Gelation of Chicken Breast Muscle Actomyosin As Influenced by Weight Ratio of Actin to Myosin

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Heat-induced gelation of reconstituted chicken breast muscle actomyosin was studied by monitoring the thermal stability and dynamic rheological properties at different weight ratios of actin (A) to myosin (M) (AM of 0:1, 1:15, and 1:1.3). Free myosin to actomyosin ratios were 0.2 and 2.4 for AM of 1:1.3 and 1:15, respectively. Addition of actin delayed the initial unfolding temperature of myosin and changed the enthalpy profile. This stabilizing effect was decreased with addition of pyrophosphate. Storage (G') and loss (G'') moduli of AM 1:1.3 sol at 30 °C were greater than those of myosin and AM 1:15 sols, but AM 1:1.3 had a higher loss tangent and lower G' at 80 °C. Pyrophosphate decreased G' of myosin and actomyosin solutions at 30 °C and increased viscous character after heating to 80 °C. Actin affected the denaturation of structural domains of myosin and possibly altered the gelation mechanism.

Keywords: *Actin to myosin ratio; chicken breast; actomyosin; DSC; dynamic testing*

INTRODUCTION

Myosin is an asymmetric molecule, consisting of two globular heads (S-1) attached to a long coiled coil rod. Asghar et al. (1985) suggested that the gelling potential of myosin was confined to the myosin rod, as S-1 exhibited poor gelling ability upon heating. Addition of actomyosin affected the gelation of myosin rod by increasing cross-link formation. Maximum gel strength in 0.6 M KCl, pH 6.0, was obtained at a myosin to F-actin mole ratio of 2.7:1, which corresponded to a weight ratio of 15:1. At this ratio, 15–20% of the total protein existed as an actomyosin complex and the remainder was free myosin (Yasui et al., 1980). Dudziak et al. (1988) reported that postrigor turkey breast myosin formed gels of greater rigidity than thigh myosin. They found that myosin to actomyosin weight ratios for breast and thigh were 3.8:1 and 6.9:1, respectively. Sano et al. (1989b) found that increases in F-actin to myosin ratio changed the rheogram of storage modulus of fish muscle actomyosin between 46 and 53 °C.

Inorganic pyrophosphate (PP_i) has been used as a nonhydrolyzable adenosine triphosphate (ATP) analog to investigate muscle contraction and the nucleotide binding site in myosin. During muscle contraction, myosin cross-bridges extending from the thick filament cyclically interact with the thin actin filaments as ATP is hydrolyzed (Huxley, 1969). Addition of PP_i was found to change both muscle fiber tension and fiber stiffness. These changes were due to cross-bridge detachment (Thomas and Cooke, 1980; Chen and Reisler, 1984; Brenner et al., 1986) or changes in cross-bridge structure upon binding (Goody et al., 1976; Padron and Huxley, 1984). It was also found that this ligand-induced dissociation of actin and myosin was enhanced by high ionic strength and low temperatures (Konrad and Goody, 1982; Biosca et al., 1986; Pate and Cooke, 1988). Pyrophosphate binds strongly to myosin with a binding constant of $2.07 \times 10^6\ M^{-1}$ and may cause local structural changes in S-1 (Nauss et al., 1969). Dissociation of actomyosin by addition of PP_i prior to heating caused a decrease in gel strength (Ishioroshi et al., 1980; O'Neill et al., 1993). Kijowski and Mast (1988) reported enhanced thermal stability of myosin in the presence of PP_i using differential scanning calorimetry (DSC).

In previous work, we reported that the dynamic rheological properties of chicken breast salt-soluble proteins, with a myosin to actin weight ratio of 1.3:1, were pH-dependent in 0.6 M NaCl during heating from 30 to 80 °C (Wang et al., 1990). The dynamic rheological properties of chicken breast myosin were different from salt-soluble proteins (Wang and Smith, 1994). The reasons for differences in the viscoelastic transitions of these two protein preparations during heating were not known. Therefore, the purpose of this paper was to understand the role of F-actin on myosin unfolding and gel development in both bound (actomyosin) and free forms. The specific objectives were to (a) determine the denaturation temperature, enthalpy changes, and dynamic rheological properties as a function of actin to myosin weight ratios during heating and (b) study the effect of free and bound F-actin (with and without PP_i) on both actomyosin unfolding and viscoelastic properties.

