Heat-Induced Interactions and Gelation of Mixtures of Bovine β-Lactoglobulin and Serum Albumin

Jacquiline Gezimati,[†] Harjinder Singh,[‡] and Lawrence K. Creamer*,[§]

Department of Food Technology, Massey University, Private Bag 11 222, Palmerston North, New Zealand, and Food Science Section, New Zealand Dairy Research Institute, Private Bag 11 029, Palmerston North, New Zealand

The storage modulus, G', and aggregation of β-lactoglobulin AB and bovine serum albumin (BSA) were measured during 60 min of heating at 70 or 75 °C in a buffer simulating the whey protein concentrate environment. BSA formed stiffer gels than β-lactoglobulin and also aggregated (shown by loss of native protein by PAGE) and polymerized (shown by loss of residual monomeric protein by SDS−PAGE) more rapidly than β-lactoglobulin. Both BSA and β-lactoglobulin formed heat-induced hydrophobically bonded aggregates which may have been intermediates in the transformation of the native proteins into the disulfide-bonded gel networks. The gelation temperature (the temperature at which an appreciable increase in G' occurred during heating at 1 °C/min) of mixtures of BSA and β-lactoglobulin at a total protein concentration of 10% (w/v) increased from ∼72 °C, for BSA alone, to ∼84 °C, for β-lactoglobulin alone. When protein solutions were heated, BSA solutions formed stiffer gels at lower concentrations and at lower temperatures. When 10% mixtures of the two proteins were heated at 75 °C, the gelling behavior of the mixture was more akin to that of BSA when BSA was the major constituent in the mixture and vice versa. The results are consistent with the formation of intertwined and commingled homopolymers of BSA and β-lactoglobulin together with some heteropolymers. The proportion of homo- and heteropolymers in each mixture is probably dependent on the heating temperature and the composition of the mixture.

Keywords: *Whey protein concentrate; hydrophobic aggregation; disulfide bonding*

INTRODUCTION

Whey protein isolates and concentrates are used in a range of food products because of the satisfactory heat-induced gelling properties they confer. Elucidation of the relative importance of the various components of these ingredients on gelation should lead to understanding how changes in composition could lead to improved product function. Clearly the concentration of the salts, the pH, and the proportions and the concentrations of the protein components are all important factors (Mulvihill and Kinsella, 1987). The denaturation and gelling properties of the major protein component, β-lactoglobulin, have been studied extensively using a number of techniques (Paulsson et al., 1986; Mulvihill and Kinsella, 1987; Stading and Hermanson, 1990; Matsudomi et al., 1991; Griffin et al., 1993; Cairoli et al., 1994; Matsuura and Manning, 1994; McSwiney et al., 1994a,b; Roefs and de Kruif, 1994; Qi et al., 1995). Recently McSwiney et al. (1994a,b) examined the gelation of β-lactoglobulin by dynamic oscillatory rheometry and assessed the pre-gelation aggregation of this protein at 10% (w/v) by gel electrophoresis under "native" conditions and in SDS buffer without and with reducing agents. They concluded that the development of rheologically significant structures was probably preceded by the formation of hydrophobically bonded aggregates and disulfide-bonded polymers.

* Author to whom correspondence should be addressed (fax +64 6 356 1476; e-mail L.Creamer@NZDRI.Org.NZ).

[†] Present address: 560 Mukonono Road, Marimba Park, Harere, Zimbabwe.

[‡] Massey University.

[§] New Zealand Dairy Research Institute.

Whey protein concentrates contain other proteins such as α-lactalbumin and bovine serum albumin (BSA), and the effect of these on the gelation of β-lactoglobulin is important for a fuller understanding of the mechanisms of gelation in whey protein systems. Hines and Foegeding (1993) found that the rate of loss of native β-lactoglobulin from solution at 80 °C was increased by the presence of BSA, indicating some synergistic effect. Matsudomi et al. (1994) found that when mixtures of BSA and β-lactoglobulin were heated at 80 °C and examined at room temperature, the gels from the mixtures were stronger, again suggesting a synergistic effect.

As a further step toward understanding the reactions that occur during the thermal gelation of whey protein concentrates and isolates, we now report the results of heating mixtures of β-lactoglobulin and BSA at 70 and 75 °C in a buffer simulating the environment of a 12% solution of whey protein concentrate (WPC).

MATERIALS AND METHODS

Proteins and Chemicals. β-Lactoglobulin (product L-2506) and BSA (product A-4378) were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were of analytical reagent grade.

