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Complexation of Whey Proteins with Carrageenan

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The formation of electrostatic complexes of whey protein (WP) and a nongelling carrageenan (CG) was investigated as a function of pH, ionic strength, temperature, and protein-to-polysaccharide (Pr:Ps) ratio. On lowering the pH, the formation of soluble WP/CG complexes was initiated at pH_c and insoluble complexes at pH_ϕ, below which precipitation occurred. The values of the transition pH varied as a function of the ionic strength. It was shown that at [NaCl] = 45 mM, the value of pH_ϕ was the highest, showing that the presence of monovalent ions was favorable to the formation of complexes by screening the residual negative charges of the CG. When $CaCl_2$ was added to the mixtures, complexes of WP/CG were formed up to pH 8 via calcium bridging. The electrostatic nature of the primary interaction was confirmed from the slight effect of temperature on the pH_ϕ. Increasing the Pr:Ps ratio led to an increase of the pH_ϕ until a ratio of 30:1 (w/w), at which saturation of the CG chain seemed to be reached. The behavior of WP/CG complexes was investigated at a low Pr:Ps ratio, when the biopolymers were mixed directly at low pH. It resulted in an increase of the pH of the mixture, as compared to the initial pH of the separate WP and CG solutions. The pH increase was accompanied by a decrease in conductivity. The trapping of protons inside the complex probably resulted from a residual negative charge on the CG. If NaCl was present in the mixture, the complex took up the Na^+ ions instead of the H^+ ions.

KEYWORDS: Whey proteins; carrageenan; electrostatic interactions; complexation

INTRODUCTION

Macromolecules are the main components of formulated food products, and the control of structural properties of proteins and polysaccharides is a wide topic of investigation (*1*). Interactions between food macromolecules can be either repulsive or attractive, underlining two opposite phenomena: biopolymer incompatibility and complex formation (*2*). Interbiopolymer complexing of positively charged proteins and anionic polysaccharides can lead to the formation of soluble or insoluble complexes (*3*). Systematic studies were carried out on the complexation behavior of proteins with synthetic polyelectrolytes (*4*−*12*) and proteins with polysaccharides (*13*−*16*). They revealed two pH-induced structural transitions due to increasing attractive interaction and depending on the ionic strength of the system, indicating that complexation was mainly electrostatically driven. The formation of soluble protein/polyelectrolyte complexes was initiated at pH_c on lowering the pH, which preceded the pH of macroscopic phase separation at pH_ϕ. Often, soluble complexes were formed at pH values above the pI of the protein; that is, the pH at which the protein is overall negatively charged (*4*, *11*, *17*). This phenomenon can be explained by the presence of positively charged patches at the surface of the protein.

Previous work in our group included the study of WP and a weak polyelectrolyte (*14*, *15*). The electrostatic interactions between the WPs and the carboxylated gum arabic led to the formation of a liquid coacervate in the pH window between 2.5 and 4.8 (*14*, *15*, *18*, *19*). It was also shown that the pH boundaries pH_c and $pH_{\phi 1}$ were mainly due to the formation of a complex between the β-lg and the gum arabic (the contribution of α-la would appear at lower pH values). The interaction of WPs with the phosphated exocellular polysaccharide EPS B40 (EPS B40) was studied as well (*16*). Now, the interaction between the WPs and another strong polyelectrolyte, CG, has been investigated. The two batches used in this study had the commercial name of λ-CG; however, because the amount of pure λ-CG was rather limited in one of the batches, the authors referred to the polysachharide as CG. CG is an anionic sulfated polysaccharide extracted from red algae (*20*). It is mainly used as a thickener/viscosity builder. Three main types of CG are used in the food industry. The λ-CG does not present pronounced gelling properties, unlike the other two commercial types of CG (i.e., ι and κ). λ-CG carries three sulfate groups, ι-CG carries two sulfate groups, and κ-CG carries one sulfate

