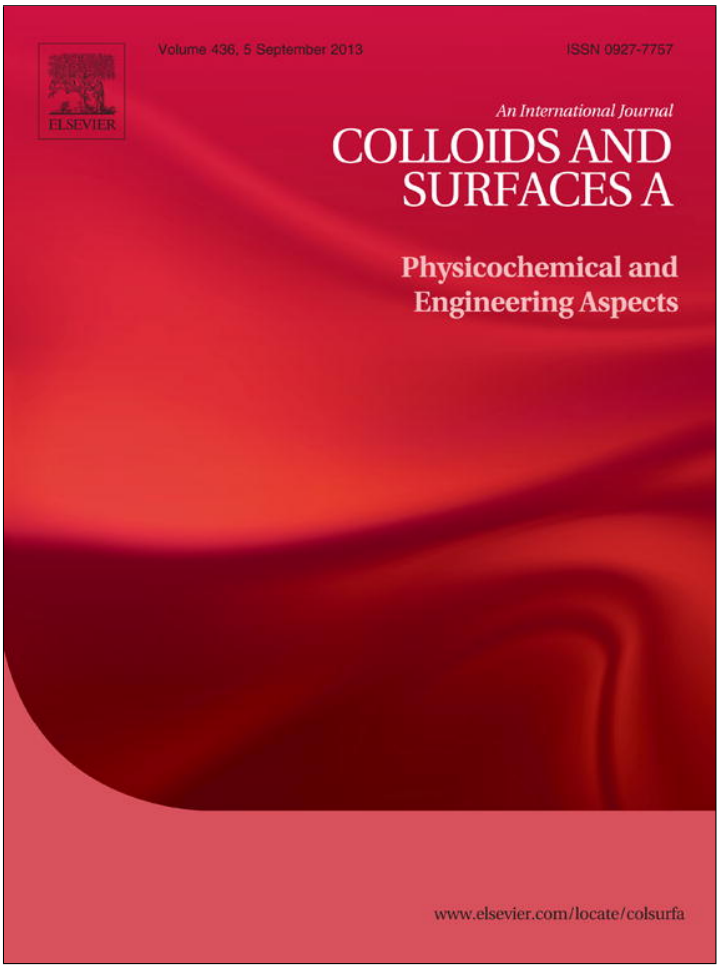

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Do low surfactants concentrations change lysozyme colloid properties?

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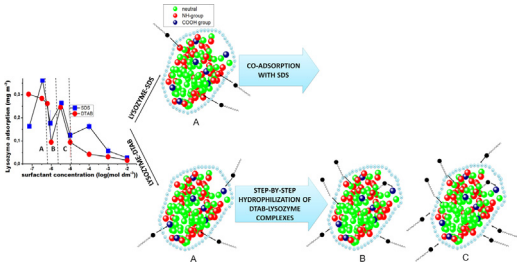
HIGHLIGHTS

- We study the adsorption of lysozyme mixed with SDS or DTAB at a PBS/octane interface.
- The surfactant concentration region lower than 5 μM is carefully described.
- The formation of the association between the protein and both SDS and DTAB occurs.
- Several hydrophobic states of associates occur at low surfactant concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

Multi-component aqueous/octane systems, which contain low-molecular-weight ionic surfactants (sodium dodecyl sulfate (SDS) or dodecyltrimethylammonium bromide (DTAB)) and lysozyme, were investigated using the scintillation phase method and the pendant drop technique. Experiments were carried out in the solutions of high ionic strength. Interfacial tension measurements reveal the appearance of local extremums for the SDS–lysozyme mixture that were attributed to changes in the nature of the SDS–lysozyme complex, which was confirmed by a radiochemical approach (determination of the distribution ratios and values of the surface excess of each component on the background of the other component). The formation of lysozyme–ionic surfactant complexes was observed for both cationic and anionic surfactants. Low surfactant concentrations of both SDS and DTAB result in the same changes of the colloidal behavior of protein. We proposed two possible mechanisms of interaction of lysozyme with cationic and anionic surfactants at low concentrations. The interactive process associated with the formation of lysozyme–ionic surfactant complexes by means of protein binding sites following by co-adsorption with SDS and by step-by-step hydrophilization and displacement from the adsorption layer by DTAB. The increase in the detergent concentration results in the usual substitution of protein from the adsorption layer that was observed by interfacial tension measurements and the radiochemical method.

1. Introduction

The importance of biopolymer–surfactants interactions is due to the relevance and widespread use of these interactions in technological applications. Mixing macromolecules and association colloids (surfactants or lipids) gives rise to rich polymorphic behaviors with the presence of molecular or micelle-like solutions, complexes, precipitates and gels [1–3].

The influence of a surfactant on protein colloidal behavior depends on both the structural stability of the protein and the molecular structure of the surfactant. The length of the hydrocarbon radical, the charge and shape of the polar part and even of the

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counterion affect the result of binding [4,5]. The principle difference in binding charged and neutral surfactants to a protein is that charged surfactants can latch onto the protein surface via strong electrostatic interactions and thus form a cluster of molecules that can induce structural changes, whereas zwitterionic surfactants must be preassembled in micelles [6].

