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Factors Associated with the Tenderness of Three Bovine Muscles

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ABSTRACT

Samples were obtained from bovine Longissimus dorsi (L), Biceps femoris (BF) and Psoas major (PM) muscles at different times of postmortem storage at 1.2°C. At day 1 postmortem, PM was the most tender muscle followed by BF then L; PM muscle also had the smallest fiber area and longest sarcomere followed by BF and L. After postmortem storage, shear force values decreased greatly for L, and only slightly for PM. There were no differences in activities of cathepsins, B, H, and L for any of the muscles. However, L muscle had the highest Ca^{++}-dependent protease activity followed by BF and PM. Thus results suggested that Ca^{++}-dependent protease activity was best determinant of tenderization resulting from postmortem storage at refrigerated temperatures.

INTRODUCTION

POSTMORTEM STORAGE of carcasses at refrigerated temperature has been known to improve meat tenderness for many years and still remains an important procedure for producing tender meat. Although improvement in meat tenderness is measurable both subjectively and objectively, the exact mechanism of improvement in tenderness as a result of postmortem storage still remains unclear. However, there appears to be general agreement that proteolysis of myofibrillar protein is the major contributor to meat tenderization during postmortem storage (Dutson, 1983; Goll et al., 1983a). Of the proteases located inside skeletal muscle, calcium-dependent proteases and lysosomal enzymes appear to be the best candidates for bringing about the tenderness changes during postmortem storage (Dutson, 1983; Goll et al., 1983a).

Calcium-dependent protease (CDP) was initially identified in skeletal muscle by Busch et al. (1972) and later purified by Dayton et al. (1976). The protease referred to as CDP in this manuscript, has a variety of other names including calcium-activated factor (Busch et al., 1972; Olson et al., 1977; Koohmaraie et al., 1984, 1986); calcium-dependent neutral proteinase (Vidalenc et al., 1983; Ducasting et al., 1985); calcium-activated protease (Suzuki et al., 1982); calpain (Murachi, 1985) and others (Goll et al., 1985). Mellgren (1980) reported the existence of a second form CDP. These two forms of the protease are now referred to as CDP-I and CDP-II, according to the sequence of elution from a DEAE-cellulose column at pH 7.5. CDP-I requires only very low concentration of calcium for 50% activation, whereas CDP-II requires much higher calcium concentration (Goll et al., 1983b). Both of these proteases are located primarily in the cytosol.

A second group of proteases that have been implicated in postmortem tenderization are lysosomal enzymes. Of thirteen reported lysosomal enzymes, only seven have been shown to exist in the lysosome of skeletal muscle cells (Goll et al., 1983a). These enzymes have an acidic pH optima and, therefore, if involved in postmortem tenderization, they are most involved once muscle approaches its ultimate pH.

To explain a basis for meat tenderization during postmortem storage, it has been postulated that one class of these proteases or the synergistic action of both classes of proteases (CDPs and lysosomal enzymes) is responsible for postmortem changes (Dutson, 1983; Goll et al., 1983a; Pearson et al., 1983; Dutson and Pearson, 1985; Greaser, 1986; Asghar and Bhatti, 1987). It is logical to assume that the class of protease responsible for postmortem aging should have higher activity in the carcasses with a high aging response and vice versa. To avoid complications resulting from variability between animals, in this study different muscles within the same carcass were used. It is well documented that different muscles within a carcass react differently to postmortem storage. Olson et al. (1976) reported that while there was a progressive decrease in shear force (increase in meat tenderness) with postmortem storage for both longissimus dorsi and semitendinosus muscles, the Psoas major was basically unaffected under the same conditions. The objective of these experiments was to relate measurements of tenderness and characteristics of muscle, including protease activities, during postmortem aging.