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group per repeating unit. The two CG batches used in this study are nongelling; the type I is a mixture of different CG types, and the type II is a pure λ-CG. CG is highly charged (much more than gum arabic or EPS B40). It is known that the molecular attraction of protein-bound NH_3^+ groups for $-OSO_3^-$ groups is much stronger than for $-CO_2^-$ groups (*21*). CGs are added to food products to enhance their textural properties, and because most food products have an acid pH, CG and protein would form complexes and alter the texture and stability of food products. Various studies on proteins/CG have already been carried out, especially on the effect of segregative interactions on the gelling properties of κ-CG (*22*−*28*). A beneficial consequence of complexation of proteins with sulfated polysaccharide is the protection afforded against loss of solubility as a result of protein aggregation during heating (*29*, *30*) or following high-pressure treatment (*31*, *32*). The protective effect is probably due to the blocking of the hydrophobic sites of the protein by the polysaccharide (*21*). Interactions of casein with CG or pectin are widely used in the control of texture and stability of some dairy products (e.g., yogurt drinks) (*27*, *28*, *33*−*36*). However, very limited work has been done on the electrostatic interaction between WPs and CG, especially λ-CG.

This work aims to investigate the complex formation of WPs and CG as a function of pH and ionic strength. Furthermore, an attempt was made to compare the results with those obtained with WP/gum arabic and WP/EPS B40. This comparison allowed us to identify system specific or generic properties of the complexes. In addition, the results can also be used for practical applications (e.g., in dairy products), so as to understand how the formation of WP/CG complexes can be tuned. Interesting results on the addition of calcium ions to induce complex formation and on the influence of temperature on the pH_ϕ allowed a clarification of the phenomenon involved in complexing. Titration experiments revealed the behavior of the complexes as ion traps, which was previously poorly recognized for this type of system.

EXPERIMENTAL PROCEDURES

Material. Bipro is a WP isolate comprised mainly of β-lg and α-la from Davisco Foods International (Le Sueur, U.S.A.). Residual WP aggregates were removed by acidification (at pH 4.75) and centrifugation (1 h at 33 000 rpm with a Beckman L8-70M ultracentrifuge, Beckman Instruments, The Netherlands). The supernatant was then freeze-dried (in a Modulo 4 K freeze dryer from Edwards High Vacuum International, U.K.). Finally, the resulting powder was stored at 5 °C. The final powder contained (w/w) 88.1% protein (N × 6.38), 9.89% moisture, 0.3% fat, and 1.84% ash (0.66% Na^+, 0.075% K^+, 0.0086% Mg^{2+}, and 0.094% Ca^{2+}). The protein content of the treated Bipro was 14.9% α-la, 1.5% bovine serum albumin, 74.9% β-lg, and 3.2% immunoglobulin.

Two batches of CG were used. The first batch (type I) was λ-CG 3830 Carravisco DLF1 from Ferdiwo (Oudwater, The Netherlands). The powder contained (w/w) 8.7% moisture, 0.64% proteins (Dumas method), and 13.2% ash (0.07% PO_4^{3-}, 0.16% Ca^{2+}, 0.077% Mg^{2+}, 3% K^+, 2.2% Na^+, 12 mg/kg Fe^{3+}, and 0.32% Cl^-). The amount of glucose was low (0.9%) and saccharose < 0.05%. The weight-averaged molar mass of the CG type I measured by SEC MALLS was 774 kDa, and the weight average radius was 49 nm. The second batch (type II) was GENU λ-CG X-7055 (BRR) from CPKelco (Lille Skensved, Denmark) and was not commercially available. Both CG samples were cold water soluble and did not gel. Stock solutions were prepared by dissolving the powder in deionized water (concentrations were set at 0.10 or 0.25% w/w). Various WP and CG mixtures were obtained by diluting the stock solutions in deionized water at the desired pH and ionic strength (use of NaCl or $CaCl_2$). The concentration of the total biopolymer (Cp) was set at 0.10 or 0.25%, the ratio of WP to CG (Pr:Ps) was varied from 1:1 to 150:1 (w/w), and the salt (NaCl or $CaCl_2$) was added to the mixtures from 0 to 1 M.

