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THE DISTRIBUTION OF PROTEOGLYCANS IN BOVINE SKELETAL MUSCLE STUDIED BY LIGHT MICROSCOPICAL METHODS

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INTRODUCTION

The major components of the extracellular matrix of skeletal muscle are collagen and elastic fibres which form a stress resistant network. A close relationship exists between collagen fibres and proteoglycans in connective tissues such as skin (Fleischmajer *et al.*, 1991) and tendon (Scott *et al.*, 1981). The proteoglycans represent a group of sulphated macromolecules with a wide diversity in structure as well as molecular size. They play important functional roles for the mechanical properties of the tissue as well as for fibre growth and organization. The proteoglycans can thus play a role in the tenderization of skeletal muscle during post-mortem storage. Very little information has until recently been available on proteoglycans in extracellular matrix of adult skeletal muscle.

We have isolated two dermatan sulphate proteoglycans from bovine *m.semimembranosus*. The two proteoglycans exhibited similar biochemical and immunological characteristics as PGL (large proteoglycan) and decorin from bovine sclera (Cøster and Fransson, 1981). Determination of the distribution patterns of the proteoglycans in muscle, is the first step in understanding the role these molecules may play in meat quality.

Using conventional histologic staining and immunohistochemical techniques we have investigated the distribution of sulphated proteoglycans in bovine *m.semimembranosus* with special emphasis on decorin and PGL. The distribution of collagen I or IV in the same samples was included for comparison.

MATERIALS AND METHODS

Pieces of *m.semimembranosus* from young bulls, obtained immediately after slaughter, were embedded in Tissue Tek O.C.T compound (Miles,U.S.A.) and frozen in liquid nitrogen. Cross-sections of 3 to 5µm thickness were cut in a cryostat, mounted on poly-L-lysine coated glass slides and fixed in cold acetone (4°C). After staining the sections were rinsed, dehydrated in ascend-ing concentrations of ethanol, cleared in xylene and mounted in Eukitt.

Staining procedures

Mayer's Haematoxylin in combination with Eosin Y (H&E)(Sigma) was used to demonstrate general tissue structure.

Staining of anionic groups was achieved using 0.05%(w/v) Alcian Blue 8GX (BDH, UK) in 0.2M acetate buffer pH5.8 containing 0.06 or 0.6M $MgCl_2$. At the lower concentration all anionic groups stain; at the higher only sulphate groups (Scott and Dorling, 1965).

Primary antibodies

Anti-Proteoglycans

Polyclonal antibodies against PGL and decorin from bovine sclera, were produced in rabbits (Cøster and Fransson, 1981) and purified by affinity chromatography, (Heinegård and Oldberg, 1989).

Anti-Collagens

Two rabbit polyclonal antibodies against bovine collagen I or IV (Cat.no. AB 749 and AB 751 Chemicon Int.) were used after pretreatment (see below). All commercial available antisera used in the present study, primary as well as secondary, were absorbed for one hour. with 10 % heat inactivated serum from newborn calf (Gibco Laboratories) and clarified by centrifugation.

Immunostaining

Before immunostaining the sections were digested with a drop of chondroiti-nase ABC lyase EC 4.2.2.4 from *proteus vulgaris* (1mg/ml) (Sigma) in 0.5M Tris-HCl pH8.0 for four hours at 37°C. After rinsing in tris buffered saline (TBS) the sections were exposed to a blocking solution containing normal swine serum (DAKO A/S) diluted 1:5 in TBS for one hour. The sections were then incubated at 4°C overnight with the primary antisera: anti-decorin 1:500, anti-PgL 1:50, anti-collagen I 1:20 or anti-collagen IV 1:20. After washing in TBS (3x10 min), the sections to be examined for decorin and collagen I or IV, were incubated with peroxidase-conjugated swine anti-rabbit IgG (1:100) (DAKO A/S) for two hours.

For the study of PGL, biotinylated swine anti-rabbit IgG (1:400) was applied for two hours followed by 45 minutes incubation with a streptavidin-ABClyase-horseradish peroxydase complex prepared according to manufacturers' recommendation (DAKO A/S). Peroxidase was revealed by use of a 0.6mg/ml solution of 3,3'diamino-benzidine (DAB) in 0.05M Tris buffer pH7.6 containing 0.03% (v/v) H_2O_2, (Graham and Karnovsky, 1966). TBS was used for dilution of the antisera. Non-specific binding of primary antibodies was checked using non-immune serum, that of the secondary antibody using dilution buffer.

Some sections were furthermore treated with 0.3% H_2O_2 in methanol for five minutes in order to block a possible endogenous peroxidase activity in the tissue. No differences were observed in the staining patterns between treated or non-treated sections.

RESULTS and DISCUSSION

The extracellular matrix of skeletal muscle consists of epi-, peri- and endomysium. The epimysium refers to the connective tissue that surrounds each muscle. The perimysium penetrates into the muscle, separating bundles of muscle fibers and carrying larger blood vessels, small lymphatics and nerves. The thin envelopes that surround the individual muscle fibers are called endomysium. These components are all clearly visible in the section of bovine *m.semimembranosus* stained with H&E (Figure 1a). In other areas of the perimysium, adipose tissue and blood vessels of different sizes are observed.

Use of Alcian Blue in the presence of 0.06M $MgCl_2$ resulted in heavy extra- and intracellullar staining of tissue components except for the fat cells (Figure 1b). However, in the presence of 0.6M $MgCl_2$ a total different staining pattern appeared (Figure 1c). At this concentration of $MgCl_2$, intracellular staining of the myofibers was no longer apparent, showing that the sulphated proteoglycans were localized in the extracellular matrix. Both peri- and endomysium stained. The perimysium displayed a wavy appearance with dark blue threads woven in. Heavy staining was furthermore associated with the blood vessels in the perimysium, especially with *lamina adventitia* of the artery wall and the basement membrane area. Immunostaining by use of antibodies against decorin showed a similar staining pattern to Alcian Blue in the presence of 0.6M $MgCl_2$. Decorin was present in the perimysium and the endomysium (Figure 1d). Accumulation of stain was furthermore noticed in the connective tissue junctions between the myofibers and in the basement membrane area (Figure 2a).

The staining pattern of PGL was quite different from that of decorin (Figure 2b). PGL was found only in distinct areas

of the perimysium whereas decorin was evenly distributed throughout the peri- and endomysium. Decorin codistributed with the collagens, especially collagen I, (Figure 2c,d) indicating a functional role related to the collagen fibers. Interactions between decorin and collagen fibers may be of great importance for the quality of meat since post-mortem degradation of the fibers may be extensively modified by decorin. PGL does not bind to collagen but rather to hyaluronan, building large aggregates of importance for water holding and swelling between the collagen fibers. Further studies are needed to elucidate this aspect.

CONCLUSION

The results from the present study showed that the extracellular matrix of bovine striated muscle contains sulphated proteoglycans evenly distributed throughout the peri- and endomysium. The localization of the individual proteoglycans, decorin and PGL, varied considerably, reflecting differing functionalities which remain to be determined. Which role decorin and PGL play for meat quality, is the subject of further investigation.

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