DRIP CONTAINS SIGNIFICANT LEVELS OF PROTEOLYTIC ENZYMES

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Background

Tenderness is one of the most important aspects of consumer satisfaction with meat. Although there is an alternative view that the release of calcium causes non-enzyme tenderisation (Takahashi, 1999), development of tenderness during the post-mortem storage of meat is most probably due to proteolytic enzymes. Enzymic degradation of extracellular connective tissue has been shown to occur (Stanton and Light, 1987; Nishimura et al, 1996), but the majority of interest is in the effects of enzymes on myofibrillar or cytoskeletal structures within the cell. The majority of attention focuses on μ-calpain as the major enzyme responsible for tenderisation (Dransfield, 1999), although the lysosomal enzymes (e.g. cathepsins) which are progressively released (Ertbjerg et al, 1999a) from the lysosomes during conditioning remain possible contributors (Taylor and Goll, 1995). The concentration of these proteases is known to vary between muscles of different fibre-type composition. It is possible that intracellular enzymes may be released into extracellular spaces to act not only on connective tissue structures but also to influence proteolysis in neighbouring cells. However, the release of enzymes into the extracellular space is not well understood.

Objective

The aim of this study is to determine the extent and time-course of intracellular protease release into the extracellular space by monitoring enzyme activity in drip. Cathepsin B + L activity was chosen to avoid complications with autolysis of calpains, especially μ-calpain.

Methods

Longissimus muscle was obtained from 12 pigs 1 hour post-mortem. Samples (c. 100g) were cut out at 6 hours post-mortem vacuum-packed and stored at 4°C for up to 13 days. The exudate in the pack was sub-sampled at days 1,3,6,9,13 post-mortem. For drip measurements, cubes of meat roughly 3.5 cm on each side, were cut out at 6 hours post-mortem, and suspended in plastic netting within polythene bags at 4°C. Small sub-samples of drip were taken from the bags at 1, 3, 6, 9 and 13 days post-mortem. From the muscles of four animals, small sub-samples of the muscle were also taken at 1, 3, 6, 9 and 13 days, homogenised and fractionated into nuclear, mitochondrial, liposomal, myofibrillar and sarcoplasmic fractions by centrifugation, as described by Ertbjerg et al. (1999b). The combined cathepsin B + L activity per ml of the sarcoplasmic fractions and of the collected drip samples was determined using the method described previously by Ertbjerg et al. (1999b).
The spatial location of cathepsin B + L in drip at the cut surface of the meat pieces was determined by a novel method. Cubes cut from the muscle at 1 and 8 days post-mortem were gently "blotted" on one cut surface (transverse to the fibre direction) on a PVDF membrane. The membrane was then immuno-labelled using a polyclonal anti-human cathepsin B antibodyraised in sheep and visualised by exposure to BCIP/NCT.

Results and Discussion

Figure 1 shows the average cathepsin B + L activity per ml in the accumulated exudate as a function of time post-mortem (n = 7). The activity gradually rises from day 1 to day 13 post-mortem, but is already quite significant on day 1. The cumulative process of drip formation means that the sub-sample on day 1 post-mortem comprised the sum of the exudate over the preceding 24 hours.

The mean activity of cathepsin B + L in the drip is compared to the mean activity in the sarcoplasmic fraction of the muscle for 4 specimens in figure 2. The sarcoplasmic fraction contains a very small proportion of the total cathepsin B + L activity in the muscle, as approximately 90% of the total activity remains within the lysosomes even after long times post-mortem (data not shown). However, the relative concentration of activity in the sarcoplasmic (soluble) phase remains roughly constant (figure 2) with time. Even at day 1, the activity in the drip is approximately one half of the level within the sarcoplasmic fraction of the muscle cells,

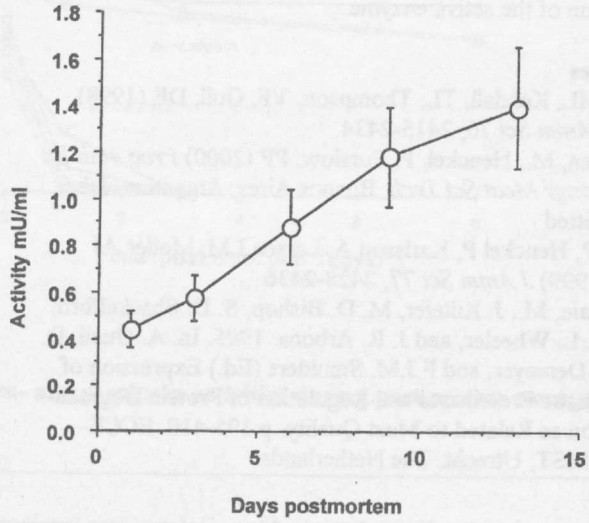

Fig 1. Mean Cathepsin B+L activity in exudate as a function of days postmortem. Bars denote + one standard error (n=7)

