Interfacial Shear Rheology of Aged and Heat-Treated β-Lactoglobulin Films: Displacement by Nonionic Surfactant

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Interfacial shear rheology of adsorbed β-lactoglobulin films (bulk protein concentration 10^{-3} wt %) has been studied over the temperature range 20−90 °C using a two-dimensional Couette-type viscometer. Effects of the type of interface (air−water, triolein−water, and *n*-dodecane−water), the pH (2.0, 5.6, 6.0, 7.0, and 9.0), and the extent of the heat treatment have been assessed via measurements of changes in the apparent interfacial shear viscosity and elasticity before and after the addition of increasing amounts of nonionic surfactant Tween 20 (polyoxyethylene sorbitan monolaurate). The highest interfacial viscosities were obtained at the *n*-dodecane−water interface and the lowest at the air−water interface. Competitive displacement of protein from the interface by Tween 20 was easier at the air−water and *n*-dodecane−water interfaces as compared to the triolein−water interface. The surface shear viscosity was higher and the displacement by Tween 20 more difficult as the isoelectric point of the protein was approached, which is in agreement with the presence of a more strongly cross-linked protein network at the interface. The effect of heat treatment was dependent on the pH of the aqueous solution. No simple relationship between the surface rheological characteristics and the ease of displacement by Tween 20 could be inferred.

Keywords: *Interfacial shear rheology; β-lactoglobulin; Tween 20; heat treatment; pH effects*

INTRODUCTION

Food emulsions typically contain two major types of surface-active substances: moderately high molecular weight proteins and detergent-like surfactants of lower molecular weight. They each stabilize interfaces by different mechanisms. Surfactants stabilize an interface via the Gibbs−Marangoni mechanism, which partly relies on the surfactant having a high degree of lateral mobility. On the other hand, many proteins stabilize an interface by forming a strong viscoelastic network in which the protein molecules are essentially immobile. It is the action of this network that slows drainage and resists stretching of the film. The two mechanisms are incompatible and addition of surfactants leads to competition between the two mechanisms which results in the displacement of protein from the interface (Murray, 1998; Murray and Dickinson, 1996).

Interfacial shear rheology of adsorbed layers, along with other interfacial properties, are useful as indicators of the structural state of adsorbed layers of proteins and mixed protein-surfactant systems at liquid−gas and liquid−liquid interfaces (Dickinson *et al*., 1990; Wilde *et al*., 1993; Hunt *et al*., 1993). They are also important for understanding the processes of foaming (Malysa *et al*., 1991; Hunt *et al*., 1993) and emulsification (Doi and Ohta, 1991; Lucassen-Reynders and Kuijpers, 1992). The rheological characteristics of adsorbed protein films as a function of film aging are a reflection of particularly the restructuring phenomena that occur after molecular adsorption (Dickinson *et al*., 1988; Murray, 1998). Interfacial shear rheology of protein films appears to be very sensitive to the detailed macromolecular structure and to the nature of intermolecular interactions in the adsorbed layer (Dickinson *et al*., 1988). The composition and structure of stabilizing layers in oil-in-water emulsions are also known to be affected by the nature and strength of protein−surfactant and protein−protein interactions in the aqueous phase and at the oil−water interface (Clark *et al*., 1994). Therefore, an understanding of those interactions is important for the systematic development and manufacture of food colloid products.

In this study, we investigate the effect of some important environmental conditions (type of interface, pH, and aging time) and a technological treatment (heating) on the interfacial shear rheology of the adsorbed milk protein β-lactoglobulin and its displacement from the interface by a nonionic surfactant Tween 20 (polyoxyethylene sorbitan monolaurate). The aim is to understand better how such conditions and treatments affect the properties of the interfacial protein film and consequently the stability of the corresponding emulsions.

MATERIALS AND METHODS

Materials. The β-lactoglobulin used was a commercial whey protein isolate (PSDI-2400) obtained from MD Food Ingredients (Vidabaek, Denmark) with a β-lactoglobulin content >95%. High purity Tween 20 was supplied by Sigma Chemical Co. (St. Louis, MO). Trisun 80-high oleic sunflower oil, supplied by Danisco Ingredients (Brabrand, Denmark), was either used as received or treated with silica to remove any surface-active impurities (e.g., monoglycerides and fatty acids), according to the method described by Gaonkar (1989). The *n*-dodecane was from Sigma Chemical Co. (St. Louis, MO). The water used for all the experiments was double distilled and had a surface tension of not less than 72 ± 0.3 mN m^{-1} at 25 °C.

