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# Gelation of Chicken Pectoralis Major Myosin And Heat-Denatured β-Lactoglobulin

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Thermal, rheological, and microstructural properties of myosin (1 and 2% protein) were compared to mixtures of 1% myosin and 1% heat-denatured β-lactoglobulin aggregates (myosin/HDLG) and 1% myosin and 1% native β-lactoglobulin (myosin/β-LG) in 0.6 M NaCl and 0.05 M sodium phosphate buffer, pH 6.0, 6.5, and 7.0 during heating to 71 °C. Thermal denaturation patterns of myosin and myosin/HDLG were similar except for the appearance of an endothermic peak at 54−56 °C in the mixed system. At pH 7.0, 2% myosin began to gel at 48 °C and had a storage modulus (*G*′) of 500 Pa upon cooling. Myosin/HDLG (2% total protein) had a gel point of 48 °C and a *G*′ of 650 Pa, whereas myosin/β-LG had a gel point of 49 °C but the *G*′ was lower (180 Pa). As the pH was decreased, the gel points of myosin and myosin/HDLG decreased and the *G*′ after cooling increased. The HDLG was incorporated within the myosin gel network, whereas β-LG remained soluble.

**KEYWORDS:** **β-Lactoglobulin; gelation; myosin; aggregates; microstructure; rheology**

## INTRODUCTION

Whey protein has been widely used as a functional food ingredient. In meat products, whey protein is used to improve color, yield, and textural quality. However, the effect of whey protein on textural attributes of meat products is often variable as commercial whey proteins do not form gels at typical meat processing temperatures (∼68−71 °C), which limits the potential for textural enhancement.

Many studies have looked at the cold-set gel properties of pre-denatured or preheated whey protein aggregates or polymers (*1*−*6*). Heating whey protein solution at low ionic strength leads to the formation of denatured soluble aggregates or polymers. Cold-set gels of the aggregates can be induced by increasing the ionic strength through the addition of salts, lowering the pH, or proteolytic digestion. The gels formed from preheated whey proteins were reported to be clearer and harder than those from untreated whey protein gels.

Vittayanont et al. (*7*) studied the heat-induced gelation of myosin and β-lactoglobulin (β-LG), the major functional components, respectively, of meat and whey protein. The addition of β-LG to myosin solutions did not increase gel hardness until the mixture was heated to 75 °C or above, the gelling point of β-LG (*7*). Vittayanont et al. (*8*) also studied gelling properties of β-LG compared to heat-denatured aggregates of β-LG (HDLG) in a high-salt buffer system. The HDLG was prepared by preheating β-LG solutions at 80 °C for 30 min at pH 7.0. At pH 6.0−7.0 and 0.6 M NaCl (optimum conditions for myosin gelation), HDLG formed a gel with a finer microstructure and with higher gel stiffness at lower protein concentration and temperature than β-LG.

The salt-induced gelation of heat-denatured aggregates of β-LG at low temperature suggests a potential application for the use of these aggregates in meat products. Salt is commonly added to meat and poultry products to extract salt-soluble protein to maximize yields and develop desired textural attributes. Hongsprabhus and Barbut (*9*) studied the textural properties and water-holding capacity of comminuted chicken and preheated whey protein isolate (WPI). They found that addition of 2% preheated WPI aggregates (prepared by heating at 80 °C for 30 min, pH 7.0) in conjunction with cold-set gelation for 6 h at 1 °C lowered cooking loss and improved hardness of the comminuted chicken after cooking to 78 °C at salt concentrations of 1.5% or below when compared to unheated WPI. More work is needed to understand interactions between whey protein aggregates and meat proteins during heating to optimize the use of whey protein in meat products. The objective of this work was to characterize the thermal transitions, rheological properties, and microstructure of chicken pectoralis major myosin and HDLG during heat-induced gelation in 0.6 M NaCl at pH 6.0, 6.5, and 7.0.

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## MATERIALS AND METHODS

**Protein Preparation.** Myosin from pre-rigor breast muscle (pectoralis major) was extracted immediately after sacrifice from 9-week-old commercial type broilers and stored in 48% saturated $(NH_4)_2SO_4$ containing 30% glycerol at −20 °C (*10*). Immediately prior to use, myosin was suspended in 0.6 M NaCl and 0.5 M sodium phosphate buffer (PBS) at pH 6.0, 6.5, or 7.0, dialyzed against three changes of the same buffer for 48 h, and centrifuged at 78000*g* to remove denatured proteins. Solutions of 1 and 2% (w/v) myosin were prepared by dilution of this stock solution with the dialysis buffer.

