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Gelation of Bovine Serum Albumin and β-Lactoglobulin; Effects of pH, Salts and Thiol Reagents

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ABSTRACT

The formation of heat-induced gels from bovine serum albumin (BSA) and β-lactoglobulin (β-Lg) under different conditions was studied. The minimum protein concentration required for formation of self-supporting gels in 100 mM Tris-HCl buffer (pH 8·0) following heating at 90°C for 15 min was 4% for BSA and 5% for β-Lg. Maximum gel hardness for both BSA and β-Lg occurred at pH 6·5. The hardness of β-Lg gels reached a maximum with the addition of 20–40 mM NaCl or 2 mM $CaCl_2$, while BSA was at a maximum with 5 mM $CaCl_2$ but were unaffected by NaCl. The hardness of β-Lg gels decreased slightly upon addition of various anions of sodium (between 50 and 100 mM). The effects followed the lyotropic series. Addition of N*-ethylmaleimide (NEM) decreased gel strength of BSA, while the gel hardness of β-Lg increased slightly with 5 mM NEM but decreased at higher NEM levels. Dithiothreitol (DTT), at 5 and 2 mM, respectively, enhanced gel hardness of BSA and β-Lg gels. Higher DTT levels significantly decreased gel hardness for both BSA and β-Lg. These results indicated that disulfide bonds are important in BSA gels, while electrostatic interactions and disulfide bonds are involved in the formation and maintenance of β-Lg gels.*

INTRODUCTION

Whey proteins as ingredients possess useful functional properties for a variety of applications in the food industry (Kinsella, 1984; Kinsella & Whitehead, 1989). The capacity to form heat-induced gels is an important

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property (Mulvihill & Kinsella, 1988). The properties of whey protein gels are affected by the chemical and physical properties of its protein components, β-Lg, α-lactalbumin and BSA. Both BSA and β-Lg can form heat-induced gels (Hillier *et al.*, 1980; Hegg, 1982; Paulsson *et al.*, 1986; Yasuda *et al.*, 1986; Mulvihill & Kinsella, 1987, Clark *et al.*, 1981). The gelation behavior of whey proteins is affected by the heating temperature and time, the protein concentration, and the conditions of the medium such as pH, salt concentration and the type of salt (Haggette, 1976; Cooper *et al.*, 1977; Hillier *et al.*, 1980; Mulvihill & Kinsella, 1987; Schmidt *et al.*, 1978; 1979). The gelling behavior is also sensitive to a critical balance between attractive and repulsive forces (Mulvihill & Kinsella, 1988).

The mechanisms responsible for the formation of the three-dimensional network of whey protein are not fully understood because of the complicated interactions among the different proteins of whey. Hence, studies of the gelation behavior of individual whey proteins may provide a better understanding of the mechanisms of gel formation by whey protein. The objective of this study was to determine the factors affecting the gel formation by individual whey proteins, β-lactoglobulin (β-Lg) and bovine serum albumin (BSA).

MATERIALS AND METHODS

Proteins and chemicals

Bovine serum albumin (BSA, fraction V, Product No. A-9647), β-lactoglobulin (β-Lg, Product No. L-2506), *N*-ethylmaleimide (NEM), and dithiothreitol (DTT) were obtained from Sigma Chemical Co., St. Louis, Missouri, USA. Proteins were desalted by dialysis prior to use and freeze-dried. Other chemicals were reagent grade.

Preparation of protein gels

A stock protein solution of 20% (w/v) was prepared in 0·1M Tris-HCl buffer (pH 8·0). The protein solution was diluted with the same buffer solution to give various protein concentrations. To prepare the protein solutions containing salts or other additives, the stock protein solution was diluted 1:1 volume ratio with the same buffer solution containing the specific salts or other additives, respectively, to give the desired final concentrations. For examination of the effect of pH on gel hardness, the protein solution (20% (w/v), dissolved in distilled water), was diluted 1:1 (volume ratio) with constant ionic strength buffer solutions. Aliquots (1 ml) of these gelling

solutions were transferred to glass vials (inside diameter 6·0 mm) which were precoated with Sigmacoat (Sigma Chemicals Co.) to facilitate subsequent removal of the gels and were then centrifuged at low speed (500 × g) to remove air bubbles. The vials containing gelling solutions were heat-sealed and then heated in a water bath at 90°C for 15 min. After heating, the vials were cooled immediately in ice water and held overnight at 4°C (Kamata *et al.*, 1988).

