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Interactions between furcellaran and the globular proteins bovine serum albumin and β-lactoglobulin

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Abstract

The interaction between the algal polysaccharide furcellaran and the globular proteins, bovine serum albumin and β-lactoglobulin was examined as a function of pH using potentiometric and turbidimetric titration and photon correlation spectroscopy. On decreasing pH, the furcellaran first formed a soluble complex with the globular proteins at a pH_c, which showed a maximum in its dependence on ionic strength. On further decrease in pH, the onset of a more substantial aggregation, as indicated by a marked increase in turbidity occurred in the vicinity of the isoelectric point of the protein. Between these pH's the protein/furcellaran complex had a characteristic average size which was larger than the isolated furcellaran chain in solution. Complexation occurred when the protein carried an average net charge of the same sign as the furcellaran.

Keywords: Furcellaran; Lactoglobulin; Serum albumin; Complexation

1. Introduction

Protein/polysaccharide mixtures are widely encountered in nature and in industrial products, including manufactured foods. The resulting microstructures which form from these mixtures is influenced by the intermolecular interactions between biopolymers. Many such systems are far from equilibrium, with kinetic arrest having a major impact on microstructure.

Attractive interactions between positively charged peptides/proteins and anionic polysaccharides can lead to gelation (MacDougall et al., 2001), where the cationic fragment acts as a crosslink; coacervation (Turgeon, Beaulieu, Schmitt, & Sancez, 2003) where the attraction of opposite charges on the biopolymers leads to phase separation/precipitation of a protein/polysaccharide mixture (Weinbreck, de Vries, Schrooyen, & de Kruif, 2003), and multilayer formation where sequential deposition of oppositely charged polymers leads to surface crosslinking and successive charge reversal at the surface and the build up of a multilayer (Decher, 1997). Proteins and polysaccharides carrying charge of the same sign, can phase separate from concentrated solution with the formation of a protein-rich and a polysaccharide-rich phase (Dickinson, 2003). It is has been found that under some conditions the polyampholyte protein can form complexes with polycations and polyanions under conditions where the average charge on the protein has the same sign as the polycation or polyanion (Park, Muhoberac, Dubin, & Xia, 1992). This interaction has mainly been examined in mixtures of a protein with a synthetic polyelectrolyte (Hallberg & Dubin, 1998; Wen & Dubin, 1997). During the titration of a polyanion/protein mixture from a high pH, as the charge on the protein is reduced there is a transition, at pH_c, where complexation of the protein and polysaccharide occurs, with the formation of a soluble complex. This transition can be probed experimentally by a range of techniques including turbidimetry (Hattori, Kimura, Seyrek, & Dubin, 2001), dynamic light scattering (Li, Mattison, Dubin, Havel, & Edwards, 1996), and potentiometry (Wen & Dubin,

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1997). In this region, complexation behaviour is reversible. As the charge on the protein is further reduced and becomes opposite in sign, another transition is observed which signifies the onset of complex coacervation. Charge is not evenly distributed over the protein surface. While the net charge on the protein may be of the same sign as that of the polyelectrolyte, oppositely charged 'patches' on the protein surface may help stabilise complex formation. The ionic strength dependence of complexation also points to the involvement of electrostatic interactions in complex formation. At low ionic strength, long range repulsive interactions between the polyampholyte and polyelectrolyte dominate. As the ionic strength increases, these interactions become progressively screened and the attractive interaction between charge patches on the polyampholyte and the polyelectrolyte become significant (Seyrek, Dubin, Tribet, & Gamble, 2003). An important entropic driving force for complex formation is the release of counterions 'associated' with the charged regions on polyampholyte and polyelectrolyte on complexation (Mascotti & Lohman, 1990; Record, Lohman, & deHaseth, 1976). Further increase in ionic strength reduces the strength of the attractive interaction (Skepo & Linse, 2002) and the entropic effect favouring complexation. A combination of these effects produces the observed maximum in the extent of complexation as a function of ionic strength. The complex may be further stabilised through other intermolecular forces. For example, in the case of the complexation of proteins and synthetic polyelectrolytes it is suggested that hydrophobic effects could also play a role (Hallberg & Dubin, 1998).

