Sulfated Glycosaminoglycans and Collagen in Two Bovine Muscles (M. Semitendinosus and M. Psoas Major) Differing in Texture

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M. semitendinosus (ST) and M. psoas major (PM) were used as models for tough and tender meat to study a possible role of sulfated glycosaminoglycans (GAGs) for muscle tenderness. The difference in texture was confirmed by Warner Bratzler shear force measurements. No significant difference in total amount of GAGs in the muscles was found. In contrast, a significant difference in the ratio of GAG/collagen was found between the two muscles. After separation of the GAGs by density gradient ultracentrifugation and ion-exchange chromatography, dermatan sulfate (DS), keratan sulfate (KS), chondroitin sulfate (CS), and heparan sulfate (HS) were identified by cellulose acetate electrophoresis after use of specific enzymes and chemical methods. The content of DS was higher in the tougher muscle (ST) than in PM, and the difference in DS content was statistically significant. Furthermore, a significant difference in the GAG composition pattern of the two muscles was found. The yield of GAGs extracted from the muscles was 77% for ST and 87% for PM. The residue after extraction was further analyzed and found to contain mainly HS. Immunohistochemical studies using antibodies against CS/DS showed a staining pattern of the perimysium of ST different from that of PM.

Keywords: *Sulfated glycosaminoglycans; meat texture; tenderness; collagen*

INTRODUCTION

Skeletal muscles exhibit great differences in textural properties both between individuals and within the same individual. For the meat industry it is a challenge to provide consumers with tender meat. Several factors ranging from conception to cooking are known to influence tenderness. A different biochemical composition of the muscles is most likely a factor of great importance, but which components contribute to the textural properties are far from clarified. Connective tissue has been shown to influence meat tenderness (Bailey and Light, 1989). Previous studies on connective tissue have focused mainly on the collagen fibers as candidates for determining tenderness, as the collagen fibers provide the muscles with tensile strength and stiffness. The collagen fibers are important constituents of the extracellular matrix. Microscopical studies have shown that fracture lines first appear in this matrix, between the collagen fibers, when tension is applied perpendicularly to the myofibers in meat (Purslow, 1974). Furthermore, an electron microscopical study has shown that this area was degraded during post-mortem storage of meat (Nishimura et al., 1996a,b).

One major component in this matrix are the proteoglycans (PG). PGs are a family of macromolecules in which one or more sulfated polysaccharide chains, the glycosaminoglycans (GAGs), are covalently linked to a protein core (Gallagher, 1989). The structure and composition of the GAG chains strongly influence the mechanical properties of connective tissue (Fransson and Cøster, 1979; Velleman et al., 1996). In previous studies the GAG composition of the epimysial layer from species such as pork and cattle was studied (Cormier et al., 1971; Nakano et al., 1996). However, these results cannot be extrapolated to the peri- and endomysium of the muscle as the content and type of PGs are determined by which forces are applied to the tissue and may vary from tissue to tissue and even within the same tissue (Vogel, 1996).

Some studies have focused on the role of PGs in muscle development and differentiation in species such as rat, rabbit, fowl, pork, sheep, and human (Andrade and Brandan, 1991; Bianco et al., 1990; Brandan et al., 1991; Campos et al., 1993; Carrino et al., 1982; Nishiumi et al., 1997; Parthasarathy et al., 1991; Velleman et al., 1996). Limited information exists on PGs in the skeletal muscle of cattle, particularly in relation to tenderness. Eggen et al. (1994) isolated decorin and described a large dermatan sulfate PG in M. semimembranosus (ST) after extraction in 4 M guanidine−HCl. Further studies showed degradation of PGs during post-mortem storage (Eggen et al., 1998). Whether GAGs may contribute to the differences in tenderness between the muscles in the same animal has not been studied. Such differences have often been ascribed variations in chilling rates for the individual muscles. More information about the structural components present in muscles differing in texture may contribute to a further understanding of the major factors influencing tenderness.

In the present study the compositions of GAGs in two bovine muscles with different textural properties were compared. The textural properties were determined by measuring Warner Bratzler (WB) shear force values. However, the connective tissue of meat is composed of fibrous and nonfibrous proteins, of which collagens and PGs are the major families. As the relative amount of fibrous and nonfibrous proteins may influence the

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textural properties, the amount of GAGs in the muscles was related to the content of hydroxyproline, which is the most widely used measure for total collagen content. The distribution of chondroitin sulfate/dermatan sulfate (CS/DS) in the two muscles was studied by immunohistochemical methods at light microscopical level.

In a study on bovine nasal cartilage it was shown that 25% of the glycoconjugates was not dissolved after extraction in 4 M guanidine−HCl (Christner et al., 1983). The amount of PG, which remained in the residue after extraction with guanidine−HCl, varied considerably depending on the tissue. The extraction yield of PG ranged from 92% in rat chondrosarcoma to 57% in the cartilage of older dogs (Inerot et al., 1978). Whether the textural properties of ST and M. psoas major (PM) would influence the extractability of PGs was also a subject of analysis.

