Purification and structural characterization of a cartilage matrix protein

Mats PAULSSON and Dick HEINEGÅRD

Department of Physiological Chemistry, University of Lund, P.O. Box 750, S-220 07 Lund 7, Sweden

(Received 12 February 1981/Accepted 7 April 1981)

The cartilage matrix protein is a major non-collagenous protein in bovine cartilage. It was purified from a 5 M-guanidinium chloride extract of bovine tracheal cartilage by sequential CsCl-density-gradient centrifugation, gel chromatography in guanidinium chloride and differential precipitation. The molecular weight of the intact protein is 148 000, determined by sedimentation-equilibrium centrifugation. It was dissociated to three subunits of molecular weight 52 000 by reduction of disulphide bonds. The cartilage matrix protein was insoluble in low-salt solutions and behaved abnormally on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The content of cysteine was high, whereas the contents of aromatic amino acids were low. The carbohydrate content was 3.9% (w/w). Glycopeptides obtained after papain digestion were heterogeneous on gel chromatography. Asparagine/aspartic acid was enriched in the purified glycopeptides, indicating the presence of *N*-glycosidic linkages to protein.

Physical properties of cartilage depend on the molecular characteristics of its major constituents, collagen and proteoglycans. Probably even more important is the molecular organization of the tissue. Multiple intermolecular interactions, some of which have been characterized (Hardingham & Muir, 1972; Heinegård & Hascall, 1974; Caterson & Baker, 1978), are probably essential for the three-dimensional structure of the tissue matrix.

The cartilage matrix protein was first identified as a component in cartilage proteoglycan preparations that had been extracted by high-speed homogenization in a low-salt buffer and purified by mild techniques (Paulsson & Heinegård, 1979). It could be separated from proteoglycans when transferred to 4 M-guanidinium chloride. It was also extracted in good yield from cartilage by 4 M-guanidinium chloride.

There are relatively few non-collagenous proteins in cartilage. In both bovine tracheal and nasal cartilages the cartilage matrix protein is one of the quantitatively dominating proteins. Another major protein, the link protein, important in proteoglycan aggregate formation, is the only non-collagenous protein that has been studied in some detail (Baker & Caterson, 1979; Périn *et al.*, 1980). The object of the present study was to purify and structurally characterize the cartilage matrix protein, as part of the overall aim of understanding the function and organization of the cartilage matrix.

Experimental

Materials

Bovine tracheal rings from young animals were obtained directly from the slaughterhouse. The tracheal cartilage was freed from surrounding tissue and perichondrium, frozen in liquid N₂ and ground frozen in a Wiley mill. This procedure was completed within a few hours after slaughter. The ground cartilage was stored frozen at –20°C until used.

Preparation of cartilage matrix protein

Extraction. A 20 g portion of ground cartilage was suspended in 200 ml of ice-cold 5 M-guanidinium chloride/5 mM-sodium phosphate buffer, pH 7.4, containing 0.1 M-6-aminohexanoic acid, 10 mM-EDTA and 10 mM-*N*-ethylmaleimide. The extraction was allowed to proceed for 20 h at 4°C under continuous stirring. Unextracted material was removed by centrifugation at 25 000 *g*_{av} for 30 min.

CsCl-density-gradient centrifugation. The extract was adjusted to a density of 1.40 g/ml by addition of solid CsCl. It was then centrifuged at 35 000 rev./min (95 000 *g*, *r*_{av} 6.886 cm) for 48 h at 18°C in the MSE 8 × 25 ml aluminium angle rotor. Tubes were frozen by immersion in liquid N₂ and cut in three parts of equal volume, while still frozen. The top fraction was dialysed overnight at 4°C against 20 vol. of 4 M-guanidinium chloride/5 mM-sodium

phosphate buffer, pH 7.4, to remove most of the CsCl.

Gel chromatography. Portions (20 ml) of the top fraction from the CsCl gradient were chromatographed on a column (3.0 cm $\times$ 145 cm) of Sephadex G-200 eluted with 4 M-guanidinium chloride/5 mM-sodium phosphate buffer, pH 7.4. Fractions of volume 10 ml were collected. Fractions (pool 1, Fig. 2a) from two chromatograms were pooled and concentrated to 20 ml by ultrafiltration (Amicon PM-10, cut-off mol.wt. 10 000). The sample was chromatographed on a column (3.0 cm $\times$ 145 cm) of Sepharose CL-6B. Elution and fraction collection was done as described above.