Interfacial Shear Rheology. The instrument used to measure the interfacial shear viscosity (η) and shear elasticity

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(G) at the fluid interface was the two-dimensional Couette-type interfacial viscometer described previously (Dickinson et al., 1985, 1987). A stainless steel biconical disk (diameter 30 mm) was suspended by a torsion wire with its edge in the plane of the fluid interface formed by the protein solution and the oil layer, which were contained in a thermostated glass dish (diameter 145 mm). The thickness of the torsion wire was 0.5 mm, and it was chosen to be 330 or 200 mm in length depending on the magnitude of the interfacial viscosity of the system being studied. Measured values of η and G were determined over a period of 24 h. For viscosity measurements, the film was subjected to intermittent shear at a steady dish rotation speed of 1.27×10^{-3} rad s^{-1} for 10−15 min until a steady-state interfacial shear stress was obtained. The interfacial stress was monitored by measuring the rotation of the bob via a light beam reflected from the bob onto a diode array. For elasticity measurements, the dish was intermittently rotated at 1.27×10^{-2} rad s^{-1} to give shear strains of the order of 1%, the corresponding maximum shear stress being achieved approximately 1 s after the movement of the dish. The values of the viscosities of the films were such that, for the torsion constant of the particular wire used, there was negligible rotation of the disk after the initial elastic response over the time required to measure the corresponding "instantaneous" elastic strain.

Prior to each experiment, the β-lactoglobulin was solubilized in buffer at 20 °C and left for 1 h at 20 °C before adding 500 mL of the protein solution to the dish. The bulk concentration of protein was 10^{-3} wt %. For the experiments at the oil−water interface, this was immediately followed by careful addition of 45 mL of the oil to the dish, to form an oil layer above the protein solution.

pH Control. The desired pH was achieved by adjusting with diluted HCl or NaOH before solubilizing the protein, for consistency with previous measurements (Roth *et al.*). The pH of the protein solution was confirmed as constant over the time of the experiments. The validity of the neutral pH results were checked by repeating some experiments at pH 7.0 using 20 mM imidazole buffer to control the pH. Within experimental error, the same results were obtained with and without buffer.

Heat Treatment. Experiments were either started with the dish thermostated at the elevated temperatures (65−90 ± 0.5 °C) right at the beginning, or they were left at 20 ± 0.5 °C for 24 h before heating rapidly to the higher set temperature used. In some experiments the systems were then cooled back down to 20 °C before continuing measurements of η and G.

Competitive Displacement with Tween 20. The ability of the surfactant Tween 20 to disrupt and displace adsorbed β-lactoglobulin films under the different conditions studied was assessed rheologically following the addition of a small amount of surfactant directly to the aqueous phase at the bottom of the dish. The addition was accompanied by gentle stirring sufficient to distribute the surfactant rapidly and uniformly throughout the solution, but not disturb the interfacial film. After 10 min, values of η and G were determined. The measurement was followed by the addition of another small amount of Tween 20 and remeasurement of η and G. The cycle was repeated until the values of η and G became approximately equal to zero, corresponding to an interface that was presumed to be fully covered by Tween 20, i.e., corresponding to complete displacement of protein, since the shear viscosities of low-molecular-weight surfactant films are negligible compared with those of globular proteins (Murray, 1998).

RESULTS AND DISCUSSION

Comparison of Different Interfaces. The effects of different types of fluid interface on the interfacial rheology and the ability of Tween 20 to displace the protein were investigated. These experiments were carried out at 20 °C and pH 5.6.

Figure 1 shows the apparent interfacial shear viscosity, η, as a function of film age at the oil−water (n-dodecane, purified triolein, and nonpurified triolein) and air−water interfaces. For these and all the other η measurements reported in this paper, the reproducibility of was ±50 mN s m^{-1}. At the hydrocarbon oil−water interface, the value of η initially increased rapidly, reaching a value of ca. 150 mN s m^{-1} after a few minutes and thereafter increased more slowly, reaching a value of ca. 500 mN s m^{-1} after 24 h. For the purified and nonpurified triolein, the time-course of η exhibited a similar trend, though the values of η were lower as compared with n-dodecane: the values of η after 24 h were ca. 250 mN s m^{-1} and ca. 80 mN s m^{-1}, respectively.

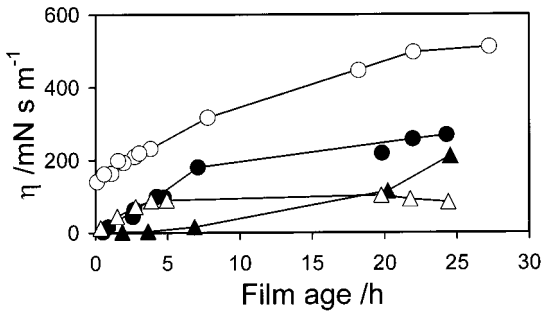

Figure 1. Effect of the type of interface on η of adsorbed β-lactoglobulin films (20 °C, pH 5.6) as a function of film age: (○) n-dodecane−water interface, (●) purified Triolein−water interface, (△) nonpurified Triolein−water interface, (▲) air−water interface.