MATERIALS AND METHODS

Sampling. Immediately after slaughter bovine ST and PM were cut into several pieces after removal of the epimysium. The pieces were collected randomly from the same medial area in the central part of the two muscles. Muscle pieces were vacuum-packed in polyethylene bags for textural analyses and powdered in liquid nitrogen for GAG analyses. For histochemical analyses, muscle pieces were embedded in O.C.T. compound (Tissue Tek 4583, Miles Inc., Elkhart, IN) for 30 min and finally frozen in liquid nitrogen.

Textural Measurements. Samples vacuum-packed in polyethylene bags were heat-treated in water baths at 70 °C for 50 min, cooled in running ice water for 50 min, frozen, and stored at −40 °C. Before analysis, the samples were thawed overnight at 4 °C and stabilized for at least 30 min at 20 °C before measurements.

Muscle slices of 1 cm were cut along the fiber direction of the muscle to give the final samples cross-section dimensions of 1 cm × 1 cm. Structures with visible fat were avoided. At least three samples from each muscle specimen were sheared in 10−12 consecutive cuts at right angles to the fiber direction with a WB shear-press device in an Instron Testing Machine (model 4202, Instron Engineering Co., High Wycombe, U.K.) (Hildrum et al., 1994). The average of the maximum force readings was used for calculation.

Hydroxyproline Determination. Nitrogen-powdered samples were assayed for hydroxyproline content according to the method of Stegeman and Stalder (1967). In brief, nitrogen-powdered samples were hydrolyzed with 30% H_2SO_4 and oxidized with 0.7% chloramine-T solution. 4-(Dimethylamino)benzaldehyde was added and the hydroxyproline content determined by measuring the absorbance at 550 nm.

Separation of GAGs from Muscles. *Extraction.* The samples powdered in liquid nitrogen were incubated in an extraction buffer containing 4 M guanidine−HCl in 0.05 M sodium acetate buffer, pH 6, with added protease inhibitors (0.1 M 6-aminohexanoic acid, 10 mM EDTA, 1 mM phenylmethanesulfonyl fluoride, 10 mM *N*-ethylmaleimide) and 2% Triton X-100 (Sajdera and Hascall, 1969; Heinegård and Sommarin, 1987). The solid-to-liquid ratio was 10 g of tissue to 150 mL of extraction buffer. The suspension was gently stirred for 16 h at 4 °C, clarified by centrifugation at 10000*g* at 4 °C for 30 min, and filtered through a nylon membrane with a pore size of 40 μm. An aliquot of the supernatants was dialyzed against distilled H_2O at 4 °C for 18 h and lyophilized to determine the content of GAGs in the extract by measuring the hexuronic acid according to the carbazole method (Bitter and Muir, 1962). Glucuronolactone was used as standard.

Density Gradient Ultracentrifugation. The supernatants were subjected to ultracentrifugation in a gradient of $CsCl_2$ with a starting density of 1.37 g/mL. Centrifugation was carried out at 140000*g* for 92 h in a Beckman ultracentrifuge, Optima L-80, by use of a Type 70 Ti fixed-angle rotor equipped with polyallomer quick seal centrifuge tubes (Opti-Seal 25 × 86 mm) (Beckman, Fullerton, CA). The gradients were collected in 5 mL fractions by puncturing the bottom of the tubes. The fractions were named D1, D2, D3, D4, D5, and D6, where D1 represented the bottom fraction. The top of the tube consisted of a red, sticky surface layer, which was discarded. The density of the fractions collected was determined with a 250 μL pipet as a pycnometer. The contents of sulfated GAGs and protein in the fractions were determined by use of the of 1,9-dimethylene blue (DMB) method (Farndale et al., 1982) and a Bio-Rad assay based on the method of Bradford (1976).

The DMB positive fractions collected after $CsCl_2$ gradient centrifugation (D1−D4) were pooled and dialyzed against distilled H_2O containing protease inhibitors as described above. The dialysis was performed at 4 °C followed by lyophilization of the samples.

Ion-Exchange Chromatography. Lyophilized samples after ultracentrifugation were chromatographed by ion-exchange chromatography on a HI-Load TM 26/10 Q-Sepharose Fast Flow column (Amersham Pharmacia Biotech, Uppsala, Sweden) by a Pharmacia FPLC system. The columns were equilibrated in 0.05 M sodium acetate buffer, pH 5.8, containing 6 M urea and 0.1 M NaCl; 100 mg of each sample dissolved in equilibration buffer was applied to the column, and bound anionic material was eluted with a gradient of NaCl ranging from 0.1 to 1.5 M. The protein elution profile was determined by monitoring the protein content automatically at 280 nm during the run. The eluate was collected in 4 mL fractions, and the content of sulfated GAGs in each fraction was measured according to the DMB assay (Farndale et al., 1982). Fractions containing sulfated GAGs were pooled, dialyzed against distilled H_2O, and lyophilized.

