Structure of Particulate Whey Protein Gels: Effect of NaCl Concentration, pH, Heating Temperature, and Protein Composition

Marleen Verheul and Sebastianus P. F. M. Roefs*

Netherlands Institute for Dairy Research (NIZO), P.O. Box 20, 6710 BA Ede, The Netherlands

In this study whey protein gels were investigated with the aim of further testing a model and ideas developed previously. Measurements on the kinetics of the denaturation/aggregation process, dynamic rheology, gel permeability, and confocal scanning laser microscopy were combined. A broad set of experimental parameters was investigated: NaCl concentration, pH, heating temperature, and protein composition. This resulted in large variations in kinetics and the gel structures formed. The kinetics of the aggregation process determine the amount of aggregated material at the gel point, whereas the primary spatial structure is directly related to this amount of aggregated protein. The primary spatial structure essentially does not change after the gel point. The protein incorporated after the gel point induces a "thickening" of the strands in the gel network and a "decoration" of the pores in the gel. The consequence is that gel permeability decreases only slightly after the gel point but that gel rigidity increases greatly.

Keywords: *Whey; protein; gelation; permeability; rheology*

INTRODUCTION

Whey is the liquid remaining after removal of caseins from milk and contains small, mainly globular proteins: the whey proteins ($\sim$6 g L^{-1} in bovine milk). The main whey proteins are β-lactoglobulin (β-lg), α-lactalbumin (α-la), bovine serum albumin (BSA), and immunoglobulins (Ig), with β-lg as the most abundant one (>50% of the total whey protein) (Walstra and Jenness, 1984). When heated, whey proteins aggregate, and under suitable conditions, gels may be formed (Mulvihill and Donovan, 1987). These heat-induced whey protein aggregates or gels can be used in the food industry for structure improvement (de Wit, 1984; Mulvihill and Kinsella, 1987). Gel structure is strongly influenced by physical conditions (Mulvihill and Donovan, 1987; Paulsson et al., 1990; Matsudomi et al., 1991; Langton and Hermansson, 1992; Foegeding et al., 1995; Verheul and Roefs, 1998). Much research has already been done on this subject and has revealed some general trends. For example, transparent gels with a fine-stranded structure are formed when there is a large electrostatic repulsion between the proteins [at low ionic strength (<0.1 M) and far from the isoelectric point of the proteins]. Under conditions of reduced electrostatic repulsion (at high ionic strength and close to the isoelectric point of the proteins) turbid, milk-white gels are formed with a particulate structure (Clark et al., 1981; Stading et al., 1993). At low ionic strength gel rigidity and gel strength can increase with ionic strength, whereas at higher ionic strength ($I > 0.1$ M) these properties generally decrease with ionic strength (Mulvihill and Kinsella, 1988; Bowland and Foegeding, 1995; Barbut, 1995).

In the present study we focus on the structure of whey protein gels and the effect of four parameters (NaCl concentration, pH, heating temperature, and protein composition) relevant to practice. We combined the kinetics of the aggregation process with the development of the gel structure. Structure development was measured by small-strain dynamic rheology and gel permeability. Gel rigidity, as measured by dynamic rheology, depends on the spatial gel structure and the number and rigidity of the bonds in the gel. Gel permeability is, as a first approximation, only dependent on the large (micrometer) scale spatial structure of the gel network (Roefs et al., 1990; Thies-Weesie et al., 1994; Verheul et al., 1998). Permeability appeared to be very useful in earlier studies and is in a practical sense related to the water-holding capacity of whey protein gels (Verheul and Roefs, 1998; Verheul et al., 1998; Bowland and Foegeding, 1995). The combination of the above-mentioned experimental techniques in a previous study gave us more insight into the gelation process of whey proteins (Verheul et al., 1998).

Heat-induced gel formation results from an aggregation process. Denatured whey proteins aggregate irreversibly and eventually form a space-filling structure: a gel. At the time a gel is formed (the gel point), only a fraction of the total amount of whey protein present has aggregated, and this fraction and the gel point strongly depend on the salt concentration (Verheul et al., 1998). At the gel point the primary spatial structure of the gel is fixed and the amount of protein aggregated determines this spatial structure. After the gel point, more protein is incorporated in the gel network, but this hardly influences the permeability, while gel rigidity strongly increases. The permeability, measured after heating times far beyond the gel point, even scales with the amount of protein aggregated at the gel point, for different initial protein concentrations (35−80 g L^{-1}) and NaCl concentrations (0.2−3.0 M) (Verheul et al., 1998). In the present study, properties of heat-induced whey protein gels were investigated under a much broader set of physical conditions than

* Author to whom correspondence should be addressed (telephone +31 318 659582; fax +31 318 650400; e-mail roefs@nizo.nl).

before, and the results are consistent with the above-described mechanism for the heat-induced gelation developed for the limited set of conditions (Verheul et al., 1998).

MATERIALS AND METHODS

Materials. Chemicals were of analytical grade. A commercial whey protein isolate (WPI) powder was used for the experiments [trade name Bipro, produced by Davisco International Inc. (USA) and purchased from Domo Food Ingredients, Beilen (The Netherlands)]. This powder contained ~89% (w/w) protein [70% (w/w) β-lg, 11% (w/w) α-la, 5% (w/w) Ig, 4% (w/w) BSA], 2% (w/w) ash, <1% (w/w) lactose, and 4% (w/w) water. Purified β-lg was prepared at NIZO from cheese whey, basically following the procedure of Maubois et al. (1987), and contained the genetic variants A and B in a nearly 1:1 ratio (Hoffmann et al., 1996). This sample contained about 88% (w/w) β-lg, 2% (w/w) α-la, 2% (w/w) nonprotein nitrogen material, 2% (w/w) ash, and 4% (w/w) water.

Methods. *Preparation of Protein Dispersions and Gels.* Protein dispersions (total protein concentrations of 35−89 g L^{-1}) were prepared by dissolving β-lg powder or WPI powder in 0.1−3.0 M NaCl solutions that were made using double-distilled water. The protein dispersions were stirred for at least 2 h, and eventually the pH of the whey protein dispersion was adjusted to the desired value using HCl or NaOH. Next, the samples were centrifuged for 10 min at 20000g and filtered using a 0.45 μm non-protein-adsorbing filter to remove insoluble materials. WPI gels were made by heating the dispersions at 65−85 °C; β-lg gels were made only at 68.5 °C.

Conversion of Native Whey Proteins. Protein dispersions were heated in test tubes at 65−85 °C for different time periods. The tubes were cooled in ice water, the pH was adjusted to 4.7 ± 0.1, and the aggregated proteins were sedimented by centrifugation for 30 min at 20000g. The concentrations of the native whey proteins in the supernatant were determined by high-performance gel permeation chromatography (HP-GPC) (Hoffmann et al., 1996).

