# Competitive Adsorption of Lecithin and β-Casein in Oil in Water Emulsions

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Competitive adsorption at the oil–water interface of egg yolk lecithin (L-α-phosphatidylcholine) and β-casein has been studied in *n*-tetradecane in water and soya oil in water emulsions (0.4 wt % protein, 20 wt % oil, pH 7). With both types of oil phase, the addition of lecithin at high lecithin/protein molar ratios prior to emulsification leads to partial displacement of protein from the interface. Much more lecithin is associated with *n*-tetradecane droplets than with soya oil droplets, and at low lecithin/protein molar ratios there is substantially more β-casein adsorbed at the hydrocarbon–water interface than at the triglyceride–water interface. The ability of lecithin to displace β-casein from the emulsion droplet surface is qualitatively similar to that found previously for a nonionic water-soluble poly(oxyethylene) surfactant.

There are two classes of molecules that have a strong tendency to adsorb at the oil–water interface in food oil in water emulsions: proteins and small-molecule surfactants (often called "emulsifiers" in the technical literature). A significant factor affecting the formation, stability, and rheology of emulsions like mayonnaise or ice cream is the distribution of proteins and surfactants between the droplet surface and the two bulk phases (Dickinson and Stainsby, 1982; Darling and Birkett, 1987; Bergenståhl and Claesson, 1990); displacement of protein from fat globules by emulsifiers, for instance, enhances fat agglomeration during ice-cream production (Barfod et al., 1991). Interactions between emulsion droplets depend on the chemical nature of stabilizing molecules adsorbed at the droplet surface (Fisher and Parker, 1988). The composition and structure of the stabilizing layer are determined by protein/surfactant competitive adsorption and by the nature of surfactant–protein interactions both at the surface and in the bulk aqueous phase (Dickinson and Woskett, 1989; Dickinson et al., 1989, 1990a).

This paper reports experimental results on the competitive adsorption of β-casein and lecithin (L-α-phosphatidylcholine) in emulsions with either soya oil or *n*-tetradecane as the dispersed phase. The protein β-casein is chosen because of its major importance in the stabilization of dairy emulsions (Walstra and Jenness, 1984). There has been detailed study of adsorbed films of pure β-casein (Graham and Phillips, 1979) and, more recently, of competitive adsorption of β-casein with other milk proteins (Dickinson et al., 1988, 1990b) and with the egg yolk protein phosvitin (Dickinson et al., 1991). The surfactant phosphatidylcholine is an important component of egg yolk and commercial soybean lecithin. The latter is a widely used ingredient in food-processing applications (Dashiell, 1989). Two different oil phases are studied here: *n*-tetradecane and soya oil. The hydrocarbon *n*-tetradecane is used to facilitate comparison with the system β-casein plus nonionic water-soluble poly(oxyethylene) surfactant (Courthaudon et al., 1991). Pure soya oil is used to make the model systems more relevant to real food products.

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## MATERIALS AND METHODS

**Materials.** Egg yolk lecithin (L-α-phosphatidylcholine) was obtained from Sigma Chemical Co. (St. Louis, MO) in the form of a solution (100 mg $mL^{-1}$) in either *n*-tetradecane (P5888) or chloroform (P2772). The phosphatide had been separated chromatographically according to a modification of the method of Singleton et al. (1965). It was shown to be a single chemical component (>99% pure) by high-pressure liquid chromatography (HPLC) using dipalmitoyl-L-α-phosphatidyl-*N,N*-dimethylethanolamine (Sigma P0399) as internal standard. The freeze-dried β-casein was prepared as described previously (Dickinson et al., 1988); analysis by fast protein liquid chromatography (FPLC) on a Mono-Q ion-exchange column gave a single sharp peak. The soya oil was a high-purity triglyceride sample from Karlshamns LipidTeknik (Stockholm, Sweden); it was shown by HPLC to be entirely free of monoglycerides, diglycerides, and free fatty acids. AnalaR grade *n*-tetradecane (>99% pure) was obtained from Sigma. All buffer solutions were prepared from AnalaR grade reagents and double-distilled water.

