Gel Formation from Industrial Milk Whey Proteins under Hydrostatic Pressure: Effect of Hydrostatic Pressure and Protein Concentration

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The effects of high hydrostatic pressure and protein concentration on the denaturation and gelation of whey protein were investigated. Industrial whey protein isolate (WPI) and whey protein concentrate (WPC) solutions (pH 6.8) at various concentrations were pressurized for 10 min at 30 °C under 200−1000 MPa. With the WPI solution, the concentration for affecting the turbidity was 1% and was 6% for the viscosity at 400 MPa, while for inducing gelation, it was 10% at 600 MPa. With the WPC solution, the viscosity changed at a concentration >12%, and gel formation began at >18% at 400 MPa. The hardness and breaking stress of pressure-induced WPI gels increased with increasing concentration of WPI (12−18%) and hydrostatic pressure, the ratings for the 20% WPC gels being one-third those of the 20% WPI gels. The solubility of proteins from the pressure-induced WPI gels decreased with increasing pressure, while that of WPC gel induced at >600 MPa remained constant at ∼50%. The microstructure of the WPI gels had a porous network form, whereas the WPC gels were irregular particulates. β-Lactoglobulin, α-lactalbumin, and serum albumin preferentially participated in pressure-induced aggregation and gelation through S−S bonding.

Keywords: *Hydrostatic pressure; whey protein isolate; whey protein concentrate; denaturation; aggregation; gelation; rheological property; microstructure*

INTRODUCTION

Because whey proteins possess outstanding physicochemical properties in their gelation and binding, they are widely used as functional ingredients in many formulations of bakery, dairy, and sausage products (Kinsella and Whitehead, 1989; Robin et al., 1993). Whey is a byproduct from cheese manufacturing and contains ∼13% protein in the dry matter. The major constituents of the whey protein of bovine milk are β-lactoglobulin, α-lactalbumin, serum albumin, and immunoglobulins (Eigel et al., 1984). The advent of improved and more cost-effective processing technology and such production procedures as ultrafiltration, reverse osmosis, and electrodialysis has resulted in a dramatic increase in the production of whey protein products (deWit and Klarenbeek, 1984; Zall, 1984; Schmidt et al., 1984; Morr and Foegeding, 1990). At present, five kinds of industrial whey proteins are produced, four of which belong to whey protein concentrate (WPC) and which are classified into four grades according to protein contents of about 35, 50, 65, and 70−90%. Similarly, whey protein isolate (WPI) contains >90% protein in the dry matter (Mulvihill, 1994).

One of the functional properties of whey proteins is their gelation, which is known to be induced by heating. There have been many studies concerning the heat-induced gelation of whey proteins (Kinsella et al., 1994.). Hydrostatic pressure has recently been shown to induce coagulation of proteins without causing chemical changes, while heating sometimes destroys covalent bonding (Hayashi, 1992). The changes in structure and/or the function of proteins under hydrostatic pressure are accompanied by the formation of hydrogen bonds, the rupture of hydrophobic interactions, and the separation of ion pairs (Cheftel, 1992). β-Lactoglobulin in whey proteins can be preferentially digested with thermolysin under pressure at 100−300 MPa (Hayashi et al., 1987; Dufour et al., 1995), and the antigenecity of β-lactoglobulin has been selectively removed (Okamoto et al., 1991; Nakamura et al., 1993). β-Lactoglobulin, which accounts for half the amount of whey protein, has been shown to aggregate at low protein concentrations by high-pressure processing (Dumay et al., 1994; Funtenberger et al., 1995). At a high protein concentration, intermolecular interaction and irreversible aggregation are induced in chymotrypsinogen (Wong and Heremans, 1988). There is, however, limited information concerning the gel formation of whey proteins under hydrostatic pressure and on how the protein concentration and hydrostatic pressure influence structural changes. The development of a new industrial processing method such as that involving hydrostatic pressure will be useful in dairy product formulation. Pressure treatment can be performed at room temperature and is energy-saving as compared to heat treatment. Foods treated by high pressure keep their original state of freshness but may have been changed in texture, and several pressure-induced foods such as strawberry jam and fruit juices have come onto the market (Hayashi, 1992; Tauscher, 1995). It is expected that gels produced from whey proteins by high pressure may have different properties from those made by heat treatment. Further funda-

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mental studies are required to understand the gelation of whey proteins under hydrostatic pressure.

The present study was undertaken to clarify the relationship between the concentration of whey proteins and the hydrostatic pressure required for gel formation by measuring the viscosity and turbidity of pressurized WPI and WPC solutions. Furthermore, the rheological properties, protein solubility, electrophoretic properties, and microstructure of pressure-induced gels from the WPI and WPC solutions were analyzed.

MATERIALS AND METHODS

WPI and WPC Preparations. WPI and WPC were gifts from the Central Research Institute of Snow Brand Dairy Industry Co. Single batches of WPI and WPC were used. WPI contained about 6.1% moisture, 89.8% protein, 1.8% ash, 1.3% lactose, and 0.5% lipids, while WPC contained about 5.5% moisture, 74.9% protein, 5.4% ash, 3.5% lactose, and 5.9% lipids, according to the manufacturer. WPI and WPC were each dissolved in a 0.05 M sodium phosphate buffer (pH 6.8) to give a solution of 1−20% (w/v) and allowed to stand overnight at 4 °C before pressurization.

