DIFFERENCES IN COMPOSITION AND SIZE OF PROTEIN-POLYSACCHARIDES EXTRACTED FROM PIG ARTICULAR CARTILAGE

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1. Introduction

The protein-polysaccharides (P-Ps) of cartilage comprise a family of closely related molecules which vary in size and chemical composition [1,2]. Recent studies of articular cartilage in this laboratory confirm this heterogeneity and indicate that it is possible to extract selectively different populations of P-Ps from this tissue [3]. Since fractional solubilization may provide information on the distribution of P-Ps within the matrix, a sequential extraction is described which yielded a series of P-P fractions graded in size and composition.

2. Materials and methods

Femoral articular cartilage from normal 9 month old pigs was pulverized after freezing in liquid nitrogen and extracted as outlined below. The powdered cartilage was suspended in cold sodium acetate (NaAc), 0.15 M, pH 6.8, and homogenized for 10 min in a small MSE homogenizer. The homogenate was filtered through lint and the P-Ps were isolated from the clear filtrate and purified by 2 precipitations with 9-aminoacridine essentially as described previously [4], giving Extract 1. The cartilage residue was then resuspended in fresh NaAc and shaken in the cold overnight, following which the suspension was filtered, and the P-Ps in the filtrate isolated and purified as above (Extract 1W). Extract 2 was obtained after homogenizing the residue in fresh NaAc for 1 hr and Extract 2W by shaking in the cold overnight. Extracts 3 and 3W were obtained subsequently in the same way. At this point low-speed homogenization was replaced by more vigorous homogenization in a larger instrument for 2 consecutive 1 hr periods, yielding Extracts 4 and 5.

Gel filtration profiles of the P-Ps were determined on columns of agarose-6% (45 × 1.8 cm), a gift of C.P.Tsiganos, and Sepharose 4B (60 × 1.8 cm), eluted with NaAc, 0.5 M, pH 6.5.

Uronic acid, total hexosamine, xylose, hexose, hydroxyproline, protein and molar ratios of glucosamine to galactosamine (G1cN:Ga1N) were determined by established methods (for ref. see [2]).

3. Results and discussion

The combined extracts contained 34% of the total tissue uronic acid. Of the P-Ps released from the tissue nearly 40% by weight were liberated during the initial 10 min period of homogenization, following which the yield dropped sharply. When the larger homogenizer was substituted for the smaller more disruption took

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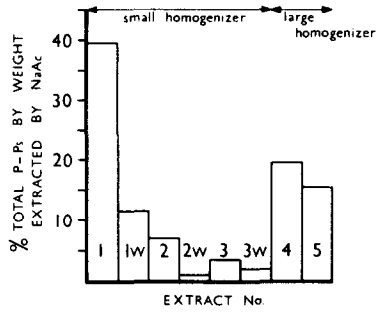

Fig. 1. Relative yields of sequential sodium acetate extracts expressed as percentage of the total amount of protein-polysaccharides (P-Ps) extracted with NaAc. See text for details of sequential extraction.

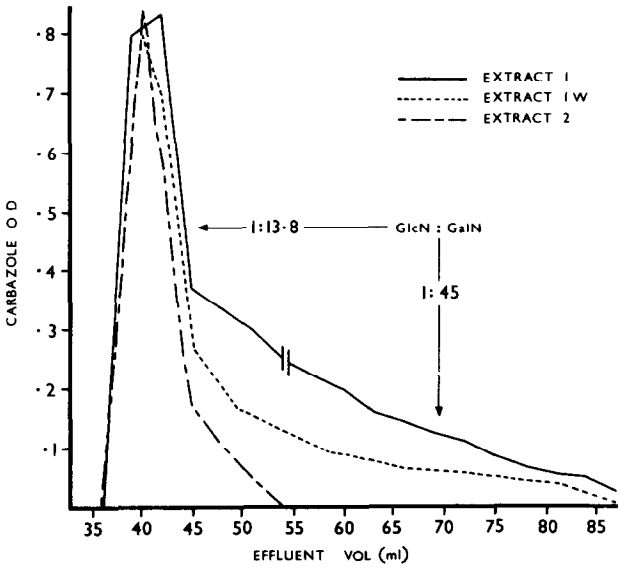

Fig. 2. Gel filtration profiles (determined by uronic acid) on agarose-6% of Extracts 1, 1W and 2. The P-Ps in Extract 1 which were excluded and retarded by the gel (interrupted line) were re-isolated and the ratios of GlcN:GalN determined.

place and the yield of P-Ps in Extract 4 rose to nearly half that in Extract 1 (fig. 1).

The uronic acid, total hexosamine, hexose and xylose contents of the P-Ps in all the extracts were similar (table 1). Hydroxyproline was not detected in any. With increasing resistance to solubilization, however, there was a steady increase in the ratio of GlcN:GalN, presumably reflecting increasing proportions of keratan sulfate in the isolated P-Ps (see ref. 3), and more vigorous homogenization produced no deviation from this trend. Only after use of the larger homogenizer, however, did the P-Ps contain somewhat more protein than those more easily extracted (table 1).

