Meat Science 119 (2016) 32–40

Contents lists available at ScienceDirect

Meat Science

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

On the water-holding of myofibrils: Effect of sarcoplasmic protein denaturation

Jiao Liu [a], Anders Arner [b], Eero Puolanne [a], Per Ertbjerg [a,*]

[a] Department of Food and Environmental Sciences, University of Helsinki, 00014 Helsinki, Finland
[b] Department of Physiology and Pharmacology, Karolinska Institutet, SE 171 77 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form 3 April 2016
Accepted 16 April 2016
Available online 19 April 2016

Keywords:
PSE
Heat denaturation
Protein aggregation
Water-holding capacity
X-ray diffraction

ABSTRACT

The role of heat-denatured sarcoplasmic proteins in water-holding is not well understood. Here we propose a new hypothesis that in PSE-like conditions denatured sarcoplasmic proteins aggregate within and outside myofilaments, improving the water-holding of denatured myofibrils. The process is compartmentalized: 1) within the filaments the denatured sarcoplasmic proteins shrink the lattice space and water is expelled; and 2) between the myofibrils and in the extracellular space, the coagulated sarcoplasmic proteins trap the expelled water from interfilamental space. The effect of sarcoplasmic proteins on the water-holding of myofibrils following incubation for 1 h at 21 to 44 °C was investigated. Our results were consistent with the new hypothesis. Myofibrils without sarcoplasm had the poorest water-holding. With increasing amount of denatured sarcoplasmic proteins, the water-holding of heat-denatured myofibrils improved proportionally. X-ray diffraction was used to measure the lattice space between the filaments. Precipitated sarcoplasmic proteins shrank ($P < 0.001$) the lattice spacing by 6.3% at 44 °C.

1. Introduction

Myofibrils take up the biggest part of the muscle fiber volume, where 85% of the myowater is trapped between the myofilaments and 15% is in extramyofibrillar space, i.e. between the myofibrils, between the fibers and between the fiber bundles (Huff-Lonergan & Lonergan, 2005). The structural changes during conversion of muscle to meat affects the distribution and mobility of myowater and drip from meat has been suggested to originate from the fluid that accumulates within the extracellular space (Pearce, Rosenvold, Andersen, & Hopkins, 2011). After slaughter, myofibrils start to shrink laterally and the cross-sectional area of muscle fibers is reduced. In agreement, Offer and Cousins (1992) observed enlarged gaps between the fibers and the fiber bundles by electron-microscopy. X-ray diffraction measurements have shown also that the lattice spacing between the filaments shrinks early post mortem (Diesbourg, Swatland, & Millman, 1988), due to the pH drop (Diesbourg et al., 1988; Irving, Swatland, & Millman, 1990) and formation of actomyosin bounds between thick and thin filaments (Offer & Knight, 1988). As a consequence of fiber shrinkage, sarcoplasm is expelled and accumulates outside the fibers, and becomes the potential drip formed afterwards.

Protein denaturation induced by low pH (<6.0) combined with high temperature (>35 °C) early post mortem has been extensively studied in order to explain the poor water-holding capacity of pale, soft and exudative (PSE) meat — for a recent review see Kim, Warner, and Rosenvold (2014b). Strong evidence of denaturation of both soluble and structural proteins in PSE meat has been shown. Thus, the total protein solubility of the sarcoplasmic fraction decreases in PSE meat (Joo, Kauffman, Kim, & Park, 1999; Sayre & Briskey, 1963; Scopes, 1964), resulting in a translocation of enzymes such as glycogen phosphorylase and creatine kinase from the sarcoplasmic to the myofibrillar fraction (Fischer, Hamm, & Honikel, 1979; Liu, Ruusunen, Puolanne, & Ertbjerg, 2014b; Zhu, Ruusunen, Gusella, Zhou, & Puolanne, 2011). Denaturation of structural proteins is indicated by decreased extractability of myofibrils as well as decreased ATPase activity of myosin in PSE meat (Penny, 1967; Warner, Kauffman, & Greaser, 1997; Wismer-Pedersen, 1959). A decrease in Ca^{2+}-ATPase activity is indicative of denaturation of myosin heads in the region of subfragment-1 (S1). Further evidence of structural alterations in the S1 unit were described in PSE-like meat with poor water-holding (Liu, Puolanne, & Ertbjerg, 2014a). The degree of myosin denaturation depends on the sarcomere length (Warner, Kerr, Kim, & Geesink, 2014; Kim, Kerr, Geesink, & Warner, 2014a), suggesting that the formation of the actomyosin rigor bond offers some protection against denaturation.

