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# Effect of Processing on the Displacement of Whey Proteins: Applying the Orogenic Model to a Real System

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Atomic force microscopy (AFM) has been used to investigate the displacement of a commercial whey protein system and the behavior as compared to that of β-lactoglobulin (Mackie, A. R.; Gunning, A. P.; Wilde, P. J.; Morris, V. J. Orogenic displacement of protein from the air−water interface by competitive adsorption. *J. Colloid Interface Sci.* **1999**, *210*, 157−166). The whey protein isolate (WPI) was displaced from an air−water interface by the surfactants Tween 20 and Tween 60. Displacement data obtained were compared with data obtained for pure β-lactoglobulin and have shown that WPI was more resistant to displacement from the air−water interface than native β-lactoglobulin. This was related to the greater surface elasticity of WPI at higher surface stresses. In the presence of Tween 20, WPI was observed to remain on the interface at surface pressures up to 8 mN/m greater than the surface pressure at which complete displacement of β-lactoglobulin was observed. Displacement of WPI and β-lactoglobulin films by the surfactant Tween 60 showed similar results. However, because of the lower surface activity of Tween 60, it was not possible to reach surface tension values similar to those obtained for Tween 20. Despite the lower surface activity of Tween 60, WPI was still observed to be present at the interface at surface pressure values greater than those by which β-lactoglobulin had been completely displaced.

**KEYWORDS: Protein; surfactant; displacement; orogenic; atomic force microscopy; interfacial properties**

## INTRODUCTION

In the food industry, the role of emulsifiers in the stabilization of foams and emulsions is of widespread technological importance. Emulsifiers can generally be classified into two main groups: large molecules, such as proteins, or small molecules, for example, surfactants. Proteins are capable of stabilizing an interface by forming an immobile viscoelastic film resistant to stress. Small molecule surfactants are highly mobile and able to migrate rapidly to areas of low concentration in order to maintain a homogeneous, stable interface. These two mechanisms are essentially mutually incompatible, and in most studies of mixed protein−surfactant systems, reduced stability has been observed as compared to the individual components. As the two types of emulsifier compete for the same interface, adsorption of surfactant into the protein film diminishes the viscoelastic network. Similarly, residual protein at the interface restricts diffusion of surfactant molecules. Consequently, conflict between these two mechanisms may cause destabilization of the foam or emulsion.

Despite the technological implications of competitive displacement between proteins and surfactants, until recently, investigations into their behavior at interfaces have been limited to techniques that consider the macroscopic behavior of the interfaces. For example, protein loading measurements (*1*, *2*), surface and interfacial tension measurements (*2*), and surface rheology (*3*−*6*) have been used, allowing little insight into the mechanisms occurring at the molecular level. In recent years, atomic force microscopy (AFM) has been used to observe the behavior of proteins and surfactants at both the air−water (*7*) and the oil−water interface (*8*), leading to the proposal of an orogenic displacement model. The model states that surfactant adsorbs at defects within the protein layer forming domains; as these domains increase in size, the protein film is compressed until it ruptures allowing protein to be displaced into the bulk phase and the surfactant to colonize the interface. In addition to AFM, alternative techniques such as Brewster angle microscopy (BAM) (*9*−*11*) and scanning near-field optical microscopy (SNOM) (*12*) have also been used to visualize protein−surfactant interactions at interfaces.

Previous work carried out using AFM has been limited to work on pure milk proteins that have been studied both individually and as a mixture. The aim of this work was to test the applicability of the generic principles of the orogenic model to real protein systems commonly used in the food industry. The displacement of a whey protein isolate (WPI) by the surfactants Tween 20 and Tween 60 has been investigated and compared with data obtained for β-lactoglobulin. In addition to the displacement data obtained from the AFM, the surface activity and rheological properties of these emulsifiers have been

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investigated and are discussed in order to understand the complex interfacial behavior of real protein systems.

## MATERIALS AND METHODS

**Materials.** WPI Bipro 95 (97.8% protein, Davisco Foods International, Inc.) and Tween 60 (Quest International, Kent, U.K.) were supplied by Unilever plc. Solutions of the WPI Bipro 95 were prepared with surface pure water obtained from an Elga Elgastat UHQ water purification system. Tween 20 was obtained as a 10% solution from Pierce (Rockford, IL). Tween 60 solutions were prepared by heating the surfactant above 60 °C, followed by addition of hot water while stirring. Once the addition of water was complete, the sample was stirred at 60 °C for 30 min and then during cooling to ambient temperature.

