Proteoglycans and Meat Quality - A Possible Role of Chondroitin/Dermatan Sulfate Proteoglycans in *Post Mortem* Degradation

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Abstract

The knowledge of components involved in *post mortem* degradation of striated muscle is of great importance for the meat industry in order to provide tender meat for the consumer. To address this problem the present study has focused on proteoglycans. Proteoglycans were extracted from meat *(M. sentimembranosus)* stored for 0, 7, 14 and 21 days *post mortem* by use of denaturing agents. The content of glycosaminoglycans (GAGs) in the extracts showed a reduction during *post mortem* storage of meat whereas the protein content showed a small increase. The reduction in GAGs could be explained by degradation of proteoglycans of high as well as low molecular mass judged by gel filtration, ion exchange chromatography and electrophoresis. Among the low molecular size PG, decorin was identified by use of antibodies and Western blotting. This collagen interacting molecule was shown to be degraded during *post mortem* storage. Furthermore it was shown by ion-exchange chromatography and electrophoresis that the degradation of decorin involved both the peptide core and the GAG side chains. Judged by immunohistochemistry the proteoglycans involved in degradation showed a widespread distribution in the extracellular matrix.

Key words: chondroitin/dermatan sulfate, decorin, aggrecan-like PG, meat texture, tenderness.

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1 exture is important for consumers acceptance of meat, and to provide tender meat is a great challenge for the meat industry. Many factors from conception of the animal to cooking procedures are shown to influence the texture, but its origins are poorly understood. Great variations in texture exist between the di fferent skeletal muscles within the individual. In addition, the same muscle from different individuals of same age and handled by the same slaughter and processing methods, varies in tenderness.

Post mortem storage of meat increases tenderness, but the components and mechanisms involved in the process are far from clarified in detail. Connective tissue, especially the collagen fibers, provides the muscle with tensile strength and may contribute to meat toughness. Some studies have focused on the collagen component showing that collagen remained unchanged during conditioning [13]. An increase m the amount of soluble OH-proline has nevertheless been observed during *post mortem* storage indicating a connective tissue breakdown [32, 53]. The nature and content of crosslinks inside the collagen fibrils have been suggested as decisive for the texture [2, 3], but attempts to establish a correlation between meat texture and muscle collagen phenotypes have been unsuccessful [35]. Several years ago it was suggested that *post mortem* ageing involved breakdown of glycoeonjugates [17, 36]. Proteoglycans (PGs), a class of glycoeonjugates, are proteins carrying a variable number of sulfated carbohydrate chains, glycosaminoglycans (GAGs) covalently attached to the central core protein. There are two major families of proteoglycans present in extracellular matrix of connective tissues. One is represented by large molecular mass PGs (> 10 *Da) which have the capacity to form large aggregates with hyaluronate [27]. The other _consists of members of lower molecular mass (around IO"[1] Da). They are present in many tissues and predominate in fibrous tissues. One of the best described members of the family is decorin, which binds to both the fibrillar collagen types I and II [30, 61], and the FACTT collagens [23] acting most likely as interfibrillar bridges [48], In addition decorin binds to fibronectin [52, 62], to growth factors as TGFp and influences protease activity and tissue metabolism [64]. Because of this mul ^functionality the proteoglycans are determinants of the physical and biological properties of tissues. The relative composition, and the structure of

the PGs varies between tissues and within tissues due to differences in mechanical stress [57], age [60], hormonal status [14,54] and changes during differentiation [22,42]. An intact structure of PG molecules is considered a prerequisite for optimal mechanical function. Nevertheless, data on proteoglycans present in the extracellular matrix of adult skeletal rnuscies are limited. Some studies have focused mainly on the role of proteoglycans in muscle development and differentiation in species such as rat, rabbit, fowl and human [1.5,9,11,12,43,44,56]. Limited information exists on proteoglycans in the connective tissue of domestic animals except for Vellernan et al. who have studied the role of proteoglycans and collagen in skeletal muscle development in sheep [55]. Proteoglycans as contributors to meat texture have never been studied. This is rather surprising considering that microscopical studies have shown that the fractureline first appears in the extracellular matrix between the collagen fibers when tension is applied perpendicularly to the myofibers in meat [46]. Furthermore, an electronmicroscopical study has shown that this area was degraded during *post mortem* storage of meat [41],

We have demonstrated by biochemical methods that the matrix of intramuscular connective tissue from adult bovine *M. semimembranosits* contains chondroitin/ dermatan sulfate proteoglycans of high and low molecular mass which were identified as decorin and aggrecan-like proteoglycans [18], Both decorin and aggrecan-Jike proteoglycans were found by immunohistological techniques to be located in connection with the collagen fibers, but showed different types of distribution [19].

