JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of pH, Salt, and Biopolymer Ratio on the Formation of Pea Protein Isolate−Gum Arabic Complexes

SHUANGHUI LIU, NICHOLAS H. LOW, AND MICHAEL T. NICKERSON*

Department of Food and Bioproduct Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, Canada S7N 5A8

Turbidity measurements were used to study the formation of soluble and insoluble complexes between pea protein isolate (PPI) and gum arabic (GA) mixtures as a function of pH (6.0−1.5), salt concentration (NaCl, 0−50 mM), and protein−polysaccharide weight mixing ratio (1:4 to 10:1 w/w). For mixtures in the absence of salt and at a 1:1 mixing ratio, two structure-forming transitions were observed as a function of pH. The first event occurred at a pH of 4.2, with the second at pH 3.7, indicating the formation of soluble and insoluble complexes, respectively. Sodium chloride (≤7.5 mM) was found to have no effect on biopolymer interactions, but interfered with interactions at higher levels (>7.5 mM) due to substantial PPI aggregation. The pH at which maximum PPI−GA interactions occurred was 3.5 and was independent of NaCl levels. As PPI−GA ratios increased, structure-forming transitions shifted to higher pH.

KEYWORDS: Pea protein isolate; gum arabic; complex coacervation

INTRODUCTION

Protein−polysaccharide interactions play significant roles in controlling the structure, texture, and stability of food systems (*1*) and coating and packaging materials (*2*). To control these interactions to give desired functionality (e.g., gel/film-forming, emulsifying, foaming), a greater understanding of the factors affecting protein−polysaccharide interactions is required (*3*). The nature of these interactions may lead to either segregative or associative phase behavior, depending on biopolymer characteristics (e.g., their charge density, type and distribution of reactive groups, size), biopolymer concentration and ratio, and solvent conditions (e.g., pH, temperature, ionic strength) (*4*−*6*). In general, segregative phase behavior occurs when both proteins and polysaccharides carry a similar charge, separating into protein- and polysaccharide-rich phases. Associative phase behavior (also termed complex coacervation) typically occurs via an electrostatic attraction between proteins and/or polysaccharides carrying an opposing charge, leading to separation into biopolymer- (also known as "coacervate-rich") and solvent-rich phases (*1*, *5*, *7*). The former is composed of electrostatically formed complexes that rearrange to form liquid coacervates with small amounts of entrapped solvent. Because these interactions are dominated by electrostatic forces, interactions are generally reversible and highly dependent upon solvent pH and salt content, although irreversibility of the formed complexes may occur if non-Coulombic interactions are significant (*8*). During complex coacervate formation, both the conformational entropy of the biopolymers and the entropy associated with solvent mixing are reduced, which offsets the enthalpic contributions associated with the release of water and counterions during complexation, leading to the stabilization of the formed coacervate phase (*8*−*10*).

Most of the studies on complex coacervation have investigated complexation between milk proteins (serum albumin) (*11*, *12*), casein (*1*, *5*, *13*), β-lactoglobulin (*10*, *14*−*16*), or whey (*2*, *6*, *17*, *18*) with anionic polysaccharides. Although the mechanisms driving complexation have not been fully elucidated, it is believed that two structure-forming transitions occur as a function of pH, associated with the formation of first soluble and then insoluble complexes via a nucleation and growth-type mechanism (*19*, *20*). In a recent paper by Turgeon et al. (*21*), advances associated with complex formation between proteins and polysaccharides were reviewed. Soluble protein−polysaccharide complexes form near the protein's isoelectric point (p*I*), as evident by a slight increase in turbidity (denoted pH_c), followed by macroscopic phase separation at $pH_{\phi 1}$ and the formation of insoluble protein−polysaccharide complexes (*1*, *5*, *22*). The critical pH (pH_c) signifies the initial non-covalent attraction experimentally detected between the protein and the polysaccharide, whereas at $pH_{\phi 1}$ macroscopic phase separation occurs as the net charge on each biopolymer becomes opposite (*23*). Conditions for complexation are considered to be optimal at a pH at which both biopolymers reach their electrical equivalence (denoted pH_{opt}), and complexation ceases at a lower pH ($pH_{\phi 2}$), where reactive groups along the polysaccharide become protonated (giving both biopolymers a similar net charge). Weinbreck et al. (*6*) and Mekhloufi et al. (*24*) using

* Corresponding author [telephone (306) 966-5030; fax (306) 966-8898; e-mail Michael.Nickerson@usask.ca.

10.1021/jf802643n CCC: $40.75
Published on Web 01/26/2009

whey protein isolate and β-lactoglobulin observed a two-step structure-forming event upon an acid titration with a similar polysaccharide as in the present study. Due to the electrostatic nature of the interactions, investigation of the effects of salt, degree of ionization, and mixing ratio (i.e., achieving conditions at which biopolymers are electrically equivalent) on these critical pH values is crucial to understanding their complexation.