**Surface Tension Measurements.** *Pendant Drop.* Samples were placed in a 1 mL glass syringe with a flat-tipped, Teflon-coated needle (2.15 mm diameter). The syringe was held in position in a metal frame, and a micrometer mount was used to control the syringe plunger. The syringe tip was suspended in a glass cuvette. A droplet of solution was expelled from the syringe until it hung from the tip of the syringe. The droplet was back illuminated, and the image of the droplet was captured using a CCD (charge coupled device) camera attached to a low magnification microscope, frame grabber, and computer. Samples were prepared from stock solutions of surfactant and/or protein. A typical measurement was carried out for 30 min and sampled every 10 s at ambient temperatures. Measurements were carried out in triplicate. The standard error was ±0.5 mN/m.

*Langmuir Trough.* Both WPI and β-lactoglobulin were spread on a PTFE (polytetrafluoroethylene) Langmuir trough (600 mm × 100 mm × 15 mm, 1 L volume; Labcon Molecular Photonics), and the surface tension was monitored using a glass Wilhelmy plate. Aliquots of the surfactants Tween 60/Tween 20 were added to the subphase, and the change in surface tension was monitored for 30 min. The average of the last 10 values was taken as the final surface tension. Measurements were carried out in triplicate, and the standard error was ±0.5 mN/m.

**Preparation of Interfacial Films.** *Langmuir Trough.* Protein films were spread and compressed where necessary to give a surface pressure of 15 mN/m. Measurements were carried out using a PTFE Langmuir trough (surface area, 0.05 $m^2$) equipped with one fixed and one movable barrier. The surface pressure of the interface was monitored by means of a glass Wilhelmy plate.

*Imaging of Films.* The surface pressure of the interface was increased by the addition of water soluble surfactant to the subphase either through a pipet or via a tube immersed below the interfacial film and attached to a syringe. At various surface pressures, the interfacial film was transferred on to freshly cleaved mica using the Langmuir−Blodgett technique (*7*).

Imaging of transferred Langmuir−Blodgett films was carried out using an East Coast Scientific AFM (ECS Ltd., Cambridge, U.K.). Images were obtained in dc (contact) mode, using Nanoprobe silicon nitride cantilevers (0.38 N/m; Veeco Instruments Ltd.). Measurements were carried out in a liquid cell under redistilled *n*-butanol (Sigma Chemicals).

**Compression Isotherms.** WPI and β-lactoglobulin films were spread on the Langmuir trough to give an initial surface coverage of 3 mg/$m^2$ and allowed to equilibrate for 30 min prior to compression. Each film was compressed to approximately one-eighth of its original surface area while monitoring the surface tension. From these data, it is possible to calculate the elastic modulus during compression using the following equation:

$$|E| = \frac{d\gamma}{d\ln A}$$

where $\gamma$ is the surface tension and $A$ is the surface area occupied by the sample.

**Surface Shear Rheology.** The surface shear rheology of both WPI and β-lactoglobulin were determined using a Bohlin CS1O controlled stress rheometer (Bohlin Instruments, Cirencester, U.K.) fitted with an interfacial geometry. The geometry was a titanium, biconical disk (angle, 4.04°; diameter, 56.7 mm), which was located at the air/water interface, in a 111.0 mm diameter glass dish. Seventy-five milliliters of sample was placed in the dish, and a 60 min time sweep at minimum stress was carried out in order to monitor the developing surface rheology, prior to a 5 min stress sweep (0.0001−0.05 N/m). A background value for water was used and subtracted from the results obtained from the protein solution. Reproducibility between measurements was found to be around 10%.

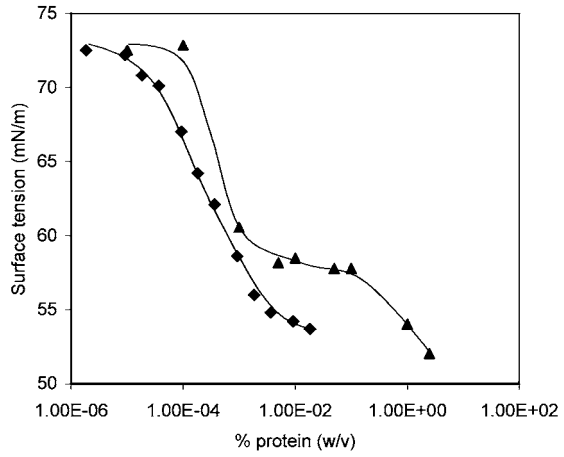


**Figure 1.** Surface tension data using pendant drop obtained for β-lactoglobulin (♦) and WPI (▲) as a function of protein concentration (w/v).

