Effect of High-Pressure Treatment of Protein on the Rheology of Flocculated Emulsions Containing Protein and Polysaccharide

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We report on the effect of high-pressure treatment (up to 7 kbar) on the rheology of concentrated emulsions containing the globular protein, bovine serum albumin (BSA), and the anionic polysaccharide dextran sulfate. Small-deformation rheological properties have been determined for oil-in-water emulsions (40 vol % *n*-tetradecane, 2.7 wt % BSA, pH 7) containing polysaccharide added after emulsion formation. In the absence of high-pressure treatment, a plot of complex shear modulus G^* at 1 Hz and 30 °C against polysaccharide concentration C_P shows a maximum in G^* at $C_P \approx 0.1$ wt % which is consistent with bridging flocculation caused by a net attractive electrostatic protein−polysaccharide interaction at the emulsion droplet surface. High-pressure treatment of BSA before emulsification, for 30 min at a pressure in the range of 400−700 MPa, leads to substantial changes in the flocculation and rheological behavior of the emulsion after polysaccharide addition but no discernible change in the emulsion droplet-size distribution prior to the addition. In contrast, heat treatment of BSA at 70−80 °C before emulsification leads to an increase in average droplet size and to changes in emulsion rheology (following addition of polysaccharide) that are qualitatively different from those found with the pressure-treated systems. These results are discussed in relation to current knowledge about effects of high-pressure processing on protein structure and functionality.

Keywords: *High-pressure processing; protein−polysaccharide interaction; bovine serum albumin; dextran sulfate; emulsion rheology; bridging flocculation*

Interactions between adsorbed and unadsorbed macromolecules in food colloids can have an important influence on the stability and textural properties (Dickinson and McClements, 1995). The stability and rheological properties of oil-in-water emulsions containing adsorbed and nonadsorbed biopolymer molecules are sensitive to the detailed nature of the biopolymer interactions, both in the bulk aqueous phase and at the surface of the droplets (Dickinson and Euston, 1991; Dickinson *et al.*, 1993, 1995; Dickinson, 1996). In a concentrated emulsion system, where there is a weak net attractive interaction between biopolymer present in the bulk aqueous phase (polysaccharide) and adsorbed biopolymer (protein) located at the surface of dispersed oil droplets, it has been found experimentally (Dickinson and Pawlowsky, 1996) that the formation of bridging flocs leads to a large change in the small-deformation rheology as compared with that of the unflocculated system. This sensitivity of rheological measurement to small changes in particle−particle and particle−polymer interactions is supported by a recent theoretical analysis (Dickinson, 1995) of flocculation in concentrated colloidal dispersions based on the idealized sticky hard sphere model (Perram and Smith, 1977; Chiew and Glandt, 1989; Regnaut *et al.*, 1995). In the present paper, we are interested in how the biopolymer interactions and emulsion rheology are affected by high pressure pretreatment of the protein.

The protein structure and interactions are sensitive to environmental solution conditions such as temperature, pH, ionic strength, *etc.* This sensitivity is exploited during the processing of food proteins by using changes in these conditions to modify protein functional properties in the formulation of gels, foams, and emulsions. Individual proteins that have become partially denatured by heat, for instance, are known to have different interactions with oil droplet surfaces and other macromolecules (*e.g.* polysaccharides) than do the untreated native proteins. A novel food preservation and processing technique that has attracted increasing attention in recent years is high-pressure processing (Hayashi, 1990; Farr, 1990; Johnston, 1992; Knorr, 1993). [The "high" pressures involved here are on the order of several hundred megapascals (*i.e.* several thousand kilobars).] While it is already established that globular protein structure is susceptible to modification by high-pressure processing, it is not known to what extent this new technology allows systematic modification of the rheology and texture of protein-stabilized emulsions. There is the possibility, for example, that the stability and rheology of emulsion systems containing protein + polysaccharide could be controlled by subjecting the protein emulsifier to high-pressure treatment prior to emulsification.

The effect of high hydrostatic pressure on the structure of proteins in aqueous solution has received considerable attention over the last few years (Hayakawa *et al.*, 1992; Masson, 1992; Cheftel, 1992; Silva and Weber, 1993; Gross and Jaenicke, 1994; Dumay *et al.*, 1994; Defaye *et al.*, 1995; Heremans, 1995; Johnston and Murphy, 1995; Galazka *et al.*, 1996b). These studies have confirmed the view that, since formation of ion pairs or hydrophobic bonds is accompanied by a substantial positive volume change (Masson, 1992), high-pressure treatment has a disruptive effect on intramolecular electrostatic and hydrophobic interactions. On the other hand, hydrogen bond formation is associated with a small (usually negative) volume change, and so hydrogen bonding is relatively insensitive to pressure. What this means in relation to globular proteins is that high pressure disrupts the tertiary and quaternary structure but has relatively little influence on the secondary structure. As with thermal treatment, the

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partial denaturation of globular proteins on application of high pressure leads to aggregation (Dumay *et al.*, 1994; Galazka *et al.*, 1996b) and ultimately, if the concentration is high enough, to gelation (Johnston, 1992).

So far, there has been little reported work on the effect of high hydrostatic pressure on protein emulsifying properties (Denda and Hayashi, 1992; Karbstein *et al.*, 1992; Galazka *et al.*, 1995, 1996a), and essentially nothing at all on protein−polysaccharide interactions. We showed recently (Galazka *et al.*, 1996a) that high-pressure treatment (up to 800 MPa) of the globular protein β-lactoglobulin leads to a loss of emulsifying capacity and a reduction in the stability of fine emulsions made at neutral pH with 0.4 wt % protein and 20 vol % oil. This was interpreted (Galazka *et al.*, 1996a) as being due to a loss of emulsifying efficiency due to pressure-induced unfolding followed by aggregation. Qualitatively similar effects of high-pressure treatment on emulsifying properties were also observed when β-lactoglobulin was replaced with commercial whey protein concentrate (Galazka *et al.*, 1995).

The present paper is concerned with the effect of high-pressure treatment of protein before emulsification on the rheology of emulsions made with bovine serum albumin (BSA). The emulsions contain various concentrations of anionic polysaccharide dextran sulfate added after emulsification under neutral pH conditions. The small-deformation rheological approach adopted for this investigation is similar to the one we used recently to probe protein−emulsifier interactions in heat-set emulsion gels (Dickinson and Hong, 1995). Dextran sulfate is chosen because it is a nongelling polysaccharide with a high pH-independent charge density and strongly repulsive polysaccharide−polysaccharide interactions. The system BSA + dextran sulfate is chosen here because it has been previously demonstrated in our laboratory [from separate measurements of surface shear viscosity (Dickinson and Galazka, 1992; Dickinson, 1996), electrophoretic mobility (Dickinson and McClements, 1995; Dickinson, 1996), emulsion stability (Dickinson and Galazka, 1991; Dickinson and McClements, 1995), and foam stability (Izgi and Dickinson, 1995)] that this biopolymer mixture forms an electrostatic interfacial complex at neutral pH and low ionic strength. Addition of dextran sulfate to a concentrated BSA-stabilized emulsion is therefore expected to induce rheological changes associated with bridging flocculation at low polysaccharide concentrations and restabilization at high concentrations. It has been shown recently (Galazka *et al.*, 1996) that the presence of dextran sulfate affects the degree of BSA unfolding during high-pressure processing. What we are interested in here is the extent to which high-pressure pretreatment of the protein influences the emulsion rheological behavior. To facilitate comparison between the effects of high-pressure treatment and those of the more familiar heat treatment, measurements are also reported here for systems where the BSA is heat-treated prior to emulsion formation. As far as we are aware, such comparative emulsion experiments have not previously been performed.