The aim of the present study was to study the macromolecular properties of chondroitin/dermatan sulfate proteoglycans in the connective tissue of meat stored for different time periods *post mortem.*

Materials and Methods

Post mortem storage

M. semimembranosus from young bulls was obtained two hours after slaughter. After removal of the epimysium, the medial, central portion of the muscle was cut into pieces. The pieces were collected randomly into four portions which were vacuum packed and stored for 0, 7, 14 and 21 days at a temperature of 15°C. At the end of the different time periods of storage, the meat samples were put in a freezer and stored at -80°C until extraction of the proteoglycans could be performed. The extraction and purification procedures used in the present experiment are the same as described previously [18],

Extraction

Before extraction of proteoglycans, the muscle pieces were powdered in liquid nitrogen and aliquots incubated in a sodium acetate buffer, pH 6.0 containing 4 M guanidine-HCL containing the following protease inhibitors: 0.1 M 6-amino hexanoic acid, 0,01 M EDTA, 1 mM phenyl methyl sulphonyl fluoride and 10 mM N-ethylmaleimide. The solid to liquid ratio was 1 g tissue (w/w) to 10 ml buffer. The suspensions were gently stirred for 16 hours at 4°C, clarified by centrifugation and the sediments reincubated with fresh extraction buffer at the same conditions as described. After centrifugation the supernatants from the two extractions were pooled. Aliquots (10 ml) of the supernatants were dialysed against distilled water and freeze dried for chemical analyses. The contents of protein and uronic acid in the samples were measured by the Bio-Rad assay [8] and the carbazole reaction assay [7], respectively. The rest of the supernatants were concentrated to 1/3 the original volume in an Amicon ultrafiltration cell under the influx of liquid nitrogen by use of a PM 30 filter.

Density gradient ultracentrifugation

The concentrated extracts were adjusted to a density of 1.37 g/ml by the addition of solid CsCI. Centrifugation was carried out for 92 hours at HO.OOOg by use of a TI-70 angle rotor equipped with Quick-Seal Centrifuge Tubes (16x76 mm). The gradients were collected in 5 ml fractions from the bottom of the tubes. The density of the fractions was determined and the content of proteins and sulfatcd GAGs were measured directly on the fractions by the Bio-Rad assay [8] and by the use of 1,9-dimethyleneblue (DMB)-method [21], respectively. GAGs were recovered in fractions with densities > 1.31 g/ml.

The fractions were furthermore examined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on gradient gels ranging from 3-12% [33] after precipitation with ethanol with a sample/ ethanol ratio of 1:5. High molecular mass PGs were mainly found in the bottom fractions (densities > 1.5 g/ml) which were rich in GAGs, but contained very little protein material.

PG material of smaller sizes were found in all fractions with densities > 1.31 g/ml). These fractions showed an increase in protein content with decreasing densities. For further fractionation of PGs, the GAG-containing fractions were pooled and concentrated to 1/3 the original volume with simultaneous removal of CsCI in the Amicon Ultrafiltration cell, using the PM 30 filter as described above,

Gel chwmatography

The concentrated samples were subjected to gel filtration on a Sepharose CL-4B column (volume 100 x 1.75 cm). As elution buffer was used 0,5 M sodium acetate pH 7.0 added 4 M guanidine-HCL and protease inhibitors described above. The eluates were collected in 4 ml fractions. The content of protein in the fractions were monitored by UV absorbance at 280 nm wavelength in a Diode Array Spectrophotometer 8452 A, Hewlett Packard, Corvallis, OR, USA. The content of sulfated polysaccharides in each fraction was measured by use of the DMB-method described above.

The eluate fractions after gel filtration with a Kav < 0.3 were pooled and called Sample], whereas eluate fractions with a Kav value > 0.3 were pooled and called Sample II.

Ion exchange chromatography

Previous studies have shown that decorin is present in Sample II with this procedure [18]. Subsequently, Sample II material obtained by gel filtration of samples after 0, 7, 14 and 21 days of storage, were subjected to ion exchange chromatography on a Pharmacia FPLC System. The experiments were carried out by use of a Mono-Q Fast Flow column (Pharmacia Fine Chemicals) equilibrated with 0.05 M sodium acetate buffer, pH 5.8, containing 6M urea and 0.1 M NaCl. Bound anionic material was eluted with a gradient of NaCl ranging from 0.1 to 1.5 M. The eluates were monitored for protein and GAGs as described. The protein content was expressed in relative absorbance (100% absorption corresponds to 0.5 UV-units). The anionic GAG-containing fractions were pooled, dialysed against distilled water and lyophilized.

Enzymatic treatment

Digestion with chondroitinase ABC lyase from Proteus Vulgaris (EC 4.2.2.4, Sigrna Chemical Corp. St. Louis, MO, USA) was carried out according to the method of Yamagata et al [63]. Digested samples were used for preparation of core protein for SDS-PAGE and for the preparation of samples for immuriohistocheniistry.

Electrophoresis and identification of decorin by Western blotting

SDS-PAGE was carried out by use of gradient gels ranging from 3-12% and the discontinuous buffer system described by Laemmli [33]. The samples were dissolved in a sample buffer containing 4% (w/v) SDS and 5% (v/v) raercaptoethanol and heated at 80°C for 10 minutes. After ihe run bands were visualized by Coomassie blue (Coomassie brilliant blue R-250, Sigma) for detection of small proteoglycans (chondroitinase ABC-digested and non-digested samples) or a silver staining technique [37] for detection of high molecular size PG.

