IS m-CALPAIN ACTIVE POST-MORTEM IN PORK?

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Keywords: pork, calpains, autolysis, muscle differences, meat tenderness

Introduction

The role of calpains in the process of meat tenderization is not fully understood. According to the literature μ- and m-calpain play an important role in this process, however, the roles of the two isoforms are still discussed. The activities of these calpains isozymes have a different rate of decrease in *post mortem* (PM) muscles. While m-calpain activity remains more or less constant, μ-calpain activity decrease as a function of time *post mortem* (Boehm et al., 1998; Dransfield, 1993). For this reason some authors consider only μ-calpain involved in the tenderization of meat (Koohmaraie et al., 1987; Veiseth et al., 2001). A role of the m-calpain in the later phase of the tenderization process has been suggested (Boehm et al., 1998). The aim of our study was to investigate the μ- and m-calpain activities in two different porcine muscles at 72 hours PM.

Materials and Methods

Italian Duroc x (Landrace x Large White) crossbred pigs (N=75; mainly castrated males) with an average live weight of 135 kg were pen-fed and finished under standard Parma-ham practice. Samples were taken 72 hours *post mortem* (PM) from *M.longissimus dorsi* (LD) (7th rib) and *M. semimembranosus* (SM). Frozen meat samples (- 80°C) were finely chopped. One g of each sample was homogenised (13.500 rpm) in 6 ml of an extraction buffer at pH 8.0 and centrifuged for 30 minutes at 4°C at a speed of 15000 x *g*. To determine the activity of μ- and m-calpain the zymography method was used. After addition of sample buffer, 15 μL supernatant was loaded in each well of casein minigels with 26 wells. Gels were run at 80 V for 3 hours at 4 ºC before incubation with calcium for one hour. After staining and destaining, the density of each band was measured and quantified relative to reference standards within each gel. The data were analysed using Proc Mixed (SAS, ver. 9.1). The model included muscle as fixed effect and animal as random effect.

Results and discussion

In most of the pork samples the determination of calpain activity showed four bands of enzymes activity (Fig 1A, B). Veiseth et al. (2001) showed that zymography can be used to differentiate and detect the activity of native and autolyzed calpains in lamb. Kent et al. (2004) used Western blotting of mice muscle and detected four calpain bands. They demonstrated that these correspond to the native and autolyzed μ- and m-calpain isoforms; this suggests that the four bands detected in our zymography gels correspond to the native and autolyzed μ- and m-calpains. To confirm this hypothesis we incubated partly purified pig muscle calpain with 20mM $CaCl_2$ at different time points (15s, 30s, 1m, 2m, 4m and 8m) in order to obtain the autolysis of the enzymes. The autolysis process was stopped using a buffer containing 60mM EDTA. The activities of the native and the autolyzed forms of calpains were evaluated with the zymography method, as described above. This experiment showed that the first and second bands co-migrated with native and autolyzed μ-calpain, respectively (Fig 1C), and the third and fourth bands co-migrated with native and autolyzed m-calpains (Fig 1D).

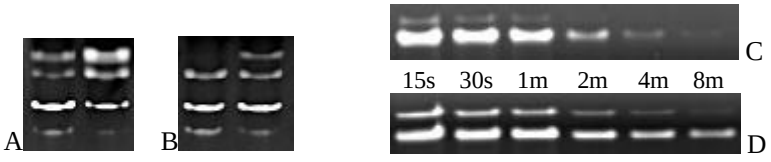

Figure 1. Casein zymography gels: A) μ- and m-calpain activity in LD pork samples 72 hours PM; B) μ- and m-calpain activity in SM pork samples 72 hours PM; C) partly purified μ-calpain and D) partly purified m-calpain from swine LD incubated with calcium at different times.

According to Veiseth et al. (2001), the concentration of calcium in the muscle *post mortem* is high enough to activate μ-calpain but it is not high enough to activate m-calpain. However, Boehm et al. (1998) described an increase in the calcium concentration in bovine muscle during prolonged ageing. It has been suggested that the activity of μ- and m-calpain is synergic: μ-calpain contributes to early *post mortem* proteolysis, while m-calpain is partially activated and contributes to *post mortem* tenderization during prolonged ageing (Dransfield, 1993).

The detection of autolyzed m-calpain activity in our study supports a role of this isoform in *post mortem* ageing of pork. The presence of autolysis-products of m-calpain 3 days *post mortem* in pork could be due to an increase in the free calcium concentration sufficient to activate m-calpain, and suggest an involvement of this enzyme in the tenderization process of pig meat.
Previous studies (Boehm et al., 1998; Dransfield, 1993) have shown that the activity of µ-calpain had a fast decrease within the first 24 hours PM; in contrary the m-calpain activity was more or less constant during the storage period. In our study on pork, surprisingly LD muscle at 72 hours PM had higher activity of native µ-calpain than native m-calpain (Fig. 2). The activity of native µ- and m-calpain was higher ($P < 0.001$) in LD muscle compared to SM (Fig. 2). Previous studies found no correlations between the activity of the two calpains isoforms in different muscles, and a possible muscle-specific difference in the calpain system has therefore been suggested. Geesink et al. (1992) studied the activity of µ-calpains in different bovine muscles; they observed that the activity of the enzyme at 43 and 67 hours PM was higher in LD than in SM muscle. Ilian et al. (2001) compared the activity of µ- and m-calpain in different ovine muscles (LD, SM, *M. psoas major* and *M. semitendinosus*) and also found higher µ- and m-calpain activity (12 and 24 hours PM) in LD compared to SM. When comparing the activity of the autolyzed µ and m-calpain we noticed a lower m-calpain activity ($P < 0.001$) in LD. To our knowledge no other studies detected and compared the activity of autolyzed µ- and m-calpain.

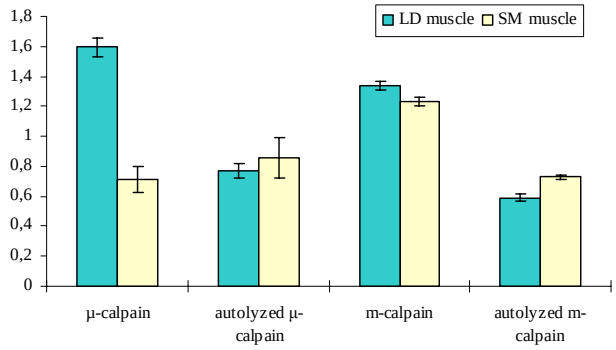

Figure 2. Comparisons between the activity of native and autolyzed µ- and m-calpain isoforms from LD and SM pig muscles 72 hours PM.

Conclusions

Day three *post-mortem* calpain showed four bands of enzyme activity on zymography gels. The two upper bands corresponded to native and autolyzed µ-calpain, and the two lower bands to native and autolyzed m-calpain. The detection of this fourth band suggests that m-calpain is active in pork *post mortem.* The activity of native µ- and m-calpain was higher ($P < 0.001$) in porcine *M. longissimus dorsi* compared to *M. semimembranosus.*

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