Dynamics of Exchange between α_{S1}-Casein and β-Casein during Adsorption at Air−Water Interface

Kamlesh Anand and Srinivasan Damodaran*

Department of Food Science, University of Wisconsin—Madison, 1605 Linden Drive, Madison, Wisconsin 53706

Competitive adsorption of α_{S1}-casein and β-casein at the air−water interface has been investigated. In single-component systems, the adsorptivities of these caseins to the air−water interface were very similar and the binding affinity of β-casein was only slightly greater than that of α_{S1}-casein. However, in a 1:1 binary mixture of α_{S1}- and β-caseins, the ratio of surface load of these caseins was 2:1 in favor of β-casein adsorption. Kinetic studies showed that α_{S1}-casein arrived first at the interface, but the late arriving β-casein was able to displace the adsorbed α_{S1}-casein from the interface and establish a new equilibrium. Exchange and displacement experiments showed that bulk phase β-casein was able to readily displace adsorbed α_{S1}- and β-casein molecules; similarly, α_{S1}-casein was also able to displace α_{S1}- and β-caseins from the interface, although to a lesser extent. The mechanism of the dynamic exchange between α_{S1}- and β-caseins is discussed.

Keywords: *Air−water interface; protein adsorption; α_{s1}-casein; β-casein*

INTRODUCTION

The foaming and emulsifying properties of a food protein isolate are influenced by its composition and the relative surface activity of each protein component. The composition of proteins at interfaces of emulsions and foams made with protein mixtures is often dominated by the highly surface active protein component of the mixture. Studies on emulsions made with sodium caseinate or α_{S1}-casein and β-casein mixtures have shown that β-casein was the predominant protein component at the interface of these emulsions (Robson and Dalgleish, 1987; Dickinson et al., 1988). Exchange experiments have shown that when β-casein was introduced into an emulsion made with α_{S1}-casein, the latter was readily displaced from the interface by the former; similarly, α_{S1}-casein also was able to displace β-casein, although to a lesser extent (Dickinson and Whyman, 1987; Dickinson et al., 1988). Although these studies have shown that caseins reversibly adsorb at an oil−water interface, neither the kinetics of adsorption of individual caseins from a mixture nor the mechanism of displacement of one casein by the other has been studied. Further, since multilayer protein films are often formed in emulsions prepared with concentrated protein solutions (e.g., 0.5%), it has been difficult to unambiguously conclude whether exchange occurs between bulk phase and primary monolayer molecules or between bulk phase and molecules in secondary or multilayer.

Both α_{S1}-casein and β-casein are believed to be highly flexible, random-coil-type proteins. Table 1 shows some of the physicochemical properties of these caseins. Several studies have indicated that β-casein is more surface active than α_{S1}-casein (Mitchell et al., 1970; Dickinson et al., 1985); this must be related to differences in the net charge and mean residue hydrophobicity. While information regarding kinetics of adsorption of β-casein at interfaces has been studied (Xu and Damodaran, 1993a), no systematic studies exist either on comparative adsorption of α_{S1}-casein and β-casein in single-component systems or on competitive adsorption of these caseins in binary systems. Thus, the objective of the present study is to systematically investigate the dynamics of adsorption, displacement, and/or exchange between α_{S1}-casein and β-casein during adsorption from a binary bulk phase to the air−water interface.

Table 1. Comparison of Selected Physicochemical Properties of α_{S1}-Casein and β-Casein[a]

property	α_{S1}-casein	β-casein
no. of amino acid residues	199	209
molecular weight	23 500	24 000
% α-helix	∼10	∼7
% β-sheet	20	13
net charge at pH 7	−21	−13
mean residue hydrophobicity (cal/mol)	1170	1330

[a] Compiled from Swaisgood (1992).

MATERIALS AND METHODS

Lyophilized α_{S1}-casein and β-casein, both from bovine milk, were purchased from Sigma Chemical Co. (St. Louis, MO). Ultrapure Na_2HPO_4, $NaHPO_4$, and NaCl were purchased from Aldrich Chemical Co. (Milwaukee, WI). [^{14}C]Formaldehyde was from New England Nuclear (Boston, MA). All other reagents used in this study were of analytical grade. Purified water from a Milli-Q ultrapure water system (Millipore Corp., Bedford, MA) with a resistivity of 18.2 mΩ cm was used in all adsorption experiments.

