# Heat-Induced Gelation of Chicken *Pectoralis Major* Myosin and *β*-Lactoglobulin

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The denaturation, aggregation, and rheological properties of chicken breast muscle myosin, *β*-lactoglobulin (*β*-LG), and mixed myosin/*β*-LG solutions were studied in 0.6 M NaCl, 0.05 mM sodium phosphate buffer, pH 7.0, during heating. The endotherm of a mixture of myosin and *β*-LG was identical to that expected if the endotherm of each protein was overlaid on the same axis. The maximum aggregation rate ($AR_{max}$) increased, and the temperature at the $AR_{max}$ ($T_{max}$) and initial aggregation temperature ($T_o$) decreased as the concentration of both proteins was increased. The aggregation profile of <0.5% myosin was altered by the presence of 0.25% *β*-LG. Addition of 0.5−3.0% *β*-LG decreased storage moduli of 1% myosin between 55 and 75 °C, but increased storage moduli ($G'$) when heated to 90 °C and after cooling. *β*-LG had no effect on the gel point of ≥1.0% myosin, but enhanced gel strength when heated to 90 °C and after cooling. After cooling, the $G'$ of 1% myosin/2%*β*-LG gels was about 1.7 times greater than that of gels prepared from 2% myosin/1% *β*-LG.

**Keywords:** *β-lactoglobulin; myosin; gelation; aggregation; denaturation*

## INTRODUCTION

Salt-soluble muscle proteins form heat-induced gels that contribute to the texture, water holding ability, binding ability, and appearance of meat products (*1*). To improve yields, reduce costs, or improve quality of meat products, meat processors often use nonmeat proteins to substitute for muscle proteins. Whey proteins possess high nutritional and functional properties and are used in meat products as binders to enhance yield and textural quality. However, results from the use of whey proteins in meat products are highly variable because of differences in the source and processing history of whey protein concentrates or isolates (*2*). These differences often affect whey protein composition and functional properties. Differences in processing conditions and formulations used to prepare different meat products also affect the functional properties of whey proteins because of changes in pH, salt concentration, heating rate, and endpoint temperature (*3*).

The functions of whey proteins in meat systems have not been clearly explained on the basis of the multicomponent nature of these proteins. Many studies have looked at mixed gel systems of salt-soluble protein and whey protein concentrate (*2*−*5* ). As myosin and *β*-lactoglobulin (*β*-LG) are the major functional proteins of meat and whey products, respectively, evaluation of these proteins in a model gel system might help further elucidate the interactions that occur when these proteins are used together. Protein gelation is a multistep process involving denaturation, aggregation, and network formation. The objective of this paper was to evaluate the formation of myosin and *β*-LG co-gels in a high salt environment by (1) observing the thermal stability of myosin and *β*-LG during heating of mixed systems, (2) investigating the influence of *β*-LG on aggregation of myosin during heat-induced gelation, and (3) monitoring rheological properties of myosin and *β*-LG in mixed protein gel systems.

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## MATERIALS AND METHODS

**Protein Preparation.** Myosin from pre-rigor breast muscle (*Pectoralis Major*) was extracted immediately after sacrifice from four 8-week-old commercial type broilers (*6*). Myosin was pooled and stored in 48% saturation ammonium sulfate with 30% glycerol (v/w) at −20 °C. Prior to use, myosin was suspended in 0.6 M NaCl, 0.05 M sodium phosphate buffer, pH 7.0 (PBS), dialyzed against three changes of the same buffer for 48 h, and centrifuged at 78 000*g* to separate denatured protein. Bovine milk *β*-LG (L0130, lot #114H0755), containing variants A and B, was purchased from Sigma (St. Louis, MO). The purity of both myosin and *β*-LG were assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis using 4% and 12% acrylamide for stacking and resolving gels, respectively (*7*). Protein concentration of stock solutions was determined by absorption using extinction coefficients ($E^{1\%}$) of 5.5 at 280 nm for myosin (*8*) and 9.55 at 278 nm for *β*-LG (*9*).

Mixed protein solutions were prepared by mixing an equal volume of each protein stock containing twice the desired concentration of each protein in PBS. The concentrations of myosin, *β*-LG, and mixed solutions used in denaturation, aggregation, and dynamic oscillatory experiments are presented in Table 1.

**Differential Scanning Calorimetry.** The thermal denaturation of myosin, *β*-LG, and myosin/*β*-LG in PBS, pH 7.0, was investigated using an MC-2 differential scanning calorimeter (DSC) (Microcal, Amherst, MA). The protein and blank solutions (PBS) were degassed in a vacuum chamber before loading 1.24 mL into the DSC. Experiments were conducted at a scan rate of 1 °C/min from 25 °C to 90 °C. Calorimetric enthalpy ($\Delta H_{cal}$) and endothermic peak or melting temperature ($T_m$) were determined from heat capacity profiles using the

10.1021/jf000774z CCC: $20.00 
Published on Web 02/22/2001

**Table 1. Concentrations of Myosin and β-Lactoglobulin (β-LG) in 0.6 M NaCl, 0.05 M Sodium Phosphate Buffer, pH 7.0, Used in Thermal Denaturation (DSC), Thermal Aggregation, and Dynamic Oscillatory Experiments**

| | protein concentration (%) | | | | |
|---|---|---|---|---|---|
| protein type | DSC experiment | thermal aggregation experiment | | dynamic oscillatory experiment | |
| myosin | 1.0 | 0.1, 0.25, 0.5, 1.0, 1.5 | | 0.5, 1.0, 1.5, 2.0 | |
| β-LG | 1.0 | 0.1, 0.25, 0.5, 1.0, 1.5 | | 1.0, 2.0, 3.0 | |
| myosin/β-LG | 1.0:1.0 | 0.1:0.1 | 0.1:0.25 | 1.0:0.5 | 0.5:1.0 |
| | | 0.1:0.25 | 0.25:0.25 | 1.0:1.0 | 1.0:1.0 |
| | | 0.1:0.5 | 0.5:0.25 | 1.0:2.0 | 1.5:1.0 |
| | | 0.1:1.0 | 1.0:0.25 | 1.0:3.0 | 2.0:1.0 |

DA-2 Data Acquisition and Analysis software provided by the manufacturer.