Bovine milk β-LG (L0130, lot 113H0755), containing variants A and B, was purchased from Sigma Chemical Co. (St. Louis, MO). Heat-denatured β-LG was prepared by heating 4% (w/v) β-LG in deionized water, adjusted to pH 7.0 with 0.1 M HCl, in a water bath at 80 °C for 30 min. The HDLG was cooled to 4 °C in an ice bath and diluted 1:1 with 1.2 M NaCl and 0.1 M sodium phosphate buffer, pH 6.0, 6.5, or 7.0, to prepare working solutions of 2.0% HDLG. Native β-LG was solubilized in PBS, pH 7.0, overnight at 4 °C.

Mixtures of myosin/β-LG and myosin/HDLG (2% total protein) were prepared by mixing together equal amounts of 2% myosin and 2% β-LG or 2% HDLG immediately before use. Protein concentrations were determined by absorption using extinction coefficients ($E^{1\%}$) of 5.5 at 280 nm for myosin (*11*) and 9.55 at 278 nm for β-LG (*12*).

**Differential Scanning Calorimetry.** The thermal denaturation patterns of 1% myosin, myosin/β-LG, and myosin/HDLG in PBS, pH 6.0 and 7.0, were determined using a differential scanning calorimeter (DSC) (MC-2, Microcal Inc., Amherst, MA). The protein and buffer solutions were degassed in a vacuum chamber before loading into 1.24 mL cells of the DSC. Experiments were conducted at a scan rate of 1 °C/min from 25 to 90 °C. Heat capacity profiles (endotherm; $C_p$ vs temperature), calorimetric enthalpy ($\Delta H_{cal}$), and endothermic peak or melting temperature ($T_m$) were obtained using the software (DA-2 data acquisition and analysis system) provided by the manufacturer.

**Rheological Properties.** A controlled stress rheometer (RS 100, Haake, Karlsruhe, Germany) equipped with a 35 mm diameter stainless steel parallel plate was used to monitor storage modulus ($G'$) and loss modulus ($G''$) during heating of 1 and 2% myosin, myosin/β-LG, and myosin/HDLG solutions. Tests were performed at a fixed frequency of 2.9 rad/s using constant stresses (producing strains from 0.1 to 0.3%) within the range of linear viscoelastic behavior. Stress sweeps were performed for each protein at 71 °C and after cooling to 25 °C to determine the linear viscoelastic range. About 1.0 mL of each protein solution was loaded between the plate and base with a gap of 1.0−1.1 mm. A few drops of maize oil (Mazola, Best Foods, CPC International, Inc., Englewood Cliffs, NJ) were used to cover the edge of the plate to prevent evaporation. The solutions were equilibrated at 25 °C for 5 min, heated to 71 or 90 °C at 1° C/min, held for 60 min at 71 °C or for 30 min at 90 °C, cooled to 25 °C within 10 or 15 min, and held at 25 °C for 5 min. Frequency (0.01−100 rad/s) sweep tests were conducted at the end of the cooling step using stress, which was controlled to produce a strain of 0.1−0.2%. The gel point was defined as the temperature at which $G'$ and $G''$ crossed over in the fixed frequency test.

**Scanning Electron Microscopy (SEM).** To prepare protein gels, 1.0 g of each protein solution (myosin, myosin/β-LG, or myosin/HDLG) was transferred into 12 mm × 75 mm glass tubes and sealed with Teflon tape. Tubes were placed in a water bath (PolyScience, model 9510, Niles, IL) programmed to heat from 25 to 71 or 90 °C at 1 °C /min and held for 60 min at 71 °C or for 30 min at 90 °C. Gels were cooled in an ice−water bath and cut into 1 × 2 × 2 mm pieces. Specimens were prefixed for 3 h in 2.0% gluteraldehyde in 0.1 M sodium phosphate buffer, pH 7.0, and postfixed overnight in 0.1% osmium tetraoxide. Fixed gels were rinsed thoroughly with 0.1 M sodium phosphate buffer, pH 7.0, and dehydrated by immersion in a graded ethanol series of 25, 50, 75, and 95% for 20 min each, followed by three 20 min changes in 100% ethanol. Gels were then dried using a Balzers critical point dryer (Balzers CPD, FL-9496, Balzers, Liechtenstein) in liquid carbon dioxide, mounted on metal stubs, and coated with a 25−30 nm gold layer in an Emscope ion-sputter coater (Emscope Laboratories Ltd., Ashford, Kent, U.K.). Microstructures was observed with a JEOL scanning microscope (model JSM-6400V, Tokyo, Japan) at a 15 mm working distance using an accelerating voltage of 12 kV and magnification of 5000×.