However, the mechanisms behind how protein denaturation relates to water-holding capacity of meat are not well understood. Different

* Corresponding author.
E-mail address: per.ertbjerg@helsinki.fi (P. Ertbjerg).

http://dx.doi.org/10.1016/j.meatsci.2016.04.020
0309-1740/

hypotheses proposed so far in literature are still debated (Huff-Lonergan & Lonergan, 2005, 2007; Kim et al., 2014b; Kristensen & Purslow, 2001). One hypothesis by Offer and Knight (1988) focuses on the influence of denaturation of myofibrillar proteins rather than sarcoplasmic proteins. Myosin denaturation before rigor was thus suggested to be the causative factor for high drip losses in PSE (Offer, 1991). Shrinkage of the myosin heads may in turn induce transversal shrinkage of the interfilamental spacing and is therefore believed to play a role in the reduced water-holding in PSE meat (Offer & Knight, 1988). Alternatively, it has been argued that the denaturation of sarcoplasmic proteins is highly related to the loss of water-holding (Bendall & Wismer-Pedersen, 1962; Sayre & Briskey, 1963) and a strong negative correlation between drip loss and sarcoplasmic protein solubility has been reported (Joo et al., 1999). These observations have stimulated speculations on how denatured sarcoplasmic proteins influence water-holding. Precipitates of denatured sarcoplasmic proteins onto the surface of myofilaments have been suggested to result in shielding of net charges, subsequently reducing the interaction between filaments and water molecules (Bendall & Wismer-Pedersen, 1962; Boles, Parrish, Huiatt, & Robson, 2002; Zhu et al., 2011). As a result part of the sarcoplasm would be expelled from myofibrils and lost as drip. However, the high drip is not necessarily causatively related to the observed denaturation of sarcoplasmic protein.

In the current study, the aim was to investigate the relative influences of denatured sarcoplasmic and myofibrillar proteins on the water-holding capacity of myofibrils. Myofibrillar protein denaturation was indicated by measuring the Ca^{2+}-ATPase activity and surface hydrophobicity. In addition the changes in the filamental space within myofibrils with or without denatured sarcoplasmic proteins were studied by x-ray diffraction analysis.

2. Materials and methods

2.1. Raw materials

Six porcine *longissimus thoracis et lumborum* (LTL) muscles were excised from different carcasses the day after slaughter for water-holding experiments (three each for the experiments described in Section 2.5 and 2.6), and four LTL muscles were excised for x-ray diffraction measurements (Section 2.7). Pigs were Norwegian Landrace × Swedish Yorkshire × Danish Landrace crosses and were CO_2 stunned, slaughtered and chilled at a commercial slaughterhouse in Finland. Muscles were transported refrigerated and arrived in the lab 24 h post mortem and were thereafter classified not to be PSE meat based on the Minolta L* values being less than 54. For all muscles the Minolta L* values were in the interval 50–54; Drip loss (24–72 h post-mortem) was 5.5–7.5%; and 3) pH_u was 5.4–5.6.

Muscles were trimmed of visible connective tissue and fat and divided into two parts. One part was frozen at 24 h post-mortem at −80 °C and later used for preparation of myofibrils. The other part was used for sampling of sarcoplasm (drip) at 4 °C from 24 h to 48 h post mortem by the gravimetric method (Honikel, 1998). The collected drip was stored at −80 °C and subsequently used together with myofibrils representing the original sarcoplasm.

The pH of drip were measured by using a Knick Portamess752 pH-meter equipped with a Mettler-Toledo Inlab 427 electrode and the values were between 5.3–5.5. The protein concentration in the drip were determined by DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA), and results were calculated from the average of duplicates.