Measurement of gel hardness

After tempering gels at 25°C for 30 min, the gels were removed from the tubes without disrupting the surface or interior of the gels (Kamata *et al.*, 1988). Gel hardness was determined on the gel sections (6·0 mm diameter × 5·0 mm height) with an Instron Universal Testing Instrument (Model 112, Instron Co.), according to the method of Mulvihill and Kinsella (1988). The gel section was compressed to either 80% (4·0 mm) or 30% (1·5 mm) of its original height, i.e. 20 and 70% compression. These were selected because they relate to the texture of gels which are important to sensory phenomena as used for texture profile analyses. The gel hardness was calculated from the force vs. deformation curves as the height of the force peak on the first compression cycle (Bourne, 1978). All analyses were done in triplicate.

RESULTS AND DISCUSSION

Effects of heating temperature and time

In order to determine the effects of temperature and time on gelation, solutions of BSA and β-Lg were prepared (10% (w/v) protein) at pH 8·0, and heated at various temperatures for 15 min. BSA solutions did not form a self-supporting gel by heat treatment below 70°C although the solutions became viscous. The hardness of BSA gels increased rapidly between 75° and 95°C (Fig. 1). β-Lg formed self-supporting gels following heat treatment at 70°C and, compared to BSA, firmer gels were obtained at lower temperatures (70–80°C) whereas at temperatures above 85°C, the hardness of BSA gels were higher than those of β-Lg. Self-supporting gels of either BSA or β-Lg were not formed following heat treatment at 65°C for 15 min. BSA is denatured by heating at >56°C (Shen, 1980) and, at pH 8·0, β-Lg is denatured at >65°C (de Wit & Klarenbeëk, 1981). These results indicated that heating temperatures above minimum denaturation temperatures of the proteins were required for gel formation by these proteins. This is consistent with the result of Paulsson *et al.* (1986). The effect of heating time

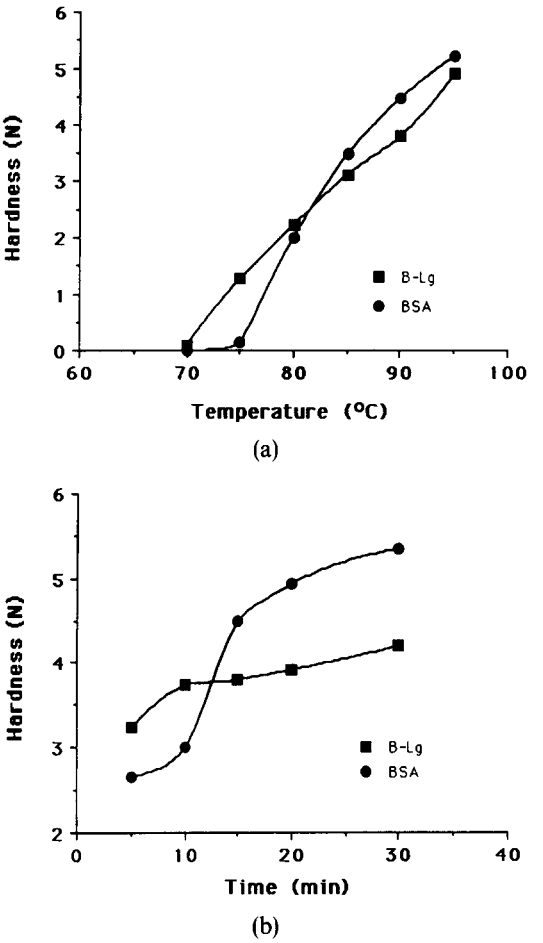


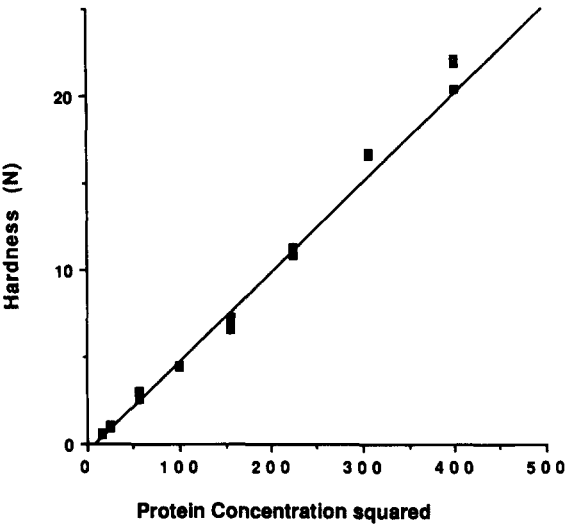
Fig. 1. Effects of heating temperature (a) and time (b) on the hardness of gels made from β-lactoglobulin (β-Lg) or bovine serum albumin (BSA) (see text for details).

at 90°C on gel hardness is summarized in Fig. 1(b). The hardness of BSA gels increased progressively with increased heating time, while that of β-Lg increased slightly after 15 min. These results indicated that the heat treatments for gel formation affected the firmness of gels. In the subsequent studies gel formation was obtained by heating for 15 min at 90°C.

