Isolation and Characterization of a Small Proteoglycan Associated with Porcine Intramuscular Connective Tissue

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In the present investigation, proteoglycan associated with porcine intramuscular collagen fibrils was isolated and characterized. The increase in the values of released uronic acid (percent) and heat solubility of collagen (percent) was observed by chondroitinase ABC digestion of intramuscular connective tissue isolated from porcine longissimus dorsi muscles, indicating that collagen fibril-associated proteoglycan was involved in the thermal stability of intramuscular collagen. A proteoglycan was purified from the adult porcine intramuscular connective tissue by successive steps of an extraction with 4 M guanidine hydrochloride, CsCl density gradient ultracentrifugation, DEAE-cellulose ion-exchange chromatography, and Sepharose CL-6B gel filtration. The isolated proteoglycan possessed an average molecular mass of 100 kDa and contained a core protein with mass of 48 kDa. Its constituent glycosaminoglycan chain was a dermatan sulfate, and the N-terminal amino acid sequence of its core protein was identical to that of small proteoglycan (PG-II) from other tissues. These results indicate that the collagen fibril-associated proteoglycan isolated from adult porcine intramuscular connective tissue was the small proteoglycan containing a dermatan sulfate chain (DS-PG-II), namely decorin.

Keywords: *Decorin; small proteoglycan; core protein; dermatan sulfate; intramuscular connective tissue*

INTRODUCTION

Intramuscular connective tissue organizing endomysium, perimysium, and epimysium contributes to background toughness of meat (Pearson and Young, 1989). The main components of intramuscular connective tissue are fibrous collagen and elastin and amorphous ground substances such as proteoglycans and glycoproteins, which are embedded in the connective tissue fibers. Studies on the effects of the intramuscular connective tissue on meat toughness have been chiefly focused on collagen because of its abundant existence and structural firmness. Meat toughness is affected by the thermal stability of collagen given by the stable intermolecular cross-linking (Bailey, 1972; Smith and Judge, 1991). However, proteoglycans have also been reported to play important roles in many organs and tissues by interacting with collagen, affecting collagen fiber diameter (Vogel and Trotter, 1987), binding to collagen fibrils at a specific site in tendon (Scott and Orford, 1981), and *in vitro* regulating collagen fibrillogenesis (Vogel and Trotter, 1987). In skeletal muscle, Nishimura *et al.* (1996) observed electron microscopically that the structural weakening of intramuscular connective tissue during post-mortem aging of beef was caused by the dissociation of proteoglycans from collagen fibrils. We have also found a significant correlation between intramuscular proteoglycan content and thermal stability of collagen involved in raw meat toughness (Nishiumi *et al.*, 1995). Therefore, proteoglycans can be assumed to play an important role in the stabilization of the intramuscular collagen matrices, thus contributing to meat toughness.

Proteoglycans are ubiquitous components of the extracellular matrix in all mammalian tissues (Heinegård and Paulsson, 1984). Early studies on tissue proteoglycans clarified the structure and function of the large proteoglycans having mainly chondroitin sulfate chains (CS-PG) from cartilage (Poole, 1986; Doege *et al.*, 1987; Paulsson *et al.*, 1987). Subsequently, small proteoglycans, the core protein of which is smaller than that of the cartilage CS-PG, have been shown to occur in a number of tissues, including bone (Fisher *et al.*, 1983), cartilage (Rosenberg *et al.*, 1985), tendon (Vogel and Heinegård, 1985), skin (Damle *et al.*, 1982), aorta (Kapoor *et al.*, 1981), placenta (Brennan *et al.*, 1984), cornea (Gregory *et al.*, 1982), and sclera (Cöster and Fransson, 1981). Most extracellular matrices contain the small proteoglycans with one or two glycosaminoglycan chains. Two members in this group, PG-I (biglycan, 150−240 kDa) and PG-II (decorin, 90−140 kDa), contain chondroitin sulfate or dermatan sulfate and have core proteins that are particularly rich in leucine and aspartic acid/asparagine (Heinegård *et al.*, 1985; Fisher *et al.*, 1987). After the glycosaminoglycan side chains are removed from both proteoglycans with chondroitinase, the masses of their core proteins exhibit 45−48 kDa (Heinegård *et al.*, 1985). The amino acid sequences of these core proteins have been determined by conventional and cDNA sequencing methods (Heinegård and Oldberg, 1989). PG-II and PG-I contain one and two consensus sequences, Ser-Gly-Xaa-Gly, respectively, which are near the N termini and are involved in binding to chondroitin sulfate or dermatan sulfate chains (Bourdon *et al.*, 1987).

