*Biochem. J.* (1983) **213**, 355–362
*Printed in Great Britain*



# Isolation and characterization of high-buoyant-density proteoglycans from bovine femoral-head cartilage

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(*Received 15 February 1983/Accepted 19 April 1983*)

Proteoglycans were extracted from bovine (15–18 months old) femoral-head cartilage. The heterogeneity of the A1D1 proteoglycan fraction was examined by gel chromatography, sedimentation velocity, sucrose rate-zonal centrifugation and $Cs_2SO_4$ isopycnic centrifugation. In all cases polydisperse but unimodal distributions were obtained. Chemical analysis of the preparation yielded a galactosamine/glucosamine molar ratio of 7:1, and $^{13}C$ n.m.r. spectroscopy showed that the chondroitin sulphate comprised equal proportions of the 4- and 6-sulphate isomers. Gel chromatography of a papain and Pronase digest of the proteoglycan indicated that the chondroitin sulphate chains had a $\bar{M}_n$ of approx. 10500. The mean buoyant density of the proteoglycan in pure $Cs_2SO_4$ was 1.46 g/ml. Physical characterization of the proteoglycan preparation in 4 M-guanidine hydrochloride, pH 7.4, by using conventional light-scattering gave a radius of gyration of 42 nm and a $\bar{M}_w$ of $0.96 \times 10^6$. Quasi-elastic light-scattering in the same solvent yielded a translational diffusion coefficient, $D^0_{20,w}$, of $5.41 \times 10^{-8}\,cm^2 \cdot s^{-1}$, and ultracentrifugation gave a sedimentation coefficient, $s^0_{20,w}$, of 12.0S. Thus from sedimentation-diffusion studies a $\bar{M}_w$ of $1.36 \times 10^6$ was calculated. The possible origins for the differences in the two molecular-weight estimates are discussed. It is concluded that the high-buoyant-density proteoglycans from bovine articular cartilage are significantly smaller than those from bovine nasal septum, and that this is largely due to the smaller size of their chondroitin sulphate chains.

Proteoglycans are responsible for the water-retention and compressive-strength properties of cartilage and occur mainly as proteoglycan aggregates. The aggregates form as a result of the binding of proteoglycan monomers to the long-chain polysaccharide hyaluronate (Hardingham & Muir, 1972), an interaction which is further stabilized by link-protein molecules (Hardingham, 1979).

Most of the comprehensive studies on which our understanding of the structure (for reviews see Paulsson & Heinegård, 1983; Hascall & Kimura, 1982) and the physico-chemical parameters of proteoglycan molecules is based have been performed on the non-articular bovine nasal-septum and pig laryngeal cartilages. These include light-scattering studies of bovine nasal-cartilage proteoglycans in 4 M-guanidine hydrochloride (Pasternack *et al.*, 1974; Kitchen & Cleland, 1978), light-scattering studies of pig laryngeal-cartilage proteoglycans in NaCl solutions (Sheehan *et al.*, 1978) and a combined quasi-elastic light-scattering and ultracentrifugal investigation of bovine nasal-septum proteoglycans in 0.15 M-NaCl (Reihanian & Jamieson, 1979). These studies all indicate that the proteoglycan monomer has an $\bar{M}_w$ of $2.3 \times 10^6$ or higher.

The chemical structures have been studied for proteoglycans from articular cartilages of bovine (Swann *et al.*, 1979; Garg & Swann, 1981; Thonar & Sweet, 1981), human (Bayliss & Ali, 1978; Roughley & White, 1980), baboon (Stanescu & Sweet, 1981) and dog (Inerot *et al.*, 1978) origins. There have also been elegant chemical (Franzén *et al.*, 1981) and immuno-electron-microscopic (Poole *et al.*, 1982) studies of the variation of proteoglycan apparent sizes, composition and distribution as a function of distance from the articular surface. However, little physico-chemical or hydrodynamic data have been presented for proteoglycans from articular cartilages. Swann *et al.* (1979) have studied the major high-density proteoglycan of bovine articular cartilage by equilibrium centrifugation, and Shogren *et al.* (1982) and Bullimore *et al.* (1981) have examined by light-

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scattering the proteoglycans of chicken limb bud and human articular cartilage respectively.