The proteins were radiolabeled with ^{14}C nuclide by reductive methylation of amino groups with [^{14}C]formaldehyde at pH 7.5 as described previously (Xu and Damodaran, 1992). The protein concentration was determined using $E^{1\%}$ values of 10.5 at 280 nm for α_{S1}-casein and 4.6 at 280 nm for β-casein (Swaisgood, 1992). The specific radioactivities of the labeled proteins were 1.3 and 0.8 mCi/mg, respectively, for α_{S1}-casein and β-casein.

The kinetics of adsorption of radiolabeled proteins at the air−water (20 mM phosphate buffer saline solution, pH 7.0, I = 0.1) interface was studied as described elsewhere (Xu and Damodaran, 1992, 1993a). Briefly, the rate of change of protein concentration at the air−water interface of radiola-

* Author to whom correspondence should be addressed [telephone (608) 263-2012; fax (608) 262-6872; e-mail sdamodar@facstaff.wisc.edu].

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beled protein solutions (120 mL) in a Teflon trough (21 × 5.56 × 1.27 cm) was monitored by measuring surface radioactivity using a rectangular gas proportional counter (8 × 4 cm) (Ludlum Measurements, Inc., Sweetwater, TX). The entire experimental setup was housed in a refrigerated incubator maintained at 25 ± 0.2 °C. A carrier gas composed of 98% argon and 2.0% propane was passed continuously through the gas proportional counter at a rate of 20 mL/min. A calibration curve relating cpm versus surface radioactivity (mCi/m^2), constructed by spreading ^{14}C-labeled β-casein on the air−water interface, was used to convert surface cpm to mCi/m^2. The surface concentration (mg/m^2) was then calculated by multiplying surface activity with specific radioactivity (mCi/mg) of the protein. The rationale for using ^{14}C-labeled β-casein to construct the cpm versus surface radioactivity (mCi/m^2) has been discussed elsewhere (Xu and Damodaran, 1993b). The contribution of bulk radioactivity to cpm was corrected using a standard curve relating cpm versus specific radioactivity of $CH_3{}^{14}COONa$ solutions. The rate of change of surface pressure was monitored by the Wilhelmy plate method using a thin sand-blasted platinum plate (1 cm width) hanging from an electrobalance (Cahn Instruments, Co., Cerritos, CA). Both surface concentration and surface pressure were monitored simultaneously for each protein solution.

In competitive adsorption experiments involving binary mixtures of α_{S1}-casein and β-casein, the following approaches were used to monitor adsorption of each protein component from the bulk phase to the air−water interface. To determine the kinetics of adsorption of α_{S1}-casein from a binary mixture of α_{S1}-casein and β-casein, ^{14}C-labeled α_{S1}-casein and unlabeled β-casein stock solutions were mixed with buffer to the required final concentration ratio. The rate of adsorption of ^{14}C-labeled α_{S1}-casein from this bulk mixture was studied by monitoring the surface radioactivity. To determine the kinetics of adsorption of β-casein from the binary mixture, a complementary adsorption experiment was performed with the bulk phase containing ^{14}C-labeled β-casein and unlabeled α_{S1}-casein at the same concentration ratio as the earlier experiment. In all competitive adsorption experiments the bulk concentration of β-casein was fixed at 1.5 mg/mL and only α_{S1}-casein concentration was varied from 0.15 to 3.0 mg/mL.

The dynamic exchange between bulk and adsorbed protein molecules of the same kind and the ability of α_{S1}-casein and β-casein to displace each other from the air−water interface were investigated as follows: First, ^{14}C-labeled protein 1 (either ^{14}C-labeled β-casein or ^{14}C-labeled α_{S1}-casein) was allowed to adsorb for 24 h. After 24 h, an aliquot of unlabeled protein 1 or protein 2 was injected into the bulk phase; surface radioactivity was then monitored for 24 h. A time-dependent decrease in surface radioactivity was taken as evidence for exchange between bulk and adsorbed molecules or displacement of one protein by the other from the interface.

RESULTS

Figure 1 shows adsorption isotherms of α_{S1}-casein and β-casein in single-component systems. Both caseins exhibited a plateau in the bulk concentration range of 1.5−4.0 mg/mL. The saturated monolayer coverage for α_{S1}-casein was about 1.7 mg/m^2, whereas it was about 1.8 mg/m^2 for β-casein. Both α_{S1}-casein and β-casein exhibited a C_{crit} value, i.e., the minimum bulk concentration above which formation of a saturated monolayer begins, of about 1.5 mg/mL. Adsorption of proteins at interfaces follows a Langmuirian behavior when the bulk concentration is below C_{crit} (Hunter et al., 1990), and under these conditions the equilibrium surface concentration, Γ_{eq}, is given by the relationship

$$\ln K - \lambda\Gamma_{eq}^{n} = \ln \frac{\Gamma_{eq}}{C(1 - a\Gamma_{eq})} \qquad (1)$$