There are relatively few papers available on characterization of the proteoglycans in skeletal muscles. Parthasarathy and colleagues (Parthasarathy and Tanzer, 1987; Parthasarathy *et al.*, 1991) isolated proteoglycans from adult rabbit skeletal muscle and demonstrated that the major proteoglycan of this tissue was a small proteoglycan containing chondroitin sulfate (CS-PG-II). Andrade and Brandan (1991) reported that rat

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leg muscles contained a chondroitin/dermatan sulfate proteoglycan, decorin (CS/DS-PG-II). Eggen *et al.* (1994) isolated a decorin containing a dermatan sulfate chain (DS-PG-II) from adult bovine skeletal muscle and showed immunohistochemically that the decorin was present in both perimysium and endomysium. Recently, the decorin was also found in ovine skeletal muscles (Velleman *et al.*, 1996). However, the amount of proteoglycans in skeletal muscle was too low to be characterized sufficiently.

In the present study, we isolated and characterized proteoglycans associated with collagen fibrils in adult porcine skeletal muscle after confirming the effect of the collagen fibril-associated proteoglycan on thermal stability of intramuscular collagen contributing to meat toughness.

MATERIALS AND METHODS

Preparation of Intramuscular Connective Tissue. Four 6-month-old Göttingen miniature pigs were obtained from the Experimental Farm, Faculty of Applied Biological Science, Hiroshima University, and slaughtered conventionally. Longissimus dorsi muscles were dissected from each carcass within 1 h at 4 °C, trimmed to remove all visible external fat and epimysium, and then stored at −30 °C. Intramuscular connective tissue was isolated according to a modification of the procedure reported by Fujii and Murota (1982). Minced muscle (approximately 300 g) was homogenized with 5 volumes (v/w) of ice-cold phosphate-buffered saline (PBS, pH 7.4) containing protease inhibitors (10 mM EDTA, 10 mM 6-aminohexanoic acid, 5 mM benzamidine hydrochloride, 1 mM phenylmethanesulfonyl fluoride). The homogenate was stirred for 12 h. Fibrous materials not passing through 0.5 mm square holes of a sieve were collected. They were extracted with 5 volumes (v/w) of PBS containing 0.6 M KCl for 12 h by stirring. Following this extraction process repeated three times, extraction with PBS containing 0.6 M KI for 12 h was repeated twice. The obtained insoluble residue, intramuscular connective tissue, was washed with distilled water thoroughly. All of the above procedures were carried out at 4 °C.

Chondroitinase ABC Digestion of Intramuscular Connective Tissue. Chondroitinase ABC (2.4 units, protease free, Seikagaku Co., Tokyo, Japan) was dissolved in 0.1 mL of 20 mM Tris-HCl buffer (pH 7.2) and stored at −80 °C. Ten milligrams of lyophilized and powdered intramuscular connective tissue was incubated at 37 °C with 0.1 mL of 0.1 M Tris-HCl buffer (pH 8.0) containing 30 mM sodium acetate and 0.024 unit of chondroitinase ABC. After a 5-h treatment at 37 °C, the heat solubility of collagen and the content of released uronic acid were determined as described in a previous paper (Nishiumi *et al.*, 1995).

Isolation and Purification of Proteoglycans from Intramuscular Connective Tissue. The prepared intramuscular connective tissue was washed with 4 M guanidine hydrochloride containing the protease inhibitors and 10 mM sodium acetate (pH 5.8), and then proteoglycans were extracted with 3 volumes (v/w) of the same solution by stirring at 4 °C for 72 h. The supernatant was collected by centrifugation at 10000*g* for 1 h.

