Meat Science 98 (2014) 124–128

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Temperature induced denaturation of myosin: Evidence of structural alterations of myosin subfragment-1

Jiao Liu, Eero Puolanne, Per Ertbjerg *

Department of Food and Environmental Sciences, University of Helsinki, 00014 Helsinki, Finland

ARTICLE INFO

Article history:
Received 7 March 2014
Received in revised form 16 May 2014
Accepted 19 May 2014
Available online 27 May 2014

Keywords:
Water-holding capacity
Chymotrypsin
Ca^{2+} ATPase activity
Surface hydrophobicity
PSE

ABSTRACT

Denaturation of myofibrillar proteins in porcine *longissimus thoracis et lumborum* muscle was investigated after pre-rigor temperature incubation at 20, 30 and 40 °C. At 24 h myofibrils were isolated and myosin was further cleaved by chymotrypsin. High temperature pre-rigor induced release of myosin S1 (subfragment-1), less ($P < 0.05$) Ca^{2+}-ATPase activity and structural alterations of the region of the myosin molecule that harbors S1. Surface hydrophobicity of myofibrils from the 40 °C group increased ($P < 0.001$), suggesting a temperature-induced structural rearrangement exposing hydrophobic groups on the surface of myofibrils which in turn may explain the reduced water-holding of PSE meat.

1. Introduction

Pale, soft and exudative (PSE) meat has high drip loss and pale appearance. During the past, studies focusing on the mechanism behind the poor water-holding capacity in PSE meat have been conducted intensively. It has been widely accepted that the poor water-holding capacity of PSE meat is highly related to muscle protein denaturation when muscle has experienced low pH and high temperature early post mortem (Bendall & Wismer-Pedersen, 1962; Fischer, Hamm, & Honikel, 1979; Scopes, 1964; Van Laack & Kauffman, 1999). Sarcoplasmic protein denaturation in PSE meat as well as in pre-rigor high temperature incubated muscle has been studied particularly with respect to the reduction in the solubility of these proteins (Joo, Kauffman, Kim, & Park, 1999; Maribo, Olsen, Barton-Gade, Møller, & Karlsson, 1998). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot provide more specific results showing that at least glycogen phosphorylase and creatine kinase translocate from the sarcoplasmic to the myofibrillar fraction (Joo et al., 1999; Liu, Ruusunen, Puolanne, & Ertbjerg, 2014; Zhu, Ruusunen, Gusella, Zhou, & Puolanne, 2011). The myosin molecule, as the major constitute of myofibrils, is a multi-functional protein. Myosin has two globular heads called S1 (subfragment-1) and each head has a site of ATPase activity and an actin binding site (Rayment & Holden, 1994). Two S1 units are connected by a S2 (subfragment-2) to a coiled-coil rod. The rods aggregate to form the backbone of the thick filament with S2 and S1 units pointing out from the surface of the backbone, and S1 unit contacting with thin filament (Miroshnichenko, Balanuk, & Nozdrenko, 2000). The importance of the interfilamental space between thick and thin filaments to the water-holding capacity of meat has been emphasized (Hamm, 1972; Offer & Knight, 1988; Puolanne & Halonen, 2010). Myofibrillar proteins are therefore crucial structural muscle proteins, and their denaturation may play a key role in the development of PSE meat. The loss of ATPase activity and the reduction of myofibril extractability were reported in PSE meat as indicators for the denaturation of myofibrils (Joo et al., 1999; Penny, 1969; Warner, Kauffman, & Greaser, 1997). Based on comprehensive modeling Offer (1991) reported that myosin denaturation increases with accelerated pH drop due to pre-rigor high temperature and is therefore highly related to the pre-rigor chilling regime. Differential scanning calorimetry studies have also shown myosin denaturation occurring in PSE meat (Stabursvik, Fretheim, & Frøystein, 1984), and temperature incubation experiments demonstrated that myosin heads are more sensitive to heat-induced denaturation than sarcoplasmic proteins and actin (Deng et al., 2002). Offer and Knight (1988) suggested that myosin denaturation likely shrinks the myofilamental lattice spacing thereby reducing the water-holding capacity. However to understand more of the structural background of myosin denaturation during PSE-like conditions this study was undertaken. The 3-dimensional structure determines the functionality of protein molecules. Any alterations in the enzyme structure can cause a lack of contact with substrates, such as the interaction of denatured myosin S1 with ATP molecules resulting in a loss of ATPase activity. Chymotrypsin cleaves the myosin S1/S2 junction in the presence

