Specific Divalent Cation-Induced Changes during Gelation of β-Lactoglobulin†

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Thermally induced gelation of β-lactoglobulin sols that contained NaCl and/or $CaCl_2$ at pH 7.0 and the rheological properties of the resulting gels were investigated. Gels containing 20 mM $CaCl_2$ were more deformable than gels containing 100 mM NaCl since a greater shear strain was required to achieve fracture. Investigating the gelation process by small-strain dynamic rheology showed that β-lactoglobulin sols containing 20 mM $CaCl_2$ gelled more rapidly and had lower gel points than similar sols containing 100 mM NaCl. Following gelation at 80 °C and cooling to 25 °C, gels containing 100 mM NaCl had greater values for G' (storage modulus) than those containing 20 mM $CaCl_2$. These cation-dependent differences in gelation and rheological properties of the gels could not be explained by cation-associated differences in structure or denaturation characteristics determined by circular dichroism measurements.

INTRODUCTION

A functional property of food proteins is the ability to form a gel structure capable of immobilizing water and solute particles. Gelation accounts for textural and water-holding properties of many food products (de Wit, 1984). Under conditions normally encountered in foods, a gel is defined as "a continuous network of macroscopic dimensions immersed in a liquid medium and exhibiting no steady-state flow" (Ziegler and Foegeding, 1990). The rheological and textural properties of food gels are related to the continuous network and the liquid medium surrounding the network. Foods such as cheese and frankfurters can be considered multicomponent protein gels, and their textural properties are determined by the gel matrix and other constituents of the food such as fat or filler particles (Tolstoguzov and Braudo, 1983).

The human perception of texture during mastication is determined, in part, by how food deforms and the force(s) required to cause fracture. Physical properties determined at deformations which cause a breakdown of the food structure are termed fracture properties. These properties are also referred to as failure or ultimate properties in polymer and material sciences. From force–deformation curves one can determine stress at fracture, which is the force (shear or tensile) per unit area, and strain at fracture, the deformation (shear or tensile) per length unit (Hamann, 1983). Previous work has shown that true shear stress at fracture correlates with the sensory texture profile note of "hardness" (force required to bite through sample with molar teeth), whereas true shear strain at fracture is associated with sensory "first bite cohesiveness" (degree to which sample deforms before it ruptures) (Montejano et al. 1985). Stress and strain at fracture are therefore fundamental properties of food materials that can be measured mechanically and are associated with human perception of texture.

In a previous study we showed that thermally formed gels made from whey protein isolate suspensions containing various concentrations of NaCl or $CaCl_2$ had similar salt-associated changes in shear stress at fracture, whereas the shear strains at fracture responded in opposite directions (Kuhn and Foegeding, 1991). Increasing NaCl concentration (20–150 mM) caused a decrease in shear strain at fracture. In contrast, increasing $CaCl_2$ concentration (5–100 mM) produced an increase in shear strain at fracture. The opposite influences of NaCl and $CaCl_2$ on shear strain at fracture were also observed with other mono- and divalent cations of chloride salts, suggesting that the effect is primarily dependent on cation valence. The cited study demonstrated that specific textural properties of whey protein isolate gels could be selectively altered by addition of monovalent or divalent cations.

Understanding the cation-associated mechanism that modulates shear strain would provide insight into the molecular mechanics of strain at fracture. Moreover, this information would serve as a basis for designing whey protein concentrates and isolates with maximal gelling abilities. Whey protein isolate contains approximately 68% β-lactoglobulin, 17% α-lactalbumin, 7% bovine serum albumin, and 7% immunoglobulin G (Morr and Foegeding, 1990). Thus, the cation-associated differences in strain at fracture could be due to the predominant protein, β-lactoglobulin, or the result of interactions among a combination of proteins. The objectives of this study were to determine (1) if the different effects of NaCl and $CaCl_2$ detected with WPI (Kuhn and Foegeding, 1991) are seen in β-lactoglobulin gels and, if so, (2) how NaCl and $CaCl_2$ specifically alter the respective gelation processes.