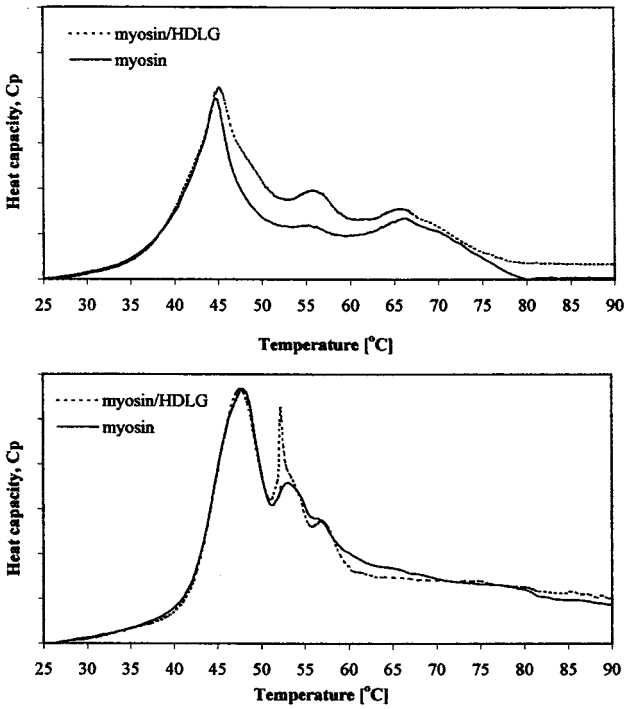


**Figure 1.** Heat capacity profiles of 1% (w/v) myosin and 1% (w/v) myosin/1% (w/v) heat-denatured β-LG (HDLG) in 0.6 M NaCl and 0.05 M sodium phosphate buffer: (A) pH 6.0; (B) pH 7.0.

**Soluble Proteins Expressed from Gels.** The 2% myosin, 4% β-LG, 2% HDLG, myosin/β-LG, and myosin/HDLG solutions (0.5 g) were heated in 1.5 mL microcentrifuge tubes using the heating schedule previously described for SEM gel preparation. After cooling, the protein gels were centrifuged at 10000*g* for 30 min. The supernatants were collected and the protein compositions compared by electrophoresis.

Electrophoresis was run in a Mini-Protein II dual slab cell (Bio-Rad Laboratories, Hercules, CA) using a tris(hydroxymethyl)aminomethane (Tris)-glycine electrode buffer, pH 8.3, containing 0.1% sodium dodecyl sulfate (SDS) (*13*). The acrylamide concentrations of stacking and resolving gels were 4 and 12%, respectively. Supernatants were diluted 1:2 with sample buffer (0.0625 M Tris, pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.2% bromophenol blue), mixed well, and heated in boiling water for 5 min immediately before use. Protein samples (10 μL) were loaded into the wells, and the gels were run at constant voltage (200 V). Gels were stained with Coomassie Brilliant Blue R250 solution (0.25%) in acetic acid/methanol/water (9:45:45 v/v/v) and destained overnight in acetic acid/methanol/water (6:4:7, v/v/v) solution. Molecular mass of the proteins was estimated by comparing relative mobilities to those of protein standards (Bio-Rad) run under the same electrophoretic conditions (*14*).

**Experimental Design and Statistical Analysis.** Three replicates were used for the rheological and electrophoresis experiments. Two replicates were used for SEM, and three digital images of each replicate gel were taken. Six replicates were used for DSC analysis. A replicate is defined as a single preparation of protein heated separately in the DSC, rheometer, or water bath (for SEM). Results are expressed as the mean ± standard error of the mean. Differences in gel point, $G'$, and tangent delta (tan $\delta$) of gels due to protein mixture or pH were statistically analyzed using one-way analysis of variance. Means were compared using Tukey's test at a significance level of 5% (*15*).

## RESULTS AND DISCUSSION

**Thermal Denaturation of Mixed Proteins.** Denaturation patterns of myosin showed three $T_m$ points at 45.0, 55.5, and 66.0 °C at pH 6.0 (**Figure 1A**). Similarly, three $T_m$ points of

