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Complexes of bovine serum albumin with sulphated polysaccharides: effects of pH, ionic strength and high pressure treatment

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Abstract

Solutions of bovine serum albumin (BSA) (5 mg ml^{-1}) subjected to high pressure processing (600 MPa for 20 min) at low ionic strength and neutral pH have shown a reduction in protein surface hydrophobicity. This decreases further in the presence of ι- or κ-carrageenan (2.5:1 weight ratio). The total calorimetric enthalpy (ΔH) for the pressure treated BSA is reduced by 50%. Addition of ι- or κ-carrageenan to BSA reduces both the denaturation temperature (T_m) and ΔH under the same treatment conditions. Size exclusion chromatography at low ionic strength has indicated a weak electrostatic interaction for BSA + ι- or κ-carrageenan at pH 7, which becomes stronger at pH 6.5. Complexation of BSA with the more highly sulphated ι-carrageenan gives a stronger electrostatic interaction than with κ-carrageenan under the same solution conditions. Replacement of ι-carrageenan with dextran sulphate (DS) gives an even stronger complex, which again suggests that the strength of complexation is dependent on the charge density on the polysaccharide. Complexation of BSA with polysaccharide at low ionic strength appears to protect the globular protein against pressure-induced aggregation. The addition of salt dissociates the protein–polysaccharide complex(es), and the protective effect of polysaccharide is then lost.

1. Introduction

Proteins and polysaccharides are the two main macromolecules found in the ingredients of a wide range of food products. These biopolymers play a significant role in the structure, stability and textural characteristics of many food colloids through their aggregation and gelling behaviour (Dickinson and Stainsby, 1982; Mitchell and Ledward, 1986). The overall structure–property relationship of food colloids depends, not only on the functional properties of the individual biopolymers, but also on the nature and strength of the mixed biopolymer interactions (Tolstoguzov, 1997).

The types of protein–polysaccharide interactions can vary widely due to wide variations in biopolymer structure and solvent conditions (pH, ionic strength, temperature). Depending on these conditions, macromolecular interactions may be specific or non specific, weak or strong, repulsive or attractive. For example, previous studies at pH 7 have demonstrated that the highly anionic polysaccharide, dextran sulphate (DS), forms an interfacial electrostatic complex with adsorbed bovine serum albumin (BSA) in both emulsions (Dickinson and Galazka, 1992) and foams (Izgi and Dickinson, 1995; Galazka et al., 1997a), but not with the adsorbed protein in equivalent β-lactoglobulin-stabilised emulsions (Dickinson and Galazka, 1991) and foams (Izgi and Dickinson, 1995; Galazka et al., 1997a).

Carrageenans are commonly used as gelling agents, thickeners and stabilizers in milk-based products (Enriquez and Flick, 1989). Like dextran sulphate, they are also sulphated polysaccharides, and the various forms of carrageenan mainly differ in the number and position of the sulphate groups on the polygalactose backbone. The order of charge density in commercially available carrageenans is: γ-carrageenan > ι-carrageenan > κ-carrageenan.

Many previous studies have been carried out on protein–carrageenan interactions, especially involving the caseins (Snoeren et al., 1976; Lynch and Mulvihill, 1996; Drohan et al., 1997), but also other proteins (Chakraborty and Randolph, 1972; Fernandes, 1996). In a recent study by Dickinson and Pawlowsky (1997), it was found that ι-carrageenan forms an electrostatic

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complex with BSA in aqueous solution at low ionic strength and pH≤7. This interaction becomes stronger at pH 6, but is dissociated in 100 mM NaCl. In oil-in-water emulsions containing these two biopolymers, strong bridging flocculation is responsible for destabilizing the emulsion at pH 6 over a certain range of carrageenan concentrations. Rheological studies also support the hypothesis that the formation of a solid-like emulsion structure is through bridging flocculation. Comparison with previous work on the BSA + DS system (Dickinson and Pawlowsky, 1996) shows that DS also induces bridging flocculation at pH 7 in a concentrated emulsion stabilized by BSA, and that there is comparable rheological behaviour in both emulsion systems. However, with the ι-carrageenan a lower concentration is required to form a bridging network, possibly because of its larger molecular size (Dickinson and Pawlowsky, 1997).

Recently, it has been demonstrated (Galazka et al., 1996, 1997b) that BSA in solution (low ionic strength, pH 7), when subjected to high pressure treatment, leads to denaturation and aggregation, which is mainly considered to be due to the formation of intermolecular disulphide bridges via–SH/–S–S–interchange. As a result of pressure treatment it is suggested that non-covalent protein–polysaccharide interactions are modified due to pressure-induced changes in electrostatic interactions and protein conformation. Studies of BSA + DS in solution under neutral conditions have suggested (Galazka et al., 1996, 1997b) that complexation of BSA with DS has the effect of protecting the BSA against extensive aggregation after high pressure treatment.