About 35% of Extract 1 was retarded by agarose-6% and when these smaller P-Ps were reisolated it was found that GlcN accounted for only about 2% of the total hexosamine whereas it accounted for 7% in P-Ps excluded from agarose-6%, indicating a higher keratan sulfate content.

Specific antiserum giving a single precipitin line against this fraction of smaller-sized P-Ps of articular cartilage showed immunological identity with one obtained by immunizing rabbits with a comparable fraction of small P-Ps isolated from pig larynx by Tsiganos and Muir [2], indicating that porcine cartilages from these two sites share common antigenic

Table 1
Analysis of fractions extracted sequentially with 0.15 M NaAc, pH 6.8.

Extract No.	Uronic acid	Hexosamine	Hexose	Xylose	Protein	GlcN:GalN
	Per cent of dry weight					
1	28.4	31.0	4.7	0.8	7.1	1:30
1W	28.6	32.2	4.3	0.9	9.9	1:18
2	28.0	31.5	5.0	0.8	9.5	1:12
2W	27.2	30.8	4.7	0.8	10.1	1:12
3	29.0	32.0	4.6	1.0	9.9	1:10
3W	27.3	–	4.6	–	10.6	–
4	28.2	30.7	5.2	0.8	13.2	1:9
5	26.8	31.4	5.1	0.9	12.5	1:8.5

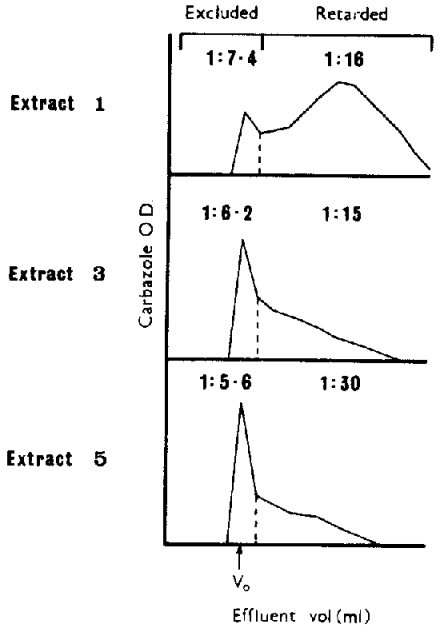

Fig. 3. Gel filtration profiles (determined by uronic acid) on Sepharose 4B of fractions from Extracts 1, 3 and 5. P-Ps were chromatographed initially on a column of agarose-6% and the P-Ps eluted in the void volume were re-isolated and applied to the column of Sepharose 4B. The excluded and retarded fractions from the latter were isolated (vertical dashed line) and hexosamine molar ratios determined.

determinants. It has already been shown that the determinant of the small P-P of pig larynx is common also to other species, whereas those of the larger laryngeal P-Ps are species-specific [2,5].

The amount of small-sized P-Ps was insignificant in all but the earliest extracts and, in particular, was not augmented by the large homogenizer. Presumably this component was completely extracted in the initial steps; it seems unlikely that significant quantities of the small components were entrapped in the matrix and their appearance in later extracts thus prevented. Despite the absence of a fraction permeating agarose-6%, however, each of these extracts possessed, amongst others, the immunological determinant characteristic of the small P-P, emphasising the close relationship of the smaller and larger P-Ps, as noted in the immunological studies of pig laryngeal cartilage [2,5].

The P-Ps from each extract which were excluded from agarose-6% were chromatographed on Sepharose 4B, which has a larger exclusion limit. The proportion of larger sized P-Ps increased steadily in successive extracts (fig. 3), so that about 5% of the total P-Ps in extract 1 and about 50% of the P-Ps in extract 5 were eluted in the void volume.

P-Ps which permeated Sepharose 4B invariably had a lower ratio of GlcN:GalN than did the larger excluded P-Ps in each extract (fig. 3), although none contained as low a proportion of GlcN as the smallest-sized P-Ps which were retarded on agarose-6%. Furthermore, although the GlcN:GalN of the fractions of P-Ps excluded from Sepharose 4B remained about 1:6 (fig. 3), the GlcN content of each whole extract rose successively as each contained a higher proportion of larger molecules. The P-Ps excluded from Sepharose 4B were themselves inhomogeneous, however, since a proportion was retarded on Sepharose 2B, which is a gel of greater porosity than 4B. In successive extracts this proportion decreased in the same way as the proportion retarded by Sepharose 4B.

Thus the P-Ps of articular cartilage appear to lie in a series of "compartments" progressively resistant to solubilization in neutral salt, and their molecular size and composition may be related to their ease of solubilization. The basis of this organization of cartilage matrix is as yet obscure, but the results are consistent with data of Stockwell and Scott [6] and Maroudas, Muir and Wingham [7], showing topographical variations in the distribution of chondroitin sulfate, keratan sulfate, and collagen in human femoral articular cartilage.

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