

Potentiometric Investigation of Specific Ionic Effects on Interactions between Bovine Serum Albumin and Weak Polyelectrolytes

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The effect of salt on the behaviour of bovine serum albumin (BSA) in solution and on the interactions between BSA and the weakly charged polyelectrolytes poly(allylamine hydrochloride) and poly(dimethylaminoethylmethacrylate) was investigated by potentiometric titrations. Titrations of pure BSA solution and of the BSA solution with the addition of polyelectrolyte were performed in the presence of different salts. Three electrolytes having the same anion and a different cation were used, lithium chloride, sodium chloride, and caesium chloride. The corresponding base served as titrant. Experiments were performed at four electrolyte concentrations. The titration curves showed a strong influence of ionic strength and the electrolyte type. The cation effect was found to be in accordance with the Hofmeister series.

INTRODUCTION

It has been known for decades that the ionic conditions (the type and concentration of the salt, the pH, *etc.*) have a strong effect on the polyelectrolyte-protein interactions in solution and at the interface,^{1,2} that is, on the formation and stability of polyelectrolyte-protein complexes³ and multilayers.⁴ Descriptions of various methods for examining such interactions can be found in the literature.⁴ Turbidimetry,^{5,6} electrophoretic light scattering,⁷ isothermal titration calorimetry,⁸ and static and dynamic light scattering^{9,10} are among the techniques used for investigating the association of proteins with synthetic polyelectrolytes in solution. For monitoring the specific ionic effects on polyelectrolyte-polyelectrolyte and poly-

electrolyte-protein interactions at the surface stagnation point optical reflectometry,^{11,12} ellipsometry,¹³ quartz crystal microbalance,¹⁴ and optical waveguide lightmode spectroscopy¹⁵ are commonly applied.

It is well known¹⁶ that the formation of protein-polyelectrolyte complexes is initiated at a critical pH value (pH_c) and that phase separation starts at another critical value (pH_ϕ). In the case of complexation between the globular protein bovine serum albumin (BSA) (having the isoelectric point at $\text{pH} \approx 4.9$) and the positively charged polyelectrolyte several pH regions can be distinguished. In the $\text{pH} < \text{pH}_c$ region no complexation takes place because of the repulsive electrostatic forces acting between the positively charged protein and the positi-

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vely charged polycation. The region $\text{pH}_c < \text{pH} < \text{pH}_\phi$ is marked by formation of soluble complexes owing to the fact that BSA is bearing more negative charge. Finally, in the $\text{pH} > \text{pH}_\phi$ region aggregation occurs.

A method which is not very often used for monitoring the protein-polyelectrolyte interactions in solution is potentiometry (or pHmetry).^{3,17} The predominantly electrostatic interactions taking place between the protein and the polyelectrolyte in solution depend on their charge densities, and, in the case of weak polyelectrolytes, can be controlled by means of the pH of solution. Accordingly, potentiometric titrations of the protein solution, pure or containing the polyelectrolyte, in different ionic conditions, could provide information complementary to that gained by other techniques. More specifically, they could provide a better insight into local, short-range interactions between protein ionophores and polyelectrolytes than other scattering or hydrodynamic methods.

Investigations into the specific ionic effects (due to the type of salt, salt concentration, *etc.*) on proteins and polyelectrolytes in solution have been going on since 1888 when Hofmeister demonstrated that the amount of salt required to precipitate a protein solution strongly depended on the type of salt used.¹⁸ Since then a number of papers have been published but a full understanding of the phenomenon has never been achieved.¹⁹

In the attempt to investigate systematically the behaviour of proteins in solution and protein-polyelectrolyte interactions we performed titrations of the BSA solution with and without the addition of polyelectrolyte using two weakly charged polyelectrolytes, poly(allylamine hydrochloride) (PAH) and poly(dimethylaminoethylmethacrylate) (PAMA). The pK_a values of these polycations were ≈ 9 (PAH) and ≈ 7.5 (PAMA) depending on ionic strength. As the electrostatic interactions between the protein and the polyelectrolyte were also dependent on ionic strength, experiments were performed at four different ionic strength values ($I_c = 0.03 \text{ mol dm}^{-3}$, $I_c = 0.1 \text{ mol dm}^{-3}$, $I_c = 0.5 \text{ mol dm}^{-3}$, $I_c = 1.0 \text{ mol dm}^{-3}$). Three electrolytes having the same anion, but a different cation (LiCl, NaCl, and CsCl) were used as supporting electrolytes. To distinguish between the effects of specific cations, the respective hydroxides (LiOH, NaOH, and CsOH) served as titrants.