The aim of this present study is to determine the effect of solution conditions and high pressure on properties of mixtures of BSA with ι- or κ-carrageenan under electrolyte conditions favouring strong electrostatic interaction. This paper is intended to complement recent surface tension and emulsion studies by Dickinson and Pawlowsky (1997) and previous solution work on the BSA + DS system (Galazka et al., 1996, 1997b). Size exclusion chromatography is used to demonstrate the presence of BSA-polysaccharide complexation, and ionic strength is adjusted to investigate the extent of the electrostatic character of the protein–protein and protein–polysaccharide interactions.

2. Materials and methods

2.1. Materials

Bovine serum albumin (globulin-free lyophilized powder, ≥99% purity, product A-7638, lot 16H9314) and dextran sulphate (500 kDa, containing 0.5–2.0% phosphate buffer salts pH 6–8, made by treatment of dextran T-500 with chlorosulphonic acid in pyridine, product D-6001, lot 112H0372) were obtained from Sigma Chemical Co. (St Louis, MO). The food grade ι- and κ-carrageenan samples were a gift from Systems Bio Industries (Carentan, France). ι-Carrageenan, made from *Eucheuma denticulatum*, was in almost pure sodium form with about 5% contamination by κ-carrageenan. Weight-average molecular weight was given by the suppliers as 560 kDa and the *z*-average hydrodynamic radius as 80 nm. The food grade κ-carrageenan, manufactured from *Eucheuma cottoni*, was in 60 mol% potassium form and 40 mol% sodium form with a small amount of contamination by ι-carrageenan (< 10%). The weight-average molecular weight was given by the suppliers as 720 kDa and the *z*-average hydrodynamic radius as 100 nm. The sulphate:glucose group relative proportions are 1:1 for DS, 1:5 for ι-carrageenan and 1:15 for κ-carrageenan, as determined by a turbidimetric method. Sulphate was determined turbidimetrically by the precipitation of barium sulphate when barium chloride was added to solutions of polysaccharide (MAFF, 1986).

1-Anilinonaphthalene-8-sulphonate (ANS) ammonium salt (316.4 Da) was purchased from SERVA (Feinbiochemica, Heidelberg, Germany). Buffer solutions (20 mM imidazole, pH 6.5; Tris-HCl 20 mM, pH 7–8 and bis-Tris 20 mM, pH 6.5) for use in the size exclusion chromatography experiments were prepared from analytical grade reagents and HPLC grade water.

2.2. Preparation of solutions

A solution of carrageenan (2 mg ml^{-1}) was prepared by dispersing the polysaccharide powder in HPLC grade water and continuously stirring at 70°C for 20 min. The solution was cooled to 25°C and 5 mg ml^{-1} BSA was dissolved in it. The pH was adjusted to a value in the pH range 7–8 by addition of 0.05 M HCl or 0.05 M NaOH. The protein alone (5 mg ml^{-1}) or a mixture of BSA (5 mg ml^{-1}) + DS (2 mg ml^{-1}) was dissolved in HPLC grade water, and the pH was adjusted to 7 or 8 by addition of HCl or NaOH. Imidazole buffer (20 mM) was used for all experiments performed at pH 6.5.

2.3. High-pressure treatment

5 ml samples of protein solution (5 mg ml^{-1}) and solutions of BSA (5 mg ml^{-1}) + ι- or κ-carrageenan or DS (2 mg ml^{-1}) at pH 6.5–8 were sealed in Cryovac bags (Cryovac–W.R. Grace Ltd., London) and subjected to a pressure treatment of 600 MPa for 20 min at ambient temperature (Galazka et al., 1996) using a prototype Stansted Food-Lab high pressure machine (Stansted Fluid Power, UK). Pressure-treated samples were kept on ice for 1–2 h before analysis.

2.4. Analytical techniques

2.4.1. Spectrofluorometry

The surface hydrophobicity of solutions of pure BSA or BSA + ι- carrageenan (2.5:1 or 5.0:1 by weight) or κ-carrageenan (2.5:1 by weight) was determined before and after pressure treatment using a solution of ANS at ambient temperature and neutral pH (Nakai et al., 1996). The fluorescence intensity was recorded on a Perkin–Elmer LS50 spectrofluorimeter (excitation 350 nm, slit 2.5 nm; emission 470 nm, slit 2.5 nm), and the relative intensity measured at 470 nm. The ANS concentration was kept constant at 2.31 μM and the BSA concentration was varied in the range 0.17–16.0 μM. Values quoted are the mean ± SD from three determinations.