Permeability Measurements. The permeability coefficient, B_{gel}, was determined from the liquid flux of an NaCl solution through glass tubes filled with a whey protein gel, due to a hydrostatic pressure gradient. B_{gel} was measured at 20 °C after the tubes with gels had cooled in ice water. There were no indications that gel structure, as measured by B_{gel}, changed much on cooling (Verheul and Roefs, 1998). The method has been extensively described before (Verheul and Roefs, 1997, 1998) and is based on the method developed by van Dijk and Walstra (1986). B_{gel} values given under Results and Discussion are mean values taken from 6 (β-lg gels) or 12 (WPI gels) readings, and errors are represented as the standard deviation of the mean value. Error bars are shown only when larger than the symbols used.

Rheological Measurements. Rheological measurements were made with a Rheometrics RFS II rheometer. A couette geometry was used (diameter cup = 17 mm, diameter bob = 16.5 mm, length bob = 12.9 mm). A whey protein dispersion of 2 mL was heated at 65−85 °C in the measuring cup of the rheometer, which was preheated to the desired temperature. The sample was covered with a layer of hexadecane oil to prevent evaporation. During the gelation process an oscillating strain was applied to the sample with a frequency of 1 rad s^{-1} and a maximum strain of 1%. The resulting time-dependent stress was recorded and used to calculate (using the software of the apparatus) G', the storage modulus, and G'', the loss modulus. After 20 or 45 h of heating, the frequency dependence of the moduli was measured over the frequency range of 10^{-2}−10^2 rad s^{-1}.

Confocal Scanning Laser Microscopy (CSLM). Protein dispersions were prepared as described above; the protein was stained before heating with Rhodamine B (~0.25 mg/g of protein), which is a fluorescent dye. Dispersions were put on special glass slides in which holes of 0.5 mm were polished and were covered with glass cover slips. The glass slides were
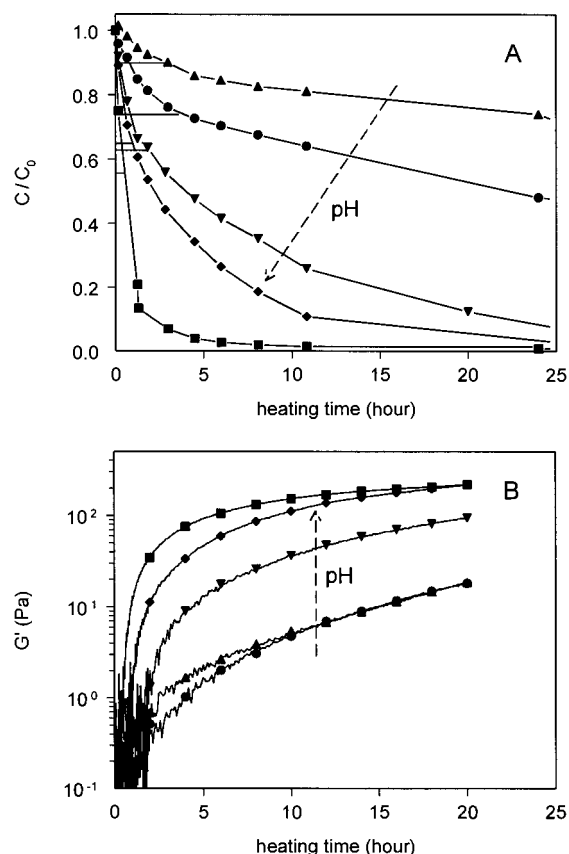

Figure 1. Fraction of nonaggregated protein (A) and G' (B) as a function of heating time at 68.5 °C for 44.5 g L^{-1} WPI and 0.5 M NaCl: (▲) pH 6.0; (●) pH 6.3; (▼) pH 6.8; (◆) pH 7.0; (■) pH 7.5. Gel points are indicated in (A) by horizontal lines; symbols on the curves in (B) are drawn to guide the eye.

put on a glass dish, in which some oil was put to improve heat transfer. The glass dish with the slides was put in a water bath and heated for 20 h at 68.5 °C. After cooling to room temperature, the whey protein gels were studied by CSLM, a technique in which the sample is scanned by a focused laser beam (Brakenhoff et al., 1988; Blonk and van Aalst, 1993; Bremer et al., 1993). In this study the CSLM facility at CDI-DLO in Lelystad (The Netherlands) was used (Bio-Rad MRC 500). The apparatus was supplied with an Ar laser and a BHS filter (488 nm). The intensity of the fluoresced light was measured by a photon multiplier via a conventional light microscope. Pictures were taken at a depth of ~10 μm from the glass surface to minimize surface effects. The thickness of an optical section was kept constant at ~0.7 μm (Brakenhoff et al., 1988).

RESULTS AND DISCUSSION

We systematically varied four parameters: the pH and NaCl concentration of the dispersing medium, the heating temperature, and the protein composition (WPI was replaced by pure β-lg). The sensitivity of gel properties to these parameters will be treated consecutively.

pH and NaCl Concentration. In Figure 1 the fraction of nonaggregated protein (Figure 1A) and G' (Figure 1B) are plotted as a function of heating time at 68.5 °C for 44.5 g L^{-1} WPI and 0.5 M NaCl at pH 6.0−7.5. Figure 1A indicates that the pH has a major influence on the kinetics of the aggregation process. At pH values closer to the isoelectric point of the main whey proteins (β-lg, α-la, BSA), the rate at which native proteins are transformed into aggregates is much lower

Structure of Particulate Whey Protein Gels

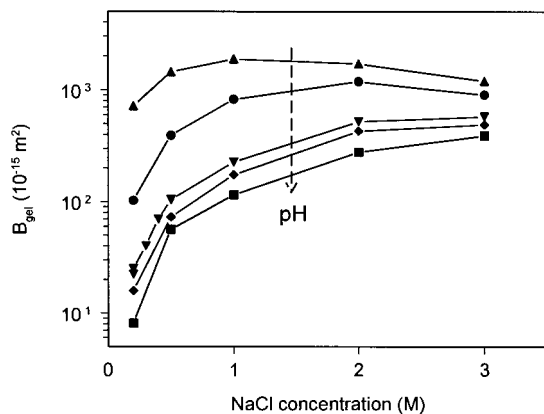*J. Agric. Food Chem.,* Vol. 46, No. 12, 1998 **4911**

Figure 2. B_{gel} versus NaCl concentration for 44.5 g L^{-1} WPI heated for 20 h at 68.5 °C: (▲) pH 6.0; (●) pH 6.3; (▼) pH 6.8; (◆) pH 7.0; (■) pH 7.5.

than at higher pH values. The gel point, here defined as the time after which $G' > 1$ Pa, decreases with increasing pH (from 6.3 to 7.5). However, the gel point at pH 6.0 is lower than at pH 6.3. At the gel point only part of the total amount of protein present in solution is aggregated, as indicated in Figure 1A. This was reported earlier for WPI gels made at different NaCl concentrations at neutral pH (Verheul et al., 1998), and it now appears to be a common feature of heat-induced whey protein gels for a broader range of conditions. Figure 1A also shows that together with aggregation rate the concentration of protein aggregated ($C_{agg} = C_0 - C_t$) at the gel point increases with pH.