where K is the equilibrium binding constant, a is the average area occupied per molecule at saturated monolayer coverage (i.e., $1/\Gamma_{eq}$ at saturated monolayer coverage), C is the bulk concentration, Γ_{eq} is the surface concentration at equilibrium, n is an exponent related to cooperativity among adsorbing protein molecules, and λ is related to an activation energy barrier for adsorption. The adsorption isotherms of α_{S1}-casein and β-casein were analyzed according to eq 1 to determine their equilibrium constants for binding to the air−water interface. The right-hand side of eq 1 was plotted against Γ^n for n = 2, 3, and 4. The lowest value of n that gave a straight line with the highest correlation coefficient was selected as its value. A good linear fit of the data was obtained with n = 2 for β-casein and with n = 4 for α_{S1}-casein. The equilibrium binding constants of α_{S1}-casein ($K_{\alpha S1}$) and β-casein ($K_{\beta C}$), determined from the intercepts, were 3.6 × 10^4 and 4.0 × 10^4 mg/(m^2 wt %), respectively. The affinity of β-casein to the air−water interface was only slightly greater than that of α_{S1}-casein.

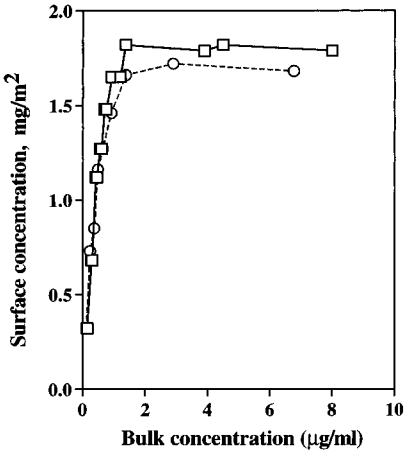

Figure 1. Adsorption isotherms of α_{S1}-casein (○) and β-casein (□) at air−water interface at 24 °C. The subphase was 20 mM phosphate-buffered saline solution, pH 7.0, I = 0.1.

Figure 2A shows rates of adsorption of α_{S1}-casein and β-casein from single-component solutions at 1.5 mg/mL protein concentration. In single-component systems adsorption commenced immediately after a fresh interface was created in the trough. Adsorption continued up to 400 min and reached equilibrium surface concentrations (Γ_{eq}) of about 1.66 mg/m^2 in the case of α_{S1}-casein and 1.8 mg/m^2 for β-casein. No decrease in surface concentration occurred when the system was left to stand for over 1100 min (Figure 2A), indicating that both α_{S1}-casein and β-casein formed a stable monolayer in single-component systems. The Γ_{eq} of α_{S1}-casein was only slightly lower than that of β-casein, although the initial rate of adsorption of α_{S1}-casein was slightly faster than that of β-casein.

Figure 2B shows the time course of adsorption of α_{S1}-casein and β-casein in a 1:1 binary mixture solution containing 1.5 mg/mL each of the proteins. The adsorption profile of α_{S1}-casein in the binary system was remarkably different from that in the single-component system: The surface concentration of α_{S1}-casein increased first to a value of 1.0 mg/m^2 within about 100 min, then decreased with time, and attained an equilibrium value of 0.6 mg/m^2 after 1000 min (Figure 2B). The Γ_{eq} of α_{S1}-casein in the binary system was much lower than its value in the single-component system. On the other hand, the surface concentration of β-casein increased continuously and reached an equilibrium value of about 1.1 mg/m^2 after 1000 min, which was also lower than its value in the single-component system.