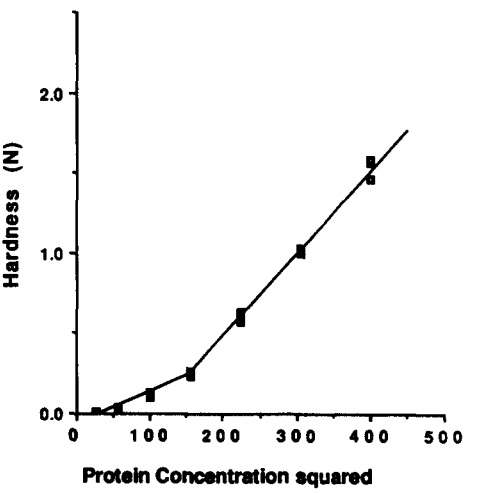
Effect of protein concentration

The hardness of heat-induced gels of globular proteins is also affected by the protein concentration (Hillier *et al.*, 1980; Clark *et al.*, 1981; Hegg, 1982; Katsuta *et al.*, 1990). The minimum concentration of protein required for gel formation is an important criterion of the gel-forming ability of specific

proteins. The hardness of gels made with various concentrations of BSA or β-Lg was measured using 20% and 70% compression. The BSA gels were transparent, had a smooth texture, and exhibited good water-holding capacity with little syneresis. The hardness of BSA gels increased exponentially to approximately the 2·2 power of protein concentration (Fig. 2). This was similar to the hardness which increased with the square of protein concentration (Ferry, 1948). When gel hardness was plotted against



(a)

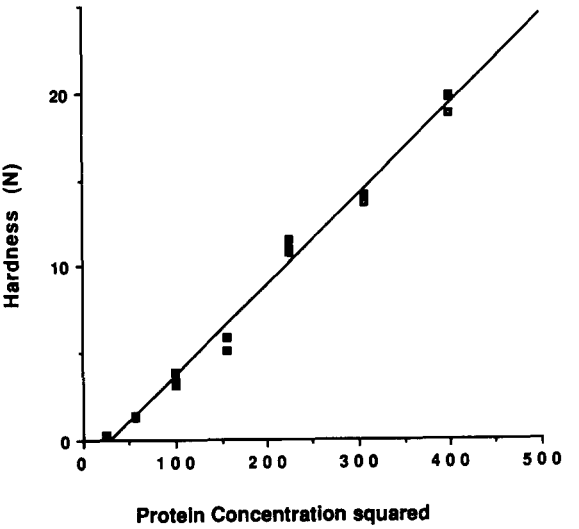


(b)

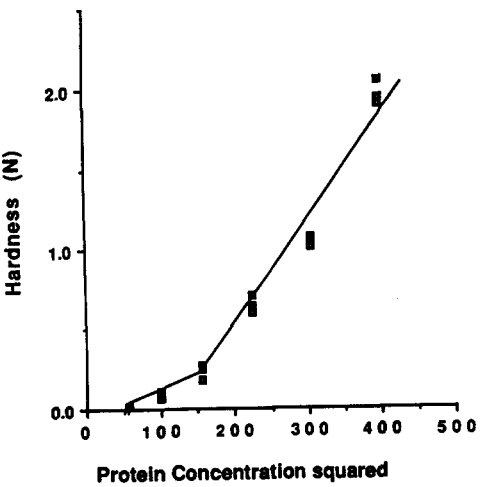
Fig. 2. The relationship between the hardness of gels and protein concentration at 70 (a) and 20% (b) compression of BSA gels, respectively.

the square of BSA concentration a linear relationship was obtained indicating that a minimum concentration of 4·4% BSA was required for gel formation (0 hardness). This is close to the BSA concentration of 4% w/v required for the formation of a self-supporting gel (Fig. 2).

At the 20% compression both BSA and β-Lg gels showed a biphasic response (Fig. 2) with a break point around 12% protein. This conceivably may reflect the involvement of differing crosslinking mechanisms which



(a)



(b)

Fig. 3. The relationship between the hardness of gels and protein concentration squared at 70 (a) and 20% (b) compression of β-Lactoglobulin gels, respectively.

form better networks and give harder gels above this concentration. For example, non-covalent forces may be the major elements involved in protein:protein interactions within the network which caused the viscous behavior below 12% protein, whereas, above that concentration, disulfide cross-linking may have become an additional mode of interaction and imparted more elastic behavior to the gels (Katsuta *et al.*, 1990). This is consistent with the reversible gelation of β-Lg (Rector *et al.*, 1989) and the rheological behavior of β-Lg gels (Katsuta *et al.*, 1990).