2.4.2. Differential scanning calorimetry (DSC)

DSC measurements were performed on a MicroCal MA2 scanning calorimeter (MicroCal Inc., Northampton, MA) at a scan rate of 1°C min^{-1}. Samples were kept under nitrogen gas at 2 bar to maintain a positive pressure in the vessel and so prevent oxidation. The endothermic peak temperature (T_m) and total calorimetric enthalpy (ΔH) were calculated using the MicroCal Origin data analysis package. Solutions of BSA (5 mg ml^{-1}) or BSA + ι- or κ-carrageenan (5 mg ml^{-1} BSA; [BSA]/[polysaccharide] = 2.5) at pH 7 were heated through the temperature range 20–85°C.

2.4.3. Size exclusion chromatography

A Sephacryl S-300 column (100×1.6 cm) was calibrated with thyroglobulin (6.7×10^5 Da), apoferritin (4.4×10^5 Da), β-amylase (2.0×10^5 Da), alcohol dehydrogenase (1.5×10^5 Da), bovine serum albumin (6.6×10^4 Da), and carbonic anhydrase (2.9×10^4 Da). Blue dextran (2.0×10^6 Da) was used to determine the column void volume. The column buffer was 20 mM Tris-HCl at pH 7–8 or bis-Tris pH 6.5, in some cases supplemented with 20–100 mM NaCl in order to assess the importance of the electrostatic contributions to the protein–protein and protein–polysaccharide interactions. BSA (5 mg ml^{-1}) and mixtures of BSA + polysaccharide (5 mg ml^{-1} BSA; [BSA]/[polysaccharide] = 2.5) were eluted from the column at a flow rate of 0.75 ml min^{-1} and detected at 280 nm.

3. Results and discussion

3.1. Surface hydrophobicity

Probe spectrofluorimetry studies of ANS binding to native and pressure-treated (600 MPa for 20 min) BSA in the absence and presence of ι- or κ-carrageenan are presented in Table 1. These experiments indicate a substantial loss in protein surface hydrophobicity of pure BSA following pressurization which is in agreement with the previous studies of Galazka et al. (1996, 1997b) and Hayakawa et al. (1992, 1996). The reduction of surface hydrophobicity in the pressure-treated sample is thought to be due to intermolecular interactions which lower the accessibility of hydrophobic groups binding to the ANS, and possibly also to the burying of some of the binding sites in the refolded, partly denatured BSA.

Table 1
Influence of high pressure treatment on the surface hydrophobicity (S_o) of native and pressurized BSA (0.17–16 μM) and mixtures of BSA (0.17–16 μM) + ι- or κ-carrageenan (2.5:1 or 5.0:1 by weight)

Sample	Treatment pressure (MPa)	S_o (absorbance units/mol of ANS)
BSA	0	275
BSA	600	126
BSA:κ-carrageenan (2.5:1)	0	148
BSA:κ-carrageenan (2.5:1)	600	85
BSA:ι-carrageenan (5:1)	0	157
BSA:ι-carrageenan (5:1)	600	98
BSA:ι-carrageenan (2.5:1)	0	67
BSA:ι-carrageenan (2.5:1)	600	35

Duration of pressure treatment was 20 min. Measurements were made in the presence of 2.31 μM ANS, pH 7, and quoted values are the averages of triplicate measurements with an estimated error ± 10%.

Addition of ι-carrageenan (BSA:ι-carrageenan weight ratio = 5:1) to the native BSA leads to a reduction in protein hydrophobicity (S_o) (untreated BSA = 275 AU/mol ANS; untreated BSA + ι-carrageenan = 157 AU/mol ANS) and at the higher ι-carrageenan concentration (BSA: polysaccharide weight ratio = 2.5) there is a further loss in protein hydrophobicity (S_o = 67 AU/mol ANS). In both cases, the protein hydrophobicity (S_o) for the pressurized mixture is lower than for the untreated mixture at the same weight ratio. Replacement of ι-carrageenan with κ-carrageenan (BSA:κ-carrageenan weight ratio = 2.5:1) leads to an increase in S_o (148 AU/mol ANS) for the untreated mixture, but this is still lower than that for the native BSA. Direct comparison with previous work on the BSA + DS system (Galazka et al., 1997b) shows a broadly comparable trend of behaviour for all mixtures, although in the BSA + DS system there is a lower value of S_o = 27 AU/mol ANS. We have demonstrated that the ANS does not bind to the polysaccharide alone, and so it is assumed that the decrease in surface hydrophobicity is mainly due to electrostatic repulsion between the two negatively charged molecules and/or possible blocking of the ANS binding sites by BSA–polysaccharide complexation. Pressurization of the biopolymer mixtures gives a further reduction in S_o , which suggests that some pressure-induced modification of the BSA occurs in the complexed state.