EXPERIMENTAL

Materials

The protein was bovine serum albumin ($M_w = 67\,000 \text{ g/mol}$, Fluka BioChemika, $\text{pH}_{\text{iep}} = 4.9$). The polyelectrolytes, poly(allylamine hydrochloride) ($M_w = 67\,000 \text{ g/mol}$, Aldrich) and poly(dimethylaminoethylmethacrylate)²¹ ($M_w = 20\,000 \text{ g/mol}$), were used as received without further purification. The supporting electrolytes were lithium chloride, LiCl (Fluka), sodium chloride, NaCl (Fluka), and caesium chlo-

ride, CsCl (Merck). Buffer solutions ($\text{pH} = 3$, $\text{pH} = 7$, and $\text{pH} = 10$) were from Kemika, Zagreb, and LiOH, NaOH, and CsOH solutions from Merck. The initial pH was adjusted with hydrochloric acid. All the polyelectrolytes were dissolved to a final concentration of 0.6 g dm^{-3} . The BSA mass concentration was $\gamma = 3 \text{ g dm}^{-3}$.

Potentiometric Titrations

Potentiometric titrations were performed with a Metrohm 713 pH-metre with a combination electrode (Metrohm) under argon at 25°C . The protein solution (γ (BSA) = 3 g dm^{-3}) without the polyelectrolyte addition was prepared by dissolving BSA in hydrochloric acid solution at a fixed salt concentration. The BSA + polyelectrolyte solutions were prepared by addition of a desired amount of polyelectrolyte to the already prepared BSA solution. Three buffer solutions ($\text{pH} = 3$, $\text{pH} = 7$, and $\text{pH} = 10$) were used for calibration of the pH-metre. In all experiments 0.1 mol dm^{-3} LiOH, NaOH, or CsOH was added to a 50 mL solution.

RESULTS AND DISCUSSION

Bovine Serum Albumin in Solution

Figure 1 shows the raw data obtained by titration of a BSA water solution with a sodium hydroxide water solution at four different ionic strengths ($I_c = 0.03 \text{ mol dm}^{-3}$, $I_c = 0.1 \text{ mol dm}^{-3}$, $I_c = 0.5 \text{ mol dm}^{-3}$, $I_c = 1.0 \text{ mol dm}^{-3}$), in the presence of sodium chloride, without the addition of polyelectrolyte. It can be observed that the titration curves shifted with the increasing background electrolyte concentrations. As expected, at higher ionic strength more sodium hydroxide was needed to reach a constant pH value since the addition of salt leads to the more pronounced compensation of negative charges on the protein surface and therefore more H^+ ion could be released.

The same trend was observed when the BSA solution was titrated in the presence of lithium chloride and caesium chloride at three different ionic strengths (not

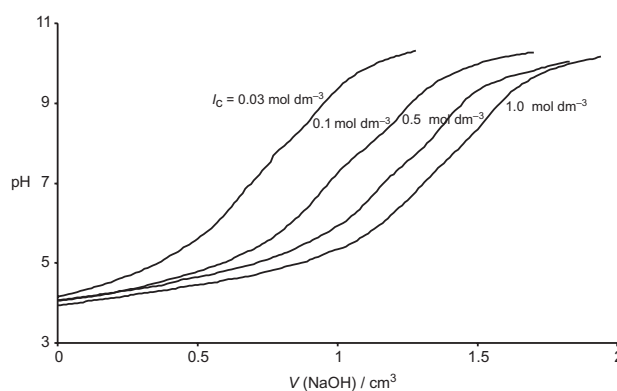


Figure 1. Potentiometric titration of the BSA solution (mass concentration = 3.0 g dm^{-3}) with sodium hydroxide in the presence of sodium chloride at four different ionic strength values.

shown here). The increase in ionic strength ($I_c = 0.03 \text{ mol dm}^{-3}$, $I_c = 0.1 \text{ mol dm}^{-3}$, $I_c = 0.5 \text{ mol dm}^{-3}$) induced a shift of the titration curve in the same direction as in the case of sodium chloride.

