Composition and Rheological Properties of β-Lactoglobulin/Pectin Coacervates: Effects of Salt Concentration and Initial Protein/Polysaccharide Ratio

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The composition and rheological properties of β-lactoglobulin/pectin coacervates have shown significant correlations with sodium chloride concentration (C_{NaCl}) and initial protein/polysaccharide ratio (r). An increase of C_{NaCl} from 0.01 to 0.21 M at $r = 5:1$ leads to the increase in both β-lactoglobulin and pectin contents in the coacervates, which can be explained in terms of salt-enhanced effect at lower salt concentrations. Further increase of C_{NaCl} from 0.21 to 0.41 M decreases the proportions of these two biopolymers in the coacervates, exhibiting salt-reduced effect at higher salt concentrations. Moreover, the stronger self-aggregation of β-lactoglobulin with increasing salt concentration gives rise to a decreasing actual protein/polysaccharide ratio in the coacervates at 0.01−0.21 M C_{NaCl} and $r = 5:1$. An increase of r from 5:1 to 40:1 often increases the actual amount of pectin chains in β-lactoglobulin/pectin coacervates, but it exhibits a maximum in β-lactoglobulin content at $r = 20:1$. A much higher storage modulus (G') than loss modulus (G'') for all β-lactoglobulin/pectin coacervates suggests the formation of highly interconnected gel-like structure. The values of G' increase as C_{NaCl} increases from 0.01 to 0.21 M, whereas a further increase of C_{NaCl} from 0.21 to 0.41 M causes G' values to decrease to much lower values. These results further disclose the salt-enhanced effect and the salt-reduced effect at low and high salt concentrations, respectively. On the other hand, increasing r from 5:1 to 40:1 favors the formation of stronger gel-like β-lactoglobulin/pectin coacervates, which mainly originates from the higher actual amount of pectin chains in β-lactoglobulin/pectin coacervates at higher r values.

Introduction

Knowledge of biopolymer interactions has important values for diverse phenomena, from biological systems like organization of living cells[1] to industrial applications such as microencapsulation,[2] protein separation and purification,[3] and processed foods.[4] Protein molecules may interact with polysaccharide chains to generate coacervation, in which a protein/polysaccharide mixed solution separates into one dense phase called coacervate and one relatively dilute macromolecular liquid phase called supernatant.[5] The general picture for coacervation between protein and anionic polysaccharide is that the primary soluble complexes are initially formed from the binding of protein molecules on polysaccharide chains at the first critical pH (pH_c), and then soluble protein/polysaccharide complexes start to aggregate into insoluble protein/polysaccharide complexes at the second critical pH ($pH_{\phi 1}$), which ultimately sediment into the dense coacervate phase.[6,7] When pH decreases down to the third critical pH ($pH_{\phi 2}$), protein/polysaccharide coacervates can dissociate into soluble complexes, even uninteracted protein molecules and polysaccharide chains.[8] Because the coacervates formed by proteins and oppositely charged polysaccharides are mainly driven by the long-range character of the electrostatic interaction, physicochemical parameters, such as pH, ionic strength, polysaccharide linear charge density, protein surface charge density, rigidity of the polysaccharide chain, and protein/polysaccharide ratio, have been demonstrated to strongly influence the formation of protein/polysaccharide coacervates.[9]

Generally speaking, the addition of salt weakens the formation of protein/polysaccharide coacervates.[10] For the system of protein with anionic polysaccharide, the critical pH values pH_c and $pH_{\phi 1}$, corresponding to the onset of soluble and insoluble complex formation, respectively, decrease with increasing salt concentration. The added salt could reduce the $pH_{\phi 1}-pH_{\phi 2}$ range for protein/polysaccharide coacervates, and the addition of excess salt may completely suppress the formation of protein/polysaccharide coacervates. Furthermore, the addition of salt also significantly influences the structure of protein/polysaccharide coacervates. Usually, a more watery and heterogeneous coacervate structure is observed at higher salt concentration.[11,12] The salt-induced less-structured protein/polysaccharide coacervates are generally explained as that the addition of microions screens the charges of protein molecules and polysaccharide chains, and therefore decreases the complex formation. However, different from the salt screening effect, some authors also noted that the added salt could enhance the formation of protein/polysaccharide complexes at certain salt concentrations,[7,13] as indicated by the increase of either pH_c or $pH_{\phi 1}$. In the micelle/polymer systems with similar electrostatic nature, it has been recently reported that the addition of salt can either enhance or reduce the formation of micelle/polymer complexes at specific salt concentrations.[14−16] The initial protein/polysaccharide ratio is another important factor in protein/polysaccharide coacervation. The values of pH_c were reported to be independent of the initial protein/polysaccharide ratio, whereas $pH_{\phi 1}$ values first increased and then stabilized with increasing initial protein/polysaccharide ratio for the coacervation between protein and anionic polysaccharide.[17] This result suggests that the soluble complexes originate from the electrostatic interaction between protein and polysaccharide, and the phase separation is induced by the aggregation of the electrostatically neutralized soluble complexes. Although the higher initial protein/polysaccharide

