Rheokinetic Analysis of Protein Films at the Air−Aqueous Subphase Interface. 2. Bovine Serum Albumin Adsorption from Sucrose Aqueous Solutions

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In this paper surface dynamic properties (surface tension and surface dilational properties) of bovine serum albumin (BSA) films adsorbed on the air−aqueous sucrose solution interface are presented, as a function of adsorption time. The experiments were performed using a superficial, sinusoidal oscillatory rheometer (ring trough), at constant temperature (20 °C). The surface rheological parameters (i.e. surface dilational modulus, elastic and viscous component, and loss tangent angle) and the surface tension were measured as a function of sucrose concentration (0, 0.25, 0.5, and 1 M) and on a mixture of ethanol (1.0 M) and sucrose (0.5 M) in the aqueous phase. The films displayed a viscoelastic behavior, which was practically elastic. At low sucrose concentration (<0.5 M) the surface rheological properties were similar to those on water, whereas at the highest sucrose concentration (1 M) these properties decreased significantly. The transient surface dynamic properties also depend on sucrose concentration in the aqueous phase. A first-order kinetic model is a satisfactory mathematical description of the BSA adsorption and unfolding at the interface. These phenomena have been related to the protein unfolding and/or protein−protein interactions in the presence of solutes (ethanol or sucrose) in the aqueous phase.

Keywords: *Superficial dilational rheology; adsorption; surface properties; bovine serum albumin; air−water interface*

INTRODUCTION

Adsorption of protein at fluid−fluid interfaces is the first step in the formation and stabilization of food emulsions and foams (Halling, 1981). Perhaps the most important application of proteins at interfaces is their use as emulsifiers or surfactants in food dispersions (Kinsella, 1976). In fact, an interfacial layer of adsorbed protein lowers the surface or interfacial tension and imparts a peculiar rheological behavior at the interface. These are important factors for emulsion and foam formation and stabilization (Bos et al., 1997; Damodaran, 1990; Dickinson, 1992). However, little is known about the structure of the interfacial layers, especially in more complex food models, in which proteins and solutes are present in the subphase.

In the preceding paper (Rodríguez Niño et al., 1997) of this series, we have studied the influence of ethanol in the subphase on the transient dynamic surface properties (surface tension and surface dilational properties) of bovine serum albumin (BSA) solutions at constant temperature (20 °C). The major conclusions were the following. The competitive adsorption of ethanol and BSA caused time-dependent changes to the surface tension and surface rheological properties. This phenomenon as well as protein−ethanol interactions reduced the surface dilational modulus as either the ethanol concentration increased or the BSA concentration decreased. These results are of practical importance because the surface dilational modulus decreased to low values at the highest ethanol concentration studied (2 M).

This work is an extension of studies on BSA adsorption from aqueous solutions that are of interest in food technology. The aim of this paper is to study the effect of sucrose in the subphase on transient dynamic surface properties of BSA adsorbed films. Due to its practical interest, the characteristics of BSA adsorbed films (the first-order rate constant for BSA adsorption) from aqueous solutions containing ethanol and sucrose have been compared, as a function of solute concentration in the subphase. The effects of ethanol and sucrose on protein adsorption are relevant to certain food formulations—such as alcoholic beverages and bakery and confectionery products—in which foams and emulsions are stabilized by proteins in the presence of these solutes (Halling, 1981; Dickinson, 1992).

EXPERIMENTAL PROCEDURES

Materials. BSA (Fluka, >96% pure), and analytical grade sucrose (Fluka, >99.5%), ethanol (Merck, >99.8%), potassium dihydrogen phosphate (Merck, 99.5%), and dipotassium hydrogen phosphate (Merck, 99%) were used without further purification. All samples were prepared using double-distilled surface chemical pure water.