Pressurization. A WPC or WPI solution was put into a Teflon tube (4 mL) with a screw cap (internal diameter of 15 mm and 22 mm in depth) and then pressurized to 200−1000 MPa with a hand-operated oil pressure generator (HR15-B2, Hikari Koatsu, Hiroshima, Japan). The temperature was kept at 30 °C by a band heater. In each experiment, the indicated pressure was achieved within 0.5−2 min, held for 10 min, and then released to atmospheric pressure within 1 min. After the pressure was released, the pressurized sample was quickly removed from the vessel and immediately analyzed at room temperature.

Measurement of Viscosity. The viscosity of each fluid sample was measured with a viscometer (DVM-E type, Tokyo Keiki, Japan), with the thermostat set at 30 °C. A standard liquid for calibrating the viscometer was employed. The sample cup was filled with 1 mL of the pressurized WPI or WPC solution, and the viscosity was measured at a rotational speed of 10−50 rpm of the conical rotor.

Measurement of Turbidity. The turbidity of each fluid sample was measured as absorbance at 570 nm with a Hitachi U-2000 spectrophotometer.

Measurement of Hardness and Breaking Stress. The hardness and breaking stress of each gel were individually measured at room temperature (∼25 °C) with a Fudoh rheometer (Rheotech RT-2005DD, Tokyo, Japan). A gel specimen was 15 mm in diameter and 22 mm in length. For the measurement of hardness, a disk-type adapter (no. 3, 5 mm in diameter and 19.6 mm^2 in area) was used at the stress range of 500 g with the sample holder moving upward and downward at the speed of 5 cm/min. For the measurement of breaking stress, a globe-type adapter (no. 4, 5 mm in diameter and 19.6 mm^2 in area) was used at the stress range of 500 g with the sample holder moving upward at the speed of 5 cm/min. The maximum force that developed separately was evaluated from the height (h) of the peak profile, and the hardness (H) was represented as $H = hg$ (g) and breaking stress (Bs) as Bs = hg (g), where g is stress range. Data were analyzed with the Rheosoft program attached to the rheometer.

Determination of Protein Concentration. The protein concentration of the supernatant obtained by centrifugation described below was determined according to the method of Markwell et al. (1978), except that the protein in the solution containing 2-mercaptoethanol was precipitated according to the method of Peterson (1977) with 2.0% sodium deoxycholate. Bovine serum albumin was used as a standard. After 4.0 mL of a 0.05 M sodium phosphate buffer (pH 6.8) was added to the wet gel from WPI or WPC (0.2 g), the solution was homogenized at 19 000 rpm for 1 min in an ice bath with an ST20 Polytron homogenizer (Kinematika), using an OD-S shaft (20 mm in diameter), and centrifuged at 3000g for 15 min. The supernatant was recovered for the determination of protein solubility and electrophoresis, and the precipitate was dissolved in a 0.05 M sodium phosphate buffer (pH 6.8) containing 2% sodium dodecyl sulfate (SDS) for electrophoresis. Protein solubility (percent) was calculated as (protein content of the supernatant/total protein content) × 100.

SDS−Polyacrylamide Gel Electrophoresis (PAGE). SDS−PAGE in the presence or absence of 2-mercaptoethanol was performed according to the method of Laemmli (1970). Each supernatant and solubilized precipitate fraction obtained for the determination of protein solubility was diluted with an equal volume of a 20 mM Tris-HCl buffer at pH 8.0 containing 2% SDS, 6 mM EDTA, 24% sucrose, and 0.2 M 2-mercaptoethanol (for Figure 5). Alternatively, the pressurized samples (0.11 g for gel and 0.37 g for liquid) were dispersed in 4 mL of a 0.086 M Tris−0.09 M glycine buffer (pH 8.0) containing 4 mM EDTA (buffer A), homogenized as described under Determination of Protein Concentration and separated into the supernatant and precipitate by centrifugation for 15 min at 20000g. The precipitate was solubilized in 2 mL of a buffer A containing 8 M urea and 0.5% SDS. The supernatant and precipitate fractions were diluted with 4 volumes of a 50 mM Tris-HCl buffer (pH 6.8) containing 150 mM EDTA, 5% SDS, and 60% sucrose, with or without 5% 2-mercaptoethanol (for Figure 6). All solubilized protein solutions were boiled for 2 min and centrifuged for 15 min at 20000g. The concentration of polyacrylamide was 5% for the stacking gel and 15% for the resolving gel with a 1-mm thickness (for Figure 5), while a linear gradient of the separating gel (SPG-520L, Atto Corp.) from 4 to 20% in polyacrylamide was used (for Figure 6). The proteins were fixed and stained in 25% methanol and 10% acetic acid containing 0.25% Coomassie brilliant blue R-250, before being destained in 25% methanol and 7% acetic acid. A kit containing α-lactalbumin (14.2 kDa), trypsin inhibitor (20 kDa), trypsinogen (24 kDa), carbonic anhydrase (29 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), ovalbumin (45 kDa), and bovine serum albumin (66 kDa) and another kit containing carbonic anhydrase (29 kDa), ovalbumin (45 kDa), bovine serum albumin (66 kDa), phosphorylase *b* (97 kDa), β-galactosidase (116 kDa), and myosin (205 kDa) (Sigma) were employed as protein standards for evaluating the apparent molecular mass.