For identification of decorin, anionic material after ion exchange chromatography was separated by SDS-PAGE using a 7.5% gel with a stacking gel of 4% in a Mini Protean Dual Slab Cell (Bio-Rad, Richmond, CA, USA). Western blotting of the SDS-PAGE separated anionic material onto a nitrocellulose membrane was performed in a Bio-Rad Trans-Blot apparatus by application of 23 mA for 45 min using a transfer buffer consisting of 25 mM Tris, 192 mM glycine and 20% (v/v) methanol pH 8.3. After transfer of PG from the polyacrylamide gel to the nitrocellulose membrane, residual binding capacity of the membrane was blocked by incubation in a solution of 1% BSA in IBS {20 mM Tris-HCl pH 7.5 with 500 mM NaCl) for 1 hour. The blocked nitrocellulose sheet was first incubated in a TBS solution containing a 1:1000 dilution of antiserum to decorin from sclera, and thereafter in a 1:3000 dilution of the alkaline phosphatase-conjugated goat anti-rabbit F(ab)2 fragment. Alkaline phosphate substrate solution was prepared by mixing 15 mg NBT (p-nitroblue tetrazolium chloride) in 0.5 ml 70% BMP (N,N-dimethyl formamide) and 7.5 mg BCIP (5-bromo-4-chloro-3-indolyl phosphate toluidine salt) in 100% BMF with 50 ml buffer consisting of 100 mM NaHCC-3 and 1.0 mM MgCb, pH 9.8. The reaction was terminated with transfer to distilled water.

Antiserum

Polyclonal antibodies for detection of decorin were raised in rabbits against decorin from bovine sclera [15]. The antibodies which had been purified by affinity chromatography at 4°C with the antigen coupled to CNBr-activated Sepharose 4B, were a kind gift from Dr. Anders Malmstr0m, University of Lund, Sweden.

A commercially available polyclonal antiserum raised in rabbits against chondroitin sulfate PG from bovine nasal cartilage after digestion with chondroitinase ABC [4] (Chemicon International Inc., Ternecula, CA, USA) was used for the immunohistochemical study of chondroitin/dermatan sulfate PGs in the muscular connective tissue.

Immunohistochemistry

Frozen tissue sections of M *semimembranosus* were cut in a cryostate, subjected to digestion with chondroitinase ABC and treated with rabbit antiserum against chondroitin sulfate diluted 1:200. Peroxidase-conjugatcd swine anti-rabbit IgG (1:100) (Dako A/S, Glostrup, Denmark) was used for detection of the antibodies. The cover slips were photographed using a Wild MPS 46/52 photo automate camera (Wild Leitz, Heerbrugg, Switzerland) with an Ectachrome 64 ASA colour film.

Results

Proteoglycans and storage

Immunohistochemistry using an antibody against CSPG after digestion with chondroitinase ABC, showed a wide spread distribution of c bond ro it in/derm atan sulfate proteoglycans in all parts of the muscle connective tissue at day zero (Figure 1.) Strong staining is observed in both the

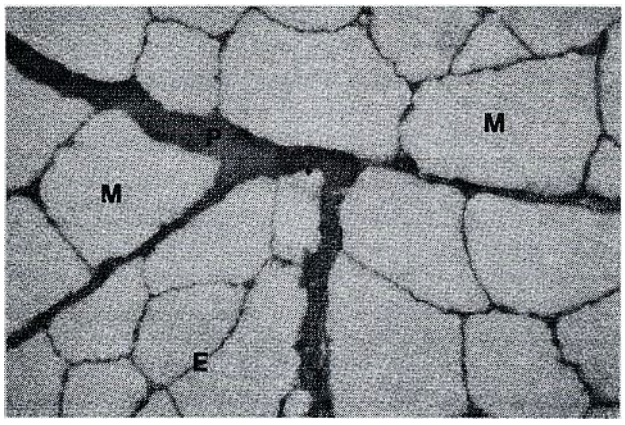

Figure I. Immunohistochemistiy of a frozen cross section of tissue from bovine M. semimernbrartosus *treated with an antibody against chondroitin-sulfate PG from nasal cartilage after digestion with chondroitinase ABC. P illustrates the perimysium, E the endomysium whereas M represents the myofiber.*

perimysial as well as the endomysial layers. The antigenic epitopes for this antibody is produced by digestion with chondroitinase ABC and are thus common for both chondroitin and dcrmatan sulfate GAGs [4]. So this antibody shows the presence of all types of chondro it in/derm atari sulfate proteoglycans present in the tissue.

The contents of protein and uronic acid in the extracts obtained from meal stored for 0, 7, 14 or 21 days post mortem are shown in Figure 2. The content of protein showed a small increase during storage whereas the content of uronic acid decreased from 1,6 mg to 0.6 mg per g nitrogen powdered meat (w/w).

