The Effect of Monoglycerides on Structural and Topographical Characteristics of Adsorbed β-Casein Films at the Air−Water Interface

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The effect of monoglycerides (monopalmitin and monoolein) on the structural and topographical characteristics of β-casein adsorbed film at the air−water interface has been analyzed by means of surface pressure (π)−area (*A*) isotherms and Brewster angle microscopy (BAM). At surface pressures lower than that for the β-casein collapse ($\pi_c^{\beta-\text{casein}}$), attractive interactions between β-casein and monoglycerides were observed. At higher surface pressures, the collapsed β-casein is partially displaced from the interface by monoglycerides. However, β-casein displacement by monoglycerides is not quantitative at the monoglyceride concentrations studied in this work. From the results derived from these experiments, we have concluded that interactions, miscibility, and displacement of proteins by monoglycerides in adsorbed mixed monolayers at the air−water interface depend on the particular protein−monoglyceride system, the interactions between film-forming components being higher for adsorbed than for spread films. The adsorbed films are more segregated than spread films, and both collapsed protein domains and monoglyceride domains in adsorbed films are smaller than for spread films.

Introduction

Two types of emulsifier or foaming agents are used in foods: low molecular weight surfactants (LMWE: mono- and diglycerides, phospholipids, etc.) and macromolecules, such as proteins and some hydrocolloids.[1,2] Although low molecular weight surfactants are more effective than proteins in reducing the interfacial tension, foams and emulsions formed by such surfactants are mostly unstable. This is because proteins, in addition to lowering interfacial tension, can form continuous viscoelastic gel-like films around oil droplets or air cells, whereas the low molecular weight surfactant cannot form such a viscoelastic film.[3−6] Thus, in foods that contain both low molecular weight and macromolecular surfactants, the stability of colloidal dispersed phases is primarily dependent on protein films adsorbed at fluid interfaces.[6,7−11]

The competitive adsorption and/or displacement between LMWE and proteins at fluid−fluid interfaces has been studied in detail in several investigations.[4,6,11−13] However, so far, little is known about the structure that biopolymers and LMWE adopt at fluid interfaces, although in practice mixtures of these emulsifiers are usually used in order to achieve an optimal effect in food formulations. Monolayers at the air−water interface are interesting systems for studying two-dimensional structures of amphiphilic substances.[5,13,14] In addition, insoluble monolayers at the air−water interface have a wide range of applications including models for dispersed systems (emulsions and foams). From a fundamental point of view, interactions, orientation phenomena, and domain structure are of particular interest. In addition, the development of intermolecular associations at the interface leads to alterations in surface properties that have measurable rheological consequences.[15,16] Although monolayer technique has been used successfully for studying the properties of mixed emulsifiers spread at the air−water interface,[13] adsorbed monolayers of mixed emulsifiers are more interesting from a technological point of view. However, there exists little information about these systems so far.[17]

The aim of this work was to analyze the effect of monoglycerides on the interfacial behavior of a model milk protein (β-casein) previously adsorbed at the air−water interface. We will considerer emulsifier (β-casein, monoglycerides, and their mixtures) adsorption, interactions, structure, and topography at the interface, as related to the formation and stability of food dispersions (emulsions and foams). This paper complements previous works on pure proteins[18−20] and protein−monoglyceride mixed monolayers[17] adsorbed at the air−water interface.

Experimental Section

Chemicals. Synthetic 1-monohexadecanoyl-*rac*-glycerol (monopalmitin, DIMODAN PA 90) and 1-mono-(*cis*-9-octadecanoyl) glycerol (monoolein, RYLO MG 19) were supplied by Danisco Ingredients (Brabran, Denmark) with over 95−98% purity. β-Casein (>99%) was supplied and purified from bulk milk from the Hannah Research Institute (Ayr, Scotland). Samples for interfacial characteristics of β-casein adsorbed films were prepared using Milli-Q ultrapure water and were buffered at pH 7. To form the mixed surface film on a previously adsorbed β-casein monolayer, monoglyceride was spread in the form of a solution, using hexane/ethanol (9:1, v/v) as a spreading solvent. Analytical-grade hexane (Merck, 99%) and ethanol (Merck, >99.8%) were used. The water used as subphase was purified by means of a Millipore filtration device (Milli-Q). A commercial buffer solution called trizma ($(CH_2OH)_3CNH_2$/$(CH_2OH)_3CNH_3Cl$, Sigma, >99.5%) was used to achieve pH 7. Ionic strength was 0.05 M in all the experiments.

Surface Film Balance. Measurements of surface pressure (π)−area (*A*) isotherms of β-casein−monoglyceride mixed films at the air−water

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interface were performed on a fully automated Langmuir-type film balance as described previously.[21] Before each measurement, the film balance was calibrated at 20 °C. For protein-adsorbed films from water, a protein solution at $5 \cdot 10^{-6}$ to $7.5 \cdot 10^{-6}$ % (wt/wt) was left in the trough and time allowed for protein adsorption at the interface. These protein concentrations were selected from previous data of the adsorption isotherm.[22] At this protein concentration in solution, the surface pressure at equilibrium is zero. In fact, after 24 h the surface pressure (π) at the maximum area of the trough was practically zero. At this point, the monoglyceride (0.01−0.05 mg) was spread at different points on the β-casein film. For pure adsorbed protein films, the maximum protein concentration in the bulk phase should be selected in order to obtain a reasonable rate of adsorption at the interface, but maintaining the surface pressure at zero.[19] On the other hand, for mixed films, at low protein concentrations in the aqueous phase we cannot observe the collapse point, especially for low monoglyceride concentrations. Thus, in these experiments, we have selected optimum conditions in order to obtain the complete $\pi-A$ isotherm of the mixed film, from the more expanded monolayer (at the higher areas) to the more condensed monolayer, at the collapse point (at the lower areas). Mixtures of particular mass ratios—expressed as the mass fraction of monopalmitin, X_{MP}, or monoolein, X_{MO}, in the mixture—were studied. The compression rate was 3.3 cm·min^{-1}, which is the highest value for which isotherms were found to be reproducible in preliminary experiments. The $\pi-A$ isotherm was measured five times. The reproducibility of the results was better than ±0.5 mN/m for surface pressure and ±0.05 m^2/mg for area.

