Isolation and Characterization of Proteoglycans from Porcine Lungs*

(Received for publication, March 17, 1978, and in revised form, July 7, 1978)

Saura Sahu and William S. Lynn

From the Departments of Biochemistry and Medicine, Duke University Medical Center, Durham, North Carolina 27710

Proteoglycans were extracted from porcine lungs with 4 M guanidinium chloride. The extract was subjected to associative density gradient centrifugation, and four equal fractions, labeled A₁ through A₄ from the bottom to the top of the gradient, were obtained. The pooled A₁ fractions containing proteoglycan aggregates were further fractionated by dissociative density gradient centrifugation to yield four equal fractions labeled A₁D₁ through A₁D₄ from the bottom to the top of the gradient. These fractions were analyzed for their protein, uronic acid, glucosamine, galactosamine, hexose, and sialic acid content. The fraction A₁D₁, with the highest buoyant density had the highest content of uronic acid and galactosamine, and lowest content of protein, indicating the enrichment of proteoglycan monomers at the bottom of the dissociative density gradient. As the density of the gradient decreased, the protein, hexoses, and sialic acid content increased, whereas uronic acid and galactosamine content decreased. The amino acid analysis showed similar composition for all four fractions with aspartic acid, serine, glutamic acid, proline, glycine, alanine, valine, and leucine as the major constituent amino acids. No hydroxyproline was detected in any of the fractions. As the buoyant density of the fractions decreased, the aspartic acid content increased and glycine content decreased.

Proteoglycans, like collagen and elastic fibers, are a major component of all connective tissues. In recent years, proteoglycans of cartilage from various sources have been isolated and characterized (1-12). Results of these studies indicate that the cartilage proteoglycans are large aggregates composed of up to 140 proteoglycan subunits linked noncovalently to a linear molecule of hyaluronic acid in association with two link proteins. According to the current structural model, the proteoglycan monomer consists of approximately 110 chondroitin sulfate and 50 keratan sulfate side chains attached covalently to a linear core protein. The average molecular weights of the proteoglycan monomer is mainly due to the variation in the size of the chondroitin sulfate-enriched region (12, 15, 16). In terms of the overall composition by weight, the cartilage proteoglycans contain approximately 86% chondroitin sulfate, 6% keratan sulfate, 8% protein, and less than 1% hyaluronic acid (2).

Composition of connective tissues of the lung is of fundamental importance to its structure and function. Since proteoglycans are one of the most important constituents of connective tissue, these macromolecules are expected to play an important role in the structure and function of this vital organ. It is not known what role, if any, these macromolecules play in the health and disease of the lung. However, their potential importance in fostering association between other components of connective tissue suggests that they are an integral part of the normal lung structure and may be altered in the pathogenesis of pulmonary diseases. Indeed, it has been suggested that lung glycosaminoglycans are of fundamental importance to the pathogenesis of several pulmonary diseases, including emphysema (17, 18) and silicosis (19). Therefore, the study of these macromolecules of the lung is of importance. The present study reports the isolation and partial characterization of the porcine lung proteoglycans.

**EXPERIMENTAL PROCEDURES**

**Materials**—Porcine lungs were obtained from a local slaughterhouse immediately following the killing of the healthy normal animals. The lungs were removed from the surrounding tissues, including bronchi, large blood vessels, and trachea. They were cut into small pieces and washed repeatedly with cold isotonic 0.9% NaCl solution (saline) until free of blood. All these steps were done at 4°C. The washed lung pieces were stored at -40°C until extraction. All chemicals used were of analytical grade unless otherwise stated. Guanidinium chloride (grade I), cesium chloride (grade I), and glucuronolactone were purchased from Sigma Chemical Co., St. Louis, Mo. All guanidinium chloride solutions were filtered through charcoal and then buffered at pH 5.8 with 0.05 M sodium acetate. Benzamidine hydrochloride hydrate and 6-aminohexanoic acid were obtained from Aldrich Chemical Co., Milwaukee, Wis. Bio-Gel A-50m and all chemicals used in the polyacrylamide gel electrophoresis were purchased from Bio-Rad Laboratories, Richmond, California. Standard hyaluronic acid, chondroitin sulfates, keratan sulfate, dermatan sulfate, heparin, and heparin sulfate were kindly supplied by Dr. M. B. Mathews and Dr. J. A. Cifonelli of the University of Chicago.

