Thermal Denaturation and Aggregation of Chicken Breast Muscle Myosin and Subfragments

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To elucidate the roles of the head and tail portions of chicken breast muscle myosin in gelation, the thermal stability and aggregation behavior of myosin and seven subfragments in 0.6 M NaCl, pH 6.5, were investigated, namely, myosin heavy chain (MHC), light chains (LC), heavy meromyosin (HMM), light meromyosin (LMM), rod, subfragment 2 (S-2), and subfragment 1 (S-1). Myosin had four independent cooperative endothermic transitions (T_m) at 47, 54, 57, and 63 °C and aggregated from 50 to 70 °C. The MHC endotherm had peaks at 46, 54, and 64 °C and aggregated between 45 and 63 °C. S-1 unfolded in a single transition, having a T_m of 47.7 °C, and aggregated from 49 to 53 °C. The rod melted between 30 and 63.3 °C and continuously aggregated over this temperature range. Initial unfolding of the rod occurred in the LMM region. S-2 was primarily responsible for denaturation and aggregation above 55 °C. Transition temperatures of 48 and 57 °C were recorded for LC; however, no aggregation occurred. The rod had the biggest influence on gel formation. Light meromyosin and S-1 contributed to gel structure at temperatures less than about 55 °C, whereas S-2 was responsible for matrix formation above 60 °C.

Keywords: *Myosin; gelation; differential scanning calorimetry; denaturation*

INTRODUCTION

Gelation of muscle proteins during thermal processing is largely responsible for the characteristic texture and cohesion of comminuted and reformed meat products. Myosin is a prerequisite for developing desired gel strength in meat model systems (Samejima et al., 1969). Fukazawa et al. (1961) have shown that myosin contributes to the binding properties and water-holding capacity of comminuted meat products. Heat-induced protein gelation has been defined as a two-step process involving unfolding of proteins followed by aggregation into a three-dimensional network. Protein unfolding and orientation of unfolded molecules during aggregation influence the development of a gel network (Ferry, 1948; Hermansson, 1978). Due to the complexity of muscle systems, simplified models composed of salt soluble proteins or purified muscle proteins have been used to provide information on the gelation process.

Myosin is a multidomain protein, and the domains can be considered independent of each other (Privalov and Gill, 1988). Originally, denaturation studies were performed using rabbit skeletal myosin; more recently, differential scanning calorimetry (DSC) studies have been conducted using poultry myosin (Liu, 1994; Wang and Smith, 1994a, 1995). Bertazzon and Tsong (1989) reported a melting range from 41 to 60 °C for rabbit muscle myosin and a calorimetric enthalpy (ΔH_{cal}) of 1715 kcal/mol in 0.5 M KCl, 1 mM EDTA, 20 mM potassium phosphate buffer, pH 7.0. Transition temperatures (T_m) of 53 °C for subfragment 2 (S-2), 52.3 and 58 °C for heavy meromyosin (HMM), 45 and 56 °C for light meromyosin (LMM), and 45 and 56 °C for the rod were reported for rabbit muscle myosin subfragments in 0.6 M KCl, 20 mM sodium phosphate buffer, pH 7.0 (Samejima et al., 1983). Shriver and Kamath (1990) reported T_m values for rabbit myosin of 41 °C for S-2 and 41 and 48 °C for HMM with an exothermic peak at 65 °C in 0.1 M KCl, pH 7.9. Wang and Smith (1994a) reported a melting range from 36 to 70 °C for chicken breast muscle myosin and a ΔH_{cal} of 2216 kcal/mol in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5. Upon deconvolution of the endotherm, 10 independent domains were reported at 44, 47, 49, 51, 53, 56, 58, 63, 67, and 70 °C. The authors could not assign each deconvoluted peak to a particular region of the myosin molecule due to lack of T_m values for chicken breast muscle myosin subfragments. Xiong et al. (1987) reported that denaturation and aggregation temperatures of muscle proteins vary depending on the species under investigation. Furthermore, aggregation temperatures of muscle proteins are also influenced by experimental conditions, such as pH, heating rate, ionic strength (Wright and Wilding, 1984), and type and concentration of phosphate (Wang and Smith, 1994b).

Wu et al. (1991) reported that chicken breast myosin in 0.5 M NaCl, 10 mM sodium phosphate buffer, pH 7.0, started to gel at 43 °C and formed a weaker gel structure when heated above 55 °C. Sano et al. (1990) reported that gel elasticity in carp muscle myosin in 0.6 M KCl, 20 mM K phosphate buffer, pH 7.0, developed between 30 and 44 °C and that gelation was attributed to the rod portion of the myosin molecule. Wang and Smith (1994a) reported that gel elasticity in chicken breast muscle myosin in 0.6 M NaCl, pH 6.5, increased at 53 °C. There appears to be confusion over the temperature at which gelation occurs and the region of the myosin molecule involved in gelation at specific temperatures.

