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Myosin heavy chain isoform composition influences the susceptibility of actin-activated S1 ATPase and myofibrillar ATPase to pH inactivation

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Abstract

The objectives of this study were to determine the influence of pH and MyHC isoforms on myofibrillar and actin-activated myosin subfragment 1 (S1) ATPase activity and the protective effect of actin. Red (RST) *semitendinosus* and white (WST) *semitendinosus* myofibrils were incubated at pH 7, 6, or 5.5 with 0 or 2 mM ATP. RST and WST S1 isolates were incubated at pH 7, 6, or 5.5 in the presence or absence of actin. Maximum calcium-activated myofibrillar and actin-activated S1-ATPase activity were then assayed at pH 7. Incubation of myofibrils with ATP caused ATPase activity of myofibrils to decrease ($p < 0.05$) with the pH of the incubation. RST myofibrils maintained a higher ($p < 0.0001$) relative activity than WST myofibrils after incubation at pH 6 with ATP. Myofibrils incubated without ATP exhibited higher ($p < 0.001$) activities than those incubated with ATP following pH 5.5 treatments. WST myofibrils had a lower ($p < 0.05$) relative activity than RST following incubation at pH 5.5 without ATP. S1 ATPase activities decreased ($p < 0.05$) with incubation pH in WST samples, but not in RST samples. WST S1 activity was higher ($p < 0.01$) in samples exposed to pH 6 and 5.5 with actin bound compared to those incubated without actin. RST S1 exhibited a higher ($p < 0.01$) relative activity than WST samples following pH 5.5 treatment with bound actin. These data show that low pH inactivates myofibrils by altering actin-activated S1 ATPase. Furthermore, these results suggest that muscles with high proportions of fast fibers are more susceptible to pH inactivation of ATPase activity and that the protective effect of actin binding to myosin is less in fast fibers.

Keywords: Myofibril; S1; Actin; ATPase; Myosin heavy chain

1. Introduction

The relationship between muscle pH, temperature, and rigor onset is critical in the development of pale, soft, exudative (PSE) pork. The PSE condition is caused by a rapid rate of postmortem glycolysis during the early postmortem period (<1 h). Numerous studies have shown that a rapid metabolism results in a low muscle pH while the carcass temperature is still high (>35 °C), resulting in excessive protein denaturation and inferior meat quality attributes (Bendall & Wismer-Pedersen, 1962; Briskey & Wismer-Pedersen, 1961; Wismer-Pedersen & Briskey, 1961). In particular, PSE has a higher degree of myofibrillar protein denaturation compared to normal muscle (Joo, Kauffman, Kim, & Park, 1999; Sayre & Briskey, 1963; Warner, Kauffman, & Greaser, 1997). The PSE condition is more likely to develop in white muscles than red muscles (Dildey, Aberle, Forrest, & Judge, 1970; Warner, Kauffman, & Russell, 1993). This is most likely the result of white, fast-twitch fibers having a more rapid pH decline than red fibers due to their inherently higher ATPase capacity and glycolytic nature. Differences in the inherent

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susceptibility of various muscle fibers types to myofibrillar protein denaturation may also account for the differential meat quality attributes between red and white muscles.

Myofibrillar ATPase activity serves as a useful indictor of myofibrillar protein denaturation (Penny, 1967a). Earlier data showed that maximum myofibrillar ATPase activity is lower in PSE muscle compared to muscle with a normal rate of pH decline (Greaser, Cassens, Briskey, & Hoekstra, 1969; Honikel & Kim, 1986; Sung, Ito, & Fukazawa, 1976) indicating that myofibrils are susceptible to differing degrees of inactivation/denaturation. Additionally, in vitro myofibrillar ATPase activity was shown to decrease with pH decline from approximately pH 6.25 to pH 5.5, with this decrease being greater in myofibrils with more fast MyHC isoforms (Bowker, Grant, Swartz, & Gerrard, 2004). Thus, it is hypothesized that this susceptibility to inactivation is related to MyHC isoform composition and its influence on myofibrillar ATPase activity.

The low water-holding capacity and high drip losses characteristic of PSE meat are partially the result of myosin denaturation prior to rigor onset (Offer, 1991; Penny, 1977). Myosin denaturation reduces the length of the myosin motor domain, which upon binding to the actin during rigor, decreases the inter-filament spacing resulting in expulsion of water into the extracellular space and consequently greater drip losses (Offer et al., 1989). Binding of actin to the motor domain during rigor onset results in the formation of rigor crossbridges which are thought to protect myosin from further denaturation by low pH (Penny, 1967a). Overall, there is a lack of data detailing the effect of MyHC isoform composition on the inherent denaturation susceptibility of myofibrils and the protective action of actin binding to myosin.

Thus, the primary objective of this experiment was to determine the influence of pH and MyHC isoforms on the myofibril apparatus by measuring the irreversible inactivation of (1) the myofibrillar ATPase activity of intact myofibrils and (2) the actin-activated S1-ATPase activity of purified S1. Secondly, the ability of rigor crossbridges to protect against inactivation was tested using two methods. One was with intact myofibrils treated at low pH under rigor and relaxed conditions in which relaxation occurred through addition of ATP. The other was to subject isolated S1 to low pH in the presence and absence of actin.