MATERIALS AND METHODS

Protein Suspensions for Rheology and Calorimetry Experiments. Bovine β-lactoglobulin A and B (1 or 3 times crystallized) was from Sigma Chemical Co. (St. Louis, MO). All the chemicals were of analytical grade. The following procedure was used to make β-lactoglobulin suspensions containing one of the following salts or salt mixtures: 20 mM $CaCl_2$, 100 mM NaCl, 5 mM $CaCl_2$ plus 100 mM NaCl, or 20 mM $CaCl_2$ plus 100 mM NaCl. Concentrated β-lactoglobulin suspensions were prepared by hydrating (250 mg/mL) in 50 mM, pH 7.0, TES buffer and filtering through a 0.45-μm filter (Nalge Co., Rochester, NY). The β-lactoglobulin solutions were then mixed in a 1:1 ratio with one of four salt solutions (40 mM $CaCl_2$, 200 mM NaCl, 10 mM $CaCl_2$ plus 200 mM NaCl, or 40 mM $CaCl_2$ plus 200 mM NaCl), also prepared in 50 mM, pH 7.0, TES buffer. These solutions

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were degassed under vacuum until bubbles stopped appearing, adjusted to pH 7.0, if necessary, with 1 N NaOH or 1 N HCl, and adjusted to 7 or 10% w/v protein concentrations using ϵ_{278} = 0.955 cm²/mg for the mixture of A and B isoforms (Bell and McKenzie, 1967).

Fracture Properties. β-Lactoglobulin gels were made by heating 10% (w/v) protein suspensions in 10 mm diameter silicon-coated (Sigmacote, Sigma) glass tubes for 30 min at 80 °C. Gels were cooled for 30 min at room temperature (23 ± 1 °C), removed from the tubes, and stored overnight at 4 °C. The following day they were equilibrated to 23 ± 1 °C, cut into 28 mm long cylinders, and mounded on plastic disks with cyanoacrylate glue (Krazy Glue, B. Jadow and Sons, Inc., New York, NY). The gel cylinders were ground into capstan shapes with a minimum diameter of 5.25 mm and twisted to failure at 2.5 rpm with a Torsion Gelometer (Gel Consultants, Raleigh, NC) according to the procedure of Kim et al. (1986). True shear stress at fracture and true shear strain at fracture were calculated from the torque and angular displacement as described by Diehl et al. (1979).

Dynamic Rheology. A Bohlin VOR rheometer (Bohlin Reologi AB, Lund, Sweden) was used for rheological measurements and heating. A concentric cylinder fixed bob and rotating cup (C 14) measuring cell (geometry) attached to a 103 g cm torsion bar (linear range 150–19 500 Pa) were used in all experiments. In thermal scanning experiments, the suspensions were heated from 25 to 80 °C at 1 °C/min, held at 80 °C for 3 h, and then cooled to 25 °C at 1 °C/min. Gels were also formed by heating from 25 to 72 °C at 1 °C/min and holding at 72 °C for 12 h. Storage moduli (G', elastic element), loss moduli (G'', viscous element), and phase angles were determined at a frequency of 0.05 Hz and maximum strain of 0.1. Gel points (GP) were determined according to the method of Steventon et al. (1991) as follows. The first 7–10 G' data points that had values above 1% of the torsion bar's linear range were analyzed by linear regression to determine the time or temperature corresponding to the extrapolated values at a G' of zero. The concentration of β-lactoglobulin in all dynamic rheology experiments was 7% (w/v).

Differential Scanning Calorimetry. A Perkin-Elmer DSC-4 (The Perkin-Elmer Corp., Norwalk, CT) differential scanning calorimeter was used to investigate the effects of NaCl and $CaCl_2$ on the thermodynamic characteristics of β-lactoglobulin. Fifty-microliter fractions containing 5 mg of protein were analyzed in hermetically sealed, stainless steel pans. A pan containing 45 μL of deionized water was used as the reference. Five or 10 replicates of each protein suspension were scanned at heating rates of 10 and 20 °C/min over the temperature range 25 to 95 °C.

Circular Dichroism. Bovine β-lactoglobulin B was from Sigma. β-Lactoglobulin solutions were prepared in solutions containing NaCl, $CaCl_2$, and no salt to compare their effects on the protein structure prior to and following heat denaturation. Protein solutions were prepared as follows. β-Lactoglobulin was first hydrated to approximately 2.5 mg/mL in deionized water. Once thoroughly mixed, the protein solution was adjusted to pH 7.0 with 0.1 and 0.01 N NaOH and filtered through a 0.45-μm syringe filter (Nalge) to remove any particulate material and clarify the solution. Following degassing under vacuum to remove entrapped air bubbles, the protein concentration was determined spectrophotometrically at 278 nm using ϵ_{278} = 0.95 cm²/mg (Bell and McKenzie, 1967). The solution was diluted to 2 mg/mL protein with deionized water, and fractions were then mixed in a 1:1 ratio with either deionized water adjusted to pH 7.0 with 0.01 N NaOH or 20 mM, pH 7.0, Tris-HCl containing either 100 mM NaCl or 20 mM $CaCl_2$. After holding overnight at 4 °C, the protein solutions were equilibrated to room temperature (23 ± 1 °C) and diluted to a final protein concentration of 250 μg/mL with the appropriate solutions.

