POSTMORTEM CHANGES IN THE CALPAIN SYSTEM IN FIVE PORCINE MUSCLES

Per Ertbjerg and Peter P. Purslow

Department of Dairy and Food Science, The Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

Background

μ-Calpain (or calpain I) and m-calpain (or calpain II) and their specific inhibitor (calpastatin) are part of the Ca^{2+}-dependent proteolytic system. The calpains have been shown to be able to disrupt the myofibril Z-line, and in vitro to degrade a range of myofibrillar proteins, e.g. troponin-T, troponin-I, desmin, filamin, C-protein, titin, nebulin, vimentin and vinculin. Meat tenderness increases during postmortem ageing, and it is generally accepted that proteolytic degradation of muscle proteins is at least partly responsible for this increased tenderness. There are several indications in literature that calpains play a significant role in the postmortem proteolysis that result in increased meat tenderness. However, the present understanding of the involvement of calpain and calpastatin in proteolysis and postmortem tenderisation is incomplete.

Objectives

To further clarify the role of calpains in postmortem tenderization, we have followed the activities of the calpain system and monitored changes in calpains using antibodies during storage of five different porcine muscles.

Methods

The muscles longissimus dorsi (LD), semitendinosus (ST), semimembranosus (SM), soleus (S) and vastus intermedius (VI) were excised approximately 60 min after slaughter from the left side of two female pigs. Subsamples of ca. five grams were vacuum packed and then frozen in liquid nitrogen. Right side samples were excised at 24 h postmortem and either frozen then or stored at 2 °C for further 3 or 8 days before devision into subsamples of five grams and freezing.

Calpains were extracted by a slight modification of the method described (Ertbjerg et al., 1999). Briefly, five grams of muscles were homogenized in 6 volumes of buffer (50 mM Tris/HCl, 5 mM EDTA, 10 mM monothioglycerol, 150 nM pepstatin A, pH 8.0). The homogenate was centrifuged (30,000 *g*, 15 min, 4°C) and the supernatant was used for determination of calpastatin activity (see below), to determine the immunological detectable calpain level (see below) and to determine μ- and m-calpain activity. A hydrophobic column (butyl-sepharose) was used to separate calpains from calpastatin. The calpain-containing eluate was loaded on a mono-Q column. μ-Calpain and m-calpain were each collected and the calpain activity was determined using casein as substrate. One unit of calpain activity was defined as an increase in absorbance at 278 nm of 1.0 per hour at 25°C. Results were corrected for noncalcium dependent proteolytic activity by using 10 mM EDTA instead of $CaCl_2$ in the incubation medium..

Calpastatin were determined using the one mL sample removed from the homogenate extract. The homogenate extract was heated at 100 °C for 5 min and centrifuged at 20,000 x *g* for 4 min. The calpastatin activity in the supernatant was determined using various dilutions in the above described casein assay containing 0.2 units of m-calpain extracted and fractionated from other samples. From observation of the decrease in absorbance at 278 nm, an inhibition curve was generated. One unit of calpastatin activity was defined as the amount that inhibited one unit of m-calpain activity.

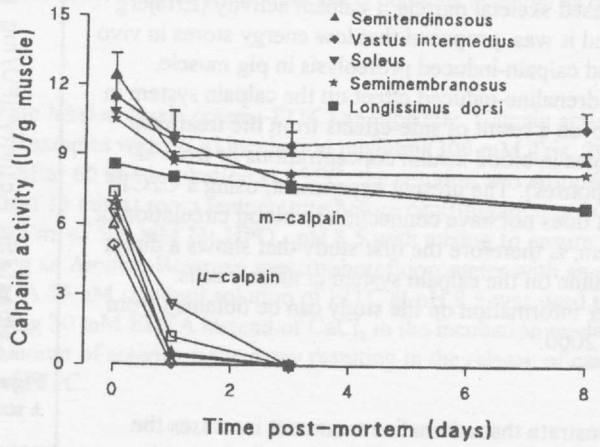

Fig. 1. Changes in m- and μ-calpain activities during postmortem storage of five muscles.

Western blots were obtained by dissolving calpains in the sarcoplasmic fraction sample buffer. Samples were heated at 100 °C for 4 min before they were loaded onto 7% Tris-Acetate gels. After electrophoresis, gels were transferred by electroelution to PVDF membranes. Primary antibodies used were monoclonal anti-μ-calpain antibody and polyclonal anti-m-calpain antibody, both labelling the 80 kDa subunit. Bound primary antibodies were labeled with horseradish peroxidase-conjugated secondary antibodies diluted 1:5,000 in PBS-Tween, for 60 minutes at 25°C. A chemiluminescent system was used to detect labeled protein bands as described by the supplier (Amersham).

Results and discussions

Results show that μ-calpain activity decreased rapidly in all muscles with postmortem storage, while m-calpain activity were only little affected by storage (Fig. 1). This pattern of decline of μ- and m-calpain activity reported here in various porcine muscles is in good agreement with the pattern of decline observed in bovine longissimus muscle (Koohmaraie, *et al.*, 1995) and bovine semimembranosus muscle (Boehm *et al.*, 1998).

