Rheological Properties and Characterization of Polymerized Whey Protein Isolates

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Whey protein polymers were formed by heating whey protein isolate solutions at 80 °C. Flow behaviors of whey protein polymers produced from different protein concentrations and heating times were comparable to various flow behaviors of hydrocolloids. Polymer formation was found to be a two-phase process. The initial protein concentration was a significant factor that determines the size and/or shape of the primary polymer in the first phase as shown by intrinsic viscosity. Heating time was a factor in determining the aggregation in the second phase as shown by apparent viscosity. Intrinsic viscosity of whey protein polymers was as high as 141.7 ± 7.30 mL/g, compared to 5.04 ± 0.20 mL/g for native whey proteins. The intrinsic viscosity and gel electrophoresis data suggested that disulfide bonds played an important role in whey polymer formation.

Keywords: *Polymerization; rheological properties; characterization; whey proteins*

INTRODUCTION

Viscosity plays a major role in consumer acceptability of liquid and semisolid type foods. Large molecular weight polymers, such as hydrocolloids, remarkably increase viscosity even at low concentration. This relates to molecular properties such as size, shape, flexibility, and hydration. This is why hydrocolloids, which are large, linear, and considerably flexible polymers (Walker, 1984), have often been used as thickening agents in food products (Damodaran, 1997).

Whey, the byproduct from cheese manufacturing, has been increasingly utilized as a source of protein and as a functional ingredient in foods. In general, several intrinsic factors that affect the functional properties of food proteins are amino acid sequence and composition, structure (secondary, tertiary), hydrophilic/hydrophobic character of protein surface, net charge and distribution, and molecular rigidity/flexibility. Due to their small molecular weight and more spherical shapes, whey proteins exhibit low viscosity and have not been utilized as a thickening agent.

The effect of heat treatment on the functionality of whey proteins has been the subject of investigation. Heating generally has a negative effect on protein solubility (deWit and Klarenbeek, 1984). Moderate heat treatment of whey protein concentrate has been shown to improve its foaming properties, whereas severe treatment results in impairment of foaming properties (Richert et al., 1974). Emulsification properties are also enhanced after moderate heating (Schmidt et al., 1984). Heating results in protein denaturation followed by aggregation. Roefs and De Kruif (1994) proposed that protein aggregation might be considered to be a polymerization process if it proceeds in a manner similar to an ordinary radical polymerization reaction, in which sulfhydryl groups play the role of the radicals. They used their model to describe the polymerization mechanism of β-lactoglobulin (β-LG) which is the major protein in whey. The polymerization model contained initiation, propagation, and termination steps. The reaction was based on thiol/disulfide exchange reactions leading to the formation of polydisperse aggregates of disulfide linked β-LG monomers. Few studies have been reported on the quantitative characterization of the size and shape of whey aggregates and particles, despite the extensive study of whey protein denaturation and aggregation. Using high-performance size exclusion chromatography and multiangle laser-light scattering detection, Hoffmann et al. (1997) reported the molecular mass distribution of heated β-LG aggregates. Upon heating of β-LG in the concentration range 10−75 g/L, aggregates having molecular masses of 4×10^6 Da and higher were formed. In this study, whey protein isolate (WPI) was heat denatured and polymerized to produce soluble polymers. The effects of protein concentration and heating time were investigated. The viscosities of the polymers were compared with those of hydrocolloids. Whey polymers were characterized by intrinsic viscosity and gel electrophoresis. The importance of disulfide bonds in polymer formation was also studied.

MATERIALS AND METHODS

Materials. WPI (90.68% protein) was obtained from Davisco Foods International (Le Sueur, MN). Hydrocolloids were provided as follows: locust bean gum, xanthan gum (Rhodigel), and guar gum (Jaguar) by Rhône-Poulenc (Washington, PA); alginate (Algin) by Kelco (Chicago, IL); and λ-carrageenan (Viscarin) by FMC (Philadelphia, PA). All chemicals were of analytical grade.

Heat Polymerization of WPI. WPI solutions at different concentrations (8, 9, 10, and 11%) were prepared in deionized (DI) water (>17 MΩ) and left overnight at 4 °C to equilibrate. Samples were brought to room temperature and transferred to closed polycarbonate tubes. The tubes were placed in a water bath at 80 °C for 1, 3, and 9 h. For apparent viscosity measurement, the samples were cooled at 21 ± 3 °C for 1.5−2 h and then held at 4 °C to reach 24 h of cooling time before

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measurement. For intrinsic viscosity and electrophoresis, the samples were cooled at 21 ± 3 °C for 2 h then diluted and analyzed.