Characterization of the CG Batches. NMR (^1H) experiments were carried out on both CG batches. It was found that the type I contained various types of CG in the following quantities: λ-CG, 9%; κ-CG, 46%; υ-CG, 20%; μ-CG, 1%; and ι-CG, 24%. Moreover, the type I does not contain any other additives. This result surprisingly revealed that the type I powder contained only a rather low amount of λ-CG and therefore was compared to a purer sample. Indeed, the ^1H NMR spectrum of type II CG showed that only λ-CG was present, and a small amount of starch (in the range of 10%) could also be detected. ζ-Potential measurements were carried out on both CG mixtures with a Zetasizer 2000 (Malvern, U.S.A.). The results showed that the ζ-potential of both CG samples was −86 mV. In most of the experiments, the type I CG was used (the commercial grade sample), unless otherwise mentioned.

Turbidimetric Titration under Acidification. Mixtures of WP and CG were acidified by dropwise addition of HCl or by adding GDL, which provided a slow acidification. The influence of the ionic strength ([NaCl] or $[CaCl_2] = 0-1$ M), the Pr:Ps (Pr:Ps = 1:1−150:1 w/w), and the Cp (Cp = 0.10 or 0.25% w/w) was studied by varying one parameter at a time. The turbidity of each sample was measured as a function of the pH with a Cary 1E spectrophotometer (Varian, The Netherlands) at a wavelength of 514.5 nm [similar to previous measurements (*14*)]. The samples were put in a 1 cm path length cuvette, and the turbidity was then measured as a function of time at 25 °C. The turbidity (τ) was defined as:

$$\tau = -\ln(I/I_0)$$

where I is the light intensity that passes through a volume of solution of 1 cm length and I_0 is the incident light intensity.

The pH_c was determined for some samples; it could be measured using DLS measurements, with a 22 mW HeNe laser at a wavelength of 632.8 nm. The sample was initially filtered with a 0.45 μm filter and centrifuged for 30 s to remove all impurities and air bubbles. The sample was placed in the cuvette housing, which was kept at a temperature of 25 °C in a toluene bath. The goniometer was set at 45°. The detected intensity was processed by a digital ALV-5000 correlator. Finally, the scattered light intensity was measured and its average was recorded every minute. The averaged intensity was used as the scattering intensity value each minute. The values of pH_c were measured graphically as the intersection point of two tangents to the curve, as described in ref *14*.

Each measurement was at least done in duplicate. Control measurements with only WP and only CG were systematically carried out under the same conditions as the mixtures of biopolymers. From all measurements, a statistical uncertainty of 0.2 pH units was calculated (*STATISTICA*, version 6; Statsoft, Inc., 2001).

Titration of One Polymer by the Other. The titration was performed by slowly adding under stirring 0.25% WP into 0.25% CG (type I). These titrations were done at pH 3 and at pH 4 on mixtures at [NaCl] = 0 and 45 mM. The Pr:Ps ratio was then recalculated, and at each ratio, the turbidity, the pH, and the conductivity of the WP/CG mixture were measured. The conductivity of the WP/CG mixture at each Pr:Ps ratio was then subtracted from the initial conductivity of the CG mixture and plotted as a function of Pr:Ps ratio. Titration curves were reproducible within the uncertainty of 0.5 Pr:Ps ratio and 0.2 pH units.

Conductivity Measurement. The conductivity of the mixtures was systematically measured with a conductivity meter LF 340 and a standard conductivity cell TetraCon 325 (Wissenschaftlich-Technische Werkstätten GmbH, Germany).

RESULTS AND DISCUSSION

Behavior of the System as a Function of pH and Salt. The interaction between WP and CG is expected to be electrostatic in nature. Indeed, because the pH influences the ionization of the protein charges, electrostatic complexes would be formed in the pH window where WP and CG are oppositely charged.

