

Polycomplexes of Bovine Serum Albumin with Poly[2-Methacryloyloxy)Ethyl]Trimethyl Ammonium Chloride

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Abstract

Complex formation between bovine serum albumin and water-soluble synthetic cationic polyelectrolyte poly[2-methacryloyloxy)ethyl]trimethyl ammonium chloride has been studied in aqueous solutions by turbidimetric and viscometric methods. It was found that the structure of polycomplex is compact and its stability strongly depends on the environment. Formation of insoluble polycomplexes is observed in solutions with low ionic strength and pH, higher than 5.0. This pH value corresponds to the isoelectric point of the protein, so at lower pH the biopolymer macromolecules gain the positive charge and not able to be bound by the positively charged macromolecules of poly[2-methacryloyloxy)ethyl]trimethyl ammonium chloride. An increase of pH within 5.0-11.0 leads to further stabilization of polycomplex because of appearance of additional negative charges on biomacromolecules, caused by ionization of acidic groups. It was found that the main forces, which are responsible for the complexation, are electrostatic interactions. The intensity of the complexation is dependent on the solution concentration, pH and ionic strength. In solutions with high ionic strength ($I=0.2, 1.0$) the mixing of the reagents does not lead to the formation of insoluble polycomplexes. The observed dependence is connected with the screening of electrostatic interactions between macromolecules of the biopolymer and synthetic polyelectrolyte by small ions present in solution.

Introduction

Complexation of proteins with water-soluble synthetic polymers in aqueous solutions is interesting from both theoretical and practical points of view. The practical applications of these processes include development of separation, enrichment and purification technologies, which allow isolating the pure biopolymers from their natural mixtures.

A number of studies have dealt with complexes between proteins and cationic [1-4], anionic [5-7], amphoteric [8] and non-ionic polymers [9, 10] in aqueous solutions with different pH and ionic strength. The complexation between proteins and oppositely charged macromolecules mainly is occurred due to ionic contacts. The environmental (pH and ionic

strength of solution, temperature, etc.) as well as the structural parameters of the interacting bio- and synthetic polymers (charge distribution, isoelectric point, etc.) considerably influence the complexation.

In the present work we have studied the complex formation between strong cationic polyelectrolyte poly[2-methacryloyloxy)ethyl]trimethyl ammonium chloride (PMADQUAT) and bovine serum albumin (BSA) in aqueous solutions with different pH and ionic strength media.

Experimental

Radiation polymerization of MADQUAT was conducted in the gamma-cell BK-1000 by the procedure described in our earlier paper [11]. The dose rate was determined with the help of Fricke dosim-

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eter and was equal to 1.8 Gy/min. The synthesized polymer was purified by double precipitation from water to isopropyl alcohol and dried at 50°C to the constant weight. The average viscosity molecular weight of PMADQUAT was determined using the relationship $[\eta]=6 \cdot 10^{-3} \cdot M^{0.72}$ taken from [7]. For experiments with complex formation we used the sample of PMADQUAT with $M_w=1.2 \cdot 10^6$ Da, obtained at irradiation dose 257 Gy for 80% conversion.

BSA with molecular weight $6 \cdot 10^4$ Da was purchased from BelNIIZM (Russia) and used without further purification

Turbidimetric titration was conducted using spectrophotometer UV2401PC (Shimadzu, Japan) at the wavelength 400 nm.

Viscometric measurements were carried out with the help of Ubbelohde viscometer, having the flow time for pure water 92 sec at $25 \pm 0.1^\circ\text{C}$.

Potentiometric measurements were done on Ion meter 3345 (Jenway, UK).

Results and discussion

PMADQUAT is a strong cationic polyelectrolyte having a high ability to form various polycomplexes with some inorganic ions [11], poly(carboxylic acids) [12], etc. However, there is little known about the complexation of PMADQUAT with proteins. To the best of our knowledge the paper of Kaibara with co-workers [13] is the only work reported in this field.

In the present work the complexation between PMADQUAT and BSA was studied by turbidimetric titration as a simple standard tool for evaluation the formation of insoluble polycomplexes. It is seen from Fig. 1 an addition of BSA to PMADQUAT solution is accompanied by appearance of turbidity indicating the formation of insoluble polycomplexes. However, these dramatic changes are observed only if the concentration of the reagent is higher than 0.001 g/dL. Probably the formation of insoluble polycomplexes requires some minimal concentration of the reagents to provoke the aggregation of polycomplex particles. The growth of the reagents concentration within 0.005-0.01 g/dL decreases the binding ability from 4.75 to 2.75 g of the protein per 1 g of PMADQUAT.

