Competitive Adsorption between Dioleoylphosphatidylcholine and Sodium Caseinate on Oil−Water Interfaces

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Oil-in-water emulsions stabilized by sodium caseinate and dioleoylphosphatidylcholine (DOPC) were studied by dynamic light scattering and electrophoresis. The initial mean droplet size of the emulsions depended strongly on the concentration of casein, but was virtually independent of DOPC. However, the emulsion stability was strongly affected by the presence of DOPC; with a DOPC: casein molar ratio of <10, the emulsions were stable, but at higher ratios, the average size of emulsion droplets increased during storage. Competitive adsorption between DOPC and casein occurred during both the formation and storage of the emulsions. Of the constituents of whole casein, β-casein interacted most with DOPC. At casein concentrations of <0.7%, β-casein was completely removed from the oil−water interface when the emulsions were stored for 48 h, but α_{s1}-casein remained on the surface even at the highest DOPC:casein molar ratio (49:1) used in this study. As well as changing the adsorption characteristics of the different caseins, DOPC also changed the hydrodynamic thickness of the adsorbed casein layer. Compared with emulsions stabilized by casein alone, the layer thickness of caseins in the emulsions formed with mixtures of caseinate and DOPC changed because of competitive adsorption between DOPC and casein at the oil−water interface.

Keywords: *Casein; emulsions; phospholipids; proteins; adsorption; emulsion stability*

INTRODUCTION

Milk proteins are good emulsifiers and therefore have been important ingredients in food emulsions. However, other surfactants, such as phospholipids, are also widely used (Graf and Meyer, 1976). The interactions between casein and surfactants of smaller molecular weight have important influences on the properties of food emulsions. From studies on the interactions between milk proteins and a variety of surfactants it has been demonstrated that many surfactant molecules can displace protein from the interfaces (de Feijter *et al.*, 1987; Courthaudon *et al.*, 1991a; Dickinson and Tanai, 1992; Heertje *et al.*, 1990; Wilde and Clark, 1993; Courthaudon *et al.*, 1991b). The degree of displacement depends not only on the specific surfactant used, but also on when it is added (i.e., before or after emulsion formation); if the surfactant is introduced before emulsification, partial displacement is found, and if the surfactant is added after emulsification, complete displacement may occur (Courthaudon *et al.*, 1991b). Among many surfactants studied, phospholipids, especially phosphatidylcholine from egg yolk (egg-PC), have been given special attention. The addition of soy lecithin to concentrated homogenized milk can increase the heat stability of the product (Muir and Sweetsur, 1992; Hardy-Lloyd *et al.*, 1986). Also, egg-PC displaces β-casein from the oil−water interface at high phospholipid-to-protein ratios (Courthaudon *et al.*, 1991a), but egg-PC is far less efficient than other surfactants such as Tween 20; even at high phospholipid:protein ratios, protein and egg-PC coexist on the interface (Courthaudon *et al.*, 1991b). Our earlier work on oil-in-water emulsions stabilized by egg-PC and casein confirmed this coexistence and showed that the stability of emulsions containing low concentrations of casein was enhanced by the presence of egg-PC (Fang and Dalgleish, 1993a). This behavior is quite different from that of other water-soluble surfactants, which tend to have a strong capacity for displacing proteins from the interface (Dickinson and Tanai, 1992; Courthaudon *et al.*, 1991b,c).

Surfactants may also affect the protein itself; for example, SDS promotes polymer formation of β-casein in solution, and there seems to be a limited number of binding sites for SDS on β-casein (Creamer, 1980). Among the different components of whole casein, β-casein is believed to be the most hydrophobic and it also gives a thicker hydrodynamic layer when adsorbed on hydrophobic surfaces (Dalgleish, 1993); this measurement can be made by dynamic light scattering combined with proteolysis of the casein (Fang and Dalgleish, 1993b, Dalgleish and Leaver, 1991). Caseins can also compete for adsorption; β-casein adsorbs more strongly on an oil−water interface than does α_s-casein, and β-casein can displace α_s-casein from the interface (Dickinson *et al.*, 1988; Nylander and Wahlgren, 1994).

