Influence of myosin heavy chain isoform expression and postmortem metabolism on the ATPase activity of muscle fibers

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Abstract

The objective of this study was to determine the effects of postmortem muscle pH and temperature declines on the actomyosin ATPase activity of muscle fibers expressing different MyHC isoforms. Using a quantitative histochemical procedure to determine ATPase activity, the maximum actomyosin ATPase activity was determined on individual fibers classified by MyHC expression. Samples were collected from the red (RST) and white (WST) semitendinosus muscles at 3 min and 24 h postmortem from electrically stimulated (ES) and control (NS) pork carcasses. In samples taken at 3 min postmortem, type I fibers had the lowest ATPase activity staining and type 2X and 2B had the highest activity staining, with type 2A fibers intermediate. Postmortem time and carcass treatment did not influence the ATPase activity staining of type I muscle fibers. ATPase activity staining of 2A fibers was lower (p < 0.001) in 24 h samples than in 3 min samples from ES carcasses. In 3 min and NS-24 h samples, RST type 2A fibers had lower (p < 0.05) activities than type 2A fibers from the WST. In type 2X fibers, ATPase activity staining decreased (p < 0.01) from 3 min to 24 h postmortem in ES carcasses. This decrease was more severe in WST 2X fibers compared to RST 2X fibers. ATPase activity staining in type 2B fibers did not decrease from 3 min to 24 h postmortem in NS carcasses. In ES carcasses, activity staining of 2B fibers decreased (p < 0.0001) with time postmortem. The results of the experiment indicate that fibers expressing fast MyHC isoforms have a higher ATPase activity early postmortem than slow muscle fibers but are more prone to inactivation by a rapid pH decline.

Keywords: Myosin heavy chain isoform; ATPase activity; Postmortem metabolism

1. Introduction

The ability of muscle fibers to hydrolyze ATP is dependent upon myosin heavy chain (MyHC) isoform expression. Single skinned fibers expressing MyHC-2B have the highest rate of ATP splitting followed in decreasing order by MyHC-2X, MyHC-2A, and MyHC-slow/I expressing fibers (Bottinelli, Canepari, Reggiani, & Stienen, 1994). Consequently, MyHC expression and biochemically determined myofibrillar ATPase activity are highly correlated to contractile properties of muscle fibers such as shortening velocity (Bárány, 1967; Eddinger & Moss, 1987; Reiser, Moss, Giulian, & Greaser, 1985; Schiaffino et al., 1988; Sweeney, Kushmerick, & Mabuchi, 1986). Besides dictating the contractility characteristics and functionality of fibers in living muscles, inherent differences in ATPase activities between muscle fiber types are thought to play a role in regulating postmortem muscle metabolism.

Accelerated rates of glycolysis early postmortem result in adverse pH and temperature conditions within porcine muscle, which lead to excessive protein denaturation and poor meat quality (Briskey & Wismer-Pedersen, 1961; Wismer-Pedersen, 1959). The rate of postmortem glycolysis is controlled by the level of ATPase activity (Scopes, 1974). Due to the abundance of myosin in muscle, myofibrillar (i.e., actomyosin) ATPase is likely a primary determinant of overall muscle ATPase activity. Therefore, it can be postulated that differential actomyosin ATPase activities,
due to MyHC isoforms, influence muscle ATP breakdown and subsequently alter postmortem glycolysis. Previous experiments have shown that MyHC isoforms differentially influence myofibrillar ATPase activity and denaturation in an in vitro system simulating postmortem muscle conditions (Bowker, Grant, Swartz, & Gerrard, 2004). Therefore, the objective of this study is to determine the effects of in vivo pH and temperature declines on the actomyosin ATPase activity of muscle fibers expressing different MyHC isoforms.

Past studies used isolated myofibrils to investigate the role of myofibrillar ATPase activity in determining postmortem glycolytic rate (Greaser, Cassens, Briskey, & Hoekstra, 1969; Honikel & Kim, 1986). Myofibril isolation procedures have been shown to result in populations of myofibrils varying in MyHC isoform expression, thus making it difficult to assess the role specific MyHC isoforms may play in determining postmortem muscle ATPase activity (Bowker, Swartz, Grant, & Gerrard, 2004). Furthermore, these methods do not distinguish whether decreases in myofibrillar ATPase activity postmortem result from the entire population of myofibrils being inactivated by pH and temperature conditions, or whether only a subset of myofibrils is affected. Additionally, the ratio of different MyHC isoforms in isolated myofibril preparations may not be reflective of that which is in muscle (Bowker et al., 2004). Such an effect would make it even more difficult to determine the influence of MyHC isoforms on postmortem ATPase activity.

