THE MECHANISM FOR TENDERISATION OF BEEF MUSCLE CAUSED BY INJECTION OF SALTS

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KEYWORDS: hot-boning, pre-rigor, beef, rapid chilling, cold-shortening, tenderness, pH, temperature, ATP, proteolysis, protein solubilization.

BACKGROUND

The original hypothesis of this work was that certain compounds (e.g., sodium pyrophosphate) could be used to inhibit or prevent the shortening of myofibrils by blocking, or competing with ATP for the ATP-binding-site between the actin and myosin proteins. This would ideally prevent contraction and maintain the muscle in a pre-rigor, tender condition, even when rapidly chilled and processed. In order to investigate whether treatments can achieve this goal, the muscles must initially be subjected to cold-shortening (CS) conditions. For CS to occur, the muscles must be chilled rapidly while still in the pre-rigor state. This is when the pH is still above about 6.2 and before the muscle temperature falls below 11°C, so that the muscle contains enough ATP to allow contraction (Bendall, 1978). Several studies indicated that various phosphate compounds can act as ligands at the actin-myosin cross-bridge sites to prevent ATP from promoting contraction and there is also evidence that various compounds, including phosphates, when injected or infused, have beneficial effects on shortening and tenderness of hot boned pork, beef and sheep muscles (for review see Geesink et al., 1994; Stevenson-Barry and Kauffman, 1995) but the degree of tenderisation obtained and detrimental effects (flavour undesirability, dark colour, etc.), as well as the mechanisms of action, remained unclear. In order to investigate the different effects, particularly effects related to contraction, it is important to distinguish between them and this can be facilitated by investigating pre- vs post-rigor effects.

OBJECTIVES

Investigate the effects of distilled water, sodium chloride and sodium pyrophosphate injected into muscle pre- and post-rigor on determinants of tenderness (rates of temperature, pH and ATP decline, ultimate pH , sarcomere length (SL), protein solubility and protein breakdown), to determine whether the effects were solely due to pre-rigor actions (i.e. prevention of contraction) or due to other effects (ionic strength, pH, enhanced proteolytic enzyme activity etc).

METHODS

The muscles in this study were treated as outlined in the companion paper titled "Tenderisation of beef muscle by injection of salts". Temperature and pH were measured in triplicate at 1, 3, 6, 10, 24 and 48 h PM on the hot-boned (HB) muscles and at 48, 72 and 96 h PM on the cold-boned (CB) muscles using an Omega pH50 portable meter with an Orion spear-tip glass pH electrode and a temperature probe inserted to a depth of 2.5 cm. Samples (~50 g) were excised from the HB muscles at 1, 3, 24 and 48 h PM and from the CB muscles at 48 h PM immediately frozen in liquid nitrogen then transferred to a -80° C freezer and later analysed for ATP using a procedure adapted from Adams (1963). ATP levels were measured using the Sigma Diagnostics Procedure No. 366-UV (Sigma Diagnostics, St Louis, MO 63178 USA). In expt. 3 hot-boned muscle lengths were measured using a ruler immediately after excising and again 48 h later and the difference was expressed as a percentage of original muscle length (% shortening). Also for expt. 3 only, small cores (approx. 0.5 cm x 2 cm) were excised at 48 h PM and fixed in glutaraldehyde solutions and later teased into individual fibres for SL measurement (Koolmees, 1986). Approx. 2 g of muscle was collected at 48 h PM and used for myofibril purification using a procedure adapted from Swartz et al. (1993) with continued Polytron homogenization rather than dounce homogenization. The average SL for five sarcomeres within one myofibril was measured, and 50 myofibrils were measured per sample. Exudate samples from steaks and purified myofibril samples were frozen for later analysis of protein concentration (Gornall et al., 1949) and SDS gel electrophoresis (Fritz et al. 1989). Proteins were transferred from an unstained gel to 0.45 µm Immobilon-P (Millipore, Bedford, MA 01730) membrane. The transfer buffer was 25 mM Tris, 192 mM glycine, 0.1% (w/v) SDS and 10 mM 2-mercaptoethanol made fresh for each transfer (adapted from Fritz et al., 1989). The transfer was run at 0.3 A current for 45 min for myosin transfers and 2 h for titin transfers. Once completed, the entire membrane was treated using the procedures outlined by Fritz and Greaser (1991) with myosin monoclonal MF-20. The secondary antibody was alkaline phosphatase anti-mouse IgG (H & L) (Promega, #S372B, Madison, WI 53711), and alkaline phosphatase (AP) color was developed by incubation in 30 ml substrate buffer (0.1 M Tris-HCl (pH 9.5), 0.1 M NaCl, 50 mM $MgCl_2$) containing 300 µl AP color reagent A (contains nitroblue tetrazolium in aqueous dimethyformamide [DMF], containing magnesium chloride) and 300 µl AP color reagent B (contains 5-bromo-4-chloro-3-indolyl phosphate in DMF) (Bio-Rad Laboratories, #170-6432, Hercules, CA 94547) for 10-30 min. Development of the reaction was stopped by rinsing in distilled water and blots were protected from light until photographed.