Much of the earlier research on these interactions focused on the behaviour of synthetic polyelectrolytes. One advantage in their study is that polyelectrolyte characteristics such as charge, charge density and its distribution, can be relatively well defined and controlled. These effects also play an important role in the behaviour of biopolymer systems, such as synovial fluid (Moss, VanDamme, Murphy, & Preston, 1997); the DNA histone complex (Luger, Mader, Richmond, Sargent, & Richmond, 1997); the interaction between prion proteins and sulphated polysaccharides (Brimacombe et al., 1999); and the behaviour of proteins at the interface in the bulk and at the surface in fabricated biopolymeric materials. Many complex foods consist of protein/polysaccharide mixtures. Food polysaccharides include both weak (pectin, alginate) and strong polyelectrolytes (carrageenan), having carboxylate and sulphate groups, respectively. Recent studies have examined the complexation of whey proteins with carrageenan (Weinbreck, Nieuwenhuijse, Robijn, & de Kruif, 2004). As part of our continuing research on the behaviour of polysaccharides in complex foods we examine the interaction between a strong polyelectrolyte of the carrageenan family (furcellaran) and the globular proteins bovine serum albumin (BSA) and β-lactoglobulin (BLG).

Furcellaran is a sulphated galactan which can be extracted from the seaweed *Furcellaria lumbricalis* (Lahaye, 2001; Truus, Vaher, Usov, Pehk, & Kollist, 1997). Sugar and methylation analysis have shown that it consists of residues of 3-linked β-D-galactopyranose and its 4-sulphate and 4-linked α-D-galactopyranose. The latter residues may exist as a 3,6-anhydro residue which may be partially sulphated at position 2. Furcellaran preparations may also contain small amounts of other sugar residues (Truus et al., 1997). Structurally, furcellaran is related to the algal polysaccharide κ carrageenan, with a major structural difference being that furcellaran has a smaller degree of sulphation (Lahaye, 2001). A characteristic of these polysaccharides is their ability to form gels in the presence of specific ions, associated with a molecular conformational change from coil to helix (Lahaye, 2001). They also have the potential to interact with oppositely charged macroions, including globular proteins. It is this latter aspect which is the focus of the current study.

2. Materials and methods

2.1. Source of materials

Furcellaria lumbricalis was collected in Kassari Bay (the Baltic Sea, Estonia) and cleaned of epiphytes. The algae were washed thoroughly with tap water and the galactans were extracted according to the method of Amimi, Mouradi, Givernaud, Chiadmi, and Lahaye (2001), using deionized-water (1.5 L) under agitation for 3 h at 95 °C. The aqueous phase was clarified by passing through a nylon cloth and then precipitated in 2.5 vol. of isopropanol, filtered and dried at room temperature. The yield of furcellaran was 37.8% w/w. Three grams of furcellaran were dissolved in 2 L milliQ water at ~100 °C. The solution was passed through diatomaceous earth (Sigma–Aldrich Co, Ltd., UK) and dialyzed. The Na^+ furcellaran was obtained by elution through an ion-exchange (Amberlite IR-120) column in the Na^+ form at 4 °C. The eluant was freeze-dried. The behaviour of this laboratory-prepared furcellaran was similar to that of a commercial sample obtained from FMC Food Ingredients. Bovine serum albumin and β-lactoglobulin, were obtained from Sigma–Aldrich Co, Ltd., UK, and used without further purification.

2.2. Characterization of furcellarans

For the determination of monosaccharide composition, the furcellaran was hydrolysed by a double-hydrolysis-reduction method as described by Stevenson and Furneaux (1991). The alditols were converted to their acetates and analysed by GC/MS as described. (Harris, Blakeney, Henry, & Stone, 1988) The sulphate content was measured colorimetrically as described by Silvestri, Hurst, Simpson, and Settine (1982). For ^{13}C NMR spectroscopy, the polysaccharide samples were dissolved in D_2O (5% w/v) and spectra were recorded in a JEOL EX spectrometer at 85 °C. Chemical shifts were calculated with reference to the C-6 signal from the galactose residue at 61.3 ppm.

Measurements of specific viscosity as a function of concentration of furcellaran (0.025–0.2% w/w) in 0.1 M NaCl were carried out using an Ubbelohde viscometer at 20 °C. The efflux time for 0.1 M NaCl was 143.7 s and no shear rate corrections were made. The instrinsic viscosity [η] was calculated from the extrapolation of specific viscosity to zero concentration.

