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Mixed Monolayers of Bovine Serum Albumin with a Nonionic Surfactant (Tween 80)

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Abstract—Mixed monolayers of bovine serum albumin (BSA) with a nonionic surfactant (Tween 80) are obtained by spreading solutions containing both components over the surface of a subphase (water with pH 6) over a wide range of solution compositions. According to compression–expansion isotherms, the mixed monolayers are of the condensed type when the BSA concentrations in the solution are far higher than or equal to the surfactant concentration. Such monolayers mostly consist of BSA–Tween 80 (1 : 1) complexes. In contrast, a BSA monolayer is of the expanded type. When Tween 80 in the solution prevails over Tween 80, the monolayers become unstable. The results of this work pertain to the monitoring of the properties of protein–surfactant mixtures and design of Langmuir–Blodgett (LB) films.

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The use of dispersions (in the food industry, detergents, biotechnologies, cosmetics, pharmacology, and medicine) creates many problems for researchers. One problem is the destabilization of a dispersion because of the incompatibility of the stabilization mechanisms of its components. An approach to this problem requires systematic investigations of the properties of mixtures and their compositions in an aqueous phase and at the surface, as well as detailed investigations of thin films (dispersion models) stabilized by such complexes.

To draw adequate conclusions, it is pertinent to use proteins whose surface properties are well known. In choosing nonionic surfactants, one should be guided by the following requirements: (1) a high probability of the compatibility of the conditions for the dispersion stabilization by mixtures of surfactants and proteins, (2) the nonexistence of data about a negative effect of the surfactant on protein conformation, and (3) the nontoxicity of the surfactant (this is especially important for the food industry, pharmacology, and medicine).

Proteins are natural macromolecular nanoparticles having a surface activity. With low-molecular-weight surfactants, proteins form associates of variable composition with different surface activities. Proper attention has not been paid to this fact. The properties of mixtures are not additive; thus, reliable control over the behavior of systems containing mixtures of a surfactant and protein is impossible.

Investigations of mixed surfactant–protein monolayers are only part of systematic investigations in this field. However, these investigations have their own value for design of biosensors based on Langmuir–Blodgett (LB) films and others.

SUBJECTS AND METHODS

We used lyophilized bovine serum albumin from Sigma and nonionic surfactant polyoxyethylene-20sorbitan monooleate (Tween 80) with the following parameters: reagent grade; molecular weight, 1308 g/mol; critical micelle concentration (CMC), 1.5×10^{-4} mol/l; hydrophilic–lyophilic balance, 15.

The protein concentration in solutions was determined spectrophotometrically at a wavelength of 280 nm. All solutions were prepared with twice distilled water (pH 6.0). Mixtures of the components were prepared by the titration of a BSA solution (with a fixed concentration of 8.5×10^{-5} mol/l) with a Tween 80 solution (1.3×10^{-3} mol/l) so that the Tween 80 concentration in the solution varied from 1.4×10^{-7} to 8.5×10^{-2} mol/l.

Compression isotherms (biaxial pressure–area diagrams) were measured with a circular Langmuir balance; the biaxial pressure was recorded by the Wilhelmy method. A 20 μl aliquot of the solution of a component or a mixed solution of various compositions was applied to the surface of a bath (the subphase was water with pH 6.0) and allowed to spread over the water–air interface for 2 min; then, the monolayer was compressed at a fixed rate of 0.01309 m^2/min. All measurements were carried out at 21°C.

RESULTS AND DISCUSSION

Tween 80 is a water-soluble nonionic surfactant. When applied to a water–air interface, it does not form stable monolayers: because of the uncontrolled transfer of Tween 80 molecules to the subphase, the recorded biaxial pressure (π, mN/m) does not correspond to rea-

sonable surface areas per Tween molecule. From surface tension measurements [1], it is known that the surface area per Tween molecule in the ultimately saturated adsorbate layer is 0.65 nm^2. At the same time, when more than 1×10^{16} molecules are applied to the bath surface, the ultimate biaxial pressure is 37–38 mN/m. This value approaches the maximal decrease in the surface tension of Tween 80 solutions upon the formation of a saturated absorbate layer.