2. Materials and methods

2.1. Muscle samples

Semitendinosus muscles were excised from both halves of five porcine carcasses within 10 min postmortem. The *semitendinosus* muscles were trimmed of excess fat and connective tissue and further subdivided into the red (RST) and white (WST) *semitendinosus*. RST samples were taken from the medial 1/3 of the *semitendinosus* muscles, and WST samples were obtained from the superficial 1/3 of the muscles.

2.2. Myofibril isolation and S1/actin protein purification

RST and WST muscle samples from three carcasses were used to isolate myofibrils according to the procedures of Swartz, Greaser, and Marsh (1993b) with slight modifications. Isolated myofibrils were stored at −20 °C after resuspension in rigor buffer (75 mM KCl, 10 mM imidazole (pH 7.2), 2 mM $MgCl_2$, 2 mM EGTA, 1 mM NaN_3, 1 mM DTT) diluted to 50% with glycerol.

RST and WST muscle samples from two carcasses were used to prepare myosin subfragment-1 (S1) by chymotryptic digestion of myosin according to the procedures of Weeds and Pope (1977) and Swartz, Greaser, and Marsh (1993a). The crude S1 from RST and WST was further purified using ion-exchange chromatography on SP-Sephadex according to the procedures of Trayer and Trayer (1988) and Swartz and Moss (1992). For each muscle, fractions homogeneous for the myosin light chain alkali 1 (LC1) isoform of S1 were pooled, adjusted to 5 mM EDTA (pH 7) and precipitated by adding ammonium sulfate to 75% saturation. The precipitate was collected by centrifugation at 10,000*g* for 20 min and stored at 4 °C until use. Following the removal of muscle for S1 isolation, the remaining WST muscle was stored at −80 °C and subsequently used for the purification of actin according to procedures described by Pardee and Spudich (1982). Due to logistical reasons and the fact that actin does not vary in isoform between RST and WST muscles, actin was only purified from the WST for the purposes of this study. Preliminary data (not shown) using actin isolated from both RST and WST muscle indicate that the source of actin does not affect the actin-activated S1 ATPase activity. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Fritz, Swartz, and Greaser (1989) with slight modifications was used to monitor the chromatographic elution profiles (not shown) and the protein purity of the myofibril, S1, and actin preparations (Figs. 1–3, respectively).

2.3. Protein determination

Myofibrillar protein concentration was estimated using the biuret assay (Gornall, Bardawill, & David, 1949) with bovine serum albumin (BSA) as a standard. Myosin, chymotrypsin, S1, and F-actin protein concentrations were determined spectrophotometrically at 280 nm with mass absorptivity values of 0.56, 2.12, 0.75,

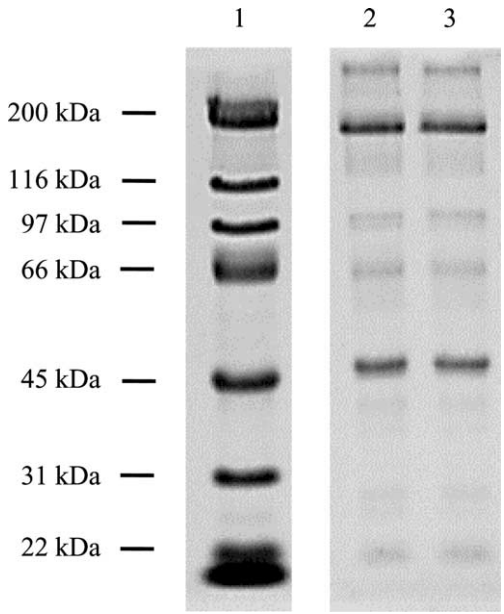

Fig. 1. Comparison of isolated RST (lane 2) and WST (lane 3) myofibrils by SDS-PAGE. Lane 1 designates molecular weight markers. RST and WST samples represent isolated myofibrils pooled from three carcasses and loaded at 8 μg protein per lane.

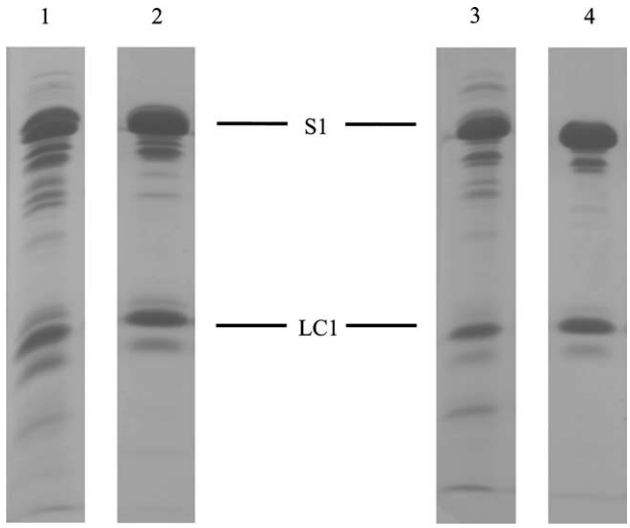

Fig. 2. Comparison of crude and purified RST and WST S1 protein by SDS-PAGE. Gel lane designations: crude RST S1 (lane 1), RST S1 after ion-exchange chromatography (lane 2), crude WST S1 (lane 3), and WST S1 after ion-exchange chromatography (lane 4). RST and WST S1 samples were pooled from two carcasses and loaded at 10 μg protein per lane.

and 1.17 ml/mg cm, respectively. Absorbance values at 280 nm were corrected for light-scattering at 320 nm.