A significant amount of experimental research and a large number of theoretical calculations exist concerning the surfactants-protein interactions at a surfactant concentration range close to the critical micelles concentration (CMC). There are several models that describe the structure of protein–sodium dodecyl sulfate (SDS) complexes (detergents that are widely used in polyacrylamide gel electrophoresis) at high SDS concentrations. The model of "rigid rod-like particles" proposed by Reynolds and Tanford [7] represents a protein–SDS complex as an ellipsoidal object, where the length of the minor axis remains constant at 1.8 nm and where the major axis length is proportional to the molecular weight of the protein. The "flexible helix model" [8] suggests that all of the SDS bond to a single protein form a single cylindrical micelle around which the hydrophilic parts of the polypeptide chain are helically located. Guo and Chen observed a polymer-like phase separation of the denatured protein solution [9]. In this model, the complex consists of a string of SDS micelles decorating the hydrophobic patches of the unfolded polypeptide chain of proteins. Neutron scattering and spectroscopic studies indicates that for complexes of SDS with some globular proteins, the structure is described by the "necklace and bead" model in which micelle-like clusters of SDS are distributed along the unfolded polypeptide backbones of proteins in the aqueous solution [10–12]. Recently, Chakraborty and Basak [13] used fluorescence and circular dichroism spectroscopy to observe that the caseins were found to adopt more ordered conformations in the presence of both anionic (SDS) and cationic (cetyltrimethylammonium bromide) surfactants. In the cited paper, the authors believe that the mechanism of protein–surfactants interactions occurs in two different types of the 'necklace and bead' model: (1) anionic surfactant molecules converge tail-first around the exposed hydrophobic surfaces of the caseins to form micelle-like structures; (2) the protein itself wraps around the micellar aggregates formed by cationic surfactant molecules that have cationic head groups in close association with the protein's negatively charged/polar residues.

The mechanism of the interactions of proteins with ionic surfactants at detergent concentrations ranging from several order of magnitude lower to at least order of magnitude higher than the CMC in the aqueous solutions can be summarized from published data [6,7,14–16] as follows: (1) at the initial region, binding to the specific sites of the protein without any significant changes in the structure is observed; (2) increasing the uptake of the surfactant is followed by changes in the tertiary structure; (3) at the CMC and above, the uptake of micelles by the protein results in the denaturation of the protein.

Narayan and co-authors studied structure of lysozyme–SDS complex by means of X-ray scattering (SAXS) [17]. It was shown that complexes formed by lysozyme and SDS micelles cannot be described by the necklace and bead model for unfolded polypeptide chin interspersed with micelles. Authors suggested that lysozyme being a small protein, can penetrate the micellar core which is occupied by flexible and disordered paraffin chains. Thus SXAS data were described by a partially embedded swollen model.

However, it is quite difficult to study the concentration range significantly below the CMC. In contrast to the surfactants in high concentration region, very low concentrations can stabilize proteins [18]. Mogensen and co-authors [19] observed that low concentrations of all types of detergents (nonionic, zwitterionic, cationic and anionic) enhance the activity of the *Thermomyces lanuginosus* lipase by more than an order of magnitude while at higher detergent concentrations, the activity declines, leveling off close to the value measured in the absence of detergent. Authors suggested that activation takes place at monomer level and occurs via a noninterfacial mechanism without the presence of a well-defined interface [20].

Another question is concerned with studies of the interaction of surfactants with proteins in a system of two immiscible liquids. Only few data have been published on the behavior of proteins at oil/water interface. The dilational rheology data for proteins at oil/water interface are reviewed if Ref. [21]. There are several processes that occur simultaneously. The most important and evident are the adsorption at the liquid/liquid interface and the distribution in the bulk of the system. Mozhaev et al. [22] have shown that even typical hydrophilic enzymes can be dissolved in a non-polar liquid alone but possess high activities in the liquid even at high temperatures. It must be emphasized that the activities of some enzymes are increased when the enzymes interact with an organic liquid at the interface [23].

To determine both the adsorption of the compound at an aqueous/organic liquid interface and the concentrations in each phase, a radiotracer method that used low-energy β-emitters shows great promise. In our previous studies, we have shown that liquid scintillation spectrometry of tritium in terms of the scintillation phase method allows measurements of the amount of a compound in a few μm subsurface layer in the aqueous phase of an aqueous/organic liquid system. The details of the experimental and the theoretical background of the method were described in Ref [24]. The nature of the organic phase determines an absolute value of the effect (the distribution ratio and the adsorption value), whereas the hydrophobicity and the surface activity as a function of the parameters mentioned above do not depend on the organic phase nature. In a previous study using this approach, we have found that the presence of ionic surfactants in the aqueous media, even at ultralow concentrations, can significantly influence the colloidal behavior of lysozyme in a water/p-xylene system [25].

The goal of this study was to reveal the influence of surfactant-protein binding on the surface behavior (interfacial tension and surface excess) of both proteins and ionic surfactants. To this end, lysozyme was mixed with SDS or dodecyltrimethylammonium bromide (DTAB) and was equilibrated with an octane phase. Properties of the surfactants are summarized in Table 1.

The system was analyzed by the pendant drop method for the interfacial tension at the liquid/liquid interface and by the scintillation phase technique to determine an exact amount of each component of the mixture in both phases and at the liquid/liquid interface.

2. Materials and methods

2.1. Materials

Lysozyme, isolated from egg whites, and sodium dodecyl sulfate (SDS) were purchased from MP Biomedicals. Dodecyltrimethylammonium bromide (DTAB) was obtained from Merk.

Adsorption experiments were performed in phosphate buffer saline (PBS) that was prepared using Na_2HPO_4 (0.008 M) and K_2HPO_4 (0.002 M). The ionic strength (finally 0.16 M) was adjusted by adding NaCl. To avoid bacterial growth, NaN_3 (0.004 M) was added to the buffer solution. The pH of the solution was adjusted to 7.2 ± 0.1 using NaOH or HCl solutions. The pH was measured by a HI 8314 portable pH meter (HANNA instruments, Romania).

n-Octane (99%) was produced by Panreac. All of these compounds were used as received. To prepare the scintillator based on n-octane, 4% of naphthalene and 0.4% of 2,5-diphenyloxazole were added to the organic liquid [24].

Table 1
The surface properties of surfactants.

