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DEGRADATION OF DECORIN IN BOVINE *M.SEMIMEMBRANOSUS* DURING *POST MORTEM* STORAGE

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INTRODUCTION

Proteoglycans (PG) are a class of macromolecules consisting of highly polyanionic carbohydrate side chains (GAGs), covalently bound to a peptide core. PGs play an important role for function and stability of extracellular matrix. The small PG, decorin, is known to interact with type I and II collagen fibrils[1] and influences the distance between the adjacent collagen fibers. Furthermore it influences the kinetics of fibril formation and binds growth factors[2,3]. We have recently isolated decorin and a large aggrecan-like PG from bovine *M.semimembranosus*[4] and demonstrated that both are present in the same areas as the collagen fibers[5]. The aim of the present study was to examine the fate of decorin in meat stored for different time intervals *post mortem* by biochemical methods.

MATERIALS AND METHODS

Post mortem storage: Bovine *M.semimembranosus* was obtained two hours after slaughter and cut into pieces. The pieces were collected randomly into four portions which were vacuum packed and stored for 0,7,14 or 21 days at a temperature of 15 °C. At the end of the different times of storage, the meat samples were kept at -80 °C until extraction could be performed. The purification procedure used in the present experiment is described previously,[4] and will be only shortly described in the present paper.

Extraction: For extraction of decorin, the muscle pieces were powdered in liquid nitrogen and aliquots incubated in a 0.05M sodium acetate buffer, pH 6.0 containing 4 M guanidine-HCL added protease inhibitors[6]. After extraction the suspensions were clarified by centrifugation and concentrated to 1/3 the original volume in an Amicon ultrafiltration cell on a PM 30 filter under the influx of nitrogen.

Density gradient ultracentrifugation: The concentrated extracts were adjusted to a density of 1.37 g/ml by the addition of CsCl. Ultracentrifugation was carried out for 92 hours at 140.000 g. The gradients were collected in 2 ml fractions. The densities of the fractions as well as the contents of GAGs and protein were determined. The GAG containing fractions were pooled and concentrated.

Gel chromatography:The concentrated samples after ultracentrifugation were subjected to gel filtration on a Sepharose CL-4B column (volume 100 X 1.75 cm) in aliquots of 4 ml. As elution buffer was used 0.5 M sodium acetate pH 7.0, added 4M guanidine-HCl and protease inhibitors[4]. The eluates were collected in 4 ml fractions and the content of GAGs in each fraction was measured by use of the DMB-method[7].

Ion exchange chromatography of Sample II after gel filtration was carried out in a Pharmacia FPLC System by use of a Mono-Q Fast Flow column after exchange of buffer to 0.05 M sodium acetate, 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 1.5M. The eluates were collected in 1 ml fractions. Each fraction was examined for DMB-positive material. The anionic material of Sample II obtained from meat after 0, 7, 14 and 21 days of storage, was lyophilized.

Electrophoresis: SDS-polyacrylamide gel electrophoresis was carried out by use of gradient gels ranging from 3-12 % and the discontinuous buffer system described by Laemmli[8]. The samples were dissolved in a sample buffer containing 4 % (w/v) SDS and 5 % (v/v) mercapto-ethanol and heated at 80 °C for 10 minutes. After the run bands were visualized by Coomassie brilliant blue R-250 staining.

Anti-decorin antibodies were a gift from Dr. Anders Malmstrøm, University of Lund, Sweden. The antibodies were produced in rabbits against decorin from bovine sclera[9] and purified by affinity chromatography.

Identification of decorin-Western blot. Anionic material from Sample II, day 0, was digested with chondroitinase ABC[4]. Digested as well as non-digested material was separated by SDS-PAGE using a 7.5 % gel. Western blotting of the SDS-PAGE separated anionic material, onto a cellulose membrane was performed in a Bio-Rad Trans-Blot apparatus by application of 23 mA for 45 min. The membrane was then incubated in a TBS solution containing a 1:1000 dilution of antiserum to decorin, and then in a 1:3000 dilution of the alkaline phosphatase-conjugated goat anti-rabbit $F(ab)_2$ fragment. Bands were visualized by an alkaline phosphate substrate solution[4].

RESULTS AND DISCUSSION

In the present study 4M guanidine-HCl was used as extractant. After density gradient ultracentrifugation, the GAG containing material was recovered in the fractions of densities >1.31 g/ml. These fractions contained only a small amount of protein. The elution profiles obtained by gel filtration of material from day 0 and day 21, are shown in figure 1. Samples from day zero separated into 4 distinct GAG containing peaks. After storage a change in the elution pattern with a lower content of GAGs was observed. For further purification the eluted material was pooled into two fractions, Sample I and Sample II, according to molecular size as illustrated in the figure. In this study further analyses were performed on Sample II which contains decorin according to previous results[4]. The results from ion exchange chromatography of Sample II are shown in figure 2. The major proportion of GAGs in Sample II from day zero was eluted at high ionic strength. After 21 days of storage the amount of highly polyanionic GAGs decreased whereas the amount of DMB-positive material in the flow-through fractions increased, indicating a decomposition of GAGs after 21 days storage. The yield of anionic material obtained from Sample II after 0, 7, 14 and 21 days of storage was 0.56 mg, 0.50 mg, 0.39 mg and 0.31 mg, respectively, expressed as mg/g nitrogen powdered meat. Judged by gel electrophoresis, the lyophilized anionic material contained several components (figure 3). A diffuse band appeared slightly above 97K. This band was hardly visible in the preparation obtained after 21 days of storage. Figure 4 shows the results after Western blotting of the anionic material from day zero before or after chondroitinase ABC digestion, separated by gel electrophoresis. After chondroitinase ABC digestion, the 97 K band disappeared and emerged as a sharp band at 43K. Both the 97K band and the 43K band were detected by anti-decorin antibodies. Subsequently, the present results showed a decomposition of decorin during *post mortem* storage.