3.2. Denaturation

DSC data for solutions of native and pressure treated BSA (5 mg ml^{-1}) and mixtures of BSA (5 mg ml^{-1}) + ι- or κ-carrageenan (2 mg ml^{-1}) at pH 7 are compared in Table 2. We note that the endothermal peak temperature T_m (70.5°C) for native and pressurized BSA (600 MPa for 20 min) are the same (within experimental error); however, pressure processing leads to a 45% reduction in the total calorimetric enthalpy ΔH. Our results are consistent with earlier data from Galazka et al. (1997b) and Hayakawa et al. (1994) which indicate that high pressure treatment causes a substantial loss of the tertiary structure.

The effect of κ-carrageenan addition to BSA leads to a small reduction in T_m (by <2°C) with a corresponding 20% decrease in ΔH. Pressurization in the presence of polysaccharide significantly reduces the value of ΔH (by 60%) and leads to a small increase in T_m (<1°C) when compared with the untreated mixture. Replacement of κ-carrageenan with ι-carrageenan leads to a slight decrease in T_m (<1°C) and ΔH ($\sim$5%) for the native mixture at the same weight ratio. High pressure treatment of the BSA + ι-carrageenan mixture induces a large drop in ΔH (to 3.1×10^4 kcal mol^{-1}) and a slight increase in T_m. The carrageenans alone do not deviate from the baseline. Recent work on the BSA + DS system (Galazka et al., 1997b) showed that DS induces such a large reduction in T_m and ΔH that pressurization has little additional effect. It was therefore suggested that BSA–DS complexation during or after pressure treatment does not allow the BSA to unfold to the same extent as is normally the case with the protein alone. Comparison with the BSA + carrageenan systems suggests that, in these cases, weak complexes are formed and that pressure treatment does cause further denaturation of the protein.

Table 2
Effect of high pressure processing on the DSC endothermic peak temperature T_m and total calorimetric enthalpy, ΔH, for BSA (5 mg ml^{-1}) and mixtures of BSA (5 mg ml^{-1}) + ι- or κ- carrageenan (2 mg ml^{-1}) at pH 7.

Sample	Treatment pressure (MPa)	T_m (°C)	ΔH (kcal/mol)
BSA	0	70.5	17.5×10^4
BSA	600	70.5	9.7×10^4
BSA + κ-carrageenan	0	69.0	14.1×10^4
BSA + κ-carrageenan	600	69.9	7.3×10^4
BSA + ι-carrageenan	0	68.5	13.3×10^4
BSA + ι-carrageenan	600	69.5	3.1×10^4
κ- or ι-carrageenan (no BSA)	600	0	0

High pressure treatment time was 20 min. Measurements were performed in duplicate with the experimental errors of ±0.5°C in T_m and error ±10% in ΔH.

3.3. Nature of the protein–polysaccharide complexation

We now turn to the size exclusion chromatography studies for the BSA alone (5 mg ml^{-1}) at pH 7 in the absence of NaCl. Fig. 1(a) for native BSA shows a single major peak at *ca.* 6.6×10^4 Da which corresponds to the monomeric protein before pressure processing. After pressure treatment at 600 MPa for 20 min, the chromatogram in Fig. 1(b) shows a broader peak with a shoulder (*ca.* 1.5×10^5 Da) at shorter elution times which corresponds to the formation of dimers. BSA has a free cysteine residue which, under appropriate conditions, can become accessible for intermolecular association with other sulfhydryl or disulphide groups to form covalent disulphide bonds. Earlier studies have shown (Galazka et al., 1996) that there is a loss of free sulfhydryl groups during pressure treatment and that the presence of DL-Dithiothreitol (DTT) in the pressure processed sample reduces the level of dimerization. It seems likely that these aggregates are due to polymerization by –S–S– bridging. It is noteworthy that, at pH 8, size exclusion chromatography studies of native BSA (not shown) suggest an increase in the apparent

