STUDIES ON LOW-MOLECULAR WEIGHT PROTEOGLYCANS ISOLATED FROM RABBIT EAR CARTILAGE

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1. Introduction

Heterogeneous populations of proteo-chondroitin sulphate complexes comprising low-molecular weight species, as minor constituents, have been reported in extracts of porcine laryngeal [1, 2] and articular [3, 4] cartilage. A proteoglycan containing only two glycosaminoglycan chains has been identified in bovine heart valves [5]. In all these instances, the experimental evidence suggests that the low-molecular weight complexes are not degradation products of larger macromolecules present in the tissues.

In the following study, the isolation of several minor constituent proteoglycans from rabbit ear cartilage is reported. Results obtained from gel filtration and analytical ultracentrifugation indicate for three of these complexes a 1:2:3 relationship in terms of both Stokes radius and molecular weight.

2. Material and methods

Ear cartilage isolated from New Zealand albino rabbits, 6–8 weeks old and weighing less than 1 kg, was dried in cold acetone and ground to a fine powder. Proteoglycan was extracted from the acetone-dried tissue by low-speed homogenization in water for 1 hr at 4°. The homogenate was fractionated by high-speed centrifugation [6] and the material contained in the supernatant collected by ethanol precipitation after addition of KAc. Further aqueous extraction of the cartilage residue did not yield any appreciable amount of hexosamine-containing material.

Gel filtration of the cartilage extract was carried out on a column of agarose-4% (bed volume 124 ml) equilibrated and eluted with KAc 1 M pH 7. Samples (20 mg), dissolved in 1.5 ml of 1 M KAc pH 7, were applied to the column. The effluent fractions (3 ml each) were monitored for glycosaminoglycan by determination of their hexuronic acid content. The column was calibrated with proteins of known Stokes radii as described [7].

Molecular weights were determined by the method of Yphantis [8] on a Spinco model E ultracentrifuge at 20°. Samples were dissolved in and dialysed against 2% KCl.

Partial specific volumes were estimated by the microdrop density method [9, 10] and corrected for charge effect according to Casassa and Eisenberg [11]. Due to the limited amounts of samples available, the accuracy of the determination of the thermodynamic interaction factor was checked by comparison with values of this quantity obtained from equilibrium dialyses of chondroitin sulphate preparations having a S content of 5.5%.

Analytical methods were used for the quantitative assay of hexosamine [12], hexuronic acid [13], hexoses [14, 15], sulphur [16] and protein [17].

3. Results

The hexosamine content of the cartilage examined in the present investigation was 3%, on a dry-weight basis. This value would suggest a proteoglycan concentration of 84 mg/g of dry tissue. Only approx. 1.4% of this material was extracted by low-speed

homogenization. The elution profile of the extract on agarose-4% is reported in fig. 1. The area under each peak was integrated and the values obtained are reported above the corresponding fractions. These values represent the amount of each macromolecular species present in the tissue, expressed as hexuronic acid. The majority of the cartilage extract (approx. 86%) was retarded by gel filtration, revealing the presence of four low-molecular weight fractions having elution volumes of 72 ml (F1), 92 ml (F2), 109 ml (F3) and 125 ml (F4). The average Stokes radii of the macromolecules in fractions 1, 2 and 3 are indicated in fig. 1. The compositions of the excluded material and of F1, F2 and F3 are reported in table 1.

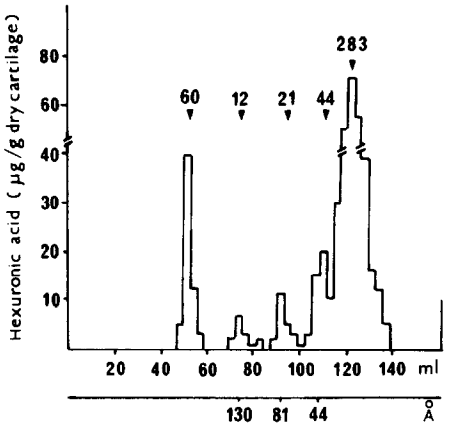

Fig. 1. Elution profile of the cartilage extract. Agarose-4% column: bed volume 124 ml, void volume 52 ml. Figures above represent areas of peaks (see text).

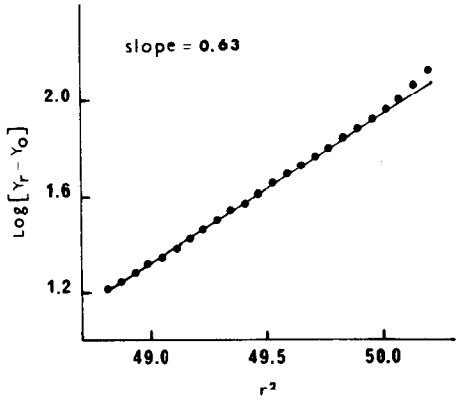

Fig. 2. Plot of fringe displacement versus distance from a sedimentation equilibrium experiment on F1. Rotor speed 12,590 rpm.

