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Surface Shear Viscosity as Affected by Protein-Surfactant Interactions

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Abstract

Surface shear rheology of adsorbed β-lactoglobulin film (bulk protein concentration 2×10^{-3} wt%) containing small-molecule surfactant (ionic water-soluble SDS or nonionic oil-soluble Sp-80) has been investigated using a two-dimensional Couette-type viscometer. The effect of surfactant concentration, pH (5.5, 6, 7, 8.5) and heat treatment (70°C, 1 h) have been evaluated by measurements of changes in the apparent surface shear viscosity. With the SDS addition, the typical shear thinning behaviour of the adsorbed protein film is observed, which is more pronounced at the interface where less amounts of the ionic surfactants are associated. The higher pH and heat treatment also result in the pronounced shear thinning character of the protein film. The presence of oil-soluble surfactant influences on the surface shear viscosity. At low surfactant concentrations ($R\leq12$), it is higher than that of the pure β-lactoglobulin film but the opposite is the case at high surfactant concentrations ($R\geq16$). This may suggest the co-operative and competitive adsorption of the protein at the oil-water interface.

Key words: Surface shear viscosity, β-lactoglobulin, SDS, Sp-80, heat treatment, pH effects

Introduction

In food emulsions, milk proteins are frequently used as emulsifiers and stabilizers, together with small-molecule surfactants such as Spans and Tweens. How proteins and small-molecule surfactants are distributed between the droplet surface and the bulk (aqueous) phase is an important factor affecting the stability and rheology of food emulsion (Dickinson and Woskette, 1989). The composition and structure of the adsorbed layer in food emulsions is affected by competition between protein and surfactant at the interface (de Feijter *et al.*, 1987), and by the nature and strength of protein-surfactant interactions both at the interface and in the bulk aqueous phase (Clark *et al.*, 1994).

Surface shear rheology is the study of the two-dimensional deformation of thin films adsorbed at a fluid interface (Dickinson *et al.*, 1988). The surface shear rheology of a protein film depends on the molecular structure of protein in the adsorbed layer (Boyd *et al.*, 1973; Graham and Phillips, 1980; Martin *et al.*, 2002), and is extremely sensitive to intermolecular interactions occurring in adsorbed film (Dickinson *et al.*, 1988). In this connection, there have been extensive studies on the surface shear rheology of an protein film or mixed films of protein+protein (Castle *et al.*, 1987; Dickinson *et al.*, 1987; Dickinson *et al.*, 1990; Murray and Dickinson, 1996). From these experiments, it is concluded that such measurements give useful and sensitive information about competitive adsorption and co-operative interactions in mixed protein films.

Surface shear viscosity of protein films shows a time-dependent character, especially with proteins having complex of secondary and tertiary structures (*e.g.*, globular proteins). The time-dependent character arises from the slow strengthening of protein-protein interactions between segments at the interface (but not in the primary adsorption layer) or thickening of the film due to slow precitation from the bulk phase (Castle *et al.*, 1987; Dickinson *et al.*, 1988).

Factors such as pH and ionic strength affect the surface shear viscosity of protein films (Graham and Phillips, 1980). Maximum values are found at the

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isoelectric point of protein. This is because of the optimization of intra- and intermolecular interaction in the film where electric repulsion and hydration are at a minimum (Dickinson *et al.*, 1988). The surface shear viscosity of films of globular proteins is also found to be dependent on the ionic strength, whereas it is not so for the disordered proteins. It appears that electrostatic interactions have a greater influence on the former system compared to the latter (Castle *et al.*, 1987).