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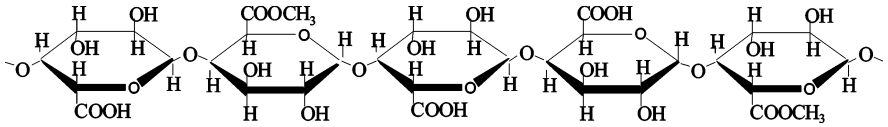

Figure 1. The typical chemical structure of pectin.

ratio often results in the higher actual protein/polysaccharide ratio in the coacervates,[11] the protein and polysaccharide contents in the coacervates also rely on other parameters such as pH and polymer molecular weight.[11,12] Thus, systematic works are still needed to further disclose the relationship between physicochemical parameters, such as salt concentration and initial protein/polysaccharide ratio, and the structure of protein/polysaccharide coacervates, because the assembly of protein molecules and polysaccharide chains in the coacervates remains quite unclear.[18,19]

Some authors have found that protein/polysaccharide coacervates were dense and structured,[11] vescicular to sponge-like, exhibiting numerous spherical inclusions of water.[20,21] By using small-angle neutron scattering (SANS), Huang et al. have demonstrated that the self-aggregation of β-lactoglobulin could significantly affect the microstructure of β-lactoglobulin/pectin coacervates.[22] The high self-aggregation ability of β-lactoglobulin promotes the formation of protein domains in β-lactoglobulin/pectin coacervates. In the present work, we continue to study the structure of β-lactoglobulin/pectin coacervates using a combination of composition analysis and dynamic rheological measurements. β-Lactoglobulin is a model globular protein with a well-known structure.[23,24] Pectin is an anionic polysaccharide[25,26] with the typical chemical structure shown in Figure 1. β-Lactoglobulin/pectin coacervates are prepared at various sodium chloride concentrations and different initial protein/polysaccharide ratios. The aim of this paper is to investigate the impacts of salt concentration and initial protein/polysaccharide ratio on the final composition and rheological properties of β-lactoglobulin/pectin coacervates.

Experimental Section

Materials. β-Lactoglobulin (lot JE 003-3-922) was obtained from Davisco Foods International, Inc. (Le Sueur, MN), and used without further purification. The powder composition was (g/100 g of powder): 5.2% moisture, 92.0% protein, 0.3% fat, and 2.5% ash. Pectin with 31% esterification obtained from Danisco A/S, Denmark, was purified by dialysis (Spectra/Por dialysis membrane with molecular weight cutoff equal to 12 000), followed by freeze-drying. The average molecular weight (M_n) of the purified pectin was about 7.0×10^5, as determined by gel permeation chromatography. Sodium chloride (NaCl, purity >99%) and standard hydrochloric acid (HCl, 0.5 N) were purchased from Fisher Scientific (Pittsburgh, PA). Milli-Q water was used in all experiments.

Preparation of β-Lactoglobulin/Pectin Coacervates. The stock solutions of β-lactoglobulin and pectin were mixed together with defined external addition of sodium chloride and initial protein/polysaccharide ratio (r). The final pectin concentration is fixed at 0.5 wt % for all β-lactoglobulin/pectin mixtures. Because the pectin molecules used have been dialyzed against DI water, they contain a negligible amount of metal ions. However, the metal ion (i.e., ~0.9% Na^+) existing in β-lactoglobulin may not be neglected. In this paper, we use the sodium chloride concentration (C_{NaCl}), which includes both the external added sodium chloride and the contribution of sodium chloride from β-lactoglobulin, to denote the total salt content in the starting β-lactoglobulin/pectin mixed solution. 0.5 N standard HCl solution was used to adjust the pH of the mixtures to 4.0. After acidification, the coacervates were collected after removal of the supernatant through centrifugation at 3000 rpm for 30 min. The yield of β-lactoglobulin/pectin coacervates varies from 3% to 7%. It should be pointed out that C_{NaCl}, r, and pH are the initial sample preparation conditions from which the coacervates are formed.