Phospholipids differ in their head groups and also the fatty acids that make up the diglyceride portion of the molecule. As part of research into the interactions of different types of phospholipids with milk proteins, we studied dioleoylphosphatidylcholine (DOPC), whose two fatty acid chains are unsaturated. The stability of the emulsions stabilized by casein and DOPC were monitored by light scattering as the emulsion aged, and the thickness of the casein layer adsorbed on the interface was studied by dynamic light scattering. The amount of casein adsorbed on the oil droplets was obtained by separating the emulsion droplets by centrifugation and then measuring the adsorbed protein by electrophoresis (SDS-PAGE). Most experiments in this work were performed under conditions similar to those in earlier studies on emulsions stabilized by egg-PC and casein to allow direct comparison of the impact of the two different phospholipids and to try to determine the important factors governing the protein/ phospholipid interaction.

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MATERIALS AND METHODS

Soybean oil, DOPC, imidazole, and TPCK-trypsin were purchased from Sigma Chemicals, St. Louis, MO, and were used without further purification. 2-Mercaptoethanol was purchased from Fisher Scientific (Mississauga, ON). Whole sodium caseinate was prepared in the laboratory by precipitating skim milk at pH 4.6, redissolving the washed precipitate to pH 7 with 0.1 N NaOH, and then freeze-drying the solution. Casein solutions were prepared in imidazole buffer (20 mM imidazole, pH 7.0) and were pre-filtered through a 0.22-μm filter (Millipore, Mississauga, ON) before use. Trypsin solution was prepared at a concentration of 1 mg·mL^{-1}.

Emulsions were made by homogenizing soybean oil (20%, w/w), casein solution, and DOPC together in a Microfluidizer (model 110S, Microfluidics Corp., Newton, MA) at an input pressure of 0.3 MPa, which corresponds to a pressure drop of 42 MPa. The DOPC was dispersed in the aqueous phase before homogenization, and, after all the ingredients were prehomogenized, each sample was circulated through the homogenizing unit 10 times before being collected. Total volumes of 10 and 20 mL of emulsion were prepared for light scattering studies and for the analysis of casein by electrophoresis, respectively. The concentration of casein was varied between 0.3 and 2% (w/w), and concentrations of 0.2 or 0.5% of DOPC were used. After the emulsions were prepared, they were analyzed the same day and then were stored at 4 °C for future measurements.

The mean volume-to-surface diameter (d_{32}), size distribution, and the surface area of the emulsion droplets were measured by light scattering with a Mastersizer X (Malvern Instruments Inc., Southboro, MA). The surface area of the emulsion measured by this method was used to calculate the surface concentration of the casein. The stability of the stored emulsions was also monitored by measuring the size distribution every day for 1 week.

The hydrodynamic thickness of the adsorbed layer of casein was measured by photon correlation spectroscopy (PCS) with a 4700 optical system with a scattering angle of 90°, attached to a 7032 correlator (Malvern Instruments). The emulsions were diluted at a ratio of 1.5 μL of emulsion to 3 mL of imidazole buffer, which had been passed through a 0.22-μm cellulose nitrate filter (Millipore Ltd.). The temperature of the samples was controlled at 25 ± 0.1 °C with a circulating water bath. The average hydrodynamic diameters of the emulsion droplets were first measured by averaging sets of 10 individual PCS runs, each lasting 1 min. Then, 1 μL of trypsin solution was added to the diluted emulsion to degrade the adsorbed protein, and the diameter was measured again under the same conditions. A new lower equilibrium size of the emulsion was reached very quickly, and the decrease in hydrodynamic radius caused by the addition of trypsin was defined as the hydrodynamic thickness of the adsorbed layer of protein (Dalgleish, 1993).