Surfactant	Mw, g/mol	CMC		HLB	pC_{20}
		In water, mM	In 0.15 M NaCl, mM		
SDS	288	7.8 [26], 8 [27], 9 [28]	4.4 [27]	40 [29]	2.5 [30]
DTAB	308	14 [28,31]	4 [31]	24.25 [32]	2.97 [33]

2.2. Tritium labeling

The labeling procedure for lysozyme, SDS and DTAB is described in Ref. [34]. Briefly, the solution of the compound was equally distributed on the walls of a glass reaction flask. The solvents were removed by lyophilizing or by air flux. The flask with the solid target was connected to a special device for gaseous tritium, which contained a W-wire filament for hydrogen activation, and the flask was filled with tritium gas after evacuation. Tritium atoms were formed at a W-wire temperature of approximately 1900 K. To achieve high specific radioactivity of the labeled compound on a background of compound stabilization, the reaction with tritium atoms was performed at room temperature of the target for 10 s using special traps cooled with liquid nitrogen. The compound was then dissolved in a protonic solvent and purified from the labile tritium (7 days dialysis for lysozyme and drying/dissolving for SDS and DTAB [34]). After chromatographic purification, the specific radioactivities were 1.7, 0.16 and 0.16 TBq/mmol for lysozyme, SDS and DTAB, respectively. Previously developed purification procedures provide tritium label in the CH-bonds of the molecule and radiochemical purity was not less than 98%. However, it must be born in mind that an actual position of tritium in the molecule is indefinite.

2.3. The distribution ratio and the adsorption measurement (scintillation phase method)

Solutions of an aqueous phase were prepared in PBS. In the mixed Lz-surfactant solutions, the protein concentration was 0.01 g dm^{-3} ($7{\cdot}10^{-7}$ mol dm^{-3}). The surfactant concentration was varied from 10^{-8} to 10^{-3} mol dm^{-3}. Adsorption experiments were performed according to the procedure developed for protein–surfactants mixtures described in Ref [25]. In brief, 3 cm^3 of scintillation liquid based on octane was added to 1 cm^3 of a [^{3}H]lysozyme–surfactant or [^{3}H]surfactant-lysozyme solution in PBS. The two-phase systems were incubated at 25 °C for 48 h. The radioactivities of the entire two-phase system (I) and 1 cm^3 (V_1) aliquot of the organic phase (I_1) were subsequently measured using liquid scintillation spectrometer Rackbeta 1215. The concentration of the labeled compound in the octane phase (c_o) and its surface excess (Γ) were calculated as

$$c_o = \frac{I_1}{\varepsilon a_{sp} V_1} \quad (1)$$

$$\Gamma = \frac{I - (I_1 V/V_1)}{\frac{1}{2}\varepsilon a_{sp} S} = \frac{I_2 - (I_1 V_2/V_1)}{\frac{1}{2}\varepsilon a_{sp} S} \quad (2)$$

In this equations I_2 is a tritium β-radiation counting rate in the two-phase system after 1 cm^3 of octane separation, V is the volume of octane in the two-phase system, $V_2 = V - V_1$, ε is the registration efficiency of the tritium β-radiation registration in the bulk of the scintillator, a_{sp} is the specific radioactivity of the tritium-labeled compound, and S is an area of liquid/liquid interface.

The equilibrium concentration of the tritium-labeled compound in the aqueous phase (c_w) was measured similarly with the concentration in the bulk of the octane phase. The distribution ratio of the compound in the system of two immiscible liquids can be calculated as $D = c_o/c_w$ if the dependence of c_o vs. c_w is an affine function.

For free protein, SDS and DTAB the distribution ratios and the adsorption isotherms were obtained using the same procedure where an aqueous phase is a solution of tritium labeled lysozyme, SDS or DTAB alone.

2.4. The interfacial tension measurement

Surface tension experiments have been performed with a pendant drop method. A 1-ml volume of an aqueous solution of either a surfactant-lysozyme mixture or a free compound and 2 ml of octane were combined for 48 h (without shaking), ensuring that each component became mutually saturated. Next, a drop of an aqueous solution was formed over 30 min in the octane phase. After 30 min the value of the surface tension stops to vary. The drop was photographed with the help of a horizontal microscope equipped with a DCM-130 digital video camera. The value of the surface tension was determined from the shape of the pendant drops using the method of numerical integration of the Young-Laplace equation and approximation of the resulting droplet profile to experimentally obtained profile according to the procedure previously described [25]. The surface tension of the aqueous/octane interface was 50 mN m^{-1}, which correlates with the data published in literature [35,36]. For the lysozyme solution at concentration 0.01 g dm^{-3}, the interfacial tension at the aqueous/octane interface was 26 mN m^{-1}.

3. Results

We have previously shown that all of the tested compounds, lysozyme, SDS and DTAB, can penetrate into the octane phase [24,37]. Fig. 1 shows the dependence of the SDS (Fig. 1 a) and DTAB (Fig. 1b) concentration in the octane phase on its concentration in PBS for the free surfactant and in a mixture with 0.01 g dm^{-3} of lysozyme. The value of the concentration involves all possible forms of the surfactant, namely, free and bound with a protein.

It can be found for free SDS and DTAB (at concentration range below 1×10^{-6} mol dm^{-3} when both surfactants believed are in monomeric form) that the distribution ratio between PBS and octane is close to 3×10^{-3} and 6×10^{-4} respectively. At higher concentrations D value for SDS decreases to 1×10^{-3} and keep close to 6×10^{-4} for DTAB. D values changes with concentration growth can be explained by self-organization of the surfactant in the bulk of the system. Unlike the system with p-xylene, where the distribution ratio of DTAB at low bulk concentrations was higher than for SDS [25], here we observed that at low concentrations D for SDS is significantly higher than for DTAB.

