ISOLATION OF A GLYCOPROTEIN FROM CARTILAGE PROTEINPOLYSACCHARIDE

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Electron microscope studies of proteinpolysaccharide preparations from epiphyseal cartilage (Fitton Jackson, 1965) support the idea that the association of several different macromolecules with other smaller molecules,to form specific three-dimensional ordered structures within the ground substance, is important in maintaining the integrity of the tissue. These studies have led to the suggestion (Partridge, Whiting and Davis, 1965) that a globular protein, distinct from the covalently bound protein of the proteinpolysaccharide complex, may be involved in the formation of the three-dimensional structure; these authors have isolated non-covalently bound protein from bovine cartilage preparations, and have determined its amino acid composition. Meyer (1966) has suggested that crude proteinpolysaccharide complexes with high molecular weights ($1\text{-}5 \times 10^6$) and high peptide content, are formed by the bridging of simpler complexes by basic proteins.

The further isolation and characterisation of such "bridging" proteins would appear to be a necessary step in the understanding of their function in the organisation of ground substance. Recent observations (Franek and Dunstone, 1966; Silpan-

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anta, Dunstone and Ogston, 1967) have shown that protein can be successfully separated from acidic proteinpolysaccharide complexes by equilibrium sedimentation in caesium chloride density gradients. By applying this method to preparations from bovine nasal cartilage, a glycoprotein has been obtained. This paper describes its isolation and preliminary characterisation.

Material and methods: The proteinpolysaccharide preparation from nasal cartilage, used in this work, is similar to the PPL fraction of Gerber, Franklin and Schubert (1960); details are given elsewhere (Franek and Dunstone, 1967).

Preparative and analytical sedimentation in density gradients were performed as described by Franek and Dunstone (1966) and by Silpananta et al (1967).

Results: When solutions of cartilage proteinpolysaccharide are sedimented to equilibrium in CsCl gradients (initial density: 1.63

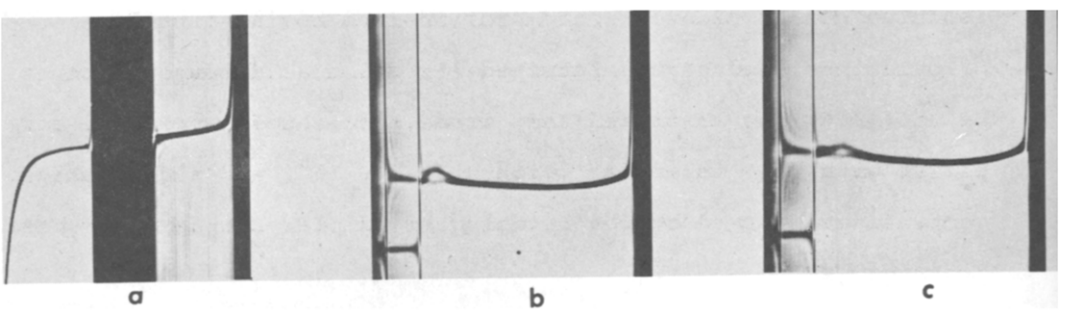

Fig.1. a. Equilibrium sedimentation of fraction A in a CsCl density gradient. Concentration: 1.02 mg protein/ml; initial density: 1.40 g/ml; phaseplate angle: 60°; photograph taken 20.5 h after reaching speed of 44,770 rpm in Yphantis-Waugh separation cell.

b,c. Velocity sedimentation of a fraction B_G. Concentration: 0.8 mg/ml; phaseplate angle: 50° (b), 45° (c); photographs taken 16 min (b), 32 min (c) after reaching speed of 50,740 rpm.

g/ml; density range: 1.52-1.75 g/ml) two main fractions are obtained (Franek and Dunstone, 1967). The fraction with density < 1.55 g/ml (A), while still containing some uronic acid, has proportionally more protein than the other, denser fraction (density > 1.65 g/ml).

Fraction A was examined in a CsCl gradient (initial density: 1.40 g/ml; density range: 1.34-1.46 g/ml; concentration 1.0 mg/ml), in an Yphantis-Waugh separation cell in the analytical ultracentrifuge. Fig.1a shows the schlieren diagram obtained at equilibrium (the separator remained stationary during sedimentation because the solution density > the density of the separator: this did not prevent the flow of solutes and thus did not affect the establishment of equilibrium throughout the cell). Material accumulated near the meniscus, density < 1.36 g/ml, and near the cell bottom, density > 1.45 g/ml. Preliminary chemical analysis of the fractions obtained from the cell showed that the upper fraction was largely protein with only a small amount of uronic acid; the lower fraction, while containing a relatively large proportion of protein, accounted for about 96% of the total uronic acid.