Precipitation. The cartilage matrix protein was precipitated after Sepharose CL-6B chromatography. Pool 3 (Fig. 3a) was dialysed against 39 vol. of 5 mM-sodium phosphate buffer, pH 7.4, for 48 h, so that a concentration of 0.1 M-guanidinium chloride was reached. The precipitate was collected by centrifugation at 25 000 g_{av} for 30 min and washed by suspension in distilled water and re-centrifugation in cycles. The supernatant was dialysed against another 39 vol. of the phosphate buffer, and the precipitate formed was recovered as described above. All procedures were at 4°C. The final supernatant was extensively dialysed against distilled water and freeze-dried.

Preparation of subunits

A 15 mg portion of cartilage matrix protein was dissolved in 5 ml of 6 M-guanidinium chloride/0.10 M-Tris/HCl buffer, pH 8.0. Dithiothreitol was added to a concentration of 10 mM and the sample was incubated at 37°C for 5 h. The reduced protein was alkylated by addition of iodoacetic acid to a final concentration of 30 mM and incubation overnight in darkness. The sample was diluted to 4 M-guanidinium chloride by addition of 2.5 ml of distilled water and chromatographed on a column (3.0 cm $\times$ 145 cm) of Sephadex G-200 eluted with 4 M-guanidinium chloride/5 mM-sodium phosphate buffer, pH 7.4. Fractions containing subunits were pooled and the material was recovered by dialysis and freeze-drying.

Preparation of glycopeptides

Papain digestion. A 10.2 mg portion of cartilage matrix protein was suspended in 1 ml of 0.1 M-sodium phosphate buffer, pH 6.5, containing 5 mM-EDTA and 5 mM-dithiothreitol. Then 4 μ l of papain (type III; Sigma Chemical Co., St. Louis, MO, U.S.A.) (enzyme/substrate ratio 1:100) was added and the sample was incubated at 60°C for 36 h. After 12 h and 24 h another 4 μ l of papain and 50 μ l of 0.1 M-dithiothreitol in the digestion buffer was added. The digest was chromatographed on a column (0.8 cm $\times$ 250 cm) of Bio-Gel P-10 eluted

with 0.5 M-pyridinium acetate buffer, pH 6.5. Fractions of volume 1.1 ml were collected.

Gel electrophoresis

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was performed as described by Neville (1971). The bottom fraction from the CsCl-density-gradient centrifugation was pretreated with chondroitinase ABC (Paulsson & Heinegård, 1979) to avoid collapse of the stacking gel. Samples were reduced with 2-mercaptoethanol unless otherwise stated. Purification was followed by electrophoresis of samples representing equal proportions of each pool. This was done on gels 8.0 $\times$ 2.5%, where the first numeral (T) designates the total amount of monomer (% w/v) and the second numeral (C) designates the amount of *NN'*-methylenebisacrylamide, expressed as a fraction (% w/w) of total monomer in the gel casting solution. A Ferguson (1964) plot was obtained by comparison of the relative mobilities of the major subunit of the cartilage matrix protein with those of reference proteins (high-molecular-weight and low-molecular-weight standard proteins; Pharmacia Fine Chemicals, Uppsala, Sweden) on gels 5–11 $\times$ 2.5%. Gels were stained for protein with Kenacid Blue R (BDH Chemicals, Poole, Dorset, U.K.).

Analytical ultracentrifugation

Preparation of samples. All samples were dissolved in 6 M-guanidinium chloride (Ultrapure; Schwarz-Mann, Orangeburg, NY, U.S.A.)/5 mM-Tris/HCl buffer, pH 7.0. In all experiments a stock solution of the highest sample concentration was dialysed for 20–40 h against 25 vol. or more of the solvent. Weighed dilutions were made with the diffusate.

Determination of molecular weights. An MSE Centriscan 75 analytical ultracentrifuge equipped with a photoelectric scanner and a 280 nm filter was used. Samples, giving 4 mm column height, were layered on fluorocarbon oil (FC 70; 3M, Minneapolis, MN, U.S.A.). High-speed equilibria were established essentially as described by Yphantis (1964). Samples with initial concentrations ranging from 0.1 mg/ml to 0.8 mg/ml were centrifuged at two or three different rotor speeds (cartilage matrix protein, 18 000, 20 000 and 22 000 rev./min; cartilage-matrix-protein subunits, 28 000 and 34 000 rev./min) at 20°C.

