Surface Coverage of β-Lactoglobulin at the Oil–Water Interface: Influence of Protein Heat Treatment and Various Emulsifiers

Eric Dickinson* and Soon-Taek Hong

Procter Department of Food Science, University of Leeds, Leeds LS2 9JT, U.K.

Competitive adsorption of β-lactoglobulin and small-molecule emulsifiers has been compared at room temperature and at 70 °C in oil-in-water emulsions (0.4 wt % protein, 20 wt % *n*-tetradecane, pH 7). Protein heat treatment leads to a higher protein surface coverage, and the coverage increases steadily with increasing emulsion storage time. Heat-treated protein is more difficult to displace from the oil–water interface by water-soluble nonionic surfactant Tween 20, as confirmed by complementary surface shear viscosity measurements. In emulsions containing anionic surfactant DATEM, the surface coverage of β-lactoglobulin is only slightly affected by the presence of emulsifier at 70 °C. Surface shear viscosity measurements are suggestive of an interfacial complex between β-lactoglobulin and DATEM. Monoglyceride present in corn oil-in-water emulsions leads to an increase in protein surface concentration at low emulsifier concentrations but a reduction at higher concentrations in agreement with earlier work. Diglyceride has no effect on the measured β-lactoglobulin surface coverage.

Keywords: *Adsorbed protein; β-lactoglobulin; protein denaturation; competitive adsorption; surface viscosity; oil–water interface; protein-stabilized emulsion*

This paper reports part of a continuing program of research into aspects of the competitive adsorption of pure milk proteins and small-molecule surfactants ("emulsifiers") at the oil–water interface in model food emulsions (Courthaudon et al., 1991a–d; Dickinson and Tanai, 1992a,b; Dickinson et al., 1993; Chen and Dickinson, 1993; Dickinson and Iveson, 1993; Chen et al., 1993). Understanding the factors affecting the structure and composition of protein/emulsifier layers around oil or fat droplets is necessary for improved control of the formation, stability, and rheology of a manufactured food emulsion such as ice cream (Goff and Jordan, 1989; Berger, 1990; Barfod et al., 1991).

The functional properties of whey proteins are of substantial and growing importance to the food industry (de Wit, 1989; Kinsella and Whitehead, 1989; Jost et al., 1990; Morr and Ha, 1993). In experiments on model oil-in-water emulsions containing just the major whey protein β-lactoglobulin, competitive displacement of the globular protein from the oil–water interface has been demonstrated (Courthaudon et al., 1991b,c; Dickinson et al., 1993; Chen and Dickinson, 1993; Dickinson and Iveson, 1993; Chen et al., 1993) for both oil-soluble and (especially) water-soluble surfactants. In addition, from some complementary surface shear viscosity measurements of β-lactoglobulin adsorbed at the oil–water interface (Dickinson et al., 1990; Courthaudon et al., 1991b; Dickinson and Iveson, 1993), we have useful information on dynamic properties of β-lactoglobulin films in the presence and absence of emulsifiers. The β-lactoglobulin monomer has an internal free sulfhydryl group which is not available for reaction with other sulfhydryl or disulfide groups under normal conditions. However, as the protein adsorbs, it partially unfolds, and the exposed sulfhydryl group becomes available for polymerization at the interface (Dickinson and Matsumura, 1991) and cross-linking by disulfide bonds between flocculated droplets (McClements et al., 1993). Increasing the temperature also leads to increased accessibility of the free sulfhydryl group, and indeed the formation of intermolecular disulfide cross-links is partly responsible for the strength of heat-set whey protein gels (Katsuta and Kinsella, 1990; Mulvihill et al., 1991).

This paper presents new experimental information on the time-dependent competitive adsorption of β-lactoglobulin and the nonionic water-soluble emulsifier Tween 20 [polyoxyethylene (20) sorbitan monolaurate] at a temperature (70 °C) corresponding to the onset of the thermal denaturation and aggregation of the protein (Xiong et al., 1993). We also present new results on the competitive adsorption of β-lactoglobulin with the ionic food-grade emulsifier DATEM (diacetyltartaric acid ester of monoglyceride) as well as the slightly polar lipid glycerol dioleate for comparison with earlier studies involving mixtures of β-lactoglobulin with glycerol monosterate (Dickinson et al., 1993) or lecithin (Dickinson and Iveson, 1993). Our ultimate objective is to gain insight into the physicochemical mechanisms involved during the thermal and mechanical processing of dairy emulsions.