In the present study the physico-chemical parameters of the high-buoyant-density proteoglycans from bovine articular cartilage are examined as a prelude to further studies on proteoglycan aggregatability and aggregate formation in articular cartilage.

## Experimental

### *Materials*

Guanidine hydrochloride (practical grade) was obtained from Sigma (Poole, Dorset, U.K.) and further purified by treatment of 8M stock solutions with activated charcoal. Benzamidine hydrochloride and papain (twice crystallized) were also from Sigma. CsCl (AnalaR), 6-aminohexanoic acid and orcinol were from BDH, Poole, Dorset, U.K. $Cs_2SO_4$, carbazole and Pronase (*Streptomyces griseus*) were from Koch–Light, Haverhill, Suffolk, U.K. Sephadex G-200 and Sephacryl S-500 were obtained from Pharmacia, Uppsala, Sweden. [$^3$H]-Acetic anhydride (500Ci/mol) was from Amersham International, Amersham, Bucks., U.K.

### *Analytical methods*

Amino acids and hexosamines were determined with a LKB 4101 amino acid analyser (LKB Biochrom Ltd., Cambridge, U.K.) after hydrolysis of the proteoglycan samples in 6M-HCl for 24h at 105°C, and in 4M-HCl for 10h at 105°C, respectively (Heinegård & Hascall, 1979). All hydrolyses were performed under $N_2$.

Uronic acid content of fractions from density-gradient centrifugation and gel chromatography was determined by the orcinol (Brown, 1946) or carbazole (Bitter & Muir, 1962) methods, with glucuronolactone as standard.

### *Proteoglycan isolation*

Bovine femoral heads and nasal septum from 15–18-month-old animals, of both sexes, were obtained from the abattoir and frozen at −30°C. Surrounding tissue was removed from the frozen heads, which were then washed briefly in water, and the cartilage was finely shaved with a Stanley Surform woodwork tool. The slivers were suspended in 10 times their weight of cold 4M-guanidine hydrochloride/0.05M-sodium acetate, pH5.8, containing the proteinase inhibitors 0.01M-EDTA, 0.005M-benzamidine hydrochloride and 0.1M-6-aminohexanoic acid (Oegema *et al.*, 1975). Extraction was performed at 6°C for 90h with stirring. The extract was filtered in the cold through glass wool. The extract and washings were dialysed against 7vol. of 0.05M-sodium acetate, pH5.8, plus proteinase inhibitors (as above).

The density of this non-diffusible material, which contained approx. 2mg of proteoglycan/ml, was adjusted to 1.61g/ml by the addition of solid CsCl. Equilibrium density-gradient centrifugation under 'associative' conditions was performed in an 8× 50ml Al angle-head rotor in a MSE Prepspin 50 centrifuge at $87000g_{av.}$ for 72h at 15°C. After centrifugation, the contents of the bottom third of each tube (A1 fraction, 87% of the uronic acid), containing the proteoglycan aggregates, were removed by siphoning from the bottom of the tube. The solution was adjusted to 4M in guanidine hydrochloride by addition of 7M-guanidine hydrochloride/0.05M-sodium acetate, pH5.8, with proteinase inhibitors, as above. The density was adjusted to 1.50g/ml by further addition of solid CsCl. 'Dissociative' density-gradient centrifugation was performed under the centrifugal conditions described above. Fractions (A1D1, approx. 90% of the uronic acid) containing the proteoglycans, with densities $\geqslant$1.59g/ml, were pooled. Orcinol assay of a papain digest of the residue showed that 91% of the uronic acid was extracted from the total cartilage.

### *$^3$H labelling of proteoglycan*

Proteoglycan aggregates (A1 fraction, approx. 130mg in 65ml) in 0.005M-sodium phosphate, pH7.4, containing proteinase inhibitors were labelled by addition of 15mCi of [$^3$H]acetic anhydride. After 5min stirring, the solution was extensively dialysed against 0.005M-phosphate, pH7.4, plus inhibitors and then concentrated by ultrafiltration. The concentrate was increased to 4M in guanidine hydrochloride and the density was adjusted to 1.49g/ml by addition of solid CsCl. After density-gradient centrifugation under 'dissociative' conditions (see under 'Proteoglycan isolation'), the proteoglycan-containing fractions, with densities >1.58g/ml, were pooled (A1D1). The incorporation of $^3$H was approx. $5\times10^5$c.p.m./mg of proteoglycan, corresponding to approx. 0.8 acetyl group per proteoglycan molecule.