Measurement of Protein Gelation. Gelation properties of β-lactoglobulin, BSA, and various protein mixtures dispersed in a buffer designed to simulate the mineral and sugar content of a 12% WPC solution were determined using a Bohlin VOR rheometer (Bohlin Reologi AB, Lund, Sweden). The buffer was composed of 2.03 g of K_2HPO_4, 0.63 g of NaCl, 2.06 g of trisodium citrate dihydrate, 1.31 g of $CaCl_2 \cdot 2H_2O$, and 8.0 g of lactose made up to 1 L and adjusted to pH 6.8. The protein solution (0.65 mL) was placed in a measuring system consisting of a cup (the outer cylinder diameter was 8.8 mm) and a bob (the inner cylinder diameter was 8 mm). A torsion bar of 4.4×10^{-3} Nm was used. The solution was covered with a

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thin layer of paraffin oil to prevent evaporation. The frequency was set to 1 Hz, and the shear strain was at 0.0206 ± 0.0005 throughout the measurement range. [Preliminary tests made by measuring G', G'', and δ as a function of heating rate, frequency, amplitude, etc. (Gezimati, 1995) allowed the choice of parameters within the linear ranges.] The protein solution was heated from 25 °C to the heating temperature (70 or 75 °C) at a rate of 1 °C/min and the temperature maintained for 60 min. The solution or gel was then cooled to 25 °C at 1 °C/min, and the temperature was maintained for 10 min. G', G'' and phase shift were recorded every 30 s for an hour from attainment of the heating temperature and for 10 min after the return to 25 °C.

Gelation Temperature. Protein solutions were heated at a rate of 1 °C/min from 25 to 90 °C, and the rheological properties and temperature were recorded at 1 min intervals.

Protein Aggregate Formation. Provided the solution gelled at an appropriate rate, a second solution of protein was given the same temperature treatment as that used in the gelation studies and aliquots were removed at 0 (when the heating temperature was just reached) 1, 2, 4, 6, 10, and 14 min (when possible) and mixed with chilled sample buffers for subsequent analysis using native and SDS−PAGE followed by densitometry using the methods of McSwiney et al. (1994a,b).

RESULTS

Nomenclature. To differentiate among the variety of forms of the proteins in these systems, classification and definition of these forms have been attempted. On the basis of PAGE results (McSwiney et al., 1994b), three classes of protein are apparent. Typically, analysis of a heated and cooled β-lactoglobulin solution by native PAGE (pH ~8.4) showed that one of the protein bands coincided with the major band of a native protein sample, and this can be operationally classified as "native-like" under PAGE conditions. Analysis of the same sample by SDS−PAGE (pH 8.4, 0.1% SDS) gave a slightly more intense band that ran as though it were monomeric. This could be called "protein dispersible to monomers in 0.1% SDS solution" or "SDS-monomeric" protein. Finally, analysis of the heated sample by SDS−PAGE after protein reduction, which converts the disulfide bonds to free thiols, gave an even more intense band that coincided with that from reduced monomer protein. This material could be called "total reducible and dispersible in SDS solution" or "total" protein.

Gelation Temperature. Heating solutions of BSA in the simulated WPC buffer from 25 to 90 °C at 1 °C/min in the Bohlin rheometer showed that the gelling temperature, defined as the temperature at which an appreciable increase in G' occurred (Figure 1), decreased with increasing protein concentration. A similar pattern of behavior was apparent for various concentrations of β-lactoglobulin, although this protein always gelled at a higher temperature and the concentration needed to form a gel was greater than for BSA (result not shown), supporting earlier results (Paulsson et al., 1986). At a constant total protein concentration of 10% (w/v) and with varied ratios of β-lactoglobulin to BSA, the gelling temperature decreased when the proportion of BSA in the mixture increased (Figure 1).

Protein Aggregate Formation. *(i) Native-like Protein Concentrations.* The results for a mixture of 5% BSA and 5% β-lactoglobulin, together with comparable solutions of individual proteins, analyzed by native PAGE are shown in Figure 2. At 70 °C, the loss of native-like BSA was greater from the mixture than from the 5% solution of pure protein (Figure 2A). A similar result was obtained at 75 °C (Figure 2B), although little