2.3. Potentiometric and turbidimetric titration

pH titrations were carried out with a Jenway pH meter equipped with a combination electrode, under N_2, at room temperature. A microburette was used to add 0.1 M HCl to 15 mL of a 3 g/L protein solution in 0.03 M NaCl with or without 0.6 g/L furcellaran. Prior to titration the pH was adjusted to 9.0 with dilute NaOH, the alkali was added slowly, through narrow bore capillary tubing, with a microburette to the stirred protein-containing solution. pH values were recorded when the change in pH with time was <0.01 pH units per minute. For turbidimetric titration, solutions of 3.0 g/L of protein and 0.6 g/L of furcellaran were prepared in 0.03 M NaCl. The initial pH was adjusted to 10.0 for BSA and 9.0 for BLG. The solution was titrated with 0.1 M HCl as described above. Changes in turbidity were monitored at 600 nm (Perkin-Elmer Lambda 15 UV/Vis Spectrophotometer, Beaconsfield, Bucks) and reported as 100 – %T. The net charge of protein was determined by titrating a 15 mL of a protein solution (3 g/L) in 0.03 M NaCl from the reported p*I*, either with 0.1 M HCl or with 0.1 M NaOH. A solvent blank was used to determine the direct contribution of HCl or NaOH to the solution pH. The protein titration data were in good agreement with published research (Cannan, Palmer, & Kibrick, 1942; Tanford, Swanson, & Shore, 1955). All experiments were performed in triplicate

2.4. Photon correlation spectroscopy

The apparatus employed was an ALV/SP-86 spectrogoniometer (ALV, Langen, Germany) equipped with a Coherent Radiation Innova 100-10 Vis Argon Ion laser operating at 0.5 W and wavelength of 514 nm. Furcellaran (1.2 g/L) and protein solution (6 g/L), at pH 9.0, were filtered through a 0.2 μm Millipore filter. The biopolymer solutions were weighed into a washed cuvette, mixed (to give concentrations of furcelleran and protein of 0.6 and 3 g/L, respectively), and the pH of the mixture adjusted through the addition of dilute HCl. After the experiment the pH was measured.

The scattered light intensity was monitored using an ALV/PM-15 ODSIII detection system at a fixed scattering angle of 90°. After amplification and discrimination, signals were directed to an ALV/5000E digital multiple-τ correlator and time–intensity correlation functions recorded, typically for 600 s duration. Size distribution functions were computed using the appropriate Windows-based ALV software which incorporated regularised inverse Laplace transform and ALV-CONTIN packages. Additional analysis was undertaken using Origin V6 (Microcal) proprietory software. All experiments were performed in triplicate.

3. Results and discussion

3.1. Furcellaran characterisation

The monosaccharide composition of the furcellaran preparation is shown in Table 1, and includes galactose; 3,6 anhydro galactose; and smaller amounts of 6-*O*-methyl galactose. The composition is similar to that obtained in a previous study and indicates that the polysaccharide structure may be somewhat more complex than that represented by a simple repeating unit (Truus et al., 1997). The sulphate content of 14.9% w/w indicates that there is on average one charge for every 2.8 monomer residues, which is in broad agreement with commonly accepted value of one charge for every three residues (Lahaye, 2001). The intrinsic viscosity of 507 mL g^{-1} indicates a relatively large molecular size in 0.1 M NaCl. The ^{13}C NMR spectra of the polysaccharide is shown in Fig. 1. The chemical shift assignments (Truus et al., 1997) indicate the presence of (1 → 3) linked β-D-galactopyranose; (1 → 4) linked 3,6

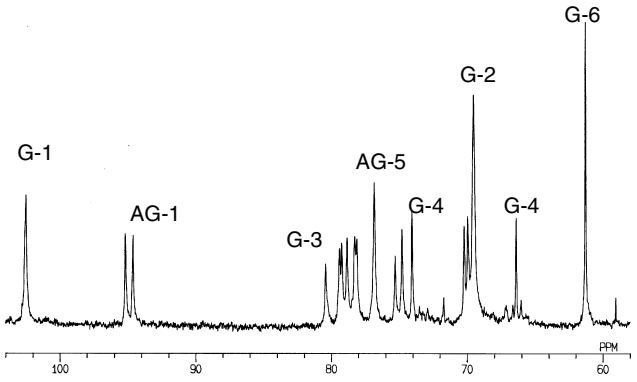

Fig. 1. ^{13}C NMR of 5% (w/v) furcellaran in D_2O at 85 °C, where AG is anydrogalactose and G galactose.