Scanning Electron Microscopy. A rectangular gel specimen (4 × 4 × 2 mm) was cut and, without any chemical fixation, dehydrated by immersion in an ethanol mixture series of 30, 50, 70, 85, 95 and 100%, and finally immersed in 97% isoamyl acetate (Chanyongvorakul et al., 1995). After dehydration, critical-point drying was performed in liquid CO_2 in a pressurized chamber. The dried sample was coated with carbon in a Hitachi E1020 10N spotter. Each sample was then examined and photographed with an S-2250N Hitachi scanning electron microscope at an acceleration voltage of 15 kV.

RESULTS

Effect of Hydrostatic Pressure on the Denaturation of WPI and WPC. A rise in the turbidity and viscosity of each pressurized protein solution was determined as an index of the denaturation and aggregation, respectively, of protein in the first stage. In this experiment, pH 6.80 was chosen because most formulated and processed foods are prepared at a nearly neutral pH value.

Turbidity. Figure 1 shows the change in turbidity for various concentrations of a WPI solution as a function of pressure. The turbidity of the WPI solution was greatly influenced by pressure and protein concentration. Even at 1% concentration, although its viscosity was not changed (Figure 2A), the turbidity of the WPI solution increased when the pressure applied exceeded 400 MPa. Although there was a slight differ-

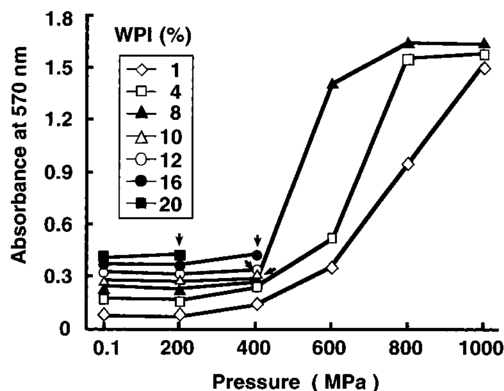

Figure 1. Effect of hydrostatic pressure on the turbidity of different concentrations of WPI solutions. WPI solution was pressurized at the indicated pressure for 10 min and, after the pressure was released, the turbidity of the fluid was measured at 570 nm. The standard deviation of three measurements was extremely small, so that no variation can be seen. Arrow shows that gel was induced at higher than the indicated pressure.

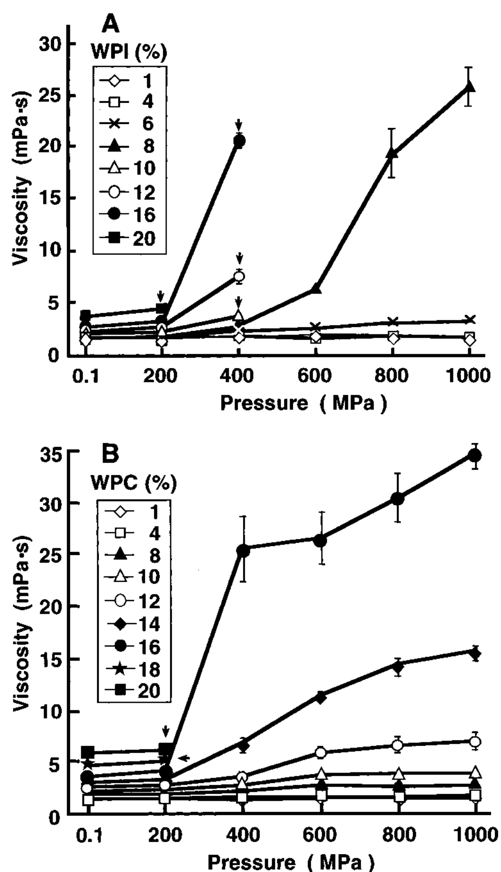

Figure 2. Effect of hydrostatic pressure on the viscosity of different concentrations of WPI (A) and WPC (B) solutions. WPI and WPC solutions were pressurized as described in Figure 1, and the viscosity of the fluid was measured. Bars indicate the standard deviation for three measurements. Arrow is as indicated in Figure 1.

ence in turbidity due to the increase in protein concentration, it remained unchanged until a pressure of >200 MPa was applied. Furthermore, WPI solutions at a concentration >10% formed a gel under a pressure >600 MPa. The 20% WPI solution, however, formed a gel when a pressure of 400 MPa was applied. The result of turbidity showed that hydrostatic pressure at 400 MPa induced the denaturation of protein in 1% WPI solution.

The change in turbidity of the 1−20% WPC solutions at 200 and 400 MPa was not determined, because they were too turbid.

Viscosity. Figure 2A shows the change in viscosity of 1−20% WPI solutions pressurized at 200−1000 MPa. No appreciable change in viscosity was observed for the 1−6% WPI solutions, even when the pressure was increased up to 1000 MPa, but was strongly apparent for the WPI solutions with ≥8% concentration at >400 MPa. The viscosity of the pressurized 8% WPI solution increased with increasing pressure, reaching 26 mPa·s at 1000 MPa. The WPI solutions of 10−16% concentration formed a gel at 600 MPa, while the 20% WPI solutions formed gels at 400 MPa. The gels formed from the 20% WPI solution at 400 MPa and from the 16% solution at 600 MPa were soft and translucent in appearance.

Curves for the viscosity of the pressurized WPC solution are shown in Figure 2B. The viscosity of 1−10% WPC solutions did not change at a pressure of 200−1000 MPa, but a change in viscosity was observed for the solutions with a concentration of ≥12%. A gel was formed in the WPC solutions of ≥18% concentration under a pressure of >400 MPa. The gels formed from the 18% WPC solution at 400 and 600 MPa were soft and milky white. WPC was more resistant to pressure-induced gelation than WPI. The result of viscosity indicated that hydrostatic pressure at 400 MPa induced the aggregation of protein in 8% WPI and 12% WPC.