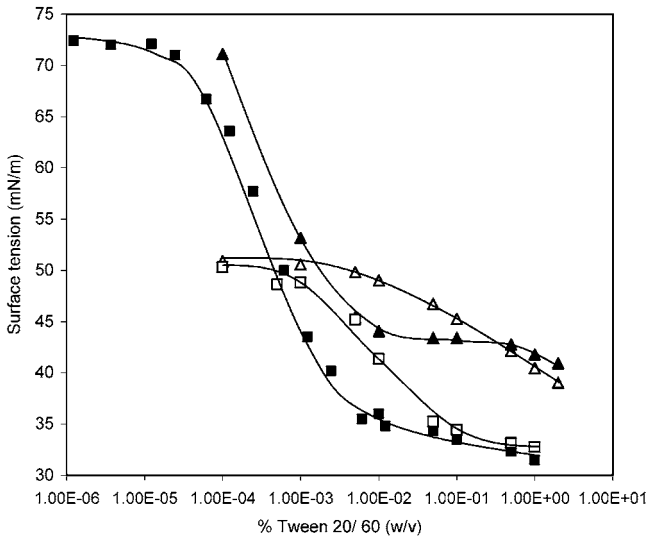


**Figure 2.** Surface tension data using pendant drop obtained for Tween 20 [2.5% WPI (w/v)], □; Tween 20 (water), ■; Tween 60 [2.5% WPI (w/v)], △; and Tween 60 (water), ▲.

## RESULTS AND DISCUSSION

Surface tension data for both WPI and β-lactoglobulin are shown in **Figure 1**. On a weight basis, it can be seen that the WPI is less surface active than pure β-lactoglobulin. This is probably because WPI contains other proteins with lower surface activities than β-lactoglobulin and, as seen in some of the AFM images, contains some aggregated protein. This may be a result of the processing of the WPI during production. Although WPI is claimed to contain mainly native protein due to the lack of heat treatment, other processes such as spray drying could well affect the protein structure and state of aggregation. Surface tension values obtained for the surfactants Tween 20 and Tween 60, in the presence and absence of WPI (2.5% w/v), are shown in **Figure 2**. This concentration represents a typical value used in food applications. The Tween 60 shows a point of inflection

at around 0.1 w/v % that is probably due to the purity of the sample, as this is a food grade product. It is interesting to note that at equivalent Tween 20 concentrations the surface tension in the presence of whey protein is greater than that obtained for the surfactant alone. This occurs up until a surfactant concentration of 0.05%, where values for both the WPI:Tween 20 measurements and Tween 20 converge. A similar trend is seen for the data obtained for the whey protein in the presence of Tween 60. Because of the lower surface activity of Tween 60 in comparison to Tween 20, the protein:Tween 60 data and Tween 60 data converge at a surfactant concentration of 0.5%, an order of magnitude greater than observed for the Tween 20 data. These data suggest that below these concentrations the WPI resists displacement by both of the surfactants studied, suggesting that both whey protein and surfactant are present on the interface at the surfactant concentrations investigated in this study.

Spread WPI and $\beta$-lactoglobulin films were both displaced by Tween 20 and Tween 60. Following spreading of protein at the interface, the surface pressure of the $\beta$-lactoglobulin film reached ~15 mN/m. A spread WPI film of equivalent concentration had a surface pressure of ~8 mN/m and was therefore compressed to give a surface pressure of 15 mN/m prior to addition of surfactant.

Langmuir−Blodgett transfers carried out prior to surfactant addition indicated uniform protein coverage. Following addition of either Tween 20 or Tween 60 to the subphase, further transfers were carried out as the surface pressure of the film increased. The initial increase in surface pressure was dramatic, although in all cases little evidence of protein displacement was seen until the surface pressure exceeded ~20 mN/m.

