# 4-P38

## CHANGES IN PROTEOGLYCANS DURING POSTMORTEM AGING OF MEAT

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**Background**

The tenderization of meat during postmortem aging depends on the relative contributions of structural changes in the myofibrils (Takahashi, 1992) and the intramuscular connective tissues (IMCT) (Bailey & Light, 1989). The IMCT is comprised mainly of collagen fibrils embedded in ground substances such as proteoglycans (PGs). Although proteoglycans are a minor part of intramuscular connective tissue in term of their proportionate dry weight in the tissue, they interact with collagen fibrils and play an important role in stabilizing extracellular matrix in tissues. We have reported the presence of decorin and chondroitin sulfate PGs in the endomysium and the perimysium of semitendinosus muscle of adult steers (Nishimura et al., 1997). Eggen et al. (1997) demonstrated the presence of aggrecan-like PGs and decorin in the perimysium. However, very little is known about changes in PGs during postmortem aging of meat, and its contribution to meat tenderization.

Recently, we demonstrated that the PGs in the perimysium are degraded during postmortem aging of beef (Nishimura et al., 1996 b), which leads to a separation of collagen fibrils in the IMCT observed by the cell-maceration/scanning electron microscopy (Nishimura et al., 1995). Eggen et al. (1998) have shown that decorin is degraded during postmortem aging of beef. Avery et al. (1998) also showed the rapid depletion of PGs in the epimysium during postmortem aging, but no reduction of tensile strength of the epimysium after removal of PGs. However, it is still unclear which kinds of PGs are degraded, and how they are decomposed during postmortem aging of meat.

**Objectives**

The aim of this study was to investigate changes in PGs during postmortem aging of meat, and to clarify the weakening mechanism of the IMCT.

**Materials and Methods**

*Muscle samples*

*Pectralis superficialis* muscle of Rhode Island Red chickens aged 30 weeks were used in this study. After bleeding, skinning and evisceration, muscle samples were dissected and sterilized by dipping them in a solution containing 1 mM $NaN_3$, and they were wrapped with polyethylene film and stored at 4°C until sampling.

*Extraction and purification of PGs*

PGs were extracted and purified by the method of Parthasarathy and Tanzer (1987) with slight modifications. About 10 g of muscle were minced finely, homogenized briefly in a microhomogenizer with 4 volumes of a solution containing 4 M guanidine hydrochloride, 10 mM sodium acetate, pH 6.0, 0.1 M 6-aminohexanoic acid, 10 mM EDTA, 1 mM benzamidine hydrochloride, and 1 mM phenylmethylsulfonyl fluoride, and extracted for 72 h at 4°C with gentle stirring. To separate PGs from other proteins, the CsCl density-gradient ultracentrifugation method was employed; the extract was adjusted with CsCl to a density of 1.35 g/ml and centrifuged at 100,000 rpm for 4 h with an ultracentrifuge. PGs were recovered from the bottom one-fourth of the gradient (D1) by puncturing the bottom of the tube. The density of CsCl containing D1 fraction was 1.40-1.48 g/ml. The D1 fraction was extensively dialyzed against a solution containing 7 M urea and 0.02 M Tris-acetate buffer, pH 7.0. After dialysis, the amount of uronic acid in the D1 fraction was determined by the procedure of Bitter and Muir (1962).

*Gel-filtration*

The concentrated sample of D1 fraction obtained by CsCl density-gradient ultracentrifugation were subjected to gel filtration on a Superose 6 HR column (volume 30x10 cm). The elution buffer contained 0.5 M sodium acetate (pH 6.0), 4 M guanidine HCl and protease inhibitors described above. The elutes were collected in 0.6 ml fractions and monitored for the amount of uronic acid.

*SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting*

Linear 2-10% polyacrylamide gradient slab gels were prepared and 20 µl of each sample was subjected to SDS-PAGE. After the run, gels were washed three times with 40% methanol and 8% acetic acid for 30 min, and subsequently stained with a solution containing 0.002% Alcian Blue, 0.001% Coomassie Brilliant Blue R-250, 40% methanol and 8% acetic acid. PGs separated by SDS-PAGE were transferred electropholetically onto nitrocellulose membranes. The membranes were treated with anti-PGs antibodies.