To determine the amount of protein adsorbed on the oil droplets, direct analysis of the emulsion droplets was used (Hunt and Dalgleish, 1994). Electrophoresis (SDS-PAGE) on a 20% homogeneous gel was performed with a PhastSystem (Pharmacia LKB Canada Ltd., Baie d'Urfé, PQ). The emulsions were divided into two parts (each of 10 mL) immediately after they were made, and one part was immediately centrifuged at 15000*g* for 1 h. The floating fraction (cream) of emulsion droplets was collected and resuspended in imidazole buffer to wash off any unbound protein, and the resuspended droplets were centrifuged again, and the cream was collected once more. The second sample of the emulsion was stored in the refrigerator for 48 h before being centrifuged in the same way to collect the cream phase. After the cream was collected, it was dried on a Whatman No. 1 filter paper and an appropriate amount of it (0.4 g) was weighed out and resuspended in 1.6 mL of water to make a dispersion containing ~20% oil phase. As long as the amount of cream is accurately known and the diluting water is also accurately measured, then it is not necessary to make exactly 20%. The dilution was done in this way to ensure that the protein concentration was in the same range as the standards. This dispersion was allowed to equilibrate for several hours, and then 150 μL was mixed with 250 μL of 20% SDS solution, 100 μL of 2-mercaptoethanol, and 100 μL of a 0.05% solution of bromophenol blue solution. This mixture was heated at 100 °C for 5 min to allow desorption and denaturation of the protein. The whole emulsion was also treated in the same manner as the redispersed cream and was run on the same gel as a standard. The gels were dried, and the intensities of the stained bands were measured with a gel scanner (Ultrascan XL, Pharmacia LKB). There sultant data were stored and analyzed with a computer, and the integrated area of the peaks representing the amount of protein detected in the cream phase was compared with the amount of protein detected in the whole emulsion, to calculate the amount of adsorbed protein. Combining the surface area of emulsion droplets with the amount of adsorbed protein allowed the surface concentration of casein to be calculated.

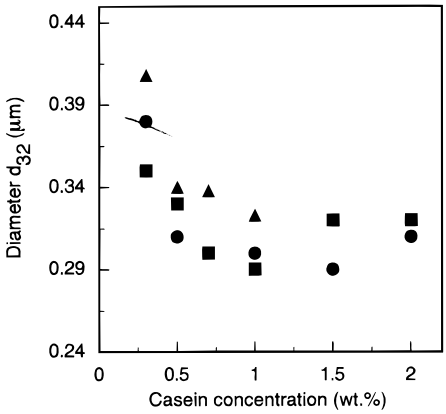

Figure 1. Mean droplet size (d_{32}) of emulsions (20%, v/v) as a function of casein concentration in the presence of 0.2% (●) and 0.5% (■) DOPC. Results obtained with emulsions stabilized by casein alone (▲) are also plotted as a reference.

RESULTS AND DISCUSSION

Emulsion Stability. The mean droplet sizes measured shortly after the emulsions were made are shown in Figure 1. The d_{32} values of emulsion droplets made with 0, 0.2, and 0.5% DOPC were similar for given casein concentrations, but varied with the concentration of casein. For freshly prepared emulsions, this dependence of d_{32} on the concentration of protein is similar to emulsions made without any lecithin under the same homogenization conditions (Fang and Dalgleish, 1993a). The presence of DOPC had a small effect on the initial droplet size in the emulsions; that is, the droplets were slightly smaller than in the absence of phospholipid.

The stability of emulsions containing DOPC was also followed as the emulsions were stored, and in some cases the particle size and size distribution were found to change depending on their composition. Emulsions made with 0.2% DOPC required at least 0.7% casein to maintain a stable mean droplet size and unchanging monomodal size distribution during storage (Figure 2a). The mean size of emulsion droplets for samples containing 0.5% or less casein increased with time, and the size distributions shifted toward a larger value (Figure 2b). For emulsions made with 0.5% DOPC, only those containing 1.5% casein or more (Figure 3a) were stable with time and had a monomodal distribution. The sample containing 1% casein was on the borderline of stability, but emulsions with 0.7% or less casein were not stable. The mean droplet size increased and the size distribution moved to larger sizes after 2 days of storage (Figure 3b).

