THE JOURNAL OF PHYSICAL CHEMISTRY B

ARTICLE

pubs.acs.org/JPCB

Formation of Protein/Surfactant Adsorption Layer at the Air/Water Interface as Studied by Dilational Surface Rheology

A. A. Mikhailovskaya,[†] B. A. Noskov,[*,†] S.-Y. Lin,[‡] G. Loglio,[§] and R. Miller[∥]

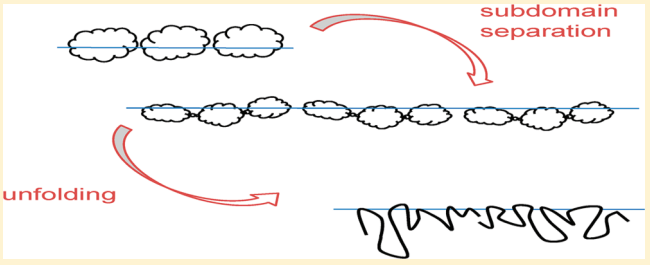
[†]Department of Colloid Chemistry, St. Petersburg State University, Universitetsky pr. 26, 198504 St. Petersburg, Russia

[‡]Chemical Engineering Department, National Taiwan University of Science and Technology, 43 Keelung Road, Section 4, Taipei, 106 Taiwan

[§]Dipartimento di Chimica Organica, Universita degli Studi di Firenze, Via della Lastruccia 13, 50019 Sesto Fiorentino, Firenze, Italy

[∥]MPI für Kolloid- und Grenzflächenforschung, Wissenschaftspark Golm, D-14424 Golm, Germany

ABSTRACT: The dynamic dilatational surface elasticity of mixed solutions of globular proteins (β-lactoglobulin (BLG) and bovine serum albumin (BSA)) with cationic (dodecyltrimethylammonium bromide (DTAB)) and anionic (sodium dodecyl sulfate (SDS)) surfactants was measured as a function of the surfactant concentration and surface age. If the cationic surfactant concentration exceeds a certain critical value, the kinetic dependencies of the dynamic surface elasticity of BLG/DTAB and BSA/DTAB solutions become nonmonotonous and resemble those of mixed solutions of proteins with guanidine hydrochloride. This result indicates not only the destruction of the protein tertiary structure in the surface layer of mixed solution but also a strong perturbation of the secondary structure. The corresponding kinetic dependencies for protein solutions with added anionic surfactants are always monotonous, thereby revealing a different mechanism of the adsorption layer formation. One can assume that the secondary structure is destroyed to a lesser extent in the latter case and hinders the formation of loops and tails at the interface. The increase of the solution's ionic strength by the addition of sodium chloride results in stronger changes of the protein conformations in the surface layer and the appearance of a local maximum in the kinetic dependencies of the dynamic surface elasticity in a relatively narrow range of SDS concentration.



INTRODUCTION

The protein conformation at liquid−fluid interfaces has been a subject of intensive discussion.[1−3] Although the main steps of some physiological processes occur in the interfacial layer, and protein adsorption layers play a crucial role in the stabilization of various natural and industrial colloidal systems, information on the changes of the protein's tertiary and secondary structure in the course of adsorption is still rather limited. If in the bulk phase one can study some details of the protein globule unfolding,[4] in surface layers even the fact of destruction of the tertiary structure after protein adsorption can be questioned. The discovery of slow protein adsorption and the formation of highly cohesive protein networks at liquid−fluid interfaces in the early period of protein adsorption studies led to the conclusion of the unfolding of some globular proteins at liquid surfaces.[5,6] The subsequent application of neutron reflectivity indicated the preservation of the protein globular structure in the surface layer.[7−12] At the same time, X-ray reflectivity[13] and circular dichroism spectroscopy[14] do not confirm the latter conclusion for some systems.

Information on the structure of adsorption layers of globular protein/ionic surfactant complexes is even more scarce. These proteins are frequently used in industry or encountered in nature together with surfactants of low molecular weight. Details of protein−surfactant interactions in the bulk phase have been studied by fluorescence probe techniques,[15−25] neutron and X-ray small-angle scattering,[17,19,21,26,27] dynamic light scattering,[19,28] circular dichroism,[15,16,21,24,25] NMR[18,29] and optical spectroscopy.[30] It is well established now that the protein globule can bind only a few surfactant monomers in special binding sites at low surfactant concentrations, but the globular structure gets destroyed at higher concentrations when the binding becomes cooperative. The increase of the surfactant concentration can result also in changes of the secondary protein structure.[14,26] However, the entire destruction of the ordered structure does not occur except for high guanidine hydrochloride (G.HCl) concentrations.[31] In spite of the special importance of surface properties of protein/surfactant solutions from the point of view of various applications, they have been studied only by a few authors, and mainly by means of surface tension measurements.[32−34] Information on globular protein/ionic surfactant interactions in the liquid surface layer relates mainly to the case when the surfactant displaces the preliminary adsorbed protein from the surface.[35,36] These studies led to the creation of the model of orogenic protein displacement. According to this

Received: May 27, 2011
Revised: July 19, 2011
Published: July 24, 2011

model, the surfactant molecules are adsorbed first at defects of the protein adsorption layer. These small surface domains expand gradually, leading to the collapse of the protein adsorption layer and to the subsequent protein displacement from the interface. The kinetics of adsorption layer formation during the simultaneous adsorption of proteins and surfactants have been studied to a lesser extent.[34,37] The main difficulties in this field are obviously connected to the limited number of experimental methods available for investigations of liquid−fluid interfaces. The surface tension and the adsorbed amount usually change monotonously as a function of the surface age, and one can extract only limited information on the changes of the macromolecules' conformation from these data.[6,32,38]

It has been shown recently that one can follow the protein unfolding in the surface layer by measuring the kinetic dependencies of the dilational dynamic surface elasticity.[39,40] This quantity increases monotonously up to high values (∼ 80 mN/m) in the course of globular protein adsorption but decreases strongly if the tertiary and secondary protein structures are destroyed. For mixed solutions of β-lactoglobulin (BLG)[39] and bovine serum albumin (BSA)[40] in the presence of a denaturant (G.HCl)) the kinetic dependencies of the dynamic surface elasticity become nonmonotonous and resemble those of flexible homopolymers[41,42] or block copolymers[43] if the G.HCl concentration exceeds a critical value.

In the present work, this approach is applied to mixed solutions of ionic surfactants (sodium dodecyl sulfate (SDS) and dodecyltrimethylammoniun bromide (DTAB)) with globular proteins (BLG and BSA), which have different tertiary and secondary structures, with the aim to study the globule unfolding in the surface layer. Previous results on BLG and BSA unfolding in G.HCl solutions[39,40] are used for comparison. The globules of BLG with a molecular weight of 18.4 kDa consist of a single domain. In aqueous solution, it resembles a flattened cone, contains two disulfide bonds, and is formed mainly of β-sheets.[24,25,44] The globule of BSA with a molecular weight of 66.7 kDa consists of three weakly bound domains. In its turn, all the domains consist of two subdomains. The secondary structure is formed mainly by α-helixes, which are stabilized by 17 disulfide bonds and contain 67% of amino acid residues.[45,46]

The interaction of both proteins with ionic surfactants in the bulk phase has been discussed in numerous papers.[15−30] It has been dicovered that ionic surfactants have a denaturating influence on globular proteins. The surfactant binding to protein molecules results gradually in the destruction of the protein globular structure and some increase of β-sheets and disordered structures at the expense of α-helices.