Table 1
Characteristics of the furcellaran

Monosaccharide composition (% w/w carbohydrate) (Mole ratio)						SO_3^- (% w/w)	[η] (ml/g)
Gal	3,6-AGal	6-*O*-MeGal	Gluc	Xyl	Man		
59 (0.56)	32 (0.34)	5 (0.044)	2 (0.019)	2 (0.023)	1 (0.009)	14.9	507

anhydro-α-D-galactopyranose; and (1 → 3) linked β-D-galactopyranose 4-sulphate.

3.2. Turbidimetric titration

The interaction between furcellaran and the globular proteins bovine serum albumin (BSA) and β-lactoglobulin (BLG) was examined by turbidimetric titration. The change in turbidity with decreasing pH for a furcellaran solution (0.6 g/L) containing either 3 g/L of BSA or BLG is shown in Fig. 2a and b, respectively. At high pH's the turbidity is essentially independent of pH. As pH is decreased the turbidity starts to show a weak dependence on pH which occurs at a pH_c. As pH is further decreased there is a marked increase in turbidity over a narrow pH range. In previous research on polyelectrolyte/protein complexes (Hattori et al., 2001; Seyrek et al., 2003) it was shown that pH_c is associated with the formation of a soluble polyelectrolyte/protein complex, and the more marked change in turbidity is associated with precipitation and complex coacervation of the polyelectrolyte with the protein. For the mixtures shown, in 0.03 M NaCl, pH_c occurs in the region of 7.5 for both BLG and BSA. At this pH, the average net charge on the protein molecule is ~−13 (Cannan et al., 1942; Fogolari et al., 2000; Tanford et al., 1955). The onset of a more marked change in turbidity, associated with the formation of larger aggregates, occurs in the region of pH 5.0 and 5.3 for BSA and BLG, respectively. As the isoionic point of the isolated proteins is ~5.3 for defatted BSA (Tanford et al., 1955) and ~5.1 for BLG (Cannan et al., 1942) this change in behaviour is occurring in the region of the isoelectric point. The more substantial aggregation and turbidity increase at lower pH's is occurring when the protein is expected to have a net positive charge (Cannan et al., 1942; Tanford et al., 1955).

At pH > 6.0 the turbidity is stable over minutes and the turbidity increase can be reversed by raising the pH. If the pH is lowered to pH 4.0, and then raised again, then for both BSA and BLG the turbidity increase is substantially reversible (>95%) indicating that the more substantial aggregation observed at pH's below the isoelectric point also involves a limited kinetic arrest. The values of pH_c showed a dependence on ionic strength as shown in Fig. 3. In dilute salt solutions in the range 0.01–0.075 M, the pH_c transition is shifted to higher pH's as the ionic strength increases and then decreases on further increase in ionic strength for both BLG and BSA. This ionic strength dependence is a generally observed feature for the complexation of polyelectrolytes and proteins (Seyrek et al., 2003). Over the range of ionic strength examined the net charge on the protein at pH_c ranges from ~−9 to ~−15 (Cannan et al., 1942; Fogolari et al., 2000; Tanford et al., 1955). At each ionic strength, the charge on the proteins, BSA and BLG, at pH_c is comparable. Based on previous research on polyelectrolyte/protein complexation, the dependence of turbidity on pH in these mixtures indicates that there is complexation between furcellaran and the globular proteins at pH's between pH_c and the isoelectric point of the protein. Below the isoelectric point of the protein there is a more marked aggregation as indicated by the substantial increase in turbidity of the mixtures.