In the present study, such problems were avoided by utilizing a quantitative histochemical technique for determining actomyosin ATPase activity on muscle cross-sections. Thus, ATPase activity was determined for individual muscle fibers identified by predominant MyHC abundance. By sampling muscles that vary in pH and temperature decline profiles at different postmortem times, the present study assessed the influence of in situ conditions and MyHC isoforms on postmortem ATPase activity of muscle fibers. The results of the experiment demonstrate the effect of MyHC isoform on the energy metabolism (ATPase activity) of muscle fibers and their susceptibility to pH/temperature inactivation, which is an indicator of muscle fiber damage.

2. Materials and methods

2.1. Animals and treatments

Six (Yorkshire × Landrace) market pigs (105 ± 4 kg) were slaughtered in the Purdue University Meat Science Teaching and Research Laboratory following standard slaughtering procedures. Exsanguination was considered 0 min postmortem. Carcasses were held at room temperature (~22 °C) until 60 min postmortem and then placed in a chill cooler (4 °C) for 24 h.

Carcasses were subjected to one of two randomly assigned treatments: electrical stimulation (ES) for 30 pulses of 450 V (2 s on and 2 s off) at 5 min postmortem (modified from Bowker, Wynveen, Grant, & Gerrard, 1999) or non-stimulated (NS) controls. ES was delivered through a 16.5 cm long steel electrode placed in the left shoulder muscles of the carcass and grounded to the rail.

2.2. pH and meat quality measurements

At 45 min and 24 h postmortem, pH measurements were taken in the longissimus dorsi (LD) muscle adjacent to the last rib using a Beckman Φ 110 ISFET pH meter with a temperature-compensated, spear-tipped KCl- gel probe (Fullerton, CA, USA). To ensure that measurements were taken near the center of the LD, the probe was inserted perpendicular to the long axis of the LD approximately 4.5 cm lateral to the midline of the carcass to a depth of 4.0–5.0 cm. At 24 h postmortem, color and firmness scores of the LD between the 10th and 11th rib were evaluated according to the National Pork Producers Council standards (NPPC, 1991). From a 2.54 cm cross-sectional slice of the LD removed at the 10th rib, water-holding capacity was determined using the drip loss method (Rasmussen & Stouffer, 1996). Drip loss was estimated from 24 h moisture loss of triplicate 7 g core samples placed in sealed plastic tubes at 4 °C.

2.3. Muscle samples

The semitendinosus muscles were excised from the carcasses at 3 min (left side of carcass) and 24 h (right side of carcass) postmortem. The semitendinosus muscles were further subdivided into the red (RST) and white (WST) semitendinosus. RST samples were dissected from the medial 1/3 of the semitendinosus muscle, and WST samples were obtained from the superficial 1/3 of the muscle. Samples were frozen in 2-methylbutane cooled in liquid nitrogen and then stored at −80 °C. Serial 10 μm cross-sections of each muscle sample were cut using a Microm HM 500M cryostat (Walldorf, Germany) at a chamber temperature of −25 °C and placed on slides coated with 0.1% poly-L-lysine. From each muscle sample (24 total), 10 serial cross-sections were used to determine MyHC isoform expression and the actomyosin ATPase activity staining of individual fibers. Six serial cross-sections were used for ATPase activity determination (three for ATPase activity and three for controls) and four serial cross-sections were used for immunohistochemical classification of muscle fiber type (one each for type I, 2A, and 2X antibodies and one for a negative control).
2.4. Quantitative histochemical determination of fiber actomyosin ATPase activity