2.2. Preparation of protein-depleted sarcoplasm

An aliquot of sarcoplasm was incubated at 80 °C for 30 min and then centrifuged at 12,000 rpm for 10 min at room temperature to remove all the sarcoplasmic proteins. After filtration to remove any visible particles, the clear solution was used as protein-depleted drip in the following experiment. The pH values of protein-depleted sarcoplasm were between 5.5–5.6. The difference compared to the original sarcoplasm could be due to the buffering capacity of sarcoplasmic proteins.

2.3. SDS-PAGE

In order to compare the protein composition between the sarcoplasm and the sarcoplasmic protein fraction within meat, as well as to prove that heating at 80 °C was efficient enough to remove all the proteins in the sarcoplasm, SDS-PAGE was used to compare the protein composition between sarcoplasm, protein-depleted sarcoplasm and sarcoplasmic protein fraction extracted from meat. SDS-PAGE was performed on the six LTL muscles (Section 2.5 and 2.6). Meat samples were mixed 1:10 (w:v) with cold rigor buffer (75 mM KCl, 20 mM Tris(hydroxymethyl)aminomethane, 2 mM $MgCl_2$, 2 mM EGTA (ethylene glycol tetraacetic acid), pH 7.0) and homogenized by an IKA Ultra-Turrax T25 homogenizer (Labortechnik, Staufen, Germany) at 13,500 rpm for 20 s. The supernatant was taken as sarcoplasmic fraction after centrifugation at 10,000*g* at 4 °C for 10 min. Protein content was determined by DC Protein Assay Kit, and results were calculated from the average of duplicates. The protein determination indicated that only 5–6% of the original amount of sarcoplasmic protein was left in the protein-depleted sarcoplasm.

After adjusting the protein level in the drip, protein-free drip and sarcoplasmic fraction, 65 μL of each sample was mixed with 25 μL NuPAGE® LDS (lithium dodecyl sulfate) sample buffer (4×) and 10 μL NuPAGE® reducing agent (Invitrogen, Carlsbad, CA). The mixture was heat treated at 100 °C for 3 min and applied onto NuPAGE® 12% Bis-Tris gels (Invitrogen). Protein amount loaded in each well was 8 μg. Gels were settled in XCell SureLock® Mini-Cell electrophoresis chamber and the electrophoresis was run at 200 V for approximately 60 min. Gels were stained in Coomassie Brilliant blue R-250 (Liu et al., 2014b) and images were captured by a digital camera (Canon 400D, Tokyo, Japan).

SDS-PAGE showed that drip had quite similar protein profile as the sarcoplasmic fraction extracted from meat. In addition, heating to 80 °C for 1 h efficiently removed the sarcoplasmic proteins that could precipitate under PSE-like conditions and left were only small amounts of heat-stable proteins (Fig. 1). Therefore, it is reasonable to use drip and 80 °C-treated drip to represent sarcoplasm and protein-depleted sarcoplasm, respectively.

2.4. Preparation of myofibrils

Meat samples were mixed 1:10 (w:v) with cold rigor buffer and homogenized by an IKA Ultra-Turrax T25 homogenizer at 13,500 rpm for 20 s. The supernatant was decanted after centrifugation at 10,000*g* at 4 °C for 10 min and the pellet was then washed twice by the same procedure but in a buffer with lower pH (75 mM KCl, 20 mM MES, 2 mM $MgCl_2$, 2 mM EGTA, pH 5.5) to bring down the pH close to 5.5. The final pellet had a pH of 5.5–5.6 and was used as sarcoplasm-free myofibrils. One batch of myofibrils was prepared from each LTL muscle. After resuspending 1 part of the pellet in 10 parts of rigor buffer, the myofibrillar protein content (mg/g of meat) was measured by RC Protein Assay kit and calculated from duplicates. The initial weight of meat used for myofibrillar protein preparation, and the pellet obtained in the end were recorded. Therefore, it is possible to calculate the myofibril protein content in the pellet by:

$$= \frac{\text{Meat weight (g)} * \text{Myofibrillar protein content} \left(\frac{\text{mg}}{\text{g}}\right)}{\text{Pellet weight (mg)}} * 100\%.$$

The myofibril protein content was around 18–21% in the pellet obtained as described above.