RESULTS AND DISCUSSION

The hot-boned muscles were chilled very rapidly and all had dropped to below pH 6.2 within 6 hours PM which ensured cold-induced toughening (see accompanying paper for tenderness results). However, the SL measurements did not show any indication of shortening (table 1). It is believed that the levels of ATP remaining in the HB samples at 48 h PM, particularly for the PPi-treated muscles was sufficient to allow relaxation in the buffers used, making the SL values meaningless. The techniques for SL measurement used in this work are similar to those used by other researchers but they appear to have limitations when used with samples with ATP remaining. It was concluded from the tenderness measurements that all of the non-injected control muscles had indeed cold-shortened (though perhaps cold-toughened would be a more appropriate description) even though these measurements of shortening did not indicate conclusively that this had happened.

There appeared to be no major effect of treatment on temperature, although the control muscles were always slightly ($P < 0.05$) warmer at 1 h PM than the injected muscles since the injected solutions were at room temperature (22-25°C) which was cooler than the muscle temperatures (~ 37°C), but for the remainder of the chilling period there were no significant ($P > 0.05$) differences between control and treated muscle temperatures (data not shown). There were significant pH effects and these are summarised in table 2. The HB PPi-injected muscles had lower ($P < 0.05$) pH values than the controls at 1 and 3 h PM, and at 6 and 10 h PM there were no significant differences (data not shown). PPi treatment elevated 24 h pH (pH_{24}) in both HB and CB muscles, whereas H_2O, as expected, did not elevate pH_{24} and NaCl elevated pH_{24} in HB muscles only. These results suggested that PPi-injection caused an initial increase in glycolysis then slowed glycolysis, in agreement with Van Hoof and Hamm (1973) who reported an increase in the rate of ATP breakdown by pyrophosphate

followed by an inhibition of glycolysis after several hours PM. This inhibition is thought to occur in the salted tissue due to the denaturation of glycolytic enzymes by the combined effect of low pH (< 6) and high ionic strength (Hamm, 1977).

Gel electrophoresis of myofibril samples showed no trends for differing protein degradation due to treatments but the proportions of proteins in the steak exudates were different; the NaCl and PPi treatments showed significant amounts of myosin, titin and titin breakdown products. The higher pH values at 24 and 48 h PM for the PPi and NaCl treatment s possibly created more favourable conditions for proteolytic enzymes, leading to more extensive breakdown of high molecular weight proteins and greater tenderisation. Troy et al. (1987) and Geesink et al. (1992) found that high ultimate pH results in more extensive breakdown of high molecular weight proteins, possibly by the enhanced activity of calpain I, corresponding to more tender meat. The increased breakdown of titin observed in western blots supports the involvement of this mechanism, hence, the increased tenderisation in the PPi treated beef was most likely due to the higher pH creating more favourable conditions for calpain I.