Taking 23 000 and 786 Da as the relative molecular masses for casein and DOPC, respectively, the DOPC:

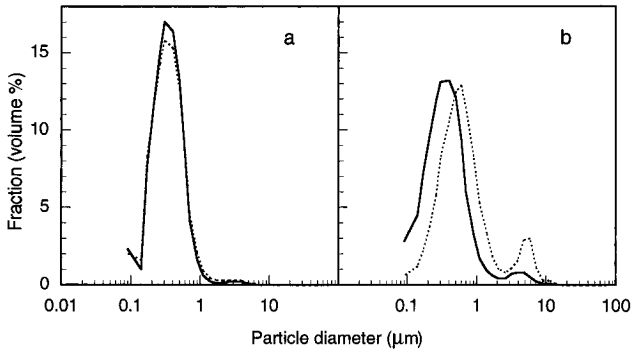

Figure 2. Size distribution of emulsion droplets containing 0.2% DOPC: (a) emulsions made with 0.7% casein; (b) emulsions containing 0.5% casein; solid lines, results obtained from fresh emulsion; broken lines, results obtained from the same emulsion that had been stored for 2 days at 4 °C before measurement.

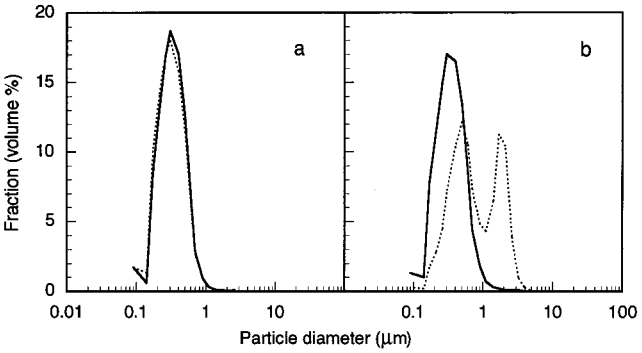

Figure 3. Size distribution of emulsion droplets containing 0.5% DOPC: (a) emulsions made with 1.5% casein; (b) emulsions containing 0.7% casein; solid lines, results obtained from fresh emulsions; broken lines, results obtained from the same emulsions which had been stored for 2 days at 4 °C before measurement.

Table 1. Molar DOPC:Casein Ratios[a]

DOPC concn (%)	casein concn 0.3%	0.5%	0.7%	1.0%	1.5%	2.0%
0.2	19.5	11.7	**8.4**	5.9	3.9	2.9
0.5	48.8	29.3	20.9	14.6	**9.8**	7.23

[a] Limiting ratios for stable emulsions are shown in bold type.

casein molar ratio can be calculated for each sample (Table 1). Combining the results of the emulsion stability from Figures 2 and 3 with the data listed in Table 1, it is evident that if the DOPC:casein molar ratio was <10, the emulsions were stable, but if the ratio was >10, they were not. These results are very different from our previous work on emulsions stabilized by casein alone (Fang and Dalgleish, 1993b) or by mixtures of casein and egg-PC (Fang and Dalgleish, 1993a). In those studies, casein at a concentration of 0.3% was sufficient to stabilize an emulsion of 20% oil in the absence of phospholipid, and the presence of egg-PC even enhanced the stability of emulsions with casein concentrations as low as 0.1%. Even though DOPC and egg-PC share a common hydrophilic head group, the present results demonstrate that DOPC behaves very differently from egg-PC in emulsions containing casein and phospholipid. In the case of competitive adsorption between casein and some small surfactants, the emulsions remain stable if not sheared after the protein is displaced (Courthaudon *et al.*, 1991b; Chen *et al.*, 1993). However, DOPC not only displaces casein from the interface, but it destabilizes the emulsion at the same time, even though displacement of the protein is not complete.