CONCLUSIONS

In the present experiment, decorin was extracted from nitrogen powdered *M. semimembranosus* after different periods of storage and fractionated by use of ultracentrifugation, gel filtration and ion-exchange chromatography. A decomposition of decorin was detected after 21 days of storage. A change in the structure of decorin may result in destabilization of the extracellular matrix and contribute to tenderization of meat during *post mortem* storage. Further studies are going on to elucidate this aspect.

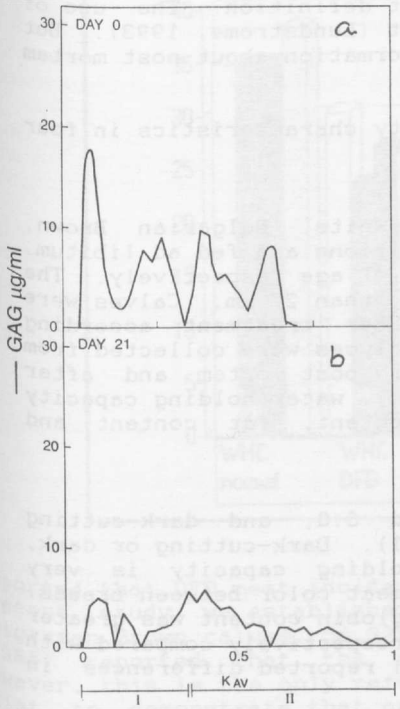

Figure 1: Sepharose CL-4B gel chromatography of material stored 0 or 21 days *post mortem*.
GAG-containing material from CsCl centrifugation subjected to gel chromatography on a Sepharose CL-4B column (100 x 1.75 cm). The solid line shows the content of GAG in the preparation from a) day 0 and b) day 21. The fractions were pooled into Sample I and II as shown in the figure.

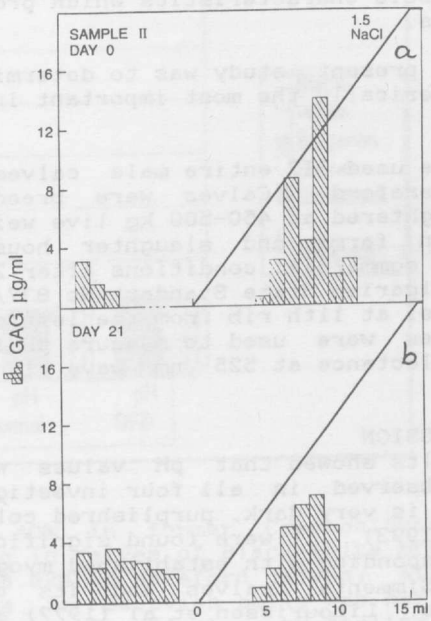

Figure 2: Mono Q-ion exchange chromatography of Sample II material obtained after 0 or 21 days of storage.
Sample II (see Fig. 1) was applied to a Mono Q-column and anionic material bound to the column was eluted by a NaCl gradient ranging from 0.1-1.5M NaCl. The bars show the content of GAG in the fractions from a) day 0 and b) day 21.

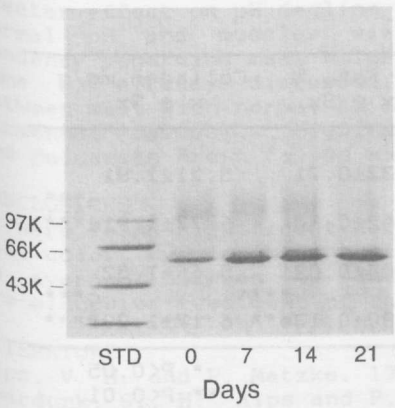

Figure 3: SDS-polyacrylamide gel electroforesis of bound anionic material of Sample II obtained after different periods of storage.
Molecular weight standard (lane 1), material after 0, 7, 14 and 21 days of storage in lane 2-5 respectively. Bands were visualized by commassie blue staining.

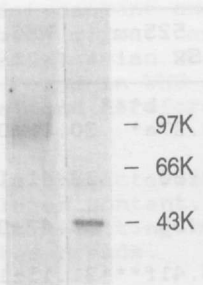

Figure 4: Western blot analysis.
Bound anionic material from day zero run on SDS-PAGE by use of a 7.5 % gel, before or after chondroitinase ABC digestion, blotted and treated with antibodies against decorin. Lane 1 shows the blot of material before chondroitinase ABC treatment. Lane 2 shows the same material after digestion.

REFERENCES

1. Scott, J.E. (1988) Biochem.J. 252, 287-297.
2. Vogel, K.G., Paulsson, M. and Heinegård, D. (1984) Biochem. J. 223, 587-597.
3. Yamaguchi, Y., Mann, D.M. and Ruoslahti, E. (1990) Nature 246, 281-284.
4. Eggen, K.H., Malmstrøm, A. and Kolset, S.O. (1994) Biochim. Biophys.Acta 1204, 287-297.
5. Eggen, K.H. and Sørensen, T. (1993) Proceed. 39th Int. Cong. Meat. Sci. Tech. Calgary, Canada S5PO5 WP.
6. Sajdera, S.W. and Hascall, V.C. (1969) J. Biol. Chem. 244, 77-87.
7. Farndale, R.W., Sayers, C.A. and Barret, A.J. (1982) Connect. Tissue Res. 19, 247-248.
8. Laemmli, U.K. (1970) Nature 227, 680-685.