**Thermal Aggregation of Protein.** Thermal aggregation of proteins was followed by turbidity measurement at 340 nm using a Lamda 20 UV−visible spectrophotometer connected to a PTP-6 peltier temperature programmer (Perkin-Elmer, Norwalk, CT). Protein solutions of 1.4 mL were placed in quartz cuvettes (1-cm path length) and sealed with tape to prevent evaporation. A blank containing the same buffer was used as the control. Solutions were equilibrated at 25 °C for 5 min, then heated to 90 °C at 1 °C/min, held at 90 °C for 30 min, and cooled to 25 °C within 15 min, followed by a 5 min holding period at 25 °C. The turbidity of the protein solutions was recorded every 0.5 min. A plot of aggregation rate (Δabsorbance/Δtime) against temperature was constructed for determination of the initial aggregation temperature ($T_o$), maximum aggregation rate ($AR_{max}$), and temperature at $AR_{max}$ ($T_{max}$). The $T_o$ was defined as the temperature at which the aggregation rate was $\geq 0.01$ min$^{-1}$.

**Small Amplitude Dynamic Oscillatory Testing.** Dynamic oscillatory tests were performed using a controlled stress rheometer (RS 100, Haake, Karlsruhe, Germany) equipped with a 35-mm-diameter stainless steel parallel plate. Storage ($G'$) and loss ($G''$) moduli were recorded continuously at 0.464 Hz using constant stresses (producing strains from 0.1 to 0.3%) within the range of linear viscoelastic behavior. Stress sweeps were performed to determine the range of linear viscoelastic behavior for each protein solution at 90 °C and after cooling to 25 °C. Protein solutions were loaded between the plate and base with a gap between 1.0 and 1.2 mm. A few drops of corn oil (Mazola, CPC International, Englewood Cliffs, NJ) were used to cover the edge of the gap to prevent evaporation. Solutions were heated using the same temperature profile used in aggregation experiments. Temperature was controlled by a circulating water bath attached to the rheometer. The gel point of protein solutions was defined as the temperature at which $G'$ and $G''$ crossed over in the fixed frequency test (*10*). Frequency sweeps (0.01−100 rad/s) were performed after cooling of the solutions, using constant stress resulting in a strain of 0.1−0.3%. Protein solutions used in sweep tests were 2% myosin, 1% myosin/1% β-LG, and 4% β-LG.

**Experimental Design and Statistical Analysis.** Three replicates of each protein solution were prepared and analyzed. Differences in aggregation and rheological properties as affected by protein type or concentration were analyzed using one-way analysis of variance. Means were compared using the Tukey-Kramer HSD test with the mean square error at 5% probability (JMP Software, Version 3.2.2, SAS Institute Inc., Cary, NC).

## RESULTS AND DISCUSSION

**Thermal Denaturation of Myosin and β-Lactoglobulin.** The enthalpy profile of 1% chicken breast myosin in PBS, pH 7.0, contained three endothermic peaks ($T_m$) at 48.5, 53.2, and 57.0 °C (Figure 1A) and was similar to those reported by other researchers (*11*, *12*). The endotherm of 1% β-LG in PBS, pH 7.0, showed a broad peak with a $T_m$ at 73.6 °C. This result was close to the $T_m$ of 73.4 °C reported by Foegeding et al. (*9*) using 10% β-LG in 0.1 M NaCl, 0.05 M 2-((tris-(hydroxymethyl)methyl)-amino) ethanesulfonic acid buffer, pH 7.0. A rapid decrease in the heat capacity of β-LG was observed at 76.0 °C. The intensity of this peak increased as the concentration of β-LG was decreased from 2% to 0.5% (data not shown). Moreover, no such phenomenon occurred when 1% β-LG in distilled water was heated at 1 °C/min (data not shown). The sudden decrease in heat capacity was previously reported in DSC studies of myosin at concentrations ≤0.523% (*12*) and was attributed to the aggregation and precipitation of the unfolded proteins at low concentration. A DSC study using 0.4% β-LG in 0.07 M phosphate buffer, pH 6.75, showed only a single peak (*13*). Therefore, the rapid decrease in heat capacity could be a result of the aggregation and precipitation of low concentrations of β-LG in PBS at high ionic strength (0.6 M NaCl). In preliminary experiments, this rapid decrease in heat capacity was not observed at higher protein concentrations. This effect has been previously reported to be concentration dependent (*6*, *12*).

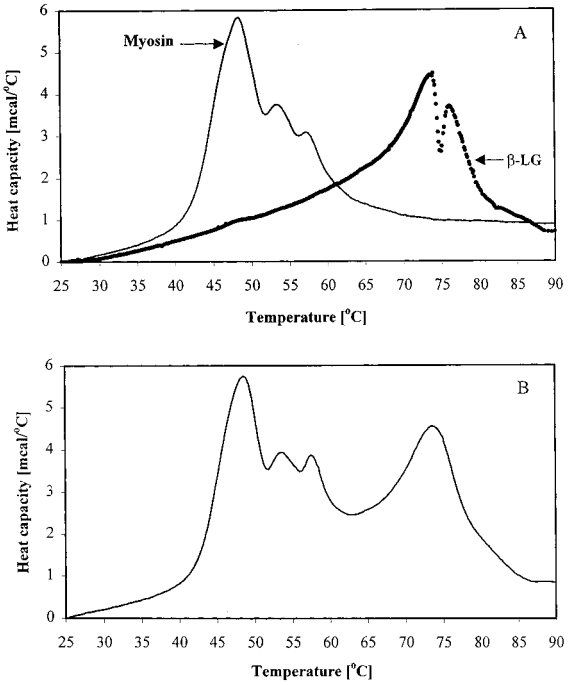


**Figure 1.** Heat capacity profiles of (A) 1% myosin and 1% β-lactoglobulin (β-LG) analyzed separately, and (B) a mixture of 1% myosin/1% β-LG mixture in 0.6 M NaCl, 0.05 M sodium phosphate buffer, pH 7.0.

