Rheology and Microstructure of β-Lactoglobulin/Sodium Polypectate Gels

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Thermally induced β-lactoglobulin (β-Lg) and sodium polypectate (SPP) composite gels or coagulum were formed at pH 6.5 and 3.5 and evaluated by dynamic rheological techniques and transmission electron microscopy (TEM). At pH 6.5, SPP-induced β-Lg gelation, resulting in the formation of opaque β-Lg/SPP and transluscent β-Lg/SPP/$CaCl_2$ gels. Co-gelation of β-Lg and SPP increased the storage modulus (G') of β-Lg/SPP/$CaCl_2$ gels during cooling, whereas ungelled SPP prevented further increases in G' of β-Lg/SPP gels. Irrespective of calcium cations, β-Lg and SPP interacted at pH 3.5 to form a grainy white precipitate that coagulated on heating. At pH 6.5, TEM micrographs revealed a nonuniform distribution of β-Lg aggregates in β-Lg/SPP gels as opposed to a homogenuous β-Lg/SPP/$CaCl_2$ gel matrix. At pH 3.5, however, micrographs indicated the formation of a curd-like β-Lg/SPP coagulum that did not form a cohesive network structure. Overall, there is a potential for using pectates or other anionic polysaccharides to modify the gelling properties of whey proteins and subsequently expand whey utilization in diverse food applications.

Keywords: *β-Lactoglobulin; sodium polypectate; gels; rheology; microstructure*

INTRODUCTION

The individual gelling properties of proteins and polysaccharides are important in providing unique textural qualities that influence consumer acceptability of many food products. Depending on processing conditions, gels possessing a wide variety of sensory attributes can be formed from a protein−polysaccharide composite system (Moritaka et al., 1980; Clark et al., 1982, 1983; Watase and Nishinari, 1983; Tolstoguzov, 1986).

The structural and rheological properties of gels formed from protein−polysaccharide mixtures are dependent on thermodynamic and structural compatibility between both macromolecules (Braudo et al., 1986; Tolstoguzov, 1986). Depending on experimental conditions, both macromolecules can gel separately in a single phase (mixed gel) or one of the macromolecules gels and the other component is dispersed as a filler (filled gel). In some cases, however, salt linkages between both macromolecules can result in the formation of complex gels. Tolstoguzov (1986) reported that sodium alginate and sodium caseinate are capable of forming complex gels under conditions where both macromolecules do not gel. In a gelatin−kappa carrageenan system, strong electrostatic forces between both macromolecules inhibited gel formation (Watase and Nishinari, 1983). Overall, the gelation properties of proteins can be modified or controlled through interactions with polysaccharides.

The overabundance of whey proteins, a byproduct of cheese processing, has made whey utilization of major environmental and economic significance to the dairy industry. Whey proteins are nutritive and functional proteins that form thermally induced gels depending on pH, salt concentration, and salt type (Mulvihill and Kinsella, 1987). β-Lactoglobulin (β-Lg), the principal whey protein, plays a major role in influencing the gelling behavior of whey proteins (Kinsella, 1984; Mulvihill and Kinsella, 1987).

Although the gelling properties of β-Lg and low-methoxy pectin (LMP) are well documented, the utilization of both macromolecules as gelling ingredients in a composite system has not been reported. The potential of utilizing LMP in a composite system with β-Lg to form gels possessing a variety of textural attributes would not only expand whey protein utilization but would also open new avenues for fabricating protein-enriched foods, such as jams and jellies or meat analogs. The objective of this research was to evaluate and characterize the rheological and microstructural attributes of gels formed from a composite system of β-Lg and the LMP sodium polypectate (SPP) at selected pH values with and without calcium cations.

MATERIALS AND METHODS

Materials. Purified β-Lg containing genetic variants A and B, and sodium polypectate were purchased from Sigma Chemical Company (St. Louis, MO). Stock solutions of 20% (w/v) β-Lg were prepared in deionized water with and without 30 mM $CaCl_2$. The pHs of the solutions were adjusted to 3.5 or 6.5 with 1 M NaOH or HCl. Equal volumes of either 2% (w/v) SPP or deionized water were mixed with the protein stock solutions, resulting in the following treatments at both pH values: β-Lg (10%), β-Lg (10%)/SPP (1%), and β-Lg (10%)/SPP (1%)/15 mM $CaCl_2$. Solutions for each treatment were divided into two portions: one portion was used for rheological measurement and the other portion was used for microstructural evaluation. β-Lactoglobulin solutions (10%) containing 25 mM NaCl were also evaluated for rheological and microstructural attributes.

Preparation of Solutions for Viscosity Measurements. Stock solutions of β-Lg (2%), with and without 3 mM $CaCl_2$, and 0.2% SPP solutions were prepared in deionized water. The pH of the stock solutions was adjusted to 6.5, and the following treatments were prepared: β-Lg (1%), β-Lg (1%)/SPP (0.1%), β-Lg (1%)/1.5 mM $CaCl_2$, SPP (0.1%), SPP (0.1%)/1.5 mM $CaCl_2$, and β-Lg (1%)/SPP (0.1%)/1.5 mM $CaCl_2$. A portion of solution from each treatment was heated to 90 °C and then cooled to 20 °C at 1 °C/min. To minimize surface gelation and lump formation during preparation of solutions containing SPP and calcium cations, SPP should be added to calcium-containing solutions and not vice versa.