The development of turbidity as a function of furcellaran concentration was probed at pH 4.0 and 7.0 for both BSA (Fig. 4a) and BLG (Fig. 4b) in 0.03 M NaCl at a fixed protein concentration of 1 g/L. At pH 7.0 for both BSA and BLG there is a slow increase in turbidity with increasing furcellaran concentration consistent with complex formation. At pH 4.0 there is a more marked increase with increasing furcellaran concentration. At pH 4.0 the BSA molecule carries a net positive charge of 26, and the BLG monomer a charge of 15. From the known molecular masses of BSA and the

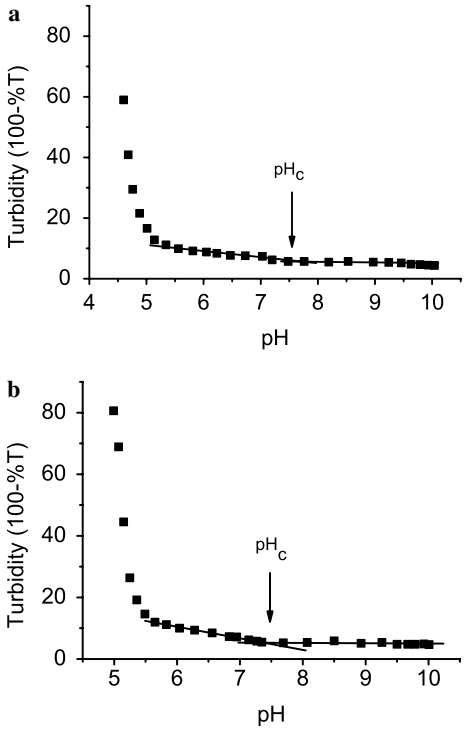

Fig. 2. (a) Turbidimetric titration of furcellaran (0.6 g/L) BSA (3 g/L) mixture in 0.03 M NaCl at 20 °C. (b) Turbidimetric titration of furcellaran (0.6 g/L) BLG (3 g/L) mixture in 0.03 M NaCl at 20 °C.

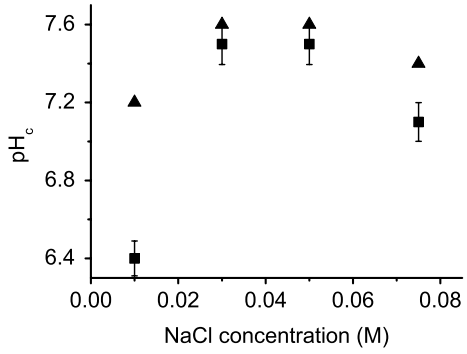

Fig. 3. Variation of pH_c with salt concentration for furcellaran/BSA (▲) and furcellaran/BLG (■) mixtures at 20 °C.

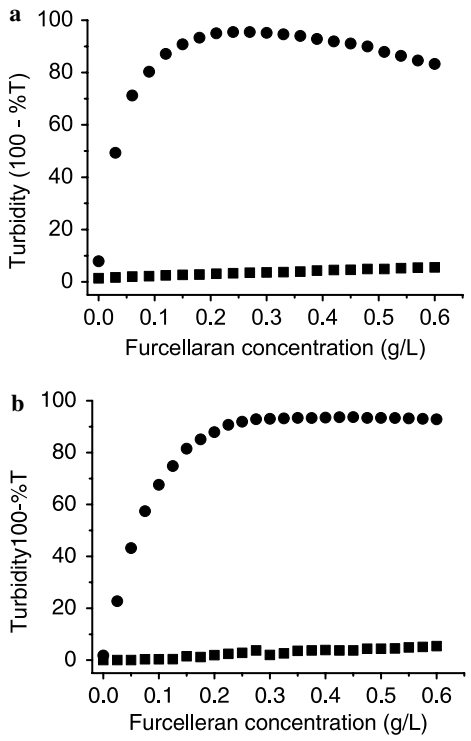

Fig. 4. (a) Development of turbidity as a function of furcellaran concentration for a furcellaran/BSA mixture at a BSA concentration of 1 g/L at pH 4 (●), and pH 7(■) at 20 °C in 0.03 M NaCl. (b) Development of turbidity as a function of furcellaran concentration for a furcellaran/BLG mixture at a BLG concentration of 1 g/L at pH 4 (●), and pH 7 (■) at 20 °C in 0.03 M NaCl.