Typical impurities in the triglyceride oil, such as fatty acids, monoglycerides, etc., will tend to adsorb more rapidly than the protein because of their lower molecular weight. Moreover, adsorbing protein is unlikely to displace lower molecular weight surfactants, though the opposite may occur; i.e., low molecular weight surfactants displace protein (de Feijter et al., 1987; Dickinson and Woskett, 1989). It is therefore interesting to observe that, during the first 5 h, no differences in η were found between the data for purified and nonpurified triolein (Figure 1). Therefore, any impurities in the triglyceride oil that may affect the adsorption/reconformation process do not appear to have any effect during this time, which at this bulk protein concentration is the time typically expected for the interface to become saturated with protein under these conditions (Makievski *et al.*, 1998).

For the air−water interface there was no initial rapid increase in η: for the first 4 h η was nearly zero and then started to increase slowly, reaching ca. 200 mN s m^{-1} after 24 h.

A lower interfacial viscosity is probably indicative of less cross-linking between protein molecules at the interface, which is possibly related to less unfolding of protein at the interface. Thus the processes of cross-linking and unfolding of β-lactoglobulin at pH 5.6 appear to be more rapid at the n-dodecane−water interface than at the triolein−water interface, and apparently more rapid at the triolein−water interface than at the air−water interface. Interfacial pressure−area per molecule isotherms of spread films have shown (Murray, 1997) that films of β-lactoglobulin (at pH 7) are considerably more expanded at the oil−water interface compared to the air−water interface, implying a greater extent of unfolding at the oil−water interface. Other recent measurements (Murray et al., 1998; Murray and Garofalakis, 1999) have suggested that adsorbed β-lactoglobulin is in a more aggregated state at the air−water interface and that it may take considerably more

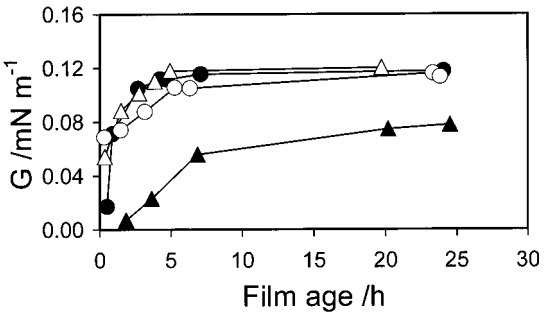

Figure 2. Effect of the type of interface on G of adsorbed β-lactoglobulin films (20 °C, pH 5.6) as a function of film age: (○) *n*-dodecane−water interface, (●) purified Triolein−water interface, (△) nonpurified Triolein−water interface, (▲) air−water interface.

time for the same protein to disaggregate and unfold compared to the protein at the oil−water interface. This may be due to the greater affinity of the hydrophobic portions of the protein for the oil phase as compared to air, which enhances the rate and extent of adsorption and unfolding, at least initially. The different results for the purified and nonpurified triolein suggest the impurities present in the nonpurified triolein prevent β-lactoglobulin from adopting the same conformation that it does in their absence.

Turning now to the time-dependent interfacial shear elasticity, G, it is seen in Figure 2 that no significant differences were observed with the different oils, unlike the data for η. (For these and all the other G measurements reported in this paper the reproducibility was ± 0.015 mN m^{-1}). Also, after 24 h, G was lower at the air−water interface ($\sim$0.07 mN m^{-1}) than at the oil−water interface ($\sim$0.12 mN m^{-1}), and for both the air−water and oil−water interfaces a plateau in G seemed to be reached after ca. 5−10 h, whereas the value of η continued to increase (see Figure 1). If the G values can be correlated with the protein-adsorbed amount, this means that there was a similar rate of adsorption at the *n*-dodecane−water and triolein−water interfaces. Thus, the differences in the η values observed with different oils might be better explained by differences in the kinetics of conformational rearrangements in the different layers. Following this line of reasoning, the reason the purity of the triglyceride oil has a significant effect on η, but little effect on G, is that the impurities at the interface affect the intermolecular bonding between the interfacial proteins, but not particularly to the adsorbed amount.

