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CATHEPSIN B+L ACTIVITY IN BEEF AS AFFECTED BY AGEING

V.M.H. BARNIER[1], H.L.J.M. Van LAACK[2] and F.J.M SMULDERS[1]

[1] Department of the Science of Food of Animal Origin, University of Utrecht, P.O. Box. 80175, 3508 TD Utrecht, The Netherlands

[2] Meat Science Research Laboratory - USDA, Beltsville, MD, United States

INTRODUCTION

One of the most obvious changes during ageing of beef is myofibrillar proteolysis and concurrent tenderisation. At least two groups of endogenous proteolytic enzymes are known to degrade myofibrillar proteins; e.g., the calpains and the lysosomal cathepsins. In the last decade numerous reports indicating the importance of calpains have been published. However, the rate of protein degradation and tenderisation do not parallel the changes in calpain activity indicating that calpain alone cannot account for the tenderisation process (Geesink, 1993).

To be able to degrade myofibrillar proteins cathepsins have to be released from the lysosomes. Results from immunohistochemical studies (Dutson and Lawrie, 1974; Kas *et al.*, 1983) and studies in which released and bound lysosomal enzyme activities were measured (Dutson *et al.*, 1980; Wu *et al.*, 1985; Kudryashov *et al.*, 1988; Pommier *et al.*, 1987) indicate that post-mortem conditions result in a release of cathepsins.

Many post-mortem factors such as low pH, high temperature (Moeller *et al.*, 1976; Dutson *et al.*, 1980; Wu *et al.*, 1985), high Ca^{++} concentration (Kas, 1983) and low ATP levels (Mellman, 1986) negatively affect the stability of lysosomal membranes.

Electrical stimulation (ES) has long been used to prevent cold shortening (Bendall, 1980; Carse, 1973; Davey *et al.*, 1976). However, even in the absence of cold shortening conditions, ES may have a positive effect on tenderness (Savell *et al.*, 1981; Ouali and Valin, 1984; Dransfield *et al.*, 1992). It has been suggested that this may be explained by an effect on post-mortem proteolysis.

Electrical stimulation induces a low pH in combination with a relatively high temperature. Both Dutson *et al.* (1980) and Pommier *et al.* (1987) observed an earlier release of lysosomal cathepsins into the cytoplasm under these conditions. Their measurements were limited to the early post-mortem period. Data on the evolution of cathepsin activity during storage are limited and conflicting. Wheeler *et al.* (1990) observed an increase in total cathepsin B+L activity during ageing, whereas Shackelford *et al.* (1991) did not observe any effect of storage time on total cathepsin B+L activity. Whipple *et al.* (1990) did not find differences in bound cathepsin B+L activity between 1 and 14 days post-mortem.

The purpose of the present study was to investigate the effect of post-mortem storage and electrical stimulation on the release of cathepsin B+L in bovine *m.longissimus*.

MATERIAL AND METHODS

Experiment 1

Eighteen bulls (age 1.5 years, carcass weight 351±31kg) were randomly assigned to three treatment groups. Six animals

were stimulated electrically during 8s (ES8), six animals were stimulated electrically during 64s (ES64) and six animals were not stimulated (NS). Electrical stimulation was applied within five minutes after bleeding (85V, 14Hz; Mitab, Simrishamm, Sweden). At about 40 minutes post-mortem, carcasses entered the chilling rooms (4.5°C air velocity $1.8ms^{-1}$ or 2.5°C, air velocity $2ms^{-1}$).

At one day post-mortem *m.longissimus* (6^{th}-10^{th} rib) was excised, divided in four parts, vacuum packaged and stored at 0 to 2°C. One part of each muscle was sampled at 1, 7 and 14 days post-mortem and cathepsin B+L activity was assessed.

Experiment 2

On each of four days, three cows were randomly selected at a commercial slaughter plant. All 12 carcasses were electrically stimulated at 20 to 30 minutes post-mortem during the dehiding process (150V, 50Hz, ca.10s). At one day post-mortem *m.longissimus* was excised, divided in four parts, vacuum packaged and stored at 0 to 2°C. One part of each muscle was sampled at 1, 2, 3 and 8 days post-mortem and cathepsin B+L activity was assessed.

Lysosomal enzyme activities

Samples for measurement of free (soluble fraction) and bound (nuclear and microsomal fractions) cathepsin B+L activity were prepared according to Wu *et al.* (1985). Activities were measured according to Etherington *et al.* (1987). In experiment 1, samples for measurement of total cathepsin B+L activity were prepared from frozen muscle following the procedure of Etherington *et al.* (1987).

