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ScienceDirect
Mendeleev Commun., 2014, **24**, 264–265
Mendeleev Communications

Dynamic surface elasticity of the mixed solutions of bovine serum albumin and synthetic polyelectrolytes

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DOI: 10.1016/j.mencom.2014.09.005

The kinetics of dynamic surface elasticity of the mixed solutions of bovine serum albumin and synthetic polyelectrolytes makes it possible to apply dilational surface rheology to the determination of the adsorption mechanism of protein/polyelectrolyte complexes and the structure of adsorption layers.

In spite of the frequent applications of mixed protein/polyelectrolyte solutions as liquid disperse systems or thin films, information on the surface properties of these solutions and on the structure of adsorption layers is scarce.[1,2] In this work the method of the dilation surface rheology[3–5] is applied to the mixed solutions of bovine serum albumin (BSA) and synthetic polyelectrolytes, poly(diallyldimethylammonium chloride) (PDADMAC) and sodium polystyrenesulfonate (PSS).

The dilational dynamic surface elasticity (DSE) of BSA/PSS and BSA/PDADMAC solutions was measured by the oscillating ring method as a function of the surface age and polyelectrolyte concentration. The pH of solutions was 3.8 or 7, whereas the isoelectric point of BSA is at pH ~ 5. The protein concentration was 8×10^{-8} mol dm^{-3}. Before the experiment, a glass ring with the sandblasted inner surface and the main axis perpendicular to the test liquid surface was partly immersed into the liquid. Ring oscillations along the axis led to the oscillations of the liquid surface area as a result of periodical changes in the meniscus shape at the inner ring surface. The ratio of the oscillation amplitudes of the surface tension and surface area, and the phase shift between the oscillations of these two quantities allowed us to calculate the real and imaginary parts of DSE. The imaginary part of DSE for the test systems was close to zero within the experimental error, and we had to take into account only the real part or the modulus of DSE.

The kinetics of the DSE modulus of BSA/PDADMAC and BSA/PSS mixed solutions at different pH and polyelectrolyte concentrations are shown in Figures 1 and 2. The surface properties of BSA solutions without polyelectrolyte are similar to those of other globular proteins: DSE monotonically increases and reaches 70 mN m^{-1}, while the surface tension decreases by about 12 mN m^{-1}.[3] The pH changes result only in slight changes in DSE. The same kinetics is typical of the mixed solutions of BSA and PDADMAC at pH below the isoelectric point of protein in a wide range of polyelectrolyte concentrations when both of the macromolecules have the same charge (Figure 1). This means that the polyelectrolyte and protein do not interact at pH below the isoelectric point and the polyelectrolyte does not influence the structure of the protein adsorption layer. At the same time, the kinetics of DSE of the mixed solutions of BSA and PSS changed strongly at pH 3.8 (Figure 2). A local maximum of DSE appeared at low PSS concentrations (2×10^{-5} and 2×10^{-4} mg ml^{-1}). This maximum disappeared with increasing polyelectrolyte concentration. At a PSS concentration of 2×10^{-3} mg ml^{-1}, the kinetic curve of DSE almost coincided with that for pure BSA solutions (Figure 2). A further increase in the concentration led to a considerable decrease in the rate of change in DSE; at a PSS concentration of 2×10^{-1} mg ml^{-1}, DSE reached only 20 mN m^{-1} in 5 h after the surface formation.

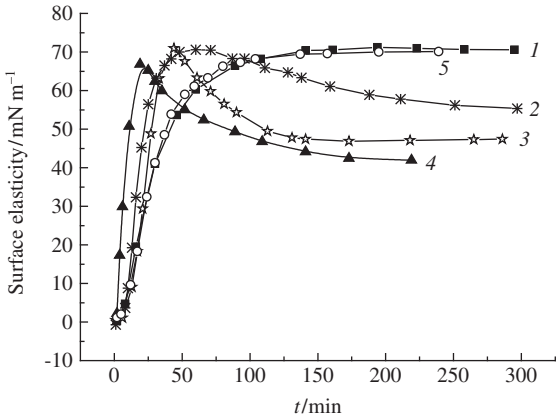

Figure 1 Kinetic curves of the modulus of the DSE of BSA/PDADMAC solutions at pH 7, BSA concentration of 8×10^{-8} mol dm^{-3}, and polyelectrolyte concentrations: (*1*) 0, (*2*) 2×10^{-5}, (*3*) 2×10^{-3} and (*4*) 2×10^{-1} mg ml^{-1}; (*5*) at pH 3.8 and PDADMAC concentration of 2×10^{-3} mg ml^{-1}.