Since Mackie and co-workers first observed the orogenic displacement of proteins using AFM (*7*), it has been understood that surfactant is able to adsorb at the interface via defects in the protein film. The images obtained for the displacement of the spread WPI and $\beta$-lactoglobulin films by each of the surfactants indicated that initial displacement is consistent with those results obtained previously (*7*). Initially, small holes are detected. Upon increasing the surface pressure further, these holes begin to expand in size. As the surfactant domains grow in size, the protein network is compressed, and gradually, the integrity of the protein network deteriorates, followed by buckling of the network, which also causes thickening of the protein film. It was not possible to measure the thickness of the WPI films during displacement due to the presence of protein aggregates at the surface. The size of the aggregates varied greatly; therefore, to exclude these meant that an objective measurement of the thickness was impossible. At higher surface pressures, the network is stretched out into filaments that eventually snap leaving small protein aggregates in a continuous surfactant layer. Mackie et al. observed that displacement of protein from the interface occurred as a function of increasing surface pressure (*7*). In this study, a similar trend is observed as can be seen from both of the images shown in **Figure 3** and the displacement curves calculated from these images shown in **Figure 4**. Therefore, although the absolute thickness of the protein film could not be determined, all other aspects unique to the orogenic displacement mechanism have been observed with WPI. The essence of the orogenic model is that through forming a network the proteins thwart displacement of individual proteins; the network has to been broken to allow removal of protein into the bulk phase. Displacement curves shown in **Figure 4** were chosen to represent the general trend of increased displacement in relation to increasing surface pressure. In some

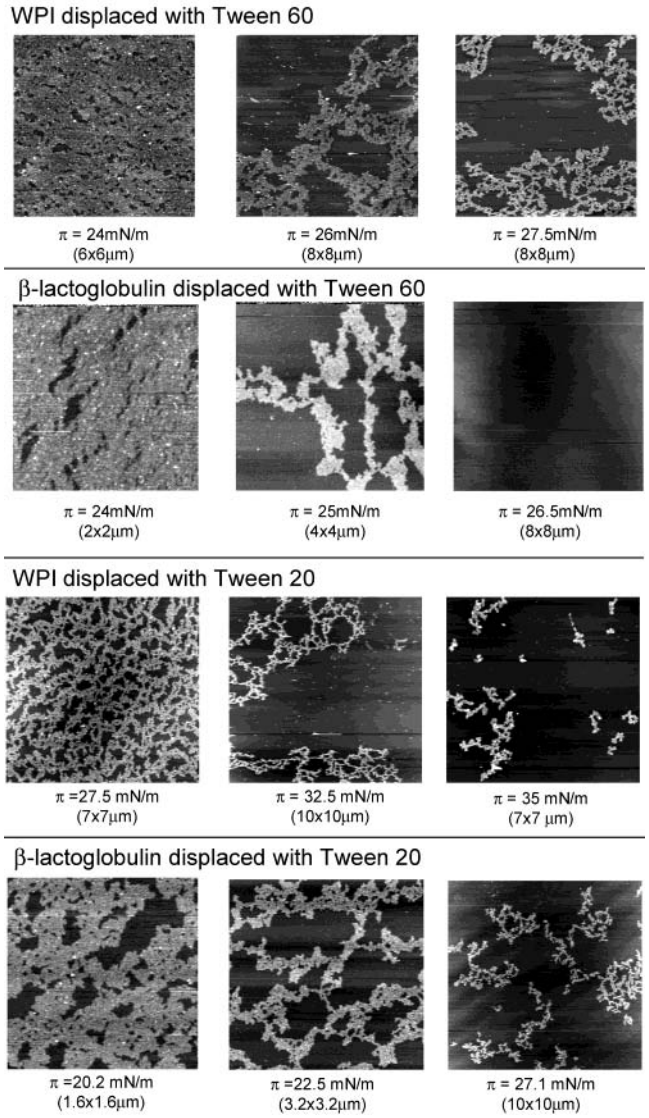


**Figure 3.** AFM images obtained from the displacement of WPI and $\beta$-lactoglobulin by the surfactants Tween 20 and Tween 60.

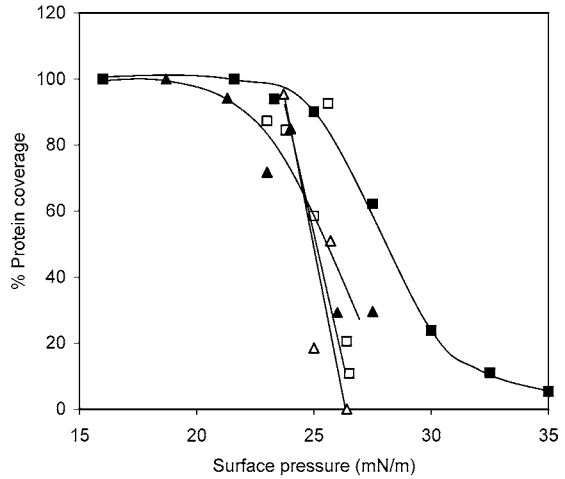


**Figure 4.** Displacement profiles of WPI and Tween 20, ■; WPI and Tween 60, ▲; $\beta$-lactoglobulin and Tween 20, □; and $\beta$-lactoglobulin and Tween 60, △.