Circular dichroism (CD) spectra were measured on a Jasco J-600 spectropolarimeter (Japan Spectroscopic Co. Ltd., Japan). Protein solutions were analyzed at 25 and 72 °C in a 0.1 mm path length, jacketed quartz cell connected to a temperature-controlled recirculating water bath. CD spectra were collected in 0.5-nm steps at a rate of 20 nm/min over the wavelength range 195–260 nm. Buffer, salts, and heating effects decreased the signal to noise ratio in the lower wavelength region, restricting acquisition of meaningful data below 195 nm. CD measurements were terminated when the photomultiplier tube voltage went below −800 mV, which corresponds to a transmittance of less than 1%. Baseline runs of each solvent solution were measured as soon after the corresponding protein runs as possible. Each protein suspension was scanned in triplicate; the CD spectra were baseline-corrected, averaged, and smoothed using a five-point running average. Molar ellipticities, Δε, correspond to [θ]/3298, where [θ] is the residue-average molar ellipticity in units of deg·cm²/dmol obtained by dividing the measured ellipticity by the residue-average molecular weight of the protein.

Table I. Fracture Properties of 10% (w/v) β-Lactoglobulin Gels

treatment	shear stress at fracture, kPa	shear strain at fracture	stress/strain[a]
100 mM NaCl	40.0 ± 2.7[b]	1.42 ± 0.04	28.2 ± 1.2
20 mM $CaCl_2$	36.6 ± 4.8	1.78 ± 0.08	20.6 ± 3.7

[a] True shear stress at fracture/true shear strain at fracture. [b] Average values ± standard deviation of two replications.

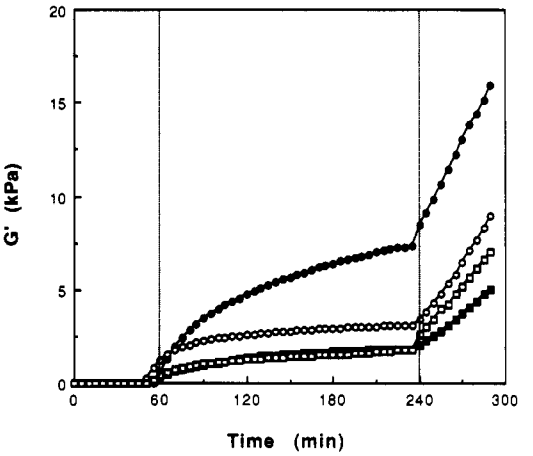

Figure 1. Changes in storage modulus (G') during heating and cooling. Solutions contained 7% (w/v) β-lactoglobulin, pH 7.0, and either 100 mM NaCl (solid circles), 20 mM $CaCl_2$ (open circles), 100 mM NaCl + 20 mM $CaCl_2$ (open squares), or 100 mM NaCl + 5 mM $CaCl_2$ (solid squares). Vertical lines separate sequential times of heating (25–80 °C at 1 °C/min), holding (80 °C for 3 h), and cooling (80–25 °C at 1 °C/min).

RESULTS AND DISCUSSION

Fracture Properties of β-Lactoglobulin Gels. Gels made from β-lactoglobulin solutions containing $CaCl_2$ had greater values for true shear strain at failure (TS strain) than those that contained NaCl (Table I). This demonstrated that the Ca^{2+}-associated increase in TS strain that was observed with whey protein isolate (WPI) gels (Kuhn and Foegeding, 1991) also occurred with β-lactoglobulin gels. Therefore, other whey proteins such as α-lactalbumin, bovine serum albumin, and immunoglobulin G are not required to produce this effect.

The force–deformation curves from β-lactoglobulin and WPI gels were linear up to the point of fracture. This allowed calculation of the "fracture shear modulus", the ratio of true shear stress (TS stress) at fracture to TS strain at fracture. Because the stress/strain relationship was linear up to the point of fracture, the fracture shear modulus would be valid for deformations at strains less than those which result in fracture. Calcium-containing gels had lower fracture shear moduli than Na^+-containing gels; i.e., they required a smaller stress to produce an equivalent deformation (Table I). This was also observed with β-lactoglobulin gels that were deformed under uniaxial compression to a true shear strain of 0.158, which was probably less than the fracture strain (Mulvihill and Kinsella, 1988). The lower shear modulus is important for the interpretation of results from empirical testing of