It seems, therefore, that η and G may be sensitive to different aspects of the film. On the other hand, the different behavior of η and G may reflect the fact that G is derived from a small-deformation, short-time experiment, whereas η is from a large-deformation, long-time experiment. The latter probably involves some disruption of the adsorbed layer structure under the imposed strain. Most protein films appear to exhibit marked shear thinning behavior under the conditions of this measurement (Murray, 1998; Murray and Dickinson, 1996). The ease of breakdown and the resistance to deformation of the adsorbed layer network, reflected by the value of η, may simply be more sensitive to the solution conditions than is the measurement of G. It should also be mentioned that studies of emulsions have suggested that slightly lower amounts of the same protein occur on triglyceride droplets than on hydrocarbon droplets (Chen and Dickinson, 1998), in which

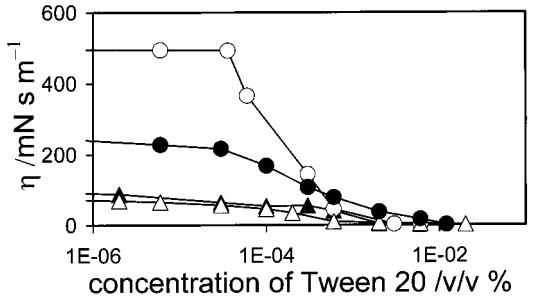

Figure 3. Effect of the type of interface on η as a function of the logarithm of the concentration of Tween 20 added to the aqueous phase beneath 24 h old adsorbed β-lactoglobulin films (20 °C, pH 5.6): (○) *n*-dodecane−water interface, (●) purified Triolein−water interface, (△) nonpurified Triolein−water interface, (▲) air−water interface.

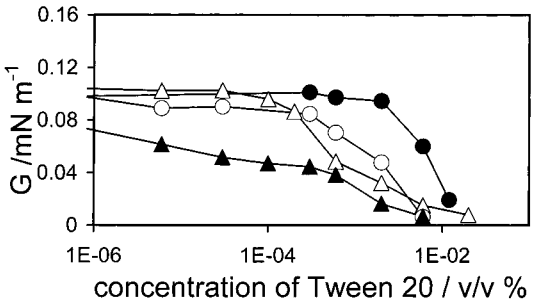

Figure 4. Effect of the type of interface on G as a function of the logarithm of the concentration of Tween 20 added to the aqueous phase beneath 24-h-old adsorbed β-lactoglobulin films (20 °C, pH 5.6): (○) *n*-dodecane−water interface, (●) purified Triolein−water interface, (△) nonpurified Triolein−water interface, (▲) air−water interface.

case it would seem that there is no clear-cut relationship between the value of G and the adsorbed amount.

Figures 3 and 4 demonstrate the displacement of β-lactoglobulin from the interface by Tween 20, in plots, respectively, of η and G as a function of the logarithm of the surfactant concentration in the aqueous phase. Ten times more Tween 20 is required to displace fully the protein from the triolein−water interface compared with the *n*-dodecane−water or air−water interfaces. Full displacement can be presumed to be indicated by a fall in the value of η to less than 1 mN s m^{-1}. For low molecular weight surfactants η is typically 2 or 3 orders of magnitude even lower than this (Murray, 1998): the points of "zero" η indicated in Figures 3 and 4 actually mean η was lower than the lowest values measurable with this technique, i.e., 0.05 mN s m^{-1}. As Tween 20 is a water-soluble nonionic surfactant, it is more likely to displace the protein by replacement rather than by solubilization (Wüstneck et al., 1996). The competitive displacement arises because the surfactant−interface interaction is stronger than the interaction between the protein (or protein−surfactant complex) and the interface (Dickinson and Woskett, 1989; Dickinson, 1998). The surfactant does not actually have to interact with the protein, but it must bind to the interface. Therefore, the implication is that the protein−oil interactions are stronger with the triolein oil.

However, an added complication with surfactant + protein systems is the possibility of protein−surfactant complexes at the interface, referred to above. A single, specific binding site for low molecular weight amphiphilic molecules occurs on β-lactoglobulin. This has

been studied by many workers (e.g., Coke et al., 1990; Wilde and Clarke, 1993), but overall it is thought that complexation with molecules such as Tween20 does not effect drastic changes in the conformation of β-lactoglobulin and therefore the surface behavior of such complexes is probably very similar to the noncomplexed protein. Thus low values of η are still best interpreted in terms of gross disruption and removal of the adsorbed protein, in whatever form.

Recent experiments using atomic force microscopy, combined with measurements of interfacial tension and interfacial rheology (Mackie et al., 1999), have suggested that β-lactoglobulin desorption by Tween 20 (from the air−water interface) proceeds by adsorption of surfactant at defects in the protein network, followed by growth of these nucleation sites, which then compress the protein network. At sufficiently high interfacial pressure the network finally fails, releasing protein that then desorbs from the interface. If this mechanism is correct, it would appear that the critical interfacial pressure needs to be higher at the triolein−water (Figures 3 and 4), which means that protein−oil interactions are stronger with triolein than with *n*-dodecane or air as the nonaqueous phase.