### *Preparation of chondroitin sulphate chains*

Freeze-dried proteoglycan (18mg) was digested with papain for 24h at 65°C in 0.2M-NaCl/0.05M-phosphate/0.01M-cysteine hydrochloride/0.05M-EDTA, pH7, followed by Pronase for 24h at 37°C in 0.067M-phosphate, pH7.4. Gel chromatography of the chains was performed on a Sephadex G-200 column (for details see legend to Fig. 1) calibrated with glucuronolactone and Blue Dextran.

### *Chromatography*

Gel chromatography of proteoglycans was performed on a Sephacryl S-500 column (131cm× 1.1cm) in 4M-guanidine hydrochloride, pH7.4, with proteinase inhibitors. The column was calibrated

with glucuronolactone and proteoglycan aggregates (run in 0.5 M-guanidine hydrochloride, pH 7.4).

*Ultracentrifugation*

Sedimentation-velocity measurements were performed in both 4 M- and 0.5 M-guanidine hydrochloride, pH 7.4, on a MSE Centriscan 75 ultracentrifuge at $30000 g_{av.}$, as described in Sheehan *et al.* (1978).

*Analytical isopycnic ultracentrifugation*

Proteoglycans were subjected to analytical isopycnic density-gradient centrifugation, after dialysis first against distilled water and then against the appropriate caesium salt, as in the procedure of Sheehan *et al.* (1981).

*Sucrose rate-zonal centrifugation*

Linear gradients were formed in 5.5 ml tubes from 10% and 50% (w/w) sucrose solutions in 0.5 M-NaCl/0.005 M-phosphate, pH 7.0, by using a two-chambered mixer. Samples (100 $\mu$l) in 0.5 M-NaCl/0.005 M-phosphate, pH 7.0, were layered on top of the gradients. Centrifugation was performed in a MSE Prepspin 50 centrifuge at $185000 g_{av.}$ and 20°C for 3.5 h in a swing-out rotor (6 × 5.5 ml, Al). Tubes were unloaded from the bottom via a capillary tube. Fractions (150 $\mu$l) were counted for $^3$H radioactivity (procedure of Nieduszynski *et al.*, 1980) and the sucrose gradient was determined with a refractometer (Bellingham and Stanley, London N.5, U.K.).

*Light-scattering*

Light-scattering intensities were measured on proteoglycans in 4 M-guanidine hydrochloride, pH 7.4, as described by Sheehan *et al.* (1978), and data were evaluated by the procedure of Tomimatsu *et al.* (1968).

Quasi-elastic light-scattering measurements were performed in a Malvern Molecular Analyser System 4300 (Precision Devices and Systems Ltd., Malvern, Worcs., U.K.) equipped with a 5 mW He–Ne laser (Coherent Radiation Ltd., Palo Alto, CA, U.S.A.). Light ($\lambda$ *in vacuo* = 632.8 nm) was focused at the centre of the sample in a small glass cuvette placed in an optical index-matching bath, which was temperature controlled at 25°C.

The photon-count autocorrelation function $g^2(\tau)$ of the scattered light (at angles between 135° and 30°) was obtained by analysis of the digitized output of the photomultiplier tube by using a Malvern type K7023 48 channel correlator and RR95 Clipping and Scaling Unit, and data were analysed on-line with a Malvern MPD 7023S microcomputer. The experimentally observed correlation function is related to the translational diffusion coefficient ($D_T$) by

$$g^2(\tau) = 1 + A \exp(-2D_T K^2 \tau)$$

where $K$ is the scattering vector

$$\frac{4\pi n}{\lambda_0} \sin^2\left(\frac{\theta}{2}\right),$$

$n$ is the refractive index of the scattering solution, $\lambda_0$ is the wavelength of the He–Ne source *in vacuo*, and $\theta$ the angle between the incident laser beam and the photomultiplier axis. The translational diffusion coefficient ($D_T$) can thus be obtained from the slope of $\ln[g^2(\tau)]$ versus $\tau$. For a polydisperse sample the correlation function becomes a sum of exponential terms, and the $\ln[g^2(\tau)]$-versus-$\tau$ line will deviate from linearity. These data were fitted to a quadratic function as derived by Pusey (1973), and the diffusion coefficient was calculated from the linear term of the quadratic fit. The diffusion coefficient extrapolated to zero angle was determined for a number of concentrations, and the translational diffusion coefficient was determined from the extrapolation to zero concentration.