The profile obtained from uronic acid measurements showed a sharp deeline in the curve between day 0 and 7. Between day 7 and 14 the curve flattened with a new decrease between day 14 and 21. The drip in the vacuum packages, measured as the weight of water lost during the storage, was between 5-7% w/w.

After density gradient ullracentrirugation GAG containing material was recovered in the fractions of densities > 131g/ml. These fractions contained only a small amount of protein material. SDS-PAGE of the bottom fractions with densities > 1.5 g/ml revealed the presence of a proteoglycan component of molecular mass > 200 kDa which hardly entered the gel (Figure 3, lane 1). High molecular mass PGs obtained from samples stored for 7, 14 and 21 days penetrated deeper into the gel (Figure 3, lanes 2,3 and 4). The degradation was evident at 7 days of storage and the migration distance increased with increasing time of storage *post mortem.* Figure 4 shows the scans of the gels illustrated in Figure 3, confirming a breakdown of large proteoglycans into components of smaller sizes.

PGs of lower molecular mass were detected in the fractions of densities > 1.31 g/ml by SDS-PAGE (results not shown}. For preparative purposes these fractions obtained from the same source of meat from different time periods, were pooled and studied by gel filtration.

From Figure 5 it is evident that the elution profile changed during 21 days of storage. The sample from day

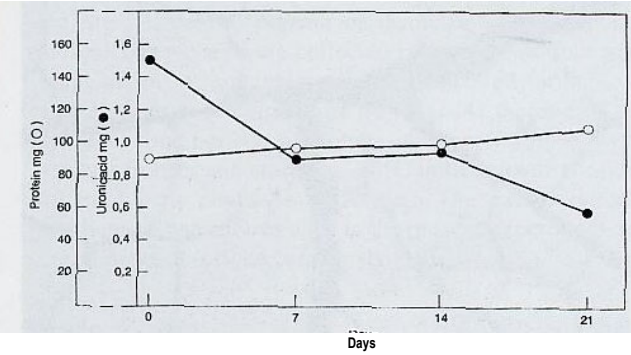

Figure 2, The content of uronic acid and protein in the guanidine-HCl extracts of meat stored for 0, 7, 14 and 21 days post mortem. The results represent the mean from four animals. •— illustrates the content of uronic acid whereas* O—O *shows the content of protein.*

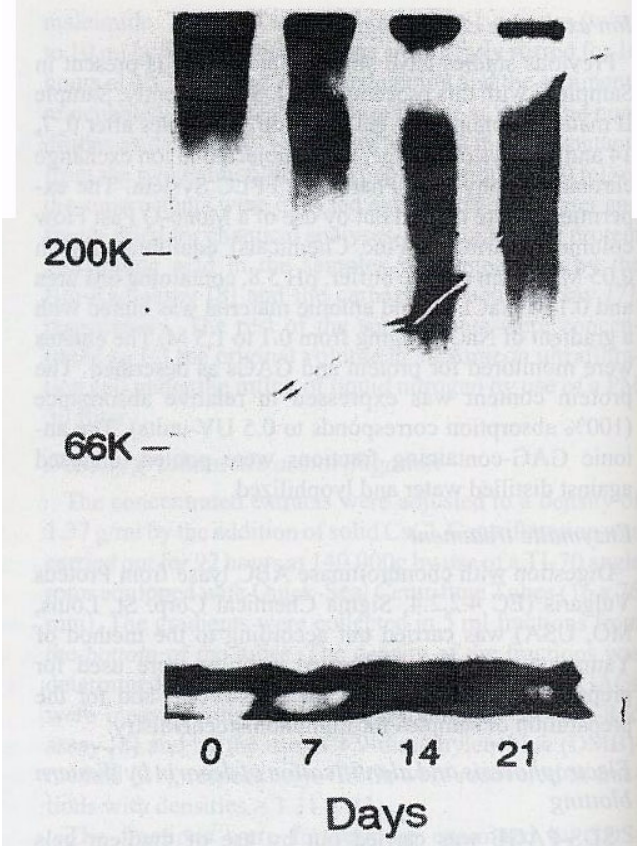

Figure 3. SDS-PAGE on gradient gels (3-12%) of the bottom fractions (densities > 1.5 g/ml) obtained after density gradient ultracentrifugation of extracts obtained after 0, 7, 14 and 21 days of post mortem storage. Proteoglycans were visualised by a silver staining technique [3 7].

0 meat separated into 4 distinct GAG containing peaks. After storage a shift towards more low molecular mass material was observed, confirming the degradation of high molecular mass PGs with increasing time observed by SDS-PAGE. In addition the total content of sulfated GAGs, was also reduced.

Polyanionic properties

The polyanionic properties of the tissue samples obtained by gel filtration (Figure 5) were compared by Mono-Q ion exchange chromatography. At day zero the larger portion of Sample II contained polyanionic material (Figure 6), and approximately 85% of the DMB-positive material in Sample II was eluted after application of the sali gradient. After 21 days of storage only 50% was eluted at high ionic strength. Furthermore, the salt concentration needed for elution of the GAG-contain ing material from day zero was 1.2 M NaCl, whereas 1 M NaCI was sufficient to elute all GAG-containing material after 21 days of storage. The material in the flow through fractions increased to approximately 50%, as can be seen in Figure 6.