Gel Preparation for Rheological Measurements. Gels were formed by heating β-Lg and SPP solutions in situ between

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0021-8561/96/1444-0086$12.00/0

the parallel plates of a Physica VM5 Rheometer (Physica USA Inc., Spring, TX) from 25 to 95 °C at 1 °C/min, and then equilibrating at 95 °C for 5 min. The heat-treated solutions were then cooled to 25 °C at a rate of 1 °C/min. The gap between the plates was set at 0.3 mm. Paraffin oil was layered over the exposed liquid (confined by an O-ring glued to the lower plate) to prevent evaporation during heating. To prevent intermixing between paraffin oil and test solutions and subsequent effect on measurements, drops of paraffin oil must be layered slowly and carefully on the exposed solution, and test solutions must be preheated to 60 °C before oscillatory measurements are taken.

Viscosity Measurements. Flowcurves of unheated and heated β-Lg and SPP solutions were obtained at 20 °C with a Physica VM5 rheometer (Physica USA) equipped with a concentric cylindrical Z1 DIN measuring system. Viscosity measurements were obtained under controlled shear rate conditions (100−300 s^{-1}) within a time interval of 2 min. Duplicate viscosity measurements were made on treatments obtained from two separate preparations.

Gel Preparation for Microstructural Evaluation. The solutions used for microstructural evaluation were placed in cylindrical screw-capped molds, and gels were formed with the same heating and cooling regime as described for rheological measurements.

Measurement of Rheological Properties of Gels. Viscoelastic characteristics of gels were evaluated by measuring storage modulus (G') and loss modulus (G'') as a function of temperature. Rheological measurements were made at a frequency of 10 rad s^{-1}, with a strain amplitude of 0.02 within the linear viscoelastic range of the gels (Mitchell, 1980). Storage and loss modulus values were recorded during heating and cooling.

Gel Microstructure. The microstructural properties of gels were examined by transmission electron microscopy with the preparation and fixation procedures of Clark et al. (1982) with minor changes. Ante-medium propylene oxide and araldite epoxy resin were substituted for acetone and Spurr's resin, respectively.

Statistical Analyses. Gels were prepared from three separately prepared solutions. Triplicate measurements of storage and loss moduli were made for each gel. Statistical significance of viscosity and loss and storage moduli means was evaluated by analysis of variance (SAS, 1988). Means were separated by Duncan's multiple range procedure.

RESULTS AND DISCUSSION

Rheological Measurements. At 20 °C, calcium cations did not influence the viscosity of β-Lg solutions but significantly increased the viscosity of SPP solutions (Figure 1a). Thickening of pectate solutions resulting from calcium−pectate interactions is well documented (Mitchell and Blanshard, 1976; Rees, 1982; Garnier et al., 1991). SPP did not affect the viscosity of unheated β-Lg solutions (Figure 1a), indicating that the significantly higher viscosity of β-Lg/SPP/$CaCl_2$ solutions compared with that of β-Lg/SPP solutions, at low shear rates (<200 s^{-1}), was due to calcium−pectate interactions. Reduction in the viscosity of SPP/$CaCl_2$ solutions by addition of β-Lg was possibly related either to β-Lg−calcium interactions that reduced the amount of calcium cations available to pectate or to interactions between β-Lg and SPP that interfered with the formation of calcium−pectate linkages. Although both possibilities are plausible, the comparable resistance of β-Lg/SPP/$CaCl_2$ and SPP/$CaCl_2$ solutions to shear thinning suggested that β-Lg did not impair the formation of calcium−pectate linkages.

Viscosity profiles of heat-treated β-Lg and SPP solutions (Figure 1b), with and without $CaCl_2$, indicated that the viscosities of β-Lg/$CaCl_2$, β-Lg/SPP/$CaCl_2$, and SPP/$CaCl_2$ solutions upon cooling were significantly higher