Two primary points were observed. First, for both surfactants, the dependence of c_o vs. c_w is not linear in a wide concentration range. The reason for this nonlinearity is the formation of new states of the compound in the aqueous phase, such as micelles, that increase the number of degrees of freedom states where the compound is distributed. Thus, equilibrium should be considered between all possible states. Second, the addition of lysozyme does not influence the SDS distribution, whereas this addition critically increases the DTAB penetration into the organic phase at low detergent concentrations. For SDS, the values for the free surfactant and mixed with lysozyme were the same. In the case of DTAB, at the

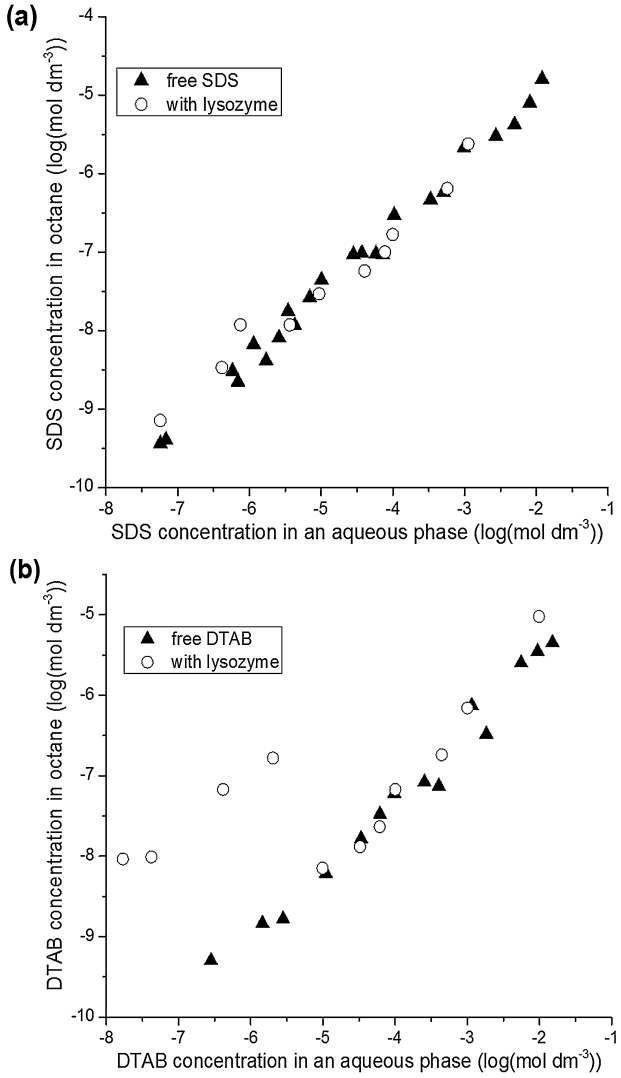

Fig. 1. The dependence of the surfactant concentration in octane on its concentration in an aqueous phase for the free (black symbols) compound and mixed with 0.01 g dm^{-3} of lysozyme (open symbols). (a) SDS, (b) DTAB. The standard deviation does not exceed 15%.

concentration ranges lower that 2×10^{-6} and at 1×10^{-4} mol dm^{-3}, we found an extremely high increase in the surfactant concentration in the organic phase. Changes in the angle of inclination of the dependence of c_o vs. c_w are concerned either with the new formations in the system (i.e., micelles) [38,39] or with surfactant transfer into the organic phase together with proteins. The last might occur at an ultralow concentration region. An experiment with labeled proteins can confirm a possible binding.

The addition of both detergents to lysozyme solutions significantly changes their distribution ratio between the water and octane phases. For the free lysozyme, D in the aqueous/octane system is $(3.3 \pm 0.2) \times 10^{-4}$ [24]. The changes of D of the lysozyme with the addition of the second component are presented in Fig. 2. As for the surfactants, the concentrations of lysozyme used for the D calculation involve all possible forms of protein: free and bound with surfactant.

A low surfactant concentration increases the distribution ratio of lysozyme up to 2 times. Both surfactants exhibit a reduction of lysozyme D at 1×10^{-6} mol dm^{-3} and D(lysozyme) growth up to 5×10^{-4} at a surfactant concentration of 5×10^{-6} mol dm^{-3} followed by a reduction with SDS (or DTAB) concentration growth. At a surfactant concentration of 1×10^{-5} mol dm^{-3}, the

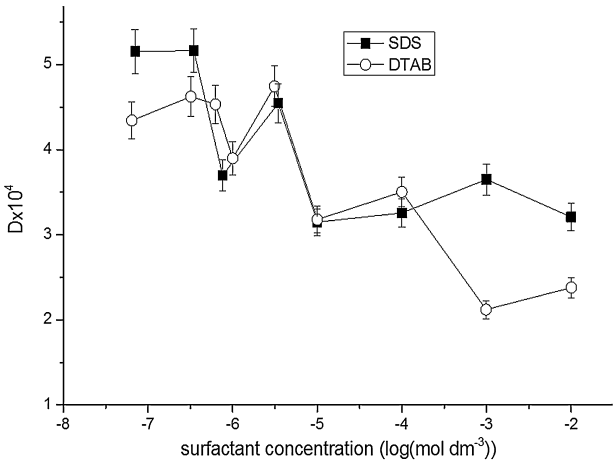

Fig. 2. The dependence of the lysozyme distribution ratio on SDS (squares) and DTAB (circles) concentrations.

distribution ratio reaches the value for free protein. A further increase of the SDS concentration does not change the D of lysozyme, whereas DTAB decreases this value by one and a half times. Because the distribution ratio is a characteristic of the hydrophobicity of the compound, we can conclude from the changes in the surfactants and the protein's D that in 0.16 M of PBS (pH 7.2 ± 0.1), lysozyme associates with both positively and negatively charged surfactants. At surfactant concentrations below 10^{-4} mol dm^{-3}, the hydrophobicities of the complexes formed with both SDS and DTAB are the same.