Few studies have been reported relating thermal aggregation of myosin and subfragments to thermal denaturation. Our objectives were to determine the temperature at which regions of the chicken breast muscle myosin molecule unfolded and subsequently

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aggregated during the gelation process. This was done by comparing DSC endotherms and aggregation profiles for myosin and subfragments. The T_m values of myosin subfragments were related to theoretically determined deconvoluted domains of myosin and used to identify the order in which domains become involved in myosin gelation.

MATERIALS AND METHODS

Chemicals. Papain, bovine pancreas α-chymotrypsin (type VII), and iodoacetate were purchased from Sigma Chemical Co. (St. Louis, MO). Other proteins and chemicals used were of the purest grade available.

Protein Purification. Breast muscle (*M. pectoralis*) myosin was extracted immediately after sacrifice from 12 week old commercial meat type broilers as described by Wang and Smith (1994c) and stored in $(NH_4)_2SO_4$ at −20 °C. The purified myosin was used to obtain subfragments. Rod was prepared as previously described by Bertazzon and Tsong (1989). LMM, HMM, and subfragment 1 (S-1) were purified following the procedures of Margossian and Lowey (1982). S-2 was purified according to the procedure of Sutoh et al. (1978). Myosin light chains (LC) and heavy chains (MHC) were obtained according to the procedure of Gaetjens et al. (1968) except purified myosin was dialyzed in 0.1 M glycine buffer, pH 11.1, 10 mM DTT, 10 mM EDTA, 2 mM ATP, 0.5 M KCl, and 2.0 M LiCl for 48 h at 4 °C with at least three changes of buffer. The dialyzed myosin solution was centrifuged at 78000*g* for 1 h at 4 °C (Beckman ultracentrifuge model L7-65, Beckman Instruments, Inc., Palo Alto, CA). The final washing step after MHC precipitation was eliminated to prevent loss of MHC, as purification was not improved. The supernatant contained the purified LC.

Prior to use, myosin or subfragments were suspended in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, and dialyzed against three changes of the same buffer for 48 h. The dialyzed protein solutions were centrifuged at 78000*g* for 1 h, to precipitate denatured protein. Protein concentrations were determined either by absorption at 280 nm or by the method of Lowry et al. (1951). The extinction coefficients ($E^{1\%}$ at 280 nm) were 5.5 for myosin (Swenson and Ritchie, 1980), 2.1 for rod (Hvidt et al., 1982), and 6.0, 3.0, 3.5, 8.3, and 0.7 for HMM, LMM, LC, S-1, and S-2, respectively (Margossian and Lowey, 1982).

Electrophoresis. Proteins were characterized by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) as described by Laemmli (1970) at acrylamide concentrations of 4% and 12% for stacking and resolving gels, respectively. The molecular weights of the protein bands were estimated by their relative mobilities as compared to those of standard molecular weight markers under the same electrophoretic conditions (Weber and Osborn, 1969). The gels were scanned with a GS 300 transmittance/reflectance scanning densitometer using GS 365W electrophoresis data system software (Hoefer Scientific Instruments, San Francisco, CA) to assess protein purity.

Thermal Stability. Thermal stability of myosin and subfragments was measured using a DSC (MC-2, Microcal Inc., Amherst, MA) with a scan rate of 1 °C/min as described by Wang and Smith (1994a). Myosin and MHC were examined at concentrations of 10.0 mg/mL, whereas LMM, rod, HMM, and S-1 were at 4.0 mg/mL, and LC and S-2 were at 2.0 mg/mL. Each protein was analyzed in duplicate. Heat capacity profiles (C_p vs temperature) were defined by a calorimetric enthalpy (ΔH_{cal}), a van't Hoff enthalpy (ΔH_{vH}), endothermic peak temperatures (T_m), and a cooperative ratio ($CR = \Delta H_{vH}/\Delta H_{cal}$) (Tsong et al., 1970; Privalov and Potekhin, 1986). Myosin endotherms were fitted into a minimum number of independent transitions, assuming a two-state unfolding process using software provided by the manufacturer, based on a least-squares fitting procedure described by Freire and Biltonen (1978).