Residual GAGs. The extraction residue was defatted in 10× volume of acetone at 4 °C for 12 h with stirring. The acetone was changed and the same procedure repeated. The defatted sediment was lyophilized, and 20 mg of the samples was digested with 0.2 mg of papain (Sigma Chemical Co., St. Louis, MO) in 0.05 M sodium phosphate buffer, pH 7.0, containing 0.05 M EDTA, 0.5 M NaCl, and 10 mM cysteine hydrochloride, at 65 °C for 24 h. After proteolysis, the content of sulfated GAGs in the samples was determined according to the DMB method (Farndale et al., 1982).

For fractionation of GAGs, the papain-digested samples were subjected to ion-exchange chromatography on a Dowex-52 column (Whatman, Maidstone, U.K.). The GAGs were eluted with 2 M NaCl in sodium acetate buffer, pH 6.0 The fractions were dialyzed against distilled H_2O at 4 °C for 24 h and lyophilized. The samples were then dissolved in 200 μL of 0.1 M Tris, pH 7.0, and examined for the content of GAGs by cellulose acetate electrophoresis.

Analyses of GAGs. *Enzymatic Procedures.* Lyophilized material after ion-exchange chromatography was dissolved in 0.1 M Tris-HCl buffer, pH 7.5, with 5 mM $CaCl_2$ at a concentration of 12 mg/mL. The samples were digested with 2 mg of protease type XIV *Streptomyces* dissolved in the same Tris-HCl buffer. The samples were incubated at 55 °C for 18 h. For identification of CS and DS, protease-treated samples were digested with chondroitinase−ABC or chondroitinase−AC according to the method of Yamagata et al. (1968). Keratan sulfate (KS) was identified by digestion with 6 mU keratanase dissolved in 0.1 M Tris-HCl, pH 7.5, and incubation for 18 h at 37 °C.

All enzymes were delivered by Sigma Chemical Co. (St. Louis, MO).

Nitrous Acid Degradation. For identification of heparan sulfate (HS), samples obtained after FPLC fractionation or Dowex-52 column chromatography of papain-digested sediments were treated with nitrous acid for 10 min at 25 °C according to the method of Shiveley and Conrad (1976). The reaction was stopped by raising the pH from 1.5 to >5 with 1 M Tris-HCl, pH 8, or 2 M Na_2CO_3.

Cellulose Acetate Electrophoresis. Electrophoresis was carried out on supported cellulose acetate sheets (Electraphor, Shandon Southern Products, Chesire, U.K.) according to the method of Stanbury and Embery (1977). To compare the GAG

Table 1. Comparison of Connective Tissue Components and WB Shear Force Values in Two Bovine Muscles, ST and PM[a]

	ST	PM
WB shear force values[b] (N)	81.7 ± 3.8	37.5 ± 4.2
OH-proline[b] (mg/g of wet wt)	1.2 ± 0.01	0.7 ± 0.01
GAG (mg/g of wet wt)	1.1 ± 0.1	1.0 ± 0.2
GAG/OH-proline[b]	0.9 ± 0.08	1.7 ± 0.3
GAG extraction yield	77 ± 16	87 ± 13

[a] All values are given as mean ± SE; number of animals tested (n) = 8 except for the calculation of GAG extraction yield, which is based on 4 animals (n = 4). [b] Two-sided paired t test showed significant difference between PM and ST ($P < 0.05$).

compositions, aliquots (3 μL) of protease-treated samples from the two muscles, ST and PM, were run on the same cellulose acetate sheet. For identification of the different GAGs, protease-digested samples were treated with specific enzymes or nitrous acid. Aliquots of treated and nontreated samples were then run on the same cellulose acetate sheet. The electrophoresis was carried out for 5 h at 10 mA in a 0.2 M calcium acetate buffer, pH 7.2. A standard mixture of commercially available GAGs containing HS, DS, chondroitin 4-sulfate (C4S), and chondroitin 6-sulfate (C6S) (Sigma Chemical Co.) at a concentration of 0.05 mg/mL was routinely run with the samples. Bands were visualized by staining with 0.05% Alcian blue (Gurr Biological Stains, BDH, Poole, U.K.) in 3% acetic acid containing 0.05 M $MgCl_2$ for 10 min. After destaining, the cellulose acetate sheets were scanned by a Agfa Arcus 2 scanner (Agfa, Brockton, MA) and the results analyzed by count function of Image-Pro Plus program (Media Cybernetics, Silver Spring, MD). The count function shows the density of black pixels within an area. Background was removed from the paper to determine the density based on image analysis software.