Table II. Rheological Properties of β-Lactoglobulin Gels[a]

treatment	G′, kPa		G″, kPa		phase angle, deg	
	80 °C	25 °C	80 °C	25 °C	80 °C	25 °C
100 mM NaCl	8.3 ± 1.2	17.2 ± 1.4	1.00 ± 0.12	1.59 ± 0.09	6.9 ± 0.37	5.3 ± 0.55
20 mM $CaCl_2$	3.5 ± 0.9	10.4 ± 2.5	0.54 ± 0.33	1.16 ± 0.30	8.5 ± 5.4	6.4 ± 0.31
100 mM NaCl + 20 mM $CaCl_2$	2.1 ± 0.4	7.3 ± 0.3	0.32 ± 0.07	0.85 ± 0.12	8.6 ± 0.67	6.7 ± 1.13
100 mM NaCl + 5 mM $CaCl_2$	2.2 ± 1.1	6.3 ± 2.6	0.32 ± 0.23	1.01 ± 0.53	7.7 ± 1.5	8.8 ± 0.93

[a] Mean value ± standard deviation of three or four replications for storage modulus (G′), loss modulus (G″), and phase angle after 3 h at 80 °C and after cooling to 25 °C.

Table III. Rheological Transitions during β-Lactoglobulin Gelation[a]

treatment	GP		$t_{50\%G'}$, min	$t_{90\%G'}$, min
	temp, °C	time, min		
100 mM NaCl	80.0	0.68 ± 0.47	33.5 ± 5.5	122.8 ± 14.2
20 mM $CaCl_2$	73.2 ± 0.29	–[b]	18.8 ± 11.8	124.8 ± 27.8
100 mM NaCl + 20 mM $CaCl_2$	76.7 ± 0.26	–	18.0 ± 4.6	127.3 ± 18.2
100 mM NaCl + 5 mM $CaCl_2$	77.8 ± 0.23	–	39.3 ± 27.0	131.7 ± 25.0

[a] Mean value ± standard deviation of three or four replications for gel point (GP) and time to achieve 50 ($t_{50\%G'}$) or 90% ($t_{90\%G'}$) of the final G′ value after holding at 80 °C. [b] Gelation occurred during heating from 25 to 80 °C at 1 °C/min.

"gel strength", where the sample geometry or testing method does not permit accurate calculations of stresses and strains. Gel strengths (usually reported as force values) determined at strains less than the fracture strain show that Ca^{2+}-containing gels are weaker than Na^+-containing gels, as judged by lower moduli. If the sample is deformed to the point of fracture, the relative strengths of the gels would depend on the testing method; either type of gel may appear to be "stronger". Torsional testing of gels allows for unambiguous measurement of stress and strain because the sample is deformed without changes in specimen shape or volume, which can result in artifacts during compression and puncture testing (Hamann and Lanier, 1986).

Dynamic Rheological Properties. Rheological transitions in protein sols during thermally induced gelation can be followed dynamically by small-strain (i.e., strains that do not cause fracture) oscillatory shear rheology. A strain level of 0.1 was chosen because it did not cause structural damage as shown by strain sweep experiments yet was high enough to allow sensitive detection of the gel point. Rheological changes that occurred during heating (25–80 °C), holding (80 °C for 3 h), and cooling (80–25 °C) were detected as changes in G′ (Figure 1). All of the gels showed an increase in G′ during the holding period; the most pronounced increase was in the gel that contained 100 mM NaCl. Moreover, the major increase in G′ occurred during cooling for all of the treatments (Figure 1). An increase in G′ upon cooling was previously reported for β-lactoglobulin (Paulsson et al., 1990), ovalbumin, and soybean isolate gels (Van Kleef, 1986). Average values for G′, G″, and phase angles are shown in Table II. Sols containing 100 mM NaCl formed gels with the highest G′ values at 80 and 25 °C. Gels produced by the 20 mM $CaCl_2$ treatment had the second highest values for G′, whereas those produced by the salt mixtures were the lowest in G′ (Table II). Phase angle values indicate the relative amounts of viscous and elastic elements in the gels. A viscous fluid has a phase angle of 90°, and that of an elastic solid is 0°. The low phase angle (all <9°) and G″ values indicate that the β-lactoglobulin gels were fairly elastic. Our values are in agreement with those of Paulsson et al. (1990), who reported loss tangent values of ~0.1 (corresponding to a phase angle of 6.4°) for β-lactoglobulin gels at pH 7.0 and concentrations of 3, 4, and 5% w/v.

