Original Research Article

Probing Effect of 6 MeV Electron Beam Irradiation on Haemoglobin Protein Using Spectroscopic Techniques

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Abstract

In this work, we study the effect of 6 MeV electron beam irradiation on the physicochemical properties of lyophilized Human Haemoglobin A (HbA). Electron beams generated from Race Track Microtron accelerator with energy 6 MeV were used to irradiate HbA at fluences of 5×10^{14} e^{-}/cm^2 and 10×10^{14} e^{-}/cm^2. Pristine and electron beam irradiated HbA were characterized using UV-visible and Fourier transform infrared spectroscopy (FTIR) spectroscopy. The interfacial tension of the aqueous solutions of HbA are also analysed by pendant drop method. Absorbance intensity, % transmittance and interfacial tension decrease with fluence. The peak position of the Soret band (λ_{soret} = 404 nm) remains unaffected by the fluences. FTIR spectroscopy confirms the changes in the secondary structure of the haemoglobin. In the amide band I, the percentage of α-helix reduced from 8% to 1%, and an increase in β-sheet (19% to 29%) and β helix (6.3% to 15%) is observed. Interfacial tension decreases from 46.0 mN/m and 44.0 mN/m with increase in irradiation dose. These finding provides realistic guideline for biological cells exposure to electron beam radiation doses.

Keywords

electron irradiation, haemoglobin, interfacial tension, UV-visible, Fourier transform infrared spectroscopy

Introduction

From the beginning of the universe, not only human beings but also each and every object on the earth has been exposed by different radiations like ionizing and non-ionizing. The effect of ionizing radiation viz. gamma radiation, X-ray radiation and electron beam radiation on biological cells is an interesting subject to understand complex physiochemical processes.[1,2] It is useful to improve cancer treatment, chemotherapy and development of biomedical devices.[3] Radiation used in cancer therapy cause changes in red blood cell – cytoskeleton, lipid membrane, phospholipid bilayer, and leakage of proteins depending on its wavelength, dose rate, and exposure time.[4] Proteins are more complex macromolecule and sensitive to external microenvironment. Impact of high energy ionizing radiation modifies the protein structure which leads to cleavage of peptide bond, protein fragmentation, protein aggregation, protein folding and unfolding as a result denaturation of protein. Moreover, radiation affects the secondary structure of proteins like α-helix, β-sheets, random coils and aggregation. Such a conformational change in protein causes abnormalities in cell structure.[5,6] Indirect effect of radiation on water causes radiolysis and generates free radicals (H_2O, H_2O^+, OH^+, H^-, H^+, O^+, and O^-) which cause damage to the cells, tissues, organs, and reactive oxygen species (ROS) alters the internal structure and functionality of macromolecules like DNA, RNA, lipid, proteins, etc.[7]

Effect of electron beam irradiation on blood and its components was used to avoid the graft vs host disease (GVHD).[8] Electron beam carries both mass and charge and hence highly interactive with body tissues and non-tissue biomaterial like forming hydrogel for artificial kidney and

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blood vessels.[9] Electron beam radiation of 6 MeV energy is also used for industrial processing, polymerization, preservation, microbial inhibition, pharmaceutical products and sterilization of medical devices viz. needles, syringes, surgical gloves, blood containers, etc.[10] In order to understand implication of electron beam (6 MeV) on macromolecules, we used haemoglobin (globular protein) as a model system which plays an important role in microcirculation. Hb protein (64 kDa) is composed of 2 α and 2 β polypeptide chain. Hb occurs inside red blood cells (RBC) with Fe moiety encapsulated with porphyrin ring which supplies oxygen to whole body.[11] Any disturbance in the physiological state of iron electronic structure affects metabolic processes.[12] Conformational changes in haem protein ($\alpha_2\beta_2$) cause disorder in human body.[13] Various biochemicals and biophysical studies viz. circular dichroism (CD), dynamic light scattering (DLS), MS-MALDI, nuclear magnetic resonance (NMR) and electrophoresis gel techniques on haemoglobin due to ionizing radiation have been reported.[14]

