Gelation of pH-Aggregated Whey Protein Isolate Solution Induced by Heat, Protease, Calcium Salt, and Acidulant

Zhi Yong Ju and Arun Kilara*

Department of Food Science, The Pennsylvania State University, University Park, Pennsylvania 16802

Effects of pH (3.0−7.0) on aggregation of whey protein solution (WPS, 18%) were investigated by examination of turbidity, aggregate size, and microstructure. As expected, maximum turbidity and aggregate sizes occurred at the isoelectric point (p*I* 5.2) of whey proteins. Lower or higher pH than the p*I* resulted in a steady decrease of the turbidity and aggregate size. Microstructure analysis revealed that the WPS at pH 5.7 contained loose and irregular aggregates with 200−400 nm sizes. From the pH (5.7)-aggregated WPS, gelation was induced by heating, hydrolyzing with a protease from *Bacillus licheniformis (*BLP), increasing ionic strength with $CaCl_2$, and quiescently acidifying with glucono-δ-lactone (GDL), respectively. The hardness, color, and microstructure of the gels so formed were determined. Micrographs of BLP- and $CaCl_2$-induced gels showed aggregates similar in size and shape to the parent aggregates in the WPS. Heat- and GDL-induced gels were structured with enlarged aggregates (500 nm and 1−2 μm). Separating the processes of formation of aggregates and gels may provide a means to manipulate the protein gel properties.

Keywords: *Whey proteins; aggregation; gelation; microstructure; pH*

INTRODUCTION

Whey proteins (WP) are increasingly utilized in many food products. The ability to form thermal gels is a major application of these proteins in foods. Understanding the mechanism of aggregation and gelation of WP is very important to further expand WP markets.

The structure of individual WP, in the molecular or aggregated state, is pH-dependent. β-Lactoglobin (β-Lg), a dominant whey protein, exists as dimers at neutral pH (Nielsen et al., 1996). Upon acidification, the dimers of β-Lg were first dissociated, and then monomers formed aggregates around the isoelectric point (p*I*; Macleod et al., 1995). At pH 3.5, the β-Lg was in equilibrium with the monomeric and dimeric forms (Macleod et al., 1995). Therefore, WP isolate (WPI) or β-Lg solutions were transparent at neutral and low pH, and were turbid around the p*I*. Langton and Hermansson (1992) found that heating the turbid solutions led to the formation of particulate gels and heating transparent solutions resulted in fine-stranded gels. The transitions between the two types of structures took place between pH 4 and 6.

Lowering the pH from neutral toward the p*I* reduces electrostatic repulsion and may have also exposed hydrophobic groups of the proteins. These changes led to protein−protein interactions to form the aggregates. Most foods have pH values around or below 6.0. Addition of the WP to such foods may first result in the formation of aggregates. Improving knowledge on the aggregation of WP at different pH levels and the resultant gelation, thus, is very important for appropriate application of WP in a food.

Besides the pH-induced aggregates, heat (Kawamura et al., 1993; Ju et al., 1997), enzymes (Otte et al., 1996a), or salts (Zhu and Damodaran, 1994) also could independently induce WP to form aggregates. Once the WP or β-Lg aggregates, the protein solution would demonstrate the functionality of the aggregates, and not that of WP molecules. The denatured solution of WP, containing heat-induced soluble aggregates, could be gelled by additions of salts (Barbut and Foegeding, 1993), acidification (Kawamura et al., 1993), or enzymatic hydrolysis (Sato et al., 1995; Ju et al., 1997). These treatments as well as heat also independently resulted in gelation of Ca^{2+}-induced aggregates of WP (Ju and Kilara, 1998b). The gel so formed had significantly different textural and rheological properties. The microstructures of the gels may be composed of the same aggregates as those in the parent solution (Nakamura et al., 1995). These facts suggest that the formation of the protein aggregates is essential for the gelation of WP. However, it is not known whether all these factors, heat, protease from *Bacillus licheniformis* (BLP), $CaCl_2$, and glucono-δ-lactone (GDL), could independently induce gelation from the pH-aggregated WP solution (WPS). The objective of this research is to investigate the effects of pH change on the aggregate size of WP, and of treatments of the various factors on gelation of the aggregates and on the hardness, color, and microstructure properties of formed gels.