The gel point (GP) occurs during the gelling process when the weight-average molecular weight of the aggregates diverges to infinity and there is a phase transition

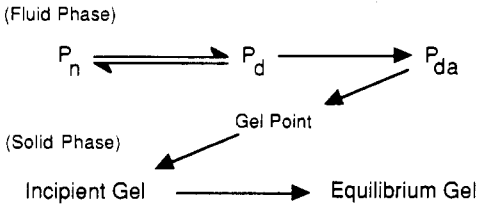

Figure 2. Thermally induced gelation reaction. Native proteins (P_n) are denatured (P_d) and then aggregate (P_{da}) into a solid gel structure.

from a viscoelastic fluid to a viscoelastic solid (Winter, 1987). Unlike investigations with chemical gels (polymers), where rheological detection of the gel point has been the subject to rigorous investigations (Winter, 1987), no standardized definition for gel point has been adopted for protein gelation. One can operationally define gel point as the time required for G′ to become greater than the noise level of the instrument. This definition has been used in other studies (Wu et al., 1991); however, this parameter can vary as a function of instrumental sensitivity and is not inherently related to the physical properties of the system. This approach was modified by Clark (1991), who defined the gel point as the time axis intercept obtained by extrapolating the rapidly rising G′ values to zero. The method of Clark (1991) was shown to be useful in applying percolation theory to protein gelation (Steventon et al., 1991) and was used to determine GPs in this study. This approach to determining GP was very precise; standard deviations for three or four replications were in the range 0.2–0.5 min (heat rate was 1 °C/min so 1 °C = 1 min), which would be in the expected range of random errors (Table III). Sols that contained 20 mM $CaCl_2$ gelled during heating from 20 to 80 °C, whereas those that contained only NaCl required 0.7 min at 80 °C (Table III). Sols that contained a mixture of salts gelled at temperatures between those that were obtained in the presence of $CaCl_2$ or NaCl exclusively.

The gelation time course was followed by determining the time required to achieve 50 or 90% of the final G′ values at 80 °C (G'_{80}). The times required to achieve 90% of G'_{80} were similar for all of the conditions, whereas sols that contained 20 mM $CaCl_2$ showed a more rapid initial gelling phase, as evidenced by the shorter times that were required to reach 50% of G'_{80} (Table III). To summarize, the presence of 20 mM $CaCl_2$ in the gelling sol resulted in an early onset of gelation with a rapid initial increase in G′ followed by a slow increase in G′ (Figure 1; Table III).

Table IV. Effect of NaCl and $CaCl_2$ on Denaturation of β-Lactoglobulin

treatment	rate, °C/min	n^a	T_o,[b] °C	T_{max},[c] °C	enthalpy,[d] kJ/mol	width at half-peak height, Δ°C
deionized H_2O	10	10	69.7 ± 1.1	77.3 ± 0.32	228 ± 35	8.3 ± 0.48
	20	10	71.4 ± 1.2	80.0 ± 0.43	211 ± 35	10.4 ± 1.08
100 mM NaCl	10	5	73.4 ± 0.16	78.8 ± 0.24	207 ± 8	6.6 ± 0.27
	20	5	74.8 ± 0.39	81.4 ± 0.16	212 ± 7	8.6 ± 0.31
20 mM $CaCl_2$	10	5	69.9 ± 0.22	75.3 ± 0.05	230 ± 23	6.9 ± 0.42
	20	5	72.1 ± 0.20	78.9 ± 0.37	232 ± 5	8.9 ± 0.50

[a] Number of replications. [b] Temperature of onset. [c] The temperature at peak maximum. [d] Enthalpy was calculated from the area under the peak.

A generalized model for thermally induced protein gelation is shown in Figure 2. In the context of this model, the differences in GP values could be due to ion-mediated effects on either the unfolding or aggregation/gelation processes or both. The presence of $CaCl_2$ may destabilize β-lactoglobulin and convert it to a denatured (P_d) state at a lower temperature. The effect of NaCl and $CaCl_2$ on denaturation was investigated using differential scanning calorimetry (DSC). To determine if the denaturation reaction was reversible, the samples were heated, cooled, and reheated to see if transitions were detected. No peaks indicative of transitions were detected during the second heating. Therefore, the proteins were considered to be irreversibly denatured under these conditions (data not shown). This is in agreement with results of Ruegg et al. (1977), which documented the irreversible denaturation of 3–6% w/v β-lactoglobulin in simulated milk ultrafiltrate buffer at pH 6.6. The "denatured state" is not necessarily a randomly coiled polypeptide. A number of studies indicate that a partially denatured "molten globule" state forms prior to more extensive unfolding (Harding et al., 1991). The molten globule state retains a high degree of secondary structure. Relative to thermal denaturation of β-lactoglobulin in deionized water as a reference reaction, the addition of 100 mM NaCl increased the "onset temperature" (T_o) but did not change the temperature at peak maximum (T_{max}) (Table IV). Addition of 20 mM $CaCl_2$ did not alter T_o and caused a 1–2 °C decrease in T_{max}. No salt-associated differences in enthalpy or peak width at half height were observed (Table IV). The width at half-peak height decreases as the degree of cooperativity in the transition process increases. The denaturation transition for β-lactoglobulin was more cooperative when salts were added to the protein solution (Table IV). This is reasonable, since salts would be expected to enhance the stabilizing hydrophobic effect (Dill, 1990; Schein, 1990). Our values for denaturation enthalpy agreed with the value of Ruegg et al. (1977) (227 ± 21 kJ/mol) but were lower than the range determined by Dannenberg and Kessler (1988) (262–277 kJ/mol). The T_o value obtained for β-lactoglobulin in deionized water (Table IV) is in close agreement with that of Imafidon et al. (1991) (69.6 °C); however, our T_{max} was 2 °C lower than theirs.