MATERIALS AND METHODS

The bovine serum albumin (BSA), dextran sulfate (5×10^5 Da), and *n*-tetradecane were purchased from Sigma Chemical Co. (St. Louis, MO). Two different samples of BSA were used: sample A (lot no. 79F9303) and sample B (lot no. 14H9349). The buffer solution was prepared from analytical grade reagents and double-distilled water. Protein was dissolved at 4.6 wt % in aqueous imidazole buffer (5 mM, pH 7) under ambient conditions. The protein solution was subjected to either high-pressure or thermal processing (or used without treatment). The thermal processing involved heating the protein solution in a water bath at set temperatures in the 70−80 °C range and then immediately cooling in ice/water.

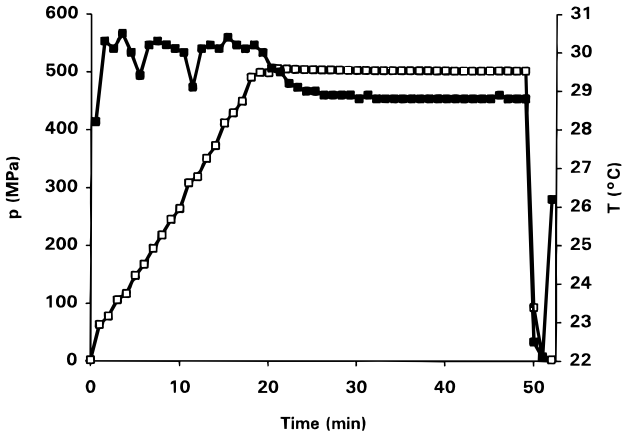

Figure 1. Change in pressure p and temperature T for a single treatment cycle with a holding pressure of 500 MPa, a dwell time of 30 min, and a maximum upper temperature setting of 30 °C: □, pressure; and ■, temperature.

High-pressure treatment was carried out using a Stansted Mark II Enhanced Mini Food Lab (Stansted Fluid Power, Essex, U.K.). This machine incorporates a pressure barrel consisting of a stainless steel liner prestressed by shrink fitting into a 2.5 % Ni/Cr alloy outer body. Pressure is generated by thrusting a plunger into the working chamber containing the "pressure fluid" (20 vol % caster oil and 80 vol % ethanol). First, the working chamber is precharged by means of a pneumatic pump, and then it is brought to the desired pressure with a hydraulic pump. At the end of the treatment cycle, the high pressure is released by retracting the plunger and then opening the release valve. The equipment has a built-in thermostating system with water circulated from a temperature-controlled tank into a heat-transfer jacket fitted to the pressure chamber. The pressure cycle is controlled automatically by a PLC program into which can be input the temperature limits (lower/upper), the treatment pressure, and the intended dwell time under pressure.

The degassed protein solution was transferred into polythene/nylon bags (Moore and Buckle, St. Helens, Lancs) which were vacuum-sealed and immersed in the pressure fluid. Prior to pressure treatment, the chamber was preheated for several hours. The temperature was normally set at 30 °C with notional lower and upper limits of −7.0 and +0.1 °C, respectively. The set pressure was in the 200−700 MPa range, and the dwell time was normally 30 min. With pressurization at an approximately linear rate of 25 MPa min^{-1}, it was possible to eliminate "adiabatic overshoot" (which might have an unpredictable effect on the protein structure) and hence to control the processing temperature to within ±1 °C from the start of the treatment cycle until the end of the dwell time. (An acceptable sudden release of pressure at the end of the cycle produces adiabatic cooling of several degrees.) Figure 1 shows time-dependent pressure and temperature profiles for a typical treatment cycle with a set pressure of 500 MPa.

Oil-in-water emulsions were prepared at room temperature using a laboratory-scale jet homogenizer (Burgaud *et al.*, 1990) operating at 30 MPa. Emulsions were made from 45 vol % *n*-tetradecane + 55 vol % aqueous phase containing 4.6 wt % BSA (pressure-treated, heat-treated, or untreated). Droplet-size distributions of emulsions were determined using a Malvern Mastersizer S2.01. To determine the distribution of protein between bulk aqueous phase and interface, a freshly prepared emulsion sample stabilized by native (untreated) BSA was centrifuged at 1×10^4 rpm for 75 min at 3 °C, the

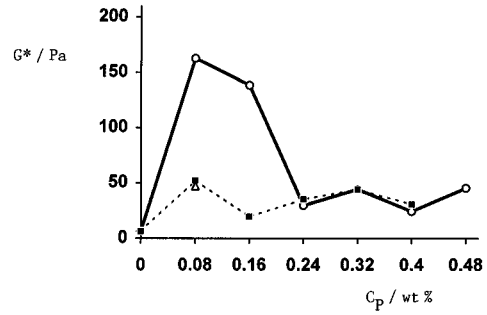

Figure 2. Effect of dextran sulfate added after emulsification on the viscoelasticity of *untreated* protein-stabilized emulsions [40 vol % oil, 2.7 wt % BSA (sample A), pH 7.0, $d_{32} = 0.55 \pm 0.01\ \mu$m]. The complex modulus G^* at 1 Hz and 30 °C is plotted against the added polysaccharide concentration C_P: ○, 5 mM imidazole; △, as ○, except sheared at 10^5 s^{-1} for 15 min; and ■, 70 mM imidazole.

concentration of protein in the resulting serum phase was determined from the absorbance at 280 nm, and the protein surface coverage was calculated assuming a specific surface area of 10.7 m^2 mL^{-1}. Dextran sulfate solutions of the appropriate concentrations were mixed with freshly prepared BSA-stabilized emulsions to give final emulsion samples (40 vol % oil, 2.7 wt % BSA) containing various concentrations of polysaccharide in the 0−0.5 wt % range.

Small-deformation dynamic viscoelastic measurements were made using a controlled stress Bohlin CS-50 rheometer with a concentric cylindrical cell (inner diameter of 14 mm, outer diameter of 15.4 mm, volume of ∼2 mL). Oscillatory measurements at 30 °C were used to determine the complex shear modulus G^* over the 10^{-3}−10 Hz frequency range. To facilitate comparison of samples with differing compositions and processing treatments, we have mainly adopted the reference frequency of 1 Hz. The maximum shear strain (0.005) was set to lie within the linear viscoelastic regime.