MATERIALS AND METHODS

Materials. A commercial WPI (BiPro), purchased from Davisco Intl. Inc. (Le Sueur, MN), was used to prepare 18% WPS (pH 7.0). Reagent grade $CaCl_2$ and acidulant, and GDL were from Sigma Chemical Co. (St. Louis, MO). The protease BLP was from Novo Nordisk A/S (Denmark).

Aggregation of WPS. A 100-mL portion of the 18% WPS was centrifuged at 10000*g* for 1 h. The pH of the supernatant (pH 7.0) was gradually lowered by adding 25% (w/v) GDL solution. During the acidification, samples were taken at selected pH's to determine turbidity and aggregate size. The GDL solution was prepared by mixing 25 g of GDL with 100 mL of distilled water and stored at room temperature for at least 48 h.

* Corresponding author. Phone: (814) 863-2963. Fax: (814) 863-6132. E-mail: kyq@psu.edu.

S0021-8561(97)01018-2 CCC: $15.00
Published on Web 04/21/1998

Turbidity and Aggregate Size. Turbidity measurements were performed at room temperature on a Ultrospec 3000 spectrophotometer (Pharmacia Biotech., Cambridge, England). The samples at varying pH (3.0−7.0) were diluted to 0.36% WPS with distilled water. The turbidity of the samples was determined as the apparent optical density at 420 nm, and distilled water was used as the reference (Otte et al., 1996a).

Aggregate sizes of the diluted samples were determined by dynamic light scattering (DLS) using a Microtrac Ultrafine Particle Analyzer (Leeds & Northup Instruments, St. Petersburg, FL). The light source was a 632.8 nm He−Ne laser, and the scattering angle was 90°. Each sample was analyzed by DLS for 3 min. The experimental determinations of turbidity and aggregate size were replicated 2 times.

Preparations of Various Gels. After the 18% WPS was adjusted to pH 5.7 with 25% GDL solution, the WPS immediately turned turbid. The aggregated WPS was then diluted to 1−15% WP with distilled water.

For heat-induced gelation, 15-mL aliquots of aggregated WPS at the varying concentrations (1−18%) were placed in 25-mL beakers, and then heated in an 80 °C water bath for 30 min. For BLP-, $CaCl_2$-, and GDL-induced gelation, 15-mL aliquots of aggregated WPS at the varying concentrations were incubated at 45 °C for 60 min immediately after addition of BLP (1% enzyme to protein, w/w), $CaCl_2$ (40 mM), or GDL powder (at ratios of 2% and 6% GDL to protein, w/w). According to preliminary tests, the two ratios of GDL were expected to result in pH 5.2 and pH 4.6 after the hydrolysis of GDL. The aggregated WPS (1−18%) without any additives were incubated at 45 °C for 60 min as controls.

In all these experiments, gelation was determined by the procedure of Morr and Foegeding (1990). Formed gels were immediately cooled to room temperature with tap water, and stored at 5 °C overnight before analysis. The gelation experiments were performed in triplicate.

Gel Hardness. Gel hardness was determined by a Texture Analyzer TA XT-2 (Texture Technologies Corp., Scarsdale, NY). The gels formed in the beaker were penetrated with a cylinder probe at 12-mm diameter. A force−time curve was obtained at a crosshead speed of 60 mm/min for a 15-mm displacement. The resulting force−time curves were analyzed using the texture profile analysis method (Bourne, 1978).

Color of Gels. The surface reflectance of four freshly prepared gels, induced by heat, BLP, $CaCl_2$, and GDL from the 18% WPS at pH 5.7, was measured with a Minolta CR-200 colorimeter (Rvamsey, NJ), using the standard CIELAB color system (L = lightness, a = red to green, b = yellow to blue). The samples in beakers were placed on a black surface, and the reflectance of the circular surface was recorded in triplicate.

Transmission Electron Microscopy. The aggregated 18% WPS (pH 5.7) was gelled by adding an equal volume of 4% molten agar at 45 °C (Glaser et al., 1980). The agar-gelled samples and four gels, induced by heat, BLP, GDL, and $CaCl_2$ from the aggregated 18% WPS were examined by transmission electron microscopy (TEM) according to the procedure of Otte et al. (1996b). Ultrathin sections (60−70 nm) were imaged and photographed using a JEOL 1200 EXII transmission electron microscope (Peabody, MA) under an accelerating voltage of 80 kV.

Statistical Analysis. Data were analyzed using ANOVA procedure from Minitab version 11.0 (State College, PA). Means were separated by Tukey's significant difference test ($p < 0.05$).