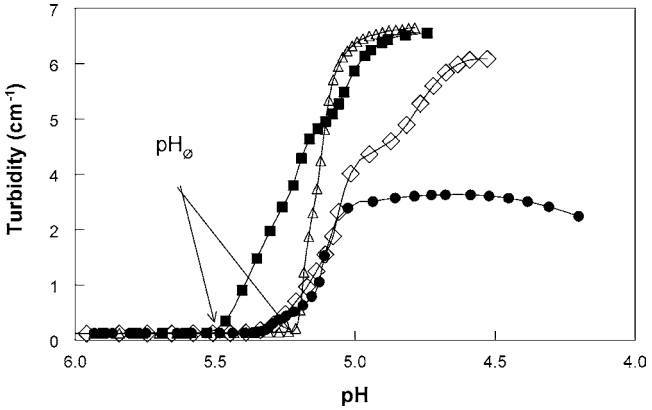

Figure 1. HCl titration at various ionic strengths of a mixture of WP/CG, Cp = 0.25%, Pr:Ps ratio = 15:1, temperature = 25 °C. [NaCl] = 0 mM (●); [NaCl] = 15 mM (△); [NaCl] = 45 mM (■); and [NaCl] = 120 mM (◇).

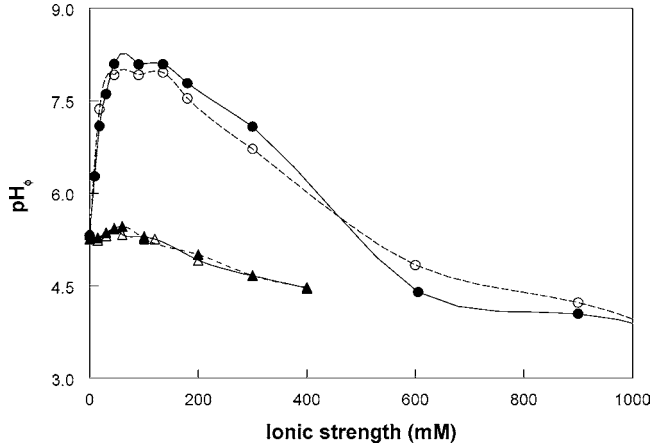

Figure 2. State diagram as a function of ionic strength of a mixture of WP/CG, Pr:Ps ratio = 15:1, Cp = 0.25%, temperature = 25 °C. Addition of NaCl (△/▲); addition of $CaCl_2$ (○/●); type I CG, open symbols; and type II CG, filled symbols. The mixture shows macroscopic phase separation below the curves.

Furthermore, the presence of ions in the solution may screen the charges of the polymer and influence the formation of complexes. Therefore, the two key parameters (i.e., pH and ionic strength) influencing the complexation between two oppositely charged polymers were varied systematically. Mixtures of WP and CG were acidified, and the turbidity of the mixtures was measured as a function of pH for various concentrations of NaCl (Cp = 0.25% and Pr:Ps = 15:1). **Figure 1** shows that the turbidity increased abruptly in the pH range of 5.5−5.2 for all samples. The blanks of WP or CG remained at a low turbidity over the whole pH range (not shown here). The pH at which the turbidity suddenly increased was defined as pH_ϕ. For pH < pH_ϕ, the mixtures became unstable and the complexes sedimented/precipitated to the bottom of the test tubes. The value of pH_ϕ varied with the [NaCl]. For [NaCl] < 45 mM, pH_ϕ shifted toward higher pH values and the maximum turbidity increased, showing an increase in number and/or size of the biopolymer complexes. A small addition of salt seemed thus to enhance the formation of complexes. Burgess mentioned that complexation could be reduced because of an unfavorable extended shape of the molecules at low salt (*37*). The result could also be explained if phase separation was a result of the charge compensation of the complexes. Below their pI, the proteins became net positively charged and bound to the sulfate groups of the CG. However, the charge density of CG is so high that electroneutrality of the complex is not fully achieved by the WP only (possibly because of a spatial packing problem). Therefore, if small microions are present in the solution, they will screen the residual negative groups of CG and thus effectively reduce repulsion between complexes and allow an effective phase separation. When the concentration of NaCl was higher than 45 mM, the pH_ϕ shifted to lower values, and for [NaCl] > 1 M, no phase separation occurred. The influence of salt as being unfavorable to complexation is a well-known phenomenon and was already reported in the 1940s (*38*). A large ionic strength is known to reduce electrostatic interactions by screening the charges of the biopolymers. Therefore, at [NaCl] > 1 M, the complexes were not formed. Between 45 mM and 1 M, complexes could be formed at more acidic pH_ϕ values, corresponding to a pH where the WP carried more charges. The interaction of ions with the complex will be described below.