Direct dissociative CsCl density gradient ultracentrifugation of the guanidine hydrochloride extract was employed to separate proteoglycans from other proteins of the muscle (Parthasarathy and Tanzer, 1987). The density of the guanidine hydrochloride extract was adjusted with CsCl to about 1.40 g/mL, and dissociated proteoglycans were prepared directly by equilibrium density gradient ultracentrifugation in a Hitachi SCP85H2 ultracentrifuge employing a RP70T rotor (156000*g*, 48 h, 10 °C). The bottom fraction (D1, density = 1.47 g/mL) was provided for further purification.

The density gradient fraction was extensively dialyzed against 20 mM Tris-acetate buffer (pH 7.0) containing 7 M urea and applied on a DEAE-cellulose column (1.7 × 25 cm, DE-52, Whatman, Maidstone, U.K.) equilibrated with the same buffer at 4 °C. After a wash with the same buffer, elution of the proteoglycans was achieved with 600 mL of a NaCl linear gradient (0−1.0 M) in 7 M urea and 20 mM Tris-acetate (pH 7.0) at a rate of 15 mL/h (one fraction, 10 mL). The content of uronic acid (Bitter and Muir, 1962) in each fraction was determined to evaluate the proteoglycan content.

Further purification of the proteoglycans was performed by Sepharose CL-6B gel filtration. The proteoglycan fraction eluting at 0.3−0.4 M NaCl on DEAE-cellulose chromatography was dialyzed, concentrated to about 7 mL by ultrafiltration, and then applied on a Sepharose CL-6B column (2 × 115 cm, Pharmacia, Uppsala, Sweden) equilibrated with 360 mL of 50 mM Tris-acetate buffer (pH 7.0) containing 4 M guanidine hydrochloride at 4 °C (one fraction, 4 mL). After dialysis, the content of uronic acid (Bitter and Muir, 1962) in each fraction was analyzed.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). SDS−PAGE was carried out on 7.5% polyacrylamide slab gels with a 3% stacking gel, according to the method of Laemmli (1970). The gels were stained with Coomassie brilliant blue R-250 for proteins and Alcian blue for glycosaminoglycans (Fisher *et al.*, 1983). Molecular weight standards (Bio-Rad, Tokyo, Japan) used were phospholylase B (112 000), bovine serum albumin (86 000), ovalbumin (52 000), carbonic anhydrase (34 400), and soybean trypsin inhibitor (28 500).

Electrophoretic Separation of the Glycosaminoglycan Chains. After the dialysis of the purified proteoglycan against 0.1 M Tris-HCl buffer (pH 8.0), the core proteins were digested with 0.01% actinase E (Kaken Seiyaku, Tokyo, Japan) at 37 °C for 2 h. The obtained glycosaminoglycan chains were dialyzed against 1 M pyridine acetate (pH 3.5) and lyophilized. Electrophoretic separation of glycosaminoglycan chains was accomplished with cellulose acetate membrane (Advantec, Tokyo, Japan) in 1 M pyridine acetate at 0.5 mA/cm for 20 min, and they were stained with 0.5% Alcian blue in 3% acetic acid (Seno and Meyer, 1963; Seno *et al.*, 1970). Glyosaminoglycan standards were purchased from Seikagaku Co. (Tokyo, Japan).

N-Terminal Amino Acid Sequencing of the Core Proteins. The purified proteoglycan fraction on the gel filtration was dialyzed against 0.1 M Tris-HCl buffer (pH 8.0) containing 30 mM sodium acetate and then digested with chondroitinase ABC. After the electrophoresis by SDS−PAGE, the core proteins were transferred to a poly(vinylidene difluoride) membrane (ATTO, Tokyo, Japan), according to the method of Matsudaira (1987), and stained with Coomassie brilliant blue. The core protein on the membrane was sequenced with an Applied Biosystems 477A protein sequencer.

Table 1. Effect of Chondroitinase ABC Digestion on the Release of Uronic Acid and the Heat Solubility of Collagen in Intramuscular Connective Tissue

treatment	released uronic acid (%)	heat solubility of collagen (%)
control[a]	19.0	6.7
chondroitinase ABC	34.0	12.8

[a] Treatment with 0.1 M Tris-HCl (pH 8.0) containing 30 mM sodium acetate without chondroitinase ABC.