The rate of decrease in the surface tension for mixed protein/PSS solutions also changed nonmonotonically with PSS concentration. The rate increased with PSS concentration at low polyelectrolyte concentrations, but it began to decrease close to a PSS

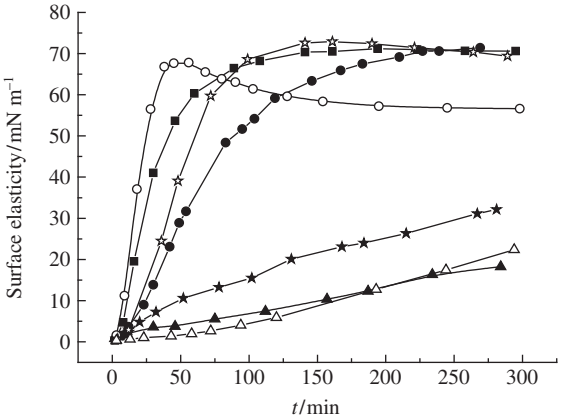

Figure 2 Kinetic curves of the modulus of the DSE of BSA/PSS solutions at pH 7 (solid symbols) and pH 3.8 (open symbols), BSA concentration of 8×10^{-8} mol dm^{-3} and polyelectrolyte concentrations 0 (squares), 2×10^{-4} (circles), 2×10^{-3} (stars), 2×10^{-1} mg ml^{-1} (triangles).

concentration of 2×10^{-4} mg ml^{-1}. We can explain this behavior by the formation of a protein/polyelectrolyte complex with a higher surface activity and a lower absolute value of the charge than those of the protein molecule. When the PSS concentration increased, the charge of the complex changed its sign.

The local maximum of the kinetic curves of DSE, which is typical of mixed BSA/PSS solutions at low pH, can also be observed in the mixed solutions of oppositely charged BSA and PDADMAC at pH 7 (Figure 1). Unlike the solutions of BSA/PSS at pH 3.8, the local maximum does not disappear and the rate of changes in the surface properties increases monotonically with polyelectrolyte concentration.

The maxima of the kinetic curves of DSE of mixed protein/polyelectrolyte solutions indicate conformational transitions in the surface layer and, most probably, the beginning of the destruction of the protein tertiary structure.[3–6] Nonmonotonical kinetic curves in Figures 1 and 2 are similar in shape to those for BSA solutions with a strong denaturant, guanidine hydrochloride (GuHCl).[3] In BSA/GuHCl solutions, the region of DSE maximum corresponds to the destruction of the globular structure of the protein and the subsequent formation of loops and tails in the surface layer. In this case, the relaxation of surface stresses becomes possible at the expense of the exchange of segments between different regions of the surface layer, and DSE starts to decrease. The results show that the protein tertiary structure starts to break down even at low polyelectrolyte concentrations of about 2×10^{-5} mg ml^{-1}. The difference between the maximum DSE modulus and a nearly equilibrium value for BSA/PSS solutions is less than the corresponding difference for BSA/PDADMAC solutions and significantly less than that for BSA solutions with the addition of strong denaturants (GuHCl, urea and ionic surfactants).[3–6] This may indicate that the addition of PSS leads only to a partial destruction of the protein tertiary structure.

