Denaturation and Aggregation of Myosin from Two Bovine Muscle Types

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The thermal behaviors of myosin from bovine vastus intermedius (VI, predominantly red muscle) and semimembranosus (SM, predominantly white muscle) at pH 6.05 (ultimate pH of VI muscle) and 5.50 (ultimate pH of SM muscle) were compared. Differential scanning microcalorimetry and turbidity measurements were used to monitor changes in myosin during heating from 25 to 80 °C at 1 °C/min. VI and SM myosin heavy chain isoforms were identified on gradient SDS-PAGE. Endotherms of VI myosin at pH 6.05 had three transition temperatures (T_m) of 45, 53, and 57 °C, whereas at pH 5.50 two transitions were observed at 42 and 59 °C. SM myosin had two T_m values of 46 and 58 °C at pH 6.05 and T_m values of 43 and 62 °C at pH 5.5. SM myosin at its ultimate pH was less heat stable than VI myosin at its ultimate pH; however, when SM and VI myosin were compared at the same pH, VI myosin was less stable.

Keywords: *Myosin; bovine; ultimate pH; fiber type; denaturation; aggregation*

INTRODUCTION

Bovine muscles are often classified by fiber type based on twitch speed (slow or fast), appearance (red or white), or metabolism (oxidative or glycolytic) (*1*). Common adult bovine skeletal muscle types are I (slow, oxidative, red), IIb (fast, glycolytic, white), and IIa (fast, oxidative, intermediate) (*2*). Most bovine muscles comprise a mixture of fiber types, although a few muscles have a high percentage of a particular fiber type. Different isoforms of both the heavy and light chains of myosin are found in the various muscle types.

Heat-induced gelation is one of the most important functional properties of muscle myofibrillar proteins and is responsible for the characteristic texture, juiciness, and yields of many processed meat products. The fiber type and the ultimate pH of the muscle have a large influence on the functional properties of the protein during processing. Numerous researchers have reported differences in the quality of products made from light (white) and dark (red) meat, although the reasons for these differences are poorly understood (*3*). Many of these differences have been attributed to myosin isoform; however, the results have been confounded by the presence of many other muscle proteins within the test system and by the lack of pH control or the use of only a single pH (*1*).

Thermally induced protein gelation occurs in three steps: unfolding, aggregation, and gel matrix formation. The properties of the final gel are dependent on the rates of protein unfolding and aggregation (*4*). A filamentous gel structure results if the rate of aggregation is slow relative to the rate of unfolding. If aggregation occurs rapidly, a coarser gel matrix is produced.

Myosin is the primary gelling protein in muscle. Although the overall contribution of myosin light chains to gel properties is very small, the influence of myosin heavy chain on gelation properties is significant (*5*).

Differences have been reported in the thermal stability, aggregation properties, and gelation properties of myosin isolated from red and white muscles of the same species. In fact, Stabursvik and Martens (*6*) reported that myosin from the same fiber type, but different animal species, had more similar denaturation properties than did myosin from red and white muscles of the same species. Myosin from white muscle denatures and aggregates at a lower temperature and forms a more rigid and elastic gel than myosin from red muscle at pH ≥6.0 in 0.6 M NaCl or KCl (*7*−*10*). This effect is independent of species. Liu et al. (*9*) reported that differences in rheological properties of red and white chicken skeletal muscle myosin gels disappeared upon cooling.

Another possible cause of variation in gelation properties of myosin extracted from muscle of different fiber types is pH. Red muscle has a higher ultimate pH than does white muscle (*11*) due to the differential accumulation of metabolic byproducts, such as lactic acid (*12*), suggesting that myosin isoforms may respond differently to low pH environments. In fact, bovine cutaneus trunci (fast, white) myosin was more soluble and less turbid at pH 5.5 than masseter (slow, red) myosin in 0.6 M NaCl, 0.04 M phosphate buffer, although solubility and turbidity were similar at pH 6.0 (*10*). In the same study, red myosin gels had greater elasticity and rigidity than gels from white myosin at pH 5.8. Conversely, chicken breast myosin gels have been reported to have higher rigidity than leg myosin gels between pH 5.2 and 6.0 in 0.6 M KCl (*13*, *14*). Thus, the effect of pH on the gelation properties of myosin isoforms is not clear. The objective of this research was to study the contribution of isoform and pH to the thermally induced unfolding and aggregation properties of myosin extracted from two bovine muscles, semimembranosus

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(SM, comprising primarily white fibers) and vastus intermedius (VI, comprising primarily red fibers).

