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Changes in Some Biophysical Properties of Rat's Hemoglobin Following Exposure to Ultra Violet Waves Irradiation

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Abstract: The present work was designed to study the effects of low doses of near (NUV) and middle (MUV) Ultraviolet irradiations on some biophysical properties of rat's hemoglobin (Hb) to clarify the variations of its structure and function.Samples of Hbwere divided into three groups: the first one was exposed to NUV radiation (48.6-291.6 x10^{-2} J/cm^2), the second group was irradiated by MUV doses (0.97-5.8 x10^{-2} J/cm^2) and the third group wasn't subjected to any radiation (control group). The first and second groups were subdivided into six subgroups according to the dose. Relative viscosity and electrical conductivity of Hb were measured besides examining the effect of temperature on the conductivity of Hb. Results revealed that, a decrease and fluctuation in relative viscosity of Hb molecule after irradiation by NUV due to the effect of exposureon the heme part of Hb whereas the bonds interaction increases with elevating the exposure dose, while in case of MUV there is an incrementin the values of relative viscosity as a result of the different degrees of Hbunfolding. The unfolding of Hbmolecule may also stay behind the increase of electrical conductivity with frequency in exposure to NUV, while in case of MUV there is a decrease of electrical conductivity values with higher photon energy which has an effect onthepermeability of cell membranewhich hasisnot completely denatured due to the effect of temperature on Hbfrom (45-50°C). It was concluded that the exposure of Hb by NUV enhanced bonds interaction and led to improvement of Hb function. Otherwise irradiation by MUV led to conformational and structural changes in Hb molecule.

Key words: Ultraviolet · Rlative Viscosity · Electricalconductivity · Hemoglobin

INTRODUCTION

The ultraviolet radiation (UV) has many harmful effects on human bodies. These effects may be divided into non-stochastic or stochastic. The non-stochastic effects are directly related to the radiant power and are generally acute, while the stochastic effects take the form of an increase in the risk of contracting certain diseases, of which the late appearance of skin cancer is the most important. The severity of the non-stochastic influences in the exposed individual varies with dose and there is a threshold below which no harms occur. These effects may be acute or late and can occur in any cell or tissue that can be exposed to UV irradiation [1, 2].

The biological damage that results from ultraviolet irradiation is initiated due to photochemical absorption by macro- molecules such as nucleic acids and proteins [3].

The present work aimed to study the effect of UV irradiation on some biophysical parameters of rat's hemoglobin to clarify the variations of its structure and function. UV irradiation ranges applied in this work were near UV (400-300 nm), middle UV (300-200 nm) additionally to explore the effect of temperature change on electrical conductivity of hemoglobin molecules.

MATERIALS AND METHODS

Hemoglobin Preparation: Blood samples of white Albino female rats, weighing 200-230g, were obtained by draining the blood from eyes into tubes containing heparin, using capillary plexus tubes. Hemoglobin was extracted by a Trivellimethod, Trivelli *et al*. [4]. For Hb concentration, an appropriate dilution with deionized water was used to adjust the concentration at room temperature (25±1°C) on the base of heme absorption band at 576 nm where the

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absorbance of Hb at 576 nm (A_{576}) = 0.5. Before carrying out anyprocedure, samples were centrifuged at 10,000 rpm for at least 30min because the rat hemoglobin contains precipitate consists of prismatic-type hemoglobincrystals [5]. These crystals affect the measurements and therefore, the centrifuged Hb samples were drained by the micropipette into other tubes.

Hemoglobin Irradiation by UV: Irradiation of Hb was carried out by a mercury arc lamp. The exposure of samples to UV irradiation in two ranges, NUV (400- 300nm) and MUV (300-200nm). The duration of irradiation was (15, 30, 45, 60, 75 and 90 min). The distance of source was 18 cm. The mercury arc lamp and the two filters used were calibrated in Egyptian National Institute of Standards (NIS), to calculate the exposure dose in joule / cm^2 (energy density in vitro) of UV irradiation.

The Following Exposure Doses Are Used: 48.6, 97.2, 145.8, 194.4, 243 and 291.6x10^{-2} J/$cm^2$for NUV and 0.97, 1.93, 2.9, 3.9, 4.8, 5.8 x10^{-2} J/cm^2 for MUV irradiation.

Samples of Hb Were Divided into Three Groups: The first one was exposed to NUV radiation (48.6-291.6 x10^{-2} J/cm^2), the second group was irradiated by MUV doses (0.97-5.8 x10^{-2} J/cm^2) and the third group wasn't exposed to any doses (control). The first and second groupswere subdivided into six subgroups according to the dose. The average value of the sample results was calculated and the statistical analysis was performed.

Measurement of Relativeviscosity of Hb: All measurements were carried out at a constant temperature of 25±1°C by Ostwald capillary viscometer. The viscosity of the solution was calculated by the following equation:

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

whereas η, d and t are viscosity, density and time of flowresprctively, for the sample (s) and water (w). The relative viscosity of Hb was calculated by measuring the time of flow, using a same viscometerof constant volume of sample and water.