Methods. The surface rheological parameters—such as surface dilational modulus and elastic and viscous components—and the surface tension were measured according to the method of Kokelaar et al. (1991) as a function of time and radial frequency. The method has been detailed in Part 1. The method involves a periodic sinusoidal interfacial expansion and compression performed in a special Langmuir trough with a cylindrical barrier. The surface dilational

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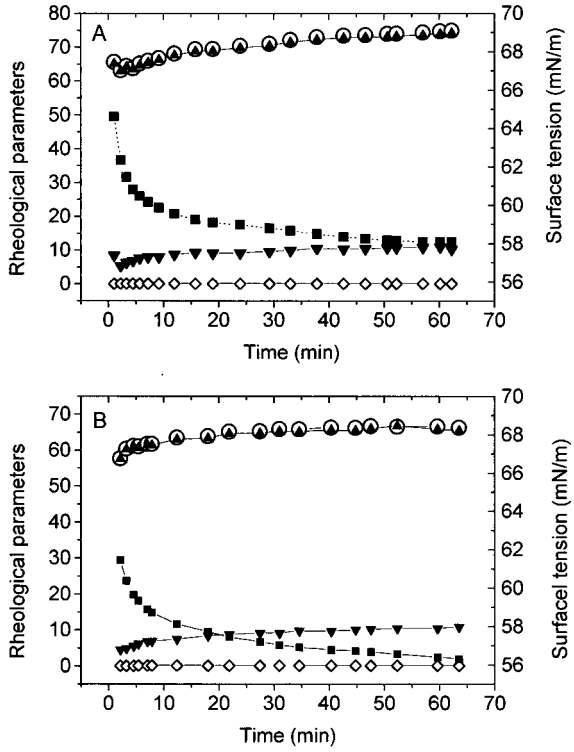

Figure 1. Time dependence of surface tension (σ, ■, mN/m), surface dilational modulus (E, ○, mN/m), surface dilational elasticity (E_d, ▲, mN/m), surface dilational viscosity ($\eta_d\omega$, ▼, mN/m), and loss angle tangent (tan θ, ◇) for BSA films adsorbed on water (A) and 0.25 M sucrose aqueous solution (B), angular frequency = 0.81 rad s^{-1}. T = 20 °C. BSA concentration = 0.1% w/w.

modulus, E, the elastic, E_d, and viscous, $\eta_d\omega$, components, and the loss angle tangent, tan θ, were derived from the small change in surface tension, measured using a glass Wilhelmy plate in permanent contact with the liquid surface, resulting from a small change in surface area, A (Lucassen and van den Temple, 1972). θ is the loss angle of the modulus, and ω is the frequency of the oscillation (rad s^{-1}). If the film is elastic, the loss angle tangent is zero.

The experiments—measurements of surface tension and surface dilational properties as a function of time—were carried out at 20 °C. All of the aqueous subphases were prepared in 50 mM phosphate buffer and adjusted to pH 7.0. To study the rate of protein adsorption, time began at the moment the BSA solution (200 mL) at 20 °C was placed in the trough. In experiments with sucrose in subphase (sucrose aqueous solutions at 0.25, 0.5, and 1.0 M) and with a mixture of 1 M ethanol and 0.5 M sucrose, protein solutions were prepared at 20 °C by stirring for 30 min and then placed in the trough. The absence of surface active contaminants in the aqueous subphase was checked. Measurements were performed at least twice. The reproducibility of the results was better than 6%.

The secondary structure of BSA was determined using circular dichroism spectroscopy. The near-UV (340−250 nm) and far-UV (260−180 nm) spectra of 1.0 mg/mL BSA in 0.1 mm path length quartz cells were obtained using a Jasco J-710 spectropolarimeter (Jasco Corp., Tokyo). The measurements were performed in the absence and presence of a 1.0 M sucrose solution. The far-UV spectra were analyzed using the Selcon method (Sreerama and Woody, 1993), fitting to three structural parameters—α-helix, β-sheet, and aperiodic.

RESULTS

Transient Surface Dynamic Properties. The effect of added sucrose at 0.25, 0.5, and 1.0 M on the surface tension and surface dilational parameters of BSA adsorbed films is shown in Figures 1−3, respectively. The surface tension decreased as the adsorption time increased, which can be associated with the amount of protein adsorbed on the interface (MacRitchie, 1978; Graham and Phillips, 1979; Damodaran and Song, 1988). However, the time dependence of the surface dilational properties, especially surface dilational modulus and surface dilational elasticity, was more complex. At the lower sucrose concentration (0.25 M), the surface dilational modulus and the elastic component increased with time until a plateau was attained. This behavior was similar to that observed with BSA adsorption on water (Rodríguez Niño et al., 1997). At sucrose concentrations >0.5 M, the surface dilational modulus and surface elastic component presented a maximum and then decreased as time progressed. The surface dilational viscosity was low but tended to increase with time, no matter what the sucrose concentration was. However, the loss angle tangent was practically zero in every case.

