Heat-Induced Denaturation and Rheological Properties of Chicken Breast Myosin and F-Actin in the Presence and Absence of Pyrophosphate

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The heat capacity profile of chicken breast myosin had four transitions at 49, 50, 57, and 67 °C with a calorimetric enthalpy (ΔH_{cal}) of 2215.8 ± 89.3. Addition of sodium pyrophosphate produced similar heat capacity profiles but reduced ΔH_{cal} to 1727.9 ± 45.4 kcal/mol. Both curves were deconvoluted into 10 two-state transitions. Using dynamic testing, storage modulus (G') of myosin increased at 53.5 °C, formed a transition peak, and increased again above 62 °C. Pyrophosphate did not change the rheogram. The F-actin endotherms had a single peak at 75.5 ± 0.4 °C, with a ΔH_{cal} of 143.4 ± 9.6. The cooperative ratio of F-actin was higher than unity, indicating intermonomer interaction. Addition of pyrophosphate to F-actin resulted in a major peak at 75.6 ± 0.5 °C and a minor peak (G-actin) at 53.3 ± 0.1 °C. Storage modulus of F-actin increased at 64.1 ± 0.9 °C; this temperature was similar to the initial unfolding temperature (64.2 °C) determined by differential scanning calorimetry.

Keywords: *Myosin; actin; pyrophosphate; differential scanning calorimetry; rheological properties; denaturation; gelation*

INTRODUCTION

Myosin, the most abundant myofibrillar protein, is composed of two heavy chains and four light chains. Each heavy chain contains a globular head or subfragment 1 (S-1) which binds actin and ATP, and a coiled-coil α-helical rod (Harrington, 1979). Myosin heavy chain is a prerequisite for developing desired gel strength in model systems (Samejima et al., 1969; Asghar et al., 1985). Monomeric G-actin is a globular shaped molecule. Polymerization of G-actin monomers forms F-actin (fibrous form). In contrast to myosin, F-actin has no gelling ability (Yasui et al., 1979, 1980) and few changes in viscoelasticity were observed upon heating (Sano et al., 1989). The currently accepted model for heat-induced protein gelation includes protein unfolding, followed by protein-protein interactions and matrix development (Ferry, 1948; Clark et al., 1981; Foegeding and Hamann, 1992). Factors influencing protein stability or interactions may affect gel properties. Therefore, basic research related to molecular properties of chicken muscle myosin and F-actin during heating will provide information on the mechanism of myosin gelation and contribute to manipulation of protein functionality in poultry products.

Differential scanning calorimetry (DSC) is a technique used to determine the thermodynamics of molecular systems. These thermodynamic parameters can be related to structural/conformational changes occurring in proteins on heating (Chowdhry and Cole, 1989). Thermal denaturation of myosin and its subfragments has been studied using DSC. Single or multiple transitions were ascribed to myosin that varied with pH, salt concentration, and species (Stabursvik and Martens, 1980; Swenson and Ritchie, 1980; Wright and Wilding, 1984; Akahane et al., 1985; Rodgers and Harrington, 1987a; Rodgers et al., 1987b; Bertazzon and Tsong, 1989, 1990a,b). The term "domain" has been defined as an independent, cooperative unit in a folded protein (Privalov, 1982; Shriver and Kamath, 1990). It has been reported that myosin rod in rabbit muscle undergoes a multistep endothermic process consisting of at least six quasi-independent structural domains within the temperature range from 41 to 67 °C (Potekhin et al., 1979; Lopez-Lacomba et al., 1989; Bertazzon and Tsong, 1990b). Some domains in the rod were further resolved into subdomains (Bertazzon and Tsong, 1990b). The DSC endotherm showed a single peak at 57 and 67 °C for G-actin and F-actin, respectively, at pH 8.0 (Bertazzon et al., 1990).

In post-rigor meat, myosin exists primarily in the bound form as actomyosin. We hypothesize that the unfolding and protein–protein aggregation mechanism of free and bound myosin is different due to interactions with actin. Pyrophosphate is often used to dissociate actomyosin (Nauss et al., 1969). The objective of this study was to monitor the denaturation temperature, enthalpy and dynamic rheological properties of chicken breast muscle myosin and F-actin during heating in the presence and absence of pyrophosphate. Examining the effects of pyrophosphate on free myosin and actin will provide some background information necessary to elucidate the complex relationship between actin and myosin during gelation.