ATPase activity of individual muscle fibers identified by MyHC isoform expression was determined using the quantitative histochemical procedure described by Van der Laarse, Diegenbach, and Hemminga (1986) and Blanco and Sieck (1992) with slight modifications. Tissue sections were pre-incubated for 10 min at 25 °C in 100 mM Tris-maleate (pH 7.2), 45 mM Pb(NO₃)₂, and 5 mM NaN₃. The ATPase reaction was initiated by placing the sections in 100 mM Tris-maleate (pH 7.2), 45 mM Pb(NO₃)₂, 5 mM NaN₃, 68 mM CaCl₂, and either 0 or 4 mM ATP at 25 °C. The reaction was stopped after 4 min by rinsing with distilled water. Tissue sections were then immersed for 30 s in PBS containing 1% sodium sulfide (pH 7.4). Finally, slides were washed two times with PBS, air-dried, and mounted in Aqua-mount (Miles Laboratories, MN) prior to observation under the microscope. Slides incubated without ATP during the enzyme reaction served as negative controls to account for baseline staining intensity. Fig. 1 illustrates the ATPase staining of the RST muscle cross-sections from 3 min and ES-24 h samples.

The staining density of individual fibers was determined from the average of three serial sections. In each muscle sample of each pig, the actomyosin ATPase activities of 12 fibers per muscle fiber type were determined. Images were captured using a Nikon DN100 digital camera (Nikon Corporation, Tokyo, Japan) mounted on a Nikon Labphot light microscope (Nikon Corporation, Tokyo, Japan). Microscopic images of stained muscle cross-sections were digitized at 8-bit resolution into 1024 × 1024 pixel element (pixel) arrays. Images were captured under fixed light intensity and image-capture conditions. The staining density of individual fibers was measured using image processing software (IPLabs, Scanalytics Inc., Fairfax, VA, USA) and normalized to the background staining on each slide.

2.5. Immunohistochemical labeling of muscle fiber type

Muscle fiber type was determined by an immunoperoxidase procedure with anti-MyHC isoform primary antibodies. Serial sections were reacted with monoclonal antibodies against MyHC type I (A4.840)(Hughes & Blau, 1992), type 2A (6B8) (Depreux et al., 2000), and type 2A/2X (SC-71)(Bottinelli, Schiaffino, & Reggiani, 1991). Primary antibodies were diluted in PBS (pH 7.4) buffer (1 and 2X at 1/100, 2A at 1/1200) and applied to slides for 1 h at 37 °C. Slides were then washed in PBS and reacted for 1 h at 37 °C with biotinylated-conjugated goat antimouse secondary antibody (IgM for I, IgG for 2A and 2X) (Jackson Immunoresearch Laboratories, West Grove, PA, USA) diluted 1/200. Slides were then washed with PBS and incubated at room temperature for 1 h with a preformed avidin/biotinylated horseradish peroxidase macromolecular complex (VECTORSTAIN® Elite ABC Kit, Vector Laboratories, Inc., Burlingame, CA, USA). Slides were washed with PBS and the 3,3’-diaminobenzidine (DAB) substrate reagent (Vector Laboratories, Inc., Burlingame, CA, USA) was incubated on the sections for 10 min. Finally, slides were washed with PBS, air-dried, and mounted in Aqua-mount prior to observation under
the microscope. Sections incubated with only the secondary antibody served as controls for non-specific reactivity.

2.6. Classification of muscle fiber types

Previous studies have shown that muscle fibers can be classified into type I, 2A, 2X, and 2B fibers as well as fibers expressing multiple MyHC isoforms (Depreux, Grant, & Gerrard, 2002; Eggert, Depreux, Schinckel, Grant, & Gerrard, 2002; Lefaucheur et al., 1998). For the current study, ATPase activity was quantified for only those fibers that could be classified as purely type I, 2A, 2X, or 2B. According to the preceding methodologies, serial cross-sections of each muscle sample were stained for ATPase activity and labeled with type I, 2A, and 2A/2X antibodies. Fig. 2 depicts immuno-stained RST and WST serial cross-sections labeled with anti-MyHC antibodies. Corresponding fields of the muscle samples were identified in each of the ATPase and immuno-stained sections such that both actomyosin ATPase activity staining and MyHC isoform expression of individual fibers could be assessed. Type I fibers were identified in RST muscle samples with the A4.840 anti-MyHC I antibody. Type 2A fibers were identified in RST and WST muscle samples with the 6B8 anti-MyHC 2A antibody. The SC-71 antibody was found to react with both 2A and 2X fibers. Thus, pure 2X fibers were located in RST and WST muscle samples by identifying only those fibers that reacted with the SC-71 antibody and not the 6B8 anti-MyHC 2A antibody. Fibers that reacted with both antibodies were not quantified for the purpose of this study. Fibers within WST samples that did not react with the A4.840 (type I), 6B8 (type 2A), and SC-71 (type 2A/2X) antibodies were classified as 2B fibers.