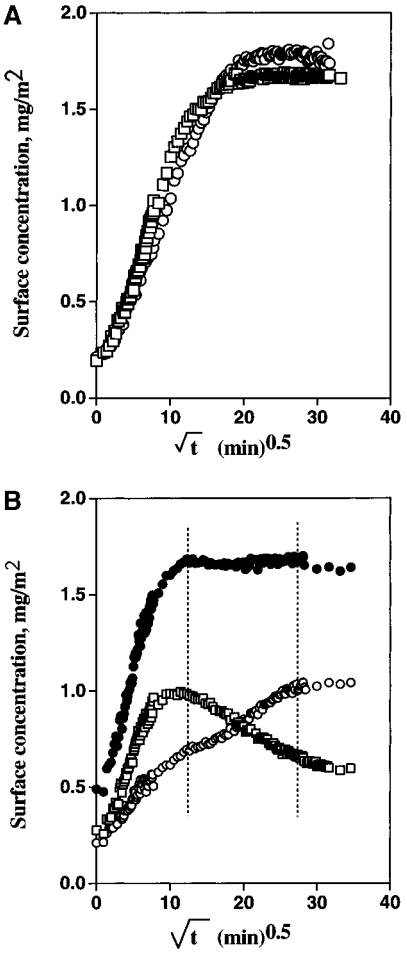

Figure 2. (A) Kinetics of adsorption of α_{S1}-casein (□) and β-casein (○) in single-component systems. Bulk phase concentration of each protein was 1.5 mg/mL. (B) Kinetics of adsorption of α_{S1}-casein (□) and β-casein (○) at air−water interface from 1:1 binary bulk solution containing 1.5 mg/mL each of the proteins. ● represents total surface concentration (obtained from the sum of □ and ○ curves) as a function of adsorption time. The vertical dotted lines denote the time zone at which displacement of α_{S1}-casein by β-casein occurs. See text for details.

The initial rate of adsorption of α_{S1}-casein in the binary system was almost the same as that in the single-component system, whereas the initial rate of adsorption of β-casein in the binary system was markedly slower than that in the single-component system, even though the bulk concentrations in both systems were the same.

Also presented in Figure 2B is the sum of the surface concentrations of α_{S1}-casein and β-casein as a function of time. The Γ_{total} reached a steady state value of 1.8 mg/m^2 within about 100 min, which was about same as the Γ of β-casein in the single-component system. This indicated that irrespective of the composition of the bulk phase, a maximum (saturated) monolayer coverage of only about 1.8 mg/m^2 could be attained for pure β-casein film or α_{S1}-casein plus β-casein mixed film at the air−water interface. For a 1:1 bulk mixture containing 1.5 mg/mL each of the proteins, the ratio of Γ_{eq} of α_{S1}-casein to β-casein at equilibrium was found to be about 1:2. This suggested that, under identical thermodynamic conditions, β-casein had a greater propensity to be at the interface than did α_{S1}-casein.

It should be noted, interestingly, that the time at which Γ_{total} reached a steady state value coincided with the time at which Γ of α_{S1}-casein reached its maximum value. Beyond this time, even though Γ of α_{S1}-casein decreased and that of β-casein increased with time, there was no significant change in Γ_{total} (Figure 2B). To determine if the decrease in Γ of α_{S1}-casein after a maximum value was reached during the course of adsorption was due to its displacement by the adsorbing β-casein molecules, the rates of adsorption of β-casein and desorption of α_{S1}-casein were compared in the time zone indicated by the dotted lines in Figure 2B. The rate of desorption of α_{S1}-casein was 0.0236 mg m^{-2} min$^{-1/2}$, which was almost the same as 0.024 mg m^{-2} min$^{-1/2}$ for the rate of adsorption of β-casein. This strongly suggested that, in the α_{S1}-casein/β-casein binary system, α_{S1}-casein adsorbed at the interface initially more readily than did β-casein; when the total surface concentration of the mixed film reached close to saturated monolayer coverage, β-casein continued to adsorb by displacing α_{S1}-casein from the interface. Since the total surface concentration of the mixed protein film remained constant from 100 to 1000 min of adsorption, for each β-casein molecule adsorbed at the interface, one molecule of α_{S1}-casein was displaced from the interface.

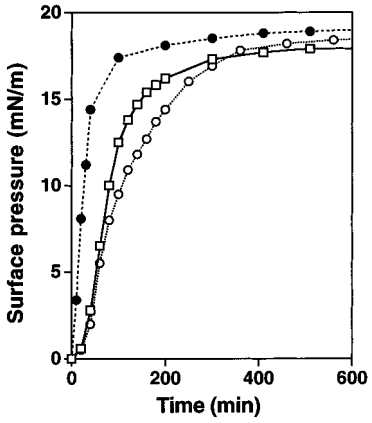

Figure 3. Changes in surface pressure during adsorption of α_{S1}-casein (□) and β-casein (○) in single-component systems and in 1:1 binary protein system (●) containing 1.5 mg/mL each.

Figure 3 shows time-dependent changes in surface pressure (π) of α_{S1}-casein, β-casein, and α_{S1}-casein plus β-casein (1:1 mixture) solutions. In single-component systems, the equilibrium surface pressure of α_{S1}-casein reached a value of about 18 mN/m, which was only slightly lower than that of β-casein. The π_{eq} of the 1:1 mixture was only slightly greater than those of the single-component systems; however, π reached its equilibrium value within a short time, obviously because of the short time needed to reach equilibrium surface concentration (Figure 2B). The similarities in the Γ_{eq} and π_{eq} values of α_{S1}-casein and β-casein in single-component systems tend to suggest that these two caseins may possess similar surface activities. However, on the contrary, the large differences in Γ_{eq} values of α_{S1}- and β-caseins in the 1:1 binary system (Figure 2B) clearly demonstrate that the surface activities of these two caseins are indeed very different.