Fig. 3 presents the dependence of the lysozyme surface excess at an aqueous/octane interface on the surfactant concentration.

For a free protein, the value of adsorption is approximately 0.15 mg m^{-2}. The addition of both surfactants at concentrations of 3×10^{-7} mol dm^{-3} results in an adsorption growth of up to 2.5 times. As for the lysozyme distribution ratio, at surfactant concentration of 1×10^{-6} mol dm^{-3}, the adsorption of protein decreases. The surface concentration of protein then increases again when the surfactant concentration reaches 3×10^{-6} mol dm^{-3}. At surfactant concentrations of approximately 1×10^{-5} mol dm^{-3}, the adsorption decreases to values close to that of a free protein. A further increase in the surfactant concentration reduces the lysozyme adsorption by as much as 3 times.

Changes in the protein surface excess and the distribution ratio indicate variations in the protein's hydrophobicity and surface

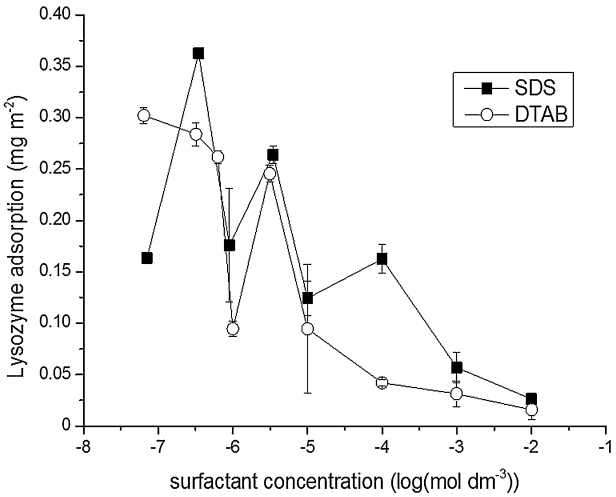

Fig. 3. Adsorption of lysozyme in the presence of SDS (circles) and DTAB (squares).

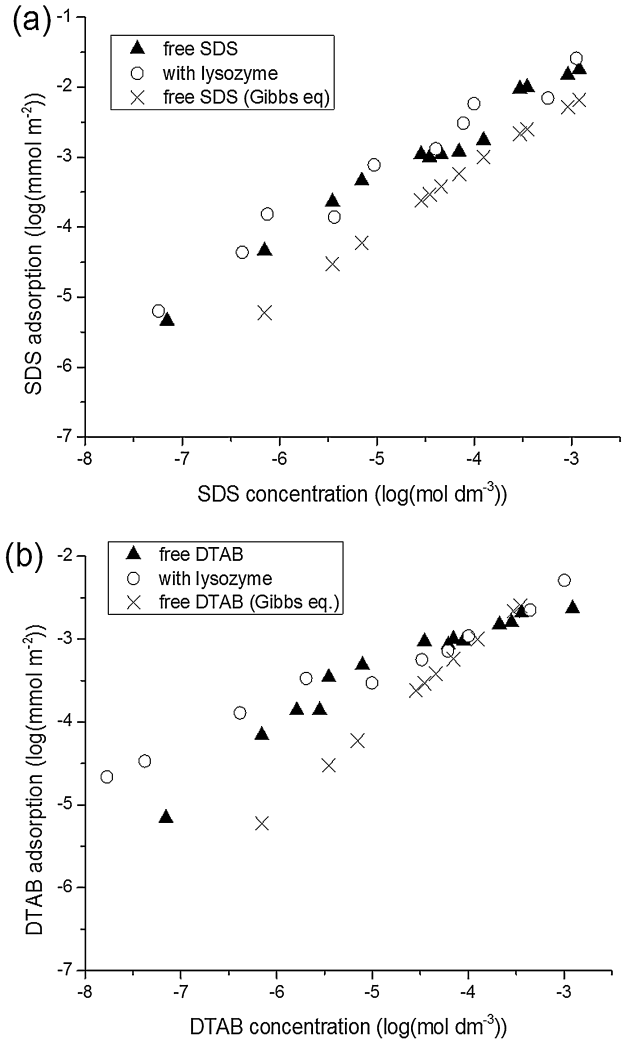

Fig. 4. Adsorption of free surfactants (black symbols) and in the mixture with lysozyme (open symbols) at the aqueous/octane interface: (a) SDS; (b) DTAB. The standard deviation does not exceed 20%. Adsorption of free surfactants calculated by Gibbs equation is presented by crosses.