MATERIALS & METHODS

Sample preparation

Eight heifers were slaughtered in the normal manner. A description of the carcasses are given in Table 1. At 45 min postmortem, one-half of each muscle (Longissimus dorsi, Biceps femoris, and Psoas major) was removed from one side of each carcass. Each muscle was then cut into samples for extraction of calcium-dependent protease I and II and their inhibitor, and for determination of lysosomal enzyme activities, and collagen (amount and solubility). Both sides were chilled at −0.8°C for 18 hr and then stored at 1.2°C for the duration of the experiment. In order to eliminate the shortening effects caused by prerigor sampling, all the additional determinations, with the exception of those immediately after death (calcium-dependent proteases and their inhibitor, lysosomal enzymes and collagen), were conducted on the intact muscles from the opposite side of the carcass.

pH and temperature determinations

Temperature and pH were determined on the intact muscles at 1, 3, 6, 9, 12 and 24 hr postmortem. Temperatures was measured with a Fluke (model 8020A) digital multimeter and temperature probe (model 80T-150). pH was measured on the intact muscles with a glass stab electrode (Orion No. 91–63) attached to an Orion ionanalyzer (model 69A).

Preparation of calcium-dependent proteases and their inhibitor

Low calcium-requiring and high calcium-requiring calcium-dependent proteases (CDP-I, CDP-II) and their inhibitor were isolated from

Table 1—Description of carcasses[a]

Live weight (kg)	496.46 ± 59.50
Hot carcass weight)kg)	301.91 ± 36.55
Fat thickness (cm)	0.70 ± 0.29
Ribeye area (cm^2)	84.60 ± 9.33
Estimated kidney, heart and pelvic fat (%)	2.73 ± 0.41
Marbling Score[b]	386.2 ± 53.7

[a] [a]Mean ± standard deviation.
[b] Devoid = 000-099; traces = 200-299; slight = 300-399; small = 400-499, etc.

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200 g of each muscle immediately after slaughter. All extraction procedures were done at 2–4°C. Muscles were trimmed of fat and connective tissue, cut into 2×2 cm pieces and homogenized in 2.5 volumes (vol/weight) 10 mM tris-HCl, pH 7.5 containing 4 mM EDTA, 50 mM NaCl and 2 mM 2-mercapetoethanol (MCE), in a Waring blender. Homogenization was done twice at low speed and twice at high speed setting, each for 30 sec with a 30 sec cooling period interspersed between the bursts. The homogenate was centrifuged at 30,000 × g_{max} for 50 min. The supernatant was filtered through two layers of cheese cloth and its pH was adjusted to 7.5 followed by centrifugation at 50,000 × g_{max} for 50 min. The supernatant of 50,000 × g_{max} was filtered through glass wool and applied to a DEAE-Sephacel Column that had been equilibrated with 5 mM tris-HCl, pH 7.4 containing 50 mM NaCl, 0.1 mM EDTA, 2 mM MCE. Columns were then washed with the same buffer to remove unbound protein, until absorbance of the outflow at 278 nm was between 0.1 to 0.4. The bound proteins were then eluted with a continuous gradient of 50–500 mM NaCl in 5 mM tris-HCl, pH 7.4 containing 0.1 mM EDTA and 2mM MCE.

Activity of CDP-I was assayed according to Koohmaraie et al. (1986) and CDP-II according to Koohmaraie et al. (1984). Incubation time was 50 min. Activities of CDP-I and CDP-II are reported as total absorbance units at 278 nm per 200g muscle.

The activity of the inhibitor was determined by pre-incubating appropriate amounts of inhibitor and enzyme at 4°C for 1 min before adding 1.5 mL reaction mixture (Koohmaraie et al., 1986) to start the reaction. After 50 min of incubation the reaction was stopped by adding an equal volume of 10% trichloracetic acid. For each fraction assayed the following measurements were made to determine inhibitor activity: (a) Enzyme alone in Ca^{++}—containing reaction mixture; (b) Fraction assayed alone in EDTA-containing reaction mixture (to determine Ca^{++}—independent activity); and (c) Enzyme and fraction (same volume as in b) in Ca^{++}—containing reaction mixture. Inhibitor activity was then determined according to the following formula:

$$\text{Inhibitor activity} = (a-b-c) \times \text{dilution factor}$$

Inhibitor activity is reported as total absorbance units at 278 nm/200g muscle in the caseinolytic assay.

Preparation of cathepsins B, H, and L

Muscle homogenates were prepared from frozen muscles according to the procedures of Moeller et al. (1976) as modified by Moeller et al. (1977). The activities of cathepsins B, H, and L were determined in the sedimentable and unsedimentable fractions with a general procedure utilizing methylcoumarylamide (Z) substrates (Barrett, 1980) as modified by Kirschke et al. (1983). The substrates used were Z-Arg-Arg-NMec for cathepsin B, Z-phe-Arg-NMec for cathepsin L, and Arg-NMec for cathepsin H. The assay conditions were the same as those of Barrett (1980). Since both cathepsins B and L have activity against Z-Phe-Arg-NMec, the activities against this substrate are reported as cathepsins B plus L activity (Kirschke et al., 1983). Activities were calculated as described by Barrett (1980) and reported as μunites/min/mg protein.