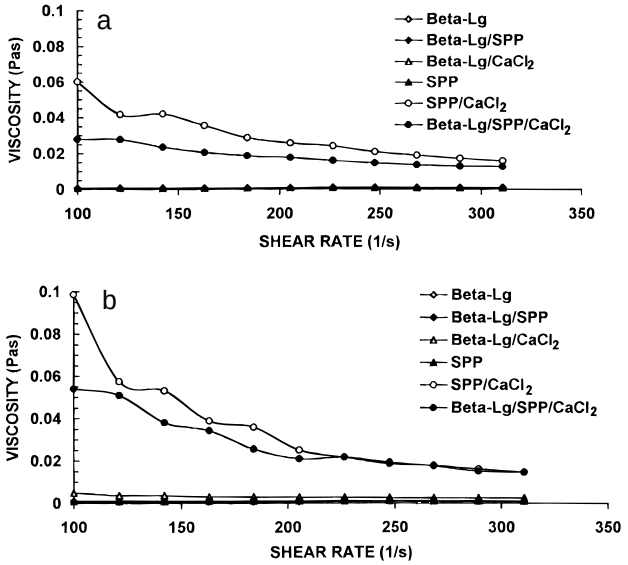

Figure 1. (a) Viscosity profiles of β-lactoglobulin, sodium polypectate, and calcium chloride mixtures at 20 °C. (b) Viscosity profiles of β-lactoglobulin, sodium polypectate, and calcium chloride mixtures after heating to 90 °C.

than those of their unheated counterparts (Figure 1a). Increase in the viscosity of β-Lg/$CaCl_2$ upon cooling was evidence for heat-induced interactions between β-Lg and calcium chloride. Heating did not have a profound effect on the viscosity of β-Lg/SPP solutions. Increase in viscosity upon cooling was observed only for β-Lg/$CaCl_2$ and SPP/$CaCl_2$, but not β-Lg/SPP solutions. This finding indicated that thickening of β-Lg/SPP/$CaCl_2$ solutions on cooling was due to both β-Lg−calcium and SPP−calcium interactions.

Changes in the value of G' of β-Lg/SPP and β-Lg/SPP/$CaCl_2$ solutions during heating at pH 6.5 are shown in Figure 2. During heating and cooling, the trends in G'' and G' were identical except that the magnitude of G'' was always smaller than that of G'. No change in G' was observed for β-Lg solutions, whereas the G′ of β-Lg/SPP and β-Lg/SPP/$CaCl_2$ solutions increased drastically during heating (Figure 2). The increase in G' of protein as well as protein−polysaccharide solutions during heating is indicative of gel formation (Clark et al., 1982, 1983). The storage modulus of β-Lg did not change during heating, indicating that 10% β-Lg solutions did not gel at pH 6.5. In the presence of 25 mM NaCl, however, gel formation was evident (Figure 2).

The inability of β-Lg solutions to form heat-induced gels was also reported by Mulvihill and Kinsella (1988) and Foegeding (1993). Mulvihill and Kinsella (1988) reported that 10% β-Lg soultions did not gel at pH 8.0 except in the presence of sodium chloride (>25 mM) and calcium chloride (5−100 mM). Foegeding (1993) indicated that high protein concentrations or long heating times are required for β-Lg solutions to gel without added salts. Lack of gelation observed in β-Lg solutions in the absence of salts was attributed to increased repulsion among denatured β-Lg strands (Harwalkar and Kalab, 1985a,b). At high ionic strengths, salts provide counterions that reduce charge repulsion among denatured β-Lg strands and therefore enhance aggregation and subsequent gelation.

On the basis of our preliminary experiments, SPP did not form a network structure in the absence of calcium cations. This finding was consistent with viscosity results that indicated that the viscosity of SPP solutions was unaffected by heat treatment in the absence of

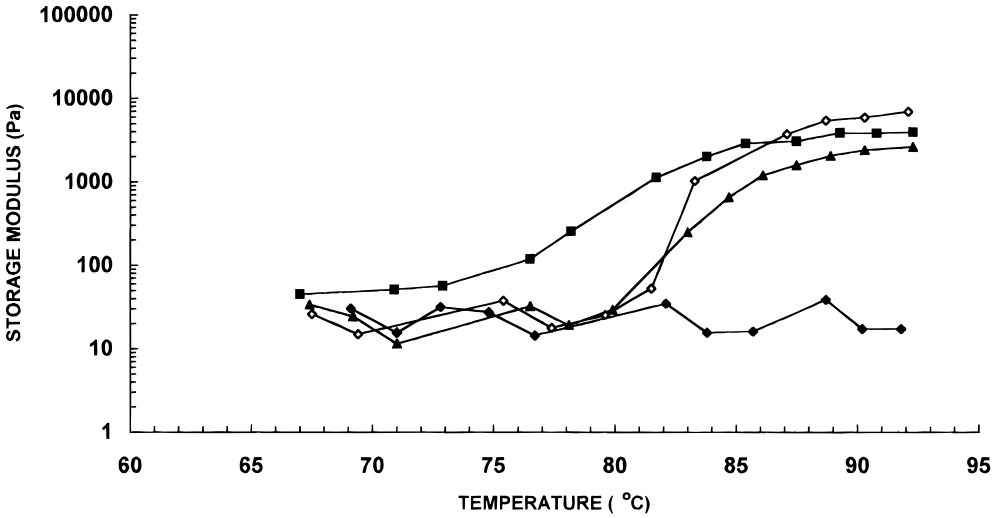

Figure 2. Changes in the storage modulus of β-Lg (◆), β-Lg/NaCl (◇), β-Lg/SPP (▲), and β-Lg/SPP/$CaCl_2$ (■) during heating at pH 6.5.

calcium cations (Figures 1a, b). Mixtures of β-Lg and SPP, however, formed a gel (Figure 2). Based on the gelling behavior of β-Lg in 25 mM NaCl (Figure 1) and the fact that β-Lg denatures at ~76 °C at pH 6.5 (De Wit and Klarenbeek, 1989), it was apparent that the sharp increase in storage modulus of β-Lg/SPP mixtures above 80 °C was due to β-Lg. Evidently, interactions between β-Lg and SPP resulted in gel formation.