The yield of anionic GAG-containing material after lyophilization decreased during storage as shown in Table 1.

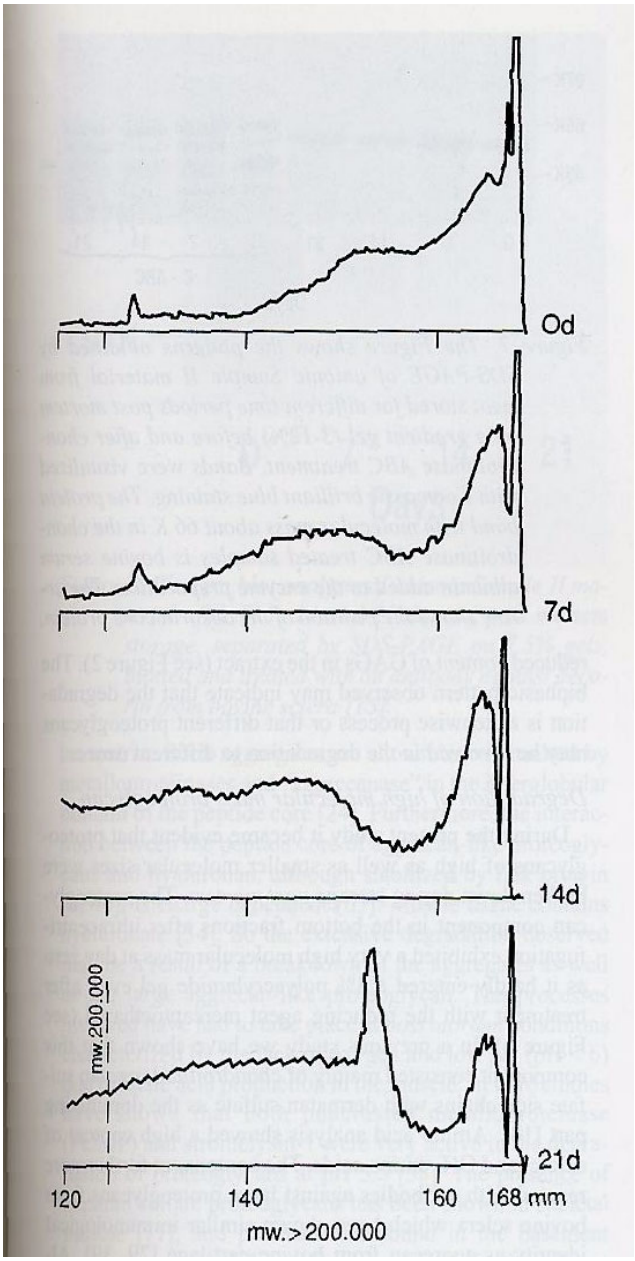

Figure 4. The densitometric scans of the gel illustrated in Figure 3, 168 mm represents the top of the gel. A standard protein of molecular mass of200 kDa is detected at 124 mm.

Changes in molecular mass

The anionic GAG-containing material from Sample II was examined by SDS-PAGE, The results obtained by use of gradient gels are shown in Figure 7. A faint band can be seen at the same position as a standard protein with a molecular mass of 97 kDa (Figure 7, lane 1). This band was hardly detectable after 21 days of storage (Figure 7, lane 4). After chondroitinase ABC treatment the broad band disappeared. A protein of molecular mass around 40 kDa appeared after enzyme treatment (indicated by arrow in Figure 7). The amount of this protein decreased during