Properties of the Pressure-Induced Gels from WPI and WPC. *Rheological Properties.* The gels formed from the 10% WPI solution at 600 MPa and from the 12% WPI and 20% WPC solution at 400 MPa were too soft to measure their textural properties with the rheometer. Figure 3 shows the hardness and breaking stress of those gels which were sufficiently firm that had been formed from 12−20% WPI and 20% WPC at different pressures. The hardness and breaking stress of the WPI gels increased with WPI concentration increasing from 12 to 18% at a constant pressure and, similarly, those of each solution at a constant concentration rose as hydrostatic pressure was increased from 600 to 1000 MPa. However, the hardness and breaking stress of the gel induced from the 20% WPI solution were both less than those of the gels from 18% WPI within the 600−1000 MPa range. The scores for the hardness and breaking stress of gels from 20% WPC solution were approximately one-third those of 20% WPI at 1000 MPa and were almost similar to those of 20% WPI at 600 MPa and to those of 14% WPI at 800 MPa. The hardness and breaking stress of gels from 20% WPC were not affected in this pressure range. In addition, no water was expelled from the gel matrix that had been induced from these WPI and WPC samples under pressure during the measurement of hardness and breaking stress.

Protein Solubility. The solubility of the protein in the pressurized 6 and 20% WPI and WPC at different pressures was determined by measuring the protein concentration in the supernatant after centrifugation. The results are shown in Figure 4 as the ratio to total protein. Most of the proteins were recovered in the supernatant of 6 and 20% WPI at 400 MPa, in which no visible precipitates were formed. The protein solubility was higher in 6% WPI and WPC than in 20% WPI and WPC. At 400 and 600 MPa, the solubility was higher in WPI than in WPC. Protein solubility in the

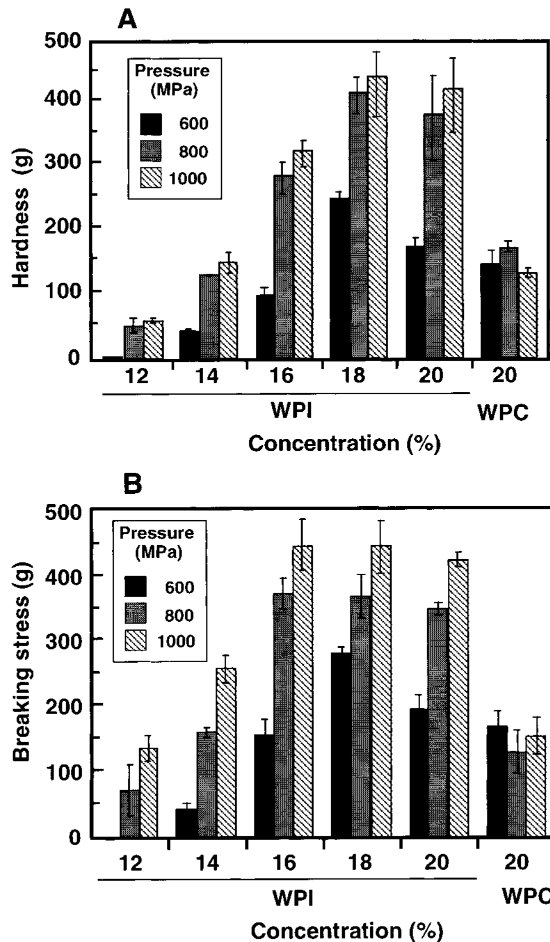

Figure 3. Hardness and breaking stress of pressure-induced gels from WPI and WPC solutions. WPI and WPC solutions were pressurized as described in Figure 1, and the textural properties were measured. Bars are as indicated in Figure 2.

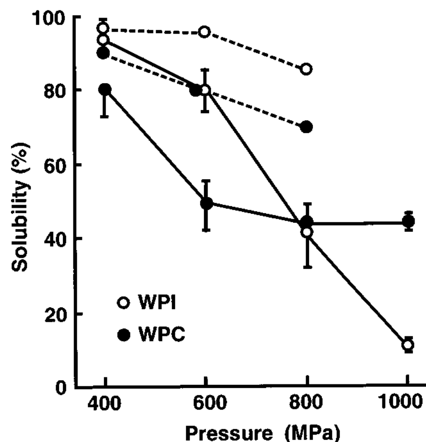

Figure 4. Protein solubility of pressurized WPI (○) and WPC (●) solutions. Pressurized 6% (- - -) and 20% (—) WPI and WPC solutions were dispersed in 0.05 M sodium phosphate buffer (pH 6.8), homogenized, and separated into the precipitate and supernatant by centrifugation for 15 min at 3000*g*. Bars are as indicated in Figure 2.