2.4. Solutions

Buffers used for treating myofibrils at different pH levels and for measuring myofibrillar ATPase activity were formulated using the program of Fabiato (1988). Base pCa 3 and pCa 9 solutions consisted of 20 mM PIPES

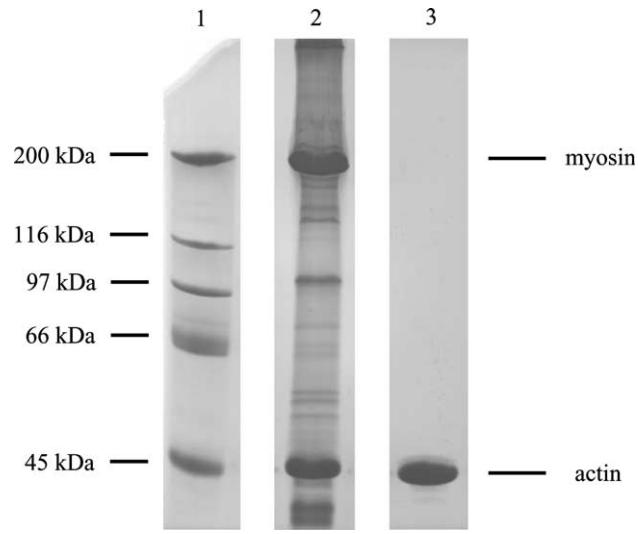

Fig. 3. SDS-PAGE of purified actin (lane 3) from WST. Lane 1 designates molecular weight markers. As a reference to monitor the actin isolation and purification procedure, intact WST muscle was denatured and applied to the gel (lane 2). Each lane was loaded with 5–15 μg of protein.

buffer (pH 7), 4 mM EGTA, 4 mM $MgCl_2$ (free), 1 mM NaN_3, 180 mM ionic strength with KCl, and either 5 (pCa 3) or 0 mM (pCa 9) $CaCl_2$. Buffers at pH 6 and 5.5 were formulated similarly but with 20 mM MES as the buffering reagent. All solutions contained 1 mM DTT and 1 mg/ml BSA. pCa 4 buffers were formulated by mixing the stock pCa 9 and 3 buffers in the proper ratio.

2.5. Myofibril treatments

Glycerinated RST and WST myofibrils were washed three times at 4 °C by repeated suspension in 5 volumes of pCa 9 buffer (pH 7, 6, or 5.5) followed by centrifugation for 5 min at 1000*g* and then resuspended in 5 volumes of fresh pCa 9 buffer at pH 7, 6, or 5.5. Each myofibril suspension was subdivided into two groups, and myofibrils were then incubated for 1 h at 39 °C with either 0 or 2 mM of MgATP in the incubation buffer. Myofibrils were incubated at various pH levels in the absence of ATP in order to determine the protective nature of the rigor crossbridges. ATP was added to the samples during incubation to test the effects of low pH when the myofibrils were in the relaxed state. All myofibrils were then washed three times at 4 °C by repeated centrifugation at 1000*g* for 5 min with resuspension in 5 volumes of pCa 9/pH 7 rigor buffer per wash. Following protein determination, myofibrils were diluted to 0.5 mg/ml and pCa 4 with pCa 3 rigor buffer.

2.6. Myofibrillar ATPase activity

The ATPase activities of the myofibrils were measured utilizing the procedures of Swartz, Zhang, and Yancey (1999) with slight modifications. Activity was

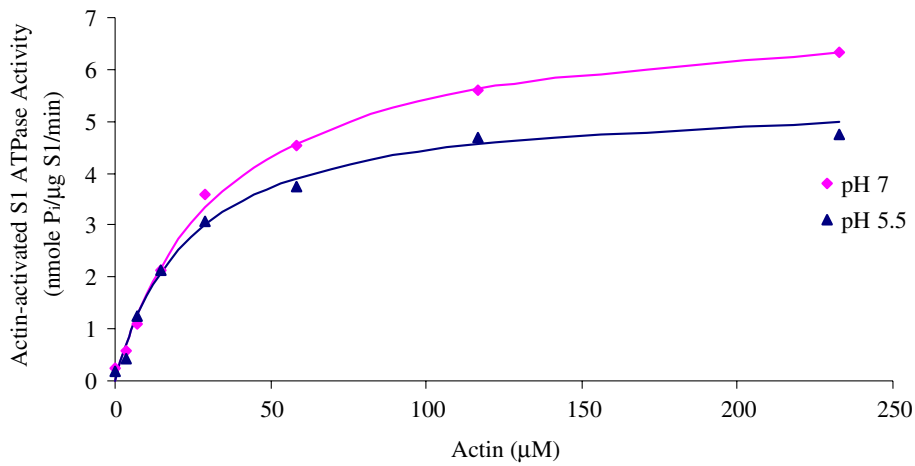

Fig. 4. Plot of actin-activated S1 ATPase activity of WST S1 versus actin concentration following treatment of S1 at pH 7 and 5.5.

