Hydrodynamics of Concentrated Proteoglycan Solutions*

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The dynamics of water transport in proteoglycan compartments has been studied in relation to osmotic flow (proteoglycan diffusion) and hydraulic permeability (proteoglycan sedimentation) in concentrated solutions of proteoglycan subunit and native proteoglycan aggregate isolated from Swarm rat chondrosarcoma. A central parameter that describes the kinetics of both types of water movement is the hydrodynamic frictional coefficient of water with proteoglycan. The frictional coefficient is markedly concentration dependent, increasing with increasing concentration, and highlights important structural features and types of organization of the proteoglycans in concentrated solutions. These include the requirements that proteoglycans in the extracellular matrix not to be immobilized but to have translational diffusive mobility and concentration gradients to be osmotically active, that chondroitin sulfate segmental mobility describing translational motion largely determines osmotic flow and hydraulic permeability of the proteoglycans, and that the proteoglycans exhibit an enhanced ability to resist flow as compared to other macromolecules. Additional dynamic studies suggest the formation of transient super-aggregate structures may occur at high concentrations which endows the proteoglycan subunit hydrodynamic properties similar to proteoglycan aggregate.

Articular cartilage is an avascular tissue in which the chondrocytes are sparsely distributed in an extracellular matrix which is synthesized by these cells. The physical properties of the cartilage are determined by its extracellular matrix. The matrix is a multicomponent system; its major components are water, type II collagen, and proteoglycan (1). The components have been generally considered to exist in a simple biphasic arrangement (2, 3) consisting of a porous network of insoluble collagen fibers filled with a soluble phase of water and proteoglycans up to 50–80 mg ml⁻¹ in concentration (4). The major proteoglycan population has a hierarchical branching organization that enables efficient packing and concentration of the anionic charges, provided by fully ionized sulfate and carboxyl groups on the repeat disaccharide units of the constituent polysaccharide chains. The proteoglycan subunit (5) exists as a large molecule in which many pendant polyionic polysaccharide chains are covalently bound to a protein core (6). Proteoglycans are normally present in the matrix as aggregates in which 20–50 PGS molecules are bound through noncovalent interactions to an hyaluronate core (7); the interactions being stabilized by link proteins (8). The significance of aggregate formation is not clearly understood although it is commonly thought that aggregates by virtue of their size, are retained within the pores of the cross-linked collagen network by a physical entrapment mechanism. Weak electrostatic and hydrogen-bonding interactions between proteoglycans and collagen, which may be facilitated by the relatively lower NaCl concentration in proteoglycan compartments (10), may augment the retention of the proteoglycans in this structure. There is a paucity of information, however, as to the microstructural organization of macromolecules in the matrix, i.e. the nature of macromolecular concentration gradients (with attendant micro-ion concentration gradients) and other regional variations, of the network structure, and of proteoglycan interactions with other matrix macromolecules and structures.

In relation to the mechanical properties of cartilage its most important component is water. The load-bearing properties of cartilage result largely from its ability to retain water (an essentially incompressible fluid) under the application of load. A correlation has been established between the proteoglycan content of the matrix and factors which reflect retained water in the matrix namely high swelling pressure and low hydraulic permeability (4, 9). A polemic exists as to the relative importance of these factors; Mow et al. (3) concluded that the frictional drag of the relative motion between water and the matrix components is the most important factor governing the tissues response to compression, whereas Maroudas et al. (4, 11) have emphasized that maintenance of tissue hydration under load can be described in terms of proteoglycan osmotic pressure. Other less substantiated claims have been made in relation to the fact that the proteoglycan itself has rigidity or acts as a mechanical “spring” within the cartilage. The investigations reported in this study set out to examine the relative influence of osmotic pressure and hydrodynamic friction in governing the movement of water in proteoglycan solutions. The results are discussed in terms of the specific features of proteoglycan structure.

EXPERIMENTAL PROCEDURES

Materials

Cesium chloride (analytical grade) was from Nova Chemicals (South Yarra, Australia). Carbazole (laboratory grade) was obtained from C.P. Chemicals (Philadelphia, Pa.). Cesium chloride (analytical grade) was from Nova Chemicals (South Yarra, Australia). Carbazole (laboratory grade) was obtained from C.P. Chemicals (Philadelphia, Pa.).

1 The abbreviations used are: PGS, proteoglycan subunit; CS, chondroitin sulfate; PGA, proteoglycan aggregate; PBS, phosphate-buffered saline; CHAPS, 3-[3-chloramidopropyl]dimethylammonium]-1-propanesulfonate; HTO, tritiated water; GdnHCl, guanidine hydrochloride.