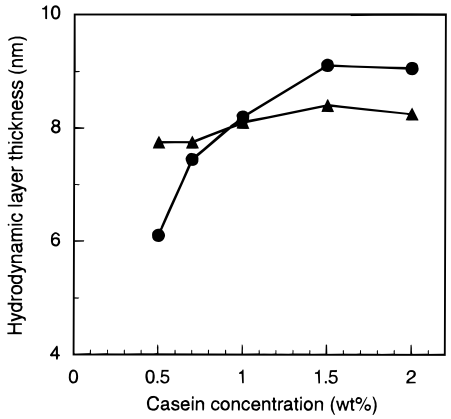

Figure 4. Change of apparent thickness of the adsorbed casein layer on the droplets in emulsions containing 0.2% DOPC, measured by trypsin treatment of the emulsion, as defined by the overall concentration of casein: (●) data obtained from fresh emulsions; (▲) data from emulsions two or more days old.

Thickness of the Adsorbed Casein Layer. The hydrodynamic thickness of the adsorbed layer of casein on the oil droplets measured with PCS also depended on the age of the emulsion. The apparent thickness of the casein layers in emulsions containing 0.2% DOPC is shown in Figure 4. In freshly made emulsions, the apparent thickness of the layer depended on the casein concentration and was similar in value to the results obtained when emulsions were made containing a similar amount of egg-PC (Fang and Dalgleish, 1993a). The thickness was slightly lower than those obtained in the absence of phospholipid, where the thickness of the casein layer increased with casein concentration until it reached a plateau value of ~9 nm (Fang and Dalgleish, 1993b). The apparent layer thicknesses measured on the day after the emulsions were prepared were very different from the results measured on the first day (Figure 4), but no further change was observed as the samples were stored for longer times (measurements were made for up to 5 days). During storage, at concentrations of casein of <1%, the thickness of the adsorbed casein layer increased from 6.1 to 7.8 ± 0.3 nm; at higher concentrations of casein, the thickness decreased from 9.1 to 8.2 ± 0.3 nm. So, during aging, the thickness of the casein layer (8 nm) became almost independent of casein concentration. The thickness of the adsorbed casein layer in emulsions containing 0.5% egg-PC is 8 nm (Fang and Dalgleish, 1993a). This dependence of the layer thickness on the emulsion age shows that interactions between DOPC and casein were not at equilibrium shortly after the emulsions were made, and that the structure of the adsorbed layer of DOPC and casein changed with time at least partly because the composition changed (see next section). The thickness of the adsorbed layer of casein reflects both the surface concentration and conformation of the adsorbed protein, so the initial thickness of adsorbed casein is an indication of the degree of competitive adsorption to the oil−water interface that is freshly created while the emulsions are formed. As the concentration of casein is increased, the molar ratio DOPC: casein decreases, and more casein is adsorbed on the surface until the surface reaches saturation, resulting in the thickest casein layer. The relatively constant

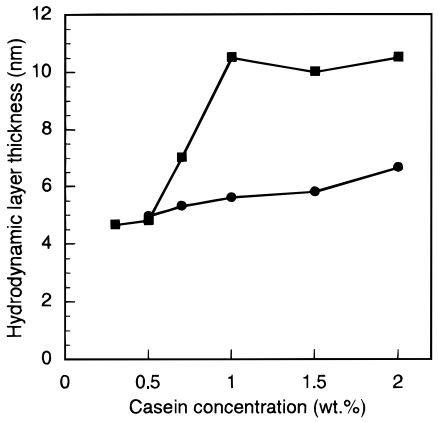

Figure 5. Change of apparent thickness of the adsorbed casein layer on the droplets in emulsions containing 0.5% DOPC, measured by trypsin treatment of the emulsion, as defined by the overall concentration of casein (●). Results obtained from emulsions stabilized by casein only (■) are also plotted as a reference.