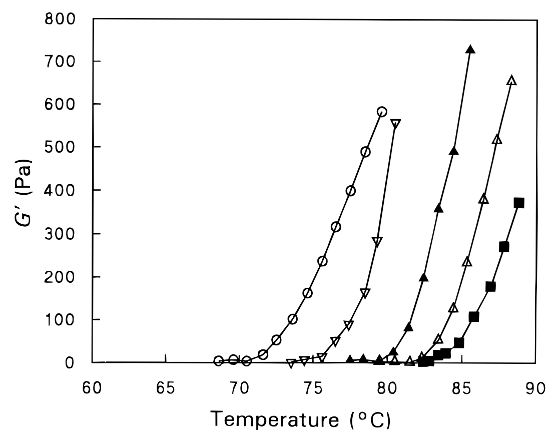

Figure 1. Gelation temperatures of 10% β-lactoglobulin (■), 10% BSA (○), and protein mixtures containing 8% β-lactoglobulin and 2% BSA (△), 5% β-lactoglobulin and 5% BSA (▲), and 2% β-lactoglobulin and 8% BSA (▽).

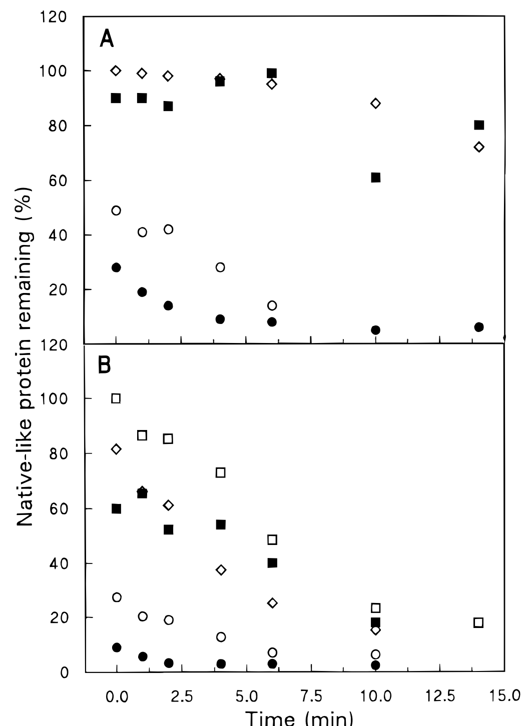

Figure 2. Effect of heating time at 70 °C (A) and 75 °C (B) on the loss of native-like β-lactoglobulin (◇, □, ■) and native-like BSA (○, ●) from a 10% β-lactoglobulin solution (◇), a 5% β-lactoglobulin solution (□), a 5% BSA solution (○), and a solution containing both 5% β-lactoglobulin (■) and 5% BSA (●).

of the native-like BSA remained by the time the solutions had attained 75 °C.

When a 10% solution of β-lactoglobulin was heated at 70 °C, the loss of native-like β-lactoglobulin was low and comparable to the loss from a mixture of 5% BSA and 5% β-lactoglobulin (Figure 2A). At 75 °C (Figure 2B) the loss was greater from the 10% β-lactoglobulin than from the 5% β-lactoglobulin solution, whereas the loss from the mixture was intermediate between that from the 5 and 10% controls. The amount of native-like protein present at the end of the heating period was less for the β-lactoglobulin in the mixture than for either the 5 or 10% control (Figure 2B) and may have been affected by the presence of BSA. It is also clear that native-like BSA is lost from the system more quickly than native-like β-lactoglobulin.

(ii) SDS-Monomeric Protein Concentrations. The results from the SDS−PAGE analysis of the protein

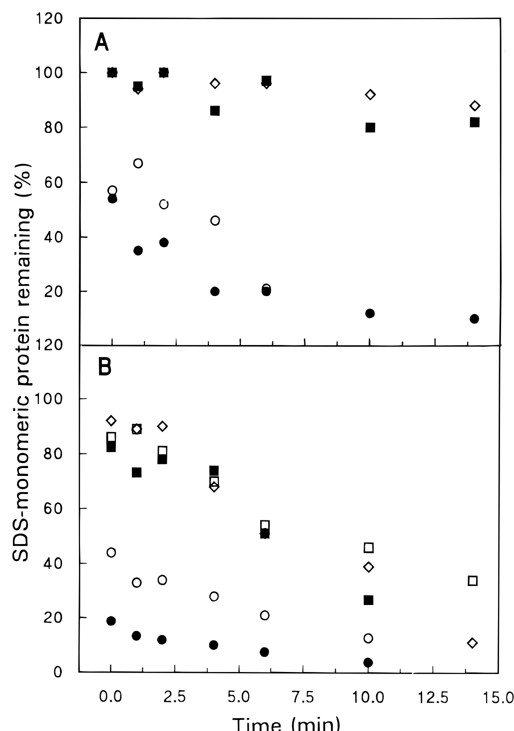

Figure 3. Effect of heating time at 70 °C (A) and 75 °C (B) on the loss of SDS-monomeric β-lactoglobulin (■, □, ◇) and SDS-monomeric BSA (○, ●) from a 10% β-lactoglobulin solution (◇), a 5% β-lactoglobulin solution (□), a 5% BSA solution (○), and a solution containing 5% β-lactoglobulin (■) and 5% BSA (●).