MATERIALS AND METHODS

Myosin Extraction. Myosin was extracted from pre-rigor SM and VI muscles of three heifers between 16 and 24 months of age as described by Swartz et al. (*15*), except myosin was precipitated in 41% $(NH_4)_2SO_4$ in the final purification step. The myosin pellet was collected by centrifugation at 8000*g* for 20 min and stored in 10% glycerol at −20 °C for future use. Purification yields were 6−8 and 10−15% of original muscle weight for SM and VI myosin, respectively.

Myosin was dissolved in 0.6 M NaCl, 0.05 M phosphate buffer (pH 6.05 or 5.50), 1 mM EDTA, and dialyzed against three changes of buffer before use. These two treatments were selected as pH 6.05 is the ultimate pH of bovine VI muscle and pH 5.50 is the ultimate pH of bovine SM muscle (*16*). Myosin was then dialyzed two more times against the same buffer, but without EDTA, and then centrifuged at 78000*g* for 1 h (model L7-65, Beckman Ultracentrifuge, Palo Alto, CA) to precipitate denatured protein. The concentration of myosin in the supernatant was determined using an extinction coefficient of $E^{1\%} = 5.5$ at 280 nm (*17*).

Electrophoresis. To determine the purity of the myosin, a Mini-Protean II dual slab cell (Bio-Rad Laboratories, Richmond, CA) was used for sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (*18*). The resolving and stacking gels contained 10 and 4% acrylamide, respectively. Molecular weight standards (12 μg, SDS-6H, Sigma Chemical Co., St Louis, MO), commercial bovine skeletal muscle myosin (5 μg, M-6643, Sigma Chemical Co.), or extracted myosin (6 μg) in a volume of <10 μL were loaded into the sample well using a syringe. The gels were run at constant voltage (200 V) for ∼50 min and then stained for 30 min with 0.25% Coomassie Brilliant Blue R250 in acetic acid/methanol/water (9:45:45, v/v/v) solution. Mobility of the extracted myosin was compared to that of the myosin standard. The molecular weights of the protein bands were estimated by their relative mobilities and compared to that of the standard molecular weights under the same electrophoretic conditions (*19*).

Electrophoresis of Myosin Isoforms. Gradient SDS-PAGE was used to visualize the myosin heavy chain isoforms present in each muscle. A 5−8% acrylamide gradient and a 30−40% glycerol gradient were used as described by Sugiura and Murakami (*20*). The stacking gel was 3.5% acrylamide with 35% glycerol.

Commercial bovine skeletal muscle myosin (Sigma) and extracted VI and SM myosin were loaded at 500 ng per well. Gels were run at 50 mV for 5 h, at 100 mV for 2 h, and then increased to 150 mV until the tracking dye reached the bottom of the gel. The electrophoretic cells were surrounded with ice during the run. Due to the length of the run (∼8 h), cold electrode buffer was added every 90 min to maintain the correct volume.

Gels were stained with Coomassie Brilliant Blue R250 in acetic acid/methanol/water (9:45:45, v/v/v) solution. Duplicate gels were also stained using a silver stain kit (161-0443, Bio-Rad). The mobility of the myosin heavy chain was compared with that of the myosin standard and literature reports of myosin isoform separations (*21*, *22*).

Western Blot Analysis. Myosin isoforms were identified by Western blot analysis. Proteins were transferred electrophoretically from SDS-PAGE gradient gels onto a nitrocellulose membrane using a Mini Trans-Blot unit (Bio-Rad) for 1.5 h at 100 V. When the transfer was complete, each membrane was washed twice with 0.15 M NaCl, 0.05 M phosphate buffer, pH 7.2, containing 0.02% (v/v) Tween 20 (PBS-Tween), then blocked with 10 mL of filtered 3% egg albumin (Sigma) in PBS (OV-PBS) for 30 min at ambient temperature, and rinsed with PBS-Tween. Membranes were incubated with three different antibodies: anti-myosin skeletal (M7523, polyclonal, Sigma), anti-myosin skeletal fast (M4276, monoclonal, Sigma), and anti-myosin skeletal slow (RPN.1168, Amersham, Arlington Heights, IL). Membranes were incubated for 30 min at ambient temperature with 10 mL of the appropriate antibody diluted 1:10 or 1:20 in OV-PBS.