To our knowledge, studies focused on understanding mechanisms for complexation involving plant proteins with anionic polysacchrides have been limited, especially as it relates to initial pea protein isolate (PPI)−gum arabic (GA) interactions (i.e., formation of soluble complexes). The aim of this study was to advance our understanding of mechanisms underlying associative phase behavior between PPI and GA by investigating the effects of salt, pH, and biopolymer weight mixing ratio on the formation of soluble and insoluble complexes. Previous work by Ducel et al. (*25*) on pea globulin−gum arabic complexation involved studying solvent and biopolymer effects under conditions showing maximum protein−polysaccharide interactions, as it relates to a potential encapsulating material, rather than investigating their effects on the formation of soluble and insoluble complexes.

Pea proteins are dominated by two globulin-type storage proteins: legumin and vicilin. The former fraction is a hexameric 11S protein (350−400 kDa) containing disulfide bridges, whereas the latter is a trimeric 7S protein with a molecular mass of 150 kDa (*25*). Gum arabic is an anionic arabinogalactan polysaccharide−protein complex, composed of three fractions. The major faction (∼89% of the total; ∼250 kDa) consists of a β-(1→3) galactopyranose (galactan) polysaccharide backbone that is highly branched with β-(1→6) galactopyranose residues terminating in arabinose and glucuronic acid and/or 4-*O*-methyl glucuronic acid units. The second fraction (∼10% of the total) is composed of an arabinogalactan−protein complex, in which arabinogalactan chains are covalently linked to a polypeptide backbone. The remaining fraction (∼1% of the total) consists of a glycoprotein similar to the arabinogalactan−protein complex, except that it has higher protein levels and different amino acid sequences (*26*). Discussions within the present study will refer to the electrostatic attraction between negatively charged glucuronic acid residues of the side chains of the major galactan polysaccharide fraction with positively charged pea proteins at pH conditions below its isoelectric point.

MATERIALS AND METHODS

Materials. Pea flour (PF) (Fiesta Flour, lot F147X, 2008) and gum arabic (GA) (Gum Arabic FT Pre-Hydrated, lot 11229, 2007) were kindly donated by Parrheim Foods (Saskatoon, SK) and TIC Gums (Belcamp, MD), respectively. The composition of the PF was 7.80% moisture, 21.78% protein (% N × 6.25), 1.00% lipid, 65.26% carbohydrate, and 4.16% ash [mineral content (w/w): 0.02% sodium, 0.88% potassium, 0.09% calcium, 0.12% magnesium, 0.34% phosphorus]. In contrast, GA was composed of 9.56% moisture, 0.86% protein (N × 6.25), 0.11% lipid, 84.28% carbohydrate, and 5.19% ash [mineral content (w/w): 0.50% sodium, 0.24% potassium, 1.03% calcium, 0.12% magnesium]. Chemical analyses on all materials were performed according to AOAC methods 925.10 (moisture), 923.03 (ash), 920.87 (crude protein), and 920.85 (lipid) (*27*). Carbohydrate content was determined on the basis of percent differential from 100%.

PPI was prepared by dissolving PF into a 0.1 M phosphate buffer (pH 8) containing 6.4% KCl at a 1:10 (w/v) ratio, followed by mixing at 500 rpm using a magnetic stirrer (Heidolph MR 3003 control stir plate, Heidolph Instruments GmbH & Co.) for 24 h at room temperature (∼21−22 °C) (*28*). Insoluble residues were then removed by centrifugation at 3840*g* for 20 min. The supernatant was dialyzed (Spectro/Por tubing, 6−8 kDa cut off, Spectrum Medical Industries, Inc.) for salt removal using Milli-Q water (Millipore Corp., Bedford, MA) at 4 °C, refreshing every 30 min until dialysis water conductivity reached 2.1 mS/cm. Desalted supernatant was freeze-dried (Labconco Corp., Kansas City, MO) and stored at 4 °C. The composition of the PPI was determined to be 8.92% moisture, 82.80% protein (% N × 6.25), 1.06% lipid, 0.75% carbohydrate, and 6.47% ash [mineral content (w/w): 0.17% sodium, 0.69% potassium, 0.08% calcium, 0.13% magnesium, 0.58% phosphorus]. PPI and GA powders were used without further purification. Biopolymer concentrations used in this study reflect the protein (PPI) or carbohydrate (GA) content rather than powder weight.