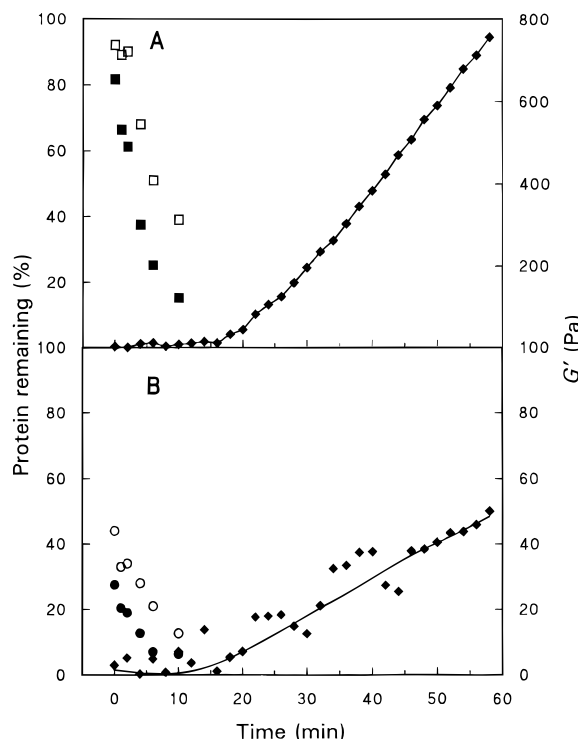

Figure 4. Effect of heating 10% β-lactoglobulin (A) or 5% BSA (B) solution at 75 °C on G' (◆) and quantities of native-like β-lactoglobulin (■), native-like BSA (●), SDS-monomeric β-lactoglobulin (□), and SDS-monomeric BSA (○).

solutions used for the results shown in Figure 2 are shown in Figure 3. At 70 °C the amount of SDS-monomeric BSA was less in the mixture than in the 5% BSA solution. A similar trend was obtained at 75 °C (Figure 3B), although the effect seemed to be greater; that is, more of the BSA was recovered from the solution containing BSA alone than from the mixture (Figure 3B). At both temperatures the amount of SDS-monomeric BSA present in each sample (Figure 3A,B) was greater than the quantity of native-like BSA present as shown in parts A and B, respectively, of Figure 2.

The loss of SDS-monomeric β-lactoglobulin from the solutions heated at 70 °C (Figure 3A) was slight and comparable to the results for the native-like protein (Figure 2A). Heating at 75 °C caused comparable rates of decrease in the SDS-monomeric β-lactoglobulin in the 5% control, the 10% control, and the mixture (Figure 3B). In all cases the loss was less than that of the native-like protein (Figure 2B). The loss of both native-like and SDS-monomeric BSA was more rapid than the losses of the comparable β-lactoglobulins.

When the samples were reacted with mercaptoethanol before SDS–PAGE analysis, the concentrations of BSA and β-lactoglobulin in all of the heated samples were indistinguishable from those of the unheated controls, showing that all of the protein was native-like, SDS-soluble, or cross-linked with disulfide bonds.

Relationship between Aggregation and *G′*. *(i) Individual Proteins.* The results for the loss of native-like and SDS-monomeric β-lactoglobulin from the 10% β-lactoglobulin solution heated at 75 °C were combined and presented with the G' results in Figure 4A. By extrapolation of the loss of native-like or SDS-monomeric protein results, it appears that there was less than 5% native-like protein and less than 20% SDS-monomeric protein present when G' started to increase (i.e. gel formation commenced) after about 18 min of heating at 75 °C (Figure 4A). When the experiment was repeated using a 5% β-lactoglobulin solution, gelation did not occur even after 60 min of heating at 75 °C, despite the essentially complete conversion of the native-like (Figure 2B) and SDS-soluble (Figure 3B) forms to the disulfide-bonded form.