assayed at pH 7 and pCa 4 in quadruplicate 0.1 ml samples at a total protein concentration of 0.1 mg/ml. The reaction was initiated by adding 80 µl of pCa 4 buffer at 2.5 mM MgATP to 20 µl of myofibrils (0.5 mg/ml in pCa 4) and stopped after 5 min with 20 µl of cold 25% trichloroacetic acid (TCA). Samples were centrifuged at 14,000*g* for 15 s to remove protein and an aliquot of supernatant was removed for the determination of inorganic phosphate (P_i) according to the procedures of Carter and Karl (1982). To account for any P_i contamination in buffers and the non-enzymatic hydrolysis of ATP, controls were included in the assay in which TCA was added prior to the myofibrils. All assays were replicated three times with freshly deglycerinated myofibril samples used for each replicate.

2.7. S1 and actin treatments

Both S1 and actin samples were individually dialyzed against one of three buffers: (a) 10 mM imidazole (pH 7) and 1 mM DTT, (b) 10 mM MES (pH 6) and 1 mM DTT, or (c) 10 mM MES (pH 5.5) and 1 mM DTT. To test the protective action of the rigor linkage between actin and the motor domain, S1 samples were also dialyzed into the various pH buffers after actin (pH 7) was added to the S1 at a ratio of 3 moles of actin per mole of S1. Samples were then incubated for 1 h at 39 °C prior to the measurement of maximum actin-activated S1 ATPase activity. All samples were assayed in quadruplicate and the assays were replicated three times with freshly dialyzed S1 and actin samples used for each replicate.

2.8. Actin-activated S1 ATPase activity

The actin-activated S1 ATPase activities of the samples were measured using the procedure of Margossian and Lowey (1978) with slight modifications. The ATPase activities of the S1 samples (pH 7, 6, and 5.5) were measured with the addition of actin (pH 7). Likewise, the activities of the S1 samples with actin bound prior to dialysis were determined with the addition of actin (pH 7). To determine the effects of pH treatment on actin, ATPase activity of S1 samples (pH 7) was measured with the addition of actin samples treated at pH 7, 6, and 5.5 for 1 h at 39 °C. The ATPase activity was measured in 50 mM imidazole (pH 7), 2.5 mM $MgCl_2$, 1 mM DTT, and 2.5 mM ATP. The total assay volume was 200 µl at 0.05 mg/ml S1 (0.42 µM) and 5 mg/ml actin (116 µM). The reaction was quenched by the addition of TCA and samples were centrifuged at 14,000*g* for 15 s prior to the supernatant being assayed for P_i according to the procedures of Carter and Karl (1982). The actin-activated S1 ATPase activities were corrected for S1-ATPase activity without actin. In S1 samples that were bound to actin prior to dialysis, the amount of actin added to determine the actin-activated S1 ATPase activity was adjusted to give a final actin concentration of 5 mg/ml in the reaction. Blanks containing actin and ATP were assayed to account for P_i present in the actin preparations and the non-enzymatic hydrolysis of ATP.

In order to determine the effects of low pH on the apparent affinity of S1 for actin in the presence of ATP, the maximum actin-activated S1 ATPase activities of S1 samples treated with pH 7, 6, and 5.5 buffers were determined at 0, 3.6, 7.3, 14.5, 29, 58, 116, and 232 µM actin at pH 7.0. Plots of activity versus actin concentration yielded a hyperbolic shaped curve to which the equation $f(x) = ax/(b + x)$ was fit using SigmaPlot software (Sigma Plot, 1994) where $f(x)$ was equivalent to activity, x to total actin concentration, a to maximum activity, and b to total actin concentration at half-maximal activity. The b value obtained from the regression is analogous to a dissociation constant referred to as K_{app}, which is essentially an inverse measure of the apparent affinity of the reaction for actin as an activator. Thus, K_{app} is expressed as µM of actin and yields insight into the binding of S1 and actin in the presence of ATP. Fig. 4 illustrates an

example of the hyperbolic curve generated from plotting actin-activated S1 ATPase activity versus actin concentration.