**Preparation of Lung Tissue for Light Microscopy**—Random samples of the lung tissue used in this study were examined by light microscopy. They were fixed in 10% formalin and stained with hematoxylin and eosin.

**Extraction of Proteoglycans**—Proteoglycans were extracted by the method of Hascally et al. (20) with 4 M guanidinium chloride in 0.05 M sodium acetate, pH 5.8, containing 0.01 M sodium EDTA, 0.1 M 6-aminohexanoic acid, and 0.005 M benzamidine hydrochloride. The extraction was done with 15 volumes of the extracting medium at 4°C for 24 h with gentle agitation. The suspension was centrifuged at 4°C for 30 min at 10,000 x g. The supernatant was decanted and the residue resuspended in 5 volumes of the above medium. After 12 h of further extraction, the suspension was centrifuged, and the supernatant was combined with the first extract. The residue was washed twice with 2 volumes of the above medium. The supernatants and the washings were combined.

**Associative Density Gradient Centrifugation**—The proteoglycan extracts with 4 M guanidinium chloride were dialyzed at 4°C for 36 h.
against 20 volumes of 0.5 M guanidinium chloride in 0.05 M sodium acetate, pH 5.8, containing 0.1 M sodium EDTA, 0.1 M 6-aminohexanoic acid, and 0.005 M benzamidine hydrochloride. Solid CsCl was added (1.0 g of solution) to this dialyzed extract containing approximately 1 mg of proteoglycan/ml to bring the density of the solution to approximately 1.6 g/ml (20). The solution was then centrifuged at 40,000 rpm for 48 h at 10°C in a Beckman SW 50.1 rotor. Finally it was divided into four equal fractions, labeled A1 through A4, from the bottom to the top of the gradient, following the notation of Heinegard (5). The flocculent material formed after the addition of CsCl was removed as a surface gel at the top of the gradient.

**Dissociative Density Gradient Centrifugation**—The bottom A1 fraction from the associative centrifugation were pooled, and the concentration of guanidinium chloride was brought to 4 M by addition of 7.5 M solution of the salt in 0.05 M sodium acetate, pH 5.8, containing the same protease inhibitors in the same concentrations as described above. The density of this solution was adjusted by adding solid CsCl (0.59 g of solution) to approximately 1.5 g/ml (20). Sufficient 4 M guanidinium chloride/Chloride solution of density 1.5 g/ml was then added to dilute the proteoglycan concentration to approximately 1 mg/ml. The solution was then centrifuged at 40,000 rpm for 48 h at 10°C in a Beckman SW 50.1 rotor. It was then divided into four equal fractions, labeled A1 through A4, from the bottom to the top of the gradient, using the notation of Heinegard (5). The fractions were pooled, dialyzed against water, and lyophilized. The dry protein was weighed at −10°C.

**Column Chromatography**—Gel filtration of proteoglycan fractions was performed on a Bio-Gel A-50m column (130×1.5 cm) equilibrated and eluted with 0.5 M sodium acetate, pH 6.8 (7). Fractions of 1.5 ml were collected. Blue dextran 2000 and glucuronolactone were used to determine the void volume (V0) and total volume (Vt), respectively.