Surface rheology can also be used to study the nature of the interfacial film (*i.e.*, protein-surfactant interaction). Courthaudon *et al.* (1991a) studied the interfacial film in systems made with β-lactoglobulin+Tween 20 by using surface shear viscometry. The film of β-lactoglobulin shows a high viscosity of *ca.* 600 mN m^{-1} s after 5 h adsorption at the planar oil-water interface, but addition of Tween 20 up to a surfactant/protein molar ratio of 1 is found to lead to a sudden drop in the surface shear viscosity without inducing any significant change in the amount of protein adsorbed in an emulsion at this surfactant concentration. Qualitatively similar results have also been found with other nonionics (Dickinson *et al.*, 1990; Chen and Dickinson, 1995a), suggesting that this behaviour is specific to non-ionic surfactants. For instance, in systems of non-ionic $C_{12}E_8$+sodium caseinate, the film adsorbed from a 0.1 wt % protein solution develops a surface shear viscosity of ca. 20 mN m^{-1} s after 24 h, but the presence of 10^{-4} wt% surfactant in the gap between the casein train segments prevents the formation of a film with any measurable surface viscosity (0.2 mN m^{-1}s). Alternatively, addition of the same concentration of surfactant below a caseinate film aged for 24 h leads to a rapid fall in the surface viscosity down to values which are too low to be detected. The results discussed above are consistent with the view that the presence of low concentrations of non-ionic surfactant disrupts protein-protein interactions in the adsorbed layer, leading to a much lower resistance to flow. A partial reduction in surface shear viscosity in the presence of surfactant has also been reported by Dickinson and Iveson (1993) for systems containing β-lactoglobulin+α-phosphatidylcholine (lecithin). It has been found that, in the presence of lecithin ($R \fallingdotseq 100$), the surface shear viscosity is reduced by about an order of magnitude as compared with that determined for protein alone. It has been suggested that interfacial complexation between β-lactoglobulin and lecithin resulting in limited protein displacement could be the reason for the partial reduction in surface shear viscosity.

As with competitive adsorption behaviour, the presence of ionic surfactants also affect the interfacial rheology, but in different way, compared to that of non-ionic surfactants. Chen and Dickinson (1995a) studied the surface shear viscosity of systems containing protein+anionic surfactant. In systems of β-lactoglobulin with SLES 2EO (sodium lauryl ether sulphate), the addition of surfactant to the 1-day-old film of β-lactoglobulin results in a shear-thinning character in the film. On the other hand, the addition of the same surfactant to a gelatin film produces a molecular rearrangement and eventually a gradual decrease of the surface viscosity. These observations are in line with results of Wüstneck and Miller (1986) who found that foam films stabilized by gelatin/SDS complexes were much thinner than those adsorbed from pure gelatin solution as a result of surfactant-induced unfolding. This effect may cause the surface shear viscosity of gelatin film to decrease.

In this paper the author presents new experimental information on the surface shear rheology for systems of β-lactoglobulin with various surfactants well studied anionic SDS (sodium dedecyl sulfate) and oil-soluble nonionic Sp-80 (sorbitan monooleate) and the results has been related to the nature of the interactions between the two molecules.

Materials and Methods

Materials

Bovine β-lactoglobulin (1.84×10^{-4} dalton, purity> 99%), SDS (sodium dodecyl sulfate), Sp-80 (sorbitan monooleate) and *n*-tetradecane (purity>99%) were obtained from Sigma Chemicals. Buffer salts were AnalaR-grade reagents.

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 surface rheometer was built in the Procter Department of Food Science at the University of Leeds. 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. 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 (SDS) was added to the aqueous phase using a syringe without causing any significant disruption to the interfacial film. In case of the oil-soluble surfactant (Sp-80), the surfactant was dissolved in the oil phase prior to the creation of interface. The surface shear viscosity η of a two-dimensional film was calculated using the following equation (Dickinson *et al.*, 1985);

$$\eta = k(b^2 - a^2)\Phi/a^2b^2\omega$$

where a is the radius of the disc, b is the radius of the dish, Φ is the steady state disc deflection, ω is the angular velocity of the dish, and k depends on the torsion constant of the wire and is used to convert the distance of deflection to an angle.

Preparation of Heat-treated Protein Solution

The native protein solution (2×10^{-3} wt% β-lactoglobulin in 2 mM bis-tris buffer, pH 7) prepared at room temperature (~20°C) was placed in a 100 mL flask. This was heated in a water bath at 70°C for 1 h, then cooled immediately to room temperature to produce the heat-treated β-lactoglobulin solution.

pH Control

The desired pH of the native protein solution was achieved by adjusting with diluted HCl or NaOH (2×10^{-3} wt% β-lactoglobulin in 2 mM bis-tris buffer).

Results and Discussion

Fig. 1 shows the influence of SDS addition on the surface shear viscosity of a -lactoglobulin film adsorbed from bulk solution (2×10^{-3} wt% protein, pH 7, 25°C) at the planar n-tetradecane-water interface (±10%). SDS was added at two different concentrations (surfactant/protein molar ratio R=1 and 16) to the aqueous subphase in contact with the 1-day-old β-lactoglobulin film. For both samples, it is found that the apparent surface shear viscosity after the addition of surfactant decreases slightly under the influence of continuous shearing, but it later recovers almost to the same value as that for the original 1-day-old β-lactoglobulin film when subsequently kept undisturbed for several hours.

