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Effect of a cationic surfactant on protein unfolding at the air–solution interface

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Kinetic dependences of the dynamic dilatational surface elasticity of solutions of the complexes of β-lactoglobulin and bovine serum albumin with dodecyltrimethylammonium bromide show that the destruction of the tertiary protein structure and the partial destruction of the secondary structure in the surface layer occur at lower surfactant concentrations than in the bulk solution.

In spite of the extensive application of protein mixtures with surfactants to the stabilization of foams and emulsions, information on the conformations of macromolecules in mixed adsorption layers remains scarce.[1] Furthermore, the extent of the destruction of the protein tertiary structure in the course of adsorption at a liquid surface is a subject under discussion.[2,3] The main problem of these studies consists in the lack of available experimental methods. The denaturation of globular proteins bovine serum albumin (BSA) and β-lactoglobulin (BLG) in concentrated solutions of guanidine hydrochloride (G.HCl) is accompanied by a transition from monotonic to non-monotonic kinetic dependences of the dynamic surface elasticity.[4,5] On the other hand, it is well known that ionic surfactants have a denaturating effect in the bulk of solution at high concentrations.[6] The aim of this work was to study the unfolding of protein globules in a surface layer of aqueous solutions containing the cationic surfactant dodecyltrimethylammonium bromide (DTAB) based on the data of dilatational surface rheology.

The dilatational surface elasticity was measured by the oscillating barrier method based on the simultaneous detection of oscillations of a solution surface area in the Langmuir trough and the induced surface tension oscillations.† The experimental procedure was described in detail elsewhere.[7]

The surface pressure π of BLG or BSA solutions at a concentration of 0.1 or 0.03 μmol dm^{-3} approaches the equilibrium values of 6.5 and 8.1 mN m^{-1}, respectively, 5 h after the creation of a fresh surface (Figure 1). The observed slow increase in the surface pressure and a noticeable induction period are the characteristic features of globular protein solutions.[9,10]

All the kinetic dependences of the surface pressure remain monotonic upon the addition of small amounts of DTAB. An increase in the surfactant concentration results in an acceleration of the surface pressure growth and a gradual disappearance of the induction period from the kinetic dependences. These effects are similar to those observed in the solutions of hydrophobically modified BLG.[11] The influence of the surfactant on the kinetics of surface pressure changes becomes appreciable at a DTAB concentration of 0.1 μmol dm^{-3}, whereas the surface tension of a pure DTAB solution of the same concentration is the same as that of pure water. This result indicates the formation of protein–surfactant complexes of relatively high surface activity leading to stronger changes in the surface pressure.

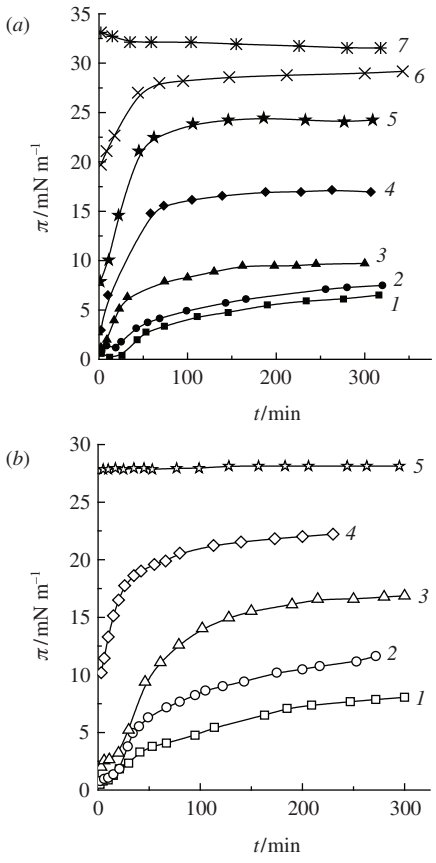

Figure 1 Kinetic dependences of the surface pressure of (*a*) BLG (0.1 μM)–DTAB solutions at various concentrations of surfactant: (*1*) 0, (*2*) 0.0001, (*3*) 0.0008, (*4*) 0.04, (*5*) 0.4, (*6*) 4 and (*7*) 14.4 mmol dm^{-3}; (b) BSA (0.03 μM)–DTAB solutions at various concentrations of surfactant: (*1*) 0, (*2*) 0.01, (*3*) 0.1, (*4*) 1 and (*5*) 10 mmol dm^{-3}.

The influence of the surfactant on the shape of the kinetic curves of the real part of the dilatational surface elasticity proves

† BSA (Sigma-Aldrich) was used as received. BLG (Sigma-Aldrich) was purified with activated charcoal according to a published method.[8] DTAB (Sigma-Aldrich) was twice recrystallized from ethyl acetate–ethanol. A phosphate buffer was prepared immediately before measurements. The ionic strength and pH of BSA or BLG solutions were 0.02 M and 6.7 or 0.01 M and 7.0, respectively.