**Table 1.** Gel Point, Storage Moduli ($G'$), and Tangent Delta (tan $\delta$) after Cooling of Myosin, 1% Myosin/1% $\beta$-Lactoglobulin (Myosin/$\beta$-LG), and 1% Myosin/1% Heat-Denatured $\beta$-LG (Myosin/HDLG) in 0.6 M NaCl, 0.05 M Sodium Phosphate Buffer, pH 7.0, Heated to 71 °C for 60 min or to 90 °C for 30 min[a]

| protein | temp (°C) | total protein concn (%) | gel point (°C) | $G'$ after cooling (Pa) | tan $\delta$ after cooling |
|---|---|---|---|---|---|
| myosin | 71 | 1 | 49.0 ± 0.17 a | 120 ± 3.5 c | 0.08 ± 0.000 b |
| | 71 | 2 | 47.8 ± 0.06 b | 500 ± 21.4 b | 0.08 ± 0.000 b |
| myosin/HDLG | 71 | 2 | 48.0± 0.12 b | 650 ± 15.6 a | 0.08 ± 0.000 b |
| myosin/$\beta$-LG | 71 | 2 | 49.0 ± 0.17 a | 180 ± 9.2 c | 0.11 ± 0.011 a |
| myosin/$\beta$-LG | 90 | 2 | 49.2 ± 0.17 a | 500 ± 16.7 b | 0.10 ± 0.011 a |

[a] Values are means of three observations ± standard error of the mean. Means followed by different letters in each column are significantly different ($p < 0.05$).

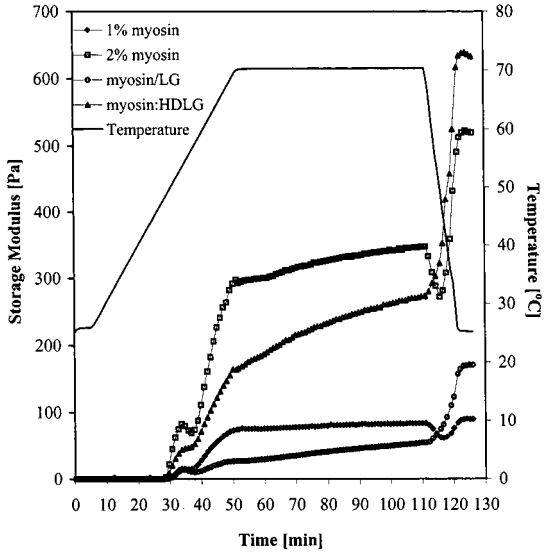


**Figure 2.** Storage moduli of myosin, 1% (w/v) myosin/1% (w/v) $\beta$-LG, and 1% (w/v) myosin/1% (w/v) heat-denatured $\beta$-LG (HDLG) in 0.6 M NaCl and 0.05 M sodium phosphate buffer, pH 7.0, during heating at 1 °C/min from 25 to 71 °C, holding at 71 °C for 60 min, and cooling to 25 °C.

48.5, 53.2, and 57.0 °C were observed at pH 7.0 (**Figure 1B**). The calorimetric enthalpies ($\Delta H_{cal}$) of myosin at pH 6.0 and 7.0 were 2138 ± 30.2 and 2310 ± 32.7 kcal/mol, respectively. Transition temperatures and calorimetric enthalpies were similar to those reported for chicken breast muscle myosin in other studies (*10*, *16*). Myosin is a multidomain protein, and transition peaks shift due to differences in ionic strength and pH of the buffer system (*17*).

The thermograms of myosin/HDLG mixtures were similar to those of myosin at the same pH, except for the increase in the transition peak at 56 °C at pH 6.0 and the appearance of an additional endothermic peak at 54 °C at pH 7.0. Because HDLG was irreversibly denatured and had no transition peak when heated in the DSC (*8*), the transition peak at 54−56 °C might be due to interactions between HDLG and myosin that shifted the transition temperature of one of the myosin domains.

**Rheological Properties.** The $G'$ of 1 and 2% myosin at pH 7.0 increased during heating and cooling (**Figure 2**). The 2% myosin solution gelled at a lower temperature (47.8 °C) than 1% myosin, which reached its gel point at 49.0 °C. The $G'$ began to increase after the gel point, formed a transition peak at 53 °C, and then increased rapidly between 58 and 71 °C before reaching a plateau when held at 71 °C. The $G'$ decreased during cooling from 71 to 46−47 °C and then increased rapidly during the remainder of the cooling period. A similar $G'$ curve was reported during heating of chicken myosin in 0.6 M NaCl, pH 6.0 (*18*). The authors noticed that the temperature corresponding to the minimum $G'$ during cooling was close to the onset temperature of myosin gelation, agreeing with our observation. Hydrophobic interactions increase with temperature between 25 and 75 °C, whereas hydrogen bonds decrease. Liu and Foegeding (*18*) attributed the decrease in $G'$ at 53 °C to the breaking of hydrogen bonds during heating and the increase in $G'$ upon cooling from 46 to 25 °C to the re-formation of hydrogen bonds. Sulfhydryl and hydrophobic interactions also play a role in myosin gelation (*19*).