G', the elastic modulus, increases as a function of heating time for all pH values investigated; only for the highest pH value does a plateau value seem to be reached after 20 h of heating at 68.5 °C (Figure 1B). The increase in G' with heating time is coupled with the increase in aggregated protein. Tan δ, the ratio between G'' and G', is small (∼0.12) for all WPI gels made at different pH values, indicative of an elastic character of the gels. Straight lines are obtained by making double-logarithmic plots of G' versus ω (10^{-2}–10^2 rad s^{-1}) for all WPI gels. The slopes of these lines do not depend on pH, and their values are relatively small (∼0.07), so relatively few structural units with relaxation times between 6×10^{-2} and 6×10^2 s are present.

The permeability coefficient, B_{gel}, of WPI gels made by heating at 68.5 °C for 20 h is depicted in Figure 2 at various pH values (pH 6.0–7.5) and NaCl concentrations (0.2–3.0 M). Because B_{gel} is, in a first-order approximation, proportional to the particle size squared for particle gels (Bremer et al., 1989), it is plotted on a logarithmic scale. At high pH B_{gel} strongly increases with increasing NaCl concentration, whereas at low pH the increase is less strong and followed by a slight decrease. At low ionic strength, B_{gel} strongly decreases with pH, whereas at high ionic strength the decrease is less pronounced. The decrease of B_{gel} with pH at 0.5 M NaCl (Figure 2) is coupled to an increase of C_{agg} at the gel point (Figure 1A). These findings are consistent with previous observations for WPI gels made at pH 6.8 and different salt concentrations (Verheul et al., 1998), that is, that (1) B_{gel} decreases only by a factor of ∼2 after the gel point, (2) B_{gel} after 20 h of heating scales with C_{agg} at the gel point for different salt concentrations with a low C_{agg} at the gel point leading to a high B_{gel}, and (3) C_{agg} at the gel point decreases with NaCl
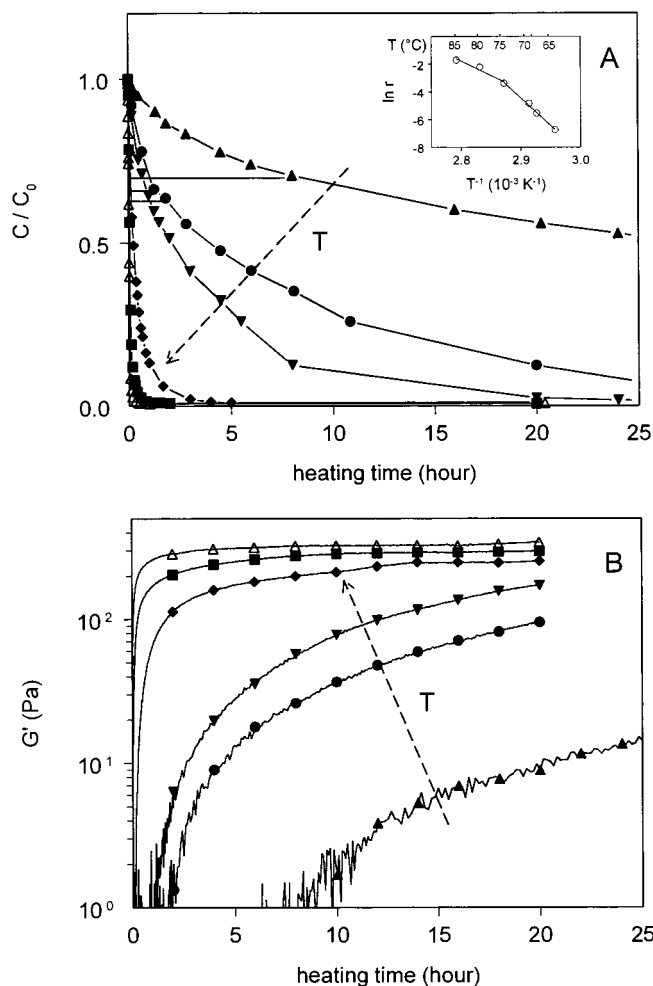

Figure 3. Fraction of nonaggregated protein (A) and G' (B) as a function of heating time for 44.5 g L^{-1} WPI at pH 6.8 and 0.5 M NaCl: (▲) 65 °C; (●) 68.5 °C; (▼) 70 °C; (◆) 75 °C; (■) 80 °C; (△) 85 °C. (Inset) ln r (r is initial reaction rate in g L^{-1} s^{-1}) versus T^{-1} (Arrhenius plot; T is heating temperature in K). Some gel points are indicated in (A) by horizontal lines; symbols on the curves in (B) are drawn to guide the eye.

concentration. The large-scale structure of whey protein gels is related to the electrostatic repulsion between and the stability/solubility of the protein molecules and aggregates. These factors, which depend on pH and NaCl concentration, determine the overall aggregation kinetics and C_{agg} at the gel point.

Heating Temperature. In Figure 3A the fraction of nonaggregated protein is depicted as a function of heating time at various heating temperatures (65–85 °C) and at pH 6.8 and 0.5 M NaCl. The conversion rate increases tremendously with temperature. The inset of Figure 3A shows an Arrhenius plot of the initial reaction rate, r, in grams per liter per second, obtained from the curves in Figure 3A. For the initial reaction rate the slope of the initial concentration decrease is taken. A discontinuity is found in the Arrhenius plot, which has been observed before for the aggregation kinetics of whey proteins in milk (Lyster, 1970; Dannenberg and Kessler, 1988; Anema and McKenna, 1996). The temperature of the discontinuity is lower in the present study (∼75 °C) than found by others (80–90 °C), which may be due to the difference in medium composition and/or the much higher protein concentration used in this study (44.5 g L^{-1}) compared to the other studies (∼6 g L^{-1}). The discontinuity is gradual rather than

4912 *J. Agric. Food Chem.,* Vol. 46, No. 12, 1998

Verheul and Roefs

abrupt and can be explained by the much stronger increase in the rate of the denaturation reaction with increasing temperature compared to the rate of the successive aggregation reactions (Hoffmann et al., 1996; Jeurnink et al., 1996).

G' increases as a function of heating time for all heating temperatures investigated (Figure 3B) and is coupled with the decrease in nonaggregated protein observed in Figure 3A. Only at high heating temperatures (>75 °C) is a plateau value reached within 20 h of heating, when all protein has aggregated. The rigidity of the gels increases with heating temperature, mainly due to an increase in the amount of aggregated protein (Verheul et al., 1998), but the plateau values of the moduli still slightly tend to increase with temperature. The gel point decreases with increasing heating temperature. C_{agg} at the gel point is readily observable only between 65 and 70 °C and hardly varies in this temperature regime (see Figure 3A).