of the experiments carried out, the displacement was observed to be heterogeneous, which resulted in a nonlinear decrease in protein coverage with increasing surface pressure (data not

shown). Heterogeneity of protein displacement has been observed previously by Patino and co-workers using BAM. During the displacement of β-casein (*10*) and WPI (*11*) by monoglycerides, some heterogeneities were observed within the monolayer. Mackie et al. also observed heterogeneity when investigating the displacement of β-lactoglobulin by Tween 20, also using BAM (*9*). The heterogeneity of the protein displacement may arise from local surfactant concentration gradients in the bulk phase immediately following its addition to the subphase. These cause local nucleation and expansion of surfactant domains, which in turn causes compression of the protein film. This then lowers the probability of surfactant domain nucleation and expansion in regions of the film not affected by the high local concentration of surfactant. The overall result is that domain nucleation is greater in some regions than others. Previous work using BAM to investigate the displacement of β-lactoglobulin from the air−water interface showed that immediately following addition of the surfactant Tween 20, heterogeneity of surfactant distribution was observed (*9*). Images obtained immediately following addition of surfactant to the subphase showed three distinct regions at the interface: a dark region of surfactant, a bright region of protein, and finally an area of intermediate brightness where the protein film was being displaced by the surfactant (*9*). These effects were avoided in this present study by ensuring even mixing of the surfactant in the subphase and by adding the surfactant in small concentration increments.

As can be seen from the AFM images obtained (**Figure 3**) and also from the displacement curves (**Figure 4**), upon displacing the WPI film with Tween 60 and Tween 20, protein is still observed at a surface pressure of 27.5 and 35 mN/m, respectively (because of the reduced surface activity of Tween 60 in relation to Tween 20, it was not possible to push the surface pressure to greater values during the time scale of the experiment). This observation is surprising given that previous studies carried out on the displacement of individual milk proteins by Tween 20 showed (*7*) that β-lactoglobulin (the dominant surface active whey protein) was completely displaced by a surface pressure of 27 mN/m (cf. **Figure 4**). Displacement of β-lactoglobulin by Tween 60 is also included in **Figure 4** and shows complete displacement by a surface pressure of 26.5 mN/m. The presence of WPI at surface pressures greater than 27 mN/m—the surface pressure by which β-lactoglobulin had been completely displaced—suggests that the WPI is more stable to displacement by surfactant than the pure native proteins. This could be due to a range of effects including enhanced interactions between the different components of whey or modification of the protein structure following production and processing of WPI. These observations are now the subject of a separate study designed to investigate these specific effects.

It is interesting to note that the morphology of the surfactant domains in the WPI is characteristic of that of the two proteins that make up the bulk of whey protein (β-lactoglobulin and α-lactalbumin) as observed previously by Mackie et al. (*7*). However, previous displacement data obtained for spread β-lactoglobulin films showed a continuous network until a surface pressure of 27 mN/m at which all of the protein had been displaced, whereas it was not possible to obtain the same data for spread α-lactalbumin films because of the increased percolation threshold (*7*), that is, the surface protein coverage at which the protein network collapses and becomes discontinuous was at an earlier stage of displacement, making subsequent measurements very difficult. It would appear reasonable then to surmise that the behavior of the whey protein is dominated by β-lactoglobulin, although this will be further discussed later. Previous AFM images obtained for mixed β-casein/β-lactoglobulin films showed that the displacement pattern observed for the mixed films was intermediary between the two extremes observed for the individual proteins (*13*). β-Casein films exhibit round or nearly round surfactant domains with a distinct boundary between the protein and the surfactant phases, whereas the surfactant domains in β-lactoglobulin films are more irregular and characteristic of stress propagation (cf. **Figure 3**). Certainly from both of the images obtained previously for individual proteins and their mixtures and those obtained for the WPI used in this study, it appears that the three stage process of orogenic displacement (adsorption, phase separation, and displacement) is generic and may be applied to commercial proteins.