To gain further insight into the specific ionic effects on the BSA behaviour we titrated the BSA solution in the presence of different supporting electrolytes of constant and equal concentration, or more precisely, those having a different cation and identical anions. Comparison of the titration curves at $I_c = 0.03 \text{ mol dm}^{-3}$ presented in Figure 2 shows differences depending on the electrolyte (*i.e.* cation) used. The trend was in accordance with the Hofmeister cation series, *i.e.* the lowest amount of the base needed to reach the plateau was observed for caesium, followed by sodium and lithium. It was therefore tempting to conclude that the responsibility for the behaviour of proteins in electrolyte solution did not lie solely with the electrostatic forces. The effect of ion hydration, and possibly even that of the dispersion forces, as suggested by Ninham and co-workers,²⁰ could also be held accountable.

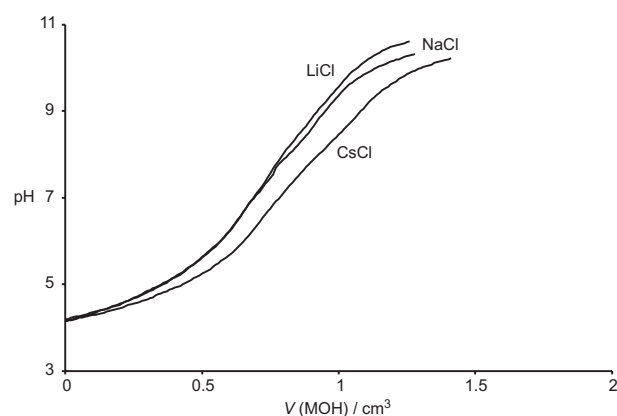


Figure 2. Potentiometric titration of the BSA solution (mass concentration = 3.0 g dm^{-3}) in the presence of different alkali metal chlorides (LiCl, NaCl, CsCl) with the corresponding hydroxides (MOH = LiOH, NaOH, or CsOH) at $I_c = 0.03 \text{ mol dm}^{-3}$.

A comparable relationship between the titration curves was established also for the other two ionic strength values ($I_c = 0.1 \text{ mol dm}^{-3}$, $I_c = 0.5 \text{ mol dm}^{-3}$). The observed trend was in accordance with the growth of the ionic cloud and with the increase in compensation of the protein charges in the Li–Na–Cs direction.

BSA-Weak Polyelectrolyte Interactions

As the aim of the study was to examine the effect caused by the type of salt and by ionic strength on the interactions between BSA and weak polyelectrolytes we repeated the titrations of the BSA solution by adding to it a weakly charged polyelectrolyte, PAH or PAMA, in the presence of background electrolytes of different concentration.