Tan δ decreases slightly from 0.13 to 0.08 with increasing heating temperature. The low values found at each temperature indicate that the gels have an elastic character. A decrease in tan δ with increasing measuring temperature has been observed before for heat-set whey protein gels (Cooney et al., 1993; Paulsson et al., 1990). The slope of a double-logarithmic plot of G' versus ω (10^{-2}–10^2 rad s^{-1}) is small (0.05–0.08) and decreases slightly with increasing heating temperature. This indicates that relatively few structural units with relaxation times between 6×10^{-2} and 6×10^2 s are present, and there are even fewer at higher heating temperatures.

In Figure 4A B_{gel} is plotted versus heating temperature (65–85 °C) at NaCl concentrations of 0.2 and 0.5 M at pH 6.8. Gels were made by heating for 20 or 45 h (at 65 °C). B_{gel} decreases slightly with temperature between 65 and 70 °C for both NaCl concentrations. In this range, the temperature of heating hardly influences the large scale spatial structure. After 45 h of heating at 65 °C B_{gel} is lower than after 20 h of heating (especially for 0.5 M NaCl). At 65 °C the rate of the overall aggregation process is low and a plateau value for B_{gel} is not reached completely within 20 h of heating. At temperatures >70 °C a large increase in B_{gel} is observed (Figure 4A) and coarser gel structures are formed. The transition in gel structure occurs at about the same temperature as where the discontinuity in the Arrhenius plot is observed (inset of Figure 3A). The transition is related to changes in the kinetics of the overall aggregation process of whey proteins. This process consists of unfolding (denaturation) reactions, which are strongly dependent on temperature, and subsequent aggregation reactions. Around 70–75 °C the rate of the denaturation reactions becomes very large and the successive aggregation reactions become rate-limiting. In contrast to lower temperatures, all protein molecules present rapidly denature and are available for successive aggregation. This will affect the nature of the aggregates formed and probably C_{agg} at the gel point. It will lead to coarser network structures with increasing B_{gel} values. In Figure 4B G' is depicted versus heating temperature at 0.5 M NaCl and pH 6.8 after 2, 20, and 45 h of heating. The transition in B_{gel} and aggregation kinetics around 70–75 °C coincide with the temperature at which G' reaches a plateau value very shortly after the beginning of heating. However, Figures 3B and 4B indicate that the value of this plateau level,
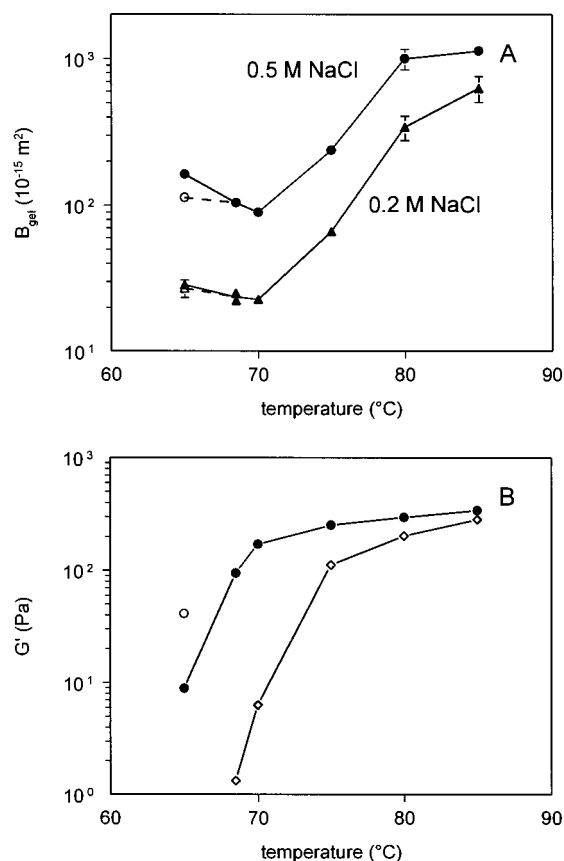

Figure 4. B_{gel} (A) and G' (B) versus heating temperature for 44.5 g L^{-1} WPI at pH 6.8: (▲) 0.2 M NaCl, heated for 20 h; (△) 0.2 M NaCl, heated for 45 h; (◇) 0.5 M NaCl, heated for 2 h; (●) 0.5 M NaCl, heated for 20 h; (○) 0.5 M NaCl, heated for 45 h. Error bars represent the standard error deviation of the mean value.

that is, when all protein has aggregated, does not show the above-mentioned transition with temperature. This is consistent with previous findings that G' mainly depends on the total amount of protein aggregated (Verheul et al., 1998). Changes in gel properties at high heating temperatures have been found by others as well (Clark, 1991; Tang et al., 1993). They attributed the changes to a loss of cross-linking or a change in network strand character but did not connect this to an alteration in the aggregation kinetics of the process.

WPI and β-lg. In Figure 5 a comparison is made between gels from WPI and from pure β-lg at pH 6.8. B_{gel} after 20 h of heating at 68.5 °C increases with NaCl concentration both for WPI and for β-lg gels (Figure 5A), but it increases much more strongly for β-lg than for WPI. At 0.2 M NaCl both type of gels have the same permeability, whereas at 0.5 M NaCl the difference is a factor of ~5. B_{gel} shows a power law dependence on the initial protein concentration for both WPI and β-lg gels at 0.2 M NaCl with different exponents (Figure 5B). CSLM pictures of 44.5 g L^{-1} WPI and β-lg gels after heating for 20 h at 68.5 °C are shown in Figure 6 for 0.1 and 0.5 M NaCl. Figure 6 demonstrates that the structures of WPI and β-lg gels are similar at 0.1 M NaCl, but at 0.5 M NaCl the β-lg gel is much coarser than the WPI gel, consistent with the permeability measurements (Figure 5). The differences in B_{gel} and gel structure observed are the result of a difference in the kinetics of the aggregation process (Verheul et al., 1998) and/or in the type of aggregates formed.
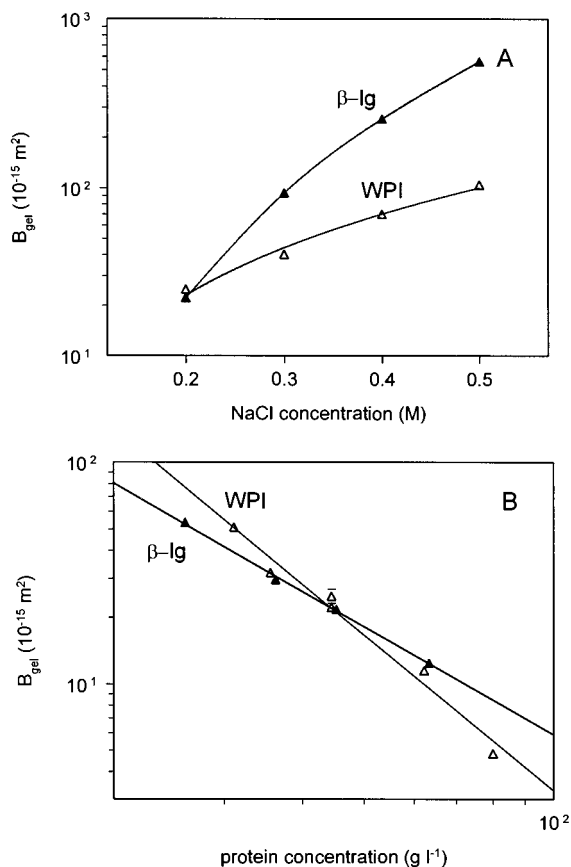
Structure of Particulate Whey Protein Gels