Preparation of Hydrocolloids Solution. Hydrocolloid solutions at different concentrations (0.25, 0.50, 0.75, and 1%) were prepared by adding the powdered hydrocolloid to vigorously stirred DI water at 60−80 °C. The solutions were stirred for 2−3 h to ensure complete hydration. Volume adjustment was made after the solutions were cooled to room temperature.

Viscosity Measurements. Measurements were made at 25 °C using a Bohlin VOR rheometer (Bohlin Reologi, Inc., Cranbury, NJ). The Bohlin C 25 concentric cylinder measuring system was used in all experiments. The measuring system consisted of a rotating cup and a fixed bob attached to a torque bar (1.82, 13.2, or 42.5 g cm). The samples were sheared from 0.185 to 116 1/s. Flow behavior was described by the power law model

$$\sigma = k\dot{\gamma}^n \qquad \text{or} \qquad \eta = k\dot{\gamma}^{n-1} \tag{1}$$

where σ = shear stress, η = apparent viscosity, $\dot{\gamma}$ = shear rate, n = flow behavior index, and k = consistency index. Data were collected from three replications. Within the same replication, three measurements were made.

Gel Electrophoresis. Unheated and heated WPI samples were analyzed by polyacrylamide gel electrophoresis (PAGE), using a vertical slab gel system (Idea Scientific, Minneapolis, MN). Samples, 3 mg/mL in DI water, for sodium docecyl sulfate (SDS)−PAGE were diluted 1:2 with Laemmli sample buffer (Bio-Rad, Hercules, CA). The Laemmli sample buffer for reducing conditions also contained 710 mM 2-mercaptoethanol (2-ME). For native (nondissociating) PAGE, samples (3 mg/mL) were diluted 1:2 with native sample buffer (Bio-Rad).

SDS−PAGE was performed according to the method of Laemmli (1970). SDS was omitted in the case of native PAGE. The resolving and stacking gels contained 12.5 and 4% acrylamide, respectively. Proteins were stained with Coomassie brilliant blue R250 staining solution (Bio-Rad) and destained with destaining solution (Bio-Rad). The relative intensity of the stained bands was determined by densitometry (Molecular Dynamics Inc., Sunnyvale, CA).

Intrinsic Viscosity. Intrinsic viscosity was measured with a Cannon-Fenske capillary viscometer immersed in a constant-temperature water bath maintained at 30 ± 0.5 °C. Stock solutions from each sample were prepared with DI water to contain 20 mg/mL protein. For viscosity measurement, the stock solution was diluted with DI water for a series of five concentrations between 2 and 14 mg of protein/mL. The specific viscosity (η_{sp}) was calculated from

$$\eta_{sp} = (t - t_0)/t_0 \tag{2}$$

where t_0 = the efflux time of water and t = the efflux time of protein solution (Kragh, 1961). On the basis of the well-known Huggins equation $[\eta_{sp}/c = [\eta] + k[\eta]^2c$, where c = the concentration of protein (g/mL)], intrinsic viscosity $[\eta]$ was determined from the extrapolation of the plot of η_{sp}/c vs c (Tanford, 1961).

The effects of disulfide and noncovalent bonds were further investigated with polymers produced from 11% WPI solutions. The stock solutions were prepared such that they contained 2% protein and 350 mM dithiothreitol (DTT) and/or 8 M urea. The stock solutions were diluted with 350 mM DTT and/or 8 M urea. The dilution concentrations and the calculations were as previously described.

RESULTS AND DISCUSSION

Apparent Viscosity. Untreated 20% protein WPI solutions were low in viscosity (Figure 1). A polymer solution formed by heating (80 °C for 1 h) an 8% protein WPI had a flow behavior similar to that of a 20% protein solution of untreated WPI. The viscosity of heated samples increased with increasing protein concentration from 8 to 11%. The flow behavior index (n) and consistency index (k) values obtained by fitting the shear stress versus shear rate data to a power law model (eq 1) are given in Table 1 for WPI solutions and in Table 2 for hydrocolloids. When $n = 1$, the solution is Newtonian and the smaller the value of n, the greater the departure from Newtonian behavior. The R^2 values from fitting the data to the power law model of all the samples range from 0.83 to 1.00. The major whey proteins, β-lactoglobulin and α-lactalbumin, are small and globular in shape; therefore, native whey protein solutions exhibit low viscosity. Heating ruptures various intramolecular bonds stabilizing the native protein structure. Above a certain temperature, the protein unfolds followed by aggregation. These aggregates or polymers are larger in size as well as more asymmetric in shape and have a larger effective volume fraction than the native molecules, resulting in an increase in viscosity.