Thermal Aggregation. Thermal aggregation of myosin and subfragments was followed by measuring the increase in absorbance at 340 nm in a Cary 3E UV/vis spectrophotometer (Varian Analytical Instruments, Sunnyvale, CA). Turbidity data was collected from 25 to 85 °C at a heating rate of 1 °C/min. The 2 mm path length cuvettes were covered with Teflon, to prevent evaporation. Myosin and MHC were at concentrations of 10.0 mg/mL, and HMM and S-1 were at 4.0 mg/mL, whereas LC, rod, S-2 and LMM were at concentrations of 2.0 mg/mL. Each protein was analyzed in triplicate.

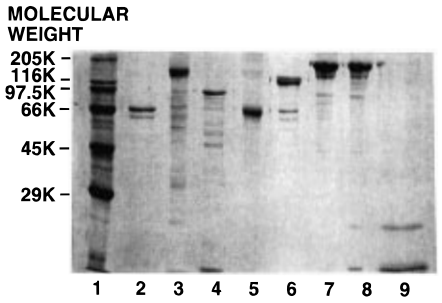

Figure 1. Electrophoretogram of myosin and subfragments characterized by sodium dodecyl sulfate−polyacrylamide gel electrophoresis: 1, high molecular weight standard; 2, LMM; 3, HMM; 4, S-1; 5, S-2; 6, rod, 7, MHC; 8, myosin; 9, LC. Gel lanes contained 5 μg of protein.

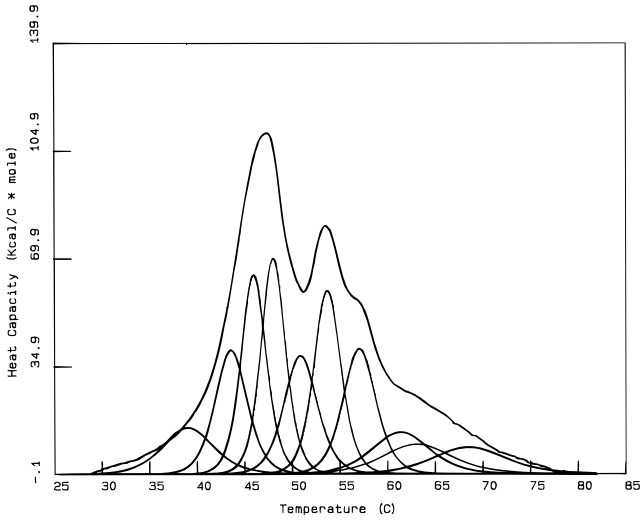

Figure 2. Heat capacity profile and deconvoluted peaks of 1% (w/v) chicken breast myosin in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, heated from 25 to 85 °C at 1 °C/min.

RESULTS AND DISCUSSION

Composition of Myosin and Subfragments. Composition of myosin and subfragments are shown in the SDS electrophoretogram (Figure 1). The presence of contaminating proteins may change the calorimetric profile. However, it was assumed that any contaminants present had little influence on the analysis, due to the low concentrations observed by SDS−PAGE. Myosin consisted of MHC with a molecular mass of 205 kDa and three LC of 16, 18, and 22 kDa (Starr and Offer, 1971). Two contaminating bands were observed; one was identified as C-protein and had a molecular mass of 140 kDa (Margossian and Lowey, 1982). The second band was not identified but had a molecular mass of 95 kDa. The MHC fraction had two high molecular mass bands of 205 and 140 kDa. The smaller band was contaminating C-protein. The rod had a molecular mass of *ca.* 120 kDa and a minor contaminating band at 70 kDa that probably was LMM (Margossian and Lowey, 1982). The LMM fraction had two bands of about 66 and 70 kDa. The 70 kDa band was LMM; the lower molecular mass band was probably a proteolytic fragment of LMM. S-2 consisted of a single band at 60 kDa (Sutoh et al., 1978). HMM had a

Table 1. Temperature and Calorimetric Enthalpy of Deconvoluted Chicken Breast Muscle Myosin Domains in 0.6 M NaCl, 50 mM Sodium Phosphate Buffer, pH 6.5, Heated from 25 to 85 °C at 1 °C/min[a]

peak	transition temp (°C)	enthalpy (kcal/mol)
1	39.6 ± 0.7	99.8 ± 11.7
2	43.7 ± 0.3	174.9 ± 5.8
3	46.2 ± 0.2	226.5 ± 3.1
4	48.1 ± 0.2	235.2 ± 6.1
5	50.9 ± 0.2	173.5 ± 7.9
6	54.0 ± 0.3	214.7 ± 14.4
7	57.4 ± 0.3	164.9 ± 32.4
8	61.5 ± 0.4	91.2 ± 14.0
9	63.1 ± 0.1	93.9 ± 0.9
10	67.2 ± 1.3	95.6 ± 7.4

[a] Mean ± standard deviation from two replicates.

