THE RELATIONSHIP BETWEEN INTEGRINS, CALPAINS AND DRIP CHANNEL FORMATION IN PORK.

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Background

One of the major quality problems faced by the Danish pork industry is the high variability seen in the water holding capacity of the meat. Within hours after slaughter, channels open between adjacent muscle fibers and muscle fiber bundles (drip channels) allowing for the loss of water from the product. There is a significant increase in the rate of channel fiber formation in meat samples which show higher levels of water loss. In beef muscle it has been previously shown that drip channels are formed due to the detachment of the cell membrane from the cell body. Cell membranes are attached to the cell body by two major types of adhesion complexes, integrin-containing focal adhesions and the dystrophin/dystroglycan complex of proteins. It has previously been shown that integrins are degraded in vitro by a family of proteolytic enzymes known as calpains. In this study, we used samples with known and varying rates of drip channel formation to determine whether the time frame of formation of the channels correlated with the rate of cell/membrane adhesion protein degradation, and whether this degradation may be due to calpain proteolytic activity.

Objectives

The purpose of this study was to investigate the fate of integrins in muscle postmortem, and to determine whether calpain-mediated integrin degradation may play a role in the formation of drip loss in pork.

Methods

Hampshire x Danish Duroc x Danish Landrace x Large White pigs (HDLY) were used, all non-carriers of the RN gene. All pigs were reared at an experimental farm and were fed a standard diet *ad libitum* to slaughter weights was 86 kg.
Drip loss analysis: A 2 cm thick slice was excised 24 hours after slaughter, suspended within a net in a plastic bag and stored at 4°C. The percent change in weight over the subsequent 48 h was taken as the drip loss, as described by Honikel (1998).
Embedding and cutting: Slices of 3 cm thickness were sequentially cut out of the LD at 3 h, 6 h, 9 h and 24 h *post mortem and* were fixed in 4% formaldehyde. The samples were embedded in Technovit 7001 (Heraeus Kulzer, Germany) as per manufacturers instructions and cut at 3 µm thickness on a RM2155 Microtome (LEICA, Nussloch, Germany) using a metal d-knife. Sections were stained with toluidin blue O.
Cryosectioning and immunostaining: Meat samples were cut into 1 cm square cubes and dropped into liquid nitrogen for 2 minutes. Each sample was cut into 6 mm sections using a Leica Frigicut cryostat (LEICA, Nussloch, Germany). To visualize cell membranes, samples were incubated in a dilution of fluorescein isothiocyanate in PBS. For immunofluoresence, the samples were rehydrated with PBS followed by blocking with 10% goat serum in PBS for 10 min. Sections were then incubated in a 1:100 dilution of anti β1-integrin monoclonal antibody (Chemicon, Temecula, CA) for 1h at RT and then in a fluorescein-conjugated secondary antibody for 1 h in the dark at RT. Immunofluorescent images were obtained using a LEICA DmIrb inverted fluorescent microscope with a coolsnap CCD (Media Cybernetics, Silver Spring, MD) camera. Exposure time for each image was 6 seconds. Images were collected and processed using Image Pro plus software (Image House, Denmark). Calpain activity was detected using Boc-leu-met-CMAC (Molecular Probes, Eugene, OR) as described by Rosser et al. (1993).

Results and discussion:

During the post-mortem process, extracellular spaces referred to as drip channels are formed in pork when the cell membrane detaches from the cytoskeleton (Figure 1). This occurs within three hours after death in animals that exhibit high drip loss (Figure 2B, with an ultimate drip loss of 11%) and within 9 hours in animals which show low drip loss (Figure 2A, with an ultimate drip loss of 4%). The difference seen in drip channel formation correlates with the degradation of the cytoplasmic domain of the β1 integrin subunit, the subunit which is responsible for the attachment of cell membranes to the cytoskeleton (Figure 2 inset). The dystrophin/dystroglycan complex is not disrupted in the time frame (data not shown).
m-Calpain is co-localised into integrin-containing adhesion complexes at the surface of muscle cells (Figure3, left) as is therefore available for activation and subsequent degradation of integrin subunits. At early time points, calpain activity can be found specifically in this region of the muscle fibre (Figure 3, right) in punctuate structures similar in size and localization to integrin-containing focal adhesions.