Brewster Angle Microscopy (BAM). A commercial Brewster angle microscope (BAM), BAM2, manufactured by NFT (Göttingen, Germany) was used to study the topography of the monolayer. The BAM was positioned over the film balance. Further characteristics of the device and operational conditions have been described elsewhere.[23,24] Measurements of the surface pressure, area, and gray level as a function of time were carried out simultaneously by means of a device connected between the film balance and the BAM. To measure the relative reflectivity (I) of the film, a previous camera calibration is necessary.[23,24] The imaging conditions were adjusted to optimize both image quality and quantitative measurement of reflectivity. Thus, generally, as the surface pressure or the protein content increased the shutter speed was also increased.

Results and Discussion

Structural and Topographical Characteristics of β-Casein Monolayer Adsorbed at the Air−Water Interface. Figure 1A shows the π-trough area isotherms for an adsorbed monolayer of β-casein after successive compressions, formed from adsorption in solution at $5 \cdot 10^{-6}$ to $7.5 \cdot 10^{-6}$ %, wt/wt. There was a difference in the π-trough area isotherms as a function of time after protein addition to the aqueous bulk phase. It can be seen that there was a shift of the π-trough area isotherms toward higher areas as the protein adsorption time increased. The π-trough area isotherm for a first compression at 30 min of adsorption time (data not shown) indicates that a low amount of protein was adsorbed at the interface, because the surface pressure at the minimum area was much lower than the equilibrium surface pressure for β-casein ($\pi_e \cong 20.9$ mN/m).[22] The maximum surface pressure also increased with the aging time. These data reveal that a long time interval of adsorption allows more β-casein to adsorb at the surface, especially from low protein concentrations in solution as those used in this work. After 23 h of adsorption time, the π-trough area isotherms after successive compressions were practically coincident (data not shown). In addition, the π-trough area isotherms at long-term adsorption were reproducible after repeated experiments using new aliquots of the protein in solution, for different protein concentrations in the range $5 \cdot 10^{-6}$ to $7.5 \cdot 10^{-6}$ %, wt/wt (data not shown). These results demonstrate that it is possible to measure reproducible π-trough area isotherms for adsorbed β-casein monolayers from low protein concentration in the bulk phase, as observed for globular proteins.[17,19] This is clear evidence that the π-trough area isotherms obtained after long adsorption time have a thermodynamic character.

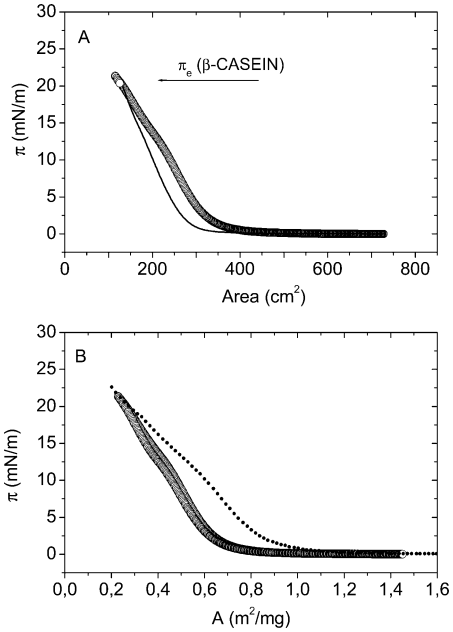

Figure 1. (A) π-Trough area isotherms for an adsorbed monolayer of β-casein after successive compressions: (—) 6.5 h, (○) 23 h. (B) $\pi-A$ isotherms for (○) adsorbed and (●) spread β-casein monolayers. Aqueous subphase at pH 7. Temperature 20 °C. The π_e of β-casein is indicated by means of an arrow.

Since the surface concentration is actually unknown for the adsorbed monolayer, the values were derived by assuming (Figure 1B) that the A values for adsorbed and spread monolayers were equal at the collapse point of the mixed film.[19,25] This assumption can be supported by the fact that for protein films, the equilibrium spreading pressure (π_e), the surface pressure at the plateau for a saturated β-casein adsorbed film,[22] and the collapse pressure for adsorbed (Figure 1A) and spread[24,26] β-casein monolayers are practically equal.

The $\pi-A$ isotherm deduced for adsorbed β-casein monolayer is more condensed than that obtained directly by spreading (Figure 1B). One speculation is that a disordered protein (like β-casein) can be adsorbed at the interface, maintaining part of its secondary and tertiary structure. However, the same protein may be more unfolded during the spreading at the interface at high mass areas.[24] Thus, the structures of the monolayers formed in the two different ways must be different, at least for adsorption from low bulk protein concentrations. As opposed to β-casein, globular proteins show a good agreement between the adsorbed and spread isotherms.[17,18,22,25,27] These results indicate that a globular protein maintains part of its native structure (in the form of loops and tails) as it is spread or adsorbed at the interface.