Heating a 10% BSA solution to 75 °C caused gelation to occur before attainment of the final temperature, and thus samples for electrophoresis were unavailable. When a 5% BSA solution was heated at 75 °C, only a weak gel formed (Figure 4B) with a G' of ~50 Pa after 60 min of heating. By extrapolation, it appeared that gelation did not commence until both the native-like and SDS-monomeric BSA had been depleted from the protein solution (Figure 4B).

Comparison of the results using the two proteins showed that, under the same conditions (75 °C, 5% w/w), native-like and SDS-monomeric structures of both BSA and β-lactoglobulin are progressively lost with the formation of disulfide-bonded aggregates. However, under these conditions BSA formed a weak gel (Figure 4A) but β-lactoglobulin did not.

(ii) Protein Mixtures. The results for loss of native-like and SDS-monomeric protein from the mixture of 5% BSA and 5% β-lactoglobulin (Figures 2 and 3) were combined and presented with the G' results in Figure 5. It can be seen that after 14 min of heating at 70 °C (Figure 5A), concentrations of both the native-like and SDS-monomeric BSA were virtually nil and the concentration of the SDS-monomeric β-lactoglobulin remaining was probably >70% of the original concentration. By extrapolation, it seems likely that some β-lactoglobulin was not cross-linked and had probably remained in the native-like and SDS-monomeric forms when G' started to increase after about 25 min of heating at 70 °C.

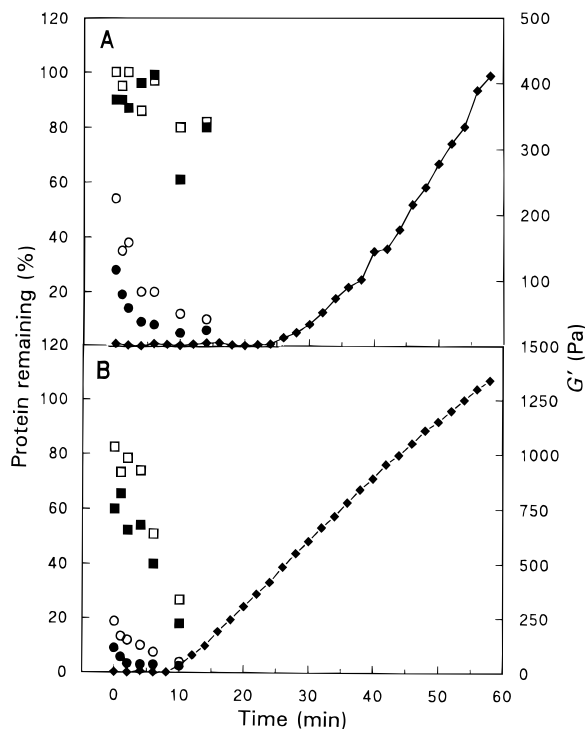

Figure 5. Effect of heating a mixture of 5% β-lactoglobulin and 5% BSA at 70 °C (A) and 75 °C (B) on G' (◆) and quantities of native-like β-lactoglobulin (■), native-like BSA (●), SDS-monomeric β-lactoglobulin (□), and SDS-monomeric BSA (○).

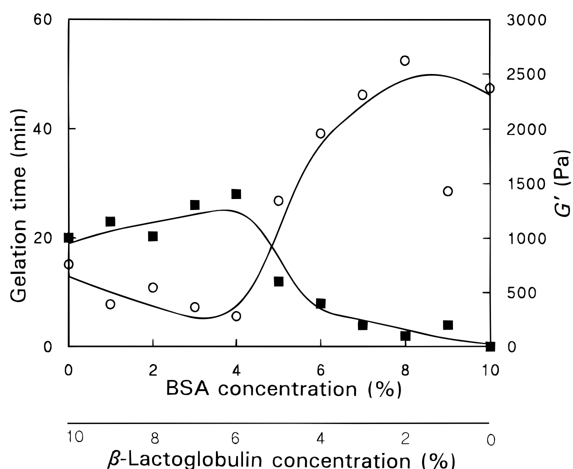

Figure 6. Effect of protein composition in mixtures of β-lactoglobulin and BSA on the gelation time (■) and G' (○) after heating at 75 °C for 60 min.

When the mixture was heated for 60 min at 75 °C (Figure 5B), the gel stiffness, G', was greater than when the heating temperature was 70 °C. G' started to increase after about 10 min of heating at 75 °C, and there were appreciable quantities of SDS-monomeric β-lactoglobulin but very little SDS-monomeric BSA remaining in the gelling system (Figure 5B).

