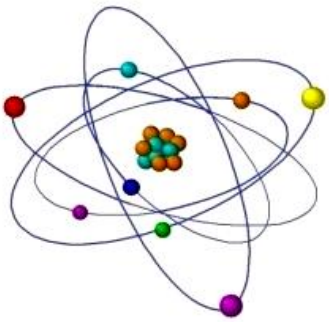
IMPACT OF GAMMA IRRRADIATION ON THE THERMODYNAMIC AND METAL BINDING PROPERTIES OF BOVIN SERUM ALBUMIN



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ABSTRACT: *Effect of gamma(γ)- irradiation on the bovin serum albumin's (BSA) global thermodynamic stability and metal binding ability has been examined. ^{137}Cs was used as a source of γ-irradiation. It has been shown that irradiation of BSA solution with doses 50Gy and 100Gy causes drastic decrease of amount of proteins molecules capable to interact and form complexes with double charged copper ions while the global stability of BSA is in less extent effected under the same irradiation conditions.*

Keywords: irradiation, bovin serum albumin, double charged copper ions, differential scanning calorimetry

INTRODUCTION

Gamma (γ) irradiation is widely applied in various fields of our life such as industry, agriculture, medicine and etc. Use of irradiation in the medicine should be specially mentioned. Gamma irradiation can be helpful if properly used but at the same time, we must remember that ionizing radiation can severely alter and damage living systems, so its misuse (Incorrect dosage, duration of irradiation and etc.) can lead to irreversible and life-threatening consequences. Therefore, it is of great importance to study the impact of gamma irradiation on biological organisms and objects at both, the molecular and cellular levels in order to protect radiation safety [1-4].

In general, the subject of our interest is the study of the effect of gamma irradiation on blood serum albumin of mammals - Human serum albumin (HSA) and bovine serum albumin (BSA). HSA and BSA (molecular weight ca. 66500Da) are the most studied representatives of monomer globular proteins in serum plasma [5-9]. Human serum albumin is structurally, as well as functionally similar with bovin serum albumin [5,8,16,17] (see fig1). Due to this similarity often in laboratories during intensive experimental work relatively cheap and easily available BSA is used instead of expensive HBA [16,17]

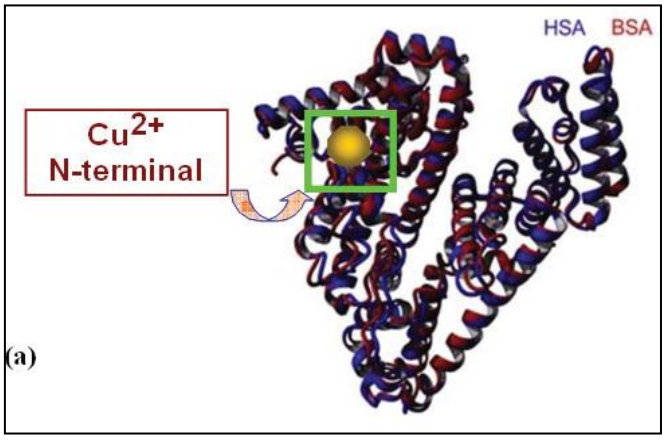


Fig.1. Structure of BSA (blue) and HAS (red) and N- terminal binding site for copper ions (yellow) sphere (according to Ref. [5,8,17])