Turbidimetric Analysis during an Acid Titration. Stock solutions (0.5%, w/w; pH 8.0) of PPI and GA were prepared by dissolving each powder in Milli-Q water under constant stirring (500 rpm) for 2 h at room temperature (21−22 °C) and then overnight at 4 °C to help facilitate protein solubility. Mixtures of PPI and GA were prepared by mixing appropriate masses of stock solutions, at the desired ratio, with Milli-Q water to achieve a total biopolymer concentration (C_p) of 0.05% (w/w) (adjusted to pH 8.0 with 0.1 M NaOH). Turbidimetric titration upon acidification was achieved via the addition of an internal acidifier [0.05% (w/w) glucono-δ-lactone, GDL] to slowly lower the mixture pH to 3.9, followed by the dropwise addition of HCl (*2*, *6*). Diluting effects were kept to a minimum using a gradient of HCl concentrations based on pH (0.05 M > pH 3.3; 0.5 M > pH 2.7; 1 M > pH 2.2; 2 M > pH 1.5). Dilution was thought to not significantly influence complex formation on the basis of findings reported under Associative Phase Behavior as a Function of NaCl and because critical pH values (pH_c, $pH_{\phi 1}$, and pH_{opt}) measured in this study corresponded to conditions at which no or minimal HCl addition occurred. For instance, within the pH range (4.2−3.5) corresponding to pH_c, $pH_{\phi 1}$, and pH_{opt}, the rise in conductivity (∼150 mS/cm) and level of dilution (<0.5%) were considered to be insignificant. In contrast, at pH 2.5 (corresponding to $pH_{\phi 2}$) the changes in conductivity (1900 mS/cm) and dilution (<3%) were much greater. The rise in conductivity measured in this study is associated with the addition of acidulants and the release of counterions during complexation (*8*−*10*). Changes in the optical density of the solution were recorded over a pH range of 6.0−1.5 using a UV−vis spectrophotometer (Mecasys Co., Daejeon, South Korea) at 600 nm using plastic cuvettes (1 cm path length). The effects of salt (NaCl) and biopolymer mixing ratio on turbidimetric acid titrations were investigated systematically, testing (a) the effect of added NaCl (0−50 mM) for a 1:1 PPI−GA mixture and (b) the effect of PPI−GA mixing ratio (1:4, 1:2, 1:1, 2:1, 4:1, 6:1, 8:1, and 10:1, on a weight-by-weight basis) in the absence of added NaCl. Homogenous PPI and GA solutions were used as blanks under the same solvent conditions and at corresponding protein or polysaccharide concentrations. Structure-forming transitions (pH_c, $pH_{\phi 1}$, and $pH_{\phi 2}$) were determined graphically as the intersection point of two curve tangents (*2*), whereas pH_{opt} corresponded to the maximum optical density at 600 nm (**Figure 1**). All measurements were made in triplicate, using separate stock solutions. All chemicals used in this study were of reagent grade and purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON).

Electrophoretic Mobility. To access the overall surface charge of the formed complexes, electrophoretic mobility (U_E) for a 2:1 PPI−GA mixed system and its corresponding homogeneous PPI and GA solutions during an acid titration from 6.5 to 1.5 was measured using a Zetasizer Nano with a MPT-2 autotitrator (Malvern Instruments, Westborough, MA). Sodium hydroxide (0.25 M) and HCl (0.25 and 0.025 M) were used as titrants to lower the pH in 0.5 pH unit increments. Samples were prepared as described in the previous section but were diluted 10-fold to a final total biopolymer concentration of 0.005% (w/w). Electrophoretic mobility (i.e., velocity of a particle within an electric field) was related to the zeta potential (ζ) using the Henry equation

$$U_E = \frac{2\varepsilon \times \zeta \times f(\kappa\alpha)}{3\eta} \quad (1)$$

where η is the dispersion viscosity, ε is the permittivity, and $f(\kappa\alpha)$ is a function related to the ratio of particle radius (α) and the Debye length (κ). Using the Smoluchowski approximation $f(\kappa\alpha)$ equaled 1.5. All measurements were made in duplicate.

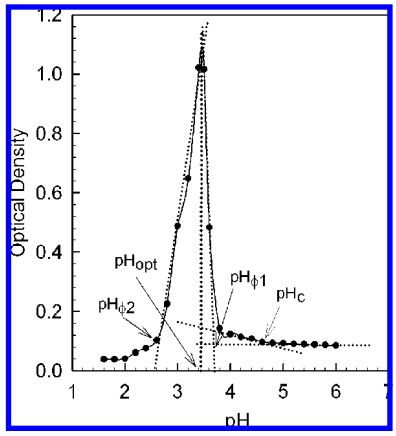

Figure 1. Critical pH transition points (pH_c, $pH_{\phi 1}$, pH_{opt}, and $pH_{\phi 2}$) corresponding to structure-forming events within a turbidity pH−acid titration were determined by extending tangent lines on either sides of inflection points. Data are representative of the 1:1 PPI−GA system in the absence of added NaCl.

Statistical Analysis. A one-way analysis of variance (ANOVA) with a Scheffe post-hoc test was used to measure statistical differences within state diagrams for each pH_c, $pH_{\phi 1}$, pH_{opt}, and $pH_{\phi 2}$ as a function of salt and biopolymer mixing ratio. All statistical analyses were performed using Systat software (SPSS Inc., ver. 10, 2000, Chicago, IL).