*Differential refractive increment*

The concentration of a stock solution of proteoglycan was determined from an extensively dialysed and freeze-dried sample. A dry weight was then measured by using a TGS-1 Thermobalance (Perkin–Elmer, Norwalk, CT, U.S.A.), and a water content of 18–20% (w/w) was recorded.

A Waters R401 Differential Refractometer (Waters Associates, Milford, MA, U.S.A.) was used after calibration at 20°C with sucrose solutions of known refractive index. A differential refractive-index increment of 0.143 ml/g was obtained.

## Results

The A1D1 proteoglycan preparation was subjected to amino acid and hexosamine analysis (see Table 1). From the galactosamine/glucosamine ratio it can be deduced that the chondroitin sulphate/keratan sulphate ratio is approx. 7.5:1 (assuming disaccharide weights of 503 and 467 for the respective sodium salts, and ignoring any possible contributions from other oligosaccharides). Analysis of the $^{13}$C n.m.r. spectrum (not shown) of this preparation confirms this, and indicates that chondroitin 6-sulphate and chondroitin 4-sulphate occur in approximately equal proportions.

The molecular size of the chondroitin sulphate chains in the proteoglycan preparation was examined on Sephadex G-200 after papain and Pronase digestion (Fig. 1). The $\bar{M}_n$ was estimated to be 10500 by interpolation into the equation $\log \bar{M}_n = 1.395 K_{av.} + 4.763$, calculated from the data of Yamaguchi (1980). Chondroitin sulphate chains from bovine nasal-septum cartilage gave a $\bar{M}_n$ of approx. 16500 by this method (Fig. 1), which

compares with the value of 18600 obtained by Yamaguchi (1980).

The inherent heterogeneity of the A1D1 proteoglycan preparation was examined in several ways.

Table 1. *Amino acid and hexosamine analyses*
Amino acid values are expressed as residues per 1000 residues. The molar ratio of D-galactosamine to D-glucosamine is 7.0. The ratio of total hexosamine to protein (w/w) is approx. 2.3. Abbreviation: n.d., not determined.

| | |
|---|---|
| Asx | 70 |
| Thr | 65 |
| Ser | 127 |
| Glx | 150 |
| Pro | 81 |
| Gly | 145 |
| Ala | 77 |
| Cys | n.d. |
| Val | 62 |
| Met | n.d. |
| Ile | 36 |
| Leu | 79 |
| Tyr | 6 |
| Phe | 33 |
| His | 19 |
| Lys | 17 |
| Arg | 32 |

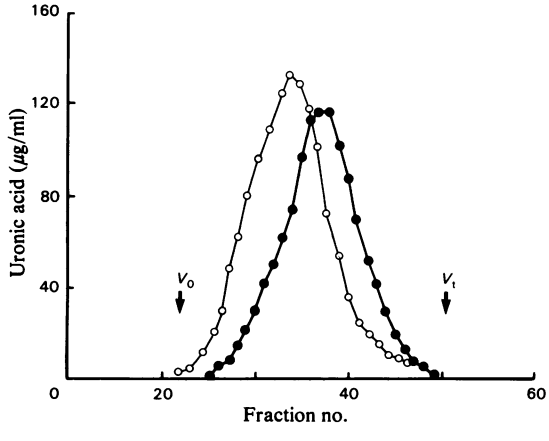


Fig. 1. *Gel chromatography of chondroitin sulphate chains on Sephadex G-200*
Papain-and-Pronase digests of bovine femoral-head proteoglycan (●) and bovine nasal-septum cartilage (○) were chromatographed on Sephadex G-200 (98 cm × 0.85 cm) eluted with 0.2 M-NaCl/0.005 M-phosphate, pH 7.4, at a flow rate of 1.1 ml/h. Fractions (1.1 ml) were assayed for uronic acid by the orcinol method. $V_0$ (void volume) and $V_t$ (total volume) were determined with Blue Dextran and glucuronolactone respectively.