indicating an early release of the enzymes from the cell. The steady rise with time in activity per ml of the accumulated drip seems to indicate that the enzyme activityis progressively concentrated in the drip as the outflow rate of cellular water diminishes with time post-mortem. To check against the possibility of large evaporative losses distorting this pattern of results, the activity in "drip" obtained by mechanically squeezing the meat with light forces at 1 – 13 days post-mortem was measured. The measurements showed a similar rising concentration of activity in the exudates (data not shown).

Figure 3 shows a low-magnification view of the "immuno-blot" against cathepsin B taken from the surface of a piece of longissimus muscle 1 and 8 days post-mortem. The staining is sufficient to show the anatomical arrangement of individual muscle fibres into bundles. At day 1 there is significant labelling within fibres and muslce fascicles, but the heaviest labelling for the enzymes occurs at the gaps between muscle fascicles, ie. the "perimysial drip channels" observed by Offer et al. (1989). At day 8 the labelling in the drip channels is even more pronounced.

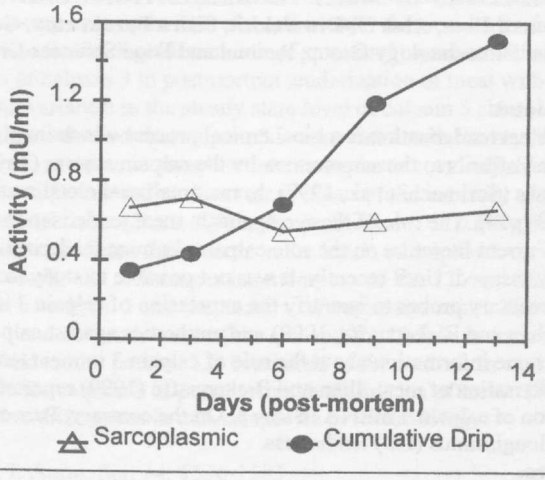

Figure 2: Comparison of the average athepsin B+L activity per ml in the sarcoplasmic fraction and in the accumulated drip from porcine longissimus (n=4).

Conclusions

Measurements of enzyme activity strongly suggest that cathepsin B + L are released into drip in significant amounts within the first day post-mortem, and increase thereafter. Immuno-blots of enzyme location on the cut surface of the meat support this view. Other enzymes, (eg. μ- and m-calpain) may be expected to be similarly mobile. This suggests that proteolytic enzymes are relatively free to migrate through the muscle tissue during conditioning, influencing the proteolysis in neighbouring cells and intervening connective tissue structures.

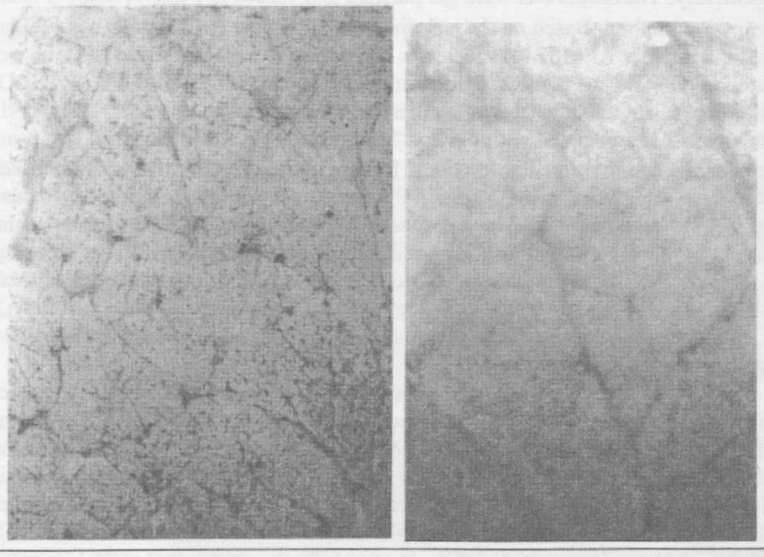

Fig. 3: Immuno-blot of cut muscle surface with anti-cathepsin B antibody. Note the relative level of staining in spaces between muscle fibre bundles increases between day 1 (left) and day 8 (right) post-mortem.

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