RESULTS AND DISCUSSION

Associative Phase Behavior as a Function of NaCl. Control of pH and ionic strength is critical to maximizing the strength of protein−polysaccharide interactions during complex coacervation. Solvent pH influences the number of charged reactive groups on the biopolymers surface, whereas the ionic strength acts to promote solubility at low concentrations, but interferes with electrostatic attractive forces at higher levels (*10*). The effects of NaCl (0−50 mM) on complexation between PPI and GA were studied during an acid titration at a 1:1 biopolymer mixing ratio in aqueous solution. Mixtures in the absence of NaCl displayed two structure-forming transitions corresponding to the formation of soluble (pH_c 4.2) and insoluble ($pH_{\phi 1}$ 3.7) complexes (**Figure 2a**). For pH values higher than $pH_{\phi 1}$, all solutions were transparent. The rise in optical density signifies an increase in size and number of PPI−GA or PPI(aggregated)−GA complexes being formed. The highest amount of biopolymer interactions occurred at pH 3.5 (pH_{opt}), followed by a disassociation of complexes near pH 2.5 ($pH_{\phi 2}$), at which both biopolymers carried a similar overall net positive charge. Under the same conditions, PPI alone gave only a weak scattering intensity (optical density < 0.2), whereas GA had none (**Figure 2a**). Ducel et al. (*25*) reported a similar pH_{opt} for a 1:1 pea globulin−GA mixture. The observed two-step structure -forming events have been previously reported for whey protein−carrageenan (*18*), whey protein−EPS B40 (*22*), whey protein−gum arabic (*6*), β-LG−gum arabic (*24*), β-LG−pectin (*19*), and gelatin−agar (*9*) systems as a consequence of increased electrostatic attractive interactions. The peak intensity for the homogeneous PPI solution at pH ∼4.0 in the current study, although minor, is thought to correspond to a rise in protein−protein aggregation. The broad nature of the peak reflects the polydispersity of the isolate and its various protein components.

At a NaCl level of 7.5 mM, optical density of the PPI blank became more pronounced as charges on the protein's surface became screened (**Figure 2b**). At NaCl levels >7.5 mM, the aggregation phenomenon dominated the scattering spectrum preventing reliable estimates of critical pH transitions (data not shown). Higher salt levels are also expected to interfere with coacervate formation. Ducel et al. (*25*) investigated the effect of NaCl on the coacervation of pea globulin and GA at a 1:1 mixing ratio (pH 3.5) and found that ionic strengths of <50 mM did not alter the process; however, they were unable to determine a critical ionic strength that inhibited coacervation due to protein precipitation. A state diagram describing changes to critical pH values for PPI−GA mixed systems as a function of NaCl is shown in **Figure 3**. PPI−GA mixtures were considered to be cosoluble at solvent pH > pH_c and <$pH_{\phi 2}$. Within the pH range (∼4.2−2.5) at which coacervation occurred, all critical pH values were found to be independent of NaCl levels at concentrations of ≤7.5 mM, despite being dominated by electrostatic forces (**Figure 3a**). Maximum PPI−GA interactions at a biopolymer ratio of 1:1 occurred at a solvent pH of 3.5 (**Figure 3b**). Singh et al. (*9*) observed similar trends for gelatin−agar mixtures, where pH_c remained independent of NaCl levels (0−200 mM) and $pH_{\phi 1}$ remained constant until reaching a critical value (∼50 mM), before shifting to a lower pH as the ionic strength increased to 200 mM. Weinbreck et al. (*18*) also observed a similar phenomenon in whey−carrageenan mixtures.

Associative Phase Behavior as a Function of Biopolymer Mixing Ratio. Biopolymer mixing ratio is critical for controlling the charge balance between proteins and polysaccharides, the intensity of interactions, and the degree of self-aggregation during complexation (*8*). The effect of PPI−GA mixing ratio (1:4 to 10:1) on complex coacervation was investigated during an acid titration in the absence of added salt and at a constant total biopolymer concentration (0.05%, w/w). Under conditions at which PPI−GA ratios were <1, the turbidity curves shifted to lower pH and the overall scattering intensity was reduced relative to the 1:1 ratio at pH_{opt} (**Figure 4a**). Interactions were almost negligible at a PPI−GA ratio of 1:4; likely due to the large concentration of GA present in the system, which is a poor scatterer by itself. Blanks for both PPI and GA solutions contributed little to the scattering spectrum (optical density < 0.2) for PPI−GA ratios <1:1 (data not shown). In contrast, at PPI−GA mixing ratios >1:1, scattering curves shifted to higher pH until reaching a mixing ratio of 4:1, after which turbidity curves overlapped (**Figure 4b**). Schmitt et al. (*10*) reported a similar phenomenon when acacia gum or β-lactoglobulin was in excess.

At PPI−GA mixing ratios of <1, the critical transition pH values (pH_c and $pH_{\phi 1}$) shifted considerably to lower pH as the protein content decreased, suggesting that interpolymer interactions were significantly reduced. Complex formation could not be detected at a PPI−GA ratio of 1:4 possibly because of the reduced protein levels present (**Figure 4a**). At mixing ratios having greater protein content, critical values shifted to higher pH as biopolymer mixing ratio increased, until reaching a ratio of 4:1 at which it then became stable, showing no statistical differences ($p > 0.05$) for PPI−GA mixing ratios up to 10:1 (**Figure 5a**).