Conclusions:

In porcine muscle, the cell membrane detaches from the cell body postmortem allowing for the opening of drip channels. Degradation of the cytoplasmic domain of β1 integrin, but not the dystrophin/dystroglycan complex, correlates to the opening of drip channels between muscle fibers in pork postmortem. Samples which show early degradation of β1 integrin have higher drip loss. m-calpain, an enzyme which has been shown to degrade β1 integrin in vitro, co-localizes with b1 integrin on muscle cell membranes. The activity of calpain at early time points can be found specifically in the region of integrin-containing focal adhesions.

Pertinent literature

Honikel, K.O. 1998. Reference methods for the assessment of physical characteristics of meat. **Meat Sci**. 49: 447-457.
Pfaff, M., Du, X., and M.H. Ginsberg. 1999. Calpain cleavage of integrin β cytoplasmic domains. **FEBS Letters**. 460:17-22.
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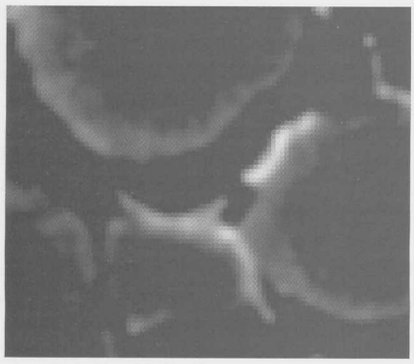
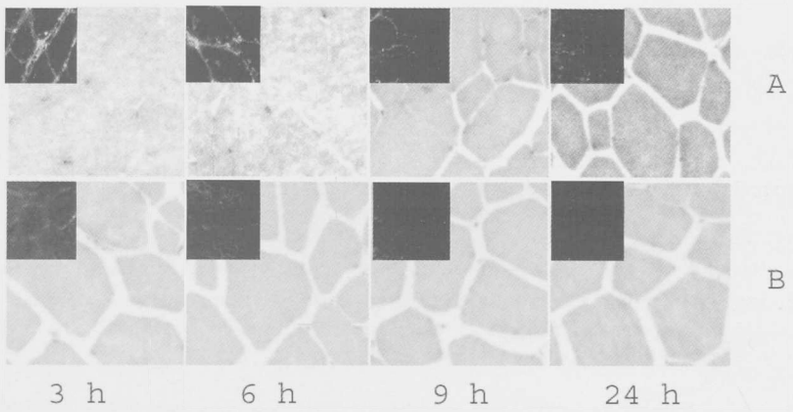


Figure 1. Immunofluorescent staining of cell membranes in pork muscle postmortem. The membranes have lifted off the muscle fiber and are clearly visible.

Figure 2. Histological staining of pork muscle. Drip channels can be readily seen in sample A at 9 hours postmortem, and in sample B at 3 hours postmortem. Inset; Immuofluorescent staining for the cytoplasmic domain of β1 integrin.



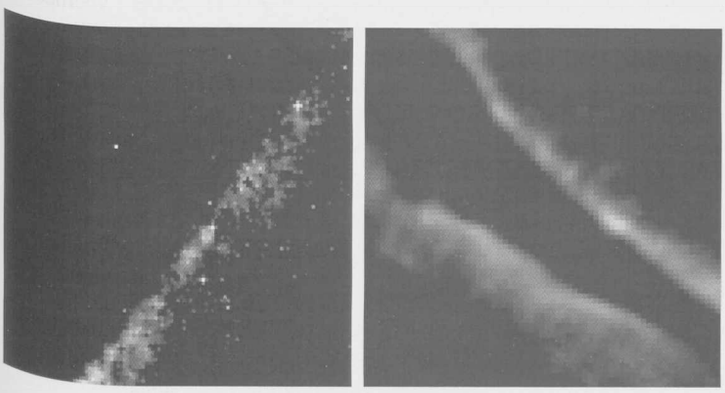


Figure 3. Left: Co-localization of β1 integrin and m-calpain in 1 h postmortem muscle samples. Right: Immunofluorescent imaging of calpain activity in postmortem muscle. The cleaved substrate can be found in punctuate regions at the cell membrane.