MATERIALS AND METHODS

Extraction of Myosin. Broiler breast muscle myosin was extracted and stored in $(NH_4)_2SO_4$ at −20 °C as described by Nauss et al. (1969). Prior to use, myosin was dialyzed against 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, with two changes of buffer. The first two dialysis buffers contained 1 mM EDTA. The dialyzed myosin solution was centrifuged at 78000g for 1 h at 4 °C (Beckman Ultracentrifuge, Model L7-65, Beckman Instruments, Inc., Palo Alto, CA) to remove insoluble proteins. Myosin was adjusted to 10 mg/mL using an extinction coefficient of $E^{1\%} = 5.5$ at 280 nm (Swenson and Ritchie, 1980).

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Purification of Actin. Acetone powder was prepared as described by Feuer et al. (1948) and stored at −20 °C for future use.

Actin was purified as described by Spudich and Watt (1971). The purified G-actin was polymerized by adding KCl to a final concentration of 50 mM, $MgCl_2$ to 1 mM, and ATP to 1 mM and stirred slowly for 2 h. The F-actin solution was dialyzed overnight against 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, prior to use. Concentration of actin was measured using an extinction coefficient of $E^{1\%} = 11$ at 280 nm (Duong and Reisler, 1987).

Polymerization of F-Actin. Actin polymerization in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5 was determined by measuring the actin concentration before centrifugation ($[A]_I$) and in the supernatant ($[A]_F$) after centrifugation at 100000*g* for 120 min (Beckman Ultracentrifuge, Model TL-100) (Yasui et al., 1982):

$$\text{polymerization } (\%) = ([A]_I - [A]_F)/[A]_I$$

Addition of Pyrophosphate. To determine the effect of pyrophosphate, myosin and F-actin solutions were brought to 5 mM sodium pyrophosphate and 1 mM $MgCl_2$ by addition of $^1/_{10}$ volume of 50 mM sodium pyrophosphate and 10 mM $MgCl_2$ stock solution.

Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) (Laemmli, 1970) was performed using a Mini-Protean II electrophoresis unit (Bio-Rad Laboratories, Richmond, CA) with stacking and separating gels of 4 and 10% acrylamide, respectively. Three micrograms of protein and 12 μg of molecular weight standards (SDS-6H, Sigma Chemical Co., St. Louis, MO) were loaded. Molecular masses were determined by comparing the relative mobilities of protein bands to those of molecular weight standards (Weber and Osborn, 1969).

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. Protein solutions were loaded in the sample cup and equilibrated at 30 °C for 3 min. Solutions were heated from 30 to 80 °C at 1 °C/min using a programmable circulating oil bath (Model MTP-6, Nelsprit Temperature Programmer, Newington, NH). The gap between upper and lower plates was between 1 and 1.5 mm. Storage (G') and loss (G'') moduli were recorded continuously at a fixed frequency of 10 rad/s and strain of 0.01. The strain was within the linear range based on the strain sweeps (0.0001−0.5) conducted at 30 and 80 °C.

Thermal Stability. Thermal stability of myosin was measured using a differential scanning microcalorimeter (MC-2, Microcal Inc., Amherst, MA) at a scan rate of 1 °C/min from 20 to 90 °C. Cell capacity was 1.24 mL. The effect of myosin concentration on calorimetric analysis was examined between 1 and 10 mg/mL. The final concentration chosen was 10 mg/mL for better peak resolution. A base line obtained by running buffer vs buffer was subtracted from the sample data files before analysis. The heat capacity profiles (C_p vs temperature) were defined by a calorimetric enthalpy (ΔH_{cal}), a van't Hoff enthalpy (ΔH_{vH}), a melting temperature (T_m) at which proteins are 50% denatured, and the cooperative ratio (CR) which was defined as $\Delta H_{vH}/\Delta H_{cal}$ (Privalov and Potekhin, 1986; Tsong et al., 1970). Because no precise molecular mass has been reported for chicken myosin and actin, the molecular masses of rabbit myosin, 5.21×10^5 (Yates and Greaser, 1983), and actin, 4.19×10^4 (Elzinga et al., 1973), were used for analysis. For proteins with CR value below unity, the endotherms were fitted into a minimal number of independent transitions, assuming a two-state unfolding process (i.e. $\Delta H_{vH} = \Delta H_{cal}$). Data analysis was done using the software provided by the manufacturer, based on a least-squares fitting procedure described by Freire and Biltonen (1978a,b).

