ISOLATION AND PURIFICATION OF DECORIN FROM BOVINE SKELETAL MUSCLE AND ITS STRUCTUAL CHANGES UNDER HIGH PRESSURE

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Abstract - High hydrostatic pressure induces a weakening of intramuscular connective tissue, which is mainly composed of collagen. Decorin, a small proteoglycan, binds to and stabilizes collagen fibrils. It has been suggested that the weakening of intramuscular connective tissue may result from alteration of the decorin-collagen interaction due to structural changes of the decorin molecule. In this study, decorin was isolated and purified from bovine skeletal muscle 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 decorin possessed an average molecular mass of 100 kDa and contained a core protein mass of 48 kDa by SDS-PAGE. The structural changes in decorin from bovine skeletal muscle were investigated by measuring fluorescence spectra under high pressure.

I. INTRODUCTION

The use of high-pressure technology in food processing has steadily increased over the past 10 years. Among products processed using high pressure, the number and variety of meat and meat products have risen dramatically worldwide (1). High pressure is also used for tenderizing meat or accelerating postmortem aging of meat (2-5). High hydrostatic pressure affects actomyosin toughness and background toughness, leading to meat tenderization. The effect of high pressure on background toughness ascribed to connective tissue, is gradually becoming clearer. Ichinoseki *et al.* (6) reported that high pressure did not degrade collagen molecules but dissociated collagen fibrils. Decorin is one of small proteoglycans and binds to and stabilizes collagen fibrils (7-8). It has been suggested that a weakening of intramuscular connective tissue may result from structural changes to decorin, leading to alteration of the decorin-collagen interaction. Komoda *et al.* (9) found a change of the native structure of decorin molecules from bovine articular cartilage under high pressure at 200-400 MPa. It is assumed that structural changes of decorin molecule are induced by high-pressure processing. In this study, decorin was isolated and purified from bovine skeletal muscle 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 structural changes of purified decorin were then analyzed by fluorescence spectra under high pressure.

II. MATERIALS AND METHODS

Longissimus dorsi muscles were dissected from each carcass of two 3-month-old Holstein steers, trimmed to remove all visible external fat and epimysium, and then stored at -30°C. Decorin was extracted and purified according to the method of Nishiumi *et al.* (7) with slight modifications. The muscles were minced finely, homogenized briefly in a Waring blender (Mauda, Japan) with 4 volumes of a solution containing 4 M guanidine hydrochloride, 1 M sodium acetate (pH 6.0), 0.1 M 6-aminohexanoic acid, 1 mM benzamidine hydrochloride, 10 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride, and extracted for 72 h at 4°C with gentle stirring. The supernatant was collected by centrifugation at 24,900 x *g* for 1 h at 4°C.

To separate decorin from other proteins of the muscle, direct dissociative CsCl density gradient ultracentrifugation was employed. The density of the guanidine hydrochloride

extract was adjusted with CsCl to about 1.37 g/mL, and ultracentrifuged (CP-80WX; Hitachi, Japan) at 156,000 x *g* for 48 h at 4°C in 40 PA tubes (Hitachi) on an angle rotor (P50-AT2; Hitachi). After ultracentrifugation, the tubes were divided into four fractions (D1-D4; D1=bottom) according to the CsCl density. The D2 fraction (density=1.43 g/mL) was subjected to further purification. The fraction was extensively dialyzed against 20 mM Tris-acetate buffer (pH 7.0) containing 7 M urea and applied to a DEAE-cellulose column (2.5 x 25 cm; Sigma, U.S.A.) equilibrated with the same buffer at 4°C. After washing the column with the same buffer, decorin were eluted with a linear gradient of 0-0.5 M NaCl in 7 M urea and 20 mM Tris-acetate buffer (pH 7.0) at a rate of 15 mL/h (one fraction, 10 mL). The content of uronic acid in each fraction was determined (10), and fractions containing decorin were pooled. Further purification was performed by Sepharose CL-6B gel filtration. The pooled fraction containing decorin was concentrated to about 10 mL by ultrafiltration, and then applied to a Sepharose CL-6B column (1.5×120 cm; Sigma) equilibrated with 420 mL of 50 mM Tris-acetate buffer (pH 7.0) containing 4 M guanidine hydrochloride. Decorin was eluted with the same buffer at 4°C at a rate of 10 mL/h (one fraction, 5 ml). The content of uronic acid in each fraction was measured and subjected to SDS-PAGE analysis.

SDS-PAGE was carried out on 7.5% polyacrylamide slab gels with a 3.75% stacking gel, according to the method of Laemmli (11). The gels were stained with Coomassie Brilliant Blue R-250 or Silver staining for proteins or Alcian blue for glycosaminoglycans (12).

Fluorescence spectra were obtained using a spectrometer (F-2500; Hitachi) fitted with a high-pressure vessel (PCI-400; Syn Corporation, Japan) and pump (TP-500; Syn Corporation). A decorin solution (0.2 mg/mL in 100 mM Tris-HCl buffer solution (pH 7.3) was subjected to a range of pressures of between 0.1 and 400 MPa at 50 MPa intervals during approximately 10 s, and fluorescence spectra were measured at each pressure 10 min after pressure was achieved. After 400 MPa compression, the decorin solution was decompressed continually at 50 MPa intervals during approximately 10 s. Fluorescence spectra between 300 and 450 nm with excitation at 280 nm were recorded for decorin during the pressurization and depressurization. Changes in the center of the spectral mass (ν) were calculated in accordance with the method of Ruan *et al.* (13),

$$(\nu) = \Sigma \nu i * Fi / \Sigma Fi$$

where νi is the wavenumber and Fi is the fluorescence intensity at νi.