Sarcomere length determination

Sarcomere lengths were measured on the intact muscles after 24 hr postmortem storage according to the procedure described by Cross et al. (1980) using the neon laser diffraction.

Collagen (amount and solubility) determination

Collagen amount and collagen solubility were determined according to procedures of Hill (1966) and Bergman and Loxley (1963).

Shear force determination

After 1 and 14 days of postmortem storage, two steaks per muscle were removed, frozen at −30°C and stored until used for Warner-Bratzler shear evaluation. Steaks were removed from the freezer and allowed to thaw at 2°C for 24 hr. The steaks were broiled on a Farberware "open-hearth" broiler and internal temperature was monitored with iron constantan thermocouple wires attached to a Honeywell 112 multipoint recorder. Steaks were turned at 40°C internal temperatures and removed from the broiler at 70°C. Steaks were then cooled for 24 hr at 2–4°C and a minimum of six cores (1.25 cm diameter by 5 cm long) were removed from each steak. Each core was sheared twice with a Warner-Bratzler shear device.

Statistical analysis

Data were analyzed by analysis of variance and the significance of the differences between means were tested by Duncan's multiple range test (Steel and Torrie, 1960).

RESULTS

pH and temperature decline

The temperature declined most rapidly in Longissimus dorsi (L) muscle and less rapidly and equally in the Bicep femoris (BF) and psoas major (PM) muscles (Table 2). The PM reached its ultimate pH first followed by BF and then L. These results demonstrate the effect of location of a muscle in the carcass on its temperature decline which in turn may explain the difference in pH decline among muscles (Newbold and Harris, 1972). Since BF and PM muscles had similar temperature but different pH decline patterns, temperature alone can not explain the differences observed in pH decline among these muscles.

Shear force evaluations

At day 1, shear force values indicated that PM was the most tender muscle, L was the toughest muscle and the BF muscle had an intermediate tenderness (Table 3). After 14 days of postmortem storage, there were no differences between shear values for the three muscles. However, the change in shear value between day 1 and day 14 was greater for L and BF than for PM, indicating a greater capacity to age in L and BF than in PM.

Sarcomere length, fiber area, and collagen

The longest sarcomeres were in PM, the shortest in L and those in BF were of intermediate length (Table 3). These re-

Table 2—Effect of postmortem storage on the temperature and pH decline of Longissimus dorsi, Biceps femoris and *Psoas major* (Mean ± S.E.M.)

	Longissimus	Biceps femoris	Psoas major
Muscle Temp (°C)			
1 hr	32.41 ± 0.24[a]	33.34 ± 0.32[b]	33.81 ± 0.15[b]
3 hr	24.48 ± 0.49[a]	29.10 ± 0.61[b]	20.14 ± 0.46[b]
6 hr	13.48 ± 0.72[a]	22.57 ± 0.65[b]	21.74 ± 0.71[b]
9 hr	7.96 ± 0.66[a]	17.57 ± 0.79[b]	16.92 ± 0.68[b]
12 hr	4.72 ± 0.60[a]	14.00 ± 0.63[b]	13.43 ± 0.95[b]
24 hr	1.16 ± 0.28[a]	6.80 ± 0.74[b]	5.80 ± 0.72[b]
Muscle pH			
1 hr	6.52 ± 0.05[a]	6.50 ± 0.06[a]	5.95 ± 0.09[b]
3 hr	6.10 ± 0.13[a]	6.10 ± 0.09[a]	5.51 ± 0.05[b]
6 hr	5.87 ± 0.12[a]	5.72 ± 0.08[a,b]	5.47 ± 0.03[b]
9 hr	5.77 ± 0.13[a]	5.61 ± 0.08[a]	5.51 ± 0.04[a]
12 hr	5.68 ± 0.12[a]	5.57 ± 0.07[a]	5.57 ± 0.06[a]
24 hr	5.63 ± 0.08[a]	5.51 ± 0.05[a]	5.57 ± 0.05[a]

[a,b] Means within the same row with different superscripts were significantly different ($P < 0.05$).