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from British Drug Houses, Australia. Benzamidine hydrochloride, \(\epsilon\)-aminocaproic acid, iodosacetic acid, N-ethylmaleimide, EDTA, whale or shark chondroitin sulfate, glucuronolactone, guanidine hydrochloride (GdnHCl), CHAPS, phenylmethylsulfonl fluoride, and bovine serum albumin (fraction V) were purchased from Sigma. Gradient grade cesium sulfate was purchased from Gallard Schlesinger, New York. Sterile dextran, dextran sulfate, Sephacryl S-500 and S-1000 were purchased from Pharmacia Biotechnology Inc., Sweden; PM-10 ultrafilters were from Amicon, and Visking dialysis tubing was purchased from Medicell International, United Kingdom. Nucleopore polycarbonate capillary pore membranes were from Nuclepore Corp. (Pleasanton, CA). Trilithium (lot 1275-133, 0.25 mCi \(\text{mg}^{-1}\) ) was from DuPont-New England Nuclear. Carrier-free sodium \([35S]SO_4\) was purchased from Gallard Schlesinger, New York. Dextran, dextran sulfate, Sephacryl S-500 and S-1000 were from Amersham, United Kingdom. Unless otherwise stated, all other reagents were of the highest grade commercially available.

Isolation and Purification of Proteoglycan Subunit, Proteoglycan Aggregate, and Chondroitin Sulfate

Proteoglycan aggregate (PGA) was purified from Swarm rat chondrosarcoma tumors that were grown in female Wistar rats following the methods of Falts et al. (12) and Kimata et al. (13). Minced tumors were extracted with 1 g of extractant per g of tissue where the extractant was 0.4 M GdnHCl, 50 mM sodium acetate, pH 5.8, which contained the protease inhibitor mixture 0.025 M EDTA, 10 mM N-ethylmaleimide, 0.1 M phenylmethylsulfonl fluoride, 1 M benzamidine hydrochloride, 0.1 M \(\epsilon\)-aminocaproic acid, and 2 mM iodosacetic acid. The PGA was purified as an \(\alpha_1\) fraction by equilibrium CsCl gradient centrifugation and then further purified from proteoglycan material on a sucrose density gradient. PGS was prepared by extracting the tumors with 4 M GdnHCl, 50 mM sodium acetate, pH 5.8, containing the inhibitor mixture described above. The proteoglycan material was then purified as a \(D_1^2\) fraction as previously described (5). \([35S]\)PGS was prepared from the PGA by a modification of the method described by Kimata et al. (14). Sterile PBS (0.14 N NaCl, 2.6 mM KCl, 1.5 mM KH\(_2\)PO\(_4\), 8.1 mM Na\(_2\)HPO\(_4\); pH 7.5) and then centrifuged. The cell pellet was incubated with trypsin at 37°C for 30 min then collagenase for 30 min. The cells were pelleted and resuspended in Dulbecco's modified Eagle's medium and pH indicator at a concentration of 0.5 \(\times\) 10\(^6\) cells/ml. Carrier-free Na\(_2\)SO\(_4\) in isotonic saline (1 M) was incubated for 24 h with 5 \(\times\) 10\(^5\) cells in culture medium. Extraction of [\(35S\)PS]PGS for 24 h at 4°C used an equal volume of 8 M GdnHCl, 100 mM acetate buffer plus inhibitor mixture (double strength). The [\(35S\)PS]PGS was then purified by equilibrium gradient centrifugation as a \(D_1\) fraction with a specific activity 1200 cpm \(\mu\)g\(^{-1}\).

The commercially supplied preparation of chondroitin sulfate was further purified to remove protein by \(\beta\)-elimination, proteolytic digestion, and trichloroacetic acid precipitation. The chondroitin sulfate was precipitated with ethanol and then dried.

All samples were dialyzed extensively against 1 M NaCl to yield the Na\(^+\) form and then dialyzed against PBS. The preparations were stored frozen in PBS.

Ultracentrifuge Experiments

Diffusion—Mutual diffusion coefficients were measured by free diffusion in a Beckman Model E analytical ultracentrifuge using the schlieren optical system. A solution of concentration \(C_1\) was layered over a slurry of minced tumor tissue \(C_0\) with \(C_0 > C_1\) in a synthetic boundary cell and the speed was reduced to 2200 rpm for PGA samples and 3400 rpm for other samples. The concentration step, \(\Delta C = C_0 - C_1\), was normally in the range of 1-3 mg \(\text{ml}^{-1}\) for routine measurements. Diffusion was analyzed at 20°C in cells with 0.5, 1.2 or 3.0-cm synthetic boundary centerpieces. The apparent diffusion coefficients, \(D_{\text{app}}\), were calculated from the broadening of the schlieren peaks by following the change in width at the half-height \((W_{0.5})\) from the relation

\[
D_{\text{app}} = \frac{(W_{0.5})^2}{166 \ln 2}
\]

where \(t\) is the time. Values of \(D_{\text{app}}\) were calculated from the slope of the plots \((W_{0.5})^2\) against \(t\) from eight pictures taken at regular time intervals. We assume that \(D_{\text{app}}\) closely approximates the differential mutual diffusion coefficient \((D)\) at the corresponding mean concentration \(C = (C_0 + C_1)/2\). This is probably an acceptable approximation considering the small step concentration gradients involved.