Statistical Analysis. Basic statistics for computing means and standard deviations and two-way analysis of variance (replication × treatment) were performed on a completely randomized design (six replicates) using MSTAT software (version C, 1990, Michigan State University, East Lansing, MI).

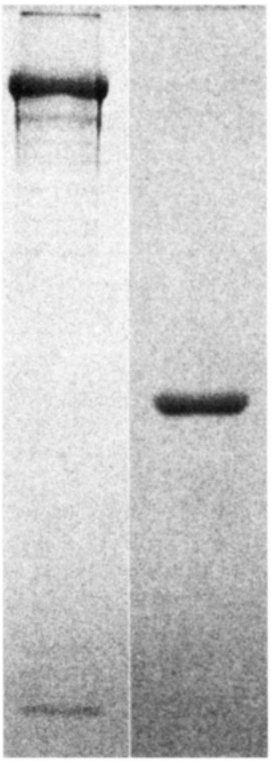

Figure 1. Sodium dodecyl sulfate−polyacrylamide electrophoresis gel of chicken breast myosin and actin: (a) myosin; (b) actin.

RESULTS AND DISCUSSION

Characterization of Myosin and F-Actin. Chicken breast muscle myosin exhibited a major band of about 205 kDa on SDS−PAGE that was identified as myosin heavy chain (Figure 1). One minor contaminating protein was present in the myosin preparation and was probably C-protein (Margossian and Lowey, 1982). Purified actin showed a single band near 45 kDa. F-Actin in the presence and absence of 5 mM PPi was 90% polymerized in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5. Contaminating proteins might change the calorimetric profiles and viscoelasticity of myosin; however, because of their low concentrations, we assumed any effects would be negligible.

Thermal Denaturation of Myosin. The effect of myosin concentration (0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5) on calorimetric analysis was examined. An exothermic process, resulting in a negative peak at about 57.5 °C was observed in solutions of 1 mg/mL myosin. Shriver and Kamath (1990) observed an exothermic peak in purified rabbit myosin S-1 (13.8 μM) at 48 °C in 0.6 M KCl, 50 mM Tris buffer, pH 8.0, as well as in heavy meromyosin (12 μM) at 65 °C in 0.1 M KCl, 50 mM Tris buffer, pH 7.9. The authors reported that the position and magnitude of this exothermic peak were variable. They suggested the exotherm resulted from aggregation and precipitation of the unfolded protein. The endothermic profiles of chicken breast myosin at 4 mg/mL and above were similar; however, better peak resolution was observed at 10 mg/mL.

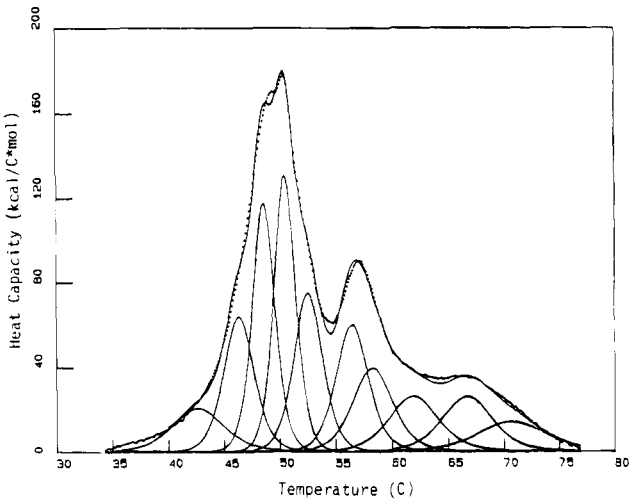

Figure 2. Heat capacity profile and deconvoluted peaks of myosin in 0.6 M NaCl, 50 mM sodium phosphate, pH 6.5. Scan rate is 1 °C/min. The dotted line is the experimental data. The theoretical endotherm and deconvoluted peaks are expressed as solid lines.