RESULTS

Effect of Proteoglycans on Heat Solubility of Collagen from Intramuscular Connective Tissue. On the preparation of the intramuscular connective tissue, 80.8% of collagen (measured as hydroxyproline) and 5.1% of proteoglycan (measured as glycosaminoglycan uronic acid) were recovered from the muscle (not shown). As shown in Table 1, the proportion of released uronic acid was 19.0% in the intramuscular connective tissue incubated with the buffer, while it was 34.0% in one incubated with chondroitinase ABC. This indicates a dissociation of the proteoglycans from intramuscular

Table 2. Distribution of Glycosaminoglycan (GAG) Uronic Acid on CsCl Density Gradient Ultracentrifugation of 4 M Guanidine Hydrochloride Extract from Porcine Intramuscular Connective Tissue

fraction	density (g/mL)	GAG uronic acid (mg/300 g of wet tissue)
D1 (bottom)	1.47	0.288
D2	1.42	0.129
D3	1.37	0.114
D4	1.32	0.150

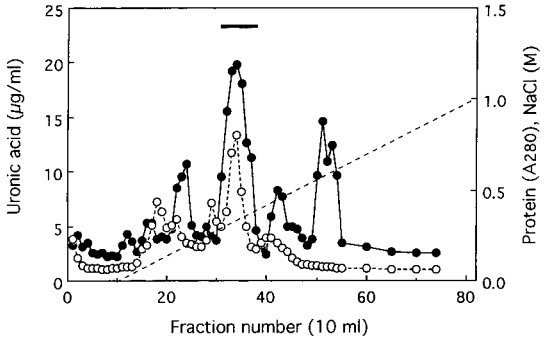

Figure 1. DEAE-cellulose ion-exchange chromatography of proteoglycan from porcine intramuscular connective tissue. The proteoglycan fraction (0.29 mg of uronic acid) obtained by CsCl density gradient ultracentrifugation was dialyzed against 20 mM Tris-acetate (pH 7.0) containing 7 M urea and applied to the DE-52 column (1.7 × 25 cm) equilibrated with the same buffer. After washing it with the equilibrating solution, a linear NaCl gradient (0−1.0 M, - - -) was started as described under Materials and Methods. The absorbance at 280 nm (○) and the uronic acid content of each fraction (●) were determined. Fractions 31−38 indicated by the bar were combined for further purification.

collagen fibrils by treatment with the enzyme. Heat solubility of collagen also increased from 6.7% to 12.8% by chondroitinase ABC digestion. These results confirm that an increase of the heat solubility of collagen is caused by a dissociation of the collagen fibril-associated proteoglycans.

Isolation of Proteoglycan from Intramuscular Connective Tissue. As much as 89% of the intramuscular connective tissue proteoglycan could be extracted with 4 M guanidine hydrochloride (not shown). The extract was partitioned on a CsCl equilibrium density gradient with a starting density of 1.40 g/mL, and four fractions were obtained with the different densities. The bottom one (D1, density = 1.47 g/mL) contained 42% of the glycosaminoglycan uronic acid (Table 2). The SDS−PAGE of each fraction showed that the D1 fraction contained a major polydisperse component the mobility of which was changed by the chondroitinase ABC digestion (not shown). When the proteoglycan derived from the D1 fraction was further purified by DEAE-cellulose chromatography, uronic acid-containing materials were eluted as four peaks at fraction numbers (NaCl concentrations) of 24 (0.20 M), 35 (0.36 M), 42 (0.47 M), and 51 (0.60 M), respectively (Figure 1). Among them, the fractions 31−38 at 0.30−0.40 M NaCl contained the characteristic polydisperse component with molecular mass of 90−110 kDa. Thus, the fractions 31−38 were subjected to Sepharose CL-6B chromatography. The large proportion of uronic acid-containing materials was recovered in fractions 50−65, shown as the proteoglycan fraction in Figure 2. However, no proteoglycans could be detected in fractions 70−98, which contained uronic acid.