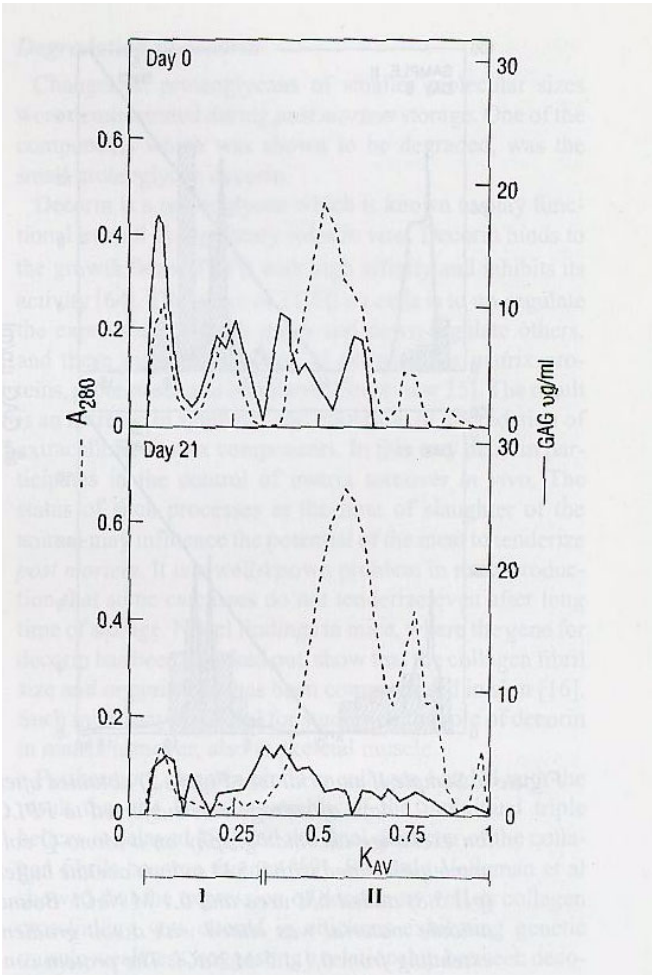

Figure 5. Gel filtration oj glycosaminoglycan containing material after CsCl-ullracentrifugatlon obtained after 0 and 21 days of storage on a Sepharose CI-4B column (100 X 1.75 cm) in a 0.5 M acetate buffer (pH7) containing 4M guunidine-HCL and protei-nase inhibitors. The eluates were collected in 4 ml fractions and monitored for the contents of gly-cosaminogiycans and protein. Fractions with K betiveen 0.3 and 1 were pooled and called Sample II as illustrated in the Figure for further purification by ion exchange chromatography.

storage (Figure 7, lanes 5, 6, 7 and 8). Imrmmologica! characterization of the 100 kDa band with a polyclonal antibody against decorin from bovine sclera [15] by the use of Western blotting identified this band as decorin, illustrated in Figure *$*. A similar reduction in the staining intensity was observed after Western blotting as after SDS-PAGE. The 40 kDa band which appeared after digestion with chondroitinase ABC has previously been identified as the protein core of decorin [18]. Accordingly, the results showed that decorin was degraded during *post mortem* storage of muscle tissue. A protein band with an apparent molecular mass around 60 kDa appeared after separation on SDS-PAGE (Figure 7). This protein was not

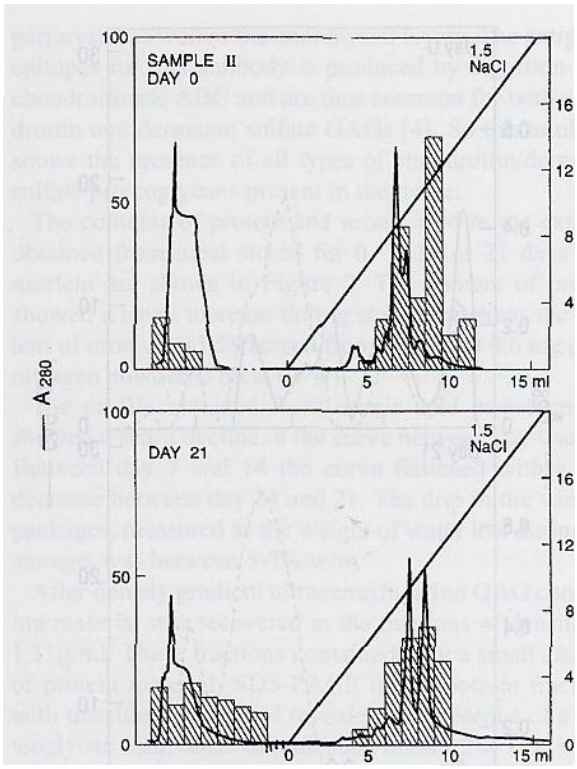

Figure 6. Sample II material (see Figure 5) obtained after 0 and 21 days of storage was subjected to FPLC ion exchange chromatvgraphy on a Mono-Q column equilibrated with 0.5 M sodium acetate buffer (pH 5.8) added 6M urea and O.I M NaCl. Bound anionic material was eluted with a salt gradient extending from 0.1-1.5 M NaCl. The protein content was monitored automatically during the run. The eluates were collected in 1 ml fractions and each fraction was analysed for the content ofg/y-cosaminoglycans [21J.

influenced by digestion with chondroitinase ABC, showing that it is not a chondroitin/dermatan sulfate PG.

Discussion

In the present study chondroitin/dermatan sulfale PGs were shown by immunohislochemistry to be distributed in all parts of the muscular connective tissue in the perimysium as well as the cndomysium. Proteoglycans were extracted from meat *(M. semimemhranosus]* after different time periods of storage by use of a denaturing agent, 4 M guanidine-HCl. Most studies in meat research have been performed with water or weak salt solutions which most likely have excluded the study of proteogly-cans.