In the presence of impurities, the competitive displacement initially appeared easier (Figures 3 and 4), but the same amount of Tween 20 was actually needed to displace all the protein (η < 1 mN s m^{-1}). This could be explained by the presence of more defects in the protein network at the start due to adsorption of these impurities at the interface, but the same interfacial pressure is ultimately required to desorb the protein completely because the impurities must be displaced by Tween20 as well as the protein. Similarly, Dickinson and Tanai (1992) have shown that the presence of glycerol monostearate (GMS) in oil-in-water emulsions is particularly effective in reducing the amount of Tween 20 required for displacement of β-casein from the interface, though GMS on its own is not an effective displacer of protein.

Both the interfacial viscosity and elasticity measurements indicate that nearly the same amount of Tween 20 is needed to displace completely the protein from the air−water and *n*-dodecane−water interfaces (Figures 3 and 4). We can observe no simple correlation between the initial value of η or G and the ease of displacement. Indeed, 10 times more Tween 20 was needed to displace β-lactoglobulin from the nonpurified triolein−water interface than from the *n*-dodecane−water interface, even though the initial value of η for the latter interface was approximately six times greater (Figure 3) and the G values were similar (Figure 4).

Effect of pH. The effect of pH on the interfacial rheology of β-lactoglobulin at the *n*-dodecane−water interface was studied. Figure 5 shows the time-dependent viscosity data for each of these pH values. After 24 h, the highest value (ca. 500 mN s m^{-1}) was obtained at pH 5.6, which is the isoelectric pH, pI, of the protein. The lowest values measured after 24 h were obtained at pH 2.0, and whereas η increased with time at the other pH values, in this case η decreased for the first 5 h from approximately 50 to 15 mN s m^{-1}, then increased again to approximately 50 mN s m^{-1} after 24 h. This type of behavior was also seen in the G measurements at pH 2.0 (Figure 6), with the values obtained being also significantly lower (ca. 0.06 mN m^{-1}) than those at the higher pH values. Values obtained at pH 6.0 and

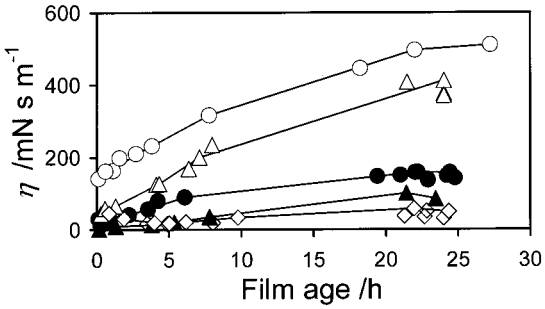

Figure 5. Effect of pH on η of adsorbed β-lactoglobulin films (20 °C) at the *n*-dodecane−water interface as a function of film age: (○) pH 5.6, (△) pH 6.0, (●) pH 7.0, (▲) pH 9.0, (◇) pH 2.0.

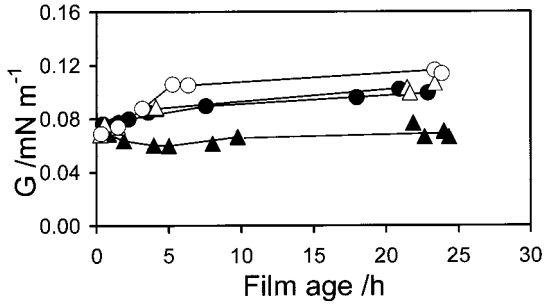

Figure 6. Effect of pH on G of adsorbed β-lactoglobulin films (20 °C) at the *n*-dodecane−water interface as a function of film age: (○) pH 5.6, (△) pH 6.0, (●) pH 7.0, (▲) pH 2.0.

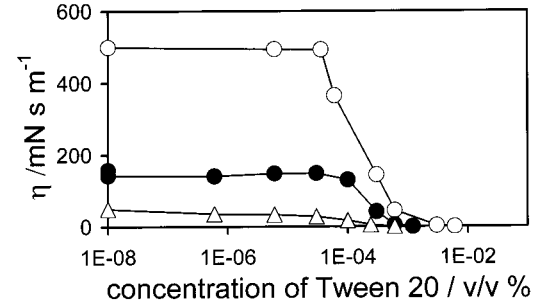

Figure 7. Effect of pH on η as a function of the logarithm of the concentration of Tween 20 added to the aqueous phase beneath 24 h old adsorbed β-lactoglobulin films (20 °C) at the *n*-dodecane−water interface: (○) pH 5.6, (●) pH 7.0, (△) pH 2.0.

7.0 were similar (0.10 mN m^{-1}) and close to the values obtained at pH 5.6 (0.11 mN m^{-1}). The occurrence of a maximum in the interfacial shear viscosity at the pI is consistent with previous results reported for a variety of proteins (Izmailova, 1979) and has been interpreted in terms of decreasing electrostatic repulsion between the adsorbed protein molecules, which allows them to pack together and associate more easily. Certainly this is the case in bulk solution. Pessen et al. (1985) have reported that dimers of β-lactoglobulin are associated in an octameric structure at the isoelectric pH. Moreover they observed a dimeric to monomeric transition as the pH was increased, and pointed out the existence of a monomeric structure also at pH 2.0.

