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Spectral Analysis for Rat's Hemoglobin Molecule

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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 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 subjected to any radiation (control group). The first and second groups were subdivided into six subgroups according to the dose. The absorption spectra and autoxidation rate of Hb were estimated. Results revealed that, the absorbance of globin increased non-significantly as affected by doses (0.97-3.9 $x10^{-2}$J/cm^2), while it decreased insignificantly at (4.8 $x10^{-2}$ J/cm^2) and significantly at the last dose (5.8 $x10^{-2}$J/cm^2). The decrement of absorbance may be due to the weakness or stretching of some bonds or starting of protein unfolding. Also, some changes were found in the heme part where the absorbance decreased significantly as affected by UV doses. The appearance of new band at 630 nm as a result of converting of ($HbFe^{2+}$) to ($HbFe^{3+}$) at dose (3.9 $x10^{-2}$J/cm^2) and at the last dose (5.8 $x10^{-2}$J/cm^2). The absorbance of Soret band at (414 nm) decreased when compared to control as affected by all doses of UV (0.97-5.8 $x10^{-2}$J/cm^2) and shifted from 414 nm to 412 nm at the last three doses (3.9, 4.8 and 5.8 $x10^{-2}$J/cm^2) to confirm the conversion of OxyHb to metHb at these doses. This is confirmed by autoxidation rate of Hb which increased with doses (0.97-2.9 $x10^{-2}$ J/cm^2) as a result of conversion of oxyHb to metHb. It was concluded that the bonds interaction of Hb molecule enhanced which led to the improvement of Hb function as affected by NUV doses, whereas MUV doses led to conformational and structural changes in the Hb molecule, specially the last three doses (3.9, 4.8 and 5.8 x 10^{-2} J/cm^2) which had a negative effect on the function of Hb.

Key words: Ultraviolet · Autoxidation · Hemoglobin · Spectrum

INTRODUCTION

Photohaemolysis of erythrocytes was studied under UVB light, (290-320 nm) region. Maximum haemolysis was obtained with rat red blood cells (RBCs), followed by human, fish, sheep, pigeon, lizard and frog RBCs. The rate of UVB-induced haemolysis was almost identical to that produced by UVC (200-290), both causing extensive damage to RBCs. On the other hand, natural sunlight or UVA (320-400 nm) caused very little damage to RBCs. The results indicated that exposure to UVB is detrimental to RBCs. And photomodification of RBCs is induced even with small increments in UVB level due to stratospheric ozone depletion [1]

Hemolysis in rat erythrocyte membrane induced by irradiation with ultraviolet (UV) light at 254 nm showed a pronounced oxygen effect. Under irradiation in vacuum, the rate of hemolysis decreased by an order of magnitude. Irradiation at 254 nm in air but not under vacuum caused the peroxidation of erythrocyte membrane lipids. These results suggest that membrane lipid photoperoxidation is one of the causative factors of UV hemolysis. Irradiation at different wavelengths showed that UV-induced lipid photoperoxidation in erythrocyte membranes developed while the antioxidant α-tocopherol was directly photooxidized [2].

MATERIALS AND METHODS

The samples of blood were collected from female albino rats (200-230g) and Hb was separated from the blood according to [3]. The samples were exposed to the UV mercury lamp at distance 18cm for (15-90 min).

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The absorption spectrum of Hb was measured by (Jasco. UV-visible spectrophotometer type V-570, made in Germany), in the range between 200 and 700 nm at 25±1°C. The concentration of Hb was adjusted by measuring the absorption of Hb at 576 nm (A_{576}=0.5). The measurements were carried out in spectroscopy lab., physics division, National Research Center (NRC), Dokki, Giza, Egypt. [4]

Measurement of the autoxidation rate of HbO_2 was carried out in water bath at temperature 37°C.The reaction was followed by scanning the wavelengths (500-700nm) and taking the reading at 630nm every 1h by 240-UV/visible spectrophotometer (made in Germany). The measurements were carried out in the biophysics lab., genetic engineering and biotechnology division, NRC, Dokki, Giza, Egypt. The ratio of concentration of HbO_2 at zero time to that after t time, which is required for the first order plot, was obtained by following absorbance at 630nm and by the equation:

$$(HbO_2)_o / (HbO_2)_t = (A_o - A_\infty)/ (A_t - A_\infty)$$

Where A_o, A_t and A_∞ are the absorbances at time o, t and complete conversion to methemoglobin respectively. [5]

RESULTS AND DISCUSSION

The absorption spectra and absorbance bands of Hb as affected by different doses of near UV (NUV) or long UV (UVA) radiation are shown in fig (1) and table (1).

