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Interactions of α-Lactalbumin and Bovine Serum Albumin with β-Lactoglobulin in Thermally Induced Gelation†

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The interactions of α-lactalbumin and bovine serum albumin (BSA) with β-lactoglobulin in mixed-protein gel systems were investigated by dynamic oscillation rheology and aggregation rates. BSA and α-lactalbumin were found to contribute to the storage modulus (G') of mixed-protein gels made primarily with β-lactoglobulin. Gels made with β-lactoglobulin alone, or combinations of α-lactalbumin and β-lactoglobulin, had similar transitions and ultimate values for G'. In contrast, the rheological transitions and properties of gels made with mixtures of β-lactoglobulin and BSA were dependent on the ratio of proteins in the mixture. Second-order aggregation rate constants of α-lactalbumin, β-lactoglobulin, and BSA heated alone at 80 °C were in the order BSA ≫ β-lactoglobulin > α-lactalbumin. The aggregation rate of α-lactalbumin increased when heated in combination with β-lactoglobulin, suggesting a coaggregation of proteins. These results indicate that rheological properties of whey protein gels can be altered by changing the ratios of constitutive proteins.

INTRODUCTION

The ability of cheese whey proteins to form gels is a major factor in their use as food ingredients. Solutions of whey protein concentrates and isolates are capable of forming viscoelastic gels after sufficient heat denaturation of the proteins (Schmidt, 1981; Kinsella and Whitehead, 1989; Ziegler and Foegeding, 1990). There are three major influences that determine the nature of the protein gel formed: (1) environmental conditions, such as pH, ionic strength, and mineral content; (2) protein type and concentration; and (3) processing conditions such as heating and cooling rates (Kinsella and Whitehead, 1989; Paulsson et al., 1990; Ziegler and Foegeding, 1990). Thermally induced protein gelation begins with heat denaturation of proteins, exposing interior regions of the proteins. The proteins aggregate through intermolecular interactions and produce a gel network when the aggregates sufficiently develop into a continuous three-dimensional structure that entraps and restricts the motion of solvent (Ziegler and Foegeding, 1990). The aggregates of heat-denatured protein molecules can be formed through intermolecular hydrophobic interactions, disulfide bonds, hydrogen bonds, or electrostatic interactions (Kinsella and Whitehead, 1989). Hydrophobic interactions (Kohnhorst and Mangino, 1985) and disulfide bonds (Matsudomi et al., 1991) are considered the strongest contributors to developing stable aggregate and gel networks in whey proteins.

There is a wealth of information on the gelation of whey proteins, but much less is known about the roles of the constitutive proteins in the gelation process. The major proteins found in cheese whey are β-lactoglobulin, α-lactalbumin, and bovine serum albumin (BSA), with β-lactoglobulin as the predominant protein (Swaisgood, 1982). Protein concentrates and isolates which are made from whey can also contain considerable amounts of immunoglobulins and proteose–peptone fractions. These protein fractions are very heterogeneous, with a wide range of molecular weights.

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An understanding of the contributions of the individual proteins to whey protein gelation would allow for whey protein concentrates and isolates to be manufactured with protein blends that produce an optimal gel for a specific food product. There has been some research on the gelation properties of β-lactoglobulin alone (Paulsson et al., 1986, 1990; Katsuta and Kinsella, 1990; Foegeding et al., 1992) but very little on mixed-gel systems with β-lactoglobulin in combination with other proteins found in whey (e.g., BSA and α-lactalbumin). What are the influences, if any, of BSA and α-lactalbumin on the gelation of β-lactoglobulin?

Matsudomi et al. (1991) evaluated effects of pH, salts, and thiol reagents on individual gelation of BSA and β-lactoglobulin. At pH 8.0, the minimum protein concentration for gelation was 4% (w/v) for BSA and 5% (w/v) for β-lactoglobulin. The maximum gel hardness was achieved at pH 6.5. Paulsson et al. (1986) used a dynamic oscillation rheometer to examine gelation properties of β-lactoglobulin, α-lactalbumin, and BSA. Heating protein solutions at 1 °C/min up to 90 °C, at pH 6.6, they found BSA capable of gelation at 2% (w/v) and β-lactoglobulin at 5% (w/v), but α-lactalbumin incapable of gelation at protein concentrations up to 20% (w/v). Gels of BSA were nearly ideally elastic and more pH dependent than β-lactoglobulin gels. The addition of α-lactalbumin to BSA significantly reduced the gelling ability of BSA, as measured by the complex modulus $|G^*|$.