MATERIALS AND METHODS

BSA (Sigma-Aldrich) was used as received. BLG (Sigma-Aldrich) was purified by the addition of activated charcoal (charcoal:BLG mass ratio is 6:1) according to the method of Clark et al.[47] The solution was stirred intensively for half an hour, and then the charcoal was removed by centrifugation.

The BSA and BLG solutions in phosphate buffer at pH 6.7 and 7.0, respectively, were prepared by dilution of a stock solution, which had been stored in a refrigerator at 2 °C not longer than one week. All measurements in this work were carried out at BSA concentrations of 0.03 μM and 0.3 μM and BLG concentrations of 0.1 μM and 0.5 μM. The solution pH was regulated by the addition of NaH_2PO_4 and Na_2HPO_4. The ionic strengths of BSA and BLG solutions were 0.02 and 0.01 M correspondingly. Triple-distilled water was used for the preparation of all solutions.

DTAB (Sigma-Aldrich) was recrystallized twice from a mixture of ethylacetate and ethanol. SDS (Sigma-Aldrich) was recrystallized twice from ethanol.

The surface tension was measured by the Wilhelmy plate method using a roughened glass plate attached to an electronic balance. Measurements of the complex dynamic surface elasticity were executed by the oscillating barrier method at a fixed frequency of 0.1 Hz. The corresponding experimental procedure was described in detail elsewhere.[41] The oscillations of the solution surface area in a polytetrafluoroethylene (PTFE) Langmuir trough were produced by a movable PTFE barrier sliding along the polished brims of the trough. A mechanical generator transformed the rotation of an electric motor into the translational motion with reversion and gave the possibility to control the oscillation amplitude and frequency. The moving part of the generator was connected to the barrier by a steel rod. The barrier glided back and forth along the Langmuir trough and produced oscillations of the liquid surface area δS with a relative amplitude of 3%. The corresponding oscillations of the surface tension γ were measured by the Wilhelmy plate method. The dynamic surface elasticity ε was determined from the oscillations of the surface tension γ and surface area according to the following relation

$$\varepsilon(\omega) = \varepsilon_r + i\varepsilon_i = \delta\gamma/\delta \ln S$$

where ε, $\delta\gamma$ and δS are complex quantities.

The elasticity modulus was determined from the ratio of the oscillation amplitudes, while the phase shift between the oscillations of the two measured parameters determined the phase angle of the dynamic surface elasticity. At frequencies less than about 0.2 Hz, the length of surface longitudinal waves far exceeds the length of the Langmuir trough, and they did not influence the surface tension oscillations in the trough. However, these oscillations are not completely uniform mainly due to the influence of the trough walls on the liquid flow. To exclude this effect, all measurements in this work corresponded to a fixed position of the Wilhelmy plate in the center of the Langmuir trough, allowing a reduction of the relative experimental error.

The imaginary part of the complex dynamic surface elasticity of mixed protein/surfactant solutions under investigation proved to be much less than the real part. Therefore only the results for the real part are discussed below.

RESULTS

The surface pressure π of BLG solutions at a concentration of 0.0001 mM and BSA solutions at a concentration of 0.00003 mM approaches the equilibrium values of 6.5 and 8.1 mN/m, respectively, during five hours after the fresh surface creation (Figures 1a and 2a). One can observe an induction period for the BLG solution when the surface pressure equals zero in error limits during about half an hour. The induction period is not noticeable for BSA solutions, but there is only a small increase in the rate of surface tension changes at the initial adsorption step (Figure 2a). The slow changes of the surface properties with surface age and the induction period are typical for solutions of many globular proteins.[5,48,49] Note that the induction period is not connected directly with the diffusion coefficient and consequently the molecular weight of the protein, but is

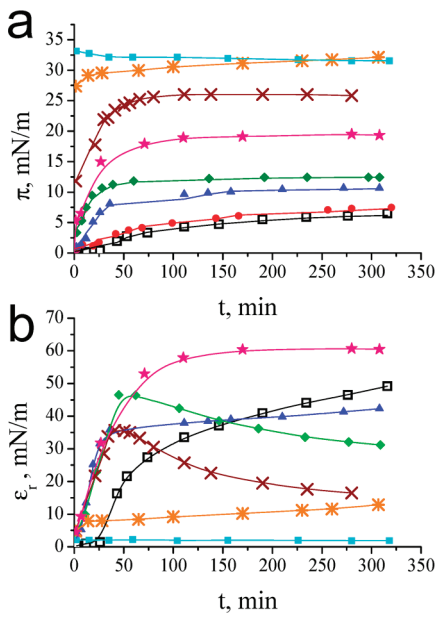


Figure 1. (a) Dynamic surface pressure and (b) dynamic surface elasticity of BLG(0.0001 mM)/DTAB solutions at surfactant concentrations of 0 (black open squares), 0.0001 (red circles), 0.0024 (blue triangles), 0.006 (dark-green diamonds), 0.08 (pink stars), 0.4 (light-green diamonds), 0.8 (brown crosses), 8 (orange snowflakes), and 14.4 mM (light-blue squares) at a frequency of 0.1 Hz. Lines are guides for the eye.

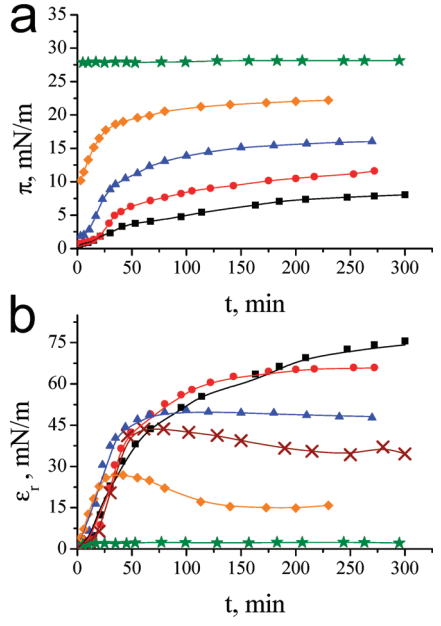


Figure 2. (a) Dynamic surface pressure and (b) dynamic surface elasticity of BSA(0.03 μM)/DTAB solutions at surfactant concentrations of 0 (black squares), 0.01 (red circles), 0.05 (blue triangles), 0.1 (brown crosses), 1 (orange diamonds), and 10 mM (dark-green stars) at a frequency of 0.1 Hz. Lines are guides for the eye.

probably determined by the equation of state of the protein adsorption layer.