The hardness of β-Lg gels increased exponentially with increasing protein concentration (Fig. 3). The minimum protein concentration for gel formation of β-Lg was around 5%. As protein concentration was increased, the number of potential interactions were enhanced and a finer network was formed. Heating at pH 8·0 causes dissociation and unfolding of β-Lg with an increase in viscosity (McKenzie, 1971; Reddy *et al.*, 1988; Rector *et al.*, 1989). The conformational changes result in gel formation via protein:protein interactions between unfolded segments of β-Lg upon cooling. Thiol:disulfide interchange may also contribute to disulfide cross-linking (Hillier *et al.*, 1980; Katsuta *et al.*, 1990; Xiong & Kinsella, 1990). Subsequent studies were conducted to determine factors influencing protein:protein interactions and gel properties.

Effect of pH

The net charge on proteins markedly affected gel hardness. BSA gels possessed maximum hardness at pH 6·5 and, as the pH was increased above 6·5, the gel strength decreased (Fig. 4). This was observed with compression to 80 and 30% of the original height of the gels. At pH values below 6·0, the gel hardness decreased markedly and white, opaque sponge-like coagula were obtained. The β-Lg gels showed the same tendency as BSA gels with maximum hardness at pH 6·5 and a rapid decrease above pH 6·5 (Fig. 4(b)). These observations indicate that a certain number of electrostatic attractive and repulsive forces between the unfolded protein molecules play an important role in gel network structure (Ferry, 1948; Hermansson, 1979; Schmidt, 1981). When the negative forces exceed a critical number, protein: protein interactions are reduced and weaker gels are obtained (Hillier *et al.*, 1980).

Effect of salts

Salt concentration and species affect gelation and gel properties (Gumpen *et al.*, 1979; Kinsella, 1982; Mulvihill & Kinsella, 1988). The addition of sodium chloride caused variable changes in the hardness of BSA and β-Lg gels

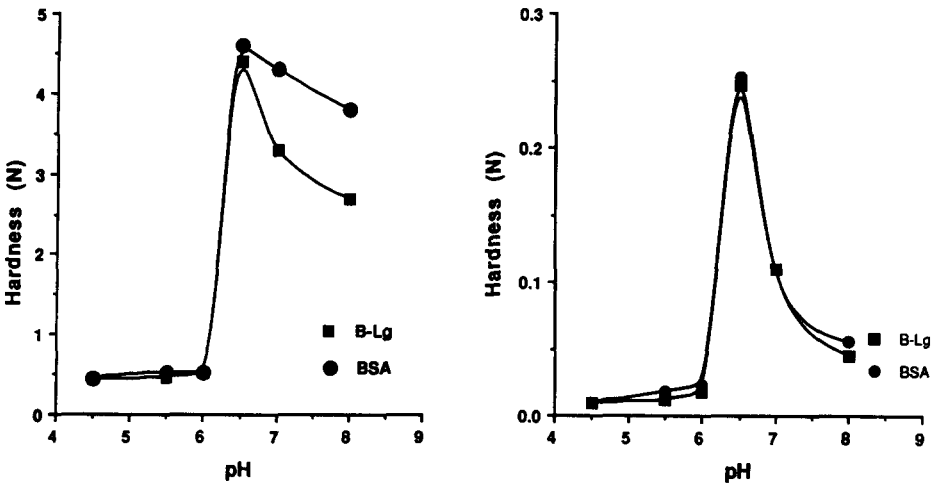


Fig. 4. The effect of pH on gel hardness of β-lactoglobulin and bovine serum albumin at 70 (a) and 20% (b) compression, respectively.

(Fig. 5). At 70% compression, the hardness of BSA gels was not affected by NaCl concentration (i.e. 4·8 to 4·9N at 0 to 400mM NaCl) whereas the maximum hardness of β-Lg gels occurred at 20mM added NaCl, and the gel strength decreased with increasing NaCl concentration up to 400mM (Fig. 5(a)). β-Lg formed transparent gels at NaCl concentration <20mM. The gels became slightly turbid and more fragile at 40mM NaCl, while at high NaCl levels the β-Lg gels were opaque coagula with sponge-like properties.

Sodium and other salts cause charge neutralization (von Hippel & Schleich, 1969; Damodaran & Kinsella, 1982). Therefore, at pH 8·0 the suppression of repulsion by counter ions enhances protein–protein interaction and the formation of a more stable gel network. At NaCl levels above 80mM, repulsive interactions between β-Lg molecules result in the collapse of the protein matrix with extensive syneresis as observed in electron micrographs (Mulvihill & Kinsella, 1988). Thus, β-Lg gels were more sensitive to NaCl concentration than BSA gels. This suggests that electrostatic interactions may be more important in the formation and stabilization of β-Lg gels at pH 8·0.