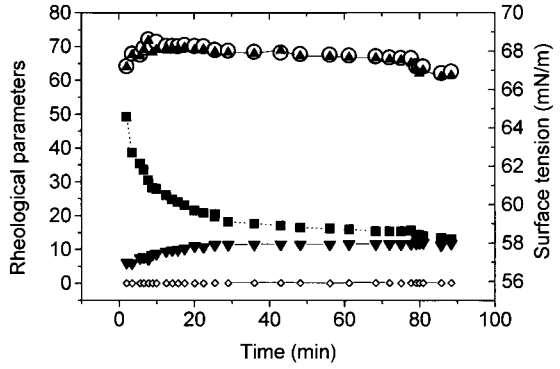

Figure 2. Time dependence of surface tension (σ, ■, mN/m), surface dilational modulus (E, ○, mN/m), surface dilational elasticity (E_d, ▲, mN/m), surface dilational viscosity ($\eta_d\omega$, ▼, mN/m), and loss angle tangent (tan θ, ◇) for BSA films adsorbed on 0.5 M sucrose aqueous solution, angular frequency = 0.81 rad s^{-1}. T = 20 °C. BSA concentration = 0.1% w/w.

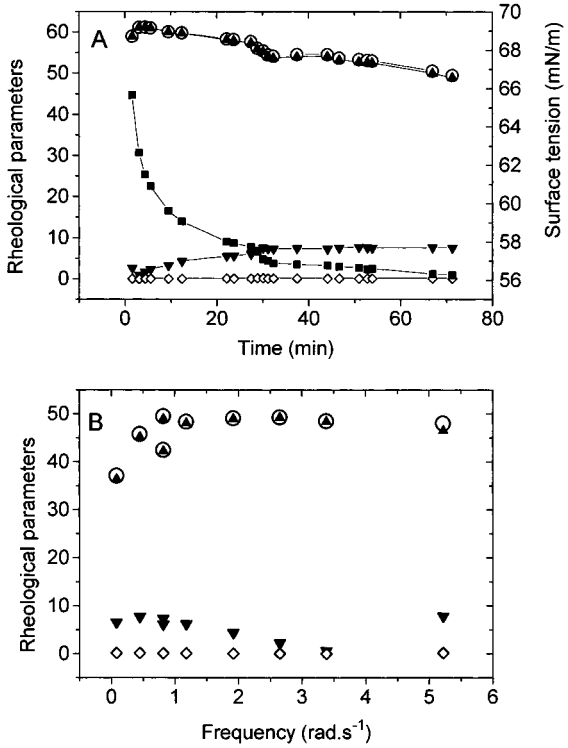

Figure 3. (A) Time dependence of surface tension (σ, ■, mN/m), surface dilational modulus (E, ○, mN/m), surface dilational elasticity (E_d, ▲, mN/m), surface dilational viscosity ($\eta_d\omega$, ▼, mN/m), and loss angle tangent (tan θ, ◇) for BSA films adsorbed on 1.0 M sucrose aqueous solution, angular frequency = 0.81 rad s^{-1}. (B) Angular frequency dependence of rheological parameters. T = 20 °C. BSA concentration = 0.1% w/w.

The effect of added sucrose on the rate of surface tension change with time during the BSA adsorption was quite different from that observed with ethanol (Rodríguez Niño et al., 1997). In fact, in the presence of sucrose, the rate of surface tension change during BSA adsorption was higher than that from ethanol aqueous solutions. Moreover, opposite to that observed with BSA adsorption from ethanol aqueous solutions, no induction period was observed in the presence of sucrose in the aqueous phase.

Surface Dilational Properties. The effect of added sucrose on surface rheological properties of BSA films shows some interesting features (Figures 1−3): (i) the values of surface dilational modulus were similar to that of surface dilational elasticity, (ii) both the surface dilational viscosity and the loss angle tangent were low, and the latter was practically zero, and (iii) the surface rheological parameters—especially surface dilational modulus and its elastic component—increased with frequency until a plateau was attained. As a consequence of this behavior, it can be concluded that the surface viscoelastic characteristics of BSA films adsorbed on aqueous sucrose solutions are practically elastic.