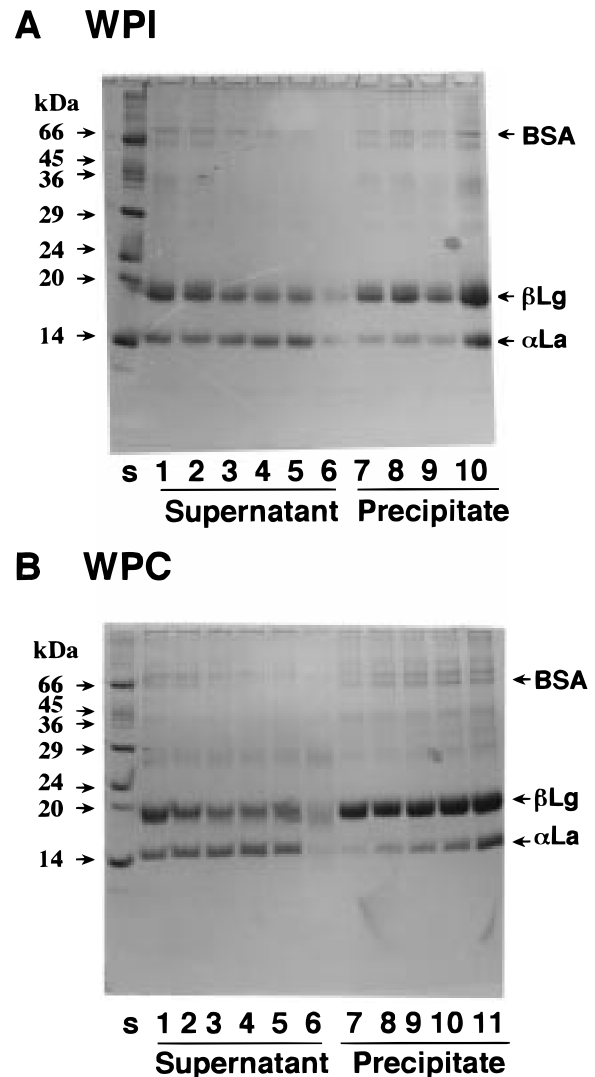

Figure 5. SDS–PAGE patterns in the presence of 2-mercaptoethanol of proteins from the precipitate and supernatant of pressure-induced and heat-induced gels from WPI (A) and WPC (B) solutions. Gels from 20% WPI and WPC solutions were treated as described in Figure 4: (A) Lane S, marker protein; lane 1, untreated WPI; lane 2, 400 MPa; lanes 3 and 7, 600 MPa; lanes 4 and 8, 800 MPa; lanes 5 and 9, 1000 MPa; lanes 6 and 10, heating for 10 min at 90 °C. (B) Lane S, marker protein; lane 1, untreated WPC; lanes 2 and 7, 400 MPa; lanes 3 and 8, 600 MPa; lanes 4 and 9, 800 MPa; lanes 5 and 10, 1000 MPa; lanes 6 and 11, heating at 90 °C for 10 min. The loaded protein was 20 μg. BSA, bovine serum albumin; βLg, β-lactoglobulin; αLa, α-lactalbumin.

pressure-induced gels from 20% WPI decreased with increasing pressure and dropped to 12% at 1000 MPa. The protein solubility of the 20% WPC gel induced at a pressure of >600 MPa remained at ~50%, indicating that a high concentration of WPC was baroprotective.

Electrophoretic Analysis. To investigate the effect of pressure on each constituent protein of WPI and WPC, pressure-induced gels from 20% WPI and WPC solutions were dispersed in 4 mL of the phosphate buffer and centrifuged. Proteins in both the supernatant and precipitate were analyzed by SDS–PAGE in the presence of 2-mercaptoethanol in comparison with the heat-induced gel, which was prepared by heating a 20% WPI or WPC solution for 10 min at 90 °C. Figure 5 indicates that such major whey proteins as β-lactoglobulin, α-lactalbumin, and serum albumin were detected in the supernatant and precipitate. It was found for the gels formed from WPI and WPC that had been pressurized at 400–1000 MPa, the ratio of α-lactalbumin to β-lactoglobulin in the precipitate was less than that in the supernatant, while the pattern from the heat-induced gel was similar to that of the untreated sample. Of the major whey proteins, β-lactoglobulin preferentially and α-lactalbumin incidentally participated in the ge-

