Isolation and Partial Characterization of Protein Polysaccharides from Rat Costal Cartilage (35143)

KUNG-YING TANG KAO, JAMES G. LESLIE, WILLIAM E. HITT, AND THOMAS H. MCGAVACK

Geriatrics Research Laboratory, Veterans Administration Center, Martinsburg, West Virginia 25401; and Department of Medicine, George Washington University School of Medicine, Washington, D. C. 20005

The heterogeneity of protein polysaccharides (PP) from cartilage has been reported by a number of investigators in the past few years. Pal *et al.* (1) separated five fractions from bovine nasal cartilage by extraction with various salt solutions and hydroxylamine. Dunstone and Franek (2, 3) have isolated three glycosaminoglucuronoglycan-containing fractions and a glycoprotein fraction from bovine nasal cartilage by preparative equilibrium sedimentation in cesium chloride density gradients, coupled with rate-zonal sedimentation in linear NaCl gradients. Herring (4) used ion-exchange chromatography to separate three fractions from protein-bound chondroitin sulfate of bovine cortical bone. Hoffman *et al.* (5), using preparatory electrophoresis, demonstrated a variable ratio of chrondroitin sulfate to protein in the protein polysaccharide fractions extracted from bovine nasal cartilage. Recently, Hascall and Sajdera (6) have also separated a protein polysaccharide complex from bovine nasal cartilage into two fractions by equilibrium centrifugation in a cesium chloride gradient formed in the presence of 4 *M* urea. Hashimoto and Ludowieg (7) separated two major PP from nucleus pulposus of whale intervertebral disc by sucrose density gradient procedure.

Ten years ago, Johnson and Schubert (8) and, later Rosenberg *et al.* (9) reported the separation of protein polysaccharide light (PPL) and protein polysaccharide heavy (PPH) fractions from human costal cartilage. No further separation and characterization has been done. Previously, we have extracted PP from rat costal cartilage and separated the chondromucoprotein (CMP) from glycoprotein (GP) by the precipitation of the latter at 55% saturation with $(NH_4)_2SO_4$ (10). The ultracentrifugal pattern of the CMP showed approximately 90% of a slow-moving component and 10% of a fast-moving component. The GP fraction contained equal amounts of two components. Recently, we have prepared three PPL fractions from human costal cartilage by a modification of the method of Pal *et al.* (1). The results are reported elsewhere (11). With the same method, we report below the isolation and characterization of two PPL fractions from the costal cartilage of rats of three different ages.

Method and Procedures. Two samples of rat costal cartilages from each age group, 6 months, 1 and 2 years, were fractionated according to the method described previously (11). Each sample was pooled from 12 to 24 rats. The purification of the PPL's on carboxymethyl (CM)-cellulose columns and all analytical methods were the same as used in the previous paper (11).

Results and Discussion. The yields (mg/g) of dried cartilage of PPL-2 were approximately 20, 20, and 30; and for PPL-3 were 10, 30, and 30, respectively, for 2 and 1 year, and 6 months of age. There were only trace amounts of the PPL-4, PPL-5, and PPL-6 fractions.

The sedimentation patterns of PPL-2 and PPL-3 after 30 min of centrifugation are shown in Fig. 1. PPL-2 and PPL-3 from 1-year- and 6-month-old rats show heterogeneity and as centrifugation was continued to 60 min, the PPL-2 sample from 2-year-old rats showed pronounced heterogeneity. This is different from the PPL-2 isolated from