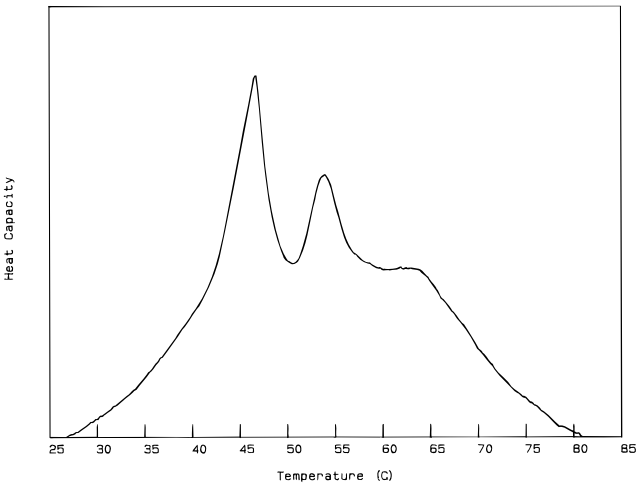

Figure 3. Differential scanning calorimetric endotherm of 1% (w/v) chicken myosin heavy chain in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, heated from 25 to 85 °C at 1 °C/min.

molecular mass of 140 kDa. S-1 had a molecular mass of 97 kDa (Margossian and Lowey, 1982). Traces of proteolytic fragments between 66 and 45 kDa were also observed in the S-1 preparation. The LC fraction contained three bands at 16, 18, and 22 kDa.

Thermal Stability and Aggregation of Myosin and Subunits. The enthalpy profile for myosin showed initial unfolding at 37 °C, with peaks at 47.5 and 54 °C and shoulders at 57.4 and 63.1 °C. The ΔH_{cal} of myosin denaturation was 1579.71 ± 106.7 kcal/mol. Deconvolution of the endotherm indicated that myosin could be reasonably fitted into 10 two-stage independent transitions or domains, each defined by a melting temperature (Figure 2). The ΔH_{cal} and T_m values for each transition were calculated (Table 1). Similar denaturation profiles for chicken breast myosin have been reported by Wang and Smith (1994a). It has been noted by several researchers that the transition occurring in myosin at 55 °C is the most important to gel formation, since gels do not attain appreciable gel strength until this temperature is reached (Ziegler and Acton, 1984; Wang and Smith, 1994a,c). Maximum elasticity of chicken breast muscle myosin in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, has been reported to occur at 65 °C (Wang and Smith, 1994c).

The endotherm of MHC was similar in shape to that of myosin (Figure 3). A large peak was noted at 46.4 °C, with a smaller one at 54.1 °C and a shoulder at 63.9 °C. The ΔH_{cal} for the MHC was 1296.64 ± 117 kcal/mol. The experimentally determined CR was <1, indicating that the MHC was also a multidomain protein. The absence of the shoulder at 57.4 °C observed

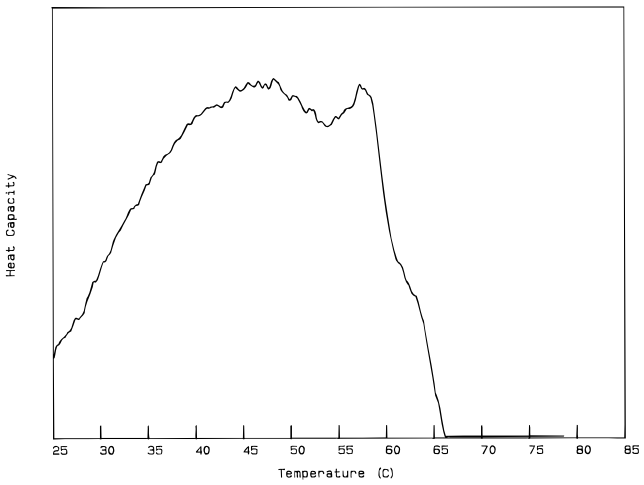

Figure 4. Differential scanning calorimetric endotherm of 0.2% (w/v) chicken breast muscle light chains in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, heated from 25 to 85 °C at 1 °C/min.

in myosin was attributed to the removal of the light chains, as the DSC endotherm for the purified light chains showed T_m values at 48.3 and 57.6 °C (Figure 4).