Both β-Lg and SPP are predominantly negatively charged macromolecules at pH 6.5, so it was unlikely that SPP induced β-Lg gelation by reducing the magnitude of repulsive forces between denatured β-Lg polypeptide strands. Instead, it is plausible that steric and electrostatic interactions between β-Lg and SPP enhanced β-Lg aggregation, resulting in gel formation. Furthermore, the observation that SPP did not influence the viscosity of 1% β-Lg solutions (Figure 1b) but enhanced thermal gelation of 10% β-Lg (Figure 2) suggested that the magnitude of β-Lg/SPP interactions depended on bulk macromolecular concentration. This proposed hypothesis is consistent with the observation that, at pH 6.5, SPP enhanced the formation of heat-induced high molecular weight (HMW) β-Lg aggregates at high (5%) but not at low (1%) β-Lg concentrations (Ndi and Luedecke, 1995). Formation of HMW protein aggregates during heating of heparin (an anionic polysaccharide) and trehalose−phosphate synthetase mixtures at pH 7.5 was also reported by Elbein and Mitchell (1975).

Calcium lowered the onset temperature range of β-Lg/SPP gelation and increased the storage modulus (Figure 2). The ability of calcium cations to influence gelation of β-Lg/SPP mixtures was expected because calcium significantly increased the viscosity of β-Lg/SPP solutions after heating via calcium−pectate and calcium−β-Lg interactions. Calcium-induced gelation of both β-Lg and SPP gelation is well documented (Mitchell and Blanshard, 1978; Mulvihill and Kinsella, 1988). At low gelling temperatures (<85 °C), the storage modulus of β-Lg/SPP/$CaCl_2$ solutions was higher than that of β-Lg/SPP solutions. At higher gelling temperatures (85−95 °C), however, the storage modulus of β-Lg/SPP and β-Lg/SPP/$CaCl_2$ gels were not significanly different. Although gelation profiles of β-Lg/$CaCl_2$ and SPP/$CaCl_2$ solutions were not conducted, viscosity measurements suggested that calcium−pectate and not calcium−β-Lg interactions accounted for the high storage modulus observed for β-Lg/SPP/$CaCl_2$ at sub-gelling temperatures. The dominance of calcium−pectate interactions over calcium−β-Lg interactions in β-Lg/SPP/$CaCl_2$ at sub-gelling temperatures is consistent with the observation that calcium cations interact preferentially with pectates over proteins at low gelling temperatures (Hughes et al., 1980). At higher temperatures, however, the storage modulus of β-Lg/SPP/$CaCl_2$ gels, approached that of β-Lg/SPP gels indicating the progressive predominance of β-Lg/SPP or β-Lg/$CaCl_2$ interactions over SPP−calcium interactions during heating. The gelation profile of β-Lg/$CaCl_2$ solutions was not determined, so the relative impact of β-Lg−pectate and β-Lg−calcium interactions on the storage modulus of β-Lg/SPP/$CaCl_2$ gels at gelling temperatures was not determined. The minimal effect of calcium−pectate interactions on the storage modulus of β-Lg/SPP/$CaCl_2$ gels at gelling temperatures was supported by the observation that the viscosity of SPP/$CaCl_2$ solutions at 20 (0.06 Pa s) and at 90 °C (0.056 Pa s) was not significantly different. In addition, studies by Garnier et al. (1991) indicated that the storage modulus of SPP/$CaCl_2$ or the magnitude of calcium−pectate interactions decreased with increasing temperature.

Changes in G' for β-Lg/NaCl, β-Lg/SPP, and β-Lg/SPP/$CaCl_2$ gels during cooling are shown in Figure 3. The G' of β-Lg/SPP gels did not change significantly after cooling to 25 °C, but the G' of β-Lg/SPP/$CaCl_2$ and β-Lg/NaCl gels increased significantly. Development of rigidity in β-Lg/SPP/$CaCl_2$ gels during cooling is consistent with the observations by Clark et al. (1982), who also reported increases in G' of bovine serum albumin (BSA)/agar gels during cooling. In protein gels, increase in gel rigidity during cooling is attributed to the reorientation and rearrangement of denatured polypeptide chains. Without calcium cations, ungelled SPP present in the β-Lg/SPP gel matrix possibly interfered with the reorientation and rearrangement of denatured β-Lg polypeptide chains and prevented further increases in G' of β-Lg/SPP gels during cooling. Differences in the storage modulus curves for β-Lg/NaCl and β-Lg/SPP during cooling indicated apparent differences in the mechanism of NaCl- and SPP-induced gelation of β-Lg. In β-Lg/SPP/$CaCl_2$ gels, increase in storage modulus during cooling possibly resulted from interactions between SPP and calcium cations that resulted in the formation of an additional calcium−SPP network struc-