The results of the $\pi-A$ isotherms (Figure 1B) with the help of the compressional coefficient (data not shown) deduced from the slope of the $\pi-A$ isotherm ($\kappa = -d\pi/dA$) indicate that adsorbed β-casein monolayers at the air−water interface adopt two different structures or condensation states and the collapse phase. The surface pressure at the transition of an adsorbed β-casein monolayer ($\pi_t^a \approx 12.1$ mN/m) is higher than for a spread monolayer ($\pi_t^s \approx 10$ mN/m).[24] At low surface pressures (at $\pi < 12.1$ mN/m), adsorbed β-casein monolayers exist as

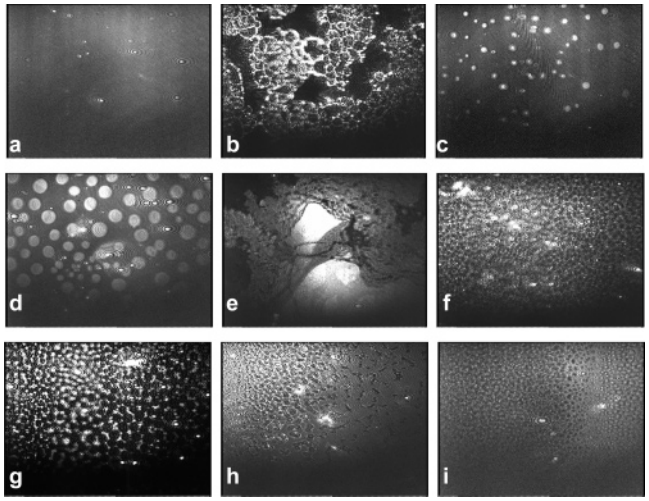

Figure 2. Visualization of β-casein−monoglyceride mixed monolayers by Brewster angle microscopy at 20 °C. (a) This picture was observed for β-casein, monoolein, monopalmitin, and β-casein−monopalmitin mixed films at $\pi < 5-7$ mN/m, or β-casein−monoolein mixed films at $\pi < \pi_c$ (β-casein). (b) β-Casein aggregates at the beginning of the compression in pure and mixed films. (c) Domains of monopalmitin in β-casein−monopalmitin mixed films at $\pi \cong 5-7$ mN/m and at $X_{MP} = 0.5$. (d) Domains of monopalmitin in β-casein−monopalmitin mixed films at $\pi > 10$ mN/m and at $X_{MP} = 0.5$. (e) The beginning of the squeezing out of β-casein by monopalmitin at $\pi \cong \pi_c$ (β-casein). (f) Microdomains of monopalmitin in β-casein−monopalmitin mixed films at $\pi > \pi_c$ (β-casein) and at $X_{MP} = 0.2$. (g) Microdomains of monopalmitin in β-casein−monopalmitin mixed films at $\pi > \pi_c$ (β-casein) and at $X_{MP} = 0.5$. (h) β-Casein−monopalmitin mixed films at the collapse point of the mixed film. (i) Two-dimensional foams in β-casein−monoglyceride mixed films at $\pi \cong 0$. The horizontal direction of the image corresponds to 630 μm and the vertical direction to 470 μm.

trains with amino acid segments located at the interface (structure **1**). At higher surface pressures (at $\pi > 12.1$ mN/m), and up to the equilibrium surface pressure, amino acid segments are extended into the underlying aqueous solution and adopt the form of loops and tails (structure **2**). The monolayer collapses at a surface pressure ($\pi_c^{\beta-\text{casein}}$) of about 21 mN/m (Figure 1B), a value close to the surface pressure at the plateau for a saturated adsorbed film and the equilibrium surface pressure.[22]

The topography (Figure 2) and, especially, the relative reflectivity (Figure 3) as a function of surface pressure obtained with adsorbed β-casein monolayers show that the domains that residues of protein molecules adopt at the air−water interface appear to be of uniform reflectivity (Figure 2a), suggesting homogeneity in thickness and film isotropy with dust (small particles from the air) causing the only visible features. The $I-\pi$ dependence (Figure 3) is characteristic for this protein and is independent of the experimental conditions adopted, either for spread[21] or adsorbed monolayers (Figure 3). The relative monolayer thickness increases with the surface pressure and is a maximum at the collapse, at the highest surface pressure. The increase in reflected light intensity with surface pressure, and especially at the monolayer collapse, suggests that an increase in the monolayer thickness from more expanded to more condensed structure and a further increase at the monolayer collapse take place. At the highest surface pressure, $I = 1.7 \cdot 10^{-6}$ au, which means that for adsorbed β-casein the monolayer thickness is of the same order of magnitude as for milk and soy globulin spread monolayers at the air−water interface.[20] Minor regions with reflectivity peaks were observed, even at the beginning of the compression (at $\pi \cong 0$ mN/m). Even for a visible "homogeneous" interface (Figure 2A), the reflectivity