The DTAB addition leads to an acceleration of the surface pressure changes and to an increase of equilibrium surface pressure for both systems. The influence of surfactant additions becomes noticeable already at concentrations about 0.0001 mM when the surface tension of the pure surfactant solution equals the value for pure water. The strong influence of small surfactant additions is obviously caused by the formation of protein/surfactant complexes with higher surface activity than that of the pure protein. The induction period for BLG/DTAB solutions decreases with the surfactant concentration and becomes imperceptible already at a DTAB concentration of about 0.005 mM. All surface tension changes at higher concentrations are within the error limits within about 100 min after the new surface creation. The approach to the equilibrium state is slower for the solutions of BSA with higher molecular weight. In this case, the surface tension reaches the equilibrium value in error limits within 100 min only if the surfactant concentrations are higher than 1 mM.

All kinetic dependencies of surface pressure remain monotonous at the increase of the DTAB concentration. At the same time, the addition of the cationic surfactant appreciably influences the shape of the kinetic curves of the dynamic surface elasticity (Figures 1b and 2b).

The kinetic dependencies of the dynamic surface elasticity of the solutions of pure proteins are also monotonous, and the induction period is noticeable for BLG solutions. The additions of the cationic surfactant at concentrations as low as 0.0001 mM (data not shown, cf. Figure 7 below) appreciably increase the rate of the surface elasticity rise at the initial adsorption step. For BLG/DTAB solutions (Figure 1b), this effect is stronger than that for BSA/DTAB solutions (Figure 2b). In spite of the fact that the negative charge of the BSA globule in neutral solutions (−18;[45,46] the isoelectric point is about 4.8[46]) is higher than the negative charge of the BLG globule (−8;[44,50] (the isoelectric point of BLG is close to 6.0[44]), the electrostatic adsorption barrier is probably lower in the former case due to the larger size of the globule, its multidomain structure, and the peculiarities of the adsorption layer structure. Therefore the formation of the complex with the oppositely charged surfactant does not lead to significant acceleration of the BSA adsorption as in the case of BLG solutions.

A significant acceleration of adsorption of BLG/DTAB complexes occurs already at surfactant concentrations of about 0.0024 mM (Figure 1b). The shape of the kinetic curves of the dynamic surface elasticity does not almost change at higher surfactant concentrations up to about 0.1 mM, or, more exactly, the upper limit of the concentration range where one can observe the monotonous kinetic dependencies of the dynamic surface elasticity is somewhere between 0.08 and 0.4 mM. The surface elasticity increases slowly for a few hours after the initial strong rise during the first 25 min of the adsorption process. The shape of the kinetic curves changes abruptly upon further increase of the DTAB concentration, thereby indicating a conformation transition in the surface layer. The kinetic dependencies of the dynamic surface elasticity become nonmonotonous: the fast increase of the surface elasticity during the initial adsorption step is replaced by a slow decrease (Figure 1b). The surfactant concentration increase results in the decrease of both the maximal value of the surface elasticity and the limit value at long surface life times corresponding to an almost equilibrium system. The kinetic dependencies become monotonous eventually when the surfactant concentration approaches the critical micelle concentration (CMC) and the surface elasticity approaches zero. The surface elasticity decrease in this case is obviously caused by

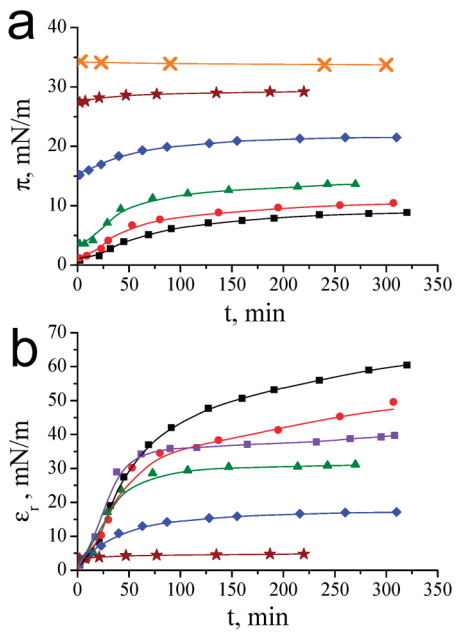


Figure 3. (a) Dynamic surface pressure and (b) dynamic surface elasticity of BLG(0.0001 mM)/SDS solutions at surfactant concentrations of 0 (black squares), 0.01 (red circles), 0.03 (violet squares), 0.1 (dark-green triangles), 1 (blue diamonds), 3 (brown stars), and 10 mM (orange crosses). Lines are guides for the eye.

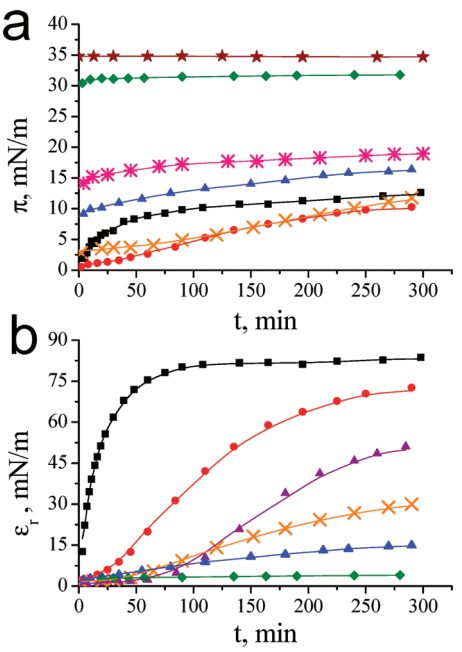


Figure 4. (a) Dynamic surface pressure and (b) dynamic surface elasticity of BSA(0.0003 mM)/SDS solutions at surfactant concentrations of 0 (black squares), 0.005 (red circles), 0.01 (purple triangles), 0.05 (orange crosses), 0.5 (blue triangles), 1 (pink snowflakes), 3 (dark-green diamonds), and 10 mM (brown stars) at a frequency of 0.1 Hz. Lines are guides for the eye.

the protein displacement from the interface and its solubilization by surfactant aggregates. This results in the adsorption layer consisting mainly of surfactant monomers.

Approximately the same changes of the kinetic dependencies of the dynamic surface elasticity occur for BSA/DTAB solutions as a result of the surfactant addition (Figure 2b). In this case, a local maximum of the kinetic dependencies appears at DTAB concentrations of about 0.05 mM. One can observe the transition from monotonous to nonmonotonous kinetic curves of the dynamic surface elasticity at various protein concentrations (data not shown).

The dynamic surface properties of the solutions of the complexes between globular proteins and surfactants depend strongly on the charge of the surfactant ion. One can discover the influence of SDS additions on the surface pressure (Figure 3a) and dynamic surface elasticity (Figure 3b) of BLG solutions only at surfactant concentrations of about 0.01 mM as in ref 34, where the authors also observed the influence of added SDS on the surface tension of BLG solutions only when the surfactant concentration exceeded the protein concentration by two decimal orders of magnitude. This result agrees with the data on the stability of BLG/surfactant complexes in aqueous solutions showing different mechanisms of the BLG interactions with cationic and anionic surfactants.[51,52] In the latter case, the surfactant anion penetrates the hydrophobic cavities of the globule and stabilizes its structure. The selectivity of the interaction is caused by a positive charge close to the cavity leading to the repulsion of the surfactant cations. Therefore the BLG molecule interacts with only one or two SDS anions in a broad concentration range and entirely preserves its tertiary structure. On the other hand, the BLG interactions with cationic surfactants result in the destruction of the globular structure at lower surfactant concentrations, leading to the binding of a larger number of surfactant molecules.

