High-Performance Liquid Chromatography Study of Water-Soluble Ternary Polyacrylamide–Metal–Protein Complexes

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ABSTRACT: Lethal or harmful effects of X-rays and gamma rays are known to be reduced by prior administration of certain "radioprotector" chemicals. In this work, complex formation of polyacrylamide with bovine serum albumin (as a model protein) in the presence of divalent copper ions (Cu^{2+}) was investigated with high-performance liquid chromatography (HPLC) before evaluating its possible use as a radioprotector. HPLC results determined the most suitable metal concentration and protein/polymer ratio for maximum complex formation between polymer and protein molecules. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **65:** 37–40, 1997

Key words: gamma rays; polyacrylamide; high-performance liquid chromatography; radioprotector

INTRODUCTION

It is a well-established phenomenon that extremely small amounts of certain chemicals taken a short time before exposure to irradiation can provide a significant measure of protection for living beings. Water-soluble polymers and their various polymer complexes have potential for radioprotective activity, since they might bond temporarily to protein side chains that are particularly radiation sensitive.[1–3] Therefore, it is very important to understand the mechanisms for protein cooperative binding with synthetic polymers, in order to assist in the development of new types of radioprotectors.

This study used high-performance liquid chromatography (HPLC) to investigate the complex species formed when polyacrylamide reacts with bovine serum albumin (BSA; as a model protein) in the presence of divalent copper ions (Cu^{2+}). It also illustrates the use of HPLC to facilitate an understanding of the formation mechanism of polymer–metal–protein complexes.

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EXPERIMENTAL

The polyacrylamide (PAM) used in this work was prepared by the polymerization of acrylamide, with, essentially, the cerium ammonium nitrate [Ce(IV)]–methionine redox initiator system reported previously.[4,5] The HPLC instrument used throughout this work contained a Waters Model 501 pump, a Waters Model U6K sample injector, a Lambda-Max Model 481 LC spectrophotometer, a Waters 746 integrator, and a 300 × 7.8 mm (inner diameter) stainless steel column packed with Protein Pak (20 μm particle size, Waters, Norwich, UK).

The mobile phase contained 0.067*M* KH_2PO_4 and 0.067*M* $Na_2HPO_4 \cdot 2H_2O$ and was adjusted to pH 7. Elution was isocratic and at a flow rate of 1 mL/min. The mobile phase and samples were

prefiltered (0.45-μm-pore-size filter, Waters). A 25-μL sample volume was injected for analysis. All of the samples were monitored at 280-nm wavelength, where BSA has a maximum absorbance. The column was maintained at ambient room temperature. Collected HPLC eluate fractions were analyzed for copper content with a Perkin–Elmer 403 Atomic Absorption Spectrophotometer (AAS).

The polymer–metal complex was prepared by dissolving $CuSO_4 \cdot 5H_2O$ (pH = 4) in water and adding 1.0*N* NaOH to pH = 7.2. Then, the organic ternary complexes were prepared by adding protein solutions to the polymer–metal complex.

RESULTS AND DISCUSSION

Polymer–Metal Complex

Water-soluble PAM–Cu^{2+} complexes were prepared by simple mixing of polymer with metal in neutral aqueous media. The composition and structure of polymer–metal complexes were controlled by adding variable concentrations of metal to aqueous solutions of polymer at a constant concentration of polymer.

The addition of a metal ion to the polymer starts to give a homogeneous solution for a certain value of copper concentration at pH 7. Although metal salt ($CuSO_4$) is not soluble at pH 7, its mixture with the polymer is soluble up to a certain copper concentration. This result confirms the idea of complex formation.

Figure 1 illustrates the typical HPLC chromatograms of PAM–Cu^{2+} complexes at different concentrations of [Cu^{2+}]. As can be observed from the figure, at relatively low concentrations of Cu^{2+}, only one peak is seen in the chromatogram. A further increase in the [Cu^{2+}] concentration leads to a bimodal distribution of components, with a decrease in the area of peak I and an increase in the area of peak II.

One may assume that peak I corresponds to a free polymer and peak II corresponds to a complex polymer–Cu^{2+}. On the basis of this assumption, the most suitable metal concentration at which maximum complex formation takes place between PAM and Cu^{2+} ions is 2.1×10^{-3} mol/L. The existence of the free polymer in the system indicates a nonrandom distribution of the copper ions between polymer chains.

In light of the HPLC results mentioned above, it can be suggested that at relatively low concentrations of Cu^{2+}, these cations are randomly distributed between adsorbing polyions and that, on further increase in Cu^{2+} concentration, the distribution changes considerably so that the system consists of two fractions: part of the polymer binds the maximal amount of Cu^{2+}, whereas the other part is in the free state. This mechanism is further confirmed by AAS analysis of the collected HPLC fractions. No copper was detected in the sample fraction, while a small amount of copper was found in the peak 2 sample fraction.