A preparative density gradient separation of fraction A was therefore carried out in a CsCl gradient (initial density 1.47 g/ml; range: 1.38-1.58 g/ml). All material with density < 1.4 g/ml (fraction B_G was collected, freed from CsCl by dialysis, and pressure dialysed to a concentration of 0.5-1.0 mg/ml.).

A similar protein fraction (fraction B_S) was obtained from fraction A by chromatography on Sephadex G-200. Material, essentially free from uronic acid and predominantly protein, was retarded by the column; uronic acid-containing material was eluted near the void volume.

TABLE 1. The chemical compositions of fractions B_G and B_S.

	Fraction B_G	Fraction B_S
	g/100 g polypeptide[1]	
Polypeptide[1]	100	100
Polypeptide[2]	70	78
Uronic acid[3]	1.5	1.0
Total hexosamine[4]	2.4	2.7
Galactosamine[5]	1.3	0.9
Hexose[6]	7.5	8.4
	moles/100 moles amino acids estimated	
Lysine	9.6	8.4
Histidine	2.7	2.7
Arginine	4.5	4.2
Aspartic acid	9.4	10.6
Threonine	5.8	5.8
Serine	5.5	5.7
Glutamic acid	13.8	11.9
Proline	5.6	5.5
Glycine	6.9	5.7
Alanine	8.7	8.0
Half Cystine	1.1	2.3
Valine	6.9	6.7
Methionine	0.6	0.9
Isoleucine	2.4	3.0
Leucine	9.7	10.5
Tyrosine	2.4	3.4
Phenylalanine	4.4	4.7

[1]Lowry, Rosebrough, Farr and Randall (1951). [2]From amino acid analysis after hydrolysis for 22 h in 6N HCl at 110°. [3]Dische (1947). [4]Cessi and Piliego (1960) after 8 h hydrolysis in 4N HCl. [5]Cessi and Serafini-Cessi (1963) after 8 h hydrolysis in 4N HCl. [6]Dubois, Gilles, Hamilton, Rebers and Smith (1956), as galactose.

The chemical compositions of fractions B_G and B_S are given in Table 1. They differ in only minor respects. In addition paper chromatographic studies (Dunstone, 1967), demonstrate the presence of glucose and galactose (Fig.2).

Fig.1b,c shows schlieren diagrams obtained during velocity sedimentation of a fraction B_G (0.8 mg/ml). The material sedimented as a single boundary ($s_{uncorr.}$ = 3.4S).

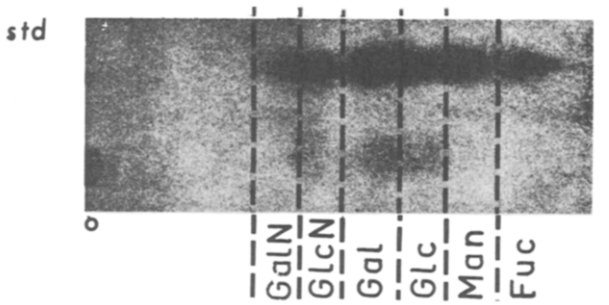

Fig.2. Paper chromatography of a fraction B_G. Hydrolysis- 7 h in 2N HCl at 100°. Solvent - Butanol:pyridine: water (6:4:3). Stain - Aniline hydrogen phthalate.

Disc electrophoresis of a fraction B_G on 10% polyacrylamide gel columns at pH 8.4, (Ornstein and Davis, 1962) showed that a large proportion of the material was contained in one band (Fig.3).

Discussion: The chemical composition of the isolated protein material (fractions B_G and B_S) suggests that it be classed as a glycoprotein (Gottschalk, 1966). The amino acid composition differs from that of the protein and of the corresponding chondromucoprotein obtained by Partridge et al (1965). The somewhat higher proportion of the basic amino acid, lysine, is more in agreement with the comments of Meyer (1966) concerning the nature of the protein, although high proportions of glutamic and aspartic acids are also present.

The carbohydrate composition has not been clearly established. Of particular interest is the presence of glucose,

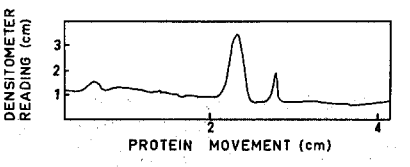

Fig.3. Polyacrylamide gel electrophoresis of a fraction B_G. The stained column was photographed, and the negative measured on a Beckman/Spinco Analytrol film densitometer.

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and reference should be made to recent reports of the presence of this monosaccharide in proteinpolysaccharide preparations from several connective tissue sources (Radhakrishnamurthy, Fishkin, Hubbell and Berenson, 1964; Dunstone, 1967).

Up to the present only small amounts of the glycoprotein material have been isolated; the methods described represent new approaches to the separation of tissue proteinpolysaccharides and it is hoped that by their use sufficient quantities of purified material may become available for the study of physicochemical interactions between the various components of connective tissue, and lead to a better understanding of their function in tissue organisation.

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