Table 1. Myosin Differential Scanning Calorimetry (DSC) Deconvoluted Endotherm Peaks and Rheological Transitions When Heated from 20 to 90 °C at 1 °C/min in 0.6 M NaCl, 50 mM Sodium Phosphate Buffer, pH 6.5[a]

storage modulus transition temp (°C)	DSC deconvoluted peaks	
	transition temp (°C)	enthalpy (kcal/mol)
	44.2 ± 0.4	148.2 ± 7.3
	47.1 ± 0.2	240.3 ± 4.8
	49.0 ± 0.2	298.0 ± 6.8
	50.7 ± 0.2	317.4 ± 17.0
53.5 ± 0.7	52.9 ± 0.3	242.4 ± 15.6
	56.4 ± 0.2	241.4 ± 10.8
59.0 ± 0.6	58.7 ± 0.5	215.7 ± 17.0
62.1 ± 0.4	62.6 ± 0.6	178.8 ± 10.4
	66.8 ± 0.4	174.5 ± 7.6
	70.8 ± 0.4	137.2 ± 17.2

[a] Means of six replications ± standard deviation.

Myosin started to unfold at 36.2 °C. The heat capacity profile was characterized by four endothermic peaks at 49.2 ± 0.2, 50.2 ± 0.1, 57.2 ± 0.2, and 66.8 ± 0.6 °C (Figure 2). The overall endothermic profile of our chicken breast myosin was different from rabbit myosin. Bertazzon and Tsong (1989) reported that rabbit myosin unfolded in a multistage process, with a peak at 46 °C and three shoulders at 43, 49, and 54 °C in 0.5 M KCl, 20 mM potassium phosphate, pH 7.0. The temperature range of denaturation of rabbit myosin was narrower than that for chicken myosin due to the presence of a transition peak above 60 °C.

The ΔH_{cal} of chicken breast myosin denaturation was 2215.8 ± 89.3 kcal/mol. The ΔH_{cal} of chicken breast myosin was greater than that of rabbit myosin (1715 kcal/mol) reported by Bertazzon and Tsong (1989). Because of the multiple transitions shown in myosin endothermic profiles, the endotherm was deconvoluted into 10 two-state transitions (i.e. $\Delta H_{vH} = \Delta H_{cal}$) to better understand the denaturation process despite the fact that these thermal transitions are apparently irreversible (Bertazzon and Tsong, 1990a). The enthalpy of each domain was calculated (Table 1).

Rabbit myosin rod contained at least six quasi-independent domains (Potekhin et al., 1979; Lopez-Lacomba et al., 1989; Bertazzon and Tsong, 1990b) and is the only fragment showing a high degree of reversibility during thermal unfolding (Bertazzon and Tsong, 1989). A single peak was observed in S-1 and light chains with T_ms of 46.3 and 51.5 °C, respectively, in 0.5 M KCl, pH 7.0, 20 mM potassium phosphate buffer, and 1 mM EDTA (Bertazzon and Tsong, 1989). Subfragment 2 (S-2) had a T_m of 48.6 °C at pH 6.45, 0.5 M KCl (Bertazzon and Tsong, 1990a), and its endotherm was fitted to three two-state transitions at 47, 48.4, and 53.8 °C with a ΔH_{cal} of 143, 145, and 114 kcal/mol, respectively (Bertazzon and Tsong, 1990b). The endotherm of light meromyosin (LMM) showed three main peaks at pH 6.4, and was fitted to five two-state transitions at 41.4, 48.7, 49.8, 55.9, and 57.6 °C with a ΔH_{cal} of 157, 78, 114, 177, and 77 kcal/mol, respectively. The lowest stability domain was found in the hinge region at the LMM/S-2 junction (Bertazzon and Tsong, 1990b).

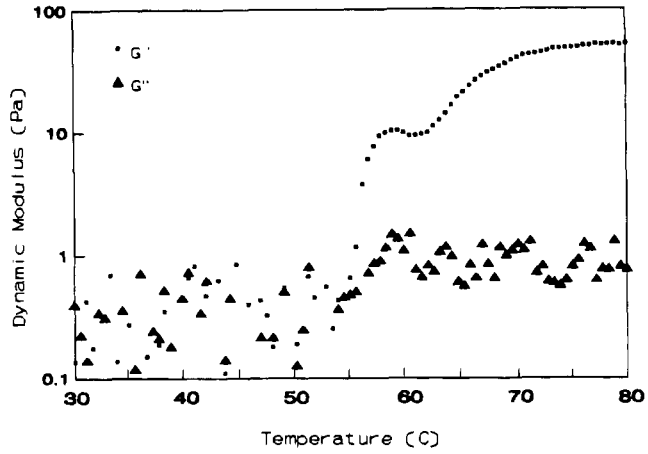

Figure 3. Representative rheogram showing storage (G') and loss (G'') moduli of myosin (10 mg/mL) heated at 1 °C/min in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5.