**Isolation of Glycosaminoglycans**—The glycosaminoglycans were isolated as previously described (21) following the method of Hardingham and Muir (7). The proteoglycan extracts were hydrolyzed at 60°C for 4 to 6 h with crystalline papain (Worthington Biochemicals, Freehold, N.J.) in 0.1 M sodium acetate, pH 5.5, containing 0.05 M EDTA and 0.01 M cysteine. The gelatin was centrifuged at 15,000 rpm for 15 min. The clear supernatant was diluted with an equal volume of water. The glycosaminoglycans were precipitated by dropwise addition of 10% cetylpyridinium chloride solution. The precipitate was centrifuged and washed once with 0.05 M solution of cetylpyridinium chloride containing 5 mM NaN3, and once with 0.05 M cetylpyridinium chloride alone. The precipitate was dissolved in a minimum volume of i-propanol and precipitated as the sodium salt after the addition of CsCl to approximately 1 mg/ml. The solution was then centrifuged at 40,000 rpm for 48 h at 10°C in a Beckman SW 50.1 rotor. It was then divided into four equal fractions, labeled A1 through A4, from the bottom to the top of the gradient, using the notation of Heinegard (5). The fractions were recycled through a second associative density gradient, and the bottom one half of these samples were pooled. These recycled A1 samples containing proteoglycan aggregates (5) were subjected to the dissociative density gradient centrifugation (20), and four equal fractions labeled A1D through A4D from the bottom to the top of the gradient were obtained. The relative amounts and composition of these four fractions are given in Table I. Approximately 4 to 6 mg of proteoglycans were obtained from 1 g of wet tissue. The proteoglycans recovered at the highest density had the lowest protein and highest uronic acid and galactosamine content. Chondroitin sulfates were the only detectable glycosaminoglycans present in this fraction (Fig. 3). Their identification was confirmed by the disappearance of the band corresponding to chondroitin sulfates on the cellulose acetate electrophoresis after digestion with chondroitinase AC and chondroitinase ABC. With the decrease in the density of the dissociative gradient, the protein, hexoses, as well as the sialic acid content increased, whereas uronic acid and galactosamine decreased. A1D2 and A1D3 fractions contained mostly hyaluronic acid and small amounts of heparin sulfate (Fig. 3). After digestion with Streptomyces hyaluronidases the major band corresponding to hyaluronic acid on the cellulose acetate paper disappeared, but a minor band corresponding to heparin sulfate could be detected. Heparin sulfate was further confirmed by its susceptibility to deamination cleavage (30). The A1D3 fraction was rich in protein, glucosamine, hexosamines, and sialic acid. This fraction was found to contain heparin sulfate (Fig. 3) which could be degraded by the action of nitric acid. The SDS polyacrylamide gel electrophoresis of A1D1 fraction showed two protein bands with apparent molecular weights of 50,000 and 65,000 (Fig. 5). These two proteins may be compared with the two hyaluronic acid-binding proteins of apparent molecular weights 40,000 and 65,000 isolated by Hascall and Heinegard (6) from bovine nasal and tracheal cartilage proteoglycans. Baker and Caterson (39) have also isolated two similar proteins of apparent molecular weights 47,000 and 51,000 from cartilage proteoglycans. Recently, Bonnet et al. (40) have shown that two such proteins with apparent molecular weights of 45,000 and 49,000 are present in bovine nasal cartilage proteoglycans. The 50,000 molecular weight protein found in the A1D2, A1D3, and 2A1D4 fractions seems to represent probably the larger of the two link proteins described by Baker and Caterson (39) as well as by Bonnet et al. (40). Whether the 65,000-
Lung Proteoglycans

FIGS. 1 (left) and 2 (right). Light microscopic sections of the lung tissue. H and E, × 500.

FIG. 3. Tracing of an electrophoretogram on cellulose acetate paper of glycosaminoglycans obtained from various lung proteoglycan fractions together with reference standards. The electrolyte system composition was 0.05 M LiCl/0.01 M HCl, pH 2.0, at a constant current of 1.0 mA/cm for 60 min. Staining was by Alcian blue. Reference standards: hyaluronic acid (1), chondroitin 4-sulfate (2), chondroitin 6-sulfate (3), heparin sulfate (4), dermatan sulfate (5), keratan sulfate (6), and heparin (7).

dalton protein present in the A1D1 fraction (Fig. 5) represents the hyaluronic acid-binding region of the proteoglycan monomer as described by Hascall and Heinegard (6) is not known.