Physiological function of serum albumins is maintenance of osmotic pressure in serum, transporting of fatty acids, amino acids, medicines, therapeutic agents, drugs, metal ions and etc [9-15]. BSA and HSA are an important transporter of the physiologically essential double charged copper (Cu^{2+}) and zinc (Zn^{2+}) ions as well as toxic nickel (Ni^{2+}) and Cd ($^{2+}$) ions in living organisms [9-18]. Electron paramagnetic resonance spectroscopy and fourier transform infrared spectroscopy was used to identify BSA/HSA specific metal binding sites and binding intensity [8,9,18]. In particular, albumins have partially selective metal binding sites with well-defined metal preferences and they can bind different amount of distinct metals. According to [9] one molecule BSA can bind up to 4 molecules of double charged copper ions. The N-terminal region of bovin serum albumin (sequence of Asp-Thr-His-Lys), provides a specific binding site for the first copper ion [5,8,9,17,18,]. As one can see from Fig.1, this chelating site is located at the peripheral area of protein. Binding sites of further Cu^{2+} ions has been marked as ``non-specific" [31]. Meanwhile, under different specific conditions metal ions binding to protein, can cause/induce proteins conformational and structural changes and eventually even aggregation [9,20-22]. Specifically, depending on the molar ratio of metal and protein ions in the solution, attachment of metal ions to protein, may impact protein's structure, induce proteins structural changes resulting in appearance of precipitate [19,20, 9]. According to authors [20] when adding double charged cobalt, nickel, ions (Co^{2+}, Ni^{2+}) to the HSA solution aggregation occurs if (Co^{2+}, Ni^{2+}) /HSA molar ratio exceeds 8, while BSA forms precipitate after binding approximately 6 or more copper ions and 8 or more zinc ions [19]. In [17] complex formation between BSA and divalent copper chloride ($CuCl_2$) in the solutions containing equal amounts (1.8×10^{-3} M) of albumin and divalent copper ions (so, [BSA]:[Cu^{2+}]= 1:1) has been studied using thermodynamic and voltammetric approach. According to the authors [17] complex formation was accompanied with a very dramatic shift in Cu^{2+} reduction process to much more negative potentials. Along with voltamperometric experiments complex formation was confirmed with combined microcalorimetric examinations [17].

The effect of gamma irradiation on the molecular structure of serum albumins is discussed in the literature [23-27], while specifically the influence of gamma irradiation on metal binding properties of serums albumins, to our knowledge, has not been studied yet. According [23] the results of distribution of specific length of Z-structures on the film surface as well as absorption and fluorescence spectroscopy and dynamic light scattering data, have shown that exposure of bovine serum albumin to gamma irradiation rays (source ^{60}Co) with doses between 1 and 200 Gy leads to minor changes in the structure of the protein. Changes happened when irradiation dose exceeds 200Gy [23,26,27]. However, according to Zarei [24] even absorbed dose 5 Gy (source ^{60}Co) causes structural changes in BSA, specifically primary structure is unaltered, while spectroscopic data revealed obvious changes in secondary and tertiary structure of protein. In the case, when source of irradiation is ^{137}Cs γ-rays, changes in the secondary structure and tertiary structure of BSA starts over irradiation doses 500 Gy, while, exposure up to the 500 Gy irradiation dose causes a minor change [25].

In present work we focused on the studies on effect of gamma irradiation on metal binding possibilities of bovin serum albumin with doubly charged copper ions (Cu^{2+}). Thermodynamic response of the BSA under irradiation conditions is also reviewed.

MATHERIALS AND METHODS

Bovin Serum Albumin (BSA), copper oxide ($CuCl_2 \cdot 2\ H_2O$), potassium Chloride (KCl) were purchased from Sigma and were used without further purification. Solution samples of bovin serum albumin (pH 6.1) were placed in plastic vials and irradiated at room temperature using ^{137}Cs as a gamma radiation source (Dose rate -1.1 Gy per minute).

The radiation doses were 50 Gy and 100 Gy, 200 Gy. Mikrocallorimetric measurements were performed with DSC instrument DASM-4A connecting to PC via the Interface unit PCI-DASM 4-A. All solutions were prepared using Milli Q water.

RESULTS AND DISCUSSIONS

Figure 2 and Figure 3 depict the zero-baseline-corrected calorimetric curves (partial heat capacity of protein versus temperature) for the temperature-induced melting of non irradiated and irradiated (with -50 Gy , 100 and 200 Gy) bovin serum albumin, correspondingly in 0.1 M phosphate buffer solution and in 0.1M phosphate buffer solutions containing 1 M NaCl. As one can see from figure 2 protein melting (endothermic) peak, as a whole, gradually shifts to lower temperatures and decreases in height when going from non irradiated to irradiated with dose 50 Gy sample and this trend continue when increasing the irradiation dose. Addition of NaCl (Figure 3) causes stabilization toward the transition temperature for non radiated [29] as well as irradiated BSA samples.