Myosin from vertebrate skeletal muscle contains two different types of LC, designated the alkali LC and the dithionitrobenzoate (DTNB) LC. Morita and Ogata (1991) found that at 35 °C, the alkali LC were partially dissociated from myosin, while the DTNB LC were not dissociated at 70 °C in 0.1 M NaCl, pH 6.0. This suggested that alkali LC are less heat stable than the DTNB LC. Thus, the first transition seen in the endotherm of purified LC at 48.3 °C was attributed to the alkali LC, whereas the DTNB light chains were probably responsible for the second transition at 57.6 °C. Bertazzon and Tsong (1989) reported a single transition at 51.1 °C for rabbit myosin LC in 0.5 M KCl, 1 mM EDTA, 20 mM potassium phosphate buffer, pH 7.0, at a heating rate of 1.5 °C/min. One reason for the difference in the results may be due to the different experimental conditions, as the temperatures at which transitions occur are directly dependent on heating rate, pH, ionic strength (Wright and Wilding, 1984), phosphate concentration (Wang and Smith, 1995), and the species from which the myosin was obtained (Xiong et al., 1987). The LC did not aggregate when heated from 25 to 85 °C, suggesting that the LC by themselves do not gel (Figure 5).

Differences in aggregation between myosin and MHC were observed (Figure 6). The MHC was less heat stable and more aggregated than intact myosin at 80 °C. Myosin aggregated in two stages. A rapid increase in absorbance was observed from 50 to 53 °C followed by a plateau from 54 to 59 °C. Another increase in turbidity occurred between 60 and 70 °C which was the point of optimum aggregation. The aggregation process for the MHC was continuous, beginning at 45 °C and reaching an optimum at 63 °C. These results suggest that LC influence the aggregation profile of myosin during heating. It is possible that the plateau from 54 to 59 °C seen during aggregation of myosin is due to the unfolding of the DTNB LC which had a T_m of 57.6 °C.

Myosin Subfragments. Myosin was hydrolyzed into smaller subfragments to determine at which temperature different regions of the MHC were denatured. Purified S-1 had an endothermic peak at 47.4 °C and an exothermic peak at 51 °C (Figure 7). Shriver and

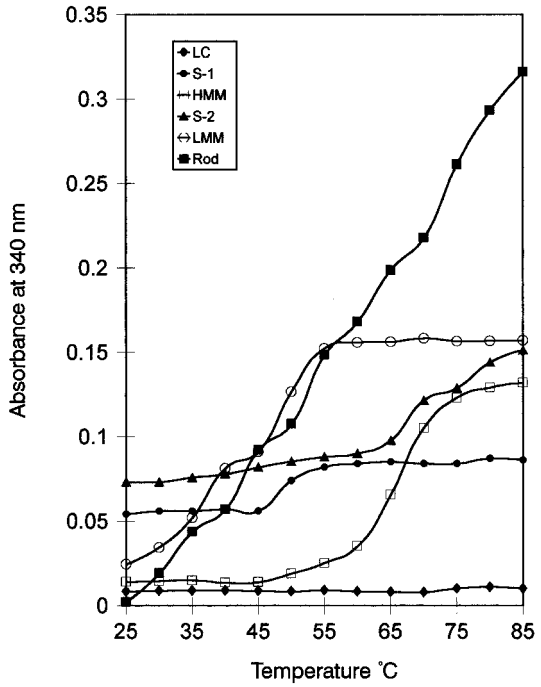

Figure 5. Turbidity profile of myosin subfragments in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5: S-1, 0.4% (w/v); HMM, 0.4% (w/v); LC, 0.2% (w/v); rod, 0.2% (w/v); LMM, 0.2% (w/v); S-2, 0.2% (w/v).

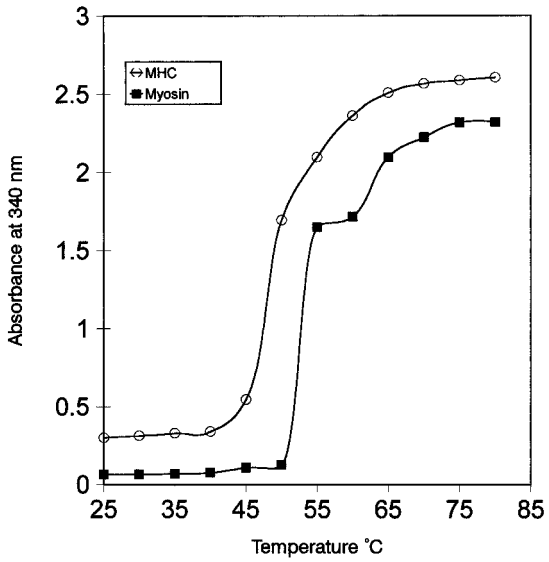

Figure 6. Effect of temperature on turbidity of 1% (w/v) chicken breast muscle myosin and MHC in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5.