The frequency dependence of the surface dilational properties at low frequencies is an indication that either a reorientation of the molecules at the interface or a diffusion of molecules between the interface and the subsurface during the compression−expansion cycle may exist. However, the existence of a plateau region at higher frequencies indicates that relaxation processes different from the reorientation or diffusion processes, if they exist, have a time scale longer than that of the compression−expansion cycle (Graham and Phillips, 1980).

Effect of Sucrose Concentration. The effect of sucrose concentration on surface dynamic properties at 30 and 60 min of adsorption time is shown in Figure 4. It can be seen that surface tension decreased with increased sucrose concentration. However, surface rheological properties, especially the surface dilational modulus and the elastic component, are practically independent of sucrose concentration at concentrations <0.5 M, but both decrease at the highest sucrose concentration studied (1.0 M). These results are different from those obtained with ethanol aqueous solutions. The surface dilational modulus values for BSA adsorbed from a 1.0 M aqueous sucrose solution are 4 and 3 times higher than those from a 1.0 M ethanol solution, at 30 and 60 min of adsorption time, respectively. The phenomena reported here must be associated with the protein−protein and protein−solute interactions as a function of aqueous phase composition, which will be discussed later.

BSA Adsorption from a Mixed Aqueous Solution of Ethanol and Sucrose. The effect of added ethanol (1.0 M) and sucrose (0.5 M) on the transient dynamic surface properties of BSA adsorbed films is shown in Figure 5. As on the aqueous solutions of 1.0 M ethanol (Rodríguez Niño et al., 1997) and on 0.5 M sucrose (Figure 2), the BSA films displayed a behavior that was practically elastic (Figure 5). It can be seen that the values of the surface rheological properties of BSA films were dominated by the presence of ethanol in the aqueous phase. The surface dilational modulus was similar to that observed on a 1.0 M ethanol aqueous solution (Rodríguez Niño et al., 1997) and was lower than that observed on a 0.5 M sucrose aqueous solution (Figure 2).

However, the presence of sucrose in the bulk phase affects mainly the BSA kinetic adsorption. As was

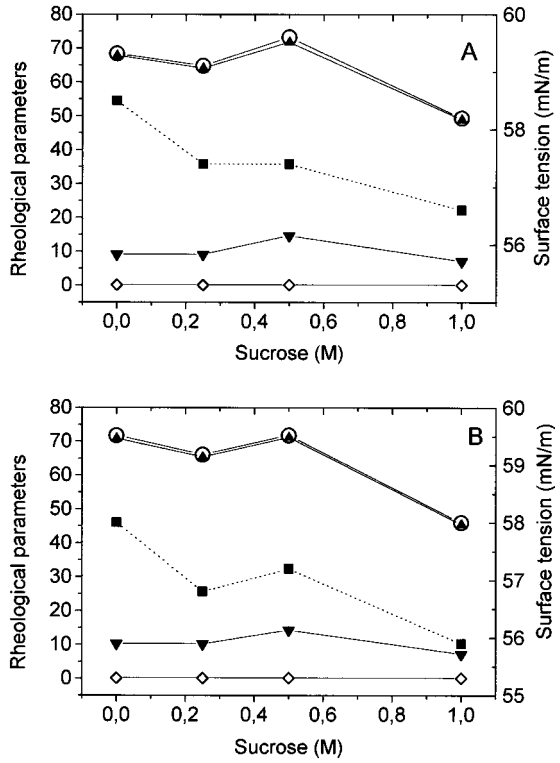

Figure 4. Sucrose concentration dependence of surface tension (σ, ■, mN/m), surface dilational modulus (E, ○, mN/m), surface dilational elasticity (E_d, ▲, mN/m), surface dilational viscosity ($\eta_d\omega$, ▼, mN/m), and loss angle tangent (tan θ, ◇) for BSA films adsorbed on sucrose aqueous solutions, angular frequency = 0.81 rad s^{-1}. T = 20 °C. Adsorption time: (A) 30 min; (B) 60 min. BSA concentration = 0.1% w/w.