Histochemistry. Pieces embedded in O.C.T. compound (Tissue Tek 4583, Miles Inc.) and frozen in liquid nitrogen were sectioned in a Leitz 1720 digital cryostat (Leica Instruments GmbH, Heidelberg, Germany) into 3−5 μm sections and mounted on poly-L-lysine-coated glass slides. These were fixed in cold acetone (4 °C), dried, and stored at −20 °C.

Immunostaining. The sections were digested with 0.03 U of chondroitinase−ABC lyase from *Proteus vulgaris* (Sigma Chemical Co.) in 0.5 M Tris-HCl, pH 7.6, containing 0.05 M NaCl for 2 h at 37 °C. After washing in Tris-HCl-buffered saline (TBS) (20 mM Tris, pH 7.5, with 0.5 M NaCl), the sections were exposed to a blocking solution containing 20% normal swine serum (DAKO A/S, Glostrup, Denmark) diluted in TBS. The sections were then incubated at 4 °C with rabbit antiserum against CS/DS (AB 1918, Chemicon International Inc. Ternecula, CA) diluted 1:200 in TBS. After washing in TBS, the sections were incubated in biotinylated swine anti-rabbit IgG (1:400 dilution) (DAKO) for 2 h followed by incubation in a streptavidin−ABC lyase−horseradish peroxidase complex for 45 min prepared according to the manufacturer's recommendation (DAKO). 3,3′-Diaminobenzidine-tetrahydrochloride (DAB) (0.6 mg/mL in 0.05 M Tris-HCl buffer, pH 7.6, with 0.03% H_2O_2) was used to detect peroxidase activity. After rinsing, the sections were dehydrated in alcohol, cleared in xylene, and mounted in Eukitt (O. Kindler GmbH & C, Freiburg, Germany). Nonspecific binding of primary antibodies was checked using nonimmune serum.

Statistical Analysis. To test whether the differences in the contents of GAGs were significant, a two-sided paired t test ($P < 0.05$) by use of Minitab statistical software was used. To test whether the difference in GAG composition pattern between ST and PM was significant, the Pearson chi-square test ($P < 0.05$) was used. Systat statistical software was used in this test.

RESULTS

Shear Force. The values varied between the two muscles (Table 1). By using this parameter it was evident that the PM muscle was significantly more tender than the ST muscle for all animals measured, although individual differences were present, as can be seen from the SE values.

Collagen. The collagen content of the two muscles differed as shown by a difference in the OH-proline content. The mean value of the OH-proline content in ST was higher than for PM (Table 1). These values represented measurements from eight animals, and the difference in OH-proline content between the two muscles was significant. Furthermore, the OH-proline content did show variations when the same muscle was analyzed in tissue preparations from several animals. In ST the range was between 0.98 and 1.38 mg/g of nitrogen-powdered material, and in PM the range was 0.45−0.8 mg/g of nitrogen-powdered material.

GAGs. Total amount of GAGs in the extracts was determined according to the carbazole method. No significant difference was found in total amount of GAGs per gram of nitrogen-powdered material between the two muscles (Table 1). The GAG content in the muscles showed animal variations, ranging from 0.78 to 1.25 mg/g of nitrogen-powdered material in ST, whereas the values in PM ranged from 0.69 to 1.4 mg/g of nitrogen-powdered material.

GAG/Hydroxyproline Ratio. In contrast to the total amount of GAGs, a significant difference was found between the two muscles by calculating the GAG content per unit of OH-proline (Table 1). For all animals studied ST contained less GAG per unit of OH-proline than PM.

Residue. The amount of sulfated GAGs in the residue after extraction was determined by use of the DMB method after papain digestion and related to the content of GAGs in the extract to calculate the extraction yield. The extraction yield was somewhat higher for PM compared to ST (Table 1). The difference observed was not significant.

Fractionation of Extracted GAGs. For further studies of the GAG components of the muscles, the extracted material was fractionated by density gradient ultracentrifugation. After ultracentrifugation, GAG-containing material was recovered in the fractions of densities >1.31 g/mL, which contained only small amounts of protein material. Figure 1 shows the distribution of GAG and protein in the different fractions. D4 represents the fraction with a density of 1.31 g/mL, whereas D1 represents the heaviest fraction of density = 1.5 g/mL. The GAG-containing fractions after ultracentrifugation were further separated by ion-exchange chromatography as illustrated in Figure 2. The GAGs were highly anionic as judged by the concentration of NaCl needed for elution, and the PGs in samples from both muscles were eluted into two GAG-containing peaks, peaks 1 and 2 (Figure 2). However, a slight difference in the GAG elution profile was observed between material from the two muscles. ST contained a higher proportion of GAG in peak 2 than did PM, but the difference was not significant (Table 2).