* Corresponding author. Tel.: +358 503183909; fax: +358 919158460.
E-mail address: per.ertbjerg@helsinki.fi (P. Ertbjerg).

http://dx.doi.org/10.1016/j.meatsci.2014.05.013
0309-1740/

of EDTA (Weeds & Pope, 1977). Previous studies in fish have used chymotrypsin digestion to detect structural alterations in the myosin molecules (Takahashi, Takahashi, & Konno, 2005; Yuan, Wang, Chen, Qu, & Konno, 2011). The chymotryptic digestion pattern is therefore able to reveal information on the structural changes in the S1 to S2 regions of myosin.

Protein hydrophobicity is a significant parameter for protein functionality because it reflects the biochemical and structural characteristics of protein molecules (Boyer, Joandel, Ouali, & Culioli, 1996). Measuring surface hydrophobicity of myosin has been used largely to determine the degree of myosin denaturation as well as myosin aggregation and gelation (Boyer et al., 1996; Chelh, Gatellier, & Santé-Lhoutellier, 2006; Visessanguan, Ogawa, Nakai, & An, 2000).

The aim of the present study was to investigate the denaturation of myofibrillar proteins in porcine *longissimus thoracis et lumborum* (LTL) muscle after pre-rigor temperature incubation at 20, 30 and 40 °C until 6 h post mortem. The denaturation was studied at the level of myosin molecule by measuring its susceptibility to chymotryptic digestion and the loss of myosin ATPase activity as well as at the level of myofibrils by measuring the surface hydrophobicity.

2. Materials and methods

2.1. Raw materials

Three female pigs were used in this study and they belonged to the cross Norwegian Landrace × Swedish Yorkshire × Danish Landrace. They all had slaughter weight around 90 kg. The three LTL muscles (one from each animal) were pre-rigor incubated at 20, 30 and 40 °C to 6 h post mortem and cold stored at 4 °C as described by Liu et al. (2014). Meat samples were collected at 24 h post mortem and frozen at −80 °C until analysis.

2.2. Preparation of myofibrils

Two grams of frozen meat was homogenized by IKA Ultra-Turrax T25 homogenizer (Labortechnik, Staufen, Germany) at 13,500 rpm for 20 s in 20 ml cold rigor buffer (75 mM KCl, 20 mM MOPS (3-[N-Morpholino]propanesulfonic acid), 2 mM $MgCl_2$, 2 mM EGTA (ethylene glycol tetraacetic acid), pH 7.0). The supernatant was discarded after centrifugation at 10,000 *g* at 4 °C for 10 min. The pellet was then washed twice more by the same process in rigor buffer. The final pellet, which was mainly myofibrils, was re-suspended in 20 ml of digestion buffer (0.12 M NaCl, 20 mM MOPS, 1 mM EDTA (ethylenediaminetetraacetic acid), pH 7.0). Protein content of myofibrillar fraction was determined by *RC DC* Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA). Results calculated from the average of two duplicates were taken as protein content.

2.3. Myofibrillar surface hydrophobicity

Myofibrils were isolated as described under 2.2 except that the final pellet was resuspended in rigor buffer. Myofibrils were isolated in duplicates. Myofibrillar surface hydrophobicity was measured as described by Chelh et al. (2006) with a slight modification. The surface hydrophobicity is proportional to the amount of bound chromophore bromophenol blue (BPB) by hydrophobic sites on the protein surface. Briefly, 1 ml of myofibril suspension was adjusted to a protein concentration of 5 mg/ml and 200 μL of 1 mg/ml BPB was added. After incubation 10 min at room temperature and centrifugation at 12,000 *g* for 5 min, an aliquot of the supernatant was diluted 10 times and the absorbance was measured at 595 nm. The surface hydrophobicity was expressed as μg BPB bound by 1 mg of myofibrillar protein.