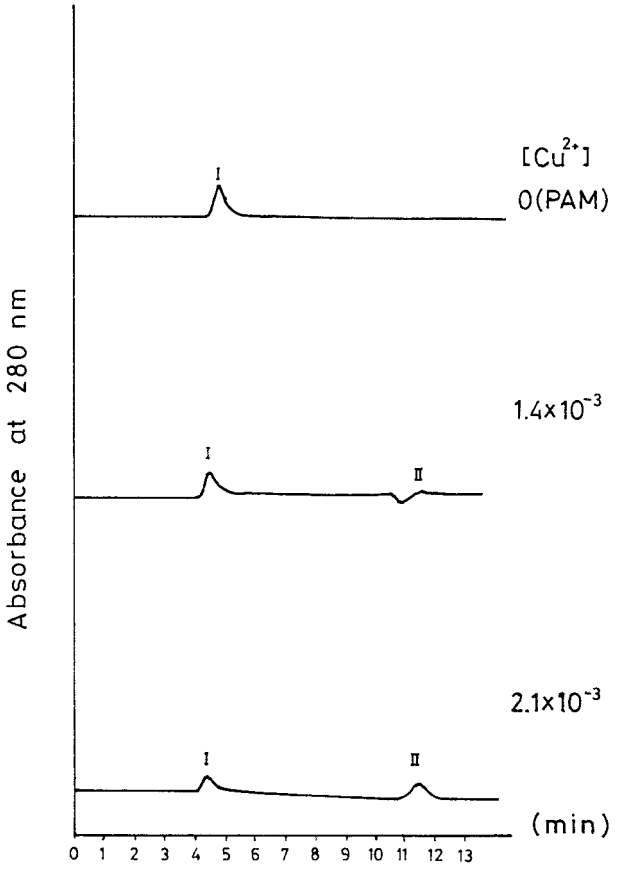

Figure 1 Original HPLC chromatograms of PAM-Cu^{2+} complexes prepared at different [Cu^{2+}] concentrations (mol/L).

Polymer–Metal–Protein Complexes

The formation of some water-soluble triple complexes with BSA in the presence of divalent copper ions (Cu^{2+}) was investigated by sedimentation analysis, turbidimetric titration, viscometry, and ultraviolet spectroscopy in neutral aqueous media, according to the method developed by Mustafaev and Kabanov.[6] Under conditions where both

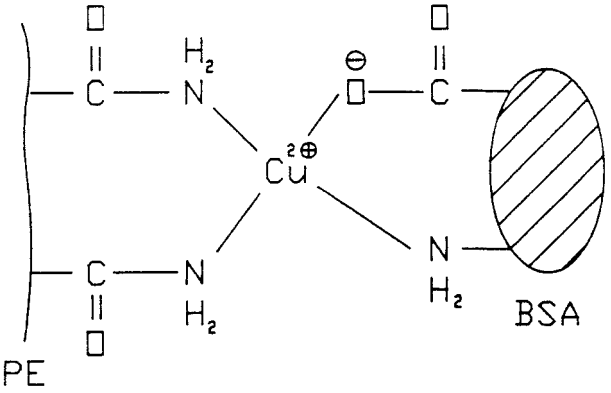

Figure 2 Schematic illustration of the hypothetical structure of the ternary PAM–Cu^{2+}-BSA complex.

polymer and protein have a similar (negative) charge, they cannot bind together without a mediator (metal ions). The divalent Cu^{2+} acts as "fasteners" between BSA globules and PAM chains to promote the formation of a soluble ternary complex which is stable under physiologic conditions.[7] The hypothetical scheme for the structure of the ternary PAM–Cu^{2+}–BSA complex is illustrated in Figure 2. Such complexes have not been previously studied by HPLC. However, the HPLC method provides an excellent opportunity for studying systems with different component ratios and elucidating some important features which characterize triple polymer–metal–protein complex formation. Hence, the complex formation of PAM–Cu^{2+}–BSA was analyzed by the HPLC method at different ratios of components (n_{BSA}/n_p).

For the ternary complexes, variable protein concentrations were added to soluble polymer-metal complexes. These triple polymer–metal–protein complexes remained soluble over a wide range of n_{BSA}/n_p concentrations, where n_{BSA} and n_p are the moles of protein (BSA) and polymer, respectively.