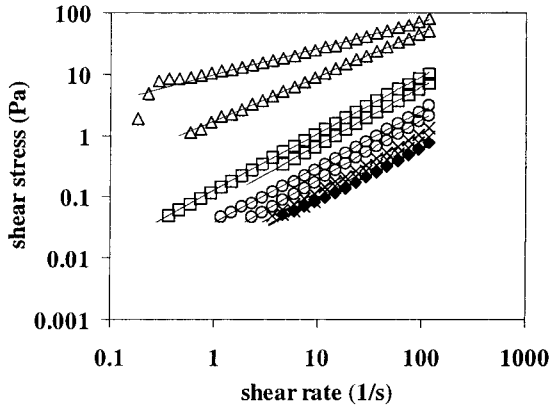

Figure 1. Comparison of flow behaviors of untreated WPI and heated WPI. Lines represent the fitted curves based on a power law model. The parallel lines for heated samples represent the range of viscosity for solutions heated for 1−9 h: (◆) 20% protein WPI dispersions untreated; (×) 8% protein WPI dispersions heated at 80 °C; (○) 9% protein WPI dispersions heated at 80 °C; (□) 10% protein WPI dispersions heated at 80 °C; (△) 11% protein WPI dispersions heated at 80 °C.

Table 1. Effect of Heat Treatment on Flow Behavior Index (n) and Consistency Index (k) of WPI Solutions

protein concn (%)	heating time (h)	n^a	k^a
8	1	0.93 ± 0.04	0.01 ± 0.00
	3	0.95 ± 0.03	0.01 ± 0.00
	9	0.94 ± 0.03	0.01 ± 0.00
9	1	0.94 ± 0.03	0.03 ± 0.00
	3	0.97 ± 0.03	0.03 ± 0.00
	9	0.95 ± 0.03	0.03 ± 0.01
10	1	0.92 ± 0.02	0.12 ± 0.01
	3	0.97 ± 0.02	0.08 ± 0.01
	9	0.96 ± 0.00	0.09 ± 0.00
11	1	0.44 ± 0.01	8.70 ± 1.09
	3	0.73 ± 0.02	1.80 ± 0.11
	9	0.75 ± 0.02	1.90 ± 0.25

[a] Mean ± standard deviation.

The effect of protein concentrations on flow behaviors of heated WPI solutions was found to be significant ($P < 0.01$ for both n and k). Increasing concentration increased viscosity (higher k). The flow behavior index of 11% protein WPI polymer dispersions indicated that they were more pseudoplastic (lower n) than polymer dispersions formed at lower concentrations. The effect of heating time within the same protein concentration

Table 2. Flow Behavior Index (*n*) and Consistency Index (*k*) of Hydrocolloids

sample	concn (%)	n^a	k^a
alginate	0.25	0.93 ± 0.01	0.04 ± 0.00
	0.50	0.82 ± 0.02	0.19 ± 0.02
	0.75	0.79 ± 0.02	0.50 ± 0.07
	1	0.71 ± 0.00	1.00 ± 0.05
guar gum	0.25	0.86 ± 0.25	0.04 ± 0.01
	0.5	0.64 ± 0.01	0.63 ± 0.02
	0.75	0.56 ± 0.01	2.04 ± 0.09
	1	0.47 ± 0.01	5.70 ± 0.49
locust bean gum	0.25	0.89 ± 0.02	0.02 ± 0.00
	0.5	0.86 ± 0.01	0.15 ± 0.01
	0.75	0.76 ± 0.01	0.67 ± 0.06
	1	0.69 ± 0.01	1.98 ± 0.13
λ-carrageenan	0.25	0.92 ± 0.02	0.04 ± 0.01
	0.50	0.90 ± 0.01	0.10 ± 0.00
	0.75	0.88 ± 0.02	0.18 ± 0.02
	1	0.79 ± 0.01	0.38 ± 0.04
xanthan gum	0.25	0.25 ± 0.01	0.93 ± 0.05
	0.50	0.22 ± 0.02	1.66 ± 0.08
	0.75	0.18 ± 0.00	2.53 ± 0.07
	1	0.18 ± 0.01	3.87 ± 0.10

[a] Mean ± standard deviation.

was found to be insignificant for 8 and 9% protein dispersions. For 10 and 11% protein dispersions, heating at 3 and 9 h did not show significant differences, but heating at 1 h was found to be significantly different ($P < 0.1$) from heating at 3 and 9 h. Prolonged heating of 10 and 11% protein dispersions decreased viscosity and lowered the degree of pseudoplasticity compared to those for dispersions heated for 1 h.