Effect of Mixture Composition on G' and Gelling Time. A series of 10% protein solutions with various ratios of BSA to β-lactoglobulin was prepared and heated at 75 °C for 60 min. The results for G' are shown in Figure 6. The G' values of gels from solutions containing 6−10% BSA were >1200 Pa, whereas those from mixtures containing 0−4% were <800 Pa. When the gelled systems were cooled and held at 25 °C for 10 min, G' increased markedly and typically the ratio of G'_{25} to G'_{75} was ~3 for the systems containing 6−10% BSA, compared with ratios of about 5 when there was 0−4% BSA in the mixture. Similar trends in G' and in the ratios of G'_{25} to G'_{70} were obtained when a selection of the experiments was repeated at 70 °C.

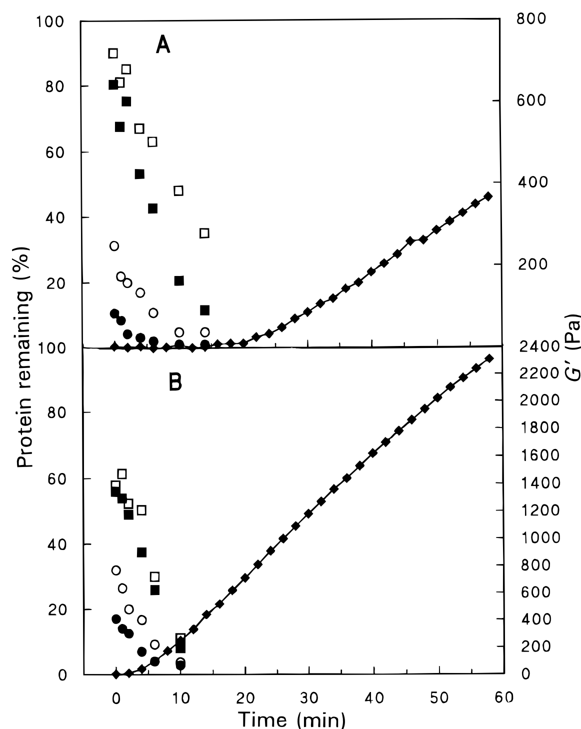

Figure 7. Effect of heating mixtures of 7% β-lactoglobulin and 3% BSA (A) and 3% β-lactoglobulin and 7% BSA (B) at 75 °C on G' (◆) and quantities of native-like β-lactoglobulin (■), native-like BSA (●), SDS-monomeric β-lactoglobulin (□), and SDS-monomeric BSA (○).

Approximate gelling times were estimated as the time taken for G' to attain 15 Pa. For example, in Figure 5A the gelling time was about 26 or 28 min, and in Figure 5B it was 10 min. It can be seen (Figure 6) that gelling times for the mixtures containing various ratios of BSA to β-lactoglobulin were 20 min or greater when the mixtures contained 0−4% BSA and were less than 10 min when the mixtures contained 6−10% BSA.

Because the mixture containing 5% of each protein behaved in an intermediate fashion (Figure 6), results from two mixtures that were typical of low and high ratios of BSA to β-lactoglobulin are shown in parts A and B, respectively, of Figure 7. The differences in gelling times are clearly shown for the 7:3 and 3:7 β-lactoglobulin to BSA mixtures in Figure 7, parts A and B, respectively. Also noticeable are the different rates of loss of the proteins from solution. For instance, after 10 min of heating, most of the β-lactoglobulin was depleted from the 3% β-lactoglobulin mixture (Figure 7B), but only 50 and 80% of the native-like and SDS-monomeric β-lactoglobulin were lost from the 7% β-lactoglobulin mixture (Figure 7A). It is also clear that the quantity of native-like or SDS-monomeric β-lactoglobulin is greater at all heating times in the mixture containing 7% β-lactoglobulin and also that the difference between the concentrations of native-like and SDS-monomeric β-lactoglobulin is greater, indicating that the disulfide cross-linking reaction is possibly slower in this mixture than in the mixture containing 3% β-lactoglobulin and 7% BSA.

By contrast the rate of loss of BSA from the mixtures appeared to be independent of the ratios of the two proteins. The rates for the BSA in this experiment were slightly lower than for the 5% BSA/5% β-lactoglobulin

mixture, but within the expected repeatability for such experiments made using different samples of protein. Similar trends were obtained when protein mixtures were heated at 70 °C.

Clearly the behavior of the mixtures was determined by the ratio of BSA to β-lactoglobulin with two rather clear types of behavior depending on whether BSA or β-lactoglobulin dominated the mixture and, hence, the behavior.

