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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,