A comparative study of proteoglycans from bovine lung, trachea, tracheal mucosa, and aorta[1]

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Summary. Proteoglycans were isolated from bovine lung, trachea, tracheal mucosa, and aorta by dissociative extraction with 4M guanidinium hydrochloride. Fractionation of these tissue extracts by cesium chloride density centrifugation and gel chromatography allowed the isolation from each extract of a high molecular weight fraction consisting mainly of proteochondroitin sulfate and hyaluronic acid.

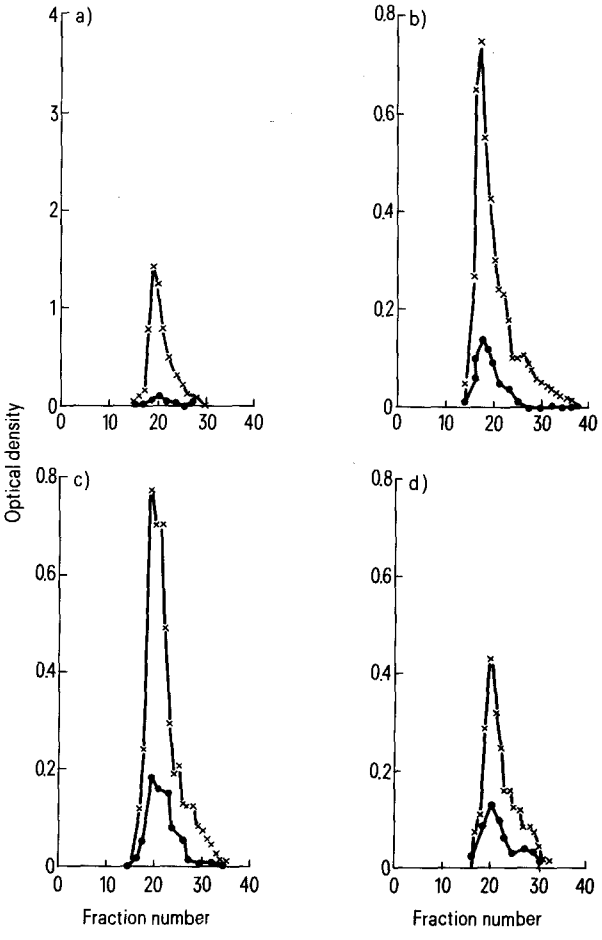
The technique of dissociative extraction of proteoglycans from tissues using high ionic strength solvents such as 4M guanidinium chloride (GuHCl) was developed to aid in the isolation of intact proteoglycans from cartilage[2-6]. Recently, this technique has been applied to other tissues such as brain[7], skin[8], and aorta[9,10]. Direct comparison of similar proteoglycans obtained from different tissues has not been studied extensively, although recent work[11] suggests that proteoglycans from different tissues may share similar protein components. To improve understanding of the similarities and differences among proteoglycans from different tissues, we have begun a comparative study of similar proteoglycans extracted from lung, aorta, tracheal mucosa, and trachea.

Materials and methods. Bovine lung, trachea, and aorta were collected in dry ice and transported while frozen to the laboratory. The tracheal mucosa was obtained from trachea by dissecting the inner mucosal membrane of trachea after partial thawing of the tissue. The tissues (150 g) were allowed to thaw in 450 ml of 4 M GuHCl with 0.02 M 2-(N-morpholino)ethane sulfonic acid (MES), pH 5.8, and the protease inhibitors[10], 0.01 M ε-aminocaproic acid, 0.01 M EDTA, 0.005 M benzamidine-HCl, 0.001 M phenylmethylsulfonyl fluoride and were homogenized in a Waring blender at top speed for 45 sec. The homogenate was allowed to shake in 250 ml plastic centrifuge bottles for 24 h at room temperature. Subsequently, the extracted residue was removed by centrifugation at 10,000 × g for 30 min.

Isolation of proteoglycans from the extract was by isopycnic CsCl centrifugation in the presence of 4 M GuHCl using an initial density of 1.35 g/ml. After dialysis against 0.1 M Tris-HCl, pH 7.0, 0.05 M $MgCl_2$ with proteinase inhibitors (no EDTA), the mixture of proteoglycans from the bottom one-sixth of the CsCl gradient was treated with 100 units each of DNAase (EP), EC 3.1.4.5, and RNAase (T1), EC 3.1.4.8, at 37 °C for 60 min to eliminate sample viscosity due to the presence of high molecular weight DNA. The digest was then separated into high molecular weight (K_{av}, 0 to 0.36) and low molecular weight (K_{av}, 0.57 to 1.0) proteoglycans by chromatography on Bio-Gel A5 (5 × 35 cm). The gel column was eluted with 0.1 M Tris-HCl, 0.15 M NaCl, 10^{-3} M EDTA, pH 7.0. The high molecular weight proteoglycans were also chromatographed on a Bio-Gel A15 column (0.7 × 45 cm).

The glycosaminoglycan (GAG) composition of the proteoglycans was determined by the method of Roblin, et al.[12]. In this method, the low molecular weight saccharides formed after enzyme digestion of aliquots containing 50 µg of uronic acid were recovered by chromatography on Bio-Gel A0.5 columns. Digests were prepared using hyaluronidase (EC 3.2.1.35) from *Streptomyces hyalurolyticus* and bovine testes (10 TRU and 560 units, respectively, in 0.1 M sodium acetate, 0.15 M NaCl, pH 5.0, 37 °C, 16 h), and chondroitinase ABC (EC 4.2.2.4), 0.05 units in 0.05 M Tris-HCl, pH 8.0, 100 µg/ml bovine serum albumin. Further characterization was by electrophoresis of GAG obtained after papain digestion of the proteoglycans on cellulose acetate in pyridine-acetic acid buffer, pH 3.2.