Cellulose Acetate Electrophoresis. To study the composition of GAG, material obtained by density gradient ultracentrifugation and ion-exchange chromatography was subjected to cellulose acetate electrophoresis. Samples from both ST and PM were separated into four major spots, 1−4 (Figure 3). Spots 3 and 4 consisted of DS and CS (Figure 4, panels a and b), whereas HS and KS were present in spots 1 and 2

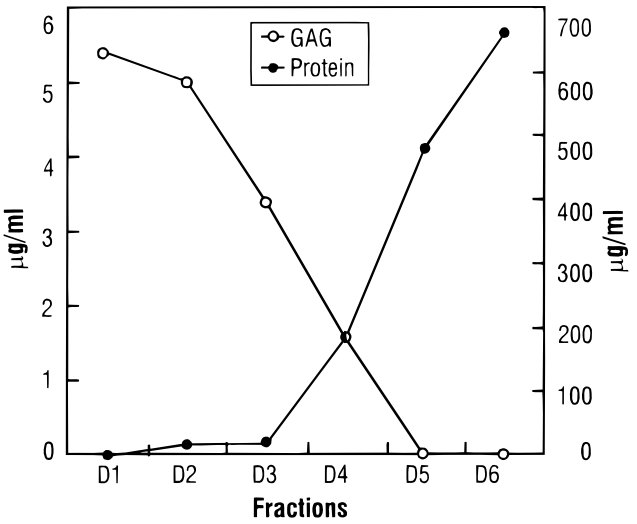

Figure 1. Content of protein and sulfated GAGs in the different fractions, marked D1−D6, after $CsCl_2$ gradient ultracentrifugation. D1 represents the bottom fraction with highest density, whereas D6 represents the top fraction.

Table 2. Content of Sulfated GAGs, Expressed in Percent, in Peaks 1 and 2 Eluted by Ion-Exchange Chromatography (See Figure 2)[a]

	peak 1 (fractions 30−36)	peak 2 (fractions 37−42)
ST	40 ± 2	59 ± 1
PM	45 ± 2	55 ± 2

[a] The content of anionic material was measured according to the DMB method. All values are given as mean ± standard error (SE). Number of animals (n) = 4.

Table 3. Contents of DS in ST and PM[a]

	ST	PM
DS[b]	127 ± 12	86 ± 21

[a] The values are based on optical density measurements of the DS spot on cellulose acetate sheets and expressed as mean ± SE. Number of sheets monitored (animals tested) = 4. [b] The difference in DS content between the two muscles (ST and DS) was statistically significant, tested by two-sided paired t test ($P < 0.05$).

Table 4. GAG Composition Pattern of ST and PM[a]

spot	1	2	3	4	total
ST	366.2	382.0	509.0	184.0	1441.2
PM	393.5	465.0	345.0	164.0	1367.5

[a] The calculations are based on optical density measurements of the Alcian blue stained GAG spots on cellulose acetate sheets. One sheet represents samples from both ST and PM from the same animal as shown in Figure 3. Values presented are the total sum of optical density measurements of four sheets (four animals). The two muscles showed significant difference in GAG composition pattern, tested by Pearson chi-square test ($\chi^2 = 39.852$, 3 degrees of freedom, $P = 0.0000001$).

(Figure 4, panels c and d). DS and HS were the dominating GAGs present. Scanning the density of the Alcian blue stained cellulose acetate sheets revealed a difference in GAG composition between the two muscles. DS was the major GAG in ST, whereas HS appeared to dominate in PM. Statistical testing showed that the difference in the content of DS between the two muscles was significant, as shown in Table 3. Furthermore, scanning of the sheets revealed a difference in the GAG composition pattern between the two muscles which was statistically significant (Table 4).

