Influence of Protein Interfacial Composition on Salt Stability of Mixed Casein Emulsions

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The influence of casein surface composition on the stability toward flocculation by sodium chloride has been investigated for oil-in-water emulsions (20 vol % oil, 1 wt % total protein) prepared with a mixture of α_{s1}-casein + β-casein. It has been observed that the poor salt stability of emulsions containing 100% α_{s1}-casein is greatly enhanced by the replacement of about one-third of the α_{s1}-casein by β-casein. With around half of the adsorbed protein layer consisting of β-casein, emulsions remain stable at ionic strengths of 2 M NaCl in the presence or absence of calcium ions (5 mM). At the point of droplet flocculation in these emulsions, most of the casein present is associated with the surface of the droplets. These results provide definitive evidence for the important influence of the compositional balance in sodium caseinate on the colloidal stability behavior of casein-based emulsions.

Keywords: *Casein; flocculation; oil-in-water emulsions; salt stability; competitive adsorption; sodium caseinate; surface composition*

Food colloids contain mixtures of protein components with different contributions to stability and texture (Dickinson and Stainsby, 1982). A widely used ingredient in dairy-type oil-in-water emulsions is the heterogeneous protein emulsifier sodium caseinate, which is prepared by adjusting acid-coagulated whole casein to pH 6.7 with sodium hydroxide, followed by pasteurization and spray-drying (Muller, 1982). The functional properties of sodium caseinate include emulsification, water-binding, fat-binding, thickening, gelation, and whipping (Kinsella, 1988; Doxastakis, 1989). So long as there is enough protein present to cover the oil–water interface completely, sodium caseinate provides excellent protection against droplet coalescence at neutral pH as a result of combined electrostatic and steric stabilization mechanisms (Dickinson, 1989; Fang and Dalgleish, 1993; Dalgleish, 1997a,b). Emulsion instability, when it does occur, is usually associated with some kind of flocculation process (Dickinson et al., 1997a). For the control of stability and rheology, a fundamental issue is the relationship between the composition of the casein emulsifier mixture and the susceptibility toward aggregation of protein-coated droplets under a variety of experimental conditions.

Proteins α_{s1}-casein and β-casein, in roughly equal proportions, make up ~75% of total bovine milk casein. The preponderance of proline residues, and the absence of disulfide cross-links, means that in solution the caseins adopt flexible disordered configurations with little ordered secondary structure. Rapid and effective accumulation of hydrophobic side chains at the oil–water interface occurs during adsorption of both these caseins, but β-casein is slightly more hydrophobic and surface-active than α_{s1}-casein (Mitchell et al., 1970; Dickinson et al., 1988). Studies of well-defined binary mixtures of the pure caseins at fluid interfaces have demonstrated (Dickinson et al., 1988; Anand and Damodaran, 1996) that β-casein in aqueous solution can displace adsorbed α_{s1}-casein—the latter can also displace the former, albeit to a lesser extent. Under conditions inhibiting protein aggregation (neutral pH, low ionic strength, no calcium ions), exchange of these monomeric caseins between the oil–water interface and bulk aqueous phase can be described by a simple equilibrium statistical model (Dickinson, 1992).

Reversible bulk/surface exchangeability is enhanced by mobility and flexibility at the interface (Dickinson and Matsumura, 1994). Exchangeability is inhibited by factors that reduce molecular flexibility or increase intermolecular association, e.g., calcium ion binding (Hunt et al., 1993). Compared with the binary mixture of pure α_{s1}- + β-caseins, the rather more aggregated state of the multicomponent sodium caseinate is reported to give slower and less extensive protein exchange (Robson and Dalgleish, 1987; Euston et al., 1995). Another factor affecting competitive adsorption is total protein content: whereas β-casein is preferentially adsorbed in emulsions made at relatively low levels of sodium caseinate, it has been found (Srinivasan et al., 1996) that the opposite is the case in emulsions containing substantial levels of unadsorbed caseinate.