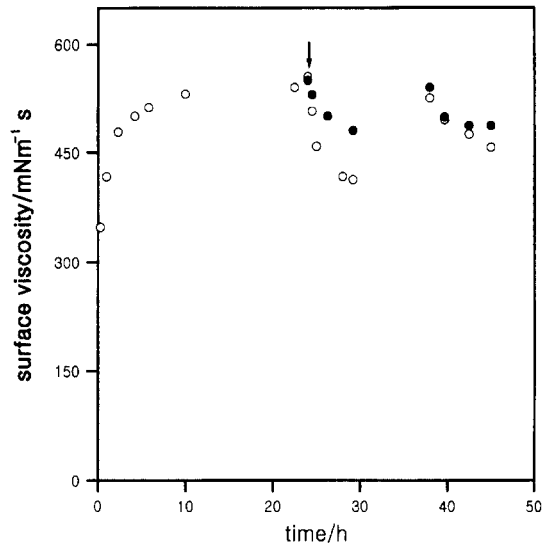

Fig. 1. Influence of SDS on surface shear viscosity of β-lactoglobulin adsorbed at *n*-tetradecane-water interface (2×10^{-3} wt% protein, 2 mM bis-tris buffer, pH 7, 25°C). Apparent surface viscosity is plotted against time for two different surfactant/protein molar ratio *R*: ○, *R*=1; ●, *R*=16. The arrow denotes the point (after 24 h) at which the surfactant is introduced into the aqueous phase.

Shear-thinning behaviour is commonly observed with adsorbed protein films (Dickinson *et al.*, 1985; Chen and Dickinson, 1995a) or protein solutions containing anionic surfactant (Greener *et al.*, 1987). This has been attributed to the slow breaking down of protein-surfactant complexes under shear (Chen and Dickinson, 1995a). In addition, it is also noted that the extent of shear thinning tends to be more pronounced in the samples of lower surfactant content (R=1). This behaviour seems to suggest that the interfacial films

Table 1. Steady-state apparent surface shear viscosity η of β-lactoglobulin adsorbed at *n*-tetradecane-water interface (2×10^{-3} wt% protein, 2 mM bis-tris buffer, pH 7, 25°C) containing various amounts of added SDS (expressed as surfactant/protein molar ratio *R*)

R	η/ mN m^{-1}s
0	560
1	540
4	550
16	540
64	450
520	210
1800	80

containing protein/surfactant complexes are less resistant to shear at lower surfactant concentrations.

It has been shown that SDS (Feijter *et al.*, 1987) or sodium lauryl ether suphate (Chen and Dickinson, 1995b) produces almost complete displacement of interfacial protein in emulsions at a surfactant concentration of ca. 0.3 wt% (R≒60 for SDS). The effect of interfacial protein displacement by the SDS on the surface shear viscosity has been investigated and the results are presented in Table 1. Steady-state surface shear viscosity is presented against the surfactant/protein molar ratio *R*. Based on the emulsion results of Feijter *et al.* (1987), it could be supposed that, even at rather low SDS concentrations, there should be a limited displacement of adsorbed β-lactoglobulin from the planar oil-water interface (*e.g.*, 30 % displacement at *R*=2). The displacement of interfacial protein usually affects the interfacial rheology in a similar way to that found with binary systems of proteins of different surface activity (Castle *et al.*, 1987; Dickinson *et al.*, 1990). In general, the interfacial rheology of binary system resembles that of the predominant component at the interface as the displacement of interfacial protein proceeds (provided no specific interaction occurs between the two). Nevertheless, what can be seen in Table 1 is that up to a surfactant/protein molar ratio *R*=16, the steady-state surface shear viscosity of the adsorbed film following SDS addition is almost the same as that for the original 1-day-old β-lactoglobulin film in the absence of added surfactant. These results suggest a strong interaction between β-lactoglobulin and SDS occurring at the interface, and this strong interfacial interaction appears to compensate for the loss of resistance to shear caused by the displacement of interfacial protein. In addition, it should be pointed out that the steady-state surface shear viscosity decreases for the first time at a protein/surfactant molar ratio *R*=64, but it never goes down to zero even at very high *R* (*i.e.*, η=80 mN m^{-1}s at *R*=1800) (see Table 1). This may be due to the nature of the surface rheology experiment which probes interfacial regions further away from the surface (Dickinson, 1992). That is, even though the interface may be fully covered with a primary SDS monolayer, it is likely that considerable amounts of protein-surfactant complex still remain in the interfacial region, interacting weakly with the primary adsorbed surfactant layer, and that this in turn affects the measured apparent interfacial shear rheology. It is interesting to note that the onset of a decrease in steady-state surface shear viscosity corresponds to the surfactant concentration at which complete displacement of interfacial protein takes place (Feijter *et al.*, 1987). It also coincides with the saturation binding concentration of SDS to β-lactoglobulin (0.9 mg SDS/mg β-lactoglobulin, corresponding to surfactant/protein molar ratio R≒58), as reported by Pitt-Rivers and Impiombato (1968).