**Emulsion Preparation.** Oil in water emulsions containing 20 wt % oil were prepared as described previously (Dickinson et al., 1987) by using a small-scale single-stage valve homogenizer operating at a pressure of 300 bar. Every emulsion sample contained the same protein content: 0.5 wt % β-casein dissolved in the aqueous phase (20 mM Bis-Tris propane buffer, pH 7) corresponding to 0.4 wt % in the emulsion as a whole. The amount of lecithin added to the oil or aqueous phase of the pre-mix prior to homogenization was varied to give an emulsifier to protein ratio *R* in the range from 0 to 100. The droplet size distribution of each freshly made emulsion was measured by using a Malvern Mastersizer S2.01. To separate the oil droplets from the aqueous serum phase, the emulsion samples were centrifuged at 15000*g* for 15 min; the cream phase was then redispersed in buffer and centrifuged again at 15000*g* for 15 min. The resulting cream and aqueous phases were analyzed for protein and lipids as described below.

**Protein Analysis.** The β-casein content of the aqueous phase was determined by the technique of pyrochemiluminescent nitrogen analysis using a Model 703C nitrogen analyzer from Antek Instruments (Dusseldorf, Germany). The choice of the method was influenced by the cloudiness of protein solutions separated from the soya oil emulsions which would have invalidated turbidometric methods and by the much smaller quantities that could be assayed as compared with the standard Kjeldahl method. The pyrochemiluminescent method involves oxidizing the sample at 1050 °C to convert all chemically bound

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nitrogen to nitric oxide. The decay to the ground state is accompanied by near-infrared light emission (650–900 nm), which is sensed by a photomultiplier tube. The method has been shown to be well correlated with Kjeldahl nitrogen determination (Ward et al., 1980; Jones and Daughton, 1985).

By use of a 10-μL septum-piercing syringe and an Antek Model 735 syringe driver, liquid samples (3–5 μL) were injected into the nitrogen analyzer through a Teflon-lined silicone rubber seal at a constant rate of 1.0 μL $s^{-1}$. Standard β-casein solutions of known concentration were used to calibrate the output from the instrument. A constant nitrogen content was deducted from each reading to allow for the nitrogen content of the buffer salts (determined by running the buffer solution in the absence of protein).

For the *n*-tetradecane in water emulsions, the protein concentrations assayed by the pyrochemiluminescent nitrogen analysis technique were found to be in good agreement with those determined by FPLC (Dickinson et al., 1988). The FPLC technique was not used in this study with the soya oil emulsions because of a problem of drift in the baseline of the chromatogram, caused by a contamination of the protein by unknown lipid, which prevented accurate protein concentration determination in the aqueous serum phase.

**Phosphatidylcholine Analysis.** The quantity of phosphatidylcholine associated with the emulsion droplets was measured by using a Spectra Physics Model SP 8770 HPLC pump (Spectra Physics, St. Albans, U.K.) and a Cunow Model DDL 21 light scattering detector (Severn Analytical, Shefford, U.K.) with dipalmitoyl-L-α-phosphatidyl-*N*,*N*-dimethylethanolamine as internal standard. A 10-μL sample of phosphatidylcholine in chloroform was injected at a flow rate of 2 mL $min^{-1}$. The eluting solvent was made up from 6 mL of aqueous solution (99% water, 1% serine buffer, pH 7.5), 48 mL of 2-propanol, 8 mL of chloroform, and 36 mL of hexane (Christie, 1986).