Figure 4 shows the effects of the ratio of bulk concentrations of α_{S1}-casein and β-casein on the rate and extent of adsorption of each protein at the air−water interface. In these experiments, the ratio of bulk concentrations of α_{S1}-casein to β-casein was varied by keeping the bulk concentration of β-casein fixed at 1.5 mg/mL and varying the concentration of α_{S1}-casein from 0.15 to 3.0 mg/mL; these concentrations corresponded

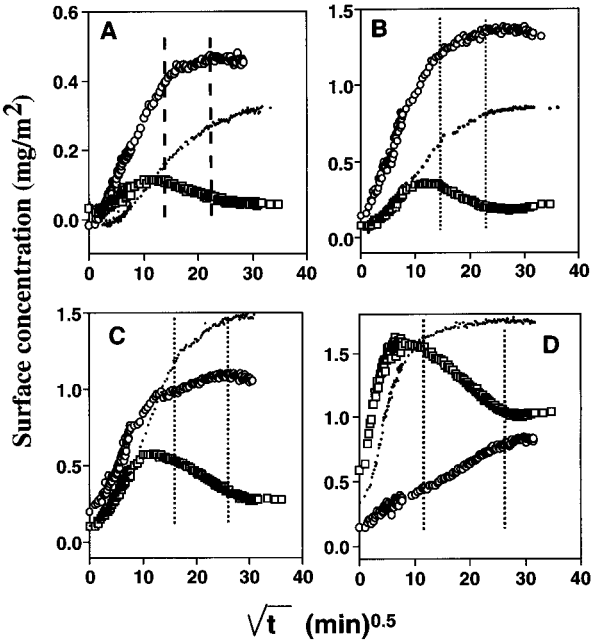

Figure 4. Kinetics of adsorption of α_{S1}-casein (□) and β-casein (○) at air−water interface from binary protein solutions containing 1.5 mg/mL β-casein and increasing concentration of unlabeled α_{S1}-casein. The dotted curves represent kinetics of adsorption of α_{S1}-casein in single-component system at bulk concentrations corresponding to those in the binary systems. The bulk concentrations of α_{S1}-casein were (A) 0.15, (B) 0.45, (C) 0.75, and (D) 3.0 mg/mL. The vertical dotted lines denote the time zones at which α_{S1}-casein is displaced by β-casein. See text for details.

to α_{S1}-casein to β-casein ratios of 0.1−2.0. Also presented in Figure 4 are the effects of bulk concentration of α_{S1}-casein on the time course of its adsorption in binary systems containing 1.5 mg/mL β-casein and in single-component system (at correponding bulk concentrations as in binary systems). In the single-component system, an increase in bulk concentration increased the extent of adsorption of α_{S1}-casein at the air−water interface. At each bulk concentration, the surface concentration of α_{S1}-casein increased linearly with square-root-of-time and reached a steady state value. In the binary systems, however, the surface concentration of α_{S1}-casein increased initially with square-root-of-time and, after reaching a certain maximum surface concentration value, it decreased linearly with square-root-of-time and finally reached a steady state value. In binary mixtures, although the Γ_{eq} of α_{S1}-casein increased with increasing bulk ratio of α_{S1}-casein to β-casein, it was significantly lower than those in the corresponding single-component systems. For example, at a α_{S1}-casein to β-casein bulk concentration ratio of 0.5 (Figure 4C), Γ_{eq} of α_{S1}-casein in the binary system was only about 0.3 mg/m^2, whereas in the single-component system it was about 1.3 mg/m^2. The initial rate of adsorption of α_{S1}-casein in both single-component and binary systems was almost the same at any given bulk concentration, indicating that its rate of adsorption to the air−water interface was not influenced by the presence of β-casein. To ascertain if the desorption phase of the kinetics curves of α_{S1}-casein reflected its displacement from the interface by the adsorbing β-casein molecules, the rates of adsorption of β-casein and desorption of α_{S1}-casein in the time zones indicated by the dotted lines in Figure 4 were compared. As shown in Table 2, the rates were very similar, suggesting that the desorption phase of α_{S1}-casein was essentially due to its displacement by β-casein from the interface.