Kamath (1990) observed an exothermic peak in purified rabbit myosin S-1 at 48 °C in 0.6 M KCl, pH 8.0, and attributed it to the aggregation and precipitation of the purified protein. The light chains were associated with S-1 (Figure 1), but T_m values were not observed at 48.3 °C (alkali LC) or 57.6 °C (DTNB LC). The T_m at 57.6 °C was not observed due to the low concentration of DTNB LC present in a 4 mg/mL sample of S-1. The T_m of S-1 at 47.4 °C was similar to that of the alkali LC, and both transitions showed up as a single peak. S-1 contributed partly to the largest peak in the myosin molecule endotherm which had a T_m of 47.4 °C. An increase in the aggregation profile of S-1 was observed from 45 to 54 °C, suggesting that the S-1 region contributed to the gelation of myosin in this temperature range (Figure 5).

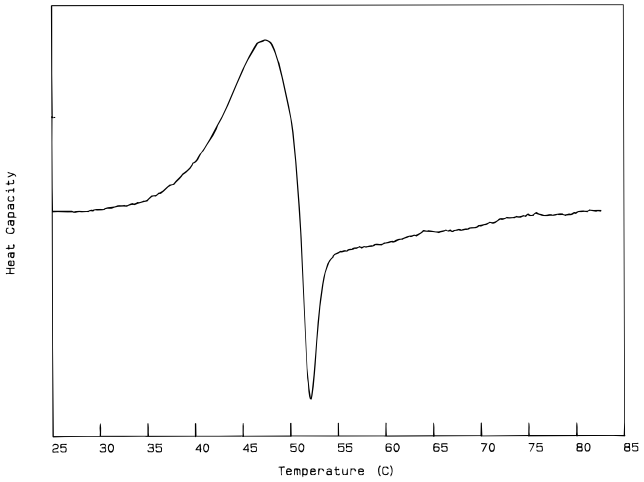

Figure 7. Differential scanning calorimetric endotherm of 0.4% (w/v) chicken breast muscle subfragment 1 in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, heated from 25 to 85 °C at 1 °C/min.

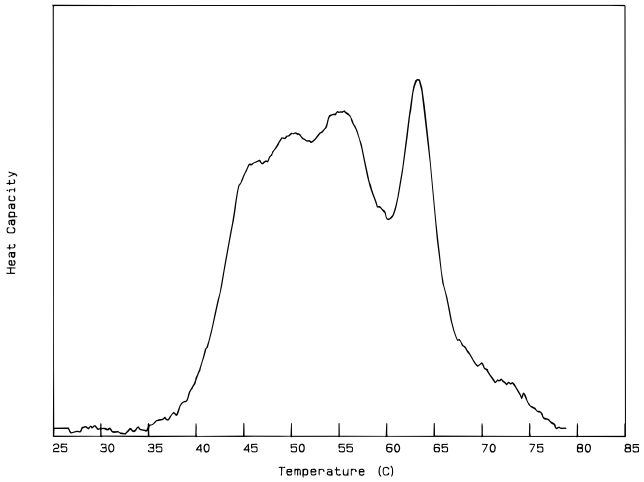

Figure 8. Differential scanning calorimetric endotherm of 0.4% (w/v) chicken breast muscle rod in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, heated from 25 to 85 °C.

The melting transition of the rod was multiphasic and took place between 30 and 63.3 °C, with four transitions at 44.5, 50, 55.7, and 63.3 °C (Figure 8). Multiphasic transitions are expected from coiled-coil proteins and suggest the existence of quasi independent melting domains within the structure (Bertazzon and Tsong, 1989). Hvidt et al. (1984) reported only slight unfolding of the rabbit muscle myosin rod over the temperature range of 1−43 °C, based on both electric birefringence and electric light scattering, which suggested that the rod was not involved in thermal unfolding at temperatures <43 °C. In contrast, our endotherm started to melt at 30 °C, and aggregation increased continuously from 30 to 85 °C (Figure 5). Thus, the rod was involved in gelation at temperatures <43 °C and probably had the biggest influence on the overall gelation process (Ziegler and Acton, 1984).

To determine unfolding transitions in the rod, LMM, S-2, and HMM were purified. These endotherms were simpler in feature compared to the rod and displayed broad melting transitions. LMM had a single peak with a T_m of 50 °C and a shoulder at 39.2 °C which corresponded to a theoretical peak in myosin (Figure 9). LMM began to aggregate at 25 °C and reached a peak maximum at 55 °C (Figure 5), which suggested that LMM was involved in the initial stages of myosin gelation.