Table 3—Shear force values, sarcomere length and fiber size characteristics determinations from Longissimus dorsi, Biceps femoris, and *Psoas major* muscles (Mean ± S.E.M.)

	Longissimus	Biceps femoris	Psoas major
Sarcomere length, day 1 (μm)	1.68±0.08[a]	2.15±0.15[b]	3.55±0.12[c]
Average fiber area, day 1 area (μm^2)	4333.62±213.37[a]	3699.17±241.15[b]	1737.45±65.51[c]
Total collagen (mg/g)	3.40±0.07[a]	6.16±0.09[b]	2.23±0.37[c]
Soluble collagen (%)	6.94±0.36[a]	5.05±0.27[b]	7.40±0.36[a]
Shear force, day 1 (kg)	8.25±0.40[a]	6.16±0.29[b]	3.99±0.27[c]
Shear force, day 14 (kg)	4.97±0.37[a]	4.70±0.53[a]	3.83±0.25[a]

[a,b] Mean with different superscripts among muscles were significantly different ($P < 0.05$).

sults are in general agreement with those reported by Herring et al. (1965), Hostetler et al. (1972), and McKeith et al. (1985).

The smallest fiber area was observed in PM whereas area was intermediate in BF and largest in L in agreement with Herring et al. (1965).

The highest collagen amount was observed in BF whereas the amount was intermediate in L and lowest in PM (Table 3). In terms of collagen solubility, PM had the highest percentage of soluble collagen, BF the least and L was intermediate (Table 3).

CDP-I, -II, their inhibitor and cathepsin activities

The highest CDP-I, and CDP-II activities were in L with those in BF intermediate and those in PM the lowest (Table 4). The CDP-inhibitor activity was the same in L and BF and lower in PM. There was approximately 2-fold more CDP-I, -II and inhibitor activity in L than PM. These results are in agreement with those reported by Olson et al. (1977) who measured calcium-dependent proteolytic activity of isoelectrically precipitated (pH 4.9) calcium-dependent protease. This fraction theoretically contains both CDP-I and CDP-II although CDP-I was not discovered at the time of their study (Olson et al., 1977). Results of our experiment indicates that for all three muscles the ratio of CDP-I:CDP-II was approximately 1:1 and the ratio of CDP-I + CDP-II:inhibitor was also approximately 1:1, as reported by Murachi (1985) in rat skeletal muscle.

Unlike the results for CDP activities, no particular pattern was observed for catheptic enzymes (Table 4). The activities of cathepsins B, H and B+L were almost identical among muscles.

DISCUSSION

THIS STUDY DEMONSTRATES that at 24 hr postmortem L, BF and PM muscles differed significantly in their shear force values, but after 14 days of aging these differences were reduced considerably due to postmortem aging. According to Marsh (1977), collagen and the myofibrillar apparatus determine tenderness. Collagen crosslinking has been observed to be of more significance than the quantity of collagen (Bailey, 1985). Data presented here indicate that amount of collagen or collagen solubility do not explain the large differences in shear force values at day 1 (Table 3). At day 1, the muscle with the largest fiber size and shortest sarcomere length has the highest shear force (Table 3). Of all the variables examined, average fiber size and sarcomere length were the only basis on which the differences between these muscles could be explained. We have consistently observed a direct relationship between fiber size and tenderness regardless of breed or sex of bovine animals (Seideman and Koohmaraie, unpublished data). However, at this point we cannot offer an explanation for the relationship between fiber size and meat tenderness, except that shortened muscles generally have larger diameters. Herring et al. (1965) reported a significant correlation (r = 0.73) between fiber diameter and tenderness and that muscles with larger fiber diameter had shorter sarcomere length.

In terms of the aging response, L had the greatest response with a lesser response in BF, and no response at all in PM at day 14. In this study we have examined the activities of catheptic enzymes, B, H, L as well as the activities of CDP-I and II in an attempt to identify which protease class might be responsible for the observed differences seen in aging response. Our results indicated that regardless of the differences seen in aging response, activities for cathepsins (B, H, and B+L) were the same for all three muscles. However, it may be possible that these enzymes could be differentially activated in vivo by higher temperature and/or lower pH (Dutson, 1983) as seen in the PM muscle, thus causing more aging response at the same enzyme concentration. In the case of CDP, activities followed the same pattern as the aging responses; L which had the highest aging response also had the highest CDP-I activity. In turn, PM, which displayed the least aging response had the lowest CDP-I activity, and BF was intermediate in both CDP-I activity and aging response. Based on the results of this and other experiments (Koohmaraie et al., 1986, 1987) it was concluded that the initial levels of CDP-I activity determine the aging response of a given muscle.