The gelation profiles of myosin/LG and myosin/HDLG were similar to those of myosin, except $G'$ did not decrease during heating at 53 °C or during cooling from 71 to 46 °C. These results suggest that the relative importance of the various chemical bonds forming the mixed protein gel network might be different from those forming the myosin gel network. Although further research is needed, disulfide bond formation and exchange have been reported to be important during the gelation of LG and may have offset the negative effects of hydrogen bond breakdown observed with myosin alone (*20*, *21*).

The $G'$ after cooling of 2% myosin was ~4 times greater than that of 1% myosin at pH 7.0 (**Table 1**). The $G'$ of myosin/$\beta$-LG (2% total protein) was greater than that of 1% myosin after heating to 71 °C and cooling, but ~3 times less than that of 2% myosin. The $G'$ of myosin/HDLG (2% total protein) was greater than that of 1% myosin throughout the heat treatment and surpassed the $G'$ of 2% myosin on cooling. The $G'$ after cooling of myosin/HDLG gels at pH 7.0 heated to 71 °C was 650 Pa or 3.6-fold greater than that of the myosin/$\beta$-LG gel and 1.3-fold greater than that of the 2% myosin gel. The $G'$ of myosin/HDLG gels heated to 71 °C was also greater than that of myosin/$\beta$-LG gel heated to 90 °C. The addition of HDLG did not affect the gel point of myosin of 48 °C. Hongsprabhus and Barbut (*9*) reported that the addition of 2% preheated WPI aggregates (prepared at 80 °C for 30 min, pH 7.0) in conjunction with cold-set gelation (6 h at 1 °C) improved the hardness of comminuted chicken after cooking to 78 °C when compared to the use of unheated WPI.

Two percent myosin and myosin/HDLG heated to 71 °C at pH 7.0 exhibited viscoelastic gel characteristics as indicated by tan delta (tan $\delta$) values of 0.08 after cooling (**Table 1**) and moduli that were independent of frequency (**Figure 3**). Polymer systems show high tan $\delta$ for dilute solutions, 0.2−0.3 for amorphous polymers, and low (near 0.01) for glassy crystalline polymers and gels (*22*). The $G'$ and $G''$ of myosin and myosin/HDLG were independent of frequency, and $G'$ was 8−10 times greater than $G''$ throughout the frequency range, typical of a strong cross-linked gel network (*23*, *24*). The $G''$ of myosin/$\beta$-LG heated to 71 °C was increased at high frequency, resulting in a smaller difference between $G'$ and $G''$.

As the pH was decreased from 7.0 to 6.0, the gel points of both myosin and myosin/HDLG decreased (**Figure 4**). The gel point of 1% myosin decreased from 49 °C at pH 7.0 to 44 °C

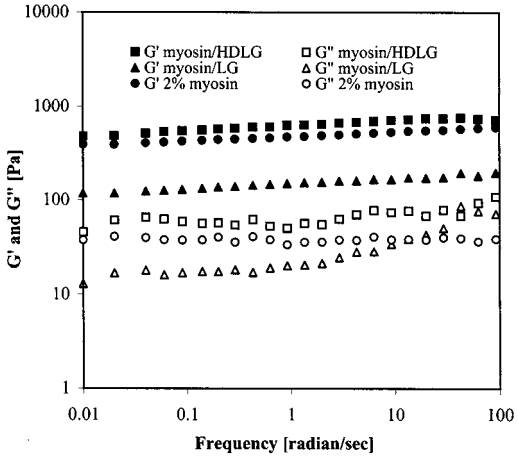


**Figure 3.** Frequency dependence of storage ($G'$) and loss ($G''$) moduli of 2% (w/v) myosin, 1% (w/v) myosin/1% (w/v) $\beta$-LG, and 1% (w/v) myosin/1% (w/v) heat-denatured $\beta$-LG (HDLG) gels in 0.6 M NaCl and 0.05 M sodium phosphate buffer, pH 7.0, at 25 °C. Gels were prepared by heating at 1 °C/min from 25 to 71 °C, holding at 71 °C for 60 min, and cooling to 25 °C.