The practical significance of competitive adsorption lies in the fact that β-casein-coated droplets are considerably more stable than α_{s1}-casein-coated ones. Therefore, any factors that affect interfacial protein composition are likely also to affect emulsion stability (Dickinson, 1997). Emulsions made with pure α_{s1}-casein have been found (Dickinson et al., 1987, 1997d) to be much more susceptible to flocculation by salts (NaCl or $CaCl_2$) than β-casein emulsions. In particular, it has been shown (Dickinson et al., 1997d) that NaCl addition produces extensive flocculation of α_{s1}-casein-coated oil droplets at ionic strength 0.1 M or above but no flocculation of droplets stabilized by β-casein or sodium caseinate. This difference in colloidal stability can be attributed to higher net charge density on the

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adsorbed α_{s1}-casein layer and better steric stabilizing efficiency of β-casein due to its greater amphiphilicity (Dalgleish, 1997a; Dickinson et al., 1997b,c). Statistical modeling of a pair of interacting casein layers (Dickinson et al., 1997c) suggests the presence of a net attraction between α_{s1}-casein-coated surfaces at ionic strengths at which β-casein-coated surfaces give a strong surface−surface repulsion, in qualitative agreement with observed differences in salt stability for the α_{s1}-casein and β-casein emulsions.

This paper presents new results on the flocculation behavior of emulsions prepared with well-defined binary mixtures of α_{s1}-casein and β-casein. The objective is to determine the precise range of adsorbed layer compositions at which emulsions are stable under conditions of high ionic strength (up to 2 M NaCl).

MATERIALS AND METHODS

Freeze-dried samples of bovine α_{s1}-casein and β-casein were prepared at the Hannah Research Institute (Ayr, Scotland) from fresh skim milk by a process of acid precipitation, washing, reprecipitation, dissolution in urea, ion-exchange chromatography, and dialysis. Purity with respect to other milk protein contaminants (1−2%) was assessed by fast protein liquid chromatography (FPLC). *n*-Tetradecane and imidazole (99%) were purchased from Sigma Chemical Co. (St. Louis, MO). Analytical grade sodium chloride and calcium chloride were obtained from Fisons Scientific (Loughborough, U.K.).

Oil-in-water emulsions (20 vol % *n*-tetradecane, 1 wt % protein emulsifier) were prepared at room temperature using a laboratory-scale jet homogenizer (Burgaud et al., 1990) operating at 300 bar with jet hole size 0.53 mm. Prior to emulsification, proteins were dissolved at a total concentration of 1.19 wt % in 20 mM imidazole/HCl buffer solution (pH 7.0) at α_{s1}-casein/β-casein ratios of 60:40, 70:30, 80:20, 90:10, and 100:0. In some cases the protein solution also contained 5 mM $CaCl_2$. Droplet size distributions and specific surface areas of emulsions were determined using a Malvern Mastersizer S2.01. Immediately prior to particle size analysis, emulsion samples were diluted into distilled water (pH close to neutral) and stirred gently in the Mastersizer circulation bath.

Salt stability was assessed by mixing emulsions 1:1 with NaCl solutions of gradually increasing ionic strength, waiting for 15 min, and then remeasuring the droplet size distribution (after dilution in water). A critical salt concentration c^* was defined as the NaCl content in the diluted emulsion (10 vol % oil) giving a sharp increase in volume−surface average diameter d_{32}.

Surface protein concentration and composition were determined according to the depletion method. Each emulsion sample was centrifuged at $10^4 g$ for 15 min at 20 °C, and the resulting serum layer was carefully removed with a syringe. The cream layer was redispersed in buffer (20 mM imidazole, pH 7.0), the emulsion sample was recentrifuged under the same conditions as before, and the second serum layer was separated and mixed with the first. After passage through a 0.22 μm Millipore filter, the aqueous serum was assayed for α_{s1}-casein and β-casein by FPLC on a Pharmacia Mono-Q ion-exchange column with a linear salt gradient of 0.2−0.5 M NaCl. Protein concentrations were calculated from integrated peak areas by reference to calibration curves of concentration against peak area. Surface protein concentration was estimated from the specific surface area and the difference between the protein content in the aqueous phase and that used to make the emulsion.

Surface protein compositional analysis was also carried out on serum phases of centrifuged emulsion samples previously treated with NaCl to concentrations just below the flocculation point (nearly c^*). These serum phases were treated with 6 M urea to inhibit protein aggregation and then dialyzed against 200 mL of 6 M aqueous urea solution to reduce the salt concentration prior to FPLC analysis.

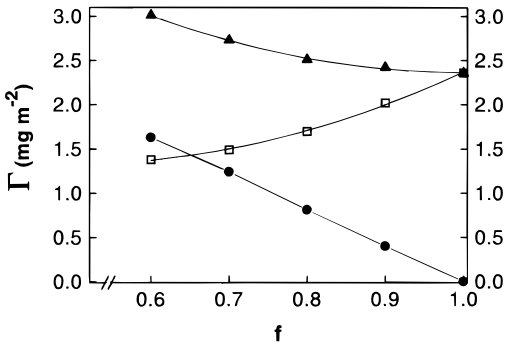

Figure 1. Influence of emulsifier composition on surface coverage in oil-in-water emulsions prepared with α_{s1}-casein and β-casein (20 vol % oil, 1 wt % protein, pH 7, ionic strength 0.02 M). The protein surface concentration is plotted against the weight fraction f of α_{s1}-casein: □, α_{s1}-casein; ●, β-casein; ▲, total protein.

