

Whereas:

- 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 Abberefractometer, manufactured by Carl Zeiss Jena / 234174, Germany. Its sensitivity is $1x10^{-4}$ illuminated a monochromatic light source.

Acid and base buffer capacities of hemoglobin were determined by titration with 0.5 mM NaOH (molecular weight 40, made in ADWIC Company) and 0.5mM HCL (molecular weight 36.46, made in ADWIC Company) respectively at constant temperature 25±1 using a magnetic stirrer in combination with digital pH meter (Kent model 7065 Type 1.691(2N3/201), Metrhom Compony, Swiss made).

Data were computed and illustrated by figures and tables.

RESULTS

Table 1 represents the changes in the Intrinsic viscosity of hemoglobin in rats irradiated by different doses of near ultraviolet waves. Intrinsic viscosity of hemoglobin slightly increased with increasing of exposure dose of near ultraviolet waves.

Table 1: Effect of different doses of near ultraviolet waves on the Intrinsic viscosity of hemoglobin in exposed rats

Dose x10^{-3}J/cm^2	Intrinsic viscosity dl/gm
0	0.1± 0.01
7.5	0.11±0.01
15	0.13± 0.01
22.5	0.11± 0.011
37.5	0.16± 0.012
45	0.13± 0.013
52.5	0.30± 0.012
67.5	0.12± 0.012

Table 2: Effect of NUV radiation on the base and acid buffer capacity of hemoglobin

Dose x10^{-3}J/cm^2	Base buffer capacity (UI)	Acid buffer capacity (UI)	Base/acid raio
0	8 ±0.83	7.04±1.6	1.14
7.5	6.6±0.99	5.04±0.9	1.31
15	6.7±1.2	5.06±0.92	1.32
22.5	10.3±0.57a	7.4±1.7	1.39
37.5	9.2±0.76	4.01±0.87a	2.3
45	12.2±0.76c	5.2±0.77	2.35
52.5	14.8±1.05c	4.1±0.9a	3.6
67.5	15.5±1.06c	4.8±0.98a	3.2

a :p< 0.05,b :p<0.01 and c: p<0.001

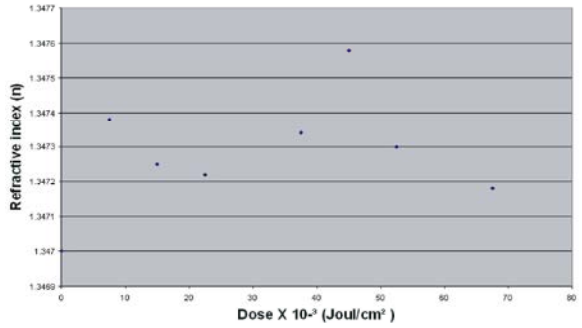

Fig. 1: Effect of different doses of near ultraviolet waves on the Refractive index of hemoglobin in exposed rats

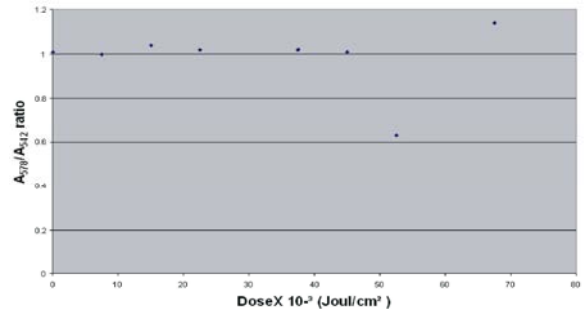

Fig. 2: The changes in A_{578} /A_{542} ratio of hemoglobin in rats exposed to near ultraviolet waves

Fig 1 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.

The changes in active sites of Hb from H^+ and /or OH^- receptors can be expressed as base or acid buffer capacity. The obtained data from table 2 revealed that as compared to control the effect of NUV doses led to a non-significant decrease in the base buffer capacity of Hb at the first two doses and the base buffer capacity started to slight increase with dose and significantly ($P<0.01$) with doses (45,52.5 and 67.5 x 10^{-3}J/cm^2).While the acid buffer capacity of Hb decreased with doses, slight at doses (37.5, 52.5 and 67.5 x 10^{-3}J/cm^2). So, the base/acid ratio reflects the changes in the H^+ and OH^- receptors showed an increase with all doses regarding control.

Fig 2 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 intrinsic 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 [11, 12].

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 [13, 14]. 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 [15, 16].

The results of buffer capacity on hemoglobin due to the effect of NUV detect a change in active sites of the molecule (H^+ and OH^-), may be explained by the appearance of new groups on the surface of the molecule, which may reflect the hydrophobic/hydrophilic ratio of the membrane. [17].

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 $45x10^{-3}$ J/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 [18].

From this study, it was concluded that exposure to near ultraviolet may lead to a disturbance in the functionof hemoglobin molecule after a critical dose ($45x10^{-3}$ J/cm^2) which should be avoid in daily life.

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