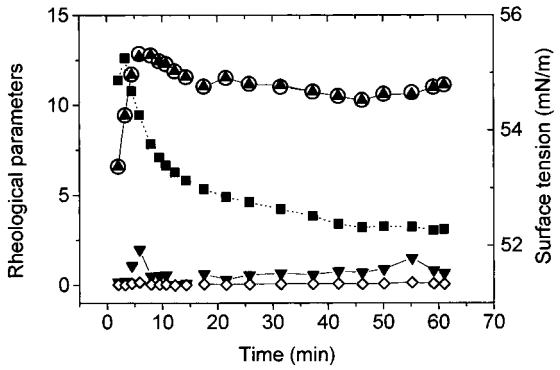

Figure 5. Time dependence of surface tension (σ, ■, mN/m), surface dilational modulus (E, ○, mN/m), surface dilational elasticity (E_d, ▲, mN/m), surface dilational viscosity ($\eta_d\omega$, ▼, mN/m), and loss angle tangent (tan θ, ◇) for BSA films adsorbed on aqueous solution of 1.0 M ethanol 0.5 M and sucrose, angular frequency = 0.81 rad s^{-1}. T = 20 °C. BSA concentration = 0.1% w/w.

previously discussed, sucrose increases the rate of surface tension change with time during the BSA adsorption. Moreover, the surface dilational modulus versus time exhibited a maximum and then decreased as the time increased, a phenomenon that could be associated with the structuring effect of sucrose on the protein molecule.

Structural Effects. The measurements by circular dichroism showed that the tertiary and secondary structures of BSA in solution agreed with previous literature values (Takeda et al., 1988) and were not significantly affected by the presence of 1.0 M sucrose (Table 1). The analysis of the secondary structure showed some very small changes showing a slight increase in secondary structure at the expense of the aperiodic content.

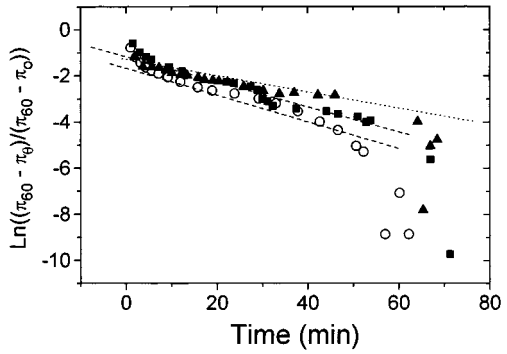

Figure 6. Effect of aqueous phase composition on the rate of BSA adsorption at the air−aqueous phase interface at 20 °C. The substrate was a phosphate buffer (pH 7.0, $I = 0.05$ M). BSA concentration = 0.1% w/w. Aqueous phase composition: (○) water; (△) 1 M ethanol; and (■) 1 M sucrose.

Table 1. Secondary Structure Composition of BSA in the Presence and Absence of 1 M Sucrose, As Determined by Far-UV Circular Dichroism

sample	α-helix (%)	β-sheet (%)	aperiodic (%)
BSA in water	55.7	7.9	36.8
BSA + 1 M sucrose	57.9	9.9	32.8

DISCUSSION

Kinetic Considerations. The fact that the time dependence of the surface tension follows the same trend as the protein surface concentration indicates that σ and E depend on the surface coverage, which is expected to increase with time (MacRitchie, 1978, 1989; Joos et al., 1992; Damodaran and Song, 1988). Due to this similarity we propound an analogy to define a first-order kinetic equation similar to that used by Tornberg (1978) and Graham and Phillips (1979) to monitor unfolding and configurational rearrangements of adsorbed protein molecules at the interface. As with other methods (Rodríguez Patino and Rodríguez Niño, 1995a,b), the diffusion step is too fast to be detected with the experimental device use in this work. The rate of these processes can be analyzed by the first-order equation

$$\ln [(\pi_{60} - \pi_{\phi})/(\pi_{60} - \pi_{o})] = -k\phi \qquad (1)$$

where $\pi = \sigma_o - \sigma$ is the surface pressure, σ_o is the subphase surface tension, π_{60}, π_0, and π_ϕ are the surface pressures at $\phi = 60$ min of adsorption time, at time $\phi = 0$, and at any time, ϕ, respectively, and k is the first-order rate constant. In practice, a plot of eq 1 usually yields two or more linear regions. The initial slope is taken to correspond to a first-order rate constant of penetration and unfolding (k_1), while the second slope is taken to correspond to a first-order rate constant of rearrangement (k_2), occurring among a more or less constant number of adsorbed molecules (Tornberg, 1978; Graham and Phillips, 1979; Suttiprasit et al., 1992).