Statistical analysis

Significance of differences was tested using Students t-test (paired comparison was allowed).

RESULTS AND DISCUSSION

In experiment 1 the effect of ES on cathepsin activity was studied. Results are included in Table 1. At one day post-mortem free cathepsin activity was higher in ES64 samples than in NS samples. These results are in agreement with those of Pommier *et al.* (1987). Surprisingly the difference between ES and NS samples disappeared during ageing, after seven days of storage activities were similar. It was unclear what may have caused the increase in free cathepsin activity. Changes in cathepsin activity in the bound fraction could not account for the increase in the soluble fraction. Another preparation procedure of samples for measurement of total cathepsin B+L activity was performed on the same samples. To determine the total activity of cathepsin B+L, a detergent (Triton X-100) was added to the extraction buffer (Etherington *et al.*, 1987). Table 1 includes the results obtained for each treatment group at one and 14 days post-mortem. Again, electrical stimulation had a significant effect on cathepsin activity. Also, there was a significant increase in total activity during ageing. This was rather unexpected. As there is no post-mortem enzyme synthesis, other processes seem to be involved. It was hypothesized that during ageing the expression of activity increases as a result of a decrease in inhibition. Possibly cystatins denatured or were otherwise affected by post mortem conditions.

The changes in cathepsin activity seemed to occur during the first seven days post-mortem. Therefore, in the second experiment, cathepsin activity during the first eight days post-mortem was investigated. The set-up of this experiment was such that a day effect on sampling and preparation procedures (and thus on results) was avoided.

The results presented in Table 2 indicate an increase of free cathepsin B+L activity during ageing. Most of the increase (76%) occurred between days one and three. These results are in agreement with Kas *et al.* (1983) who observed that

the majority of lysosomes became permeable or were completely damaged, usually by the third day of meat storage.

In contrast to experiment 1, there was no increase of activity in the bound fraction. Nevertheless, changes in bound activity could not account for the increase in the soluble fraction.

The results of these two experiments indicate that soluble cathepsin activity increases during ageing. A possible explanation for this increase is a decrease in the level of inhibition by cystatins. However, Shackelford *et al.* (1991) estimated cystatin levels by measuring cathepsin B+L activity before and after removal of cystatin by affinity chromatography and no changes in cystatin levels were observed (Shackelford *et al.*, 1991).

The fact that we observe an increase in total as well as free activity, strongly suggests that cystatins are involved. Further research is needed to establish if inhibition by cystatins is reduced as a result of a decrease in cystatin levels or as a result of physical chemical changes affecting the interaction between cathepsins and cystatins.

CONCLUSIONS

The present study indicates that free cathepsin B+L activity increases during ageing and that electrical stimulation accelerates this process. Further research is needed to assess which processes are responsible for the increase in cathepsin B+L activity during ageing and their possible role in post mortem tenderisation.

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Table 1. The effect of electrical stimulation during 8s (ES8) or 64s (ES64) at various times post-mortem on the cathepsin B+L activity (nmol/min/g muscle) as assessed at 1, 7 and 14 days post-mortem (experiment 1).

Fraction Trait	Day 1 (n=6)	Day 7 (n=6)	Day 14 (n=6)
Free			
NS	0.21±0.04[a]	1.02±0.30[b]	0.98±0.08[b]
ES8	0.25±0.11[a]	1.11±0.26[b]	0.13±0.26[b]
ES64	0.41±0.10[a]	1.12±0.22[b]	1.10±0.15[b]
Bound			
NS	0.25±0.09[a]	0.10±0.13[b]	0.10±0.02[b]
ES8	0.26±0.13[a]	0.15±0.06[b]	0.12±0.04[b]
ES64	0.32±0.17[a]	0.14±0.05[b]	0.16±0.05[b]
Total			
NS	3.37±0.18	ND	3.81±0.54
ES8	3.53±0.65	ND	3.86±0.84
ES64	3.89±0.54[a]	ND	4.13±0.44[b]

[a,b] Between columns, means with superscripts not containing a common letter differ significantly ($P<0.05$). ND=not determined.

Table 2. Cathepsin B+L activity (nmol/min/g muscle) after 1, 2, 3 and 8 days post-mortem (experiment 2).

Fraction	Free	Bound
Day 1 (n=12)	1.51±0.85[a]	0.35±0.17
Day 2 (n=12)	1.97±0.98[b]	0.39±0.22
Day 3 (n=12)	2.34±0.93[c]	0.44±0.19
Day 8 (n=-12)	2.61±0.86[c]	0.44±0.25

[a,b,c] Between columns, means with superscripts not containing a common letter differ significantly ($P<0.005$).