Determination of sedimentation coefficients. Samples containing 0.8–3.2 mg of cartilage matrix protein or subunits/ml were centrifuged at 59 000 rev./min at 20°C. The photoelectric scanner was used at 280 nm. The position of the boundary was determined as the *r* co-ordinate of the half-height of the tracing. The sedimentation was

followed to the bottom of the cell, corresponding to a minimum distance of 8 mm.

Determination of the apparent partial specific volume. The apparent partial specific volume of cartilage matrix protein in 6M-guanidinium chloride was experimentally determined by the $^2\text{H}_2\text{O}$ method (Thomas & Edelman, 1971) and calculated from the amino acid composition as described by Lee & Timasheff (1974). With both procedures a value of 0.730 ml/g was obtained.

Analytical procedures

Protein contents were determined as the absorbance of solutions at 280 nm.

Amino acid composition was determined by using an automatic amino acid analyser after hydrolysis in 6M-HCl under argon at 110°C for 24 and 72 h. Cysteine and methionine were determined as cysteic acid and methionine sulphone respectively, after performic acid oxidation. Tryptophan was determined by the spectroscopic method of Edelhoch (1967).

Hexosamines were determined by using an automatic amino acid analyser after hydrolysis in 4M-HCl under argon at 100°C for 10 h.

Hexuronic acid in column effluents was determined by an automated version (Heinegård, 1973) of the carbazole reaction (Bitter & Muir, 1962).

Analytical separations of neutral sugars were performed by a modification (S. Lohmander, personal communication) of the borate complex ion-exchange procedure of Walborg & Kondo (1970). Samples were hydrolysed in 2M-trifluoroacetic acid for 3 h at 100°C. Neutral sugars in column effluents were determined by an automated version of the orcinol/ H_2SO_4 procedure, essentially as described by Kesler (1967).

Sialic acid was determined by the periodate/resorcinol procedure of Jourdain *et al.* (1971). When column effluents were analysed an automated version was used (Lohmander *et al.*, 1980).

Results

Preparative procedure

Proteoglycan contents and absorbance at 280 nm of the fractions from the CsCl-density-gradient-equilibrium centrifugation were determined (Fig. 1a). The distribution of proteins in the fractions was studied by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Fig. 1b). The proteoglycans were almost quantitatively recovered in the bottom fraction, as indicated by the uronic acid. Most of the 280 nm absorbance detected in the bottom two fractions was due to the proteinase inhibitors and contaminations in the CsCl used. The top fraction contained very little uronic acid, but most of the non-proteoglycan protein could be

Fig. 1. (a) CsCl-density-gradient centrifugation of the cartilage extract and (b) sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the fractions obtained. Experimental details are given in the text. (a) The samples were diluted with 9 vol. of distilled water before the determination of uronic acid and protein contents. —, A_{530} (carbazole reaction); —, A_{280} ; ●—●, density (g/ml). (b) The sample from fraction 1 was digested with chondroitinase ABC to remove chondroitin sulphate chains before electrophoresis. All samples were treated with 2-mercaptoethanol.

recovered from this fraction. Among the proteins in this fraction, the subunits of the cartilage matrix protein showed as a prominent band with an apparent molecular weight of about 60 000.