Heating Mixtures Containing More than 10% Protein. When the protein concentration in mixtures was increased from 10% by addition of either BSA or β-lactoglobulin (control) and the change in G' determined at 70 or 75 °C, BSA was found to be more effective than additional β-lactoglobulin in decreasing gelling time and increasing G' after 60 min of heating (Gezimati, 1995).

DISCUSSION

When β-lactoglobulin was heated and the cooled samples were examined by electrophoresis, the quantity of native-like protein was found to decrease more rapidly as a consequence of heating (Figures 2 and 4A) than the quantity of SDS-monomeric β-lactoglobulin (Figures 3 and 4A). There was also protein that was not dispersed by SDS, but after addition of mercaptoethanol, a reducing agent, to any of the reaction mixtures, all of the β-lactoglobulin was dispersible to monomers as shown by SDS−PAGE (Gezimati, 1995). These results confirm the suggestion (McSwiney et al., 1994a,b) that in the reaction mixtures there is native-like β-lactoglobulin, covalently cross-linked (disulfide-bonded) β-lactoglobulin, and a third type of β-lactoglobulin that does not disperse to give native-like monomers in PAGE sample buffer [∼0.05 M Tris (tris[hydroxymethyl]aminomethane)/HCl at pH ∼8.4] but does disperse in the presence of 0.1% SDS to give monomeric β-lactoglobulin. Questions arise as to how this protein is associated and what is (are) the conformation(s) of the associated protein. One possibility is that several protein molecules associate together to give aggregates that are stable at 70−75 °C and remain stable at room temperature at a pH of ∼8.4. It seems likely that these aggregates that are dispersible in SDS solution at pH 8.4 are held together by some type of hydrophobic interaction. A second possibility is that once some β-lactoglobulin has formed into disulfide-bonded polymers, native-like β-lactoglobulin associates with the covalent aggregate and is only dissociated from the polymer by SDS. Prior to, or as a consequence of, this association, the native β-lactoglobulin may change conformation.

When BSA solutions were heated, cooled, and examined by electrophoresis (Figures 2B, 3B, and 4B), aggregates similar to those previously identified in β-lactoglobulin solutions (McSwiney et al., 1994a,b) were apparent. This suggests that both BSA and β-lactoglobulin responded in a similar way to heat treatment except that BSA began to aggregate (and gel) at a lower temperature (Figure 1). This is expected on the basis that the thermal transition temperature for BSA is lower than that of β-lactoglobulin under a range of conditions at neutral pH (de Wit and Klarenbeek, 1984). A further similarity is that both BSA and bovine β-lactoglobulin each contain a free thiol group, cysteine 34 for BSA (Carter and Ho, 1994) and cysteine 121 for β-lactoglobulin (Hambling et al., 1992). It seems likely that the BSA aggregates observed in the present study are similar to those reported by Matsudomi et al. (1993) to occur in solutions of *N*-ethylmaleimide-treated BSA that had been heated at 80 °C for 30 min.

The three-dimensional structures of the proteins that are seen in native PAGE as "native" β-lactoglobulin or BSA are probably similar to the known crystal structures for these proteins (Hambling et al., 1992; Carter and Ho, 1994), although they may have been different at the gelation temperatures. However, the structures of the proteins that are not observed in native PAGE but are observable in SDS−PAGE (i.e. the hydrophobic aggregates) may not be folded into native-like conformations. Hirose (1993) speculated that intermediates during the formation of gels, foams, and emulsions may be in the "molten globule" conformation, as did McSwiney et al. (1994b) for the hydrophobic aggregates formed in heated β-lactoglobulin solutions. More recently Tani et al. (1995) observed that some characteristics of the heat-denatured aggregates of BSA, hen egg white lysozyme, and ovalbumin were consistent with these proteins being in the molten globule state using the criteria of Kuwajima (1989) and Ptitsyn (1995). Characteristics of this state are the loosened tertiary structure but largely retained secondary structure, giving greater access to regions of the protein molecules that are inaccessible in the native protein.

The present results for each protein can be considered in terms of the formation, at the heating temperatures, of hydrophobic aggregates that have some of the characteristics of the molten globule state. If two or more protein molecules can form an aggregate with a hydrophobic region that spans the protein boundaries, then within this low-polarity environment certain reactions, such as the thiol−disulfide interchange, might take place more readily, even if only for statistical reasons. In such an aggregate it would be expected that peptide segments of one protein molecule would be able to commingle with segments of other protein molecules within the putative hydrophobic core of the aggregate. This would allow the occasional close juxtaposition of thiol and disulfide groupings with the possibility of the interchange reaction occurring. Similarly, the formation of the stable hydrophobic aggregates, detected by different electrophoretic behavior, may also result from the close juxtaposition of appropriate peptide segments.