J. Agric. Food Chem., Vol. 46, No. 12, 1998 **4913**

Figure 5. Comparison of B_{gel} of WPI gels ($\triangle$) and β-lg gels ($\blacktriangle$) at pH 6.8 after heating for 20 h at 68.5 °C: (A) B_{gel} versus NaCl concentration for 44.5 g L^{-1} protein solutions; (B) B_{gel} versus protein concentration at 0.2 M NaCl.

In Figure 7A the fractional concentration of native protein is plotted as a function of heating time for pure β-lg and WPI at pH 6.8 and 0.5 M NaCl. The aggregation process is different for both dispersions. At the gel point C_{agg} for the pure β-lg gel is much smaller than for the WPI gel. Thus, the primary spatial structure is formed with less protein in the case of pure β-lg, and this will lead to the coarser structure with the higher gel permeability, as observed in Figures 5 and 6. In the case of WPI the four proteins present all take part in the aggregation process. The inset of Figure 7A shows the concentration decrease of the individual whey proteins in WPI. At short heating times α-la, Ig, and BSA contribute significantly to the amount of aggregated protein, and although they constitute only ~22% of the total weight of protein (78% is β-lg), they make up 55% of the aggregated protein at the gel point. Gel formation obviously occurs at a larger concentration of mixed WPI aggregates than of pure β-lg aggregates, leading to a lower permeability. For WPI gels we have found previously that B_{gel} decreases after the gel point by not more than a factor of ~2. Figure 7B shows that B_{gel} of β-lg gels does not change after 20 h of heating. For both type of gels we think that a primary spatial structure is formed that does not change essentially after the gel point (Verheul and Roefs, 1998; Verheul et al., 1998).

General Discussion. Structural properties of whey protein gels are monitored by various techniques. Microscopical techniques (light microscopy, scanning electron microscopy, transmission electron microscopy, CSLM) provide information on the microstructure [e.g.,

Clark et al. (1981), Langton and Hermansson (1992), Bremer et al. (1993), Stading et al. (1993), Walkenström (1996), and Verheul and Roefs (1998)]. Gel rigidity (the moduli G' and G'') is assessed in small strain rheological measurements and depends on the total number and rigidity of the bonds in the gel [e.g., Paulsson et al. (1990) and Stading et al. (1993)]. Large deformation measurements provide information on the gel strength (i.e., stress or force at fracture) and the deformation at fracture [various kinds of fracture tests are used [e.g., Mulvihill and Kinsella (1988), Matsudomi et al. (1991), Foegeding et al. (1995)]]. For heat-set protein gels we have introduced permeability measurements (Verheul and Roefs, 1998), which, like microscopical methods, probe the gel microstructure but in a noninvasive way.

The gel properties assessed by the various experimental techniques (microstructure, gel rigidity, gel strength, deformation at fracture) are often not mutually related (Foegeding et al., 1998), which makes comparison to literature data complicated. Moreover, gel properties strongly depend on medium composition and the temperature/time history of heating.

In this study medium composition was chosen such that (with one exception) particulate gels were formed. Differences in gel rigidity and gel structure, as found under various physical conditions, are related to the kinetics of the aggregation process. The kinetics are influenced by the stability of the proteins against denaturation (Blundell and Johnson, 1976; Paulsson et al., 1985), the solubility of the native proteins (Blundell and Johnson, 1976, Tanford, 1961) and protein aggregates, the reactivity of thiol groups (Dunnill and Green, 1965; Shimada and Cheftel, 1989), and the repulsive charge of the proteins.