Samples of the top fraction were chromatographed on a Sephadex G-200 column eluted with 4M-guanidinium chloride/5 mM-sodium phosphate buffer, pH 7.4 (Fig. 2a). The protein was eluted in three major peaks. The middle peak contained more sialic acid, compared with the other peaks, indicating a higher oligosaccharide content. Fractions corresponding to the peaks were pooled as indicated, and samples were reduced and analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Fig. 2b). The major portion of the cartilage matrix protein was present in the first pool and was eluted in or close to the void volume. This fraction also contained a number of other high-molecular-weight proteins. Some of these appeared to be of collagenous nature, as revealed by the reddish staining obtained with Kenacid Blue R (McCormick *et al.*, 1979). The second pool contained a predominant protein appearing as a broad diffuse band with an apparent molecular weight of 70 000–90 000. The electrophoretic mobility and polydispersity are similar to the behaviour of the hyaluronic acid-binding region fragment of cartilage proteoglycans, isolated by trypsin digestion of proteoglycan aggregates (Heinegård & Hascall, 1974). This material reacted strongly with an antiserum directed against the isolated hyaluronic acid-binding region (results not shown) and therefore appears to represent free hyaluronic acid-binding region, probably fragmented from the cartilage proteoglycans by the physiological proteolytic catabolism. Caputo *et al.* (1980) have isolated a molecule with similar electrophoretic mobility from the Swarm rat chondrosarcoma. The third pool contains two proteins with apparent molecular weights of 49 500 and 45 500, mobilities characteristic of the link proteins (Keiser *et al.*, 1972). A faint band present below the 45 500-mol.wt. link protein probably represents the third link protein, described by Baker & Caterson (1979). In addition, an intensely stained protein with an apparent molecular weight of 35 000 is present.

After concentration by ultrafiltration the first pool was rechromatographed on Sepharose CL-6B eluted with 4M-guanidinium chloride/5 mM-sodium phosphate buffer, pH 7.4 (Fig. 3a). The chromatogram showed three distinct peaks and a leading shoulder on the intermediate peak. Fractions corresponding to the peaks were pooled and equal portions were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Fig. 3b). The cartilage matrix protein was eluted in the third pool, together with a protein that stained like collagen. The first two pools contained high-molecular-weight material,

Fig. 2. (a) Sephadex G-200 gel chromatography of the top fraction from the CsCl-density-gradient centrifugation of the cartilage extract and (b) sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of pooled fractions obtained

Experimental details are given in the text. (a) A 20 ml portion of the top fraction (Fig. 1a) was dialysed against 4M-guanidinium chloride/5 mM-sodium phosphate buffer, pH 7.4, and applied to the column. The chromatography was performed as described in the text. Fractions were pooled as indicated by the bars. —, A_{280} ; ----, A_{620} (sialic acid). V_0 , Void volume; V_t , total volume. (b) The samples correspond to the pools from the Sephadex G-200 chromatogram as indicated by numbers. The samples were treated with 2-mercaptoethanol before electrophoresis.

Fig. 3. (a) Sepharose CL-6B gel chromatography of the cartilage-matrix-protein-containing pool from the Sephadex G-200 chromatography and (b) sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of pooled fractions obtained

Experimental details are given in the text. (a) The first pools from two Sephadex G-200 chromatograms (Fig. 2a) were concentrated by ultrafiltration and applied to the column. Elution and fraction collection was as described in the text. Fractions were pooled as indicated by the bars. —, A_{280} ; ----, A_{620} (sialic acid). V_0 , Void volume; V_t , total volume. (b) The samples correspond to the pools from the Sepharose CL-6B chromato-

some of which did not enter the 8% polyacrylamide gels.

The two proteins present in the third pool of the Sepharose CL-6B chromatogram differed significantly in their solubility at lowered ionic strength. Most of the cartilage matrix protein was precipitated when the guanidinium chloride concentration was lowered to 0.1M by dialysis, whereas all of the other protein remained in solution (Fig. 4a). The small amount of precipitate formed when the guanidinium chloride concentration was lowered to 2.5 mM was a mixture of the two proteins (Fig. 4b). The supernatant contained virtually only the other protein (Fig. 4c). After being desalted the fractions were freeze-dried. Approx. 17mg of the first precipitate, 3mg of the second precipitate and 29mg of soluble protein were obtained per 10g of cartilage wet weight.

Homogeneity

The purity of the preparation of cartilage matrix protein was determined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis on gels $7 \times 2.5\%$ and Sepharose CL-6B gel chromatography in 1% sodium dodecyl sulphate/50 mM-sodium bicarbonate buffer, pH 7.5 (results not shown). On electrophoresis the protein migrated as an apparently homogeneous band and could be quantitatively shifted to the position of the subunits when reduced, which was taken as a criterion of homogeneity. On visual inspection of the stained gel the subunits displayed some microheterogeneity. Two very closely spaced bands could be seen. The more intensely staining band had the higher mobility. These findings show that the cartilage matrix protein is an oligomer, most probably disulphide-bonded. Both the intact and the reduced matrix protein were eluted as single peaks on Sepharose CL-6B chromatography in the presence of sodium dodecyl sulphate (results not shown). Neither low-molecular-weight nor high-molecular-weight contaminants were present.