When the flow behaviors of WPI polymer dispersions were compared to those of hydrocolloids, it was found that 8% protein polymer dispersions were comparable to 0.25% locust bean gum. The flow behavior of 9% protein polymer dispersions was comparable to that of 0.25% alginate and 0.25% λ-carrageenan dispersions. The viscosity of 10% protein polymer dispersions heated for 1 h appeared to be close to that of 0.50% alginate and 0.50% locust bean gum dispersions, whereas 3 and 9 h of heating produced polymers with flow behaviors similar to that of 0.50% λ-carrageenan dispersions. The flow behavior of 11% protein WPI dispersions heated for 1 h was comparable to that of 1% guar gum dispersions. Heating 11% protein dispersions for 3 and 9 h resulted in a very similar flow pattern, which was comparable to that of 1% locust bean gum dispersions.

Several studies have shown that heating whey proteins results in the formation of soluble aggregates or polymers. It has been generally accepted that the intermolecular SH/S−S interchange reactions and hydrophobic interactions are mainly responsible for polymer formation (Li-Chan, 1983; Shimada and Cheftel, 1989; Zhu and Damodaran, 1994). Above a critical concentration and under the proper conditions (pH and ionic strength), gelation may occur (Kinsella and Whitehead, 1989; Ziegler and Foegeding, 1990). Rheological properties of polymers are related to the size and shape of molecules. In this study, polymerization results in whey polymer dispersions having flow behaviors similar to those of hydrocolloids. However, the concentrations of protein used were still at least 10 times higher than those of hydrocolloids that produce a similar viscosity. This indicates that whey polymers are still much smaller and/or more spherical in shape compared to hydrocolloids.

Characterization of WPI Polymers. Characterization of WPI polymers was performed by gel electrophoresis and intrinsic viscosity analyses. The apparent viscosity data showed no significant difference between heating at 3 and 9 h; therefore, we chose to analyze whey polymers formed by heating for 1 and 3 h only.

Gel Electrophoresis. Several studies indicated that upon heating β-LG above 65 °C and at neutral or elevated pH, polymerization or aggregation occurred mainly via disulfide cross-linking (Hoffmann and Van Mil, 1997; McSwiney et al., 1994). Roefs and De Kruif (1994) proposed that the aggregation model for β-LG is analogous to a radical-addition polymerization reaction. The initial step involves the activation of β-LG monomer in such a way that the free thiol group becomes reactive (B*). The reactive thiol group of B* then reacts via a thiol/disulfide exchange reaction in the propagation step. The termination step occurs when the two reactive intermediates (B_x*) react with each other to form a polymer. The study by Prabakaran and Damodaran (1997) supported the polymerization model; however, they found that propagation occurred only when the reactive dimer concentration reached a critical level. α-Lactalbumin does not form aggregates when it is heated alone. When heated in the presence of β-lactoglobulin or bovine serum albumin (BSA), α-lactalbumin interacts with each of the other two proteins and forms aggregates or gels by means of both disulfide and hydrophobic interactions (Hines and Foegeding, 1993; Gezimati et al., 1997; Dalgleish et al., 1997). BSA has also been found to interact with β-lactoglobulin (Gezimati et al., 1996; Matsudomi et al., 1994).

Native or nondissociating PAGE revealed that whey monomers (β-LG, α-LA, and BSA) completely disappeared after heating 8, 9, 10, and 11% WPI solutions at 80 °C for 1 h (Figure 2A) or 3 h (data not shown). Bands of high molecular weight polymers appeared on top of the gel, indicating that all monomers were involved in polymer formation. The majority of polymers were unable to enter the 4% stacking gel, suggesting that the molecular weight of these polymers were >1 million (Utsumi et al., 1984).

SDS−PAGE patterns of the 1 h heated samples were analyzed under reducing and nonreducing conditions to determine the significance of intermolecular disulfide bonds (Figure 2B,C). Electrophoresis under denaturing (SDS) and nonreducing conditions (Figure 2B) showed that only weak bands of β-lactoglobulin and α-lactalbumin monomers appeared in the heated samples. These bands were apparent to the eyes but were below the sensitivity of the densitometer. When separated under denaturing (SDS) and reducing conditions (with 2-mercaptoethanol), heated and unheated samples had identical patterns. The samples heated for 3 h also had the same results (data not shown). This suggested that disulfide bonds were largely responsible for the formation of these high molecular weight polymers or that SDS did not make the polymers small enough to enter the gel. The finding of the importance of disulfide bonds is in agreement with previous studies on heat-induced aggregation of whey proteins (Shimada and Cheftel, 1989; Li-Chan, 1983; Zhu and Damodaran, 1994).