Calpastatin (inhibitor) activity in porcine muscles decreased slower than μ-calpain activity during storage (Fig. 2), again in agreement with reports on bovine muscles. Calpastatin activity were less and the proteolytic potential (estimated by the μ-calpain to calpastatin ratio early after slaughter) were greater in the two fast-twitch white muscles (LD and SM) than in ST, S and VI. Therefore, the calpain-mediated proteolysis can be predicted to occur faster in LD and SM in porcine muscle. This interpretation is confirmed by Christensen *et al.*, 2000, reporting that of desmin and troponin-T are degraded faster in porcine LD and SM than in ST, S and VI. Desmin and troponin-T are both known to be substrates for the calpains.

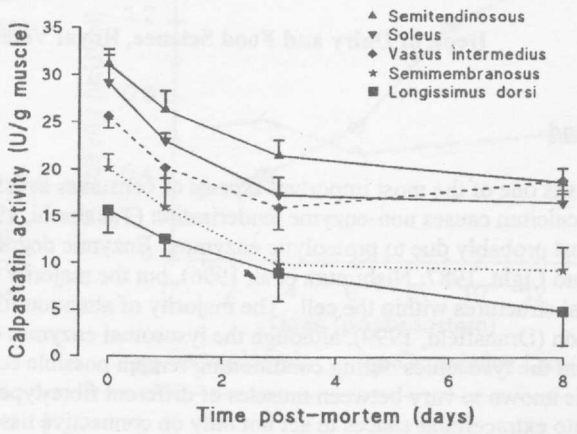

Fig. 2. Changes in calpastatin activities during postmortem storage of five muscles.

To increase the understanding of the involvement of these enzymes in postmortem protein degradation, we investigated the level of the catalytic subunit of μ- and m-calpain using immunological techniques. The immunological dectectable amount of m-calpain (Fig. 3A) and μ-calpain (Fig. 3B) in a soluble fraction was only little affected by storage. The measured *protein level* of m-calpain therefore largely reflected the measured *activity* during storage, while μ-calpain did not reflect the observed decrease in *activity* with postmortem storage (see Fig. 1). The decrease in μ-calpain activity during storage is therefore not caused by extensive autolytic degradation of the active enzyme.

Conclusions

μ-Calpain activity decreased rapidly in all muscles with postmortem storage, while m-calpain were only little affected. The activity of the inhibitor, calpastatin, were lower in two fast-twitch white muscles (LD and SM) than in ST, S and VI. Immunoblot showed that the observed decrease in μ-calpain activity during storage is not caused by extensive autolytic degradation of the active enzyme.

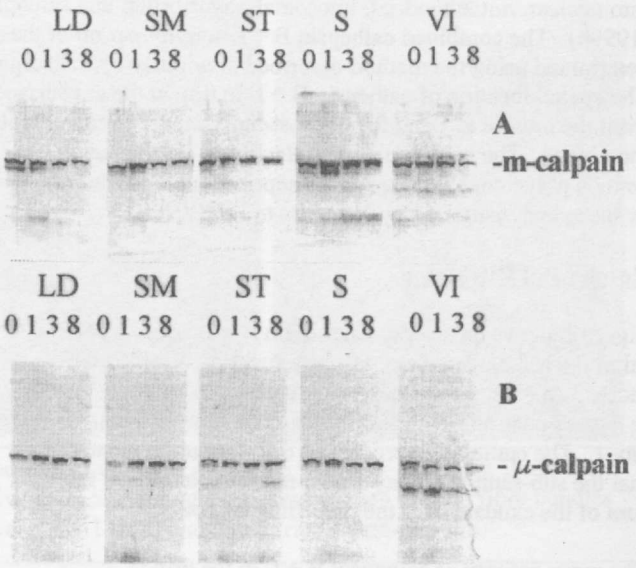

Fig. 3. Western blot analysis of A) m-calpain and B) μ-calpain during storage.

References

Boehm, ML, Kendall, TL, Thompson, VF, Goll, DE (1998) *J Anim Sci* 76, 2415-2434

Christensen, M., Henckel, P, Purslow, PP (2000) *Proc 46th Int Congr Meat Sci Tech*, Buenos Aires, Argentina, submitted

Ertbjerg P, Henckel P, Karlsson A, Larsen LM, Møller AJ (1999) *J Anim Sci* 77, 2428-2436

Koohmaraie, M., J. Killefer, M. D. Bishop, S. D. Shackelford, T. L. Wheeler, and J. R. Arbona. 1995. In: A. Ouali, D. I. Demeyer, and F.J.M. Smulders (Ed.) Expression of Tissue Proteinases and Regulation of Protein Degradation as Related to Meat Quality. p 395-410. ECCEAMST, Utrecht, The Netherlands.