Gel chromatography of the proteoglycans in 4 M-guanidine hydrochloride, pH 7.4 (conditions that should minimize self-association), on Sephacryl S-500 (see Fig. 2) yielded a unimodal peak with mean $K_{av.}$ of 0.26. A similar unimodal peak was observed in sedimentation-velocity experiments in 4 M-guanidine hydrochloride (results not shown). Rate-zonal centrifugation of radiolabelled proteoglycan in 10–50% (w/w) sucrose gradients also gave rise to a single peak (Fig. 3).

Analytical isopycnic density centrifugation in pure $Cs_2SO_4$ and CsCl was used to assess the homogeneity of the preparation and its contamina-

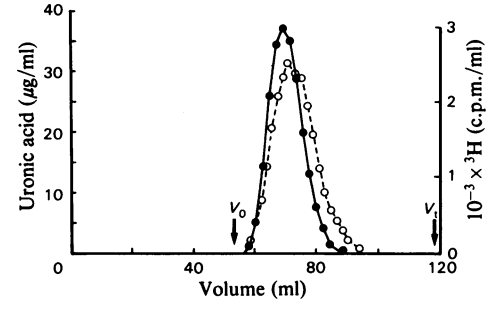


Fig. 2. *Gel chromatography of proteoglycan on Sephacryl S-500*
Proteoglycan was chromatographed on Sephacryl S-500 (131 cm × 1.1 cm) eluted with 4 M-guanidine hydrochloride/0.005 M-phosphate, pH 7.4, containing proteinase inhibitors, at a flow rate of 6.5 ml/h. Fractions (2.2 ml) were assayed for uronic acid by the orcinol method (●) or counted for $^3H$ radioactivity (○).

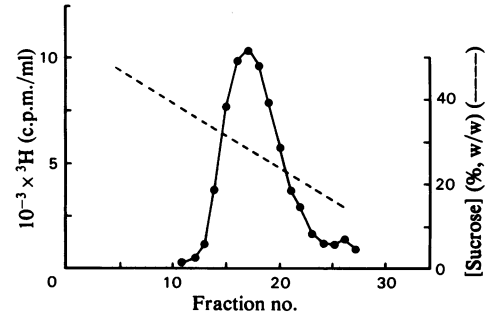


Fig. 3. *Sucrose rate-zonal centrifugation of proteoglycan*
[$^3H$]Proteoglycan (30 μg) was centrifuged in a gradient of 10–50% (w/w) sucrose/0.5 M-NaCl/0.005 M-phosphate, pH 7.0, at 185000 $g_{av.}$ for 3.5 h at 20°C (see the Experimental section for further details).

tion with DNA. The experiments were monitored at 280nm, at which the absorbance per unit weight of DNA is approx. 100 times that of proteoglycan. In pure CsCl the proteoglycans have a buoyant density, $\rho$, >1.87g/ml, and the data (Fig. 4) indicate little contamination with DNA. In $Cs_2SO_4$ the proteoglycans band as a unimodal, but polydisperse, distribution about a mean buoyant density, $\rho$, of 1.46g/ml (Fig. 4, inset). This is distinctly less than that for bovine nasal proteglycans, for which $\rho \simeq 1.55$ g/ml (J. K. Sheehan, unpublished work). All of these experiments demonstrate a unimodal but polydisperse distribution of proteoglycans, and consequently the A1D1 preparation was not further fractionated.

The A1D1 proteoglycan preparation in 4M-guanidine hydrochloride was examined by conventional light-scattering, and the data are presented as a Zimm plot (Fig. 5). By using the measured value of 0.143ml/g for the refractive-index increment of proteoglycan, in this solvent at a wavelength of 435.8nm, a $\bar{M}_w$ of $0.96 \times 10^6$ and a radius of gyration, $R_G$, of 42nm were determined.

A further estimate of the proteoglycan molecular weight was made by the sedimentation-diffusion method. Quasi-elastic light-scattering measurements of the translational diffusion coefficient, $D_T$, of proteoglycan in 4M-guanidine hydrochloride were made at 25°C, and at several angles, and the angular dependence of $D_T$ was found to be very small. A typical photon-count auto-correlation function for proteoglycan is shown in Fig. 6. The diffusion coefficients were extrapolated to zero angle and are shown plotted against proteoglycan