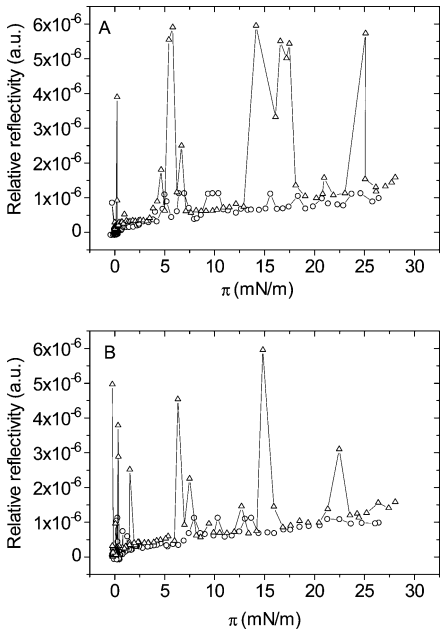

Figure 3. Relative reflectivity (arbitrary units) as a function of surface pressure during the (A) compression and (B) expansion for an adsorbed β-casein monolayer on buffered water at pH 7 and at 20 °C. Shutter speed (*s*): (○) $^1/_{50}$, (△) $^1/_{250}$.

peaks (Figure 3), which are an indication of the local heterogeneity of the interface at a microscopic level, are due to the existence of isolated interfacial regions with folds or aggregations (Figure 2b) of collapsed β-casein formed at the higher surface pressures. These heterogeneities were present at the interface during the monolayer expansion, even at the lower surface pressures (Figure 3). The domains of collapsed β-casein for adsorbed monolayers (Figure 2b) were not observed for spread monolayers.[21] At a microscopic level, the compression−expansion cycle was reversible, because the film adopted practically the same states (Figure 3).

Structural and Topographical Characteristics of *β*-Casein−Monopalmitin Mixed Monolayers Adsorbed at the Air−Water Interface. Mixtures of particular mass ratios—ranging between 0 and 0.5, expressed as the mass fraction of monopalmitin in the mixture, X_{MP}—were studied. The amount of spread monoglyceride was calculated on the basis of the mass of previously adsorbed ***β***-casein, which was calculated form the adsorbed ***π***−*A* isotherm. Thus, as opposed to spread monolayers,[21] for adsorbed monolayers the mixtures with mass fractions higher than $X_{MP} = 0.5$ saturate the interface, under the experimental conditions used in this work.

The surface pressure as a function of the trough area for β-casein + monopalmitin mixed films during a compression−expansion cycle is shown in Figure 4A. As for pure β-casein adsorbed film, the actual $\pi-A$ isotherm for β-casein + monopalmitin mixed films was derived by assuming[17] that the *A* value for adsorbed and spread monolayers was equal at the collapse point (Figure 4B,C). This assumption can be supported by the fact that the surface pressure at the collapse point for adsorbed (Figure 4) and spread[21] mixed films is practically equal to that for the pure monoglyceride. This procedure and the comparison between adsorbed and spread $\pi-A$ isotherms are illustrated in Figure 4(B and C) for two representative mass fractions of monopalmitin in the mixture.

From the $\pi-A$ isotherms for β-casein + monopalmitin mixed films (Figure 5A), it can be seen that there was a monolayer expansion as the monopalmitin concentration in the mixture was

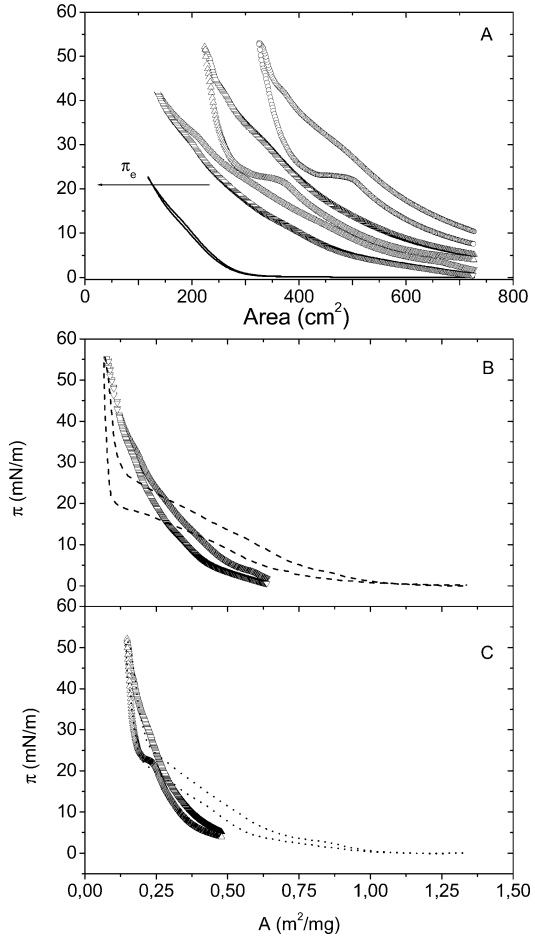

Figure 4. (A) Surface pressure−trough area isotherms (compression−expansion curves) for adsorbed β-casein−monopalmitin mixed monolayers on buffered water at pH 7 and at 20 °C. Mass fraction of monopalmitin in the mixture (X): (—) 0, (▽) 0.2, (△) 0.4, and (○) 0.5. (B) $\pi-A$ isotherms for (▽) adsorbed and (- - -) spread[21] β-casein−monopalmitin mixed monolayers at $X = 0.2$. (C) $\pi-A$ isotherms for (△) adsorbed and (·····) spread [21] β-casein−monopalmitin mixed monolayers at $X = 0.4$. The π_e of β-casein is indicated by means of an arrow.

increased, especially at higher surface pressures. That is, the $\pi-A$ isotherm is displaced toward higher A as the concentration of monopalmitin in the mixture increases. At surface pressures higher than that for β-casein collapse, the $\pi-A$ isotherm for mixed monolayers was parallel to that of monopalmitin. These data are in agreement with those deduced for spread β-casein + monopalmitin[21] and β-lactoglobulin + monopalmitin[28−30] mixed films.