2.7. Statistical analysis

The SAS general linear model procedure was used to perform analysis of variance on ATPase activity staining data (SAS, 1985) with ATPase staining density as the dependent variable and time of sampling, carcass treatment, muscle, and fiber type as the independent variables. Pig was included in the model to account for animal-to-animal variation. Since the ES treatment was applied to

![Fig. 2. Immunolocalization on serial cross-sections of MyHC-I on RST (a), MyHC-2A on RST (b), MyHC-2X on RST (c), MyHC-I on WST (d), MyHC-2A on WST (e), and MyHC-2X on WST (f).](image-url)
carcasses after the 3 min muscle samples were taken, the data from 3 min samples was pooled across carcass treatment. All values are reported as LSMEANS ± SE. Statistical significance was set at \( p < 0.05 \).

3. Results

3.1. Effect of carcass treatment on pork quality

To simulate PSE development, early postmortem ES was applied to hasten postmortem pH decline. The meat quality characteristics of NS and ES carcasses are displayed in Table 1. ES carcasses had lower (\( p < 0.05 \)) pH values at 45 min postmortem, lower (\( p < 0.05 \)) color and firmness scores, and higher (\( p < 0.05 \)) drip loss than control carcasses. Thus, the ES treatment was effective in generating the rapid postmortem pH decline and subsequent quality attributes observed in PSE.

3.2. Effect of MyHC isoform expression on ATPase activity staining

The ATPase activities of RST and WST muscle fibers expressing different MyHC isoforms are shown in Table 2.

Table 1
Meat quality characteristics of longissimus muscles of NS and ES carcasses (LSMEANS ± SE)

<table>
<thead>
<tr>
<th></th>
<th>NS carcasses</th>
<th>ES carcasses</th>
</tr>
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<tbody>
<tr>
<td>pH&lt;sub&gt;4&lt;/sub&gt;</td>
<td>6.47 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.92 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH&lt;sub&gt;45&lt;/sub&gt;</td>
<td>5.47 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.47 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Color score&lt;sup&gt;x&lt;/sup&gt;</td>
<td>2.8 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Firmness score&lt;sup&gt;y&lt;/sup&gt;</td>
<td>3.5 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Drip loss (%)</td>
<td>4.5 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.6 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

<sup>a,b</sup> LSMEANS within a row with different superscripts differ significantly (\( p < 0.05 \)).
<sup>x,y</sup> National Pork Producers Council color and firmness standards (scale 1–5).

In 3 min samples, type I fibers had an approximately twofold lower (\( p < 0.0001 \)) activity staining than type 2A fibers in RST or WST. A lower (\( p < 0.05 \)) ATPase activity staining was measured in type 2A fibers in RST compared to WST. Type 2X and 2B fibers had higher (\( p < 0.05 \)) activities than type I and 2A fibers. Type 2X fibers in RST and WST did not differ in ATPase activity staining in 3 min samples. In NS-24 h samples, type I and 2A fibers in RST had lower (\( p < 0.01 \)) activities than 2X in RST and 2A in WST. Furthermore, WST 2A and RST 2X fibers had lower (\( p < 0.05 \)) activities than WST 2X and 2B fibers in NS-24 h samples. In ES-24 h samples, ATPase activity staining did not differ between type I and 2A fibers from either muscle. ATPase activity staining of muscle fibers increased in the order of type 2B < WST 2X < RST 2X fibers in ES-24 h samples. The ATPase activities of type I and 2A fibers were intermediate between but not significantly different from type 2B and WST 2X fibers in ES-24 h samples. Compared to slow fibers, muscle fibers expressing fast MyHC isoforms generally had greater ATPase activities early postmortem and in non-stimulated muscle at 24 h, but had similarly low activity in ES muscle at 24 h postmortem.