The compression isotherm for a BSA monolayer on the water surface (pH 6.0) displayed in Fig. 1 is an S-shape curve typical of protein monolayers, in agreement with the related literature. For this protein, we know how the compression isotherm depends on the pH of the subphase [2], and its collapse pressure is 20 mN/m. Extrapolation of the linear portion of the isotherm to $\pi = 0$ gives a value of 110 nm^2 for the area per protein molecule; this value is substantially larger than the area occupied by a BSA molecule in a saturated surface layer. The equivalent sphere radius of a protein molecule R (nm) can be determined from

$$R = 0.067\sqrt[3]{M}, \quad (1)$$

where M is the protein molecular weight, equal to 67000 g/mol for BSA [3].

The radius of a BSA molecule (R_{BSA}) is 2.7 nm; the surface area of the projection of a protein molecule on the surface in an ultimately filled monolayer is 23 nm^2. Thus, a BSA monolayer is of the expanded type; probably, it has a cage or cluster structure, matching the models of [4].

Let us proceed with the properties of mixed BSA–Tween 80 monolayers. The compositions of the mixtures applied to the subphase surface are listed in Table 1. The compositions of the solutions spread over the subphase surface are listed in Table 2 (the solutions are numbered in accordance with the numbering used in Table 1). The monolayer compression isotherms for the individual components and their mixtures of various compositions are displayed as the π–A diagrams in Fig. 2. The curves are numbered to match the numbering of the systems in Tables 1 and 2. From Fig. 2, it follows that the positions and trends of the isotherms are strongly affected by the component ratio (see Table 1): the isotherms are displaced toward smaller areas compared to the BSA monolayer isotherm (Fig. 2, curve *8*); they also differ from the compression isotherm for the Tween 80 surface layer (Fig. 2, curve *9*). We should stress that the monolayer compression isotherms (Fig. 2, curves *1–4*) are well reproduced and show reversible hysteresis in compression–expansion cycling.

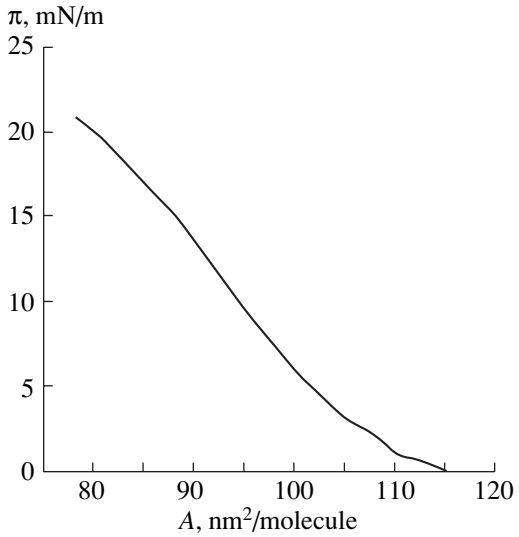

Fig. 1. Compression isotherm for a BSA monolayer (subphase, distilled water; pH 6.0; $t = 21$°C).

Figure 3 displays the same monolayer compression isotherms as in Fig. 2 (solid curves *1–4*), but in the π (mN/m)–A (area per BSA molecule, nm^2) space. Extrapolating the initial linear portions of the isotherms

Table 1. Substance amounts of BSA, Tween 80, and their mixtures applied to the subphase surface to form a surface layer

Solution no.	Applied compound				BSA/Tween 80 ratio
	BSA		Tween 80		
	moles	molecules	moles	molecules	
1	1.71×10^{-9}	1.029×10^{15}	2.85×10^{-12}	1.71×10^{12}	600
2	1.71×10^{-9}	1.029×10^{15}	1.71×10^{-11}	1.029×10^{13}	100
3	1.71×10^{-9}	1.029×10^{15}	1.71×10^{-10}	1.029×10^{14}	10
4	1.71×10^{-9}	1.029×10^{15}	1.71×10^{-9}	1.029×10^{15}	1
5	1.71×10^{-9}	1.029×10^{15}	1.71×10^{-8}	1.029×10^{16}	0.1
6	1.71×10^{-9}	1.029×10^{15}	1.71×10^{-7}	1.029×10^{17}	0.01
7	1.71×10^{-9}	1.029×10^{15}	1.71×10^{-6}	1.029×10^{18}	0.001
8	1.71×10^{-9}	1.029×10^{15}	–	–	–
9	–	–	2.04×10^{-8}	1.22×10^{16}	–