Table 1. Percentage of α_{s1}-Casein Adsorbed at the Oil−Water Interface as a Function of the Weight Fraction f of α_{s1}-Casein in the Emulsifier Mixture in (A) Freshly Prepared Oil-in-Water Emulsions (20 vol % Oil, 1 wt % Protein, pH 7, Ionic Strength 0.02 M) and (B) Emulsions after Addition of NaCl to Just below the Critical Salt Concentration c^*

	% α_{s1}-casein adsorbed[a]	
f	(A)	(B)
1.0	58	85
0.9	56	90
0.8	53	93
0.7	52	82
0.6	56	100

[a] Estimated experimental error ±5%.

RESULTS AND DISCUSSION

Fine oil-in-water emulsions (20 vol % *n*-tetradecane, 1 wt % total casein, pH 7, ionic strength 0.02 M) were prepared under identical homogenization conditions using different fractions of α_{s1}-casein, f, in the emulsifier mixture. Values of mean droplet sizes of fresh emulsions were $d_{32} = 0.51 \pm 0.01$ μm for $f = 1.0$, 0.9, and 0.8, $d_{32} = 0.53$ μm for $f = 0.7$, and $d_{32} = 0.58$ μm for $f = 0.6$. The trend toward slightly larger droplets in the β-casein-rich systems is consistent with a recent separate study of emulsions prepared with the pure α_{s1}- and β-caseins (Dickinson et al., 1997d).

Figure 1 shows the total protein surface concentration in the fresh emulsions, and the surface concentrations of the individual proteins α_{s1}-casein and β-casein, as a function of the emulsifier composition f. Increasing the proportion of β-casein in the emulsifier mixture leads to a gradual increase in total protein surface coverage from $\Gamma = 2.4$ mg m^{-2} at $f = 1.0$ to $\Gamma = 3.0$ mg m^{-2} at $f = 0.6$. The origin of this effect lies in the very strong tendency for adsorption of β-casein in emulsions under these low concentration conditions (Courthaudon et al., 1991). In fact, no measurable quantity of β-casein was detected in the serum phase after the emulsion samples were centrifuged, indicating that all of the available β-casein was adsorbed at the interface. Hence, $\Gamma(\beta)$ increases linearly with $1 - f$, and so, as less α_{s1}-casein comes off the surface than β-casein goes on, the overall protein surface concentration also increases. Table 1 shows that the percentage of α_{s1}-casein located at the

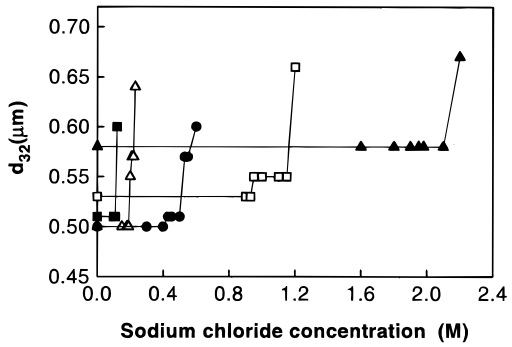

Figure 2. Onset of flocculation of emulsions with increasing sodium chloride concentration. Apparent average droplet size d_{32} is plotted against salt concentration for various values of the weight fraction f of α_{s1}-casein in the emulsifier mixture: ▲, 0.6; □, 0.7; ●, 0.8; △, 0.9; ■, 1.0.

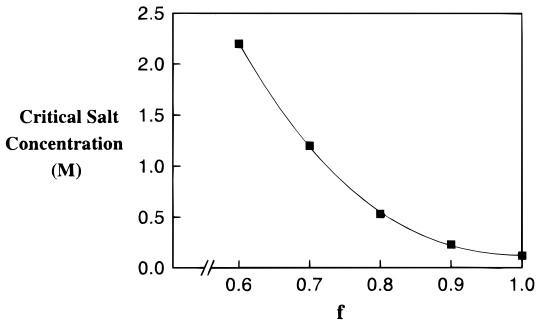

Figure 3. Flocculation stability diagram for mixed casein emulsions at pH 7. Critical salt concentration c^* required just to induce flocculation is plotted against the weight fraction f of α_{s1}-casein in the emulsifier mixture.

surface of the droplets in the freshly prepared emulsions at low ionic strength is constant at 55 ± 3%, irrespective of the proportion of β-casein in the emulsifier mixture. Preferential adsorption of β-casein over α_{s1}-casein during emulsification is clearly indicated in Figure 1 by the fact that $\Gamma(\beta)$ exceeds $\Gamma(\alpha_{s1})$ in the emulsion with the 60:40 ratio of α_{s1}-casein to β-casein. This is in close agreement with earlier work (Dickinson et al., 1988).