3.3. Effects of carcass treatment and postmortem time on ATPase activity staining

Table 2 also shows the effects of carcass treatment and postmortem time on the ATPase activities of muscle fibers expressing different MyHC isoforms. Postmortem time of sampling and carcass treatment did not significantly affect the ATPase activity staining of type I muscle fibers. With regards to RST 2A fibers, ATPase activity staining was twofold lower (\( p < 0.001 \)) in 24 h samples than in 3 min samples, regardless of carcass treatment. In WST 2A fibers, ATPase activity staining was twofold lower (\( p < 0.01 \)) in ES-24 h samples compared to 3 min and NS-24 h samples. In RST 2X fibers, ATPase activity staining was lower (\( p < 0.01 \)) in 24 h samples than in RST 2A fibers in WST. Type 2X fibers in RST and WST did not differ in ATPase activity staining in 3 min samples. In NS-24 h samples, type I and 2A fibers in RST had lower (\( p < 0.01 \)) activities than 2X in RST and 2A in WST. Furthermore, WST 2A and RST 2X fibers had lower (\( p < 0.05 \)) activities than WST 2X and 2B fibers in NS-24 h samples. In ES-24 h samples, ATPase activity staining did not differ between type I and 2A fibers from either muscle. ATPase activity staining of muscle fibers increased in the order of type 2B < WST 2X < RST 2X fibers in ES-24 h samples. The ATPase activities of type I and 2A fibers were intermediate between but not significantly different from type 2B and WST 2X fibers in ES-24 h samples. Compared to slow fibers, muscle fibers expressing fast MyHC isoforms generally had greater ATPase activities early postmortem and in non-stimulated muscle at 24 h, but had similarly low activity in ES muscle at 24 h postmortem.

Table 2
ATPase activity (LSMEANS ± SE) of RST and WST muscle fibers classified by MyHC isoform expression

<table>
<thead>
<tr>
<th>Muscle</th>
<th>MyHC expression</th>
<th>ATPase activity (staining intensity)</th>
<th>3 min&lt;sup&gt;A&lt;/sup&gt;</th>
<th>NS-24 h&lt;sup&gt;b&lt;/sup&gt;</th>
<th>ES-24 h&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RST</td>
<td>1</td>
<td>36.4 ± 2.5&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>39.9 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.2 ± 2.6&lt;sup&gt;xy&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>RST</td>
<td>2A</td>
<td>72.0 ± 4.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.2 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.1 ± 6.8&lt;sup&gt;x&lt;/sup&gt;</td>
<td>40.7 ± 3.9&lt;sup&gt;xy&lt;/sup&gt;</td>
</tr>
<tr>
<td>WST</td>
<td>2A</td>
<td>88.1 ± 5.8&lt;sup&gt;y&lt;/sup&gt;</td>
<td>80.4 ± 10.9&lt;sup&gt;y&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RST</td>
<td>2X</td>
<td>104.7 ± 6.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.5 ± 6.8&lt;sup&gt;x&lt;/sup&gt;</td>
<td>71.1 ± 7.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.4 ± 4.7&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td>WST</td>
<td>2X</td>
<td>111.1 ± 5.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>102.0 ± 8.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WST</td>
<td>2B</td>
<td>111.5 ± 7.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.7 ± 6.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>28.6 ± 6.0&lt;sup&gt;a&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>A</sup> Values represent mean of 72 individual fibers.
<sup>B</sup> Values represent mean of 36 individual fibers.
<sup>x,y</sup> Values within a row with different superscripts differ significantly (\( p < 0.05 \)).
<sup>a,b</sup> Values within a column with different superscripts differ significantly (\( p < 0.05 \)).
samples had twofold higher (\(p < 0.0001\)) ATPase activities than ES-24 h samples, while type 2B fibers had a fourfold lower (\(p < 0.0001\)) activity in ES samples taken at 24 h compared to both 3 min and NS-24 h samples. Thus, fibers expressing fast MyHC isoforms demonstrated a greater loss in enzymatic capacity due to a rapid pH decline.

4. Discussion

Inferior pork quality is known to develop as the result of rapid rates of glycolysis generating adverse pH and temperature conditions early postmortem (Briskey & Wismer-Pedersen, 1961; Wismer-Pedersen, 1959). Several recent studies have demonstrated that MyHC isoforms may account for some of the variation observed in pork quality (Depreux et al., 2002; Eggert et al., 2002). In general these studies suggest that negative meat quality attributes are associated with changes in the relative abundance of 2B MyHC. The mechanism by which this occurs has not been elucidated, however. The current study investigated the possibility that the type of MyHC isoform plays a key role in determining meat quality by regulating postmortem ATPase activity and by influencing a muscle’s susceptibility to adverse pH and temperature conditions.