The kinetics of the aggregation process determine the gel point, the amount of material aggregated at the gel point, and, thus, the primary spatial structure. This spatial structure is largely fixed at the gel point, as B_{gel} changes by only a factor of ~2 after the gel point. B_{gel} and C_{agg} at the gel point are related for particulate WPI gels according to a power law found for different initial protein concentrations and NaCl concentrations at pH 6.8 (Figure 8; open symbols and drawn line) (Verheul et al., 1998). When gel structure changes from particulate to fine-stranded, experimental points will deviate from the master curve (Verheul et al., 1998): gels made in 0.1 M NaCl have slightly lower B_{gel} values (see in Figure 8 the four triangles below the drawn line at high C_{agg}). As observed in Figure 8 results of WPI at different pH values and heating temperatures (65−70 °C) from this study fall on this master curve of B_{gel} versus C_{agg} at the gel point. Even a β-lg gel (initial protein concentration = 44.5 g L^{-1}, C_{agg} at the gel point = ~8 g L^{-1}) coincides with the master curve of Figure 8. This confirms that B_{gel} and the primary spatial structure for particulate whey protein gels are uniquely correlated to C_{agg} at the gel point. Furthermore, the findings in this study confirm the suggestion of Langton and Hermansson (1992) that the addition of salt (NaCl) has an effect on spatial structure similar to that of lowering the pH; NaCl concentration and pH are exchangeable physical parameters. The effects of NaCl concentration and pH on gel hardness (gel strength) are, however, different. Gel hardness decreases with NaCl concentration after a maximum found at NaCl concentrations varying from 20 (Matsudomi et al., 1991) and 50−75 (Kuhn and Foegeding, 1991) to 200 mM NaCl (Mulvihill
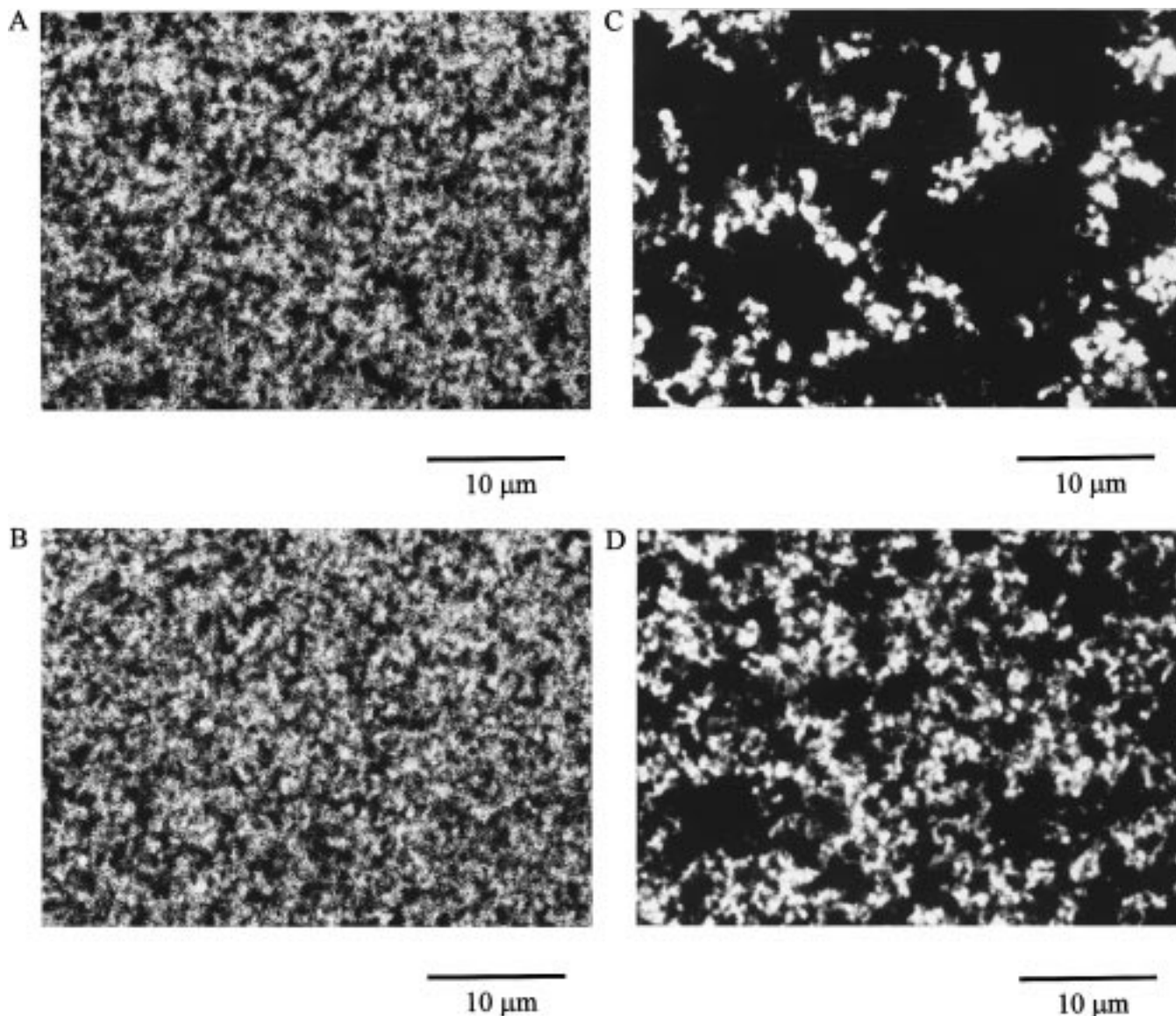
Figure 6. CSLM pictures of WPI gels and β-lg gels made of 44.5 g L^{-1} protein solutions heated for 20 h at 68.5 °C: (A) β-lg gel, 0.1 M NaCl, pH 6.8; (B) WPI gel, 0.1 M NaCl, pH 7.1; (C) β-lg gel, 0.5 M NaCl, pH 6.8; (D) WPI gel, 0.5 M NaCl, pH 6.9.

and Kinsella, 1988; Mulvihill et al., 1990) but increases when pH is lowered from 8 to 6−6.5 (Matsudomi et al., 1991; Stading and Hermansson, 1991). The effect of pH, however, refers to fine-stranded and not to particulate gel structures, because no extra salt was added. By using microscopical techniques, similar microstructures were found for β-lg and whey protein gels (Langton and Hermansson, 1992); these gels with both particulate and fine-stranded structures were made at low salt concentration and in a much broader pH region than in the present study. Langton and Hermansson (1992) reported a more expanded pH region of particulate gel structure for whey proteins than for β-lg. From this study we can conclude that this will be (partly) due to the higher salt content of the commercial whey protein concentrate compared to the highly purified β-lactoglobulin sample they have used.

At high heating temperatures ($\geq$75 °C) the correlation between B_{gel} and C_{agg} does not seem to exist anymore, as an unexpectedly high permeability is found (see Figure 4A). The latter observation agrees with microscopical findings that particulate gel structures become coarser at higher heating temperature in the range of

75−95 °C (Hermansson, 1986). Because of the high aggregation rates it was in this case not possible to determine the gel point accurately and to relate this to B_{gel}.

Results of G' versus C_{agg} at different pH values, heating temperatures, and heating times are combined with results from the earlier study in Figure 9 (Verheul et al., 1997). The curves are constructed by combining results of aggregation kinetics with rheological measurements during heating (for example, from parts A and B of Figure 1). The curves all have a similar shape and are close together, except for 0.1 M NaCl, pH 6.8 (previous work), and 0.5 M, pH 7.5. Differences are caused by the lower amounts of protein aggregated at the gel point and stronger interparticle forces at increasing salt concentrations. G' is mainly determined by the total amount of protein incorporated in the gel network for the gels investigated and seems not to depend on the spatial structure. This assumption is supported by computer simulations of particle gels formed from aggregating particles with flexible bonds, which show little sensitivity of storage modulus to large scale structure (Whittle and Dickinson, 1997). It also
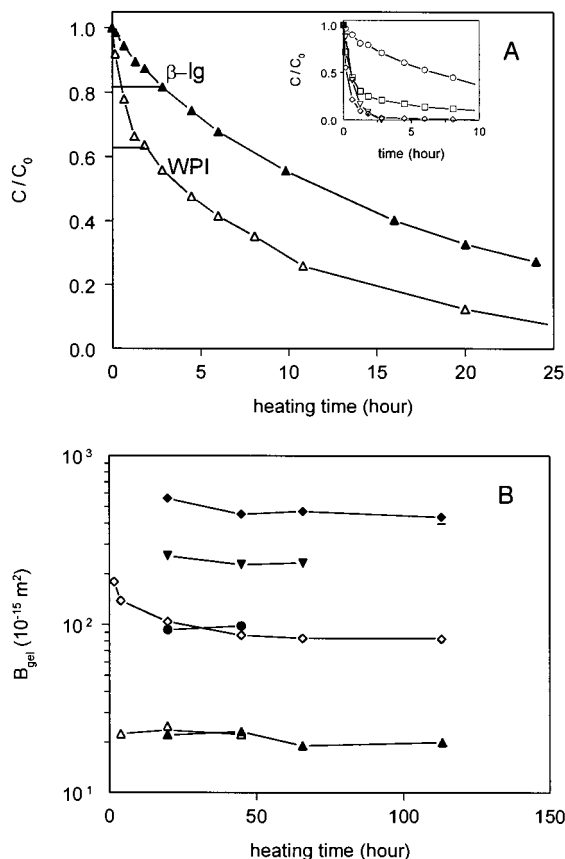
Structure of Particulate Whey Protein Gels

