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Spectroscopic study of gamma irradiation effect on the molecular structure of bovine serum albumin

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ABSTRACT

The effect of gamma radiation on the molecular structure, size distribution and surface charge of bovine serum albumin (BSA) was studied by spectroscopic techniques. The first structure of non-irradiated and irradiated BSA were investigated by UV-Vis spectroscopy and electrophoresis (SDS– PAGE). Additionally, the secondary and tertiary structural changes of BSA were studied by circular dichroism (CD) and fluorescence spectroscopy, respectively. Dynamic light scattering (DLS) and electrophoretic light scattering (ELS) was used for clarify of size and surface charge of BSA. The results indicate that the first structure of BSA is preserved, while the secondary and tertiary structures have changed significantly. The result of CD studies shows an 8% decrease in α-helix and an increase in other secondary structures for irradiated BSA in comparison to non-irradiated ones. Moreover, DLS and ELS displayed the decrease in the size and surface charge of irradiated BSA. The aggregation of irradiated BSA was also confirmed by fluorescence spectroscopy. The ELS results as an additional data confirm the aggregation of protein. The results demonstrate that doses close to therapeutic ones can lead to structural changes in macromolecules as well as the aggregation of polypeptide chain but without the fragmentation.

1. Introduction

Proteins are important biomolecules that play different roles in selectivity and specificity of living organisms. Their conformations are prone to change under a variety of external conditions such as chemical alterations (pH, metal ions and denaturants) and as well as physical alterations (radiations and temperature), which consequently affected the protein function [1–4]. Among the different proteins in living organism, bovine serum albumin (BSA) is particularly interesting as it holds certain advantages. BSA is a single-chain transporting protein which has molecular weight around ~66 kDa [4] and the number of amino acids of BSA has frequently been cited between 582 and 607 according to literatures [5,6].

BSA has several important physiological and pharmacological functions. It transports metals, fatty acids, cholesterol, pigments, and drugs. It is a key element in the regulation of osmotic pressure and distribution of fluid between different compartments [4].

Radiations as the physical alteration can damage cells in two ways; i) direct effect, through damaging DNA and other cellular targets, ii) indirect effect, through producing reactive oxygen species (ROS) [7]. ROS contain hydroxyl radicals (the most damaging), superoxide anion radicals, hydrogen peroxide and other oxidants which are generated by different environmental stress such as radiation. Exposure of biological organism in ionized radiation, caused ROS production in issue environment [8]. Since 70% volume of biological organism composed of water molecules that radiolysis via absorbing radiation energy and produce ROS. Generally, ROS make biological damage to vital cellular bio-molecules such as DNA, proteins and lipids [9]. Biological damages initiate by free radicals and proceeded through a variety of mechanisms. One of the damaging mechanism is cell membrane damage which is created by lipid peroxidation and protein aggregation [10]. The chemical changes that caused by irradiation to biopolymers such as proteins, leads to fragmentation, cross-linking, aggregation, and oxidation radicals generated by the radiolysis of water [11–13].

H. Schuessler and et al. [14], have reported that BSA was cleaved by the oxidative destruction of proline residues. These studies show

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that, after irradiation of proteins in solution, covalent cross-linkages are formed between free amino acids or peptides [15].

Determination and analysis of the secondary structure of proteins is investigated using FTIR [16], ATR-FTIR [17], ATR-IR [5], nuclear magnetic resonance (NMR) [18], Far UV region (190–250 nm) circular dichroism (CD) [19,20], Vacuum-Ultraviolet CD (VUVCD) [21] and Raman spectroscopy methods [22]. The near UV region (240–360 nm) CD and Fluorescence spectroscopy can provide information of tertiary structure. Changes in the local environment of tryptophan residues can be followed by changes in the emission spectra [23].

CD have limitation in ultraviolet (VUV) region between 100 and 200 nm because of the strong absorption of light by oxygen at these wavelengths. The short-wavelength limit of CD spectroscopy can be extended by the new generation of CD equipped with vacuum. Vacuum Ultraviolet CD (VUVCD) spectrophotometers using synchrotron radiation as an intense light source was developed to spread the short-wavelength limit [24–26]. The secondary structures of 15 globular proteins were investigated in the wavelength region from 160 to 260 nm under a high vacuum by VUVCD [27]. Matsuo et al. elucidated the structure of three proteins (metmyoglobin, staphylococcal nuclease, and thioredoxin) in the native and desaturated statues [28]. Although, the application of VUVCD recently is growing, conventional CD is still a common method.