It is not known if BSA or α-lactalbumin interacts with β-lactoglobulin to produce a mixed-protein gel matrix or gels separately to form two interpenetrating gel networks. Furthermore, there is a lack of understanding about protein–protein interactions in the aggregation process that forms the gel network. Elfagm and Wheelock (1978) used size exclusion chromatography to show that free α-lactalbumin disappeared to a greater extent when heated with β-lactoglobulin than when heated alone, at temperatures above 77 °C and in the pH range 6.4–7.2. Several researchers have investigated the rates of denaturation or aggregation of whey proteins under a variety of conditions. De Wit (1990) found a nearly second-order aggregation rate for β-lactoglobulin in whey protein concentrate, measuring loss of proteins through size exclusion chromatography, and a pseudo-first-order aggregation rate for β-lactoglobulin in whey permeate. Dannenberg and Kessler (1988) found that β-lactoglobulin and α-lactalbumin denatured at orders of 1.5 and 1.0, respectively, at pH 4.6. The whey proteins have different abilities to denature and aggregate. Ruegg et al. (1977) used differential scanning calorimetry (DSC) to determine the thermal transition temperature and degree of renaturation of BSA, β-lactoglobulin, and α-lactalbumin. The transition temperatures at pH 6.6 were 62 (BSA), 65 (α-lactalbumin), and 73 °C (β-lactoglobulin). There was no detectable renaturation of BSA and β-lactoglobulin but approximately 80–90% of α-lactalbumin renatured. Paulsson (1990) used DSC to measure the thermal denaturation and stability of BSA, β-lactoglobulin, and α-lactalbumin, finding that β-lactoglobulin thermostability was minimally altered over a wide range of pH values. Bovine serum albumin and α-lactalbumin were comparable in heat stability and most stable at neutral pH.

The primary objective of this study was to determine if BSA or α-lactalbumin contributes to the gelation of β-lactoglobulin and thus participates positively or negatively in gelation of whey protein concentrates and isolates.

MATERIALS AND METHODS

Protein Gelation. The proteins used were as follows: 3× crystallized β-lactoglobulin, containing a mixture of variants A and B (lot 51H7210); calcium-depleted α-lactalbumin, type III (lot 128F8140); and fatty acid-free bovine serum albumin (lot 118F-0056). All proteins were purchased from Sigma Chemical Co. (St. Louis, MO). The mixed-protein gels were prepared in molar ratios, β-lactoglobulin predominating, of 5:1, 10:1, and 20:1. An additional mixed gel of 50:1 was evaluated for β-lactoglobulin and BSA. Molecular weights of 18 362 for β-lactoglobulin, 14 174 for α-lactalbumin (Swaisgood, 1982), and 66 000 for BSA (Peters, 1985) were used to calculate weight percentages of the protein mixtures based on molar ratios. Protein concentrations of α-lactalbumin and β-lactoglobulin were determined spectrophotometrically at 280 nm using $\epsilon_{280} = 0.94$ cm^2 mg^{-1} for β-lactoglobulin and 2.01 cm^2 mg^{-1} for α-lactalbumin (Swaisgood, 1982). For BSA, $\epsilon_{279} = 0.667$ cm^2 mg^{-1} (Peters, 1985) was used.

A Bohlin VOR dynamic oscillation rheometer (Bohlin Reologi, Inc., Cranbury, NJ) was used to measure rheological properties of the solutions and gels during heating, holding at isothermal conditions, and cooling. A Bohlin C-14 concentric cylinder fixture was used for the measuring system. The cup inner diameter was 15.4 mm, and the bob diameter was 14 mm. Bob height was 21 mm and depth 6.9 mm, with a bottom cone angle of 150°. A 103.33 g cm torsion bar was used in all experiments. The Bohlin VOR operates at a prescribed strain amplitude and determines torque and the phase difference between shear stress and strain oscillations. Through trigonometric identities, it calculates storage modulus (G', the elastic element) and loss modulus (G'', the viscous element). Frequency sweeps and strain sweeps were run to establish testing conditions. The G' response was found to be linear within a range of oscillation amplitudes of 0.2–25%. Amplitude was set at 10% for all measurements, which produced a strain of 0.02–0.025. A frequency of 0.05 Hz was used for measurements.

Protein solutions were 7% protein (w/v) in 50 mM TES buffer, pH 7.0, with 100 mM NaCl. The solutions were stirred for 1 h at 22 °C and aspirated for 1 h at the same temperature, and the pH was readjusted to pH 7.0 and 1 M HCl or 1 M NaOH. The UV absorbance at 280 nm was checked before and after aspiration to confirm no significant change in solution concentration. Solution samples of 2.1 mL were used. For all studies, the samples were heated at 1 °C/min, from 25 to 80 °C, and held for 180 min at 80 °C. The samples were cooled from 80 to 25 °C at the rate of −1 °C/min. Protein solutions were covered with a layer (~5 mm thick) of vegetable oil to prevent evaporation during heating. Measurements were taken at 1-min intervals during the heating/hold/cooling cycles. All treatments were replicated at least twice.

