Presence of the Basement Membrane Component—Heparan Sulfate Proteoglycan—in Bovine Lens Capsules

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Heparan sulfate proteoglycan was extracted from bovine lens capsules by 0.45 M NaCl/2 M urea and purified using ion-exchange chromatography and gel filtration. The proteoglycan was found to consist of protein and carbohydrate in a ratio of 75 to 25. The estimated average molecular weight of the heparan sulfate proteoglycan eluted by 0.2 M NaCl on a diethylaminoethyl (DEAE)-cellulose column was 400 kilodaltons (kDa) and that of its glycosaminoglycan was 18.8 kDa. The amino acid composition of the proteoglycan was quite similar to that of the bovine glomerular basement membrane.

Keywords proteoglycan; heparan sulfate proteoglycan; basement membrane; lens capsule; glycosaminoglycan; collagen

Introduction

It is well known that basement membranes are thin extracellular matrices that underlie endothelial and epithelial cells in various connective tissues.¹⁾ The molecular components of a basement membrane are predominantly composed of heparan sulfate proteoglycan (HSPG), type IV collagen¹⁾ and laminin.²⁾ HSPG is mainly distributed in the basal lamina of the basement membrane.

HSPG is difficult to isolate from the basement membrane because it is a small component of tissues and is virtually insoluble due to interaction with components such as laminin, type IV collagen and other macromolecules.¹⁾ However, the isolation of HSPG has been recently attempted from some tissues such as Engelbreth-Holm-Swarm (EHS) sarcoma,³⁻⁵⁾ glomerular basement membrane^{6,7)} and other tissues.^{8,9)} From these results, the estimated molecular weights of HSPG and its heparan sulfate side chain were in the broad range of 71000—750000 and 14000—40000, respectively. However, the biological function of HSPG in tissues remains obscure at present.

In the present paper, bovine lens capsules were used for the isolation of HSPG because it is easy to deal with and contains few other components except for basement membrane components as compared with renal glomeruli.¹⁰⁾ Therefore, to elucidate whether or not HSPG from lens capsules shares a common structure with HSPG in the other tissues previously described, I tried to characterize HSPG from lens capsules, which will enable me to gain further insight into the role of HSPG in the basement membrane.

Materials and Methods

Materials Fresh normal bovine eyes were obtained from a local slaughterhouse. Phenylmethanesulfonyl fluoride (PMSF), *N*-ethylmaleimide (NEM), pepstatin A and pronase E were purchased from Sigma Chemical Co. Dermatan sulfate (DS, from hog skin), chondroitin-4-sulfate (CS, from whale cartilage), hyaluronic acid (HA, from hog skin), heparin (HP, from hog intestine), heparan sulfate (HS, from bovine kidney), chondroitinase ABC (from *Proteus vulgaris*) and AC II (from *Arthrobacter aureus*) and heparitinase (from *Flavobacterium heparinum*) were from Seikagaku Kogyo Co., Tokyo. Cellulose acetate membrane (Separax) was from Jookoo Sangyo Co., Ltd., Tokyo.

Isolation of HSPG from Bovine Lens Capsules All procedures for the extraction of proteoglycan were carried out at 4 °C. Fresh normal lens capsules (30 g wet weight) obtained from 500 bovine eyes immediately after sacrifice were washed three times with 50 mM Tris-HCl buffer, pH 7.6, containing proteinase inhibitor.¹¹⁾ After washing, the residue was extracted six times with 0.45 M NaCl, 2 M urea and 50 mM Tris-HCl buffer, pH 7.6,

containing proteinase inhibitors. The extracts were diluted with 2 M urea, 50 mM Tris-HCl buffer, pH 7.6, to obtain a final NaCl concentration of 0.05 M. They were directly applied to a diethylaminoethyl (DEAE)-cellulose column (3 × 10 cm) equilibrated with 2 M urea, 0.05 M NaCl in 50 mM Tris-HCl buffer of pH 7.6. After washing the column with the same buffer, the proteoglycan was eluted with 0.3 M NaCl in the same buffer. For a further purification, the 0.3 M NaCl eluate was dialyzed against 7 M urea in 50 mM Tris-HCl of pH 7.6 and was rechromatographed on a DEAE-cellulose (1 × 13 cm) with 7 M urea in 50 mM Tris-HCl buffer of pH 7.6, and then eluted with a stepwise increase in the NaCl concentration. Alcian blue positive fractions were recovered from fractions eluted with 0.15 M and 0.2 M NaCl elutions.