Electrophoretic behaviour

In previous work (Paulsson & Heinegård, 1979) we have used sodium dodecyl sulphate/polyacrylamide-gel electrophoresis on gels $8 \times 2.5\%$ to assign apparent molecular weights to the subunits of the cartilage matrix protein. In the present study it was found that the apparent molecular weights could vary considerably with acrylamide concentration.

gram as indicated by numbers. The samples were treated with 2-mercaptoethanol before electrophoresis.

Fig. 4. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of fractions obtained by differential precipitation of the cartilage matrix protein

Experimental details are given in the text. The samples were treated with 2-mercaptoethanol before electrophoresis. (a) First precipitate; (b) second precipitate; (c) soluble protein.

As a control, a Ferguson (1964) plot of $\log R_F$ versus the gel concentration was constructed for the major subunit of the cartilage matrix protein and for a variety of reference proteins (Fig. 5). The relative mobility of the matrix protein is not solely a function of its molecular weight, since the plot does not extrapolate to the point of convergence for the reference proteins. The major subunit of the cartilage matrix protein forms a sodium dodecyl sulphate complex of higher apparent free electrophoretic mobility than the reference proteins used (Fig. 5). It is possible that the cartilage matrix protein binds sodium dodecyl sulphate in excess of the 1.4 g/g bound by most proteins (Reynolds & Tanford, 1970). It should be stressed that the abnormal migration on electrophoresis invalidates attempts to determine molecular weights of the cartilage matrix protein subunits by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis.

Determination of molecular weight

Results obtained from several sedimentation-

Fig. 5. Ferguson plot of $\log R_F$ versus gel concentration (T%) obtained by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the subunits of the cartilage matrix protein and reference proteins

Experimental details are given in the text. For each electrophoretic run approx. $1 \mu\text{g}$ of each protein was used. All samples were treated with 2-mercaptoethanol before electrophoresis. Curve a, lactate dehydrogenase subunit (mol.wt. 36 000); curve b, ovalbumin (mol.wt. 43 000); curve c, catalase subunit (mol.wt. 60 000); curve d, serum albumin (mol.wt. 67 000); curve e, phosphorylase b (mol.wt. 94 000). $\bigcirc$ — $\bigcirc$, Cartilage matrix protein subunits.

equilibrium centrifugations are summarized in Fig. 6. By extrapolation to zero concentration the molecular weight of the cartilage matrix protein was determined to be 148 000 (± 2000) and that of the subunits to be 52 000 (± 2000). The apparent molecular weights exhibit a negative concentration-dependence, more markedly at higher speeds. This is expected, as the actual protein concentration at equilibrium is then higher. Plots of $\ln c$ versus r^2 were linear, indicating the absence of polydispersity and aggregation.

Determination of sedimentation coefficients

Results from velocity sedimentations of cartilage matrix protein and its subunits are depicted in Fig. 7. The values of $s_{20,w}^0$ were 4.41 S and 2.01 S respectively. The values of $s_{20,w}$ exhibit a negative concentration-dependence.

Protein composition

The amino acid composition (Table 1) was calculated from results obtained by hydrolysis for 24 and 72 h. The recoveries of serine and threonine were extrapolated to zero time. For other amino acids the highest value of the two was chosen. The cartilage

Fig. 6. *Sedimentation-equilibrium centrifugation of the cartilage matrix protein and its subunits*
Experimental details are given in the text. Upper part (cartilage matrix protein): ●—●, 22 000 rev./min; ■···■, 20 000 rev./min; ○—○, 18 000 rev./min. Lower part (cartilage matrix protein subunits): ●—●, 34 000 rev./min; ○—○, 28 000 rev./min.

matrix protein was found to be enriched in glutamic acid/glutamine and aspartic acid/asparagine. The contents of tryptophan and tyrosine are low and that of phenylalanine is moderate, as is also indicated by the low molar u.v. absorbance. The cysteine content is relatively high. Hydroxyproline could not be detected, indicating that cartilage matrix protein is non-collagenous.