It was difficult to assign chicken muscle myosin domains to specific transition temperatures based on the literature because of different endotherm patterns and melting temperatures observed between species (Potekhin et al., 1979; Lopez-Lacomba et al., 1989; Bertazzon and Tsong, 1990b; Shriver and Kamath, 1990). The first domain with T_m of 44.2 °C was attributed to unfolding of the hinge region. Most results from the literature showed S-1 and light chains were denatured below 55 °C and might correspond to the second through the fifth domains in our study. Stabilizing effects caused by the binding of actin to S-1 might help verify the identity of these domains. The domains above 55 °C were probably due to regions of the myosin rod. Further investigations are necessary for accurate assignment of myosin domains.

Viscoelasticity of Myosin. In 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, the torque generated by myosin was very low at temperatures below 53 °C, resulting in scattered data points for G' and G''. Storage modulus of myosin increased sharply at 53.5 °C, formed a transition peak at 59 °C, decreased slightly between 59 and 62 °C, and then increased gradually until 80 °C, showing development of gel elasticity (Figure 3). In contrast, G'' data points were scattered and did not change throughout heating. By comparing the rheological transitions of G' to the DSC deconvoluted peaks, the first four DSC domains (T_ms of 44.2, 47.1, 49, and 50.7 °C) were unfolded prior to development of gel elasticity. Rheological testing detected changes in G' during unfolding of the domain with a T_m of 53 °C. The unfolding of domains with T_ms of 56.4, 58.7 and 62.6 °C might be responsible for the plateau in G' observed from 59 to 62 °C in the myosin rheogram.

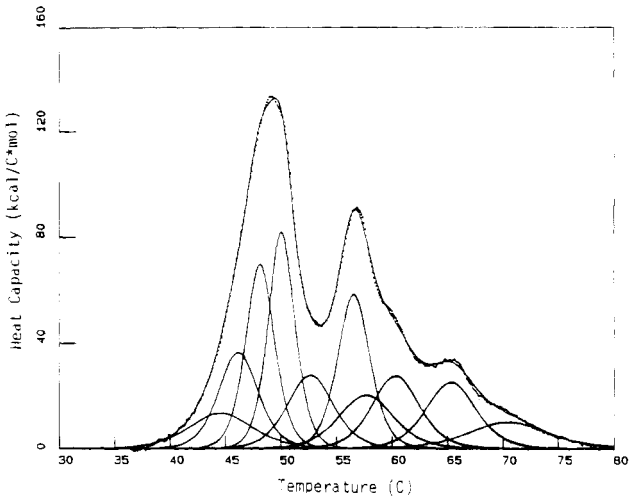

Figure 4. Heat capacity profile and deconvoluted peaks of myosin in 5 mM sodium pyrophosphate, 1 mM $MgCl_2$, 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5. Scan rate is 1 °C/min. The dotted line is the experimental data. The theoretical endotherm and deconvoluted peaks are expressed as solid lines.

Table 2. Myosin Differential Scanning Calorimetry (DSC) Deconvoluted Endotherm Peaks and Rheological Transitions in 5 mM Pyrophosphate When Heated from 20 to 90 °C at 1 °C/min in 0.6 M NaCl, 50 mM Sodium Phosphate Buffer, pH 6.5[a]

storage modulus transition temp (°C)	DSC deconvoluted peaks	
	transition temp (°C)	enthalpy (kcal/mol)
	44.1 ± 0.4	100.6 ± 4.5
	46.1 ± 0.2	173.8 ± 4.8
	48.0 ± 0.2	241.0 ± 2.1
	50.0 ± 0.2	257.3 ± 5.0
53.1 ± 0.3	52.4 ± 0.3	163.7 ± 8.5
	56.3 ± 0.2	221.0 ± 7.6
	57.4 ± 0.2	159.7 ± 23.3
61.2 ± 0.7	60.3 ± 0.2	164.0 ± 9.3
62.9 ± 0.2	64.9 ± 0.2	151.7 ± 5.8
	69.6 ± 0.6	101.4 ± 9.8

[a] Means of six replications ± standard deviation.