Composition Analysis. The compositions of β-lactoglobulin/pectin coacervates were analyzed using the method similar to the previously published procedures.[11,12] Water amount in the coacervates was determined at least twice by dry-weighting. To determine the concentrations of β-lactoglobulin and pectin in the coacervates, β-lactoglobulin/pectin coacervates were first dissolved in sodium phosphate buffer of pH 7.4. Next, the contents of β-lactoglobulin and pectin were determined using the size exclusion chromatography (SEC) system (DIONEX Ultimate 3000) connected with a ZORBAX GF-450 gel filtration column and a UV detector, with the absorbance measured at 280 and 214 nm, respectively. The contents of β-lactoglobulin and pectin in the coacervates were finally calculated according to the β-lactoglobulin and pectin concentration calibration curves. For comparison, β-lactoglobulin contents were also determined via a UV spectrophotometer (Cary Eclipse) with the absorbance measured at 280 nm, and the results were consistent with SEC measurements.

Rheological Measurements. Rheological measurements were performed on a strain-controlled rheometer (ARES, TA Instruments, New Castle, U.S.) fitted with parallel plate geometries (25 or 50 mm in diameter). β-Lactoglobulin/pectin coacervate samples were loaded onto the plate for 10 min to allow the stresses to relax and the samples to reach thermal equilibrium. Storage modulus (G') and loss modulus (G'') were measured while the frequency was being varied from 0.1 to 100 rad/s. Beforehand, stain sweep tests were carried out to determine the proper conditions of rheological measurements.

Results

First, the contents of pectin, β-lactoglobulin, H_2O, as well as the actual protein/polysaccharide ratio (r') in β-lactoglobulin/pectin coacervates prepared at various sodium chloride concentrations (C_{NaCl}) and initial protein/polysaccharide ratio (r) have been determined by a combination of UV, size exclusion chromatography, and dry-weighting methods. The composition results listed in Table 1 suggest that the protein/polysaccharide coacervates are flexible systems,[11] which can adapt to external parameters such as C_{NaCl} and r. Although the initial pectin and the protein concentrations in the initial mixed solutions are the same, both the actual pectin and the protein contents in β-lactoglobulin/pectin coacervates exhibit an increscent tendency as C_{NaCl} increases from 0.01 to 0.21 M, but a reversed variation is observed when C_{NaCl} further increases from 0.21 to 0.41 M. Moreover, actual pectin concentrations in β-lactoglobulin/pectin coacervates exhibit an increasing variation when r increases from 5:1 to 40:1, indicating that the higher r will provide protein molecules and pectin chains more chances to interact and form protein/polysaccharide coacervates, and cause a larger amount of pectin to settle in the coacervate phase.

The dynamic rheological measurements have been employed to further investigate how the salt concentration and initial protein/polysaccharide ratio influence the rheological properties of β-lactoglobulin/pectin coacervates. Figure 2a shows the typical result of small deformation oscillatory measurement of β-lactoglobulin/pectin coacervates prepared at $C_{NaCl} = 0.02$ M and $r = 5{:}1$. The complex viscosity is observed to decrease