The gel point and initial gelation rate were determined according to a modified method of Foegeding et al. (1992). The linear equation from a plot of G' vs time (or temperature for samples which gelled during the 25–80 °C heating) for the first 10 data points above 1% of the torque bar range (~700 Pa) was used to calculate the time or temperature corresponding to a G' of 150 Pa. This value represents the minimal sensitivity of the torsion bar and was called "GP_{150}". The initial rate of gelation was determined from the slope of the linear equation, with units of pascals per minute. This method was used for all protein solutions except 7% α-lactalbumin. The initial gelation rate of α-lactalbumin was determined from a linear equation fit to the data for the last 10 min of holding at 80 °C, and GP_{150} was not calculated due to inaccuracies associated with weak gels.

Protein–Protein Interactions/Aggregation Rates. The interactions of mixed proteins were determined by the aggregation rates of these proteins when heated separately and together. The aggregation rates were determined by loss of monomeric protein, as measured by size exclusion chromatography (SEC) using a FPLC chromatography system (Pharmacia-LKB Biotechnology, Piscataway, NJ), over time during isothermal heating. The SEC column was a Superose 12 HR 10/30 prepacked column (Pharmacia-LKB Biotechnology) with dimensions of 10 × 300 mm and a molecular weight separation range of 1000–300 000. The mobile phase was 50 mM TES buffer, pH 7.0, with 0.04% sodium azide, and the flow rate was 20 mL/h. The FPLC system consisted of a Pharmacia P-500 pump (Pharmacia-LKB Biotechnology),

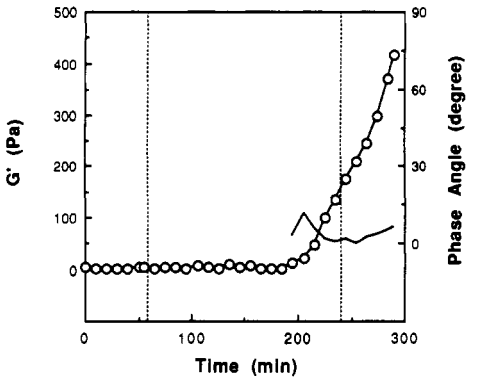

Figure 1. Changes in storage modulus (G') (circles) and phase angle (line) of a 7% (w/v) α-lactalbumin solution. Vertical lines separate sequential times of heating (25–80 °C at 1 °C/min), holding (80 °C for 3 h), and cooling (80–25 °C at 1 °C/min). Protein solutions were in 50 mM TES buffer, at pH 7.0, containing 100 mM sodium chloride.

a V-7 valve with 25-μL sample loop (Pharmacia-LKB Biotechnology), a single-path UV-1 detector and control unit (Pharmacia-LKB Biotechnology) with 280-nm filter, and a Shimadzu CR601 Chromatopac integrator (Shimadzu Instruments, Columbia, MD).

Protein samples were 1% (w/v) total protein in solution, except where noted, in 50 mM TES buffer, pH 7.0, with 100 mM NaCl. The proteins used were the same as in the rheology portion of the study that was previously described. Mixtures that contained two protein species were equimolar for each protein, with a total protein content of 1% (w/v). Samples were degassed by aspiration prior to heat treatment for a minimum of 30 min. One-milliliter aliquots of protein solutions were heated at 80 °C for 180 min in 13 × 100 mm borosilicate culture tubes (Fisher Scientific, Raleigh, NC) by immersion in a constant-temperature water bath. The samples were immediately cooled by immersion in 22 °C water. The suspensions were filtered through Gelman Acrodisc LC 13 PVDF 0.45-μm filters (Fisher Scientific) to remove large protein aggregates prior to injection into the FPLC SEC column. Samples were run in triplicate.

The rate of aggregation at 80 °C was determined by loss of monomeric protein, as measured by the chromatogram of the protein as it eluted from the SEC column and the resulting peak area absorbance. The development of a very large peak in the exclusion volume of the column represented the growth in the protein aggregates. The rates of monomer loss were calculated by both first-order and second-order kinetics, using the equations

$$\text{first order} \qquad [A] = [A]_0 e^{-kt} \tag{1}$$

$$\text{second order} \qquad (1/[A]) - (1/[A]_0) = kt \tag{2}$$

where $[A]_0$ is the concentration of the monomer protein prior to heat treatment and [A] the concentration after a specified time t. The rate constant, k, is calculated from the slope of the plot $\ln([A]/[A]_0)$ vs t for first order and $(1/[A]) - (1/[A]_0)$ vs t for second order.