Effect of Pyrophosphate (5 mM) on Myosin. Addition of 5 mM pyrophosphate (PPi) increased the initial unfolding temperature of myosin to 37.7 °C. The first two transitions of myosin observed without PPi merged into one peak, resulting in three endothermic peaks at 48.9 ± 0.1, 56.7 ± 0.2, and 65.1 ± 0.5 °C. A shoulder appeared at 59.8 ± 0.1 °C (Figure 4). The ΔH_{cal} was decreased to 1727.9 ± 45.4 kcal/mol as compared to myosin alone, indicating PPi destabilized the molecule. The endotherm was fitted into ten deconvoluted peaks (Table 2). If we assume the order of domain unfolding did not change, most domain T_ms and enthalpies decreased due to addition of PPi.

Scattered data points were observed in G' and G'' below 53 °C for myosin with PPi (Figure 5). Storage modulus started to increase at about 53 °C and reached a maximum at 61.2 °C. The temperature of this peak maximum was higher than that observed in myosin alone (59 °C). A slight decrease in G' occurred between 61 and 63 °C and then G' increased again. Loss modulus showed little change throughout heating.

When comparing rheological transitions to DSC deconvoluted peaks, four domains unfolded prior to appearance of rheological detectable structure. This finding was similar to myosin alone. The rheological transition at 61.2 °C occurred after more than 50% of the eighth domain was unfolded (T_m = 60.3 °C). The

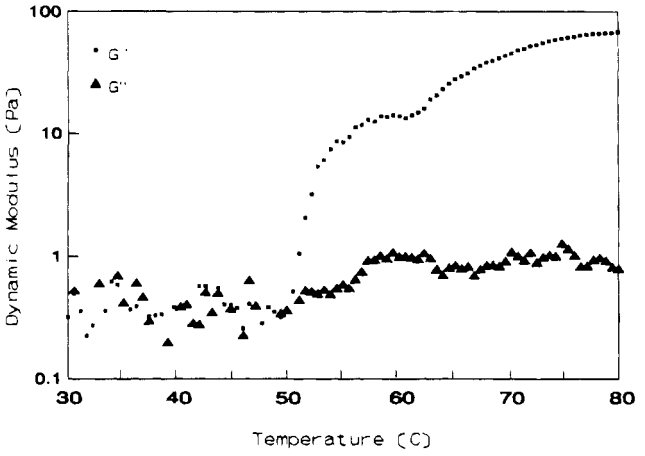

Figure 5. Representative rheogram showing storage (G') and loss (G'') moduli of myosin (10 mg/mL) heated at 1 °C/min in 5 mM sodium pyrophosphate, 1 mM $MgCl_2$, 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5.

temperature of the second increase in G' (around 63 °C) corresponded to the unfolding of the eighth and ninth domains of myosin. For myosin without PPi, fewer domains needed to unfold before this second increase in G' occurred (Table 1). It is possible that PPi binding increased the negative charge of myosin and thus the repulsive forces between molecules, so the initial myosin interaction was inhibited. The gelation process might require more protein to unfold for exposure of hydrophobic groups and subsequent interactions to overcome repulsive forces.

Lopez-Lacomba et al. (1989) observed similar endothermic profiles for rabbit myosin rod in phosphate (0.20 M) and pyrophosphate buffers (0.15 M), 0.5 M KCl, pH 6.5–9.0. They reported some stabilization in the first endothermic peak (below 50 °C) and lower enthalpy for denaturation in pyrophosphate buffer which agreed with our findings. Hamai and Konno (1989) reported the binding of PPi destabilized the light chains–heavy chain binding, resulting in the formation of aggregates of light chain-deficient heavy chains. The authors suggested dissociation of light chains was due to structural changes around the light chain binding site. Based on their results, the decrease in enthalpy of chicken breast myosin in the presence of PPi might be due to structural changes of S-1. The decreases in most T_ms and enthalpies of domains also suggested that thermal stability of S-1 influenced unfolding of other domains in myosin.