Table 1. Compositions of β-Lactoglobulin/Pectin Coacervates Prepared at Various Sodium Chloride Concentrations C_{NaCl} and Initial Protein/Polysaccharide Ratios r

r	C_{NaCl} (M)	pectin %	β-lactoglobulin %	H_2O %	actual protein/polysaccharide ratio (r')
5:1	0.01	1.1 ± 0.2	8.0 ± 0.1	90.9 ± 0.6	7.3
5:1	0.02	1.2 ± 0.2	8.2 ± 0.1	90.6 ± 0.5	6.8
5:1	0.06	2.3 ± 0.1	11.8 ± 0.1	85.9 ± 0.6	5.1
5:1	0.11	4.9 ± 0.1	17.4 ± 0.1	77.7 ± 0.8	3.6
5:1	0.21	6.3 ± 0.1	21.6 ± 0.2	72.1 ± 0.8	3.4
5:1	0.36	3.8 ± 0.1	15.9 ± 0.1	80.3 ± 0.8	4.2
5:1	0.41	0.7 ± 0.2	9.5 ± 0.1	89.8 ± 0.8	13.6
20:1	0.11	6.9 ± 0.2	29.4 ± 0.1	63.7 ± 0.8	4.3
40:1	0.11	7.9 ± 0.2	26.7 ± 0.1	65.4 ± 0.8	3.4

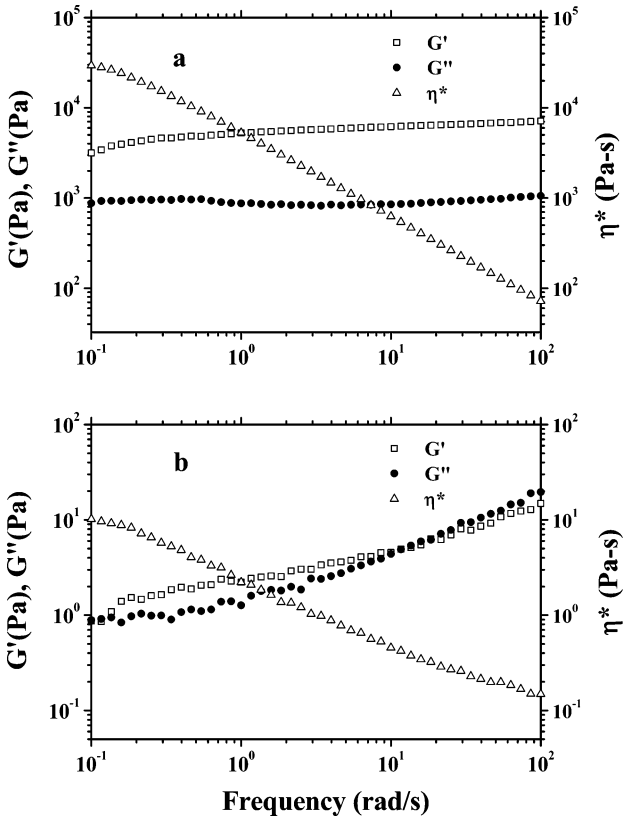

Figure 2. (a) The complex viscosity η^*, the storage modulus G', and the loss modulus G'' versus angular frequency for β-lactoglobulin/pectin coacervates prepared at C_{NaCl} = 0.02 M and r = 5:1; (b) the complex viscosity η^*, the storage modulus G', and the loss modulus G'' versus angular frequency for 2% pectin solution at C_{NaCl} = 0.02 M.

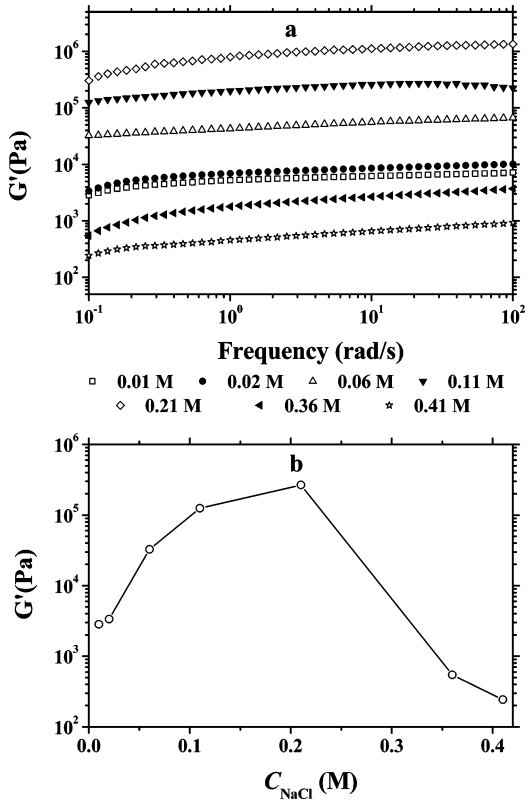

Figure 3. (a) The storage modulus G' curves for β-lactoglobulin/pectin coacervates prepared at r = 5:1 and various sodium chloride concentrations. (b) The variation of storage modulus G' values at 0.1 rad/s frequency as a function of sodium chloride concentration.