Unlike the BLG/SDS system, small SDS additions to BSA solutions result in a significant decrease of the rate of surface pressure changes with surface age (Figure 4a). This effect is obviously a consequence of the increase of the BSA globule charge as a result of its interaction with surfactant molecules. It is well-known that the adsorption of several SDS monomers on the globule surface occurs already at low surfactant concentrations in special hydrophobic binding pockets.[53] The positively charged aminoacid residues in these specific sites are close to the hydrophobic domains. The results of different experimental methods do not agree in the number of the specifically bound SDS monomers; however, this number is probably between three and nine.[53−55] The strong increase of the negative globule charge due to the specific SDS binding leads to an increase of the electrostatic adsorption barrier, and consequently to a decrease of the adsorption rate. The further increase of the SDS concentration results in a partial shielding of the electrostatic interactions in the system due to the increase of the solution ionic strength. The adsorption barrier begins to decrease, and the rate of the surface pressure increase exceeds the corresponding value for pure protein solutions already at surfactant concentrations higher than about 0.2 mM (Figure 4a).

The kinetic dependencies of the dynamic surface elasticity of solutions of the complexes between globular proteins and SDS also differ strongly from the corresponding results for solutions of protein complexes with a cationic surfactant (Figures 3b and 4b). First, these dependencies prove to be monotonous in the whole surfactant concentration range up to micellar solutions. Second, the dynamic surface elasticity together with the surface pressure change significantly slow down with increasing surface age. The surface elasticity of BLG/SDS solutions does not reach the limit value during 5 h after the fresh surface formation, at least

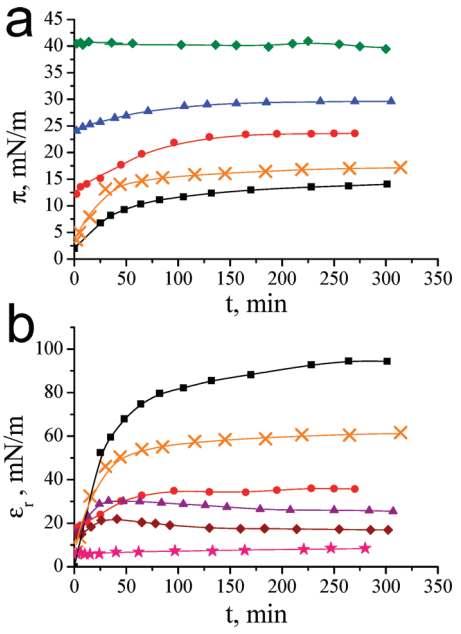


Figure 5. (a) Dynamic surface pressure and (b) dynamic surface elasticity of BLG(0.0001 mM)/SDS solutions at surfactant concentrations of 0 (black squares), 0.008 (orange crosses), 0.03 (red circles), 0.04 (purple triangles), 0.07 (brown diamonds), 0.1 (blue triangles), and 0.7 mM (dark-green diamonds) containing 0.4 M NaCl at a frequency of 0.1 Hz. Lines are guides for the eye.

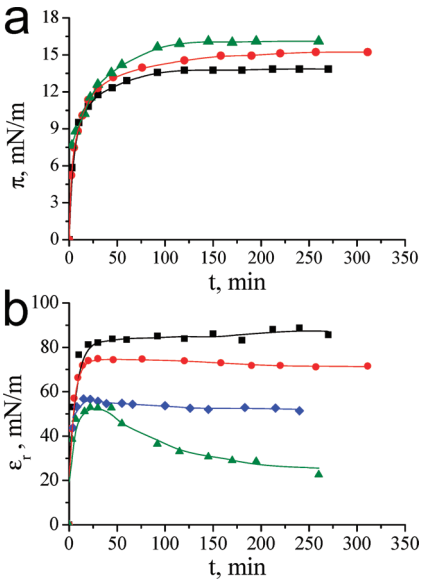


Figure 6. (a) Dynamic surface pressure and (b) dynamic surface elasticity of BSA(0.0003 mM)/SDS solutions at surfactant concentrations of 0 (black squares), 0.0003 (red circles), 0.001 (blue diamonds), and 0.01 mM (dark-green triangles) containing 0.4 M NaCl at a frequency of 0.1 Hz. Lines are guides for the eye.

at SDS concentrations lower than about 0.1 mM (Figure 3b). For BSA/SDS solutions, the concentration range corresponding to noticeable changes of the dynamic surface elasticity in a few hours after the surface formation is significantly broader. Apparently, the low surface elasticity (∼4 mN/m) of the solution with an SDS concentration of 3 mM within 5 h after the surface formation is connected with the significant deviation of the system from equilibrium (Figure 4b). The observed distinctions in the rate of surface property changes between the systems with cationic and anionic surfactants are probably caused by the differences in the value of the electrostatic adsorption barrier for globules. The barrier increases for proteins in interaction with anionic surfactants and decreases when the protein forms a complex with a cationic surfactant. In the latter case, the decrease of the repulsion between the complexes can also result in the aggregation and subsequent sedimentation of the large aggregates.[52,56] However, this effect was not observed in this work, probably due to too low protein concentrations.

The increase of the solution ionic strength at the addition of an inorganic salt must lead to the shielding of electrostatic interactions in the system and, consequently, to a decrease of the adsorption barrier. The rate of changes of surface properties really increases upon addition of sodium chloride to BLG/SDS and BSA/SDS solutions (Figures 5 and 6), and at a salt concentration of 0.4 M one can clearly observe a local maximum of the kinetic dependency of the dynamic surface elasticity. In this case, all changes in surface pressure are in the error limits for surface ages exceeding 2 h (Figures 5a and 6a). The rate of increase of the dynamic surface elasticity for BLG/SDS solutions also increases abruptly at SDS concentrations less than about 0.03 mM. The kinetic dependencies of the dynamic surface elasticity become nonmonotonous at higher surfactant concentrations, and this quantity begins to decrease slowly after a strong increase at the initial adsorption step (Figure 5). The shape of the kinetic curves for BSA/SDS solutions changes strongly with increasing surfactant concentration (Figure 6). If the difference between the maximum value and the limit value at long surface life times is close to the error limits at the SDS concentration of about 0.05 mM, the limit value is 2 times lower than the maximum at a surfactant concentration of 0.1 mM. At higher SDS concentrations, the solutions become cloudy due to aggregation. For BLG/SDS solutions, all changes of the surface elasticity are smoother, and a slightly noticeable local maximum appears only in a narrow SDS concentration range (between 0.04 mM and 0.15 mM).