All "b" solutions were dialyzed against PBS prior to their use, whereas "c" solutions were obtained by dilution of the b solution with the same buffer. Tritium probe diffusional experiments were performed to determine the initial activity gradients of salt in the diffusion experiments. Diffusion boundaries formed from solutions prepared in this way have previously been demonstrated to give rise to normal diffusion transport (15, 16).

Sedimentation—Subsequent to the diffusion experiment described above, the speed of the rotor was increased to 44,000 rpm to sediment the material. For very slow sedimenting material maximal speeds of 60,000 rpm were used. The displacement of the sedimentation boundary with time was recorded photographically at regular intervals. Generally, at high macromolecule concentrations, which were of experimental interest, the sedimentation coefficient was very low in magnitude. In some concentrated solutions, no sedimentation was observed over an 8-h period. Errors associated with radiol dilution effects were assumed to be negligible. Furthermore, substantially similar results were obtained by performing the sedimentation experiment either as described above or by sedimenting the material away from the meniscus. This demonstrates that errors associated with hydrostatic pressure effects exerted by the column of solvent above the solvent-solution boundary were also negligible in relation to the magnitude of the sedimentation coefficient.

HTO Transport in Proteoglycan Solutions—A transport apparatus of similar design to that described by Linder et al. (17) and developed by Sundeloef (18) was used to measure HTO transport. The analysis was similar to previous studies of HTO transport in dextran polysaccharide solutions (19).

Membrane Transport—Transport of [\(35S\)PGS through Nucleopore capillary pore membranes was performed with the membrane fixed between two identical cylindrical chambers (15 mm diameter and 7 mm deep) of a perspex cell. The solutions in the chambers were not stirred. The concentration of PGS on both sides of the membrane was equal with one side initially containing trace quantities of [\(35S\)PGS]PGS transport of [\(35S\)PGS]PGS to the other side was observed over a period of 72 h. Each transport measurement was made in quadruplicate. The [\(35S\)PGS]PGS was fractionated on Sephacryl S-500 prior to use in this experiment; a similar profile to that shown in Fig. 1B was obtained and fractions 44-65 were pooled for subsequent transport measurements.

Preparation of Solutions—The preparation of concentrated polysaccharide or proteoglycan solutions was done by ultrafiltration using an Amicon ultrafilter and PM-10 membrane. The highest concentrations we were able to prepare for PGA and PGS were \(-38\) mg \(\text{ml}^{-1}\) and \(-40\) mg \(\text{ml}^{-1}\), respectively; these solutions were extremely viscous and difficult to transfer to the ultrafiltration, the polysaccharide solution was dialyzed against PBS to ensure thermodynamic equilibrium of simple electrolyte. All dilutions of the proteoglycan solutions warranted gentle mixing at 4°C for at least 24 h prior to use.

Analytical Procedures—Uronic acid was determined by an automated carbazole method (20). Radioactivity was determined using 1.0-ml aqueous samples with 8.0 ml of a scintillant mixture as described by Fox (21), and recorded on an LKB Wallac 1214 Rack Beta scintillation counter. Dry weights of proteoglycan preparations that were extensively dialyzed against water were measured by heating samples over \(\text{Po}_2\) at 60°C and 130-Fa pressure (under vacuum) until constant weight was obtained. Solution densities were measured on a DMA 55 density meter (Anton Paar, Austria) equipped with Haake F4-K Cryostat (Haake, Federal Republic of Germany) that controlled the temperature to 20.0 \(\pm\) 0.02°C. Refractive index measurements were performed on a Brice-Phoenix differential refractometer at a wavelength of 546 nm (Phoenix Precision Instrument Co., Philadelphia) using NaCl solutions as standards. The physical constants and concentration conversion factors associated with the various polysaccharide fractions are described in Table 1.

Integrity of Proteoglycan Preparations—The chromatographic profile of the various proteoglycan fractions are shown in Fig. 2, and the distribution of material in the PGA preparation (curves 1, Fig. 12) was clearly confined to material eluting at or near the void volume (unmeasured) of Sephacryl S-1000 column and was easily distinguished from PGS that was well included into the column (curves 3, Fig. 1). The aggregate proteoglycans were relatively stable as indicated by its behavior in the ultracentrifuge even when it was stored frozen and subsequently used. Only in the most prolonged processing involving a concentration to \(-25\) mg \(\text{ml}^{-1}\), by ultrafiltration, followed by anal-

\[\text{(WXh)} = \frac{c}{\Delta c} \quad \text{where} \quad c = (C_0 + C_1)/2\]
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**Summary of physicochemical constants for the various polysaccharide preparations in the Na\(^+\) form**

| Sample designation | PGA | PGS | CS |
|--------------------|-----|-----|----|
| Uronic acid mass   | 3.14| 3.29| 2.85|
| Partial specific volume/ ml g\(^{-1}\) | 0.546\(^a\) | 0.54\(^a\) | 0.54\(^a\) |

\(^a\) Calculated from molecular formula.
\(^b\) Measured in water.
\(^c\) From Ref. 5.