MATERIALS AND METHODS

Materials. Bovine β-lactoglobulin (genetic variants A and B, purity >99 wt %) was obtained from Sigma Chemical Co. (St. Louis, MO). Commercial-grade DATEM (17% esterified tartaric acid; major fatty acids—palmitic and stearic) and dioleate (81% diglycerides, 15% monoglycerides, 4% triglycerides; major fatty acids—oleic (80%) and linoleic (10%); equilibrium mixture of 40% 1,2- and 60% 1,3-diglycerides) were donated by Grindsted Products (Brabrand, Denmark). Monoglycerides (purity >99 wt %), Tween 20, and *n*-tetradecane (purity >99 wt %) were purchased from Sigma. Commercial corn oil was purchased from the local supermarket. The oil was made free from surface-active impurities by passing it twice through a Florisil column (50 g of oil to 4 g of 100-mesh Florisil at each pass) according to the method of Gaonkar (1989). Buffer salts were AnalaR-grade reagents. Table 1 gives molecular masses of protein and emulsifiers.

Emulsion Preparation. Emulsions were prepared with both native β-lactoglobulin and heat-treated β-lactoglobulin. The native protein was a 0.5 wt % solution in bis-tris buffer (20 mM, pH 7) prepared at room temperature. This was placed

* Author to whom correspondence should be addressed.

0021-8561/94/1442-1602$04.50/0

Table 1. Assumed Molecular Masses Used To Calculate Values of Surfactant/Protein Molar Ratio *R*

material	molec mass (g mol^{-1})
β-lactoglobulin (monomer)	18363
Tween 20	1228
DATEM	569
monoglyceride (glycerol monostearate)	358
diglyceride (glycerol dioleate)	540

in a 100-mL flask in a water bath at 70 °C for 30 min and then cooled immediately to room temperature to produce the heat-treated sample. The oil phase and the aqueous protein solution were mixed together with a high-speed stirrer and then the premix was converted into a fine oil-in-water emulsion (20 wt % oil, 0.4 wt % β-lactoglobulin) using a small-scale single-stage valve homogenizer (Dickinson et al., 1987) operating at room temperature and a pressure of 280 ± 20 bar. A Malvern Mastersizer S2.01 was used to determine the volume–surface average droplet diameter d_{32} and the specific surface area (total area per unit mass of emulsion).

Protein Displacement from Emulsion Droplets. The freshly made emulsion was divided into several aliquots. Immediately following emulsion formation, or after a delay of 1 h, a known amount of water-soluble surfactant (Tween 20 or DATEM) was added to each aliquot, at room temperature or 70 °C, to give the required surfactant-to-protein molar ratio R. In emulsions containing the oil-soluble surfactants (mono- or diglycerides), the emulsifier was dissolved (or dispersed) in the oil phase prior to emulsification. The emulsion samples containing the surfactant (oil-soluble or water-soluble) were left for 1 h at room temperature or 70 °C to allow competitive adsorption to proceed. Each sample was then centrifuged at 20 °C and 15000g for 15 min to separate the oil droplets from the aqueous serum phase. The aqueous phase was then withdrawn with a syringe and filtered with low-protein-binding filters (0.22-μm Millipore). The β-lactoglobulin concentration in the aqueous phase was mainly determined by fast protein liquid chromatography (FPLC) as described previously (Dickinson et al., 1989). As a consistency check, some protein determinations were also carried out according to the Kjeldahl method (Shin, 1983) assuming a nitrogen factor of 6.38. The protein surface concentration (mass per unit area) was inferred from the known specific surface area and the measured amount of protein in the serum phase after centrifugation.

Surface Shear Viscometry. The surface shear viscosity at the interface between n-tetradecane and the dilute aqueous protein solution (2×10^{-3} wt % β-lactoglobulin in 2 mM bis-tris buffer) was determined using the Couette-type surface rheometer described previously (Dickinson et al., 1990). The stainless steel biconical disk (diameter of 30 mm) was suspended by a torsion wire with its edge in the plane of the fluid interface between the protein solution (370 mL) and the oil (70 mL) contained in a glass dish (diameter of 145 mm) thermostatically controlled at 25 ± 1 °C or 70 ± 1 °C. Apparent surface viscosity was determined at fixed time intervals over a period of 2 days at a dish rotation speed of 1.27×10^{-3} rad s^{-1}. After the protein film was aged for 24 h, water-soluble surfactant was added to the aqueous phase using a syringe without causing any significant disruption to the interfacial film.