The hardness of both BSA and β-Lg gels was markedly affected by $CaCl_2$ concentration (Fig. 5). BSA formed elastic, completely clear gels with $CaCl_2$ concentration up to 5mM, but above 10mM the gels became turbid and did not recover once deformed. The β-Lg gels formed with $CaCl_2$ up to 2mM were more elastic and transparent, but above 5mM the gels became opaque and possessed sponge-like properties.

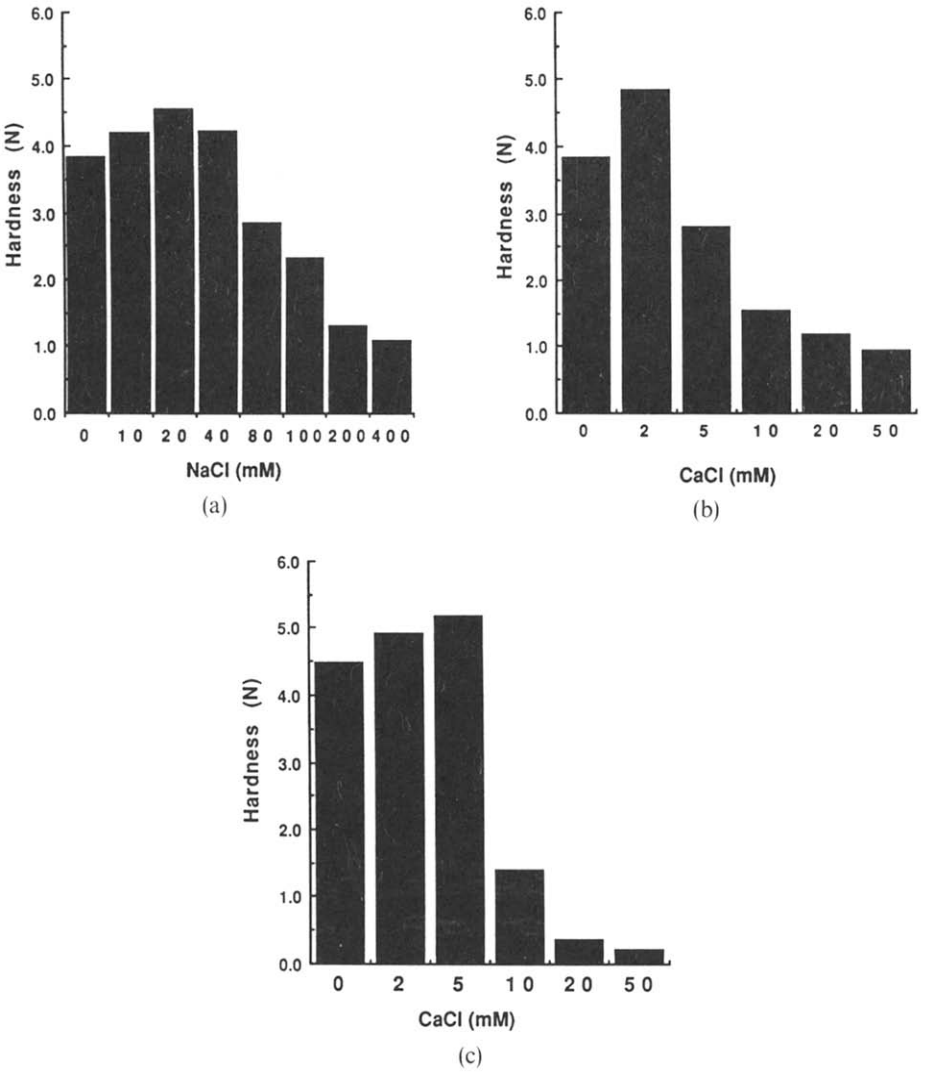


Fig. 5. The effects of sodium chloride on the hardness of β-lactoglobulin (a) and calcium chloride on the hardness of β-lactoglobulin (b) and bovine serum albumin (c).

The $CaCl_2$ was much more effective than NaCl in increasing gel strength of BSA and β-Lg, as reported by others (Schmidt *et al.*, 1979; Dunkerley & Zadow, 1984; Mulvihill & Kinsella, 1988). The calcium ions may engage in calcium bridging between negatively charged groups on adjacent unfolded protein molecules and thereby strengthen the gel matrix. However, once this level, i.e. 2 and 5 mM for β-Lg and BSA, respectively, was exceeded, the calcium bridging became excessive and the matrix collapsed yielding a coagulum (Mulvihill & Kinsella, 1988).

TABLE 1
Effect of Anions on Gel Hardness of BSA and β-Lg at 70% Compression

Anion	*Concn (mM)*	*Gel hardness (N)*	
		BSA	*β-Lg*
SO_4^{2-}	50	4·86	1·27
	100	5·13	0·51
Cl^-	50	4·51	3·83
	100	4·52	2·10
Br^-	50	4·34	3·81
	100	4·30	2·31
I^-	50	4·23	3·81
	100	4·23	2·53
SCN^-	50	4·20	3·80
	100	4·14	3·65

All samples (10% w/v protein) were prepared with the sodium salt at pH 8·0.