Measurement of Electrical Coductivity (σ) of Hb: The measurements of electrical conductivity of Hbwere carried out at room temperature (25±1°C), at frequencies ranging from 42 Hz to 300 kHz to calculate conductivity (σ) of Hb, Siemens/cm(S/cm).

Effect of Temperature on Un-Irradiated Hb Control Group: The measurements of electrical conductivity of Hb were carried out at range of temperature 22-60°C, at frequencies ranging from 42 Hz to 1MHz. The temperature controller sensor was placed directly above the sample in contact with its terminal to determine the sample temperature. Five minutes were needed to reach the stability of temperature. The cell used was calibrated using standard material (air) with stable cell constant (La^{-1}) = 0.12987.

Data were presented as mean ± S.D. (Student's t –test)

RESULTS AND DISCUSSION

Relative Viscosity of Hemoglobin Irradiated by NUV (400-300nm): Figure (1) shows the value of relative viscosity of Hb which was fluctuated when irradiated by NUV radiation, but all values were lesser than control and these changes were insignificant as compared to non-irradiated samples. The fluctuation and decrease in relative viscosity of Hb when irradiated by NUV may be due to the effect of NUV on the heme part of Hb where the bonds interaction increases with the dose beside a decrease in size of Hbmolecule [1].

Relative Viscosity of Hbirradiated by MUV (300-200nm): Figure (2) illustrates the effect of MUVirradiation on the relative viscosity of Hb. It was found that the relative viscosity of Hb increased insignificantly at doses of 0.97, 4.8 and 5.8 x10^{-2} J/cm^2, significantly ($P<0.01$) at (1.93 x10^{-2} J/cm^2) and slightly at 2.9 and 3.9 x10^{-2} J/cm^2 when irradiated by MUV as compared to control. Enhancement of relative viscosity of Hb, when irradiated by MUV referred to the effect of weakness in heme bonds and occurance of different degrees of protein unfolding. (Table 1).

Table 1: Effect of MUVirradiation (300-200 nm) on relative viscosity of Hb

Dose x10^{-2} J/cm^2	Relative viscosity of Hb
$Ctrl_{n=6}$	1.22±.008
$0.97_{n=6}$	1.22±.005
$1.93_{n=6}$	1.24±.005[ii]
$2.9_{n=6}$	1.229±.012[i]
$3.9_{n=6}$	1.238 ±.009[i]
$4.8_{n=6}$	1.223±010
$5.8_{n=6}$	1.229±.070

n = no. of samples

i: $P< 0.05$ slightly significant,

ii: $P< 0.01$ moderately significant.

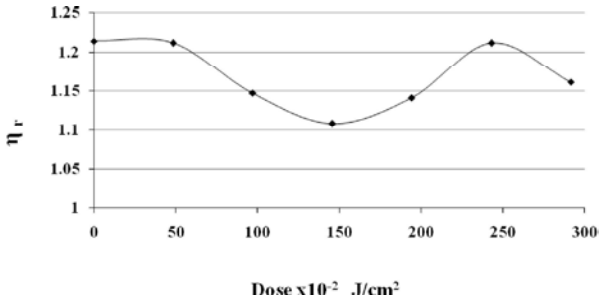

Fig. 1: Effect of NUV radiation on the relative viscosity of Hb

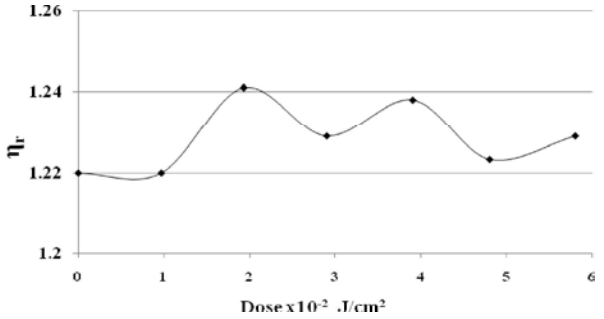

Fig. 2: Influence of MUV irradiation on the relative viscosity of Hb

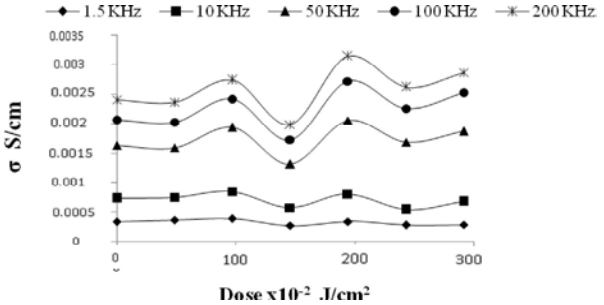

Fig. 3: Conductivity of Hbirradiated by different doses of NUV for each value of frequency

The decrease of relative viscosity of Hbwhen irradiated by NUV may be as a result of the effect of UV on the heme part of Hb whereas the bonds interaction increased with the applied doses besides the drop in size of molecule. The increase of relative viscosity of Hb when irradiated by MUV referred to the effect of weakness inheme bonds and occurrence of different degrees of protein unfolding which leads to increase insize of molecule. Abd-Elmotaleb [6] reached same results and noticed that the lesser values of viscosity forHbmolecules was due to influence of NUV-irradiation and could be as a result of the indirect effect of NUV on the protein parts. Raymond and Victor [7] confirmed that the relative viscosity of protein molecules increases during unfolding as a result of larger size of molecule.