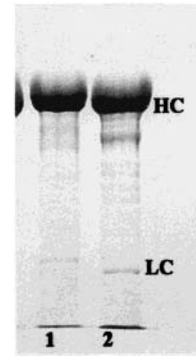

Figure 1. Bovine SM (lane 1) and VI (lane 2) myosin in an SDS-PAGE gel. HC, heavy chain; LC, light chains.

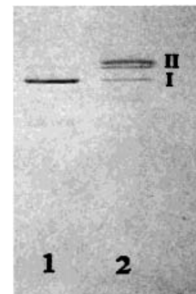

Figure 2. Heavy chain isoforms (I, II) of bovine SM and VI heavy chain myosin in a gradient sodium dodecyl sulfate−polyacrylamide (5−8%)/glycerol (30−40%) electrophoretic gel: (lane 1) VI myosin; (lane 2) SM myosin.

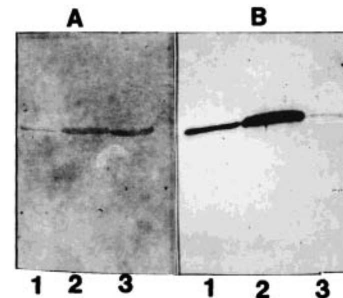

Figure 3. Western blot of bovine SMand VI myosin heavy chain visualized using (A) anti-myosin polyclonal antibodies or (B) anti-fast myosin monoclonal antibodies: (lane 1) bovine myosin standard; (lane 2) SM; (lane 3) VI.

PBS-Tween was used to remove unbound antibody from the membrane, and then 10 mL of goat anti-rabbit (for polyclonal 1:2000) and anti-mouse (for monoclonal 1:500) IgG peroxidase conjugate (Organdon Teknika, Durham, NC) diluted in OV-PBS was added to the membrane and incubated at ambient temperature for 10 min. Membranes were washed with OV-PBS, and bound peroxidase activity was determined (*23*).

Differential Scanning Calorimetry (DSC). A differential scanning microcalorimeter (MC-2, Microcal Inc., Amherst, MA) was used to measure the thermal stability of SM and VI myosin at a concentration of 10 mg/mL as described by Wang and Smith (*24*). Buffer solutions were run before each protein run to obtain a baseline for calculations. VI and SM myosins from three different extractions were each tested at pH 5.50 and 6.05 during heating from 25 to 80 °C at a rate of 1 °C/min. The following parameters were obtained from the heat capacity profile: initial transition temperature (T_o), endothermic peak temperatures (T_m), calorimetric enthalpy (ΔH_{cal}), and van't Hoff enthalpy (ΔH_{vH}).