The effects of different anions of sodium salts on hardness of BSA and β-Lg gels are summarized (Table 1). The hardness of BSA gels showed no significant differences though the trend followed the order: $SO_4^{2-} \gg Cl^- > Br^- > I^- > SCN^-$. The turbidity of BSA gels increased in order of $SO_4^{2-} > Cl^- > Br^-$, while the BSA gels made with I^- and SCN^- remained clear and transparent at both concentrations. The sulfate ion decreased the hardness of β-Lg gels while other anions at 50 mM caused negligible differences for gel hardness, compared to gel made without these anions. Anion concentration of 100 mM resulted in weaker β-Lg gels and the gel hardness decreased in order of $SO_4^{2-} > Cl^- > Br^- > I^- > SCN^-$. These results suggest that hardness and transparency of β-Lg gels followed the lyotropic series of anions at 100 mM and that β-Lg is apparently more sensitive to these salts during heating than BSA.

Effect of thiol reagents on gel hardness

Many investigators have reported that thiol groups and disulfide bonds play an important role in the heat-induced gelation of whey proteins (Hillier *et al.*, 1980; Dunkerley & Zadow, 1984; Mori *et al.*, 1982; Utsumi & Kinsella, 1985; Zirbel & Kinsella, 1989; Shimada & Cheftel, 1989). Covalent cross-linking of protein molecules by disulfide bonds can be induced by thiol oxidation and/or by thiol-induced disulfide interchange reaction which is enhanced at alkaline pH (Sawyer 1968; Ananthanarayanan *et al.*, 1977; Hillier *et al.*, 1980).

The effects of thiol reagents on the hardness of BSA and β-Lg gels made

with 10% (w/v) protein and at pH 8·0 were studied. BSA has one thiol group and 17 disulfide bonds per monomer (Spencer & King, 1971). When NEM was added to the protein solution, there was a marked decrease in the hardness of BSA gels especially at >2 mM NEM (Table 2). The BSA gels formed were transparent, fragile and less elastic than control gels.

β-Lg has one thiol group and two disulfide bonds per monomer (Braunitzer *et al.*, 1973; Whitney *et al.*, 1976). When NEM was added to β-Lg, the hardness of β-Lg gels increased slightly up to 5 mM NEM and then dramatically decreased at >10 mM NEM.

TABLE 2
Effect of Thiol Reagents *N*-ethylmaleimide and Dithiothreitol on the Hardness of Gels formed from β-Lactoglobulin and Bovine Serum Albumin

Concentration of reagent (mM)	*Hardness of gels (N)*			
	N-*ethylmaleimide*		*Dithiothreitol*	
	BSA	*β-Lg*	*BSA*	*β-Lg*
0·0	4·655	3·346	4·655	3·350
2·0	3·026	3·500	6·001	4·500
5·0	0·766	3·601	6·381	0·724
10·0	0·247	3·051	5·252	0·701
20·0	0·202	0·170	2·287	0·683
50·0	0·149	0·090	2·640	0·371

Gels made with 10% protein, pHs following heating at 90°C for 15 min. Hardness upon 70% compression.

NEM blocks the free thiol groups, which inhibits their participation in thiol–disulfide interchange interactions between protein molecules. The BSA gel was apparently much more sensitive to blockage of the thiol group than β-Lg which required 20 mM to reduce the formation of a strong gel matrix. The reason for the disparities between the effects on BSA and β-Lg is not apparent because the single free thiol groups in both proteins should be equally accessible to the reagent. The data confirm that thiol disulfide interchange and perhaps thiol oxidation by forming intermolecular cross-links are important in the formation of gels (Hillier *et al.*, 1980; Xiong & Kinsella, 1990).

DTT which reduces disulfide bonds in proteins caused BSA gels to become more turbid with increasing DTT levels during heating. The

hardness of these gels increased up to 5 mM DTT and then decreased, when DTT exceeded 10 mM (Table 2). The BSA solutions gelled without heating after addition of 20 and 50 mM DTT, but these gels possessed gel strengths around 50% of the values of BSA gels formed without DTT. The transparency of heat-induced gels was not significantly affected by the addition of DTT. However, above 2 mM DTT fragile gels of very low compressive strength were obtained. The DTT reduced the disulfide bonds of BSA and facilitated unfolding of the polypeptide and enhanced protein: protein interactions. These initially contributed to an increase in gel strength but, following more extensive reduction, interactions may have become excessive and coagula were formed rather than structured gel networks.