MATERIALS AND METHODS

Extraction of Myosin and Actin. Breast muscle (pectoralis major and pectoralis minor) myosin and actin were extracted immediately after sacrifice from 8-week-old commercial meat-type broilers as described by Nauss et al. (1969) and Spudich and Watt (1971), respectively, and stored in $(NH_4)_2\ SO_4$ at −20 °C. Prior to use, myosin was dialyzed against 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, with two buffer changes and centrifuged at 78000g for 1 h (Beckman ultracentrifuge, Model L7-65, Beckman Instruments, Inc., Palo Alto, CA). Actin was polymerized by adding KCl to a final concentration of 50 mM, $MgCl_2$ to 1 mM, and ATP to 1 mM with slow stirring for 2 h and dialyzed against 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, overnight. Protein concentration was determined using an extinction coefficient of $E^{1\%} = 5.5$ at 280 nm for myosin (Swenson and Ritchie, 1980) and 11 for actin (Duong and Reisler, 1987).

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Characterization of Actin to Myosin Weight Ratio. Purified actin and myosin were mixed to prepare solutions of different actin (A) to myosin (M) weight ratios (0:1, 1:15, and 1:1.3). The volume ($volume_I$) and concentration ($[myosin]_I$) of myosin added to each actomyosin solution were recorded for later estimation of free to bound myosin ratio. Free myosin, actomyosin, and F-actin in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, in each solution were quantified by centrifuging (Beckman ultracentrifuge, Model TL-100) at 100000*g* for 2 h (Yasui et al., 1982). Protein absorbance in the supernatant ($abs_{supernatant}$) was measured at 280 nm after centrifugation and subtracted from the absorbance of unpolymerized G-actin. The volume of supernatant was recorded ($volume_F$). The degree of F-actin polymerization was based on the results of our previous study (Wang and Smith, 1994). Absorbance attributed to actin (abs_{actin}) was estimated by multiplying the original concentration of actin by (100 − percent of polymerization) × $E^{1\%}$. The difference was the absorbance due to myosin (abs_{myosin}): $abs_{supernatant} - abs_{actin} = abs_{myosin}$.

Free myosin in the supernatant after centrifugation ($[myosin]_F$) was quantified using an $E^{1\%}$ of 5.5. The free to bound myosin ratio was calculated as follows:

$$\text{free myosin to bound myosin ratio} = \frac{[\text{myosin}]_F \times \text{volume}_F}{[\text{myosin}]_I \times \text{volume}_I\,(\text{mL}) - [\text{myosin}]_F \times \text{volume}_F\,(\text{mL})}$$

To evaluate the effect of pyrophosphate, the protein solution was brought to 5 mM sodium pyrophosphate and 1 mM $MgCl_2$ by addition of a 0.1 volume of 50 mM sodium pyrophosphate and 10 mM $MgCl_2$ stock solution. Final pH of myosin was adjusted using 0.1 N HCl or NaOH, if necessary.

Dynamic Rheological Properties. Oscillatory dynamic measurements were performed using a Rheometrics fluid spectrometer (RFS-8400, Rheometrics, Inc., Piscataway, NJ) fitted with a 50 mm diameter parallel plate apparatus and 100 g cm transducer. Storage (G') and loss (G'') moduli were recorded continuously at a fixed frequency of 10 rad/s and a strain of 0.01 while heating from 30 to 80 °C at 1 °C/min as described by Wang and Smith (1994). Protein concentration was 10 mg/mL. Phase angle ($\delta = \tan_{-1}[G''/G']$) was used to show the relative viscoelastic properties. It is 0° for a pure solid and 90° for a pure liquid. Storage modulus and phase angle of myosin (10 mg/mL) at 30 and 80 °C using the same buffer condition and heated at the same rate were taken from Wang and Smith (1994).