In Figures 7 and 8, the competitive displacement with Tween 20 after 24 h at the *n*-dodecane−water interface at different pH has been again assessed by plotting η and G, respectively, as a function of the logarithm of the concentration of Tween 20 added to the aqueous phase. These plots show that, to displace all the protein from the interface, a higher concentration of Tween 20

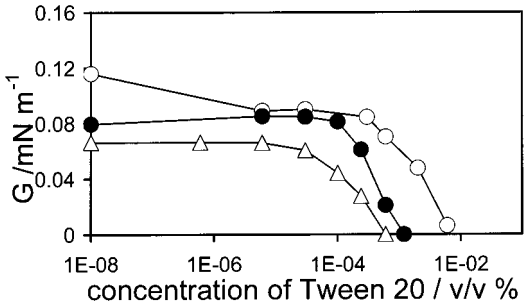

Figure 8. Effect of pH on G as a function of the logarithm of the concentration of Tween 20 added to the aqueous phase beneath 24-h-old adsorbed β-lactoglobulin films (20 °C) at the n-dodecane−water interface: (○) pH 5.6, (●) pH 7.0, (△) pH 2.0.

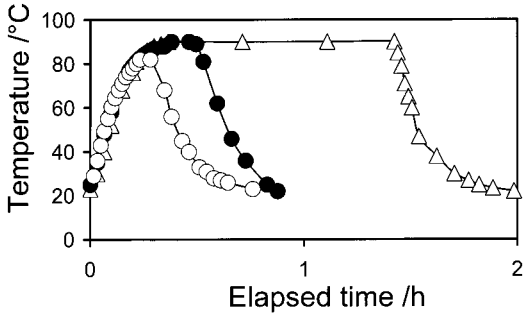

Figure 9. Temperature versus time for the different heat treatments used after 24 h at 20 °C: (○) 82 °C, 5 min; (●) 90 °C, 5 min; (△) 90 °C, 60 min.

is required at pH 7.0 than at pH 2.0, and an even higher concentration at pH 5.6 than at pH 7.0. These differences are possibly related to different degrees of accessibility of the interface to Tween 20, due to the different degrees of association of the protein at different pH values, as described above. Thus, at pH 5.6 the stronger and more compact protein network more effectively prevents the molecules of Tween 20 from penetrating the interfacial protein layer by steric hindrance.

Effect of Heat Treatment. The influence of heat treatment on competitive adsorption of milk proteins and emulsifiers is relevant to the processing of various dairy colloids, especially ice cream. At room temperature, protein unfolding at the interface can lead to the formation of permanent (disulfide) cross-links after prolonged aging (Dickinson and Matsumura, 1991), but heating is expected to accelerate this process. A more cross-linked film is likely to have higher interfacial viscosity and also to be more difficult to displace. However, as with the heat treatment of various proteins in solution, if cross-linking and aggregation is too extreme, gelation is inhibited as the network structure is lost (Kilara and Sharkasi, 1986). In the same way, excessively heated interfacial protein may effectively become insoluble and should be easier to displace. Previous work carried out in this laboratory with transglutaminase cross-linked β-lactoglobulin films (Færgemand et al., 1997) is in agreement with this, η falling with excessive cross-linking.

The effects of various heat treatments on the shear rheology of β-lactoglobulin films at the n-dodecane−water interface at pH 6.0 were examined. Figure 9 shows the temperature as a function of time in experiments where, after 24 h at 20 °C, the temperature of the system was increased to a set value for a specified time, and then decreased again to 20 °C. Three sets of

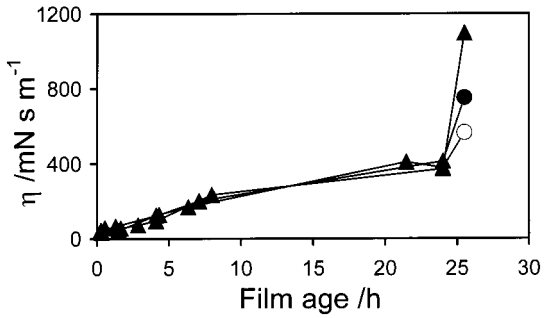

Figure 10. Effect of different heat treatments on η of adsorbed β-lactoglobulin films (20 °C, pH 6.0) at the n-dodecane-water interface as a function of film age. The last point on each curve is the value of η after cooling back down to 20 °C: (○) 82 °C 5 min; (●) 90 °C, 5 min; (▲) 90 °C, 60 min.