DISCUSSION

The main feature of the obtained experimental results consists of the appearance of a local maximum in the kinetic dependencies of the dynamic surface elasticity when the surfactant concentration is in a certain concentration range (Figures 1b, 2b, 5b, and 6b). The viscoelasticity of the adsorption layers of the protein/surfactant complexes at low surfactant concentrations resembles the properties of the layers of synthetic polyelectrolytes: the real part of the dynamic surface elasticity is much higher than the imaginary part, and the elasticity modulus increases monotonously with the increase of surface age approaching high values (60−80 mN/m).[42,57,58] The kinetic dependencies of the surface elasticity become nonmonotonous upon further increase of the surfactant concentration and resemble in this case the corresponding results for solutions of nonionic flexible polymers[41] and nonglobular proteins.[59] The local maximum of the dynamic surface elasticity for the previously studied systems corresponds to the transition from a thin almost two-dimensional adsorption film to a thicker three-dimensional structure when the tails and loops of the macromolecules protrude into the bulk phase forming the distal region of the surface layer. In this case, the relaxation of surface stresses can proceed at the expense of the

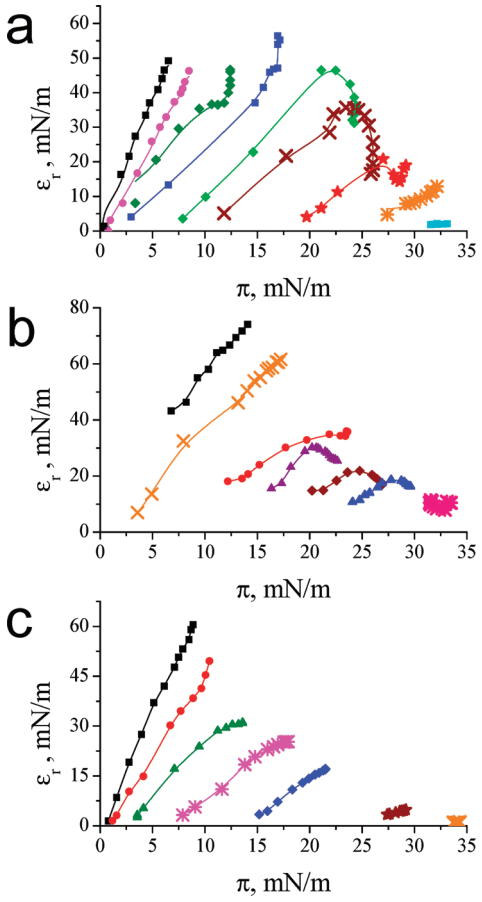


Figure 7. Surface elasticity of (a) BLG(0.0001 mM)/DTAB solutions at surfactant concentrations of 0 (black squares), 0.0002 (magenta circles), 0.006 (dark-green diamonds), 0.04 (blue squares), 0.4 (light-green diamonds), 0.8 (brown crosses), 4 (red stars), 8 (orange snowflakes), and 14.4 mM (cyan circles); (b) BLG(0.0001 mM)/SDS solutions at surfactant concentrations of 0 (black squares), 0.008 (red circles), 0.03 (blue triangles), 0.04 (dark-green diamonds), 0.07 (orange stars), 0.1 (brown crosses), and 0.2 mM (pink snowflakes) containing 0.4 M NaCl; and (c) BLG(0.0001 mM)/SDS solutions at surfactant concentrations of 0 (black squares), 0.01 (red circles), 0.1 (dark-green triangles), 0.3 (magenta snowflakes), 1 (blue diamonds), 3 (brown stars), and 10 mM (orange orange) versus surface pressure at a frequency of 0.1 Hz. Lines are guides for the eye.

segment exchange between the proximal and distal regions, and the surface elasticity begins to decrease after a local maximum.[60,61]

The nonmonotonous kinetic dependencies of the dynamic surface elasticity have been discovered recently for globular protein solutions containing a denaturant (G.HCl).[39,40] The tertiary and secondary structures of globular proteins are destroyed in concentrated G.HCl solutions. In this case, one can assume that the unfolded macromolecules become flexible chains that have to form tails and loops in the surface layer. The destruction of the protein globular structure has also been investigated recently by means of neutron and X-ray reflectivity in the surface layer of aqueous solutions of BLG,[11] lysozyme,[12] and ribonuclease A.[62] It was shown that the destruction of the BLG tertiary structure occurs in the surface layer at lower denaturant concentrations than in the bulk solution.

Although it is well-known that conventional ionic surfactants also possess a denaturing activity and the tertiary protein structure gets destroyed gradually under the action of surfactant molecules,[15−30] the secondary structure is preserved to a certain extent, even close to the CMC.[15,21−25] This means that one cannot apply the ideas developed for flexible polymers[41,60] to the molecules of globular proteins in the bulk phase of surfactant solutions. In the case of BSA solutions, the cationic surfactant binding leads at first to a separation of the domain III from the remaining part of the molecule and to the loosening of the bounds between its subdomains.[19] Finally, the unfolded and elongated BSA molecule consists of six relatively separated subdomains, which preserve their secondary structure to a significant extent. A globule unfolding occurs when the surfactant binding begins to follow the cooperation mechanism, thereby leading to the creation of new sites for binding. Different authors give close values of the molar ratios [surfactant]/[protein] corresponding to the destruction of the protein tertiary structure. The range is between 70 and 180 for BSA/DTAB solutions.[28,63] The changes of the tertiary structure of BLG/DTAB complexes in the bulk phase were discovered at [DTAB]/[BLG] values of 10−30 and 50−100,[23] whereas the corresponding conformational transition for the complexes between dodecyltrimethylammonium chloride (DTAC) and BLG was observed when [DTAC]/[BLG] > 150.[24] The BLG secondary structure does not change significantly in the course of interactions with the surfactants in the bulk phase. One can observe only an increase of the contribution of α-helices at the expense of the decrease of the β-sheet contribution at surfactant concentrations up to the CMC of pure surfactant.[22−25]

The transition to the nonmonotonous kinetic dependencies for both protein/cationic surfactant solutions under investigation in this work occurs in the range of [DTAB]/[protein] ratios from 3000 to 4000. These high ratios are due to the very low protein concentrations (much lower than the CMC) in this work as compared with those used in the studies of the bulk properties. (Figures 1b and 2b). The results of circular dichroism spectroscopy indicate the preservation of the main features of the protein secondary structure at surfactant concentrations up to the CMC[15,21−25] and, consequently, the limited flexibility of the protein molecules in the bulk phase. This was the reason why some authors[64,65] called into question the possibility of the protein molecules to wrap around surfactant micellar-like clusters, as it was assumed in the framework of the necklace model, which is frequently used to describe the surfactant cooperative binding by the proteins.[18,66] Therefore it seems improbable that the appearance of the local maximum of the dynamic surface elasticity is caused by the mere adsorption from the solution of relatively flexible protein molecules that are able to form loops and tails in the surface layer. One can assume when explaining the experimental results that the partial destruction of the secondary protein structure occurs in the surface layer. The Fourier transform infrared reflection absorption spectroscopy really shows that the secondary structure changes in the course of the globular protein adsorption at the liquid−gas interface, and the contributions of the unordered structure increases,[67] i.e., the interface has a denaturating influence on protein molecules. On the other hand, it has been found recently that the destruction of the secondary structure in the surface layer occurs at lower denaturant concentrations than in the bulk phase.[11,39,40] It is thereby probable that the partial destruction of the secondary structure in the surface layer results in an increase of the flexibility of protein molecules, a loop and tail formation, and consequently the appearance of a local maximum of the kinetic dependencies of