The type of microion present in the solution was studied by comparing the effect of NaCl and $CaCl_2$ on the complex formation of WP and CG. The valency of the microions had already been reported as an important parameter in biopolymer interactions (*37*). Acid titration was carried out on mixtures of WP/CG at a Cp = 0.25%, and Pr:Ps = 15:1 for NaCl and $CaCl_2$ in the range of ionic strength 0−400 mM. Two different sources of CG were compared as follows: type I and type II. From the acid titration, the value of pH_ϕ was determined (as in **Figure 1**) for each sample (in duplicate). The average value of pH_ϕ is plotted in **Figure 2** for mixtures of WP/type I CG and WP/type II CG at various ionic strengths (using NaCl or $CaCl_2$). The values of pH_ϕ varied with the ionic strength but were similar for both CG batches. When NaCl was added, the complexes were formed at pH values near or below the pI of the protein with a slightly higher pH_ϕ at [NaCl] = 45 mM, as was discussed above. On the other hand, the addition of $CaCl_2$ showed that complexes were formed at pH values higher than the pI. For [$CaCl_2$] = 45−140 mM, complexes were formed around pH 8. Blanks with only WP or CG remained clear and stable, showing that the complexes were formed at high pH only in the presence of a mixture of WP/CG. This result suggested that another type of interaction occurred in the presence of $CaCl_2$. It was supposed that ion bridging took place between the divalent calcium ion (Ca^{2+}) and the negatively charged WP and CG molecules. An electrostatic complex at neutral pH occurs between κ-CG and κ-casein in the absence of specific cations (*39*). Complexation also occurs between κ-CG and α_{s1}-casein (*40*) or β-casein (*41*) but in the latter case only in the presence of Ca^{2+}, through the formation of calcium bridges.

Temperature. In general, a temperature change will influence the biopolymer/biopolymer interactions by changing the Flory−Huggins interaction energy. If other enthalpic interactions—in addition to the Coulombic interactions—would be involved, the temperature would also have an influence. Low temperatures favor hydrogen bond formation, and hydrophobic interactions are enhanced by temperature increase. In this study, the turbidimetric titration was carried out at various temperatures in the range of 5−50 °C for three different samples. The values of pH_ϕ are presented as a function of temperature in **Figure 3**. In the range studied, temperature had a small effect on the complex formation. A slight increase of pH_ϕ could be noticed at low temperatures. Kaibara et al. studied the effect of temperature (*5*) on complexation of bovine serum albumin and cationic polyelectrolytes. They found that pH_c and pH_ϕ were not temperature-dependent. Here, it can be concluded that the primary interaction between WP and CG was thus mainly electrostatic in nature but may include entropic contributions, as described in the Overbeek and Voorn theory, due to the