BLG monomer (66 and 18.3 kD, respectively), and the measured sulphate content of furcellaran, charge balance would occur at furcellaran concentrations of ~0.22 and ~0.48 g/L for BSA and BLG, respectively. The observed maximum in turbidity as a function of furcellaran concentration, which is observed for BSA, occurs in the region of charge balance. For BLG there is a broad plateau in the expected region of charge balance. For the interaction of synthetic polyelectrolytes with globular proteins, similar behaviour is observed (Wen & Dubin, 1997). At pH's where the polyelectrolyte and protein carry a net charge opposite in sign, the observed maximum in turbidity as a function of polyelectrolyte concentration can be much sharper (Wen & Dubin, 1997). Indeed at higher concentrations of polyelectrolyte the turbidity falls markedly as the aggregates redisperse. One origin of these differences in behaviour with furcellaran is that the synthetic polyelectrolytes have a relatively high charge density per mass of polymer compared to furcellaran.

The extent of aggregation of polymers in the pH range below pH_c was further investigated by photon correlation spectroscopy.

3.3. Photon correlation spectroscopy

Photon correlation spectroscopy was used to further characterise the interaction between furcellaran and the globular proteins, BSA and BLG, through the determination of an effective particle size from measurements of a translational diffusion coefficient D_t. For a particle in solution subject to Brownian motion, the translational diffusion coefficient is related to the measured scattered light intensity correlation function $g^{(2)}\tau$ by the expression

$$g^{(2)}\tau = 1 + \exp(-2D_t k_s^2 \tau), \quad (1)$$

where k_s, the scattering vector, is given by

$$k_s = \frac{4\pi n}{\lambda} \sin\frac{\theta}{2}, \quad (2)$$

where n is the refractive index of the solution, θ, the scattering angle and λ the wavelength of light. For the diffusion of particles in dilute solution, the hydrodynamic radius of the particle, R_h, may be obtained from

$$D_t = kT/6\pi\eta R_h, \quad (3)$$

where, η, is the solvent viscosity and k and T have their usual meanings.

The published X-ray crystal structure of serum albumin indicates a heart-shaped molecule which can be approximated to an equilateral triangular prism with sides of 8 nm and a depth of 3 nm (Carter & Ho, 1994). Under conditions of neutral pH BSA has an axial ratio of approximately 2.66. The conformation of BSA shows a pH dependence (Carter & Ho, 1994), with the native form being found between pH's 4 and 8. Size exclusion chromatography of BSA solution showed that it was largely unaggregated (>93%). For a 0.6% w/w solution of BSA in 30 mM sodium chloride at pH 5.4 and 20 °C, the measured diffusion coefficient was 5.7×10^{-11} m^2s^{-1}. The measured value of D_t is in good agreement with published research (Gaigalas, Hubbard, McCurley, & Woo, 1992; Meechai, Jamieson, & Blackwell, 1999), with a calculated hydrodynamic radius of 4.2 nm.

The conformation of BLG is pH dependent (Sawyer, Kontopidis, & Wu, 1999; Verheul, Pedersen, Roefs, & de Kruif, 1999). The monomeric unit has a molecular mass of 18.3 kDa. At pH 3.5 the protein dimerizes. This dimerization is dependent on ionic strength and protein concentration with higher ionic strength and concentrations stabilising the dimer to repulsive interactions at lower pH's (Aymard, Durand, & Nicolai, 1996; Sakurai, Oobatake, & Goto, 2001). At pH 4.6 the dimers may further aggregate to form an octamer. At pH 7.5 the protein conformation undergoes a reversible transition which leads to a swelling of the monomeric unit (Qin et al., 1998; Tanford, Bunville, & Nozaki, 1959). There are also some reports that above pH 7.5 the dimers may dissociate into monomeric units (Witz, Timasheff, & Luzzati, 1964). At higher pH's there is the potential for a time-dependent irreversible aggregation. At pH 7.9 the measured value of D_t for a 0.6% w/w BLG solution in 0.03 M NaCl was 7.5×10^{-11} m^2s^{-1}, in agreement with previous measurements carried out under similar conditions (Beretta, Chirico, & Baldini, 2000; Le Bon, Nicolai, Kuil, & Hol-

lander, 1999; Phillies, Benedek, & Mazer, 1976), with a calculated hydrodynamic radius of 3.2 nm. As the pH was reduced the presence of a slower diffusive process in the autocorrelation function was observed, which was attributed to a limited aggregation, in agreement with published research (Takata, Norisuye, Tanaka, & Shibayama, 2000). At pH's of 5.5 and 4.2 a more substantial aggregation was noted, related to the phase separation/precipitation of BLG from aqueous solution.