We present in Figure 6 hypothetical $\pi-A$ isotherms for mixed monolayers calculated on the basis that only monopalmitin is present at the air−water interface. It must be emphasized that, because of this assumption, in Figure 6 the area in the X-axis is not the true area per unit mass of mixed film but the apparent area. For this reason, the X-axis in Figure 5A (for true area per unit mass of mixed film) is different from that in Figure 6 (for apparent area per unit mass of monoglyceride in the mixture). We can see that, supposing that the mixed monolayers are dominated by monopalmitin (Figure 6A), the $\pi-A$ isotherms for monopalmitin and β-casein-monopalmitin mixed monolayers at surface pressures higher than that for the β-casein collapse ($\pi_c^{\beta-\text{casein}}$) are practically coincident. In contrast to the above data, $\pi-A$ isotherms calculated on the basis that the mixed monolayers are dominated by β-casein (data not shown) are totally different from those for pure components under all

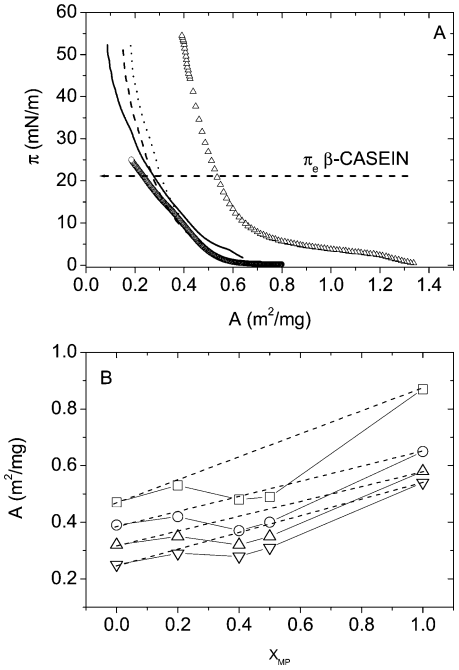

Figure 5. (A) Surface pressure−area isotherms (compression curves) for adsorbed β-casein−monopalmitin mixed monolayers on buffered water at pH 7 and at 20 °C. Mass fraction of monopalmitin in the mixture (X): (○) 0, (—) 0.2, (- - -) 0.4, (·····) 0.5, and (△) 1.0. (B) Area as a function of mass fraction of monopalmitin in the mixture (X_{MP}) and surface pressure (mN/m): (□) 5, (○) 10, (△) 15, and (▽) 20. The π_e of β-casein is indicated by means of an arrow.

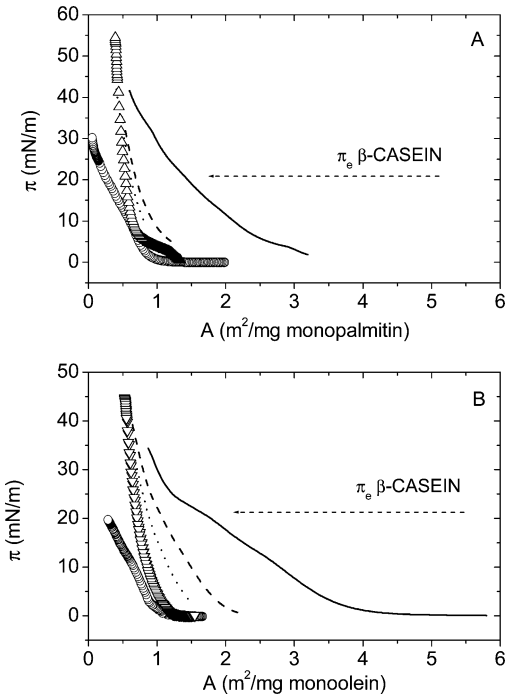

Figure 6. Surface pressure−area isotherms (compression curves) for adsorbed (A) β-casein−monopalmitin and (B) β-casein−monoolein mixed monolayers on buffered water at pH 7 and at 20 °C. Mass fraction of monoglyceride in the mixture (X): (○) 0, (—) 0.2, (- - -) 0.4, (·····) 0.5, and (△, ▽) 1.0. The mass area was calculated on the basis that only the monoglyceride was adsorbed on the interface. The π_e of β-casein is indicated by means of an arrow.

experimental conditions. These results suggest that at $\pi > \pi_c^{\beta-\text{casein}}$ a protein displacement by the monoglyceride from the air−water interface takes place. At $\pi < \pi_c^{\beta-\text{casein}}$, both β-casein and monopalmitin coexist at the interface, and the adsorbed and

spread $\pi-A$ isotherms (i.e., the monolayer structures) are different (Figure 4B,C).

The interactions between film-forming components in mixed monolayers can be studied from the point of view of miscibility by means of the excess area, $A_{exc} - A_{exc} = A - (A_1 \cdot X_1 + A_2 \cdot X_2)$, where A is the mass area at a given surface pressure for the mixed monolayer, A_1 and A_2 are the mass areas of pure components, and X_1 and X_2 are the mass fractions of pure components in the mixed monolayer. For adsorbed β-casein−monopalmitin mixed monolayers (Figure 5B), the excess area was negative at high concentrations of monopalmitin in the mixture ($X_{MP} > 0.2$), especially at low surface pressures. These results and those deduced from $\pi-A$ isotherms prove that adsorbed β-casein and monopalmitin form a mixed monolayer at the air−water interface with attractive interactions between film-forming components, at surface pressures lower than that for the β-casein collapse ($\pi_c^{\beta-casein} \approx 21$ mN/m). The attractive interactions in the mixed film decrease with the surface pressure, as the film-forming components adopt a similar liquidlike structure. At the highest surface pressures, at the collapse point of the mixed film, immiscibility between monolayer-forming components is deduced because the collapse pressure of mixed monolayers is similar to that of pure monoglyceride monolayer (Figure 5A).