The effect of ionic strength on the apparent average droplet diameter d_{32} of emulsions of different emulsifier composition is shown in Figure 2. Droplet size distributions were measured 15 min after freshly prepared emulsion samples were mixed 1:1 with NaCl solutions of gradually increasing concentration. Replicate experiments showed that the point of destabilization could be identified with a reliability of ~0.05 M. Figure 3 shows the resulting stability plot of critical salt concentration c^* versus total casein emulsifier composition f. Over the time scale of the particle size analysis (a couple of minutes), the salt-induced aggregation was therefore found not to be highly reversible, insofar as flocs produced at ionic strengths above c^* were not immediately dissociated on extensive dilution in water in the Mastersizer. (This was confirmed qualitatively by light microscopy.) Nevertheless, the aggregates formed at high ionic strength do become disrupted on extended subsequent storage at low ionic strength, especially in the presence of stirring. Some reversal of salt-induced flocculation of α_{s1}-casein emulsion systems over a time scale of tens of minutes was reported previously (Dickinson et al., 1987).

We can see from Figure 3 that only a relatively small amount of β-casein is required to improve drastically the salt stability of the pure α_{s1}-casein emulsion system.

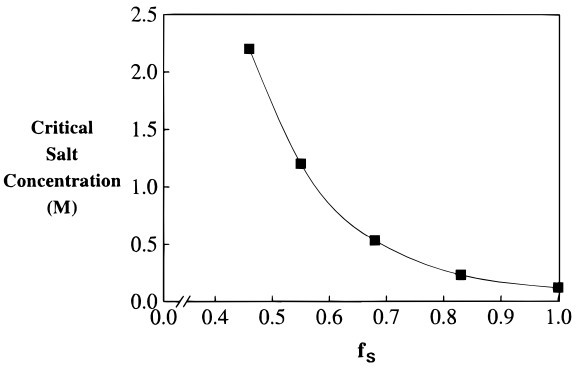

Figure 4. As Figure 3, except c^* is plotted against the weight fraction f_S of α_{s1}-casein in the adsorbed layer at the surface of the droplets.

In particular, a 70:30 mixture of α_{s1}-casein + β-casein is stable toward flocculation at ionic strengths up to 1 M. Figure 4 shows the stability diagram plotted in the form of c^* versus interfacial casein emulsifier composition f_S, based on the surface concentration data reported in Figure 1. This plot suggests that excellent stability toward flocculation by electrolyte (i.e. $c^* > 2$ M) is achieved when roughly half of the adsorbed protein layer consists of β-casein.

One complicating factor in the above interpretation is that it refers to emulsions that were prepared and analyzed with respect to surface composition at low ionic strength (0.02 M). However, it is possible that the enhanced electrostatic screening during the salt stability test could itself perturb the equilibrium between the adsorbed and unadsorbed caseins, thereby changing the surface composition at the point of flocculation. To address this issue, we repeated the surface composition analysis experiments on emulsion samples with ionic strength adjusted by NaCl addition to a level just below c^*. We observed, as before, that all of the β-casein present was located in the adsorbed layer; that is, none remained in the serum phase after centrifugation. In this case, however, the amount of α_{s1}-casein in the serum phase was found to be substantially lower (0−18%) than that in the low ionic strength original emulsion samples (42−48%) (see Table 1). At salt concentrations above c^*, we may infer that essentially all of the casein present in these systems is associated with the surface of the emulsion droplets, although this is difficult to confirm definitively. For the condition just below c^*, Figure 5 shows the resulting surface protein concentrations. Comparison with the data in Figure 1 indicates that the total protein surface coverage in the NaCl-adjusted emulsion samples is rather higher (3.3−3.6 mg m^{-2}) than that in the fresh emulsion samples (2.4−3.0 mg m^{-2}). It is clear therefore that, in these mixed casein systems, the effect of raising the ionic strength up to the flocculation point (c^*) is to increase the amount of α_{s1}-casein at the droplet surface. This is in agreement with a recent theoretical description of adsorbed α_{s1}-casein (Dickinson et al., 1997b). As the ionic strength is raised, the screening of charges on the polyelectrolyte residues in the theoretical model allows more compact packing of the segments and hence an increase in the adsorbed monolayer amount. This has a greater effect on the outer tail region of the modeled layer, which is more accessible to the free ions (Dickinson et al., 1997b). It should be noted that the experimental NaCl concentration inducing flocculation of the α_{s1}-casein emulsion ($c^* < 0.2$ M) is much lower