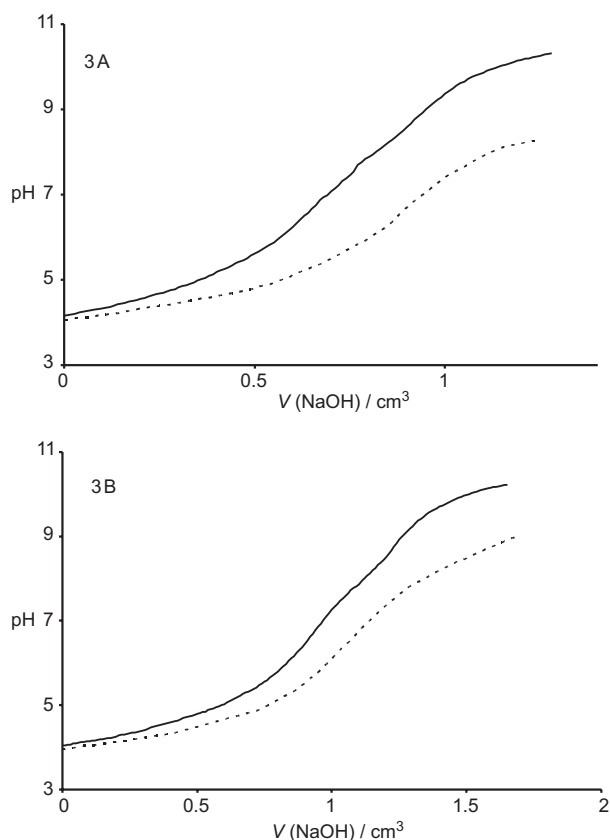


Figure 3. Potentiometric titration of the BSA solution (mass concentration = 3.0 g dm^{-3} ; full line) and of the BSA + PAH solution (mass concentration = 0.6 g dm^{-3} ; dotted line). Ionic strength $I_c = 0.03 \text{ mol dm}^{-3}$ (Figure 3A) and $I_c = 0.1 \text{ mol dm}^{-3}$ (Figure 3B).

When the BSA + PAH solution was titrated with sodium hydroxide solution in the presence of sodium chloride (Figures 3A and 3B) the difference in the titration curves observed at different sodium chloride concentrations that appeared at $\text{pH} = \text{pH}_c$ (the pH at which the formation of soluble complexes starts) was much more pronounced at a lower ionic strength ($I_c = 0.03 \text{ mol dm}^{-3}$, Figure 3A) than at a somewhat higher ionic strength ($I_c = 0.1 \text{ mol dm}^{-3}$, Figure 3B), and was not observable at $I_c = 1.0 \text{ mol dm}^{-3}$. The finding was in accordance with the conclusion²² that salt addition elevates the point of soluble complex formation. The reason for the effect of the added polyelectrolyte to have become negligible at a very high salt concentration was, predominantly, the screening of the electrostatic forces due to the presence of excess salt.

The same trend was observed for the titration of the BSA + PAH solution with lithium hydroxide solution in the presence of lithium chloride, as well as for the titration of BSA + PAH with caesium hydroxide solution in the presence of caesium chloride (neither shown here). It can be concluded that the PAH addition influenced the acidity of the titrable groups on BSA and that the effect decreased with the increasing salt concentration in the presence of all investigated electrolytes.

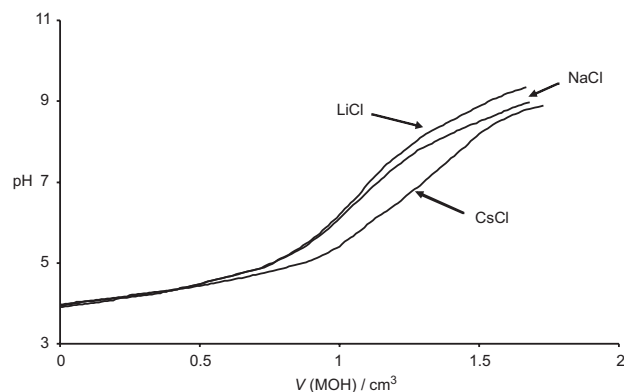


Figure 4. Potentiometric titration of the BSA (mass concentration = 3.0 g dm^{-3}) + PAH (mass concentration = 0.6 g dm^{-3}) solution in the presence of different salts (LiCl, NaCl, or CsCl) with the corresponding hydroxides (MOH = LiOH, NaOH, or CsOH). Ionic strength $I_c = 0.1 \text{ mol dm}^{-3}$.

Another interesting point of investigation concerned the effect of different electrolytes (*i.e.* different cations) on BSA titration in the presence of PAH as compared to their effect on the titration of pure BSA solution (Figure 2). As expected, in both cases the effect of the background electrolyte was the same. Again, as seen in Figure 4, the curves followed the trend of the Hofmeister series although those obtained in the presence of lithium chloride and sodium chloride were comparable up to the $\text{pH} \approx 7.5$. This is another evidence that not only the electrostatic forces but also the size of ions and their hydration play an important role in the interactions between BSA and PAH.