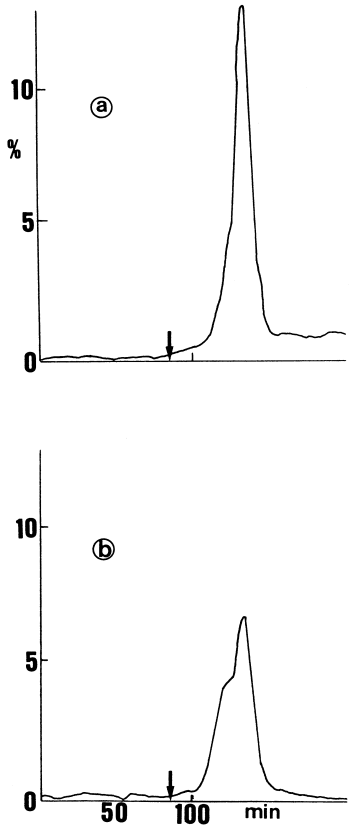

Fig. 1. Influence of high pressure treatment on size exclusion chromatograms of BSA (5 mg ml^{-1}) at neutral pH, in absence of NaCl. The absorbance was measured at 280 nm as a function of the elution time: (a) untreated; (b) pressure treated at 600 MPa for 20 min. Arrow indicates position of the void volume.

molecular size to *ca.* 1.5×10^5 Da, implying that BSA dimerizes by the oxidation of sulfhydryl groups into –S–S–bonds (Janatova et al., 1968). Pressure-treatment (600 MPa for 20 min) of the same sample showed a slightly broader peak which indicated some small change in protein structure without significant aggregation.

Fig. 2 shows chromatograms for the mixed system of BSA (5 mg ml^{-1}) + κ-carrageenan (2 mg ml^{-1}) at pH 6.5, 7.0, and 8.0 in the absence of NaCl. Chromatogram (a) for the untreated mixture at pH 8 shows a single broad peak at *ca.* 1.5×10^5 Da corresponding to dimerized BSA with a very small peak at *ca.* 7×10^5 Da which could indicate minimal interaction between BSA and κ-carrageenan. Chromatogram (d) for the pressure-treated (600 MPa for 20 min) mixture at a pre-pressure pH 8 shows that the apparent molecular size corresponding to the main peak has slightly increased, which is suggestive of some small change in protein structure during pressure treatment. Chromatogram (b) for the BSA + κ-carrageenan at pH 7 shows a narrower major peak corresponding to monomeric BSA and a small peak *ca.* 7×10^5 Da indicating very weak attractive interaction between the protein and polysaccharide. Reducing the pH to 6.5 (Fig. 2(c)) gives a narrow strong main peak and a distinct small peak which indicates the formation of a weak protein–polysaccharide complex. The effects of pressure treatment on the BSA + κ-carrageenan systems at pH 7 and pH 6.5 are shown in Fig. 2(e) and (f), respectively. We note that the major peak (*ca.* 6.6×10^4 Da) becomes smaller, and that the second peak at shorter elution times is clearly enhanced, corresponding to the formation of strong protein-polysaccharide complexes as the pH is decreased. It is also possible that, during treatment, some 'free' denatured protein could have become associated with the complex and thus have become part of the high molecular weight fraction.