RESULTS AND DISCUSSION

Aggregation of WPI Solution. *Turbidity.* The original 18% WPS (pH 7.0) was brown and transparent. During acidification at room temperature, the WPS visually turned beige at pH 5.7, and appeared white in a range of pH 5.5−4.4. The white solution became beige and translucent at pH 4.2, and returned to its original brown and transparent state at pH <3.5.

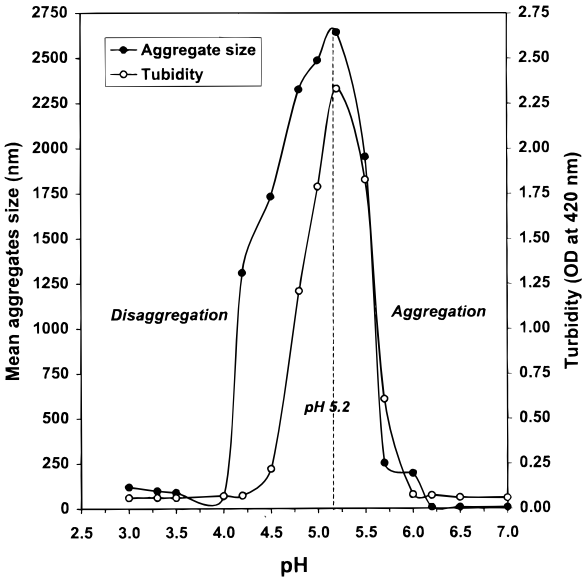

Figure 1. Turbidity (OD at 420 nm) and mean aggregate sizes of 18% whey protein solution at varied pH values (3.0−7.0). The aggregate sizes were determined by dynamic light scattering.

Figure 1 shows the results of measured turbidity and aggregate size during gradual acidification of the WPS from pH 7.0 to 3.0. The turbidity of the WPS did not change until the pH approached 6.0. Acidification from pH 6.0 to 5.2 led to remarkable increases in turbidity, and from pH 5.2 to 4.2 led to rapid decreases in turbidity. As expected, maximum turbidity occurred at the pI (pH 5.2) of whey proteins, where the turbidity was 38 times higher than that of original WPS.

After the acidification, the turbidity of WPS (pH 6.5−3.0) was also followed for a 3-h period at room temperature. No change in the turbidity was observed (result not shown), suggesting that the pH-induced WP aggregation was a rapid process, and was dissimilar to the Ca^{2+}-induced WP aggregation which was a time-dependent process (Zhu and Damodaran, 1994; Ju and Kilara, 1998a).

Size of Aggregates. DLS detected that control WPS (pH 7.0) had mean particle sizes of 8 nm (Figure 1). Upon lowering the pH of the control WPS, the globular proteins gradually aggregated, and the measured mean aggregate size continuously increased until approaching a maximum of 2.645 μm at pH 5.2 (Figure 1). Further lowering of the pH from 5.2 to 4.0 led to a steady decrease of the mean aggregate size, indicating that the maximum sized aggregates, formed at pH 5.2, gradually disaggregated. This result was consistent with the observation on turbidity (Figure 1).

The increases in turbidity and aggregate size from pH 6.0 to the pI reflected a continuous aggregation process, while their decreases from the pI to 4.0 indicated a gradual disintegration of the formed aggregates. The aggregation and disaggregation may be mediated by electrostatic interactions.

Further lowering the pH from 4.0 to 3.0 slightly increased the mean aggregate size (Figure 1). Macleod et al. (1995) reported a similar result on the aggregation of β-Lg. The smaller particle sizes measured at pH 4.0 than at pH 3.0 was explained by the transition between the monomers and dimers, approaching equilibrium at pH 3.5 (Macleod et al., 1995).

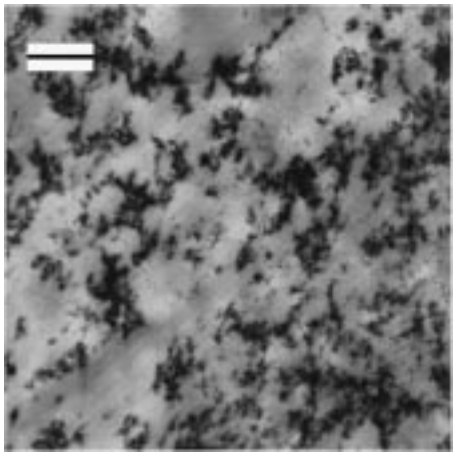

Figure 2. Transmission electron micrograph of pH (5.7)-aggregated whey protein isolate solution. The bar represents 500 nm.