The objective of the present work is that spectroscopic investigation of electron beam irradiation (6 MeV) effect particularly at 2 fluences, that is, 5×10^{14} e^-/cm^2 and 10×10^{14} e^-/cm^2 on HbA by UV-visible spectroscopy and FTIR spectroscopy. In addition, interfacial tension measurement technique (IFT) is used to confirm the ability of irradiated HbA to adsorb at air/water interface to understand the protein stability. The result of the study is useful in terms of knowledge and safety of 6 MeV electron beam handling and risk management concerns in the health of workers, technicians and radiologists.

Materials and Methods

The lyophilized haemoglobin A was purchased from Sigma Aldrich used for the study of electron beam irradiation. Pure powdered haemoglobin protein was used without further purification.

Electron Beam Irradiation

6 MeV energy Race Track Microtron accelerator[15] was used for haemoglobin irradiation. Schematic of Race Track Microtron Accelerator is shown in Figure 1.

The samples, cut in 1×1 cm^2 polythene bag, were mounted on the faraday cup. The electron beam energy, beam diameter and beam current values were 6 MeV, 16 mm and 100 nA, respectively. During the experiment, the pressure of the irradiation chamber was at 10^{-6} mbar. The current indicator connected to the faraday cup was used to read the counts for desired fluence. The irradiation carried out with 2 fluences measured the electron fluence, that is, 5×10^{14} e^-/cm^2 (5k) and 10×10^{14} e^-/cm^2 (10k). The irradiated samples were further characterized by UV-visible spectroscopy, FTIR spectroscopy and interfacial tension measurement.

Characterization

UV Visible Spectroscopy

Haemoglobin protein solution was made using .9 % NaCl saline (pH: 7.00). All solutions were freshly prepared with dilution of 1:50. UV-visible measurement was obtained using JASCO-V-670 spectrophotometer, within spectral range of 200–700 nm.

Fourier Transform Infrared Spectroscopy

Lyophilized HbA solution were mixed with potassium bromide (KBr), compressed it and record the spectra in the range of 1000–1800 cm^{-1} with JASCO-FTIR-6100 spectrophotometer. Percentage area (%) calculated from Gaussian fitted curve was used to provide the information of secondary structure (α-helix, β-sheets, β-turn, random coli and aggregate) of haemoglobin protein.

Interfacial Tension Measurement

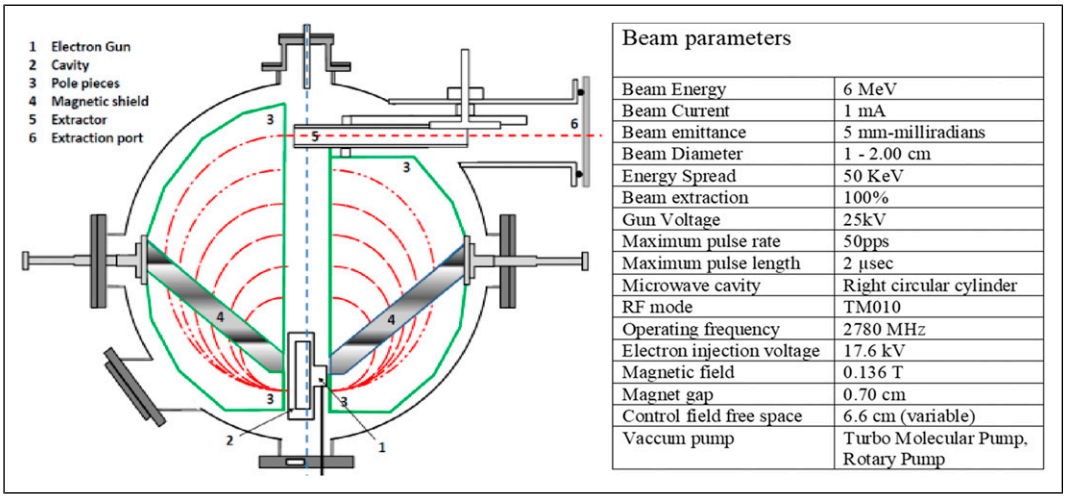
The activity and functionality of haemoglobin protein was determined by using interfacial tension. Solution of lyophilized HbA powder (1 mg/mL) was prepared. By using pendant drop method, interfacial tension of Hb protein is measured with SCA 20 software. After each measurement, syringe and needle was cleaned by washing solution (Detergent soap, IPA) in a sonicator bath. The interfacial tension (IFT) of water was performed after each sample measurement to avoid the contamination. (Instrument was calibrated using milli Q water before each set of experiment at 25°C)