RESULTS AND DISCUSSION

We first summarize the results for emulsions made with the native (untreated) protein. Figure 2 shows the complex shear modulus G^* at 1 Hz and 30 °C for *n*-tetradecane-in-water emulsions [40 vol % oil, 2.7 wt % BSA (sample A), 5 mM imidazole, pH 7] as a function of the added polysaccharide concentration C_P. As reported elsewhere (Dickinson and Pawlowsky, 1996), it is evident that the elastic modulus of the emulsion passes through a maximum at a dextran sulfate concentration of *ca.* 0.1 wt % (*i.e.* at an effective polymer surface coverage of 0.2−0.3 mg m^{-2}). Taken together with separate evidence for interfacial protein−polysaccharide complexation (Dickinson and Galazka, 1991, 1992; Dickinson and McClements, 1995; Dickinson, 1996), this emulsion rheology behavior is consistent with weak gelation by a bridging flocculation mechanism (Dickinson and Eriksson, 1991). The presence of nonequilibrium polymer-bridged particles is indicated in Figure 2 by the disruption of the flocculated network at $C_P = 0.08$ wt % following extensive high-speed shearing (10^5 s^{-1} for 15 min). (Changes due to simple shear thinning of polysaccharide in the aqueous phase are not relevant here; a 0.5 wt % dextran sulfate solution is essentially Newtonian over a very wide range of shear rates.) While both macromolecules carry a net negative charge at neutral pH, it would appear that positively charged patches on the protein interact attractively at low ionic strength with the highly charged backbone of the polysaccharide (Tolstoguzov, 1986). The electrostatic character of the emulsion flocculation is demonstrated in Figure 2 by the substantial reduction

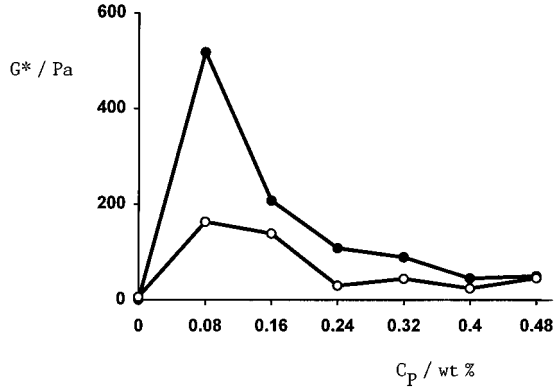

Figure 3. Comparison of different batches of BSA on the viscoelasticity of *untreated* protein-stabilized emulsions (40 vol % oil, 2.7 wt % BSA, 5 mM imidazole, pH 7.0) containing dextran sulfate. The complex modulus G^* at 1 Hz and 30 °C is plotted against the added polysaccharide concentration C_P: ○, sample A (lot no. 79F9303); and ●, sample B (lot no. 14H9349).

in G^* at $C_P = 0.08$ and 0.16 wt % on increasing the buffer salt concentration from 5 to 70 mM. More detailed information on the shear rheology of the emulsions containing *untreated* BSA + polysaccharide can be found elsewhere (Dickinson and Pawlowsky, 1996).

Protein analysis of the serum phase after centrifugation has shown that only *ca.* 20% of the BSA present in the emulsion is adsorbed at the oil−water interface; the remaining 80% is distributed in the aqueous phase between the droplets. This protein distribution corresponds to an inferred BSA surface coverage of 1.2 mg m^{-2}, which is typical of that commonly found for a globular protein monolayer (Dickinson, 1992). This means that the rheology of the emulsion in the presence of added dextran sulfate is determined not only by interaction between polysaccharide and BSA on the emulsion droplets but also (and possibly to a greater extent) by the nature of the protein−polysaccharide interactions in bulk aqueous solution.

The dependence of the rheological properties of these concentrated emulsions on globular protein structure is illustrated by the sensitivity to variations in commercial sample batch numbers. Figure 3 compares plots of G^* against C_P for emulsions separately prepared with BSA sample A (lot no. 79F9303) and BSA sample B (lot no. 14H9349). Each numerical values of G^* given in Figure 3 (and elsewhere in other G^*-C_P plots) is an average based on at least two independent measurements (standard error of ±15%). While the results for the two BSA samples are in qualitative agreement, the absolute values of the elastic modulus with protein sample B were found to be systematically higher (well outside the estimated combined nonsystematic error), especially at the polymer content ($C_P \approx 0.1$ wt %) corresponding to maximum flocculation. This may be associated with the slightly different emulsifying capacity of the two protein batches as reflected in the values of their average emulsion droplet sizes, *i.e.* $d_{32} = 0.63 \pm 0.02\ \mu$m for sample A and $d_{32} = 0.55 \pm 0.01\ \mu$m for sample B. The differences in functionality for the BSA samples could be attributable to various factors, *e.g.* contamination of the protein with lipid or different degrees of denaturation/aggregation during protein separation/purification. Variations in surface functional properties between different batches of notionally "pure" proteins have also been found previously with β-lactoglobulin (Dickinson and Iveson, 1993; Clark *et al.*, 1995).

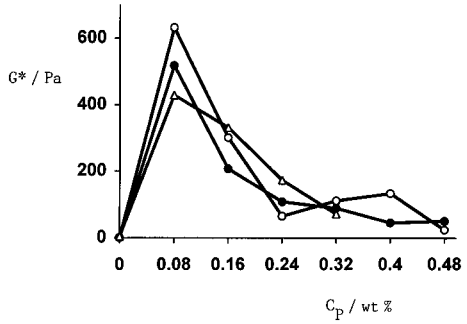

Figure 4. Effect of high-pressure treatment of BSA before emulsification on the viscoelasticity of protein-stabilized emulsions [40 vol % oil, 2.7 wt % BSA (sample B), 5 mM imidazole, pH 7.0] containing dextran sulfate. The complex modulus G^* at 1 Hz and 30 °C is plotted against the added polysaccharide concentration C_P: ●, no treatment; ○, 200 MPa; and △, 300 MPa.

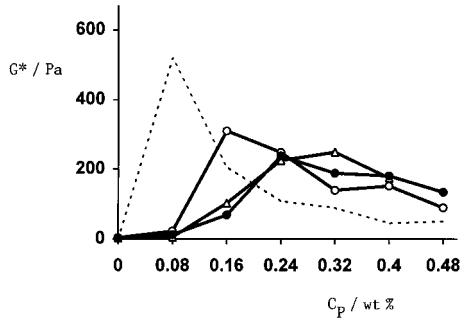

Figure 5. Effect of high-pressure treatment of BSA before emulsification on the viscoelasticity of protein-stabilized emulsions [40 vol % oil, 2.7 wt % BSA (sample B), 5 mM imidazole, pH 7.0] containing dextran sulfate. The complex modulus G^* at 1 Hz and 30 °C is plotted against the added polysaccharide concentration C_P: - - -, no treatment; ○, 400 MPa; △, 500 MPa; and ●, 700 MPa.

In what follows, therefore, where direct comparisons are made between different emulsion compositions and processing conditions, we have ensured that all the relevant comparative experiments were carried out with the same batch of protein (and polysaccharide).