Characterization of the Proteoglycan. SDS−PAGE of proteoglycan isolated from porcine intramuscular connective tissue revealed one band with an average mass of 100 kDa (range 90−110 kDa; Figure 3, lane A), which was stained with Alcian blue as well as Coomassie brilliant blue. After digestion with chondroitinase ABC, the mobility of this band was changed to the position of an apparent molecular mass of 48 kDa (Figure 3, lane B). Both results suggested that this proteoglycan was a small proteoglycan PG-II.

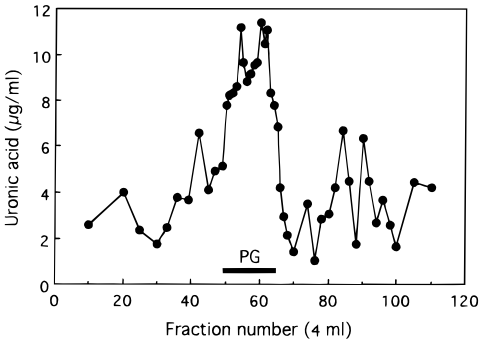

Figure 2. Sepharose CL-6B gel filtration column chromatography of proteoglycan from porcine intramuscular connective tissue. The proteoglycan fractions 31−38 (0.3−0.4 M NaCl concentration) obtained by DEAE-cellulose chromatography were dialyzed, concentrated, and then applied to the Sepharose CL-6B column (2 × 115 cm) equilibrated with 50 mM Tris-acetate (pH 7.0) containing 4 M guanidine hydrochloride as described under Materials and Methods. The uronic acid content of each fraction (●) was determined. PG, proteoglycan fraction.

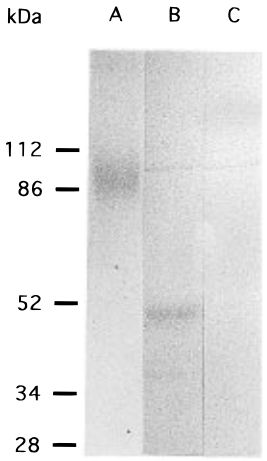

Figure 3. SDS−PAGE of purified small proteoglycan and chondroitinase ABC digested core prtein. The gel was run under a reducing condition and was stained with Coomassie brilliant blue. Lane A, intact proteoglycan; lane B, proteoglycan treated with chondroitinase ABC ; lane C, chondroitinase ABC.

Glycosaminoglycan chains were electrophoretically analyzed after the removal of the core protein by treatment with actinase E (Figure 4). The glycosaminoglycan chains from the present PG-II showed a band that possessed slower mobility than chondroitin 4- and 6-sulfate standards, and their mobility completely coincided with that of dermatan sulfate standard.

The amino acid sequence analysis of the DS-PG-II from porcine intramuscular connective tissue was performed after its digestion by chondroitinase ABC (Table 3). The N-terminal sequence of eight residues was identical to that of PG-II from other tissues (Pearson *et al.*, 1983; Vogel and Fisher, 1986; Day *et al.*, 1987; Choi *et al.*, 1989; Parthasarathy *et al.*, 1991).

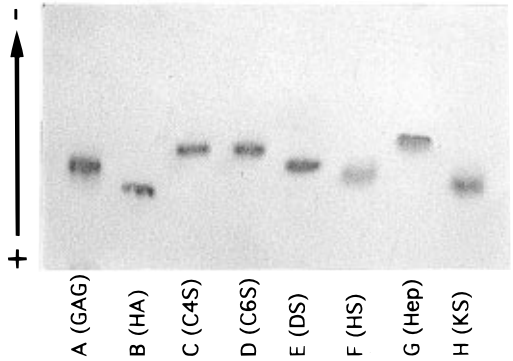

Figure 4. Electrophoretogram of glycosaminoglycan chains on cellulose acetate membrane in 1 M pyridine acetate. Lane A, glycosaminoglycan chain of small proteoglycan isolated from porcine intramuscular connective tissue (GAG); lanes B−H, hyaluronic acid (HA), chondroitin 4-sulfate (C4S), chondroitin 6-sulfate (C6S), dermatan sulfate (DS), heparan sulfate (HS), heparin (Hep), and keratan sulfate (KS) standards, respectively.