In dilute NaCl solution, algal galactans of the κ-carrageenan family exist as expanded flexible coils. In the presence of NaI a coil to helix transition is observed, and the presence of K^+ ions can additionally lead to the aggregation of helices. The D_t of furcellaran in 0.03 M NaCl solution (0.6% w/w) at pH 7.4 was $5.2 \times 10^{-12}\,m^2s^{-1}$, and similar to ($6.9 \times 10^{-12}\,m^2s^{-1}$) a κ-carrageenan preparation in 0.1 M NaCl, with a weight average molecular weight of 421 kDa (Meunier, Nicolai, & Durand, 2001).

Photon correlation spectroscopy of mixtures of furcellaran and BSA or BLG, at pH's above pH_c showed the presence of two diffusive processes corresponding to the translational diffusion of the furcellaran macromolecule and the globular protein. As the pH was lowered below pH_c there was a marked reduction in the slower process. The faster process had diffusion coefficients comparable to that of the globular protein in solution, although determination of D_t was complicated due to the polydisperse nature of the system. For both BSA and BLG mixtures with furcellaran, the hydrodynamic radius, R_h, of the components was calculated from D_t. The dependence of R_h on pH is shown in Fig. 5a and b for BSA/furcellaran and BLG/furcellaran mixtures, respectively. Above the pH_c determined by turbidimetric titration, the hydrodynamic radius of the polymers in the mixture is comparable to that of the pure polymer in solution under the same conditions. Below pH_c there is a marked stepped increase in the hydrodynamic radius of the furcellaran component. Although the total intensity of the scattered light continues to increase with decreasing pH, at pH's between pH_c and the pH at which the onset of coacervation/precipitation is observed, the hydrodynamic radius of the furcellaran component does not show a strong pH dependence. Above pH_c the furcellaran in solution can be approximated to an equivalent sphere with an R_h, ~32 nm. In the region below pH_c, where it is proposed that the furcellaran forms a complex with the globular protein, this size had increased to ~135 nm for the furcellaran/BLG mixture, and ~115 nm for the furcellaran/BSA mixture. Various models could be considered for the structure of the complex. If the globular protein could bind to more than one furcellaran chain it is possible that it could act as a crosslink. This crosslinking process would lead to the formation of large aggregates. The relatively well defined size of the BLG and BSA furcellaran complexes over a range of pH's argues against this proposal. An alternative view is that the globular proteins 'decorate' the furcellaran chain. Polysaccharides in general are fairly stiff molecules with a relatively long persistence length, it is

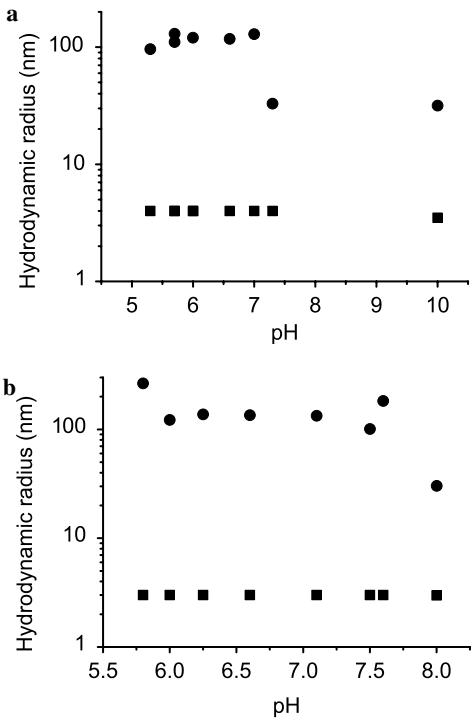

Fig. 5. (a) Plot of hydrodynamic radius (R_h) versus pH for a mixture of BSA (3 g/L) and furcelleran (0.6 g/L) where (■) is the R_h of the faster component and (●) the R_h of the slower component. (b) Plot of hydrodynamic radius versus pH for a mixture of BLG (3 g/L) and furcellaran (6 g/L) where (■) is the R_h of the faster component and (●) the R_h of the slower component (●).

therefore unlikely that the furcellaran chain can completely wrap around the globular protein but rather that part of the globular protein surface can interact with the polysaccharide chain and that the polysaccharide chain becomes decorated with globular protein. This interaction then results in an effective increase in size of the equivalent sphere which represents the behaviour of the complex in solution. The related increase in size could result from a more expanded/extended conformation, or a denser, less free-draining, conformation of the complex compared to the furcellaran chain in solution. The maintenance of particle size below pH_c while the intensity of scattered light is increasing, suggests competition between different processes and a progressive complexation with decreasing pH in this region. For polyelectrolyte/protein complexation both an increase (Ball et al., 2002; Grymonpre, Staggemeier, Dubin, & Mattison, 2001) and a decrease (Weinbreck et al., 2003) in particle size of the complex relative to that of the polyelectrolyte have been observed. The origins of these differences in behaviour are not clear.

