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Effects of Cu^{2+} on stability and composition of water soluble ternary polyacrylic acid-Cu^{2+}-protein complexes against radiation damage

M. I. Mastafaev[1,2], A. S. Saraç[2], A. Y. Erkol[3], S. Bayülken[3], B. Dinçer[3], E. Sezer[2]

[1] Baku State University, Chemistry Department, Baku, Azerbaijan
[2] Istanbul Technical University, Chemistry Department, 80626 Maslak, Istanbul, Turkey
[3] TAEA, Çekmece Nuclear Research and Training Center, Istanbul, Turkey

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Summary

The formation of water soluble ternary complexes of polyacrylic acid (PAA) and bovine serum albumin (BSA) in the presence of divalent copper ions was investigated at the neutral pH. Aqueous solutions of PAA, BSA, and their mixture (PAA-BSA, PAA-Cu^{2+}-BSA) have been irradiated with ^{60}Co γ-rays and the changes occurred have been measured by high performance liquid chromatography (HPLC) and spectrophotometry. Addition of Cu^{2+} ions to PAA-BSA mixture have been shown to protect the PAA and BSA components of ternary PAA-Cu^{2+}-BSA complexes against radiation damage. A structural model of ternary polycomplexes was proposed and the mechanism of observed protection effect was discussed.

Introduction

Water-soluble synthetic polymers are known to exhibit broad and various activities with biological systems (1). Series of polyelectrolytes (PE) increase immunoresponse to the immunizing antigen and thus produce an adjuvant effect (2). Water-soluble PE and their various PE complexes have potential possibility of radioprotective activity (3). One of the mechanism of the action of PE in biological systems is the cooperative interaction of PE with the biopolymer components of organism. This idea have been based on the results of experiments in PE-protein and PE-cell systems (2). It is remarkable that the higher immunologically active PE also have a radioprotective properties. In this work, the complexes of water soluble PE with metal ions and ternary PE-metal-protein complexes were synthesized and gamma radiolysis of these complexes have been investigated at various irradiation conditions, before evaluating their possible usage as a radioprotector.

Experimental

PAA has been synthesized and fractionated as explained in the literature. The molecular weight of the fractions used in this study were 70.000. BSA (Bovine Serum Albumin) was purchased from the Sigma Chem. Comp., and the $CuSO_4.5H_2O$ used was purchased from Merck.The molecular masses of proteins and the fraction compositions of the complexes were estimated by gel filtration chromatography using Protein-Pak 300 SW column (300 x 7.8 mm) . A Waters Model 501 HPLC was run in a buffer containing 100 mM potassium phosphate, pH 6.8, and 0.1 M NaCl at a flow rate of 1.0 ml/min at room temperature. The eluate was monitored at 280 nm. To produce a polymer-metal complex, $CuSO_4.5H_2O$ (pH=4) solution dissolved in phosphate buffer (pH=7.2). The pH values were adjusted with 1 M NaOH to desired pH. The ternary complexes have been prepared by means of adding protein solutions to the polymer-metal complex (PMC) solution. The aqueous solutions of PAA, PAA-Cu^{2+}, BSA, and PAA-Cu^{2+}-BSA were aerated or saturated with N_2 and N_2O before irradiation. γ-radiolysis has been performed by using ^{60}Co γ-source (Picker 9V). The dose rate has been 39.3 Gy.h-1 as determined by Fricke dosimetry. A Shimadzu Model 160A spectrophotometer has been used for spectrophotometric analyses. The optical density changes have been measured at 230 nm for PAA and PAA-Cu^{2+} and at 280 nm for BSA and PAA-Cu^{2+}-BSA.

Results And Discussion

The formation of the water-soluble ternary complexes of PAA and BSA in the presence of divalent copper ions (Cu^{2+}) was investigated by the method developed by Mustafaev et al. (4). HPLC studies on homogeneous systems at different ratios of the components and other our unpublished physicochemical results (electrophoresis, cyclic voltammetry analysis, etc.) suggests the following hypothetical scheme for the structure of the ternary PAA-Cu^{2+}-BSA complex (Fig. 1).