CONCLUSIONS

The HB muscles in this study were chilled rapidly and were subjected to CS conditions, i.e. the pH was still above 6.2 before the muscle temperature fell below 11°C (Bendall, 1978). The SL values obtained did agree with previous workers for conventionally boned meat but did not provide any evidence that CS occured. It was concluded that the levels of ATP remaining in the muscles at 48 h PM when the SL samples were removed, were sufficient to permit relaxation in the bathing mediums. The mechanism of tenderisation appeared to involve at least four factors that are listed here: 1. Disruption of muscle structure Injection of fluids may have disrupted muscle structure in a manner similar to blade tenderization, and/or, caused swelling and protein hydration which caused enhanced postmortem tenderization (as was observed for H_2O injection). 2. Solubilization of high molecular weight proteins by NaCl, and to a greater extent, PPi, may have disrupted myofibrillar structure and caused tenderization. 3. Increased ionic strength combined with high pH early postmortem may have inhibited glycolysis leading to higher ultimate pH. 4. Increased ionic strength and elevated ultimate pH may have enhanced activity of proteolytic enzymes resulting in extensive breakdown of high molecular weight proteins and more tender meat.

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TABLE 1: Sarcomere lengths, ATP levels and % shortening of muscles at 48 h after postmortem chilling.

Trait:	Sarcomere lengths														
Muscle:	Biceps femoris		Semimembranosus						Biceps femoris		Semimembranosus				
Treatment:	Control	PPi	Control	H_2O	NaCl	PPi	LSD		Control	PPi	Control	H_2O	NaCl	PPi	LSD
Homogenized									**ATP levels**						
Hot-boned (HB)[1]	1.99^{ab}	1.93^{abc}	1.87^{c}	2.01^{a}	1.95^{abc}	1.90^{bc}	0.09	HB[1]	0.4	0.9	0.4	0.4	0.5	0.8	0.4
Cold-boned (CB)[2]	1.76^{c}	1.82^{bc}	1.87^{ab}	1.92^{a}	1.93^{a}	1.90^{ab}	0.09	CB[2]	0.3	0.4	0.3	0.3	0.3	0.3	0.1
HB + CB[3]	1.87	1.81	1.86			1.89	0.15	CB[3]	0.4	0.4	0.4			0.4	0.1
Fixed & Teased								HB[3]	0.4	0.8	0.4			0.9	0.4
HB + CB[3]	1.81	1.80	1.74			1.79	0.24	**% shortening**							
								HB[3]	10	10	29			14	4

[abc]Least squares means in the same row with differing superscripts differ ($P < 0.05$). N = 6 animals per boning x muscle x treatment interaction group. [1]Expt. 1. [2]Expt. 2. [3]Expt. 3. Sarcomere length units were µm and ATP units were µmol/g fresh tissue.

TABLE 2: pH values of muscles (at 24 h PM for HB and averaged across 48, 72 and 96 h PM for CB).

Muscle:	Biceps femoris		Semimembranosus						Overall Time Means			
Treatment	Control	PPi	Control	H_2O	NaCl	PPi	LSD			48 h	72 h	96 h	LSD
Hot-boned (HB)[1]	5.64^{b}	5.90^{a}	5.64^{b}	5.57^{b}	5.75^{ab}	5.83^{a}	0.15						
Cold-boned (CB)[2]	5.53^{b}	5.80^{a}	5.53^{b}	5.51^{b}	5.53^{b}	5.76^{a}	0.07		CB[2]	5.67^{a}	5.63^{a}	5.53^{b}	0.04
HB[3]	5.75^{b}	5.94^{a}	5.64^{b}			5.95^{a}	0.09						
CB[3]	5.41^{b}	5.78^{a}	5.41^{b}			5.76^{a}	0.07		CB[3]	5.58	5.55	5.67	0.04

[abc]Least squares means in the same row with differing superscripts differ ($P < 0.05$). N = 6 animals per boning x muscle x treatment interaction group. [1]Expt. 1. [2]Expt. 2. [3]Expt. 3.