Results and Discussion

UV Visible Spectroscopy

Structural and micro-environmental changes of proteins were studied by using UV-Visible absorption spectroscopy. Figure 2 showed the UV-Visible absorption spectra of lyophilized human haemoglobin A in the presence and absence of electron beam irradiation within the spectral range of 200–800 nm.

Electron beam irradiation results indicated that when electron dose is increased, peak position is shifted from 220 nm to 214 nm with hyperchromic blue shift due to the slight folding of protein structure. Electron beam irradiation changes the haemoglobin microenvironment around amide bonds. The absorbance at wavelength 271 nm decreased due to intermediate state was more folded comparatively to native state of haemoglobin. This indicates that red shift in protein structure occurred in irradiated haemoglobin. Due to the electron irradiation protein structure is changed, a significant hyperchromicity appears for all the haemoglobin bands for all fluences. The major change in the haem group occurred due to iron oxidation. This oxidation results in a



Beam parameters	
Beam Energy	6 MeV
Beam Current	1 mA
Beam emittance	5 mm-milliradians
Beam Diameter	1 - 2.00 cm
Energy Spread	50 KeV
Beam extraction	100%
Gun Voltage	25kV
Maximum pulse rate	50pps
Maximum pulse length	2 μsec
Microwave cavity	Right circular cylinder
RF mode	TM010
Operating frequency	2780 MHz
Electron injection voltage	17.6 kV
Magnetic field	0.136 T
Magnet gap	0.70 cm
Control field free space	6.6 cm (variable)
Vaccum pump	Turbo Molecular Pump, Rotary Pump

Figure 1. Schematic view diagram of Race-Track Microtron accelerator and its associated electron beam parameters.