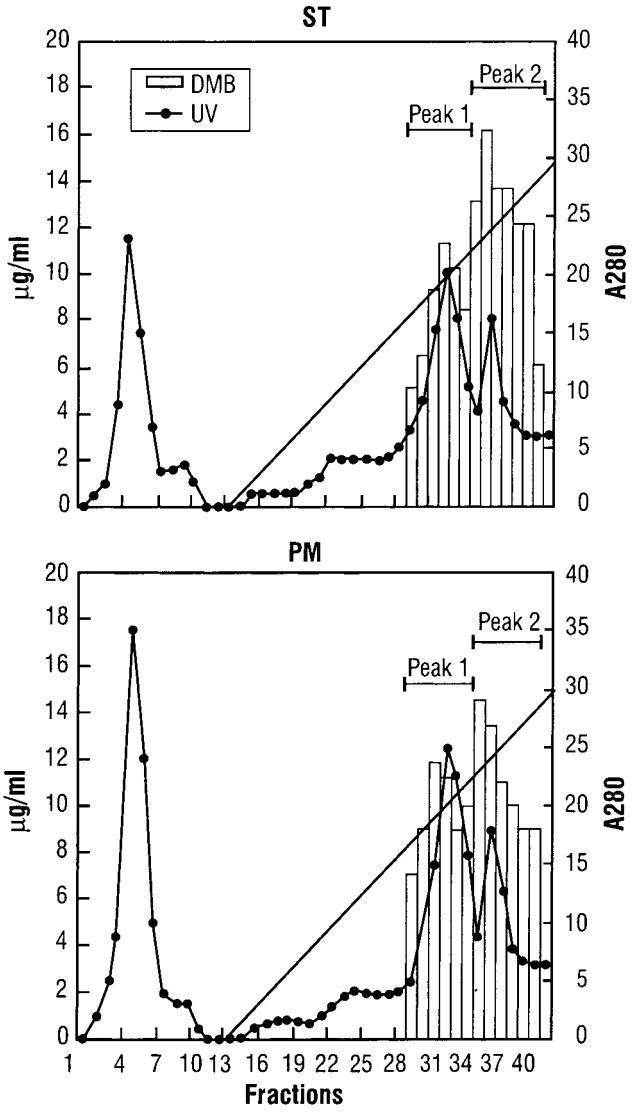

Figure 2. Ion-exchange chromatography of samples obtained from ST and PM after $CsCl_2$ ultracentrifugation on a Q-Sepharose Hiload column in 0.05 M sodium acetate buffer containing 6 M urea and with a gradient ranging from 0.1 to 1.5 M NaCl. The eluted material was collected in 4 mL fractions and monitored for the content of protein and GAGs.

Characterization of GAGs in the Sediment. As shown in Figure 5, lanes 1 and 3, one large spot with the same mobility as HS was found in the sediment from both ST and PM after cellulose acetate electrophoresis. The large spot was identified as HS by the HNO_2 method, which leads to depolymerization of heparin and HS-type of GAGs (Figure 5, lanes 2 and 4). A faint spot with mobility similar to that of CS/DS could be traced mainly in the residue from ST. Accordingly, HS was the main GAG in the residues from both ST and PM.

Histology. The perimysium and endomysium layers could be clearly outlined by staining with an antibody against CS and DS. For both muscles DS and CS were evenly distributed in both the endomysium and the perimysium. As parts c and d of Figure 6 show, individual muscle fibers were surrounded by thin envelopes of endomysium, and both muscles showed accumulation of staining in the junctions of the endomysial layers. The distribution of epitopes recognized by the antibody in the perimysium differed between the two muscles. As Figure 6a shows, the perimysium of ST showed a wavy appearance. In contrast, PM showed

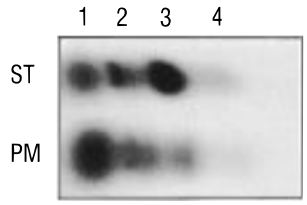

Figure 3. Cellulose acetate electrophoresis of a GAG preparation from ST (lane 1) and PM (lane 2) after fractionation by $CsCl_2$ ultracentrifugation, ion-exchange chromatography, and protease treatment.

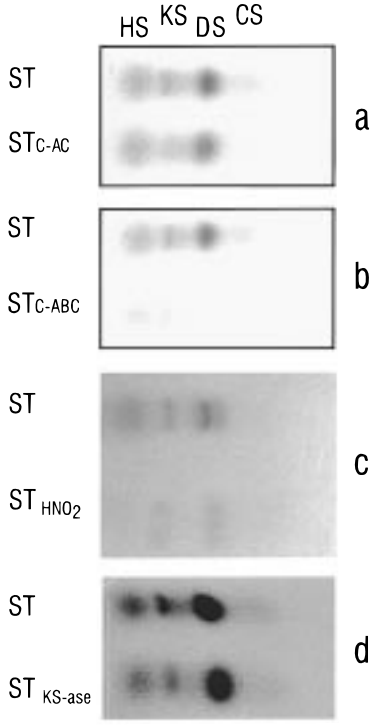

Figure 4. Cellulose acetate electrophoresis of samples from ST after fractionation by $CsCl_2$ ultracentrifugation, ion-exchange chromatography, and protease treatment: (a) identification of CS after chondroitinase−AC treatment; (b) identification of DS and CS after chondroitinase−ABC treatment; (c) identification of HS by HNO_2 treatment; (d) identification of KS after keratanase treatment. Lane 1 in each panel represents untreated samples, whereas lane 2 represents material after treatment.

a more diffuse staining pattern of the perimysium (Figure 6b).

DISCUSSION

The aim of the present study was to compare the content and composition of GAGs in muscles with different textural properties. ST and PM were used as models for tough and tender muscles at the day of slaughter. Differences in textural properties between the individual animals were noted as measured by WB shear force, but the values in PM were consistently lower than in ST in all animals studied.