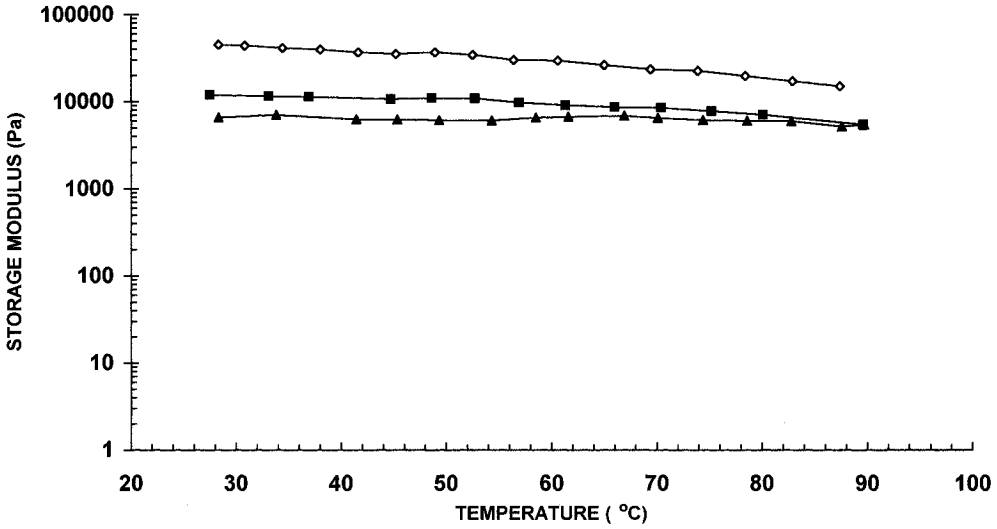

Figure 3. Changes in the storage modulus of β-Lg/NaCl (◇), β-Lg/SPP (▲), and β-Lg/SPP/$CaCl_2$ (■) during cooling at pH 6.5.

Table 1. Visual Assessment of Heat-Treated β-Lg, β-Lg/SPP, and β-Lg/SPP/$CaCl_2$ Mixtures at pH 6.5 and 3.5

sample mixture	pH 6.5	pH 3.5
β-Lg	no gel	weak transparent gel
β-Lg/NaCl	transparent smooth gel	slightly turbid smooth gel
β-Lg/SPP	opaque (milky white) smooth gel	opaque grainy coagulum + clear less viscous phase
β-Lg/SPP/$CaCl_2$	translucent coarse gel	opaque grainy precipitate + clear viscous phase

ture. Formation of additional calcium−pectate linkages during cooling was supported by the observation that the viscosity of SPP/$CaCl_2$ solutions increased significantly upon cooling (Figure 1a,b). Co-gelation of β-Lg and SPP subsequently led to a further increase in the G' of β-Lg/SPP/$CaCl_2$ gels during cooling. The possibility of calcium cations crosslinking both macromolecules and forming additional linkages that would also increase the storage modulus of β-Lg/SPP/$CaCl_2$ during cooling cannot be disregarded.

At pH 3.5, gelation of 10% β-Lg was not observed during heating. On cooling from 90 to ~70 °C, however, G' increased from 40 to 500 Pa, indicating the formation of a weak network structure. The storage modulus of 500 Pa did not change on cooling the β-Lg solutions from 70 to 20 °C. Heating of β-Lg/SPP solutions at pH 3.5 did not result in the formation of a cohesive gel network. Instead, a curd-like coagulum or β-Lg/SPP complex, distinctly separated from a clear upper solution, was formed. Complex formation between β-Lg and SPP at pH 3.5, detrimental to gelation, was attributed to electrostatic interactions between positively charged β-Lg and negatively charged SPP molecules. In a gelatin/kappa-carrageenan system, strong electrostatic interactions between both macromolecules were also reported to prevent aggregation of gelatin helices and subsequently inhibited gelation (Watase and Nishinari, 1983). Addition of calcium ions to the β-Lg/SPP mixtures did not significantly improve gelation, although the viscosity of the clear solution phase was increased.

Visual Assessment. The visual appearances of heat-treated β-Lg, β-Lg/SPP, and β-Lg/SPP/$CaCl_2$ mixtures were remarkably different (Table 1). Evidently, SPP modified the texture and appearance of β-Lg gels with and without calcium cations. Whereas β-Lg/NaCl gels at pH 6.5 were transparent, β-Lg/SPP gels were opaque. Visual differences between β-Lg/NaCl and β-Lg/SPP gels at pH 6.5 further suggested differences in the mechanism of NaCl- and SPP-induced β-Lg gelation. On a broader perspective, variations in the visual appearance of heat-treated β-Lg, β-Lg/SPP, and β-Lg/SPP/$CaCl_2$ mixtures indicated the potential of using SPP to modify the gel appearance of whey proteins and possibly open new avenues for whey utilization.