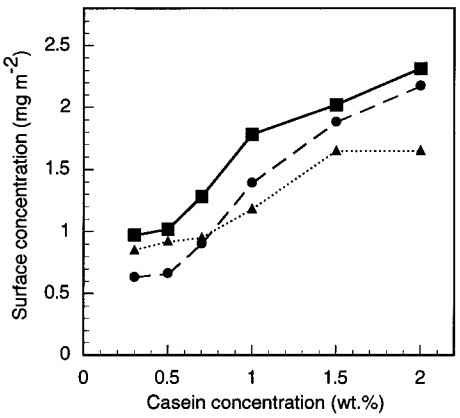

Figure 6. Surface concentration of casein as a function of total casein concentration for emulsions containing 0.2% DOPC. Results from emulsions made in the absence of DOPC (■) are shown as a reference. (●) Results from fresh emulsions; (▲) results from emulsions stored for 2 days at 4 °C.

value of layer thickness with casein concentration in aged emulsions may indicate that the presence of DOPC adjusted the surface concentration of casein to a similar level at all casein concentrations, or that it forced all casein molecules to adopt a particular conformation. In addition to this, DOPC selectively removes β-casein from the interface (see next section), and it is known that β-casein forms the thickest adsorbed layer of all of the caseins (Dalgleish, 1993). Removal of this protein could partly explain the decrease in hydrodynamic radius of the emulsions at high concentration of casein, but not the increase that is measured at low concentrations of protein.

The apparent thickness of the casein layer in emulsions containing 0.5% DOPC is shown in Figure 5. The apparent layer thickness of the adsorbed casein was as low as 5−6 nm even at high casein concentration; this is the lower plateau value of an adsorbed casein layer required to stabilize an emulsion in the absence of phospholipids (Fang and Dalgleish, 1993a). Unlike the results at 0.2% DOPC, the layer thickness did not depend on the age of the emulsion, even though some of the emulsions were unstable, especially when treated with trypsin. Emulsions containing 0.5% casein or lower could not be measured because an increase in size was observed after trypsin was added, and we had to reduce the amount of trypsin used for the other concentrations of casein to allow the measurements to be made. Because of the instability caused by casein breakdown, the errors in the layer thickness in these emulsions (Figure 5) may be larger than normal. We know that the surface concentration of casein (see below) is comparable to that in emulsions made with 0.5% egg-PC, where there is a thicker layer at most concentrations of casein (Fang and Dalgleish, 1993b), showing again the difference in effect between the two phospholipids. The coexistence of surfactant and protein on the interface can dramatically change the surface shear viscosity of the adsorbed film (Chen *et al.*, 1993; Dickinson *et al.*, 1993). Such an effect could at least partly arise from the changes in the structures of the adsorbed layers of protein as described here. However, even if protein is not displaced, the structure of the adsorbed layer can change (Dickinson *et al.*, 1990).

Surface Concentration of Casein. The surface concentration of casein adsorbed to oil droplets in emulsions containing 0.2% DOPC is shown in Figure 6. When the emulsions were analyzed within a few hours after they were made, the surface concentration of casein (Γ) as a function of the total casein concentration in the emulsions containing DOPC had a similar profile to, but a lower value than, the emulsions stabilized by casein alone. Even at low casein concentrations ($\leq$0.5%), not all of the casein was adsorbed to the oil droplets, in contrast to the behavior in emulsions containing casein and egg-PC. Even at the low casein content, there was competitive adsorption between casein and DOPC. In emulsions stored for 48 h at 4 °C, the surface concentration of casein was changed. At casein concentrations >0.7%, the surface concentration decreased with time and, because the droplet sizes in the emulsions were stable in this concentration range, the decrease in casein surface concentration arises because less casein is actually adsorbed to the interface. For emulsions containing <0.7% casein, the surface concentration of casein increased with time. In this case, the actual amount of the casein adsorbed did not change significantly, but an increase in droplet size (which gives a decrease in surface area) occurred as a result of the instability of these emulsions (Figure 2). The apparent increase in surface concentration of casein was simply a result of a similar amount of protein covering a smaller surface area. The profiles in Figure 6 help to explain the changes in the thickness of the casein layer shown in Figure 4. When the emulsions were fresh, there were considerable differences in the surface concentrations of caseins between emulsions containing 0.3% and 2.0% casein, and the thickness of the casein layer reflected this by reaching a plateau value of 9 nm and a lower value of ~6 nm. However, because the surface concentration of casein changed with time, the thickness of the adsorbed layer changed accordingly, with an increase in Γ at low total casein concentration resulting in a thicker casein layer, and a decrease in Γ at higher casein concentration leading to a thinner casein layer.

