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The intracellular distribution of small heat shock proteins in post-mortem beef is determined by ultimate pH

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Abstract

This study analysed *Longissimus dorsi* muscles from 39 Angus or Angus × Limousin bulls to determine the small heat shock protein (sHSP) dynamics in beef aged at 15 °C. Using quantitative ELISA we determined that alpha β-crystallin and HSP20 were present at higher levels in muscles from Angus bulls. sHSP levels peaked at 0.5 and 3 h post-mortem for HSP20 and alpha β-crystallin respectively. Intermediate pH (pH 5.7–6.3) beef had the highest level of alpha β-crystallin expression at 3–6 h post-mortem. Low pH (<5.7) meat had no detected soluble alpha β-crystallin and had diminished HSP27 at 22 h post-mortem. The transition of sHSP between a soluble to an insoluble phase was demonstrated by pH titration of a sarcoplasmic fraction. The effect of ultimate pH upon sHSP distribution in post-mortem muscle may influence final meat quality characteristics.

Keywords: Ultimate pH; Tenderness; Heat shock protein; Alpha β-crystallin; HSP27; HSP20; HSP70

1. Introduction

The conversion of muscle into meat to obtain optimal eating quality is a complex process. Export of prime beef from NZ to distant markets as a fresh chilled product requires balancing meat processing time and temperature to control the effects of product oxidation (affecting colour and flavour), proteolysis (contributing to tenderness and drip loss) and the microbiological shelf-life. Consumers consider that the tenderness of beef is often the most desirable prerequisite of acceptable quality (Jeremiah, 1982) and meat tenderisation is generally regarded to be enzymatic by nature. The contribution of muscle endopeptidases (Ouali et al., 2006; Sentandreu, Coulis, & Ouali, 2002) like cathepsins (De Duve, Pressman, Gianetto, Wattiaux, & Appelmans, 1955) and calpains (Guroff, 1964) to the tenderising process has been investigated in much detail over the past two decades and their roles have been demonstrated (Koohmaraie & Geesink, 2006; Sentandreu et al., 2002). However questions remain as to why the rate of meat tenderisation is so variable in red meat. The muscle glycogen content at slaughter has a profound effect upon muscle ultimate pH (pH_u) (Young, West, Hart, & Van Otterdijk, 2004) and this can then influence enzymatic activity and meat quality.

Intermediate and high pH ($pH_u > 5.7$) beef from bulls is frequently observed in New Zealand because nearly all bull beef is derived from a pasture-fed production system (low calorie diet) and stresses experienced close to slaughter can result in high and variable shear force values (Lowe, Devine, Wells, & Lynch, 2004). Understanding what causes the shear force variation in bull beef is an issue for the meat industry because the inconsistent quality of intermediate pH meat is value lost that might otherwise be realised if the mechanisms are understood.

Small heat shock proteins (sHSP) alpha β-crystallin, HSP20 and HSP27 have been identified as prominent components of living muscle necessary for cell maintenance and repair. However, no role for sHSP has yet been associated

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to post-mortem muscle and meat quality. Heat shock proteins have a homeostatic function in living tissues, stabilising unfolded proteins, assisting with refolding of denatured proteins and preventing protein aggregation (Liu & Steinacker, 2001). Cells and tissues up-regulate expression of HSP in response to stress. All cells constitutively express HSP to operate as molecular chaperones, associating with newly synthesised unfolded proteins, and assisting with protein folding. HSP70 is the prototype HSP that has been most studied in muscle and meat (Jin et al., 2006; van Laack, Faustman, & Sebranek, 1993). Proteome studies of muscle have also identified some small heat shock proteins (sHSP) that are up-regulated in post-mortem muscle (Hwang, Park, Kim, Cho, & Lee, 2005; Jia et al., 2006; Sayd et al., 2006). Alpha β-crystallin is a sHSP that has been associated with desmin protein filament assembly in muscle (Der Perng et al., 1999; Perng, Wen, Van Den Ijssel, Prescott, & Quinlan, 2004). This class also includes HSP27 that functionally associates with HSP20 and alpha β-crystallin in muscle (Fontaine, Sun, Benndorf, & Welsh, 2005; Gusev, Bogatcheva, & Marston, 2002; Sugiyama et al., 2000) to refold denatured proteins. Recent studies have identified that the down-regulation of some sHSP including alpha β-crystallin and HSP27 is correlated with improved tenderness, juiciness and flavour in bull beef (Bernard et al., 2007). This paper sets out to determine the dynamics of sHSP in ischemic bull beef muscle during its conversion to meat and relates these dynamics with ultimate pH.