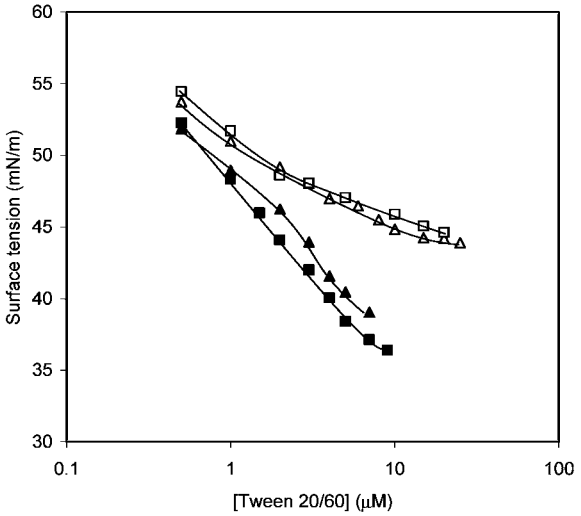


**Figure 5.** Surface tension data using pendant drop obtained for WPI displaced by Tween 60, △; WPI displaced by Tween 20, ▲; β-lactoglobulin displaced by Tween 60, □; and β-lactoglobulin displaced by Tween 20, ■.

Surface tension measurements were carried out on spread films of both WPI and β-lactoglobulin in the presence of Tween 60 and Tween 20. In this case, the surface tension values were monitored following addition of aliquots of surfactant to the subphase of the trough and the data obtained are shown in **Figure 5**. The decrease in surface tension of both of the protein films when displaced with Tween 60 is similar, indicating that the Tween 60 is able to reach the interface over a similar time scale, irrespective of the protein at the interface. Tween 20 reduced the surface tension in the presence of β-lactoglobulin to a greater degree than it did for WPI, however, the difference was very small.

Spread films of WPI and β-lactoglobulin were compressed using a Langmuir trough, and the elastic modulus was calculated. The surface elastic modulus during compression of spread and adsorbed films of individual milk proteins was calculated as reported previously (*7*) and is shown in **Figure 6**. At higher extents of compression (less than one-fifth of original area), it was not possible to obtain reliable elastic modulus data, as the noise was too high. Therefore, data are only shown up to a surface pressure of 30 mN/m. As can be seen from the data shown in **Figure 6**, the decreases in elastic modulus with surface pressure for the two proteins are similar. These decreases in elastic modulus relate to the collapse of the protein monolayers suggesting a gradual displacement of the less hydrophobic regions of the protein molecules from the air−water interface (*7*). Furthermore, in the absence of surfactant, there was no

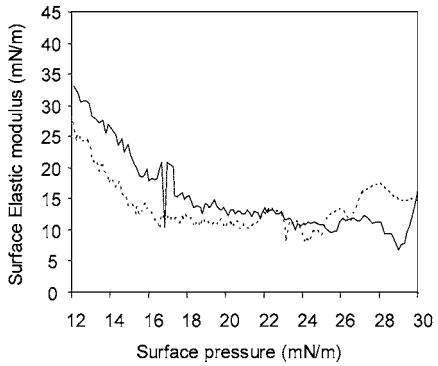


**Figure 6.** Compression of spread $\beta$-lactoglobulin (- - -) and WPI (—) films.

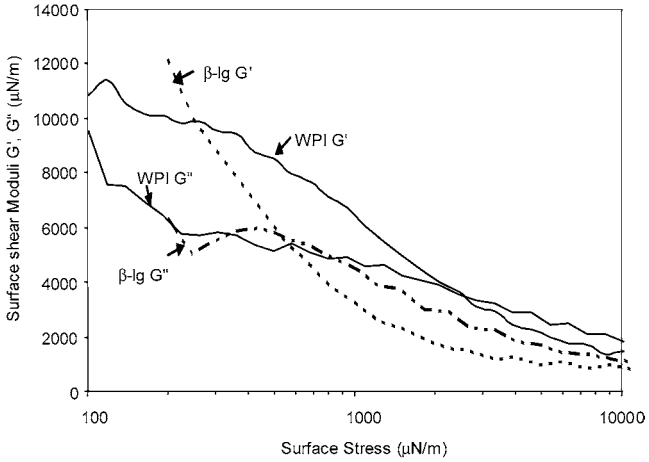


**Figure 7.** Interfacial shear elastic ($G'$) and loss ($G''$) moduli for WPI and $\beta$-lactoglobulin as a function of applied shear stress.

evidence of a further collapse around a surface pressure of 30 mN/m where much of the displacement is known to occur (cf. **Figure 4**). This suggests that the breakdown of the protein network may occur at lower surface pressures. In addition, it also shows that we still do not fully understand the precise physical properties of the protein network that resists the displacement process.