The surface concentrations of casein in emulsions containing 0.5% DOPC are shown in Figure 7. Like the emulsions containing 0.2% DOPC, the initial surface concentrations of casein, measured on the same day they were prepared, were systematically lower than those in the control emulsions stabilized by casein alone, even at very low casein concentrations where all of the casein was adsorbed on the surface in the control emulsions. The surface concentration of casein changed with time

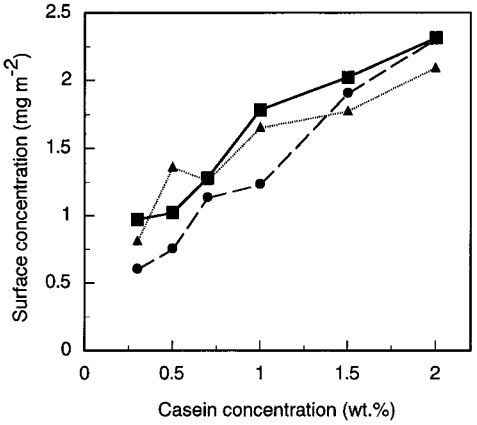

Figure 7. Surface concentration of casein as a function of total casein concentration for emulsions containing 0.5% DOPC. Results from emulsions made in the absence of DOPC (■) are shown as a reference. (●) Results from fresh emulsions; (▲) results from emulsions stored for 2 days at 4 °C.

in a similar manner to that for 0.2% DOPC; there was an increase in Γ at lower casein concentrations and a decrease in Γ at higher casein concentration. Below a total casein concentration of 1%, Γ was increased to the value obtained in the control emulsions, whereas above 1.5%, Γ was decreased. As we know from the series using 0.2% DOPC, the increase in Γ at lower concentrations of casein is a result of the decrease in the total surface area of the emulsion droplets because of the instability of the emulsion caused by the presence of DOPC. Though the casein displacement is time dependent, the experiments on emulsion stability (Figure 3) also show that this interaction is nearly completed after 48 h and the total surface area of the emulsion droplets stabilizes after this time. Combining the results from both the stability (Figure 3) and casein surface concentration (Figure 7), it seems that the emulsions formed with 1% casein or less became stable (via coalescence) once the casein surface concentration reached a similar value to the emulsions made without DOPC. The possibility was considered that some of the effects were the result of storage of the emulsions at 4 °C. Therefore, some experiments were conducted completely at room temperature, and essentially the same results were obtained, showing that the cooling did not have a significant effect or had one that was rapidly reversible.

Displacement of *β*-Casein by DOPC. In previous research on emulsions containing egg-PC and whole casein (Fang and Dalgleish, 1993a) there was no indication of preferential displacement or adsorption of any individual casein (whole casein contains four proteins α_{s1}, *β*, α_{s2}, and *κ* in the approximate concentration ratio 4:4:1:1). However, emulsions containing DOPC showed a different behavior in this respect. The electrophoresis patterns, even when the emulsions were analyzed shortly after they were formed, showed that the relative amount of *β*-casein adsorbed to the oil droplets was less than its proportion in the whole casein. During storage either at room temperature or at 4 °C, more of the *β*-casein was removed from the surface. This removal occurred at all casein concentrations, but was most easily seen in emulsions with low casein concentration. A typical gel (SDS-PAGE) obtained with emulsions containing 0.5% DOPC and 0.5 or 0.7% casein is shown in Figure 8. It is evident that the band corresponding to *β*-casein had nearly disappeared from the emulsion droplets of the 2-day-old emulsion, and a smaller