2. Materials and methods

2.1. Animals

Thirty-nine 22-month-old Angus ($N = 16$) or Angus × Limousin ($N = 23$) bulls were slaughtered in two batches with a one month interval. Animals were kept in lairage overnight prior to slaughter and were stunned by percussive puncture. Immediately after bleeding, a 1 g biopsy sample was collected from the *Longissimus dorsi* (LD). After carcass dressing (30 min post-mortem) LD was cut form the carcass. LD samples were separately packed into sealed tough plastic bags containing a weight and were then submerged in a water bath maintained at 15 °C. Muscle pH and temperature was recorded at 1–2 h intervals until *rigor*. Ten grams of muscle samples cut from the LD at 0.5, 1, 3, 6 and 22 h post-mortem were snap-frozen in liquid nitrogen and stored at −80 °C until analysed.

2.2. Tenderness measurements

The LDs were kept in the 15 °C water bath until 22 h post-mortem when the meat pH decline had ceased. The LDs were then removed and cut into five equally sized loins and vacuum packed. These samples were aged at 15 °C for 0, 0.5, 1, 2, 4, and 7 days *post rigor*. At each time interval, one sample from each LD was opened and the muscle pH was recorded. The samples were cooked in a 100 °C water bath until an internal temperature of 75 °C was reached, as determined with a thermocouple, and were then placed into an ice-water slurry. When cool, 10 mm × 10 mm cross-section samples ($n = 10$) were sheared using a MIRINZ tenderometer and the values were averaged to obtain a shear measurement (N) for each loin sample.

2.3. Meat sample preparation

Frozen meat samples were pulverized under liquid nitrogen in a plastic bag using a pestle. One gram of frozen meat fragments (FMF) were resuspended in 5 ml of ice-cold extraction buffer (25 mM HEPES pH 7.5, 0.1% Triton X-100, 5 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 1 mM PefaBloc, 1 μg/ml pepstatin, leupeptin, aprotonin) and homogenised on ice for one minute using a handheld Ultra-Turrax T8 (IKA Labortechnik) to produce a whole muscle homogenate (WMH). A proportion of WMH was centrifuged at 20,000*g* for 10 min at 4 °C and the supernatant collected (sarcoplasmic fraction).

2.4. Titration of meat pH

One gram of FMF was resuspended in either water or water + Complete Protease Inhibitors (Roche) and were homogenised as described above. An equal volume of meat homogenate was mixed with pH buffer (100 mM MES buffer pH 7.4, 6.4, 6.2, 6.0, 5.8, 5.6, 5.4) and incubated for 10 minutes at 15 °C. The sample pH was re-measured and then soluble and insoluble fractions were separated by centrifugation at 20,000*g* at 4 °C for 10 min. The insoluble pellet was re-suspended in PBS to the equivalent volume of supernatant. Soluble and insoluble fractions were diluted 1:10 in PBS, and 5 μl of each were mixed with 2× Laemmli buffer and proteins were separated on 15% SDS-glycine polyacrylamide gels.

2.5. Measuring muscle protein content

A WMH was diluted 1:4 into SDS extraction buffer (30 mM Tris–HCl, pH 8.0, 3% SDS, 10 mM EDTA and 10 mM DTT) was mixed and then heated to 50 °C for 30 min. Samples were centrifuged at 16,000*g* for 5 min and the supernatant transferred to a fresh tube. Total protein extracts were diluted 1:50 into PBS and a protein concentration was determined using a BCA protein assay (Pierce) with a BSA calibration.

2.6. Immunoblots

SDS-PAGE was performed using 15% acrylamide gels and glycine buffers using reagents and a mini-protean gel system supplied by Biorad. Proteins were electroblotted onto PVDF membranes (Immobilon) and were blocked in 2% skimmed milk powder in TBS (blotto) for 1 hour at room temperature (RT, ~20 °C). Monoclonal antibodies