Protein–Polysaccharide Complexes from Adult Human Tracheal Cartilage

By R. M. MASON*

Department of Biochemistry, University College, Cardiff CF1 1XL, U.K.

(Received 13 July 1970)

Several reports have described the isolation of protein–polysaccharide complexes (proteoglycans) from human costal and articular cartilage (Rosenberg, Johnson & Schubert, 1965; Kröz & Buddecke, 1967) corresponding to the protein–polysaccharide light (PPL) and heavy (PPH) fractions isolated previously from bovine nasal cartilage (Gerber, Franklin & Schubert, 1960). The PPL fraction has been the protein–polysaccharide most studied so far and when isolated from human adult costal cartilage its composition differs considerably from that of bovine origin (Pedrini, 1969). A major difference in the complexes from the two sources is the greater keratan sulphate content of the human PPL fraction and this is in keeping with the high proportion of this glycosaminoglycan found in adult human hyaline cartilages (Mathews & Glagov, 1966). Thus the study of protein–polysaccharides from mature human cartilage, rather than from bovine tissues, may yield much needed information about the role of keratan sulphate in the extracellular matrix. This communication is concerned with the characterization of protein–polysaccharide complexes from mature human tracheal cartilage, a tissue that has not previously been extensively studied. The keratan sulphate content of this tissue attains maximum values by the beginning of the fourth decade and thereafter remains constant (Mason, 1970).

Tracheas were collected from 20 subjects (aged 45–75 years) within 24 h of death and stored at -20°C in 80% (v/v) ethanol until required. After separation of the cartilage rings from surrounding tissue they were pooled, homogenized in ethanol and recovered as a dry cartilage powder (Schubert, 1965). Portions of this preparation were then extracted by high-speed homogenization in water (Schubert, 1965), a procedure that includes a second homogenization of the insoluble material, after which the extraction of soluble protein–polysaccharide is virtually complete (cf. Rosenberg *et al.* 1965). Soluble protein–polysaccharide from the tracheal cartilage was then fractionated into PPL and PPH fractions by high-speed centrifugation (Schubert, 1965).

* Present address: Department of Biochemistry, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.

Hexuronic acid, galactosamine and glucosamine contents of the PPL, PPH and residue fractions were determined after proteolysis and subsequent removal of cationic material from the digests by passage through Amberlite IRC-50–IR-120 resin columns (Mason, 1970). Glycosaminoglycans were hydrolysed in 4.0M-HCl at 100°C for 8 h before hexosamine analysis. Degradation of hexosamine during hydrolysis was corrected for as described by Mason (1970). Protein was determined by the biuret method and nitrogen was determined as described by Koch & McMeekin (1924). Amino acid analysis (including hydroxyproline) was carried out with the Technicon AutoAnalyzer after hydrolysis under N_2 (6.0M-HCl at 110°C for 24 h). The amino sugars were well separated from amino acids by using the gradient described by Robinson & Smith (1967). Analytical ultracentrifugation was carried out with a Spinco model E ultracentrifuge as described by Gerber *et al.* (1960). Optical-rotation measurements were made with a Bendix N.P.L. automatic polarimeter.

The yields and compositions of the PPL, PPH and residue fractions from adult tracheal cartilage are given in Table 1. The recovery of 5.5% of the dry cartilage weight as PPL fraction is similar to the 5.8% recovered from the costal cartilage of a 46-year-old subject by Rosenberg *et al.* (1965) after two successive extractions of the tissue. The composition of tracheal-cartilage PPL fraction (Table 1) is similar to that reported for PPL fraction isolated from the costal cartilages of subjects aged 20–40 years (Buddecke, Kröz & Lanka, 1963), but it contains more chondroitin sulphate as judged from its galactosamine and uronic acid contents than does PPL fraction from costal cartilages of 51- and 70-year-old subjects (Pedrini, 1969; Pedrini & Pedrini-Mille, 1968). Apart from a considerably higher glycine content the amino acid composition of the tracheal-cartilage PPL fraction is also similar to that reported for PPL fraction isolated from the costal cartilages of 51- and 70-year-old subjects. Although glycine is a prominent component ($21.2\mu\text{mol}/100\mu\text{mol}$ of amino acid) of the peptide stub attached to chondroitin sulphate isolated from papain digests of tracheal cartilage, serine is equally prominent ($21.6\mu\text{mol}/100\mu\text{mol}$). The high glycine content of the PPL

Table 1. *Yields and chemical compositions of adult tracheal-cartilage proteoglycans*

(a) Yield (g/100g dry wt. of protein-polysaccharide)			
	PPL fraction	PPH fraction	Residue fraction
Yield (g/100g wt. of cartilage) ...	5.52	11.40	64.00
Galactosamine (free base)	13.3	3.3	1.6
Glucosamine (free base)	7.7	4.4	2.0
Glucuronic acid (lactone)	11.6	2.7	1.1
Protein	29.4	66.0	—
Nitrogen	5.2	7.0	—
Hydroxyproline	0.2	1.9	—
$[\alpha]_D^{25}$	-27.0°	—	—
α_{20}^0	10.3S	—	—
(b) Amino acid composition* ($\mu\text{mol}/100\mu\text{mol}$ of amino acid residues)			
Amino acid	PPL fraction	PPH fraction	Residue fraction
Hydroxyproline	Trace	4.0	—
Aspartic acid	7.4	7.3	—
Threonine	6.4	4.6	—
Serine	6.6	4.9	—
Glutamic acid	12.7	10.6	—
Proline	7.8	6.4	—
Glycine	15.3	17.0	—
Alanine	7.4	7.3	—
Valine	6.6	5.5	—
Methionine	0.7	1.3	—
Isoleucine	2.8	3.0	—
Leucine	9.1	8.8	—
Tyrosine	3.8	3.4	—
Phenylalanine	3.7	3.9	—
Lysine	3.0	4.8	—
Histidine	1.9	2.1	—
Arginine	4.8	5.3	—

* No corrections were applied for amino acid losses during hydrolysis.

fraction is therefore probably not associated solely with its high chondroitin sulphate content.