It is assumed that there is a relationship between total collagen content and textural quality parameters as assessed by compressive force measurements, in particular between animals of the same age (Bailey and Light, 1989). Our studies supported this as ST, with the higher shear force value, contained significantly more collagen than PM. However, even if a correlation between collagen content and textural quality has been

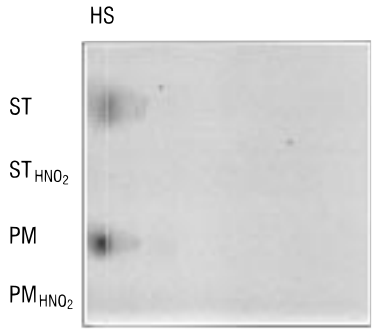

Figure 5. Cellulose acetate electrophoresis of GAGs in the residue after extraction, obtained by papain digestion and fractionation by Dowex-52 chromatography. Lanes 1 and 3 represent untreated samples of ST and PM, respectively, whereas lanes 2 and 4 represent HNO_2-treated samples of ST and PM.

observed, other studies have shown that the collagen content alone cannot be considered as a reliable parameter for textural properties (Bailey and Light, 1989).

In the present study the amount of GAGs was calculated by measuring the hexuronic acid content of the supernatant after extraction according to the carbazole method (Bitter and Muir, 1962). By this method the GAGs covalently bound to the peptide core in the PGs as well as hyaluronic acid will be measured. No significant difference in the total amount of GAGs was found when the two types of muscles were compared. In a previous study no significant correlation between hexosamine content of muscle from bulls, steers, and cows and degree of tenderness as measured by shear force has been reported (Cormier et al., 1971).

In the present study the amount of GAGs was related to the OH-proline content. The ratio showed a significant difference between the two muscles. ST showed a lower GAG content per unit of OH-proline than PM. As the OH-proline represents mainly the collagen fibers and the GAG represents the matrix around the fibers, the GAG/OH-proline ratio could be an indicator of the density of the connective tissue of the muscle. In a previous study on softening of the uterine cervix in pregnant women just before delivery, a larger decrease in the OH-proline content than in the content of GAGs was shown (Uldbjerg et al., 1983). In addition, it has been suggested that with fewer GAGs available to form a network around the collagen fibers, the formation of intramolecular cross-linkages in collagen could increase (Cormier et al., 1971; Velleman et al., 1996). This would most likely influence the textural properties of the tissue. The present results indicate that the ratio between GAG and collagen may be a parameter which deserves further attention in relation to texture.

Comparison of the elution profile obtained by ion-exchange chromatography of samples from ST and PM after ultracentrifugation showed that the GAGs were eluted as two peaks. Furthermore, the GAG preparation from ST showed a higher content of GAG in the most anionic peak (Figure 2). Although the difference was not statistically significant, the results indicate different charge properties in the GAG populations in the two muscles (Table 2). Such differences may influence interactions with other components in the tissue.

Cellulose acetate electrophoresis of the pooled GAG-containing fractions from the two muscles after ion-exchange chromatography revealed a different compo-