Intrinsic Viscosity. Intrinsic viscosities of untreated WPI and WPI polymers are given in Table 3. Intrinsic viscosity, [η], is a characteristic property of an isolated polymer molecule in a given solvent. It is a measure of the hydrodynamic volume occupied by the polymer and

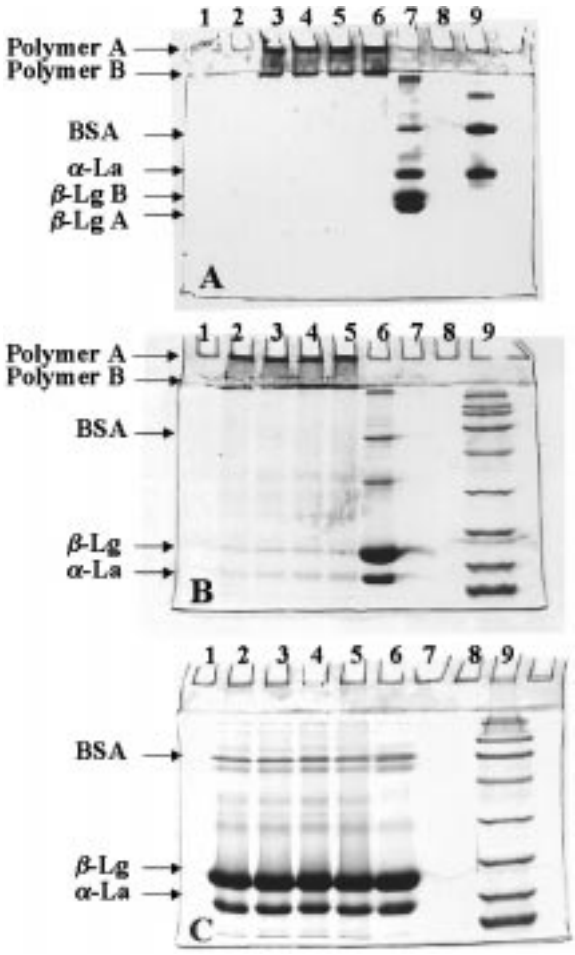

Figure 2. Heat-induced polymerization of WPI: (A) Native (nondissociating) PAGE patterns of heated WPI. Well 9 contains molecular weight markers. Well 7 contains native WPI. Wells 3, 4, 5, and 6 contain 8, 9, 10, and 11% protein WPI dispersions heated at 80 °C for 1 h, respectively. (B) Nonreduced SDS−PAGE patterns of heated WPI. Well 9 contains molecular weight markers. Well 6 contains native WPI. Wells 2, 3, 4, 5 contain 8, 9, 10, and 11% protein WPI dispersions heated at 80 °C for 1 h, respectively. (C) Same as (B), but electrophoresis was run after the samples were reduced with 710 mM β-mercaptoethanol.

can be used to detect the conformational changes of individual proteins (Morris, 1984; Yang, 1961). On the bais of the Einstein value, spherical proteins will have [η] around 3.4 mL/g regardless of their molecular weight (Ross-Murphy, 1994). The intrinsic viscosity of untreated WPI had the average value of 5.04 ± 0.20 mL/g, which is >3.4 because it represents the average hydrodynamic volume of several whey proteins including immunoglobulins, which have a more rodlike shape. In addition, some aggregation during WPI production could contribute to the higher intrinsic viscosity. It is interesting to note that the plots of η_{sp}/c vs c of whey polymers show negative slopes (Figure 3). Apparent viscosity over the range of 24−700 1/s showed no shear thinning (data not shown). Therefore, it is possible that the polymers undergo dissociation when they are diluted such that their shapes are more asymmetrical. However, the more likely explanation is that whey polymers behave as polyelectrolytes such as ionic polysaccharides. Upon dilution, the decreasing ionic strength leads to an increase in the repulsive forces within the polymer backbone, resulting in the expansion of the molecule; thus, the reduced viscosity (η_{sp}/c) increases. The most

