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Complexation between lysozyme and sodium poly(styrenesulfonate): The effect of pH, reactant concentration and titration direction

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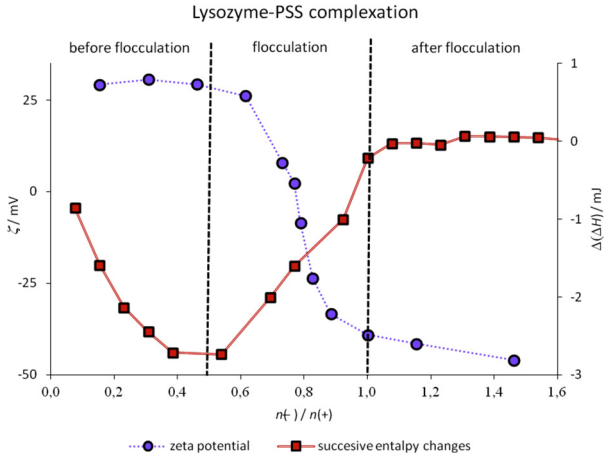
HIGHLIGHTS

- Lysozyme–PSS complexation was examined at various pH and reactant concentrations.
- At lower charge ratios of reactants stable colloid particles were formed.
- At higher charge ratios larger particles (floccules) appeared.
- Complexation resulted in negative enthalpy changes.

GRAPHICAL ABSTRACT



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ABSTRACT

It is well-known that mixing of aqueous solutions of oppositely charged proteins and polyelectrolytes leads to the formation of aggregates often called protein–polyelectrolyte complexes. In this study, we investigated the interactions between aqueous solutions of lysozyme and sodium poly(styrenesulfonate) (PSS) as the model system for investigating protein–polyelectrolyte complexation processes. The experimental methods used in this study were isothermal titration microcalorimetry, electrophoretic mobility (*i.e.* zeta potential determination) and particle size (*i.e.* hydrodynamic radius) measurements. The effect of pH, reactant (protein and polyelectrolyte) concentration and titration direction (addition order) on lysozyme–PSS complexation was investigated at $\theta = 25\,^{\circ}C$ and at ionic strength $I_c = 10^{-2}$ mol dm^{-3}.

At all three examined pH values (pH = 3.1, 4.6 and 7.5) at low charge ratios a stable colloidal suspension was obtained in which charged colloid particles, whose sign of charge corresponds to that of the titrand, were present. On the other hand, at higher charge ratios large particles (floccules) appeared (with the hydrodynamic radius in the range 2–7 μm). These floccules were also charged and, at first, their charge sign was equal to that of the titrand ($|\zeta| = 30$–50 mV). However, upon further addition of the titrant the charge of the floccules reversed as a result of the surplus of titrant molecules in the corona.

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The change in pH leads to the significant change in charge ratios at which the onset of flocculation is observed. In all examined cases the charge ratio at which zeta potential starts to change corresponds to the onset of flocculation determined by DLS. The reaction heat effects were determined and it was shown that in all examined cases complexation resulted in measurable negative enthalpy changes.

1. Introduction

It is already known for decades that interactions between opposite charged polyelectrolytes in solution often result in formation of polyelectrolyte complexes [1–3] predominantly due to the electrostatic attraction between the oppositely charged chains. The formation of such complexes and their properties are determined by various experimental conditions (*i.e.* pH, ionic strength, type of added supporting electrolyte, polyelectrolyte concentration, addition order, *etc.*) [4–6]. If instead of a polyelectrolyte a protein is used, the formed complexes are commonly called protein–polyelectrolyte complexes [7–14]. Various experimental methods could be used for studying the formation and properties of such complexes. For example, the influence of complexation between small proteins and negatively charged polyelectrolytes on thermodynamic stability of proteins was investigated by means of differential scanning calorimetry [7]. In that study lysozyme, chymotrypsin and glyceraldehyde-3-phosphate dehydrogenase were used as model proteins, while as negatively charged polyelectrolytes poly(acrylic) acid (PAA), poly(methacrylic) acid (PMA), poly(N-ethyl-4-vinylpyridinium) bromide (PEVP), sodium poly(anetholesulfonate) (PAS), potassium poly(vinylsulfate) (PVS), sodium poly(styrene sulfonate) (PSS) were used. Denaturation temperatures of complexes formed were found to be lower than that of pure protein, with the exception of PSS. In the case when PSS was used, the increase in protein denaturation temperature was observed. Moreover, the effect of PSS on lysozyme was so significant that the protein melting peak was not observed.