In the globin part, the absorbance of aromatic amino acids band at 276 nm showed a high significant increase with doses till the dose (243x10^{-2} J/cm^2), while at the last dose (291.6 x10^{-2} J/cm^2), the absorbance decreased significantly accompanied by a slight shift to shorter wavelengths as compared to control [6]. In the heme part, the absorbance increment was remarked, insignificantly, at the iron-nitrogen band (542 nm) with dose until (243 x10^{-2} J/cm^2) while a non-significant decrease in absorbance was observed at the last dose (291.6 x10^{-2} J/cm^2) as compared to control. The same trend was found in heme-heme interaction band at 576nm [7]. This finding was reflected in 576/542 ratio which it was more than one at (control, 48.6 and 194.4x10^{-2} J/cm^2), equals one at (145.8 x10^{-2} J/cm^2) and less than one at (0.97, 243 and 291.6 x10^{-2} J/cm^2). The diminished value of 576/542 ratio at the last dose (291.6 x10^{-2} J/cm^2) was accompanied by the appearance of new band at 630nm. A non-significant increase in absorbance was obtained at Soret band (414nm) until the dose (243 x10^{-2} J/cm^2), while the absorbance decreased insignificantly at the last dose (291.6 x 10^{-2} J/cm^2) accompanied by a change in the half width of this peak as compared to control. [8,9] A slight increment in absorbance of globin-heme interaction band at 340 nm was observed with all doses of NUV radiation as compared to control. Effect of MUV doses (0.97-5.8 x10^{-2} J/cm^2) on absorption spectra of Hb as compared to control is shown in fig (2) and table (2).

In the globin part, the aromatic amino acids band at 276 nm showed an increment in absorbance non-significantly at the first three doses (0.97, 2.9 and 3.9 x10^{-2} J/cm^2) and equal to control at (1.93 x10^{-2} J/cm^2), while the absorbance decreased insignificantly at the dose (4.8 x10^{-2} J/cm^2) and slight significantly at the last dose (5.8 x10^{-2} J/cm^2). In the heme part, the iron-nitrogen band at (542 nm) and the heme-heme interaction band at (576 nm) had the same trend, where the absorbance decreased non-significantly till the dose (3.9 x10^{-2} J/cm^2), slight significantly at (4.8 x10^{-2} J/cm^2) and high significantly at the last dose (5.8 x10^{-2} J/cm^2) as compared to control [6,8]. So, the 576/542 ratio, which was more than one at control, showed a decrement with all doses but still more than one.

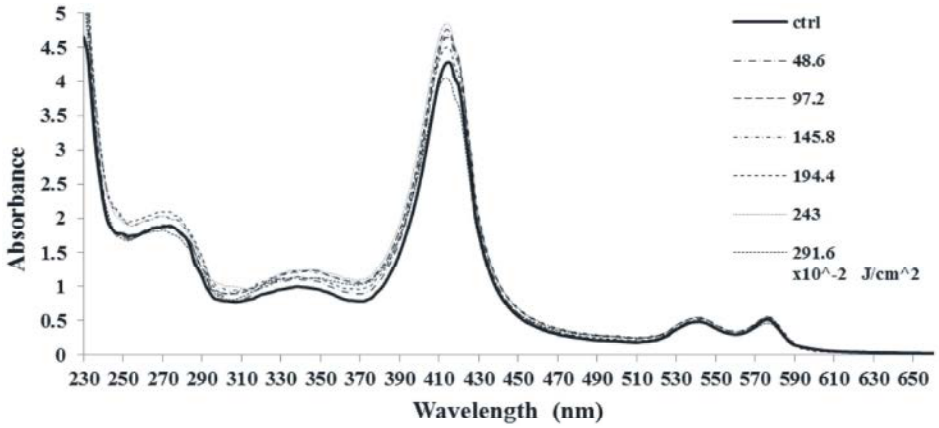

Fig. 1: Absorption spectrum of Hb affected by NUV radiation.

