The effect of exogenous μ-calpain on the mechanical properties of single muscle fibres extended to fracture

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Introduction

Postmortem degradation of myofibrillar proteins and its relationship to meat tenderness have since long been the subject of considerable research. Often the techniques used in these studies are SDS-PAGE, Western Immunoblotting or microscopy. However, these techniques does not enable determination of the structural and mechanical changes which occur during ageing and which are responsible for improved tenderness. To our knowledge, only one study of the effect of conditioning on the mechanical properties of muscle fibres have been performed (Mutungi et al., 1996). According to Mutungi et al. (1996) conditioning for 3 days resulted in a 50% decrease of the breaking strength of muscle fibres as compared to breaking strength at 1 day postmortem. The protease μ-calpain has been implicated as the major causative agent for many of the proteolytic changes that occur as meat is aged (Goll et al., 1991; Uytterhaegen et al., 1994). Our preliminary results demonstrate that μ-calpain has a major effect on the mechanical properties of the muscle fibre component of meat.

The objective of this study, was to investigate the direct effect of proteolysis by μ-calpain on the tensile breaking strength of single muscle fibres isolated from bovine *Semitendinosus* muscles.

Material and Methods

Semitendinosus muscles were obtained from one Friesian heifer (2-2½ year old) 24 hours postmortem (p.m.). Muscle samples (weighing approximately 10 g) were cut, vacuum packed and slowly frozen at –20 °C. Samples were stored at this temperature until use. The procedure for tensile test of single muscle fibres broadly followed the methodology of Mutungi et al. (1996). Small strips of muscle was carefully dissected from the frozen samples and placed in a dissection solution containing 50 mM Tris-base (pH 7.5), 100 mM NaCl, 221 mM Mannitol and 5 mM EDTA. Single fibres were isolated under a binocular dissecting microscope at room temperature. The isolated fibres were rapidly transferred onto aluminium templates (4 mm wide and 10 mm long) so they extended across a gap 2-3 mm cut in the centre of the plate and attached to the aluminium plate by gluing the fibre ends onto the plate with cyanoacrylate adhesive glue. Care was taken not to stretch the fibre during the transfer process. The free length of the fibre was measured using vernier calipers and the diameter was measured using a high-power microscope. The fibres were incubated in a incubation solution containing 50 mM Tris-base (pH 7.5), 100 mM NaCl, 221 mM mannitol, 0.75 mM $CaCl_2$, 15 mM DTT and μ-calpain (40 μg/ml) for 24 hours at room temperature. Controls were treated identically, except that an incubation solution without μ-calpain was added to the fibres. Incubation was stopped by transferring the fibres into a solution containing 50 mM Tris-base (pH 7.5), 100 mM NaCl, 221 mM Mannitol and 5 mM EDTA. The aluminium plate with the fibre glued onto it was then attached between two screw-up clamps, one attached to a motor and the other to an isometric force transducer of a small mechanical testing device (Lewis & Purslow, 1989). The side-pieces of the aluminium plate bridging the gap was carefully cut leaving the fibre hanging between the two plate ends. Fibres were stretched at a constant rate of 14 μm/s until fracture. Loads and extensions were monitored using a chart recorder and a 16 bit A/D converter (National Instruments) running under LabView data acquisition software. Tensile tests were performed on a total of 7 fibres per treatment.

Results and Discussion

Table 1 shows the effect of μ-calpain on the tensile breaking strength and strain of single muscle fibres isolated from the bovine *Semitendinosus* muscle. The values of strength are expressed as breaking stress, i.e. breaking load per unit cross-sectional area. As shown in table 1, the mechanical properties of single muscle fibres were dramatically decreased by addition of exogenous μ-calpain. Addition of μ-calpain led to an approximately 82% decline in the forces needed to fracture single muscle fibres and the strain values dropped by approximately 72% as compared to control fibres. The observed decrease in breaking strength and strain after incubation with exogenous μ-calpain is in agreement with changes in mechanical properties of single muscle fibres observed after ageing of meat (Mutungi et al., 1996).

Figure 1 shows the stress-strain curves for the incubated and control muscle fibres. The stress-strain curves of both μ-calpain treated and control fibres were r-shaped, with an early steep rise in stress followed by a yield point at strains between 5 and 20% for control fibres and 5 and 10% for μ-calpain treated fibres. It also appears from figure 1 that relatively large fibre to fibre variations occurred, especially in the control fibres. For this reason, we have chosen to show the individual stress-strain curves. The variations may be due to factors such as fibre type.

We have now shown that μ-calpain mechanically weakens the myofibrillar component of meat by direct measurements of the mechanical properties of isolated muscle fibres. The concentration of μ-calpain used in this study is probably higher than the

concentration of μ-calpain within the meat and we therefore expect a more pronounced weakening of the muscle fibres in vitro than in vivo.

The exact site of action of μ-calpain on myofibrils is unclear. We speculate that nebulin plays a role in maintaining the tensile strength of the muscle fibres. Nebulin is located in the I-band region running longitudinally to the muscle fibre direction in close proximity to the actin filaments. Structural studies using electron microscopy have reported that fragmentation of the myofibril often takes place adjacent to Z-lines (Gann & Merkel, 1978). This fragmentation could result from degradation of nebulin. However, the mechanical importance of this protein still needs to be investigated. The specific role of desmin for the tensile strength and integrity of myofibrils have been investigated using desmin knockout mice (Li et al., 1997). It was found that absence of desmin resulted in mice which were weaker and fatigued more easily. This protein therefore also seems to play a major role for the mechanical properties of the muscle fibres and will be the subject of further study.

We believe that the sarcolemma is permeable to the exogenous added μ-calpain and that the μ-calpain is diffusing into the muscle fibres and degrading proteins/structures which are important in maintaining structural integrity. However, we can not rule out the possibility that the mechanical weakening of muscle fibres is due to μ-calpain acting from the outside i.e., degrading structures on the surface of the sarcolemma. Further work is needed to clarify this.

Conclusions

Addition of exogenous μ-calpain dramatically weakens the tensile strength of single muscle fibres. Future studies will aim to clarify which structures/proteins are influenced by the μ-calpain and how the proteolytic degradation of these proteins affect the mechanical properties of the myofibrillar component of meat.

Pertinent literature

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Table 1. The influence of μ-calpain on the breaking stress and strain of single muscle fibres isolated from bovine *Semitendinosus* muscle. Values are given as means ± S.E. (n=7).

	μ-calpain	control
Breaking stress (kPa)	51.19 ± 14.79	288.17 ± 54.80
Breaking strain (%)	16.03 ± 4.40	58.21 ± 11.27

Figure 1. The influence of μ-calpain on the stress-strain curves of seven single muscle fibres extended until fracture. Stress-strain curves of (a) control fibres and (b) μ-calpain treated fibres.