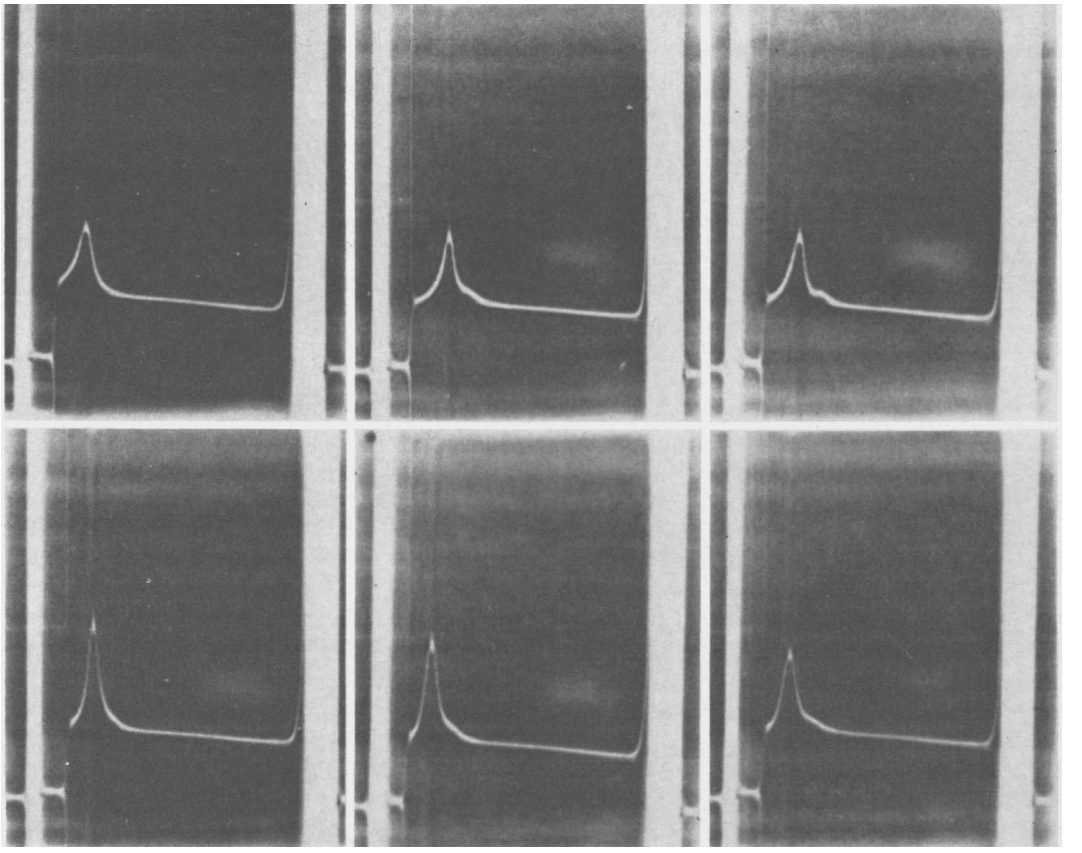

FIG. 1. Sedimentation patterns of PPL-2 (top) and PPL-3 (bottom) of rat costal cartilage. Samples were obtained from rats at 2 years (left); 1 year (middle); and 6 months (right) of age. PPL concentrations were 7 mg/ml of 0.1 *N* KCl. The sedimentation patterns were run at 25° in a Beckman-Spinco Model E Centrifuge. Pictures were taken 30 min after reaching 59,780 rpm and a phaseplate angle of 60°.

human costal cartilage (11), but similar to the PPL-2 isolated from bovine nasal cartilage (1). The sedimentation coefficients ($s_{20,w}$) for the PPL-2 and PPL-3 from rat costal cartilage, determined at protein concentrations of 5, 7, and 9 mg/ml, were 4.92 and 3.93, respectively.

The chemical composition of PPL-2 and PPL-3 of rat costal cartilage is shown in Table I. In comparison to the PPL preparations (11) from human cartilage, aged 50–70 years, there was a higher content of uronic acid and a very low glucosamine/galactosamine ratio in the rat costal cartilage preparation. These differences can be attributed to the high content of keratan sulfate in aged human cartilage.

In Table II, our data on the amino acid composition of PPL-2 and PPL-3 of rat costal cartilage is shown and for comparison,

TABLE I. Composition of PPL-2 and PPL-3 of Rat Costal Cartilage (g/g of dried wt).

	PPL-2	PPL-3
No. of pools	5	6
Protein	0.324 ± 0.006[a]	0.121 ± 0.007
Uronic acid	0.221 ± 0.004	0.270 ± 0.005
Hexose	0.054 ± 0.002	0.061 ± 0.002
Hexosamine	0.248 ± 0.010	0.272 ± 0.011
Glucosamine/galactosamine	0.06/1.00	0.03/1.00

[a] Mean ± SE, reflecting variability of sample pools. Each pool was comprised of 12–24 rats.