Gel chromatography (Fig. 6) was used to assess the size of the proteoglycans as well as the effect of combining samples from the top and middle of the dissociative gradient on the proteoglycan monomers. Most of the proteoglycan aggregates, A1, were excluded from the Bio-Gel A-50m column, whereas most of the monomers, A1D1, were included in the column. However, a small fraction of A1 was included in the column, and a small portion of A1D1 was excluded from the column. The fractions from the middle of the dissociative gradient increased the size of the proteoglycan monomers (Fig. 6, f and g). The protein-rich fraction, A1D1, changed the elution of the
Lung Proteoglycans

FIG. 5. SDS polyacrylamide gel electrophoresis of AID4 fraction obtained from pig lungs (showing the presence of two proteins indicated by the arrows) (left). Reference standards (right) are ovalbumin (43,000) and bovine cross-linked albumins (66,000, 132,000, 198,000, and 264,000, obtained from Sigma Chemicals). The gels were stained with Coomassie blue.

proteoglycans very little by itself (Fig. 6h), although in the presence of the middle fractions, AID2 and AID3, it did increase the size of the proteoglycan monomers (Fig. 6, i and j). It, therefore, appears that an active component was present in the middle fractions, which contain hyaluronic acid.

The amino acid composition of four fractions, AID1 through AID4, are somewhat similar but not identical (Table II). The major constituent amino acids are aspartic acid, serine, glutamic acid, proline, glycine, alanine, valine, and leucine. No hydroxyproline or hydroxylysine was detected in any of these fractions. The lung AID1 fraction differs in its amino acid composition from that of the cartilage AID1 fraction (5) especially in its higher content of aspartic acid and alanine and lower content of serine and proline. The amino acid composition of the AID1 fraction is quite different from those of collagen and elastin. It is similar to the amino acid composition of the "structural glycoproteins" present in the connective tissue matrix (41). Our results agree with the previously published reports that the content of aspartic acid increases and that of glycine decreases with the decrease in buoyant density of the proteoglycan fractions (7, 42, 43).

The proteoglycans isolated from the lung tissue are comparable to those of aorta (44). The 4 M guanidinium chloride extracts of bovine aorta contained chondroitin sulfates, hyaluronic acid, and heparin sulfate. The proteoglycans of aorta interacted with hyaluronic acid and cardiovascular connective tissue proteins to form aggregates.

The results described in this report clearly demonstrate that cartilage-free lung tissue contains cartilage-like proteo-
glycans. The recent report of Norling et al. (45) showing the synthesis of cartilage-like proteoglycans by the human glial cells in culture supports our finding that the ability to synthesize cartilage-like proteoglycans is not confined to the cartilage cells alone. However, the exact cellular source of the cartilage-like proteoglycans in the cartilage-free lung tissue remains to be determined.