Table 3. Effect of Heat Treatment on Intrinsic Viscosity of WPI

sample	heating time (h)	intrinsic viscosity (mL/g)
native WPI	0	5.04 ± 0.20
8%	1	54.7 ± 3.00,[a] 75. 7 ± 3.00[b]
8%	3	56.8 ± 2.60,[a] 72.7 ± 2.60[b]
9%	1	67.3 ± 5.46,[a] 87.5 ± 5.46[b]
9%	3	69.8 ± 4.88,[a] 87.1 ± 4.88[b]
10%	1	90.6 ± 8.87,[a] 107.1 ± 8.87[b]
10%	3	90.1 ± 3.67,[a] 105.6 ± 3.66[b]
11%	1	123 ± 6.92,[a] 140.1 ± 6.92[b]
11%	3	121 ± 7.31,[a] 141.7 ± 7.30[b]
β-lactoglobulin		3.4[c]
BSA		3.7[c]
xanthan gum		7534[d]
guar gum		589[e]
alginate		2000[f]
λ-carrageenan		850−900[g]

[a] Mean ± standard deviation. Values obtained from the extrapolation of η_{sp}/c vs c. [b] Mean ± standard deviation. Values obtained from simplified Fuoss equation (eq 3). [c] Tanford (1961). [d] Dhami et al. (1995). [e] Doublier and Launay (1981). [f] Smidsrød (1970). [g] Ahmad and Williams (1992).

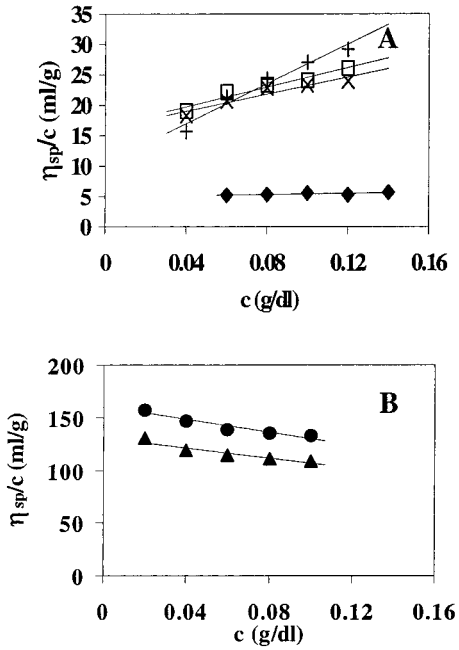

Figure 3. Plot showing intrinsic viscosity of native WPI and WPI polymers (11% protein heated at 80 °C for 1 h) with and without DTT and urea: (A) ◆, native WPI; (□, native WPI with DTT and urea; +, WPI polymers with DTT; ×, WPI polymers with DTT and urea); (B) ▲, WPI polymers; ●, WPI polymers with urea. Lines represent fitted curves.

frequently used calculation for intrinsic viscosity of polyelectrolytes is the simplified Fuoss equation

$$c/(\eta_{sp}) = (1 + B_F c^{1/2})/[\eta] \quad (3)$$

where B_F is the Fuoss constant (Launay et al., 1986). It should be noted that intrinsic viscosity of polyelectrolytes will depend on the ionic strength of the solvent during dilution. For consistency, WPI polymers were diluted with water as untreated WPI.

Whey polymers showed an increase in intrinsic viscosity ranging from 72.7 ± 2.60 mL/g for 8% protein to 141.7 ± 7.30 mL/g for 11% protein. This indicated that polymerization produced larger molecules with less spherical shapes. In addition, the electroviscous effect of polyelectrolytes also contributed to an increase in

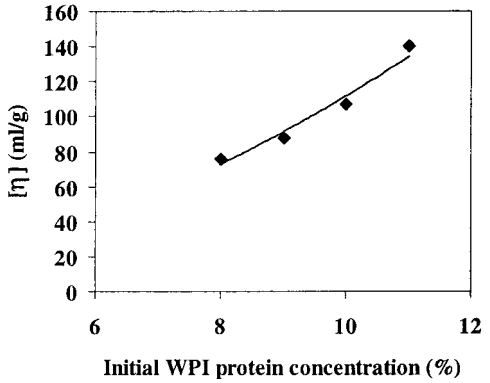

Figure 4. Relationship between the initial WPI protein concentration and intrinsic viscosity. $y = 1.4x^{1.9}$, $R^2 = 0.97$.