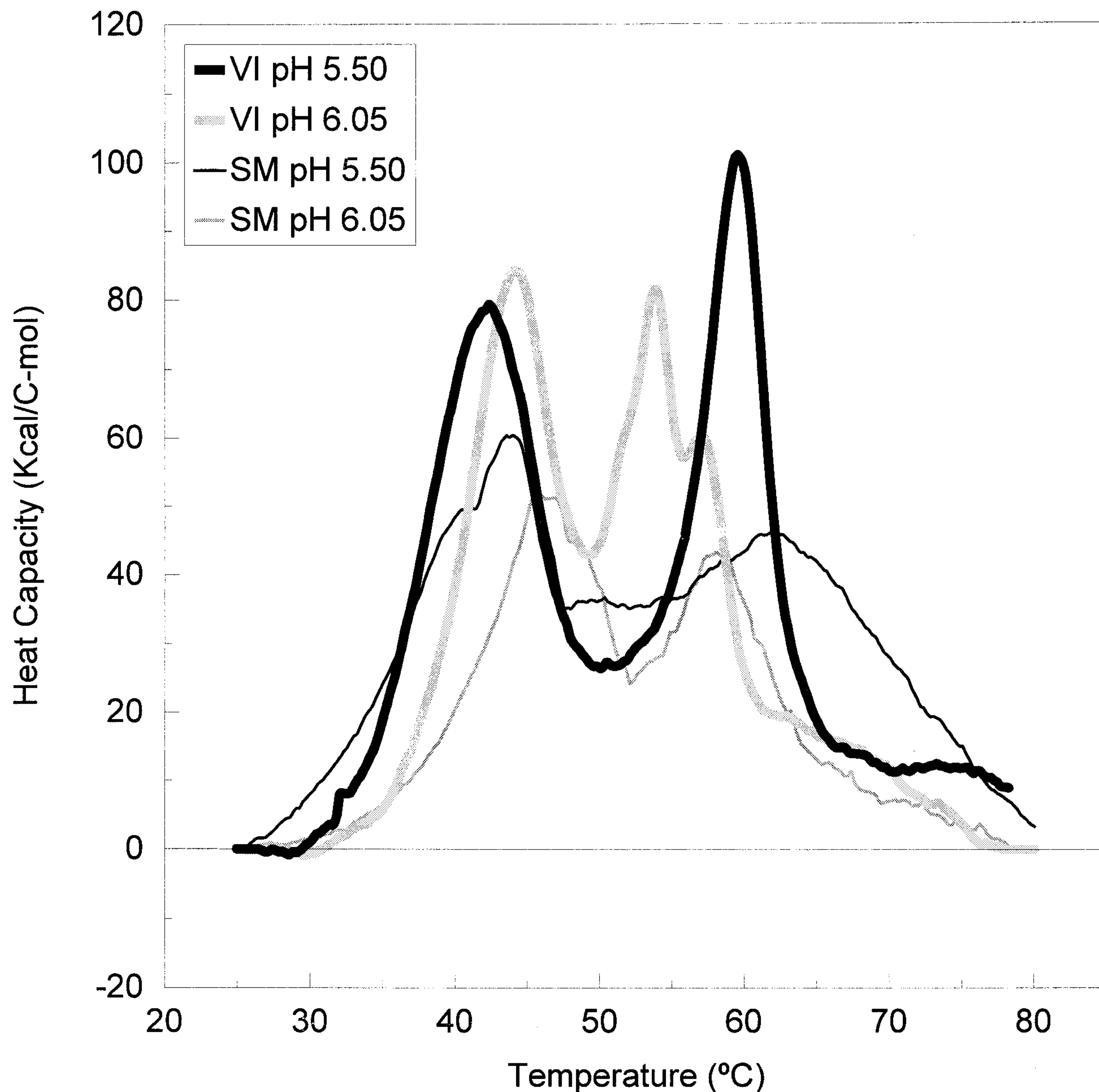

Figure 4. DSC endotherms of bovine SM and VI myosin (10 mg/mL) in 0.6 M NaCl, 0.05 M sodium phosphate buffer at pH 5.50 and 6.05. Scan rate was 1 °C/min.

Each curve was deconvoluted to estimate the minimum number of independent two-state transitions ($\Delta H_{cal} = \Delta H_{vH}$) during the unfolding of myosin (*25*, *26*) using DA-2 Data Acquisition and Analysis software (Microcal, Inc). A nonlinear least-squares minimization method was used iteratively until the square sum of residual was $<10^{-10}$.

Turbidity. Protein aggregation of myosin (5 mg/mL) during heating from 25 to 80 °C at a rate of 1 °C/min was followed by measuring the increase in absorbance at 340 nm in a Cary 3E UV–vis spectrophotometer (Varian Analytical Instruments, Sunnyvale, CA) equipped with a temperature controller and multicell block. Preliminary experiments with 2.5, 5, and 10 mg/mL myosin solutions showed that 5 mg/mL gave the best absorbance range during the heating period. Turbidity of VI and SM myosin solutions at pH 5.50 ± 0.10 and 6.05 ± 0.05 was measured. Data were transferred to Excell (ver. 5.0, Microsoft Corp., Redmond, WA), and first-derivative analysis was performed. The aggregation onset temperature (defined as the lowest temperature at which a change in absorbance of 0.05/°C was recorded), maximum aggregation rate, temperature at the maximum aggregation rate, and the maximum aggregation temperature were determined from the differential change in absorbance as a function of temperature.

Experimental Design and Statistical Analysis. A completely randomized design with three replications was used for DSC and turbidity measurements. A two-way ANOVA was performed to test the significance between myosin isoforms and pH (*27*). Tukey's test was used to test the significant difference among calorimetric enthalpy means at $P < 0.05$.

RESULTS AND DISCUSSION

Characterization of Myosin. Myosin was separated by SDS-PAGE to evaluate purity (Figure 1). One major band corresponding to myosin heavy chain at ~205 kDa and other bands at ~20 kDa, corresponding to myosin light chains, were identified. When the gel was overloaded with protein, a minor contaminating band at ~150 kDa was identifed as C-protein (*28*).