3.4. Potentiometric titration

The interaction between the globular protein and the furcellaran was also probed by potentiometric titration. In Fig. 6 is shown the measured pH as a function of added HCl over the pH range 9–4 for BSA (a) and BLG (b) in

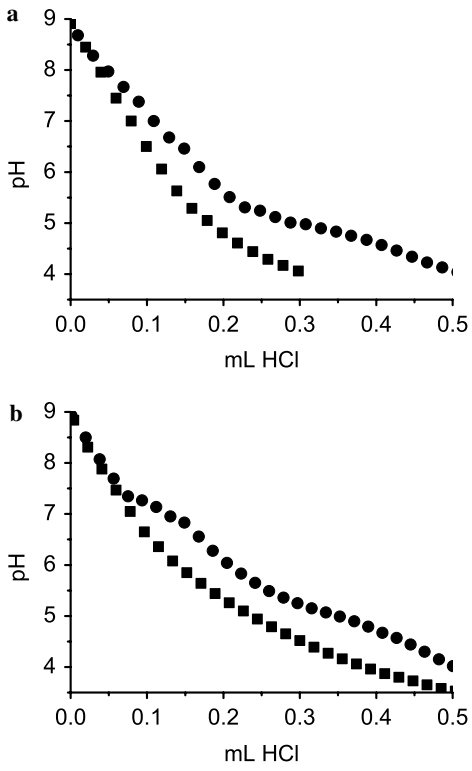

Fig. 6. (a) Potentiometric titration of 15 mL of BSA (3 g/L) (■); and 15 mL of a BSA/furcellaran mixture (3 and 0.6 g/L) (●) in 0.03 M NaCl at 20 °C. (b) Potentiometric titration of 15 mL of BLG (3 g/L) (■); and 15 mL of a BLG/furcellaran mixture (3 and 0.6 g/L) (●) in 0.03 M NaCl at 20 °C.

0.03 M NaCl. The titration curves for the globular proteins are in agreement with published research (Cannan et al., 1942; Tanford et al., 1955). For the furcellaran/BSA mixture there is a gradual divergence in the titration curve at a pH in the region of ~7.5–8.0. For BLG there is a more marked divergence at pH ~ 7.5. For both proteins there is another change in the titration behaviour in the region of pH 5.0–6.0, associated with a marked increase in turbidity on precipitation. In previous research (Wen & Dubin, 1997), the transition at the higher pH was associated with complex formation at pH_c. It was proposed that the binding of charged groups of the polyelectrolyte to proteins results in changes in local environment of the charged residues on the protein and in the pK_a of the titrable groups of the protein (Mattison, Brittain, & Dubin, 1995). This is consistent with ligand binding studies on proteins. For example, the binding of the phosphate ion to ribonuclease A increases the basicity of some of the histidine residues (Antosiewicz, Mccammon, & Gilson, 1996). The values of pH_c obtained by turbidimetric and potentiometric titration differ somewhat, perhaps as a result of their different physicochemical origins.

4. Conclusions

It has been shown that the algal polysaccharide furcellaran can form soluble complexes with the globular proteins, bovine serum albumin and β-lactoglobulin. The complexation occurs at a pH where the protein carries a net negative charge. With increasing ionic strength, as the charge on the polymers becomes screened, the attractive interaction between the negatively charged furcellaran and positively charged patches on the protein becomes more favoured. Further increase in ionic strength then reduces the strength of this interaction. The observed ionic strength dependence is consistent with observation and indicates that the release of counterions associated with the protein on complexation, is an important driving force for complex formation. The onset of complex coacervation/precipitation occurs in the region of the isoelectric point of the protein and becomes increasingly favoured as the protein acquires a net positive charge, opposite in sign to that of the furcellaran.

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