A NEW HYPOTHESIS EXPLAINING THE INFLUENCE OF SARCOPLASMIC PROTEINS ON THE WATER-HOLDING OF MYOFIBRILS

J. Liu, E. Puolanne and P. Ertbjerg

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

Helsinki, Finland

Abstract – There is some consensus in literature that heat-induced denaturation of structural proteins in the myofibrils reduces the water-holding capacity of the meat, which may partly explain the poor water-holding of PSE meat. The role of heat-denatured sarcoplasmic proteins in water-holding is, however, not well understood. Here we propose a new hypothesis that in PSE-like conditions denatured sarcoplasmic proteins aggregate, and form a protein network connected with structural proteins within the myofilaments. This network binds water and restricts shrinkage of the filament lattice and thereby improves the water-holding capacity of myofibrils. In the current study we tested the hypothesis by investigating the effect of denatured sarcoplasmic proteins on the water-holding of myofibrils. Our results on the water-holding capacity of myofibrils were consistent with the new hypothesis. Myofibrils without the presence of sarcoplasm had the poorest water-holding. However, in presence of denatured sarcoplasmic proteins, the water-holding capacity of heat-denatured myofibrils improved significantly. Myofibrils showed similar heat-induced denaturation with or without the presence of sarcoplasm as indicated by Ca^{2+} ATPase activity while the surface hydrophobicity was higher ($P < 0.001$) in the presence of denatured sarcoplasmic proteins.

Key Words – PSE, denaturation, aggregation, network

I. INTRODUCTION

Protein denaturation induced by low pH combined with high temperature post mortem has been studied intensively in order to explain the poor water-holding capacity of pale, soft and exudative (PSE) meat. Strong evidences of denaturation of both soluble and structural proteins in PSE meat have been shown in literature [1, 2, 3].

However, there has been quite different hypothesis proposed so far to understand the mechanisms underlining how protein denaturation relates to water-holding capacity of meat. One acknowledged hypothesis by Offer & Knight [4] emphasizes the importance of the denaturation of proteins within myofibrils. The shrinkage of myosin heads reduces the interfilamental spacing, which decreases the water-holding of myofibrils. Another well-known hypothesis is based on Hamm's electrostatic repulsion theory. Precipitates of denatured sarcoplasmic proteins onto the myofilaments have been speculated to result in shielding of net charges, subsequently reducing the interfilamental spacing and inducing more drip loss [5, 6]. However, it has been questioned if the denaturation of sarcoplasmic proteins is able to explain the loss of water-holding [4, 7], since the sarcoplasmic proteins are soluble and mainly globular proteins, which tend to coagulate and trap water upon heat denaturation [8].

We here propose a different role for the denatured sarcoplasmic proteins in water-holding capacity of myofibrils as illustrated in Fig.1. In conditions of PSE, sarcoplasmic proteins start to aggregate and bind to the surface of myofilaments. These denatured sarcoplasmic proteins form a network with myofilaments. The protein aggregates and the formed network bind water and restrict the shrinkage, resulting in improved water-holding capacity of myofibrils.

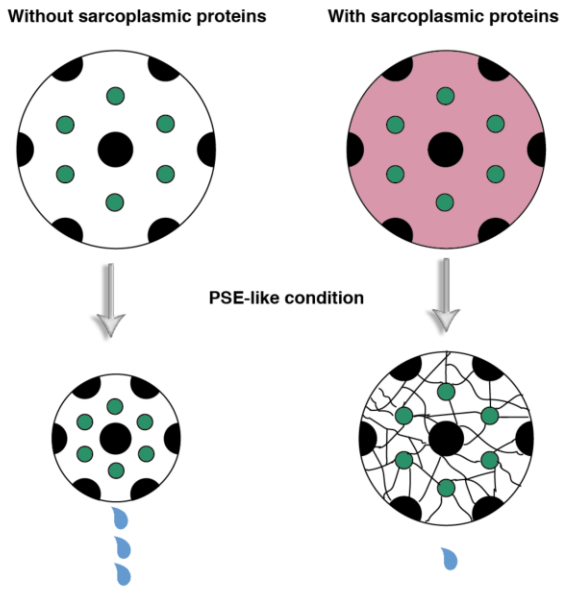

Fig.1. Hypothesis explaining the effect of sarcoplasmic proteins on changes in the water-holding capacity of myofibrils due to the combination of low pH and high temperature. Sarcoplasmic proteins (pink) denature and form aggregates between thick (black circle) and thin (green circle) filaments. The denatured sarcoplasmic proteins form a network that restricts the shrinkage and thus improves the water-holding capacity of myofibrils.