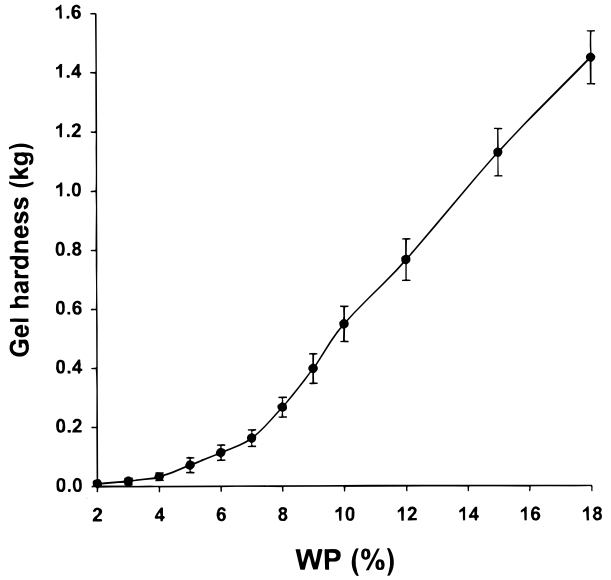

Figure 3. Thermal gel hardness as a function of whey protein concentration. The gelation was induced by heating (80 °C, 30 min) pH (5.7)-aggregated whey protein solution at varying protein concentrations (1−18%).

Aggregation was detected at pH 6.2 using DLS, whereas the turbidity method revealed aggregation at pH 6.0 (Figure 1). Also, lowering the pH from 6.0 to 5.7 led to a limited increase in mean aggregate sizes (196−252 nm, Figure 1), while the turbidity increased to a much greater extent in this pH range. This inconsistency may be ascribed to a major increase in aggregate concentration in the initial aggregation since turbidity is a function of both size and concentration of aggregates.

Stading and Hermansson (1990) have shown by turbidity measurements that β-Lg existed as aggregates in solution in the pH range of 4.0−6.0. Further heating the turbid solution at this pH range resulted in particulate gels. The microstructures of these gels showed that aggregate sizes increased with pH decreases from 6.0 to the p*I*, and decreased with the pH lowered from the p*I* to 4.0. This observation was precisely consistent with the determination of aggregate size in the WP solution at varying pH (Figure 1).

Microstructure of Aggregates. TEM revealed that the microstructure of the WPS at pH 5.7 was indeed

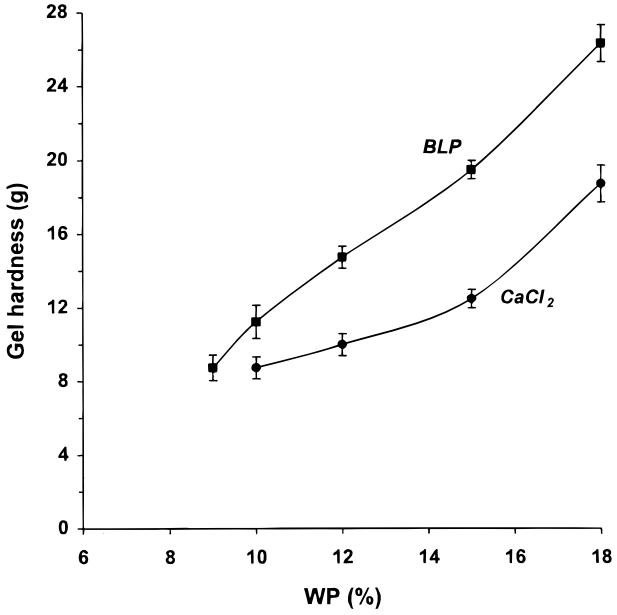

Figure 4. Protease- and calcium-induced gel hardness as a function of whey protein concentration. The gels were induced by the protease from *Bacillus licheniformis (*BLP) and calcium chloride from pH (5.7)-aggregated whey protein solution.