Because ATPase activity drives glycolysis (Scopes, 1974), it is important to understand how postmortem actomyosin ATPase activity is influenced by pH decline as well as MyHC isoforms. The maximum actomyosin ATPase activity of a fiber at any given time postmortem is indicative of the fiber’s potential to hydrolyze ATP and influence glycolytic rate. Besides potential effects on energy metabolism, diminished ATPase activity within a fiber indicates susceptibility to irreversible pH effects, which may have ultra-structural implications affecting meat quality characteristics, such as myosin denaturation causing shrinkage in myofilament lattice spacing and diminished water-holding capacity. Evidence for pH effects is supported by data showing that myofibrillar ATPase activity is significantly lower in myofibrils isolated at 24 h postmortem from muscles exhibiting poor meat quality compared to myofibrils from muscles of normal quality (Warner, Kauffman, & Greaser, 1997). Thus, postmortem alterations in myofibrillar ATPase activity are in part functions of the postmortem pH and temperature decline of the muscle.

It is not known, however, how MyHC isoforms mediate the interactions of myofibrillar ATPase activity, postmortem pH and temperature conditions, and meat quality development. Early studies showed that myofibrillar ATPase activity decreases with time postmortem in carcasses undergoing a rapid pH decline and that activity remains constant or increases with time postmortem in carcasses undergoing a normal pH decline (Greaser et al., 1969). From these data, the effects of MyHC isoform composition on postmortem myofibrillar ATPase activity could not be easily assessed, however, due to the fact that myofibril preparations have a heterogeneous population of MyHC isoforms (Bowker et al., 2004). Therefore, the objective of the current study was to utilize a quantitative histochemical ATPase assay to determine the effects of MyHC isoform on the actomyosin ATPase activity of individual fibers from muscles undergoing different rates of postmortem pH decline. For such a study it was imperative to have (1) a method for subjecting muscle fibers to different rates of pH decline, (2) a method to determine MyHC isoform expression, and (3) a method to measure the ATPase activity of individual MyHC identified muscle fibers.

To generate a rapid muscle pH decline, carcasses were subjected to electrical stimulation early postmortem (<5 min). Similar to past studies (Bowker et al., 1999; Hammelman et al., 2003), the ES treatment resulted in carcasses with lower pH at 45 min postmortem, lower color and firmness scores, and higher drip loss values than control (NS) carcasses (Table 1). Treated and control carcasses did not differ in pH at 24 h postmortem. Thus, ES effectively caused a more rapid pH decline early postmortem resulting in higher drip loss and poor meat quality. Past data provide some indication of the early postmortem characteristics of the RST and WST. In non-electrically stimulated muscles, Beecher, Briskey, and Hoekstra (1965) demonstrated that the pH of the RST was 0.1–0.2 pH units higher than the WST from 0 min to 24 h postmortem. Likewise, data from Wynveen (1999) show that even in carcasses with an accelerated pH decline due to ES, muscles with predominately red fibers (quadriceps femoris) maintain higher pH levels than white muscles (biceps femoris and semimembranosus) over 24 h postmortem.

In the current study, muscle fiber type was determined by using monoclonal antibodies to identify the predominant MyHC isoform expression. As expected RST muscles had a high proportion of type I and 2A muscle fibers, whereas WST muscles had a higher proportion of type 2X and 2B fibers (Fig. 2). Since insignificant amounts of 2B myosin are expressed in porcine RST (Lefaucheur et al., 1998), the RST data set only contains type I, 2A, and 2X fibers. Likewise, the WST data set only contains type 2A, 2X, and 2B fibers, since the WST has a very low proportion of type 1 muscle fibers.