Microstructural Evaluation. Micrographs of β-Lg/NaCl and β-Lg/SPP gels at pH 6.5 are shown in Figure 4. Visually, β-LG/SPP gels appeared opaque, whereas β-Lg/SPP/$CaCl_2$ gels were translucent. To provide additional insights on the relationship between gel microstructure and gel clarity, transparent β-Lg gels formed in 25 mM NaCl (β-Lg/NaCl gels) were also evaluated. Earlier studies have also shown that relatively transparent β-Lg gels are formed in the presence of low concentrations (25−150 mM) of monovalent cations (Mulvihill and Kinsella, 1988; Foegeding, 1993). The microstructure of transparent β-Lg/NaCl gels (Figure 4a) was homogeneous and characterized by a uniform distribution of fine protein aggregates densely associated in the gel matrix. Opaque β-Lg/SPP gels, however, displayed a nonhomogeneous gel microstructure consisting of distinct clusters of protein aggregates (Figure 4b,d). Differences in the microstructure of β-Lg/NaCl and β-Lg/SPP gels not only confirm that gelation of β-Lg in β-Lg/SPP mixtures was induced by SPP but also indicate that the mechanism of salt-induced gelation of β-Lg differs from SPP-induced gelation In the presence of calcium ions, homogeneity of gel microstructure was restored, resulting in the formation of translucent β-Lg/SPP/Ca gels (Figure 4c).

Research on the microstructure of turbid and transparent BSA protein gels was also reported by Clark et al. (1981). Turbid BSA gels (pH 6.5, 125 mM NaCl) were characterized by long-range density fluctuations of protein aggregates, whereas transparent BSA gels, formed in the absence of NaCl, possessed a finer and more uniform distribution of protein aggregates. Results from the present study and observations by Clark et al. (1981) indicate that gel clarity is related to the homogeneity and aggregate size of the gelled matrix.

In a composite system consisting of β-Lg, SPP, and calcium ions, the homogeneity of the gel microstructure depended on the interactions that occurred among the