2.9. Statistical analysis

In order to assess the loss in ATPase activity following the various pH incubation treatments, the maximum activity (assayed at pH 7) was analyzed as both an absolute activity and as the activity relative to the pH 7 control treatments. Expressing data relative to pH 7 allowed for a more direct comparison of the magnitude differences of the treatment effects between RST and WST samples, which are inherently different in ATPase activity. The general linear model procedure of SAS was utilized to perform analysis of variance on the myofibrillar ATPase activity, actin–S1 ATPase activity, and K_{app} data (SAS, 1985). The model tested the effects of muscle, pH, and rigor state during incubation (myofibrils ±ATP; S1 ±actin). Significance level was set at $p < 0.05$.

3. Results

3.1. SDS-PAGE analysis of samples

In this study, the isolated myofibrils from the three carcasses exhibited similar ATPase activities (data not shown) and electrophoretic profiles and were therefore pooled. Fig. 1 shows that isolated RST and WST myofibril preparations were similar in purity level. Likewise, the S1 samples from the two carcasses exhibited similar ATPase activities (data not shown) and electrophoretic banding patterns. Fig. 2 demonstrates the similar level of purification that was achieved between the RST and WST S1 samples following ion-exchange chromatography. SDS-PAGE analysis with high protein loads indicated a high level of purity within the actin samples utilized for the actin-activated S1 ATPase assays in this experiment (Fig. 3). Differences between the purity of RST and WST samples (both myofibrils and S1) were minor and it was concluded that they had little if any effect on the outcomes of this study.

3.2. Effect of pH and ATP on the inactivation of myofibrils

Table 1 shows the maximum myofibrillar ATPase activities (absolute activity and activity relative to pH 7) of myofibrils after exposure to various pH levels at 39 °C in the presence and absence of ATP. For both absolute activity and activity relative to pH 7, there was a significant ($p < 0.05$) interaction between muscle and the pH and rigor state (±ATP) during incubation. The absolute ATPase activity values demonstrate that in both the RST and WST myofibrils, the activity was significantly higher in myofibrils incubated at pH 7 without ATP (rigor state) compared to myofibrils incubated at pH 7 with ATP (relaxed state). In both RST and WST myofibrils in the presence of ATP, enzymatic capacity relative to pH 7 diminished with the pH of the incubation buffer. RST myofibrils maintained a higher ($p < 0.0001$) relative ATPase activity than WST myofibrils after incubation at pH 6 with ATP. After exposure to pH 5.5 with ATP, both RST and WST myofibrils exhibited a 10-fold lower activity than myofibrils incubated at pH 7. In RST samples, incubation in the absence of ATP lowered ($p < 0.01$) activity to the same degree in myofibrils treated at pH 6 and 5.5. The presence or absence of ATP in pH 6 RST samples did not affect relative ATPase activity, however, RST myofibrils treated at pH 5.5 in the absence of ATP demonstrated a sevenfold higher ($p < 0.0001$) relative ATPase activity compared to myofibrils incubated at pH 5.5 with ATP. The ATPase activity of WST myofibrils relative to pH 7 decreased ($p < 0.01$) with the pH of the incubation buffer lacking ATP. In WST myofibrils, the absence of ATP in the incubation buffer increased ($p < 0.0001$) relative ATPase activity by approximately 1.6-fold following pH 6 incubation and ninefold following pH 5.5 incubation compared to treatments including ATP. Following pH 6 treatments in the absence of ATP, RST and WST myofibrils had similar activities relative to their pH 7 treated myofibrils. Following pH 5.5 treatments in the absence of ATP, however, WST myofibrils exhibited a lower ($p < 0.001$) relative activity than RST myofibrils. Thus, even while in the rigor state, WST

Table 1
Maximum myofibrillar ATPase activity (LSMEANS) following treatment of RST and WST myofibrils at pH 7, 6, or 5.5 at 39 °C with 0 or 2 mM ATP

Muscle	Myofibril incubation conditions		ATPase activity (nmole P_i/μg protein/min)	
	ATP (mM)	pH	Absolute activity	Activity relative to pH 7.0 treatment
RST	0	7.0	$0.34^{e,f}$	1.00^{e}
		6.0	0.27^{c}	0.78^{d}
		5.5	$0.29^{c,d}$	0.84^{d}
	2	7.0	0.20^{b}	1.00^{e}
		6.0	0.18^{b}	0.87^{d}
		5.5	0.02^{a}	0.12^{a}
WST	0	7.0	0.51^{g}	1.00^{e}
		6.0	0.39^{f}	0.78^{d}
		5.5	$0.31^{d,e}$	0.62^{c}
	2	7.0	0.37^{f}	1.00^{e}
		6.0	0.18^{b}	0.49^{b}
		5.5	0.03^{a}	0.07^{a}
	SEM		0.01	0.04

[a–g] Within a column, LSMEANS without a common superscript letter differ ($p < 0.05$).

myofibrils were more sensitive to pH-induced inactivation than RST myofibrils. Furthermore, pH treatment with ATP enhanced this pH induced inactivation in both RST and WST, with the ATP effect being greater in WST myofibrils.