III. RESULTS AND DISCUSSION

The density and uronic acid content of each fraction after centrifugation is shown in Table 1. As the target, decorin was contained in the D2 fraction, in which a small size proteoglycan (about 100 kDa) was shown by SDS-PAGE. The D2 fraction was then applied to DEAE-cellulose ion-exchange chromatography for further purification. Uronic acid-containing materials were eluted as four peaks at fraction numbers (NaCl concentrations) of 17 (0.17 M), 19 (0.20 M) 25 (0.26 M), 32 (0.33 M), respectively (Fig. 1). Among them, fractions 30-40 at 0.30-0.41 M NaCl contained decorin with a molecular mass of 100 kDa (Fig. 2), and were subjected to Sepharose CL-6B gel filtration chromatography. A large proportion of the uronic-acid containing materials was recovered in fractions 22-30, shown as the decorin fraction in Fig. 3.

SDS-PAGE of isolated decorin from bovine skeletal muscle revealed one band with an average mass of 100 kDa (Fig. 4), which was stained with Silver staining. After chondroitinase ABC treatment, a molecular mass of 48 kDa was confirmed, which is the core protein derived from decorin (data not shown). These results suggest that decorin was purified from bovine skeletal muscle. However, further experiments such as electrophoretic separation of the GAG chains and N-terminal amino acid sequencing of the core protein was required.

At present, structural changes of decorin from bovine skeletal muscle are measured by fluorescence spectra under high pressure.

Table 1. Distribution of glycosaminoglycan (GAG) uronic acid on CsCl density gradient ultracentrifugation of 4 M guanidine hydrochloride extract from bovine skeletal muscle

fraction	density (g/mL)	GAG uronic acid (mg/100 g meat)
D1 (bottom)	**1.51**	**28.9**
D2	**1.43**	**3.0**
D3	**1.37**	**2.2**
D4	**1.33**	**2.6**

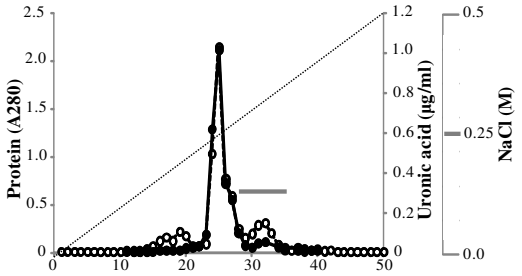

Fig. 1. DEAE-cellulose ion-exchange chromatograph of decorin from bovine skeletal muscle. The absorbance at 280 nm (○) and the uronic acid content of each fraction (●) were determined. Fractions 31-38, indicated by the bar, were combined for further purification.

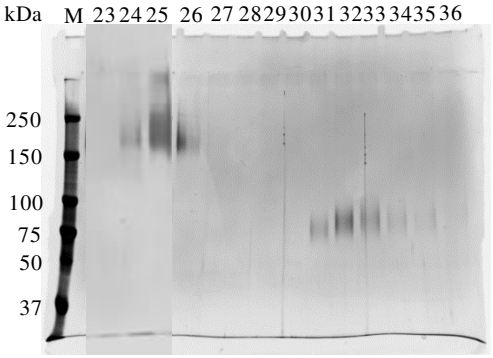

Fig. 2. SDS-PAGE after application of DEAE-cellulose ion-exchange chromatography. The gel was stained with Silver staining. M, molecular weight marker; 23-36, fraction numbers.

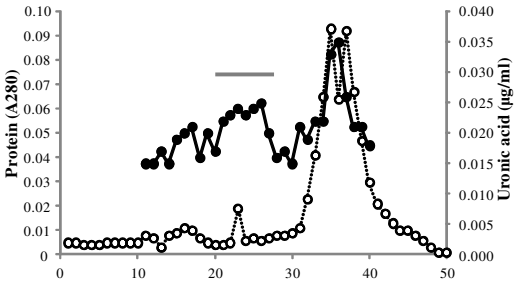

Fig. 3. Sepharose CL-6B gel filtration chromatograph of decorin from bovine skeletal muscle. The absorbance at 280 nm (○) and the uronic acid content of each fraction (●) were determined. Decorin was contained in fractions 22-30, indicated by the bar.

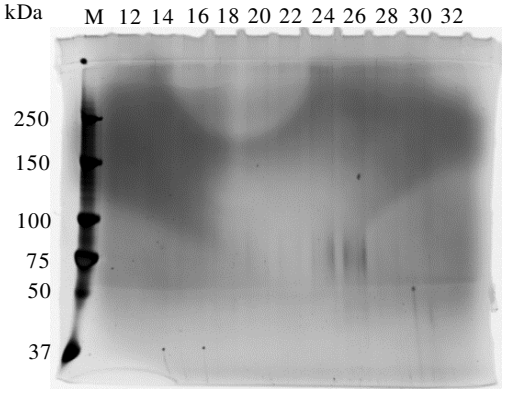

Fig. 4. SDS-PAGE after application of Sepharose CL-6B gel filtration. The gel was stained with Silver staining. M, molecular weight marker; 12-32, fraction numbers.

IV. CONCLUSION

Isolation and purification of decorin from bovine skeletal muscle was carried out 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. Purified decorin was characterized and alterations in its molecular structure under high pressure was analyzed.

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