The amount of uronic acid containing GAGs in the extracts decreased during storage, showing that a decomposition of proteoglycans had occurred. The measured amount of uronic acid represents the total amount of the different proteoglycans present in the sample at the different times of measurements. It is not possible to suggest the contribution of the separate proteoglycan families to the

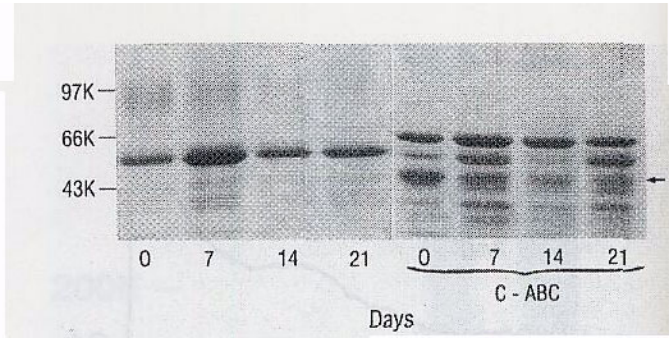

Figure 7. The Figure shows the patterns obtained by SDS-PAGE of anionic Sample I! material from meat stored for different time periods post mortem on a gradient gel (3-12%) before and after chondroitinase ABC treatment. Bandy were visualized with Coomassie brilliant blue staining. The protein band with molecular mass about 66 K in the chondroitinase ABC treated samples in bovine serum albumin added to the enzyme preparation. The arrow shows the position of the decorin core protein.

reduced content of GAGs in the extract (see Figure 2). The biphasic pattern observed may indicate that the degradation is a stepwise process or that different proteoglycans may be involved in the degradation to different times.

Degradation of high molecular mass proteoglycan

During (he present study it became evident that proteoglycans of high as well as smaller molecular sizes were broken down during storage *post mortem.* The proteoglycan component in the bottom fractions after ultracentri-fugation exhibited a very high molecular mass at day zero as it hardly entered a 3% poly aery 1 amide gel even after treatment with the reducing agent mercaptoethanol (sec Figure 3). In a previous study we have shown that this component consisted mainly of chondroitin/dennatan sui-fate side chains wilh dermatan sulfate as the dominating part f I 8]. Amino acid analysis showed a high content of acidic- and OH- amino acids. The component furthermore reacted with antibodies against large proteoglycans from bovine sclera which have shown similar immunological identity as aggrecan from bovine cartilage [29, 39]. Although the exact identity of this component has not been established yet, it belongs most likely to the large aggregating family of PGs [25]. Members of this family have in common the ability to bind to hyaluronan, allracting water molecules and building large aggregates in ihe cxtraccllu-

Table I. Anionic material obtained by FPLC chromatography of Sample II expressed as mg/g nitrogen powdered meat.

	0 DAYS	7 DAYS	14 DAYS	21 DAYS
Sample II	0.560mg	0.500mg	0.390 mg	0.310mg

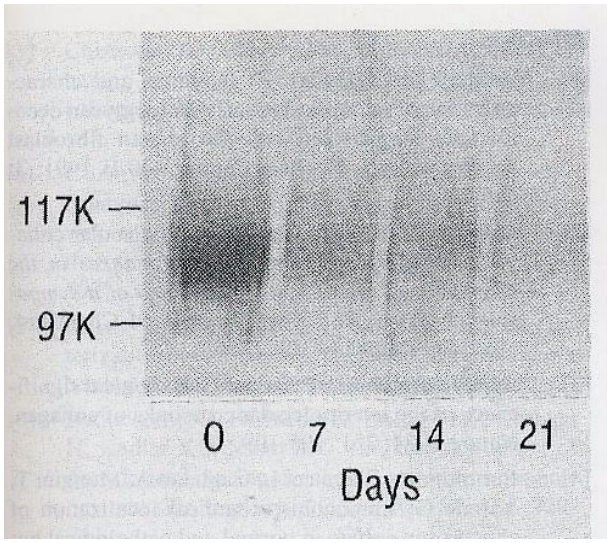

Figure 8. Western blot analysis ofanionic Sample II material after 0, 7, 14 and 2] days of post mortem storage, separated by SDS-PAGE on 7.5% gels, blotted and treated with an antibody against decorinfi'om bovine sclent [15],

lar matrix. Such aggregates are shown to be attacked by metalloproteinases and "aggrecanase" in the interglobular domain of the peptide core [24], Furthermore, the interaction between the peptide core of aggrecan-like proteogly-cans and hyaluroiian, although stabilized by link protein [6, 40], is charge dependent [65]. Muscle tissue contains hyaluronate [34]. So the extensive degradation observed may be a result of a breakdown of the aggregates as well as the large aggrecan-like proteoglycan. The processes involved have had to take place *at post mortem* conditions characterized by trie lack of oxygen and low pH (pH < *6)* due to lactic acid production in the muscle. In vitro studies have shown that both punctuated metalloproteinase (PUMP) and stromelysin-1 were very active in the degradation of protcoglyeans at pH 5.5 [38]. The presence of heparan sulfate proteoglycans has been shown in skeletal muscle [11], and perlecan was found in the basement membrane area of the endomysium [19]. In the present study guanidine-HCl was used as extractant for the proteoglycans. To check the efficiency of the extraction solution, the residue (insoluble in 4M guanidine-HCl) was digested with papain, and the content of GAGs studied by electrophoresis on cellulose acetate sheets after fractionation on DE-52 anion exchanger. The residue contained only GAGs of similar mobility as heparan sulfate [Eggen et al, unpublished results]. To what extent the heparan sulfate proteoglycans present in skeletal muscle arc degraded *duringpas/ mortem* storage, remains to be clarified. For the fractiona-lion and characterization of heparan sulfate proteoglycans, detergents should most likely be included in ihe extraction solution to improve solubilization of the residue.