Electrical Conductivityhb Irradiated by NUV (400-300nm): Fig. 3 shows the change of electrical conductivity (σ) owing to irradiation of Hb by different doses of NUV irradiation from 48.6 to 291,6 J/cm^2 for each value of the applied frequencies. It is clear from this figure that the conductivity is enhanced as the frequency reaches greater values. Therefore the electrical conductivity ofHb irradiated by NUV increased with frequency. It was fluctuated at higher values of frequency from 50 to 200 kHz. This increment in conductivity may be due to Hb molecule unfolding and associated with enlargment in the size of molecule.

These results are in agreement with the results obtained by Moussa [8] which found that, the electrical conductivity of Hb of rats exposed to microwave irradiation was significantly increased as compared to control.

Electrical Conductivity of Hb Irradiated by MUV (300-200 nm): Figure 4. Illustrates the effect of *MUV* doses on the electrical conductivity of Hb as irradiated by doses in the range from 0.97 to 5.8x10^{-2} J/cm^2 for each value of the applied frequencies. It was clear that conductivity was decreased at higher frequencies compared to control. This decrement in conductivity may be due to adverse effects of MUV which have shorter wavelength with higher photon energy. These findings are in agreement with the results obtained by Selim *et al*. [9] which explained the drop in conductivity due to the permeability damage of cell membrane associated with the subsequent loss of ions, electrolytes and intracellular components.

Effect of Temperature on the Electrical Conductivity of Unirradiated Hb: Fig. (5) illustrates the effect of temperature on conductivity of unirradiated Hb at different frequencies in the range from 42Hz to 1MHz. Conductivity showed an increment with temperature until 50°C. From 50-60°C, it was found non-appreciable change in conductivity. It may be as a result of half denaturation of Hb at 45-50°C. Jansson and Swenson [10] confirmed that after 50°C, Hb molecule can't return to the native state and it suffers from the aggregation at about 60°C. Kinderlerer *et al*. [11] mentioned that if the hemoglobin was heated to a temperature exceeding 50°C, a hysteresis effect was observed on cooling.

Also, Jansson and Swenson [10] investigated the dynamical behavior of both the hemoglobin and its surrounding water during the denaturation process. They observed that the unfolding and aggregation

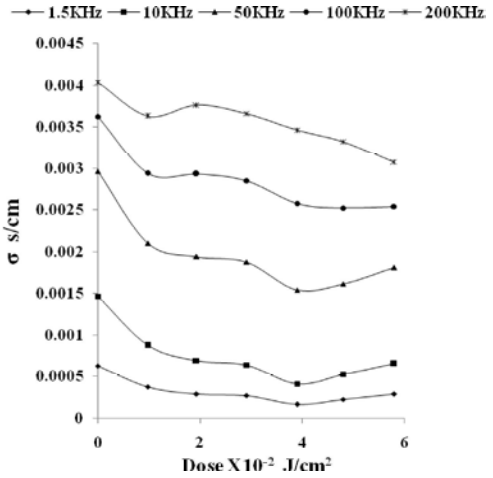

Fig. 4: Effect of MUV doses on conductivity of Hbfor each value of frequency

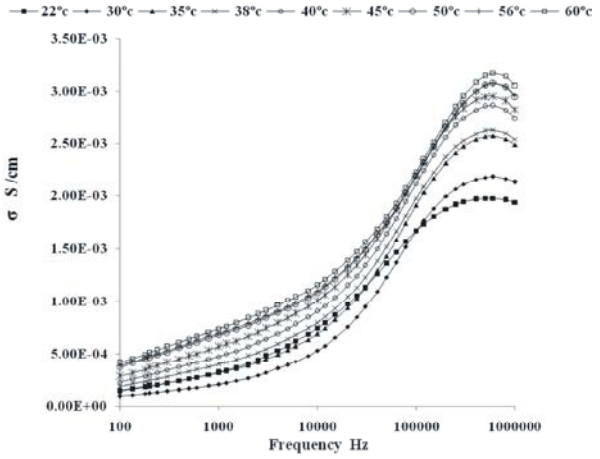

Fig. 5: Effect of temperature on conductivity of unirradiatedHbatdiffrent frequencies

processes are substantially overlapping under the effect of changing temperature. The unfolding process occurs in the approximate temperature range from 42 to 72°C, whereas the aggregation process starts around (57-62°C) and is completed at 87°C [12].

CONCLUSION

Irradiation ofHb molecule by NUV may lead to the enhancement of Hb function. Whereas its irradiation by MUV caused conformational and structural changes in Hb molecule.

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