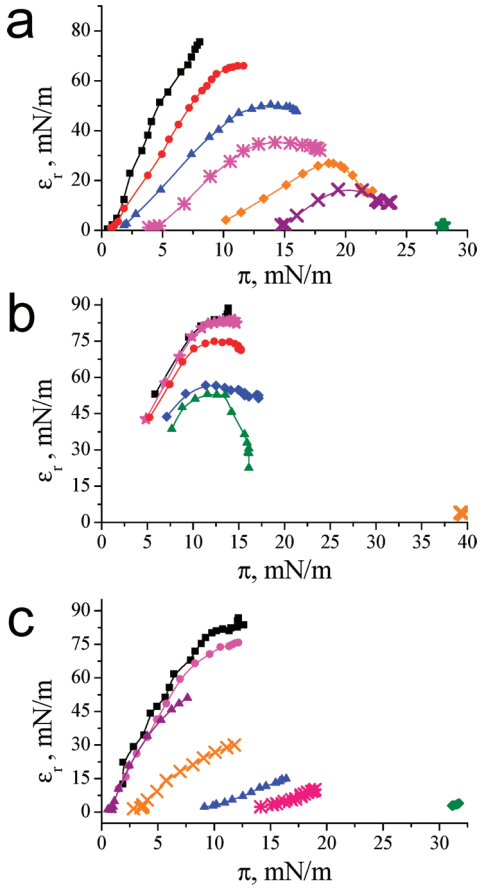


Figure 8. Surface elasticity of (a) BSA(0.03 μM)/DTAB solutions at surfactant concentrations of 0 (black squares), 0.01 (red circles), 0.05 (blue triangles), 0.3 (magenta snowflakes), 1 (orange diamonds), 3 (purple crosses), and 10 mM (dark-green stars; (b) BSA(0.0003 mM)/SDS solutions at surfactant concentrations of 0 (black squares), 0.0001 (magenta stars), 0.0003 (red circles), 0.001 (blue triangles), 0.01 (dark-green diamonds), and 0.5 mM (orange crosses) containing 0.4 M NaCl; and (c) BSA(0.0003 mM)/SDS solutions at surfactant concentrations of 0 (black squares), 0.002 (magenta circles), 0.01 (purple triangles), 0.05 (orange crosses), 0.5 (orange stars), 1 (blue triangles), and 3 mM (dark-green diamonds) versus surface pressure at a frequency of 0.1 Hz. Lines are guides for the eye.

the surface elasticity when the surfactant concentration is high enough and the [protein]/]DTAB] ratio exceeds about 3000 (Figures 1b and 2b). The molecular flexibility increases at any further surfactant concentration increase, and the peak in the kinetic dependencies of the dynamic surface elasticity becomes more pronounced. It is noteworthy that changes in the shape of the kinetic curves of the dynamic surface elasticity of protein/surfactant solutions under the influence of both substances (DTAB and G.HCl) proved to be rather similar. 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 decrease after the local maximum when the surface pressure reaches a critical value of about 19 mN/m for BLG solutions and about 14 mN/m for BSA solutions. These peculiarities of the investigated systems become especially evident if one considers the surface elasticity as a function of the surface pressure (Figures 7a and 8a, cf. also Figure 4 of ref 39 and Figure 8 of ref 40). The surface activity of the protein/surfactant

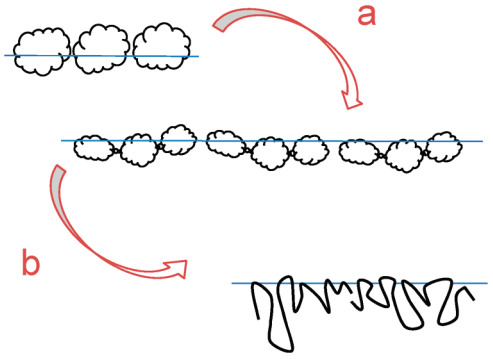


Figure 9. The scheme of protein unfolding at the air/solution interface for the solutions containing SDS and NaCl.

complex increases together with the adsorbed amount of the free surfactant molecules as the surfactant concentration increases further of, and the local maximum of the surface elasticity shifts to higher surface pressures. The dynamic surface elasticity values of 14 and 19 mN/m are probably characteristic for the investigated proteins and correspond to the onset of the loop and tail formation in the adsorption layer of unfolded BSA or BLG molecules (BSA/surfactant and BLG/surfactant complexes). In a similar manner, the value of 6 mN/m is characteristic for β-casein molecules and corresponds to the onset of the protrusion of N-terminals into the subphase as loops and tails.[59] The local maxima of the kinetic dependencies of the dynamic surface elasticity of protein/SDS/0.4 M NaCl solutions also correspond to surface pressures of 14 and 19 mN/m (Figures 7b and 8b).

Although one can also observe the transition from monotonous to nonmonotonous kinetic dependencies of the dynamic surface elasticity with increasing surfactant concentrations for protein/SDS/0.4 M NaCl solutions, this feature arises at lower surfactant concentrations than for protein/DTAB solutions. On the other hand, a significant drop of the surface elasticity precedes this transition in the former case but not in the latter one. The maximal value of the dynamic surface elasticity of BLG/DTAB solutions corresponding to the near equilibrium state does not decrease up to DTAB concentrations slightly less than 0.4 mM (Figure 7a), thereby indicating the preservation of the globular protein structure in the surface layer. The dynamic surface elasticity drops abruptly only at higher concentrations, and the kinetic dependencies prove to be nonmonotonous as a result of the increase of the macromolecular flexibility due to the destruction of both the tertiary and secondary protein structures. The dependencies of the dynamic surface elasticity on the surface pressure for protein/SDS/0.4 M NaCl solutions are close to the results for BSA/DTAB solutions (Figure 8a). In this case, all the dependencies remain monotonous at small DTAB concentrations, but the surface elasticity decreases appreciably in the whole range of surface pressure. A further increase of the SDS concentration results in the appearance of a local maximum, but the maximum surface elasticity does not exceed approximately 35 mN/m. It is possible to explain this behavior if one assumes that the destruction of the protein tertiary structure in the surface layer precedes the destruction of the secondary structure with increasing surfactant concentration. The main elements of the secondary structure are preserved during the first step of the adsorption film formation (Figure 9). In this case, the adsorption layer becomes looser as compared with the layer of adsorbed compact globules, and the dynamic surface elasticity decreases.

The destruction of the secondary protein structure begins at even higher surfactant concentrations. The macromolecules become more flexible and form loops and tails in the surface layer (Figure 9), thereby leading to the abrupt decrease of the surface elasticity and the appearance of the local maximum. The consecutive destruction of the tertiary and secondary structures was also observed in bulk phases of protein solutions containing G.HCl.[68] The hydrophobic interactions between the components are probably insufficient for protein/SDS solutions without inorganic salt to destroy the secondary structure and to increase the protein molecule flexibility. As a result, all experimental dependencies become monotonous (Figures 7c and 8c). The increase of the solution ionic strength due to the salt addition leads to stronger hydrophobic interactions in the system and shielding of the electrostatic interactions between charged amino acid residues. The latter effect can also contribute to the increase of the effective flexibility of the macromolecules.