Gel Filtration HS obtained from alkali and pronase-treated⁸⁾ HSPG was dissolved in 1.0 M NaCl containing 50 mM Tris-HCl buffer of pH 7.6. The solution was applied to a Sephadex G-200 column (1.25 × 65 cm) and then eluted with 1.0 M NaCl containing the above mentioned buffer. The molecular weight of HS was estimated using the calibration curve reported by Wasteson.¹²⁾

Electrophoresis Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) using 5% gels was performed as previously reported.¹³⁾ After electrophoresis, the gels were stained with 0.1% coomassie brilliant blue in 5% methanol-10% acetic acid for protein with 0.1% toluidine blue in 3% acetic acid for glycosaminoglycans (GAG)¹⁴⁾ or with periodic acid Schiff's reagent (PAS) for glycoprotein.¹⁵⁾ The reduction of the samples was done with 0.1 M dithiothreitol. The determination of the GAG components was carried out by two-dimensional electrophoresis on cellulose acetate membrane¹⁶⁾ and then stained with alcian blue 8 GX to identify the HS component in combination with heparitinase and nitrous acid.¹⁷⁾

Analytical and Chemical Procedure Amino acid analysis was performed by a JEOL-6AS autoanalyzer after the hydrolysis of a sample of protein with 6 M HCl in an evacuated sealed tube at 110 °C for 24 h.¹⁸⁾ Uronic acid was measured by a carbazole method with D-glucuronolactone as the standard.¹⁹⁾ The protein content was determined using a modification of Lowry's method with bovine serum albumin as the standard.²⁰⁾ Hexosamine analysis was performed by a JEOL-6AS autoanalyzer after the hydrolysis of a sample of GAG with 5 M HCl at 110 °C for 5 h.

Results and Discussion

The whole of the lens is enclosed by a homogenous capsule rich in glycoprotein which represents a specialized remnant of the epithelial basement membrane. The lens capsules (30 g wet weight) from 500 bovine eyes were treated according to the procedures described in Materials and Methods. The yields of proteoglycan were 2.57 mg of 0.15 M NaCl fraction and 5.08 mg of 0.2 M NaCl fraction from DEAE-cellulose rechromatography. Fig. 1 shows SDS-PAGE patterns of the subfractions eluted with a stepwise increase in the NaCl concentration from DEAE-cellulose chromatography, and then stained with coomassie brilliant blue. The broad band stained with a dye is one of the typical features of proteoglycan. Furthermore, both gel 4 and gel 5

Fig. 1. SDS-PAGE Pattern of HSPG from Bovine Lens Capsules

SDS-PAGE (5% gel) was performed according to the method of Laemmli.¹³⁾ 1, bovine type I collagen; 2, 0.05 M NaCl fraction from the second DEAE-cellulose column; 3, 0.1 M NaCl fraction; 4, 0.15 M NaCl fraction; 5, 0.2 M NaCl fraction. The molecular weight markers were: fibronectin (440000 (a) and 220000 (b)), β -galactosidase (116000 (c)), bovine serum albumin (66000 (d)), BF (buffer front).

Fig. 2. The Two-Dimensional Electrophoresis Pattern of a GAG Component in 0.2 M NaCl Eluate

A GAG preparation from the 0.2 M NaCl eluate was subjected to two-dimensional electrophoresis on a cellulose acetate membrane and was stained with 0.1% alcian blue. Small arrow indicates the spot position of the sample.

were stained as the same broad bands of macromolecules with toluidine blue for GAG (data not shown).

The GAG component of the 0.2 M NaCl fraction was determined by two-dimensional electrophoresis on a cellulose acetate membrane (Fig. 2). The GAG released with alkali and pronase treatment was electrophoresed in the position of HS used as the standard. Furthermore, the GAG was digested with heparitinase and deaminated with sodium nitrite but not with chondroitinase ABC. From these results, I have concluded that the proteoglycan of the 0.2 M fraction may have only the HS chain as the GAG. The 0.15 M fraction was also entirely composed of HS (data not shown).