The uniqueness of this study hinges upon measuring ATPase activity of individual muscle fibers, classified by MyHC expression, at various times postmortem. With the ATPase technique used in this study, \(P_i\) released by enzymatic hydrolysis of ATP reacts with lead nitrate to
form a lead-phosphate precipitate localized at the site of cross-bridge cycling (Meijer, 1970). The precipitated phosphate ions are then exchanged for sulfide ions to form a brown-colored lead-sulfide precipitate (Fig. 1). Past studies have demonstrated that the assay is specific for actomyosin ATPase activity and have verified that the amount of precipitate (i.e., staining intensity) linearly increases with time of incubation and is highly correlated to MyHC isoform expression (Blanco & Sieck, 1992; Van der Laarse et al., 1986; Van der Laarse, Diegenbach, & Maslam, 1984). In preliminary trials, the authors measured the effect of titrating ATP concentration and reaction time on staining intensity and found results similar to previous studies. To verify the linearity of the assay, response curves were generated by plotting staining intensity versus ATP concentration and reaction time (data not shown). For the purposes of this study, reaction time and ATP concentration were set at levels resulting in ATPase activities that fell within the linear portion of these curves.

To gain insight into the effects of MyHC isoform and postmortem pH decline on the actomyosin ATPase activity of individual fibers, the maximum ATPase activity staining of individual MyHC identified fibers was determined from muscles undergoing differing rates of postmortem metabolism. Given that fast isoforms of MyHC inherently possess higher ATPase activities, it was expected that fast fibers would have higher ATPase activities than slow fibers early postmortem. Using the same ATPase assay as the current study, Blanco and Sieck (1992) showed that maximum actomyosin ATPase activity staining significantly increased in the order of type I < 2A < 2B fibers in rat gastrocnemius muscle. Likewise, ATPase activity staining was shown to increase in the order of type I < 2A < 2X fibers in human vastus lateralis muscle (Han, Proctor, Geiger, & Sieck, 2001). Thus, in the current experiment it was of no surprise that in muscle samples collected at 3 min postmortem ATPase activity staining was highest in 2X and 2B fibers.

The increased susceptibility of white muscles to undergo a rapid postmortem pH decline and develop poor meat quality (Briskey & Wismer-Pedersen, 1961; Warner, Kaufman, & Russell, 1993) suggests that fibers expressing fast MyHC isoforms would be more prone to loss of ATPase activity due to ES. Data from the current experiment generally support this hypothesis as the greatest losses in ATPase activity from 3 min to 24 h postmortem were observed in fast fibers (especially 2B fibers) from carcasses that were electrically stimulated. Furthermore, the ATPase activity staining of type I fibers did not significantly change from 3 min to 24 h postmortem, regardless of carcass treatment. Thus, fibers expressing fast MyHC isoforms seem to be more susceptible to ATPase inactivation than slow fibers when exposed to a rapid postmortem pH decline.

Besides demonstrating that ATPase activity varies from fiber type to fiber type within a muscle, the results of this experiment suggest that muscle of origin may also influence the ATPase activity of certain muscle fiber types early postmortem as well as their response to postmortem pH decline. For example, type 2A fibers from the RST had a slightly lower ATPase activity staining than WST 2A fibers at 3 min postmortem. Likewise, 2X fibers from the WST were more severely inactivated by ES compared to RST 2X fibers. Interestingly, type 2A and 2X fibers from the RST demonstrated a decrease in activity from 3 min to 24 h in NS samples, whereas type 2A and 2X from the WST did not. It can be speculated that such variations in ATPase activity from muscle to muscle may be the result of different cooling rates. Hypothetically, the deep portion (RST) of the semitendinosus muscle could have cooled down slower than the superficial (WST) portion, thus causing a higher degree of ATPase inactivation in NS-24 h samples. Under this hypothesis, however, it is difficult to explain why the ATPase activity staining of RST 2X fibers in ES-24 h samples is as high as NS-24 h samples. Similarly, Blanco and Sieck (1992) found that the kinetic parameters of 2B fibers differed when measured in the deep and superficial regions of the gastrocnemius. These researchers postulated that such results could be due to differences in enzyme concentration or myosin light chain isoform composition. Further research is needed to explain the muscle to muscle variation that exists in the ATPase activity between fibers expressing identical MyHC isoforms.

5. Conclusions

Overall, the postmortem actomyosin ATPase activities of individual muscle fibers are greatly influenced by MyHC isoform and pH decline. Fibers expressing fast MyHC isoforms, especially 2B, have a higher ATPase activity early postmortem but are more susceptible to inactivation by a rapid pH decline. These data suggest that differences in MyHC isoform composition contribute to the variations observed in postmortem energy metabolism, susceptibility to adverse pH and temperature effects, and ultimately meat quality in porcine muscle.

References