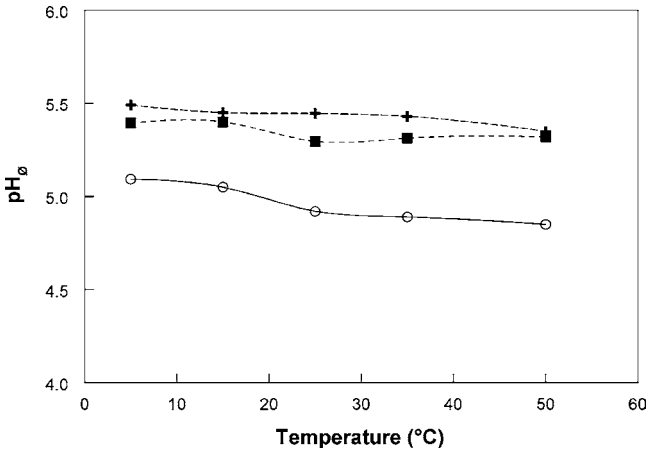

Figure 3. State diagram as a function of temperature of a mixture of WP/CG, Pr:Ps = 15:1. Cp = 0.25%, [NaCl] = 45 mM (+); Cp = 0.1%, [NaCl] = 45 mM (■); and Cp = 0.1%, [NaCl] = 0 mM (○).

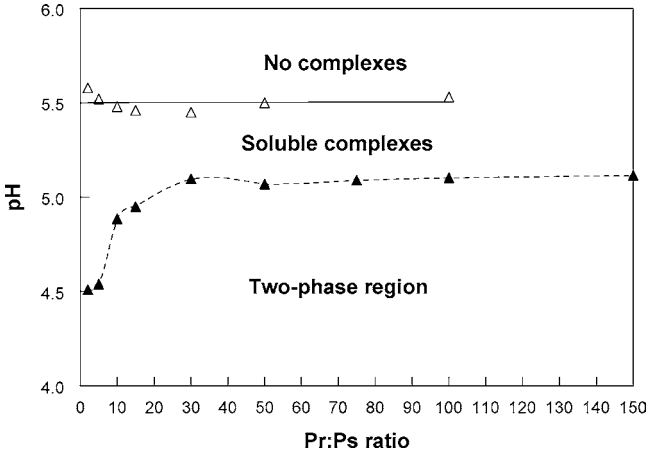

Figure 4. State diagram as a function of Pr:Ps of a mixture of WP/CG, [NaCl] = 0 mM, Cp = 0.1%, temperature = 25 °C; pH_ϕ (▲), pH_c (△).

release of counterions (*42*). Indeed, adding large amounts of salt suppressed the complexation of primary complexes. Recent work by Girard et al. reported that from the binding isotherms of the pectin to the β-lg, the formation of two kinds of complexes has been evidenced (*43*). The first ones were soluble intrapolymeric complexes, whose formation was driven by enthalpy gain (binding stoichiometry of 6−8). The second ones were based on the aggregation of the previous ones and were mostly controlled by entropy (binding stoichiometry of 15−16.5).

Pr:Ps. The amount of WP molecules available per polysaccharide chain is obviously of importance in the electrostatic complex formation. There is at each pH a specific ratio for which electroneutrality of the complex is reached (*13*). Here, a mixture of WP/CG at Cp = 0.1% and without any addition of NaCl was studied at various Pr:Ps ratios. For each Pr:Ps ratio, acid titration coupled to turbidity and light-scattering measurements allowed the determination of pH_ϕ and pH_c. pH_c was defined as the pH at which soluble complexes were formed. The value of the pH_c could be found by performing light-scattering measurements under slow acidification (more sensitive than turbidity measurements). The values of pH_c and pH_ϕ are plotted in a state diagram as a function of the Pr:Ps ratio in **Figure 4**. Above pH_c, the polymers were negatively charged and thus did not interact. Already at pH > pI of the WP (pI β-lg = 5.2), there was a strong interaction as pH_c > pI. The probable reason was the presence of positive patches and in addition charge fluctuations of the protein near the pI (*4*, *11*, *17*). Then, below pH_ϕ, insoluble complexes were formed and phase separation occurred. The pH_c remained constant around pH 5.5 for all ratios. This result suggested that the formation of soluble complexes occurred between a single polyelectrolyte chain and a given amount of proteins. On the other hand, pH_ϕ increased up to a Pr:Ps = 30:1, where pH_ϕ then stabilized. The dependence of pH_ϕ on the Pr:Ps ratio could be explained if phase separation was induced by the aggregation of the soluble complexes, which reached electroneutrality. Indeed, the charge compensation of the WP/CG occurred at a higher pH if more protein molecules were available per polysaccharide chain (larger Pr:Ps). For Pr:Ps > 30:1, the pH_ϕ remained stable, indicating that saturation of the CG had probably taken place. A saturation ratio of 30:1 (w/w) corresponds to a Pr:Ps ratio of 1300:1 (mol/mol), meaning that 1300 molecules of WP would be complexed to one CG chain at pH 5.1.