The estimated average molecular weight of the 0.2 M NaCl fraction (HSPG) was 400 kilodaltons (350–450 kDa) on the basis of SDS-PAGE and was similar to that of anterior capsules from calf lens.¹⁰⁾ However, the sugar content (Table II) differed from that previously reported.²¹⁾ The reason for these differences are not known. The sugar composition of

TABLE I. Amino Acid Composition of Subfractions from Bovine Lens Capsules

Amino acid ^{a)}	0.15 M NaCl fraction	0.2 M NaCl fraction	Bovine glomerular basement membrane ^{b)}
Hydroxyproline	—	—	6
Aspartic acid	80	70	82
Threonine	55	54	49
Serine	83	86	79
Glutamic acid	124	122	116
Proline	71	93	100
Glycine	131	138	120
Alanine	70	77	80
1/2 Cystine	21	24	61
Valine	69	65	57
Methionine	15	11	11
Isoleucine	34	22	21
Leucine	79	67	64
Tyrosine	23	20	20
Phenylalanine	27	28	25
Histidine	28	30	20
Hydroxylysine	—	6	2
Lysine	25	19	25
Arginine	65	68	62

a) The values of amino acid residues are given per 1000 amino acid residues. b) Quoted from reference 5.

TABLE II. The Proteoglycan Composition of the 0.2 M NaCl Fraction from DEAE-Cellulose Chromatography

Components	$\mu\text{mol/mg}^a)$
Glucosamine ^{b)}	0.12
Galactosamine ^{b)}	0.02
Hexuronic acids ^{c)}	0.15
Total amino acids ^{d)}	1.18

a) Values are expressed per dry weight of proteoglycan. b) Determined by the amino acid analyzer.¹¹⁾ c) Determined by the carbazole method.¹⁹⁾ d) Determined from amino acid residues (Table I).

the GAG of the HSPG was notable for its high content of glucosamine. HSPG was found to consist of protein and carbohydrate in a ratio of about 75 to 25. The SDS-PAGE pattern of HSPG did not change with or without a reduction of the samples with dithiothreitol. Recently, the isolation of HSPG has been tried from other tissues. Accordingly, the molecular weight of proteoglycan from tetracarcoma derived endodermal cells,^{22,23)} which are known to synthesize an extracellular matrix containing the basement membrane molecules, is 400 kDa and quite similar to that of the HSPG obtained from the lens capsules. It has been recently reported that the molecular weight of HSPG from EHS sarcoma was estimated to be 7.5×10^5 Da.³⁾

In order to determine the molecular weight of the HS chain of HSPG, its GAG released with alkali and pronase treatment was applied to a Sephadex G-200 column equilibrated with 1.0 M NaCl/50 mM Tris-HCl buffer of pH 7.6. The average molecular weight of the lens capsule-HS chain was estimated to be 18800 using the calibration curve reported by Wasteson.¹²⁾

The results presented in Table I summarize the amino acid compositions of the two proteoglycan subfractions eluted with 0.15 and 0.2 M NaCl on a DEAE-cellulose column and that of HSPG from the bovine glomerular basement membrane.⁶⁾ The amino acid compositions of

both fractions from the lens capsules were quite similar to that of the HSPG of the bovine glomerular basement membrane, showing high contents of glutamic acid and glycine, but different from those reported for other basement membrane components such as laminin,²⁾ type IV collagen¹⁾ and nidogen.²⁴⁾

The biological function of HSPG is not well understood at present. However, it seems most likely that HSPG may serve as a regulator of the penetration of charged macromolecules in the basement membranes. Furthermore, it has been recently reported that HSPG is often present at the junction of the attachment fibrils and the matrix network^{25,26)} and that a basal lamina proteoglycan sulfate on the surface of skeletal muscle fibers associates with aggregates of acetylcholine receptors.²⁷⁾ Now it is very interesting how HSPG contributes to the transparency of the lens capsule.

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