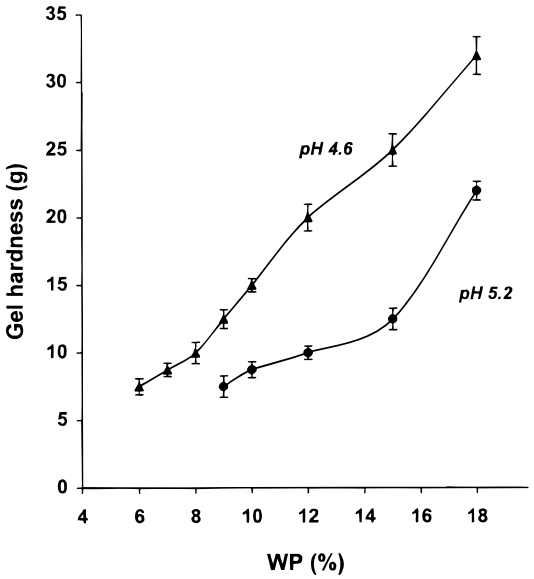

Figure 5. Effects of whey protein (WP) concentration on glucono-δ-lactone-induced gelation and gel hardness. The gelation was induced from pH (5.7)-aggregated WP solution by quiescent acidification to pH 4.6 or pH 5.2 at 45 °C.

composed of loose and irregular aggregates (Figure 2). The sizes of the aggregates were in the range of 200−400 nm, which was in line with the observation on the mean aggregate size (252 nm) by DLS (Figure 1). The background of this micrograph (Figure 2) showed dark materials, which might be unaggregated globular proteins or small protein aggregates, suggesting an inconsistent or incomplete aggregation at this pH. This aggregated WPS was used as a raw material for heat-, BLP-, $CaCl_2$-, and GDL-induced gelation.

Gelation of pH-Aggregated WPS. The various concentrations (1−15%) of WPS, which were diluted from 18% pH (5.7)-aggregated WPS, should contain the same sized aggregates. No sediment was observed in these diluted WPS during 3 days of storage at ambient

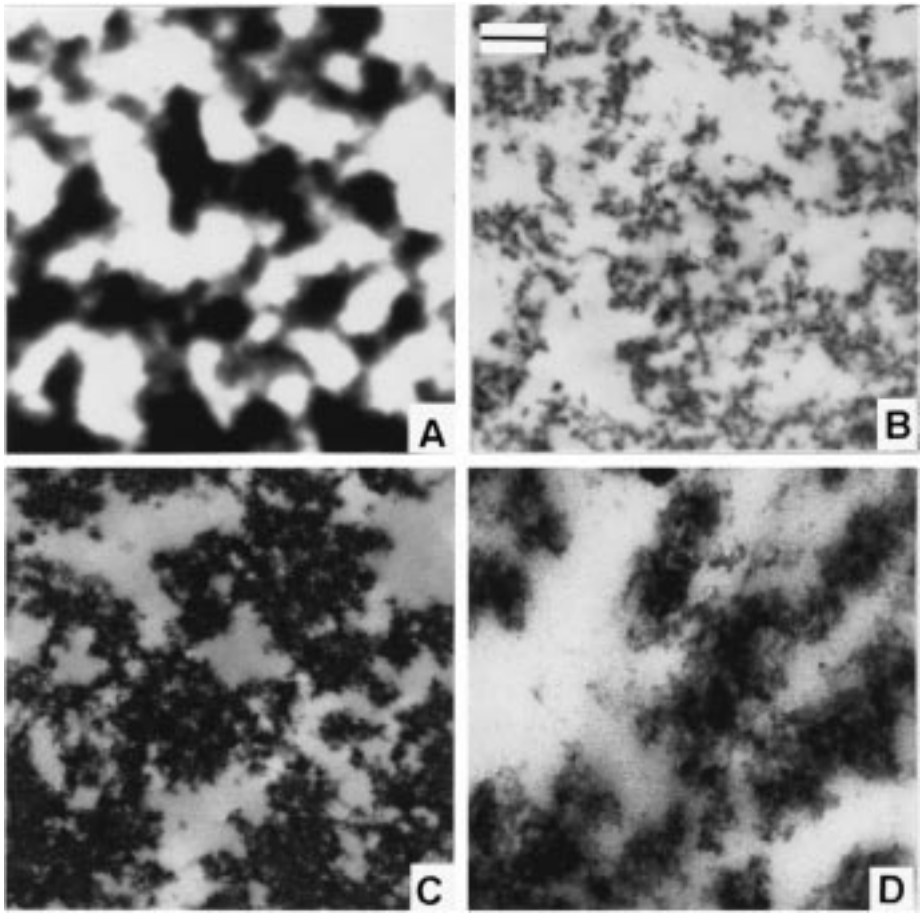

Figure 6. Transmission electron micrographs of four gels induced from 18% pH (5.7)-aggregated whey protein isolate solution by heating at 80 °C, 30 min (A), and by incubating at 45 °C for 60 min with 1% (enzyme/protein, w/w) *Bacillus licheniformis* protease (B), 40 mM $CaCl_2$ (C), or glucono-δ-lactone to pH 5.2 (D). The bar represents 500 nm.