Thermal Stability. Thermal stabilities of actin and actomyosin solutions of different ratios were measured using a differential scanning microcalorimeter (DSC) (MC-2, Microcal Inc., Amherst, MA) with a scan rate of 1 °C/min (Wang and Smith, 1994). The endotherm of myosin (10 mg/mL) under the same conditions was taken from Wang and Smith (1994). Concentrations of 5 mg/mL total protein were used for actomyosin because its high viscoelasticity caused problems in degassing and injection. Heat capacity profiles (C_p vs temperature) were defined by endothermic peak temperatures and changes in heat capacity (ΔC_p) (Tsong et al., 1970; Privalov and Potekhin, 1986). All data acquisition and analysis software were provided by the manufacturer.

Statistical Analysis. All statistics were performed using log-transformed data because of the heterogeneous variance existing within each treatment combination (Gill, 1987). The heterogeneous variance was stabilized but could not be totally removed after data transformation. A two-factor (actomyosin ratio and PP_i) completely randomized design with six replicates was used within each temperature (30 or 80 °C) (MSTAT Software, version C, 1989, Michigan State University, East Lansing, MI). Two main factors significantly interacted; therefore, the comparison for one factor was tested within each level of the other factor. Nine comparisons were made at 30 and 80 °C. Bonferroni *t*-statistics were used to test the significant difference of comparisons among means.

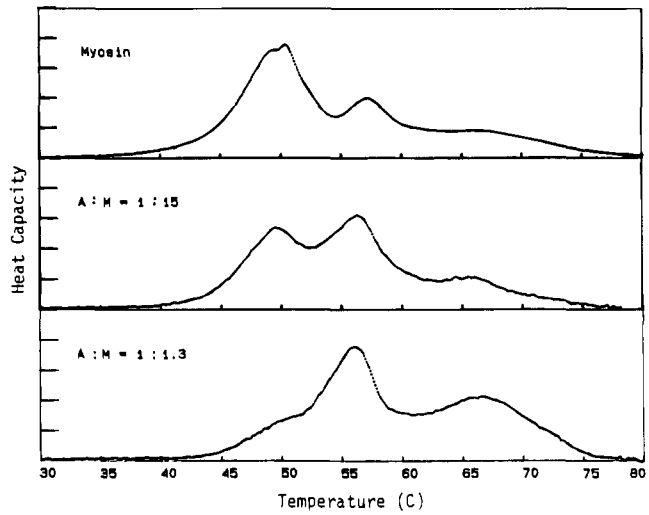

Figure 1. Effect of actin (A) to myosin (M) weight ratio on myosin denaturation in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, heated at 1 °C/min.

Table 1. Enthalpic Transitions of Actomyosin (AM) at Different Actin to Myosin Weight Ratios in 0.6 M NaCl, 50 mM Sodium Phosphate Buffer, pH 6.5, Heated at 1 °C/min[a,b]

	transition temperatures (°C)				
	initial	peak 1	peak 2	peak 3	peak 4
	Without Pyrophosphate				
myosin	36.2	49.2	50.2	57.2	66.8
	(0.4)	(0.2)	(0.1)	(0.2)	(0.6)
AM 1:15	38.5		49.8	56.7	66.5
	(0.9)		(0.2)	(0.3)	(0.2)
AM 1:1.3	42.2		50.2	56.1	66.5
	(0.3)		(0.2)	(0.1)	(0.1)
	With Pyrophosphate				
myosin	37.7	48.9	56.7	59.8	65.1
	(0.3)	(0.1)	(0.2)	(0.1)	(0.5)
AM 1:15	38.3	49.5	56.7	59.9	64.5
	(0.4)	(0.2)	(0.1)	(0.1)	(0.3)
AM 1:1.3	38.6	50.5	56.8	59.7	66.1
	(0.7)	(0.2)	(0.2)	(0.1)	(1.4)

[a] Protein concentration: myosin, 10 mg/mL; AM, 5 mg/mL. [b] Number in parentheses represents standard deviation of means.

RESULTS AND DISCUSSION

Characterization of Actin to Myosin Weight Ratio. Free myosin to bound myosin (actomyosin) ratio was 0.2:1 for AM 1:1.3 (w/w) and 2.4:1 for AM 1:15 (w/w) in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, after correction for unpolymerized actin. In the presence of PP_i, free myosin to bound myosin ratio was 5.5:1 in AM 1:1.3 and 19:1 in AM 1:15 due to the dissociating effect of PP_i.