While data in Table 1 refer to the steady-state apparent surface shear viscosity of the β-lactoglobulin film, the dynamic structural change of the interfacial film following surfactant addition, especially at high surfactant concentrations (*R*≥64), appears to be complicated, as suggested by the complex behaviour of surface shear viscosity. Fig. 2 shows the influence of SDS addition on the surface shear viscosity of a β-lactoglobulin film at pH 7 as a function of time. In contrast to Fig. 1, SDS was added at very high concentration to the aqueous subphase in contact with the 1-day-old β-lactoglobulin film (surfactant/protein molar ratio *R*=64, 520 and 1800). For all samples studied here, it can be seen that addition of surfactant to the 1-day-old β-lactoglobulin film leads to a small decrease in surface shear viscosity, followed by an increase within the next 3 to 7 hours to a value higher than that for the original 1-day-old β-lactoglobulin film.

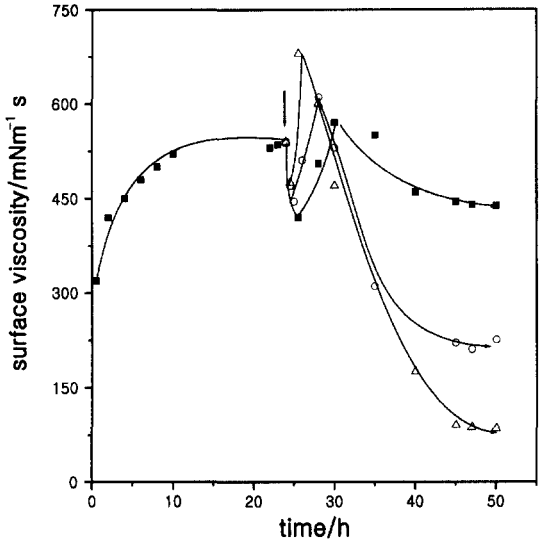

Fig. 2. Influence of SDS on surface shear viscosity of β-lactoglobulin adsorbed at *n*-tetradecane-water interface (2×10^{-3} wt% protein, 2 mM bis-tris buffer, pH 7, 25°C). Apparent surface viscosity is plotted against time for three different surfactant/protein molar ratio *R*: ■, *R*=64; ○, *R*=520; △, *R*=1800. The arrow denotes the point (after 24 h) at which the surfactant is introduced into the aqueous phase.