RESULTS AND DISCUSSION

We first consider the effect of heat treatment of β-lactoglobulin on its competitive adsorption with Tween 20 in n-tetradecane-in-water emulsions. The data in Figure 1 refer to protein surface coverage Γ as a function of surfactant/protein molar ratio R. After addition of Tween 20 to the freshly made emulsion ($d_{32} = 0.71$ μm), the sample was left at room temperature or 70 °C prior to centrifugation and protein analysis by FPLC. We see that at both temperatures there is a gradual reduction in protein surface coverage with increasing amount of emulsifier added. But, at each value of R, the protein surface concentration is higher in the system containing

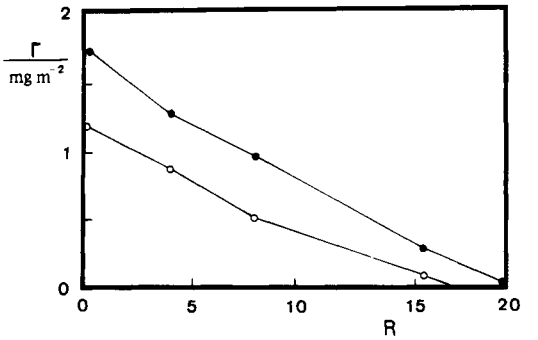

Figure 1. Competitive displacement of β-lactoglobulin by Tween 20 from the oil–water interface in emulsions (0.4 wt % protein, 20 wt % oil, 20 mM bis-tris buffer, pH 7) stored for 1 h at two different temperatures. Protein surface concentration Γ is plotted against surfactant-to-protein molar ratio R: (○) room temperature; (●) 70 °C.

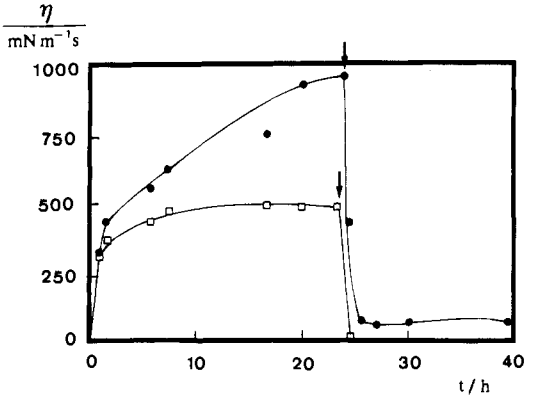

Figure 2. Influence of Tween 20 on surface shear viscosity of β-lactoglobulin adsorbed at n-tetradecane–water interface at pH 7. The protein bulk phase concentration is 2×10^{-3} wt %. Apparent surface viscosity η is plotted against time t: (□) 25 °C; (●) 70 °C. The arrow denotes the point (24 h) at which the emulsifier ($R = 1$) is added to the aqueous subphase.

the heat-treated β-lactoglobulin. Complete displacement occurs in the native β-lactoglobulin emulsions at $R \approx 17$ and in the heat-treated emulsions at $R \approx 20$. This observation that heat-treated β-lactoglobulin is more difficult to displace from the oil–water interface by Tween 20 is in agreement with the work of Das and Kinsella (1990). The latter authors attributed the increased amount of partially denatured heat-treated β-lactoglobulin adsorbed on the droplets to an increase in protein surface hydrophobicity. On the basis of earlier work by Dickinson and Matsumura (1991), we know that the β-lactoglobulin in an aged emulsion is partially polymerized by disulfide bonds, and this process is likely to be substantially accelerated at the surface of droplets in a heat-treated system, thereby possibly affecting the ease of displacement by water-soluble surfactant.

Figure 2 shows complementary competitive displacement experiments carried out at the planer n-tetradecane–water interface at neutral pH. Time-dependent surface shear viscosity η is plotted for adsorbed layers of β-lactoglobulin at 25 and 70 °C. In each case the aqueous phase protein concentration is 2×10^{-3} wt %. After 24 h, Tween 20 is added to the aqueous subphase at a concentration corresponding to $R = 1$. We see that the surface viscosity of the β-lactoglobulin film at 70 °C is higher and more time-dependent than that at 25 °C. At 25 °C, the addition of surfactant ($R = 1$) leads to a sudden drop in surface viscosity to a very low value as reported previously (Courthaudon et al., 1991b). At 70 °C, there is also a sudden drop but the value levels off

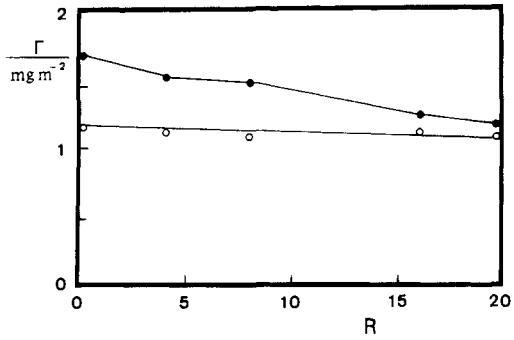

Figure 3. Competitive displacement of β-lactoglobulin by DATEM from the oil–water interface in emulsions (0.4 wt % protein, 20 wt % oil, 20 mM bis–tris buffer, pH 7) stored for 1 h at two different temperatures. Protein surface concentration Γ is plotted against surfactant-to-protein molar ratio R: (○) room temperature; (●) 70 °C.