Furthermore, thiol groups generated via the reduction of a limited number of disulfide bonds at low concentrations of DTT may have participated in thiol–disulfide interchange reactions to form intermolecular cross-links in the gel network which increased the strength of BSA protein gels made with 2–5 mM DTT. Conceivably the higher levels of DTT caused the lowering of gel strength because of extensive and excessive reduction of disulfide bonds.

Treatment with DTT had a similar effect on β-Lg gels. The gel strength initially increased with increasing levels of DTT and then decreased at higher concentrations of DTT. The decline in gel strength of β-Lg was much more dramatic than with BSA, once the concentration of DTT exceeded the optimum for gel hardness. The larger decrease in gel strength may reflect the fact that β-Lg has fewer disulfide bonds. The decrease in gel strength for both proteins occurred at approximately the same percent reduction of the proteins' disulfides (18·3% for β-Lg and 19·7% for BSA). Schmidt *et al.* (1979) reported similar results with whey protein where gel strength attained a maximum at 9·7 mM cysteine and decreased at high levels of cysteine. Shimada and Cheftel (1988) suggested that partial reduction of disulfide bonds in the whey proteins enhanced subsequent interactions between exposed hydrophobic regions. The interactions of hydrophobic regions induced by limited reduction of disulfide bonds may be also important in the formation of gels from BSA and β-Lg.

These data indicate that electrostatic interactions and thiol disulfide interactions are critical in the formation and stabilization of BSA and β-Lg gels and indicate that both of these proteins can contribute to the properties of whey protein gels.

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REFERENCES

Ananthanarayanan, V. C., Ahmad, F. & Bigelow, C. C. (1977). The denaturation of β-lactoglobulin A at pH 2. *Biochim. Biophys. Acta.*, **492**, 194.

Bourne, M. C. (1978). Texture profile analysis. *Food Technology*, **32**(2), 62.

Braunitzer, G., Chen, R., Schrank, B. & Stangl, A. (1973). The sequential analysis of β-lactoglobulins. *Hoppe-Seylers Z. Physiol. Chem.*, **354**, 867.

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

Cooper, H. R., Hughes, J. R. & Matthews, M. E. (1977). Application of 'response surface methodology' to the evaluation of whey protein gel systems. *New Zealand J. Dairy Sci. & Technol.*, **12**, 248.

Damodaran, S. & Kinsella, J. E. (1982). Effects of ion on protein conformation and functionality. In *Food Protein Deterioration Mechanisms and Functionality*. American Chemical Society. Symposium Series 206, ACS, New York, p. 327.

de Wit, J. N. & Klarenbeek, G. (1981). A differential scanning calorimetric study of the thermal behavior of bovine β-lactoglobulin at temperatures up to 160°C. *J. Dairy Res.*, **48**, 293.

Dunkerley, J. A. & Zadow, J. G. (1984). The effect of calcium and cysteine hydrochloride on the firmness of heat coagula formed from cheddar whey protein concentrates. *Aust. J. Dairy Technol.*, **39**, 44.

Ferry, J. (1948). Protein gels. *Adv. Protein Chem.*, **4**, 1.

Gumpen, S., Hegg, P. O. & Martens, H. (1979). Thermal stability of fatty acid–serum albumin complexes studied by differential scanning calorimetry. *Biophys. Acta*, **574**, 189.

Haggette, T. O. R. (1976). The whipping, foaming and gelling properties of whey protein concentrates. *New Zealand J. Dairy Sci. & Technol.*, **11**, 244.

Hegg, P. O. (1982). Conditions for the formation of heat-induced gels of some globular food proteins. *J. Food Sci.*, **47**, 1241.

Hermansson, A. M. (1979). Aggregation and denaturation involved in gel formation, ed. A. Pour-El, ACS Symposium Series 92, American Chemical Society, Washington, DC, p. 81.

Hillier, R. M., Lyster, R. L. & Cheeseman, G. C. (1980). Gelation of reconstituted whey powders by heat. *J. Sci. of Food and Agr.*, **31**, 1152.

Kamata, Y., Rector, D. & Kinsella, J. E. (1988). Influence of temperature of measurement on creep phenomena in glycinin gels. *J. Food Sci.*, **53**, 589.

Katsuta, K., Rector, D. & Kinsella, J. E. (1990). Viscoelastic properties of whey protein gels: Mechanical model and effects of protein concentration on creep *J. Food Sci.*, **52**(2) 516–21.

Kinsella, J. E. (1982). Relationship between structure and functional properties of food proteins. In *Food Protein*, ed. P. F. Fox & J. J. Condon, Applied Science, London.

Kinsella, J. E. (1984). Milk proteins: Physiochemical and functional properties. *Crit. Rev. Food Sci. & Nutr.*, **21**(3), 197.