Table 2. Rate of Displacement of α_{S1}-Casein and Rate of Adsorption of β-Casein during Desorption Phase of α_{S1}-Casein in Binary Systems[a]

α_{S1}-casein/β-casein bulk ratio	rate of desorption of α_{S1}-casein (mg m^{-2} min$^{-1/2}$)	rate of adsorption of β-casein (mg m^{-2} min$^{-1/2}$)
0.1	0.0047	0.0099
0.3	0.0161	0.0153
0.5	0.0184	0.0115
1.0	0.0236	0.0240
2.0	0.0316	0.0238

[a] The rates were calculated from the slopes of the curves of Figure 4 in the time zone indicated by the vertical dotted lines.

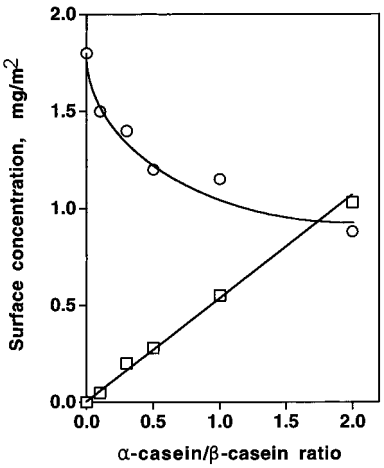

Figure 5. Relationships between surface concentrations of α_{S1}-casein (□) and β-casein (○) and the ratio of bulk concentrations of α_{S1}-casein and β-casein.

Figure 5 summarizes the relationships between Γ_{eq} of α_{S1}-casein and β-casein and the bulk ratio of α_{S1}-casein to β-casein. The Γ_{eq} of β-casein decreased and that of α_{S1}-casein increased with increasing bulk ratio of α_{S1}-casein to β-casein. The Γ_{eq} values of α_{S1}-casein and β-casein in the mixed film were equal when the bulk ratio of α_{S1}-casein to β-casein was 2.

To elucidate if a relationship existed between Γ_{eq} values of α_{S1}-casein and β-casein in mixed protein films, the number of α_{S1}-casein molecules in the mixed film at equilibrium (calculated from its surface concentration) was plotted against the number of β-casein molecules in the film. The plot exhibited a linear relationship with a slope of −1.04 (Figure 6). The value of the slope confirmed that, at monolayer surface coverage, adsorption of one molecule of β-casein at the interface displaced one molecule of α_{S1}-casein or vice versa. This can occur only when the areas occupied by both α_{S1}-casein and β-casein molecules are exactly the same.

Figure 7 shows surface pressures (π) of mixed casein films formed at various ratios of bulk concentrations of α_{S1}-casein to β-casein. It is noteworthy that the protein films formed at bulk concentration ratios 0.5−2.0 exhibited higher surface pressure than those that formed at lower concentration ratios. Probably the mixed casein films formed at 0.5−2.0 bulk concentration ratios possessed optimum protein−protein interactions at the interface and thus exerted a maximum reduction in surface tension.

To gain further information on the dynamic exchange between α_{S1}-casein and β-casein at the air−water interface, experiments were performed involving exchange between similar proteins and displacement of one protein by the other under equilibrium adsorption

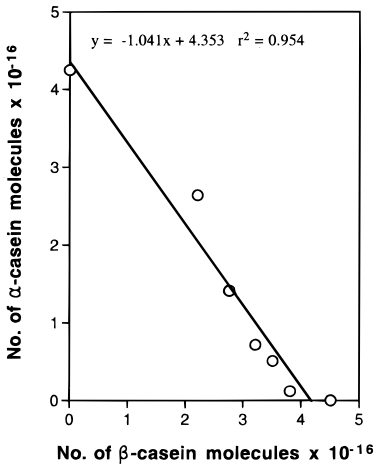

Figure 6. Correlation between the numbers of molecules of α_{S1}-casein and β-casein in adsorbed binary protein films formed from bulk solutions containing different ratios of α_{S1}-casein and β-casein.

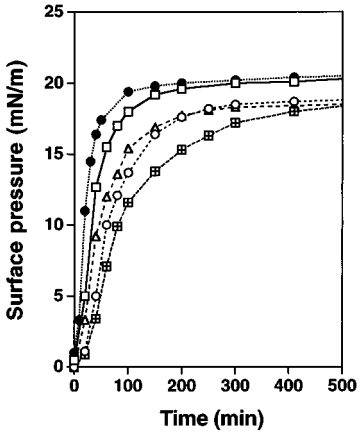

Figure 7. Changes in surface pressure with time of binary protein solutions containing 1.5 mg/mL β-casein and varying amounts of α_{S1}-casein. The bulk concentrations of α_{S1}-casein were (crossed box) 0.15, (○) 0.45, (△) 0.75, (□) 1.5, and (●) 3.0 mg/mL.