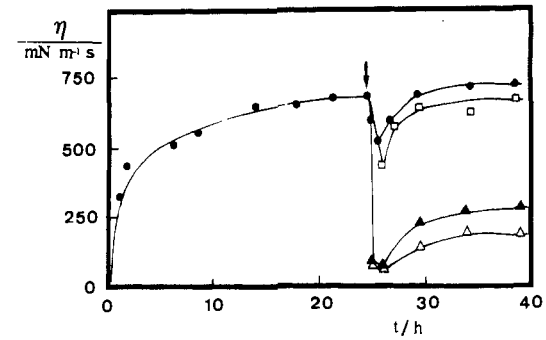

Figure 4. Influence of DATEM on surface shear viscosity of β-lactoglobulin adsorbed at n-tetradecane–water interface at pH 7. The temperature is 50 °C, and the protein bulk phase concentration is 2×10^{-3} wt %. Apparent surface viscosity η is plotted against time t: (●) $R = 1$; (□) $R = 4$; (▲) $R = 8$; (△) $R = 16$. The arrow denotes the point (24 h) at which the emulsifier is added to the aqueous subphase.

after a few hours to a finite substantial surface shear viscosity of $\eta \approx 70$ mN m^{-1} s, suggesting that there is still a significant amount of protein at the interface. These results are consistent with those from the emulsion experiments insofar as they imply a greater difficulty of displacement of the heat-treated protein.

Whey protein thermal denaturation is a complex process that is not yet fully understood for individual proteins in solution (Morr and Ha, 1993), never mind for proteins adsorbed at the surface of emulsion droplets. What is clear, however, is that two distinct stages are involved in solution: protein unfolding and protein aggregation. These two stages have different responses to such variables as pH, protein concentration, and temperature. In the present study carried out at neutral pH, we chose to compare the native β-lactoglobulin with that heat-treated at 70 °C because it appears that 70 °C corresponds to a transition temperature in the denaturation process (de Wit and Swinkels, 1980). Above 70 °C, the nature of the denaturation behavior starts to change due to the onset of the aggregation process, as indicated by the transition temperature of the differential scanning calorimetry curve. That is, at 70 °C we get the maximum degree of reversible unfolding of the β-lactoglobulin molecules with the minimum of irreversible aggregation.

At the oil–water interface, the local concentration of protein molecules is very much higher than in a typical protein solution; in addition, even at room temperature, the molecules are much more unfolded than in bulk solution. Both of these factors favor a greatly increased rate of protein aggregation at the emulsion droplet surface compared with that in solution. Whereas sulfhydryl residues are mainly responsible for formation of strong protein–protein interactions, also of substantial importance are electrostatic interactions involving ε-amino and carboxyl groups, as well as hydrophobic interactions involving nonpolar side chains. At 70 °C we expect that the rate of formation of intermolecular interactions in the adsorbed layer to be greatly enhanced as compared with the rate at room temperature. This is reflected in the strongly time-dependent surface shear viscosity of β-lactoglobulin at 70 °C shown in Figure 2. The structure of the adsorbed protein layer seems likely to correspond closely to that of a highly dense two-dimensional gel.