Note that apart from the protein/surfactant complexes at sufficiently high surfactant concentrations, for example, higher than 0.1 mM for BSA/DTAB solutions, the adsorption layer also contains free surfactant monomers. This follows from the almost zero values of the dynamic surface elasticity during the initial adsorption step when the surface pressure deviates significantly from zero (Figure 8a). In this case, the adsorption layer can be microheterogeneous, and the relation between the kinetic dependencies of the dynamic surface elasticity and the protein structure can become more complex. However, the main changes of the experimental dependencies for solutions containing 0.4 M sodium chloride occur in the concentration range where the influence of free surfactant monomers is negligible.

The local maximum of the dynamic surface elasticity of BSA/SDS/0.4 M NaCl solutions arises already at SDS concentrations close to 0.0003 mM, which is almost two decimal orders of magnitude lower that the corresponding concentration for BLG/SDS/0.4 M NaCl solutions. This result agrees with the high stability of BLG/SDS complexes in the range of low surfactant concentrations[S1,S2] and with the higher stability of the BLG adsorption layers to mechanical perturbations as compared with the layers of BSA.[67] The dynamic surface elasticity decreases abruptly after the local maximum by almost 50% at an SDS concentration of 0.01 mM (Figure 6b). This peculiarity can indicate the higher extent of the secondary structure destruction relative to that for BLG/SDS/0.4 M NaCl solutions. Unfortunately, the measurements at higher concentrations are difficult because of the formation of large aggregates in the solution.

CONCLUSION

The kinetic dependencies of the dynamic surface elasticity of BLG/DTAB and BSA/DTAB solutions proved to be close to the corresponding results for mixed solutions of these proteins and G.HCl:[39,40] the dependencies became nonmonotonous if the denaturant (surfactant or G.HCl) concentration exceeded a certain critical value. This result indicates the destruction of both the tertiary structure and the secondary structure, at least partly, in the surface layer under the action of the cationic surfactant. In this case, the flexibility of the protein molecules increases, and they begin to form loops and tails in the surface layer. This triggers a new mechanism of the relaxation of surface stresses, and the dynamic surface elasticity decreases after a local maximum. For BLG/SDS and BSA/SDS solutions, the protein secondary structure is not destroyed to a sufficient extent, and therefore the kinetic dependencies of the dynamic surface elasticity remain monotonous in the whole investigated concentration range. The surface elasticity decreases with increasing surfactant concentration as a result of the unfolding of compact globules in the surface layer. The addition of sodium chloride to protein/SDS solutions leads to the strengthening of hydrophobic interactions between the components and the stronger destruction of the protein secondary structure. The kinetic dependencies of the dynamic surface elasticity also become nonmonotonous in this case.

AUTHOR INFORMATION

Corresponding Author

*Phone: +7(812)4284093; fax: +7(812)4286939; e-mail: borisanno@rambler.ru.

ACKNOWLEDGMENT

The authors are grateful to Mrs. A. Latnikova for measurements of the dynamic surface elasticity of some BLG/surfactant solutions. The work was financially supported by the St. Petersburg University (a research grant), the Russian Foundation of Basic Research (RFBR No. 11-03-00801_a), and the National Science Counsel of Taiwan (joint project RFBR No. 09-03-92002-HHC_a).

REFERENCES

(1) Maldonado-Valderrama, J.; Rodríguez, J. M. *Curr. Opin. Colloid Interface Sci.* **2010**, *15*, 271–282.
(2) Prakash, S.; Matouschek, A. *Trends Biochem. Sci.* **2004**, *29*, 593–600.
(3) Murray, B. S. *Curr. Opin. Colloid Interface Sci.* **2011**, *16*, 27–35.
(4) Buchner, J., Kiefhaber, T. *Protein Folding Handbook*; Eds.; Wiley-VCH: Weinheim, Germany, 2005, Vols. 1−5.
(5) Graham, D. E.; Phillips, M. C. *J. Colloid Interface Sci.* **1979**, *70*, 403–414.
(6) Graham, D. E.; Phillips, M. C. *J. Colloid Interface Sci.* **1980**, *76*, 227–239.
(7) Lu, J. R.; Su, T. J.; Thomas, R. K.; Penfold, J.; Webster, J. *Langmuir* **1999**, *15*, 6975–6983.
(8) Lu, J. R.; Su, T. J.; Thomas, R. K. *J. Colloid Interface Sci.* **1999**, *213*, 426–437.
(9) Lu, J. R.; Su, T. J.; Howlin, B. J. *J. Phys. Chem. B* **1999**, *103*, 5903–5909.
(10) Taylor, D. J. F.; Thomas, R. K.; Penfold, J. *Adv. Colloid Interface Sci.* **2007**, *132*, 69–110.
(11) Perriman, A. W.; Henderson, M. J.; Holt, S. A.; White, J. W. *J. Phys. Chem. B* **2007**, *111*, 13527–13537.
(12) Perriman, A. W.; Henderson, M. J.; Evenhuis, C. R.; McGillivray, D. J.; White, J. W. *J. Phys. Chem. B.* **2008**, *112*, 9532–9539.
(13) Postel, C.; Abillon, O.; Desbat, B. *J. Colloid Interface Sci.* **2003**, *266*, 74–81.
(14) Damodaran, S. *Anal. Bioanal. Chem.* **2003**, *376*, 182–188.
(15) Gelamo, E. L.; Tabak, M. *Spectrochim. Acta, Part A* **2000**, *56*, 2255–2271.
(16) Ding, Y.; Shu, Y.; Ge, L.; Guo, R. *Colloids Surf., A* **2007**, *298*, 163–169.
(17) Shweitzer, B.; Zanette, D.; Itri, R. *J. Colloid Interface Sci.* **2004**, *277*, 285–291.
(18) Turro, N. J.; Lei, X.; Ananthapadmanabhan, K. P.; Aronson, M. *Langmuir* **1995**, *11*, 2525–2533.
(19) Lee, C. T.; Smith, K. A.; Hatton, T. A. *Biochemistry* **2005**, *44*, 524–536.