Kinsella, J. E. & Whitehead, D. (1989). Proteins in whey: Chemical, physical and functional properties. *Adv. Food and Nutr.*, **33**, 343.

McKenzie, H. A. (1971). β-Lactoglobulins. In *Milk Proteins*. ed. H. A. McKenzie, Academic Press, Vol. 2, p. 503.

Means, G. E. & Feeney, R. E. (1971). *Chemical modification of proteins.* Holden Day, Inc., London.

Mori, T., Nakamura, T. & Utsumi, S. (1982). Gelation mechanism of soybean 11S gobulin: Formation of soluble aggregates as transient intermediates. *J. Food Sci.*, **47**, 26.

Mulvihill, D. M. & Kinsella, J. E. (1987). Gelation characteristics of whey proteins and β-lactoglobulin. *Food Technol.*, **41**, 102.

Mulvihill, D. M. & Kinsella, J. E. (1988). Gelation of β-lactoglobulin: Effects of sodium chloride and calcium chloride on the rheological and structural properties of gels. *J. Food Sci.*, **53**, 231.

Paulsson, M., Hegg, P. O. & Castberg, H. B. (1986). Heat-induced gelation of individual whey proteins. A dynamic rheology study. *J. Food Sci.*, **51**, 87.

Rector, D. J., Kella, N. K. & Kinsella, J. E. (1989). Reversible gelation of whey proteins: Melting, thermodynamics and viscoelastic behavior. *J. Text. Studies*, **20**(4), 457.

Reddy, J. M., Kella, N. K. D. & Kinsella, J. E. (1988). Structural and conformational basis of the resistance of β-lactoglobulin to peptic and chymotryptic digestion. *J. Agric. Food Chem.*, **36**, 737.

Sawyer, W. H. (1968). Heat denaturation of bovine β-lactoglobulin and relevance of disulfide aggregation. *J. Dairy Sci.*, **49**, 323.

Schmidt, R. H., Illingworth, B. L., Ahmed, E. M. & Richter, R. L. (1978). The effect of dialysis on heat-induced gelation of whey protein concentrate. *J. Food Proc. Preserv.*, **2**, 111.

Schmidt, R. H., Illingworth, B. L., Deng, J. C. & Cornell, J. A. (1979). Multiple regression and response surface analysis of the effect of calcium chloride and cysteine on heat-induced whey protein gelation. *J. Agric. Food Chem.*, **27**, 529.

Schmidt, R. H. (1981). Gelation and coagulation. In *Protein Functionality in Foods.* ed. J. P. Cherry, American Chemical Society, Washington, DC.

Shen, J. (1980). Abstract of Papers, 179th National Meeting of the American Chemical Society, Houston, TX, 23–28 March.

Shimada, K. & Cheftel, J. C. (1988). Texture characteristics, protein solubility, and sulfydryl group/disulfide bond content of heat-induced gels of whey protein isolate. *J. Agric. Food Chem.*, **36**, 1018.

Shimada, K. & Cheftel, J. C. (1989). Sulfydryl group/disulfide bond interchange reactions during heat-induced gelation of whey protein isolate. *J. Agric. Food Chem.*, **37**, 161.

Shimizu, M., Saito, M. & Yamauchi, K. (1985). Emulsifying and structural properties of β-lactoglobulin at different pHs. *Agric. Biol. Chem.*, **49**, 189.

Spencer, E. M. & King, T. P. (1971). Isoelectric heterogeneity of bovine plasma albumin. *J. Biol. Chem.*, **246**, 201.

Utsumi, S. & Kinsella, J. E. (1985). Forces involved in soy protein gelation: Effect of various reagents on the formation, hardness and solubility of heat-induced gels made from 7S, 11S, and soy isolate. *J. Food Sci.*, **50**, 1278.

von Hippel, P. H. & Schleich, T. (1969). Effect of salts on the structure and conformational stability of macromolecules in solution. In *Structure and Stability of Biological Macromolecules.* Marcel Dekker, New York, p. 47.

Whitney, R. McL., Brunner, J. R., Ebner, K. E., Farrell, H. M., Josephson, R. V., Moor, C. V. & Swaisgood, H. E. (1976). Nomenclature of the proteins of cows milk: Fourth revision. *J. Dairy Sci.*, **59**, 785.

Xiong, Y. L. & Kinsella, J. E. (1990). On the mechanism of urea-induced whey protein gelation. *J. Agric. Food Chem.* (38) (in press).

Yasuda, K., Nakamura, R. & Hayakawa, S. (1986). Factors affecting heat-induced gel formation of bovine formation of bovine serum albumin. *J. Food Sci.*, **51**, 1289.

Zirbel, F. & Kinsella, J. E. (1989). Effects of thiol reagents and ethanol on strength of whey protein gels. *Food Hydrocolloids*, **2**(6), 467.