Table 1: Effect of NUV radiation on the absorption spectrum of Hb

	Bands of Hb						
Dose x10^{-2} J/cm^2	Aromatic amino acid λ =276nm	Globin-heme interaction λ =340nm	Soret band (oxyHb) = λ 414nm	Nitrogen-iron band = λ 542nm	Heme-heme interaction λ=576nm	MetHb λ =630nm	576/542 ratio
$0_{n=6}$	1.86±0.047	0.99±0.04	4.28±0.18	0.489±0.007	0.5±0.0013	0.037±0.03	1.023
$48.6_{n=6}$	1.87±0.018	1.09±0.13	4.66±0.83	0.521±0.082	0.528±0.09	0.032±.0003	1.014
$97.2_{n=6}$	1.88±0.027	1.21±0.16^{i}	4.76±0.8	0.544±0.08	0.542±0.083	0.042±0.005	0.996
$145.8_{n=6}$	1.99±0.021^{iii}	1.24±0.13^{i}	4.7±0.9	0.546±0.05	0.546±0.05	0.039±0.02	1
$194.4_{n=6}$	2.05±0.01^{iii}	1.13±0.16	4.51±0.95	0.499±0.084	0.502±0.084	0.034±0.008	1.006
$243_{n=6}$	1.99±0.027^{iii}	1.23±0.21^{i}	4.86±0.95	0.549±0.089	0.545±0.091	0.038±0.012	0.99
$291.6_{n=6}$	1.75±0.2^{iii}	1.13±.3	4.045±1.13	0.475±0.1	0.455±0.11	0.052±0.021	0.96

n= no. of samples

i: P<0.05 slightly significant, ii: P<.01 moderately significant, iii: P<.001 highly significant

Table 2: Effect of MUV radiation on the absorption spectrum of Hb

	Bands of Hb						
Dose x10^{-2} J/cm^2	Aromatic amino acid λ =276nm	Globin-heme interaction λ =340nm	Soret band (oxyHb) = λ 414nm	Nitrogen-iron band = λ 542nm	Heme-heme interaction λ=576nm	MetHb λ =630nm	576/542 ratio
$0_{n=6}$	1.62±0.09	0.97±0.009	4.49±0.21	0.49±0.009	0.5±0.008	0.024±0.012	1.02
$0.97_{n=6}$	1.65±0.13	0.966±0.042	4.48±0.23	0.48±0.025	0.5±0.027	0.022±0.006	1.018
$1.93_{n=6}$	1.62±0.122	0.95±0.07	4.29±0.4	0.474±0.028	0.489±0.035	0.022±0.021	1.012
$2.9_{n=6}$	1.68±0.14	.985±0.052	4.39±0.3	0.471±0.027	0.479±0.032	0.031±0.012	1.014
$3.9_{n=6}$	1.68±0.19	.982±0.073	4.27±0.3	0.467±0.031	0.468±0.037	0.032±0.002	1.0036
$4.8_{n=6}$	1.56±0.112	0.96±0.06	4.38±0.5	0.455±0.031^{i}	0.459±0.042^{i}	0.029±0.009	1.008
$5.8_{n=6}$	1.48±0.09^{i}	0.92±0.07	4.13±0.4	0.442±0.032^{iii}	0.443±0.04^{iii}	0.036±0.01	1.003

n= no. of samples i: P<0.05 slightly significant, ii: P<.01 moderately significant iii: P<.001 highly significant.