The endotherm of myosin and β-LG mixtures was identical to that expected if the endotherm of each protein was overlaid on the same axis (Figure 1B), suggesting that each protein denatured independently and did not interfere with the denaturation of the other. The rapid decrease in heat capacity was not observed in mixed solutions of myosin and β-LG as total protein

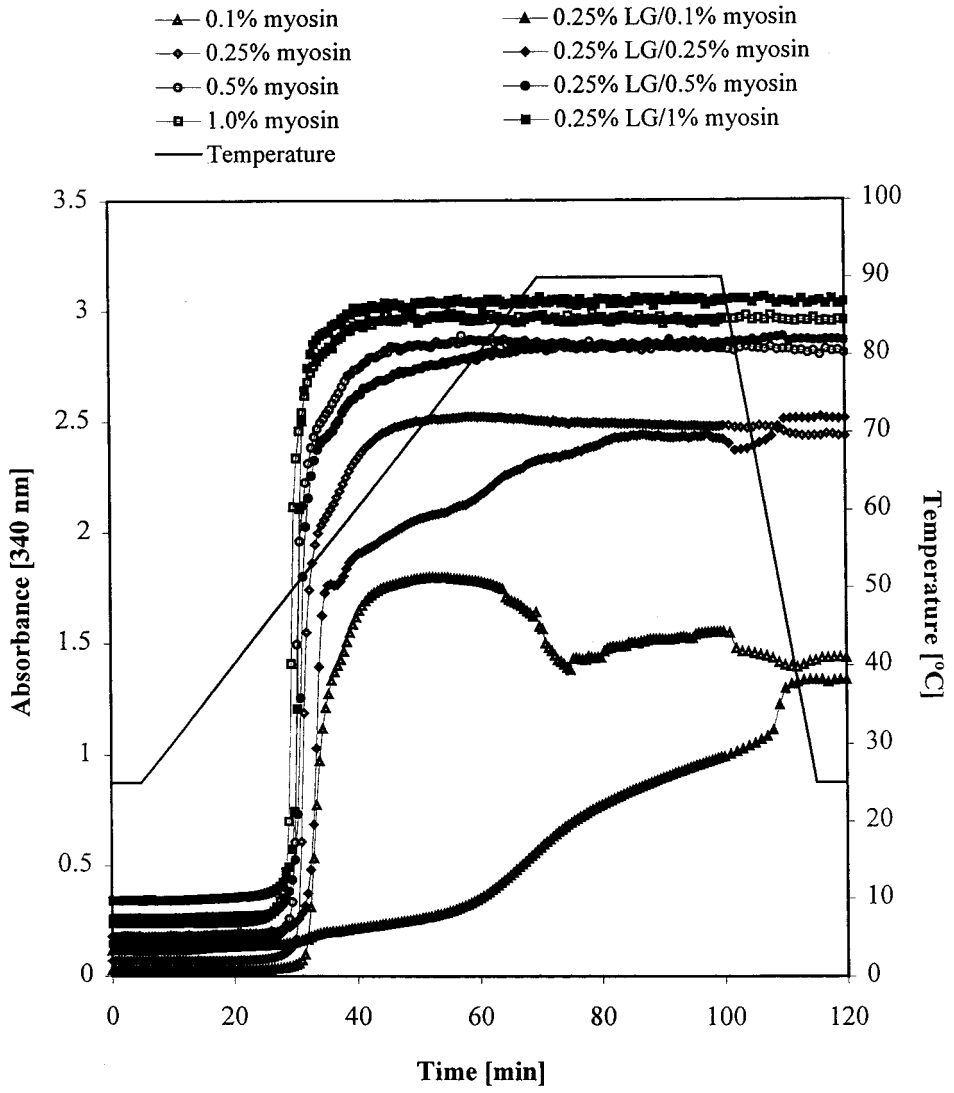


**Figure 2.** Aggregation of 0.1−1.0% myosin and mixed solutions of myosin with 0.25% β-lactoglobulin (β-LG) in 0.6 M NaCl, 0.05 M sodium phosphate buffer, pH 7.0, during heating at 1 °C/min from 25 to 90 °C, holding at 90 °C for 30 min, and cooling to 25 °C.

**Table 2. Initial Aggregation Temperature ($T_o$), Temperature at the Maximum Aggregation Rate ($T_{max}$), and Maximum Aggregation Rate ($AR_{max}$) of Myosin and Mixed Solutions of 0.25% β-Lactoglobulin (β-LG) and 0.1−1.0% Myosin in 0.6 M NaCl, 0.05 M Sodium Phosphate Buffer, pH 7.0, during Heating from 25 to 90°C, Holding at 90°C for 30 min, and Cooling to 25°C**

| protein type | myosin concentration (%) | $T_o$ (°C) | $T_{max}$ (°C) | $AR_{max}$ (min$^{-1}$) |
|---|---|---|---|---|
| myosin | 0.1 | $50.6 \pm 0.2^b$ | $53.2 \pm 0.3^b$ | $0.44 \pm 0.051^b$ |
| | 0.25 | $49.2 \pm 0.4^c$ | $51.7 \pm 0.7^c$ | $1.07 \pm 0.077^d$ |
| | 0.5 | $46.3 \pm 0.2^d$ | $50.1 \pm 0.2^{cd}$ | $1.36 \pm 0.150^e$ |
| | 1.0 | $45.8 \pm 0.7^d$ | $49.9 \pm 0.6^d$ | $1.61 \pm 0.136^f$ |
| | 1.5 | $45.3 \pm 0.2^d$ | $49.3 \pm 0.2^d$ | $1.60 \pm 0.167^f$ |
| 0.25% β-LG/myosin | 0.1 | $77.5 \pm 0.5^a$ | $90^{1a}$ | $0.03 \pm 0.002^a$ |
| | 0.25 | $49.2 \pm 0.7^c$ | $54.2 \pm 0.3^b$ | $0.71 \pm 0.180^c$ |
| | 0.5 | $46.3 \pm 0.3^d$ | $52.3 \pm 0.5^{bc}$ | $1.35 \pm 0.180^e$ |
| | 1.0 | $46.1 \pm 0.2^d$ | $50.9 \pm 0.5^{cd}$ | $1.79 \pm 0.250^f$ |