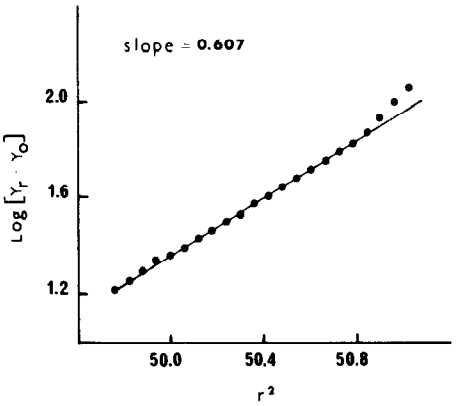

Fig. 3. Plot of fringe displacement versus distance from a sedimentation equilibrium experiment on F2. Rotor speed 15,220 rpm.

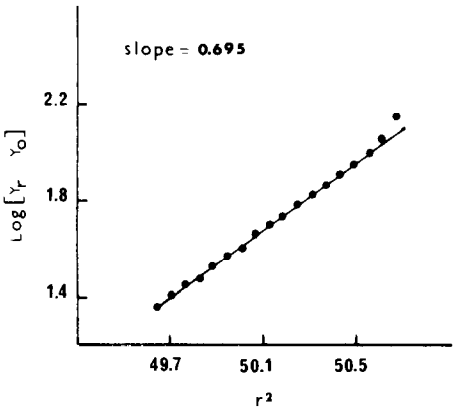

Fig. 4. Plot of fringe displacement versus distance from a sedimentation equilibrium experiment on F3. Rotor speed 23,150 rpm.

Table 1
Chemical analyses of individual fractions isolated by chromatography on agarose-4%.

Fraction ml collected	Excluded 45–60	1 65–80	2 85–100	3 105–115
Protein	14.7	16.1	17.2	15.1
Galactosamine (as free base)	24.2	22.9	20.9	24.3
Hexuronic acid	29.0	28.6	29.8	29.1
Hexose	4.9	4.3	4.2	5.9
Sulphur	4.4	4.2	4.2	4.0

Values are expressed as percentages of ash and moisture-free samples.

Owing to contamination from F4, F3 was rechromatographed prior to molecular weight determination. Figs. 2, 3 and 4 show typical plots of the natural logarithms of the differences between fringe displacements (Y_r) and the zero-concentration level (Y_0) against the square of the distances from the centre of rotation (r) obtained for F1, F2 and F3. Averaged values of slopes were calculated by least squares treatment of significant experimental points. The operating speeds are indicated in the legends to the figures. A mean partial specific volume (0.647 $cm^3\ g^{-1}$) was used in the calculation of the molecular weight of the three fractions, owing to the close agreement between experimental data obtained for different samples. The solvent density was 1.011 $g\ cm^{-3}$. The calculated values of $\bar{M}_w$ for F1, F2 and F3 were 118,900, 77,500 and 38,400 respectively.

4. Discussion

The chemical compositions of the gel-excluded material and of the three gel-retarded fractions referred to as F1, F2 and F3 indicate that all these macromolecular species are chondroitin sulphate-protein complexes. At present, F4 has not been fully characterized this being mainly due to contamination of the hexosamine-containing material with protein and anthrone-reacting compounds.

Bentley and Rokosová [18] have isolated from rabbit ear cartilage, previously digested with papain, three chondroitin sulphate fractions two of which had molecular weights in the range $1.6–2.5 \times 10^4$, as calculated from their serine contents. The molecular weight of F3 (3.84×10^4 daltons) is therefore consistent with a model in which two glycosaminoglycan chains are linked to a short polypeptide. The other two macromolecular species, F2 and F1, could equally be interpreted as comprising four and six chondroitin sulphate chains respectively. It is worth noticing that in spite of the chondroitin sulphate polydispersity detected by Bentley and Rokosová [18], the $\log (Y_r - Y_0)/r^2$ plots reported in figs. 2, 3 and 4 reveal a high degree of monodispersity for all three fractions. This could be in keeping with the postulated existence in cartilage of two pools of proteoglycan only one of which is metabolically active [18].

The Stokes radii of F3, F2 and F1 equally indicate a 1:2:3 relationship which is consistent with the model of chondroitin sulphate-protein complexes in which the polysaccharide chains are covalently bound to the protein core at more or less regular intervals [19]. From the information which is at present available, it is impossible to speculate on the biological role of these low-molecular weight proteoglycans, but they could represent subunits of larger complexes similar to those already reported in bovine nasal cartilage [7, 20]. In this case elongation of the core would occur by polymerisation of subunits through bonds of non-peptide nature as previously proposed [20].

Acknowledgements

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