RESULTS

Rheology of Protein Gels. When protein solutions gel, they are converted from a viscoelastic fluid to a viscoelastic gel. When this happens, the storage modulus (G') increases and the phase angle decreases (Hamann, 1991). A gel of α-lactalbumin was formed (Figure 1), albeit a weak gel in comparison to β-lactoglobulin and BSA gels (Figure 2), after α-lactalbumin was held at 80 °C for over 120 min. The storage modulus (G') of the α-lactalbumin gel rose from essentially zero to over 400 Pa with a 180-min hold at 80 °C and a subsequent cooling to 25 °C. Concomitant with the rise in the storage modulus, the phase angle dropped to nearly zero and then experienced a slight rise with cooling. The phase angle is an index of the viscoelasticity of a material. A phase angle of 90° would be a purely viscous fluid, and a phase angle of 0° would be a purely elastic solid.

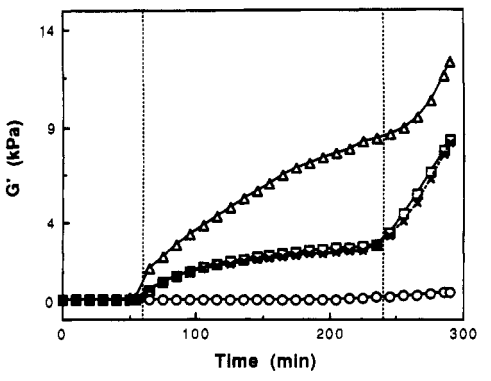

Figure 2. G' development of 7% (w/v) solutions of β-lactoglobulin (squares), α-lactalbumin (circles), bovine serum albumin (triangles), and whey protein isolate (×). Solution conditions and heating, holding, and cooling were as outlined in Figure 1.

Table I. Gel Point$_{150}$ and Initial Gelation Rate[a]

protein	molar ratio	gel point$_{150}$ temp, °C	gel point$_{150}$ time, min	initial gelation rate, Pa min^{-1}
β-lactoglobulin		79.4 ± 0.57		40 ± 2.5
α-lactalbumin		ND[b]	ND	3.3 ± 1.5
BSA		70.0 ± 0.71		66 ± 3.2
β-lactoglobulin/ α-lactalbumin	5:1	80	2.4 ± 0.55	50 ± 0.0
	10:1	80	2.8 ± 1.0	44 ± 6.0
	20:1	80	3.5 ± 0.14	41 ± 0.9
β-lactoglobulin/ BSA	5:1	80	0.75 ± 0.11	109 ± 2.1
	10:1	80	1.8 ± 0.24	81 ± 2.1
	20:1	80	1.6 ± 0.59	87 ± 4.5
	50:1	80	1.9 ± 0.31	51 ± 0.85

[a] Mean values ± standard deviations for two to four replications. [b] Not determined. The GP_{150} values were not calculated due to inaccuracies associated with weak gels.

The relative patterns of β-lactoglobulin, α-lactalbumin, BSA, and whey protein isolate (WPI) gelation are shown in Figure 2, and the gel points (GP_{150}) are seen in Table I. β-Lactoglobulin gelled at 79.4 °C, developed most of the final G' of the 180 min 80 °C hold in the first 60 min, continued with weak to moderate G' development in the remaining 120 min at 80 °C, and then rapidly developed the G' during the cooling phase from 80 to 25 °C. The BSA gelled earlier than β-lactoglobulin, with a GP_{150} of 70 °C. The G' developed rapidly and consistently during the 80 °C hold, increased at a somewhat more rapid rate during the cooling cycle, but was not as rapid as β-lactoglobulin.

One concern with using pure proteins from scientific suppliers is that they are not representative of food-grade proteins. To address this point, a solution of whey protein isolate (Bipro, Le Sueur Isolates, Le Sueur, MN) was adjusted to 7% (w/v) total protein and gelled as outlined for other proteins. The gelling pattern of the whey protein isolate was identical to that of β-lactoglobulin (Figure 2), suggesting that there were no major differences between sources of protein that would question the validity of using β-lactoglobulin supplied by Sigma. The relative contribution of constitutive proteins will be discussed in subsequent sections.