Carbohydrate composition

Attempts to determine hexosamine contents in hydrolysates of the intact protein were unsuccessful, as the relatively small amounts of hexosamine were incompletely separated from non-hydrolysed peptides. Therefore cartilage matrix protein was digested with papain, and the glycopeptides were purified by

Fig. 7. *Sedimentation-velocity centrifugation of the cartilage matrix protein and its subunits*
Experimental details are given in the text. ●, Cartilage matrix protein; ○, cartilage matrix protein subunits.

Table 1. *Amino acid composition of the cartilage matrix protein*
Experimental details are given in the text.

	Composition (residues/1000 residues)
Asx	92.8
Thr	47.5
Ser	82.5
Glx	112.1
Pro	36.3
Gly	77.5
Ala	83.9
Cys	26.6
Val	88.2
Met	13.9
Ile	45.9
Leu	82.4
Tyr	19.1
Phe	39.5
His	20.0
Lys	63.7
Arg	65.3
Trp	2.7
Hyp	Not detected

Bio-Gel P-10 gel chromatography (Fig. 8). The heterogeneous population of glycopeptides was eluted in the included volume of the column. Two components were partially resolved. They were

Table 2. *Carbohydrate composition of the cartilage matrix protein*
Experimental details are given in the text.

	Composition			
	Intact protein		Glycopeptides	
	(nmol/mg)	(% of total)	(nmol/mg original wt.)	(% of total)
Amino acids*	7906.1	100.0	647.0	100.0
Asx	767.7	9.7	115.5	17.8
Ser	682.0	8.6	50.8	7.9
Thr	392.8	5.0	38.9	6.0
GlcN			85.5	
GalN			34.1	
Man			74.1	
Gal			9.5	
Fuc			15.3	
Xyl			<0.4	
Glc			Trace	
AcNeu			Not detected	

* The data do not take into account the contents of cysteine, methionine and tryptophan.

Fig. 8. *Bio-Gel P-10 gel chromatography of the papain digest of the cartilage matrix protein*

Experimental details are given in the text. The column was calibrated with hyaluronic acid oligosaccharides (Hascall & Heinegård, 1974) containing the numbers of monosaccharide residues indicated in the Figure. UA indicates the elution position of glucuronic acid. The carbazole peak at V_t is due to the dithiothreitol present in the sample. The glycopeptides were pooled as indicated by the bar. —, A_{420} (orcinol reaction); ---, A_{530} (carbazole reaction). V_0 , Void volume; V_t , total volume.

pooled as indicated, recovered in a quantitative manner and used for quantitative determination of hexosamine and neutral sugar. The material that was eluted in the void volume of the column reacted in the carbazole procedure. It probably represents glycosaminoglycans from a small contamination (1–2%, w/w) of proteoglycans in the preparation.

The contents of sugars and of relevant amino acids in the glycopeptides are given in Table 2. The total carbohydrate content of cartilage matrix protein was 3.9% (w/w). The predominating carbohydrates are glucosamine and mannose. This, taken together with the fact that aspartic acid/asparagine is markedly enriched in the glycopeptides, indicates that a major proportion of the oligosaccharides probably are of the high-mannose type and are bound to protein by an *N*-glycosidic linkage between glucosamine and asparagine (Kornfeld & Kornfeld, 1980). There is, however, a considerable amount of galactosamine present, a sugar not normally found in this type of oligosaccharide.

Discussion

Structural proteins of the connective-tissue matrix interact with various other tissue components. For the detailed characterization of such interactions the participating molecules are needed in pure form. The insolubility of many of the matrix molecules makes their purification and characterization difficult and often necessitates the use of chaotropic solvents. Some of the matrix molecules, e.g. the proteoglycans, the link proteins and presumably the cartilage matrix protein (Paulsson & Heinegård, 1979), do, however, recover their ability to interact when renatured. Therefore it is possible to extract the molecules with guanidinium chloride, purify them with the use of chaotropic solvents and then study their interactions when renatured.

The early attempts to purify the cartilage matrix protein were hampered by pronounced non-specific aggregation of proteins. This aggregation could be

prevented by including 10 mM-*N*-ethylmaleimide, to block free thiol groups, in the extraction buffer. It is therefore probable that the aggregation was due to disulphide exchange between proteins in the denatured state (Tanford, 1961).