In order to evaluate the conformation changes, which occur in the system we studied the complexation by viscometric method. PMADQUAT as a strong polyelectrolyte of high molecular weight is characterized by high value of reduced viscosity (Fig. 2). However, upon addition of BSA the reduced viscosity falls

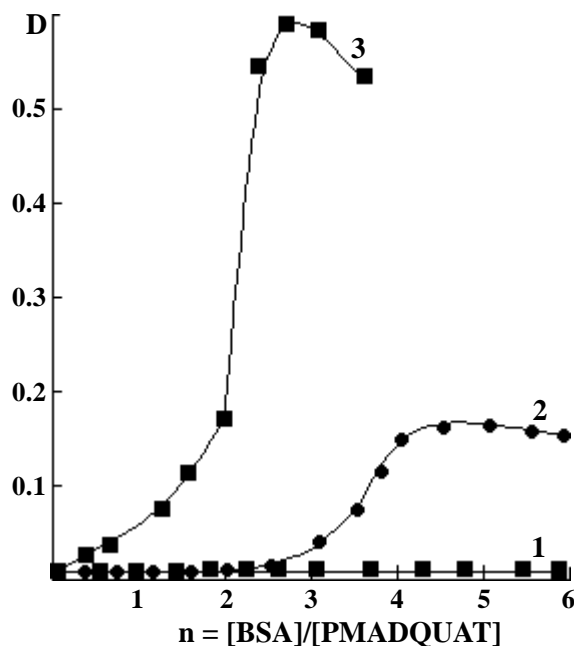


Fig. 1. Turbidimetric titration curves of PMADQUAT solutions by BSA. $C_{\text{BSA}}=C_{\text{PMADQUAT}}=0.001$ (1), 0.005 (2), 0.01 g/dL (3)

down drastically indicating the formation of compact particles of polycomplexes. For comparison the addition of water to PMADQUAT solution does not lead to such considerable changes of the reduced viscosity.

For clarification the mechanism of interaction be-

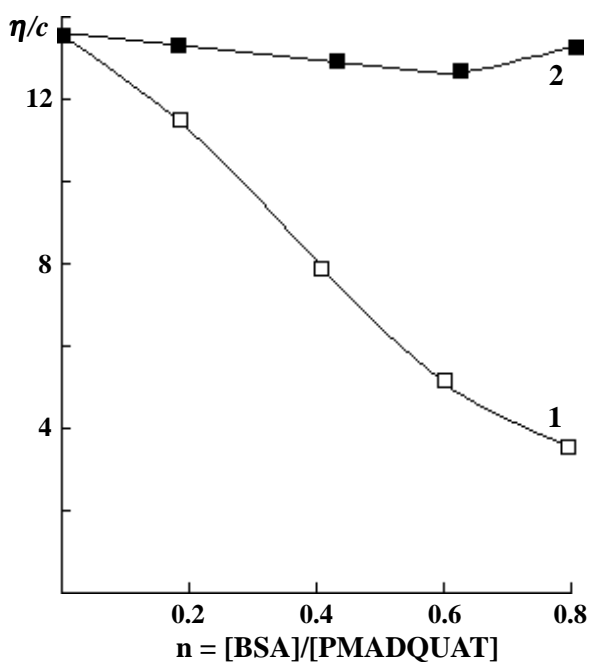


Fig. 2. Viscometric titration curves of PMADQUAT solutions by BSA (1) and by water (2). $C_{\text{BSA}}=C_{\text{PMADQUAT}}=0.03$ g/dL

tween PMADQUAT and BSA we studied the effect of pH and ionic strength of solution on the complexation. For this purpose the aqueous solutions of PMADQUAT and BSA (with concentration 0.05 g/dL) were mixed with the ratio 1:2.4. The turbidity of the mixture was measured as a function of pH, which was regulated by addition of negligibly small amounts of 0.1 M solutions of HCl and NaOH. The dependence of turbidity on pH is plotted in Fig. 3. It is clearly seen that the insoluble polycomplex completely disappears at pH lower than 5.0. This pH value corresponds to the isoelectric point of the protein, so at lower pH the biopolymer macromolecules gain the positive charge and are not able to be bound by the positively charged macromolecules of PMADQUAT. An increase of turbidity within pH 5.0-11.0 is connected with appearance of additional negative charges on biomacromolecules, caused by ionization of acidic groups. It should be noted that an ionization of the strong polyelectrolyte PMADQUAT is practically not changed in these conditions. Hence, one can conclude that the main driving force of the complexation between PMADQUAT and BSA is electrostatic contacts between oppositely charged groups of both polymers.