An example of the application of eq 1 to monitor the kinetics of penetration and unfolding of BSA molecules at the interface—in the period after that affected by diffusion—is shown in Figure 6, as a function of aqueous phase composition. We find, for all the experiments of BSA adsorption, one or two linear regions. Because protein adsorption measurements are very time-consuming (Rodríguez Patino and Rodríguez Niño, 1995a,b), no attempt was made to correlate the experimental data to the second rearrangement step of previously adsorbed BSA molecules.

To summarize the effect of aqueous phase composition and BSA concentration on the kinetics of BSA adsorption, the first-order rate constants derived from eq 1 are shown in Table 2. The fit of experimental data to the model was made at an interval of time based on the best linear regression coefficient (LR), which is also included in Table 1. As a general rule, it can be seen that the rate of BSA adsorption increases when the BSA concentration in the bulk phase is increased, which agrees with previous data in the literature (Tornberg, 1978; Graham and Phillips, 1979). The rate of BSA adsorption also depends on the aqueous phase composition. It can be seen (Table 2) that the rate of BSA adsorption is higher from water than from all of the aqueous solutions of ethanol and sucrose concentrations <0.5 M. The rate of BSA adsorption was increased from aqueous solutions of 1.0 M sucrose.

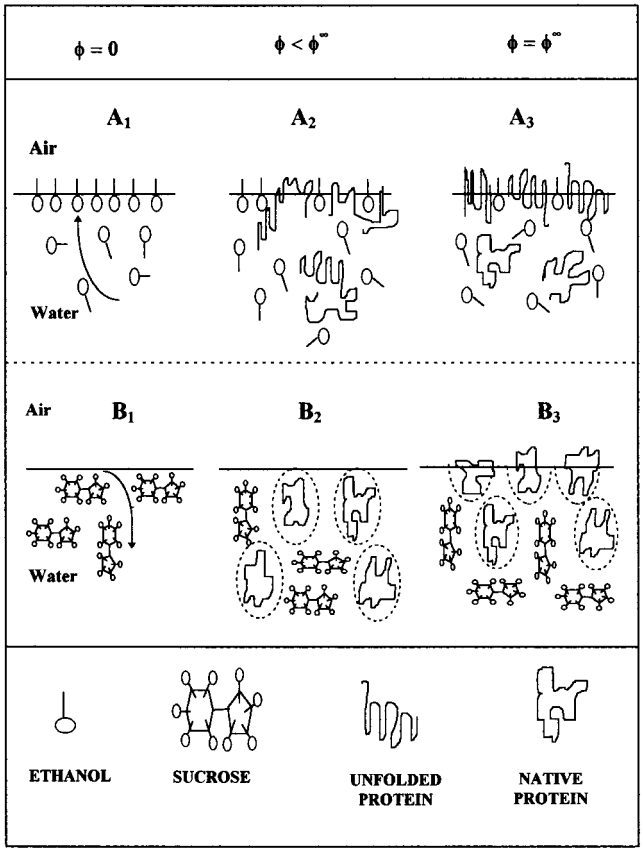

Figure 7. Models for BSA arrangement at the interface region and in the bulk aqueous phase in the presence of ethanol (A) and sucrose (B), as a function of adsorption time: at the beginning ($\phi = 0$); at any adsorption time ($\phi < \theta$); at steady state ($\phi = \infty$). For an explanation see the text.

The discussion of the effect of aqueous phase composition on BSA adsorbed films—as a function of time and at steady state—can be made on the basis of the molecular model drawn on Figure 7.