[1] $T_{max}$ occurred during holding at 90 °C. [a−f]Values are means ± standard deviations of three observations. Means with different superscripts in each column are significantly different ($p < 0.05$).

concentration was greater. Wang and Smith (*14*) reported that interactions between myosin and actin led to changes in the denaturation profile of actomyosin when compared to pure myosin and actin or when the proteins were dissociated with phosphate.

**Thermal Aggregation.** Aggregation patterns of myosin (Figure 2) had a sigmoidal shape similar to those reported by previous researchers (*11*, *12*). The turbidity increased rapidly to about 67 °C regardless of concentration, then remained constant or decreased slightly, suggesting that myosin aggregation was complete.

The initial aggregation temperature of myosin ($T_o$) decreased ($p < 0.05$) from 50.6 to 46.3 °C as the protein concentration was increased from 0.1 to 0.5% (Table 2). The maximum aggregation rate ($AR_{max}$) increased and the temperature at maximum aggregation rate ($T_m$)

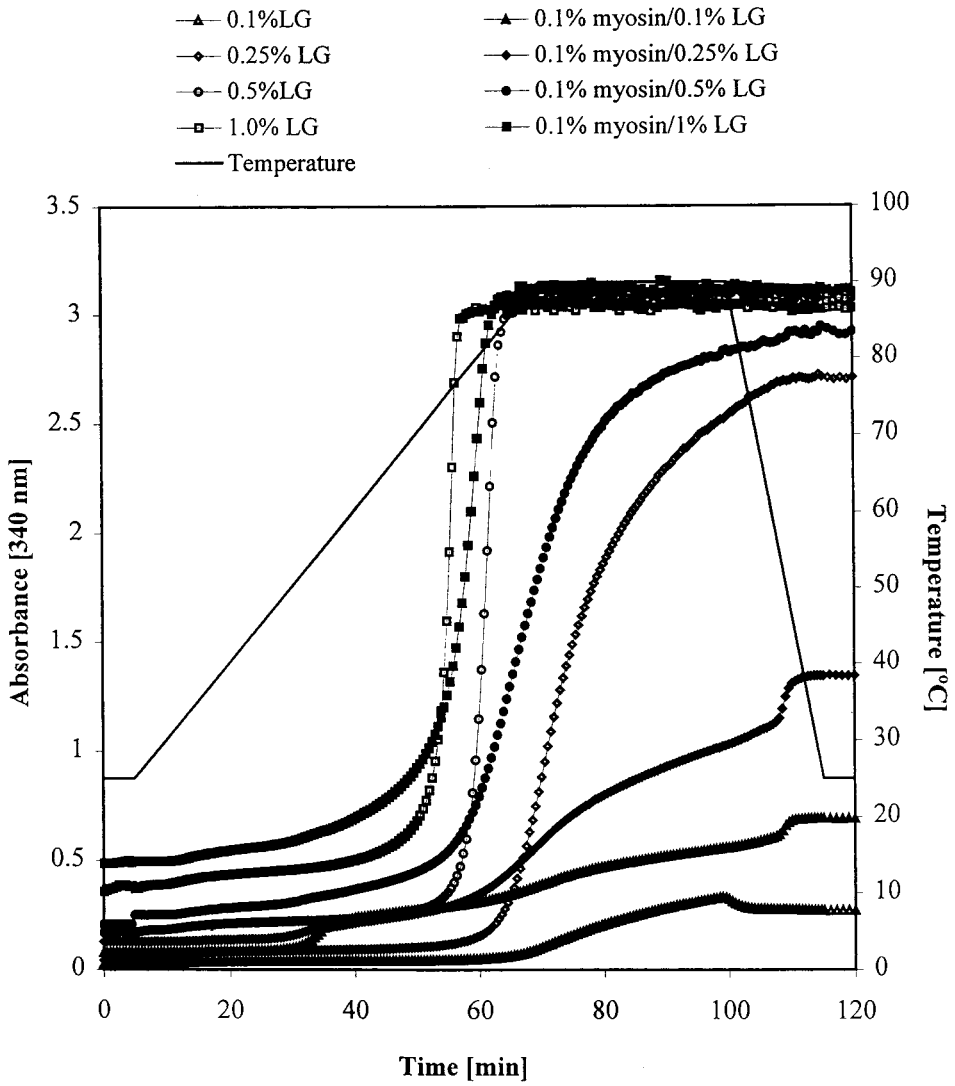


**Figure 3.** Aggregation of 0.1−1.0% $\beta$-lactoglobulin ($\beta$-LG) and mixed solutions of $\beta$-LG with 0.1% myosin in 0.6 M NaCl, 0.05 M sodium phosphate buffer, pH 7.0, during heating at 1 °C/min from 25 to 90 °C, holding at 90 °C for 30 min, and cooling to 25 °C.