Mixed gels of β-lactoglobulin and α-lactalbumin are shown in Figure 3. With mixtures of β-lactoglobulin and α-lactalbumin from 5:1 to 20:1, there were small or no differences in the G' patterns of gelation during both the 180-min hold at 80 °C and the cooling cycle. The mixed-protein gels developed G' in a manner similar to that of the pure β-lactoglobulin gel and then experienced rapid

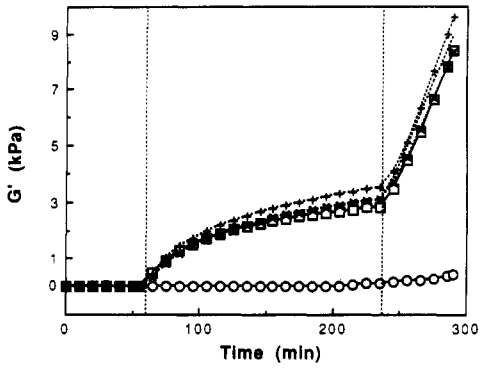

Figure 3. G' development of 7% (w/v) solutions of β-lactoglobulin (squares), α-lactalbumin (circles), and mixtures of β-lactoglobulin and α-lactalbumin. The mixtures contained β-lactoglobulin and α-lactalbumin in respective molar ratios of 5:1 (+), 10:1 (×), and 20:1 (···). Solution conditions and heating, holding, and cooling were as outlined in Figure 1.

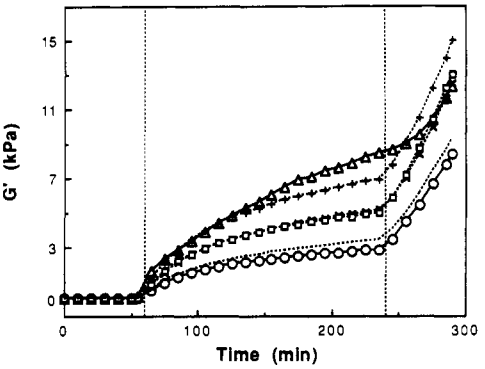

Figure 4. G' development of 7% (w/v) solutions of β-lactoglobulin (circles), bovine serum albumin (BSA) (triangles), and mixtures of β-lactoglobulin and BSA. The mixtures contained β-lactoglobulin and BSA in respective molar ratios of 5:1 (+), 10:1 (×), 20:1 (squares), and 50:1 (···). Solution conditions and heating, holding, and cooling were as outlined in Figure 1.

development of G' in the cooling cycle. The GP_{150} values for the β-lactoglobulin/α-lactalbumin mixed gels were greater than for β-lactoglobulin alone and appeared to increase as the amount of β-lactoglobulin increased (Table I).

Mixed gels of β-lactoglobulin and BSA (Figure 4) showed patterns of gelation characteristic of β-lactoglobulin, with modulations reflecting the strong gelling ability of BSA. The mixtures of β-lactoglobulin and BSA, ranging from 5:1 to 50:1, developed G' during the 180-min 80 °C hold in a pattern similar to that of β-lactoglobulin but with increased G' proportional to the amount of BSA in the original protein solution. The β-lactoglobulin/BSA mixtures developed most of their final G' of the 80 °C hold in the first 60–90 min and then experienced a slower rate of G' development. The mixed-protein pattern, similar to β-lactoglobulin gelation, continued in the cooling cycle with the β-lactoglobulin/BSA mixtures having linear and rapid G' development during this stage. The GP_{150} values for the β-lactoglobulin/BSA mixtures were greater than values for individual proteins, and there was no apparent difference associated with protein stoichiometry (Table I).

As mentioned previously, the gelling pattern of whey protein isolate was identical to that of β-lactoglobulin (Figure 2). The relative concentration of each protein in the isolate was found to be approximately 20% BSA, 61% β-lactoglobulin, and 19% α-lactalbumin, as determined by FPLC-SEC and whey protein standards. On a molar basis, these percentages reflect ratios of 10.8:1 for β-lactoglobulin to BSA, 2.5:1 for β-lactoglobulin to α-lactalbumin, and 4.4:1 for α-lactalbumin to BSA. It appeared that the β-lactoglobulin gelling pattern is seen in both binary and ternary protein mixtures when β-lactoglobulin is the predominant protein.

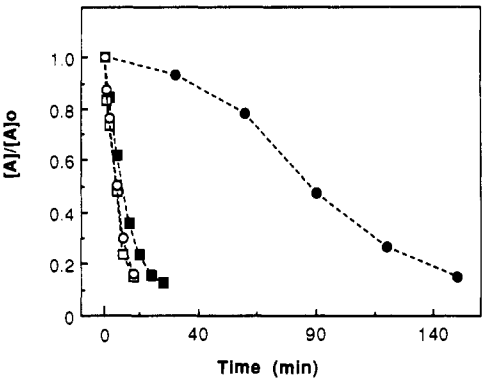

Figure 5. Relative losses of β-lactoglobulin (0.56% w/v, squares), α-lactalbumin (0.44% w/v, circles), and an equivalent mixture of the two proteins at 80 °C, as determined by the percent of remaining monomeric protein over time. Proteins heated individually are indicated by solid symbols and those in the mixture by open symbols. Proteins were in solutions containing 50 mM TES buffer, pH 7.0, with 100 mM sodium chloride.