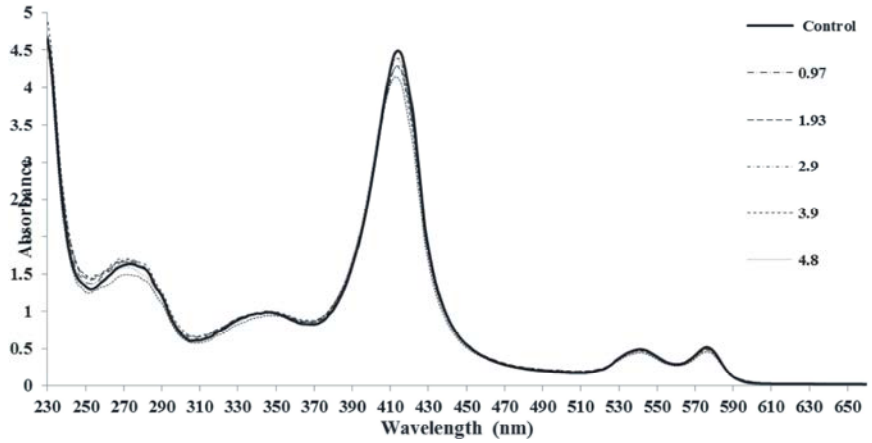

Fig. 2: Effect of MUV radiation on the absorption spectrum of Hb.

The intensity of a new band at 630 nm went hand in hand with the value of the 576/542 ratio which this band appeared at the doses (3.9 and 5.8 x10^{-2} J/cm^2) [10]. At Soret band, 414 nm, the absorbance decreased with all doses insignificantly as compared to control and there was a shift to shorter wavelengths in this band, fig (2), at the last three doses (3.9, 4.8 and 5.8 x10^{-2} J/cm^2). An inappreciable effect of doses was observed in the globin-heme interaction band at (340nm), where the absorbance increased at (2.9 and 3.9 x10^{-2} J/cm^2) and decreased at (0.97, 1.93, 4.8 and 5.8 x10^{-2} J/cm^2) non-significantly. [11,12].

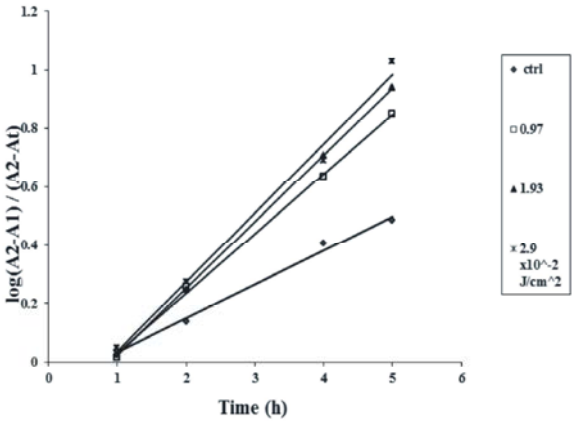

Fig. 3: Effect of UV radiation on the autoxidation of Hb.

Fig (3) illustrated the oxidation of Hb versus time (h) as affected by UV radiation. It was found that the autoxidation of Hb increased gradually with doses of UV radiation.It may be due to the dependance of UV mechanism on the production of reactive oxygen species (ROS) [13,14] confirmed that the autoxidation of hemoglobin was increased when the solution of hemoglobin was irradiated by UV radiation under aerobic conditions. The oxidation of hemoglobin was not observed when hemoglobin had been irradiated with UV ray under anaerobic conditions. The rate of oxidation of oxyhemoglobin induced by UV irradiation was dependent on the changes in pH, i.e., it was accelerated at acidic pHs. The result suggested that active oxygen produced by UV irradiation may be involved in the oxidation of hemoglobin [15, 16].

Also, the UVB converts oxyHb to metHb. and the superoxide radical (O_2^-) is produced when oxyhemoglobin autoxidizes and the active species (O_2^-) and H_2O_2 produced from it in the absence of catalase or superoxide dismutase, cause oxidation of further hemoglobin, [13,15]

CONCLUSIONS

An increment of autoxidation rate of Hb with doses (0.97-2.9 $x10^{-2}$ J/cm^2) as a result of conversion of oxyHb to metHb and an improvement of Hb function due to NUV irradiation, whereas MUV doses led to conformational and structural changes in the Hb molecule, specially the last three doses (3.9, 4.8 and 5.8 x 10^{-2} J/cm^2) which had a negative effect on the function of Hb.

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