A similar trend was evident for pH_{opt} as a function of biopolymer mixing ratio (**Figure 5b**); however, its corresponding maximum optical density was observed at 2:1 PPI−GA ratio before declining and reaching a plateau at ratios of 6:1 to 10:1 (**Figure 5c**). These results suggest that the greatest amount of PPI−GA interactions occurred at a mixing ratio of 2:1. It was assumed that at this ratio proteins became saturated with GA chains and, at mixing ratios above 2:1, proteins were in excess as evident by a rise in protein−protein aggregation in the turbidity spectrum of the protein blank (data not shown). The

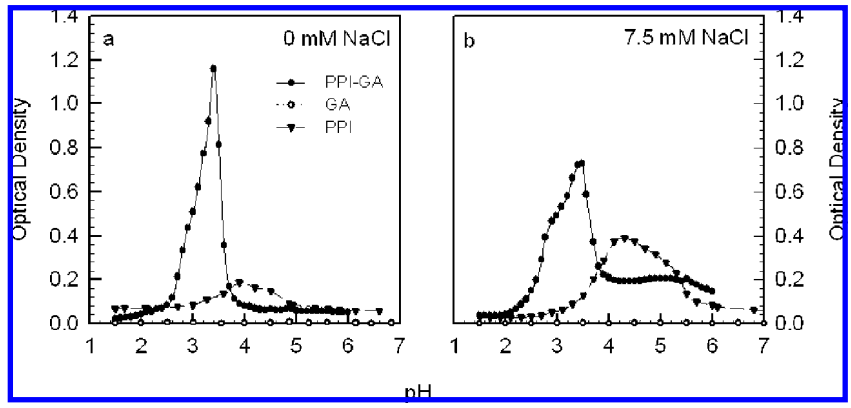

Figure 2. Turbidity curve of a homogeneous and mixed (1:1 ratio) PPI−GA system as a function of pH, in the absence (**a**) and in the presence of 7.5 mM NaCl (**b**).

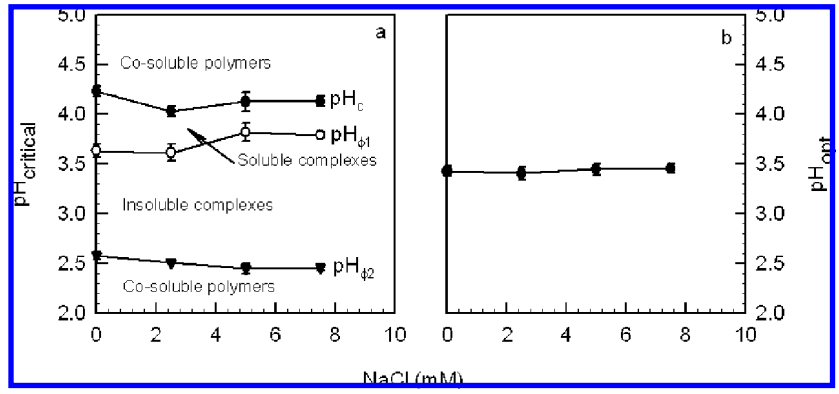

Figure 3. Phase diagrams of critical pH values associated with structure-forming events (**a**) at optimal pH conditions for biopolymer interactions (**b**) in a PPI−GA mixture (1:1 ratio) as a function of pH and NaCl ($n = 3$).

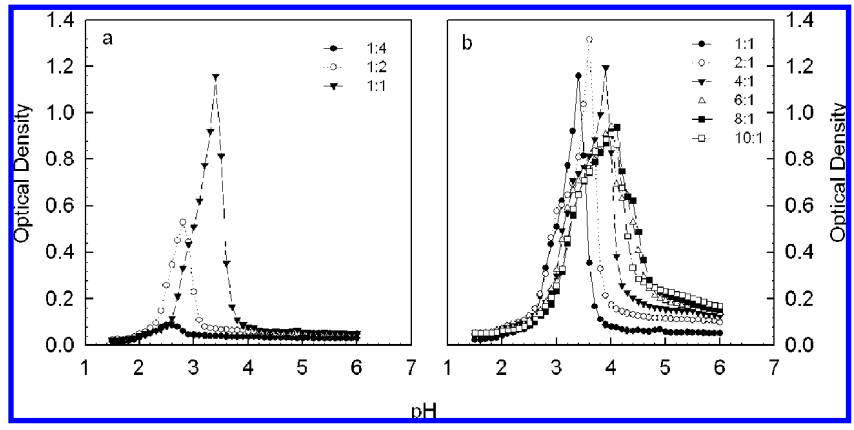

Figure 4. Turbidity curve of a PPI−GA mixture as a function of pH and biopolymer mixing ratio.

increased contribution from PPI aggregates during complex formation at higher mixing ratios is also shown by shifts in $pH_{\phi 1}$ and pH_{opt} closer to the isoelectric point of PPI, as the mixing ratio increased from 1:1 to 4:1. This phenomenon can be attributed to an increase in the number of proteins interacting with the same number of GA chains, as a result of reduced protein charge (*10, 29*). Electrophoretic mobility measurements for a 2:1 PPI−GA ratio estimated a net neutral surface charge of formed complexes (zeta potential = 0 mV) occurred at a solvent pH of 3.57 (**Figure 6**), which was equivalent to the pH_{opt} of the system (**Figure 5b**). At pH >3.57, formed complexes carried a net negative charge (zeta potential < 0 mV) as the charge contribution from the GA dominates over that of PPI despite being the less prevalent biopolymer present. In contrast, at pH <3.57 a net positive charge of complex occurs (zeta potential > 0 mV) when the positive charge contribution from PPI dominates, as glucuronic acid residues on the GA start to become protonated. Neutrality of homogeneous PPI and GA solutions occurred at solvent pH values of 5.60 and 1.88, respectively, and corresponded to the isoelectric point of the PPI and the protonation of the carboxyl group on the GA molecule, respectively.