DSE of mixed BSA/PSS solutions at pH 7 and low PSS concentrations ($\leqslant 2\times10^{-4}$ mg ml^{-1}) is similar to the results obtained for pure protein solutions (Figure 2). An increase in the concentration of polyelectrolyte leads to a significant deceleration of DSE changes. If experimental data on DSE are plotted as a function of surface pressure (Figure 3), all the curves for a broad range of PSS concentrations coincide within the limits of experimental error with those for pure protein solutions. This fact suggests that the adsorption layer structure does not change upon the addition of a polyelectrolyte in this pH range. The surface properties are determined by the adsorbed protein globules regardless of the PSS concentration. The polyelectrolyte forms a soft inner skin around the protein globule. The skin is easily subjected to deformations when the protein/polyelectrolyte complex goes from the bulk phase into the surface layer and it does not affect the surface properties after that. At the same time, BSA molecules in this case form a strong network of intermolecular bonds at the liquid–gas interface, largely keeping their tertiary structure.[7,8]

PSS does not possess any surface activity in the test concentration range. Therefore, we assume the formation of complexes between BSA and PSS at pH 7 in order to explain the effect of polyelectrolytes on the dynamic surface properties of the solutions of similarly charged BSA. These complexes can be formed due to the interaction between the polyelectrolyte and some parts of the globule surface, which have the charge opposite to the overall charge of the protein molecule. The high flexibility of PSS molecules facilitates interactions between the components. The complexes have a large negative charge, and they are probably stabilized by hydrophobic interactions. Indeed, it is well known that the surface of BSA globules has hydrophobic patches and the polystyrene chain is highly hydrophobic.[9–11]

In the case of more rigid and less hydrophobic PDADMAC, the complex formation does not occur at pH values lower than the isoelectric point when the components have the same charge. In this case, PDADMAC does not influence the kinetics of DSE of BSA solutions (Figure 1).

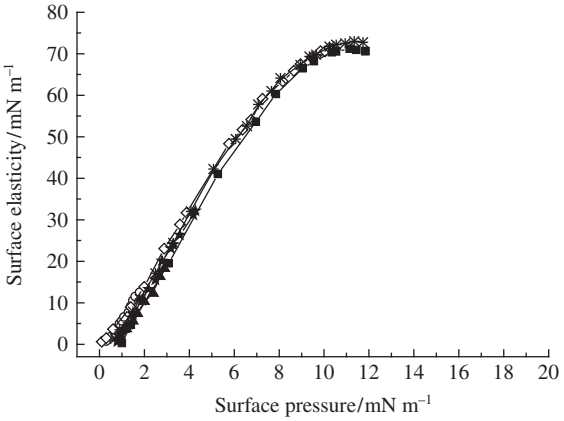

Figure 3 Modulus of the DSE of BSA/PSS solutions *vs*. surface pressure at pH 7, BSA concentration of 8×10^{-8} mol dm^{-3}, and PSS concentrations 0 (black squares), 2×10^{-5} (snowflakes), 2×10^{-4} (diamonds), 2×10^{-3} (stars), 2×10^{-2} (circles), 2×10^{-1} mg ml^{-1} (triangles).

The addition of a polyelectrolyte mainly affects the surface properties. The formation of BSA/PSS complexes at pH 7 increases the total charge of adsorbing particles and hence the adsorption electric barrier. In this case, the addition of a polyelectrolyte should cause the deceleration of globule adsorption resulting in a change in the kinetics of DSE (Figure 2).

The measurements of surface dilatational rheological properties allowed us to estimate the effect of protein–polyelectrolyte interactions on the state of protein globules at the liquid–gas interface. If the components have opposite charges, the tertiary structure of BSA changes in the adsorption layer leading to a local maximum in the kinetic curve of DSE. If the polyelectrolyte and BSA are similarly charged, the possibility of complex formation depends on the flexibility of the polyelectrolyte chain, and the main effect of the polyelectrolyte consists in a strong decrease in the rate of change in the surface properties.

The authors acknowledge St. Petersburg State University for a research grant 12.38.241.2014. This work was also supported by the Russian Foundation for Basic Research (project no. 14-03-00670) and the National Science Councel of Taiwan (joint project RFFI_NSC no. 12_03_92004_HHC_a).

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Received: 9th January 2014; Com. 14/4284