In this study, we investigated the effect of gamma radiation in the absorbed dose of 5 Gy on the BSA as the most abundant protein in blood plasma. The first, secondary and tertiary structures of BSA, as well as its size distribution and surface charge were studied. UV-Vis spectroscopy, Dynamic light scattering (DLS), electrophoretic light scattering (ELS), circular dichroism (CD), electrophoresis (SDS–PAGE), and fluorescence spectroscopy are used to monitor structure changes of BSA. In this study, we investigated the effect of gamma radiation in the absorbed dose of 5 Gy on the BSA as the most abundant protein in blood plasma. Additionally, the secondary and tertiary structural changes of BSA were studied by circular dichroism (CD) and fluorescence spectroscopy, respectively. Dynamic light scattering (DLS) and electrophoretic light scattering (ELS) was used for clarify of size and surface charge of BSA.

2. Materials and experiments

2.1. Reagents and apparatus

Bovine serum albumin (BSA) (used without further purification), tetramethylethylendiamine (TEMED), acrylamide, acrylamide-bis, tris base, coomassie brilliant blue, bromophenol blue, methanol, acetic acid, sodium chloride (NaCl), sodium hydroxide (NaOH), 2-mercaptoethanol, ammonium persulfate, isopropyl alcohol, iso-butanol, ethylene diamine tetra acetic acid (EDTA), glycerol, glycine, potassium dihydrogen phosphate (KH2PO4) and potassium hydrogen phosphate (K2HPO4), acetonitrile were purchased from Merck (Darmstadt, Germany). Sodium dodecyl sulfate and Formic acid were purchased from Sigma chemical company (USA). The solutions were prepared in deionized double distilled water (Barnstead, Nano pure infinity, USA) and all experiments were carried out at room temperature.

Spectroscopic measurements were performed using UV-Vis spectrophotometer (Carry 100, Varian, Australia), spectrofluorometer (Carry edipse, varian, Australia), Circular Dichroism Spectrometer Model-215 (215, Aviv, USA). Zeta sizer Nano, ZS, (Malvern Instruments, the United Kingdom) was used for surface charge determination and measuring of the relative sizes of the BSA before and after radiation. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), (Sino Biological Inc, Korea) and HPLC, hypersil-bds-c18-hplc-columns-4 × 125 mm was applied for investigation of the first protein structure. Each BSA sample was prepared at least three times and then scanned by Uv-vis, CD and fluorescence spectrophotometer.

2.2. Sample irradiation

One-milligram of BSA was dissolved in 1 ml of 10 mM phosphate buffer (pH 7.0) and it was placed in glass vials and irradiated at room temperature using 60Co γ-rays with dose rate of 2.68 Gy/sec, in atomic energy organization (Tehran, Iran). In this process, BSA absorbed gamma-radiation at dose of 5 Gy.

2.3. UV-Vis spectroscopy

UV optical spectra of irradiated and non-irradiated BSA were recorded at room temperature in a 1-cm quartz cell by DU 800 spectrophotometer in the wavelength region 200–400 nm. The reported UV spectra were the average of three scans that the error in the reading was less than 1% (Standard error estimation).

2.4. Fluorescence spectroscopy

The fluorescence emission intensity of the irradiated BSA was measured using a spectrofluorometer equipped with 1.0 cm quartz cells and a thermostat bath. The BSA solutions were excited at 280 nm and the emission spectra were recorded from 300 to 440 nm.

2.5. Circular dichroism spectroscopy (CD)

The CD absorption spectra were recorded on a circular dichroism Spectrometer under nitrogen atmosphere. Quartz cells have path length of 1 and 0.1 cm for near and far region respectively. The protein concentrations were 0.2 and 0.5 mg/ml for far and near region, respectively. All the experiments were run at room temperature. The scanning speed was 200 nm/min. The CD measurements of irradiated BSA solutions were made in the range of 190–260 nm. All of the CD spectra were baseline-subtracted by using a spectrum of the solvent obtained under the same experimental conditions. Data analyzed using CDNN 2.1 (Nerve Network) software.

2.6. DLS and ELS measurements

DLS experiments were carried out at room temperature with a Zeta sizer Nano ZS. For each sample; the spectra were recorded with 30 scans at a time. The scattering intensity data were processed using the instrumental software to obtain the hydrodynamic diameter and the size distribution of sample. DLS data processing is dependent to fluctuation of scattered light intensity by dissolved particles at a fixed scattering angle. All experiments were performed at a $\theta = 90^{\circ}$ and $\lambda = 514$ nm.

ELS measurements were made on a Zeta sizer Nano ZS at room temperature. For each sample, the spectra were recorded with 100 scans at a time.

2.7. Electrophoretic analysis

Protein damage was evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS– PAGE). Electrophoresis of proteins was performed on 12% acrylamide resolving gel with 6% acrylamide stacking gel that was poured on top of the resolving gel. In the upper part of the stacking gel, the gel comb was placed 40 μl of each protein solution samples were mixed with sample buffer and were boiled in water for 4 min, then each of the samples in the