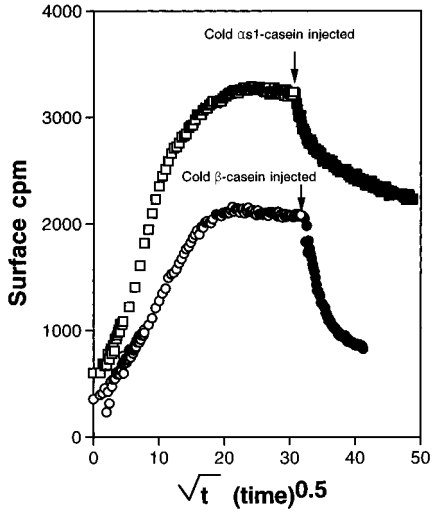

Figure 8. Exchange between adsorbed ^{14}C-labeled protein and bulk phase unlabeled protein of the same kind: (□) α_{S1}-casein; (○) β-casein. The arrow represents the time at which the unlabeled protein was injected (1.5 mg/mL final concentration) into the ^{14}C-labeled solution.

conditions. Figure 8 shows the results of exchange experiments in which ^{14}C-labeled α_{S1}-casein was first allowed to adsorb for 1100 min at the air−water interface from a 1.5 mg/mL bulk solution, after which time an aliquot of unlabeled α_{S1}-casein was injected (1.5 mg/mL final concentration) into the bulk phase. The surface cpm of the adsorbed [^{14}C]-α_{S1}-casein decreased rapidly and reached a new equilibrium value after 2500 min. This decrease in surface cpm was not due to a decrease in surface concentration but to a dynamic exchange between adsorbed [^{14}C]-α_{S1}-casein and bulk phase unlabeled α_{S1}-casein molecules. Similiar behavior also was seen for β-casein (Figure 8).

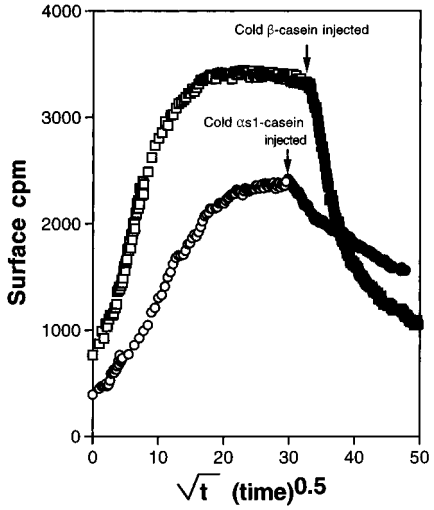

Figure 9. Displacement of ^{14}C-labeled α_{S1}-casein by bulk phase unlabeled β-casein (□) and displacement of ^{14}C-labeled β-casein by bulk phase unlabeled α_{S1}-casein (○). See text and Materials and Methods for details.

Figure 9 shows the results of displacement experiments in which ^{14}C-labeled β-casein was first allowed to adsorb for 1100 min from a 1.5 mg/mL bulk solution, after which time unlabeled α_{S1}-casein was injected (1.5 mg/mL final concentration) into the bulk phase. The surface cpm of adsorbed [^{14}C]-β-casein decreased with time and reached a new equilibrium value after 2500 min. Similarly, when unlabeled β-casein was injected (1.5 mg/mL) into the bulk phase of a ^{14}C-labeled α_{S1}-casein solution (1.5 mg/mL), the surface cpm of the adsorbed [^{14}C]-α_{S1}-casein decreased rapidly with time and reached a new equilibrium value after 2500 min. The data clearly indicated that the bulk phase α_{S1}-casein molecules were able to displace adsorbed β-casein molecules and vice versa. On the basis of these results, it is reasonable to expect that dynamic displacement and exchange should be taking place between bulk phase α_{S1}-casein and β-casein and adsorbed α_{S1}-casein and β-casein molecules under equilibrium conditions as well as during adsorption.

DISCUSSION

Although protein adsorption at interfaces is the first and foremost important step in formation of protein-stabilized foams and emulsions, the factors that affect rates of coadsorption from bulk protein mixtures, interfacial composition, and stability of mixed protein films at interfaces are not well understood. Earlier studies on protein adsorption from binary protein systems involving globular/globular or globular/random coil proteins, viz., β-casein−lysozyme (Xu and Damodaran, 1994), β-casein−serum albumin (Cao and Damodaran, 1995), and lysozyme−serum albumin (Anand and Damodaran, 1995), have shown that the protein

composition of interfacial films formed in these systems was kinetically controlled, not thermodynamically controlled. That is, the protein that arrived first at the interface adsorbed first and could not be displaced by the late arriving protein component. Further, a random coil protein could not displace an adsorbed globular protein, and a globular protein could neither displace nor exchange with an adsorbed globular protein nor displace a random coil protein from an interface. In other words, in the cases of globular/globular and globular/random coil protein binary systems, adsorption essentially followed a noncompetitive (in a thermodynamic sense), irreversible mechanism.