Myosin heavy chain isoforms from VI and SM myosin preparations were separated using gradient PAGE (Figure 2). The commercial bovine skeletal myosin standard contained a slower migrating doublet, identified as IIa and IIb, and a faster migrating band

identified as type I myosin (*21*). The VI myosin preparation contained one major band with the same mobility as the faster band of the myosin heavy chain standard, suggesting this preparation contained primarily type I myosin heavy chain. A faint band at the location of types IIa and IIb myosin was also observed. SM myosin contained three heavy chain bands. The fastest band was present in SM myosin in lower concentrations as compared with the other two bands, suggesting SM myosin contained primarily types IIa and IIb, with a smaller amount of type I myosin.

Western blotting with three antibodies that recognize the heavy chain of myosin (anti-myosin, anti-fast myosin, and anti-slow myosin) was performed to confirm these results. As expected, anti-myosin antibody reacted with all heavy chain bands from VI and SM myosin Figure 3A). The anti-fast myosin reacted with the SM myosin but only weakly with VI heavy chain myosin (Figure 3B), confirming that VI myosin contained only a small amount of the fast isoform. The anti-slow myosin antibody was prepared against rat protein and had very poor affinity for bovine muscle. Even so, a weak reaction with the VI myosin was noted (data not shown), indicating the presence of the slow myosin heavy chain isoform.

Thermal Stability of Myosin. DSC was used to investigate the influence of pH on the endothermic transitions of myosin from bovine VI (red) and SM (white) muscles. The calorimetric enthalpies (ΔH_{cal}) of the VI and SM myosin preparations at pH 5.5 did not differ and were ~255−550 kcal/mol greater than the ΔH_{cal} at pH 6.05, indicating both myosin preparations had greater conformational stability at the lower pH. The isoelectric point of myosin is ~5.3. Thus, the formation of myosin oligomers at pH 5.5 may have helped to stabilize myosin against thermally induced denaturation (*29*). Results are in contrast to those of Egelandsdal et al. (*7*), who reported that bovine cutaneous trunci (white) and M. masseter (red) had greater enthalpies at pH 6.0 than at pH 5.5 in 0.6 M NaCl, with the highest enthalpy being observed in white myosin at pH 6.0. The ΔH_{cal} of VI myosin at pH 6.05 was 1496 kcal/mol and was similar to that reported for broiler gastrocnemius (leg) myosin (1588 kcal/mol) at pH 6.0 (*9*).

The endotherms of VI and SM myosin were different and showed multiple transitions at pH 5.50 and 6.05 (Figure 4) indicative of a protein with multiple unfolding domains (*24*). At pH 5.50, VI myosin had sharp transition peaks at 42 and 59 °C, whereas SM myosin had broad short peaks. The heat capacity profile of SM myosin, pH 5.5, showed two peaks at 43 and 62 °C with two shoulders at 41 and 58 °C. Although the first major transition occurred at about the same temperature for both myosin preparations at pH 5.50, the second major transition occurred ~3 °C higher for SM when compared to VI myosin.

At pH 6.05, VI myosin had three transition peaks at 44, 53, and 57 °C. The endothermic curve of SM at 6.05 showed a broad peak at 46−47 °C with a shoulder at 48 °C and another broad peak at 58 °C. The first major transition occurred at a lower temperature for VI myosin than SM myosin at pH 6.05, whereas the final transition occurred at about the same temperature.

The heat capacity profiles indicated that the thermal transitions of VI and SM myosin occurred over a wider temperature range at pH 5.50. The first myosin transition peak occurred at a lower temperature and the final major peak occurred at a higher temperature at pH 5.50, when compared to transitions at 6.05 in both VI and SM myosin. Egelandsdal et al. (*7*) found the same trend with pH for bovine masseter (red) and cutaneous trunci (white) myosin.

The endotherms of each protein were deconvoluted into a minimum number of independent two-state transitions. At both pH values, VI and SM myosins were found to contain 10 domains or regions of the molecule that unfolded independently during heating, agreeing with results reported for the unfolding of chicken breast muscle myosin in 0.6 M NaCl, pH 6.5 (*24*, *30*). The transition temperature and enthalpy of each domain of VI (Table 2) and SM (Table 3) myosin were determined. The transition temperatures of VI and SM myosin domains were similar at the same pH. As the pH was decreased from 6.05 to 5.50 the transition temperatures of the first four domains decreased, whereas the transi-

Table 1. Calorimetric Enthalpy, Onset Transition Temperature (T_0), and Peak Transition Temperatures (T_{1-3}) of Bovine SM and VI Myosin (10 mg/mL) Endotherms Determined by DSC in 0.6 M NaCl, 0.05 M Phosphate Buffer, pH 5.50 or 6.05[a]

muscle	pH	enthalpy (kcal/mol)	transition temp (°C) T_0	T_1	T_2	T_3
SM	5.50	1972 ± 321[a]	25 ± 1.7	43 ± 0	62 ± 0.1	
SM	6.05	1416 ± 503[b]	31 ± 0	46 ± 0.2	58 ± 0	
VI	5.50	1744 ± 82[a]	30 ± 1.3	42 ± 0.4	59 ± 0.9	
VI	6.05	1496 ± 57[b]	32 ± 0	43 ± 0.1	53 ± 0.5	57 ± 0.1

[a] Mean ± standard deviation from three replicates. Means followed by the same superscript are not different ($P > 0.05$).