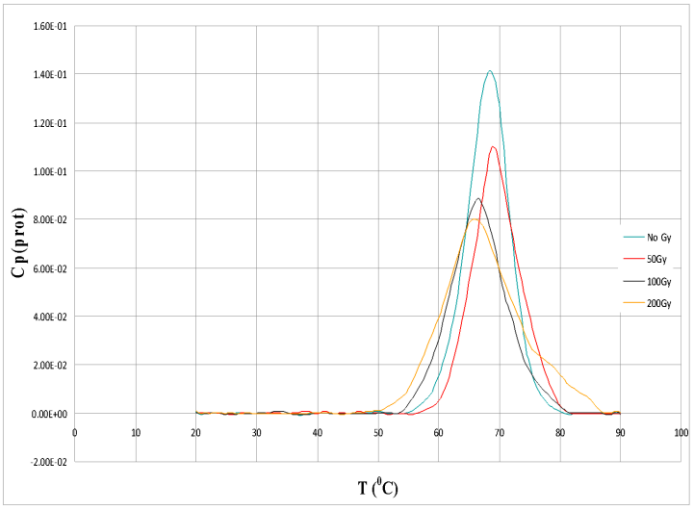


Fig. 2. The DSC melting curves of bovin serum albumin in 0.1 M PBS solution. From top to bottom: non- irradiated and irradiated with doses 50 Gy, 100 Gy and 200 Gy samples

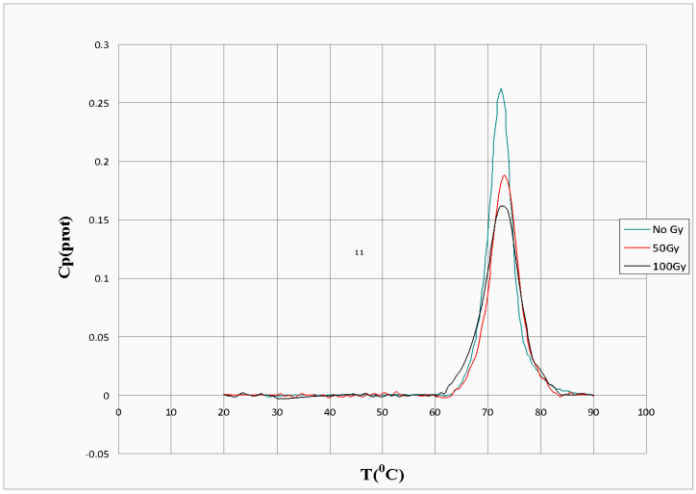


Fig.3. The DSC melting curves of bovin serum albumin in 0.1 M PBS +1 M NaCl. From top to bottom: non- irradiated and irradiated with doses 50 Gy, 100 Gy samples

Thermodynamic parameters of thermal melting for non irradiated and irradiated albumin, in pure phosphate buffer solution and phosphate buffer solution containing 1M NaCL, calculated from curves depicted in Fig. 2 and fig. 3 are gathered in Table 1.

Table 1. Thermodynamic parameters for thermal melting of non- irradiated and irradiated BSA in 0.1 M PBS and 0.1M PBS+ 1M NaCl solutions

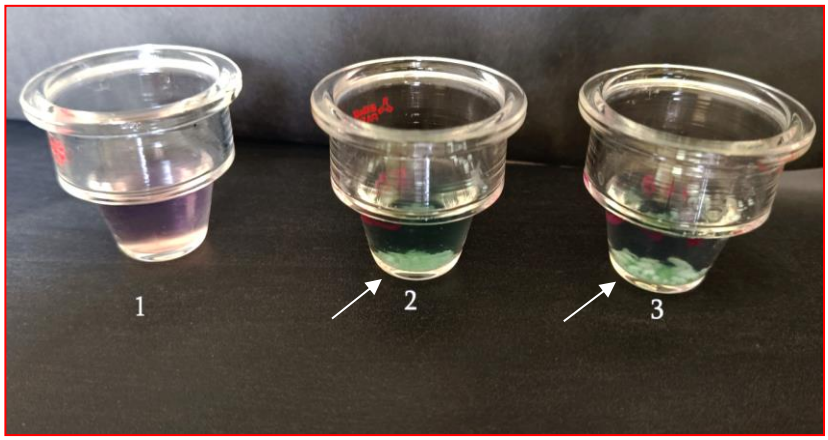
solution	Irradiation dose [Gy]	Tm, [^{0}C]	ΔTm, [^{0}C]	ΔHcal, (arbitrary units)
0.1 M PBS	0	68.46	8.11	1.32
	50	67.9	8,72	1.05
	100	66.58	10.35	1.01
	200	65.89	13.02	1.19
0.1 M PBS + 1M NaCl	0	72.67	5.03	1.68
	50	73.4	6.09	1.39
	100	73.11	7.46	1.39