Fig. 1. a, chromatographic profile of PGA (---) (graph 1) and \(^{35}S\)PGS (---) (graph 3) on Sephacryl S-1000 (column dimensions, 50 x 1 cm) in 0.5 m sodium acetate, 0.05 m sodium sulfate, and 0.01\% CHAPS, pH 7.0. PGA fractions were monitored for uronic acid and \(^{35}S\)PGS fractions were monitored for radioactivity. Graph 2 represents the PGA sample that was concentrated to ~25 mg ml\(^{-1}\), used in centrifuge for diffusion analysis at that concentration, recovered, diluted, and then re-applied to the column. Each fraction was 0.72 g, profile of PGS on Sephacryl-500 (column dimensions, 67.5 x 5 cm) in PBS. The PGS fractions were monitored for uronic acid. Each fraction was 12.3 g.

A Summary of Equations Describing Molecular Transport

**Diffusional Transport**—We will be developing arguments in this study which suggest that the diffusive mobility of the proteoglycan is critical for the manifestation of its properties. We start by describing the usual diffusion equation of Fick's Law, i.e.

\[
J_1 = -D_1 \frac{\partial C_1}{\partial x} \frac{\partial \Pi}{\partial x} \tau_{eq}
\]

where \(J_1\) is the flux of component 1 (which is proteoglycan), \(D_1\) the mutual diffusion coefficient, \(T\) the temperature, \(P\) the pressure, and \(\frac{\partial C_1}{\partial x}\) the concentration (mol cm\(^{-3}\)) gradient. We can rewrite Equation 1 in another form which now includes the osmotic pressure gradient \(\frac{\partial \Pi}{\partial x}\) and the effective diffusion coefficient \((D_{eq})\).

\[
J_1 = \left(1 - \frac{\phi_1}{\phi_2}\right) \frac{D_{eq}}{f_{12}} \frac{\partial \Pi}{\partial C_1} \tau_{eq} = -D_1 \frac{\partial C_1}{\partial x}
\]

where

\[
(D_{eq}) = RT/\phi_{12}
\]

and

\[
D_1 = \left(1 - \frac{\phi_1}{\phi_2}\right) M_1 f_{12} \frac{\partial \Pi}{\partial C_1} \tau_{eq}
\]

where \(\phi_1\) is the volume fraction of 1, \(f_{12}\) is the hydrodynamic frictional coefficient between the proteoglycan and solvent (component 2), \(C_1\) the concentration in g ml\(^{-1}\), and \(\mu_1\) in the chemical potential of solvent.

The nature of \(f_{12}\) in relation to the structure of the proteoglycan is little understood. We do know that in dilute solution we have the Stokes-Einstein equation which relates \(f_{12}\) to the hydrodynamic radius of the diffusing solute \(R_0\).

\[
f_{12} = 6\pi n_0 R_0\eta
\]

where \(n_0\) is Avogadro's number and, \(\eta_0\) the viscosity of solvent.

**Osmotic Flow**—It has now been demonstrated that there is a great deal of similarity between osmotic flow and diffusional flow (23). We have derived the equation of osmotic flow of water \((J_w)\) across a semipermeable membrane (impermeable to component 1) as the following (23).

\[
J_w = \frac{\nu_1 (1 - \phi_1) M_1 f_{12}}{\phi_2} \frac{\partial \Pi}{\delta x} \tau_{eq}
\]

where \(\nu_1\) is the partial specific volume of 1, \(\phi_1\) and \(M_1\) the volume fraction and molecular weight, respectively, of 1, \(K\) a geometric factor associated with the membrane and \(\Delta \Pi\) is the overall gradient in osmotic pressure. In summary, the important factors associated with the osmotic flow caused by proteoglycan are the osmotic pressure gradient, the geometric nature of the membrane, and the diffusional mobility of the proteoglycan as embodied in the \(f_{12}\) term. The \(f_{12}\) factor is a new consideration in the manifestation of osmotic flow.

The interpretation of this equation may be made in the following way. If an inert semipermeable membrane is interposed at the interface between the proteoglycan solution and solvent (or by analogy a collagen matrix retaining the proteoglycan) then osmotic flow will be governed by two processes in series namely: 1) the diffusive volume-exchange process associated concentration gradients of proteoglycan on the solution side of the membrane and 2) the hydraulic flow of water through the membrane (independent of proteoglycan concentration).