Figure 3 shows a series of HPLC chromatograms of the triple PAM–Cu^{2+}–BSA complexes at the different component ratios (n_{BSA}/n_p). As shown from the chromatograms, the distribution of the ternary mixtures revealed a multimodel character. The retention values of the peaks corresponding to mixture products are insignificantly different from those of individual BSA and PAM macromolecules. However, the areas of peaks 1, 2, and 3 are considerably different from those of individual PAM and BSA molecules. It should be pointed out that an increase in the n_{BSA}/n_{PAM} ratio (weight concentration: PAM, 0.05 g/mL; [Cu^{2+}],

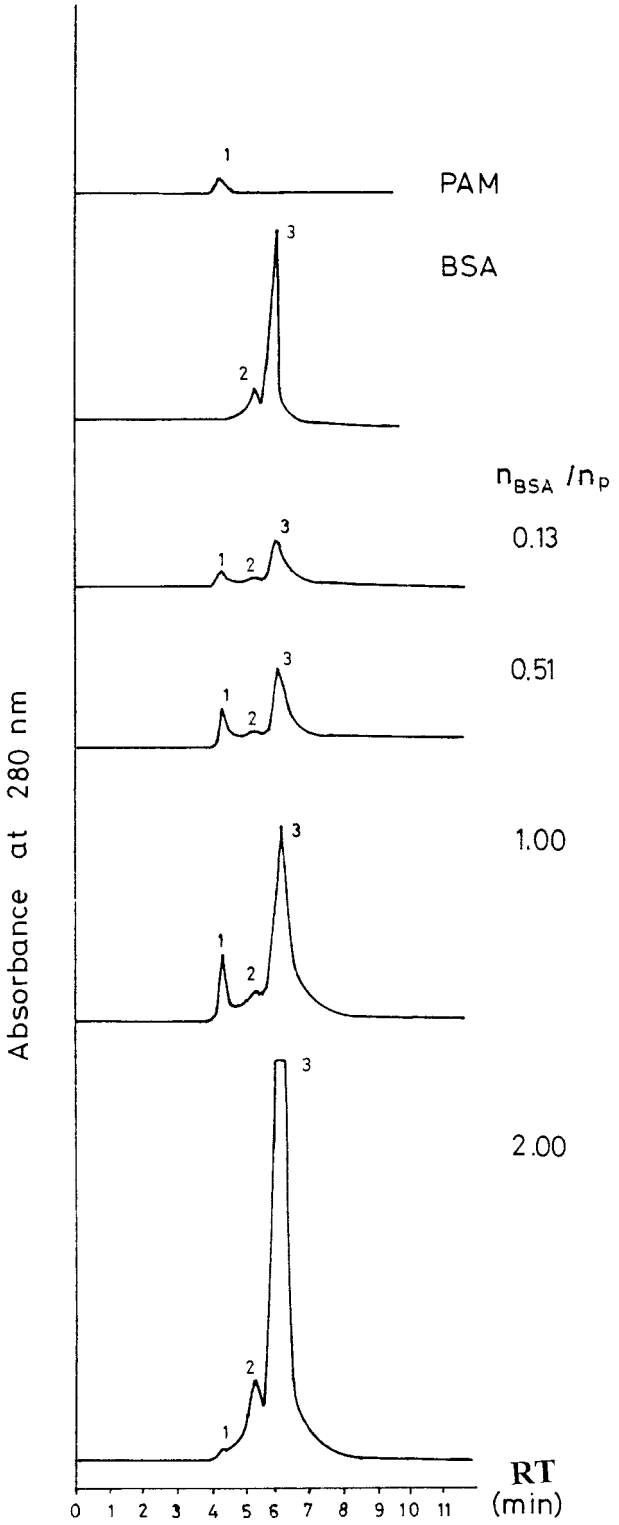

Figure 3 HPLC chromatograms of PAM–Cu^{2+}–BSA complex at different n_{BSA}/n_p ratios, [Cu^{2+}] = 1.4×10^{-3} mol/L.

2.1×10^{-3} mol/L are kept constant) initially leads to an increase in the area of the 1 and a decrease in the areas of peak 2 and 3. These results probably indicate that protein binding by PAM takes place and appears as peak 1 of the mixture, while peaks 2 and 3 correspond to the free dimer and monomer, respectively, of BSA. On the basis of these results, the following mechanism can be suggested:

$$n\,\text{PAM-Cu}^{2+} + m\text{BSA} \Leftrightarrow (\text{PAM-Cu}^{2+})_n\text{-BSA}_m \overset{\times\text{BSA}}{\Rightarrow} (\text{PAM-Cu}^{2+})_n\text{-BSA}_{m+x} \quad (1)$$

On further increase in the n_{BSA}/n_{PAM} ratio ($n_{BSA}/n_{PAM} > 1$), a considerable decrease in the area of peak 1 was observed. This result may indicate that the formation of ternary complexes is partially destroyed because of increasing concentration of protein in the mixture. In light of the HPLC results, the most suitable n_{BSA}/n_{PAM} ratio at which maximum complex formation takes place between polymer and protein molecules in the presence of metal ions is 1.00.

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