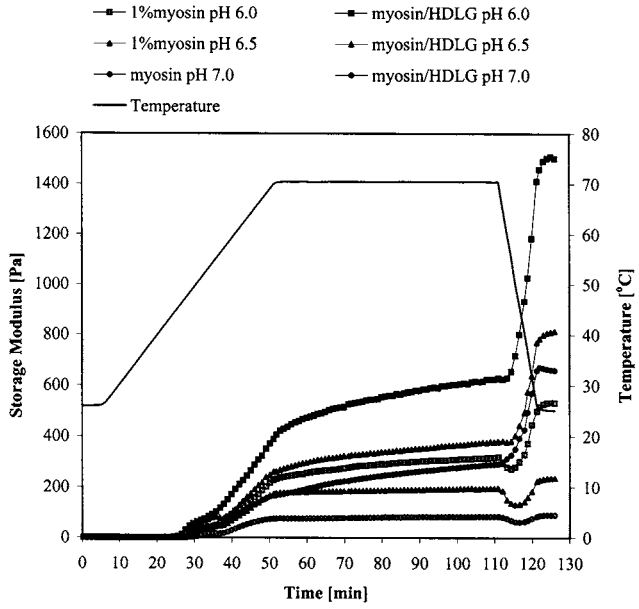


**Figure 4.** Storage modulus of 1% (w/v) myosin and 1% (w/v) myosin/1% (w/v) heat-denatured $\beta$-LG (HDLG) in 0.6 M NaCl and 0.05 M sodium phosphate buffer, pH 6.0, 6.5, and 7.0, during heating at 1 °C/min from 25 to 71 °C, holding for 60 min, and cooling to 25 °C.

at pH 6.0 (**Table 2**). Similarly, the gel point of myosin/HDLG decreased from 48 °C at pH 7.0 to 43.5 °C at pH 6.0. Because the gel points of 1% myosin and myosin/HDLG were similar at the same pH, it can be concluded that pH mainly affected the gel point of myosin in the mixed protein system. Wang et al. (*25*) reported that chicken salt-soluble protein reached the first $G'$ transition at a lower temperature at pH 5.5 than at pH 6.5 and 7.5.

The $G'$ after cooling increased as the pH was decreased for both myosin and myosin/HDLG gels. The $G'$ after cooling of 2% myosin at pH 6.0 (2350 Pa) was 4.7 times greater when compared to that at pH 7.0 (500 Pa). Maximum gel rigidity of myosin from chicken breast was found at pH 5.4−5.9 in 0.6 M KCl (*25*, *26*). The $G'$ after cooling of myosin/HDLG gels at pH 6.0 was ~2.3 times greater than that of the gel at pH 7.0. Interestingly, the $G'$ after cooling of myosin/HDLG (2% total protein) was greater than that of 2% myosin at pH 7.0 but less than that of 2% myosin at pH 6.0. Because disulfide bond formation/exchange reactions decrease with pH (*20*), this finding suggests that disulfide bonds may contribute to myosin/HDLG network formation.

**Table 2.** Gel Point, Storage Moduli, and Tangent Delta (tan $\delta$) after Cooling of Myosin and 1% Myosin/1% Heat-Denatured $\beta$-Lactoglobulin (Myosin/HDLG) Gels Heated at 71 °C for 60 min in 0.6 M NaCl, 0.05 M Sodium Phosphate Buffer, pH 6.0, 6.5, and 7.0[a]

| property | pH | 1% myosin | 2% myosin | myosin/HDLG |
|---|---|---|---|---|
| gel point (°C) | 6.0 | 44.2 ± 0.17 c | 40.8 ± 0.17 c | 43.5 ± 0.06 c |
| | 6.5 | 47.2 ± 0.17 b | 46.3 ± 0.17 b | 46.8 ± 0.17 b |
| | 7.0 | 49.0 ± 0.17 a | 47.8 ± 0.06 a | 48.0 ± 0.06 a |
| storage moduli (Pa) | 6.0 | 510 ± 13.3 a | 2350 ± 38.6 a | 1482 ± 17.3 a |
| | 6.5 | 235 ± 2.3 b | 580 ± 17.3b | 781 ± 16.7 b |
| | 7.0 | 120 ± 3.5 c | 500 ± 21.4 b | 650 ± 9.2 c |
| tan $\delta$ | 6.0 | 0.08 ± 0.006 b | 0.08 ± 0.006 | 0.09 ± 0.006 |
| | 6.5 | 0.1 ± 0.011 ab | 0.07 ± 0.006 | 0.09 ± 0.006 |
| | 7.0 | 0.12 ± 0.006 a | 0.08 ± 0.006 | 0.08 ± 0.006 |

[a] Values are means of three observations ± standard error of the mean. Means in each column within each property followed by different letters are significantly different ($p < 0.05$).

**Gel Microstructure.** When heated at 71 °C, 2% myosin formed a fine-stranded protein network composed of small globular aggregates at pH 6.5 and 7.0 (**Figure 5**). The myosin network at pH 6.0 contained thicker, smoother strands than those formed at a higher pH. A similar structure has been previously reported in rabbit myosin gels formed in 0.6 M KCl (*28*) and in salt-soluble protein extracted from chicken skeletal muscle (*29*, *30*).