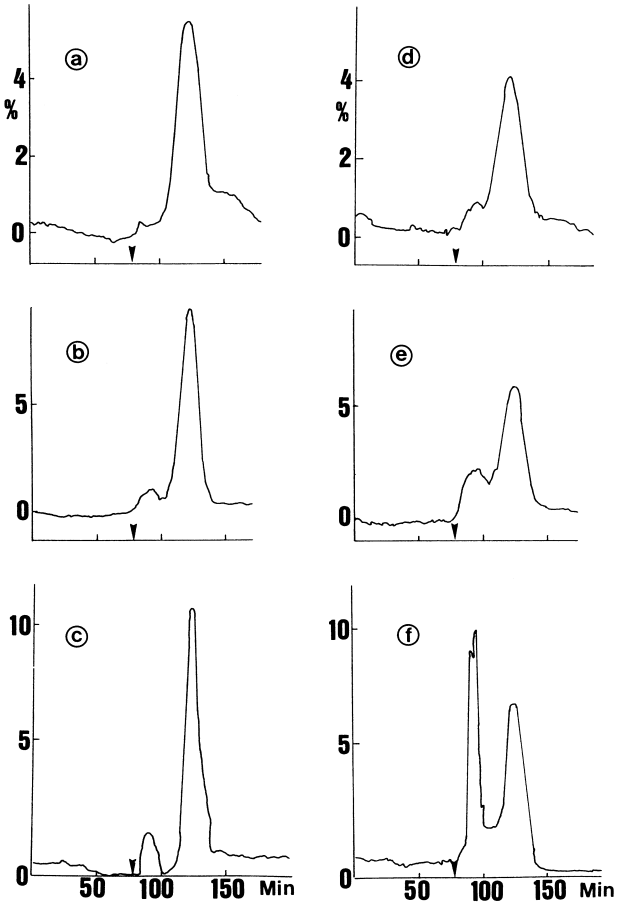

Fig. 2. Influence of high pressure and pH (6.5, 7.0 and 8.0) on size exclusion chromatograms of mixtures of BSA (5 mg ml^{-1}) + κ-carrageenan (2 mg ml^{-1}) in the absence of NaCl. The absorbance was measured at 280 nm as a function of the elution time: (a) untreated at pH 8; (b) untreated at pH 7; (c) untreated at pH 6.5 in 20 mM imidazole buffer; (d) pressure-treated (600 MPa for 20 min) at pH 8; (e) pressure-treated (600 MPa for 20 min) at pH 7; (f) pressure-treated (600 MPa for 20 min) at pH 6.5 in imidazole buffer. Arrow indicates position of the void volume.

We now move to size exclusion chromatography data for BSA (5 mg ml^{-1}) + ι-carrageenan (2 mg ml^{-1}). Fig. 3 shows the effect of pH and high pressure treatment on the elution profile in the absence of added NaCl. Chromatogram (a) for the untreated mixture and chromatogram (d) for the pressurized mixture at pH 8 both show a single peak at *ca.* 1.5×10^5 Da with no evidence of protein–polysaccharide complexation. Reducing the pH to 7.0 (Fig. 3(b)) and 6.5 (Fig. 3(c)) for the unpressurized samples leads to chromatograms with a strong second peak at shorter elution times corresponding to a substantial fraction of protein–polysaccharide complex(es) of molecular weight *ca.* 6×10^5 Da. Chromatograms for the pressure-treated samples at pH 7.0 (Fig. 3(e)) and pH 6.5 (Fig. 3(f)) show even stronger broad second peaks corresponding to very distinct protein–polysaccharide complex(es) of molecular weight *ca.* 6×10^5 Da, together with a now rather small peak corresponding to monomeric BSA. Experiments repeated in a pressure resistant buffer (bis-Tris HCl, pH 7, 20 mM) gave identical results to those performed in water, which suggests that the formation of the stronger complex(es) during high pressure processing was not simply due to the induced change in pH during the treatment. As with the BSA + κ-carrageenan systems, it again cannot be excluded that additional 'free' denatured protein becomes stuck to the complex during treatment.

To investigate further the character of the BSA-ι-carrageenan interactions, we consider the effect of adding 20 mM NaCl to the mixed system. Fig. 4(a) again presents the chromatogram of the native mixed system at pH 7 in the absence of NaCl. The elution profile shows the monomeric peak at *ca.* 6.6×10^4 Da with a second peak corresponding to protein–polysaccharide complex(es) of molecular weight *ca.* 6×10^5 Da. The net attraction at pH 7 is most probably due to interactions

between the highly charged backbone on the polysaccharide and patches of positively charged residues on the protein surface. Increasing the ionic strength by 20 mM (Fig. 4(b)) leads to the presence of just one main peak at *ca.* 6.6×10^4 Da corresponding to monomeric BSA. This change is consistent with the protein–polysaccharide complex being held together by electrostatic interactions; when simple salt ions are present to screen the local molecular charges, the complex dissociates.

The effect of higher salt concentrations on the mixed BSA + DS systems at pH 7.0 is shown in Fig. 5. Chromatogram (a) for the mixture treated at 600 MPa for 20 min in the absence of NaCl shows a strong narrow peak corresponding to high molecular weight protein–polysaccharide complex(es) of *ca.* 5×10^5 Da with a second very small peak corresponding to residual monomeric BSA. Chromatogram (c) for the pressurized mixture in the presence of 20 mM NaCl shows two distinct main peaks at *ca.* 5×10^5 Da and 6.6×10^4 Da which distinctly correspond to protein–polysaccharide complex(es) and BSA, respectively. Increasing the ionic strength to 100 mM NaCl (Fig. 5(b)) in the pressurized sample leads to a smaller peak at *ca.* 5×10^5 Da and a larger broader peak at *ca.* 6.6×10^4 Da, which suggests that the added salt induces reversible dissociation of some of the complexed BSA to monomeric and oligomeric forms. These data, and associated earlier work (Galazka et al., 1997b), suggest that, at higher ionic strengths, where the electrostatic BSA–DS interaction is screened, high pressure causes protein aggregation both in the absence and presence of the polysaccharide. Chromatogram (d) for the untreated BSA + DS mixture in the presence of 100 mM NaCl shows a very strong peak corresponding to monomeric BSA together with a minor high-molecular-weight peak, probably corresponding to some residual BSA–DS complex.