The initial gelation rate was slightly faster for BSA than for β-lactoglobulin. In contrast, α-lactalbumin had an initial gelation rate 10-fold slower than the other proteins (Table I). Mixtures of α-lactalbumin and β-lactoglobulin gelled at a rate similar to that of β-lactoglobulin alone. In contrast, the addition of BSA to β-lactoglobulin caused an increase in initial gelation rate. This was most apparent in the 5:1 treatment where the rate was greater than twice that of β-lactoglobulin alone (Table I). There was an overall trend for the rate to decrease because β-lactoglobulin represented a higher proportion of the mixture with BSA.

Protein–Protein Interactions. Aggregation rates of individual whey proteins, heated separately and together, were used to determine if these proteins interact with each other in the aggregation process that leads to gelation. Protein concentrations were used that resulted in 1:1 molar ratios of the mixed proteins and a total concentration of 1 or 2%. The concentrations corresponding to individual proteins within the mixtures were then used for determining aggregation rates of single-protein solutions. For example, a 1% protein solution with a 1:1 molar ratio of β-lactoglobulin and α-lactalbumin resulted in a concentration of 0.56% (w/v) β-lactoglobulin and 0.44% (w/v) α-lactalbumin. A total protein concentration of 0.56% was then used in determining the aggregation rate of β-lactoglobulin alone. Mixed-protein solutions of BSA and β-lactoglobulin resulted in a wide disparity of actual protein by mass because of the much larger molecular weight of BSA compared to β-lactoglobulin. BSA was 78% of the total protein mixture. Figure 5 shows the relative ($[A]/[A]_0$) remaining monomeric protein of α-lactalbumin and β-lactoglobulin when heated separately and together. As can readily be seen by this figure, α-lactalbumin aggregated very slowly when heated alone. When α-lactalbumin was heated in the presence of an equimolar quantity of β-lactoglobulin, the aggregation rate was similar to that of β-lactoglobulin (Figure 5). BSA aggregated very rapidly when heated at 80 °C, as seen in Figure 6. The rate of BSA aggregation did not appear to be affected by the presence of equimolar quantities of β-lactoglobulin. Aggregation of β-lactoglobulin was faster in the presence of BSA than when alone at a level of 0.22% (w/v).

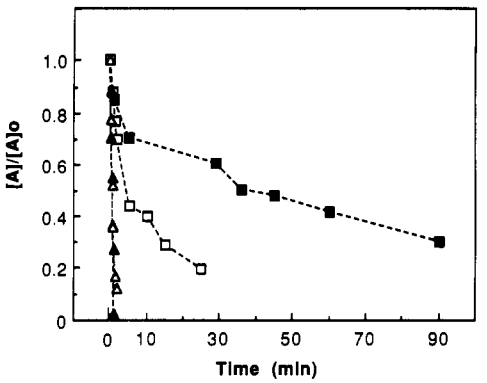

Figure 6. Relative losses of β-lactoglobulin (0.21% w/v, squares), bovine serum albumin (0.79% w/v, triangles), and an equivalent mixture of the two proteins at 80 °C, as determined by the percent of remaining monomeric protein over time. Proteins heated individually are indicated by solid symbols and those in the mixture by open symbols. Protein solutions (w/v) were in 50 mM TES buffer, pH 7.0, with 100 mM sodium chloride.

Table II. Second-Order Rate Constants of Loss of Monomeric Protein at 80 °C

protein	concn, % w/v	rate constant, $\mu M^{-1}\ s^{-1}$	r^2
β-lactoglobulin	0.56	0.00914	0.983
α-lactalbumin	0.44	0.00105	0.873
BSA	0.78	0.541	0.959
β-lactoglobulin	0.22	0.00320	0.970
mixture (1:1)	2		
β-lactoglobulin	1.13	0.0167	0.979
α-lactalbumin	0.87	0.0153	0.906
mixture (1:1)	1		
β-lactoglobulin	0.56	0.0136	0.971
α-lactalbumin	0.44	0.0141	0.985
mixture (1:1)	1		
BSA	0.78	0.555	0.995
β-lactoglobulin	0.22	0.0219	0.986