2.4. Chymotryptic digestion of myofibrils

The myofibrillar fractions from the three temperature incubations were adjusted to 10 mg/ml in digestion buffer before digestion at 30 °C for 1 h with 1/60 (*w*/*w*) of 2 mg/ml α-chymotrypsin (Sigma-Aldrich, St. Louis, MO) in 1 mM HCl and 2 mM $CaCl_2$. The reaction was terminated by adding 1 mM (final concentration) phenylmethylsulfonyl fluoride (PMSF) (Sigma-Aldrich). Supernatant 1 (Sup1), containing peptides released during the chymotrypsin incubation, was separated by centrifugation of digested myofibrils at 10,000 *g* at 4 °C for 10 min. At this step a major part of the myosin had been cleaved but myosin S1 was still attached to actin through rigor bonds. The next step intended to break the bond between actin and S1 by adding ATP. S1 was detached from actin by shaking the pellet after re-suspending it in the same volume of digestion buffer with 5 mM adenosine triphosphate (ATP), and 10 mM $MgCl_2$ at room temperature for 30 min. Supernatant 2 (Sup2), containing proteins/peptides released following ATP addition, was collected after centrifugation at 18,000 *g* at 4 °C for 2 h. The pellet (P) was then re-suspended in double volume of digestion buffer. The protein content of Sup1, Sup2 and P was determined (2.2).

2.5. Ca^{2+} ATPase assay

Ca^{2+}-ATPase activity was measured according to Silva, Sparrow, and Geeves (2003) with slight modification. Protein fractions were diluted in reaction buffer (2 mM EDTA, 2 mM $CaCl_2$, 20 mM MOPS, 30 mM KCl, pH 7.0) 200 (MF), 100 (Sup1), 40 (Sup2) and 50 (P1) times. The ATPase reaction was run at 25 °C for 30 min by adding 0.067 mM ATP. The reaction was stopped by mixing with same volume of 1 M ice-chilled perchloric acid (PCA). The inorganic phosphate (Pi) liberated during the ATPase reaction was determined by the malachite green method (Kodama, Fukui, & Kometani, 1986). Results were analyzed in duplicates and expressed as nmol Pi generated per min per mg of protein. The fractions Sup2 and P contained some Pi originating from the reaction in the previous step with ATP to break the myosin S1-actin bond. In order to minimize the background, Sup2 and P were washed free of Pi. This was done by mixing Sup2 with cold saturated ammonium sulfate (1:15 (*v*/*v*)) and settling on ice for 5 min before centrifugation at 18,000 *g* at 4 °C for 40 min. The pellet was then reconstituted in reaction buffer. Fraction P was washed once in 20 volumes of reaction buffer.

2.6. SDS-PAGE

For MF, Sup1, Sup2 and P fractions from three temperature incubations, 32 μL of proteins was mixed with 12.5 μL NuPAGE® LDS (lithium dodecyl sulfate) Sample Buffer (4×) and 5 μL NuPAGE® Sample Reducing Agent (10×) (Invitrogen, Carlsbad, CA). The mixture was heat treated at 100 °C for 3 min and applied onto NuPAGE® 12% Bis-Tris gels (Invitrogen). Each well was loaded with 10 μg protein. Gels were settled in a XCell SureLock® Mini-Cell electrophoresis chamber and the electrophoresis was run at 200 V for approximately 60 min in MOPS Buffer (Invitrogen). Gels were stained in Coomassie Brilliant blue R-250 (Liu et al., 2014) and images were captured by a digital camera (Canon 400D, Tokyo, Japan).

2.7. Western blot

Western blot was performed for all the samples to blot glycogen phosphorylase and creatine kinase as described by Liu et al. (2014).

2.8. Statistical analysis

Analysis of variance (ANOVA) was done by JMP® 9.0.0 (JMP, Cary, NC) using the Fit Model platform with the main effect of incubation temperature. Animal number was defined as a random factor.