J. Agric. Food Chem., Vol. 46, No. 12, 1998 **4915**

Figure 7. (A) Fractional concentration of native (nonaggregated) protein as a function of heating time at 68.5 °C for 44.5 g L^{-1} protein solutions at pH 6.8 and 0.5 M NaCl: (▲) β-lg; (△) WPI. Gel points are indicated by horizontal lines. (Inset) Fractional concentration of the individual whey proteins in WPI: (○) β-lg; (▽) α-la; (◇) Ig; (□) BSA. (B) B_{gel} versus heating time at 68.5 °C for WPI gels (open symbols) and β-lg gels (solid symbols) for 44.5 g L^{-1} protein solutions at pH 6.8 and NaCl concentrations of (▲ and △) 0.2 M, (●) 0.3 M, (▼) 0.4 M, and (◆ and ◇) 0.5 M.
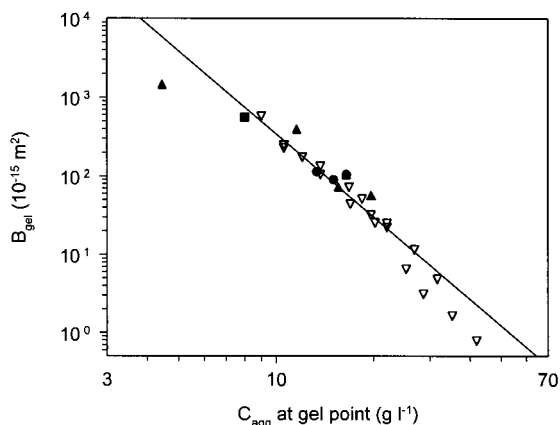
Figure 8. B_{gel} after heating for 20 h as a function of C_{agg} at the gel point; results from the present study: 44.5 g L^{-1} WPI, 0.5 M NaCl at various pH values (pH 6.0–7.5) at 68.5 °C (▲); 44.5 g L^{-1} WPI, 0.5 M NaCl, pH 6.8, at various heating temperatures (65–70 °C) (●); 44.5 g L^{-1} β-lg, 0.5 M NaCl, pH 6.8, at 68.5 °C (■); previous results with 35–80 g L^{-1} WPI, pH 6.8, and NaCl concentrations between 0.1 and 3.0 M (▽) (Verheul et al., 1998).

indicates that the (rate of) conversion of native protein should be taken into account if one interprets the evolution of G′ as a function of (heating) time (Paulsson et al., 1990; Stading et al., 1990; Tang et al., 1993, 1995).
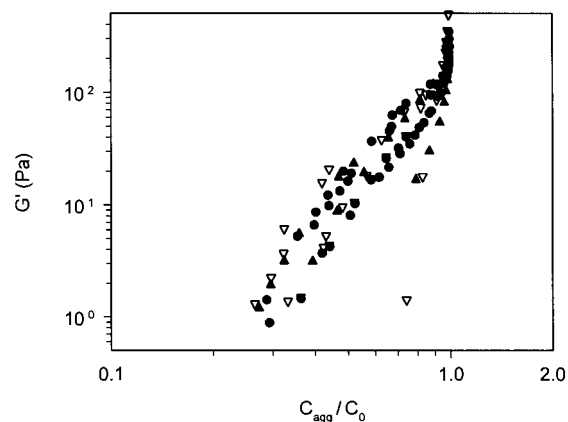
Figure 9. G′ versus total fraction of aggregated protein during heating for 44.5 g L^{-1} WPI; results from the present study (at 0.5 M NaCl) at various pH values (pH 6.3–7.5) at 68.5 °C (▲) and various heating temperatures (65–85 °C) at pH 6.8 (●); previous results (Verheul et al., 1998) at various NaCl concentrations (0.1–3.0 M) (▽).

At 0.5 M NaCl, pH 7.5 (as at 0.1 M NaCl, pH 6.8), the particulate gel structure is close to the transition to a fine-stranded structure and the G′ curve differs.

In conclusion, results from this study confirm that the mechanism for the heat-induced gelation of whey proteins developed for a limited set of conditions is also valid in a much broader, practically relevant range of conditions. Particulate, heat-induced whey protein gels are formed via a seemingly universal denaturation/aggregation/gelation mechanism. C_{agg} at the gel point determines the large scale spatial structure of these gels and is dependent upon experimental conditions (NaCl concentration, pH, heating temperature, protein composition). The permeability after a long heating time scales with C_{agg} at the gel point for various conditions investigated. The protein that is incorporated in the gel network after the gel point gives rise to a "thickening" of the strands in the gel and a "decoration" of the pores in the gel. Consequently, permeability decreases only slightly (by a factor of ~2), but gel rigidity increases strongly after the gel point (by a factor of ~200). No relation is seen between large scale spatial structure and gel rigidity, but a correlation between the total amount of protein that is contributing to the network and gel rigidity is clearly present.

LITERATURE CITED

Anema, S. G.; McKenna, A. B. Reaction kinetics of thermal denaturation of whey proteins in heated reconstituted whole milk. *J. Agric. Food Chem.* **1996**, *44*, 422–428.

Barbut, S. Effects of calcium level on the structure of preheated whey protein isolate gels. *Lebensm.-Wiss. -Technol.* **1995**, *28*, 598–603.

Blonk, J. C. G.; van Aalst, H. Confocal scanning light microscopy in food research. *Food Res. Int.* **1993**, *26*, 297–311.

Blundell, T. L.; Johnson, L. N. Crystallization of proteins. In *Protein Crystallography*; Academic Press: New York, 1976.

Verheul and Roefs

Bowland, E. L.; Foegeding, E. A. Effects of anions on thermally induced whey protein isolate gels. *Food Hydrocolloids* **1995**, *9*, 47−56.

Brakenhoff, G. J.; van der Voort, H. T. M.; Spronsen, E. A.; Nanninga, N. 3-Dimensional imaging of biological structures by high-resolution confocal scanning laser microscopy. *Scanning Microsc.* **1988**, *2*, 33−40.

Bremer, L. G. B.; van Vliet, T.; Walstra, P. Theoretical and experimental study of the fractal nature of the structure of casein gels. *J. Chem. Soc., Faraday Trans. 1* **1989**, *85*, 3359−3372.

Bremer, L. G. B.; Bijsterbosch, B. H.; Walstra, P.; van Vliet, T. Formation, properties and fractal structure of particle gels. *Adv. Colloid Interface Sci.* **1993**, *46*, 117−128.

Clark, A. H. Structural and mechanical properties of biopolymer gels. In *Food Polymers, Gels and Colloids*; Dickinson, E., Ed.; Royal Society of Chemistry: Cambridge, U.K., 1991.