These results are qualitatively similar to previous results on complex formation of WP/gum arabic and WP/EPS B40 (*14*, *16*). Gum arabic carries carboxylic groups and EPS B40 phosphate groups. The ζ-potential of the CG is two times larger than the ζ-potential of the EPS B40 and three times larger than the ζ-potential of the gum arabic. Indeed, we found that EPS B40 bound less WP than CG and gum arabic less still. Therefore, the interaction between WP and CG is much stronger than for the other two polysaccharides, and pH_c (pH_c 5.5) is also higher than the pH_c of EPS (pH_c 5.3) and gum arabic (pH_c 5.2). Nevertheless, the influence of parameters such as ionic strength, Pr:Ps ratio, and pH is qualitatively similar for all of the systems studied.

Then, WP/CG complexes were formed differently by adding WP slowly into the CG mixture at pH 3 and pH 4 for [NaCl] = 0 mM and [NaCl] = 45 mM. The formation of the complexes was monitored by turbidity measurement as a function of the Pr:Ps ratio. **Figure 5a** shows that the turbidity increased faster at pH 3 than at pH 4 and at [NaCl] = 45 mM than at [NaCl] = 0 mM. These results were very consistent with previous findings. At pH 3, the WP carried more charges and the electrostatic interaction was thus enhanced as compared to pH 4. From results described above, it was also demonstrated that a salt concentration of 45 mM enhanced the formation of complexes, shifting the pH_ϕ to higher pH values (see **Figures 1** and **2**). However, during the experiment, the pH and the conductivity of the mixtures were monitored in parallel and the results, plotted in **Figure 5b**, were surprising. Indeed, if WP was slowly added into a CG mixture of the same pH, the pH of the mixture increased and the conductivity decreased. The pH increased up to +0.3 pH unit for mixtures at pH_{ini} 3 and +1.4 pH units at pH_{ini} 4 for mixtures at [NaCl] = 0 mM. When NaCl was added to the sample, the pH increase was less dramatic. This experiment revealed that when complexes were formed, in the case of low Pr:Ps, the complexes bound the ions available in the mixture. If NaCl was present, then Na^+ ions were incorporated in the complexes. However, if the amount of positive ions was too limited, the H^+ ions were trapped within the complexes, leading to a pH increase of the mixture. Furthermore, if extra WPs were added (higher Pr:Ps ratio), then the complexes incorporated the WP and released the positive ions, the pH slowly decreased and finally reached its initial value, and the conductivity of the sample became close to the conductivity of the WP mixture (corresponding to a change in conductivity of −325 μS/cm at [NaCl] = 0 mM pH 3 and −290 μS/cm at [NaCl] = 45 mM pH 4). The ion uptake seemed to be needed to screen the excess negative charges of the CG. Similar results