The lecithin was extracted from the emulsion droplets as follows. A 0.5-g sample of cream phase was shaken with 12 mL of a 2:1 (by volume) chloroform/methanol solution and 3 mL of 1 wt % aqueous KCl solution. After centrifugation, the supernatant (water/methanol mixture) containing water-soluble contaminants (notably β-casein previously adsorbed at the oil–water interface) was discarded. The subnatant was shaken with 6 mL of a 1:1 methanol/water solution, and after centrifugation, the supernatant was discarded. The resulting subnatant contained the lipids (phosphatidylcholine and triglycerides; no monoglycerides, diglycerides, or free fatty acids) in a 9:1 chloroform/methanol solution. The solution was filtered and evaporated under nitrogen at 40 °C, and phosphatidylcholine was separated from the triglycerides by using a Sep-Pak silica cartridge (Waters Associates, Milford, MA) (Bitman et al., 1983). The lipid mixture was dissolved in ca. 1 mL of a 1:1 diethyl ether/hexane solution and applied to the silica cartridge. Triglycerides were eluted with 40 mL of a 1:1 diethyl ether/hexane solution, and phosphatidylcholine was eluted with 20 mL of methanol and then 20 mL of a 5:3:2 methanol/chloroform/water solution. The resulting solution was evaporated under nitrogen at 40 °C, and the phosphatidylcholine for HPLC analysis was finally dissolved in chloroform to give a concentration in the range 1–5 μg $μL^{-1}$.

## RESULTS AND DISCUSSION

In the absence of lecithin [throughout this paper "lecithin" means "pure phosphatidylcholine (>99%)"] droplet size distributions of the *n*-tetradecane in water and soya oil in water emulsions were found to be very similar. The volume-surface average droplet diameter was $d_{32}$ = 0.81 ± 0.05 μm. The effect on $d_{32}$ of various concentrations of lecithin in the emulsion pre-mix is shown in Figure 1 for the two types of oil phase. With *n*-tetradecane, incorporation of lecithin into the oil phase (or aqueous phase) prior to homogenization was found to produce a lowering of the average droplet size for values of the emulsifier/protein molar ratio *R* above ca. 6, reaching $d_{32}$ = 0.53 ± 0.03 μm at *R* = 100. With soya oil, however, the presence of lecithin did not lead to any reduction in droplet size. Rather, there was evidence of a slight increase

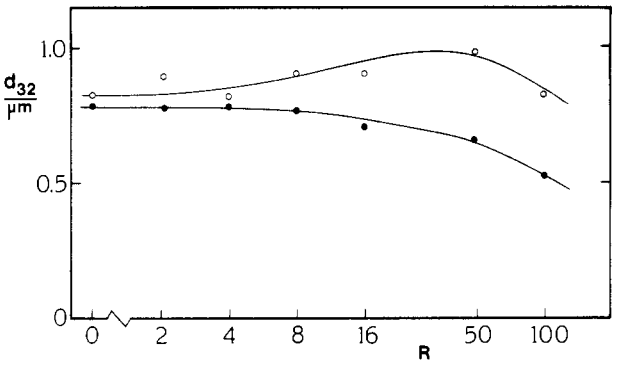


**Figure 1.** Effect of emulsifier on droplet size in β-casein-stabilized emulsions (20 wt % oil, 0.4 wt % protein, pH 7) made by valve homogenization at 300 bar. The volume-surface average diameter $d_{32}$ is plotted as a function of the lecithin/protein molar ratio *R*: (●) *n*-tetradecane; (○) soya oil.

in $d_{32}$ up to a maximum value of $d_{32} \approx 1.0$ μm at $R \approx 50$, though it should be said that the increase is only just outside the estimated experimental uncertainty.

Under the emulsification conditions employed (low protein load, severe homogenization), almost all of the available protein was adsorbed at the droplet surface in the *n*-tetradecane in water emulsion (20 wt % oil) made with β-casein (0.4 wt %) in the absence of added surfactant. That is, only ca. 5% of the total β-casein present in the emulsion was found in the aqueous serum phase after centrifugation. On the contrary, in the equivalent soya oil in water emulsion, only ca. 60% of the β-casein was adsorbed. As the droplet size distributions for $R = 0$ are the same for the two types of oil, this means that the protein surface concentration is considerably lower for the soya oil than for the *n*-alkane. The actual protein surface coverage values calculated from the results are 1.05 ± 0.1 and 1.75 ± 0.1 mg $m^{-2}$, respectively. This difference is not thought to be due to the presence of surface-active lipid impurities in the soya oil, since it was confirmed by HPLC that there were no mono- or diglycerides present in the sample. The difference in protein surface coverage must therefore be a reflection of an intrinsic difference between the triglyceride–water interface and the hydrocarbon–water interface. Possibly, the most hydrophobic side chains of the β-casein are able to penetrate further into the nonpolar hydrocarbon phase than they can into the more polar triglyceride phase. Certainly, the free energy change for protein adsorption at the triglyceride–water interface (interfacial tension ≈ 25 mN $m^{-1}$) is much lower than at the hydrocarbon–water interface (interfacial tension ≈ 50 mN $m^{-1}$) (Fisher et al., 1985, 1987).