The extent of increase is in the order of increasing surfactant concentrations *i.e.*, 'R=1800 system'>'R=520 system'>'R=64 system'. As time elapses, surface shear viscosity decreases again to an extent which is dependent on the surfactant concentration. The higher the surfactant concentration, the lower is the steady-state viscosity. This behaviour seems to reflect the displacement of interfacial protein as well as the complicated protein-surfactant interfacial interactions and slow interfacial molecular rearrangement. Qualitatively similar results were reported previously by Chen and Dickinson (1995a) for gelatin films containing various amounts of added SLES 2EO. It is generally agreed that an adsorbed film of globular protein consists of molecules in various degrees of unfolding, with the more unfolded ones being located closer to the interface. When SDS is introduced into the system, it penetrates towards the interface binding with protein. This may induce extensive further unfolding of protein (Ray and Chatterjee, 1967; Jones, 1992), leading to the additional exposure of some functional groups (such as free thiol groups and hydrophobic residues, *etc.*), which would take part in intra- and intermolecular protein interactions. The formation of such interactions at the interface is mainly responsible for the interfacial rheology of an adsorbed film of globular protein (Graham and Phillips, 1979), which may be enhanced in the presence of SDS. Such enhancement may in turn affect the interfacial rheology because of increased number of mechanically important cross-links. This explains the observed increase in surface shear viscosity following surfactant addition shown in Fig. 2. With the systems containing higher surfactant concentrations, a more pronounced increase could be expected since there are more additional exposed functional groups available for such interactions. However, at high surfactant concentrations, the full development of protein/SDS complex at the interfacial regions eventually results in the solubilization of protein molecules into isolated surfactant micelles (Dickinson and Hong, 1997) thereby inhibiting protein-protein cross-linking. This is reflected by a decrease in the surface shear viscosity after the initial increase following surfactant addition. As mentioned earlier, at R≒60, the interfacial protein may be displaced completely from the interface. The protein/surfactant complex itself could also be displaced from the interface as complexation proceeds, probably due to the hydrophilic nature of the complex arising from hydrophobic interactions between SDS and protein, especially at high surfactant concentration (Ray *et al.*, 1967; Jones and Wilkinson, 1976). This means that there may be a imaginary slip plane consisting of a surfactant monolayer at the interface. And the presence of a slip plane in the interfacial region, where shear force is applied, may cause the resistance to shear to decrease. This is reflected in the lower steady-state surface shear viscosity for systems containing a high concentration of surfactant (R≥64). In addition, it must be pointed out that the surface shear viscosity (after surfactant addition) shows a time-dependent character. This suggests slow structural molecular rearrangement at the interface involving the formation of protein/surfactant complexes and the displacement of interfacial protein or protein/surfactant complexes.

The preparation of food emulsions is often carried out at relatively high temperatures. Most food proteins are susceptible to heat denaturation during such processing.

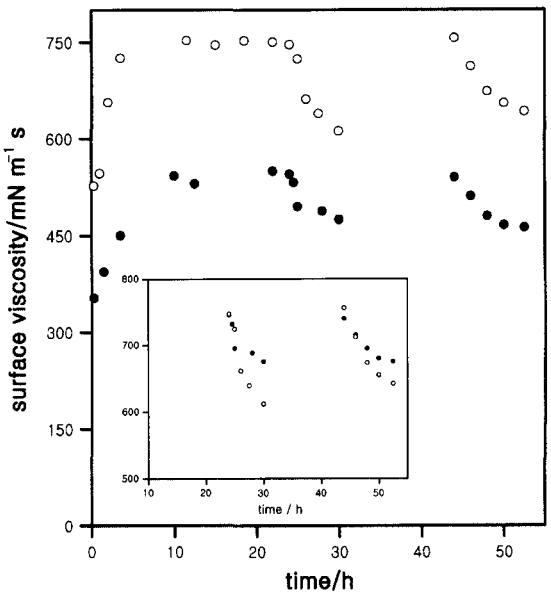

Fig. 3. Comparision of shear-thinning behaviour of native and heat-denatured β-lactoglobulin film after addition of SDS. The protein was adsorbed at *n*-tetradecane-water interface (2×10^{-3} wt% protein, 2 mM bis-tris buffer, pH 7, 25°C). Apparent surface viscosity is plotted against time for the two systems: ● , native protein: ○ , heat-denatured protein (70°C, 1 h). For comparison, inset illustrates the results with native protein shifted to give the same value as that of heat-denatured protein.