It is clearly visible from table1, that in 0.1 M PBS solutions there is small but distinct destabilization regarding the transition temperature, when going from non- irradiated to irradiated with dose 50 Gy sample , *Tm*, viz., 68, 46 ^{o}C for the non-irradiated BSA sample, versus 67.9 ^{o}C for the BSA sample irradiated with 50 Gy dose, and this tendency continuous with increasing irradiation doses.

The over-all melting enthalpy, Δ*Hcal* is decreasing gradually, while values of peak width at the half height (Δ*T*) are increasing, when going from non irradiated to radiated with doses 50 Gy, 100 Gy and 200 Gy samples.

It can be seen from the table 1, that adding 1 M NaCl to the 0.1M PBS solution of BSA causes some stabilization of transition temperature [29,30], in comparison with pure 0.1M PBS solution for non irradiated, as well as for irradiated samples. Meantime in 1 M NaCl + 0.1M PBS solutions, tendency for changes of Δ*Hcal* and Δ*T* remains the same (as in 0.1M PBS solution) when going from non irradiated to irradiated with doses 50 Gy, 100Gy samples. In overall, this kind of calorimetric behavior is characteristic for the "molten-globule" (MG) or "moltenglobule-like" states in which the protein's tertiary structure is still compact and native-like, but rather labialized [27].

So, based on calorimetric investigation results we can tell that gamma irradiation under above mentioned experimental conditions does not cause remarkable changes in BSA structure and stability this conclusion is in agreement with literature data [23-27].



Picture 1. Glass cells containing mixture of BSA and $CuCl_2$ solutions. In cell 1- BSA solution was non irradiated; In cell 2 and cell 3 BSA solution was irradiated, correspondingly with dose 50Gy and 100 Gy (see text below)

Picture 1 shows three glass cells: all three glass cells contain mixture of (1.8×10^{-3} M) bovin serum albumin solution and (1.8×10^{-3} M) double charged copper chloride solution ([$CuCl_2$] / [BSA] = 1:1). Difference is that in the cell 1 BSA solution was not irradiated prior to mixing with $CuCl_2$ solution, while in other two cases (cells 2 and 3) BSA solution was irradiated correspondingly with dose 50 Gy and 100 Gy before mixing with double charged copper chloride. Meantime, solution in the cell 1 has pink colour and is without sediment, while in the cells 2 and 3 solution has green-blue colour and contains white precipitate (indicated by arrows). It should be noted, that water solution of bovin serum albumin (non irradiated as well as irradiated) has light yellow colour, while $CuCl_2$solution has greenish blue colour.

As it was shown in [17] and discussed in the introduction, complex formation between BSA and double charged copper ions is accompanied with colour changes: when blending equal concentrations (1.8×10^{-3} M) of non irradiated, light yellow BSA solution and bluish-green $CuCl_2$solution, mixture turns to pink (picture1, cell 1).

It can be seen from Picture 1 (cell2 and cell 3), that in contrast with case of non-irradiated BSA (cell 1), adding divalent copper chloride solution to the irradiated (dose 50 Gy and 100 Gy) BSA solution of the same concentration ([$CuCl_2$] / [BSA] = 1:1) results in forming a white precipitate, while the solution retains the characteristic greenish-blue color of divalent copper chloride.