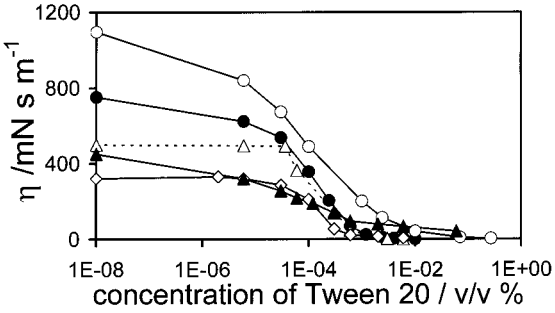

Figure 11. Effect of different heat treatments on η as a function of the logarithm of the concentration of Tween 20 added to the aqueous phase beneath adsorbed β-lactoglobulin films (20 °C, pH 6.0) at the n-dodecane−water interface: (○) 24 h at 20 °C, 60 min at 90 °C, then cooled to 20 °C; (●) 24 h at 20 °C, 5 min at 90 °C, then cooled to 20 °C; (△) 24 h at 20 °C (no heat treatment); (▲) 24 h at 65 °C then cooled to 20 °C; (◇) 24 h at 65 °C.

heating conditions were investigated. The first procedure involved heating to 82 °C for 5 min, the second to 90 °C for 5 min, and the third to 90 °C for 60 min. Measurements of η and G were made before and after heat treatment, and the subsequent ability of Tween 20 to displace protein from the interface was then assessed. Figure 10 shows η as a function of film aging at 20 °C for the three experiments: in each case, η reached a value of ca. 400 mN s m^{-1} after 24 h. The last point on each curve is the value after completion of the heat treatment, i.e., after cooling back down to 20 °C. We see that the higher the temperature and the longer the heating time, the higher was the final value of η. However, the value of G did not seem to be much affected and remained constant at around 0.110 mN m^{-1} whatever the heat treatment (data not shown).

At temperatures above ca. 70 °C, dissociation of the dimers of β-lactoglobulin occurs in solution followed by slow, irreversible aggregation of the protein. It seems that after the dimer to monomer dissociation, exchanges of disulfide bonds are responsible of the production of oligomers, which finally lead to the formation of aggregates. Hydrophobic interactions, enhanced by the higher temperature, are also thought to play an important role in the formation of aggregates (Fox and McSweeney, 1998). Therefore, the higher η obtained are probably the result of such aggregation at the interface, perhaps made easier by the already partly unfolded state of the molecules at the interface.

Figure 11 illustrates the competitive displacement by Tween 20 after each heat treatment. Data obtained after two different heat treatments have also been added. The

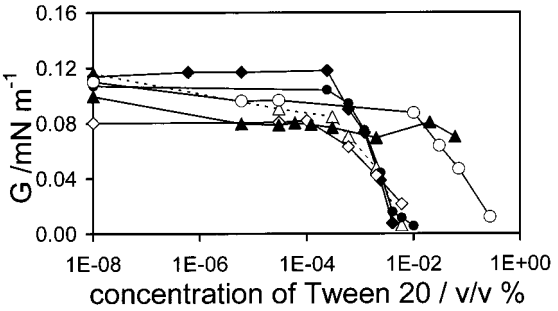

Figure 12. Effect of different heat treatments on G as a function of the logarithm of the concentration of Tween 20 added to the aqueous phase beneath adsorbed β-lactoglobulin films (20 °C, pH 6.0) at the *n*-dodecane−water interface: (○) 24 h at 20 °C, 60 min at 90 °C, then cooled to 20 °C; (●) 24 h at 20 °C, 5 min at 90 °C, then cooled to 20 °C; (△) 24 h at 20 °C (no heat treatment); (▲) 24 h at 65 °C then cooled to 20 °C; (◇) 24 h at 65 °C; (◆) 24 h at 20 °C, 5 min at 82 °C, then cooled to 20 °C.

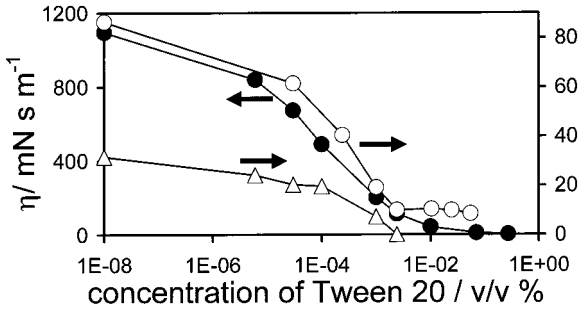

Figure 13. Effect of pH on η as a function of the logarithm of the concentration of Tween 20 added to the aqueous phase beneath a heat-treated β-lactoglobulin film at the *n*-dodecane−water interface. The heat treatment was 24 h at 20 °C, 60 min at 90 °C, followed by a cooling to 20 °C: (○) pH 9.0, (●) pH 6.0, (△) pH 2.0. The arrows indicate the corresponding η scale.