Thermal Denaturation of F-Actin. Chicken breast muscle F-actin in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, started to unfold at 64.2 ± 0.8 °C and had a single sharp peak at 75.5 ± 0.4 °C with a ΔH_{cal} of 143.4 ± 9.6 kcal/mol and a ΔH_{vH} of 179.2 ± 15.3 kcal/mol (Figure 6). For a simple two-state transition, the concentrations of intermediates between native and denatured states are very low, and ΔH_{cal} is close to or equal to ΔH_{vH}. A cooperative ratio (CR = $\Delta H_{vH}/\Delta H_{cal}$) greater than 1 indicates intermolecular interaction. Proteins with CR below unity indicate one or more significant intermediate states in the overall process (Chowdhry and Cole, 1989; Privalov and Potekhin, 1986; Donovan, 1984; Tsong et al., 1970). The cooperative ratio (CR) of actin was 1.25. Bertazzon et al. (1990) reported the ΔH_{cal} of rabbit F-actin was 162 ± 10 kcal/mol, with a T_m at 67.0 ± 0.5 °C and a CR of 1.4 in 2 mM HEPES, 1 mM Na-ATP, 50 mM KCl, 0.2 mM $CaCl_2$, 2 mM $MgCl_2$, and 0.5 mM mercaptoethanol at pH 8.0. Reducing the pH to 6.4 shifted T_m to 74.0 ± 0.3 °C, with

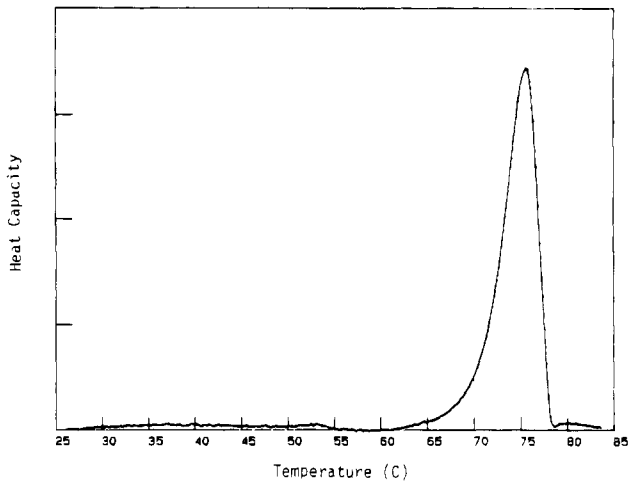

Figure 6. Heat capacity profile of actin in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, heated at 1 °C/min.

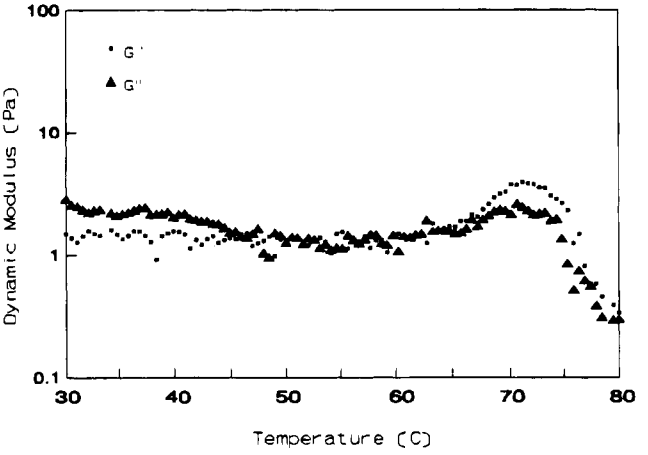

Figure 7. Representative rheogram showing storage (G') and loss (G'') moduli of actin (6 mg/mL) heated at 1 °C/min in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5.

a ΔH_{cal} of 189 ± 10 kcal/mol and a CR of 1.51. The authors suggested that the higher CR value implied interaction among actin monomers in the filament. The higher T_m and lower ΔH_{cal} for F-actin in our study might be due to species differences and buffer conditions. The lower CR in our study might also result from differences in degree of actin polymerization.

Viscoelasticity of F-Actin. Storage modulus (G') of F-actin started to increase at 64.1 ± 0.9 °C; loss modulus (G'') increased at 63.4 ± 1.2 °C (Figure 7). Both G' and G'' reached a maximum at about 71−72 °C, then decreased. Little change in viscoelasticity of F-actin occurred during heating as indicated by G' and G'' below 7 Pa. This suggested F-actin did not form gels upon heating, which agreed with the results of Yasui et al. (1979, 1980) and Sano et al. (1989). By comparing the rheogram of F-actin to its endotherm, the temperature at which G' increased (64.1 °C) was close to that of the initial unfolding temperature (64.2 °C). Both G' and G'' decreased (71−72 °C) before reaching the T_m as determined by DSC (75.5 °C). These results suggested F-actin unfolding was responsible for the initial changes in rheological properties; however, an elastic gel matrix did not form.