The objective of the aggregation rate experiment was to investigate if aggregation of the proteins proceeded at different rates in the presence of another protein than when alone. When in the presence of β-lactoglobulin, α-lactalbumin aggregated much more quickly than when heated alone (Table II). Alone, α-lactalbumin had a second-order aggregation rate constant of $1.05 \times 10^{-3}\ \mu M^{-1}\ s^{-1}$. In the presence of an equimolar quantity of β-lactoglobulin, the aggregation rate constant of α-lactalbumin increased approximately 13-fold to $1.41 \times 10^{-2}\ \mu M^{-1}\ s^{-1}$. When heated together, α-lactalbumin ($1.41 \times 10^{-2}\ \mu M^{-1}\ s^{-1}$) and β-lactoglobulin ($1.36 \times 10^{-2}\ \mu M^{-1}\ s^{-1}$) had similar aggregation rate constants. The aggregation rate of β-lactoglobulin did not increase substantially (~1.5-fold) when heated in the presence of α-lactalbumin (Table I). BSA aggregated very rapidly in comparison to β-lactoglobulin and α-lactalbumin. The BSA rate constants were similar when heated alone or in the presence of 0.22% β-lactoglobulin. The rate constant of β-lactoglobulin increased from 3.20×10^{-3} to $2.19 \times 10^{-2}\ \mu M^{-1}\ s^{-1}$ when heated in the presence of equimolar BSA.

DISCUSSION

We found that α-lactalbumin was capable of gelation, at 7% (w/v) in a pH 7.0 solution, when heated for an extended period of time. Paulsson et al. (1986) reported that α-lactalbumin was incapable of gelation at concentrations up to 20% (w/v). The crucial difference between studies is that we maintained the temperature of the α-lactalbumin solution for a 3-h hold at 80 °C, whereas the Paulsson et al. (1986) study heated the protein solutions at a rate of 1 °C/min up to 90 °C. The aggregation and initial gelation rates of α-lactalbumin (Tables I and II) confirmed that it was slow to aggregate and gel and thus required extensive hold times. It should also be noted that the α-lactalbumin gel was very weak; β-lactoglobulin gels formed under identical conditions had G' values 20-fold higher than those of α-lactalbumin gels.

On the basis of both mixed-gel rheology and aggregation rates of α-lactalbumin and β-lactoglobulin, it was apparent that α-lactalbumin interacted with β-lactoglobulin in forming mixed-protein gels. Gels of β-lactoglobulin and α-lactalbumin, in respective molar ratios from 5:1 to 20:1, appeared to be rheologically similar to gels made of β-lactoglobulin and had similar initial gelation rates. The aggregation rate of α-lactalbumin increased 13–14-fold when heated in the presence of β-lactoglobulin. This substantial increase in the aggregation of α-lactalbumin during heating may be due to an interaction between denatured α-lactalbumin and β-lactoglobulin molecules. There is evidence that α-lactalbumin is readily heat-denatured but has a much greater tendency to renature rather than form aggregates (Ruegg et al., 1977). Although the mechanism is unclear, it seems probable that the interaction of denatured α-lactalbumin with denatured β-lactoglobulin molecules was kinetically or thermodynamically more favorable than renaturation. There was no evidence that α-lactalbumin in whey protein products had a negative influence on gelation at pH 7.0.

BSA was a fast gelling protein, as seen by both the initial gelation rate and the aggregation rate constant. In mixed-protein gels, in molar ratios of β-lactoglobulin to BSA from 5:1 to 50:1, the overall changes in G' with temperature were similar to those of β-lactoglobulin. However, BSA contributed to the G' of the gel during formation and increased the initial gelation rate. Aggregation of BSA was very rapid at 80 °C, with rates approximately 50–100 times that of β-lactoglobulin. The aggregation rate constant of β-lactoglobulin increased approximately 7-fold in the presence of equimolar quantities (a 3.5:1 weight ratio of BSA to β-lactoglobulin) of BSA. The pattern of β-lactoglobulin monomer loss when heated with BSA suggested a relatively rapid aggregation rate in the initial 90–120 s. The rapid loss of β-lactoglobulin coincided with the presence of denatured BSA, and the rate slowed quickly when the monomer concentration of BSA was essentially depleted (Figure 6). Differential scanning calorimetry has shown that BSA denatures at 62 °C and β-lactoglobulin at 73 °C (Ruegg et al., 1977). The lower thermal stability and faster aggregation rate for BSA suggest that the contribution of other proteins to gelation of predominantly BSA solutions will depend on their thermal stability. It is apparent that BSA is a strong gelling protein and a positive contributor to mixed-protein gels. A whey protein isolate, with a relatively high amount of BSA, had G' development similar to that of β-lactoglobulin (Figure 2). These results suggest that α-lactalbumin and BSA do not detract from the gelation qualities of whey protein products and that BSA contributes to G' values.