Table 1. Color Index and Hardness of Gels Induced by Various Factors from GDL-Aggregated Whey Protein Isolate Solution (pH 5.7, 18% Protein)

factor	gel hardness (g)	color		
		L	*a*	*b*
heat	$1450 \pm 85^{d\,a}$	91.62 ± 0.18^{d}	-1.39 ± 0.07^{c}	4.99 ± 0.08^{b}
BLP	26 ± 3^{b}	66.28 ± 0.43^{b}	-1.56 ± 0.07^{c}	11.04 ± 0.51^{c}
$CaCl_2$	18 ± 3^{a}	60.10 ± 2.60^{a}	-2.41 ± 0.31^{b}	3.75 ± 0.28^{a}
GDL	32 ± 5^{c}	78.95 ± 0.57^{c}	-3.11 ± 0.01^{a}	4.85 ± 0.09^{b}

[a] Means within rows followed by the same letter are not significantly different ($p < 0.05$).

temperature, suggesting that the protein aggregates were colloidally stable.

Upon heating (80 °C, 30 min), even 2% WPS formed a self-supported gel (Figure 3). With the increase of WP concentration, gel hardness rapidly increased, especially in the range of 7−18% WP (Figure 3). The pH value of these gels still was 5.7, which implied that the heat treatment did not affect the pH of WPS.

Additions of BLP (1% E,S) and $CaCl_2$ (40 mM) to the aggregated WPS (1−18%) resulted in gelation of >8% and >9% WPS within 1 h incubation at 45 °C, respectively (Figure 4). The BLP- and $CaCl_2$-induced gels were white and opaque like the heat-induced gels. However, these gels were soft and thixotropic. Even 18% WP gels had only less than 26 g of hardness (Figure 4), which was over 50 times weaker than the hardness of heat-induced gels at 18% WP. The BLP-induced gels were significantly stronger than $CaCl_2$-induced gels at comparable protein concentrations ($p < 0.05$). The control WPS at pH 5.7 did not gel under the same incubation (45 °C, 1 h). Addition of $CaCl_2$ and BLP hydrolysis slightly reduced pH values (5.56 and 5.58) of the aggregated WPS initially at pH 5.7.

When GDL powder was added to the WPS followed by a quiescent incubation at 45 °C, the GDL was gradually hydrolyzed and released gluconic acid, which resulted in the acidification of the solution. This process was similar to yogurt fermentation and created a continuous aggregation in situ. Manipulating the added GDL amount could control the final resultant pH values. All of the added GDL was hydrolyzed within 40 min at 45 °C as evidenced by the stable pH. Such treatments resulted in gelation of >6% aggregated WPS at pH 4.6, and in gelation of >9% aggregated WPS at pH 5.2 (Figure 5). The gels formed at pH 4.6 were significantly stronger than the gels formed at the p*I* (5.2) of WP at comparable protein concentrations ($p < 0.05$). In addition, an interesting phenomenon was observed in that the gels formed at pH 4.6 or 5.2 could collapse or be dissolved as the pH was further lowered to 3.0 through more additions of GDL.

Microstructures of Gels. The four gels, induced by heat, BLP, $CaCl_2$, and GDL from the same aggregated 18% WPS (pH 5.7), were all white and opaque. The hardness, color values (L = lightness; a = red to green; b = yellow to blue), and microstructures of these gels are shown in Table 1 and Figure 6, respectively.