Table 2. Compositions of solutions applied the bath surface

Solution no.	c_{BSA}, mol/l	$c_{Tween\ 80}$, mol/l
1	8.5×10^{-5}	1.4×10^{-7}
2	8.5×10^{-5}	8.5×10^{-7}
3	8.5×10^{-5}	8.5×10^{-6}
4	8.5×10^{-5}	8.5×10^{-5}
5	8.5×10^{-5}	8.5×10^{-4}
6	8.5×10^{-5}	8.5×10^{-3}
7	8.5×10^{-5}	8.5×10^{-2}
8	8.5×10^{-5}	–
9	–	1×10^{-3}

to $\pi = 0$, for the areas per molecule (isotherms *2–4* in Fig. 3) we obtain a value close to the surface per BSA molecule in the ultimately filled monolayer (23–28 nm^2/molecule). This result implies that when BSA prevails over Tween 80, the monolayers consist of the protein or the protein complex with Tween 80 in which the tertiary structure of the protein does not significantly change. To understand more details of the composition of the monolayer, let us consider recent investigations of the interaction between BSA and Tween 80.

It was demonstrated in [5] that the phase behavior of mixtures is dictated by the component ratio. When Tween 80 prevalence is tenfold (mol/mol), the systems are separated into macroscopic phases. For lysozyme mixtures with sodium dodecylsulfate (an ionic surfactant), it was shown in [6] that the protein precipitates when binding eight to ten surfactant molecules; the dissolution occurs with a progress in the surfactant concentration and protein binding within a very narrow range of these parameters.

Recently [7], the properties of mixed solutions of BSA and Tween 80 were comprehensively studied using a set of methods (surface tension measurements, UV and IR spectroscopy, fluorescence quenching, and others). The main results of these investigations that directly pertain to the interpretation of our biaxial pressure isotherms are as follows. When the protein prevails over Tween 80 in the solution, the components form a 1 : 1 complex, whose solubility is 10^{-5} mol/l. Complex formation induces the hydrophobization of BSA molecules; as a result, the surface activity of the 1 : 1 complex becomes five times the surface activity of BSA and two times the surface activity of Tween 80. With the prevalence of Tween 80, particles of variable composition are formed; the average binding of Tween 80 to the protein is about ten Tween 80 molecules per protein molecule.

Thus, curves *1–4* in Fig. 3 can be interpreted in the context of the formation of the BSA–Tween 80 complexes with the composition 1 : 1 (mol/mol), whose solubility is about 1×10^{-5} mol/l. The interpretation of curves *5–7* in Fig. 2 is now difficult.

The formation of the 1 : 1 complex can be written as

$$P + S = PS. \quad (2)$$

Here, P and S, respectively, are the protein and nonionic surfactant in equilibrium with the PS complex.

Inasmuch as the solubility of the complex is far lower than the solubilities of the individual compo-