Gelation at 72 °C. The DSC results indicate that salts do not substantially alter the thermodynamics of denaturation. However, small changes in T_o and T_{max} suggest that $CaCl_2$ lowers the temperature (or time) required for conversion of β-lactoglobulin to a structure that is prone to intermolecular aggregation. To determine whether this was the result of a kinetic or thermodynamic limitation, sols were heated to 72 °C and held for 12 h. The results show that β-lactoglobulin sols containing 100 mM NaCl can form gels at 72 °C (Figure 3). This demonstrates that if the reaction is given sufficient time to occur, there is no structural (thermodynamic) limitation to gelation. The

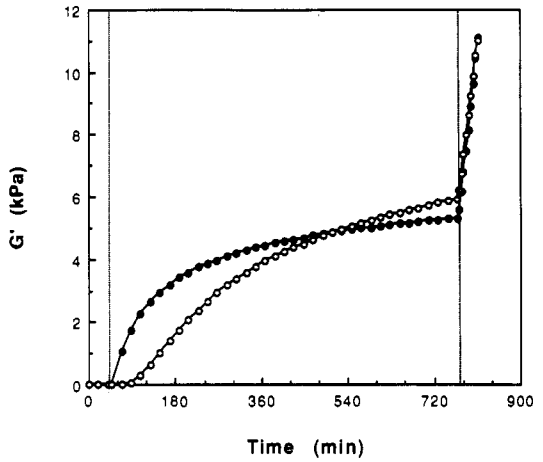

Figure 3. Changes in storage modulus (G') during heating at 72 °C. Solutions contained 7% w/v β-lactoglobulin, pH 7.0, and either 100 mM NaCl (open circles) or 20 mM $CaCl_2$ (solid circles). Vertical lines separate sequential times of heating (25–72 °C at 1 °C/min), holding (72 °C for 12 h), and cooling (72–25 °C at 1 °C/min).

time course of the gelation process is altered by the salts. Relative to sols that contained $CaCl_2$, those that contained NaCl required more time to reach the gel point and began to gel at a slower rate. However, after about 8 h, the G'_{72} value of the NaCl-containing gels surpassed those of the $CaCl_2$-containing gels (Figure 3). The G' values did not plateau, indicating that an equilibrium gel matrix did not form during the 12-h heating. This was also observed by Paulsson et al. (1990) when β-lactoglobulin gels were held at 90 °C for 16 h. It is interesting to note that the cation-associated difference in G'_{72} disappeared when the gels were cooled to 25 °C. Thus, the chemical interactions that lead to the differences at 72 °C either do not occur or are overshadowed by other factors that are not significantly affected by cation valence at 25 °C (Figure 3). This is not surprising since protein stability is the result of a balance between large internal and external forces that can change drastically with temperature and are intrinsically dependent on the nature of the solvent (Schein, 1990; Dill, 1990).