Effect of Pyrophosphate (5 mM) on F-Actin. The endotherm of F-actin with PPi exhibited one major peak at 75.4 ± 0.5 °C and a minor peak at 53.3 ± 0.1 °C, even though actin with and without pyrophosphate was 90% polymerized as measured by centrifugation (Figure

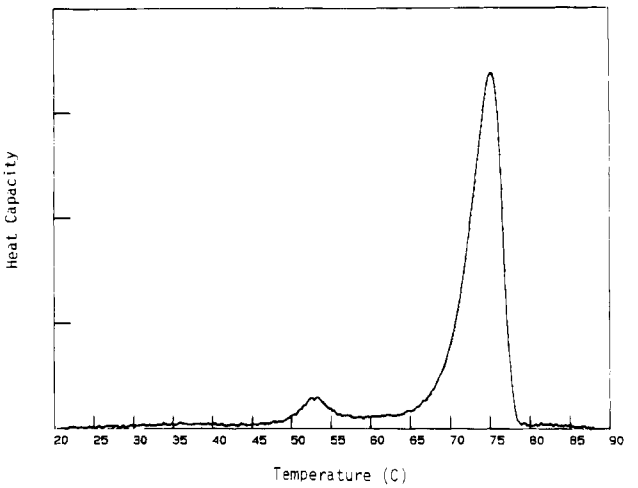

Figure 8. Heat capacity profile of actin in 5 mM sodium pyrophosphate, 1 mM $MgCl_2$, 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, heated at 1 °C/min.

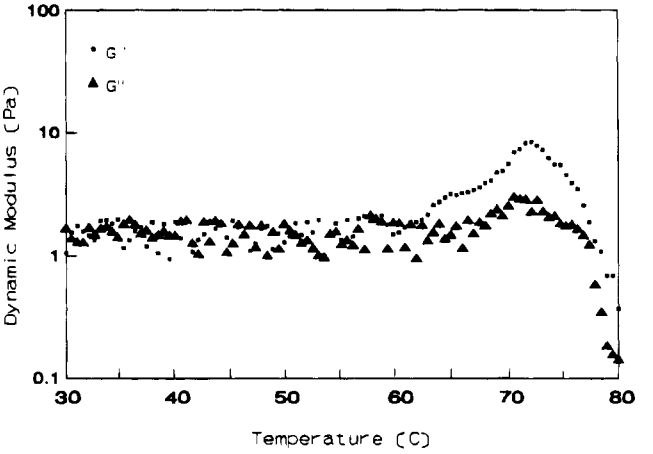

Figure 9. Representative rheogram showing storage (G') and loss (G'') moduli of actin (6 mg/mL) heated at 1 °C/min in 5 mM sodium pyrophosphate, 1 mM $MgCl_2$, 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5.

8). This minor peak with a T_m of 53 °C was assigned to G-actin (Bertazzon et al., 1990). The reason for the existence of G-actin in the presence of PPi is not clear but suggests depolymerization of F-actin by PPi. Storage modulus of F-actin with PPi increased at 62.2 ± 0.7 °C. This temperature was similar to the initial unfolding temperature measured by DSC (61.5 ± 2.4 °C); loss modulus started to increase at 64.0 ± 0.6 °C (Figure 9).

Conclusion. Myosin unfolded at 36 °C and had four transitions with a cooperative ratio below unity. Addition of PPi resulted in a similar endotherm to myosin alone, but increased the initial unfolding temperature of myosin and reduced calorimetric enthalpy. Myosin with and without pyrophosphate was deconvoluted into 10 domains. Compared to myosin alone, more domains were unfolded prior to the development of gel elasticity in the presence of pyrophosphate. Addition of pyrophosphate inhibited initial myosin aggregation. The endotherm of F-actin showed a single peak with CR value above unity, indicating intermonomer interaction. The temperature at which G' increased was close to that of the initial unfolding. Addition of pyrophosphate to F-actin resulted in partial depolymerization as evidenced by the existence of a G-actin peak at 53 °C. Unlike myosin, addition of PPi had no influence on viscoelasticity of F-actin. Actin did not form elastic gels after heating.

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LITERATURE CITED

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