For β-lactoglobulin, 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 behaviour starts to change due to the onset of the aggregation process. Fig. 3 compares the shear-thinning properties of the interfacial film containing protein/surfactant complex. Two protein solutions were used in this investigation-(i) native protein and (ii) heat-denatured protein (70°C, 1 h). SDS was added at a surfactant/protein molar ratio R=4 to the aqueous subphase in contact with the 1-day-old native or heat-denatured β-lactoglobulin film. When one set of results is shifted so as to give the same value for the 1-day-old β-lactoglobulin film (see inset), it clearly shows that interfacial film adsorbed from the heat-treated bulk protein solution tends to exhibit a more shear-thinning surface shear viscosity following surfactant addition. Such behaviour could be partly related to the binding capacity of protein to surfactant. It has been reported (Oakes, 1976) that thermally denatured BSA binds less to SDS than does native BSA, due to the formation of aggregates resulting in loss of apolar sites available for SDS-binding. In an earlier part of this article (Fig. 1), it has been shown that less surfactant binding produces more pronounced shear-thinning surface shear viscosity. Taken together with these observations, more pronounced shear-thinning behaviour may therefore be expected for systems containing heat-denatured protein. In addition, it can be seen that the surface shear viscosity of the heat-denatured β-lactoglobulin film is higher than that of the native one. Similar results have been reported by Roth et al. (2000), which have been attributed to the enhanced formation of intermolecular interactions (mainly via disulfide bonds and hydrophobic interactions) in the adsorbed layer, leading to a greater amount of cross-linking.

The results discussed so far refer to the case of surfactant added to the system after the interfacial protein film has already been formed. It is interesting also to consider the case where adsorption takes place from the protein/surfactant mixture. Fig. 4 shows the surface shear viscosity of interfacial film adsorbed from the β-lactoglobulin/SDS mixture at the planar oil-water interface as a function of adsorption time (2×10^{-3} wt% protein, pH 7, 25°C). SDS was mixed with protein prior to creating the interface so as to give a surfactant/protein molar ratio R=4. As found with the pure protein system, at the beginning of adsorption (up to $t \fallingdotseq 10$ h), the apparent surface shear viscosity increases with adsorption time. But with further elapsing of time, it levels off reaching a value of ca. 235 mN m^{-1}s, which is lower than that for the 1-day-old pure β-lactoglobulin film ($\eta \fallingdotseq 540$ mN m^{-1}s). The lower surface shear viscosity appears to reflect the poorer adsorption of protein at the interface from the mixture. This poorer adsorption of protein is consistent with results of Wahlgren and Arnebrant (1991) who found the less adsorption of protein at a solid surface when adsorbed from a mixture of protein+surfactant. There can be two explanations for the observed lower adsorption of protein. The first relates to the nature of the β-lactoglobulin/SDS complex. As mentioned earlier, the binding of surfactant to proteins may change their

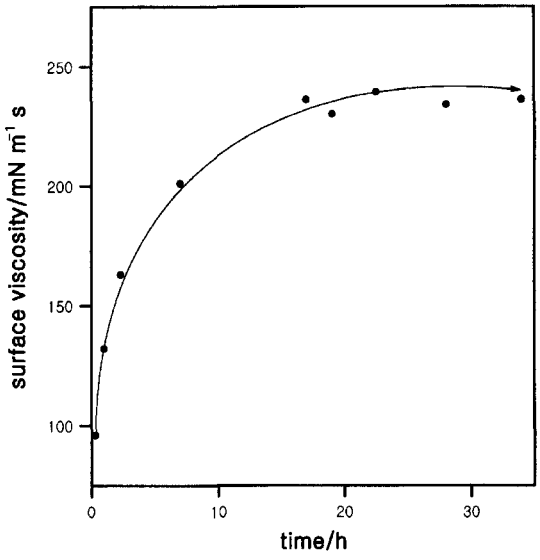

Fig. 4. The time-dependent surface shear viscosity of β-lactoglobulin film at the planar oil-water interface adsorbed from the mixture of the protein and SDS (2×10^{-3} wt% protein, 2 mM bis-tris buffer, pH 7, 25°C). SDS was mixed with the protein prior to creating interface so as to give surfactant/protein molar ratio *R*=4.