Results and discussion. Isopycnic CsCl centrifugation to separate proteoglycans from unbound protein was performed under dissociative conditions (4 M GuHCl)[5]. Results are given in table 1. Relatively more of the total tissue uronic acid was extracted from the lung than other tissues, indicating that the proteoglycans in the lung are more loosely associated with insoluble connective tissue elements such as insoluble collagen and elastin. Most of the uronic acid material, but only 1-2% of the extracted protein, sediments in CsCl fractions 1 and 2 (density > 1.5 g/ml). All proteoglycans except those from lung tissue contain 3-5 times more uronic acid than protein. The extra protein found in the lung fractions is in part noncovalently bound since after chromatography on Bio-Gel A5, the protein to uronic acid ratios of these purified lung proteoglycans are similar to the ratios found for the other tissues (table 2).



Bio-Gel A15 column chromatography of the high molecular weight material from a Bio-Gel A5 column (see text). The column was eluted with 0.1 M Tris-HCl, 0.15 M NaCl, 0.001 M EDTA, pH 7.0. a) proteoglycans from trachea, b) lung, c) tracheal mucosa, and d) aorta. V_o was at fraction 17 and V_t at fraction 45. ×-×, uronic acid, A_{530}[13]; ●-●, protein, A_{630}[14].

Table 1. Isopycnic CsCl centrifugation of 4 M GuHCl bovine tissue extracts

Fraction number	Density g/ml	Tissue (μg extracted per g wet tissue)							
		Tracheal mucosa		Trachea		Aorta		Lung	
		Uronic* acid	Protein*	Uronic acid	Protein	Uronic acid	Protein	Uronic acid	Protein
1	1.640	105	33	3011	440	219	72	184	133
2	1.564	100	38	1752	580	122	81	92	156
3	1.460	41	1378	882	2422	64	1067	23	3200
4	1.412	17	2800	441	3467	32	1778	15	5442
5	1.361	17	4667	75	4533	19	2267	15	6533
Residue (not extracted)		147		3400		130		30	
Total amount extracted (%)		66		65		78		92	

* Uronic acid was determined by the m-hydroxydiphenyl method of Blumenkrantz and Asboe-Hansen[13]. ** Protein was measured by the Lowry test[14].

Table 2. Glycosaminoglycan composition of proteoglycans from Bio-Gel A5 fractions

	High molecular weight (K_{av} = 0 to 0.36)				Low molecular weight (K_{av} = 0.57 to 1.0)		
	Lung	Tracheal mucosa	Aorta	Trachea	Lung	Tracheal mucosa	Aorta
Percent recovered	83	85	86	100	17	15	14
Uronic acid: protein ratio	4.8	4.3	3.5	7.9	3.6	2.8	3.9
Hyaluronic* acid	28	26	14	ND*	16	23	20
Chondroitin sulfates	69	69	83	100	24	55	57
Dermatan sulfate	3	5	3	ND	12	ND	11
Heparin, heparan sulfate	ND	ND	ND	ND	48	22	12

* Numbers are percents of total uronic acid material. ** ND = not detected, less than 1% of the input uronic acid material.

From all of the tissues, the high molecular weight uronic acid material consisted of chondroitin sulfate proteoglycan and hyaluronic acid mainly (table 2), whereas heparan sulfate and heparin were found in lower molecular weight fractions. The fact that the high molecular weight fraction from Bio-Gel A5 chromatography eluted with a K_{av} of 0 to 0.34 on Bio-Gel A15 (figure 1), implies that components exist with molecular weights greater than 1×10^6 daltons. Because gel chromatography was performed under associative conditions (0.15 M NaCl), otherwise low molecular weight proteoglycans could form high molecular weight aggregates which would elute in the column void volume. Whether or not these species are aggregates similar to those found in cartilage[2] is currently under investigation.

The proteoglycans from different tissues were similar with respect to glycosaminoglycan composition (table 2). Except for the trachea, which as expected from other studies is mainly chondroitin sulfate (our studies did not measure the keratan sulfate content of these tissues), the high molecular weight fraction from Bio-Gel A5 consisted mainly of chondroitin-4/6 sulfate (70–80%) and hyaluronic acid (15–25%). Dermatan sulfate accounted for only 3–5% of the proteoglycan material in this fraction. In the low molecular weight fractions, 2-fold more heparan sulfate and heparin was found in the lung compared with the other tissues. Dermatan sulfate was not found in low molecular weight material from tracheal mucosa. Non-extracted uronic acid material contained only chondroitin sulfate and heparan sulfate in a ratio of approximately 3 to 1 (residue from the trachea was only chondroitin sulfate).

Although our results are still preliminary, they do suggest that similar proteoglycan species can be obtained from 4 quite dissimilar tissues. The high molecular weight proteochondroitin sulfate found in cartilaginous tissues is also found in the lung, aorta, and mucosa and is associated with similar amounts of protein in these tissues. Whether or not these protein moities have sequences in common is currently under investigation. The present studies show that of the proteoglycans isolated by 4 M guanidinium chloride extraction, only the chondroitin sulfate proteoglycans have high molecular weights. The iduronic acid-containing species, dermatan sulfate, heparan sulfate, and heparin, have molecular weights of at least an order of magnitude lower.

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