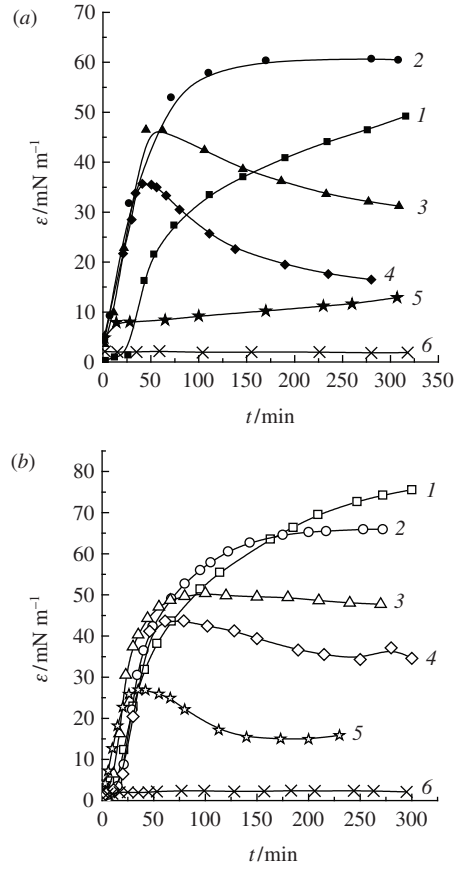

Figure 2 Kinetic dependences of the dilatational surface elasticity of (*a*) BLG (0.1 μM)–DTAB solutions at various concentrations of surfactant: (*1*) 0, (*2*) 0.08, (*3*) 0.4, (*4*) 0.8, (*5*) 8 and (*6*) 14.4 mmol dm^{-3} at the frequency of 0.1 Hz; (*b*) BSA (0.03 μM)–DTAB solutions at various concentrations of surfactant: (*1*) 0, (*2*) 0.01, (*3*) 0.05, (*4*) 0.1, (*5*) 1 and (*6*) 10 mmol dm^{-3} at the frequency of 0.1 Hz.

to be more significant (Figure 2). The imaginary part of the test solutions is close to zero within the limits of experimental error. In the case of pure protein solutions, the surface elasticity increases monotonically and reaches high values (~75 mN m^{-1} for BSA and ~50 mN m^{-1} for BLG). The effect of the surfactant at concentrations of up to 0.1 or 0.05 mmol dm^{-3} in the case of BLG or BSA solutions, respectively, consists in an adsorption acceleration. This is obviously due to the formation of protein–surfactant complexes with higher surface activity than that of pure protein. A further increase in the DTAB content results in a change in the shape of the kinetic curves of surface elasticity. This value increases for 50–60 min after the surface formation, passes through a local maximum and slowly decreases after that. An increase in the surfactant concentration leads to a decrease in both the maximum surface elasticity and its limit value at high surface ages corresponding to the almost equilibrium system. The kinetic dependences are monotonic, and the surface elasticity approaches zero when the surfactant concentration approaches the CMC of DTAB solutions. This elasticity decrease is obviously caused by the transition of protein molecules from the surface to the bulk of solution due to protein solubilization by micelles. In this case, the adsorption layer mainly consists of surfactant monomers.

The local maximum of the kinetic dependences of surface elasticity for the solutions of non-ionic flexible polymers indicates the conformational transition related to an increase in the adsorption layer thickness as a result of the formation of loops and tails (the distal region of the surface layer).[12] In this case, the relaxation of surface stresses can proceed at the expense of segment exchange between the proximal and distal regions of the surface layer and the surface elasticity begins to decrease.[13] Similar kinetic dependences have been found recently for the mixed solutions of globular proteins and G.HCl.[4,5] Both the tertiary and secondary structures of globular proteins are destroyed in concentrated G.HCl solutions, the macromolecules unfold and form flexible chains leading to the formation of tails and loops in the surface layer. The destruction of a globular structure in the surface layer proceeds at lower denaturant concentrations than in the bulk of solution. Similar conclusions on the globular structure destruction of BLG, lysozyme and ribonuclease A in the surface layer of G.HCl solutions have been drawn recently from data on X-ray and neutron reflectivity.[14–16]

The conventional ionic surfactants also destroy the tertiary structure of proteins leading to globule unfolding,[17,18] and they have almost no effect on the protein secondary structure even at concentrations close to the CMC.[19–21] The denaturating activity of surfactants reveals itself as a result of cooperative interactions between protein macromolecules and surfactant ions. For BSA solutions, the range of [DTAB]:[protein] molar ratios corresponding to the destruction of the protein tertiary structure is 70–180,[18,22] and for the solutions of BLG–DTAB complexes it is 10–30 and 50–100,[20] whereas in the case of solutions of complexes between BLG and dodecyltrimethylammonium chloride (DTAC) the conformation transition is observed at [DTAC]:[BLG] > 150.[21] The BLG secondary structure does not change significantly when the protein interacts with surfactants in the bulk phase. Only an increase in the contribution of α-helices at the expense of a decrease in the contribution of β-sheets was observed.[20,21]