Heat-Induced Gel. TEM examination of the heat-induced gel revealed a well-linked network (Figure 6A). The protein aggregates seemed fused together in the

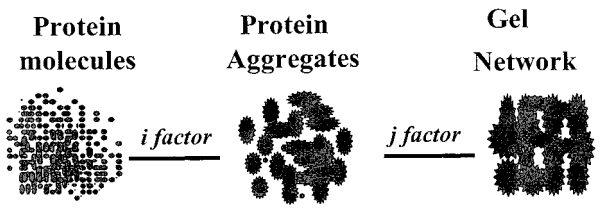

Figure 7. Schematic representation of whey protein gelation mechanism. The aggregate and gel formations in two separating stages are induced by *i* and *j* environmental factors, respectively. The *i* and *j* could be heat, acid, salt, and protease etc.

molten state and existed in chains. Edges of individual aggregates were difficult to distinguish. The chains were 500 nm in width, which might also be a reflection of the aggregate sizes (Figure 6A). This dimension in the gel at pH 5.7 was intermediate to the β-Lg aggregates in thermal gels formed at pH 6.0 (300 nm) and pH 5.5 (1−2 μm; Langton and Hermansson, 1992). The particular microstructure (Figure 6A) demonstrated a higher resistance to penetration and a propensity for blocking the passage of light (Table 1).

BLP-Induced Gel. A micrograph of the BLP-induced gel (Figure 6B) contained aggregates similar in shape and size to those in the parent WPS (Figure 2). This may indicate that the role of the enzyme was just to promote connections of the pH-induced aggregates. Hydrolysis may have occurred on the surface of protein aggregates that changed their stability, and resulted in the interaction of the protein aggregates. The loose and irregular aggregates were linked together by fine strings with few junctions (Figure 6B), which might result in the soft (Table 1) and viscous gel.

$CaCl_2$-Induced Gel. Aggregates in the microstructure of the $CaCl_2$-induced gel (Figure 6C) formed clumps. The aggregates in the clumps were well associated, but few linkages among the clumps could be visualized. Also, the aggregates were slightly larger and denser than the parent aggregates. This might be due to hydration, as a result of the calcium salt concentration. Such a structured gel had the lowest hardness and "*L*" value (Table 1) of the four gels.

GDL-Induced Gel. This gel was induced by quiescently acidifying the aggregated WPS from pH 5.7 to 5.2. Its microstructure was composed of large aggregates (1−2 μm; Figure 6D). The sizes were similar to those in thermal WP or β-Lg gel at pH 5.2 (Otte et al., 1996b; Langton and Hermansson, 1992). However, the aggregates in the GDL-induced gel alone were looser and less dense than those in the thermal gels. This gel was significantly harder and more white than BLP- and $CaCl_2$-induced gels ($p < 0.05$, Table 1).

The process of whey protein gelation was, thus, separated into two distinct stages: pH induced aggregates from the protein molecules; and the various factors induced gels from the aggregates. These factors, heat (Parris et al., 1997), enzyme (Otte et al., 1996a), salt (Zhu and Damodaran, 1994), and acidulant (Figure 1), independently induced formation of aggregates from WP molecules, and also induced formation of gels from the aggregates induced by heat (Nakamura et al., 1995), and by calcium salt (Ju and Kilara, 1998b). From these, the mechanism of WP gelation may be schematically depicted as in Figure 7. Therefore, WP companies may develop preaggregated protein products with novel and consistent functionality such as microparticulated WP concentrate to be a fat substitute (Singer and Dunn, 1990), and whey protein texturizer containing heat-induced protein aggregates that gel under cold conditions (Thomsen, 1995).

The aggregate size was a function of pH or salt concentration either in solution (Figure 1; Macleod et al., 1995; Ju and Kilara, 1998a,b) or in gels (Langton and Hermansson, 1992; Barbut, 1995; Mulvihill and Kinsella, 1988). Also, gel hardness and microstructure changed with particle or aggregate size, and with gelling factors (Nakamura et al., 1995; Parris et al., 1997; Otte et al., 1996a; Zhu and Damodaran, 1994; Ju et al., 1997; Ju and Kilara, 1998a,b). These results provide approaches to predict and to manipulate the formation of protein aggregates and the properties of gels.

ABBREVIATONS USED

β-Lg, β-lactoglobulin; BLP, protease from *Bacillus licheniformis*; DLS, dynamic light scattering; GDL, glucono-δ-lactone; p*I*, isoelectric point; TEM, transmission electron microscopy; WP, whey protein; WPI, WP isolate; WPS, WP solution.

LITERATURE CITED

Barbut, S. Effect of sodium level on the microstructure and texture of whey protein isolate gels. *Food Res. Int*. **1995**, *28*, 437−443.