Myosin/$\beta$-LG gels heated to 71 °C at all three pH values contained coarser, thicker protein strands with larger voids (**Figure 6**) when compared to 2% myosin gels at the same pH. The amount of myosin in myosin/$\beta$-LG gels was half of that in 2% myosin gels. Moreover, soluble $\beta$-LG was washed out during fixing as indicated by the dark color of the fixing solution, resulting from interactions between protein and osmium tetraoxide. Thus, the large void spaces observed in myosin/$\beta$-LG gels could be caused by the lower functional protein concentration because $\beta$-LG did not gel and participate in network formation at 71 °C.

Myosin/HDLG gels had a finer protein network and smaller voids when compared to myosin/$\beta$-LG gels at all pH values. The protein networks in myosin/HDLG gels were denser and had higher $G'$ values after cooling than myosin/$\beta$-LG gel networks, indicating that more proteins were incorporated within the gel networks.

The microstructures of myosin/HDLG gel networks contained a homogeneous granular structure at pH 6.5 and 7.0, similar to that of 2% myosin gels at the same pH (**Figures 5** and **6**). At pH 6.0, myosin/HDLG gels had larger voids and a more uneven network when compared to 2% myosin gels, indicating that interactions of myosin and HDLG at pH 6.0 resulted in a more disordered network structure and lower $G'$. There were no distinct phase separated regions in the gel structure, and the matrix of myosin/HDLG gels was as dense as that of 2% myosin gels, suggesting a coupled network in which both proteins participated in network formation (*31*).

**Soluble Proteins Expressed from Gels.** Myosin LC-1 (16.5 kDa) and LC-3 (22.0 kDa) were detected in supernatant of myosin gels heated to 71 °C (**Figure 7**, lane 9), whereas LC-2 (18.6 kDa) was also detected when myosin gels were heated to 90 °C (lane 5) as expected (*32*). All three light chains were

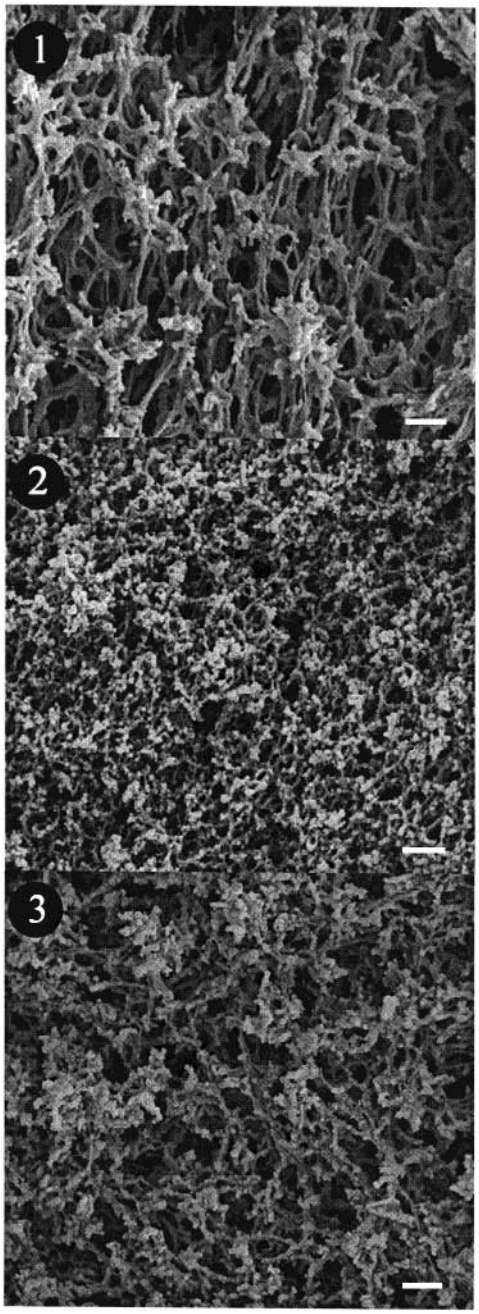


**Figure 5.** Scanning electron microscopic images of 2% (w/v) myosin gels heated to 71 °C in 0.6 M NaCl and 0.05 M sodium phosphate buffer: (1) pH 6.0; (2) 6.5; (3) 7.0 at 5000× magnification. Bar = 1 $\mu$m.

present in unheated myosin (lane 2). Myosin light chains have been identified in supernatant expressed from mixed protein gels of myosin and 11S soy protein (*33*), chicken breast muscle salt-soluble protein and whey protein or soy protein isolate (*34*), and chicken breast saltt-soluble protein and $\beta$-LG (*35*). Myosin heavy chain was not detected in the supernatants of myosin and mixed protein gels, suggesting that it was a principal contributor to network formation.