Pedrinì (1969) has shown that adult costal-cartilage PPL fraction is not electrophoretically homogeneous and can be partially separated into two distinct protein-polysaccharides, one associated predominantly with chondroitin sulphate and the other with keratan sulphate. Thus although tracheal-cartilage PPL fraction sediments as a single peak in the ultracentrifuge it is likely to be a complex aggregate of protein-polysaccharides and possibly glycoproteins. The differences noted above in the hexuronate and galactosamine contents of PPL-fraction compounds prepared from different tissues of subjects of approximately the same age therefore probably reflect differences in the proportion of the chondroitin sulphate-protein moiety present in the respective complex. Similarly changes in the hexuronate and hexose contents and in the physical parameters of PPL fraction prepared from costal cartilage from subjects of different ages

(Rosenberg *et al.* 1965; Kröz & Buddecke, 1967) may be due to changes in the ratio of the two types of protein-polysaccharide forming the complex at different ages.

Although adult tracheal-cartilage PPL fraction constitutes only a small proportion of the tissue it contains 34.5% of the total galactosamine found in the PPL, PPH and residue fractions from the cartilage. By comparison it contains only 19.2% of the total glucosamine. The galactosamine and glucosamine contents are a reliable index to the proportion of chondroitin sulphate and keratan sulphate respectively present in the protein-polysaccharide preparations.

Tracheal-cartilage PPH fraction is certainly a heterogeneous preparation. Analytical ultracentrifugation showed that it separated into a rapidly sedimenting major peak (at 12590 rev./min) and a rather less prominent material that sedimented at high velocity (52640 rev./min). The fraction contains a high proportion of protein, and estimates

derived from its hydroxyproline content indicate that up to 14.5% of the preparation may be present as collagen. Although only a small proportion of the PPH-fraction dry weight is attributable to hexosamine the fraction contains 17.5% of the total galactosamine and 22.8% of the total glucosamine.

The low percentage composition of the tracheal-cartilage residue fraction with respect to galactosamine and glucosamine also tends to obscure the actual amount of glycosaminoglycan associated with the insoluble portion of the tissue. Of the total galactosamine found in the three tissue fractions 48.0% is associated with the residue. Moreover 58.0% of the total glucosamine is present in the residue fraction.

The distribution of galactosamine and glucosamine between the highly soluble (PPL), poorly soluble (PPH) and totally insoluble (residue) fractions of adult cartilage as determined in the present investigation indicates the relative association of chondroitin sulphate and keratan sulphate in the fractions. The localization of most of the keratan sulphate in the poorly soluble and totally insoluble fractions is of particular interest. Tsiganos & Muir (1969) have reported that, of the heterogeneous population of soluble protein-polysaccharides isolated from porcine laryngeal cartilage, larger molecules contain more keratan sulphate than smaller ones. One explanation put forward was that keratan sulphate-protein may hold together variable numbers of chondroitin sulphate-proteins, so forming larger complexes. The localization of a large part of the total keratan sulphate in the residue fraction of adult human cartilage suggests that it

may play a role in the formation of insoluble cartilage matrix. One way in which this could be achieved would be if keratan sulphate-proteins were to be involved in the formation of progressively larger macromolecular complexes by aggregation with other non-polysaccharide matrix proteins (cf. Hascall & Sajdera, 1969).

The author is indebted to the Medical Research Council for a Junior Research Fellowship held during the course of this work.

- Buddecke, E., Kröz, W. & Lanka, E. (1963). *Hoppe-Seyler's Z. physiol. Chem.* **331**, 196.
Gerber, B. R., Franklin, E. C. & Schubert, M. (1960). *J. biol. Chem.* **235**, 2870.
Hascall, V. C. & Sajdera, S. W. (1969). *J. biol. Chem.* **244**, 2384.
Koch, F. C. & McMeekin, T. L. (1924). *J. Am. chem. Soc.* **46**, 2066.
Kröz, W. & Buddecke, E. (1967). *Hoppe-Seyler's Z. physiol. Chem.* **348**, 665.
Mason, R. M. (1970). *Biochem. J.* (in the Press).
Mathews, M. B. & Glagov, S. (1966). *J. clin. Invest.* **45**, 1103.
Pedrini, V. (1969). *J. biol. Chem.* **244**, 1540.
Pedrini, V. & Pedrini-Mille, A. (1968). In *The Chemical Physiology of Mucopolysaccharides*, p. 139. Ed. by Quintarelli, G. London: J. and A. Churchill Ltd.
Robinson, D. S. & Smith, M. (1967). *Proc. 5th Technicon Symposium*, p. 57. Chertsey: Technicon Instrument Co.
Rosenberg, L., Johnson, B. & Schubert, M. (1965). *J. clin. Invest.* **44**, 1647.
Schubert, M. (1965). In *Methods in Carbohydrate Chemistry*, vol. 5, p. 106. Ed. by Whistler, R. L. London: Academic Press (Inc.) Ltd.
Tsiganos, C. P. & Muir, H. (1969). *Biochem. J.* **113**, 885.