Thermal Denaturation. The enthalpy profile of myosin showed initial unfolding at 36 °C and four transitions at 49, 50, 57, and 67 °C (Wang and Smith, 1994), which was significantly altered in the presence of F-actin (Figure 1). An AM ratio of 1:15 increased the initial unfolding temperature by 2 °C as compared to myosin alone. Increasing the F-actin content to an AM ratio of 1:1.3 stabilized myosin by an additional 4 °C (Table 1). Only three peaks were observed in both AM 1:15 (50, 57, and 67 °C) and AM 1:1.3 (50, 56, and 67 °C). The heat capacity of the broad peak at 50 °C decreased with addition of F-actin and was probably shifted to a higher temperature. Increases in heat capacity were observed at both 57 and 66.5 °C with F-actin.

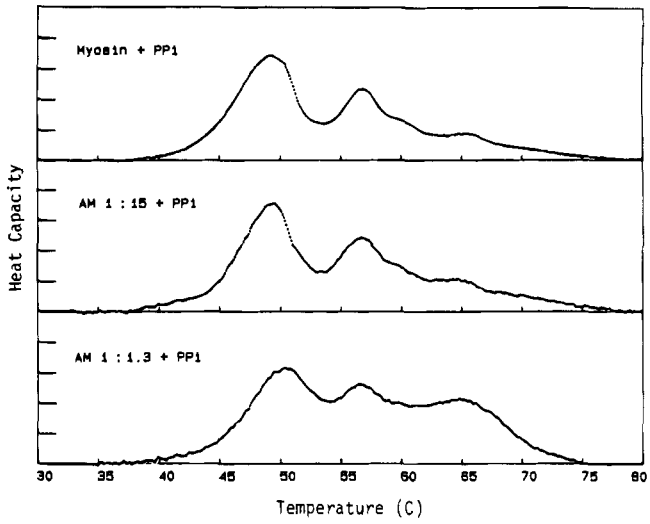

Figure 2. Effect of actin (A) to myosin (M) weight ratio on myosin denaturation in the presence of 5 mM sodium pyrophosphate (PP_i), 1 mM $MgCl_2$, 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, heated at 1 °C/min.

In the presence of PP_i, myosin showed four transition peaks at about 49, 57, 60, and 65 °C and F-actin had little effect on myosin denaturation as indicated by similar endothermic profiles for myosin, AM 1:15, and AM 1:1.3 (Figure 2). The initial unfolding temperature of myosin, AM 1:15, and AM 1:1.3 did not differ and averaged 38 °C (Table 1). The broad peak at 49 °C observed for myosin slightly increased to 50.5 °C at AM 1:1.3. A more significant increase in heat capacity occurred at 66 °C and was similar to that observed without PP_i.

When F-actin was bound to myosin, myosin initial unfolding temperature was increased and heat capacity of the broad peak at 50 °C decreased. These effects did not occur in the presence of PP_i when F-actin was dissociated from myosin. As actin binds the S-1 region of myosin head (Mornet et al., 1979) and interacts with myosin light chains (Sutoh, 1982, 1983), it is possible that the stability of myosin S-1 and light chains was increased due to actin binding. Thus, our results suggested that part of the broad peak at 50 °C in myosin was due to the unfolding of S-1 and light chains. This peak disappeared and seemed to become superimposed on the peak at 57 °C upon binding of F-actin. Initial unfolding temperature was influenced by actin–myosin binding. If the segment with the lowest stability is the myosin hinge region at the junction of heavy and light meromyosin (Burke et al., 1973), then our results suggest that binding of actin to myosin also affects the thermal unfolding of some regions in the myosin rod.

The increase in peak height at 66–67 °C with increasing concentrations of actin occurred both with and without PP_i; thus, this peak was not due to shift of the 50 °C peak which occurred only in the absence of PP_i. The peak at 66–67 °C was probably due to denaturation of F-actin under both conditions. On the basis of our previous study (Wang and Smith 1994), F-actin was partially dissociated to G-actin in the presence of PP_i, and G-actin started to unfold at 49 °C with a T_m of 53.3 °C. Thus, the higher heat capacity at about 53 °C in AM 1:1.3 compared to the other solutions might be partially due to unfolding of G-actin.