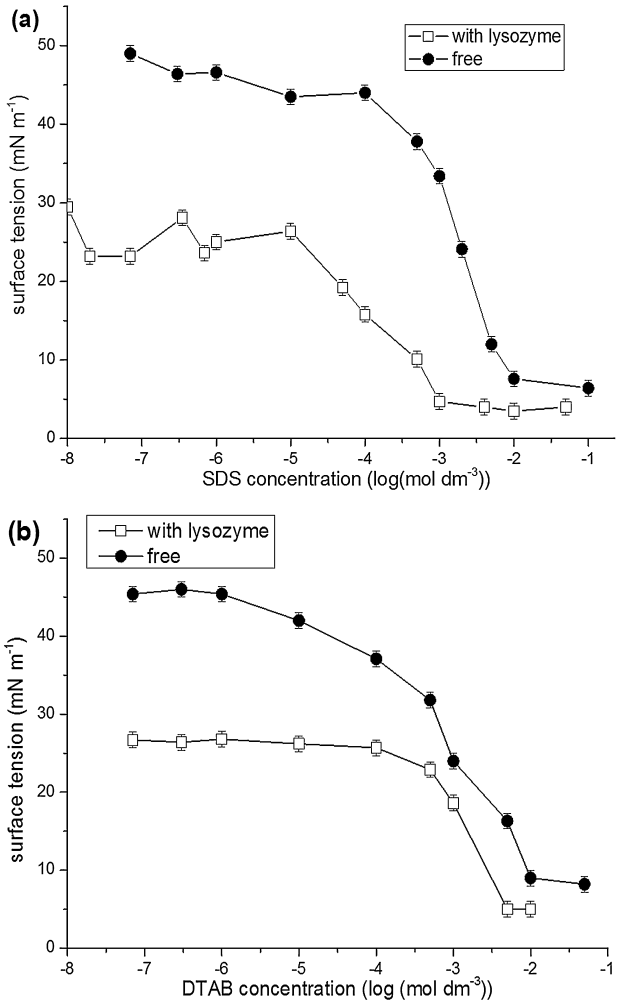

Fig. 5. The surface tension at the aqueous/octane for lysozyme–surfactant mixtures (open symbols) and free surfactants (black symbols): (a) SDS; (b) DTAB. For the free lysozyme, at the aqueous/octane interface, the surface tension at a concentration of 0.01 g dm^{-3} is 26 mN m^{-1}.

activity with the surfactant addition. These variations are indirectly confirmed by the formation of a lysozyme–surfactant composite. We can suppose that bindings with both surfactants at low concentrations of detergents increase the nonpolarity of the protein binding sites that promotes the protein's rise to the liquid/liquid interface [23].

The adsorption of free and mixed surfactants is presented in Fig. 4. The surface tension changes with the surfactant concentration are shown in Fig. 5.

For both free SDS and DTAB the values of adsorption obtained using scintillation phase method close or exceed one calculated from the surface tension (Fig. 5, free surfactants) according to the Gibbs equation. The result of the calculation is presented by crosses on Fig. 4. This is a usual observation, when the data obtained for surface active compounds with the help of radiotracer method and tensiometry are compared [40]. This observation is explained by the thickness of the registration zone of the subsurface region where tritium labeled compound can be detected and which is exceeds layer to which formation the interfacial tension is sensitive.

As the surfactant concentration in the octane phase (Fig. 1), the adsorption of SDS in the presence of protein was close to the free surfactant adsorption. The adsorption of DTAB in the presence of lysozyme exceeds the surface excess of the free surfactant at concentration ranges below 5×10^{-5} mol dm^{-3}. It must be born in mind that the amount of surfactant is not negligible in the region that we considered with both methods that is confirmed by the scintillation phase approach. So the dots presented at Fig. 5 linked with lines.

The addition of DTAB does not influence the interfacial tension at concentrations lower than 1×10^{-4} mol dm^{-3}. In this region, the interfacial tension is controlled by lysozyme (the value for a free protein is 26 mN m^{-1}). At high DTAB concentrations, the decrease in the surface tension is stronger than for free DTAB.

In the case of SDS, we observed several peaks at SDS concentrations lower than 5×10^{-5} mol dm^{-3}. The addition of 1×10^{-8} mol dm^{-3} of SDS to 0.01 g dm^{-3} of lysozyme results in a 15% increase of the interfacial tension. These data are in agreement with the trend observed previously for an aqueous/p-xylene interface [25]. The increase in SDS concentration of up to 1×10^{-7} mol dm^{-3} reduces the surface tension. We then observed another increase in surface tension at intermediate SDS concentrations. The same result was observed by Lad et al. [41] and Green et al. [42] for an aqueous/air surface. Our data show that further increases in the SDS concentration lead the reduction of surface tension but achieve values of the free surfactant at SDS concentrations that are an order of magnitude lower.

4. Discussion

The key processes that occur in the systems of two immiscible liquids are the adsorption of the compound in the interface and mass transfer through the layer. The combination of the radiochemical method with the pendant drop technique followed five parameters in the system of a lysozyme–ionic surfactant aqueous solution/octane, namely, the interfacial tension at the liquid/liquid interface, the distribution ratio of each component in the mixture and each component's adsorption at the liquid/liquid interface on the background of the other component. The primary feature of these results is the appearance of local extremums in the dependences of the protein distribution ratio, the protein's adsorption and the surface tension of the mixtures on the surfactant concentration in the aqueous phase. Each of these extremums is attributed to the changing nature of the protein–surfactant complex [42]. The principle mechanism of interaction of a protein–ionic surfactant of an opposite charge is completely described for an aqueous/air interface [41,43–47]. Here, we can compare this mechanism with one in presence of organic phase.

Let us first emphasize that all experiments described in this work were conducted in phosphate buffer saline (pH 7.2 ± 0.1) with an ionic strength of 0.16 M. Lysozyme has an isoelectric point at pH 11, and thus at pH 7.2 ± 0.1, it has a net positive charge of approximately 8 [41,47,48]. However, in presence of salts protein net charge can vary. At pH below the isoelectric point chloride ions binding is rather strong and results in increase of protein net charge [49]. Steri et al. showed that at pH 7 at low ionic strength (0.01 M phosphate buffer) lysozyme has a positive electrophoretic mobility (0.15×10^{-8} m^2 V^{-1} s^{-1}), while the increase of ionic strength to 0.15 M NaCl results in a negative electrophoretic mobility about -0.2×10^{-8} m^2 V^{-1} s^{-1} [50]. Our measurements of the electrophoretic mobility of lysozyme solution in the buffer and at concentration used in this study by means of a Zetasizer nano series (Malvern Instruments) also indicate the electrophoretic mobility of -0.2×10^{-8} m^2 V^{-1} s^{-1}, but the data obtained here were of poor quality because of low protein concentration. However, possible negative net charge of protein should be taken into account for the data explanation. Low protein concentration is also a reason why UV-spectrometry and dynamic light scattering failed for our studies.