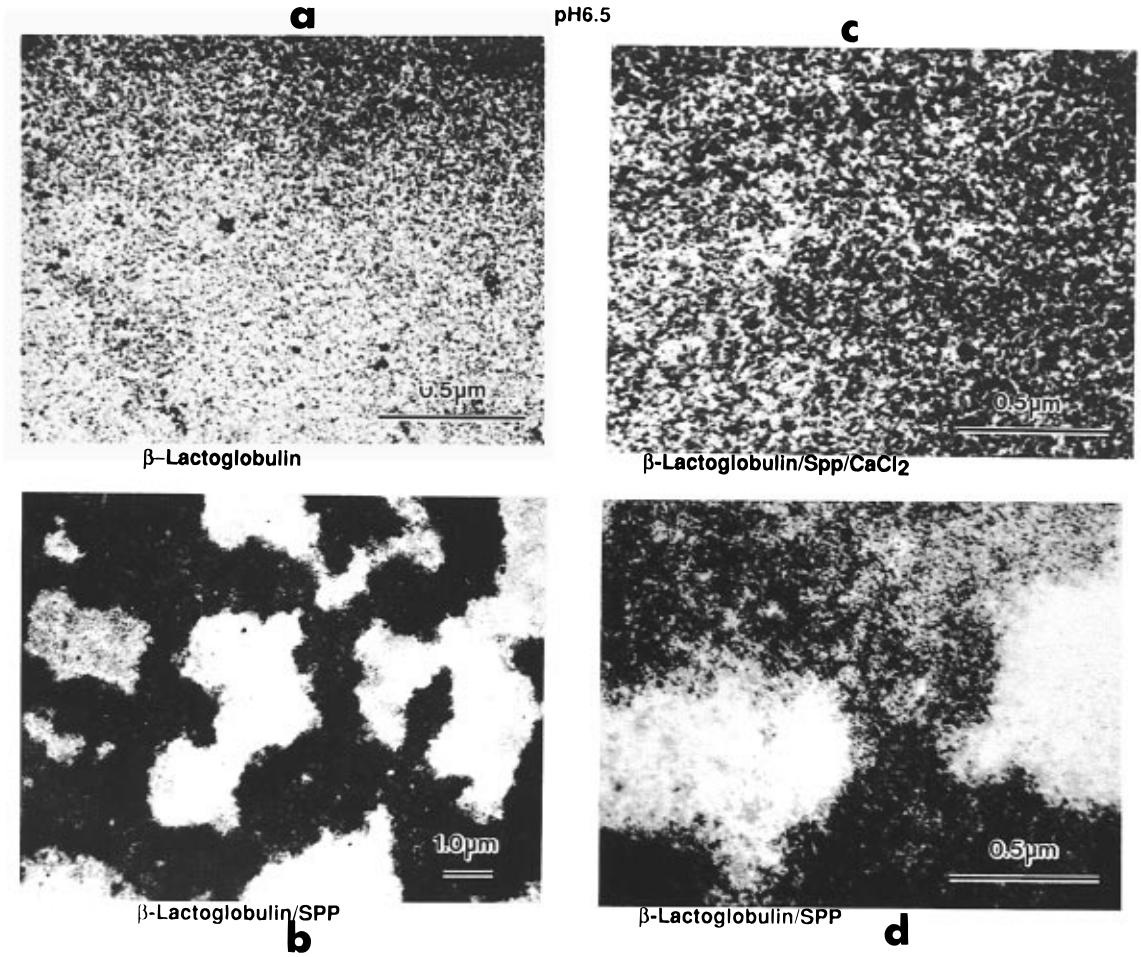

Figure 4. Gel microstructure at pH 6.5: (a) β-Lg/NaCl (60000×); (b) β-Lg/SPP (10000×); (c) β-Lg/SPP/ $CaCl_2$ (60000×); and (d) β-Lg/SPP (60000×). (Figure is reproduced at 75% of its original size.)

constituents of the system. At pH 6.5, the net charge on both SPP and β-Lg (pI = 5.3) is negative. Electrostatic and steric repulsive forces between both macromolecules at pH 6.5 probably resulted in β-Lg molecules aggregating in clusters (Figure 4b,d). Because SPP does not gel in the absence of calcium ions, the presence of ungelled SPP dispersed in a nonuniform distribution β-Lg aggregates possibly accounted for the opaque appearance of β-Lg/SPP gels.

The effectiveness of calcium in redispersing β-Lg aggregates (Figure 4c) clearly indicated that electrostatic interactions played a major role in β-Lg aggregation in the presence of SPP at pH 6.5. Calcium cations can bind to negatively charged groups on both SPP and β-Lg and reduce charge repulsion between both macromolecules, thus promoting homogeneity of gel microstructure. Translucency of β-Lg/SPP/$CaCl_2$ gels can therefore be attributed to gelled SPP entrapped in a uniform matrix of large protein aggregates.

Based on the microstructure of β-Lg/SPP (Figure 4b,d), a mechanism for SPP-induced β-Lg gelation is proposed. Without calcium cations, repulsive forces between β-Lg and SPP resulted in nonuniform or clustered aggregation of β-Lg molecules. Clustered aggregation of β-Lg indicated that repulsive forces between β-Lg and SPP increased the local concentration of β-Lg, thus enhancing aggregation during heating. This proposed mechanism is in agreement with the fact that by increasing the concentration of β-Lg, gelation occurred without salts being added (Foegeding, 1993).

The microstructure of slightly turbid β-Lg/NaCl gels formed at pH 3.5 was characterized by a uniform distribution of fine-grained protein aggregates (Figure 5a). At pH 3.5, the net charge on β-Lg is positive, whereas that on SPP is negative. Strong attractive forces between β-Lg and SPP resulted in the formation of a white β-Lg/SPP precipitate that coagulated but did not gel on either heating or cooling. Strong electrostatic attractive forces between gelatin and kappa-carrageenan have also been reported to result in precipitate formation that inhibited gelation (Watase and Nishinari, 1983). Evaluation of β-Lg/SPP "gel" microstructure at pH 3.5 revealed a nonuniform distribution of a grainy-type coagulum surrounded by loose β-Lg aggregates (Figure 5c). The presence of loose β-Lg aggregates indicated an excess of β-Lg over SPP in the system and is consistent with the observation that insoluble complexes of globular proteins with anionic polysaccharides contain an excess of protein (Tolstoguzov, 1986).

Phase separation was observed in β-Lg/SPP/$CaCl_2$ gels at pH 3.5; a clear viscous upper phase and an opaque "gelled" lower phase (Figure 5b,d). The viscous upper phase had a lower density of protein aggregates (Figure 5b) compared with the "gelled" lower phase (Figure 5d). A higher concentration of densely aggregated β-Lg molecules in the lower than in the upper phase indicated that complex formation between β-Lg and SPP was predominant in the lower phase. Visual assessement of the heat-treated β-Lg/SPP/$CaCl_2$ mixtures supported microstructural evaluations because an opaque grainy white precipitate, characteristic of β-Lg/SPP complexes, was observed only in the lower phase (Table 1) but not in the upper phase.