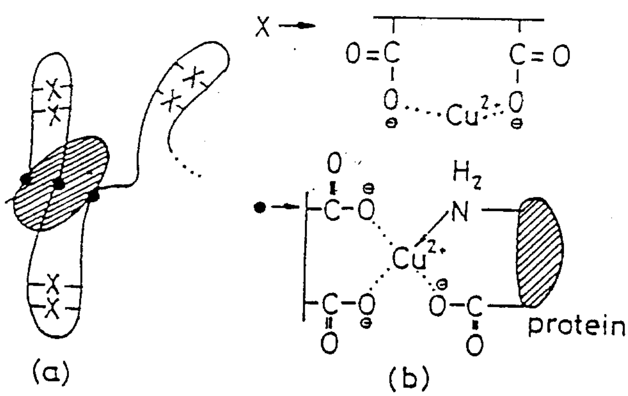

Fig 1. Schematic illustration of (a) the hypothetical structure of the ternary PAA-Cu^{2+}-BSA complex (crosses denote copper ions) and (b)the formation of chelate units between the functional groups of PAA and the protein antigen with participation of copper ions .

The divalent Cu^{2+} act as "fasteners" between the BSA globules and PAA chains and promote the formation of a soluble ternary complex which is stable under physiological conditions. Polycomplex has a looplike organization and excessive ionized groups of PAA form an extensive hydrophilic area on the protein surface which promotes the solubility of the polycomplex particles.

The spectrophotometric results of the irradiation experiments are presented in Table 1. As it can be seen from comparison of the results of optical densities of irradiated and unirradiated solutions in aerated conditions, unlike the solutions of free components (PAA, BSA, PAA-Cu^{2+}) for the ternary mixture PAA-Cu^{2+}-BSA the values of %OD in the dose up to 1.2 kGy are changed insignificantly.

A significant decrease in the radiation change of the values of % OD of these mixture was observed for solutions containing N_2. A percentage change of optical densities in N_2O saturated solutions of BSA was higher than aerated and N_2 saturated solutions (Table 1). Although the radiation-chemical changes were measured by UV-Vis spectrophotometry, more detailed information was obtained by the method of HPLC.

The HPLC results of the irradiated and unirradiated PAA, PAA-Cu^{2+}, and BSA solutions in O_2 atmosphere are shown in Fig. 2a.

No change was observed in BSA solutions irradiated at low doses (up to 0.655 kGy). However, the values of retention time (RT) and form of these peaks (heterogenecity) of BSA solutions irradiated at 1.044 kGy significantly differ from unirradiated protein solutions. For the PAA and PAA-Cu^{2+} solutions deformation of the peaks was observed at the higher irradiation dose (1.2 kGy). On the basis of these results, as well as other from earlier investigations (5,6), it can be proposed that BSA and PAA undergoes degradation and crosslinking at this dose. Similarly, denaturation and aggregation have been obtained with irradiation of proteins such as bovine and human serum albumines, egg albumin, casein and β-lactoglobulin (6).

Figure 2b compares HPLC results of the irradiated and unirradiated PAA, PAA-Cu^{2+} and BSA solutions with those of PAA-BSA and PAA-Cu^{2+}-BSA mixtures at the high irradiation dose (1.2 kGy). The chromatograms obtained for the irradiated solutions of

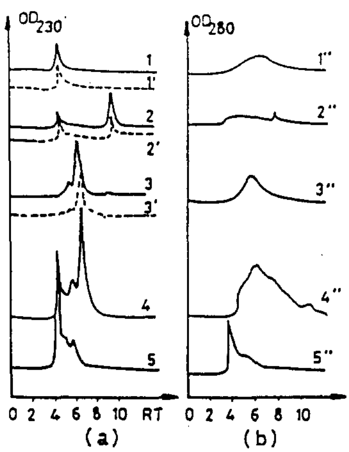

Fig. 2. HPLC results of unirradiated and irradiated solutions of PAA (1), PAA-Cu^{2+} (2), BSA(3), PAA-BSA(4), PAA-Cu^{2+}-BSA (5') in the presence of O_2; 1, 2, 3, 4, 5 (Unirradiated samples); 1', 2', (1.044 kGy); 3' (0.675 kGy) 1", 2", 3",4",5" (1.2 kGy) $[Cu^{2+}]=1.388x10^{-3}$ M, $c_{PAA}=c_{BSA}=0.1$ g/dl

Table.1 Percentage changes in optical density values {%OD=[(ΔOD/OD)*100]} in γ- radiolysis (medium: aerated)