To investigate the nature of the protein-protein interactions induced in the mixed biopolymer systems by high pressure, DTT was added to various pressure-treated samples: BSA alone, and BSA + κ-or ι-carrageenan or DS (not shown). Addition of 5 mM DTT to pure BSA reduced the level of dimerization, supporting

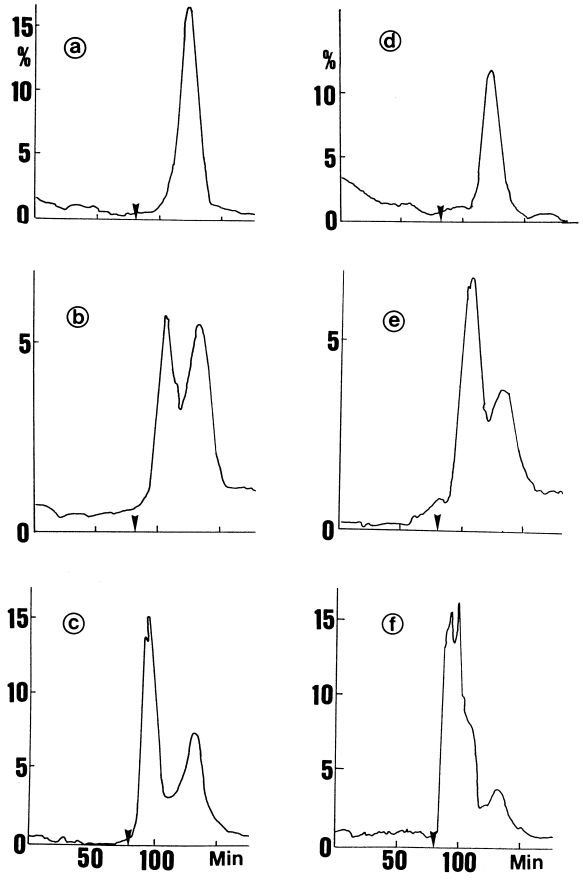

Fig. 3. Influence of high pressure and pH (6.5, 7.0 and 8.0) on size exclusion chromatograms of mixtures of BSA (5 mg ml^{-1}) + ι-carrageenan (2 mg ml^{-1}) in the absence of NaCl. The absorbance at 280 nm is plotted against the elution time: (a) untreated at pH 8; (b) untreated at pH 7; (c) untreated at pH 6.5 in 20 mM imidazole buffer; (d) pressure-treated (600 MPa for 20 min) at pH 8; (e) pressure-treated (600 MPa for 20 min) at pH 7; (f) pressure-treated (600 MPa for 20 min) at pH 6.5 in imidazole buffer. Arrow indicates position of the void volume.

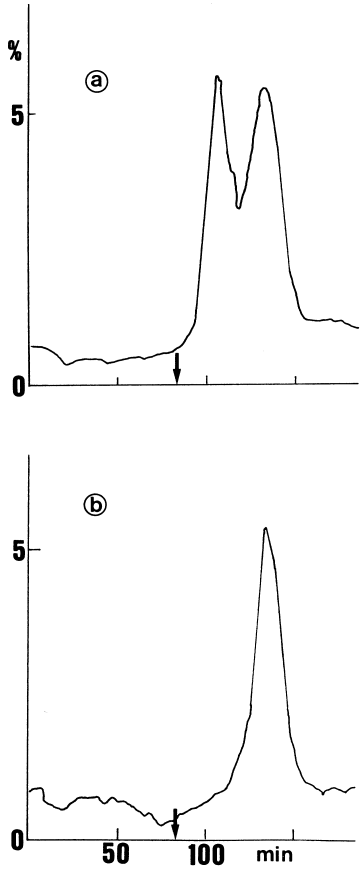

Fig. 4. Effect of ionic strength on size exclusion chromatography of mixture of BSA (5 mg ml^{-1}) + ι-carrageenan (2 mg ml^{-1}) at pH 7. The absorbance at 280 nm is plotted against the elution time: (a) absence of NaCl; (b) addition of 20 mM NaCl. Arrow indicates position of the void volume.