nearly linearly with frequency, showing the general shear thinning phenomenon. Remarkably, the storage modulus (G') is more than 3 times greater than the loss modulus (G''), wherein the two moduli are almost independent of frequency at $\omega > 1$ rad/s. The significantly higher G' than G'' at all frequencies measured indicates that β-lactoglobulin/pectin coacervates form a highly interconnected gel-like network structure, which agrees with the rheological properties of simple coacervates of gelatin.[27] For comparison, Figure 2b presents the dynamic rheological measurement results of pure 2.0% pectin solution, which is slightly higher than the actual pectin content in β-lactoglobulin/pectin coacervate (r = 5:1) prepared at the same salt concentration (C_{NaCl} = 0.02 M). It is noted that the 2% pure pectin solution shows the typical viscoelastic behavior of the polymer solution and has much smaller G' and G'' values as compared to the coacervate with similar pectin content. In addition, 2% pure pectin solution exhibits more elastic property at lower frequency region and more viscous property at higher frequency region. Therefore, the elastic behavior of β-lactoglobulin/pectin coacervate should result mainly from the interactions between β-lactoglobulin molecules and pectin chains.

All β-lactoglobulin/pectin coacervate samples are found to have much higher G' than G'' values, showing mainly elastic behaviors. Figure 3a presents the results of G' as a function of frequency for β-lactoglobulin/pectin coacervates prepared at r = 5:1 and C_{NaCl} = 0.01, 0.02, 0.06, 0.11, 0.21, 0.36, and 0.41 M. To clearly see the salt effect on the values of G', Figure 3b gives the variation of G' values at 0.1 rad/s frequency as a function of C_{NaCl}. As C_{NaCl} increases from 0.01 to 0.21 M, the values of G' become higher. In contrast, further increase of C_{NaCl} from 0.21 to 0.41 M causes G' values to decrease to much smaller values. Because the higher G' value usually indicates the stronger network structure, the variation of G' values with C_{NaCl} suggests that a tighter coacervate structure is favored at salt concentration up to 0.21 M C_{NaCl}. However, further increase

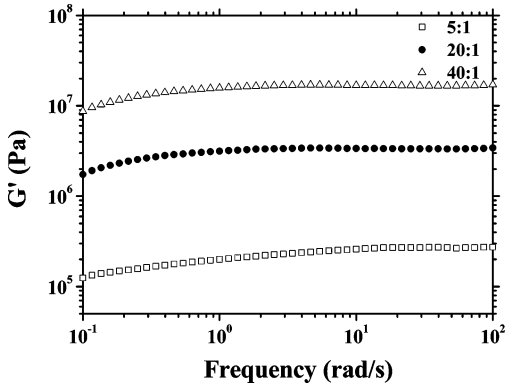

Figure 4. The storage modulus G' versus frequency curves for β-lactoglobulin/pectin coacervates prepared at $C_{NaCl} = 0.11$ M and various initial protein/polysaccharide ratios.

of salt concentration leads to a looser coacervate structure at C_{NaCl} between 0.21 and 0.41 M. Furthermore, the values of G' for β-lactoglobulin/pectin coacervates prepared at $C_{NaCl} = 0.11$ M and $r = 5{:}1$, 20:1, and 40:1 given in Figure 4 indicate that a more compact network structure is favorable for β-lactoglobulin/pectin coacervates with higher r.

Discussion

Our previous turbidimetric titration results[28] revealed that the pH ranges for the coacervation between β-lactoglobulin and pectin were significantly dependent on sodium chloride concentration (C_{NaCl}) and the initial protein/polysaccharide ratio (r). In general, an increase of r promotes the β-lactoglobulin/pectin coacervation. However, the effects of salt are more complicated. The addition of salt at lower salt concentrations promotes the β-lactoglobulin/pectin coacervation, whereas the higher amount of added salt often hinders the formation of β-lactoglobulin/pectin coacervates. In an effort to explore the structure of β-lactoglobulin/pectin coacervates, the present study focuses mainly on the effects of salt concentration and initial protein/polysaccharide ratio on the composition and rheological properties of β-lactoglobulin/pectin coacervates.