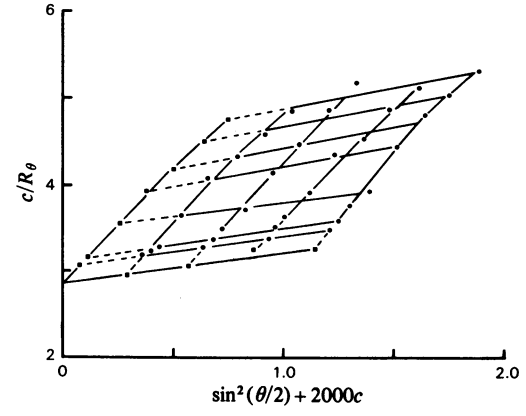


Fig. 5. *Zimm plot for proteoglycan*
Light-scattering-intensity measurements were performed on proteoglycan (0.14, 0.29, 0.43 and 0.57mg/ml) in 4M-guanidine hydrochloride/0.005M-phosphate, pH7.4. $R_\theta$ is the Rayleigh ratio at a scattering angle $\theta$ and $c$ is the proteoglycan concentration. The optical constant, $K$, is $3.631 \times 10^{-7}$ml$^2 \cdot$g$^{-2} \cdot$cm$^{-4}$. The plot yields a $\bar{M}_w$ of $0.96 \times 10^6$ and a $R_G$ of 42nm.

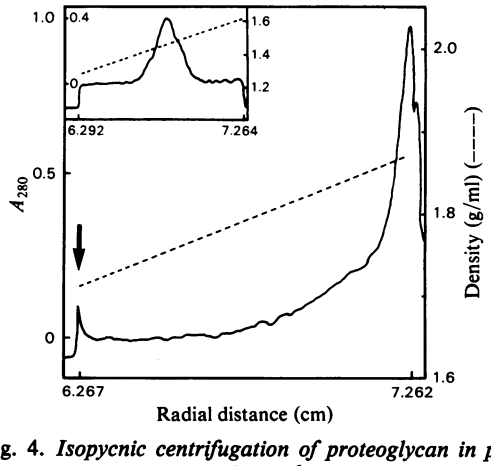


Fig. 4. *Isopycnic centrifugation of proteoglycan in pure caesium salts*
Proteoglycans were centrifuged in CsCl (loading density 1.79g/ml, 183000$g_{av.}$) and, shown inset, in $Cs_2SO_4$ (loading density 1.46g/ml, 221000$g_{av.}$) at 20°C for 24h. Proteoglycan was monitored by $A_{280}$. The arrow indicates the banding position of DNA in CsCl.

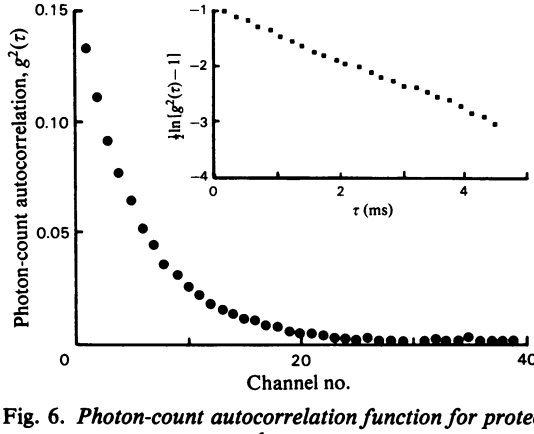


Fig. 6. *Photon-count autocorrelation function for proteoglycan*
A typical plot obtained with proteoglycan (2.56mg/ml) in 4M-guanidine hydrochloride/0.005M-phosphate, pH7.4 at 25°C. The scattering angle is 35°, and the time delay of each channel is 180$\mu$s. The insert shows the semilog plot of $[g^2(\tau)-1]$ as a function of sample time ($\tau$), which yields the translational diffusion coefficient, $D_T$, for this angle and concentration (see the Experimental section).