Table 2. Transition Temperature (T_o) and Calorimetric Enthalpy of Deconvoluted Bovine VI Myosin Domains in 0.6 M NaCl, 0.05 M Phosphate Buffer, pH 5.50 or 6.05, When Heated from 25 to 80 °C at 1 °C/min[a]

peak	pH 5.50 T_o (°C)	pH 5.50 enthalpy (kcal/mol)	pH 6.05 T_o (°C)	pH 6.05 enthalpy (kcal/mol)
1	35.0 ± 3.0	123.9 ± 13.3	40.0 ± 0.8	116.0 ± 3.2
2	39.0 ± 1.5	171.8 ± 10.8	43.2 ± 0.9	154.7 ± 10.1
3	41.9 ± 0.9	193.1 ± 10.9	45.1 ± 0.8	148.4 ± 23.7
4	44.4 ± 0.5	185.6 ± 22.5	46.8 ± 2.0	162.2 ± 7.1
5	48.4 ± 0.4	141.6 ± 20.6	48.4 ± 0.6	127.8 ± 14.5
6	53.4 ± 0.4	151.7 ± 23.7	52.5 ± 1.0	162.1 ± 61.1
7	57.1 ± 0.5	196.4 ± 19.2	54.1 ± 0.6	181.3 ± 64.3
8	59.7 ± 0.2	236.7 ± 28.4	57.7 ± 0.2	195.6 ± 6.8
9	63.7 ± 1.6	151.3 ± 14.5	64.2 ± 1.1	125.5 ± 7.4
10	72.5 ± 1.5	120.1 ± 11.9	70.7 ± 1.3	110.7 ± 9.7

[a] Mean ± standard deviation of three replicates.

Table 3. Transition Temperature (T_o) and Calorimetric Enthalpy of Deconvoluted Bovine SM Myosin Domains in 0.6 M NaCl, 0.05 M Phosphate Buffer, pH 5.50 or 6.05, When Heated from 25 to 80 °C at 1 °C/min[a]

peak	pH 5.50 T_o (°C)	pH 5.50 enthalpy (kcal/mol)	pH 6.05 T_o (°C)	pH 6.05 enthalpy (kcal/mol)
1	33.6 ± 3.0	130.3 ± 18.8	40.3 ± 2.2	114.4 ± 49.1
2	38.5 ± 0.4	183.2 ± 23.3	43.3 ± 1.6	134.1 ± 64.8
3	42.0 ± 0.1	204.2 ± 30.9	46.1 ± 0.6	186.4 ± 42.5
4	44.8 ± 0.2	203.0 ± 16.1	49.2 ± 0.9	138.6 ± 61.9
5	49.4 ± 0.2	181.3 ± 19.1	50.2 ± 1.6	107.2 ± 82.4
6	53.9 ± 0.3	179.0 ± 19.0	51.8 ± 2.6	133.9 ± 54.0
7	58.3 ± 0.3	190.5 ± 18.1	56.2 ± 0.7	166.9 ± 43.6
8	62.5 ± 0.2	199.1 ± 16.7	59.1 ± 0.8	188.0 ± 48.7
9	66.9 ± 0.2	186.2 ± 18.1	62.7 ± 1.7	135.6 ± 39.4
10	72.3 ± 0.4	152.1 ± 17.0	68.5 ± 2.8	101.5 ± 25.2

[a] Mean ± standard deviation from three replicates.