3.3. Effect of pH and actin on the inactivation of S1

To determine if pH influences S1 directly, maximum actin-activated S1 ATPase activity was determined following pH treatment of S1 at 39 °C in the presence and absence of actin at 39 °C (Table 2). For both absolute activity and activity relative to pH 7, there was a significant ($p < 0.05$) interaction between muscle and the pH and rigor state (±actin) during incubation. Relative activity did not differ between RST S1 incubated at pH 5.5 and 7, but was slightly lower ($p < 0.0001$) when incubated at 6 in the absence of actin. With WST S1, activity relative to pH 7 decreased ($p < 0.001$) with pH of incubation in the absence of actin. RST samples had an approximately twofold higher ($p < 0.001$) relative activity than WST samples after treating S1 at pH 5.5 in the absence of actin. Thus, S1 from the WST is more susceptible to pH-induced inactivation of actin-activated S1 ATPase activity than RST S1. With regards to the RST, the relative activity only slightly decreased ($p < 0.05$) following exposure to pH 6 and 5.5, regardless of whether or not actin was bound to the S1 during the pH treatment. WST S1 with actin present during the pH treatment demonstrated that relative activity was not different between pH 7 and 6 treated samples, but that treatment at pH 5.5 resulted in a lower ($p < 0.0001$) activity even when actin was bound to the S1 during incubation. Within WST samples, relative activity was higher ($p < 0.01$) in S1 samples exposed to pH 6 and 5.5 with actin present as compared to S1 samples exposed to those pH conditions in the absence of actin. Thus from relative activity data, WST S1 was more sensitive to pH induced inactivation than RST S1, with actin having only a modest protective on S1. With both WST and RST S1 samples, there was a substantial decrease in the absolute actin-activated S1 ATPase activity when S1 samples were incubated at pH 7 without actin compared to when S1 samples were incubated with actin. Absolute activity values show nearly a twofold difference with the presence and absence of actin indicating that actin and the rigor crossbridge may protect against thermal inactivation more than pH inactivation of ATPase activity.

Table 2
Maximum actin-activated S1 ATPase activity (LSMEANS) following treatment of RST and WST S1 at pH 7, 6, or 5.5 at 39 °C in the presence or absence of actin

Muscle	S1 incubation conditions		ATPase activity (nmole P_i/μg S1/min)	
	Actin	pH	Absolute activity	Activity relative to pH 7.0 treatment
RST	+	7.0	3.88^{h}	1.00^{f}
		6.0	3.26^{e}	$0.84^{c,d}$
		5.5	3.57^{g}	0.92^{e}
	−	7.0	2.16^{c}	1.00^{f}
		6.0	1.86^{b}	0.86^{d}
		5.5	2.16^{c}	1.00^{f}
WST	+	7.0	5.30^{j}	1.00^{f}
		6.0	5.10^{i}	0.96^{f}
		5.5	3.41^{f}	0.64^{b}
	−	7.0	2.63^{d}	1.00^{f}
		6.0	2.13^{c}	0.81^{c}
		5.5	1.47^{a}	0.56^{a}
	SEM		0.05	0.02

[a–j] Within a column, LSMEANS without a common superscript letter differ ($p < 0.05$).

3.4. pH effect on actin

Table 3 shows how incubating actin at different pH levels at 39 °C influenced actin-activated S1 ATPase activity. Compared to pH 7, incubating actin at pH 6 or 5.5 only decreased ($p < 0.001$) relative activity from 1.0 to approximately 0.9. The pH treated actin did not differentially influence the relative actin-activated activity of RST or WST S1 fragments. Therefore, the ability of actin to activate S1 ATPase was not severely influenced by pH.

3.5. Effect of pH treatment on K_{app}

Fig. 4 shows an example of the effect of actin concentration on the actin-activated S1 ATPase activity of S1 treated at pH 7 and 5.5. Fitting hyperbolic curves to this data allowed for the estimation of the maximum actin-activated S1 activity and the K_{app} for actin. Data fit lines with R^2 values ranging from 0.952 to 0.998. K_{app} values are shown in Table 4. Investigation of the K_{app} values relative to pH 7 in the RST samples, shows that pH 5.5

Table 3
Maximum actin-activated S1 ATPase activity (LSMEANS) of RST and WST S1 following treatment of actin at pH 7, 6, or 5.5 at 39 °C

Muscle	Actin incubation conditions	ATPase activity (nmole P_i/μg S1/min)	
	pH	Absolute activity	Activity relative to pH 7.0 treatment
RST	7.0	2.83^{b}	1.00^{c}
	6.0	2.49^{a}	0.88^{b}
	5.5	2.48^{a}	0.88^{b}
WST	7.0	4.21^{e}	1.00^{c}
	6.0	3.49^{c}	0.83^{b}
	5.5	3.79^{d}	0.90^{b}
	SEM	0.08	0.03

[a–e] Within a column, LSMEANS without a common superscript letter differ ($p < 0.05$).