The addition of sucrose to water increases the surface tension (Rodríguez Patino and Martín, 1994), which indicates that sucrose has no affinity for the interface (Figure 7-B_1) but exerts a strong cohesive force on water molecules. This phenomenon could be associated with the fact that protein molecules are preferentially hydrated in the presence of sucrose (Crowe et al., 1987; Lee and Timasheff, 1981). That is, sucrose increases the energy barrier between the native and denatured states of proteins (Crowe et al., 1987) and is excluded from the immediate domain of protein (Figure 7-B_2) and the interface (Figure 7-B_3), in aqueous solutions. The results obtained in this work at sucrose concentration of 1 M (Table 2) supported and strengthened a previous hypothesis on the BSA adsorption rate in the presence

Table 2. Characteristic Parameters for Adsorption of BSA at the Air−Aqueous Subphase Interface in the Presence and Absence (Rodríguez Patino and Rodríguez Niño, 1995a,b) of Convection, at 20 °C

system	convection, $k_1 \times 10^3$, min^{-1} (LR)	no convection, $k_1 \times 10^3$, min^{-1} (LR)
BSA (1% w/w)−water		30.7 (0.999)
BSA (0.1% w/w)−water	56.7 (0.989)	31.9 (0.997)
BSA (1×10^{-2}% w/w)−water		18.8 (0.999)
BSA (1×10^{-3}% w/w)−water		16.0 (0.998)
BSA (1×10^{-4}% w/w)−water		18.3 (0.996)
BSA (0.1% w/w)−0.1 M ethanol	16.8 (0.975)	
BSA (0.1% w/w)−0.5 M ethanol	26.9 (0.979)	
BSA (0.1% w/w)−1 M ethanol	29.3 (0.973)	24.0 (0.999)
BSA (1×10^{-2}% w/w)−1 M ethanol		6.74 (0.979)
BSA (2×10^{-2}% w/w)−1 M ethanol	22.3 (0.995)	
BSA (3×10^{-3}% w/w)−1 M ethanol	9.55 (0.919)	
BSA (0.1% w/w)−0.25 M sucrose	47.6 (0.998)	
BSA (0.1% w/w)−0.5 M sucrose	33.6 (0.973)	36.7 (0.990)
BSA (0.1% w/w)−1 M sucrose	72.0 (0.980)	

of sucrose (Rodríguez Patino and Rodríguez Niño, 1995a,b): that is, if sucrose limits protein unfolding and protein−protein interactions, the reduction in protein aggregation allows more protein to be involved in the film formation. An increase in the protein adsorption rate in the presence of sucrose has been observed previously (MacRitchie and Alexander, 1961; Rodríguez Patino and Rodríguez Niño, 1995a,b).

However, the results obtained on aqueous solutions at sucrose concentrations <0.5 M were unexpected (Table 2). The complexity of the study on the effect of sucrose in the aqueous phase on the rate of protein adsorption is due to the fact that different phenomena are associated with this process. In fact, the rate of BSA adsorption could also depend on the effect of sucrose on the viscosity of the bulk phase. The BSA adsorption could diminish as the bulk phase viscosity increases in the presence of sucrose (Lide, 1995). We have observed recently (Rodríguez Niño, 1997) that the first step for BSA diffusion from the bulk phase to the interface is also lower in the presence of sucrose. So, competition may exist between the reduction in protein aggregation in the presence of sucrose—which allowed more protein to be involved in film formation—and the reduction in the rate of BSA adsorption—due to the high viscosity of the bulk phase. These data suggest that the higher rate of BSA adsorption and the stability of protein molecules against denaturation are the predominant phenomena at higher sucrose concentrations in the aqueous phase.

The effect of ethanol on BSA adsorption was discussed in Part 1. The addition of ethanol to water decreased the surface tension (Rodríguez Patino and Martín, 1994), which indicates that ethanol has an affinity for the interface (Figure 7-A_1). This could be associated with the competitive adsorption of BSA on an ethanol film (Figure 7-A_2). However, BSA has a higher affinity than ethanol for the interface due to its higher hydrophobicity. This phenomenon as well as protein−ethanol interactions (Figure 7-A_2,-A_3) could explain the lower rate of BSA adsorption with ethanol in the subphase and the higher rate of BSA adsorption with increased BSA concentration in the bulk phase (Table 2).