Figure 2 shows how the presence of lecithin in the emulsion pre-mix affects the amount of protein adsorbed. Similar results were obtained irrespective of whether the lecithin was first dissolved (dispersed) in the oil phase or the aqueous phase. The protein surface concentration $\Gamma_p$ in Figure 2 is plotted against the lecithin/protein molar ratio *R*. With either *n*-tetradecane or soya oil, there is no change in $\Gamma_p$ at low lecithin concentrations, but there is a substantial reduction in $\Gamma_p$ at high concentrations. With *n*-tetradecane, $\Gamma_p$ remains unchanged for $R \lesssim 6$ (≲0.1 wt % lecithin); with soya oil, $\Gamma_p$ remains unchanged for $R \lesssim 20$ (≲0.3 wt % lecithin). With *n*-tetradecane, the protein surface concentration begins to go down in Figure 2 at roughly the same *R* value as does the average droplet size in Figure 1. It is interesting to note that the difference in protein surface coverage between the hydrocarbon droplets and the triglyceride droplets becomes less significant as the surfactant content increases, and indeed at $R = 100$ the coverage is almost the same for the two oil

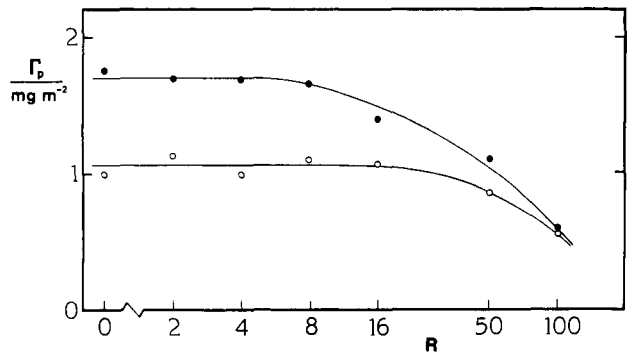


**Figure 2.** Protein surface coverage in emulsions containing added emulsifier. The surface concentration $\Gamma_p$ of $\beta$-casein is plotted as a function of the lecithin/protein molar ratio $R$: (●) $n$-tetradecane; (○) soya oil.

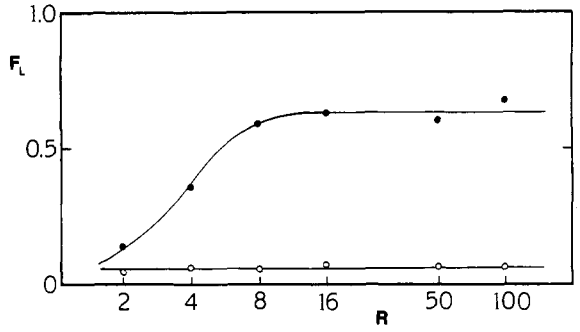


**Figure 3.** Distribution of emulsifier in protein-stabilized emulsions. The fraction $F_L$ of lecithin associated with the oil droplets is plotted as a function of the lecithin/protein molar ratio $R$: (●) $n$-tetradecane; (○) soya oil.

phases (though the total amount of protein adsorbed is greater for the hydrocarbon droplets because they are smaller).