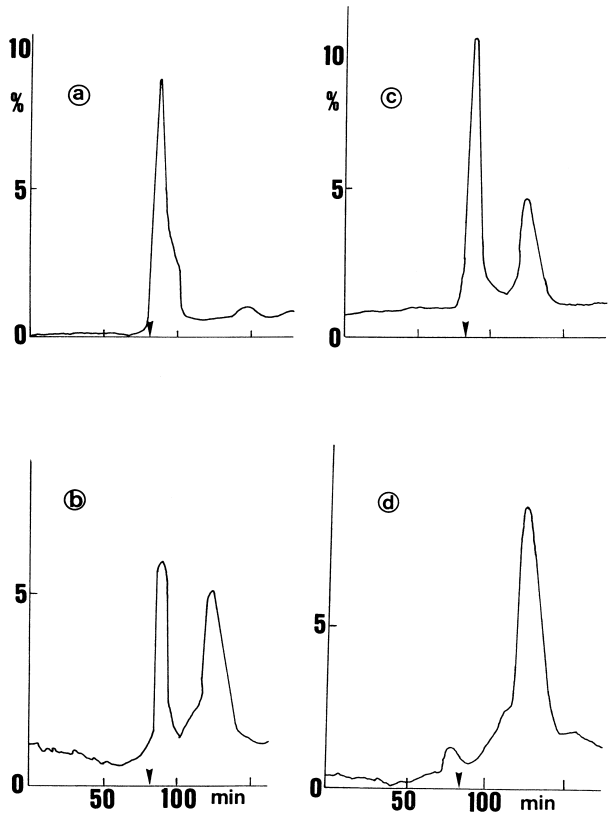

Fig. 5. Effect of salt concentration and high pressure processing on size exclusion chromatography of mixture of BSA (5 mg ml^{-1}) + DS (2 mg ml^{-1}) at pH 7. The absorbance at 280 nm is plotted against the elution time: (a) absence of NaCl and pressure-treated at 600 MPa for 20 min; (b) presence of 100 mM NaCl and treated at 600 MPa for 20 min; (c) presence of 20 mM NaCl and pressure treated at 600 MPa for 20 min; (d) untreated in presence of 100 mM NaCl. Arrow indicates position of the void volume.

the view that disulphide-mediated polymerization occurs during or after high pressure treatment. In similar experiments with the mixed biopolymers, however, the protein–polysaccharide complex(es) were unaffected. This supports the hypothesis that complexation with polysaccharide at low ionic strength protects the protein against aggregation caused by –S–S– bridging during high pressure treatment.

Based on the new experimental data presented in this paper and results from previous studies (Galazka et al., 1997b), it would seem that the application of pressure treatment probably leads to the dissociation of the electrostatic protein–polysaccharide complex. During maintenance of high pressure the globular protein becomes partly denatured and so it exposes more charged groups. Finally, during pressure release, attractive electrostatic BSA–polysaccharide interactions are reformed more strongly than ever, and recomplexation of the unfolded BSA with the polysaccharide occurs which then protects the denatured BSA against protein–protein aggregation. The mechanism involving

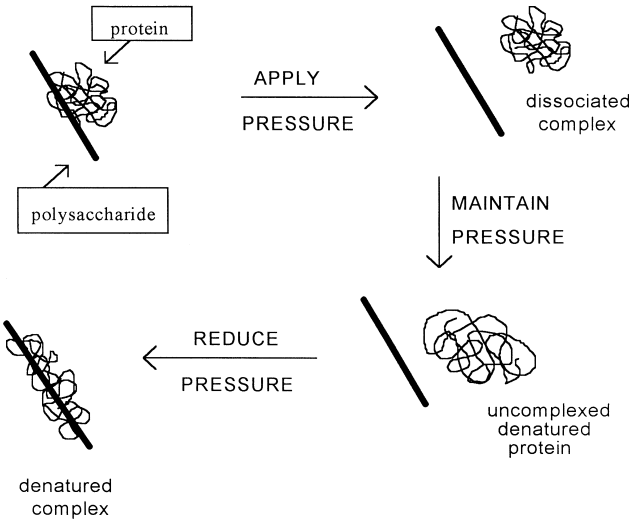

Fig. 6. Schematic representation of the effect of high pressure processing on the electrostatic protein-polysaccharide complex. Application of pressure leads to the dissociation of the biopolymers, the globular protein unfolds and exposes more charged groups during maintenance of pressure, and the complex of pressure-unfolded BSA with DS reforms during pressure release.

complex dissociation is illustrated diagrammatically in Fig. 6.

4. Conclusions

It has been clearly demonstrated that, like dextran sulphate, both ι- and κ-carrageenan can form an electrostatic complex with BSA at low ionic strength and pH$\leq$7.0. The interaction of BSA with the carrageenans considerably strengthens at pH 6.5, and the strength of protein–polysaccharide complexation appears to be related to the density of sulphate group on the polysaccharide ($\iota > \kappa$). It seems that complexation with anionic polysaccharide has the effect of protecting the globular protein against loss of functionality due to –S–S– bridge formation during or after high pressure treatment.

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