We can turn now to a consideration of the effect of protein high-pressure treatment on the rheology of emulsions containing BSA + dextran sulfate. Figure 4 shows a plot of G^* against C_P for emulsions prepared with BSA (sample B) subjected to high-pressure treatment for 30 min at 200 or 300 MPa before homogenization. Also plotted again in Figure 4 for comparison are the results obtained with the untreated protein. We can readily see that, like the untreated sample, both of the pressure-treated samples give a maximum elastic modulus of $G^* = 500 \pm 100$ Pa at $C_P \approx 0.1$ wt %. (As experiments were carried out only for certain discrete values of C_P, small differences in the values of G^* at the maxima should not be regarded as significant.) What this implies is that protein treatment within the relatively low pressure range of 0−300 MPa, prior to emulsification, has no marked effect on the rheology of these mixed biopolymer oil-in-water emulsions. However, for treatment pressures of 400 MPa and above, the position becomes rather different. This is illustrated in Figure 5 which shows equivalent results for emulsions made with high-pressure-treated BSA at 400, 500, or 700 MPa; also plotted again are data for the (untreated) control. We see that the rheological results for the emulsions made with protein treated at these higher pressures are qualitatively different: the maximum in

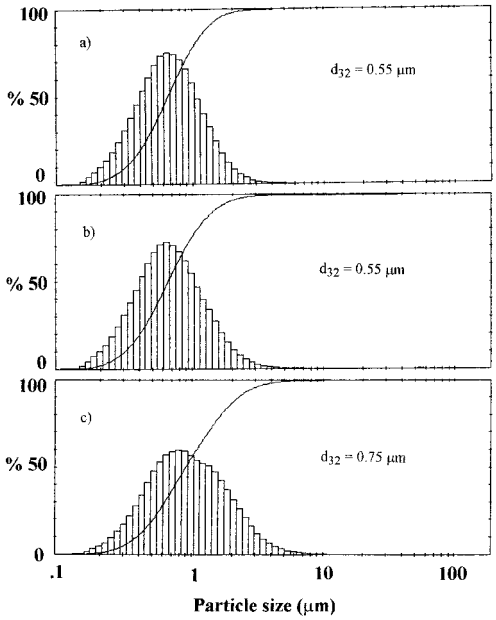

Figure 6. Effect of high-pressure or thermal treatment of BSA before emulsification on the droplet-size distributions of protein-stabilized emulsions [40 vol % oil, 3.0 wt % BSA (sample B), 5 mM imidazole, pH 7.0] *before* addition of polysaccharide: (a) no treatment, (b) 700 MPa for 30 min, and (c) 80 °C for 10 min.

$G^*(C_P)$ is shifted to higher C_P values, the value of G^* at the maximum is reduced, and the value of G^* at large C_P is increased. Since a generally similar shift of rheological behavior (not shown here) has been observed also with emulsions made with BSA sample A subjected to various pressure treatments, it can be assumed that the trends in Figures 4 and 5 reflect genuine changes in emulsion rheology which go well beyond differences arising simply from protein batch variability. What these results show is that, following high-pressure treatment of the protein emulsifier, addition of more of the polysaccharide is necessary to form the equivalent network structure in the emulsion.

Despite the fact that BSA high-pressure treatment (≥400 MPa) clearly leads to changed emulsion rheology in the presence of added polysaccharide (Figure 5), there was found to be no discernible effect of the pressure treatment on the emulsion droplet-size distribution (*before* addition of polysaccharide), as illustrated in Figure 6a,b. If the pressure treatment does have some small influence on the amount of soluble protein available for emulsification, then there is clearly enough total protein present in these systems not to have an effect on the total surface area created during homogenization. The average droplet diameter for all emulsion samples made with pressure-treated protein was found to be $d_{32} = 0.55 \pm 0.01$ μm. So the change in emulsion flocculation and rheology caused by the BSA pressure treatment cannot be directly attributable simply to the change in emulsion droplet size. Presumably, there is some unfolding or partial denaturation of the globular protein caused by treatment at 400 MPa (and above) which, although having no significant influence on the emulsifying capacity, is sufficient to subtly affect the nature of the protein−polysaccharide interaction, either at the interface or in the bulk aqueous phase between the emulsion droplets. A change in structure of the globular protein around 400 MPa is not unexpected, since pressures in the 300−500 MPa range have been reported (Heremans, 1992; Silva and Weber, 1993) for the onset

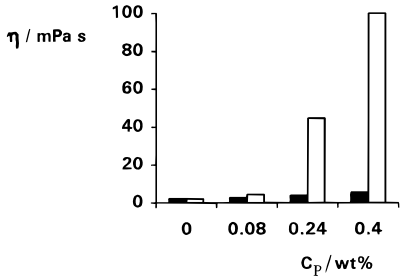

Figure 7. Effect of protein high-pressure treatment on the shear viscosity at 30 °C of solutions of BSA (sample B, 3.8 wt %) + dextran sulfate (no emulsion droplets). The chart shows the apparent viscosity η at a shear rate of 0.14 s^{-1} for three non-zero values of the polysaccharide concentration C_P: filled bars, 300 MPa; and open bars, 500 MPa.

of denaturation of single-chain proteins. On the other hand, Hayakawa and co-workers found little gradual denaturation of BSA at pressures up to 400 MPa (Hayakawa *et al.*, 1992).

Shear viscosities of *solutions* (no emulsion droplets) of high-pressure-treated BSA (300 or 500 MPa) + dextran sulfate are shown in Figure 7. The protein concentration is the same as in the aqueous phase of the 40 vol % oil emulsions. A large difference in mixed biopolymer solution rheology for the two protein pretreatment pressures can clearly be seen. The data in Figure 7 are consistent with greater resistance to flow due to partial BSA denaturation at 500 MPa and consequently very much stronger protein−polysaccharide interactions in the mixed biopolymer aqueous phase. The increase in G^* with dextran sulfate concentration in Figure 5 for a C_P of ≥0.1 wt % can therefore be attributed to an increase in the apparent viscosity η of the emulsion continuous phase arising from the high-pressure-induced development of protein−polysaccharide interactions in the aqueous continuous phase as well as at the surface of the droplets. We note also from Figure 7 that the mixed biopolymer solutions prepared from 300 MPa-treated BSA do not show any sign of a maximum in η at $C_P = 0.08$ wt % to mirror the maximum in G^* found in the corresponding emulsions. This is further confirmation of our interpretation that the maximum in G^* in Figure 4 (and in Figure 2) is indeed attributable to flocculation of the emulsion droplets and is not simply due to a change in the rheology of the emulsion continuous phase arising from the presence of the polysaccharide.

The absence of bridging flocculation in the emulsion made with BSA treated at 500 MPa with a low added dextran sulfate concentration (C_P = 0.08 wt %) is illustrated in Figure 8 by the plot of the frequency dependence of storage and loss moduli, G' and G''. The strongly viscous character of the rheology ($G'' \sim G'$) at low frequencies, together with the strong overall frequency dependence of G' and G'', is indicative of a viscoelastic suspension or very weak gel-like structure and is qualitatively different from the strong gel-type behavior (G' essentially independent of frequency, $G'' \ll G'$) found with the native protein under similar conditions (Dickinson and Pawlowsky, 1996). Also shown in Figure 8 are frequency-dependent G' and G'' plots for the 500 MPa-treated BSA emulsion with C_P = 0.4 wt %. The substantially stronger emulsion network with the larger addition of polysaccharide is indicated by the dominant elastic contribution ($G' \sim 10\ G''$) and the weaker frequency dependence than at $C_P = 0.08$ wt %.

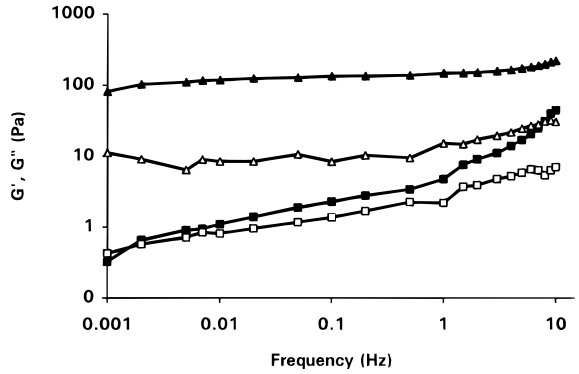

Figure 8. Effect of polysaccharide concentration on the dynamic shear rheology of protein-stabilized emulsions [40 vol % oil, 2.7 wt % BSA (sample B), 5 mM imidazole, pH 7.0] containing pressure-treated protein (500 MPa) and dextran sulfate. Storage and loss moduli, G' and G'', are plotted against frequency on a log−log scale: ■ and □, $C_P = 0.08$ wt %; and ▲ and △, $C_P = 0.4$ wt %. Filled and open symbols refer to G' and G'', respectively.