Barbut, S.; Foegeding, E. A. Ca^{2+}-induced gelation of preheated whey protein solutions. *J. Food Sci*. **1993**, *58*, 867−871.

Bourne, M. C. Texture profile analysis. *Food Technol*. **1978**, *32*, 62−66.

Glaser, J.; Carroad, P. A.; Dunkley, W. L. Electron microscopic studies of casein micelles and curd microstructure in cottage cheese. *J. Dairy Sci*. **1980**, *63*, 37−48.

Ju, Z. Y.; Kilara, A. Aggregation induced by calcium chloride and subsequent thermal gelation of whey protein isolate. *J. Dairy Sci*. **1998a**, in press.

Ju, Z. Y.; Kilara, A. Properties of gels induced by heat, protease, calcium salt and acidulant from Ca^{2+}-aggregated whey protein isolate. *J. Dairy Sci*. **1998b**, in press.

Ju, Z. Y.; Otte, J.; Zakora, M.; Qvist, K. B. Enzyme-induced gelation of whey proteins, effect of protein denaturation. *Int. Dairy J*. **1997**, *7*, 71−78.

Kawamura, F.; Mayuzumi, M.; Nakamura, M.; Koizumi, S.; Kimura, T.; Nishiya, T. Preparation and properties of acid-induced gel of whey protein. *Nippon Shokuhin Kogyo Gakkaishi* **1993**, *40*, 776−782.

Langton, M.; Hermansson, A.-M. Fine-stranded and particulate gels of β-lactoglobulin and whey protein at varying pH. *Food Hydrocolloids* **1992**, *5*, 523−539.

Macleod, A.; Fedio, W. M.; Ozimek, L. Aggregation of β-lactoglobulin as a function of pH and temperature. *Milchwissenschaft* **1995**, *50*, 666−669.

Morr, C. V.; Foegeding, E. A. Composition and functionality of commercial whey and milk protein concentrates and isolates. *Food Technol*. **1990**, *44*, 100−112.

Mulvihill, D. M.; Kinsella, J. E. Gelation of β-lactoglobulin. Effects of sodium chloride and calcium on the rheological and structural properties of gels. *J. Food Sci*. **1988**, *53*, 231−236.

Nakamura, T.; Sato, K.; Koizumi, S.; Kawachi, K.; Nishiya, T.; Nakajima, I. Preparation and properties of salt-induced gel of whey protein. *Nippon Shokuhin Kagaku Kaishi* **1995**, *42*, 1−6.

Nielsen, B. T.; Singh, H.; Latham, J. M. Aggregation of bovine β-lactoglobulins A and B on heating at 75 °C. *Int. Dairy J*. **1996**, *6*, 519−527.

Otte, J.; Ju, Z. Y.; Færgeman, M.; Lomholt, S.; Qvist, K. B. Protease-induced aggregation and gelation of whey proteins. *J. Food Sci*. **1996a**, *61*, 911−915, 923.

Otte, J.; Ju, Z. Y.; Skriver, A.; Qvist, K. B. Effect of limited proteolysis on the microstructure of heat-induced whey protein gels at varying pH. *J. Dairy Sci.* **1996b**, *79*, 782−790.

Parris, N.; Hollar, C. M.; Hsieh, A.; Cockley, K. D. Thermal stability of whey protein concentrate mixture, aggregate formation. *J. Dairy Sci.* **1997**, *80*, 19−28.

Sato, K.; Nakamura, M.; Nishiya, T.; Kawanari, M.; Nakajima, I. Preparation of a gel of partially heat-denatured whey protein by proteolytic digestion. *Milchwissenschaft* **1995**, *50*, 389−392.

Singer, N. S.; Dunn, J. M. Protein microparticulation: the principle and the process. *J. Am. Coll. Nutr.* **1990**, *9*, 388−397.

Stading, M.; Hermansson, A.-M. Viscoelastic behavior of β-lactoglobulin gel structure. *Food Hydrocolloids* **1990**, *4*, 121−135.

Thomsen, B. Whey protein texturizer. *Food Technol. Int. Eur. 1995* **1995**, 39−42.

Zhu, H.; Damodaran, S. Effects of calcium and magnesium ions on aggregation of whey protein isolate and its effect on foaming properties. *J. Agric. Food Chem.* **1994**, *42*, 856−862.

Received for review December 1, 1997. Revised manuscript received March 12, 1998. Accepted March 17, 1998.

JF9710185