**Table 3. Initial Aggregation Temperature ($T_o$), Temperature at Maximum Aggregation Rate ($T_{max}$), and Maximum Aggregation Rate ($AR_{max}$) of $\beta$-Lactoglobulin ($\beta$-LG) and Mixed Solutions of Myosin and 0.1−1.0% $\beta$-LG in 0.6 M NaCl, 0.05 M Sodium Phosphate Buffer, pH 7.0, during Heating from 25 to 90°C, Holding at 90°C for 30 min, and Cooling to 25°C**

| protein type | $\beta$-LG concentration (%) | $T_o$ (°C) | $T_{max}$ (°C) | $AR_{max}$ (min$^{-1}$) |
|---|---|---|---|---|
| $\beta$-LG | 0.1 | 84.5 ± 0.5$^a$ | 90[1]$^a$ | 0.02 ± 0.002$^a$ |
| | 0.25 | 79.3 ± 1.0$^b$ | 89.3 ± 0.7$^{ab}$ | 0.21 ± 0.099$^b$ |
| | 0.5 | 73.5 ± 0.5$^d$ | 84.0 ± 1.6$^b$ | 0.57 ± 0.046$^c$ |
| | 1.0 | 63.5 ± 0.5$^f$ | 78.3 ± 0.2$^c$ | 0.76 ± 0.025$^d$ |
| | 1.5 | 63.0 ± 0.5$^f$ | 77.3 ± 0.2$^c$ | 0.77 ± 0.058$^d$ |
| 0.1% myosin/$\beta$-LG | 0.1 | 85 ± 0.6$^a$ | 89.8 ± 1.0$^a$ | 0.02 ± 0.003$^a$ |
| | 0.25 | 77.5 ± 0.5$^c$ | 90[1]$^a$ | 0.03 ± 0.002$^a$ |
| | 0.5 | 70.0 ± 0.4$^e$ | 88 ± 0.2$^{ab}$ | 0.11 ± 0.006$^{ab}$ |
| | 1.0 | 63.2 ± 0.8$^f$ | 79.5 ± 0.1$^{bc}$ | 0.38 ± 0.002$^{bc}$ |

[1] $T_{max}$ occurred during holding at 90 °C. $^{a-f}$Values are means ± standard deviations of three observations. Means with different superscripts in each column are significantly different ($p < 0.05$).

decreased with increasing concentrations of myosin from 0.1 to 0.5%. Above 0.5%, concentration had no ($p > 0.05$) effect on either $AR_{max}$, $T_m$, or $T_o$ of myosin. The $AR_{max}$ of 0.1% myosin was 0.44 min$^{-1}$, much lower than 1.96 min$^{-1}$ as reported by Liu et al. (*12*) using 0.07% chicken *Pectoralis* myosin and heating isothermally at 55 °C in 0.6 M NaCl, pH 6.0. Differences in rates are probably due to differences in pH of the protein solutions and heating profiles. The aggregation pattern of 0.1−1.5% $\beta$-LG (Figure 3) was similar to that of myosin except that $\beta$-LG began to aggregate at a higher temperature and had a lower $AR_{max}$ than myosin. The $T_o$ of $\beta$-LG decreased from 84.5 °C at 0.1% protein to 63.5 °C at 1.0% protein (Table 3). Initial aggregation of 1% $\beta$-LG was detected 10 °C lower than its $T_m$ (73.6 °C). Prabakaran and Damodaran (*15*) reported that small $\beta$-LG aggregates were first formed via sulfhydryl−disulfide interchange reactions between two reactive monomers (molten globules) between 60 and 65 °C. The small aggregates further associated into larger aggregates in

**Table 4. Gel Point, Storage Moduli (*G′*), and Tangent Delta (tan *δ*) of Myosin and Mixed Protein Solutions of Myosin and *β*-Lactoglobulin (*β*-LG) in 0.6 M NaCl, 0.05 M Sodium Phosphate Buffer, pH 7.0, during Heating from 25 to 90°C, Holding at 90°C for 30 min, and after Cooling to 25°C**

| protein type | protein concentration (%) | gel point (°C) | *G′* at 53 °C (Pa) | *G′* at 73 °C (Pa) | *G′* after cooling (Pa) | tan *δ* after cooling |
|---|---|---|---|---|---|---|
| myosin | 0.5 | $50.3 \pm 0.01^{b}$ | $4.8 \pm 0.7^{b}$ | $29 \pm 5.6^{b}$ | $40.4 \pm 1.0^{a}$ | $0.16 \pm 0.03^{a}$ |
| | 1.0 | $49.3 \pm 0.40^{c}$ | $17.0 \pm 2.0^{c}$ | $83 \pm 6.3^{c}$ | $119 \pm 6.0^{ab}$ | $0.12 \pm 0.01^{ab}$ |
| | 1.5 | $48.5 \pm 0.15^{d}$ | $36.5 \pm 5.0^{d}$ | $147 \pm 20.0^{d}$ | $205 \pm 49.0^{bc}$ | $0.11 \pm 0.02^{ab}$ |
| | 2.0 | $47.8 \pm 0.10^{e}$ | $76.0 \pm 6.2^{e}$ | $306 \pm 4.0^{f}$ | $501 \pm 37.0^{e}$ | $0.08 \pm 0.02^{b}$ |
| 1% *β*-LG/myosin | 0.5 | $51.0 \pm 0.06^{a}$ | $2.5 \pm 0.2^{a}$ | $4.7 \pm 1.0^{a}$ | $341 \pm 21.0^{d}$ | $0.09 \pm 0.01^{ab}$ |
| | 1.0 | $49.3 \pm 0.15^{c}$ | $16.0 \pm 0.6^{c}$ | $32 \pm 1.0^{b}$ | $507 \pm 29.0^{e}$ | $0.10 \pm 0.01^{ab}$ |
| | 1.5 | $48.4 \pm 0.20^{d}$ | $42.0 \pm 2.5^{d}$ | $92 \pm 15.0^{c}$ | $730 \pm 33.0^{f}$ | $0.11 \pm 0.01^{ab}$ |
| | 2.0 | $47.7 \pm 0.20^{e}$ | $88.3 \pm 5.1^{f}$ | $208 \pm 31.0^{e}$ | $835 \pm 46.0^{g}$ | $0.10 \pm 0.01^{ab}$ |
| 1% myosin/*β*-LG | 0.5 | $49.2 \pm 0.40^{c}$ | $16.0 \pm 0.8^{c}$ | $49 \pm 2.4^{bc}$ | $235 \pm 12.0^{c}$ | $0.11 \pm 0.01^{ab}$ |
| | 1.0 | $49.3 \pm 0.15^{c}$ | $15.5 \pm 0.6^{c}$ | $32 \pm 1.0^{b}$ | $507 \pm 29.0$e | $0.10 \pm 0.01^{ab}$ |
| | 2.0 | $49.6 \pm 0.05^{c}$ | $15.0 \pm 3.0^{c}$ | $23 \pm 2.0^{ab}$ | $1447 \pm 76.0^{h}$ | $0.09 \pm 0.02^{ab}$ |
| | 3.0 | $49.7 \pm 0.30^{c}$ | $14.0 \pm 0.9^{c}$ | $17 \pm 1.3^{ab}$ | $3{,}367 \pm 51.0^{i}$ | $0.09 \pm 0.01^{ab}$ |