Weinbreck et al. (*18, 22*), for whey protein (aggregate-free)-polysaccharide, and Girard et al. (*19*), for β-lactoglobulin (aggregate-free)-pectin systems, reported a constant pH_c as a function of biopolymer mixing ratio, suggesting the formation of soluble complexes occurred between a single polysaccharide chain and a given amount of protein. In contrast, in the present study pH_c was found to be dependent upon a biopolymer mixing ratio of <4:1, which skews from the current view in literature (*18−20, 22*). Determination of the pH_c is based on the first detectable evidence of biopolymer interactions occurring within

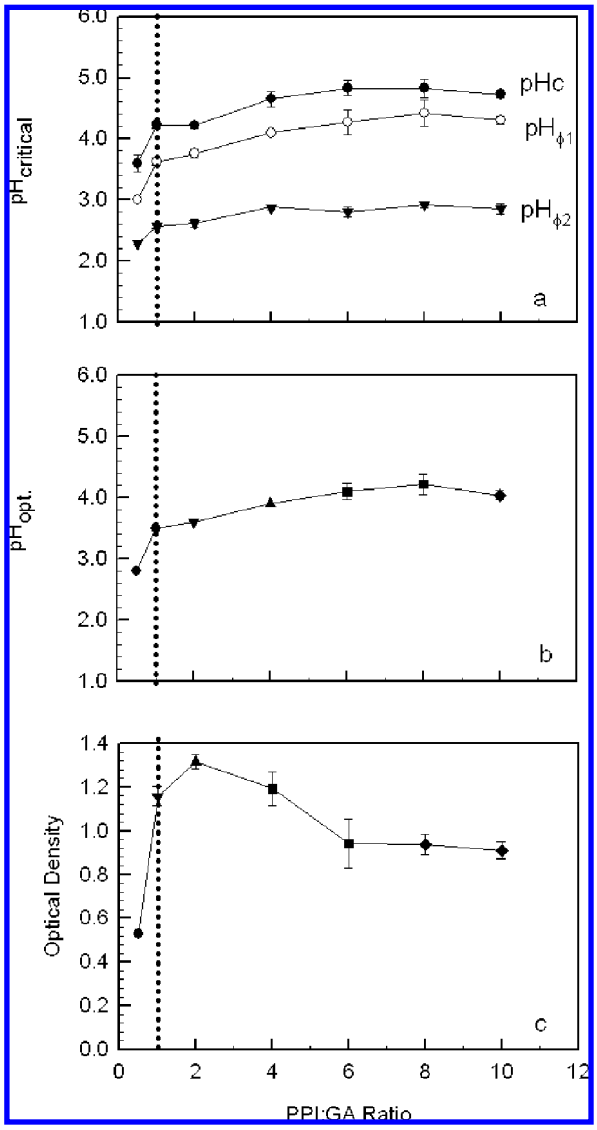

Figure 5. Phase diagram of critical pH values associated with structure-forming events (**a**), optimal pH conditions for biopolymer interactions (**b**), and peak optical density (**c**) in PPI−GA mixtures as a function of pH and biopolymer ratio. (The dotted line represents the 1:1 PPI−GA baseline ratio, $n = 3$.)

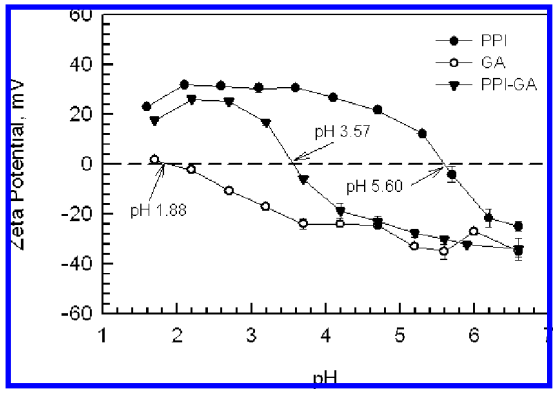

Figure 6. Zeta potential (mV) values for homogeneous and mixed PPI−GA systems at a 2:1 biopolymer mixing ratio in the absence of NaCl, as a function of pH ($n = 2$).

the scattering spectrum (as noted by a small inflection point within a turbidimetric vs pH curve during acidification). In the current study, protein−protein aggregates seem to play a significant role during complexation. In **Figure 2a**, optical density for the homogeneous PPI system is ∼0.2 within the pH region of 3.5−4.2, effectively overlapping within the region where complexes initially form for the 1:1 mixing ratio. Findings suggest that during complexation, gum arabic chains are interacting with protein−protein aggregates rather than single (or a few) molecules. Similar observations were made for homogeneous PPI systems corresponding to higher mixing ratios, except optical densities were >0.2 (not shown), suggesting the protein−protein aggregates were larger at the time of initial complexation (at pH_c). It is presumed, as a result, that a mixing ratio dependence of pH_c emerges at low ratios. At mixing ratios of >4:1, protein−protein aggregates are thought to reach a critical size leading to similar turbidimetric pH profiles (**Figure 4**) and constant critical values. Singh et al. (*9*) using type-A and type-B gelatin with agar also reported a similar trend whereby a strong dependence of pH_c was found with gelatin−agar mixing ratios of <2, followed by independence of pH_c at mixing ratios of >2. Of note, this was despite showing NaCl independence for low NaCl levels (also similar to the current study). However, Singh et al. (*9*) provide no explanation for the phenomenon. Evidence of a mixing ratio dependence of pH_c in the literature is limited and warrants further investigation to help elucidate mechanistic differences from the well-studied milk protein−polysaccharide complexation.