Table 4. Effect of DTT and Urea on Intrinsic Viscosity of WPI

sample	intrinsic viscosity (mL/g)
native WPI	5.04 ± 0.20^a
native WPI with DTT and urea	18.5 ± 0.65^a
heated 11% 1 h	140.1 ± 6.92^b
heated 11% 1 h with DTT	14.0 ± 0.53^a
heated 11% 1 h with urea	170.7 ± 7.39^b
heated 11% 1 h with DTT and urea	17.0 ± 1.05^a

[a] Mean ± standard deviation. Values obtained from the extrapolation of η_{sp}/c vs c. [b] Mean ± standard deviation. Values obtained from the simplified Fuoss equation (eq 3).

intrinsic viscosity. However, when compared to the intrinsic viscosities of hydrocolloids (Table 3), whey polymers are still much smaller and/or more spherical. These changes in physical properties, that is, size, shape, and polyelectrolyte property, could have an effect on the functionality of whey polymers such as gelation, emulsification, and foaming. A plot of the initial concentration of WPI placed in the viscometer versus $[\eta]$ indicated that intrinsic viscosity increased tremendously with concentration (Figure 4). The relationship between the initial WPI concentration (c) and intrinsic viscosity, $[\eta]$, may be written as

$$[\eta] = 1.4c^{1.9}, \qquad R^2 = 0.97 \tag{4}$$

The role of noncovalent interactions and disulfide bonds in the formation of whey polymers was observed by adding urea and/or dithiothreitol (DTT) to 11% protein polymer dispersions, which were the biggest polymers in this study. The data are shown in Table 4. Native WPI in the presence of DTT and urea had an intrinsic viscosity of 18.5 ± 0.65 mL/g. The overall effect of DTT and urea on native whey proteins was more unfolding or asymmetrical or random coil molecules, indicated by an increase in intrinsic viscosity to 18.5 mL/g. Intrinsic viscosity of whey polymers increased in the presence of 8 M urea. This result implies that breaking noncovalent bonds, which causes unfolding, leads to more asymmetrical molecules. Adding DTT alone to break the disulfide bonds reduced the intrinsic viscosity values to $\sim 1/10$ the value of WPI polymers. This result manifested the importance of disulfide bonds in WPI polymer formation. In the presence of DTT and urea, the intrinsic viscosity values of untreated WPI and whey polymers were similar (Table 4 and Figure 3A). This confirmed that the polymers were linked by disulfide bonds and noncovalent bonds without the formation of iso-amide bonds. The negative slope of the plot of η_{sp}/c vs c of whey polymers, in the presence or absence of urea (Figure 3B), suggested polyelectrolyte behavior. Whey polymers in the presence of DTT (Figure 3A) showed positive slope, indicating no polyelectrolyte behavior. DTT broke the disulfide bonds, which probably connected the polymer in the linear direction. That might have resulted in smaller molecules, which did not show polyelectrolyte behavior.

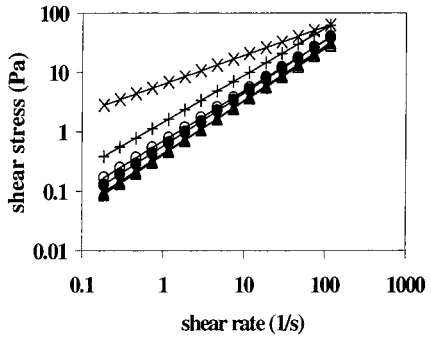

Figure 5. Effect of cooling temperature and/or cooling time on flow behaviors of whey polymers formed from 11% (w/v protein) WPI solutions were heated at 80 °C: (△) heated for 1 h, cooled at 21 ± 3 °C for 2 h; (▲) heated for 3 h, cooled at 21 ± 3 °C for 2 h; (○) heated for 1 h, cooled at 21 ± 3 °C for 6 h; (●) heated for 3 h, cooled at 21 ± 3 °C for 6 h; (×) heated for 1 h, cooled at 21 ± 3 °C for 2 h, then held at 4 °C for a total of 24 h of cooling; (+) heated for 3 h, cooled at 21 ± 3 °C for 2 h, then held at 4 °C for a total of 24 h of cooling. Lines represent the fitted curves based on a power law model.

Heating at 1 and 3 h did not produce any difference ($P < 0.01$) in intrinsic viscosity of whey polymers. This result did not agree with the apparent viscosity data for 10 and 11% WPI, where there was an effect of heating time (Table 1). However, the intrinsic viscosity measurement was performed after WPI polymers were cooled to 21 ± 3 °C for 2 h, whereas the apparent viscosity measurement was performed after cooling to 21 ± 3 °C for 1.5−2 h and then holding at 4 °C until the total cooling time was 24 h. Thus, it may be speculated that polymer formation occurs in two phases, which are cooling temperature and/or cooling time dependent.