To explain the data obtained using scintillation phase method and tensiometry lets consider the distributions of the COOH- and NH-group-contained amino acid residues in the lysozyme molecule that schematically shown in Fig. 6. For this scheme we used only coordinates of the amino acids residues from protein data bank (PDB code: 2LYZ).

4.1. Lysozyme–SDS system

At low surfactant concentrations, the protein and surfactant form complexes by means of electrostatic interactions between positively patches of protein and SDS. For an aqueous/octane interface, we observed an increase of both the distribution ratio and the adsorption of the protein at SDS concentrations lower than 10^{-6} mol dm^{-3}. The decrease of the interfacial tension in this concentration range also indicates the hydrophobization of the globule. The maximum in the lysozyme adsorption on a background of high value of the lysozyme's distribution ratio is observed at an SDS concentration of 3.5×10^{-7} mol dm^{-3} (the SDS/protein molar ratio in the bulk of an aqueous phase is 0.5). The SDS/protein molar ratio at the interface in this point is 1.7. However, the dependence of the interfacial tension vs. the SDS concentration shows an increase from 23 to 28 mN m^{-1} (the interfacial tension for a free protein at 0.01 g dm^{-3} is 26 mN m^{-1}). This increase could be due to two effects, the first effect is the formation of a lysozyme–SDS

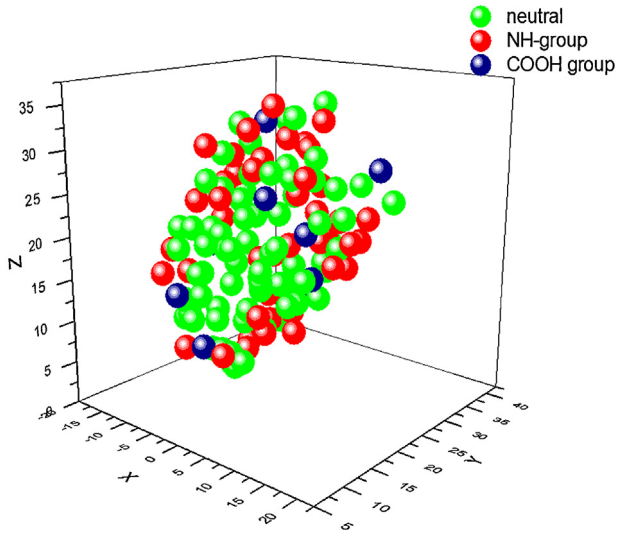

Fig. 6. Scheme of the COOH- and NH-group-contained amino acid residue distributions in the lysozyme molecule [51].

complex with a compact conformation that is possible for low SDS concentrations [52]. Such complexes are rather hydrophobic to adsorb at the interface and penetrate the bulk of the organic phase but decrease the interfacial tension, similar to a free protein [53]. Another possible situation is the structural changes of the SDS–lysozyme complex at the aqueous/octane interface. de Aguiar [54] shows that at a droplet water/alkane interface, the alkane molecules are parallel oriented and the SDS alkyl tail can lie parallel on the interface. Thus, we can suppose that at an aqueous/octane interface, the alkyl tail can interact with the hydrophobic protein residues. The initial hydrophobic interaction results in the increase of the interfacial tension. At an SDS concentration of 9×10^{-7} mol dm^{-3} (the SDS/protein molar ratio in the bulk of an aqueous phase is 1.28), the SDS/protein molar ratio at the interface is 12. The interfacial tension, the protein distribution ratio and the adsorption decrease to values close to one for a free protein, which means that a lysozyme–SDS complex at the interface is still charged. It has to be emphasized, that for p-xylene interface in this concentration range we observed that interfacial tension and the distribution ratio were higher than for free protein, but protein's adsorption was lower than in the case of free lysozyme [25]. In this concentration range the adsorption of free SDS at the aqueous/p-xylene and aqueous/octane interfaces are close to each other [40], while the adsorption of free lysozyme at the aqueous/p-xylene interface is higher than one at the aqueous/octane interface [24]. Presence of the protein does not influence SDS adsorption. In this concentration range for both interfaces we observed co-adsorption of lysozyme and lysozyme–SDS complex, that control the interfacial tension. An increase in the surfactant concentration of up to 1×10^{-5} mol dm^{-3} results in a decrease of the lysozyme adsorption at the interface and the lysozyme's distribution ratio on the background of the interfacial tension growth. At this SDS concentration, the protein–surfactant complex is less soluble than a free protein, and the concentration of free SDS is too low to decrease the interfacial tension. The same situation is described for an aqueous/air interface [41,42]. A further increase in the SDS concentration results in the usual reduction of the interfacial tension with the decrease of the lysozyme concentration in the subsurface layer and a constant distribution ratio that is close to that of a free protein. As for an aqueous/air interface [43] at a SDS concentration of 1×10^{-4} mol dm^{-3}, the increase in the protein surface excess does not lead to significant changes of the interfacial tension of the mixture. However, at a SDS concentration of 10^{-3} mol dm^{-3}, the

SDS is situated in the adsorption layer in a free form and bound to a protein.

In summary, electrostatic interactions between positive charged sites of protein and the surfactants at low SDS concentrations (lower than 1×10^{-6} mol dm^{-3}) result in the hydrophobization of the protein. An increase in the SDS concentration starts hydrophobic interactions. However, at an SDS amount of approximately 3×10^{-6} mol dm^{-3}, we observed the formation of a very hydrophobic composition that was analogous with that observed at the aqueous/air surface at a detergent concentration of approximately 1×10^{-4} mol dm^{-3}. A further increase in the SDS concentration results in the hydrophilization of the protein and decreases its surface amount first to the protein level and then lower.