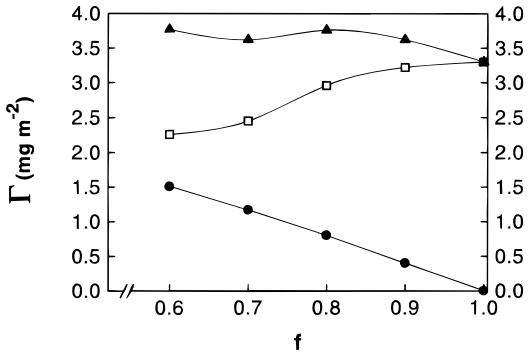

Figure 5. Influence of emulsifier composition on the surface coverage in emulsions adjusted by addition of salt to an ionic strength just below that required to induce flocculation. The protein surface concentration is plotted against the weight fraction *f* of α_{s1}-casein in the emulsifier mixture: □, α_{s1}-casein; ●, β-casein; ▲, total protein.

than that required to induce visible precipitation of a 0.5 wt % aqueous solution of α_{s1}-casein (i.e. >0.9 M). For this reason, as well as for reasons based on the theoretical modeling, we believe that the increased adsorbed amount of α_{s1}-casein near the flocculation point in Table 1 is probably not attributable to incipient multilayer formation at the oil−water interface.

It is well-known that the stability behavior of casein-based emulsions can be sensitive to calcium ion content (Dickinson et al., 1987; Agboola and Dalgleish, 1996; Dalgleish, 1997b). To check this point, some emulsions were prepared similarly to previously but with 5 mM $CaCl_2$ dissolved in the aqueous protein solution prior to emulsification. Compared with systems without any $CaCl_2$ added before emulsification, the values of c^* observed for this new set of emulsion systems were lower by ~0.05 M at $f = 1.0$ and 0.9 but more-or-less the same within experimental error for $f \leq 0.8$. Hence, while the presence of calcium ions at this level gives even poorer salt stability for the nearly 100% α_{s1}-casein emulsions, it has little influence on the sensitivity toward added NaCl in the more stable systems [$f \leq 0.8$, $\Gamma(\beta) \sim 1$ mg m^{-2}]. This trend is qualitatively consistent with the greater calcium sensitivity of α_{s1}-casein as compared to β-casein.

CONCLUSIONS

We have demonstrated that the poor salt stability of model α_{s1}-casein emulsions can be improved substantially by replacing a small fraction of the α_{s1}-casein emulsifier by β-casein. In particular, a critical salt concentration of $c^* > 2$ M is achievable at an emulsifier composition giving an adsorbed protein layer composed of ~50% β-casein in the initial emulsion before addition of salt. This mirrors the same excellent stability toward high ionic strengths typically exhibited by commercial sodium caseinate emulsions. Taken together with recent theoretical predictions (Dickinson, 1997; Dickinson et al., 1997c), these experimental observations seem to indicate that adsorbed β-casein may play a crucial role in sodium caseinate emulsions in protecting α_{s1}-casein-coated droplets against flocculation by electrolytes. The same conclusion seems applicable to casein-stabilized emulsions made with calcium-enriched sodium caseinate.

This study confirms the considerable sensitivity of the colloid stability properties of casein-based emulsions to the composition of the proteinaceous emulsifier. In this paper we have considered only the major components α_{s1}-casein and β-casein, whereas the properties of sodium caseinate will undoubtedly also be influenced to some extent by contributions from α_{s2}-casein and κ-casein. Also, we have considered here only systems of low protein/oil ratio, whereas different behavior may occur in systems containing large amounts of unadsorbed protein. Little is known about the supramolecular organization of multicomponent casein mixtures at emulsion interfaces, especially in concentrated protein systems. To develop further the interpretation of the emerging experimental data, it would be valuable to have available a statistical description of the self-assembly and competitive adsorption behavior in these mixed casein systems.

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