We turn now from the nonionic water-soluble Tween 20 to the anionic water-soluble DATEM. Figure 3 shows the experimental results for competitive protein displacement as a function of amount of added emulsifier. In fact, in contrast to Tween 20 (Figure 1), DATEM produces rather little displacement of β-lactoglobulin at room temperature or 70 °C. The surface coverage at 70 °C falls from $\Gamma = 1.7$ mg m^{-2} at $R = 0$ to $\Gamma = 1.25$ mg m^{-2} at $R = 20$. At room temperature the measured surface coverage remains constant at $\Gamma \approx 1.2$ mg m^{-2} irrespective of the amount of emulsifier. The melting point of the sample of DATEM used in this study was 45°, and so the reason for the insignificant effect of DATEM at room temperature is probably due to resolidification of the emulsifier in the emulsion during room temperature storage. Previous work with the anionic surfactant sodium dodecyl sulfate (SDS) has shown (Dickinson and Woskett, 1989; Dickinson, 1991) that it is less effective in displacing disordered protein (gelatin or caseinate) from the oil–water interface because of strong SDS–protein molecular complexation in bulk solution and at the interface. Complexation of DATEM with β-lactoglobulin could therefore be the explanation for the poor competitive displacing power of this emulsifier. Certainly, in the bakery industry, the so-called "dough conditioning" action of DATEM and other anionic emulsifiers (e.g., sodium stearoyl-2-lactylate) is supposed to involve some sort of cereal protein–emulsifier complexation. Maybe a similar kind of interaction is taking place in our model β-lactoglobulin emulsions.

Figure 4 shows the time-dependent surface shear viscosity of β-lactoglobulin at the planar n-tetradecane–water interface (2×10^{-3} wt % protein, pH 7, 50 °C) with DATEM added to the aqueous subphase after 24 h. The effect of the added emulsifier is to reduce the measured viscosity in the short term (≲1 h) but to a much lesser extent than found with Tween 20 (Figure 2). The rapid fall in surface viscosity is then followed by a slow recovery over the next 10–15 h. The larger the quantity of added emulsifier, the lower the limiting steady-state viscosity reached. At low added DATEM concentrations ($R = 1$ or 4) the surface viscosity in the mixed DATEM/protein system after the recovery is the same as that for the pure protein system immediately prior to the addition of the anionic surfactant. These surface rheology results are consistent with some interaction between β-lactoglobulin and DATEM at the oil–water interface together with some finite but limited protein displacement.

In β-lactoglobulin emulsions stored at 70 °C, the amount of protein associated with the oil–water interface increases gradually with storage time as illustrated in Figure 5. This gradual accumulation of partially denatured β-lactoglobulin at the droplet surface is consistent with the slow thermally induced aggregation of β-lactoglobulin in aqueous solution under similar

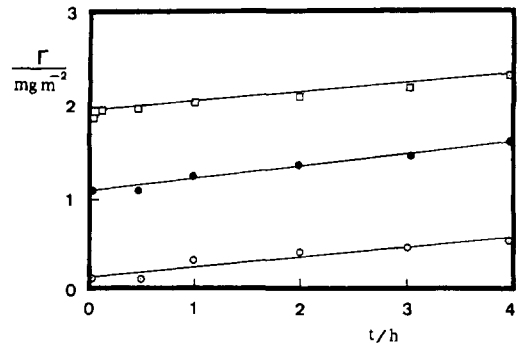

Figure 5. Time-dependent surface concentration $\Gamma(t)$ of β-lactoglobulin at 70 °C in *n*-tetradecane oil-in-water emulsions (0.4 wt % protein, 20 wt % oil, pH 7): (□) no emulsifier present; (○) Tween 20 added to fresh emulsion ($R = 14$); (●) Tween 20 added to 1-h-old emulsion ($R = 14$).

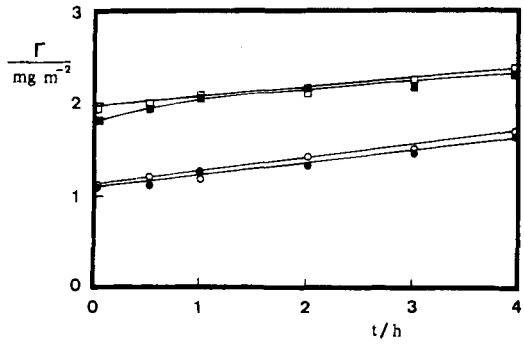

Figure 6. Comparison of time-dependent surface coverages of β-lactoglobulin at 70 °C in emulsions as determined by FPLC and Kjeldahl methods: (□, ■) no emulsifier present; (○, ●) Tween 20 added to 1-h-old emulsion ($R = 14$); (○, □) FPLC technique; (●, ■) Kjeldahl technique.

conditions (Xiong et al., 1993). It also helps to explain the strongly time-dependent surface shear viscosity of β-lactoglobulin at 70 °C shown in Figure 2. In the presence of Tween 20, there is also a similar rate of increase in the amount of protein associated with the emulsion droplets at 70 °C, although the absolute values of protein surface coverage are substantially lower (see Figure 5). The surface coverages reported in Figure 5 (and also in Figures 1 and 3) are based on serum phase protein concentrations determined by FPLC. To ensure that the trends noted in Figure 5 were not a reflection of loss of protein in the analysis procedure due to protein aggregation, it was decided to check these experiments independently using the Kjeldahl method. Figure 6 shows a comparison of the FPLC results with those obtained using the Kjeldahl technique for samples with and without added emulsifier. Within the experimental error, the two sets of measurements are in exact agreement, which gives us confidence that there is indeed a gradual accumulation of β-lactoglobulin at the surface of the droplets with storage time at 70 °C.