In the case of $pH_{\phi 1}$, Weinbreck and coauthors (*18, 22*) observed a shift to higher pH until a critical biopolymer mixing ratio was reached, at which it then became stable. The movement of $pH_{\phi 1}$ to higher pH as a function of increasing biopolymer ratio reflects the greater amount of protein molecules available per polysaccharide chain (*22*). Schmitt et al. (*10, 16*) reported that the presence of β-lactoglobulin aggregates during coacervation contributed to the complexity of β-lactoglobulin−acacia gum interactions, as non-Coulombic interactions became more pronounced. In the present study, the shift in critical pH transitions as biopolymer mixing ratio was increased from 1:1 to 4:1 is attributed to the presence of PPI aggregates in the formed complexes. Although not measured in the present study, the effect of increasing the total biopolymer concentration (at a constant biopolymer mixing ratio) on the coacervation process is well documented (*6, 8, 30*). Weinbreck et al. (*6*) found in whey protein−gum arabic systems that increasing the total biopolymer concentration caused a shift in $pH_{\phi 1}$ to higher values, until an upper concentration limit was reached. Afterward, coacervation is suppressed, which is thought to be due to the thermodynamic incompatibility of the mixture as biopolymers compete for solvent (*8, 30*) and/or a higher content of released counterions in solution that function to reduce electrostatic attractive forces between biopolymers based on charge screening (*6*).

Conclusion. Findings from this study described the effect of salts and biopolymer mixing ratio on the formation of soluble and insoluble complexes involving pea protein isolate and gum arabic. Critical structure-forming transitions (pH_c, $pH_{\phi 1}$, pH_{opt}, and $pH_{\phi 2}$) were found to be independent of NaCl at ≤7.5 mM, whereas at concentrations between 7.5 and 50 mM, protein−protein aggregation overwhelmed protein−polysaccharide interactions. Critical structure-forming transitions were found to shift to higher pH as protein content increased to a PPI−GA ratio of 4:1. Maximum biopolymer interactions occurred at a ratio of 2:1.

ACKNOWLEDGMENT

We give special thanks to Yuanlong Cao for providing technical assistance throughout this study.

Financial assistance for this study was provided by the Natural Science and Engineering Research Council of Canada, Saskatchewan Agriculture Development Fund, and the Advanced Foods and Materials Network.

LITERATURE CITED

(1) deKruif, C. G.; Tuinier, R. Polysaccharide protein interactions. *Food Hydrocolloids* **2001**, *15*, 555–563.

(2) Weinbreck, F.; Tromp, R. H.; deKruif, C. G. Composition and structure of whey protein/gum arabic coacervates. *Biomacromolecules* **2004**, *5*, 1437–1445.

(3) Dickinson, E. Stability and rheological implications of electrostatic milk protein−polysaccharide interactions. *Trends Food Sci. Technol.* **1998**, *9*, 347–354.

(4) Chourpa, I.; Ducel, V.; Richard, J.; Dubois, P.; Boury, F. Conformational modifications of α-gliadin and globulin proteins upon complex coacervates formation with gum arabic as studied by raman microspectroscopy. *Biomacromolecules* **2006**, *7*, 2616–2623.

(5) Turgeon, S. L.; Beaulier, M.; Schmitt, C.; Sanchez, C. Protein−polysaccharide interactions: phase-ordering kinetics, thermodynamic and structural aspects. *Curr. Opin. Colloid Interface Sci.* **2003**, *8*, 401–414.

(6) Weinbreck, F.; de Vries, R.; Schrooyen, P.; de Kruif, C. G. Complex coacervation of whey proteins and gum arabic. *Biomacromolecules* **2003**, *4*, 293–303.

(7) Braudo, E. E.; Plashchina, I. G.; Schwenke, K. D. Plant protein interactions with polysaccharides and their influence on legume protein functionality—a review. *Nahrung* **2001**, *45*, 382–384.

(8) Ye, A. Complexation between milk proteins and polysaccharides via electrostatic interactions: principles and applications—a review. *Int. J. Food Sci. Technol.* **2008**, *43*, 406–415.

(9) Singh, S. S.; Siddhanta, A. K.; Meena, R.; Prasad, K.; Bandyopadhyay, S.; Bohidar, H. B. Intermolecular complexation and phase separation in aqueous solutions of oppositely charged biopolymers. *Int. J. Biol. Macromol.* **2007**, *41*, 185–192.

(10) Schmitt, C.; Sanchez, C.; Thomas, F.; Hardy, F. Complex coacervation between β-lactoglobulin and acacia gum in aqueous medium. *Food Hydrocolloids* **1999**, *13*, 483–496.