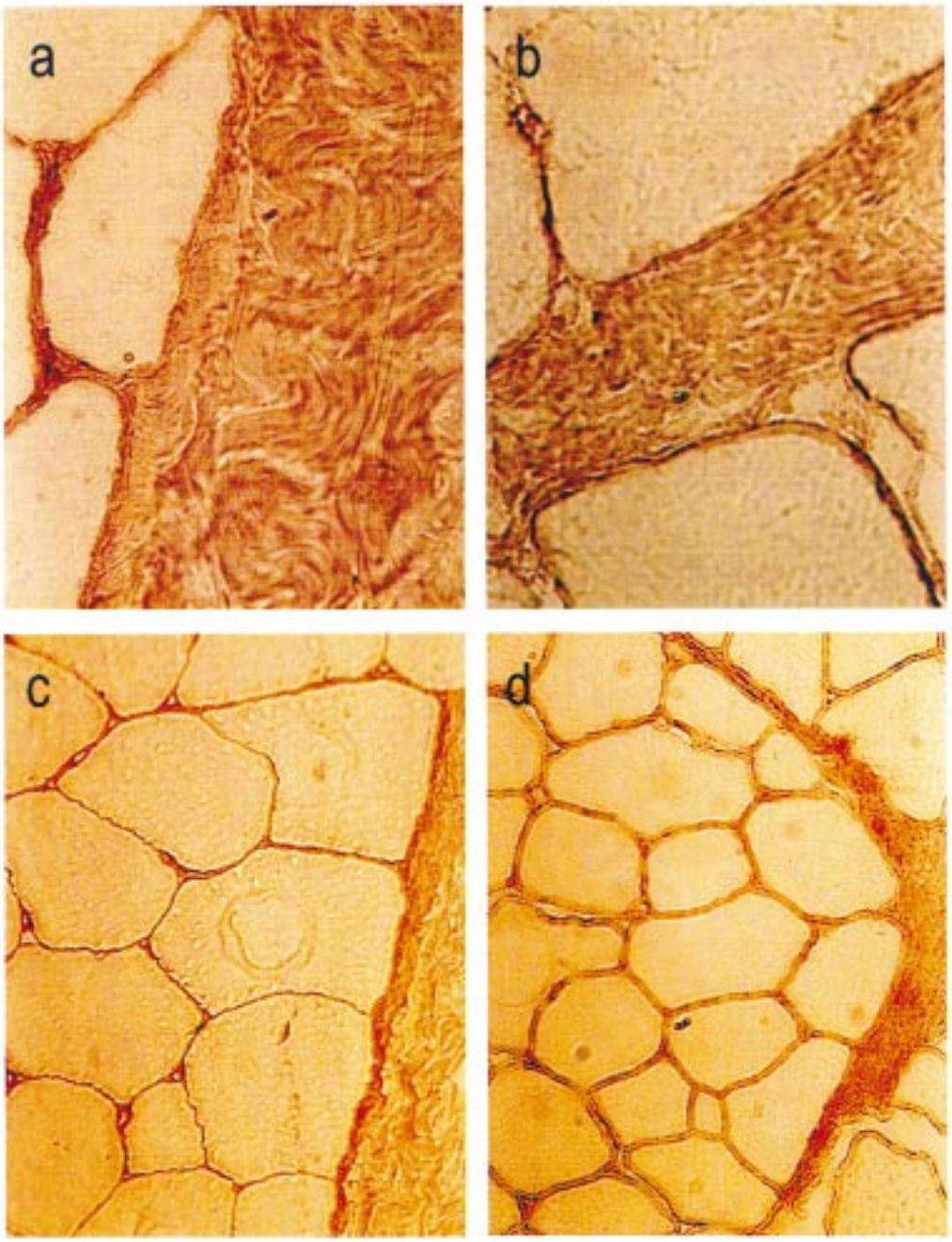

Figure 6. Immunohistochemistry: (a, b) perimysium at 1250× magnification; (c, d) the endomysium around each muscle fiber and a small part of the perimysium at 500× magnification. Frozen tissue sections of ST (a and c) and PM (b and d) were stained with antibodies against CS and DS. (Figure is reproduced here at 75% of the original.)

sition of GAGs in ST and PM. Considering the individual GAG, a significant difference was found in the content of DS (Table 3). In a study by Fransson and Cøster (1979), DS was shown to be able to self-aggregate in vitro, dependent on the content of iduronic acid (IdoUA) in the galactosaminoglycan side chain. IdoUA in DS is shown to take up more than one ring confirmation, so that the polymer configurations are potentially more varied than those of other GAGs. Furthermore, if IdoUA is $^4C_1$ in a twofold helix, DS exhibits the closest grouping of charges of any of the monosulfated GAGs (Scott, 1992).

DS has previously been shown to be the major GAG in *bovine* ST (Eggen et al., 1994). In that study a small molecular size DS−PG identified as decorin and a DS−PG of high molecular size, probably belonging to the aggrecan/versican family, were described. Small DS−PG associate with collagen fibrils (Scott and Hughes, 1986), and electron microscopical studies have shown DS filaments to bridge the gaps between the parallelly arranged collagen fibrils (Scott, 1992). Such interactions influence the mechanical properties of the tissue (Fransson, 1976; Pins et al., 1997; Scott and Thomlinson, 1998).

The extraction yield was high for both muscles, indicating that the major proportion of GAG was extracted by the methods used. The lower extraction yield of ST and the minor amount of DS sometimes detected in the residue by cellulose acetate electrophoresis are most likely the result of a more densely organized connective tissue in this muscle than in PM. This was in agreement with the microscopical study, showing more tightly arranged fibers in the perimysium in ST than in PM (Figure 6).

The residue after extraction from both muscles contained mainly HS. Only minor amounts of CS/DS could be traced in the residue from ST by cellulose acetate electrophoresis, so the content of GAG in the residue supports the suggestion that DS is the major GAG in ST, whereas HS seems to dominate in PM.

The biochemical identification of HS and KS in the present study is in agreement with previous histological and biochemical studies, which have detected HSPG in bovine skeletal muscles (Campos et al., 1993; Nishimura et al., 1996a,b; Eggen et al., 1997). HS chains in PGs such as perlecan and syndecans are described to participate in the assembly of basement membranes interacting with other components such as laminin, nidogen, and fibronectin (Isemura et al., 1987; Battaglia et al., 1992). The source of KS identified in the present study may be fibromodulin, which has been detected by immunohistochemical methods in skeletal muscle (Eggen et al., 1997) or from large aggrecan-like PGs, which are known to carry KS chains (Heinegaard and Axelsson, 1977).

The total GAGs measured thus represent different PG types with different functional roles ranging from small collagen-interacting PGs such as decorin and fibromodulin to large aggregating PGs. Although significant differences were found between PM and ST in the amount of DS as well as in the GAG composition pattern in the present study, further studies on the content of the different PGs that are the source of the GAGs described are needed. This would provide more information about how PGs could influence meat tenderness.

On the basis of the present findings, it may be argued that differences in texture at the time of slaughter are related to differences in the composition of GAGs and collagen in the connective tissue of muscles.

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