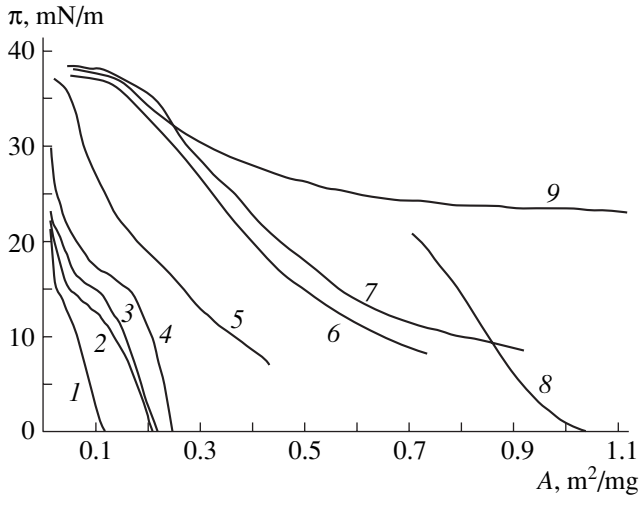

Fig. 2. Compression isotherms for BSA and Tween 80 mixed monolayers at various BSA/Tween 80 ratios (mol/mol) in the spread solution: (*1*) 600, (*2*) 100, (*3*) 10, (*4*) 1, (*5*) 0.1, (*6*) 0.01, (*7*) 0.001, (*8*) BSA, and (*9*) Tween 80.

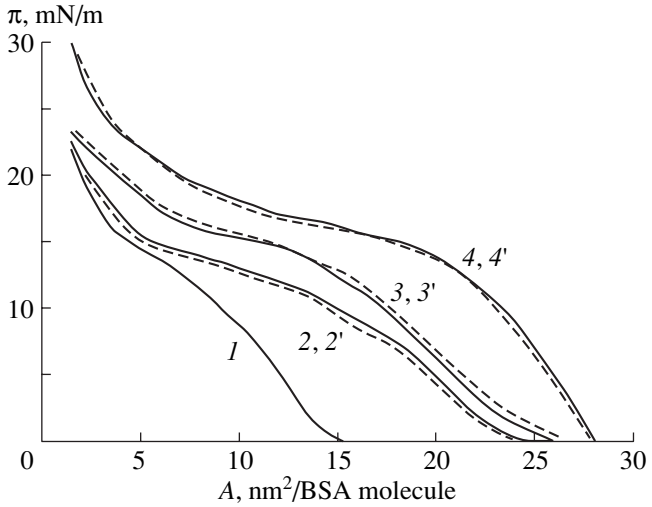

Fig. 3. Compression isotherms for BSA and Tween 80 mixed monolayers normalized to the area per BSA molecule (solid curves) and per BSA–Tween 80 complex (1 : 1) (dashed lines) at various BSA/Tween 80 ratios (mol/mol) in the spread solution: (*1*) 600, (*2*, *2*') 100, (*3*, *3*') 10, and (*4*, *4*') 1.

nents, reaction (2) is shifted toward the complex, and the concentration of the complex is determined by the concentration of the deficient component.

The dissociation constant of the complex (K_d) is

$$K_d = [\mathrm{P}]_0[\mathrm{S}]_0/[\mathrm{PS}], \quad (3)$$

$$[\mathrm{P}] = [\mathrm{P}]_0 - [\mathrm{PS}] \text{ and } [\mathrm{S}] = [\mathrm{S}]_0 - [\mathrm{PS}]. \quad (4)$$

Here, $[P]_0$ and $[S]_0$ are the initial concentrations of BSA and Tween 80, respectively.

In view of equalities (4), Eq. (3) writes

$$K_d = ([\mathrm{P}]_0 - [\mathrm{PS}])([\mathrm{S}]_0 - [\mathrm{PS}])/[\mathrm{PS}]. \quad (3a)$$

When the protein concentrations are high compared to the Tween 80 concentrations (when $[P]_0 \gg [PS]$), Eq. (3a) transforms to

$$K_d = [\mathrm{P}_0][\mathrm{S}]_0 - [\mathrm{PS}]/[\mathrm{PS}] \quad (4)$$

and [PS] can be expressed through

$$[\mathrm{PS}] = [\mathrm{P}]_0[\mathrm{S}]_0/(K_d + [\mathrm{P}]_0). \quad (5)$$

In cases where $K_d \ll [\mathrm{P}]_0$ (which is likely valid for BSA concentrations on the order of 10^{-5} and 10^{-4} mol/l), Eq. (5) can be written in the form of

$$[\mathrm{PS}] = [\mathrm{P}]_0[\mathrm{S}]_0/[\mathrm{P}]_0. \quad (6)$$

K_d can be ignored in cases where its value is at least one order of magnitude or more lower than $[\mathrm{P}]_0$ (when $K_d \leq 10^{-6}$ mol/l). At higher K_d values, the stability of the complex is insignificant. Thus, we have determined the conditions under which the concentrations of the complex are close to the Tween 80 concentrations in its binary mixture with BSA.