[a–i] Values are means of three determinations ± standard deviation. Means with different superscripts in each column are significantly different ($p < 0.05$).

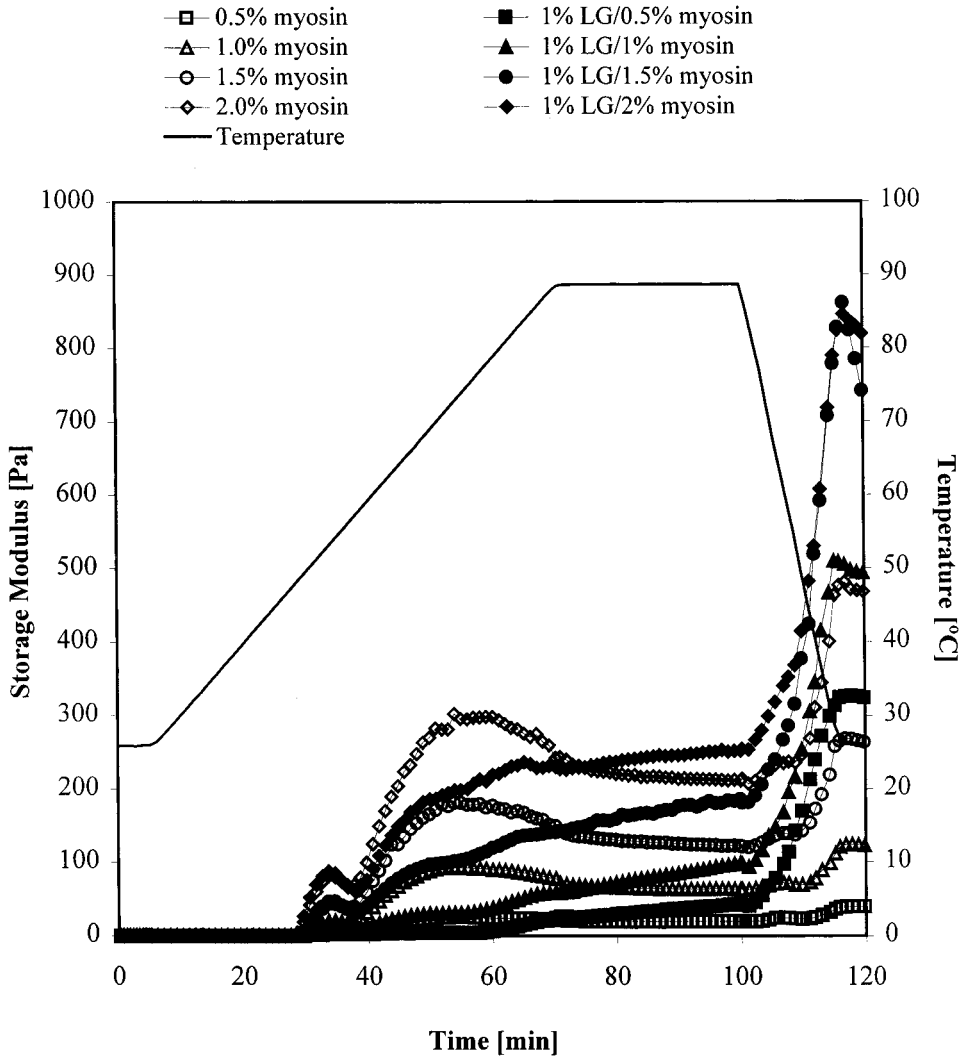


**Figure 4.** Storage moduli of myosin and mixed solutions of myosin and 1.0% *β*-lactoglobulin (*β*-LG) in 0.6 M NaCl, 0.05 M sodium phosphate buffer, pH 7.0, during heating at 1 °C/min from 25 to 90 °C, holding at 90 °C for 30 min, and cooling to 25 °C.

the propagation stage when the aggregation rate reached a maximum.

The $AR_{max}$ increased and $T_{max}$ decreased ($p < 0.05$) as the *β*-LG was increased from 0.1 to 1.0%. Many studies have shown that the rate of *β*-LG aggregation depends on the reactive *β*-LG monomer concentration (*16*−*18*). The aggregation of *β*-LG and myosin was not studied at concentrations higher than 1.5% because both proteins formed opaque gels with absorbance values greater than 5.