When the reactions of two different proteins in admixture leading to gel formation are considered, the crux of the matter is that the various proteins associate, aggregate, and interact so that the thiol of one protein can be placed in an appropriate position in relation to the disulfide of the other protein immediately prior to the thiol−disulfide interchange reaction. There is unlikely to be fruitful reaction between the proteins if one of them does not unfold or undergo a conformational change that would be measured as the thermal transition. One possible scenario is that the proportion of each protein in the hydrophobic aggregate is dependent on the relative abundance of each protein, the thermal transition temperature of the protein, and the temperature of reaction. At lower temperatures (e.g. ≤70 °C) the number of β-lactoglobulin molecules that undergo the thermal transition is less than the number of BSA molecules, and hence BSA will be the dominant species in the aggregates and the gel formed will be largely comprised of BSA molecules. As the BSA is depleted from the system, the remaining solution will contain more β-lactoglobulin than BSA in native-like states and the newly formed aggregates would contain a greater

proportion of β-lactoglobulin. By contrast, during high-temperature gelation more of each protein will form aggregates and the rates of depletion of the two proteins will be more comparable. The rate of heating would be very important; if it is slow enough, then even at high temperatures BSA would be depleted first, followed by β-lactoglobulin aggregates.

Hines and Foegeding (1993) examined the effect of added BSA on the gelation of β-lactoglobulin at 80 °C in 7% (w/v) protein solutions in a 50 mM TES (*N*-tris-[hydroxymethyl]methyl-2-aminoethanesulfonic acid) buffer containing 100 mM added NaCl and adjusted to pH 7.0. They found that G' after 3 h of heating was greatest for BSA and least for β-lactoglobulin and intermediate for the mixtures. These results, which were derived using a buffer system that did not contain lactose, are in good qualitative agreement with the results from the present study, in which lactose was included in the buffer, and suggest that the formation of lactosyl β-lactoglobulin under the heating conditions of the present study (Maubois et al., 1995; R. G. Burr, J. P. Hill, and C. H. Moore, personal communication) but not in the absence of lactose (Hines and Foegeding, 1993) did not alter gelation behavior markedly.

Hines and Foegeding (1993) also used size exclusion chromatography to determine the loss of native protein from 1% (w/v) protein solutions in pH 7.0 TES buffer heated in small glass tubes at 80 °C and found that native β-lactoglobulin in a 1:3.5 weight ratio (1:1 molar ratio) mixture with BSA was aggregated with a second-order rate constant that was nearly 7 times greater than when β-lactoglobulin was alone in solution and that the aggregation rate for BSA was unaltered by the presence of β-lactoglobulin. It is not possible to make a direct comparison with our own results because of the differences in experimental conditions. Examination of Figure 7B, in which the BSA to β-lactoglobulin weight ratio is about 2.3, indicates that when the mixture attains 75 °C, most of the BSA was polymerized, whereas 40% of the β-lactoglobulin was polymerized. By comparison, when the protein ratio was reversed (Figure 7A), most of the β-lactoglobulin was still native when the mixture reached 75 °C. Examination of the electrophoresis results for other mixtures (Gezimati, 1995) showed similar trends with early loss of native β-lactoglobulin when the weight ratio of BSA to β-lactoglobulin was greater than 1:1. Thus, our results, as do those of Matsudomi et al. (1994), support the findings of Hines and Foegeding (1993), and together they support the notion that BSA and β-lactoglobulin form heteropolymer gels under appropriate conditions. However, there does not appear to be any direct evidence of disulfide cross-links between BSA and β-lactoglobulin molecules.

CONCLUSIONS

The major pathway during gelation of β-lactoglobulin, BSA, or protein mixtures may involve formation of noncovalently linked aggregates followed by disulfide-bonded aggregates, leading to the formation of a three-dimensional gel network. In mixtures of BSA and β-lactoglobulin, BSA seems to influence the loss of native β-lactoglobulin, whereas the presence of β-lactoglobulin does not seem to influence the loss of native BSA from the protein mixtures, probably as a consequence of the lower thermal transition temperature of BSA. When protein mixtures were heated at 70 °C, BSA apparently formed the main gel network prior to β-lactoglobulin aggregation and gelation to form interpenetrating protein strands, whereas at 75 °C, both proteins may form interpenetrating gel networks with some interstrand linkages.

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