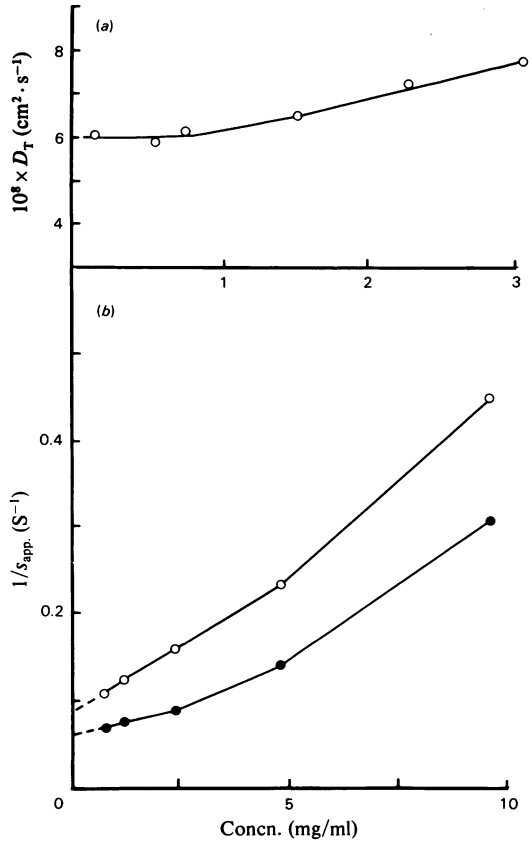


Fig. 7. *Concentration-dependence of the diffusion and sedimentation data for proteoglycan*
Concentration-dependence of (*a*) the translational diffusion coefficient, $D_T$ (cm$^2 \cdot$s$^{-1}$), of proteoglycan in 4M-guanidine hydrochloride/0.005M-phosphate, pH7.4 at 25°C, and (*b*) the reciprocal apparent sedimentation coefficient for proteoglycan in (○) 4M- and (●) 0.5M-guanidine hydrochloride/0.005M-phosphate, pH7.4, at 30000 $g_{av.}$ and 20°C. In (*a*) each point represents the extrapolate of $D_T$ to zero angle.

concentration in Fig. 7(*a*). Sedimentation-velocity experiments on proteoglycan in both 4M- and 0.5M-guanidine hydrochloride were performed at 20°C, and the reciprocal sedimentation-coefficient data are shown in Fig. 7(*b*). The calculated sedimentation coefficients were determined to be 12.0S and 16.7S in 4M- and 0.5M-guanidine hydrochloride respectively. The weight-average molecular weight of the proteoglycan was calculated to be $1.36 \times 10^6$ by substituting into the Svedberg equation and using a value of 0.55ml/g for $\bar{v}$ (Hascall & Sajdera, 1970). However, if it is assumed that the hydrodynamic characteristics of

Table 2. *Physical parameters of A1D1 proteoglycan*
All values relate to 4M-guanidine hydrochloride/0.005M-phosphate, pH7.4, as solvent. Values in parentheses are those corrected to water at 20°C.

| | | |
|---|---|---|
| $s^0_{20}$ | 12.0S | (17.3S) |
| $D^0_{20}$ | $5.41 \times 10^{-8}$cm$^2 \cdot$s$^{-1}$ | ($6.8 \times 10^{-8}$cm$^2 \cdot$s$^{-1}$) |
| $R_G$ | 42nm | |
| $\bar{M}_w$ | $0.96 \times 10^6$ | By light-scattering |
| $\bar{M}_w$ | $1.36 \times 10^6$ | By sedimentation-diffusion* |

* A value of 0.55ml$\cdot$g$^{-1}$ was used for $\bar{v}$ in 4.0M-guanidine hydrochloride.

the proteoglycans are unchanged in going from 4M- to 0.5M-guanidine hydrochloride, as found by Hascall & Sajdera (1970) for bovine nasal cartilage proteoglycans, a value for $\bar{v}$ of 0.53ml/g may be calculated from the sedimentation coefficients above. This value would give a molecular weight of $1.29 \times 10^6$. The major physical parameters for this proteoglycan preparation are summarized in Table 2.

## Discussion

The A1D1 proteoglycan population from bovine femoral heads has been shown to have a unimodal distribution of hydrodynamic sizes, but two independent measures of its weight-average molecular weight have yielded different values, i.e. $0.96 \times 10^6$ (light-scattering) and $1.36 \times 10^6$ (sedimentation-diffusion). The conventional light-scattering molecular-weight determination depends on accurate estimates of the proteoglycan concentration, the differential refractive-index increment and the referencing of intensity measurements via a benzene standard. The errors in the light-scattering determination are probably 15%. The sedimentation-diffusion molecular-weight determination is independent of proteoglycan concentration, but does depend on the sedimentation and diffusion coefficients, and the partial specific volume. As previously noted, a value of 0.55ml/g (Hascall & Sajdera, 1970) was used for the partial specific volume, and it is important to observe that the $\bar{M}_w$ estimated via the Svedberg equation depends critically on $\bar{v}$. It seems unlikely that the sedimentation–diffusion $\bar{M}_w$ determination is in error by more than approx. 20%. Furthermore, it is noteworthy that Shogren *et al.* (1982) determined a higher $\bar{M}_w$ by sedimentation–diffusion ($1.8 \times 10^6$) than by light-scattering ($1.42 \times 10^6$), for proteoglycans from chick limb bud in 4M-guanidine hydrochloride. In summary, the origin of the discrepancy remains unexplained, but the average value of $1.16 \times 10^6$ is reconcilable with the errors.