**Hydraulic Permeability**—D'Arcy (24) introduced the coefficient \(k\), the hydraulic permeability coefficient, to describe the relationship between flow in response to a mechanical pressure gradient \(\Delta P\) across a membrane of thickness \(\Delta x\) as the following.

\[
J_w = \frac{k \Delta P}{m_0 \Delta x}
\]

Measurement of this hydraulic permeability can be made by sedimentation velocity experiments. In sedimentation the "porous plug" of polymer material moves through the solvent in exactly the opposite way to a normal hydraulic flow experiment; i.e. water pushed through a polymer membrane. A direct relationship between \(k\) and the sedi-
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mentation coefficient, $S_1$, has been derived (25) as

$$k = \frac{\eta S_1}{C_1 \left(1 - (v_1/v_2)\right)}$$

(6)

which as shown previously (22), may be re-expressed in terms of the frictional coefficient $f_{12}$, since

$$S_1 = (1 - \phi_1)(1 - \rho v_1)M_1/f_{12}$$

(7)

then with $(1 - \phi_1) = (1 - \rho v_1)/(1 - (u_1/\rho_1))$

gives

$$k = \frac{(1 - \phi_1)\eta_2 M_1}{C_1} f_{12}$$

(8)

where $\rho$ is the solution density. Equations 8 and 2b highlight the dual nature of the hydrodynamic frictional coefficient, $f_{12}$. It describes the diffusional mobility of the diffusing solute and also describes the hydraulic permeability of water pushed through the same solution, the latter could be viewed as the viscous dissipation of the flow of water over the polymer. It has been established that $f_{12}$ evaluated from diffusion and sedimentation experiments of various polymer preparations are quantitatively similar (22).

Balance of Osmotic and Mechanical Pressures

The balance of osmotic and mechanical pressures on proteoglycan retaining systems, such as cartilage, can be described in terms of an equation analogous to that describing sedimentation-equilibrium in the ultracentrifuge such that

$$J_x = I_xL_x^o(\Delta P - \Delta I)$$

(9)

where the $L_x$ coefficients describe (i) the geometric (pore size) factor of the system ($L_x^o$) and (ii) concentration-dependent diffusive mobility of the osmotically active solute ($L_x^o/\alpha M_1/f_{12}$).

RESULTS

Mutual Diffusion—The concentration dependence of the mutual diffusion coefficient of the various polysaccharide preparations is shown in Fig. 2. At low concentrations, the relative differences in the diffusion coefficients are marked with CS having a diffusion coefficient 2 orders of magnitude greater than the PGA sample. The diffusion coefficient of PGA is, as expected, very low (~$10^{-9}$ cm$^2$ s$^{-1}$) in dilute solution. As the concentration is increased the diffusion coefficient for all samples increases markedly and converges to molecular weight independence up to concentrations of 40 mg ml$^{-1}$. This approach to molecular weight independence at high concentrations has also been observed with different molecular weight fractions for the uncharged polysaccharide dextran (22). At ~25 mg ml$^{-1}$ polysaccharide concentration the relative difference in the diffusion coefficient between CS and PGA is negligible; the PGA is now moving with a diffusion coefficient of ~$10 \times 10^{-7}$ cm$^2$ s$^{-1}$. The relatively rapid movement of PGA was probably not due to any significant degradation as shown by 1) diluting the 25 mg ml$^{-1}$ PGA sample to 0.33 mg ml$^{-1}$ and observing a single peak during sedimentation which had a sedimentation coefficient of 43.75 S and 2) re-analyzing the same sample on Sephadryl S-1000 (Fig. 1) after the diffusion run; approximately 80% of this sample was still aggregate (according to its column profile) and 20% as PGS. At high concentrations (greater than 45 mg ml$^{-1}$) the PGS coefficients may diverge from those measured for CS.

Sedimentation Velocity—The concentration dependence of the sedimentation coefficient is shown in Fig. 3. At low concentrations the $S_1$ values are markedly different for each of the preparations; the extrapolated $S_1$ to infinite dilution for PGA was in the range of 60–100 S which is in accord with reported $S_1$ of other aggregate preparations (26). As the concentration is increased, $S_1$ decreases markedly and not only approaches molecular weight independence but approaches very low values indeed (<0.2 S). The low sedimentation coefficient can be confirmed through analysis of Equation 2b. For example, calculating $S_1$ at a PGS concentration of 50 mg ml$^{-1}$ through evaluation of the $f_{12}$ term in Equation 2b with $D_1 \sim 16 \times 10^{-7}$ cm$^2$ s$^{-1}$ and using the osmotic data of Urban et al. (28) and Equation 7 will give a sedimentation coefficient of ~0.35 S as compared with the experimentally observed interpolated value of 0.25 S.
The calculated values of hydraulic permeability, \( k \), from Equations 7 and 8 using \( S_1 \) from Fig. 3 and shown in Fig. 4 demonstrate that on a volume fraction basis, PGS (and PGA although not shown) is most efficient in retarding water flow as compared to other macromolecules, namely globular albumin, uncharged dextran, and polyanionic dextran sulfate at salt concentrations similar to the PBS. Values of \( k \) for dextran sulfate is demonstrated to decrease with decreasing ionic strength which reflects the influence of the "primary charge effect" on \( f_{iz} \) and hydraulic permeability (29). However, dextran sulfate behavior at 0.01 M NaCl is still not significantly different from PGS. In fact the extraordinary effectiveness of proteoglycans in restricting fluid flow clearly demonstrates that apart from established shape/charge effects (29), there are other structural features of the proteoglycans associated with their resistance to flow. These features are similar to those found for chondroitin sulfate solutions. The results may suggest that the chemical and structural features of the CS chains may be important in giving rise to higher resistance to hydraulic flow of water.