Viscoelastic Properties. In a previous study using the same buffer conditions (Wang and Smith, 1994), we observed that the G' of myosin increased sharply at 53.5 °C to a peak at 59 °C, decreased slightly between 59 and 62 °C, and then increased gradually to 80 °C; G'' data points were scattered and did not change throughout heating. In the presence of PP_i, a similar rheogram was observed except the transition temperatures were higher than for myosin without PP_i (Wang and Smith, 1994).

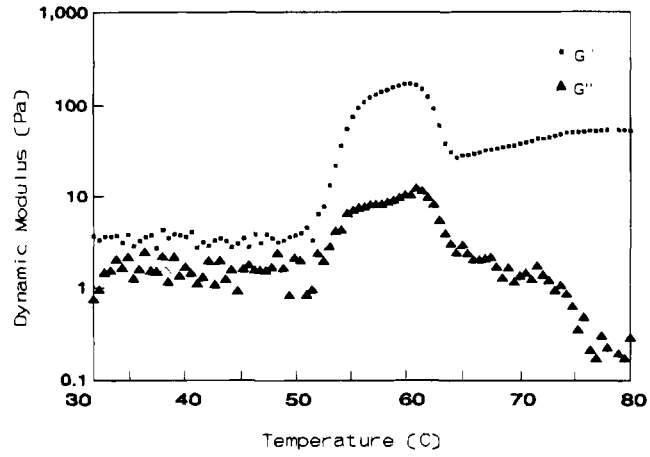

Figure 3. Representative rheogram of storage (G') and loss (G'') moduli of 10 mg/mL actomyosin at actin to myosin weight ratio of 1:15, heated at 1 °C/min in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5.

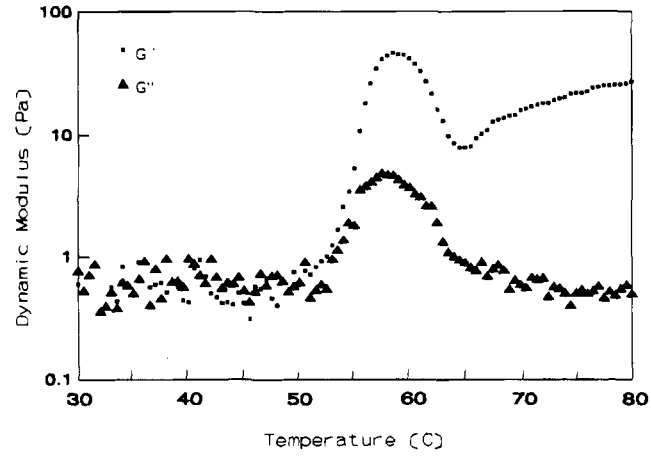

Figure 4. Representative rheogram of storage (G') and loss (G'') moduli of 10 mg/mL actomyosin at actin to myosin weight ratio of 1:15, heated at 1 °C/min in 5 mM sodium pyrophosphate, 1 mM $MgCl_2$, 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5.

Initial transitions in G' and G'' were observed at 49 and 50 °C, respectively, in AM 1:15 solutions (Figure 3), which were lower than myosin alone. The initial G' transition was close to the temperature of the first DSC endothermic peak at 49.8 °C. As previously discussed, this endothermic peak probably resulted from the denaturation of S-1, suggesting that the unfolding of myosin S-1 was partially responsible for the increase in myosin viscoelasticity. Storage modulus of AM 1:15 increased to a maximum at 59 °C, decreased rapidly from 59 to 63.6 °C, and then increased again. Loss modulus followed a similar pattern except it decreased above 63 °C. Gel elasticity increased consistantly after most of the myosin molecule was unfolded as determined by DSC.