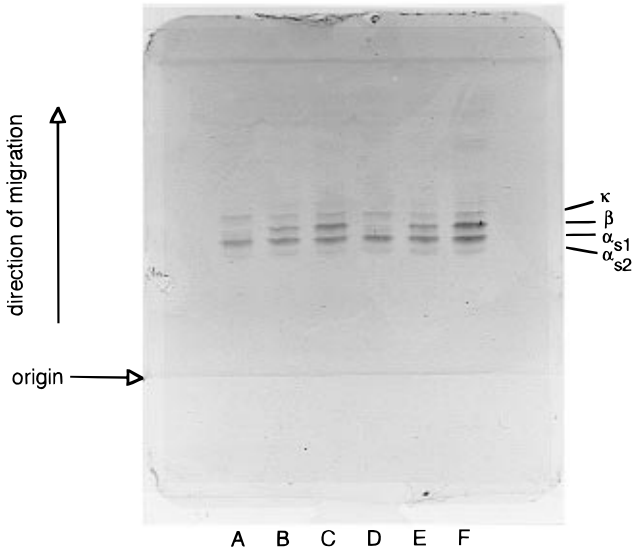

Figure 8. Typical SDS-PAGE gel of proteins obtained from emulsions containing 0.5 and 0.7% casein and 0.5% DOPC: (A) 0.5% casein, droplets isolated from 2 day old emulsion; (B) 0.5% casein, droplets isolated from fresh emulsion; (C) 0.5% casein, whole emulsion; (D) 0.7% casein, droplets isolated from 2-day-old emulsion; (E) 0.7% casein, droplets isolated from fresh emulsion; (F) 0.7% casein, whole emulsion. In order of increasing mobility, the bands are from α_{s2}-, α_{s1}-, *β*-, and *κ*-caseins (determined by comparison with standards).

amount of *β*-casein was observed on the first day cream sample compared with the emulsion as a whole. *β*-Casein is believed to be the most hydrophobic casein, and competitive adsorption studies on *β*-casein and α_s-casein have demonstrated that *β*-casein is preferentially adsorbed on the interface (Dickinson *et al.*, 1988). The results with DOPC and casein indicated the opposite: *β*-casein was preferentially removed from the interface by DOPC, and α_s- and *κ*-caseins were not significantly affected. No complete displacement of all of the caseins occurred even at the highest DOPC:casein molar ratio (which was 49:1, with 0.3% casein and 0.5% DOPC), indicating that the removal of *β*-casein by DOPC from the interface did not simply arise from general competition between all proteins and DOPC. It is probable that a specific interaction occurs between DOPC and *β*-casein that forms a DOPC−*β*-casein complex that is more hydrophilic and less surface active than either of its components. Analogous complexes have been defined for caseins and both mono- and diglycerides (Doxastakis and Sherman, 1984). This complex formation is analogous to the observation (Creamer, 1980) that SDS promotes *β*-casein polymer formation in solution and there is a limited number of binding sites on the protein for the surfactant molecule. It is known that *β*-casein and *β*-lactoglobulin have different affinities for sucrose esters with different chains; *β*-casein has the highest affinity to unsaturated chains, but *β*-lactoglobulin binds better to saturated esters (Clark *et al.*, 1992). Both chains on DOPC are unsaturated; therefore, it is not surprising that *β*-casein has a high affinity for DOPC. It is more remarkable that α_s-casein seems to have little affinity. If *β*-casein is removed from the interface in the form of a DOPC−casein complex, the binding site for DOPC on *β*-casein should presumably be located on the hydrophobic part of the protein, so that the complex formed becomes hydrophilic and leaves the interface for the aqueous phase.

CONCLUSION

Compared with egg-PC, DOPC is a less favorable co-surfactant for casein-stabilized oil-in-water emulsions. The initial size of the emulsion droplets is not markedly affected by DOPC, but DOPC caused a time-dependent increase in the droplet size when the DOPC:casein molar ratio was >10. This instability of the emulsions results from the decrease of the amount of adsorbed casein on the oil−water interface because of competitive adsorption with DOPC and selective removal of the β-casein fraction. This selective displacement probably results from specific binding of DOPC to β-casein; if the binding site is located on the hydrophobic part of the β-casein, the resulting DOPC−β-casein complex could be hydrophilic in nature and desorb from the oil droplets. The hydrodynamic thickness of the layer of adsorbed casein was also significantly modified by the presence of DOPC on the interface; this change in thickness coincided with the change in surface concentration with time because of the DOPC−casein interaction.

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