Figure 3 shows how the distribution of lecithin between the emulsion droplets and the aqueous continuous phase changes with the amount of surfactant present in the system. The fraction $F_L$ of lecithin associated with the cream phase is plotted against the lecithin/protein molar ratio $R$. Data for the two types of oil phase are distinctly different. With soya oil, less than 10% of the surfactant was found to be associated with the emulsion droplets; i.e., over 90% is dissolved (dispersed) in the aqueous phase. With $n$-tetradecane, however, there is a consistent increase in the fraction of the total phospholipid present which is associated with the droplets as $R$ is increased, with the results leveling off to $F_L \approx 0.6$ at $R \gtrsim 10$. The large proportion of the phospholipid residing on the aqueous side of the oil–water interface is not surprising in view of the known tendency of phosphatidylcholine to form lamellar mesophases and vesicles in aqueous media (Bergenståhl and Claesson, 1990) and to produce lipid–protein complexes with excellent emulsifying properties (Nakamura et al., 1988). The larger values of $F_L$ for the $n$-tetradecane in water emulsions than for the soya oil in water emulsions are presumably a reflection of the lower solubility of lecithin in triglyceride oil than in hydrocarbon oil. It should be noted, however, that the data presented in Figure 3 do not distinguish between lecithin in the bulk oil phase and lecithin adsorbed at the interface. Any lecithin weakly associated with the interface (e.g., as phospholipid bilayers loosely attached to the droplet surface) might be swept into the aqueous serum during the centrifugal separation process. For the $n$-tetradecane in water emulsions, it is interesting that the value of $R$ at which the lecithin content in the oil phase levels off is roughly the same as that at which protein begins to be

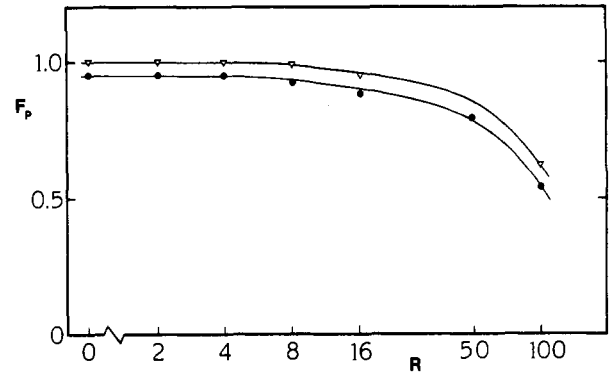


**Figure 4.** Comparison of protein displacement behavior of lecithin and $C_{12}E_8$ in $n$-tetradecane in water emulsions (20 wt % oil, 0.4 wt % protein, pH 7). The fraction $F_P$ of $\beta$-casein adsorbed is plotted as a function of the surfactant/protein molar ratio $R$: (●) lecithin; (▽) $C_{12}E_8$.

displaced from the interface (Figure 2) and at which $d_{32}$ begins to decrease (Figure 1). For the soya oil in water emulsions, it is clear from Figure 3 that little of the phospholipid is strongly adsorbed at the droplet surface. This is probably the reason for the rather modest effect of added lecithin on the protein surface coverage at the triglyceride–water interface (Figure 2) and on the soya oil droplet size (Figure 1).

At neutral pH, phosphatidylcholine is a zwitterionic molecule carrying zero net charge. One of the purposes of the present study was to investigate whether the competitive adsorption behavior of lecithin with protein is similar to that of a simple nonionic surfactant like $C_{12}E_8$ (octaoxyethylene glycol $n$-dodecyl ether), even though the former is a double-chain surfactant tending to form lamellar mesophases and the latter is a single-chain surfactant with a large head-group tending to form normal micelles. Figure 4 shows a plot of the fraction $F_P$ of $\beta$-casein adsorbed at the surface of $n$-tetradecane emulsion droplets as a function of the molar ratio $R$ of surfactant (lecithin or $C_{12}E_8$) to protein. Experimental details relating to the $C_{12}E_8$ plus $\beta$-casein system are reported elsewhere (Courthaudon et al., 1991). We see from Figure 4 that the general trend is remarkably similar for the two surfactants. Approximately 40% of the originally adsorbed protein is displaced from the interface at surfactant concentrations corresponding to $R = 100$. So, even though the thermodynamic phase behavior of the two surfactants is quite different, the competitive adsorption behavior with protein at the emulsion droplet surface is rather similar. This means that the thermodynamic description of competitive adsorption developed for synthetic poly(oxyethylene) surfactants (Dickinson and Woskett, 1989; Dickinson et al., 1990a) is valid also for a food emulsifier like egg yolk lecithin.