In order to confirm this statement we evaluated the effect of solution ionic strength on the complexation (Fig. 4). It is seen from the figure the formation of insoluble polycomplexes is disturbed by the presence of low molecular weight electrolyte (NaCl) in solution. In solutions with high ionic strength ($I=0.2$,

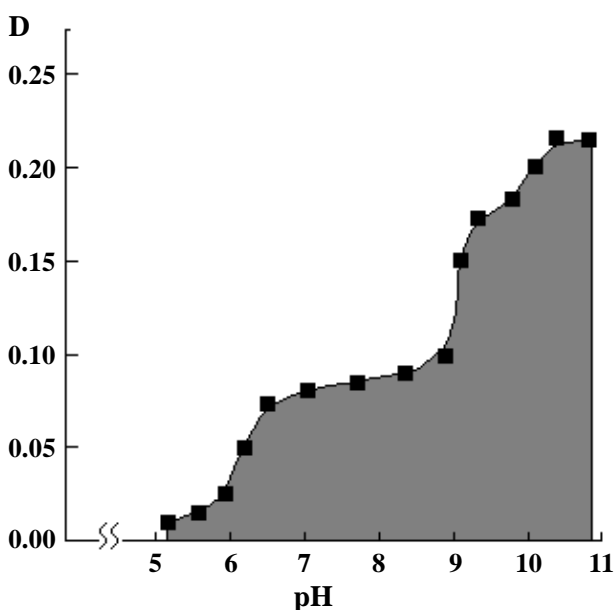


Fig. 3. Optical density of BSA-PMADQUAT (2.4:1) polycomplex as a function of pH. $C_{\text{BSA}}=C_{\text{PMADQUAT}}=0.05$ g/dL.

1.0) it completely disappears and the mixing of the reagents does not lead to the appearance of turbidity. The observed dependence is connected with the screening of electrostatic interactions between macromolecules by small ions present in solution. The obtained results are in good agreement with the work of Kabanov et. al. [3], who observed the dissociation of polycomplexes between BSA and poly-4-vinyl-N-pyridinium bromide at $I=0.2$.

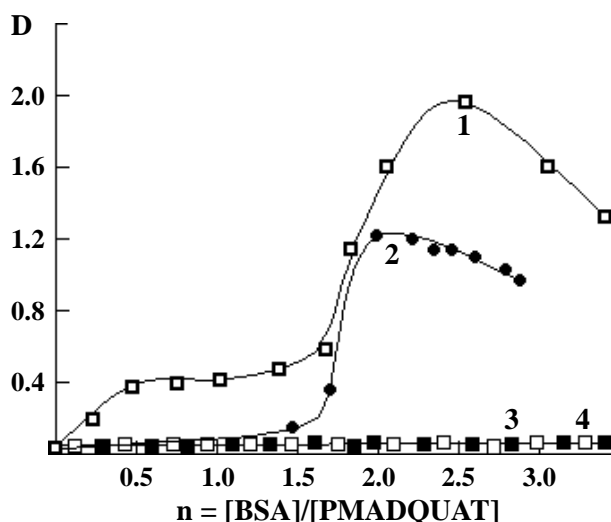


Fig. 4. Turbidimetric titration curves of PMADQUAT solutions by BSA in solutions with different ionic strength: 0 (1), 0.05 (2), 0.2 (3), 1 (4). $C_{\text{BSA}}=C_{\text{PMADQUAT}}=0.05$ g/dL.

Conclusions

Interaction between strong cationic polyelectrolyte poly[2-methacryloyloxyethyl]trimethyl ammonium chloride (PMADQUAT) and bovine serum albumin (BSA) in aqueous solutions results in the formation of insoluble polycomplexes stabilized mainly by electrostatic forces. The stability/solubility of polycomplexes is greatly dependent on pH and ionic strength of the solution. Below the isoelectric point of the protein the polycomplex is destroyed because the macromolecules have the charge of the same sign. The dissociation of polycomplexes is also observed in solutions with ionic strength higher than 0.2 because of screening effects.

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