*Immunohistochemistry*

Frozen tissue sections were cut using a cryostat at -25°C, fixed with a solution containing 10% formalin and phosphate-buffered saline (PBS), pH 7.2 for 5 min, treated with primary antibodies against various PGs for 30 min at 37°C. After extensive washing for 30 min with PBS, they were incubated with the secondary antibodies, FITC-conjugated anti-mouse IgG for 30 min at 37°C. The sections were then washed with PBS, mounted in Perma Fluor Permanent Aqueous mounting medium, and examined under an Olympus BH-2 microscope.

**Results and Discussion**

The total amount of uronic acid in D1 fraction decreased rapidly to 17% of the initial value within 6 hours postmortem, followed by a gradual decrease up to 72 hours postmortem (Figure 1). SDS-PAGE of D1 fraction obtained by CsCl density-gradient ultracentrifugation revealed the presence of PGs components of molecular mass (> 2,000 KDa) in the muscle immediately postmortem. The density of this band decreased with time postmortem, and it could be almost undetectable after 72 hours postmortem. A band with molecular mass of about 20 kDa was stained with Alcian Blue at immediately postmortem. Its density increased up to 12 hours postmortem and decreased thereafter. Disappearance of the band with a high molecular mass (> 2,000 kDa) suggests that PGs have decomposed into small fragments. The band with a lower molecular mass of about 20 kDa, which increased up to 12 hours, is probably a degradation product of the high molecular weight PGs. Figure 2 shows the elution profiles obtained by gel filtration of D1 fraction from muscles at immediately, 6 and 72 hours postmortem. The large proportion of uronic acid-containing materials was recovered in the fractions 13-20 (peak I) in the muscle immediately postmortem. The amount of uronic acid in the fractions 13-20 decreased up to 6 hours postmortem. This peak was not detectable in the profile obtained by gel filtration from muscle 72 hours postmortem. This result suggests the decomposition of PGs with high molecular mass. Western blot analysis of the fractions 13-20 obtained by gel filtration revealed that this fraction contains chondroitin sulfate (CS) PGs, but not decorin, dermatan sulfate PGs and heparan sulfate PGs. Immunohistochemical analysis showed that CSPGs were located mainly in the endomysium, and its stainability decreased with time postmortem. These results suggests that CSPGs with high molecular mass is degraded during postmortem aging of chicken. A decomposition of CSPGs may result in destabilization of the extracellular matrix of muscle and contribute to tenderization of meat during postmortem aging.

**Literature**


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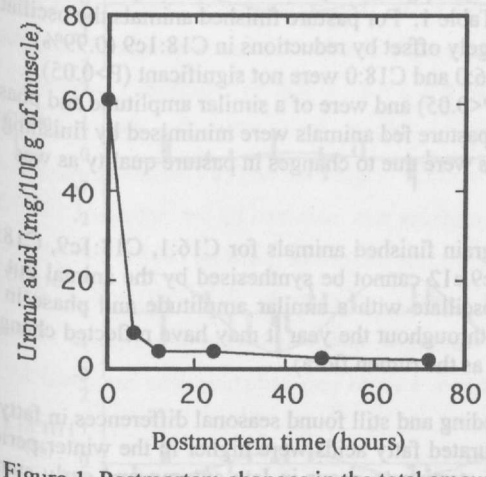


Figure 1. Postmortem changes in the total amount of PGs in chicken pectralis superficialis muscle.

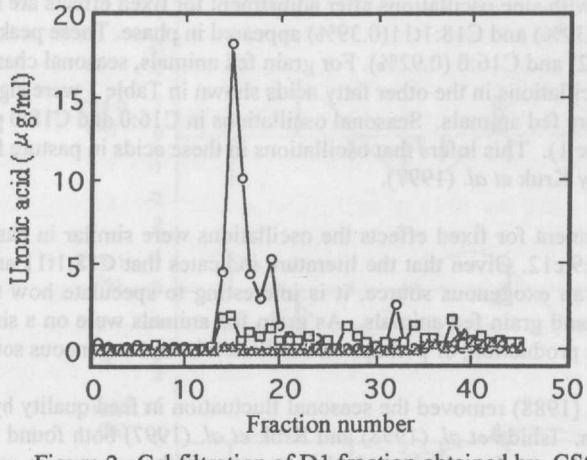


Figure 2. Gel filtration of D1 fraction obtained by CSCl density-gradient ultracentrifugation from chicken pectralis superficialis muscle stored at 0 (○), 6(□), 72(△) hours.