In the reported structural study of protein/polysaccharide coacervates,[11] the higher salt concentration usually led to a more watery coacervate structure, and the total biopolymer concentrations in protein/polysaccharide coacervates decreased monotonously with the increase of salt concentration, which was ascribed to the screening of the electrostactic interaction between protein and polysaccharide. In the present system, the increase of the total contents of protein and polysaccharide in β-lactoglobulin/pectin coacervates at 0.01−0.21 M C_{NaCl} and the reversed case at 0.21−0.41 M C_{NaCl} indicate either enhanced or reduced salt effects at different salt concentrations, in agreement with our and other peoples' previous works.[7,13,28] Our dynamic rheological results, which show that the values of G' increase as C_{NaCl} increases from 0.01 to 0.21 M but decrease to much lower values with the further increase of C_{NaCl} from 0.21 to 0.41 M, also support the salt-enhanced effect at low salt concentrations and the salt-reduced effect at higher salt concentrations.

The protein molecules are essentially amphoteric polyelectrolytes containing both positive and negative charges. Therefore, there simultaneously exists electrostatic attraction and electrostatic repulsion between the charges in protein molecules and polysaccharide chains. According to Dubin's model of electrostatic interaction of protein with polyeletrolyte,[13,29] the electrostatic attraction and electrostatic repulsion may be related to the average distance between the protein's positive sites and the polysaccharide's negative sites (R_+), the average distance between the protein's negative sites and the polysaccharide's negative sites (R_-), and the Debye length (R_d) by the following equation:

$$U = -\frac{Q_p}{2\epsilon}\left(\frac{Q_+}{R_+}e^{-R_+/R_d} - \frac{Q_-}{R_-}e^{-R_-/R_d}\right) \quad (1)$$

where U is the potential energy for the electrostatic interaction, Q_p is the number of charges of the polysaccharide segments associated with the protein molecules that contain Q_+ positive charges and Q_- negative charges, and ϵ is the dielectric constant. If Q_+, Q_-, R_+, and R_- are independent of salt concentration, the presence of salt leads to Coulombic screening through influencing R_d (at room temperature, $R_d \approx 0.3/\sqrt{C_{NaCl}}$). At lower salt concentrations, during β-lactoglobulin/pectin coacervation, there may be $R_+ < R_d < R_-$, and the addition of salt is mainly to screen electrostatic repulsion, instead of disturbing the electrostatic attraction between β-lactoglobulin and pectin. Therefore, the total interaction will be enhanced with increasing C_{NaCl}. This salt-enhanced effect thus increases both β-lactoglobulin and pectin contents in β-lactoglobulin/pectin coacervates when C_{NaCl} increases from 0.01 to 0.21 M. More pectin chains available in the coacervates at higher salt concentration may be one of the key factors (the other is water content) that cause the higher elasticity of the coacervates. In addition, higher salt concentration can screen the residual negatively charged groups in pectin chains in the coacervates. This effect may partially contribute to the observed salt-enhanced G' for β-lactoglobulin/pectin coacervates at 0.01−0.21 M C_{NaCl}. On the contrary, when C_{NaCl} is above 0.21 M, $R_d < R_+ < R_-$ could make both electrostatic attraction and repulsion be screened significantly because of the higher amount of salt, as shown in most of the previously published works.[7,10] Therefore, the increase in C_{NaCl} from 0.21 to 0.41 M leads to a more watery structure for β-lactoglobulin/pectin coacervates that contain less amount of protein and polysaccharide. The salt-reduced effect then causes β-lactoglobulin/pectin coacervates to have a looser network structure with a smaller storage modulus G' at 0.36 and 0.41 M C_{NaCl}.