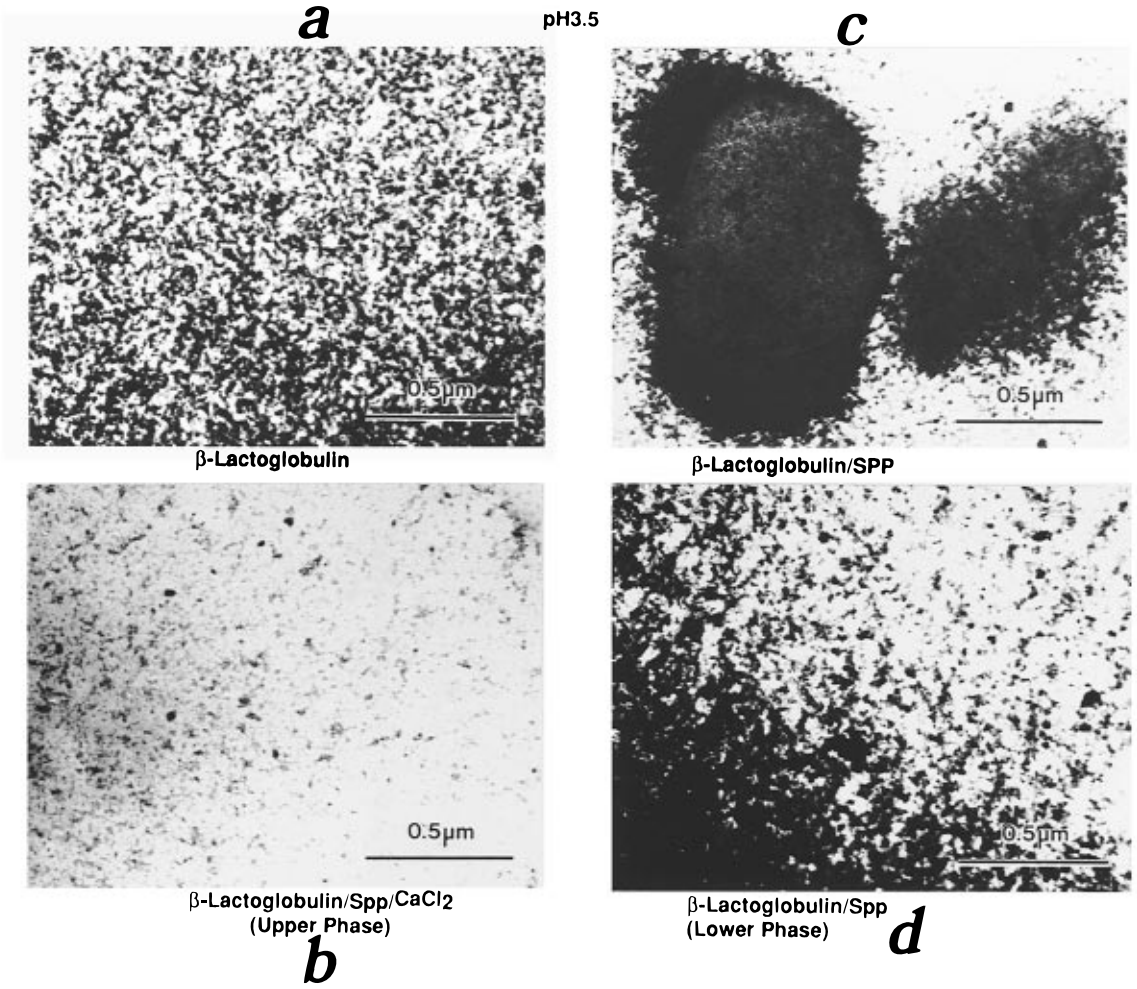

Figure 5. Gel microstructure at pH 3.5: (a) β-Lg (60000×); (b) β-Lg/SPP/$CaCl_2$ (60000×, upper phase); (c) β-Lg/SPP (60000×); and (d) β-Lg/SPP/ $CaCl_2$ (60000×, lower phase). (Figure is reproduced at 75% of its original size.)

The calcium-binding capacity of β-Lg is negligible at pH values <5.0 (Patocka and Jelen, 1991), indicating that calcium cations would bind preferentially to pectate. Apparently, interaction between calcium and SPP reduced the magnitude of complex formation between β-Lg and SPP in the lower phase (Figure 5d). Consequently, no distinct coagulum was observed in the microstructure of β-Lg/SPP/$CaCl_2$ mixtures as opposed to that present in β-Lg/SPP mixtures (Figure 5c).

Although the macromolecular composition of the upper and lower phases was not determined, microstructural evaluation revealed that a lower concentration of β-Lg, SPP, and calcium ions was present in the upper viscous phase than in the "gelled" lower phase (Figure 5b, d). This observation is consistent with reports indicating that phase separation in protein−anionic polysaccharide mixtures results in one of the phases (coacervate phase) containing a higher concentration of both macromolecules (Tolstoguzov, 1986).

Summary. At pH 6.5, SPP induced thermal gelation of β-lactoglobulin, resulting in the formation of an opaque white gel. The texture and appearance attributes of the β-Lg/SPP gels were influenced by calcium cations. Irrespective of calcium cations, SPP interacted with β-Lg to form a precipitate that did not gel. Evidently, gelation of β-Lg/SPP mixtures was favored at pH 6.5 but not 3.5. Results from the present study indicate the potential of using anionic polysaccharides to modify whey protein gelation and expand whey utilization in diverse food systems.

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Received for review September 21, 1995. Accepted October 13, 1995.[⊗]

JF9501204

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1995.