The chemical characteristics of bovine articular-cartilage proteoglycans (Table 1) show differences from those of proteoglycans from non-articular cartilage. Thus the hexosamine/protein ratio of 2.3 (w/w) contrasts with the value of 4.5 (w/w) found with bovine nasal-septum proteoglycans (Heinegård, 1977), which indicates that this articular-cartilage proteoglycan contains twice the protein content (see also Franzén *et al.*, 1981). The amino acid composition resembles that of aggregating nasal-septum proteoglycans (Heinegård & Hascall, 1979), but the gross chondroitin sulphate/keratan sulphate ratio of 7.5:1 contrasts with values of 18.8 for bovine nasal septum (Heinegård, 1977) and 12.5 for pig laryngeal proteoglycans (Hardingham & Muir, 1974) of young adult animals, and is consistent with the data obtained by Murata & Bjelle (1980) in an age-dependence study. A higher keratan sulphate content in articular cartilage was also obtained by Franzén *et al.* (1981). The ratio of chondroitin 4-sulphate to 6-sulphate of approx. 1 obtained from this 15–18-month-old bovine articular proteoglycan preparation compares with values of 1.49, 0.34 and 0.14 for 3-month-, 18-month- and 8-year-old bovine articular-cartilage proteoglycans respectively, obtained by Murata & Bjelle (1980). However, this represents a higher proportion of chondroitin 6-sulphate than is found in bovine nasal-septum proteoglycans, i.e. chondroitin 4-sulphate/6-sulphate ratios of 4.19 (Murata & Bjelle, 1976) and 6.7 (Hjerpe *et al.*, 1979). The mean buoyant density of this articular proteoglycan in pure $Cs_2SO_4$ was found to be 1.46 g/ml, which contrasts with the value of 1.55 g/ml for bovine nasal-septum proteoglycan. This difference may perhaps be attributed to higher proportions of keratan sulphate, chondroitin 6-sulphate and protein in bovine articular proteoglycan.

In conclusion, the most notable differences between the bovine articular A1D1 proteoglycans and those from nasal septum reside both in their molecular weights and in their buoyant densities in pure $Cs_2SO_4$. In the articular-cartilage proteoglycans the weight-average molecular weight lies between $1 \times 10^6$ and $1.4 \times 10^6$, with a $R_G$ of 42 nm, but in bovine nasal-septum proteoglycans a value of $2.3 \times 10^6$ and a corresponding $R_G$ of 57 nm (Pasternack *et al.*, 1974) is typical, as it is also for the pig laryngeal proteoglycan monomer (Sheehan *et al.*, 1978). The molecular weight of the chondroitin sulphate chains is found to be about 10500 ($\bar{M}_n$) for the articular proteoglycan (see also Garg & Swann, 1981), which compares with approx. 20000 ($\bar{M}_n$) for bovine nasal-septum proteoglycans (Hopwood & Robinson, 1973; Wasteson, 1969; Yamaguchi, 1980). Since the bovine articular proteoglycan has approximately half the $\bar{M}_w$, double the protein content and half the chondroitin sulphate chain length of nasal-septum proteoglycans, it is clear that the diminished molecular mass of the articular proteoglycan results from the smaller chondroitin sulphate chain sizes.

We thank Dr. T. N. Huckerby for performing the $^{13}C$ n.m.r. spectroscopy, Mr. H. Morris for the amino acid analyses, Dr. I. Carlstedt for helpful discussions and the Department of Chemistry, University of Lancaster, for the use of the light-scattering, refractometer and electrobalance equipment. We thank the Arthritis and Rheumatism Council, the Medical Research Council and the Cystic Fibrosis Research Trust for support.

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