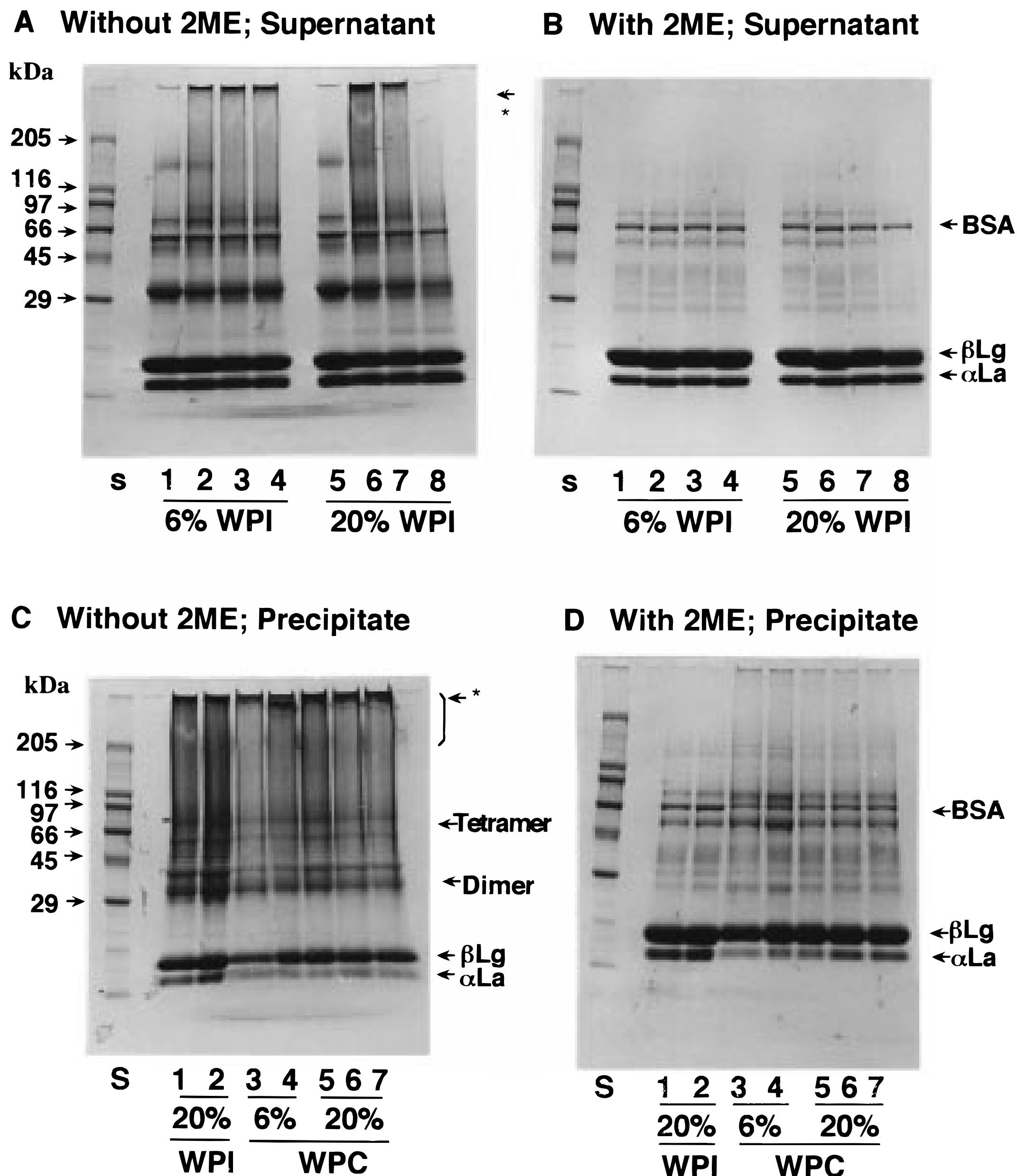

Figure 6. SDS–PAGE patterns with linear gradient gel in the presence (B and D) and absence (A and C) of 2-mercaptoethanol for proteins in the supernatant (A and B) and precipitate (C and D) of pressure-induced gels from WPI and WPC solutions. The supernatant in (A) and (B) shows the fraction soluble in 0.086 M Tris–0.09 M glycine buffer, pH 8.0, containing 4 mM EDTA (buffer A). The precipitate in (C) and (D) shows the fraction insoluble in buffer A but soluble in buffer A containing 8 M urea and 0.5% SDS. Patterns of supernatants from WPC are not shown. (A and B) Lanes 1 and 5, no pressure treatment; lanes 2 and 6, 400 MPa; lanes 3 and 7, 600 MPa; lanes 4 and 8, 800 MPa. (C and D) Lanes 2, 4, and 7, 800 MPa; lanes 1, 3 and 6, 600 MPa; lane 5, 400 MPa. Abbreviations are as indicated in Figure 5. The arrow with a star shows the bands of large aggregates. The loaded protein was 20 μg for (B) and (D), 40 μg for (A) and (C). Dimer and tetramer show those of β-lactoglobulin (βLg). 2ME, 2-mercaptoethanol.

lation of WPI and WPC by pressure at 400–1000 MPa, suggesting that aggregation of β-lactoglobulin and β-lactoglobulin/α-lactalbumin were formed.

The aggregates or gels induced from 6 and 20% WPI at different pressures were dispersed in 4 mL of the Tris–glycine buffer, separated into their soluble and insoluble fractions, and the proteins in each fraction were analyzed by SDS–PAGE with or without a reducing agent. No visible precipitate was obtained from 6% WPI at 400–800 MPa or from 20% WPI at 400 MPa. Nonreducing SDS–PAGE, with the results compared with those of a sample without the pressure treatment, indicated large aggregate bands that stayed on the top of PAGE (the arrow with a star in Figure 6A, lanes 2–4 and 6–8) from the pressurized samples, whereas they were not on the SDS–PAGE patterns in the presence of a reducing agent (Figure 6B). Similar results were obtained for WPC (data not shown). Furthermore, the formation of aggregates from WPI and WPC was profoundly detected in the insoluble fraction (the arrow with a star in Figure 6C), but not on the SDS–PAGE patterns in the presence of reducing agent (Figure 6D). Bands corresponding to the dimer and tetramer of β-lactoglobulin were also detected in the absence of 2-mercaptoethanol (Figure 6C), the intensity of the bands of β-lactoglobulin, α-lactalbumin, and bovine serum albumin by SDS–PAGE being higher in the presence of a reducing agent than in its absence (Figure

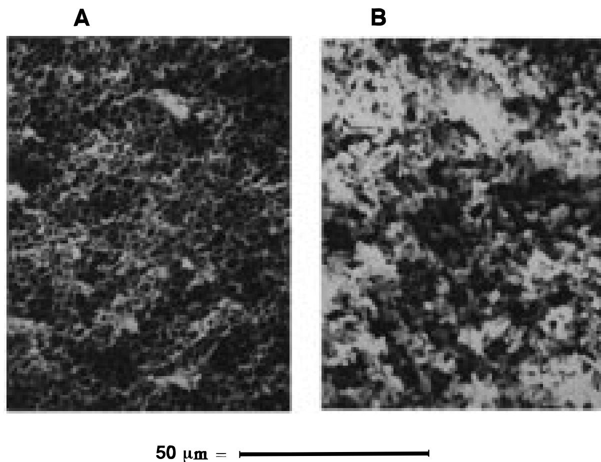

Figure 7. Scanning electron microscopic pictures of pressure-induced gels from WPI (A) and WPC (B) solutions. A 20% solution of WPI and WPC in a 0.05 M sodium phosphate buffer, pH 6.8, was pressurized for 10 min at 800 MPa and at 30 °C.