**Effective Diffusion Coefficient**—The effective diffusion coefficient defined in Equation 2a may be evaluated from the sedimentation coefficient data using Equation 7, and its concentration dependence for both PGS and PGA is shown in Fig. 5a. The very low effective diffusion coefficient, which decreases with concentration, is matched by the corresponding apparent Stokes radius shown in Fig. 5b which shows a very large increase with increasing concentration. While \( (D_{el})_0 \) or \( (R_{h})_{app} \) is markedly concentration dependent we note that \( (R_{h})_{app} \) for PGS at high concentration (>10 mg ml\(^{-1}\)) approaches \( (R_{h})_{app} \) for PGA in dilute solution. On this basis, concentrated PGS could be viewed as hydrodynamically equivalent to the dilute hydrodynamic volume of PGA. Preliminary experiments do suggest that the marked concentration dependence of \( (D_{el})_0 \), is reflected in \([\text{35S}]\)PGS transport across capillary pore (400-nm diameter) membranes over a PGS concentration range of 0.4-12 mg ml\(^{-1}\). The results shown in Table II demonstrate that for systems with equal concentrations of PGS on both sides of the membrane the quantitative transport of \([\text{35S}]\)PGS is decreased markedly on increasing the PGS concentration. Note that it has been previously demonstrated (15, 22) that the diffusional ex-

**Fig. 4.** The hydraulic permeability \( k \), as calculated from the sedimentation velocity data (Equations 7 and 8) for PGS (graph 2), albumin (5), dextran (M, ~500,000) (4), and dextran sulfate (M, ~500,000) dialyzed against 0.15 M NaCl (3) and 0.01 M NaCl (1). Values of \( k \) of albumin and the dextrans are from Comper (Comper, W. D. (1987) Biochem. Phys., submitted for publication).

**Fig. 5.** Concentration dependence of \( (D_{el})_0 \) (a) and \( (R_{h})_{app} \) (b) calculated from sedimentation data (Equations 2a, 3, and 7). PGS (●); and PGA (○).
change in this type of experiment is quantitatively similar to \((D_{m}t)\). While the membrane results are consistent, in part, with the decrease in \((D_{m}t)\) with concentration they also highlight that, in a dynamic sense, the PGS molecule at high concentrations appears larger to the pores of the membrane.

**HTO Transport**—Analysis of HTO transport in a PGS solution of 41 mg ml\(^{-1}\) gave a diffusion coefficient of HTO of \(182 \times 10^{-7}\) cm\(^2\) s\(^{-1}\) which was 96\% of its value in the absence of PGS. The small reduction is similar to that obtained in other polymer solutions (19) and can be accounted for by steric hindrance effects and not by any marked alteration in water structure in the vicinity of the proteoglycan.

**DISCUSSION**

The magnitude and the rate of increase with concentration of the mutual diffusion coefficient of CS, PGS, and PGA is considerably larger than that observed for its uncharged counterpart, dextran (22). This may be understood in terms of the parameters described in Equation 2b. The osmotic friction term for these polyelectrolytes is essentially determined by the influence of the polyanion on the micron-ion distribution in its vicinity (the Donnan effect (10, 27, 28)) and thus we would expect molecular weight-independent behavior. This has been shown by Urban et al. (28) who demonstrated that the magnitude and concentration dependence of osmotic pressures of bovine nasal cartilage CS, PGS, PGA (measured by an equilibrium dialysis technique) were essentially identical over a wide range of concentration when compared on the basis of anionic charge concentration. We would expect similar behavior in this study except that our comparisons of diffusion data are made on the basis of total mass concentrations. A small difference in osmotic behavior between CS and the proteoglycans would then be anticipated as the concentration of anionic charge/molecular mass is about 10-15\% higher for the CS as compared to PGS and PGA. This is mainly due to the presence of protein and N- and O-linked oligosaccharides in the chondrosarcoma proteoglycan structures (30). While the nature of the frictional coefficient in Equation 2b is not well understood (in contrast to osmotic pressure) it is evident from the sedimentation studies that the \(M_I/f_{12}\) will be essentially molecular weight independent in semidilute solutions. This has also been shown for dextran sedimentation (22). All these considerations are then consistent with the observed molecular weight independence of \(D_I\) for CS, PGS, and PGA at high concentrations. The actual increase in \(D_I\) with concentration is due to the predominance of the \((\partial[I]/\partial C_I)\) term in Equation 2b over the frictional term, \(f_{12}\).