Table 3. Comparison of N-Terminal Amino Acid Sequences of Small Proteoglycans (PG-II) from Intramuscular Connective Tissue (IMCT), Skeletal Muscle, Tendon, Cartilage, Bone, and Skin

source	N-terminal sequence
porcine IMCT	D-E-A-X-G-I-G-P-
rabbit skeletal muscle[a]	D-E-A-X-G-I-G-P-D-E-L-
bovine tendon[b]	D-E-A-S-G-I-G-P-E-E-H-
bovine cartilage[c]	D-E-A-X-G-I-G-P-E-E-H-
bovine bone[d]	D-E-A-S-G-I-G-P-E-E-H-
bovine skin[e]	D-E-A-S-G-I-G-P-E-E-

[a−e]Data from Parthasarathy *et al.* (1991), Vogel and Fisher (1986), Choi *et al.* (1988), Day *et al.* (1987), and Pearson *et al.* (1983), respectively.

DISCUSSION

By using the method of Fujii and Murota (1982) for the isolation of intramuscular connective tissue, we successfully obtained high yields of highly purified connective tissue. Approximately 95% of the uronic acid was removed from the muscle by the isolation process of the intramuscular connective tissue. We considered that the remainder, about 5% of the uronic acid, appeared to be the proteoglycan tightly associated with the intramuscular collagen fibrils. A harmonic increasing of the heat solubility of collagen and released uronic acid by treatment with chondroitinase ABC indicates that the collagen fibril-associated proteoglycan, particularly its glycosaminoglycan, plays an important role in the thermal stability of intramuscular collagen. Although we have proposed a possible contribution of proteoglycans to the thermal stability of the intramuscular collagen in a previous paper (Nishiumi *et al.*, 1995), our present study confirmed that the collagen fibril-associated proteoglycan was directly involved in the thermal stability of collagen.

Proteoglycans were effectively extracted from adult porcine intramuscular connective tissue with 4 M guanidine hydrochloride and purified by the successive steps including CsCl density gradient ultracentrifugation, DEAE-cellulose ion-exchange chromatography, and Sepharose CL-6B gel filtration. From a mixture of muscle proteoglycans, a predominant collagen fibril-associated proteoglycan was isolated. This proteoglycan possessed a small size (90−110 kDa), which migrated to 48 kDa after the chondroitinase ABC treatment. It also contained a dermatan sulfate chain at the fourth residue from the N terminus of the core protein, which is characterized as a DS-PG-II. The DS-PG-II isolated in the present study highly resembled other PG-II, which had already been characterized in various tissues. The proteoglycan fragment treated with chondroitinase ABC had a molecular mass of about 48 kDa, which was close to the typical average mass range of 45−48 kDa reported (Heinegård *et al.*, 1985) for the core proteins of small proteoglycans. The N-terminal amino acid sequence of eight residues in the present study was identical to that of PG-II from skeletal muscle (Parthasarathy *et al.*, 1991), tendon (Vogel and Fisher, 1986), cartilage (Choi *et al.*, 1989), bone (Day *et al.*, 1987), and skin (Pearson *et al.*, 1983). Some researchers (Pearson *et al.*, 1983; Day *et al.*, 1987; Vogel and Fisher, 1986) have demonstrated that the fourth residue from the N terminus of PG-II is a serine residue, which is an attachment site for the glycosaminoglycan chain. The fact that no amino acid could be identified at the fourth residue in our sequence suggests that this residue also seemed to be a glycosylated one in our preparation.