Surface shear rheology was used to compare the mechanical properties of the interfacial films. The storage modulus ($G'$) and the loss modulus ($G''$) were measured as a function of increasing shear. Prior to carrying out the stress sweep for each of the protein films, $\beta$-lactoglobulin had a higher viscoelasticity than the WPI film, as can be seen from **Figure 7**. With increasing stress, the $\beta$-lactoglobulin film breaks down at a stress of 400 $\mu$N/m, whereas the breakdown of the WPI film is more gradual. It is possible that WPI films increased resistance to stress may prevent its displacement at lower surface pressures. Surface shear rheology is more sensitive to the interactions between adsorbed proteins, as opposed to surface dilational rheology, which is more sensitive to compositional and structural factors (*4*). WPI is more resistant to surfactant at the later stages of displacement. There was little difference between WPI and $\beta$-lactoglobulin in their compressional elasticity. The main surface rheological differences appear to be in their surface shear responses, especially at higher stresses. Therefore, the surface shear results suggest that the interactions between the adsorbed WPI proteins are stronger than those between the adsorbed $\beta$-lactoglobulin molecules. This suggests that the reason the WPI is more difficult to displace during the later stages of domain expansion is the number or strength of the intermolecular interactions at the interface, resisting further expansion of the domains.

Bipro 95 is described as a native whey protein isolated by selective ion exchange from a pasteurized sweet whey that has been concentrated and spray dried. Although the product is described as undenatured, it is likely that some aspect of the isolation process may affect its characteristics in comparison to the native proteins. de Wit compared properties such as solubility, foamability, emulsifying activity, and gelation for several industrially prepared whey proteins (*14*), and Bipro behaved in a manner similar to $\beta$-lactoglobulin in terms of solubility, foamability, and emulsifying activity although it was only half as effective as $\beta$-lactoglobulin in terms of its gelation properties. Although $\beta$-lactoglobulin is reportedly stable at temperatures at which pasteurization is carried out, it is possible that some denaturation may have occurred. Mulvihill and Fox (*15*) reported that partial heat denaturation of $\beta$-lactoglobulin may enhance the proteins interfacial activity to values similar to those observed for caseins; they also reported that $\alpha$-lactalbumin is more surface active following denaturation (*15*). Although we know from **Figure 1** that the surface activity of the WPI used in this study is not dissimilar to that observed for $\beta$-lactoglobulin (in fact it is slightly lower), it is possible that during processing of the WPI some protein interactions at the interface (electrostatic, hydrophobic, etc.) have changed resulting in enhanced resistance of the protein to displacement at increased surface pressures. Furthermore, if the surface activity of $\alpha$-lactalbumin has been changed in a manner that increases its interaction with $\beta$-lactoglobulin, this may explain the presence of the WPI at surface pressures greater than those by which $\beta$-lactoglobulin has been completely displaced, and the greater resistance to stress exhibited by the WPI film in comparison to $\beta$-lactoglobulin.

## SUMMARY

Surface tension measurements obtained for the surfactant−protein samples showed that their surface tension values were higher than those obtained for surfactant alone until the data converged at higher surfactant concentrations, suggesting that WPI is resistant to displacement and remains at the interface. Surface tension measurements show that $\beta$-lactoglobulin was more surface active than WPI. However, surface rheology showed that although the $\beta$-lactoglobulin film was more elastic prior to the stress sweep, the WPI film was more resistant to increasing stress than the $\beta$-lactoglobulin film. It is possible that this increased resistance to stress may allow the WPI film to be more resistant to the expansion of the surfactant domains and thus to retain its integrity at higher surface pressures than those observed for $\beta$-lactoglobulin. Despite the WPI Bipro 95 being described as undenatured, previous work and those results obtained during the course of this study indicate that processing of the WPI may have resulted in modification of its properties. Furthermore, the greater resistance to stress exhibited by the WPI, as measured by surface shear rheology, indicates that the protein molecules within the WPI are interacting in a different manner to that observed for pure $\beta$-lactoglobulin. Whether this is a result of one or several of the component proteins having been denatured is unclear, but certainly, it would appear that processing has had an effect. In conclusion, despite the marked difference in behavior between $\beta$-lactoglobulin and WPI, the three main stages used to describe the orogenic mechanism, adsorption, phase separation, and displacement, observed previously for both individual proteins and their mixtures, have, for

the first time, been observed for a commercial protein, confirming that the orogenic displacement mechanism is a generic process.