Finally, it was interesting to compare the behaviour of different weakly charged polyelectrolytes when titrated with the base in the presence of BSA. For that purpose, we performed additional experiments using PAMA as a weakly charged polycation. The experiments were carried out in the presence of sodium chloride at

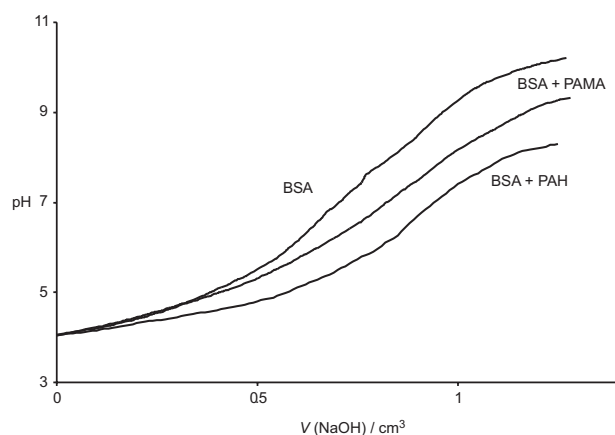


Figure 5. Potentiometric titration of pure BSA solution (mass concentration = 3.0 g dm^{-3}) and of the BSA + PAH or PAMA (mass concentration = 0.6 g dm^{-3}) solutions in the presence of sodium chloride. Ionic strength $I_c = 0.03 \text{ mol dm}^{-3}$.

three different ionic strengths ($I_c = 0.03 \text{ mol dm}^{-3}$, $I_c = 0.1 \text{ mol dm}^{-3}$, $I_c = 0.5 \text{ mol dm}^{-3}$). The titration curves of pure BSA solution and of the BSA solutions containing PAH and PAMA obtained at $I_c = 0.03 \text{ mol dm}^{-3}$ are shown comparatively in Figure 5.

It is evident that the type of polyelectrolyte also influenced the curves. For all the three background electrolyte concentrations the curves followed the same pattern. The difference between them, which was more pronounced at lower electrolyte concentration, indicated again that the effect of polyelectrolyte addition grew stronger with the rising salt concentration. The difference exhibited by the BSA + PAH/PAMA curves was the consequence of the difference in pK_a values of these weakly charged polyelectrolytes. At the certain pH, PAH is strongly charged than PAMA which leads to the higher pH_c value in the case of BSA-PAH complexation when compared to BSA-PAMA complexation.

CONCLUSIONS

The results of this work allow to conclude that potentiometric titrations can provide valuable information about the relationship between the type of electrolyte (in this case the type of cation) and its concentration, and the behaviour of proteins in solution. They proved that the protein behaviour and the interactions between the protein and the polyelectrolyte were influenced by ionic strength. Moreover, they showed that the complexation between BSA and a polycation (PAM or PAMA) yielded different titration curves and also, that the formation of protein-polyelectrolyte complexes was dependent on the type and concentration of electrolyte. They confirmed that the cations used (Li^+ , Na^+ , and Cs^+) followed the Hofmeister series.

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SAŽETAK

Potencijometrijsko ispitivanje specifičnih ionskih efekata na interakcije između albumina iz goveđeg seruma i slabih polielektrolita

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Metodom potencijometrijske titracije sustavno je ispitan utjecaj različitih soli na ponašanje albumina iz goveđeg seruma (BSA) u otopini, kao i na interakcije između BSA i slabih polielektrolita poli(alilamin hidroklorida) (PAH) i poli(dimetilaminoetil metakrilata) (PAMA). Izvedene su titracije otopine čistog BSA i otopine BSA s dodatkom polielektrolita u prisutnosti različitih soli. Upotrijebljena su tri elektrolita koji sadrže isti anion, a različiti kation, litijev klorid, natrijev klorid i cezijev klorid. Titracije odgovarajućim alkalijским hidroksidima su izvedene pri četiri različite koncentracije elektrolita. Usporedbom titracijskih krivulja uočen je značajan utjecaj ionske jakosti i vrste elektrolita. Utjecaj kationa bio je u skladu s Hofmeisterovim nizom.