Further experiments were conducted to investigate the two-phase polymerization. Whey polymer (11% protein) formed by heating at 80 °C for 1 and 3 h were cooled at 21 ± 3 °C, and the apparent viscosity was measured at 25 °C after 2 and 6 h as in previous intrinsic viscosity experiments. The dispersions were also cooled at 21 ± 3 °C for 2 h and then held at 4 °C. Their apparent viscosity was measured again at 25 °C when the total cooling time reached 24 h as in previous apparent viscosity experiments. The polymers from 1 and 3 h of heating had similar flow behaviors when cooled at 21 ± 3 °C for 2 and 6 h (Figure 5). The result, in agreement with intrinsic viscosity data, indicates that the same primary polymers are formed in the first step of polymerization for both heating times. Flow behaviors after holding at 4 °C and the total of 24 h of cooling of the two polymers were different, with polymers from 1 h of heating showing higher viscosity and higher pseudoplasticity. This, in agreement with previous apparent viscosity results, suggests that different final polymers are formed from different heating times.

Figure 6 represents a model of polymer formation and illustrates the effect of disulfide and noncovalent bonds. In the first phase, heating WPI solutions leads to an unfolding of whey protein monomers, exposing the buried sulfhydryl and hydrophobic groups and activat-

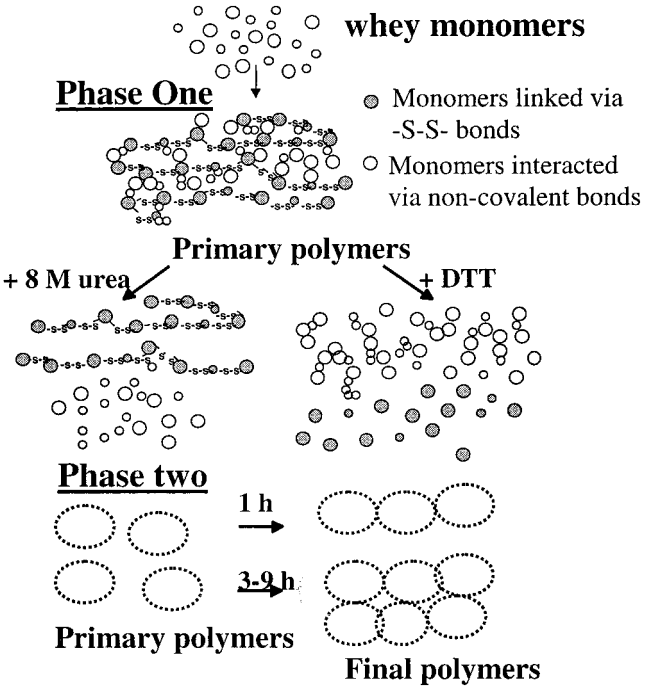

Figure 6. Model for polymer formation and the effect of disulfide and noncovalent bonds.

ing the molecule to a reactive structure. The activated monomers then form primary polymers mainly via sulfhydryl/disulfide interchange reaction and to a lesser extent via hydrophobic interactions. In the second phase, these polymers then aggregate via noncovalent interactions, forming a final polymer. Different heating times at the same protein concentration produce the same primary polymer, as indicated from intrinsic viscosity, SDS−PAGE under nonreducing conditions, and apparent viscosity after cooling at 21 ± 3 °C for 2 and 6 h. This also suggests that sulfhydryl/disulfide exchange occurs completely in the first phase. Prolonged heating results in more second-step association, in such a manner that the overall molecule becomes more symmetrical, whereas the samples heated for 1 h are more asymmetrical due to lesser degree of association. That is why samples heated for 1 h have higher viscosity and more psuedoplasticity. Because the change in flow behaviors was more pronounced in samples held at 4 °C and the total of 24 h cooling, it was speculated that hydrogen bonding was largely responsible for second-phase polymerization.

Conclusion. Whey protein polymers exhibited high viscosity and had flow behaviors similar to those of hydrocolloids. It appears that polymerization is a two-phase process. In the first phase, whey monomers interact via sulfhydryl/disulfide exchange reaction and via noncovalent interactions to form a primary polymer. The primary polymers then associate via noncovalent bonds, forming the secondary polymer. The initial protein concentration plays a major role in the size of the primary polymers, whereas heating time determines the extent of aggregation in the second phase. The effect of the changes in the physical properties of whey polymers from native whey protein on functionality will be further investigated.

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