6B,D). The interaction between β-lactoglobulin and α-lactalbumin seemed to be responsible for a part of the large aggregate. In addition, besides dimer of β-lactoglobulin, a complex between β-lactoglobulin and α-lactalbumin was found immunologically to be contained in the 32 kDa band (unpublished data). On the other hand, reducing SDS−PAGE indicated no band corresponding to large aggregates (≥20.5 kDa), implying that they had been reduced to the monomer. There was no distinct difference between WPI and WPC in 6 and 20% concentration at 400−800 MPa. These results suggest that aggregates through S−S bonding were formed in samples pressurized at 400−1000 MPa. β-Lactoglobulin and α-lactalbumin predominantly contributed to the pressure-induced gel formation by exchanging −SH and S−S bonding.

Microstructure. The microstructure of the pressure-induced gels from the 20% WPI and WPC solutions at pH 6.80 under 800 MPa was observed by scanning electron microscopy (Figure 7). The microstructures of the gels induced from WPC and WPI are markedly different: The WPI gel had a porous network structure like that of a honeycomb, whereas the WPC gel consisted of an irregular granular network like that of coral.

In addition, Figure 8 shows the effect of pressure from 400 to 1000 MPa on the microstructure of pressure-induced gels from 20% WPC and WPI at pH 6.8. The honeycomb structure of the gels from 20% WPI appears more clearly at 600 and 1000 MPa than at 400 MPa, and the pore size of the fine-stranded network became larger. With the gels from WPC, increasing the pressure resulted in a coarser structure with larger aggregates. The difference in microstructure between these gels from 20% WPI and WPC solutions seems to have been reflected in the difference of their hardness and breaking stress shown in Figure 3.

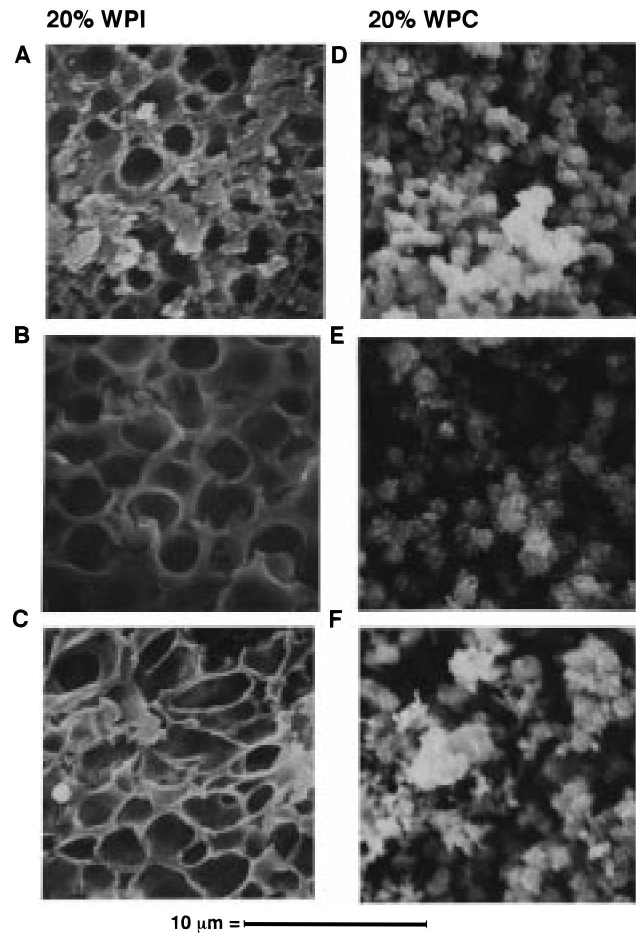

Figure 8. Effect of pressure on the microstructure of pressure-induced gels from WPI (A−C) and WPC (D−F). A 20% WPI and WPC solution in a 0.05 M sodium phosphate buffer, pH 6.8, was pressurized for 10 min at 400 (A and D), 600 (B and E), and 1000 MPa (C and F) and at 30 °C.

DISCUSSION

Whey protein concentration and pressure were very important factors for inducing denaturation, aggregation, and especially the gelation of WPC and WPI under hydrostatic pressure (Figures 1−3). Changes in the turbidity and viscosity determined as an index of denaturation and aggregation of protein in a WPI solution were detected at 1 and 6% concentrations, respectively, under hydrostatic pressure of >400 MPa. Partial unfolding and aggregation of the isolated β-lactoglobulin at a low concentration (2.5 or 5%) have been induced by pressure processing at 450 MPa at neutral pH (Dumay et al., 1994; Funtenberger et al., 1995). Furthermore, gelation of WPI occurred with a 10% concentration at 600 MPa and with 12% at 400 MPa, and with 18% for WPC at 400 MPa. High hydrostatic pressure is known to induce protein denaturation by altering the delicate equilibrium between the interactions that stabilize the folded conformation of native protein (Masson, 1992). With increasing WPI concentration and pressure, the degree of denaturation and the number and size of the aggregates of the denatured protein were increased, and subsequent gelation of the protein was caused. Gelation was found to have occurred by the intermolecular association of β-lactoglobulin, β-lactoglobulin/α-lactalbumin, and bovine serum albumin at a high concentration by pressurization (Figures 5 and 6).