During β-lactoglobulin/pectin coacervation, one must consider the unique character of self-aggregation for β-lactoglobulin molecules. At normal physiological pH, β-lactoglobulin mainly exists in the dimer form.[23,24] At pH close to pI of 5.2, β-lactoglobulin dimers have been reported to easily aggregate into oligomeric structure.[30,31] At the more acidic pH (i.e., 4.0), although there exists an equilibrium between β-lactoglobulin monomers and dimers, the addition of salt will shift the equilibrium to the dimeric side.[32,33] Accordingly, the higher salt concentration will promote the self-aggregation of β-lactoglobulin molecules at pH 4.0 because of the salt screening of protein charges, as supported by bigger turbidity values of protein solutions at higher salt concentrations in our turbidimetric titrations.[28] As shown in Table 1, the real protein/polysaccharide ratios (r') in the coacervates are observed to decrease with increasing C_{NaCl} from 0.01 to 0.21 M, which is opposite to the changes of total amounts of protein and polysaccharide. This result can be explained by the higher degree of self-aggregation of β-lactoglobulin induced by higher salt concentration, which will weaken β-lactoglobulin molecules to bind on pectin chains. Moreover, some β-lactoglobulin aggregates may remain in the solution instead of being involved in the formation of coacer-

vates. The higher is the salt concentration, the larger is the amount of β-lactoglobulin remaining in the solution. High enough salt concentration may completely suppress β-lactoglobulin/pectin coacervate formation.

Composition analysis shown in Table 1 suggests that the increase of r from 5:1 to 40:1 causes a higher actual pectin amount in β-lactoglobulin/pectin coacervates, which could be understood by the larger degree of complexation at higher r. However, the actual β-lactoglobulin content reaches a maximum in β-lactoglobulin/pectin coacervates at $r = 20{:}1$ instead of 40:1. This observation can also be ascribed to the self-aggregation of β-lactoglobulin molecules. At $r = 40{:}1$, higher concentration of β-lactoglobulin molecules has a higher tendency of forming larger β-lactoglobulin aggregates, which will not only promote the formation of protein domains in the coacervates but also weaken their ability to bind with pectin chains to generate coacervates. Other distinct evidence of the self-aggregation of β-lactoglobulin molecules is that the actual protein/polysaccharide ratio r' in the coacervates is lower at $r = 40{:}1$ than 20:1.

In the pectin gel, divalent cations such as Ca^{2+} often act as bridges between pairs of carboxyl groups of different pectin chains, leading pectin chains to form a network structure.[34] The formation of gel-like network in β-lactoglobulin/pectin coacervates is definitely different from the gelation of pectin. On the basis of large numbers of experiments[35] in agreement with the Veis−Aranyi model,[36] the formation of β-lactoglobulin/pectin coacervates can be described as follows: At pH 7.4 of initially mixed β-lactoglobulin/pectin solution, there is no interaction between β-lactoglobulin molecules and pectin chains, due to the strong electrostatic repulsion between the negatively charged β-lactoglobulin molecules and pectin chains. It should be noted that pectin chains may entangle with each other to some extent in this situation because the initial pectin concentration of 0.5 wt % is higher than its overlap concentration (about 0.3 wt %).[37] When the β-lactoglobulin/pectin mixture is acidified, some β-lactoglobulin molecules may electrostatically bind to pectin chains to form β-lactoglobulin/pectin complexes, even when β-lactoglobulin molecules carry the same net charges as pectin chains because of the heterogeneous distribution of protein charges.[38] These β-lactoglobulin/pectin complexes are soluble because of the excess amount of negative charges of pectin chains. At pH equal to 4.0 below pI (5.2), β-lactoglobulin molecules carry opposite charges to pectin chains. The strong electrostatic attraction will make β-lactoglobulin molecules closely bind to pectin chains, and thus neutralize the charges of pectin chains. In this case, initially soluble β-lactoglobulin/pectin complexes will aggregate with each other to form insoluble complexes, and ultimately sediment into dense coacervate phase. Thus, the network structure of protein/polysaccharide coacervates originates from the aggregation of pectin chains bound with β-lactoglobulin molecules. The elastic properties of β-lactoglobulin/pectin coacervates then mainly result from the contribution of pectin chains. A higher amount of pectin chains will aggregate tightly with each other to form β-lactoglobulin/pectin coacervates with higher elasticity, as suggested by our composition and rheology measurements of β-lactoglobulin/pectin coacervates. At the same time, β-lactoglobulin, although mainly contributing to the viscous components, serves as the junction points (nodes) of the coacervate network.