REFERENCES

1. Hascall, V. C., and Sajdera, S. W. (1969) J. Biol. Chem. 244, 2384-2396
2. Sajdera, S. W., and Hascall, V. C. (1969) J. Biol. Chem. 244, 77-87
3. Hascall, V. C., and Sajdera, S. W. (1970) J. Biol. Chem. 245, 4920-4930
4. Mathews, M. B. (1971) Biochem. J. 125, 39-46
5. Heinegård, D. (1972) Biochim. Biophys. Acta 285, 181-192
6. Hascall, V. C., and Heinegård, D. (1974) J. Biol. Chem. 249, 4232-4241
7. Hardingham, T. E., and Muir, H. (1974) Biochem. J. 139, 565-581
8. Thyberg, J., Lohmander, S., and Heinegård, D. (1975) Biochem. J. 151, 157-166
9. Rosenberg, L., Wolfenstein-Todel, C., Margolis, R., Pal, S., and Strider, W. (1976) J. Biol. Chem. 251, 6439-6444
10. Hardingham, T. E., Ewings, R., and Muir, H. (1976) Biochem. J. 157, 127-143
11. Pearce, R. H., and Grimmer, B. J. (1976) Biochem. J. 157, 753-760
12. Heinegård, D. (1977) J. Biol. Chem. 252, 1980-1989
13. Hascall, V. C., and Riolo, R. L. (1972) J. Biol. Chem. 247, 4529-4536
14. Hascall, V. C., Riolo, R. L., Hayward, J., and Reynolds, C. C. (1972) J. Biol. Chem. 247, 4521-4528
15. Heinegård, D., and Hascall, V. C. (1974) J. Biol. Chem. 249, 4250-4256
16. Heinegård, D., and Axelsson, I. (1977) J. Biol. Chem. 252, 1971-1979
17. Laros, C. D. (1972) Respiration 29, 442-457
18. Laros, C. D., Kuyper, C., and Janssen, H. (1972) Respiration 29, 468-477
19. Wusteman, F. S., Gold, C., and Wagner, J. C. (1972) Am. Rev. Resp Dis 106, 116-118
20. Hascall, V. C., Oegema, T. R., Brown, M., and Caplan, A. I. (1976) J. Biol. Chem. 251, 3511-3519
21. Sahu, S., and Lynn, W. S. (1978) Biochem. J. 172, 565-568
22. Sano, N., Amo, K., and Kondo, K. (1977) Anal. Biochem. 37, 197-202
23. Curwen, K. D., and Smith, S. C. (1977) Anal. Biochem. 79, 291-301
24. Breen, M., Weinstein, H. G., Blakic, J., Borcherding, M. S., and Sitting, R. A. (1976) in Methods in Carbohydrate Chemistry (Whistler, R. L., and BeMiller, J. N., eds) pp. 101-115, Academic Press, New York
25. Sahu, S., and Lynn, W. S. (1979) Biochem. J. 177, 153-158
26. Weber, K., and Osborn, M. (1975) in The Proteins (Neurath, H., and Hill, R. L., eds) Vol. I, pp. 179-223, Academic Press, New York
27. Saiio, H., Yamagata, T., and Suzuki, S. (1968) J. Biol. Chem. 243, 1536-1542
28. Yamagata, T., Saito, H., Hubuchi, O., and Suzuki, S. (1968) J. Biol. Chem. 243, 1523-1535
29. Ohya, T., and Kaneva, V (1970) Biochin Biophys Acta 198, 607-609
30. Lindahl, U., Backström, G., Jansson, L., and Hallen, A. (1973) J. Biol. Chem. 248, 7324-7341
31. Dische, Z. (1947) J. Biol. Chem. 167, 189-198
32. Bitter, T., and Muir, H. (1982) Anal. Biochem. 130, 330-334
33. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
34. Resig, J. L., Strominger, J., and Leloir, L. (1955) J. Biol. Chem. 217, 599-604
35. Sahu, S., and Lynn, W. S. (1977) Biochim. Biophys. Acta 489, 307-317
36. Oegema, T. R., Hascall, V. C., and Dziewiatkowski, D. D. (1976) J. Biol. Chem. 251, 3511-3519
37. Jourdan, G. W., Dean, L., and Roseman, S. (1971) J. Biol. Chem. 246, 430-435
38. Yemm, E. W., and Willis, A. J. (1954) Biochem. J. 57, 508-514
39. Baker, J., and Caterson, B. (1977) Biochim. Biophys. Res. Commun. 77, 1-10
40. Bonnet, F., Perin, J. P., and Jolles, P. (1978) Biochim. Biophys. Acta 532, 242-248
41. Anderson, J. C. (1976) Int. Rev. Connective Tissue Res. 7, 251-322
42. Tsiganos, C. P., Hardingham, T. E., and Muir, H. (1971) Biochim. Biophys. Acta 229, 529-534
43. Lohmander, S. (1975) Eur. J. Biochem. 57, 549-559
44. McMurtrey, J., Radhakishnamurty, B., and Benesch, G. (1977) Fed. Proc. 36, 644
45. Norling, B., Ghimelius, B., Westermark, B., and Wasteson, A. (1978) Biochim. Biophys. Res. Commun. 84, 914-921
Isolation and characterization of proteoglycans from porcine lungs.
S Sahu and W S Lynn

*J. Biol. Chem.* 1979, 254:4262-4266.

Access the most updated version of this article at [http://www.jbc.org/content/254/10/4262](http://www.jbc.org/content/254/10/4262)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/254/10/4262.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/254/10/4262.full.html#ref-list-1](http://www.jbc.org/content/254/10/4262.full.html#ref-list-1)