first of these corresponds to aging the film at 65 °C for 24 h before cooling down to 20 °C and the second to the same aging of the film at 65 °C but without cooling to 20 °C. The behavior of the interfacial viscosity with no heat treatment (i.e., at a constant temperature of 20 °C) is indicated by the dashed line. Two different types of behavior can be identified. At 65 °C, or after heating at 90 °C for 5 min before cooling down to 20 °C, the results were similar to those obtained without heating the interface, in that approximately the same amount of Tween 20 (10^{-2} v/v%) was needed to fully displace the protein. At 90 °C for 60 min, or after 65 °C for the first 24 h before cooling to 20 °C, at least 100 times more Tween 20 was apparently required to displace the protein (i.e., to reduce η to <1 mN s m^{-1}). This can be explained in terms of the increase in the level of aggregation and strength of the protein−protein interactions resulting from the elevated temperature and/or the duration of the treatment. Dickinson and Hong (1994) also observed that heat treated β-lactoglobulin was more difficult to displace from the oil−water interface by Tween 20, and heat treatment increased the surface coverage of the protein on emulsion droplets. Differences in the structure and aggregation state of denatured β-lactoglobulin have already been shown to depend on the temperature and the duration of the heat treatments (Fang and Dalgleish, 1997). Fang and Dalgleish (1997) observed that, although heating at lower temperatures (70 and 80 °C) for a longer time causes extensive denaturation, as does heating at a higher temperature (90 °C) for a short time, the structure of the denatured protein as determined by FTIR is different for the two cases. However, again we observe that the relationship between η and the ability to displace the protein is not simple. It is apparently easier to displace protein after 5 min at 90 °C than after 24 h at 65 °C, even though the value of η for the first case is nearly twice as high.

The corresponding sets of values of G following these different heat treatments are shown in Figure 12. The trend of the results is in qualitative agreement with that shown in Figure 11. Only the treatments at 90 °C for 60 min and at 65 °C for 24 h induced a noticeable change in the concentration of Tween 20 needed to displace completely the protein from the interface. However, in these two cases, the beginning of the displacement (as indicated by the decrease of η) seems to occur at a higher concentration of Tween 20 (ca. 10^{-2} v/v%), compared to the concentration at which η starts to fall (ca. 10^{-5} v/v%).

The effect of heating at 90 °C for 60 min has also been studied at pH 9.0 and 2.0. In both cases, the values of η were the same before and after the treatment (ca. 30 mN s m^{-1} at pH 2.0 and ca. 85 mN s m^{-1} at pH 9.0). However, the G value was each time lower after the heat treatment (two times lower at pH 9.0 and three times lower at pH 2.0; data not shown). Figure 13 shows values of η as a function of the logarithm of the concentration of Tween 20 in the aqueous phase. At pH 2.0, the heat treatment seems to have no effect on the ability of Tween 20 to displace β-lactoglobulin. At pH 9.0, complete displacement was apparently impossible even at 0.1 v/v% Tween 20, with η first falling and then remaining constant at ca. 10 mN s m^{-1} at concentrations of Tween 20 greater than 2×10^{-3} v/v%. At this pH and temperature the thiol group of β-lactoglobulin is known to be more reactive (Kella and Kinsella, 1988), and therefore, rapid covalent cross-linking is more likely to happen. Chen and Dickinson (1993) have shown that β-lactoglobulin is less likely to be displaced by Tween 20 when the protein is polymerized.

CONCLUSIONS

Understanding what occurs during the processing of dairy colloids is important industrially. The influence of solution conditions and heating on the characteristics of a model interfacial system of β-lactoglobulin has been studied, as well as the influence on the ease of competitive displacement by Tween 20.

The type of fluid interface has been found to have a significant influence on the interfacial rheology, and therefore presumably the interfacial structure adopted by β-lactoglobulin, which in turn affects the ease of protein displacement by the low molecular weight surfactant. The highest η values were obtained at the *n*-dodecane-water interface. The displacement by Tween 20 was easier at the air−water and *n*-dodecane−water interfaces, rather than at the triolein−water interface. The presence of impurities in triolein seems to have an effect on η only after several hours.

The surface viscosity increases as the isoelectric pH is approached, and the displacement becomes more difficult, which is in agreement with there being a more structured network at the interface at this pH.

Heat treatment has an effect on both the rheological characteristics and on the ease of displacement by the emulsifier, but no simple relationship between them

could be established. The effect of the heat treatment was found to depend on the pH of the aqueous solution. Finally, it appears that, even though the quantities η and G are both intimately connected with the nature and strength of the interactions that occur at the interface, η seems more sensitive overall to the film properties.

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