Degradation of decorin

Changes in proteoglycaus of smaller molecular sizes were demonstrated during *post mortem* storage. One of the components which was shown to be degraded, was the small proteoglycan decorin.

Decorin is a proteoglycan which is known to play functional as well as regulatory roles *in vivo.* Decorin binds to the growth factor TGFp with high affinity and inhibits its activity [64]. The effect of TGFp on cells is to up-regulate the expression of some genes and down-regulate others, and these include the genes of extracellular matrix proteins, proteinases and inhibitors [For review 25]. The result is an increase in synthesis and inhibition of degradation of extracellular matrix components. In this way decorin participates in the control of matrix turnover *in vivo*. The status of such processes at the time of slaughter of the animal may influence the potential of the meat to tenderize *post mortem.* It is a well-known problem in meat production that some carcasses do not tenderize even after long time of storage. Novel findings in mice, where the gene for decorin has been knocked out, show that the collagen fibril size and organization has been compromised in skin [16]. Such mice may be useful for studies on the role of decorin in matrix turnover, also in skeletal muscle.

Furthermore, decorin binds to collagen I and II with the result that the lateral assembly of the individual triple helices is delayed [58] and the final diameter of the collagen fibrils become thinner [59]. Recently Velleman et al showed that the expression of decorin as well as collagen crosslinking was altered in chickens exhibiting genetic muscle weakness, suggesting a relationship between decorin expression and collagen crosslinking [56], The interactions of decorin with extracellular components are mediated with the peptide core as well as the GAG chain. Decorin is shown to bind to the d and e band in the collagen fibril with its peptide core [45, 51]. An affinity constant of 3.3x10" M" hasbeenmeasurcd(Hv//ro[10].GAGchains were not necessary for the interactions but influenced the process [28].

It has been suggested that there are two binding sites for collagen on the protein core of decorin, one in the N-terminal, the other in the C-terminal region. Recently Scott [50] showed by rotary shadowi ng electron microscopy that the core protein of decorin was horseshoe shaped, probably attached to two parallel neighboring collagen molecules in the fibril, in this way decorin could help in stabilizing the collagen fibrils, A tight fit between decorin and collagen I was furthermore found by Weber et al [61] who proposed a more open structure of the decorin molecule allowing greater access to binding sites available in the inner concave surface. Such a structure should increase the capacity of the decorin molecule to form favourable contact points with other proteins.

A stabilizing effect of decorin on the collagenous network could furthermore be obtained by interactions of the GAG chains with the FAC1T collagen Type XIV [23]. Decorin in bovine skeletal muscle carries derma tan sulfate

side chain [18]. Dermatan sulfate side ehains are shown to be able to self associate, making thicker GAG chains as seen in cornea [26, 49], This may influence the interconnection between the collagen fibrils, A single GAG chain has also been shown to be able to covalently cross link different protein moieties [20], Recently, a mixture of decorin and the other small leucine-rich PGs, fibroinodulin and biglycan, were shown to interact with hyaluronate in vitro under isotonic conditions [47], Similar interactions were proposed to take place *in vivo* where regions of hyaluronate are exposed between the collagen molecules. Small proteoglycans such as decorin, present on the surface of collagen fibrils could in this way act as mediators allowing interactions between the fibrillar network and the hyaluronate of the interspersed proteoglycan aggregates.

Furthermore, decorin colocalizes and interacts with fibronectin in the cell binding domain of the molecule, exhibiting antiadhesive properties against cultured human fibroblasts [52, 62]. The results in the present study indicate that the degradation of decorin involved both the carbohydrate side chains and the protein core. The anionic charge of the decorin molecule is provided by sulfate and carboxyl groups in the carbohydrate side chains. The reduction in the amount of anionic material and negatively charged ions needed to elute the molecules observed in the ion exchange chromatography, is most likely a result of loss of these groups in the molecules.

After treatment with chondroitinase ABC a core protein of molecular size 40kDa appeared (see Figure 7, lane 5), previously identified as the protein core of decorin [18]. Only traces of this core protein could be detected in samples after storage. No protein bands of intermediate molecular sizes (100 kDa < mol. mass > 40 kDa) appeared in tlie gel (Figure 7, lanes 2, 3 and 4). A degradation of both the peptidc core and the carbohydrate side chains will effect the interconnections with the other matrix components described above.

The mechanical strength of meat will depend on the breaking strength of the individual components and the interactions between the components. The breakdown of aggrecan-like PGs and decorin found in the present study will cause a weakening of the interactions between the components of the extracellular matrix and influence the integrity of the structural network. The result is most likely a change in the textural properties of the skeletal muscle.

Further studies are in progress in our laboratory on the role of proteoglycans in meat texture.

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