The gelation of whey proteins also is known to be induced by heating, and heat-induced gelation has been extensively studied [see Monahan et al. (1995)]. These studies have demonstrated that the gelation of whey protein is affected by intrinsic factors such as the composition and concentration of the proteins and by extrinsic factors such as the heating temperature, pH, ionic strength, and divalent cations (Kinsella et al., 1994). The possible forces involved in the thermally

induced network formation of protein are hydrogen bonds, electrostatic (ionic) bonds, hydrophobic interactions, and intermolecular disulfide linkages (Kinsella and Whitehead, 1989; Monahan et al., 1995). Hydrostatic pressure as an extrinsic variable alters the three-dimensional structure of proteins and leads to the disruption of electrostatic bonds and hydrophobic interactions and the formation of hydrogen bonds (Masson, 1992). These bonds induce conformational, structural, and hydration changes in proteins (Masson, 1992). Funtenberger et al. (1995, 1997) and Tanaka et al. (1996) have reported that high molecular weight aggregates (dimer to hexomer) of isolated β-lactoglobulin at 450 MPa were induced by the formation of intermolecular S−S bonds, which were caused by SH−SS interchange or by other oxidation reaction. The formation of intermolecular S−S bonds has been reported in heat-induced gelation [see Kinsella and Whitehead (1989), Monahan et al. (1995), and Iametti et al. (1995)]. Aggregates or gels with low hardness and breaking stress ($\leq$50 g) and the high protein solubility of WPI induced at relatively low pressure and protein concentration (Figures 3 and 4) are stabilized not only by hydrogen bonds, electrostatic bonds, and hydrophobic interactions but also by protein−protein interaction through disulfide linkages, as shown in the SDS−PAGE patterns with or without the reducing agent (Figure 6). The free, highly reactive −SH group of Cys 121 of β-lactoglobulin may have been directly involved in intermolecular disulfide interchange with other −SH groups in the pressurized whey proteins.

A marked difference in pressurizing effect was found between WPC and WPI, the protein concentration required for gelation being similar between heating and pressurizing treatments. Heat-induced gel from dialyzed WPI was formed in the concentration range of 9−10.5% at pH 6.5−8.0 and at 90 °C (Rector et al., 1991). However, protein concentration $\geq$7.5% was required for the formation of strong gel from WPC solutions by heating for 10 min at 100 °C and at pH 7.0 (Schmidt, 1981). A minimum protein concentration for heat-induced gelation was relatively higher in WPI than in WPC (Morr and Ha, 1993). For pressure-induced gelation, on the other hand, concentrations of 10% for WPI and 18% for WPC under the pressure of 400 MPa at pH 6.8 were required. The protein concentration of WPC required for pressure-induced gelation corresponds to that in 15% WPI. A minimum protein concentration for pressure-induced gelation was higher in WPC than in WPI, and it was the reverse of heat treatment. This means that ingredients other than protein in WPC affected the pressure-induced gelation. The irregular particulate network observed in the pressure-induced gel from WPC was similar to that of heat-induced gels from WPC [11%, pH 6.8, 1 h at 100 °C (Sone et al., 1983)] and isolated β-lactoglobulin [12%, pH 6.0, 30 min at 85 °C (Langton and Hermansson, 1992); 9.8−14.6%, pH 6.9−7.0, 40 min at 87 °C (Zasypkin et al., 1996)]. This is another difference between heat- and pressure-induced gels, since the network structure of pressure-induced gels from isolated β-lactoglobulin was very similar to that of WPI (unpublished results; Zasypkin et al., 1996). These differences may be caused by not only the lower protein concentration in WPC than in WPI but also the higher contents of lactose, lipids, and inorganic materials in WPC than in WPI. Lactose may act as a baroprotective agent against pressure-induced unfolding and aggregation (Cheftel, 1992; Dumay et al., 1994). Aggregation and gelation of whey proteins are likely to be affected by difference in these ingredients. It is possible that a new complex might form with fat, ion, or lactose by hydrophobic interaction accompanied with a negative volume change.

Understanding of the formation of a whey protein gel by hydrostatic pressurizing should give further basic information for the practical utilization of whey protein. The production of (hydroxymethyl)furfural and loss of available lysine resulting from heating WPC (Li-Chan, 1983) may not be caused by pressurization. Further research with respect to extrinsic factors is necessary to clarify the effects of pressure on gelation at a molecular level and is underway.

CONCLUSION

With the WPI solution, the concentration for affecting the turbidity was 1% and was 6% for the viscosity at 400 MPa, while for inducing gelation it was 10% at 600 MPa. With the WPC solution, the viscosity changed at a concentration >12%, and gel formation began at >18% at 400 MPa. The hardness and breaking stress of pressure-induced gels from WPI increased with increasing WPI concentration (12−18%) and hydrostatic pressure. These ratings for the 20% WPC gels were one-third those of the 20% WPI gels. The microstructure of the WPI gels had a porous network form, whereas the WPC gels were irregular particulates. β-Lactoglobulin predominantly participated in pressure-induced aggregation and gelation, in which intermolecular S−S bonding is likely to play a critical role.

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