Effect of Convection on the Rate of BSA Adsorption. The effect of convection due to the sinusoidal movement of the ring in the interface and in the bulk phase, on the value of k_1, can be analyzed from the data presented in Table 2. The first-order rate constants derived from experiments in the absence of convection (Rodríguez Patino and Rodríguez Niño, 1995a,b) are also included in Table 2. It can be seen that the rate of BSA adsorption is higher in the presence of convection. However, the solutes in the bulk phase (ethanol and sucrose) decreased the rate of BSA adsorption in the presence of convection. The behavior of BSA adsorption from a 1.0 M sucrose aqueous solution is an exception. It must be emphasized that an important difference for the BSA adsorption due to the convection is related to the effect of sucrose on the kinetics of BSA adsorption. In fact, without convection, the rate of BSA adsorption increased in the presence of sucrose, independent of the sucrose concentration (Rodríguez Patino and Rodríguez Niño, 1995a,b). This phenomenon is opposite to that observed in this work in the presence of convection, at sucrose concentrations <0.5 M. The convection has been used (Part 1) to explain the smaller lag period during BSA adsorption from alcoholic solution as compared with previous data in a surface tensiometer (Dussaud et al., 1994; Rodríguez Patino and Rodríguez Niño, 1995a,b). So, it can be concluded that convection affects the rate of BSA adsorption at the interface, depending on the reagent present in the aqueous phase and its concentration.

Rheological Consequences. The decrease in the surface dilational properties at the highest adsorption time in aqueous sucrose solutions (Figures 2 and 3) could be associated with decreased protein−protein interactions and/or the effect of sucrose on the molecular structure of the protein (Figure 7-B_2,-B_3). This effect could be due to direct interaction with the protein or indirect effects on the structure and properties of the solvent or a combination of these mechanisms (Timasheff et al., 1976). That is, a change in the protein conformation after protein adsorption could affect the values of surface rheological properties and their evolution with time. We know from the structural studies that the structure of the BSA is not significantly affected by the presence of 1.0 M sucrose in solution.

As deduced from the low value of the loss angle tangent (Figures 1−3 and 5) and the frequency dependence of surface dilational properties (Figure 3), the BSA films adsorbed from aqueous sucrose solutions displayed a viscoelastic behavior which is essentially elastic over the range of frequencies studied. This behavior, which is characteristic of insoluble lipids (Rodríguez Niño et al., 1996), strengthened the previous hypothesis on the effect of sucrose on protein structure at the interface. In fact, if sucrose favors the protein adsorption at the interface and reduces protein−protein interactions, the viscoelastic characteristics of the film should be essentially of elastic nature.

The reduction of protein−protein interactions at the interface (Figure 7-B_3) could also explain the reduction of superficial dilational modulus as the sucrose concentration increases in the bulk phase, especially at the highest sucrose concentration (Figure 4). That is, BSA can be adsorbed in a native configuration stabilized by the presence of sucrose.

Finally, the competitive adsorption of BSA in the presence of ethanol, as well as protein−ethanol interactions, as stated in the molecular models in Figure 7-A_1−A_3, is supported by the significant reduction in the surface dilational modulus of BSA films adsorbed from ethanol solutions (Part 1) and mixtures of ethanol and sucrose (Figure 5).

CONCLUSIONS

In this series (Parts 1 and 2) we have studied the effect of aqueous phase composition on the surface dynamic properties of BSA adsorbed films. From a rheological point of view, the films displayed viscoelastic behavior which was practically elastic. The solutes (ethanol and sucrose) in the subphase produced a significant reduction in the surface dilational modulus as either the solute concentration increased or the BSA concentration decreased. From a kinetic point of view, the rate of surface tension change during BSA adsorption can be described by a first-order model. The discussion of the effect of the solutes has been based on the first-order rate constant of the unfolding of adsorbed BSA molecules. We found that the rate of BSA adsorption decreased with ethanol in the subphase and at sucrose concentrations <1.0 M, but it increases significantly at the highest sucrose concentration studied (1.0 M). The time dependence of the surface rheological properties depended on the solute present in the subphase. With ethanol, the surface dilational modulus increased with time and tends to a plateau. However, with sucrose in the subphase at 0.5 and 1 M, the surface dilational modulus increased with time, described a maximum, and then decreased as time progressed.

Further, surface tension and surface rheological properties give complementary information about BSA adsorption and interactions at the interface as a function of aqueous phase composition. The kinetics of the process can be established from surface tension data, while the existence of interactions at the interface is well-described by the surface rheological properties. However, the complexity of the study of the effect of solutes in the aqueous phase on the rate of protein adsorption and the existence of interactions at the interface requires more systematic and quantitative experimental data. This analysis is currently under way and will be published in future papers.

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