In view of the aforesaid, let us consider isotherms *1–4* in Fig. 2 in the context of the formation of the 1 : 1 BSA–Tween 80 complex in the solutions applied to the water–air interface and in the context of the properties of this complex (the most important properties are the increased surface activity and solubility). These properties are responsible for the displacement of all components other than the BSA–Tween 80 complex from the monolayer. According to Eq. (6), the concentrations of the 1 : 1 complex in the solution that match isotherms *1–3* in Fig. 2 are equal to the Tween 80 concentrations (1.7×10^{-7}, 8.5×10^{-7}, and 8.5×10^{-6} mol/l, respectively).

The concentration of the 1 : 1 complex in solution 4 can roughly be equated to the component concentrations ($[\mathrm{P}]_0 = [\mathrm{S}]_0 \approx 8.5 \times 10^{-5}$ mol/l). However, the solubility of the 1 : 1 complex is 10^{-5} mol/l. Therefore, the properties of the monolayer will be dictated by the concentration of the 1 : 1 complex if its concentration in the spread solution is equal to its solubility, i.e., 10^{-5} mol/l. The associated particles of the complex do not have a surface activity [7], and they can partially be removed from the surface. The ordinate intercepts (m^2/mg) cut by the extrapolated linear portions of the isotherms can be normalized to the weight and concentration of the 1 : 1 complex. The areas per molecule in the monolayers that correspond to isotherms *2–4* are 26–27 nm^2/molecule and are close to the sizes of BSA molecules (24–28 nm^2/molecule [8]). Isotherm *1* in Fig. 3 shows that for the BSA–Tween 80 complex, the area is 13–15 nm^2/molecule, proving the collapse of the monolayer of the 1 : 1 complex. The results of the analogous calculations for the whole trend of isotherms *2–4* are illustrated by dashed curves *2'–4'* in Fig. 3. Roughly, curves *2'–4'* follow the trend of isotherms *2–4*. This fact signifies that the monolayers are mainly constituted by 1 : 1 complexes. In the surface layer whose compression is described by isotherm *4* in Fig. 3, there are also surface-inactive particles, which are associates of 1 : 1 complex molecules surrounded by surface-active BSA–Tween 80 complex molecules. This assumption interprets the fact that isotherm *4* is displaced toward larger areas relative to almost coincident isotherms *2* and *3*. This interpretation is verified by the calculations of the monolayer compressibility (π/A) for the initial portions of the isotherms ($\pi \leq 10$ mN/m). This value (2.2 pN molecule m^{-3}) is maximal for isotherm *4*. The respective value for the compression isotherm of the BSA monolayer is 0.6 pN molecule m^{-3}. For isotherms *2* and *3*, an intermediate value is obtained (1.0–1.5 pN molecule m^{-3}).

The inflections on isotherms *1–4* in Fig. 3 signify the restructuring of the condensed monolayers of the 1 : 1 BSA–Tween 80 complex and the formation of surface films in accordance with the models developed in [4]. Another piece of evidence in favor of the prevalence of protein–BSA complexes in the monolayer is provided by the high limit biaxial pressures, which reach 35 mN/m: the relevant value for expanded BSA monolayers under the conditions where protein molecules do not bind hydrophobic surfactants is 20 mN/m.

In summary, our investigations of the mixed monolayers of BSA and Tween 80 confirm previous inferences about the formation of a 1 : 1 surface-active complex in the solution; the solubility of this complex is about 10^{-5} mol/l. This investigation is part of the systematic investigations of the interaction of proteins with surfactants in mixed solutions and at interfaces. The results of this work are to be used in design of condensed LB films of BSA, whose promise was mentioned in [9].

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