The effects of different concentrations of myosin mixed with 0.25% *β*-LG on protein aggregation is illustrated in Figure 2. Addition of 0.25% *β*-LG increased $T_o$ of 0.1% myosin from 50.6 to 77.5 °C and decreased $AR_{max}$ from 0.44 to 0.03 $min^{-1}$ (Table 2). When myosin concentration was increased above 0.25%, the $T_o$, $AR_{max}$,

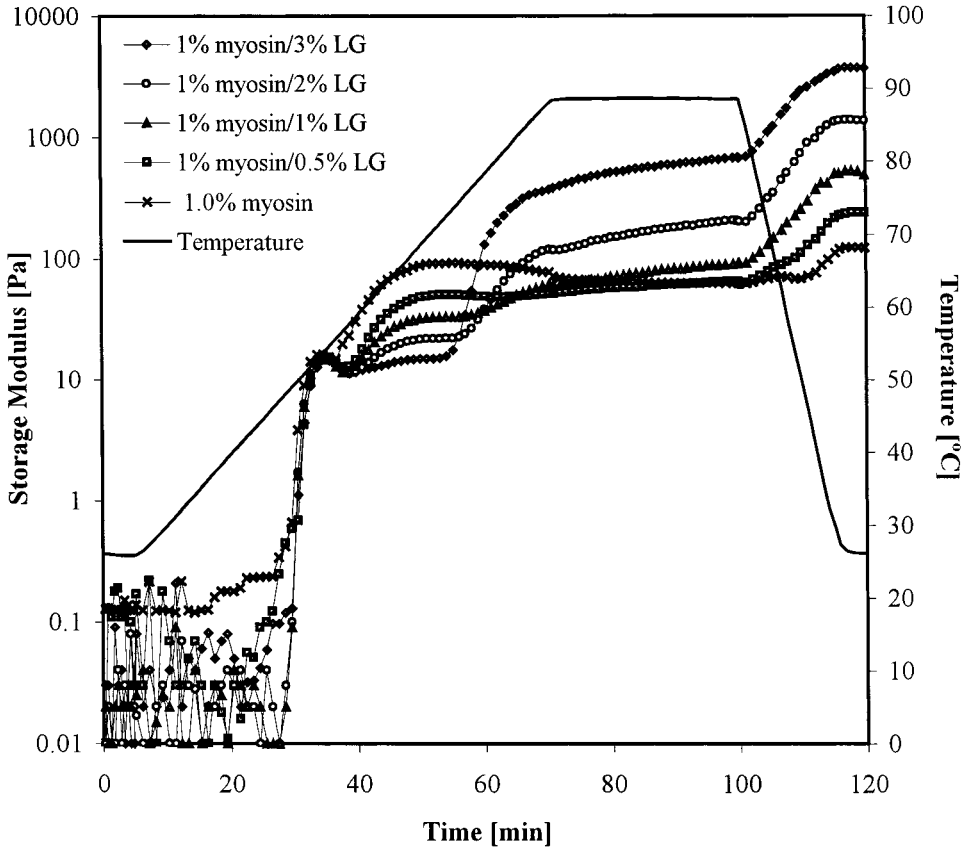


**Figure 5.** Storage moduli of myosin and mixed solutions of 1.0% myosin and 0.5−3% $\beta$-lactoglobulin ($\beta$-LG) in 0.6 M NaCl, 0.05 M sodium phosphate buffer, pH 7.0, during heating at 1 °C/min from 25 to 90 °C, holding at 90 °C for 30 min, and cooling to 25 °C.

and $T_{max}$ of the mixed systems containing 0.25% $\beta$-LG were not different ($p < 0.05$) from those of the same myosin concentration. The results indicated that 0.25% $\beta$-LG altered the aggregation pattern of myosin at concentrations below ≤0.25% myosin.

The aggregation patterns of mixed protein solutions containing 0.1% myosin and 0.1−1.0% $\beta$-LG were compared to those of 0.1−1.0% $\beta$-LG (Figure 3). Mixed solutions of 0.1% myosin and either 0.1 or 0.25% $\beta$-LG exhibited two aggregation steps. The first aggregation step at about 51 °C was attributed to myosin aggregation and occurred at 51 °C in the mixture containing 0.1% myosin and 0.1% $\beta$-LG. The second aggregation step at 85 °C in the same mixture was attributed to $\beta$-LG.

The results suggested that both proteins aggregated independently in mixed systems of low protein concentration. When $\beta$-LG concentration was increased above 0.25%, aggregation of the mixed protein solutions exhibited a pattern more typical of $\beta$-LG aggregation. However, $AR_{max}$ of all the mixed proteins were lower than that of $\beta$-LG at the same concentration. Results suggested that both proteins influenced the aggregation pattern of the other and the relative proportion of each protein determined the magnitude of the effects.

**Rheological Properties.** The gel point of myosin in PBS, pH 7.0, decreased ($p < 0.05$) from 50.3 to 47.8 °C as the concentration was increased from 0.5 to 2.0% (Table 4). The gel points of $\beta$-LG solutions in PBS, pH 7.0, decreased from 79 °C at 0.5% protein to 73 °C at 3% protein. The rheological properties of 1−4% $\beta$-LG could not be determined during holding at 90 °C or cooling because of shrinkage of the gel.

The rheograms of $G'$ during heating contained two peaks at myosin concentrations of 1% or greater (Figure 4). The first transition peak was observed at about 53 °C and a second peak occurred at about 73 °C. The $G'$, stiffness of gels at each transition peak, increased as the concentration of myosin was increased. Similar rheograms have been previously reported during the heat-induced gelation of chicken breast muscle myosin (*12*), rabbit *Psaos* major myosin (*19*), and chicken breast muscle salt-soluble proteins (*11*). Smyth et al. (*11*) suggested that light meromyosin (LMM) and S-1 of chicken breast myosin were responsible for aggregation below 55 °C, whereas S-2 unfolded and aggregated above 55 °C. Therefore, the first peak of $G'$ possibly reflected rigidity of the network formed by LMM and S-1; whereas the aggregation of S-2 led to the increase of $G'$ in the second peak.

The $G'$ of myosin decreased above 75 °C and during holding at 90 °C. In contrast, Liu et al. (*12*) reported that $G'$ of chicken breast myosin in 0.6 M NaCl, pH 6.0, increased during holding at 75 °C. Syneresis was observed during holding at 90 °C, suggesting extensive intramolecular interactions within the protein networks. The $G'$ of myosin at each concentration after cooling was 1.4−1.6 times greater than $G'$ at 73 °C, indicating that the formation of hydrogen bonds may have enhanced the final rigidity or stiffness of the gels.