4.2. Lysozyme–DTAB system

At a pH of 7.2 and ionic strength of 0.16 M, lysozyme possess a negative electrophoretic mobility because of chloride ions binding the initially positive charged protein [50]. As a consequence, not only anionic aspartate and glutamate side chains are available for binding with the surfactant. Thus, under the experiment conditions, the system lysozyme–DTAB can be considered as a system contained protein and opposite charged ionic surfactant. In general water-soluble proteins show lower affinity to for cationic surfactants and the cooperative binding step starts at higher concentrations of cationic relative to anionic surfactants [55]. However, at low surfactants concentrations we observed the same tendencies for lysozyme adsorption at the aqueous/octane interface (see Fig. 3). Also the similar trends for cationic and anionic surfactants were observed for the aqueous/p-xylene interface [25]. The increase in D of both the protein and the surfactant at low DTAB concentrations indicates the formation of hydrophobic complexes between the protein and the DTAB. A hydrophobic complex can also form by means of interaction of DTAB with negatively charged amino acids residues (region where DTAB concentration is lower than 1×10^{-6} mol dm^{-3}). The reduction of the lysozyme D and its adsorption at a DTAB concentration of 1×10^{-6} mol dm^{-3} most likely results from the hydrophilization of the protein surface by the surfactant molecules. The lysozyme adsorption at this point decreases from 0.25 to 0.1 mg m^{-2}. Previously we observed hydrophilization of lysozyme–DTAB complex at the same concentration range in the aqueous/p-xylene system [25]. However in the system with p-xylene adsorption of mixed lysozyme was lower than for free protein in the whole region of DTAB concentrations from 7×10^{-8} to 7×10^{-6} mol dm^{-3}. Such difference maybe because of difference of HLB of organic phases. Such protein–DTAB behavior does not strongly influence the interfacial tension, which is approximately 26 mN m^{-1}. However, the surface excess of DTAB is identical with that of the free surfactant, and there are 32 DTAB molecules per lysozyme in the subsurface layer. Because the interfacial tension in this point is still controlled by the protein, our data suggest that DTAB is bound to the protein and free DTAB probably does not co-adsorb at the liquid/liquid interface. The interaction of the hydrophobic parts of molecules causes the hydrophilization of the protein, resulting in the adsorption decrease. A further increase in the DTAB concentration of up to 3×10^{-6} mol dm^{-3} leads to growth of both the lysozyme surface excess and its distribution ratio. The increase in the DTAB concentration reduces both the distribution ratio of the protein and its adsorption. This phenomenon apparently results from hydrophobic interaction between DTAB and lysozyme, making the complex step by step more hydrophilic and, as a result, reducing its surface-activity. At a DTAB concentration of approximately 10^{-3} mol dm^{-3}, the DTAB is situated in the adsorption layer in the free form and bound to the protein.

Thus, at ultra-low DTAB concentrations we observed the hydrophobization of the protein that increases its surface activity. The increase of DTAB concentration corresponded to the hydrophilization of the protein surface because of hydrophobic interactions between the protein and the detergent. Such attraction most likely results in some softening in the protein structure. Unfortunately, the concentration of protein is too low to determine such changes by fluorescent spectrometry or by CD but leads to an increase in the availability of negatively charged amino acids residues to interact with surfactants by electrostatic attraction or to the formation of Van der Waals bonds between amino acids residues and detergents had-groups. Thus, we have found that both positively and negatively charged surfactants at low concentrations similarly change the colloidal behavior of lysozyme in an aqueous/octane system but that the mechanism of interactions might be different.

5. Conclusions

The competitive behavior of lysozyme with two ionic surfactants (SDS and DTAB) in water/octane systems was studied with the help of radiotracer and pendant drop techniques. The adsorption and distribution ratio of lysozyme in the mixture at detergents concentration lower than 10^{-5} mol dm^{-3} were the same for both SDS and DTAB, which indicates that the hydrophobicity and the surface activity of the complexes formed by protein and both detergents are the same despite of different mechanism of the formation. At higher surfactants concentrations, the free surfactant substitutes a protein–surfactant complex from the liquid/liquid interface. For a lysozyme–DTAB system at detergent concentration lower than 1×10^{-6} mol dm^{-3}, a hydrophobic complex with lysozyme is formed by interaction with the negatively charged amino acids residues of protein. Such interactions do not change the interfacial tension at an aqueous/octane interface. A further increase in the DTAB concentration results in the hydrophilization of the protein globule attended by both the distribution ratio and an adsorption decrease. In the case of SDS, the mechanism of the interaction with lysozyme at an aqueous/octane system is close to one described for a water/air surface. However, at low SDS concentrations, we observed several states distinguished by hydrophobicity and surface activity. The formation of a complex with less solubility than an unbound protein in the system with octane is observed at a SDS concentration of 10^{-5} mol dm^{-3}, which is one order of magnitude lower than for water/air surface. However, in this concentration range, the amount of SDS is too low to reduce the interfacial tension. At a high surfactant concentration, the SDS substitutes protein from the liquid/liquid interface and controls the interfacial tension.

The main features of this study are that at low ionic surfactants concentrations and high ionic strength the effect of both cationic and anionic surfactant to lysozyme is looks the same, but binding occurs by different sites. The mechanisms involved either co-adsorption of two different species or the formation of complexes with the surfactants. Our data suggests that at low surfactants concentrations the interactive process associated with the formation of lysozyme–ionic surfactant complexes by means of protein binding sites following by co-adsorption with SDS or by step-by-step hydrophilization and displacement from the adsorption layer by DTAB.

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