One of the reasons why scientists already for years investigate complexation between proteins and polyelectrolytes of the opposite charge is due to the numerous possible applications in which these complexes could be involved such as drug release [8] and bioseparation [9]. Studies in the field of charged nanoparticles as carriers of proteins [8] are also performed with the aim to determine the influence of negatively charge nanoparticles covered with PSS and poly(lactid-co-glycolid) (PLGA) on the increase in lysozyme abilities and on the improvement of lysozyme release. It was shown that the increase in pH value causes the decrease in ζ potential of PLGA/PSS nanoparticles, which is due to the increase in dissociation degree of sulfonic acid on the surface of nanoparticles. The conclusion is that the increase in negative charge density enables more binding sites for positively charged protein.

The interest in the study of protein–polyelectrolyte interactions is also driven by the new polyelectrolyte architectures, such as polyelectrolyte multilayers and polyelectrolyte–protein multilayers [10,11]. Mathew et al. investigated [10] the adsorption of proteins (lysozyme and bovine serum albumin) on chitosan/PSS multilayer membranes. They concluded that adsorption capacity of such multilayer membranes depends on experimental conditions (*e.g.* pH) and that the lysozyme secondary structure is preserved in broad pH range.

The influence of charge density, reactant concentrations, partial polyion chain hydrophobicity and ionic strength on lysozyme–PSS complex composition was investigated by Cousin and Gummel [12–14] by means of Small Angels Neutron Scattering (SANS) and electrophoresis. At charge ratios close to 1 and at concentrations below overlapping concentration dense globular complexes (which they call primary complexes) of about 100 Å were formed. The core of primary complexes was neutral and excess charge was localized at the surface. When negative charge is slightly in excess, surface of primary complexes has a negative charge because of PSS corona around the primary complexes. But when positive charge is in excess, the surface has positive charge and lysozyme is in the corona. They showed also that the ionic strength influences lysozyme–PSS complexation, because it affects their size and gets the active enzyme in contact with solvent [14]. The core of primary complexes is frozen so that the proteins in the core are protected from the influence of solvent and they are in their native conformation. The primary globule structure depends on charge ratio. If PSS is not in excess the globules have well-defined surface. But if PSS (negative charge) is in excess the surface is fuzzier and PSS chains are dangling out from the surface.

The interactions between proteins and polyelectrolytes are also of interest for researchers dealing with the adsorption of biological or biomimetic structures onto polyelectrolyte multilayers. Such investigations could enable additional progress in the field of biosensing surfaces, tissue engineering or drug delivery. In the literature [15–18], several examples of investigation of adsorption of various proteins on previously formed multilayers could be found. Müller et al. [15] examined the sorption of human serum albumin on poly(ethyleneimine)/poly(acrylic acid) multilayers, while Gergelly et al. [16] analyzed the adsorption of the same protein on poly(L-lysine)/poly(glutamic acid) multilayer. The secondary structure of bovine serum albumin (BSA) and hen egg white lysozyme (HEL) adsorbed onto poly(allylammonium hydrochloride) (PAH)/poly(styrenesulfonate) multilayers was examined by Schaaf et al. [17]. The differences in adsorption behavior between two investigated proteins were explained on the basis of protein–polyelectrolyte interactions, which are influenced mostly by the nature and the strength of the ionic interactions between the polyelectrolyte–protein contact surfaces.

Although the calorimetric data could also be of considerable importance to researchers concerned with energetics of formation of interpolyelectrolyte and polyelectrolyte–protein complexes in solution and at the surface, there are not many calorimetric investigations that could be found in the literature [19–22]. In most of these studies the interpolyelectrolyte neutralization was found to be isoenthalpic. In our previous study [22] about complexation between polyallylammonium (PAH) cation and PSS anion we used microcalorimetry (together with dynamic light scattering, electrokinetic and spectrophotometric experiments) to systematically exam that process in aqueous solutions of binary 1:1 sodium electrolytes.

The aim of the presented study is to extend the approach applied earlier for complexation between PAH cation and PSS anion [22] on complexation between polyelectrolytes and proteins. Therefore, we have decided to systematically study the complexation between lysozyme and PSS by means of microcalorimetry, dynamic light scattering (DLS) and electrokinetics. The main goal of the study was to investigate the effect of pH and reactant concentration on complexation with special emphasis on complexation thermodynamics. Moreover, since until now published experiments were performed only by titrating lysozyme with PSS, and not *vice versa*, we decided to study the above-mentioned process in both titration directions.