Because the molecular masses of LC-2 (18.6 kDa) and $\beta$-LG (18.4 kDa) were similar, it was difficult to determine which protein was responsible for the 18 kDa band in the mixed gels; however, some generalizations can be made on the basis of the relative amount of material at 18 kDa in myosin versus mixed gels. $\beta$-LG remained soluble in myosin/$\beta$-LG gels heated to 71 °C as indicated by the heavy band at 18−20 kDa (lane 8). The band at 18 kDa was only slightly darker in the myosin/$\beta$-LG

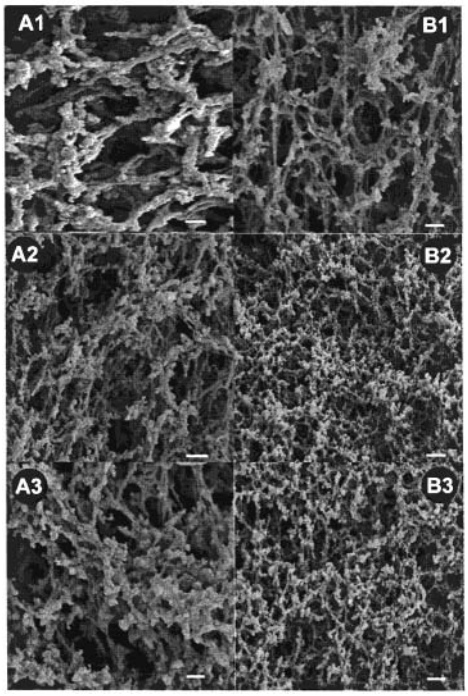


**Figure 6.** Scanning electron microscopic images of (A) 1% (w/v) myosin/1% (w/v) $\beta$-LG and (B) 1% (w/v) myosin/1% (w/v) heat-denatured $\beta$-LG gels heated to 71 °C in 0.6 M NaCl and 0.05 M sodium phosphate buffer: (1) pH 6.0; (2) 6.5; (3) 7.0 at 5000× magnification. Bar = 1 $\mu$m.

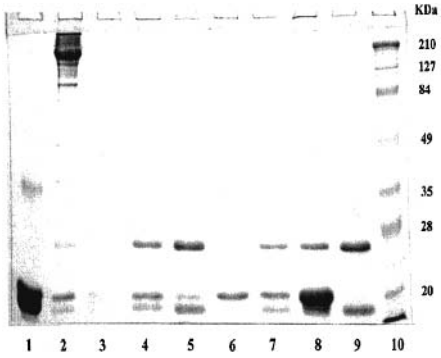


**Figure 7.** Representative electrophoretogram of proteins expressed by centrifugation at 10000*g* from 2% (w/v) myosin, 4% (w/v) $\beta$-LG, 2% (w/v) heat-denatured $\beta$-LG (HDLG), 1% (w/v) myosin/1% (w/v) $\beta$-LG, and 1% (w/v) myosin/1% (w/v) HDLG gels heated to 71 and 90 °C. Each lane was loaded with 10 $\mu$L of sample: lane 1, unheated $\beta$-LG; lane 2, unheated myosin; lane 3, $\beta$-LG heated to 90 °C; lane 4, myosin/$\beta$-LG gels heated to 90 °C; lane 5, myosin gels heated to 90 °C; lane 6, HDLG heated to 71 °C; lane 7, myosin/HDLG heated to 71 °C; lane 8, myosin/$\beta$-LG heated to 71 °C; lane 9, myosin heated to 71 °C; lane 10, broad range standard molecular weight markers.

gels heated to 90 °C (lane 4) than in the myosin gel heated to 90 °C, indicating that most of the $\beta$-LG was incorporated into the network of the mixed protein gel. The 18 kDa band was slightly darker in myosin/HDLG gels heated to 71 °C (lane 7) than in myosin alone (lane 9), again suggesting only a small amount of $\beta$-LG was found in the supernatant. Similar results were found in HDLG heated to 71 °C (lane 6), indicating that a small amount of $\beta$-LG monomers did not participate in network formation. Interactions between HDLG and myosin contributed to a denser protein network microstructure and a greater $G'$ of myosin/HDLG gels heated to 71 °C, when compared to myosin/$\beta$-LG gels heated to 71 and 90 °C.

**Conclusions.** To begin to understand how whey protein polymers function in meat products, we used a model system to study the thermal denaturation, rheological properties, and microstructures of the gels of mixtures of heat-denatured polymers of β-LG and myosin, the major functional whey and meat proteins. When heated to 71 °C, HDLG interacted with myosin during heating to enhance gel formation. A better understanding of interactions between whey and meat proteins may allow for increased use of whey protein in meat products and in a variety of other applications in which protein gelation at low temperature is desirable.

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