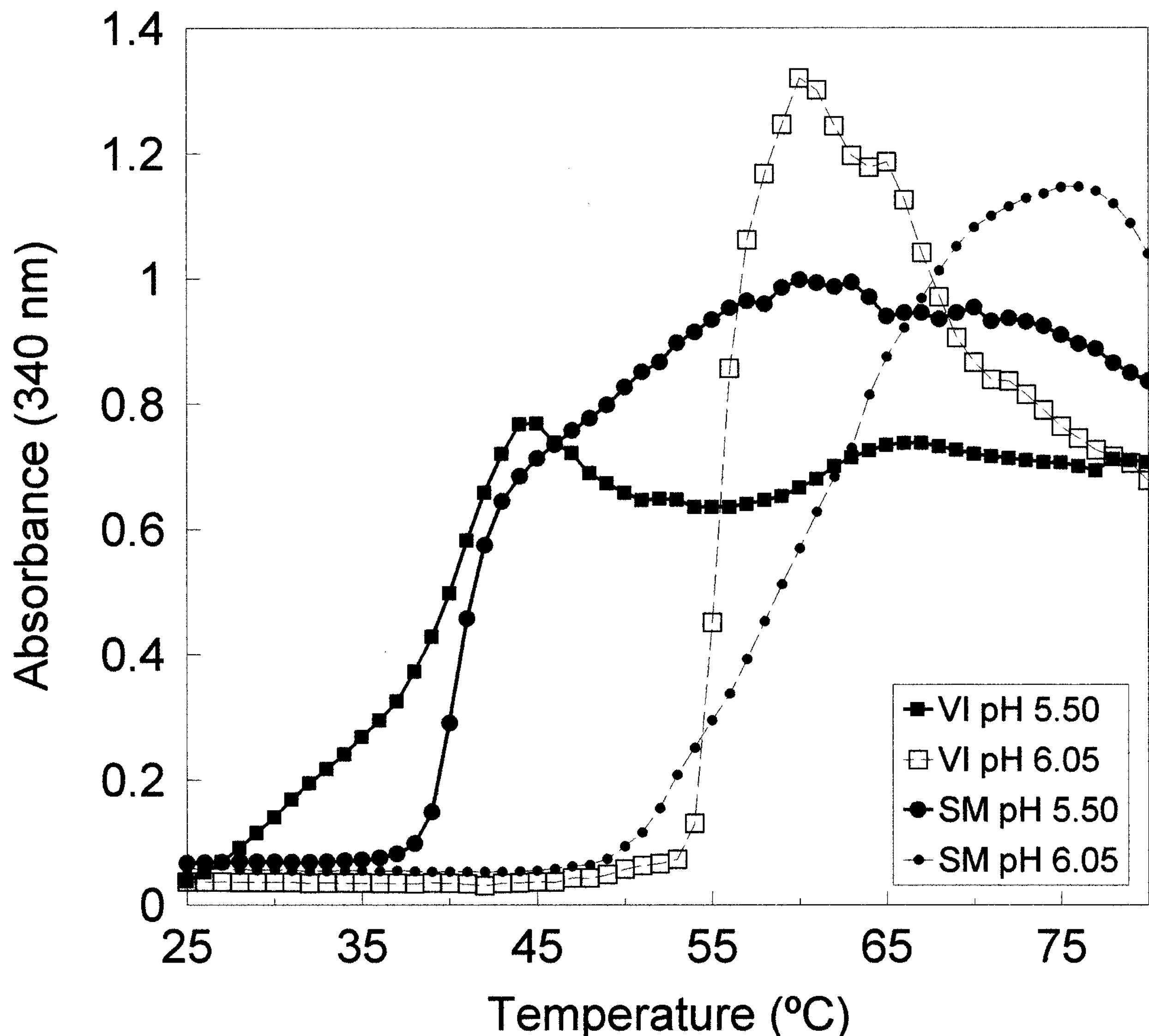

Figure 5. Turbidity (340 nm) of bovine SM and VI myosin (5 mg/mL) in 0.6 M NaCl, 0.05 M sodium phosphate buffer, pH 5.50 and 6.05, heated from 25 to 80 °C at a rate 1 °C/min.

Table 4. Aggregation Properties of Bovine SM and VI Myosin (5 mg/mL) during Heating from 25 to 80 °C at 1 °C/min in 0.6 M NaCl, 0.05 M Phosphate Buffer, pH 5.50 or 6.05[a]

muscle	pH	onset temp (°C)	max aggregation rate (A_{max}) (absorbance/min)	temp at A_{max} (°C)	max absorbance at 340 nm	temp of max absorbance (°C)	absorbance at 80 °C
SM	5.50	38.8 ± 0.5	0.18 ± 0.0	40.5 ± 0.6	1.03 ± 0.12	62.5 ± 6.6	0.83 ± 0.10
SM	6.05	50.0 ± 1.0	0.07 ± 0.01	58.0 ± 1.4	1.35 ± 0.04	75.5 ± 0.6	1.04 ± 0.03
VI	5.50	28.0 ± 0.0	0.09 ± 0.01	41.5 ± 0.6	0.88 ± 0.11	52.0 ± 11.1	0.71 ± 0.31
VI	6.05	55.3 ± 1.0	0.44 ± 0.07	55.5 ± 0.6	1.15 ± 0.08	62.0 ± 2.2	0.68 ± 0.08