Clark, A. H.; Judge, F. J.; Richards, J. B.; Stubbs, J. M.; Suggett, A. Electron microscopy of network structures in thermally-induced globular protein gels. *Int. J. Pept. Protein Res.* **1981**, *17*, 380−392.

Cooney, M. J.; Rosenberg, M.; Shoemaker, D. F. Rheological properties of whey protein concentrate gels. *J. Texture Stud.* **1993**, *24*, 325−334.

Dannenberg, F.; Kessler, H. G. Reaction kinetics of the denaturation of whey proteins in milk. *J. Food Sci.* **1988**, *53*, 258−263.

de Wit, J. N. Functional properties of whey proteins in food systems. *Neth. Milk Dairy J.* **1984**, *38*, 71−89.

Dunnill, P.; Green, D. W. Sulphydryl groups and the N−R conformational change in β-lactoglobulin. *J. Mol. Biol.* **1965**, *15*, 147−151.

Foegeding, E. A.; Bowland, E. L.; Hardin, C. C. Factors that determine the fracture properties and microstructure of globular protein gels. *Food Hydrocolloids* **1995**, *9*, 237−249.

Foegeding, E. A.; Li, H.; Bottcher, S. R. Gelation of globular proteins. In *Phase/State Transitions in Foods; Chemical, Structural, and Rheological Changes*; Rao, M. A., Hartel, R. W., Eds.; Dekker: New York, 1998; pp 253−271.

Hermansson, A.-M. Water and Fat holding. In *Functional Properties of Food Macromolecules*; Mitchell, J. R., Ledward, D. A., Eds.; Elsevier Applied Science Publishers: London, 1986; pp 273−314.

Hoffmann, M. A. M.; Roefs, S. P. F. M.; van Mil, P. J. J. M.; Verheul, M.; de Kruif, C. G. Aggregation of β-lactoglobulin studied by *in situ* light scattering. *J. Dairy Res.* **1996**, *63*, 423−440.

Jeurnink, T.; Verheul, M.; Cohen Stuart, M.; de Kruif, C. G. Deposition of heated whey proteins on a chromium oxide surface. *Colloids Surf.* **1996**, *6*, 291−307.

Kuhn, P. R.; Foegeding, E. A. Mineral effects on whey protein gelation. *J. Agric. Food Chem.* **1991**, *39*, 1013−1016.

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.

Lyster, R. L. J. The denaturation of α-lactalbumin and β-lactoglobulin in heated milk. *J. Dairy Res.* **1970**, *37*, 233−243.

Matsudomi, N.; Rector, D.; Kinsella, J. E. Gelation of bovine serum albumin and β-lactoglobulin; effects of pH, salts and thiol reagents. *Food Chem.* **1991**, *40*, 55−69.

Maubois, J. L.; Pierre, A.; Fauquant, J.; Piot, M. Industrial fractionation of main whey proteins. *Bull. Int. Dairy Fed.* **1987**, *212*, 154−159.

Mulvihill, D. M.; Donovan, M. Whey proteins and their thermal denaturation−a review. *Irish J. Food Sci. Technol.* **1987**, *11*, 43−75.

Mulvihill, D. M.; Kinsella, J. E. Gelation characteristics of whey proteins and β-lactoglobulin. *Food Technol.* **1987**, *41*, 102−111.

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

Mulvihill, D. M.; Rector, D.; Kinsella, J. E. Effects of structuring and destructuring anionic ions on the rheological properties of thermally induced β-lactoglobulin gels. *Food Hydrocolloids* **1990**, *4*, 267−276.

Paulsson, M.; Hegg, P.-O.; Castberg, H. B. Thermal stability of whey proteins studied by differential scanning calorimetry. *Thermochim. Acta* **1985**, *95*, 435−440.

Paulsson, M.; Dejmek, P.; van Vliet, T. Rheological properties of heat-induced β-lactoglobulin gels. *J. Dairy Sci.* **1990**, *73*, 45−53.

Roefs, S. P. F. M.; de Groot-Mostert, A. E. A.; van Vliet, T. Structure of acid casein gels 1. formation and model of gel network. *Colloids Surf.* **1990**, *50*, 141−159.

Shimada, K.; Cheftel, J. C. Sulfhydryl group/disulfide bond interchange reactions during heat-induced gelation of whey protein isolate. *J. Agric. Food Chem.* **1989**, *37*, 161−168.

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

Stading, M.; Hermansson, A.-M. Large deformation properties of β-lactoglobulin gel structures. *Food Hydrocolloids* **1991**, *5*, 339−352.

Stading, M.; Langton, M.; Hermansson, A.-M. Microstructure and rheological behaviour of particulate β-lactoglobulin gels. *Food Hydrocolloids* **1993**, *7*, 195−212.

Tanford, C. Thermodynamics. In *Physical Chemistry of Macromolecules*; Wiley: New York, 1961.

Tang, Q.; McCarthy, O. J.; Munro, P. A. Oscillatory rheological study of the gelation mechanism of whey protein concentrate solutions: effects of physicochemical variables on gel formation. *J. Dairy Res.* **1993**, *60*, 543−555.

Tang, Q.; McCarthy, O. J.; Munro, P. A. Oscillatory rheological study of the effects of pH and salts on gel development in heated whey protein concentrate solutions. *J. Dairy Res.* **1995**, *62*, 469−477.

Thies-Weesie, D. M. E.; Philipse, A. P. Liquid permeation of bidisperse colloidal hard-sphere packings and the Kozeny-Carman scaling relation. *J. Colloid Interface Sci.* **1994**, *162*, 470−480.

van Dijk, H. J. M.; Walstra, P. Syneresis of curd. 2. One-dimensional syneresis of rennet curd in constant conditions. *Neth. Milk Dairy J.* **1986**, *40*, 3−30.

Verheul, M.; Roefs, S. P. F. M. Structural properties of heat-set whey protein gels. In *Food Colloids−Proteins, Lipids and Polysaccharides*; Dickinson, E., Bergenståhl, B., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1997.

Verheul, M.; Roefs, S. P. F. M. Structure of whey protein gels, studied by permeability, scanning electron microscopy and rheology. *Food Hydrocolloids* **1998**, *12*, 17−24.

Verheul, M.; Roefs, S. P. F. M.; Mellema, J.; de Kruif, C. G. Power law behavior of structural properties of protein gels. *Langmuir* **1998**, *14*, 2263−2268.

Walkenström, P. Phase distribution of mixed biopolymer gels in relation to process conditions. Ph.D. Thesis, SIK, Göteborg, Sweden, 1996.

Walstra, P.; Jenness, R. Proteins. In *Dairy Chemistry and Physics*; Wiley: New York, 1984.

Whittle, M.; Dickinson, E. Brownian dynamics simulation of gelation in soft sphere systems with irreversible bond formation. *Mol. Phys.* **1997**, *90*, 739−757.