The approach of the \(M_I/f_{12}\) term to molecular weight independence does suggest that the flexibility and diffusive mobility of segments of the CS chains and its associated counterions are critical in determining \(f_{12}\) for both PGA and PGS. It is emphasized that the segmental mobility referred to here represents translational motion, and is subject to marked concentration dependence. It is of interest to note that in another related study, Torchia et al. (31) established from \(^{13}\)C nmr studies that chondroitin sulfate gelation in cartilage is relatively free, i.e., independent of aggregation and placement of the proteoglycans within the collagen network of cartilage. While there is no direct relationship between translational motion studied here and that segment motion measured by \(^{13}\)C nmr (interpreted on the basis of rotational diffusion (31)) it is clear that both show molecular weight independence.

In discussing the factors associated with volume flow in cartilage described in Equation 9, a number of interesting features emerge. First, the role of osmotic flow in cartilage is envisaged to be important in two situations namely (i) to oppose the tendency of water being pushed out of cartilage under compressive load and (ii) as the only mechanism by which water is reimbibed into the tissue once the mechanical load is removed (i.e. \(\Delta P = 0\) in Equation 9). For osmotic flow to occur, there must be osmotic or concentration gradients of osmotically active material (namely proteoglycan) which drive the diffusional exchange with water. If the osmotically active material is immobilized (i.e. \(D_I = 0\)) then no osmotic flow will occur. We also note that osmotic flow should be faster for low molecular weight material as compared to high molecular weight material (23). To a certain extent, these considerations seem paradoxical to the presence of PGA in cartilage rather than PGS. However, we have demonstrated in this study that all the parameters governing osmotic flow due to the proteoglycan as embodied in Equation 4 are molecular weight independent.

A further feature of proteoglycan solutions examined in this study is their high resistance to hydraulic flow of water (i.e. with \(\Delta P \gg \Delta \varphi\) in Equation 9) as demonstrated in the sedimentation experiments. These studies highlighted the relative influence of hydraulic permeability as compared to osmotic pressure in resisting water flow. For example, bovine nasal cartilage proteoglycan solution of 50 mg ml\(^{-1}\) has an osmotic pressure of \(~4.7 \times 10^{-6}\) dyne cm\(^{-2}\) (28). During the course of the sedimentation experiment the proteoglycan will experience a centripetal force (32) of \((1 - \rho_v)\varphi_0 \omega^2 r^2/2\), where \(\omega\) is the rotor speed in radians s\(^{-1}\) and \(r\) the radius to the compartment containing the proteoglycan solution; this force is calculated to be \(~1 \times 10^{-6}\) dyne cm\(^{-2}\). The resistance to flow, subject to this relatively large \(\Delta P\) value, therefore, clearly lies with molecular parameters contributing to the frictional coefficient \(f_{12}\) rather than osmotic pressure. Actual values for hydraulic permeability of cartilage are reported to be in the range of \(~1 - 6 \times 10^{-15}\) cm\(^3\) s g\(^{-1}\) (Ref. 4 and earlier references). When these values are multiplied by the viscosity of water, namely 0.01 poise, then they can be compared directly with those in Fig. 4. For example, a PGS concentration of 50 mg ml\(^{-1}\) gives a \(k\) of \(~10^{-14}\) cm\(^2\), whereas that of cartilage containing \(~50\) mg ml\(^{-1}\) of proteoglycan is \(~3 \times 10^{-13}\) cm\(^2\) s g\(^{-1}\) or \(~3 \times 10^{-16}\) cm\(^2\). These values seem to be in the right order and differences may be accommodated by the influence of the collagen network as embodied in the term \(L_p\) in Equation 9 and the specific concentration distribution of proteoglycans in cartilage.

The significance of the concentration dependence of \(M/f_{12}\) or \(k\) can also be appreciated in the following model of cartilage. A simplified cartilage structure could be viewed as an array of porous spaces, each "space" surrounded by a network of collagen which retains but does not immobilize the proteoglycan within the space. When these units are subject to mechanical compression, water will be ultrafiltered out of the space in a particular direction (of least resistance) which will

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### Table II

| Proteoglycan concentration/ mg ml\(^{-1}\) | Percent transfer* of \([^{35}S]PSG\) |
|------------------------------------------|-----------------------------------|
| Solution containing \([^{35}S]PSG\) Cold solution | Percent transfer* of \([^{35}S]PSG\) |
| 0.47 | 0.47 | 27.4 ± 2.9 |
| 12.40 | 12.40 | 2.2 ± 0.9 |

*Errors are given as ± standard deviation. Measurements were made in quadruplicate. Note that maximum transfer can only be 50% as the two compartments were of equal volume.
Hydrodynamics of Proteoglycan Solutions

| TABLE III | Some physicochemical properties of proteoglycans |
|-----------|-----------------------------------------------|
| (D)v | (S)v | Hydrodynamic radii/μm | Critical concentration/mg ml⁻¹ | Molecular weight/10⁶ |
| 10⁻⁹ cm² s⁻¹ | 10⁻⁶ s | | | |
| PGS | 4 | 55.5 | 6.8-28.6 | 2.0-8.4 | 2.6 |
| PGA | 0.2 | 20 | 80.5 | 0.07-0.28 | 0.02-0.08 | 211 |