## CONCLUSIONS

The orogenic displacement mechanism appears to be generic. The WPI was more resistant to displacement in comparison to $\beta$-lactoglobulin. Displacement studies have shown that displacement of proteins is dependent on the surface activity of the surfactant. Complete displacement of WPI was shown in the presence of Tween 20. However, in the presence of the less surface active Tween 60, it was not possible to reach high enough surface pressures to completely displace the WPI. Surface tension measurements suggest that the protein resists displacement by both surfactants at the surfactant concentrations investigated in this study. Surface shear rheology shows that the WPI film broke down at a greater stress than that observed for $\beta$-lactoglobulin films, suggesting that interactions between protein molecules are greater for the WPI film than those observed for $\beta$-lactoglobulin.

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## LITERATURE CITED

(1) Chen, J.; Dickinson, E. Time-dependent competitive adsorption of milk proteins and surfactants in oil-in-water emulsions. *J. Sci. Food Agric.* **1993**, *62*, 283−289.

(2) De Feijter, J. A.; Benjamins, J.; Tamboer, M. Adsorption displacement of proteins by surfactants in oil-in-water emulsions. *Colloids Surf.* **1987**, *27*, 243−266.

(3) Benjamins, J.; Lucassen-Reynders, E. H. In *Studies in Interface Science, Vol. 7—Proteins at Liquid Interfaces*; Mobius, D., Miller, R., Eds.; Elsevier Science: Amsterdam, 1998; pp 341−384.

(4) Bos, M.; van Vliet, T. Interfacial and rheological propertied of adsorbed protein layers and surfactants: A review. *Adv. Colloid Interface Sci.* **2001**, *91*, 437−471.

(5) Courthadon, J. L.; Dickinson, E.; Matsumura, Y. Competitive adsorption of beta-lactoglobulin + Tween 20 at the oil−water interface. *Colloids Surf.* **1991**, *56*, 293−300.

(6) Murray, B. S.; Dickinson, E. Interfacial properties and the dynamic properties of adsorbed films of food proteins and surfactants. *Food Sci. Technol. Int.* **1996**, *2*, 131−145.

(7) Mackie, A. R.; Gunning, A. P.; Wilde, P. J.; Morris, V. J. Orogenic displacement of protein from the air/water interface by competitive adsorption. *J. Colloid Interface Sci.* **1999**, *210*, 157−166.

(8) Mackie, A. R. Orogenic displacement of protein from the oil/water interface. *Langmuir* **2000**, *16*, 2242−2247.

(9) Mackie, A. R.; Gunning, A. P.; Ridout, M. J.; Wilde, P. J.; Rodriguez Patino, J. In situ measurement of the displacement of protein films from the air/water interface by surfactant. *Biomacromolecules* **2001**, *2*, 1001−1006.

(10) Rodriguez Patino, J.; Carrera Sanchez, C.; Rodriguez Nino, R. Analysis of $\beta$-casein—Monopalmitin mixed films at the air−water interface. *J. Agric. Food Chem.* **1999**, *47*, 4998−5008.

(11) Rodriguez Patino, J.; Rodriguez Nino, R.; Carrera Sanchez, C.; Cejudo Fernandez, M. Whey protein isolate—Monoglyceride mixed monolayers at the air−water interface. Structure, morphology, and interactions. *Langmuir* **2001**, *17*, 7545−7553.

(12) Gunning, A. P.; Mackie, A. R.; Kirby, A. R.; Morris, V. J. Scanning near field optical microscopy of phase separated refions in a mixed interfacial protein (BSA)-surfactant (Tween 20) film. *Langmuir* **2001**, *17*, 2013−2018.

(13) Mackie, A. R.; Gunning, A. P.; Ridout, M. J.; Wilde, P. J.; Morris, V. J. Orogenic displacement in mixed $\beta$-lactoglobulin/$\beta$-casein films at the air/water interface. *Langmuir* **2001**, *17*, 6593−6598.

(14) de Wit, J. N. Nutritional and functional characteristics of whey proteins in food products. *J. Dairy Sci.* **1998**, *81*, 597−608.

(15) Mulvihill, D. M.; Fox, P. F. In *Developments in Dairy Chemistry—4*; Fox, P. F., Ed.; Applied Science: London, 1989; pp 131−172.

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