Two potential explanations may be offered for the specific effects of divalent cations on gelation: (1) the cations may alter the denatured state and thus stabilize a structure that is more prone to aggregation, or (2) the protein structures denature in a similar manner and divalent cations mediate the association process in a different way from monovalent cations. This point was investigated by measuring the circular dichroism (CD) spectrum of β-lactoglobulin sols at 25 and 72 °C. The CD spectra of the native proteins (at 25 °C) did not indicate that there were any salt-dependent differences in secondary structure (Figure 4). Thermally induced changes

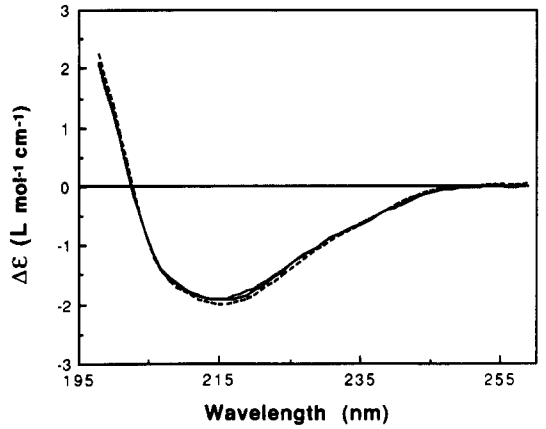

Figure 4. Circular dichroism spectra at 25 °C. Suspensions contained 250 μg/mL β-lactoglobulin B, pH 7.0, in deionized water (dashed line), 50 mM NaCl (solid line), or 10 mM $CaCl_2$ (dotted line).

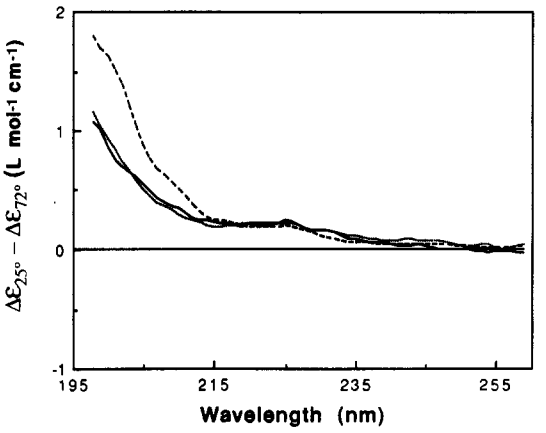

Figure 5. Circular dichroism difference spectra for native and denatured β-lactoglobulin B. The difference spectra were calculated by subtracting the 72 °C spectra from the 25 °C spectra. Solutions contained 250 μg/mL β-lactoglobulin B, pH 7.0, in either deionized water (dashed line), 50 mM NaCl in deionized water (solid line), or 10 mM $CaCl_2$ in deionized water (dotted line).

in secondary structure were detected by subtracting the 72 °C spectra from those that were obtained at 25 °C to produce the difference spectra shown in Figure 5. There is a detectable difference in how the β-lactoglobulin secondary structures change when samples were heated at 72 °C in the presence of added salt or water. However, curves measured in the presence of mono- and divalent cations were essentially identical, indicating that the respective secondary structures were probably not significantly different. The CD data were obtained using salt concentrations corresponding to half those used in the rheological studies to minimize spectral interferences (i.e., 50 mM vs 100 mM NaCl and 10 mM vs 20 mM $CaCl_2$). At lower protein concentrations (14 μm during CD measurement vs 3.8 mM in determining dynamic rheological properties) the aggregation rate will be slowed by a factor that will also depend on the concentration of involved cation species. It is unlikely that this would reduce the ability of divalent cations to induce changes in secondary structure since the molar ratios of salt to protein were higher in the CD studies ($C_{ion}/C_{protein}$ was 3676 for Na^+ and 735 for Ca^{2+}) than in the rheological studies ($C_{ion}/C_{protein}$ was 26 for Na^+ and 5.3 for Ca^{2+}). However, one cannot discount the possbility that a structural difference exists that is not detected by CD. Aggregation may be the result of a structural alteration which imparts a relatively subtle change in chirality and occurs in a large percentage of the population or a relatively large structural change that only occurs in a minor population that must form prior to entering the aggregation pathway. Recent ^1H NMR experiments indicate that the former possibility does occur (H. Li, C. C. Hardin, and E. A. Foegeding, unpublished data), although a causative role of this phenomenon in the aggregation process has not been unequivocally documented.

Conclusion. β-Lactoglobulin gels show an increase in true shear strain at fracture in the presence of Ca^{2+}, a phenomenon that was previously observed with whey protein isolate gels (Kuhn and Foegeding, 1991). This demonstrates that other whey proteins such as α-lactalbumin, bovine serum albumin, and immunoglobulin G are not required to produce this effect. When 20 mM $CaCl_2$ is present in β-lactoglobulin sols, the gelation process changes. Ca^{2+}-containing sols had lower gel points and gelled more rapidly than similar sols that contained 100 mM NaCl. The rheological properties of the gels are also cation-dependent. After gelation at 80 °C and cooling to 25 °C, gels that contained 100 mM NaCl had greater G' values than those that contained 20 mM $CaCl_2$. These differences in the gelation process and the rheological properties of the resulting gels were not due to cation-associated differences in the initial stages of the denaturation processes or substantive differences in secondary structures detected by circular dichroism spectroscopy. Thus, cations appear to differentially affect some relatively subtle aspect of the aggregation process and thereby produce gels with different rheological and fracture properties.