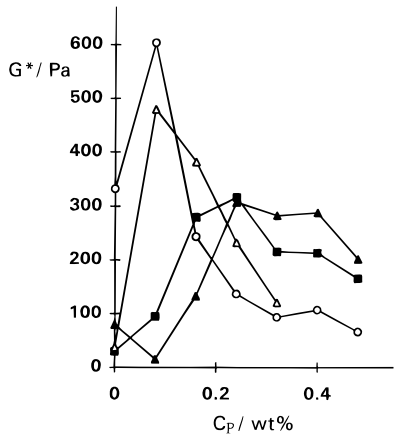

Figure 9. Viscoelasticity at 10 Hz of protein-stabilized emulsions [40 vol % oil, 2.7 wt % BSA (sample B), 5 mM imidazole, pH 7.0] containing dextran sulfate. The complex modulus G^* at 30 °C is plotted against the polysaccharide concentration C_P: ○, no treatment; △, 300 MPa; ■, 400 MPa; and ▲, 700 MPa.

Although we have chosen here mainly to compare the effects of dextran sulfate concentration on G^* at the single frequency of 1 Hz, we can also confirm that qualitatively similar trends are obtained at other frequencies. For example, Figure 9 shows G^* *versus* C_P at the higher frequency of 10 Hz for emulsions made with BSA pretreated at 0, 300, 400, and 700 MPa. The evolution of the function $G^*(C_P)$ with increasing pressure is clearly similar to that at 1 Hz (see Figures 4 and 5).

While we cannot offer an unequivocal interpretation of the effect of high-pressure treatment (≥400 MPa) on the rheological behavior in Figure 5 (or Figure 9), it seems likely that there at least are two contributory mechanisms operating. On the one hand, the partially unfolded BSA can interact with polysaccharide in the emulsion continuous phase, thereby increasing the viscoelasticity as indicated in Figure 7. Additionally, there may be simultaneous interaction of polysaccharide with protein adsorbed on two adjacent droplets, *i.e.* bridging flocculation. A possible explanation for weaker bridging (lower G^* maximum shifted to higher C_P) with the high-pressure-treated BSA is that, due to the partially unfolded state of the protein, more dextran sulfate must attach to BSA in the bulk phase (and at the interface) before load-bearing bridges are formed.

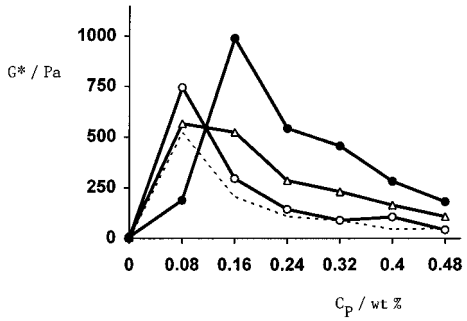

Figure 10. Effect of thermal treatment of BSA before emulsification on the viscoelasticity of protein-stabilized emulsions [40 vol % oil, 2.7 wt % BSA (sample B), 5 mM imidazole, pH 7.0] containing dextran sulfate. The complex modulus G^* at 1 Hz and 30 °C is plotted against the added polysaccharide concentration C_P: - - -, no treatment; ○, 70 °C for 10 min; △, 75 °C for 10 min; and ●, 80 °C for 10 min.

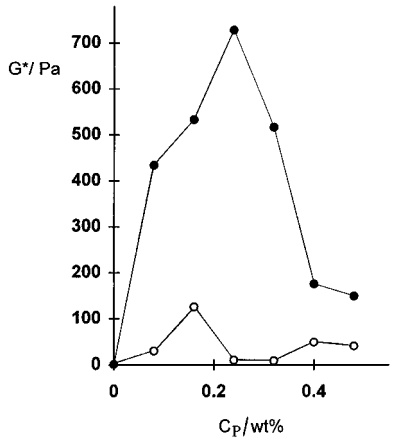

Figure 11. Comparison of shear rheology of emulsions (40 vol % oil, 2.7 wt % BSA, 5 mM imidazole buffer, pH 7.0) stabilized by heat-treated BSA (80 °C for 10 min, sample A) with that for equivalent solutions of heat-treated BSA + dextran sulfate. The complex modulus G^* at 1 Hz and 30 °C is plotted against the polysaccharide concentration C_P: ●, emulsion; and ○, solution.

At this point, it seems pertinent to draw attention to the rather close similarity between the plot of G^* against C_P for the polysaccharide-containing emulsions made here with BSA subjected to 500 MPa (or higher) (Figure 5) and the equivalent plot reported by us elsewhere (Dickinson and Pawlowsky, 1996) for similar emulsions made with sodium caseinate at pH 7. The casein-stabilized emulsions exhibited no bridging flocculation effect at low dextran sulfate concentrations, but rheological evidence was found (Dickinson and Pawlowsky, 1996) for substantial reversible electrostatic protein−polysaccharide interaction at higher polysaccharide contents ($C_P \geq 0.5$ wt %). While the exact nature of the casein−polysaccharide interaction remains unclear, it is tempting for us to speculate that the application of high hydrostatic pressure (≥400 MPa) to BSA leads to a partial unfolding of the globular protein molecule, thereby eliminating the specific BSA−dextran sulfate attraction interaction which induces bridging flocculation at low C_P and hence making the rheological behavior of polysaccharide-containing emulsions at higher C_P more like that of emulsions containing the disordered casein.

The most common method of food protein denaturation is heating. So it seems appropriate here for us to study also the effect of protein thermal treatment on the rheology of the same emulsions containing BSA + dextran sulfate. Some results, in the form of plots of G^* *versus* C_P, are shown in Figure 10. We see that pre-emulsification heat treatment for 10 min at 70−80 °C does lead to changes in emulsion rheology, but the trend is qualitatively rather different from that found with the pressure treatment. While the position of the maximum in G^* is shifted to higher polysaccharide concentrations with increasing treatment temperature (the interpolated value for 75 °C presumably lies between the values for 70 and 80 °C), the magnitude of G^* near the maximum is increased by the heat treatment, in contrast to the pressure treatment (≥400 MPa) which was found to give a reduction in G^* (see Figure 5).

Apart from the differences in the forms of the G^* *versus* C_P plots, some other differences between the heat-treated and pressure-treated experiments were observed. First, there was a tendency for a broadening in the freshly prepared emulsion droplet-size distribution (no added polysaccharide) with increasing temperatures of treatment. Figure 6c shows the distribution for the emulsion made with BSA heated at 80 °C for 10 min. The increase in average droplet size, from $d_{32} =$ 0.55 μm with the native protein to $d_{32} = 0.75$ μm with the heat-treated protein, appears significant when it is recalled that pressure treatment at 700 MPa for 30 min led to no change in average emulsion droplet size (see Figure 6b).