TABLE II. Amino Acid Composition of PPL-2 and PPL-3 of Rat and Human Costal Cartilage and Bovine Nasal Cartilage (residues/1000 residues).

	PPL-2			PPL-3		
	Costal		Nasal	Costal		Nasal
Amino acid	Rat[a]	Human[b]	bovine[c]	Rat[a]	Human[b]	bovine[c]
Cystine (½)	15.1	17.2	8	8.2	11.8	Trace
Methionine	10.8	8.0	6	15.2	4.7	3
Hydroxyproline	6.1	2.4		Trace		
Aspartic acid	92.3	95.7	80	74.5	79.7	68
Threonine	92.6	88.0	53	89.6	80.6	60
Serine	96.1	80.4	93	140.9	91.3	127
Glutamic acid	120.6	129.2	129	127.5	138.1	132
Proline	82.6	85.0	121	78.3	88.9	89
Glycine	115.0	94.1	133	139.4	107.7	126
Alanine	63.0	69.1	74	61.2	79.0	83
Valine	76.5	78.7	62	64.9	76.4	63
Isoleucine	38.9	39.8	36	33.2	43.9	40
Leucine	72.9	74.8	71	81.8	77.6	84
Tyrosine	16.6	26.6	15	9.4	21.5	11
Phenylalanine	27.8	35.1	35	29.7	36.4	34
Hydroxylysine[d]	0.8	3.5		Trace	2.6	
Lysine	29.6	29.9	30	22.8	22.3	29
Histidine	11.6	11.1	13	12.4	13.0	19
Arginine	44.9	51.3	43	29.2	43.3	33

[a] Average value from 4 analyses of PPL-2 and 6 analyses of PPL-3 obtained from rats of all age groups. Variations among samples were less than 5% for abundant amino acids and greater for amino acids in small amounts. Samples were hydrolyzed in 6 *N* HCl, 110°, 16 hr and analyzed with a Technicon amino acid analyzer. Corrections were made for destruction during hydrolysis (12).

[b] Ref. (11).

[c] Ref. (1).

[d] Similar to PPL-2 preparations from human costal cartilage (11), an unidentified Ninhydrin peak (X), 3 to 4 times greater than hydroxylysine, occurred half way between hydroxylysine and ornithine in the chromatogram of PPL-2. This unknown peak may be the same as the one reported by Shentall *et al.* (13).

comparable data on human costal cartilage (11) and bovine nasal cartilage (1) is included. As compared to the preparations from bovine nasal cartilage, the PPL's of rat costal cartilage contain greater amounts of ½ cystine and threonine and less proline, alanine, and phenylalanine. The absence of hydroxyproline in the PPL-3 shows that it is essentially free of collagen peptides as is the case for the PPL-3 of human costal cartilage (11); however, the PPL-2 of rat costal cartilage contains bound-collagen peptides. The principal differences in the amino acid composition of PPL obtained from rat costal cartilage and human costal cartilage are a greater amount of glycine and methionine and a lower amount of tyrosine, phenylalanine, and arginine in the PPL of rat costal cartilage. A much greater amount of serine was present in the PPL-3 from rat costal cartilage (140 residues/1000 residues) than that from human costal cartilage (91 residues/1000 residues).

The present study agrees with the work of Steven *et al.* (14) and that of our previous work (11), in that some of the PPL of costal cartilage contains bound-collagen peptides.

Summary. 1. Two protein polysaccharide fractions, PPL-2 and PPL-3, were isolated from the costal cartilage of rat, 6 months, 1

and 2 years old.

2. The PPL-3 sedimented as a single component in the samples prepared from 2-year-old rats. In contrast, the PPL-2 from 2-year-old rats and PPL-2 and PPL-3 from 6-months- and 1-year-old rats were heterogeneous.

3. The chemical compositions (g/g of dried wt) protein: uronic acid:hexose: hexosamine are 0.324:0.221:0.054:0.248 and 0.121:0.270:0.061:0.272 for PPL-2 and PPL-3, respectively. Ninety-five % of the hexosamine was galactosamine in both preparations.

4. The amino acid composition of rat costal cartilage is compared with that of human costal cartilage and bovine nasal cartilage.

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