An initial transition at 52 °C for both G' and G'' was observed in AM 1:15 in the presence of PP_i during heating (Figure 4). The maximal transition occurred at 57 and 56.5 °C for G' and G'', respectively. Storage

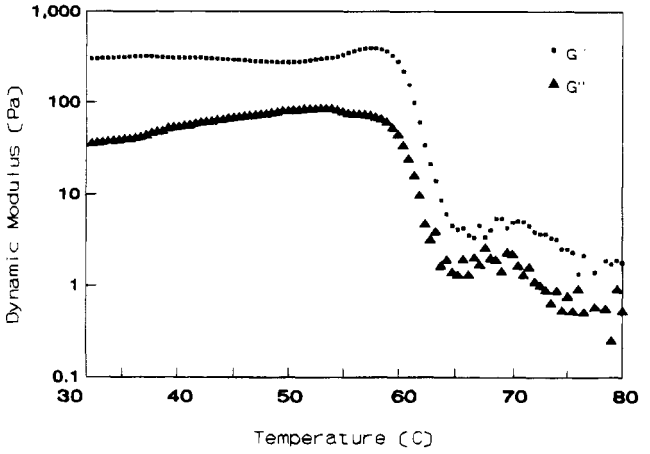

Figure 5. Representative rheogram of storage (G') and loss (G'') moduli of 10 mg/mL actomyosin at actin to myosin weight ratio of 1:1.3, heated at 1 °C/min in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5.

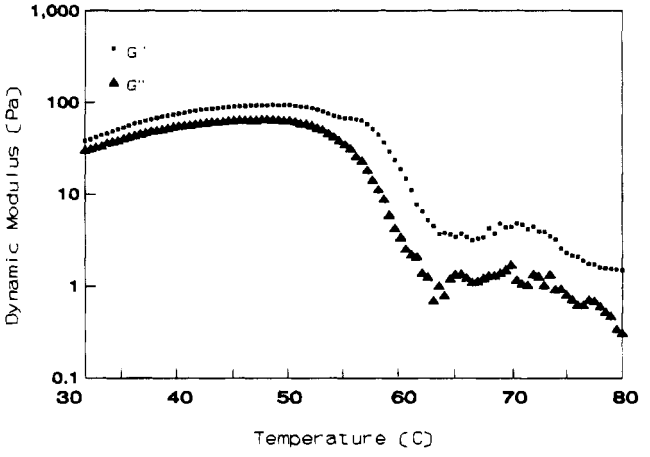

Figure 6. Representative rheogram of storage (G') and loss (G'') moduli of 10 mg/mL actomyosin at actin to myosin weight ratio of 1:1.3, heated at 1 °C/min in 5 mM sodium pyrophosphate, 1 mM $MgCl_2$, 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5.

modulus began to increase again at 63 °C, while G'' decreased slightly after 62 °C. A similar relationship between DSC endotherms and viscoelasticity was observed with and without PP_i. When actomyosin was dissociated by PP_i, a larger decrease in both G' and G'' occurred after reaching peak maximum than in AM 1:15 without PP_i. This transition did not occur when myosin alone was heated under the same conditions with and without PP_i (Wang and Smith, 1994).

Addition of F-actin to an AM ratio of 1:1.3 changed the rheogram significantly. The initial G' and G'' below 50 °C were greater (Figure 5) than myosin and AM 1:15, probably due to the presence of F-actin (Wang and Smith, 1994) and formation of actomyosin. A sharp decrease in G' and G'' occurred between 57 and 65 °C, and the magnitude of change was larger than that observed in AM 1:15 solutions. In the presence of PP_i, the G' below 50 °C was lower whereas G'' did not change much as compared to solutions without PP_i (Figure 6). This suggested that the formation of actomyosin complex influenced the magnitude of G' in protein solutions at low temperatures. A decrease in storage modulus was also observed between 57 and 62 °C. Loss modulus gradually decreased until 50 °C and then rapidly decreased. This transition occurred at a lower temperature than myosin without PP_i (around 57 °C). No rheological transitions in AM 1:1.3 were observed below

Table 2. Percent Confidence of Mean Differences in Comparisons at 30 °C[a,b]

comparisons	log G'	log(phase angle)
AM Ratio Effect		
M vs AM15	***	N
M vs AM13	***	***
AM15 vs AM13	***	***
MP vs AMP15	N	N
MP vs AMP13	***	N
AMP15 vs AMP13	***	N
Pyrophosphate Effect		
M vs MP	N	N
AM15 vs AMP15	***	N
AM13 vs AMP13	***	***

[a] Abbreviations: M = myosin; AM15 = actin to myosin ratio of 1:15 (w/w); AM13 = actin to myosin ratio of 1:1.3 (w/w); P = with pyrophosphate. [b] *** = 99.99% confidence, ** = 99.98% confidence, and * = 99.9% confidence that means are different. N = nonsignificant difference at 95%.