The remaining experimental results to be reported in this paper relate to possible competitive adsorption of β-lactoglobulin with glycerol dioleate at ambient temperature. As diglycerides are relatively weakly polar and of low surface activity, one might assume that they would have little capacity for competitively displacing proteins from the oil–water interface, although it seems that this supposition has not previously been tested experimentally. It also seems important to compare the effect of diglyceride to that of monoglyceride to give some useful estimate of experimental reproducibility and precision. Figure 7 shows the protein surface coverage Γ and the average droplet diameter d_{32} as a function of surfactant-to-protein molar ratio R for corn

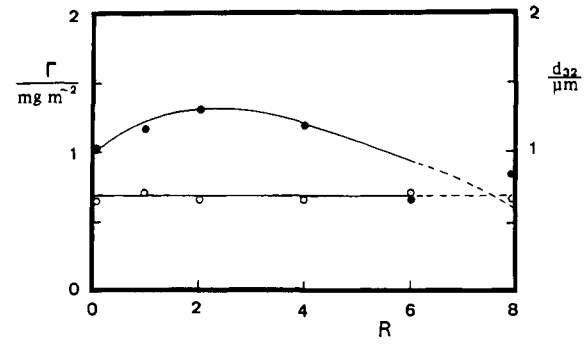

Figure 7. Influence of monoglyceride present before homogenization on protein surface coverage Γ and average droplet size d_{32} for corn oil-in-water emulsions (0.4 wt % β-lactoglobulin, 20 wt % oil, pH 7). Surface coverage (●) and average droplet size (○) are plotted against surfactant/protein molar ratio R. The dashed line denotes emulsions containing solidified emulsifier at ambient temperature.

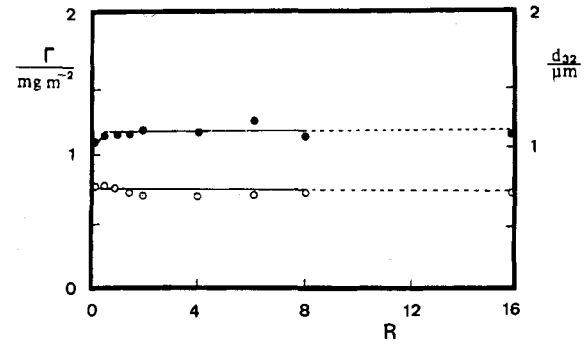

Figure 8. Influence of diglyceride present before homogenization on protein surface coverage Γ and average droplet size d_{32} for corn oil-in-water emulsions (0.4 wt % β-lactoglobulin, 20 wt % oil, pH 7). Surface coverage (●) and average droplet size (○) are plotted against surfactant/protein molar ratio R. The dashed line denotes emulsions containing solidified emulsifier at ambient temperature.

oil-in-water emulsions (0.4 wt % β-lactoglobulin, 20 wt % oil, pH 7) containing monoglyceride. Within the experimental error, the presence of monoglyceride in the triglyceride oil during emulsification has no effect on the resulting emulsion droplet size distribution, but it does have a small effect on the protein surface concentration. At small monoglyceride concentrations (up to $R \approx 2$) there is an increase in the amount of protein associated with the droplets (by 20–30%) and then a modest decrease again at higher concentrations. A similar kind of behavior was found previously (Dickinson et al., 1993) with glycerol monostearate in soybean oil-in-water emulsions and Span 80 (sorbitan monooleate) in *n*-tetradecane-in-water emulsions. The results for emulsions with monoglyceride replaced by glycerol dioleate are shown in Figure 8. We can see that, within the experimental error, there is no change in either surface coverage Γ or average droplet size d_{32} on addition of diglyceride to the oil phase. These results therefore provide a convincing demonstration that the protein surface coverage in triglyceride oil-based food emulsions is unaffected by the presence of dissolved diglycerides, especially, as is usually the case, when monoglycerides are also present.