Table 4
K_{app} (LSMEANS) for actin for the actin-activated S1 ATPase activity following treatment of RST and WST S1 at pH 7, 6, or 5.5

Muscle	S1 incubation conditions	K_{app} (μM) for actin	
	pH	Absolute K_{app}	K_{app} relative to pH 7.0 treatment
RST	7.0	40.9[e]	1.00[c]
	6.0	38.0[d,e]	0.93[b]
	5.5	36.3[c,d]	0.89[b]
WST	7.0	34.1[c]	1.00[c]
	6.0	29.9[b]	0.88[b]
	5.5	24.9[a]	0.73[a]
	SEM	1.13	0.03

[a–e] Within a column, LSMEANS without a common superscript letter differ ($p < 0.05$).

and 6.0 treated S1 resulted in slightly lower ($p < 0.05$) K_{app} values than S1 treated at pH 7. In WST samples, however, the K_{app} for actin significantly decreased ($p < 0.01$) with the pH at which the S1 was incubated in the order of pH 7 > 6 > 5.5. Thus, WST S1 samples had lower ($p < 0.01$) K_{app} values (relative to pH 7) than RST samples after treatment at pH 5.5. Absolute values for K_{app} reveal that regardless of pH treatment, S1 isolated from RST muscle exhibited higher ($p < 0.01$) K_{app} values than WST samples.

4. Discussion

The pH and temperature conditions that muscle proteins are exposed to early postmortem play key roles in determining meat quality. This is evidenced by the fact that PSE muscle has a higher degree of sarcoplasmic and myofibrillar protein denaturation, as defined by protein solubility, than muscle with a normal rate of postmortem pH decline (Joo et al., 1999; Sayre & Briskey, 1963; Warner et al., 1997). In an in vitro study measuring ATPase activity and protein solubility, Penny (1967a) showed that myosin denatures as temperature is increased from 32 to 45 °C and pH is lowered from 6.2 to 5.3, which are within the range of conditions observed in PSE carcasses early postmortem. Denaturation of myosin in particular has the most profound effects on water-holding capacity and drip loss (Offer, 1991; Penny, 1969). In the current study, loss of ATPase activity was used as the marker for protein denaturation because enzymatic activity is a more sensitive indicator of myosin denaturation than solubility (Penny, 1967a) and because ATPase activity is intimately linked to the energy metabolism of the muscle.

Maximum ATPase activity of myofibrils is dependent on the pH and temperature conditions while in the carcass. Myofibrils isolated at 24 h postmortem from pork carcasses with a rapid pH decline early postmortem have reduced ATPase activity (Greaser et al., 1969). Likewise, Penny (1967b) demonstrated that myofibrils isolated from rabbit muscle held at 37 °C for 4 h had significantly reduced ATPase activity. Because isolated myofibrils were used in these studies, it could not be conclusively determined if low pH conditions reduced ATPase activity by altering the myosin molecule or by influencing thin filament and its regulatory proteins. In the latter scenario, the capacity for Ca^{2+}-activation of the myofibrils could be reduced thus lowering the apparent maximum calcium-activated myofibrillar ATPase activity.

In the current study, we tried to determine if low pH influences the activity of the myosin motor domain or the thin filament that activates it. To simulate early postmortem pre-rigor conditions, myofibrils were incubated at various pH levels in the presence of ATP. Maximum ATPase activity of myofibrils exposed to pH 6 and 5.5 at 39 °C was significantly reduced in both RST and WST myofibrils, with WST myofibrils being more susceptible to irreversible effects at pH 6 (Table 1). Such decreases in the maximum myofibrillar ATPase indicate that irreversible alterations to the myofibril apparatus occurred, but it could not be definitively determined whether or not the pH effects were on the thin filament, the motor domain, or some other region of the myosin molecule such as the S2.

In order to test the hypothesis that low pH directly influences the motor domain, isolated S1 was treated at different pH levels and the actin-activated S1 ATPase activity was determined. Table 2 shows that pH 6 and 5.5 incubation of WST S1 diminished maximum actin-activated S1 ATPase activity. The actin-activated S1 ATPase activity of RST S1 was relatively unaffected by the pH treatments, however. Table 3 shows that pH effects on actin most likely do not account for a significant portion of the observed reduction in ATPase activity. Overall, these data demonstrate that low pH inactivates ATPase activity by directly affecting the motor domain of the myosin molecule, with the effect being more severe in samples with a higher proportion of fast MyHC isoforms.

At first glance, the adverse conditions (low pH, high temperature) seemed to have had a less detrimental influence on S1 compared to myofibrils, suggesting that pH effects on the S1 head account for only part of the pH-induced inactivation of myofibrillar ATPase activity. Thus, it could be postulated that decreased maximum myofibrillar ATPase activity may be partly due to the influence of pH on regulatory proteins associated with the thin filaments of myofibrils, which would interfere with Ca^{2+}-activation. The exaggerated loss of ATPase activity in the myofibrils compared to the S1 samples, however, may also be the result of ATP binding to the motor domain. This hypothesis needs to be investigated more thoroughly as S1 heads in the current study were not subjected to the various pH treatments in the

presence of ATP, thus direct comparisons cannot be made between myofibril and S1 data.