55 °C with or without PP_i. It is possible that the high G' and G'' at low temperatures masked the initial transitions of myosin results or the transition was undetectable due to insufficient myosin. A minor rheological transition at about 70 °C was observed in AM 1:1.3 with and without PP_i. This peak was probably due to the denaturation of F-actin, as PP_i had little effect on the DSC transition temperature (Wang and Smith, 1994).

Sano et al. (1989) reported a similar effect of F-actin on fish muscle myosin in the temperature range of 46–53 °C and proposed that the altered transitions resulted from the dissociation of myosin molecules from actin filaments, the fragmentation of the actin filament, and the subsequent breakdown of gel matrices. According to our results, F-actin caused a sharp decrease in G' and G'' in the temperature range of 57–65 °C regardless of whether actin was free or bound to myosin, suggesting this change was due to the presence of F-actin rather than to the dissociation of actomyosin or breakdown of the gel network. Because this decrease (57–65 °C) occurred before the initial unfolding temperature of F-actin determined by DSC (Wang and Smith, 1994), further investigations are needed to clarify the influence of F-actin on myosin gelation.

Effect of Pyrophosphate and Actin to Myosin Weight Ratio on Viscoelastic Properties. Even though moderate heterogeneous variance still existed, the confidences of mean differences in G', G'', and phase angle due to AM ratio and PP_i at 30 °C (Table 2) and 80 °C (Table 3) were strong in most contrasts ($\alpha < 0.01$).

Effect of Weight Ratio. Heating to 80 °C increased gel elasticity of myosin and AM 1:15 as indicated by a high G' (Figure 7) and low phase angle (Figure 8). Ishioroshi et al. (1980) reported that a 15:1 myosin to F-actin weight ratio resulted in the highest gel strength in 0.6 M KCl, pH 6.0. However, we observed no difference in either G' or phase angle between gels prepared with myosin alone or AM 1:15. Different heating conditions and buffer environments might have a different effect on gel properties. Also, the chicken breast AM 1:15 prepared in our lab contained 29% actomyosin which was greater than the 15–20% actomyosin reported by Ishioroshi et al. (1980).

Actomyosin 1:1.3 had more elastic character at 30 °C than myosin and AM 1:15 due to the presence of F-actin (83% existed as actomyosin). However, AM 1:1.3 did not form as good a gel network at 80 °C as indicated by a higher phase angle when compared to those for myosin and AM 1:15. This suggested a negative effect of F-actin

Table 3. Percent Confidence of Mean Differences in Comparisons at 80 °C[a,b]

comparisons	log G'	log(phase angle)
AM Ratio Effect		
M vs AM15	N	N
M vs AM13	***	***
AM15 vs AM13	***	***
MP vs AMP15	***	N
MP vs AMP13	***	***
AMP15 vs AMP13	***	***
Pyrophosphate Effect		
M vs MP	N	**
AM15 vs AMP15	***	***
AM13 vs AMP13	***	***

[a] Abbreviations: M = myosin; AM15 = actin to myosin ratio of 1:15 (w/w); AM13 = actin to myosin ratio of 1:1.3 (w/w); P = with pyrophosphate. [b] *** = 99.99% confidence, ** = 99.98% confidence, and * = 99.9% confidence that means are different. N = nonsignificant difference at 95%.