* Values obtained at infinite dilution can only be regarded as approximate, particularly for the aggregate preparation.
* Calculated from Equation 3.
* From Ref. 34 where Dv = R²/6πηhR₀ h, where R₀ is the radius of gyration.
* Critical concentration corresponds to a close packed system of non-overlapping coils where the molecule is arbitrarily viewed as occupying a sphere of radius R or a cube of side R which gives size to upper and lower bounds, 3Mᵢ/4πR³ < C* < Mᵢ/πR².
* From sedimentation and diffusion data.

| TABLE IV | Molecular volumes corresponding to the dilute hydrodynamic volume and fully extended core structures |
|-----------|------------------------------------------------------------------------------------------------|
| Volume dilute hydrodynamic | Fully extended cylindrical structure |
| mg 10⁻¹⁵ ml | 10⁻¹⁹ ml |
| PGS | 21.8 | 2.46⁵ |
| PGA | 1.75 × 10⁶ | 2.51 × 10⁹ |

* Calculated using the R₀ values in Table III.
* Calculated by assuming extended length of ~400 nm (where CS chains are situated) and radius of 14 nm which is based on the R₀ value for CS (Mᵢ ~ 26,000) from interpolated data of Fig. 2 of ~8 nm.
* Calculated by assuming length of 8000 nm and radius of 100 nm.

simultaneously give rise to a build up of proteoglycan concentration on the face of the porous wall. The permeability of the proteoglycan compartment will decrease dramatically for a very small change in proteoglycan concentration in accord with the variation of h in Fig. 4. The application of mechanical load will result in a pattern of concentration gradients in the tissue corresponding to the array of porous columns. When the load is released, the proteoglycan concentration gradients relax by diffusion (therefore the requirement for proteoglycan diffusive mobility) and in so doing osmotically reanimates water into the porous network and tissue as a whole. In summary, the results of hydraulic permeability, at least when ∆P > ∆L, would be in agreement with the conclusions of Morrow et al. (3) concerning factors governing the "fluid/solid viscoelastic properties of the tissue in compression." On the other hand, at low ∆P the influence of osmotic pressure will be marked (Equation 9) as demonstrated by Maroudas and Bannon (11).

Up to this stage we have considered the hydrodynamics of proteoglycan solutions in terms of molecular weight-independent quantities, such as osmotic pressure and translational segmental mobility. Another important aspect that will influence proteoglycan hydrodynamics, especially in relation to proteoglycan retention and organization in extracellular matrices, is the effective size of the molecule and the magnitude of the f₁₂ term. It is appreciated that in constructing proteoglycan solutions above their critical concentrations (Table III) it is necessary, purely through space filling requirements, that the proteoglycan must occupy a reduced volume (see also Ref. 33) as compared to its hydrodynamic volume at infinite dilution. The conformational changes involved are not clear, however. We envisage two factors of importance namely (a) the flexibility of the protein core for PGS or the hyaluronate core for PGA and (b) the balance between the internal and external osmotic pressures in relation to the domains of the molecule (note that the HTO transport studies described earlier demonstrated that water in the hydrodynamic volume of the proteoglycan is unstructured and will therefore be subject to osmotic forces). The osmotic pressure of the internal hydrodynamic domain of the proteoglycan will be directly related to the concentration of CS chains within the domain. We know from the structure of chondrosarcoma PGS (30) that the mean concentration of CS in its hydrodynamic volume is ~1.2 mg ml⁻¹. A fully extended molecule has a cylindrical structure, with a reduced volume (Table IV) and an average cylindrical domain concentration of CS of ~11 mg ml⁻¹. The variation in the mean radial concentration of CS extending from the protein core is not known. It is likely that with increasing concentration of PGS the proteoglycan will initially prefer to take on a more restricted extended conformation without changing the average cylindrical domain concentration of CS. Conformational changes resulting in increasing the cylindrical domain CS concentration through chain folding or the intermeshing of CS chains from different molecules are more likely to occur at higher concentrations. The extended conformation with restricted translational and global rotational mobility due to the presence of other molecules is consistent with the behavior of f₁₂ from sedimentation analysis which suggested a molecule undergoing substantial intermolecular contacts. We speculate that the proteoglycans may create transient aggregate or multipolyion structures. This interpretation would also be consistent with the apparent increase in the dynamic size of the proteoglycan in relation to pores in a membrane. We would also predict similar changes in the proteoglycan aggregate (Table IV). There is a growing body of work that suggests that concentration-dependent aggregation, in the form of loose aggregates (35) or liquid-crystal-type structures (36), through the sharing of counterions or counterion clouds, is a general property of polyelectrolytes.

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