The fact that, upon expansion and further compression, the $\pi-A$ isotherms were repetitive (data not shown) suggests that the protein reenters the mixed monolayer and supports the idea that the protein remains underneath the monoglyceride film either through hydrophobic interactions between protein and lipid or by local anchoring through the monoglyceride layer.[17,21,31] However, for adsorbed β-casein−monopalmitin mixed monolayers, a first-order-like phase transition was observed upon the monolayer expansion (Figure 4A) at surface pressures close to the equilibrium surface pressure of β-casein−with a degenerated plateau in the $\pi-A$ isotherm. Interestingly, this plateau is more evident and more extended at higher concentrations of monopalmitin in the mixture. This result suggests that the readsorption of previously displaced β-casein has a kinetic character,[17] which was not evident for spread mixed films.[21] Moreover, the readsorption of previously displaced β-casein is hindered as the concentration of monopalmitin in the mixture increases.

The evolution with the surface pressure of BAM images (Figure 2) and relative reflectivity (Figure 7) gives complementary information, at a microscopic level, on the structural characteristics and interactions of adsorbed β-casein−monopalmitin mixed monolayers, as deduced from $\pi-A$ isotherms (Figure 5). Briefly, the results reported here suggest that, in β-casein−monopalmitin mixed films, islands of protein and monoglyceride do exist at the air−water interface, but the interactions between them depend on the surface pressure and the composition of the mixed film. At $\pi < \pi_c^{\beta-casein}$, a mixed monolayer of monopalmitin and β-casein may exist (Figure 2c) with small domains of monopalmitin (with a liquid condensed structure at $\pi > 5$ mN/m) uniformly distributed on the homogeneous β-casein layer. The circular domains of liquid condensed monopalmitin were more numerous as the surface pressure increased (Figure 2d). At the collapse point of β-casein, a characteristic squeezing out phenomena of β-casein by monopalmitin was observed (Figure 2e). At $\pi > \pi_c^{\beta-casein}$, the mixed monolayers were practically dominated by monopalmitin molecules. That is, at higher surface pressures, collapsed β-casein residues (bright region) may be displaced from the interface by monopalmitin molecules. This squeezing out phenomenon is observed in BAM images (Figure 2f,g) with small liquid condensed domains of monopalmitin (dark circle) floating over a sublayer of collapsed residues of β-casein (bright region). However, the topography of the mixed film also proves the existence of some degree of interactions between film-forming components, because the size of monopalmitin liquid condensed domains in the mixed film are smaller than that for a pure monopalmitin monolayer.[23] In addition, both regions are not clearly separated because of some miscibility between them, a phenomenon that was not observed for spread mixed films. Another topographical characteristic of the adsorbed film (not observed in spread mixed film[21]) was the presence of short fractures in the monolayer at the collapse point of the mixed film (Figure 2h), which are characteristic of protein−monoglyceride adsorbed films.[17] Finally, after the expansion at $\pi \approx 0$, the monolayer undergoes breaking of the collapse structure up to a 2D foam structure (Figure 2i).

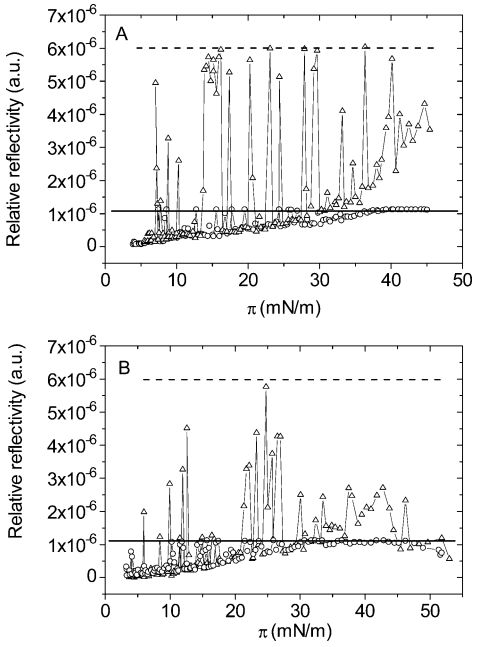

Figure 7. Relative reflectivity (arbitrary units) as a function of surface pressure during the compression for adsorbed β-casein−monopalmitin mixed monolayers on buffered water at pH 7 and at 20 °C. Monopalmitin mass fraction in the mixture: (A) 0.25 and (B) 0.5. The maximum reflectivity for monopalmitin (continuous line) and adsorbed β-casein (discontinuous line) pure monolayers are included in plots (A) and (B). Shutter speed (*s*): (○) $^1/_{50}$, (△) $^1/_{250}$.

These results have shown that over the entire range of existence of the mixed film the monolayer presents some heterogeneity, because domains of monopalmitin and β-casein residues are present during the monolayer compression−expansion cycle, giving *I*-peaks of collapsed β-casein with high relative film thickness (Figure 7). Interestingly, the reflectivity of the mixed film at $\pi > \pi_c^{\beta-casein}$ is higher than that for pure monopalmitin (Figure 7). These results strengthen the hypothesis that for adsorbed β-casein + monopalmitin mixed films some degree of attractive interactions between film-forming components do exist at a microscopic level, giving a mixed film with greater thickness compared with those of pure components. At $\pi < \pi_c^{\beta-casein}$, the segregation between LC monopalmitin domains and β-casein gives a mixed film with lower thickness compared with those of pure components.