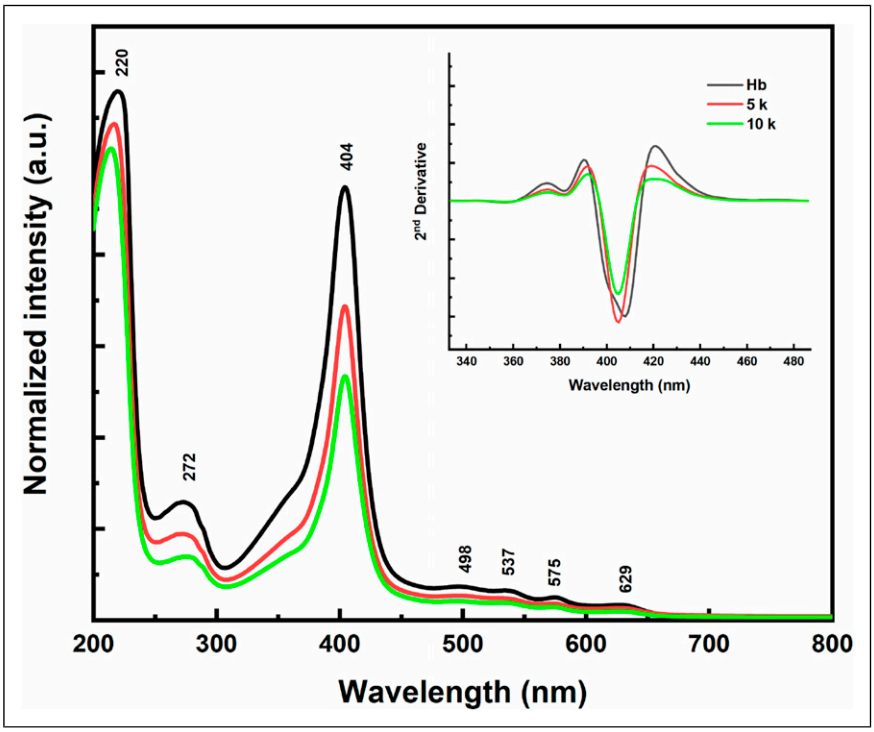


Figure 2. UV-Visible spectra for ▬ lyophilized haemoglobin A at 6 MeV energy electron beam irradiated at fluences of ▬ 5 k = 5 × 10^{14} e^{-}/cm^{2} and ▬ 10 k = 10 × 10^{14} e^{-}/cm. Inset presents second derivative of UV-Visible spectra of haemoglobin A in the range of 330–480 nm indicating change in Soret band.

loss of biological activity of haemoglobin bands which are as follows: 570 nm (haem–haem interaction band), 550 nm (Fe-N in porphyrin nitrogen iron bonds in porphyrin), 404 nm (Soret band), 350 nm (globin-haem interaction band) and 271 nm (protein band) and 220 nm (carboxylic acid moieties).[16,17] Decrease in absorbance indicates a partial loss of haemoglobin molecule stability. Also, electron radiation disrupted the haem groups resulting in the decrease of the absorbance at the Soret band. It causes a slight breakdown of the polypeptide chain and break down of the covalent bonds. However, the maximum absorption of Soret band is decreased after electron irradiation, while maximum absorption wavelength remains unchanged. It indicates that the interaction does not affect the structure of the haem group. Disruption of haem group breakdown of polypeptide chain and covalent band is caused by electron

beam radiation which is reflected in decrease in absorbance at the Soret band peak. Absorption at Soret peak position may provide information about conformational changes in the haem group. Therefore, UV-visible spectroscopy is a useful tool for conformational study of the haem region. Destruction of molecules was observed for increasing electron fluences. This confirms the widely accepted conclusion that electron beam radiation causes molecular and subsequently cellular damage.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy was used to compare the structural information of haemoglobin irradiated with 6 MeV energy electron beam. Conformational changes in protein structure are characterized by functional groups in the FTIR spectra. FTIR spectroscopy is a useful tool to check the conformational integrity of the haem proteins. This is due to its sensitivity to the chemical composition and architecture of molecules. Conformational changes in the secondary structure of the haemoglobin polypeptide chain are observed in the amide I and amide II bands by FTIR after irradiation and compared with a pristine haemoglobin sample. The amide I band (1700-1600 cm^{-1}) is caused by C = 0 stretching and the amide II band is assigned to a combination of N–H bending and C–N stretching vibration.[18,19] Figure 3 showed the FTIR spectra of lyophilized human haemoglobin A treated with electron beam irradiation within the spectral ranges of 1000–1800 cm^{-1}.

The transmission mode FTIR spectrum of pre-irradiated haemoglobin showed the peaks at wavenumber 1687.75 cm^{-1} (C = O), 1548.60 cm^{-1} (N–H bending and C–N stretching), 1449.31 cm^{-1} (C = C), 1394.40 cm^{-1} (N = O), 1331.65 cm^{-1} (C–O–H), 1169 cm^{-1} (C–O) and 1105 cm^{-1} (C–O) and their % transmittance were 12.46, 19.20, 35.98, 36.49, 38.83, 54.30 and 52.84, respectively. Whereas the percentage transmission of electron irradiated (6 MeV) haemoglobin is found to decrease at the fluences of 5×10^{14} e^{-}/cm^2 and 10×10^{14} e^{-}/cm^2, respectively. This result showed that the 6 MeV electron beam induces the conformational changes in haemoglobin. Diminishing the intensity and changes in the shape of amide I and amide II indicated the denaturation of haemoglobin, as well as change in protein secondary structure was observed more in amide I as compared to amide II.