The cartilage matrix protein is a non-collagenous protein consisting of polypeptide chains that migrate as two closely spaced bands on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Uneven distribution of the oligosaccharides among the protein subunits, however, would easily explain the microheterogeneity observed. Intact disulphide bonds are essential for the oligomeric structure. The molecular weights obtained for the cartilage matrix protein and its subunits, 148 000 (± 2000) and 52 000 (± 2000) respectively, are consistent with a trimeric structure of the intact protein.

It is as yet difficult to envisage the exact role of the cartilage matrix protein in the function of the tissue. From recoveries of purified protein, it can be estimated to constitute 1–2% of the tissue dry weight. This, taken together with its marked tendency to co-fractionate with proteoglycans (Paulsson & Heinegård, 1979), indicates that it is an integral part of the cartilage extracellular matrix.

This work was supported by the Swedish Medical Research Council (grants 05668 and 5739), Kock's Stiftelser, Konung Gustaf V:s 80-årsfond, Alfred Österlunds Stiftelse, Svenska Sällskapet för Medicinsk Forskning and by the Medical Faculty, University of Lund. M. P. was the recipient of a graduate training grant from the Medical Faculty, University of Lund. We are grateful to Dr. S. Lohmander for performing neutral sugar analysis and to Dr. J. Sheehan for valuable advice on analytical ultracentrifugation.

References

- Baker, J. & Caterson, B. (1979) *J. Biol. Chem.* **254**, 2387–2393
- Bitter, T. & Muir, H. (1962) *Anal. Biochem.* **4**, 330–334
- Caputo, C. B., MacCallum, D. K., Kimura, J. H., Schrode, J. & Hascall, V. C. (1980) *Arch. Biochem. Biophys.* **204**, 220–233
- Caterson, B. & Baker, J. (1978) *Biochem. Biophys. Res. Commun.* **80**, 496–503
- Edelhoch, H. (1967) *Biochemistry* **6**, 1948–1954
- Ferguson, K. A. (1964) *Metab. Clin. Exp.* **13**, 985–1002
- Hardingham, T. E. & Muir, H. (1972) *Biochim. Biophys. Acta* **279**, 401–405
- Hascall, V. C. & Heinegård, D. (1974) *J. Biol. Chem.* **249**, 4242–4249
- Heinegård, D. (1973) *Chem. Scr.* **4**, 199–201
- Heinegård, D. & Hascall, V. C. (1974) *J. Biol. Chem.* **249**, 4250–4256
- Jourdain, G. W., Dean, L. & Roseman, S. (1971) *J. Biol. Chem.* **246**, 430–435
- Keiser, H., Shulman, H. J. & Sandson, J. I. (1972) *Biochem. J.* **126**, 163–169
- Kesler, R. B. (1967) *Anal. Chem.* **39**, 1416–1422
- Kornfeld, R. & Kornfeld, S. (1980) in *The Biochemistry of Glycoproteins and Proteoglycans* (Lennarz, W. J., ed.), pp. 1–34, Plenum Press, New York
- Lee, J. C. & Timasheff, S. N. (1974) *Arch. Biochem. Biophys.* **165**, 268–273
- Lohmander, L. S., De Luca, S., Nilsson, B., Hascall, V. C., Caputo, C. B., Kimura, J. H. & Heinegård, D. (1980) *J. Biol. Chem.* **255**, 6084–6091
- McCormick, P. J., Chandrasekhar, S. & Millis, A. J. T. (1979) *Anal. Biochem.* **97**, 359–366
- Neville, D. (1971) *J. Biol. Chem.* **246**, 6328–6334
- Paulsson, M. & Heinegård, D. (1979) *Biochem. J.* **183**, 539–545
- Périn, J.-P., Bonnet, F., Pizon, V., Jollès, J. & Jollès, P. (1980) *FEBS Lett.* **119**, 333–336
- Reynolds, J. A. & Tanford, C. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **66**, 1002–1007
- Tanford, C. (1961) *Physical Chemistry of Macromolecules*, pp. 638–639, John Wiley and Sons, New York
- Thomas, J. O. & Edelstein, S. J. (1971) *Biochemistry* **10**, 477–482
- Walborg, E. & Kondo, L. E. (1970) *Anal. Biochem.* **37**, 320–329
- Yphantis, D. A. (1964) *Biochemistry* **3**, 297–317