Another difference between the heat-treated and pressure-treated experiments relates to the observed visual appearance of the concentrated protein solution (4.6 wt %) prior to emulsion preparation. It was noticed that the viscosity of the protein solution increased markedly with increasing temperature, with the solution being transformed into a clear weak gel at 80 °C. No such obvious thickening or gelation was noticed following the most severe of the high-pressure treatments. These combined observations with the heat-treated protein of a poorer emulsifying capacity and the onset of gelation are indicative to us that, under the conditions employed in these experiments, the thermal processing treatment produces a much greater state of BSA aggregation than does the high-pressure treatment.

The effect of dextran sulfate on the rheology of an emulsion prepared with heat-treated BSA appears quite different from its effect on the rheology of an aqueous *solution* containing a mixture of the same heat-treated protein with dextran sulfate. This is illustrated in Figure 11 for systems containing BSA preheated to 80 °C for 10 min prior to emulsification and/or mixing with polysaccharide. In the mixed biopolymer solution containing heat-treated protein, there is a maximum in G^* at $C_P \approx 0.15$ wt % which does not occur in the equivalent system containing the native protein. So, even though the maximum in G^* for the solution is much lower than that for the emulsion, it would seem that the magnitude of the latter is explicable, at least in part, to enhanced contributions from protein−polysaccharide interactions in the aqueous phase arising from thermally induced BSA unfolding and aggregation.

Further evidence for a distinct difference in the state of flocculation on adding polysaccharide to the emulsion prepared with high-pressure-treated BSA (≥400 MPa), as compared with that prepared with native BSA, is given in Figure 12. The apparent average droplet diameter d_{32}* of the emulsion *after* addition of polysaccharide is plotted against C_P. We know from earlier work (Dickinson and Galazka, 1992; Dickinson, 1996)

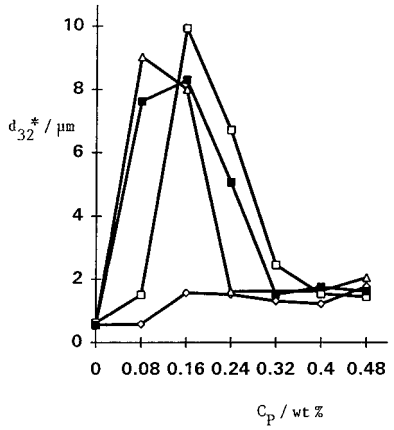

Figure 12. Effect of high-pressure or thermal treatment of BSA before emulsification on the apparent average droplet diameter d_{32}* of protein-stabilized emulsions [40 vol % oil, 2.7 wt % BSA (sample B), 5 mM imidazole, pH 7.0] *after* addition of dextran sulfate: ■, no treatment; □, 75 °C for 10 min; △, 200 MPa for 30 min; and ◇, 700 MPa for 30 min.

that dextran sulfate addition does not induce droplet coalescence, and so we must interpret the very large increase (by more than 1 order of magnitude) in d_{32}* (to *ca.* 8 μm) for the untreated BSA emulsion, as C_P increases from 0 to 0.1−0.2 wt %, as being due to the formation of large bridging flocs in the concentrated emulsion which are not dissociated on diluting the emulsion prior to particle sizing (Dickinson and McClements, 1995). The subsequent large decrease in this apparent average droplet size (to d_{32}* of <2 μm) with increasing C_P to values of 0.3−0.4 wt % is consistent with steric restabilization of the droplets by the protein−polysaccharide complex, as discussed previously (Dickinson and Galazka, 1992; Dickinson, 1996); this in turn is the reason for the lower shear moduli of the concentrated emulsions (Figures 2−4) in this polysaccharide concentration range. We can see also in Figure 12 that qualitatively the same bridging flocculation behavior is found for the emulsion made with BSA which had been pressure-treated at just 200 MPa. However, for the emulsion made with BSA pressure-treated at 700 MPa, there is no strong evidence for bridging flocculation in the data in Figure 12; while d_{32}* does increase somewhat for a C_P of ≥0.16 wt %, there is certainly no large peak in d_{32}*(C_P) and the values of d_{32}* never exceed 2 μm at any polysaccharide concentration.

One might ask why the effects of the high-pressure treatment appear larger in the particle size plot (Figure 12) than in the equivalent rheological plot (Figure 5 or 9). The explanation probably lies in the large background contribution of the continuous phase, arising from bulk phase protein−polysaccharide interactions, to the overall concentrated emulsion rheology. Under the high dilution conditions of the particle-sizing measurements, such interactions do not play any role, which means that in Figure 12 the bridging contribution involving interfacial protein−polysaccharide interactions is highlighted, whereas in Figure 5, it is partially masked by bulk phase biopolymer interaction contributions.

For completeness, also shown in Figure 12 are d_{32}* data for an emulsion made with heat-treated BSA (75 °C for 10 min); here again, there is a big bridging flocculation peak evident, but it is shifted to a slightly higher value of C_P than for the native BSA system (in apparent qualitative agreement with the trend of the rheology measurements in Figure 10). Taken as a whole, the droplet aggregation behavior indicated by the data in Figure 12 is entirely consistent with our interpretation that the rheological behavior of the polysaccharide-containing emulsions made with the protein treated at the higher pressures (≥400 MPa) is qualitatively different from that for emulsions made with the native protein, the heat-treated protein, or the protein treated at a relatively low pressure (≤300 MPa).

Other investigators have referred to relatively limited changes in conformation for "pressure-denatured" BSA as compared to the thermally denatured protein. For instance, Hayakawa *et al.* (1992) reported a loss of only 3% in α-helix content after pressure treatment at 600 MPa, as compared with a loss of 44% on heating at 80 °C. In agreement with our findings, it appears from previous workers (Denda and Hayashi, 1992; van Camp and Huyghebaert, 1995) that pressure processing at these levels does not lead to changes in BSA emulsifying capacity or solubility. On the other hand, at higher treatment pressures (≥800 MPa), substantial changes in secondary structure (loss of α-helices) have been reported (Hayakawa *et al.*, 1994; Galazka *et al.*, 1996b), as well as substantial reductions in protein surface hydrophobicity (Galazka *et al.*, 1996b). Such changes in BSA structure induced by these high pressures would be expected to have implications for the emulsifying properties. Aggregation/gelation and loss of emulsifying capacity due to thermal treatment of BSA is, of course, well-known in the biochemistry and food science literature (Lin and Koenig, 1976; Kato *et al.*, 1983; Yasuda *et al.*, 1986).

One factor that could be relevant to the processing-induced changes reported here (and elsewhere in the literature) is the much more pronounced (reversible) reduction in pH during pressure treatment as compared with that during heat treatment. The buffer used here, 5 mM imidazole at pH 7 (at 20 °C), was chosen deliberately to be of low molarity in order to maximize the strength of the electrostatic BSA−dextran sulfate interactions in the neutral pH emulsion systems. Nevertheless, such a low concentration buffer is rather more susceptible to lowering of pH during high-pressure treatment due to the electrostriction effect (Neuman *et al.*, 1973). Reductions of up to 0.3 pH unit per 100 MPa have recently been reported (Funtenberger *et al.*, 1995), depending on the ionic strength and chemical nature of the buffer salts. Reductions in pH can also occur, of course, on changing the temperature, and indeed, at 80 °C, the pH of our heat-treated BSA protein solution was found to be reduced to 6.4 ± 0.1. In a separate experiment, when pH was held constant during thermal processing (80 °C for 10 min) and during subsequent cooling by the addition of NaOH/HCl, the BSA solution did not visibly aggregate or gel, and the effect of dextran sulfate on the emulsion rheology was found to be very different from that for emulsions made with BSA heat-treated at uncontrolled pH (see Figure 13). However, an additional complication in interpreting the results in Figure 13 is the gradual increase in ionic strength (Na^+ and Cl^-) required to keep the pH strictly constant during the thermal processing. The presence of the extra salt ions will tend to screen out the attractive electrostatic protein−polysaccharide interactions and hence to reduce the values of G*. So we cannot be sure that the large difference in G*(C_P) between the two sets of data in Figure 13 for emulsions made with the heat-treated BSA is due wholly to the pH conditions during thermal processing.