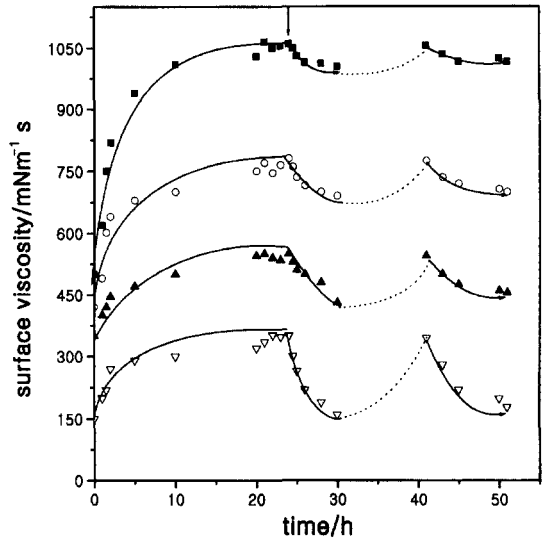

Fig. 5. Influence of pH on surface shear viscosity of β-lactoglobulin film containing SDS (2×10^{-3} wt% protein, 2 mM bis-tris buffer, pH 7, 25°C). Apparent surface viscosity is plotted against time for four different systems: ■, pH=5.5; ○, pH=6; ▲, pH=7; ▽, pH=8.5. The arrow denotes the point (after 24 h) at which the surfactant is introduced into the aqueous phase to give surfactant/protein molar ratio *R*=4.

hydrophobicity the protein/surfactant complex being more hydrophilic than pure protein, which simultaneously increases the net negative charge of the complex due to the binding of negatively charged surfactant. This behaviour may change the adsorptivity of the complex at the interface, resulting in less adsorption. It is also possible that uncomplexed surfactant would be predominantly adsorbed at the interface due to faster diffusion, probably interrupting further protein adsorption. According to Courthaudon *et al.* (1991b), the presence of water-soluble surfactant during emulsification caused protein surface concentration to decrease. The latter speculation could to some extent help to explain these findings.

Fig. 5 shows the effect of pH on the time-dependent surface shear viscosity of the β-lactoglobulin film containing SDS. The surfactant SDS was added to the aqueous subphase in contact with the 1-day-old β-lactoglobulin film at surfactant/protein molar ratio *R*=4. It can be seen that the surface shear viscosity of 1-day-old β-lactoglobulin film (*i.e.*, the protein film aged for 24 h) increases with decreasing pH. At pH 5.5, the surface shear viscosity is observed to be about three times higher than that at pH 8.5. As the protein has a net negative charge above the pI and the negativity increases with pH (Greener *et al.*, 1987), this can be attributed to lower electrostatic repulsion between the adsorbed protein molecules, leading to a denser packing at the interface. Similar results have been reported by Grahm and Phillips (1980) for BSA film and Roth *et al.* (2000) for β-lactoglobulin film. There have also been differences in shear thinning character in Fig. 5. It is less pronounced when measured at lower pH. As discussed earlier, the shear thinning character is less pronounced at the interface where more amounts of the SDS are associated. These results, therefore, suggest that at lower pH, there is a substantial interaction at the interface between the adsorbed protein and SDS. The binding of SDS to β-lactoglobulin depends on the environmental conditions such as pH and ionic strength (Magdassi and Kamyshny, 1996). Binding is greater at low pH (*i.e.*, at pH close to the isoelectric point) and decreases with increasing pH, since the portion of charged groups of the

protein molecules is increased (Jones and Wilkinson, 1976; Jones, 1992).

In contrast to the effect of ionic water-soluble surfactant, the presence of a small amount of oil-soluble surfactant at the oil-water interface can influence on the surface rheology. The effect of the presence of oil-soluble surfactant Sp-80 on the surface shear viscosity of the adsorbed pure β-lactoglobulin film has been studied. Fig. 6 shows the time-dependent surface shear viscosity of a β-lactoglobulin film with the surfactant present before the creation of the oil-water interface. The amount of Sp-80 is expressed as the surfactant/protein molar ratio *R*. At low surfactant concentrations ($R \leq 8$), the surface shear viscosity of the film increases with time, followed by a decrease over the following days and shows a plateau value on the third day. The initial increase in the surface viscosity is observed even more time-dependent than that of the pure -lactoglobulin film. These results suggest that the Sp-80 has both a synergistic and a competitive effect on the protein adsorption at the oil-water interface. According to Dickinson *et al.* (1993), the presence of a small amount