For interpretation of above-mentioned results, we should bear in mind that, as it was shown in [19], one molecule of BSA can bind up to a maximum of 5 atoms of divalent copper ions. A further increase in copper to BSA ratio ([Cu^{2+}] : [BSA] ≥ 6) causes a changes in the protein's structure resulting in formation of proteins deposit[19]. Therefore, to explain appearance of precipitate in the mixture of equal concentrations (1.8×10^{-3}M) of $CuCl_2$ solution and the irradiated BSA solution (Picture 1, cell 2) we have to assume, that (after irradiation of 1.8×10^{-3}M BSA solution) the real ratio of divalent copper ions to BSA ions

([Cu 2+] : [BSA]) is not 1:1, but is at least 6 (or more). In other words, we have to assume, that irradiation of (1.8 x 10^{-3}) M BSA solution with a dose of 50 Gy effects BSA molecules metal binding sites and causes the decrease of the number of 'active' BSA molecules, which have the ability to bind copper ions, at least 6 times. So, the real concentration of "active" BSA molecules capable of binding metal ions, (as a result of irradiation of 1.8 x 10^{-3} M BSA solution with a dose of 50 Gy), is probably a maximum (3 10^{-4})M (1.8 10^{-3} M/ 6 = 3 10^{-4}M), i.e. approximately a maximum of 16,67% of the original concentration.

To check the assumption we take into account, that mixture of BSA and $CuCl_2$ has pink color and is without precipitate when ratio[$CuCl_2$] : [BSA] is equal to 1:1[17,19] and suppose that irradiated with a dose of 50 Gy (1.8 x 10^{-3})M BSA solution contains maximum 16, 67 % of the original concentration of BSA molecules with metal binding affinity (i.e. approximately 3 10^{-4}M**)**. So**,** we added (2 10^{-4} M) $CuCl_2$ solution to the irradiated with a dose of 50 Gy (1.8 x 10^{-3}) M BSA solution.

The mixture acquired a pink color (there was no precipitate) (not shown) as expected. When a similar experiment was performed on BSA solution irradiated with a dose of 100 Gy, abundant precipitation was observed, which indicates that much less than 16% of BSA molecules in the solution irradiated with a dose of 100 Gy have the ability to bind metal ions.

From the comparison of the impact of gamma rays on the general thermodynamic stability of protein with its impact on metal bindings possibility of BSA one can see that under γ-irradiation with doses 50 and 100 BSA molecules undergo relatively minor general structural changes (destabilization), while under same conditions about 84% of protein molecules loses their ability to bind copper ions. In other words, irradiation induces different effect on proteins global thermodynamic stability and on the local, protein- metal (N-terminal site - Cu^{2+}) interaction. It should be mentioned, that it is not mandatory that under different stabilizing/ destabilizing conditions, protein's local ligand- metal bond stability follow the path of global thermodynamic stability. For example, in the case of Cytochrome C in the presence of destabilizing agent [28] protein's local ligand- metal bond (Met 80- Fe^{3+}) stability was retained while global conformation was severely altered [28]. According to authors this probably was due the fact that porphyrin in Cytochrome C with triple charged iron ions was buried inside the protein and was not easily reachable for destabilizing agent urea [28].

Taking into account high penetrating ability of gamma rays [31,32] it is hardly believable that copper binding site (N-terminal, see fig.1) due to it's location at the periphery of protein, is easier damageable under influence of gamma rays than BSA molecules global thermodynamic stability in whole. More plausible explanation is that radicals generated under radiolysis of water molecules [32,33], in the course of gamma irradiation of BSA water solution, are responsible for drastic deterioration of chelating possibilities of protein molecules. This assumption will be the subject of our future investigations.

CONCLUSIONS.

Comparing the results of microcalorimetric investigations of biovin serum albumins thermodynamic stability before and after of irradiation of proteins solutions (source 137Cs, doses 50 Gy and 100Gy) on the one hand, and analyzing the BSA- metal binding possibility changes (under same irradiation conditions) on the other hand, we came to the conclusion that gamma irradiation affects both, the global stability of protein and protein – metal binding properties but in a in different extent. Specifically, gamma irradiation of BSA solutions with dose 50 Gy results in decrease of number of molecules capable to bind double charged copper ions down to approximately maximum 16 %; increase of radiation dose (up to 100 Gy) causes the further drastic decrease in number of 'complex formation capable' BSA molecules. Meanwhile, under the same conditions, γ-irradiation induces minor changes in the structure and global stability of BSA molecules.

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