CONCLUSIONS

From the data presented here, together with results previously obtained in our laboratory, we are in a position to categorize a wide range of surfactants according to their ability to displace β-lactoglobulin from the oil–water interface. The most effective displacers appear to be the water-soluble nonionic emulsifiers:

polyoxyethylene ethers (e.g., $C_{12}E_8$), polyoxyethylene derivatives of sorbitan esters (e.g., Tween 20), or sucrose esters (e.g., sucrose monolaurate). When the nonionic emulsifier has a short *n*-alkyl chain (e.g., sucrose monocaprylate), however, it is much less effective as a displacer (Bireau and Dickinson, unpublished results). Also very effective as displacers are the zwitterionic phospholipids, e.g., phosphatidylcholine and phosphatidylethanolamine. Much less effective for β-lactoglobulin displacement are the water-soluble anionic emulsifiers: *n*-alkyl sulfates (e.g., SDS) or tartaric esters of monoglycerides (e.g., DATEM). Also much less effective at low concentrations are the oil-soluble emulsifiers: polyoxyethylene ethers (e.g., $C_{12}E_2$), sorbitan esters (e.g., San 80), and monoglycerides (e.g., GMS). Totally ineffective as protein displacers are the diglycerides.

We have demonstrated in this paper that heat treatment of β-lactoglobulin (at 70 °C) makes it more difficult to displace from the emulsion droplet surface or the planar oil–water interface by water-soluble nonionic surfactant. Other factors that also make competitive displacement more difficult are aging of the protein film at the surface prior to addition of emulsifier and reduction of pH toward the protein's isoelectric point (Chen and Dickinson, 1993). These factors also affect the stability of β-lactoglobulin-coated emulsion droplets with respect to flocculation and coalescence under quiescent and orthokinetic conditions. Understanding the relationship between competitive displacement of proteins and controlled emulsion destabilization is the objective of the next stage of our research program.

LITERATURE CITED

Barfod, N. M.; Krog, N.; Larsen, G.; Buchheim, W. Effects of Emulsifiers on Protein/Fat Interaction in Ice-cream Mix during Ageing. *Fat Sci. Technol.* **1991**, *93*, 24–29.

Berger, K. G. Ice-cream. In *Food Emulsions*, 2nd ed.; Larsson, K., Friberg, S. E., Eds.; Dekker: New York, 1990; pp 367–444.

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.

Chen, J.; Dickinson, E.; Iveson, G. Interfacial Interactions, Competitive Adsorption and Emulsion Stability. *Food Struct.* **1993**, *12*, 135–146.

Courthaudon, J.-L.; Dickinson, E.; Dalgleish, D. G. Competitive Adsorption of β-Casein and Non-ionic Surfactants in Oil-in-Water Emulsions. *J. Colloid Interface Sci.* **1991a**, *145*, 390–395.

Courthaudon, J.-L.; Dickinson, E.; Matsumura, Y.; Clark, D. C. Competitive Adsorption of β-Lactoglobulin + Tween 20 at the Oil–Water Interface. *Colloids Surf.* **1991b**, *56*, 293–300.

Courthaudon, J.-L.; Dickinson, E.; Matsumura, Y.; Williams, A. Influence of Emulsifier on the Competitive Adsorption of Whey Proteins in Emulsions. *Food Struct.* **1991c**, *10*, 109–115.

Courthaudon, J.-L.; Dickinson, E.; Christie, W. W. Competitive Adsorption of Lecithin and β-Casein in Oil-in-Water Emulsions. *J. Agric. Food Chem.* **1991d**, *39*, 1365–1368.

Das, K. P.; Kinsella, J. E. Effect of Heat Denaturation on the Adsorption of β-Lactoglobulin at the Oil/Water Interface and on Coalescence Stability of Emulsions. *J. Colloid Interface Sci.* **1990**, *139*, 551–560.

de Wit, J. N. Functional Properties of Whey Proteins. In *Developments in Dairy Chemistry 4*; Fox, P. F., Ed.; Elsevier Applied Science: London, 1989; pp 285–321.

de Wit, J. N.; Swinkels, G. A. M. A Differential Scanning Calorimetric Study of the Thermal Denaturation of Bovine β-Lactoglobulin. *Biochim. Biophys. Acta* **1980**, *624*, 40–50.

Dickinson, E. Competitive Adsorption and Protein–Surfactant Interactions in Oil-in-Water Emulsions. In *Microemulsions and Emulsions in Foods*; El-Nokaly, M., Cornell, D., Eds.; ACS Symposium Series 448; American Chemical Society: Washington, DC, 1991; pp 114–129.

Dickinson, E.; Iveson, G. Adsorbed Films of β-Lactoglobulin + Lecithin at the Hydrocarbon–Water and Triglyceride–Water Interfaces. *Food Hydrocolloids* **1993**, *6*, 533–541.