This study has shown some differences between the hydrocarbon–water and triglyceride–water interfaces, in terms of both the concentration of protein adsorbed and the distribution of lecithin between the phases. Nevertheless, it is clear that the general phenomenon of competitive displacement of milk protein by lecithin occurs at both interfaces. And it is noteworthy that, at high lecithin concentrations, the protein surface concentration is essentially the same in the two cases.

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LITERATURE CITED

Barfod, N. M.; Krog, N.; Larsen, G.; Buchheim, W. *Fat Sci. Technol.* **1991**, *93*, 24–29.

Bergenståhl, B. A.; Claesson, P. M. In *Food Emulsions*, 2nd ed.; Larsson, K., Friberg, S. E., Eds.; Dekker: New York, 1990; pp 41–96.

Bitman, J.; Wood, D. L.; Hamosh, M.; Hamosh, P.; Mehta, N. R. *Am. J. Clin. Nutr.* **1983**, *38*, 300–312.

Christie, W. W. *J. Chromatogr.* **1986**, *361*, 396–399.

Courthaudon, J.-L.; Dickinson, E.; Dalgleish, D. G. *J. Colloid Interface Sci.* **1991**, in press.

Darling, D. F.; Birkett, R. J. In *Food Emulsions and Foams*; Dickinson, E., Ed.; Royal Society of Chemistry: London, 1987; pp 1–29.

Dashiell, G. L. In *Lecithins: Source, Manufacture and Uses*: Szuhaj, B. F., Ed.; American Oil Chemists' Society: Champaign, IL, 1989; pp 213–224.

Dickinson, E.; Stainsby, G. *Colloids in Food*; Applied Science: London, 1982.

Dickinson, E.; Woskett, C. M. 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. 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. *Food Hydrocolloids* **1988**, *2*, 397–405.

Dickinson, E.; Mauffret, A.; Rolfe, S. E.; Woskett, C. M. *J. Soc. Dairy Technol.* **1989**, *42*, 18–22.

Dickinson, E.; Euston, S. R.; Woskett, C. M. *Prog. Colloid Polym. Sci.* **1990a**, *82*, 65–75.

Dickinson, E.; Rolfe, S. E.; Dalgleish, D. G. *Int. J. Biol. Macromol.* **1990b**, *12*, 189–194.

Dickinson, E.; Hunt, J. A.; Dalgleish, D. G. *Food Hydrocolloids* **1991**, *4*, 403–414.

Fisher, L. R.; Parker, N. S. In *Advances in Food Emulsions and Foams*; Dickinson, E., Stainsby, G., Eds.; Elsevier Applied Science: London, 1988; pp 45–90.

Fisher, L. R.; Mitchell, E. E.; Parker, N. S. *J. Food Sci.* **1985**, *50*, 1201–1202.

Fisher, L. R.; Mitchell, E. E.; Parker, N. S. In *Food Emulsions and Foams*; Dickinson, E., Ed.; Royal Society of Chemistry: London, 1987; pp 230–241.

Graham, D. E.; Phillips, M. C. *J. Colloid Interface Sci.* **1979**, *70*, 403–439.

Jones, B. M.; Daughton, C. G. *Anal. Chem.* **1985**, *57*, 2320–2325.

Nakamura, R.; Mizutani, R.; Yano, M.; Hayakawa, S. *J. Agric. Food Chem.* **1988**, *36*, 729–732.

Singleton, W. S.; Gray, M. S.; Brown, M. L.; White, J. L. *J. Am. Oil Chem. Soc.* **1965**, *42*, 53–56.

Walstra, P.; Jenness, R. *Dairy Chemistry and Physics*; Wiley: New York, 1984.

Ward, M. W. N.; Owens, C. W. I.; Rennie, M. J. *Clin. Chem.* **1980**, *26*, 1336–1339.

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