The reason PM muscle had no aging response, even though its CDP activity was about 50% of the L muscle, is not known. Our previous work (Koohmaraie et al., 1987) has clearly demonstrated that about 50% of the aging response is completed by 24 hrs of postmortem aging. Since in this experiment the earliest measurements of tenderness were made at 24 hr postmortem, it is possible that we failed to measure the true aging response of PM muscle (improvement in its tenderness during the first 24 hr of postmortem aging). When the data on temperature decline for each muscle were compared, the PM muscle was at a high temperature during the early postmortem period (Table 2), which would increase the aging response during the first 24 hr period (Smith et al., 1976; Lochner et al., 1980; Dutson, 1983). The pH of the PM muscle was lower than that of the other muscles, particularly in the initial 6 hrs postmortem, which may also be related to the higher temperature during that 6 hr period. Data presented by Koohmaraie et al. (1986) has shown that the effect of CDP-I on the myofibrils was not appreciably lowered by lowered pH when the temperature remained at 25°C. Sarcomere length may also be related to the aging response in that the muscles with the shorter sarcomere lengths had a greater aging response (Table 3). At present no mechanism for a relationship between sarcomere length and aging response can be proposed, however Dutson et al. (1976) demonstrated greater ultrastructural alterations of z-lines when both PM and sternomandibularis muscles were shortened.

If CDP-I activity is responsible for postmortem changes in the muscle, then its inactivation or unfavorable conditions for its activation should prevent postmortem changes in the muscle. Also activation of CDP-I or generation of favorable conditions for its activation should accelerate the postmortem changes. We are now addressing this particular point by attempting to manipulate animals and/or carcasses so that CDP-I would not be activated and then examining postmortem changes in these carcasses.

Table 4—Ca^{++}-dependent proteases, their inhibitor and catheptic enzyme activity in Longissimus dorsi, Biceps femoris, and *Psoas major* muscles (Mean ± S.E.M.)*

	Longissimus	Biceps femoris	Psoas major
CDP-I[d]	91.35 ± .57[a]	60.63 ± 4.57[b]	49.70 ± 2.83[c]
CDP-II[e]	108.02 ± 2.89[a]	79.87 ± 4.19[b]	50.40 ± 2.94[c]
Inhibitor[f]	152.44 ± 4.10[a]	148.57 ± 9.97[a]	90.20 ± 4.70[b]
Cathepsin B[g]			
Unsedimentable fraction	17.25 ± 1.74[a]	22.09 ± 2.37[a]	22.08 ± 1.40[a]
Sedimentable fraction	1.80 ± 0.26[a]	1.63 ± 0.36[a]	2.23 ± 0.41[a]
Cathepsin H[g]			
Unsedimentable fraction	48.83 ± 0.62[a]	45.73 ± 5.98[a]	42.99 ± 5.13[a]
Sedimentable fraction	10.23 ± 0.52[a]	9.04 ± 1.06[a]	9.67 ± 1.23[a]
Cathepsin L+B[g]			
Unsedimentable fraction	41.58 ± 2.92[a]	41.78 ± 5.32[a]	37.55 ± 2.16[a]
Sedimentable fraction	3.80 ± 0.53[a]	3.98 ± 0.80[a]	4.71 ± 0.57[a]

* n = 8 for all determinations except for CDP-I, -II and inhibitor (n = 4).

[a,b,c] Means within the same row with different superscripts differ ($P < 0.05$).

[d] Low calcium-requiring calcium-dependent protease A_{278}/200g muscle (caseinolytic assay).

[e] High calcium-requiring calcium-dependent protease A_{278}/200g muscle (caseinolytic assay).

[f] Inhibitor of CDP-I and CDP-II A_{278}/200g muscle (inhibition of casein hydrolysis by CDP-II).

[g] μunits/min/mg protein.

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