Structural and Topographical Characteristics of β-Casein−Monoolein Mixed Monolayers Adsorbed at the Air−Water Interface. The structural characteristics of adsorbed β-casein−monoolein mixed monolayers were essentially different from

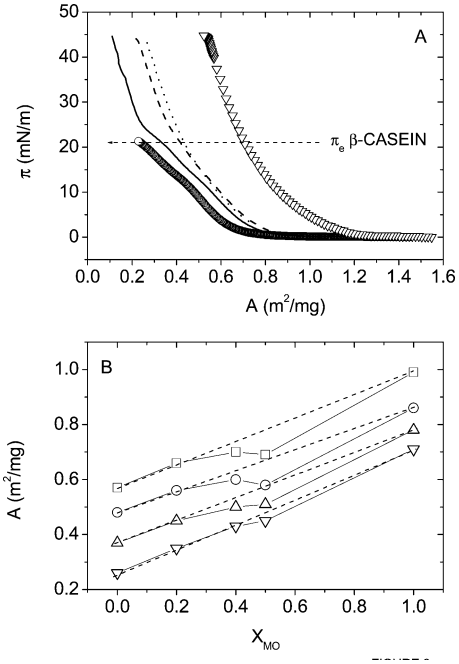

Figure 8. (A) Surface pressure−area isotherms (compression curves) for adsorbed β-casein−monoolein mixed monolayers on buffered water at pH 7 and at 20 °C. Mass fraction of monoolein in the mixture (X): (○) 0, (—) 0.2, (- - -) 0.4, (·····) 0.5, and (▽) 1.0. (B) Area as a function of mass fraction of monoolein in the mixture (X_{MO}) and surface pressure (mN/m): (□) 5, (○) 10, (△) 15, and (▽) 20. The π_e of β-casein is indicated by means of an arrow.

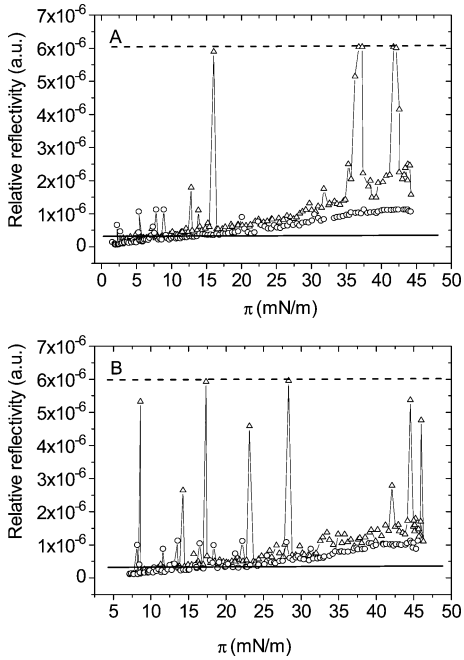

Figure 9. Relative reflectivity (arbitrary units) as a function of surface pressure during the compression for adsorbed β-casein−monoolein mixed monolayers on buffered water at pH 7 and at 20 °C. Monoolein mass fraction in the mixture: (A) 0.25 and (B) 0.5. The maximum reflectivity for monoolein (continuous line) and adsorbed β-casein (discontinuous line) pure monolayers are included in plots (A) and (B). Shutter speed (s): (○) $^1/_{50}$, (△) $^1/_{250}$.

those of monopalmitin in the mixture, as deduced from $\pi-A$ isotherms (Figure 8A) and excess area (Figure 8B). Briefly, as expected,[21] β-casein−monoolein mixed films (Figure 8) at surface pressures lower than that for β-casein collapse ($\pi_c^{\beta-\text{casein}} \cong 21$ mN/m) adopt a liquidlike expanded structure, as for pure components. There was a monolayer expansion due to the presence of monoolein in the mixture. At $\pi > \pi_c^{\beta-\text{casein}}$, the $\pi-A$ isotherm for mixed monolayers was practically parallel to that of monoolein. At these experimental conditions, the hypothetical $\pi-A$ isotherms for mixed monolayers calculated on the basis that only monoolein is present at the air−water interface tend to that of pure monoolein monolayer (Figure 6B). From the point of view of miscibility (Figure 8B), the excess area was practically zero at low concentrations of monoolein in the mixture, but it falls to negative values at higher monoolein concentrations in the mixed film, especially at low surface pressures. However, the excess area was lower compared with β-casein−monopalmitin mixed films (Figure 5B). These results prove the existence of minor attractive interactions between monoolein and β-casein at the air−water interface, especially at the lower surface pressures. At the highest surface pressures, at the collapse point of the mixed film, immiscibility between monolayer-forming components is deduced because the collapse pressure of mixed monolayers is similar to that of pure monoolein monolayer (Figure 8A).