[a] Mean ± standard deviation from three replicates.

tion temperatures of the last five domains increased in both myosin preparations. The pH did not affect the thermal transition of the fifth domain. Overall, the thermal transitions of the first four domains were more sensitive to changes in pH than the last five domains, as a greater average change in transition temperature was observed. Smyth et al. (*30*) attributed the thermal transitions of the first three domains to the unfolding of the rod region of the myosin molecule, which is highly influenced by pH (*31*).

At the same pH, fewer differences were observed in the domain transition temperatures between the myosin types. When SM and VI myosins were compared at pH 5.50, there was a <2 °C difference in transition temperature in 8 of the 10 domains. Domains 8 and 9 had higher transition temperatures in SM as compared to VI myosin. At pH 6.05, only three domains differed by >2 °C, domains 4, 7, and 10, suggesting that the transition temperatures of myosin were more affected by pH than isoform.

Protein Aggregation. Protein aggregation of VI and SM myosin was followed by measuring the increase in turbidity during heating (Figure 5). The initial aggregation temperature for both SM and VI myosin was lower at pH 5.50 than at pH 6.05 and may be due to oligomer formation as the pH approaches the isoelectric point of myosin (~5.3). Aggregation of VI myosin began at 28 °C, 11 °C below that of SM myosin at pH 5.50 (Table 4). At pH 5.50, the maximum aggregation rate of both myosins occurred at ~41 °C. At pH 6.05, SM and VI

myosin did not begin to aggregate until 50 and 55 °C, respectively. The temperatures at which the maximum aggregation rate was recorded (56−58 °C) were similar for SM and VI myosins at pH 6.05 and were at least 15 °C higher than those observed at pH 5.50.

SM myosin had a higher turbidity than VI myosin at the temperature of maximum turbidity and at the end of the heating cycle (80 °C) at both pH 5.50 and 6.05, indicative of the presence of a greater number of aggregates and/or larger aggregates in the SM preparations. Maximum turbidity was observed at 62 °C for VI myosin at pH 6.05, which was the same temperature of maximum turbidity observed for SM myosin at pH 5.50. SM myosin did not demonstrate maximum turbidity until heated to 76 °C at pH 6.05.

Relationship between Unfolding and Aggregation. Differences in the DSC endotherms and aggregation properties indicated that pH influenced the unfolding and aggregation properties of both SM and VI myosins. Initial increases in heat capacity and the temperature at which turbidity was first observed to increase were lower at pH 5.50 for both myosin preparations and may be due to aggregation of myosin near its isoelectric point. The gelation onset temperature for VI and SM salt soluble proteins was also lower at pH 5.50 than at pH 6.05 (*32*). At pH 6.05, the initial change in heat capacity occurred ∼19−23 °C before an increase in turbidity was observed. At pH 5.50, the initial changes in both heat capacity and turbidity occurred almost simultaneously for VI myosin, but a lag time of 13 °C was observed for SM myosin. The rate of protein aggregation relative to the rate of denaturation influences the degree of organization in a gel structure (*4*). Gels prepared from VI and SM myosin should have different rheological properties at each pH, agreeing with results previously reported for salt soluble proteins of prepared from VI and SM muscle (*16*).

The maximum aggregation rate occurred during the unfolding of domain 3 for both VI and SM myosin at pH 5.5, suggesting that the denaturation of domains in the rod region of myosin was very important (*30*). At pH 6.05, the maximum aggregation rate occurred after the unfolding of domain 7 for both myosin preparations, indicating that most of myosin domains were unfolded prior to aggregation. Thus, denaturation of LMM, subfragments 1 and 2 were necessary prior to the formation of aggregates at pH 6.05 (*30*).

Taken together, these results suggest that different gel structures will be formed due to differences in the mechanisms of myosin unfolding and aggregation caused by isoform and pH. In general, pH caused greater differences than isoform in the unfolding and aggregation properties of beef skeletal muscle myosin. Results suggest that it may be advantageous to adjust the pH to optimize cooking yields and functional attributes when different proportions of red and white muscles are used in a meat formulation.

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