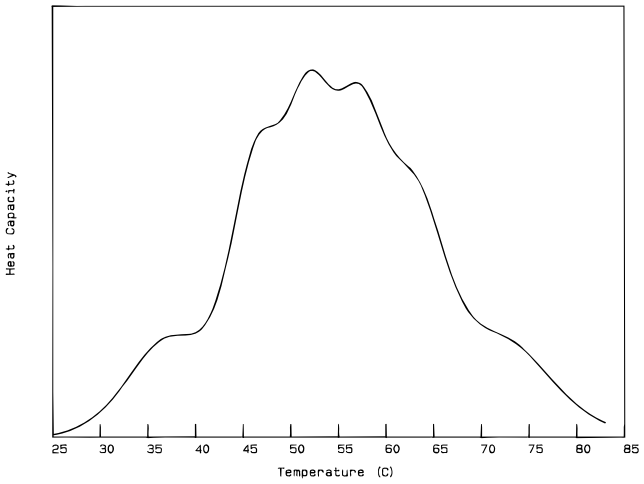

Figure 9. Differential scanning calorimetric endotherm of 0.4% (w/v) chicken breast muscle light meromyosin in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, heated from 25 to 85 °C at 1 °C/min.

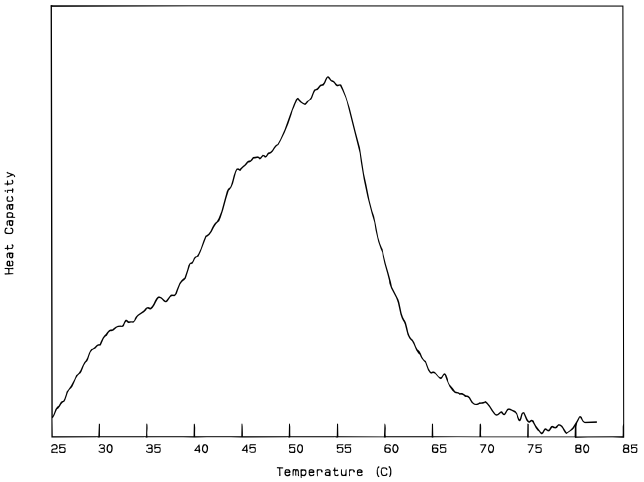

Figure 10. Differential scanning calorimetric endotherm of 0.2% (w/v) chicken breast muscle subfragment 2 in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, heated from 25 to 85 °C at 1 °C/min.

Several different T_m have been reported for rabbit skeletal muscle LMM. Wright and Wilding (1984) reported T_m values of 40 and 60.2 °C in 0.96 M KCl, pH 6.0. Samejima et al. (1983) reported T_m values of 45 and 56 °C in 0.6 M KCl, pH 7.0. Bertazzon and Tsong (1990) recorded values of 42, 45, 51, and 55 °C in 0.5 M KCl, pH 6.75. This wide variation in T_m illustrates the sensitivity of muscle proteins to changes in buffer conditions.

The S-2 region started to melt at 25 °C and reached a peak maximum at 54.1 °C, with shoulders at 46.8 and 50.9 °C (Figure 10). Bertazzon and Tsong (1990) reported T_m values for rabbit S-2 at 44, 49, and 56 °C in 0.5 M KCl at pH 6.43. The shoulder at 46.8 °C corresponded to a theoretical peak in the deconvoluted myosin and a peak in the MHC. The shoulder at 50.9 °C matched peaks in the deconvoluted myosin, rod, and LMM, illustrating that regions of both LMM and S-2 unfold at the same temperature. This observation prevented us from attributing the domain at 50.9 °C in the deconvoluted myosin molecule to a particular region in the rod. The S-2 domain at 54.1 °C was also identified in myosin and helical subfragments (MHC, rod, HMM) which contain the S-2 region (Table 2). S-2 aggregated from 60 to 85 °C.

Table 2. Transition Temperatures of Chicken Breast Myosin and Subfragments in 0.6 M NaCl, 50 mM Sodium Phosphate Buffer, pH 6.5, Heated from 25 to 85 °C at 1 °C/min[a]

	peak no.									
	1	2	3	4	5	6	7	8	9	10
myosin[b]	39.6 (0.7)	43.7 (0.3)	46.2 (0.2)	47.5 (0.2)	50.9 (0.2)	54.0 (0.3)	57.4 (0.3)	61.5 (0.4)	63.1 (0.1)	67.2 (1.3)
myosin				47.5 (0.2)		54.0 (0.3)	57.4 (0.3)		63.1 (0.1)	
MHC			46.4 (0.3)			54.1 (0.4)			63.9 (0.6)	
rod			44.5 (0.1)		50.0 (0.2)	55.7 (0.1)			63.3 (0.1)	
LMM	39.2 (0.1)				51.6 (0.6)					
HMM				47.5 (0.4)		54.8 (0.2)	58.6 (0.5)		62.7 (0.2)	
S-2			46.8 (0.3)		50.9 (0.1)	54.1 (0.2)				
S-1				47.4 (0.1)						
LC				48.3 (0.4)			57.6 (0.4)			