The amide I and II band of irradiated haemoglobin appeared at 1687.75 cm^{-1} and 1548.60 cm^{-1}, respectively, which has negligible shift to native state of haemoglobin spectrum. Interaction of electron beam with C = O and C–N groups in the protein polypeptide chain causes changes in secondary structure of haemoglobin.

To find the best Gaussian-shaped curves that fit the original haemoglobin FTIR spectra, a curve fitting procedure was used (for more detail see ES1). The spectral ranges from 1615–1637 cm^{-1}, 1638–1648 cm^{-1},1649–1660 cm^{-1},1660–1680 cm^{-1} and 1680–1692 cm^{-1} in the amide I was attributed to β-sheet, random coil, α-helix, β-turn and β-antiparallel structures, respectively. The % area of each secondary structure of human haemoglobin A was calculated by the relative area of their respective component band shown in Figure 4.

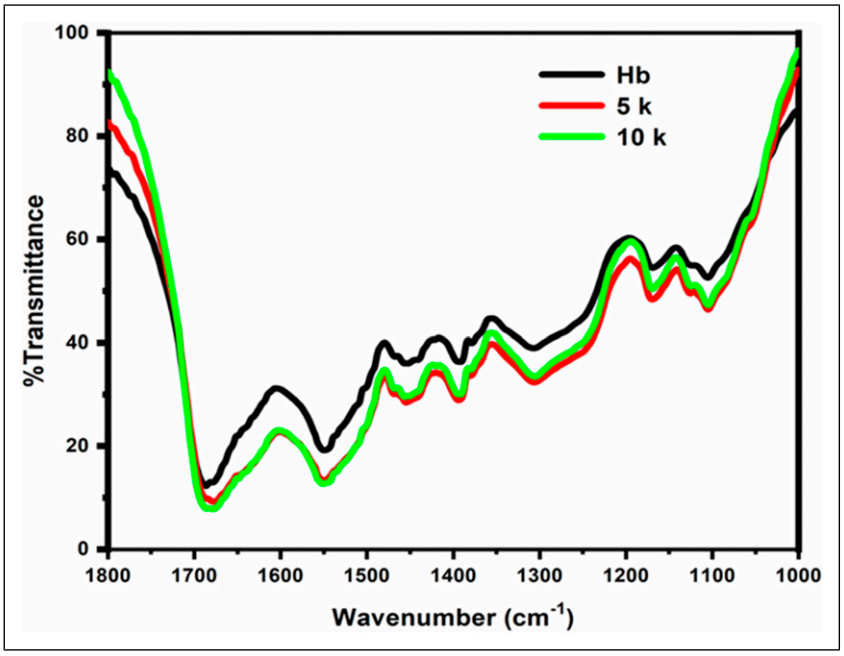


Figure 3. FTIR spectra of — lyophilized haemoglobin A irradiated at 6 MeV energy electron with different fluences — 5 k = 5×10^{14} e^{-}/cm^2 and — 10 k = 10×10^{14} e^{-}/cm^2.

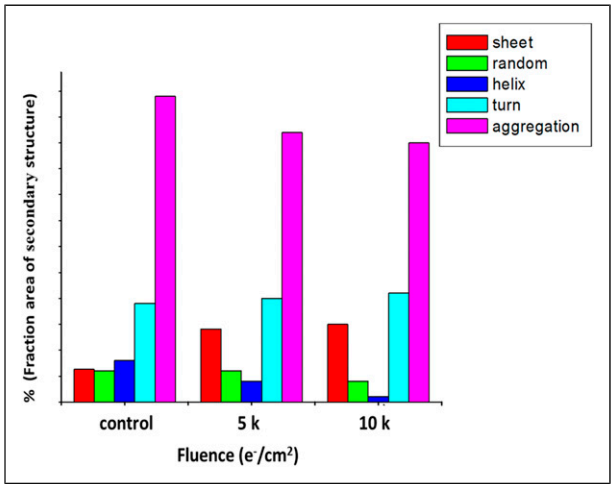


Figure 4. Percentage fraction area of secondary structure of lyophilized human haemoglobin A irradiated at 6 MeV energy electron beam.