BAM images for adsorbed β-casein−monoolein mixed monolayers (Figure 2a) were different from those described above for adsorbed β-casein−monopalmitin mixed monolayers (Figure 2). In fact, at $\pi < \pi_c^{\beta-\text{casein}}$ (≈21 mN/m), the topographies of pure components and the mixed monolayer are practically identical, because in this region, both components and the mixed monolayer form an isotropic (homogeneous) monolayer without any difference in the domain topography (Figure 2a). At surface pressures near and after β-casein collapse, BAM images (data not shown) demonstrated that monoolein and β-casein molecules adopted an isotropic structure in the mixed monolayer with some white regions, which correspond to the presence of a thicker β-casein collapsed monolayer. At the higher surface pressures, and especially at the collapse point, the topography of the mixed monolayer was dominated by the presence of small domains of collapsed β-casein and monoolein at the interface.

These results and those for β-casein + monopalmitin mixed films prove that at a microscopic level (Figure 2) (i) some degree of interaction between monoglycerides and β-casein in adsorbed films does exist at the air−water interface, and (ii) a monoglyceride monolayer spread on a previously adsorbed β-casein film was unable to completely displace β-casein molecules from the air−water interface, even at the highest surface pressures.

The $I-\pi$ plots for the adsorbed β-casein−monoolein mixed monolayers are shown in Figure 9. These results are essentially similar to those recorded for adsorbed β-casein−monopalmitin mixed monolayers (Figure 7). Thus, the same reasoning as above can be applied here.

Displacement of Adsorbed β-Casein by Monoglycerides from the Air−Water Interface. The displacement of adsorbed β-casein by monoglycerides from the air−water interface depends on the protein−monoglyceride system. From the hypothetical isotherms shown in Figure 6, we define a displacement surface pressure (π_d) as the minimum surface pressure above which the $\pi-A$ isotherms for monoglyceride and monoglyceride−protein mixed monolayers are coincident. Thus, at low π_d, protein displacement by the monoglyceride is facilitated. In Figure 10, we show the π_d as a function of the mixture composition for different β-casein−monoglyceride mixed films. At low monoglyceride concentration (at $X = 0.2$), the β-casein displacement is easier for monoolein than for monopalmitin. In fact, under these conditions, it is necessary to compress the mixed film up to the π_e value of the monoglyceride, which is higher for monopalmitin than for

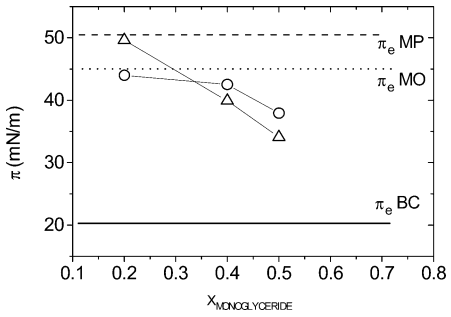

Figure 10. The displacement surface pressure of β-casein adsorbed film by (△) monopalmitin and (○) monoolein spread at the air−water interface as a function of mass fraction of monoglyceride in the mixture. The horizontal lines represent the equilibrium surface pressure of pure β-casein (—, π_e β-casein), monopalmitin (- - -, π_e MP), and monoolein (·····, π_e MO) films at 20 °C and at pH 7.

monoolein. However, the opposite is observed at higher monoglyceride concentrations. In fact, at $X \geq 0.4$ the π_d value for β-casein−monopalmitin mixed films is lower than that for β-casein−monoolein mixed films. That is, under these conditions, the β-casein displacement is easier for monopalmitin than for monoolein. However, higher monoglyceride concentrations in the mixture are necessary for a quantitative displacement of β-casein from the interface, as observed for β-casein−monoglyceride spread films.[21] That is, the capacity of a monoglyceride for β-casein displacement from the air−water interface is easier for spread[21] than for adsorbed (Figure 10) monolayers. Finally, the displacement of a protein by a monoglyceride from the air−water interface is easier for β-lactoglobulin−monoglyceride[17] than for β-casein−monoglyceride (this work) mixed monolayers.

Conclusions

From the results derived from these experiments, it can be concluded that interactions, miscibility, and displacement of β-casein by monoglycerides (monopalmitin and monoolein) in adsorbed mixed monolayers at the air−water interface depend on the particular β-casein−monoglyceride system. β-Casein−monoglyceride mixed monolayers form a practically immiscible monolayer at the air−water interface at surface pressures higher than that for the β-casein collapse (at $\pi_c^{\beta-\text{casein}} \approx 21$ mN/m). However, at lower surface pressures, attractive interactions between β-casein and monoglycerides were observed, especially for β-casein−monopalmitin mixed monolayers. At higher surface pressures, the collapsed β-casein is partially displaced from the interface by monoglyceride (either monopalmitin or monoolein). The β-casein displacement by monoglycerides is not quantitative at the monoglyceride concentrations studied in this work and at the highest surface pressure, at the collapse point of the mixed monolayer. The displacement of a protein from the air−water interface by monoglycerides is different for spread and adsorbed films and is easier for β-lactoglobulin− than for β-casein−monoglyceride mixed monolayers. Some differences between adsorbed and spread mixed films are as follows: (i) The interactions between film-forming components are higher for adsorbed than for spread mixed films, (ii) the adsorbed films are more segregated than spread films, and (iii) the collapsed β-casein domains in adsorbed films are smaller than for spread films. All these phenomena are a consequence of the increased interactions between components at the interface in the former. Finally, (iv) the readsorption of previously displaced β-casein (or β-lactoglobulin) is slower for adsorbed than for spread films. These results have direct relevance to product processing (specially for foam formation and stabilization), as a model study for extrapolation to more complex real systems (food foams and emulsions), in the specific areas of biopolymer science and biological and biomedical physicochemistry and physiology (i.e., for pulmonary surfactant systems),[32−34] and in general for understanding interactions of lipids with proteins.

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