In the rheological experiments, the molar ratio of β-lactoglobulin to α-lactalbumin or BSA varied from 5:1 to 50:1 (Table I). These ratios were picked to show how adjusting the concentrations of specific proteins in whey alters the rheological properties of gels. In the aggregation rate experiments, a 1:1 molar ratio of proteins was used because the goal was to see if one protein altered the aggregation kinetics of the other. With homogeneous mixing, a 1:1 ratio means that there is an equal distribution of molecules in solution. If diffusion differences are

assumed to be minimal, the probability of two similar or two different proteins forming aggregates should depend on the specific chemical properties associated with each protein.

The rate constants of aggregation of the whey proteins were determined by a second-order model. However, α-lactalbumin aggregation may have been better modeled as first order. The first-order equation had a much higher coefficient of determination (r^2) than the second-order equation. First-order r^2 was 0.972, whereas second-order r^2 was 0.873. The other aggregation processes were modeled better by a second-order model as evidenced by r^2 values and similarity of rate constants determined at two (1 and 2%) protein concentrations. The first-order rate constants showed an obvious concentration effect, decreasing from 0.761 to 0.162 s^{-1} for β-lactoglobulin and from 0.929 to 0.154 s^{-1} for α-lactalbumin when the concentrations were doubled. As seen by Table II, there was a minimal concentration effect when modeled as a second-order reaction. It was not surprising that different systems may have different tendencies toward certain models, and that may have been the situation with α-lactalbumin. However, for comparison purposes, all rate constants in Table II were reported as second order.

CONCLUSIONS

Thermally induced gelation of protein solutions containing mixtures of β-lactoglobulin, α-lactalbumin, and bovine serum albumin showed aggregation rate constants, G' values, and initial gelation rate constants that indicated an interaction among proteins during gelation. Gels formed from β-lactoglobulin/α-lactalbumin mixtures were similar to gels formed with β-lactoglobulin alone, not reflecting the poor gelling ability of α-lactalbumin. β-Lactoglobulin/bovine serum albumin mixtures formed gels with greater G' values than β-lactoglobulin alone. These results demonstrate that β-lactoglobulin, α-lactalbumin, and bovine serum interact favorably in mixed-protein gels.

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LITERATURE CITED

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

De Wit, J. N. Thermal stability and functionality of whey proteins. *J. Dairy Sci.* **1990**, *73*, 3602–3612.

Elfagm, A. A.; Wheelock, J. V. Interaction of bovine α-lactalbumin and β-lactoglobulin during heating. *J. Dairy Sci.* **1978**, *61*, 28–32.

Foegeding, E. A.; Kuhn, P. R.; Harden, C. C. Specific divalent cation-induced changes during gelation of β-lactoglobulin. *J. Agric. Food Chem.* **1992**, *40*, 2092–2097.

Hamann, D. D. Rheology: A tool for understanding thermally induced protein gelation. In *Interactions of Food Proteins*; Parris, N., Barford, R., Eds.; ACS Symposium Series 454; American Chemical Society: Washington, DC, 1991; pp 212–227.

Katsuta, K.; Kinsella, J. E. Effects of temperature on viscoelastic properties and activation energies of whey protein gels. *J. Food Sci.* **1990**, *55*, 1296–1302.

Kinsella, J. E.; Whitehead, D. M. Proteins in whey: chemical, physical, and functional properties. *Adv. Food Nutr. Res.* **1989**, *33*, 343–438.

Kohnhorst, A. L.; Mangino, M. E. Prediction of the strength of whey protein gels based on composition. *J. Food Sci.* **1985**, *50*, 1403–1405.

Matsudomi, D.; 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.

Paulsson, M. Thermal denaturation and gelation of whey proteins and their adsorption at the air/water interface. Ph.D. Dissertation abstract, The University of Lund, Lund, Sweden, 1990.

Paulsson, M.; Hegg, P.; Castberg, H. B. Heat-induced gelation of individual whey proteins. A dynamic rheological study. *J. Food Sci.* **1986**, *51*, 87–90.

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

Peters, T., Jr. Serum albumin. *Adv. Protein Chem.* **1985**, *37*, 161–245.

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

Schmidt, R. H. Gelation and coagulation. In *Protein Functionality in Foods*; Cherry, J. P., Ed.; ACS Symposium Series 147; American Chemical Society: Washington, DC, 1981; pp 132–147.

Swaisgood, H. E. Chemistry of milk protein. In *Developments in Dairy Chemistry*; Fox, P. F., Ed.; Elsevier Applied Science Publishers: London, 1982; Vol. 1, pp 1–59.

Ziegler, G. R.; Foegeding, E. A. The gelation of proteins. *Adv. Food Nutr. Res.* **1990**, *34*, 204–298.

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