Dickinson, E.; Matsumura, Y. Time-dependent Polymerization of β-Lactoglobulin through Disulphide Bonds at the Oil–Water Interface in Emulsions. *Int. J. Biol. Macromol.* **1991**, *13*, 26–30.

Dickinson, E.; Tanai, S. Protein Displacement from the Emulsion Droplet Surface by Oil-Soluble and Water-Soluble Surfactants. *J. Agric. Food Chem.* **1992a**, *40*, 179–183.

Dickinson, E.; Tanai, S. Temperature Dependence of the Competitive Displacement of Protein from the Emulsion Droplet Surface by Surfactants. *Food Hydrocolloids* **1992b**, *6*, 163–171.

Dickinson, E.; Woskett, C. M. Competitive Adsorption between Proteins and Small-molecule Surfactants in Food Emulsions. In *Food Colloids*; Bee, R. D., Richmond, P., Mingins, J., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1989; pp 74–96.

Dickinson, E.; Murray, A.; Murray, B. S.; Stainsby, G. Properties of Adsorbed Layers in Emulsions Containing a Mixture of Caseinate and Gelatin. In *Food Emulsions and Foams*; Dickinson, E., Ed.; Royal Society of Chemistry: London, 1987; pp 86–99.

Dickinson, E.; Rolfe, S. E.; Dalgleish, D. G. Competitive Adsorption in Oil-in-Water Emulsions containing α-Lactalbumin and β-Lactoglobulin. *Food Hydrocolloids* **1989**, *3*, 193–203.

Dickinson, E.; Rolfe, S. E.; Dalgleish, D. G. Surface Shear Viscosity as a Probe of Protein–Protein Interactions in Mixed Protein Films Adsorbed at the Oil–Water Interface. *Int. J. Biol. Macromol.* **1990**, *12*, 189–194.

Dickinson, E.; Owusu, R. K.; Tan, S.; Williams, A. Oil-Soluble Surfactants have Little Effect on Competitive Adsorption of α-Lactalbumin and β-Lactoglobulin in Emulsions. *J. Food Sci.* **1993**, *58*, 295–298.

Gaonkar, A. G. Interfacial Tensions of Vegetable Oil/Water Systems: Effect of Oil Purification. *J. Am. Oil Chem. Soc.* **1989**, *66*, 1090–1091.

Goff, H. D.; Jordan, W. K. Action of Emulsifiers in Promoting Fat Destabilization during the Manufacture of Ice-cream. *J. Dairy Sci.* **1989**, *72*, 18–29.

Jost, R.; Dannenberg, F.; Gummy, D. Action of Milk Proteins in Emulsions of Various Food Systems. In *Proceedings of the XXIII International Dairy Congress*, Montreal, Oct 8–12, 1990; International Dairy Federation: Brussels; Vol. 2, pp 1481–1491.

Katsuta, K.; Kinsella, J. E. Effects of Temperature on Viscoelastic Properties and Activation Energies of Whey Protein Gels. *J. Food Sci.* **1990**, *55*, 1296–1302.

Kinsella, J. E.; Whitehead, D. M. Proteins in Whey: Chemical, Physical and Functional Properties. *Adv. Food Nutr. Res.* **1989**, *33*, 343–360.

McClements, D. J.; Monahan, F. J.; Kinsella, J. E. Disulfide Bond Formation Affects Stability of Whey Protein Isolate Emulsions. *J. Food Sci.* **1993**, *58*, 1036–1039.

Morr, C. V.; Ha, E. Y. W. Whey Protein Concentrates and Isolates: Processing and Functional Properties. *Crit. Rev. Food Sci. Nutr.* **1993**, *33*, 431–476.

Mulvihill, D. M.; Rector, D.; Kinsella, J. E. Mercaptoethanol, N-Ethylmaleimide, Propylene Glycol and Urea Effects on Rheological Properties of Thermally Induced β-Lactoglobulin Gels at Alkaline pH. *J. Food Sci.* **1991**, *56*, 1338–1341.

Shin, H. S. *Food Analysis*; Hyung Kwang Press: Seoul, 1983; pp 81–87.

Xiong, Y. L.; Dawson, K. A.; Wan, L. Thermal Aggregation of β-Lactoglobulin: Effect of pH, Ionic Environment, and Thiol Reagent. *J. Dairy Sci.* **1993**, *76*, 70–77.

Received for review January 21, 1994. Revised manuscript received April 27, 1994. Accepted May 12, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, June 15, 1994.