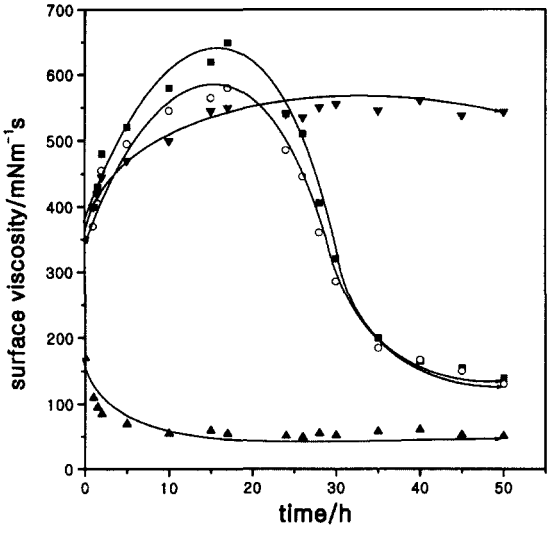

Fig. 6. The time-dependent surface shear viscosity of β-lactoglobulin film at the planar n-tetradecane-water interface (2×10^{-3} wt% protein, 2 mM bis-tris buffer, pH 7, 25°C). Oil-soluble surfactant Sp-80 was dissolved in the oil phase prior to creating interface. Apparent surface viscosity is plotted against time for four different surfactant/protein molar ratio R: ▼, *R*=0; ■, *R*=4; ○, *R*=8; ▲, *R*=64.

of this oil-soluble surfactant can induce an increase in the surface coverage of β-lactoglobulin on emulsion oil droplets and results in a smaller average droplet size. But the opposite effect is found at a high surfactant concentration. Similar results for systems containing 'diglycerides' has also been reported by Dickinson and Hong (1994). These results suggest that at low Sp-80 concentrations, the presence of oil-soluble surfactant at the oil-water interface tend to drag more protein molecules to the interface due to the interactions between surfactant and protein molecules which then enables β-lactoglobulin to form a more viscous film at the interface. The subsequent decrease in surface shear viscosity during the following days may be due to the displacement of β-lactoglobulin from the interface because of additional penetration of oil-soluble surfactant in the oil-water interface or interfacial molecular rearrangement (Dickinson and Hong, 1994). For a very high concentration (R=64), the surface viscosity starts to decrease at the beginning of the measurement (≥0.5 h). At this concentration, a saturated monolayer of the surfactant can be formed immediately after the creation of the interface. This may result in a extremely low surface viscosity.

The results for the influence of the surfactant concentration (Sp-80) on the apparent surface shear viscosity of the film are shown in Table 2. The apparent

Table 2. Apparent surface shear viscosity η of β-lactoglobulin adsorbed at *n*-tetradecane-water interface (2×10^{-3} wt% protein, 2 mM bis-tris buffer, pH 7, 25°C) containing oil-soluble surfactant Sp-80 (expressed as surfactant/protein molar ratio *R*)

R	η/ mN m^{-1}s
0	480
0.5	550
1	620
2	570
4	545
8	530
12	510
16	450
32	270
64	70

surface shear viscosity has been measured at 10 h after the creation of the interface. It can be seen that at relatively low surfactant concentrations (R≤12), the measurements are found to be higher than that of the pure β-lactoglobulin film: the maximum value is observed at surfactant/protein molar ratio R≒1, indicating a strong cooperative effect with β-lactoglobulin at the oil-water interface. This means that the presence of a small amount of oil-soluble surfactant may be beneficial to the formation of a strong protein layer at the oil-water interface, which would increase the emulsion stability against a coalescence. At high surfactant concentrations ($R \geq 16$), however, the apparent surface shear viscosities are found to be lower, probably due to the competitive displacement of interfacial protein. This would, in turn, result in deterioration of the emulsion stability.

Conclusions

Surface rheology is extremely sensitive to intermolecular interactions occurring in adsorbed film. Therefore it may be used to monitor protein-surfactant interactions at the oil-water interface. We have shown the effect of addition of some surfactants (ionic water-soluble SDS or non-ionic oil-soluble Sp-80) on the surface shear viscosity of protein film. As for the ionic surfactant, the results are generally in line with the one previously reported for similar systems (*i.e.*, a shear thinning behaviour). In addition, the shear thinning is more pronounced at the interface where less amounts of the ionic surfactants are associated. This suggests that the interfacial film containing protein-surfactant complexes formed at lower surfactant concentrations is less resistant to shear. The presence of oil-soluble surfactant can also influence on the surface shear viscosity, but in different way compared to that of ionic surfactant. At low surfactant concentrations, it is higher than that of the pure β-lactoglobulin film but the opposite is the case at high surfactant concentrations, indicating a synergistic and a competitive effect on the protein adsorption at the oil-water interface. This behaviour may implicate for the emulsion stability. Understanding the relationship between the surface shear viscosity and emulsion stability is the final objective of our research.

Acknowledgment

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