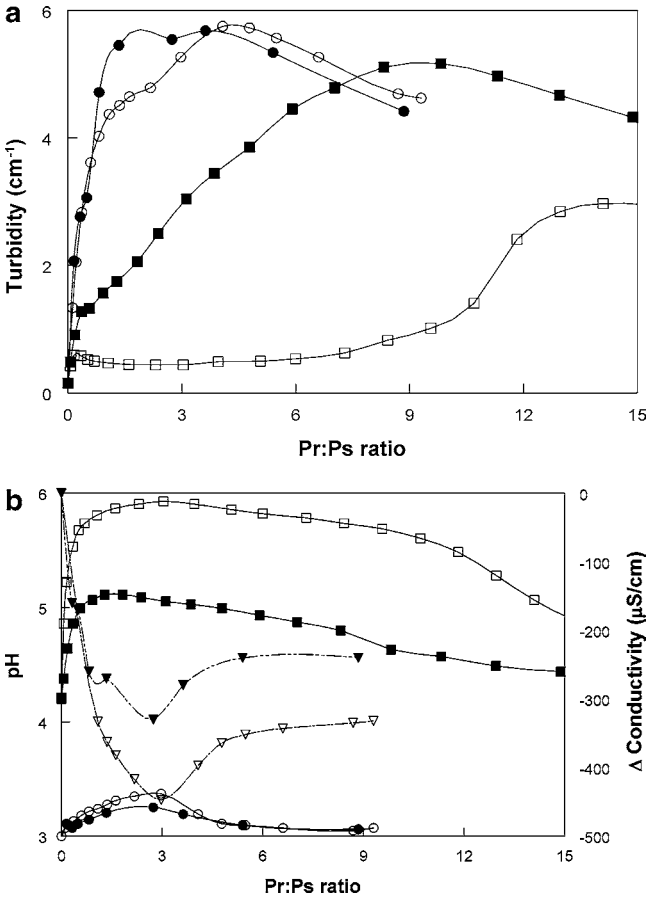

Figure 5. (**a**) Turbidity of a WP/CG mixtures as a function of Pr:Ps ratio, Cp = 0.25%, temperature = 25 °C. pH 3, [NaCl] = 0 mM (○); pH 3, [NaCl] = 45 mM (●); pH 4, [NaCl] = 0 mM (□); pH 4, [NaCl] = 45 mM (■). (**b**) pH and conductivity variation as a function of Pr:Ps ratio, Cp = 0.25%, temperature = 25 °C. pH_{ini} 3, [NaCl] = 0 mM (○) (▽); pH_{ini} 3, [NaCl] = 45 mM (●) (▼); pH_{ini} 4, [NaCl] = 0 mM (□); pH_{ini} 4, [NaCl] = 45 mM (■). pH evolution (■, □, ●, ○); evolution of conductivity (▽, ▼).

were already reported in the case of xanthan/chitosan complex gel by Ikeda et al. (*44*); the main reason of this phenomenon was described as a tendency to increase a number of ionic pairs between reacting polyelectrolytes. In the particular case of WP/CG studied here, it might be possible that the protons were taken up by the WP, as it would lead to an increase of its positive net charge. By adding further WP, the buffer capacity of the mixture was increased, and more WP molecules were able to react with the CG, and the pH returned to its initial value.

CONCLUSIONS

The formation of WP/CG complexes resulted from electrostatic interactions. The slight effect of temperature on the pH formation of insoluble complexes highlighted that the interaction was mainly Coulombic in nature. Whether soluble or insoluble complexes were formed depended on various parameters such as pH, ionic strength, and Pr:Ps ratio. In the presence of calcium ions, complexes could be formed up to neutral pH via calcium binding. If the system was supplemented with 45 mM NaCl, the formation of insoluble complexes was enhanced and complexes were formed close to and below the pI of the protein. CG being a highly negatively charged molecule, the presence of microions promoted the formation of electroneutral complexes by screening the residual negative charges of the complex. If salt was present in an insufficient quantity, the complexes incorporated the protons, which resulted in a pH increase of the mixture. Comparing the results to previous work done with carboxylated and phosphated polysaccharide, it seems justified to conclude that the intensity of the WP/polysaccharide interaction correlated with the ζ-potential of the polysaccharide, CG > EPS B40 > gum arabic, which paralleled the stoichiometry of the complexes.

ABBREVIATIONS USED

α-la, α-lactalbumin; β-lg, β-lactoglobulin; Cp, total biopolymer concentration; DLS, dynamic light scattering; EPS B40, exocellular polysaccharide B40; GDL, glucono-δ-lactone; CG, carrageenan; NMR, nuclear magnetic resonance; pH_c, pH at which soluble complexes are formed; pH_ϕ, pH at which insoluble complexes are formed; pH_{ini}, initial pH; pI, isoelectric point; Pr:Ps, protein-to-polysaccharide ratio; τ, turbidity; WP, whey protein.

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