Quantitative analysis of the secondary structure of pristine and irradiated haemoglobin showed that the α-helix decreased from 8% to 1% and β-turn and β-sheet increased from 19% to 29% and 6.3% to 15%, respectively. This observation indicates that 6 MeV electron beam irradiation damages the haemoglobin protein structure due to an increase in β-sheet structure and a decrease in α-helical structure.

Interfacial Tension Measurement

The interfacial tension measurement is used to confirm the physiological properties of haemoglobin irradiated with 6 MeV energy electron beam. IFT of lyophilized human haemoglobin A is decreased with an increase in electron fluences. Interfacial tension of virgin haemoglobin was 46.05 mN/m and it was decreased to 45.07 mN/m and 44.03 mN/m with the electron fluences 5×10^{14} e^{-}/cm^2 and 10×10^{14} e^{-}/cm^2, respectively. As seen in Figure 5, interfacial tension measurement of lyophilized human haemoglobin A is in the presence and absence of electron beam irradiation using pendant drop method.[20]

Electron beam with 6 MeV energy causes changes in polypeptide group, amide groups and secondary structures (α-helix, β-sheets, etc.) which alters the surface activity of haemoglobin. Hence, rate of adsorption of Hb protein at air/water interface decreases, and lower the interfacial tension at fluence of 5k and 10k. These results confirm that electron beam irradiation affect the function of haemoglobin. The present study is a prototype where the irradiation (6 MeV) has been carried out and limited to lyophilized haemoglobin powder for 2 electron beam fluences. However, dose-dependent cellular studies provide more information about the effect of electron beam irradiation on a biological system. Therefore, detailed study of electron beam irradiation on cells in vitro and in vivo can also be carried out in future.

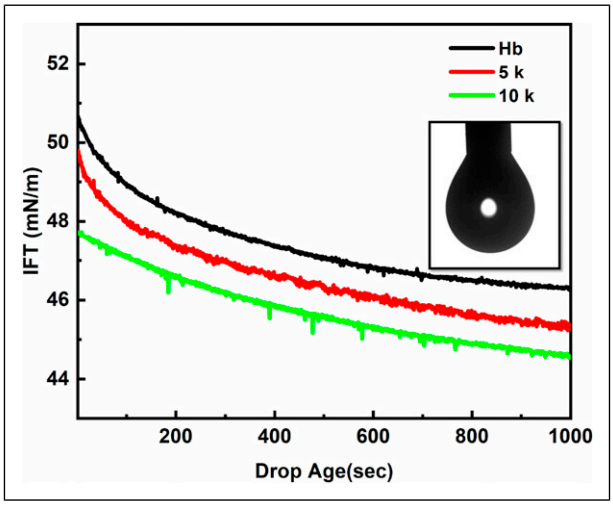


Figure 5. Interfacial tension of — lyophilized haemoglobin A and 6 MeV energy electron beam irradiated at fluences — 5 k = 5×10^{14} e^{-}/cm^2 and — 10 k = 10×10^{14} e^{-}/cm^2. Inset presents the pendant drop of haemoglobin.

Conclusions

As probed by the spectroscopic techniques such as UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) and interfacial tension measurement, the irradiation of 6 MeV electron beam is found to yield conformational and structural changes in haemoglobin protein. The structure, function and the adsorption ability of haemoglobin can be tuned by optimizing the electron beam parameters such as the energy and fluence. Therefore, the electron beam irradiation can be used as a fascinating tool to tailor the bio samples at molecular level.

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Author Contributions

S.H.: Conceptualization, Methodology, Validation, Investigation, Data curation, writing-original draft; S.D.: Providing electron beam facility; A.B.: Writing and Editing, Supervision; G.K.: Data curation, writing-review and editing, Supervision.

Declaration of Conflicting Interests

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Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Supplemental Material

Supplemental material for this article is available online.

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