(11) Glisenan, P. M.; Richardon, R. K.; Morris, E. R. Associative and segregative interactions between gelatin and low-methoxy pectin: Part 1. Associative interactions in the absence of Ca^{2+}. *Food Hydrocolloids* **2003**, *17*, 723–737.

(12) Kaibara, K.; Okazaki, T.; Bohidar, H. B.; Dubin, P. K. pH-induced coacervation in complexes of bovine serum albumin and cationic polyelectrolytes. *Biomacromolecules* **2000**, *1*, 100–107.

(13) Tuinier, R.; Rolin, C.; de Kruif, C. G. Electrosorption of pectin into casein micelles. *Biomacromolecules* **2002**, *3*, 632–638.

(14) Wang, X.; Lee, J.; Wang, Y.-W.; Huang, Q. Composition and rheological properties of β-lactoglobulin/pectin coacervates: effects of salt concentration and initial protein/polysaccharide ratio. *Biomacromolecules* **2007**, *8*, 992–997.

(15) Girard, M.; Turgeon, S. L.; Gauthier, S. F. Interbiopolymer complexing between β-lactoglobulin and low- and high-methylated pectin measured by potentiometric titration and ultrafiltration. *Food Hydrocolloids* **2002**, *16*, 585–591.

(16) Schmitt, C.; Sanchez, C.; Despond, S.; Renard, D.; Thomas, F.; Hardy, J. Effect of protein aggregates on the complex coacervation between β-lactoglobulin and acacia gum at pH 4.2. *Food Hydrocolloids* **2000**, *14*, 403–413.

(17) Weinbreck, F.; Rollema, H. S.; Tromp, R. H.; de Kruif, C. G. Diffusivity of whey protein and gum arabic in their coacervates. *Langmuir* **2004**, *20*, 6389–6395.

(18) Weinbreck, F.; Nieuwenhuijse, H.; Robijn, G. W.; de Kruif, C. G. Complexation of whey proteins with carrageenan. *J. Agric. Food Chem.* **2004**, *52*, 3550–3555.

(19) Girard, M.; Sanchez, C.; Laneuville, S. I.; Turgeon, S. K.; Gauthier, S. F. Associative phase separation of β-lactoglobulin/pectin solutions: a kinetic study by small angle static light scattering. *Colloids Surf. B: Biointerfaces* **2004**, *35*, 15–22.

(20) Sanchez, C.; Mekhlouf, G.; Renard, G. Complex coacervation between β-lactoglobulin and acacia gum: a nucleation and growth mechanism. *J. Colloid Interface Sci.* **2006**, *299*, 867–873.

(21) Turgeon, S. L.; Schmitt, C.; Sanchez, C. Protein−polysaccharide complexes and coacervates. *Current Opin. Colloid Interface Sci.* **2007**, *12*, 166–178.

(22) Weinbreck, F.; Nieuwenhuijse, H.; Robijn, G. W.; de Kruif, C. G. Complex formation of whey proteins: exocellular polysaccharide EPS B40. *Langmuir* **2003**, *19*, 9404–9410.

(23) de Vries, R.; Weinbreck, F.; de Kruif, C. G. Theory of polyelectrolyte adsorption on heterogeneously charged surfaces applied to soluble protein−polyelectrolyte complexes. *J. Chem. Phys.* **2003**, *118*, 4649–4659.

(24) Mekhloufi, G.; Sanchez, C.; Renard, D.; Guillemin, S.; Hardy, J. pH-Induced structural transitions during complexation and coacervation of β-lactoglobulin and acacia gum. *Langmuir* **2005**, *21*, 386–394.

(25) Ducel, V.; Richard, J.; Saulnier, P.; Popineau, Y.; Boury, F. Evidence and characterization of complex coacervates containing plant proteins: application to the microencapsulation of oil droplets. *Colloids Surf. A: Physicochem. Eng. Aspects* **2004**, *232*, 239–247.

(26) Dror, Y.; Cohen, Y.; Yerushalmi-Rozen, R. Structure of gum arabic in aqueous solution. *J. Polym. Sci.: Part B: Polym. Phys.* **2006**, *44*, 3265–3271.

(27) *Official Methods of Analysis*, 17th ed.; AOAC: Arlington, VA, 2003.

(28) Crévieu, I.; Bérot, S.; Guéguen, J. Large scale procedure for fractionation of albumins and globulins from pea seeds. *Nahrung* **1996**, *40*, 237–244.

(29) Koh, G. L.; Tucker, I. G. Characterization of sodium carboxylmethylcellulose−gelatin complex coacervation by chemical analysis of the coacervate and equilibrium fluid phases. *J. Pharm. Pharmacol.* **1988**, *40*, 309–312.

(30) Schmitt, C.; Sanchez, C.; Desobry-Banon; Hardy, J. Structure and technofunctional properties of protein−polysaccharide complexes: a review. *Crit. Rev. Food Sci. Nutr.* **1998**, *38*, 689–753.

Received for review August 27, 2008. Revised manuscript received November 17, 2008. Accepted December 4, 2008.

JF802643N