To test this new hypothesis, we compared the influence of the denatured sarcoplasmic and myofibrillar proteins on water-holding capacity of myofibrils. In addition, myofibrillar protein denaturation was analyzed by measuring the surface hydrophobicity.

II. MATERIALS AND METHODS

2.1. Raw materials

Three post-mortem *longissimus thoracis et lumborum* (LTL) muscles from different individual carcass of cross Norwegian Landrace x Swedish Yorkshire x Danish Landrace were used in the present study. Drip collected from 24 h to 48 h post mortem was stored at -80 °C and after thawing used to represent sarcoplasm. Meat samples were taken at 24 h post mortem, frozen at -80 °C and subsequently used for preparation of myofibrils.

Myofibrils were extracted as described by Liu et al. [3] except the last two washes were done in another buffer (75 mM KCl, 20 mM MES (2-(N-Morpholino) ethanesulfonic acid hydrate, 4-Morpholineethanesulfonic acid) hydrate, 2mM $MgCl_2$, 2 mM EGTA (ethylene glycol tetraacetic acid), pH 5.5) to bring down the pH close to 5.5. These myofibrils were used as sarcoplasm-free myofibrils. Myofibrils preparation was done once from each LTL muscle.

2.2 Treatments

Only the myofibrils and drip originated from the same muscle were mixed in the following procedures. Three different experimental lines were used affecting the amount of denatured sarcoplasmic proteins and their location within the myofibrils:

1) Myofibrils without the presence of sarcoplasmic proteins during temperature incubations were used as a reference for the effect on water-holding of heat-denaturation of myofibrillar proteins. The myofibrils were incubated at 25, 35, 38, 41, and 44°C for 1 h. After cooling, unheated drip was mixed with myofibrils (w:v=1:1) by the same procedure as below.

2) The effect of denatured sarcoplasmic proteins on the water-holding of myofibrils when denatured outside the myofibrils was studied. Myofibrils and drip were placed independently in different test tubes and incubated at 25, 35, 38, 41, and 44°C for 1 h. After incubation, all the tubes were cooled down at 4 °C for 10 min before mixing myofibrils and drip (w:v=1:1) by the same process as described below.

3) The effect of denatured sarcoplasmic proteins on the water-holding of myofibrils when denatured inside the filament lattice was studied. Myofibrils and drip (w:v=1:1) were mixed by an IKA Ultra-Turrax T25 homogenizer at 13,500 rpm for 3 s in a test tube. Then the tube was sealed and stored at 4 °C overnight for a full diffusion of sarcoplasmic proteins into myofibrils. On the next day, tubes were incubated at 21, 35, 38, 41, and 44°C for 1 h and then cooled down at 4 °C for 10 min.

For all the groups triplicates were done for each temperature.

2.3. Water-holding capacity

The water-holding of myofibrils was measured by centrifuging the "myofibril-drip mixture" at 2400 g for 5 min followed by decanting of the supernatant. The water-holding capacity was calculated as percentage of water loss =

$$\frac{Weight\ difference\ before\ and\ after\ centrifugation}{Weight\ before\ centrifugation - weight\ of\ tube} x\ 100$$

2.4. Myofibrillar surface hydrophobicity

The pellet left from the water-holding measurement was washed once in rigor buffer in order to remove soluble proteins. Surface hydrophobicity was measured by the bromophenol blue (BPB) method [6]. Each myofibrillar re-suspension was measured in duplicate and the average was taken as the myofibrillar surface hydrophobicity.

III. RESULTS AND DISCUSSION

Incubating myofibrils extracted from post-rigor meat at temperature from 21 to 44 °C significantly reduced the water-holding capacity of myofibrils. Myofibrils without denatured sarcoplasmic proteins showed increasing water loss with increasing incubation temperature and thus had the poorest water-holding (Fig. 2, Green). The addition of sarcoplasmic proteins which had been denatured outside the myofibrils improved the water-holding (Fig. 2, Red). When the sarcoplasmic proteins were denatured inside the myofilament lattice together with myofibrils, the water-holding was significantly improved not only compared to myofibrils without denatured sarcoplasmic proteins but also compared to the situation where the sarcoplasmic proteins denatured outside the myofibrils (Fig. 2, Blue).