It has been found that various forms of both large and small proteoglycans are present in adult skeletal muscle as well as other tissues. The former has a molecular mass of more than 150 kDa, has heparan sulfate chains, and is mainly localized in the basement membranes (Anderson and Fambrough, 1984; Brandan and Inestrosa, 1987). In addition, the presence of large proteoglycans carrying dermatan sulfate chains in adult bovine skeletal muscle (Eggen *et al.*, 1994) and chondroitin sulfate chains in embryonic chick muscle (Carrino and Caplan, 1982; Carrino *et al.*, 1984) has also been reported. These findings suggest that the multiple forms of the large proteoglycans are required for the functions of the skeletal muscles. The latter is PG-II, decorin, which corresponds to 44−67% of total proteoglycans present in the skeletal muscle (Parthasarathy and Tanzer, 1987; Andrade and Brandan, 1991; Eggen *et al.*, 1994). The PG-II is localized mainly in the epimysium, perimysium, and, to a lesser extent, endomysium (Fernández *et al.*, 1991; Eggen *et al.*, 1994) and is associated with intramuscular collagen fibrils (Nishimura *et al.*, 1996). In the present study, it was shown that a small proteoglycan associated with porcine intramuscular collagen fibrils was a decorin carrying a dermatan sulfate chain, DS-PG-II. PG-II has been isolated from adult rabbit skeletal muscle and shown to contain a chondroitin sulfate chain (Parthasarathy and Tanzer, 1987). Rat leg muscle PG-II contains a chondroitin/dermatan sulfate chain (Andrade and Brandan, 1991), and adult bovine skeletal muscle PG-II contains a dermatan sulfate chain (Eggen *et al.*, 1994). It is possible, then, that a difference in the glycosaminoglycan chain of the PG-II as described above seems to be caused by either species differences or the differences in the physiological age of animals used in these investigations, as a consistent increase of dermatan sulfate in place of a decrease in chondroitin sulfate with bovine growth was found (Nishimura and Takahashi, 1995). Further information must be provided to determine why the glycosaminoglycan variations occur and what kinds of functional roles those variations play.

On the other hand, the core proteins of the PG-II from bone, cornea, sclera, and tendon have been demonstrated to show considerable indentity (Heinegård *et al.*, 1985). Bovine skeletal muscle PG-II was also demonstrated to cross-react with a polyclonal antibody against sclera PG-II (Eggen *et el.*, 1994). Furthermore, identical

amino acid sequences at the N terminus have been found in the PG-II isolated from skin (Pearson *et al.*, 1983), tendon (Vogel and Fisher, 1986), skeletal muscle (Parthasarathy *et al.*, 1991), bone (Day *et al.*, 1987), and cartilage (Choi *et al.*, 1989), which is in agreement with the one in the present DS-PG-II. However, as the sequnece of the DS-PG-II provided only eight residues from the N terminus, a determination of a longer sequence must be defined for an exact comparative study of PG-II core proteins from different sources.

In the present study, we were able to isolate collagen fibril-associated DS-PG-II, which contributed to the thermal stability of intramuscular collagen, from adult porcine skeletal muscle. PG-II and other small proteoglycans, fibromodulin and corneal keratan sulfate proteoglycan, appear to have functional roles in regulating collagen fibrillogenesis and organization in the matrix (Hedbem and Heinegård, 1989). These small proteoglycans, particularly PG-II, but not PG-I and large proteoglycans, have been shown to bind collagen fibrils at a specific site (Scott and Orford, 1981), to inhibit collagen fibril formation (Vogel *et al.*, 1984), and to diminish the final diameter of collagen fibril formed during *in vitro* fibrillogenesis (Vogel and Trotter, 1987). Brown and Vogel (1989) demonstrated that the interaction of the PG-II with collagen fibrils was through the direct protein−protein binding of the core protein to collagen. In a study on the interaction between DS-PG-II isolated from human uterine cervix and type I collagen from rat skin, the presence of two binding sites was also suggested (Uldbjerg and Danielsen, 1988). Although the constituent glycosaminoglycan chains of proteoglycans, as visualized by transmission electron microscopy, appear to be associated exclusively with the periphery of the collagen fibrils, most frequently in the gap region, isolated glycosaminoglycan chains were not shown to bind to the fibril *in vitro* (Scott *et al.*, 1986). Recently, Weber *et al.* (1996) proposed the three-dimensional structure of human PG-II and the binding of the collagen molecule to the PG-II core protein. Thus, the core protein of PG-II binds directly to the collagen molecule and thus influences the fibrillogenesis. On the other hand, a protective function shown by the glycosaminoglycans with intramuscular collagens against proteolytic attacks of lysosomal enzymes (Wu *et al.*, 1981) and a decrease of the thermal stability of collagen with the resolution of the glycosaminoglycans as shown in the present study suggest that the glycosaminoglycan chain of PG-II may also take part in the structural stabilization of the collagen matrices.

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