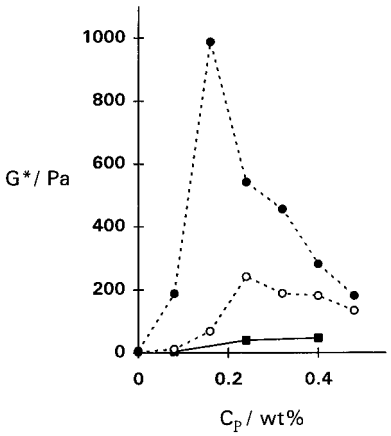

Figure 13. Effect of pH control during thermal processing of the protein (80 °C for 10 min) on the viscoelasticity of protein-stabilized emulsions [40 vol % oil, 2.7 wt % BSA (sample B), 5 mM imidazole, pH 7.0] containing dextran sulfate. The complex modulus G^* at 1 Hz and 30 °C is plotted against the polysaccharide concentration C_P: ●, uncontrolled pH during heat treatment; ■, pH held at 7.0 during heat treatment; and ○, uncontrolled pH during high-pressure treatment (700 MPa).

Whereas the pressure-processed protein solution remains at pH 7.0 ± 0.2 *following* the treatment, we do not know precisely the extent of the pH reduction *during* processing. Nevertheless, we can refer here to the recent study of Funtenberger *et al.* (1995) on the state of aggregation of β-lactoglobulin when dissolved in various buffers (but *not* imidazole) and subjected to high pressure. In low-molarity and nonbaroresistant buffers (like that used in the present study), they detected no loss of solubility and little aggregation, in contrast to the behavior found with pressure-resistant buffers. The reason for this behavior is not fully established. It could be associated with the effect of pH on the kinetics of protein unfolding. The authors suggest that the drastic drop in pH during pressure treatment protects against the formation of intermolecular disulfide bonds. Nevertheless, recent work (Galazka *et al.*, 1996b) on unbuffered BSA solutions subjected to a pressure of 800 MPa indicates that protein aggregation after high-pressure processing is caused, at least in part, by the formation of disulfide bridges.

CONCLUSIONS

We have demonstrated that the effect of anionic dextran sulfate on the small-deformation rheological behavior of concentrated BSA-stabilized emulsions at pH 7, as expressed by the change in complex modulus G^* at 1 Hz, is consistent with a bridging flocculation mechanism induced by electrostatic protein−polysaccharide interaction at the emulsion droplet surface. While high-pressure treatment of the protein prior to emulsification leads to no detectable change in emulsion droplet-size distribution before polysaccharide addition, treatment at pressures in the 400−700 MPa range does lead to qualitative changes in the effect of added polysaccharide on the emulsion rheology and the flocculation behavior. These changes are shown to be clearly different from those induced by protein heat treatment at 70−80 °C. Although the reason for the difference is not definitively established, it is possible that it is associated in some way with the effect on protein denaturation and aggregation of pH changes induced during pressure processing.

This rheological study of a mixed biopolymer emulsion has shown that high-pressure processing has potential for influencing the nature of protein−polysaccharide interactions and hence for controlling the viscoelasticity and stability of emulsions containing these macromolecules. In the system studied here, electrostatic protein−polysaccharide interaction at neutral pH is favored by the low ionic strength and the high charge density on this particular anionic polysaccharide. In principle, however, similar flocculation effects should also occur in mixtures of globular proteins with common food grade polysaccharides of lower charge density, although probably under different conditions of pH and ionic strength. In these preliminary experiments, the protein was treated with hydrostatic pressure before emulsion formation, and the polysaccharide was added after emulsion formation. The rheological and stability behavior of similar systems may become significantly modified, however, if the same high pressure were to be applied to a mixed solution of protein + polysaccharide prior to emulsification, or if the high pressure were to be applied directly to the emulsion, either before or after addition of the polysaccharide. Such aspects will be explored in future work.

LITERATURE CITED

Burgaud, I.; Dickinson, E.; Nelson, P. V. An improved high-pressure homogenizer for making fine emulsions on a small-scale. *Int. J. Food Sci. Technol.* **1990**, *25*, 39−46.

Cheftel, J.-C. Effects of high hydrostatic pressure on food constituents: an overview. In *High Pressure and Biotechnology*; Balny, C., Hayashi, R., Heremans, K., Masson, P., Eds.; Colloque INSERM/John Libbey: Montrouge, France, 1992; Vol. 224, pp 195−209.

Chiew, Y. C.; Glandt, E. D. Continuum percolation and pair-connectedness function in binary mixtures of strongly interacting particles. *J. Phys. A* **1989**, *22*, 3969−3979.

Clark, D. C.; Husband, F.; Wilde, P. J.; Cornec, M.; Miller, R.; Krägel, J.; Wüstneck, R. Evidence of extraneous surfactant adsorption altering adsorbed layer properties of β-lactoglobulin. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 1991−1996.

Defaye, A. B.; Ledward, D. A.; MacDougall, D. B.; Tester, R. F. Renaturation of metmyoglobin subjected to high isostatic pressure. *Food Chem.* **1995**, *52*, 19−22.

Denda, A.; Hayashi, R. Emulsifying properties of pressure-treated proteins. In *High Pressure and Biotechnology*; Balny, C., Hayashi, R., Heremans, K., Masson, P., Eds.; Colloque INSERM/John Libbey: Montrouge, France, 1992; Vol. 224, pp 333−335.

Dickinson, E. Structure and composition of adsorbed protein layers and the relationship to emulsion stability. *J. Chem. Soc., Faraday Trans.* **1992**, *88*, 2973−2982.

Dickinson, E. On flocculation and gelation in concentrated particulate systems containing added polymer. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 4413−4417.

Dickinson, E. Mixed biopolymers at interfaces. In *Biopolymer Mixtures*; Harding, S. E., Hill, S. E., Mitchell, J. R., Eds.; Nottingham University Press: Nottingham, U.K., 1995; pp 349−372.

Dickinson, E.; Eriksson, L. Particle flocculation by adsorbing polymers. *Adv. Colloid Interface Sci.* **1991**, *34*, 1−29.

Dickinson, E.; Euston S. R. Stability of food emulsions containing both protein and polysaccharide. In *Food Polymers, Gels and Colloids*; Dickinson, E., Ed.; Royal Society of Chemistry: Cambridge, U.K., 1991; pp 132−146.

Dickinson, E.; Galazka, V. B. Emulsion stabilization by ionic and covalent complexes of β-lactoglobulin with polysaccharides. *Food Hydrocolloids* **1991**, *5*, 281−296.

Dickinson, E.; Galazka, V. B. Emulsion stabilization by protein−polysaccharide complexes. In *Gums and Stabilisers for the Food Industry*; Phillips, G. O., Wedlock, D. J., Williams, P. A., Eds.; IRL Press: Oxford, U.K., 1992; Vol. 6, pp 351−362.