ABBREVIATIONS USED

C_{ion}, ion concentration; $C_{protein}$, protein concentration; DSC, differential scanning calorimetry; G', storage modulus; G'', loss modulus; GP, gel point; P_n, native protein; P_d, denatured protein; P_{da}, denatured and aggregated protein; TES, *N*-[tris[hydroxymethyl]methyl]-2-aminoethanesulfonic acid; Tris-HCl, tris[hydroxymethyl]aminomethane hydrochloride; T_{max}, temperature at peak maximum; T_o, onset temperature; WPI, whey protein isolate.

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LITERATURE CITED

Bell, K.; McKenzie, H. A. The isolation and properties of bovine β-lactoglobulin C. *Biochim. Biophys. Acta* **1967**, *147*, 109–122.

Clark, A. H. Structural and mechanical properties of biopolymer gel modulus. In *Food Polymers, Gels and Colloids*; Dickinson, E., Ed.; Special Publication 82; Royal Society of Chemistry: London, 1991; p 322.

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.

Diehl, K. C.; Hamann, D. D. Structural failure in selected raw fruits and vegetables. *J. Texture Stud.* **1979**, *10*, 371–400.

Dill, K. A. Dominant forces in protein folding. *Biochemistry* **1990**, *29*, 7133–7155.

Hamann, D. D. Structural Failure in Solid Foods. In *Physical Properties of Foods*; Peleg, M., Bagley, E. B., Eds.; AVI Publishing: Westport, CT, 1983; p 351.

Hamann, D. D.; Lanier, T. C. Instrumental methods for predicting seafood sensory texture quality. In *Seafood Quality Determination*; Elsevier Science Publishers: Amsterdam, 1986; p 123.

Harding, M. M.; Williams, D. H.; Woolfson, D. N. Characterization of a partially denatured state of a protein by two-dimensional NMR: Reduction of the hydrophobic interactions in ubiquitin. *Biochemistry* **1991**, *30*, 3120–3128.

Imafidon, G. I.; Ng-Kwai-Hang, K. F.; Harwalkar, V. R.; Ma, C.-Y. Differential scanning calorimetric study of different genetic variants of β-lactoglobulin. *J. Dairy Sci.* **1991**, *74*, 2416–2422.

Kim, B. Y.; Hamann, D. D.; Lanier, T. C.; Wu, M. C. Effects of freeze-thaw abuse on the viscosity and gel-forming properties of surimi from two species. *J. Food Sci.* **1986**, *51*, 951–956.

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

Montejano, J. G.; Hamann, D. D.; Lanier, T. C. Comparison of two instrumental methods with sensory texture of protein gels. *J. Texture Stud.* **1985**, *16*, 403–424.

Morr, C. V.; Foegeding, E. A. Composition and functionality of commercial whey and milk protein concentrates and isolates: A status report. *Food Technol.* **1990**, *44* (4), 100–112.

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.

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

Ruegg, M.; Moor, M.; Blanc, B. A calorimetric study of the thermal denaturation of whey proteins in simulated milk ultrafiltrate. *J. Dairy Res.* **1977**, *44*, 509–520.

Schein, C. H. Solubility as a function of protein structure and solvent components. *BioTechniques* **1990**, *8*, 308–317.

Steventon, A. J.; Gladden, L. F.; Fryer, P. J. A percolation analysis of the concentration dependence of the gelation of whey protein concentrates. *J. Texture Stud.* **1991**, *22*, 201–218.

Tolstoguzov, V. B.; Braudo, E. E. Fabricated foodstuffs as multicomponent gels. *J. Texture Stud.* **1983**, *14*, 183–212.

Van Kleef, F. S. M. Thermally induced protein gelation: Gelation and rheological characterization of high concentrated ovalbumin and soybean protein gels. *Biopolymers* **1986**, *25*, 31–59.

Winter, H. H. Transient Networks. Evolution of rheology during chemical gelation. *Prog. Colloid Polym. Sci.* **1987**, *75*, 104–110.

Wu, J. Q.; Hamann, D. D.; Foegeding, E. A. Myosin gelation kinetic study based on rheological measurements. *J. Agric. Food Chem.* **1991**, *39*, 229–236.

Ziegler, G. R.; Foegeding, E. A. The gelation of proteins. *Adv. Food Nutr. Res.* **1990**, *34*, 203–298.

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