On the basis of composition analysis and rheological measurement of β-lactoglobulin/pectin coacervates, the schematic pictures of the microstructures of β-lactoglobulin/pectin coacervates prepared at $r = 5$ and different salt concentrations are presented in Figure 5. Structure A denotes the microstructure of β-lactoglobulin/pectin coacervates at $C_{NaCl} = 0.01$ M. In this case, because the electrostatic interaction between β-lactoglobulin molecules and pectin chains is relatively weak, the amount of protein molecules and pectin chains is relatively small in the coacervates. Most β-lactoglobulin dimers are separately bound on the pectin chain network, even though some neighboring β-lactoglobulin dimers may aggregate with each other to form small protein domains. Because of the salt-enhanced effect, the increase of C_{NaCl} from 0.01 to 0.21 M causes the relatively larger amount of β-lactoglobulin molecules and pectin chains to exist in β-lactoglobulin/pectin coacervates. A large amount of β-lactoglobulin dimers at high salt concentration can easily self-aggregate into larger protein domains in the coacervates. Dynamic rheological measurements have demonstrated that an increase of C_{NaCl} from 0.01 to 0.21 M will cause the formation of tighter coacervate networks with higher G' values. This case is illustrated in structure B, which corresponds to β-lactoglobulin/pectin coacervates at $C_{NaCl} = 0.21$ M. Furthermore, structure C corresponds to the coacervate structure at much higher salt concentrations (i.e., $C_{NaCl} = 0.41$ M). In this case, the salt-reduced effect allows a much smaller amount of protein molecules and pectin chains to interact to form the coacervates. The high salt concentrations will not only promote the formation of β-lactoglobulin aggregates with larger sizes, but also weaken the binding between β-lactoglobulin and pectin, as suggested by the significantly lower G' values at $C_{NaCl} = 0.41$ M. The increase of protein domain sizes in β-lactoglobulin/pectin coacervates with increasing salt concentration is supported by our recent small-angle neutron scattering studies.[22] It should be mentioned that the mesh sizes we propose in Figure 5 are hypothetical, and further work using fluorescence recovery after photobleaching (FRAP) is still undergoing to better understand the microenvironments of the coacervates.

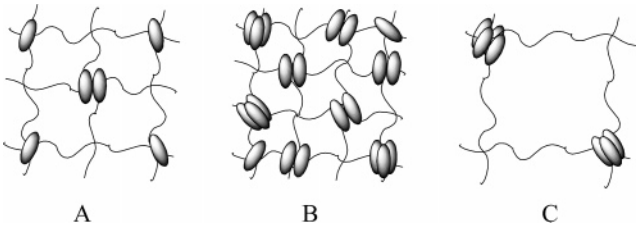

Figure 5. Schematic of possible microstructures of β-lactoglobulin/pectin coacervates prepared at $r = 5{:}1$ and different salt concentrations. (A) $C_{NaCl} = 0.01$ M; (B) $C_{NaCl} = 0.21$ M; (C) $C_{NaCl} = 0.41$ M. Ellipse denotes the dimer of β-lactoglobulin.

Conclusions

Different from the commonly accepted monotonous salt-reduced effect, the compositions and rheological properties of β-lactoglobulin/pectin coacervates prepared at $r = 5{:}1$ behave differently below and above the critical salt concentration of 0.21 M C_{NaCl}: When $C_{NaCl} < 0.21$ M, the salt-enhanced effect favors the formation of the coacervate structure with higher β-lactoglobulin and pectin contents, exhibiting a stronger gel strength. In contrast, at $C_{NaCl} > 0.21$ M, the salt-reduced effect leads to β-lactoglobulin/pectin coacervates with more water content and weaker elasticity. The higher degree of self-aggregation of β-lactoglobulin also contributes partially to the much weaker network structure of β-lactoglobulin/pectin coacervates when C_{NaCl} increases above 0.21 M, as well as favors the formation of protein domains in the coacervates, which are responsible for the decrease of actual protein/polysaccharide

ratio in β-lactoglobulin/pectin coacervates at C_{NaCl} lower than 0.21 M. On the other hand, an increase of r from 5:1 to 40:1 causes a higher pectin content in β-lactoglobulin/pectin coacervates with stronger elasticity. Our results suggest that, although polysaccharide chains contribute significantly to the strength of protein/polysaccharide networks, it is the synergetic effects of both proteins and polysaccharides that determine the real network structure of protein/polysaccharide coacervates.

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