[a] Protein concentrations: 1.0% (w/v) myosin and 0.4% (w/v) LMM, subfragment 1 (S-1), heavy meromyosin (HMM), and rod; 0.2% (w/v) light chains (LC) and subfragment 2 (S-2). Transition temperatures are the mean ± standard deviation of two replicates. [b] Transition temperatures of deconvoluted chicken breast myosin domains.

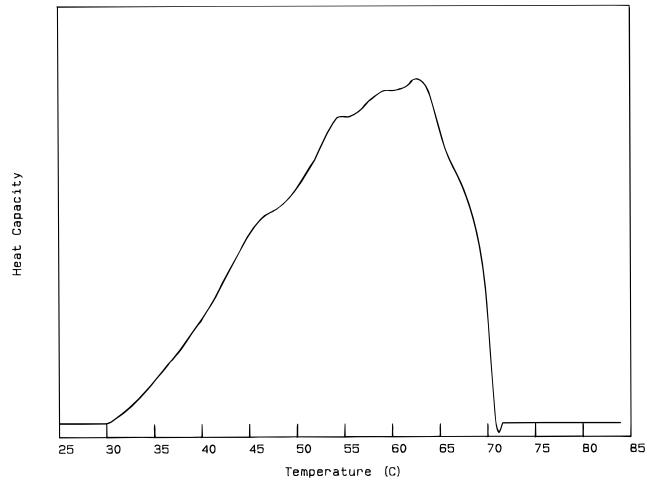

Figure 11. Differential scanning calorimetric endotherm of 0.4% (w/v) chicken breast muscle heavy meromyosin in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5, heated from 25 to 85 °C at 1 °C/min.

HMM started to melt at 25 °C and continuously unfolded until a peak maximum was reached at 62.7 °C. Shoulders were recorded at 47.5, 54.8, and 58.6 °C (Figure 11). The shoulder at 47.5 °C was attributed to the unfolding of the globular head and alkali LC (Table 2). The peak at 62.7 °C was assigned to the myosin rod, while the shoulder at 54.8 °C was assigned to the S-2 region. The DTNB LC were responsible for the shoulder at 58.6 °C (Table 2). Samejima et al. (1983) reported T_m values of 52.5 and 58 °C for rabbit muscle HMM in 0.6 M KCl, pH 7.0, whereas Wright and Wilding (1984) reported T_m values of 47 and 53 °C in 0.96 M KCl, pH 6.0. The aggregation profile for HMM showed a slight increase in absorbance from 45 to 60 °C, due to aggregation of the S-1 region. The largest increase in aggregation was observed between 60 and 75 °C, due to the aggregation of the helical region (S-2) of HMM (Figure 5).

Endotherms produced without deconvolution show the overall melting of a protein and do not indicate all the independent transitions taking place. All subfrag-

ments with the exception of S-1 had cooperative ratios <1 and were characterized as multidomain proteins. The transition at 39.2 °C was observed in deconvoluted myosin and attributed to the LMM region (Table 2), even though it was not observed in the rod or MHC. We were unable to associate the deconvoluted myosin peaks at 61.5 and 67.2 °C to a particular region of the molecule. It is possible that these peaks would be detected if the rod or MHC was deconvoluted. Bertazzon and Tsong (1990) reported six independent transitions for the deconvoluted rabbit myosin rod in 0.5 M KCl, pH 6.45.

The denaturation behavior of myosin was very complex. Since each thermal transition appeared to be associated with a discrete region of the myosin molecule, this behavior was interpreted in terms of the intrinsically different thermal stabilities (and therefore structures) of each region. It was concluded that thermal denaturation preceded thermal aggregation. The rod was the principal myosin subfragment involved in gelation. LMM and S-1 were responsible for denaturation and thermal aggregation below 55 °C, while S-2 unfolded and aggregated primarily above 55 °C. The alkali LC were less heat stable than the DTNB LC but did not aggregate, suggesting that the LC themselves do not gel. However, when associated with MHC, they influenced the thermal stability and aggregation profile of myosin. By manipulating time- and temperature-processing parameters to selectively alter myosin domain unfolding, the meat industry can enhance texture and yield of comminuted and reformed meat products.

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