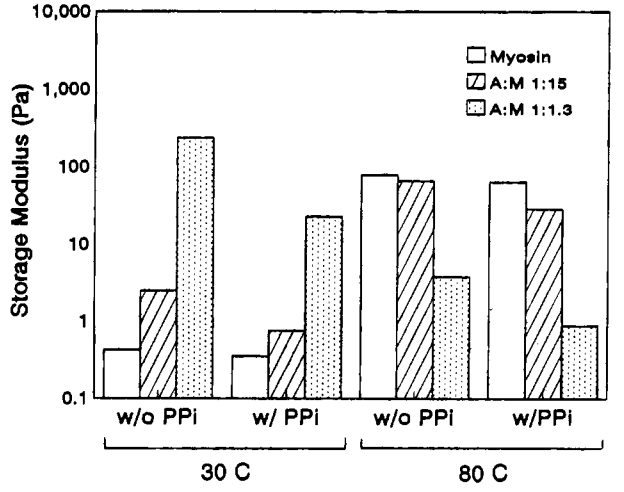

Figure 7. Effect of actin to myosin weight ratios and pyrophosphate (PP_i) on storage modulus at 30 and 80 °C in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5. Protein concentration was 10 mg/mL.

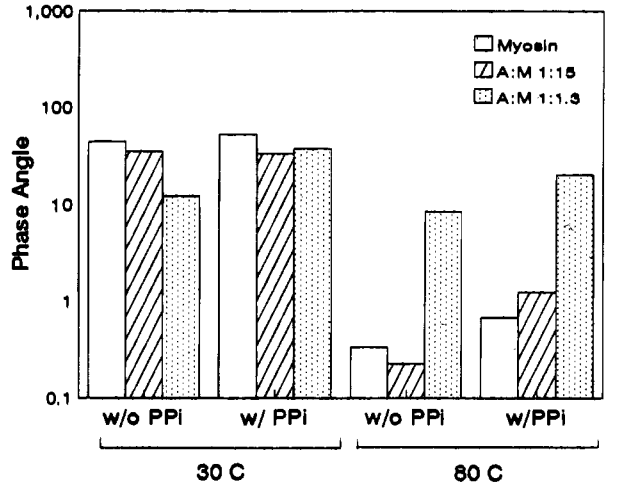

Figure 8. Effect of actin to myosin weight ratios and pyrophosphate (PP_i) on phase angle at 30 and 80 °C in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5. Protein concentration was 10 mg/mL.

on myosin gelation; however, we cannot exclude the possibility that a lower concentration of myosin in an AM 1:1.3 system might also affect gel properties.

In the presence of PP_i, AM 1:1.3 had higher G' than myosin and AM 1:15 at 30 °C; however, no differences were observed in the phase angle. The results suggested that F-actin influenced the viscoelastic properties of myosin at 30 °C. Differences in phase angle between all solutions were decreased due to dissociation; the G' of myosin and AM 1:1.5 solutions was also similar. After heating to 80 °C, the G' for AM 1:15 was lower than for myosin. Actomyosin 1:1.3 had the lowest G' and phase angle in the presence of PP_i, suggesting that F-actin had a negative effect on gelation even when dissociated from myosin.

Effect of Pyrophosphate. According to our previous study (Wang and Smith, 1994), addition of PP_i to F-actin caused a decrease in phase angle, indicating more elastic character. Addition of PP_i did not change G' of myosin at 30 and 80 °C (Figure 7), but the phase angle at 80 °C increased (Figure 8). For AM 1:15, PP_i caused a decrease in G' at 30 °C due to actomyosin dissociation. When heated to 80 °C, G' of AM 1:15 decreased and a higher phase angle was observed. AM 1:1.3 with PP_i at both 30 and 80 °C had a lower G' and higher phase angle than without PP_i. These observations indicated that PP_i-induced dissociation of actomyosin decreased the elastic character of the actomyosin gel (increased phase angle). Even with a small amount of free F-actin (AM 1:15), a significant increase in viscous character was observed. This increased viscous character was attributed to the rheological character of free F-actin as well as the effect of PP_i on myosin alone.

Conclusion. Interaction between actin and myosin not only stabilized S-1 and light chains but also some domains in the myosin rod. Delay of S-1 and light chain unfolding seemed to interfere with denaturation of myosin rod. This stabilizing effect decreased in the presence of PP_i due to dissociation of actomyosin. It was possible that the stabilized myosin domains altered the gelation mechanism and gel properties. However, F-actin, regardless of whether it was free or bound to myosin, altered the rheogram between 57 and 65 °C. Free F-actin decreased gel elasticity more than bound F-actin. The actin to myosin ratio has a large influence on the gelation properties of muscle proteins and may be one factor that should be considered when meat processors select meat in least-cost formulation calculations.

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