(20) Vasilescu, M.; Angelescu, D.; Almgren, M.; Valstar, A. *Langmuir* **1999**, *15*, 2635–2643.
(21) Gull, N.; Chodankar, S.; Aswal, V. K.; Sen, P.; Khan, R. H.; Kabir-ud-Din *Colloids Surf., B* **2009**, *69*, 122–128.
(22) Taheri-Kafrani, A.; Bordbar, A.; Mousavi, S. H.-A.; Asgari-Mobarakeh, A.; Haertlé, T. *Agric. Food Chem.* **2008**, *56*, 7528–7534.
(23) Taheri-Kafrani, A.; Asgari-Mobarakeh, E.; Bordbar, A.; Haertlé, T. *Colloids Surf., B* **2010**, *75*, 268–274.
(24) Viseu, M. I.; Carvalho, T. I. *Biophys. J.* **2004**, *86*, 2392–2402.
(25) Viseu, M. I.; Melo, E. P.; Carvalho, I.; Correia, R. F. *Biophys. J.* **2007**, *93*, 3601–3612.
(26) Aswal, V.; Chodankar, S.; Kohlbrecher, J.; Vavrin, R.; Wagh, A. *Phys. Rev. E* **2009**, *80*, 011924.
(27) Santos, S. F.; Zanette, D.; Fischer, H.; Itri, R. *J. Colloid Interface Sci.* **2003**, *262*, 400–408.
(28) Valstar, A.; Almgren, M.; Brown, W.; Vasilescu, M. *Langmuir* **2000**, 922–927.
(29) Chen, A.; Wu, D.; Johnson, C. S. *J. Phys. Chem.* **1995**, *99*, 828–834.
(30) Wei, X.; Chang, Z.; Liu, H. *J. Surfactants Deterg.* **2003**, *6*, 107–112.
(31) Santra, M. K.; Banerjee, A.; Krishnakumar, S. S.; Rahaman, O.; Panda, O. *Eur. J. Biochem.* **2004**, *271*, 1789–1797.
(32) Wüstneck, R.; Krägel, J.; Miller, R.; Wilde, P. J.; Clark, D. C. *Colloids Surf., A* **1996**, *114*, 255–265.
(33) Krägel, J.; O'Neil, M.; Makievski, A. V.; Michel, M.; Leser, M.; Miller, R. *Colloids Surf., B* **2003**, *31*, 107–114.
(34) Pradines, V.; Fainerman, V. B.; Miller, R. *J. Phys. Chem. B* **2009**, *113*, 745–751.
(35) Gunning, P. A; Mackie, A. R.; Gunning, A. P.; Wilde, P. J.; Woodward, N. C.; Morris, V. J. *Food Hydrocolloids* **2004**, *18*, 509–515.
(36) Morris, V. J.; Gunning, P. A. *Soft Matter* **2008**, *4*, 943–951.
(37) Pradines, V.; Fainerman, V. B.; Aksenenko, E. V.; Krägel, J.; Wüstneck, R.; Miller, R. *Langmuir* **2011**, *27*, 965–971.
(38) Pereira, L. G. C.; Théodoly, O.; Blanch, H. W.; Radke, C. J. *Langmuir* **2003**, *19*, 2349–2356.
(39) Noskov, B. A; Grigoriev, D. O.; Latnikova, A. V.; Lin, S.-Y.; Loglio, G.; Miller, R. *J. Phys. Chem. B* **2009**, *113*, 13398–13404.
(40) Noskov, B. A.; Mikhailovskaya, A. A.; Lin, S.-Y.; Loglio, G.; Miller, R. *Langmuir* **2010**, *26*, 17225–17231.
(41) Noskov, B. A.; Akentiev, A. V.; Bilibin, A. Y.; Zorin, I. M. *Adv. Colloid Interface Sci.* **2003**, *104*, 245–271.
(42) Noskov, B. A. *Curr. Opin. Colloid Interface Sci.* **2010**, *15*, 229–236.
(43) Noskov, B. A.; Lin, S.-Y.; Loglio, G.; Rubio, R. G.; Miller, R. *Langmuir* **2006**, *22*, 2647–2652.
(44) Wong, D.; Camirand, W.; Pavlath, A.; Parris, N.; Friedman, M. *Crit. Rev. Food Sci. Nutr.* **1996**, *36*, 807–844.
(45) Peters, T. J. *Adv. Colloid Interface Sci.* **1985**, *37*, 161–245.
(46) Carter, D. C.; Ho, J. X. *Adv. Protein Chem.* **1994**, *45*, 153–203.
(47) Clark, D. C.; Husband, F.; Wilde, P.; Cornec, M.; Miller, R.; Krägel, J.; Wüstneck, R. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 1991–1996.
(48) Cornec, M.; Cho, D.; Narsimhan, G. *J. Colloid Interface Sci.* **1999**, *214*, 129–142.
(49) Tripp, B. C.; Magda, J. J.; Andrade, J. D. *J. Colloid Interface Sci.* **1995**, *173*, 16–27.
(50) Sawyer, L.; Kontopidis, G. *Biochim. Biophys. Acta* **2000**, *1482*, 136–148.
(51) Magdassi, S.; Vinetsky, Y.; Relkin, P. *Colloids Surf., B* **1996**, *6*, 353–362.
(52) Waninge, R.; Paulsson, M.; Nylander, T.; Ninham, B.; Sellers, P. *Int. Dairy J.* **1998**, *8*, 141–148.
(53) Bhattacharya, A. A.; Grüne, T.; Curry, S. *J. Mol. Biol.* **2000**, *303*, 721–732.
(54) Nielsen, A. D.; Borch, K.; Westh, P. *Biochim. Biophys. Acta* **2000**, *1479*, 321–331.
(55) De, S.; Girigoswami, A.; Das, S. *J. Colloid Interface Sci.* **2005**, *285*, 562–573.
(56) Lu, R.-C.; Cao, A.-N.; Lai, L.-H.; Xiao, J.-X. *J. Colloid Interface Sci.* **2006**, *299*, 617–625.
(57) Noskov, B. A.; Nuzhnov, S. N.; Loglio, G.; Miller, R. *Macromolecules* **2004**, *37*, 2519–26.
(58) Noskov, B. A.; Loglio, G.; Miller, R. *Adv. Colloid Interface Sci.* **2011**, DOI: 10.1016/j.cis.2011.02.010.
(59) Noskov, B. A.; Latnikova, A. V.; Lin, S.; Loglio, G.; Miller, R. *J. Phys. Chem. C* **2007**, *111*, 16895–16901.
(60) Noskov, B. A. *Colloid Polym. Sci.* **1995**, *273*, 263–270.
(61) Noskov, B. A.; Akentiev, A. V.; Loglio, G.; Miller, R. *J. Phys. Chem. B* **2000**, *104*, 7923–7931.
(62) Hüsecken, A. K.; Evers, F.; Czeslik, C.; Tolan, M. *Langmuir* **2010**, *26*, 13429–13435.
(63) Kelley, D.; McClements, D. J. *Food Hydrocolloids* **2003**, *17*, 73–85.
(64) Mir, M. A.; Gull, N.; Khan, J. M.; Khan, R. H.; Dar, A. A.; Rather, G. M. *J. Phys. Chem. B* **2010**, *114*, 3197–3204.
(65) Takeda, K.; Moriyama, Y. *J. Phys. Chem. B* **2007**, *111*, 1244.
(66) Chakraborty, A.; Seth, D.; Setua, P.; Sarkar, N. *J. Phys. Chem. B* **2006**, *110*, 16607–16617.
(67) Martin, A. H.; Cohen Stuart, M. A; Bos, M. A; van Vliet, T. *Langmuir* **2005**, *21*, 4083–4089.
(68) Galantini, L.; Leggio, C.; Pavel, N. V. *J. Phys. Chem. B* **2008**, *112*, 15460–15469.