A transition to the non-monotonic kinetic dependences for solutions containing two test proteins occurs at the [DTAB]:[protein] ratios from 3000 to 4000 (Figure 2). The results obtained by circular dichroism spectroscopy indicate the preservation of the protein secondary structure in this concentration range and, consequently, a limited flexibility of protein molecules in the bulk phase.[21,23,24] Therefore, it seems improbable that the appearance of a local maximum of dynamic surface elasticity is caused by the adsorption of relatively flexible protein molecules, which are able to form loops and tails in the surface layer, from the solution. Thus, the partial destruction of the secondary protein structure occurs in the surface layer. FTIR spectroscopy showed that the secondary structure changes in the course of globular protein adsorption at the liquid–gas interface and the contributions of the unordered structure increase; *i.e.*, the interface exerts a denaturating effect on protein molecules.[19] On the other hand, the destruction of the secondary structure in a surface layer occurs at lower denaturant concentrations than in the bulk phase.[4,5,14] It seems probable that the partial destruction of the secondary structure in the surface layer results in a growth of the flexibility of protein molecules, the formation of loops and tails and, consequently, the appearance of a local maximum in the kinetic dependences of the surface elasticity when the surfactant concentration is high and the [DTAB]:[protein] ratio exceeds 3000 (Figure 2). Note that the local maximum of the surface elasticity appears at a low surfactant concentration when the protein displacement by the surfactant seems improbable. The molecular flexibility increases with surfactant concentration, and a peak in the kinetic curves of the dynamic surface elasticity becomes more pronounced. Note that changes in the shapes of the kinetic curves of the dynamic surface elasticity of protein–surfactant solutions are similar in both cases of the increase of DTAB and G.HCl concentrations. If the denaturant (G.HCl or DTAB) concentration is sufficiently high to destroy the tertiary and secondary protein structures, the dynamic surface elasticity starts to reduce after the local maximum when the surface pressure reaches a critical value of about 19 or 14 mN m^{-1} for BLG or BSA solutions, respectively.

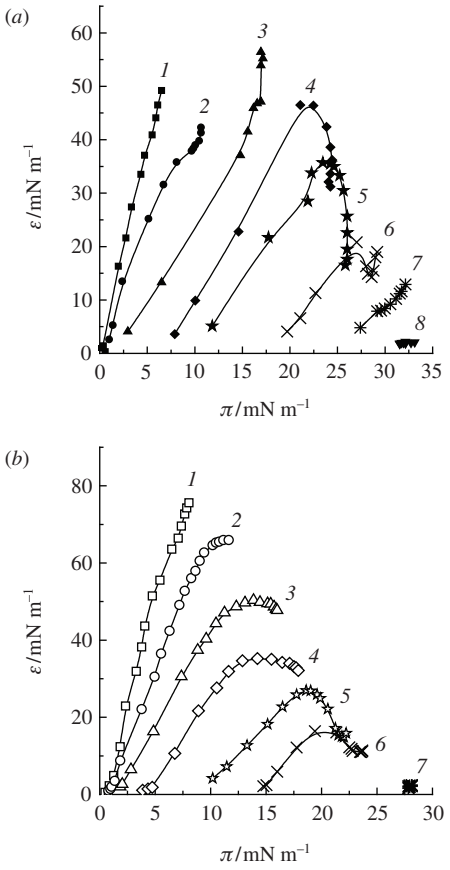

Figure 3 Dependences of the dilatational surface elasticity on the surface pressure of (*a*) BLG (0.1 μM)–DTAB solutions at various surfactant concentrations: (*1*) 0, (*2*) 0.0024, (*3*) 0.04, (*4*) 0.4, (*5*) 0.8, (*6*) 4, (*7*) 8 and (*8*) 14.4 mmol dm^{-3} at the frequency of 0.1 Hz; (*b*) BSA (0.03 μM)–DTAB solutions at various surfactant concentrations: (*1*) 0, (*2*) 0.01, (*3*) 0.05, (*4*) 0.3, (*5*) 1, (*6*) 3 and (*7*) 10 mmol dm^{-3} at the frequency of 0.1 Hz.

These peculiarities of the investigated systems become evident if one considers surface elasticity as a function of surface pressure (Figure 3, *cf.* refs. 4 and 5). The surface activity of the protein–surfactant complex increases together with the adsorbed amount of free surfactant molecules at the further increase of the surfactant concentration and the local maximum of the surface elasticity shifts to higher surface pressures. The dynamic surface elasticities of 14 and 19 mN m^{-1} are, probably, characteristic of the test proteins, and they correspond to the onset of loop and tail formation in the adsorption layer of unfolded BSA and BLG molecules, respectively (BSA–surfactant and BLG–surfactant complexes). In a similar manner, a value of 6 mN m^{-1} is characteristic of β-casein molecules, and it corresponds to the onset of the protrusion into a subphase of N-terminals of the molecules as loops and tails.[25]

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