Dickinson, E.; Iveson, G. Adsorbed films of β-lactoglobulin + lecithin at the hydrocarbon−water and triglyceride−water interfaces. *Food Hydrocolloids* **1993**, *6*, 533−541.

Dickinson, E.; Hong, S.-T. Influence of water-soluble nonionic emulsifier on the rheology of heat-set protein-stabilized emulsion gels. *J. Agric. Food Chem.* **1995**, *43*, 2560−2566.

Dickinson, E.; McClements, D. J. *Advances in Food Colloids*; Blackie: Glasgow, U.K., 1995; Chapter 3.

Dickinson, E.; Pawlowsky, K. Rheology as a probe of protein−polysaccharide interactions in oil-in-water emulsions. In *Gums and Stabilisers for the Food Industry*; Phillips, G. O., Williams, P. A., Wedlock, D. J., Eds.; Oxford University Press: Oxford, U.K., 1996; Vol. 8, pp 181−191.

Dickinson, E.; Goller, M. I.; Wedlock, D. J. Creaming and rheology of emulsions containing polysaccharide and nonionic or anionic surfactants. *Colloids Surf. A* **1993**, *75*, 195−201.

Dickinson, E.; Goller, M. I.; Wedlock, D. J. Osmotic pressure, creaming, and rheology of emulsions containing nonionic polysaccharide. *J. Colloid Interface Sci.* **1995**, *172*, 192−202.

Dumay, E. M.; Kalichevsky, M. T.; Cheftel, J.-C. High-pressure unfolding and aggregation of β-lactoglobulin and the baroprotective effects of sucrose. *J. Agric. Food Chem.* **1994**, *42*, 1861−1868.

Farr D. High pressure technology in the food industry. *Trends Food Sci. Technol.* **1990**, *1*, 14−16.

Funtenberger, S.; Dumay, E.; Cheftel, J.-C. Pressure-induced aggregation of β-lactoglobulin in pH 7.0 buffers. *Lebensm.-Wiss. Technol.* **1995**, *28*, 410−418.

Galazka, V. B.; Ledward, D. A.; Dickinson, E.; Langley, K. R. High pressure effects on emulsifying behavior of whey protein concentrate. *J. Food Sci.* **1995**, *60*, 1341−1343.

Galazka, V. B.; Dickinson, E.; Ledward, D. A. Effect of high pressure on the emulsifying behaviour of β-lactoglobulin. *Food Hydrocolloids* **1996a**, *10*, 213−219.

Galazka, V. B.; Sumner, I. G.; Ledward, D. A. Denaturation of β-lactoglobulin and bovine serum albumin by high pressure. *Food Chem.* **1996b**, in press.

Gross, M.; Jaenicke, R. Proteins under pressure: the influence of high hydrostatic pressure on structure, function and assembly of proteins and protein complexes. *Eur. J. Biochem.* **1994**, *221*, 617−630.

Hayakawa, I.; Kajihara, J.; Morikawa, K.; Oda, M.; Fujio, Y. Denaturation of bovine serum albumin (BSA) and ovalbumin by high pressure, heat and chemicals. *J. Food Sci.* **1992**, *57*, 288−292.

Hayakawa, I.; Kanno, T.; Tomita, M.; Fujio, Y. Application of high pressure for spore inactivation and protein denaturation. *J. Food Sci.* **1994**, *59*, 159−163.

Hayashi, R. Application of high pressure to food processing and preservation: philosophy and development. In *Engineering and Food*; Spiess, W. E. L., Schubert, H., Eds.; Elsevier Applied Science: London, 1990; Vol. 2, pp 815−826.

Heremans, K. From living systems to biomolecules. In *High Pressure and Biotechnology*; Balny, C., Hayashi, R., Heremans, K., Masson, P., Eds.; Colloque INSERM/John Libbey: Montrouge, France, 1992; Vol. 224, pp 37−44.

Heremans, K. High pressure effects on biomolecules. In *High Pressure Processing of Foods*; Ledward, D. A., Johnston, D. E., Earnshaw, R. G., Hasting, A. P. M., Eds.; Nottingham University Press: Nottingham, U.K., 1995; pp 81−97.

Izgi, E.; Dickinson, E. Determination of protein foam stability in the presence of polysaccharide. In *Food Macromolecules and Colloids*; Dickinson, E., Lorient, D., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1995; pp 312−315.

Johnston, D. E. High pressure. In *The Chemistry of Muscle Based Foods*; Johnston, D. E., Knight, M. K., Ledward, D. A., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1992; pp 298−307.

Johnston, D. E.; Murphy, R. J. Some changes to the properties of milk proteins caused by high-pressure treatment. In *Food Macromolecules and Colloids*; Dickinson, E., Lorient, D., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1995; pp 134−140.

Karbstein, H.; Schubert, H.; Scigalla, W.; Ludwig, H. Sterilization of emulsions by means of high pressure. In *High Pressure and Biotechnology*; Balny, C., Hayashi, R., Heremans, K., Masson, P., Eds.; Colloque INSERM/John Libbey: Montrouge, France, 1992; Vol. 224, pp 345−347.

Kato, A.; Osako, Y.; Matsudomi, N.; Kobayashi, K. Changes in the emulsifying and foaming properties of proteins during heat denaturation. *Agric. Biol. Chem.* **1983**, *47*, 33−37.

Knorr, D. Effects of high hydrostatic pressure processes on food safety and quality. *Food Technol.* **1993**, *47* (6), 156−161.

Lin, V. J. C.; Koenig, J. L. Raman studies of bovine serum albumin. *Biopolymers* **1976**, *15*, 203−218.

Masson, P. Pressure denaturation of proteins. In *High Pressure and Biotechnology*; Balny, C., Hayashi, R., Heremans, K., Masson, P., Eds.; Colloque INSERM/John Libbey: Montrouge, France, 1992; Vol. 224, pp 89−99.

Neuman, R. C.; Kauzmann, W.; Zipp, A. Pressure dependence of weak acid ionization in aqueous buffers. *J. Phys. Chem.* **1973**, *77*, 2687−2691.

Perram, J. W.; Smith, E. R. Competitive adsorption via the Percus−Yevick approximation. *Proc. R. Soc. London, Ser. A* **1977**, *353*, 193−220.

Regnaut, C.; Amokrane, S.; Heno,Y. On the analysis of the effective adhesion in colloidal dispersions in the sticky hard sphere model. *J. Chem. Phys.* **1995**, *102*, 6230−6240.

Silva, J. L.; Weber, G. Pressure stability of proteins. *Annu. Rev. Phys. Chem.* **1993**, *44*, 89−113.

Tolstoguzov, V. B. Functional properties of protein−polysaccharide mixtures. In *Functional Properties of Food Macromolecules*; Mitchell, J. R., Ledward, D. A., Eds.; Elsevier Applied Science: London, 1986; pp 385−415.

van Camp, J.; Huyghebaert, A. High pressure-induced gel formation of a whey protein and haemoglobin protein concentrate. *Lebensm.-Wiss. Technol.* **1995**, *28*, 111−117.

Yasuda, K.; Nakamura, R.; Hayakawa, S. Factors affecting heat-induced gel formation of bovine serum albumin. *J. Food Sci.* **1986**, *51*, 1289−1292.

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