Dose(Gy)	PAA	PAA-Cu^{2+}	BSA	PAA-Cu^{2+}- BSA
33.34	0.04	0.65	2.28	0.02
100.03	2.12	1.92	5.71	0.09
133.37	5.16	4.09	8.24	0.12
471.94	11.69	6.51	11.41	0.53
655.12	15.37	9.01	26.35	8.50
675.28	16.49	10.2	38.01	8.81
1036.66	17.08	-	49.43	9.74
1044.91	17.19	-	51.40	10.35
*655.12	10.25	3.31	18.41	2.23
**655.12	16.40	9.12	56.76	6.59

*Medium in N2 ** Medium in N2O

PAA-BSA mixture clearly demonstrates the formation of a new form of the protein and macromolecules at this dose. The behaviour of BSA upon irradiation in the presence of PAA macromolecules do not change essentially. The denaturation and aggregation by the crosslinking way of macromolecules in mixture takes place as in the case of individual components. At the same time, as can be seen from the results (Fig. 2b), the behaviour of ternary PAA-Cu^{2+}-BSA mixture after irradiation was not significantly different from unirradiated solutions. Although the shapes of the peaks and RT values remained the same, the peak areas decreased upon irradiation.

Addition of Cu^{2+} to PAA-BSA mixture reduces the extent of radiation-induced change of the protein and PAA macromolecules in the particules of ternary polycomplexes. This phenomenon can be considered to "protect" (or stabilization) of the macromolecules against radiation damage. Preservation of native structure of BSA in ternary PAA-Cu^{2+}-BSA complexes upon irradiation was observed and this was confirmed by the immunological methods recently. Injection of irradiated ternary PAA-Cu^{2+}-BSA complexes to animals resulted on the production of BSA-specific antibodies (unpublished results).

Mechanism : The effect of high energy radiations on water (7) may be summarized as:

$$H_2O \rightarrow 2.7\,(e^-_{aq}) + 2.7\,(OH\bullet) + 0.55\,(H\bullet) + 0.45\,(H_2) + 0.71\,(H_2O_2)$$

where the numbers before chemical symbols represents G-values. Larger yields of HO_2 and O_2^- are formed in aerated solutions by reaction of e^-_{aq} and H with oxygen (8)

$$e^-_{aq} + O_2 \rightarrow O_2^- \quad \text{and} \quad H\bullet + O_2 \rightarrow HO_2\bullet$$

With radiation, changes in the primary structure of proteins involved oxidation of SH groups, partial deamination, decarboxylation and oxidation of phenol radicals and radicals of the heterocyclic amino acids. The principal reaction of oxygenated aqueous solutions of proteins is:

$$RCONH\text{-}CHR_2 + O_2 + H_2O \rightarrow RCONH_2 + RCOR + H_2O_2$$

Irradiation of polymers in the presence of oxygen can give rise to peroxides, which may subsequently lead to degradation and crosslinking of the polymer chain. The higher change in optical density of BSA solutions saturated with N_2O could be due to the increased yield of OH radicals in N_2O saturated solutions, where e^-_{aq} is converted to an equivalent amount of OH radical:

$$e^-_{aq} + N_2O \xrightarrow{+H_2O} OH\bullet + OH^- + N_2$$

The mechanism underlying the protection effect in the mixture of PAA-Cu^{2+}-BSA might be related to complexation of copper in polyelectrolyte complex (PEC) with superoxide anion (O_2^-) and the formation of the following equilibrium:

$$PEC\bullet Cu^{1+} + O_2 \rightleftharpoons PEC\bullet Cu^{2+}\ \ O_2^- \ — \ PEC\bullet Cu^{1+} + O_2$$
$$PEC\bullet Cu^{2+} + O_2^- \rightleftharpoons$$

Copper ions may also react with OH and HO_2 (9):

$$\bullet OH + Cu^{2+} \rightarrow Cu^{3+} + OH^- \text{ and } HO_2 \bullet + Cu^{2+} \rightarrow Cu^{1+} + O_2 + H^+$$

Besides, one can notice that when polyanion macromolecules reacts with the protein globules to mask a group that is particularly radiation sensitive and may act as a "sacrificial" protective agent. Both physical (charge and energy transfer) and chemical (reacting the amino acids with carbonyl compounds and Cu, recombination of forming radicals, "repair effect" , etc.) protection can occur in this system. Stabilization of PAA chains in ternary polycomplexes was partially realized by Cu^{2+}. As shown earlier, the degradation of aqueous solutions of high-molecular-weight polymethacrylic acid caused by HO_2 radicals formed during irradiation is also inhibited by the protective-SH compounds, amines, alcohols, cyanides, etc. (5).

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