According to a model proposed by Offer (1991), pH and temperature are most vital to myosin denaturation and drip loss prior to rigor onset. Penny (1967a) demonstrated that the rate of acto-myosin ATPase inactivation at 35 °C and pH 5.7 was less than that of purified myosin alone. Thus, it was assumed that actin binding protects myosin from further denaturation. In our study, S1 was subjected to various pH conditions either alone or while bound to actin in order to determine the protective effect of actin on myosin. Table 2 demonstrates that WST S1 bound to actin is less susceptible to irreversible loss of ATPase activity than S1 incubated alone, especially at pH 6. A less dramatic effect was observed in the RST due to the fact that RST S1 was less susceptible to pH inactivation than WST S1 even in the absence of actin. The absolute activity data from Table 2 also supports the idea of the rigor crossbridge protecting the myosin molecule from denaturation. The absolute activity following S1 incubation at pH 7 without actin was nearly twofold lower than with actin, indicating that actin binding protects enzyme activity from thermal inactivation to an even a greater degree than pH inactivation. Thus, actin-activated S1 ATPase activity data from this study confirm the protective action of actin binding to myosin.

Furthermore, the protective effect of the rigor bond was tested in RST and WST myofibrils by subjecting them to various pH and temperature conditions in the presence and absence of ATP. Data in Table 1 indicate that myofibrils incubated in the absence of ATP were less susceptible to irreversible pH inactivation than myofibrils incubated with ATP. The lower relative activity observed in WST compared to RST myofibrils after pH 5.5 incubation without ATP indicates that actin had less protective effect on myosin molecules comprised of fast MyHC isoforms. These results are in agreement with those of Van Laack and Lane (2000) in which they used protein solubility as the marker for protein denaturation and found that myofibrils incubated with ATP were more susceptible to denaturation at similarly low pH levels and high temperatures.

Definitive data to determine whether the decrease in ATPase activity after exposure to low pH was the result of partial denaturation of myosin while bound to actin or simply the denaturation of unbound myosin heads is still lacking. Sarcomere length was not evaluated in myofibril preparations, so differences in thick and thin filament overlap may have resulted in varying quantities of unbound myosin heads exposed during the low pH incubation. At complete filament overlap, Cooke and Franks (1980) determined that 94–100% of myosin heads are bound to actin. Denaturation of unbound myosin in myofibrils or S1 isolates during the pH treatment may have led to an underestimation of the protective effect of actin.

The enzymatic capacity of the myosin molecule is enhanced by the binding of actin to the S1 head, which increases the rate of product release. Thus, it is intuitive that pH effects on actin and myosin binding most likely alter overall ATPase activity. The current study demonstrates that K_{app} for actin decreases when S1 heads are subjected to low pH (Table 4). Furthermore, the results indicate that WST S1 samples require less actin for activation of S1 ATPase activity and are more pH sensitive than RST samples. In this study, K_{app} was influenced by low pH to a lesser degree than the observed maximum ATPase activities, which is consistent with past data (Sung, Ito, & Izumi, 1981). In porcine muscle with a normal rate of pH decline, Sung et al. (1981) determined that the V_{max} and K_{app} of acto-heavy meromyosin (HMM) ATPase decreased with postmortem storage time, with the decrease being larger in the V_{max} parameter. Furthermore, they found that the V_{max} of acto-HMM ATPase activity sampled at 24 h postmortem was substantially lower in PSE muscle compared to normal muscle, but that K_{app} did not significantly differ. The higher degree of change observed in the K_{app} parameter in the current experiment is likely due to the fact that isolated S1 was exposed to low pH conditions in vitro. By sampling acto-HMM ATPase in muscle at 24 h postmortem, it is possible that the myosin was exposed to less harsh conditions than in vitro conditions due to the highly organized structure of the sarcomere and the existence of myosin in thick filaments. Overall, the K_{app} values only differed over an approximately 16 μM range indicating that from a kinetic standpoint the differences in K_{app} probably do not account for the large alterations observed in actin-activated S1 ATPase activity with the various pH treatments. pH and muscle differences observed in K_{app} for actin, however, may be indicative of differential actin and myosin binding which may ultimately play a role in determining rigor bond formation and influence water-holding capacity.

5. Conclusions

These data show that inactivation of actin-activated myosin ATPase activity by high temperature and low pH conditions is in part due to the irreversible altering of the motor domain of the myosin molecule. Rigor bond formation was found to provide a measure of protection against these changes, however. These data demonstrate that myofibrils and S1 from muscle containing a high proportion of white muscle fibers are more susceptible to pH inactivation and denaturation than those from predominantly red muscle. These data suggest that in an in vitro environment, fast MyHC isoforms are more susceptible to inactivation/denaturation than slow MyHC isoforms at pH and temperature conditions characteristic of postmortem muscle. Thus, motor

domain isoform likely explains a major component of the difference in the influence of pH and temperature on inactivation of motor domain ATPase activity. This would imply that MyHC isoform composition plays an important role in determining the susceptibility of muscle to postmortem pH effects that are deleterious to meat quality. In vivo studies are needed, however, to determine if conditions within postmortem muscle that inactivate muscle fiber ATPase activity occur in a fiber type dependent manner.

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