In mixed protein solutions containing 0.5% myosin and 1% $\beta$-LG, the gel point increased and the $G'$ of the first transition peak decreased when compared to that of 0.5% myosin. When myosin was increased to 1.0% or above in the mixed systems, the addition of 1% $\beta$-LG had no effect on the gel point or the $G'$ of the first

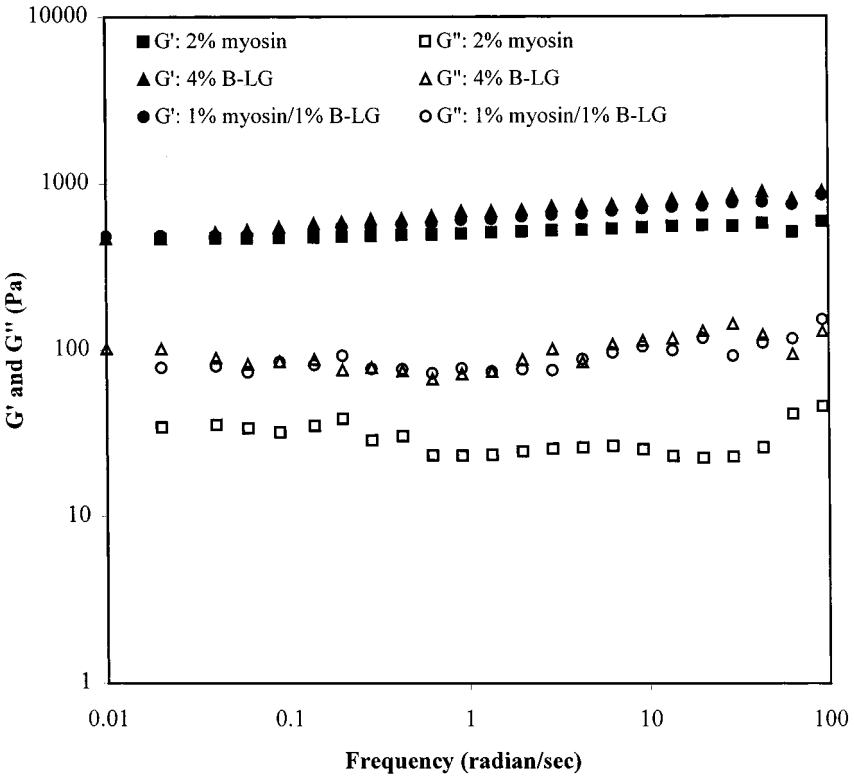


**Figure 6.** Frequency dependence of storage ($G'$) and loss ($G''$) moduli of 2% myosin, 4% β-lactoglobulin (β-LG), and 1% myosin/1% β-LG gels in 0.6 M NaCl, 0.05 M sodium phosphate buffer, pH 7.0, measured at 25 °C. Gels were prepared by heating at 1 °C/min from 25 to 90 °C, holding at 90 °C for 30 min and cooling to 25 °C.

transition of myosin. The $G'$ at 73 °C and after cooling of mixed solutions increased as myosin concentration was increased, but the $G'$ was still lower than that of myosin at the same concentration. The results suggested that in mixed systems containing ≥1% myosin, β-LG had no effect on the gel point but altered the $G'$ of myosin during heating between 53 and 73 °C. At 90 °C, the $G'$ of all mixed solutions were greater than those of myosin at the same concentration due to the gelation of β-LG. Hung and Smith (*4*) reported that addition of 4% whey protein concentrate increased the magnitude of the first $G'$ transition temperature, and increased the stiffness of 12% chicken salt-soluble protein in 0.6 M NaCl, pH 7.0, when heated to 90 °C.

A log scale was used in Figure 5 to compare gels made from 1% myosin with mixed gels containing 1% myosin and varying concentrations of β-LG. The $G'$ of mixed protein gels were lower than those of myosin until temperatures of about 78 to 90 °C were reached. The final $G'$ of co-gels from 1% myosin and 0.5%, 1%, 2%, and 3% β-LG were 2, 4, 12, and 28 times greater, respectively, than the $G'$ of 1% myosin. It was noticed that $G'$, after cooling of 1% myosin/2% β-LG gels, was 1447 Pa, about 1.7 times greater than that of the 2% myosin/1% β-LG co-gel. This suggested that the stiffness of co-gels might be controlled by combining appropriate proportions of myosin and β-LG. The increased stiffness in the mixed gels was the result of β-LG gelation at temperatures above 80 °C. The β-LG aggregates may have filled up the voids within the myosin gel matrix to form a filled gel structure, enhancing the stiffness of the co-gels.

Myosin at 1.0% protein or above and all myosin/β-LG gels exhibited viscoelastic gel characteristics as indicated by the low tan δ values at 25 °C (Table 4). Strong gels were formed from 2.0% myosin, 4% β-LG, and a mixture of 1% myosin/1% β-LG (Figure 6) as $G'$ and $G''$ were independent of frequency and $G'$ was 8−10 times greater than $G''$ (*20*). Myosin had a more elastic gel structure than myosin/β-LG gels as indicated by the larger difference between $G'$ and $G''$ (*21*).

In summary, myosin formed a gel network beginning at about 53 °C, whereas β-LG remained soluble until its gel point of 73−79 °C in 0.6 M NaCl, 0.05 M sodium phosphate buffer, pH 7.0. In systems containing both proteins, β-LG altered the aggregation and network formation profiles of myosin when heated between 53 and 73 °C. At higher temperatures, β-LG began to aggregate which enhanced the $G'$ of the mixed gels held at 90 °C. The additive effect expected from mixtures of β-LG and myosin solutions was not achieved until the protein mixture was heated to the gelling point of β-LG. These findings may help explain why whey proteins do not always improve the textural attributes of meat products that are usually processed to 71 °C or below.

## ACKNOWLEDGMENT

This paper is based in part on the Ph.D. dissertation of M. Vittayanont.

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Received for review June 27, 2000. Revised manuscript received January 2, 2001. Accepted January 10, 2001.

JF000774Z