The myofibrils showed similar heat-induced denaturation with or without the presence of sarcoplasm as indicated by Ca^{2+}-ATPase activity (results not shown).

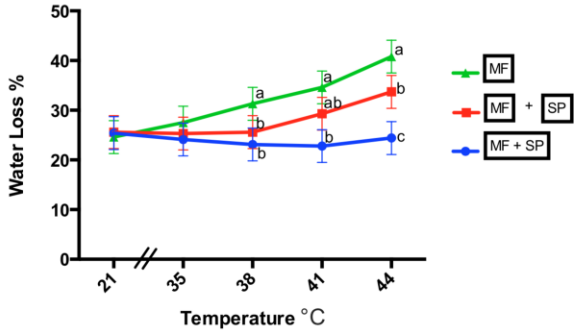

Fig.2. Water loss of myofibrils following temperature incubation for 1 h. Green: myofibrils were heated alone and mixed with unheated sarcoplasmic proteins; Red: myofibrils and sarcoplasmic proteins were heated separately and mixed afterwards; Blue: myofibrils were heated with sarcoplasmic proteins. Least square means with standard error are plotted. [ab]LSmeans within temperature with the same letter do not differ ($P > 0.05$).

Taken together, the current results therefore point to that myofibrillar rather than sarcoplasmic protein denaturation is the driving force for the decreased water-holding observed in PSE-like conditions, as suggested by Offer & Knight [8]. The present study furthermore adds to the understanding of the role of sarcoplasmic protein denaturation in water-holding of meat. Bendall & Wismer-Pedersen suggested more than half a century ago a mechanism whereby denatured sarcoplasmic proteins contribute negatively to water-holding [3]. However, instead of influencing the water-holding of myofibrils negatively by shielding the charges on the filaments, we here demonstrate that denatured sarcoplasmic proteins improve the water-holding capacity. In addition, the location where the denatured sarcoplasmic proteins precipitate seems to determine how much the water-holding can be improved. The sarcoplasmic proteins, when denatured inside the filament lattice, improved water-holding more than when denatured outside myofibrils. A possible explanation could be that sarcoplasmic proteins start to unfold and aggregate with themselves and/or with myofibrillar proteins around them driven by hydrophobic interaction. We propose a hypothesis (Fig. 1) that the aggregated sarcoplasmic proteins build a network between the filaments. The network of

denatured protein creates a supporting structure for water to be trapped and restricts transversal shrinkage of the myofibrils.

Surface hydrophobicity is measured as an indicator of protein denaturation. In the present study, temperature incubation at 21 to 44 °C significantly increased the surface hydrophobicity of myofibrils (Fig. 3), which for myofibrils alone is in parallel with the decreased water-holding of myofibrils (Fig. 2), in agreement with Liu *et al.* [2]. In presence of denatured sarcoplasmic proteins, the myofibrillar surface hydrophobicity increased ($P < 0.001$), suggesting that denatured sarcoplasmic proteins contributed relatively more to the increase of surface hydrophobicity than denatured myofibrillar proteins.

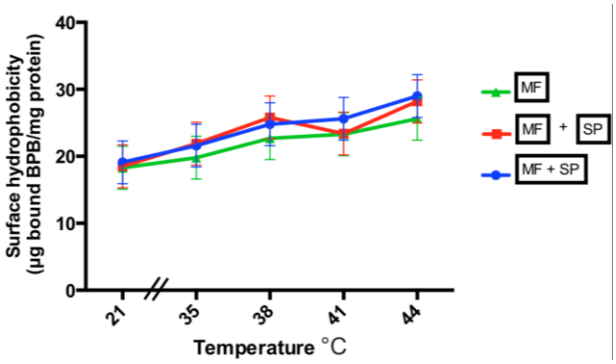

Fig.2. Surface hydrophobicity of myofibrils upon heating at 21, 35, 38, 41 and 44 °C. Blue: myofibrils were heated with sarcoplasmic proteins; Red: myofibrils and sarcoplasmic proteins were heated separately and mixed afterwards; Green: myofibrils were heated alone and mixed with unheated sarcoplasmic proteins. Least square means with standard error are plotted.

IV. CONCLUSION

We here propose a new hypothesis: In conditions of heat-induced protein denaturation, such as occurring in PSE, a network of denatured sarcoplasmic protein is formed between the filaments within the myofibrils. This network improves the water-holding capacity of myofibrils. The results show increased water-holding with increasing sarcoplasmic protein denaturation and are in agreement with the proposed hypothesis.

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