In contrast to the above binary protein systems, the results presented in this paper seem to indicate that adsorption of α_{S1}-casein and β-casein from binary solutions to the air−water interface follows a reversible, thermodynamically controlled competitive adsorption mechanism. The α_{S1}-casein that arrives at the interface first is not irreversibly bound to the interface; the late arriving β-casein displaces the adsorbed α_{S1}-casein. However, careful examination of the results reveals several anomalous behaviors. First, in single-protein systems, the adsorptivities of α_{S1}- and β-caseins to the air−water interface were very similar; the equilibrium binding constants of α_{S1}- and β-caseins to the air−water interface differed only marginally. Yet, in the 1:1 binary mixture system, the ratio of equilibrium surface concentrations of these two caseins was 2:1 ratio in favor of β-casein adsorption, implying β-casein was more surface active than α_{S1}-casein. This suggests that small differences in equilibrium binding constants can exert a large influence on surface activities of proteins. Second, we see that while the initial rate of adsorption of α_{S1}-casein was the same both in the 1:1 mixture and in the single-component systems, the rate of adsorption of β-casein was slower in the 1:1 mixture than in the single-protein system. This fundamental change in the adsorption behavior of β-casein occurs because of the high negative charge of α_{S1}-casein in the mixed film. At any given surface concentration during the course of adsorption, the net charge density (per unit area) of an α_{S1}- plus β-casein mixed film at the interface is greater than that of the β-casein alone film and lesser than that of the α_{S1}-casein alone film. Thus, the strong electrostatic repulsion between the α_{S1}- plus β-casein mixed film and the approaching β-casein molecule seems to slow down the rate of adsorption of β-casein.

Although β-casein adsorbs at a slower rate than α_{S1}-casein does, it is able to displace α_{S1}-casein from the interface and thus becomes the dominant protein in the binary film. β-casein is considered to be more surface active than α_{S1}-casein (Robson and Dalgleish, 1987). If we assume that both of these caseins are random-coil-type flexible proteins and that they experience no conformational constraints to unfold/spread at the interface, then the high surface activity of β-casein must arise from its high mean residue hydrophobicity (Table 1). The small difference in mean residue hydrophobicity, i.e., 1330 *versus* 1170 cal/mol, seems to be sufficient to cause a large difference in interfacial adsorption.

Previous studies on lysozyme/β-casein and BSA/β-casein binary systems have shown that β-casein was unable to displace either lysozyme or BSA from the air−water interface (Xu and Damodaran, 1994; Cao and Damodaran, 1995). It should be pointed out that neither lysozyme nor BSA is as surface active as α_{S1}-casein, and therefore differences in binding affinities alone cannot be responsible for displacement of α_{S1}-casein by β-casein. One possible explanation might be that, since α_{S1}-casein is a highly flexible polymer, the adsorbing β-casein molecules might sequentially desorb the adsorbed segments of α_{S1}-casein from the interface. This may not be possible in the cases of globular lysozyme and BSA, which may require simultaneous desorption of all adsorbed segments.

We propose that, in addition to relative binding affinities, the thermodynamic (in)compatibility of mixing of the proteins in a two-dimensional film also may play a role in the exchange or displacement of one protein by the other in binary systems. Since α_{S1}- and β-caseins are flexible polymers belonging to a similar class of proteins, they may be thermodynamically compatible with each other (Polyakov et al., 1986). This thermodynamic compatibility may allow α_{S1}-casein to completely mix with β-casein (and vice versa) at the interface and thereby displace α_{S1}-casein purely on the basis of differences in affinities to the interface. On the other hand, caseins and globular proteins, such as BSA and lysozyme, are dissimilar in several physicochemical properties and are known to be thermodynamically incompatible and undergo phase separation in solution (Polyakov et al., 1979, 1986). The thermodynamic incompatibility between caseins and globular proteins might prevent mixing of β-casein into a saturated monolayer of BSA or lysozyme film, and vice versa, and thus prevent displacement of one by the other. In other words, thermodynamic compatibility or incompatibility among proteins might be the fundamental mechanism by which displacement and exchange between proteins might occur at interfaces.

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