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Diffusion of tritiated water in chondroitin sulfate solutions

Kym C. Lyons and Wayne D. Comper *

Biochemistry Department, Monash University, Clayton, Victoria 3168 (Australia)

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Abstract

Water transport processes, such as titriated water exchange diffusion and hydraulic flow resistance, show molecular weight independence in semi dilute solutions of chondroitin sulfate and other linear polysaccharides yet cannot be directly related through the Onsager reciprocal relationship. Relationships can be invoked, however, with the recognition that the critical segment lengths of the linear polymers determining frictional interaction with water are different. The relatively fast process of exchange diffusion is associated with small chain segments that are independent of the chemical nature and the higher order structure of the polymer. Transport with relatively longer relaxation time, such as hydraulic conductivity, is associated with longer chain segments that manifest specific secondary and tertiary structures. This is particularly the case with chondroitin sulfate as it is very effective at flow resistance because of its interresidue hydrogen bonding.

Keywords: Chondroitin sulfate–water interaction; Articular cartilage; Flow resistance; Tritiated water; Diffusion; Hydraulic conductivity

1. Introduction

The process of water flow relative to chondroitin sulfate is important in governing the biomechanical properties of articular cartilage, particularly its compressive resistance [1]. We have previously established that chondroitin sulfate, together with other glycosaminoglycans with alternating β-(1 → 3) and β-(1 → 4) glycosidic linkages, show unusually high flow resistance when compared to other polysaccharides and synthetic polymers [2,3]. The enhanced resistance appears to be specifically associated with the structural properties of the polysaccharide chain rather than its charge. This high flow resistance identifies a unique structure–function relationship for chondroitin sulfate in load-bearing tissues, such as cartilage.

The viscous dissipation or flow of water over the chondroitin sulfate chain can be described by a binary hydrodynamic frictional coefficient. This coefficient can be directly related to the concentration dependent reduction in water self-diffusion exerted by the polysaccharide chain through the Onsager reciprocal relationship. Therefore studies of the self-diffusion of water in the presence of polysaccharide should give an independent estimate of the hydraulic flow resistance offered by the polysaccharide. It was apparent from previous work [4], however, that the obstruction offered by chondroitin sulfate chain to tritiated water (HTO) diffusion was not significantly different to that offered by other polymers studied, namely dextran and albumin. On the

* To whom correspondence should be addressed.

other hand, flow resistance measured by sedimentation velocity analysis was markedly different.

This study sets out to comprehensively examine the tritiated water diffusion in chondroitin sulfate solutions and compare it to the reduction exerted by other polymers.

2. Theory

We designate the polymer component as component 1, the solvent-water as component 2 and tritiated water as component 2*. The exchange diffusion or tracer diffusion of tritiated water may be derived [5], with the condition that $c_{2*} \ll c_2$ where c_i is the molar concentration of i, such that

$$D_2^* = RT/(f_{2*2} + f_{2*1}) \quad (1)$$

where f_{ij} is the binary frictional coefficient defined as a function of relative velocities of i and j [6].

At this stage, it is important to recognise the significance of the molar concentration term of component 1. We have previously shown that HTO diffusion in semi-dilute solutions of dextran is molecular weight independent [7] thus demonstrating that there is a critical segment length of the polysaccharide chain that is effective in obstructing the diffusion of HTO. Similar results have been obtained in studies of hydraulic flow resistance [4]. All these experiments demonstrate that for each type of measurement there must be an effective molecular weight or molar concentration term of the solute and that this is not represented by the molecular weight of the individual molecule. Accordingly we will describe the effective molecular weight and molar concentration of solute as M_1^d and c_1^d, respectively, for HTO diffusion and M_1^k and c_1^k for hydraulic flow/sedimentation studies.

Since components 2 and 2* are essentially identical, it has commonly been assumed that we may replace f_{2*1} by f_{21}. Using the reciprocal relationship

$$c_i f_{ij} = c_j f_{ji} \quad (2)$$

then from eq. (1)

$$D_2^* = RT/\left[f_{22} + \left(c_1^d f_{12}/c_2\right)\right] \quad (3)$$

The value of f_{22} can be obtained by diffusion analysis of HTO in the absence of component 1 where

$$D_2^{*o} = RT/f_{22} \quad (4)$$

It is assumed that isotope interaction is not affected by the presence of component 1. Rearranging eqs. (3) and (4) we obtain the reduced diffusion coefficient,

$$D_2^*/D_2^{*o} = 1/\left[\left(c_1^d f_{12}/c_2 f_{22}\right) + 1\right] \quad (5)$$

This expression then relates the reduced diffusion coefficient of water to the hydrodynamical frictional coefficient, f_{12}, that is common to mutual diffusion and sedimentation. We have previously identified the specific hydraulic conductivity, k, obtained from sedimentation-velocity experiments as [3],

$$k = 10^3(1-\phi_1)^2\eta_2/c_1^k f_{12} \quad (6)$$

where ϕ_1 is the volume fraction of component 1 and η_2 is the solvent viscosity. By comparison of eqs. (5) and (6), it is clear that the concentration dependence of D_2^*/D_2^{*o} and k should be similar. Previous studies by us [4] demonstrated, however, that this was not the case. The present study aims to establish these differences further with a more comprehensive set of data and to analyse the data in terms of the effective molecular weights of the chain segments offering obstruction to water movement.

3. Materials and methods

3.1. Materials

Poly(ethylene glycol) ($M_W \sim 20{,}000$) (No. 130 044119K) was from Fluka (Switzerland). Chondroitin sulfate (whale/shark) (Lot No. 81F-3887) was from Sigma (St. Louis, MO). Dextran T500 ($M_W \sim 500{,}000$) was from Pharmacia (Uppsala, Sweden). Tritiated water (Lot No. 26561669) 25

mCi/ml) was from Dupont (Boston, MA). Unless otherwise stated, all other reagents were of the highest grade commercially available.

3.2. Methods

3.2.1. Tritiated water exchange diffusion

These measurements were carried in specially designed cylindrical diffusion cells at a temperature of 20°C as previously described [4]. The analysis was similar to previous studies of water exchange diffusion in dextran polysaccharide solutions [7]. The diffusion is measured across an initial boundary formed by shearing together two cylindrical compartments containing the solutions. The total amount of diffusing solute, Q, transported across the boundary during time t is given by

$$Q^2 = A^2 S_0^2 Dt/\pi \quad (7)$$

where S_0 corresponds to the initial concentration of the labelled material in one of the chambers, A is the cross-sectional area of the diffusion compartments and D is the measured diffusion coefficient. Each measurement of the HTO diffusion coefficient in the polymer solution was taken over a range of four different time points. All experiments were performed in duplicate. The probable error in the reduced diffusion coefficient was equal to or less than 0.03. The most significant error would be in concentration estimations which would be of the order of ±5%.

The results of tests to validate that the HTO diffusion coefficient measurement is independent of HTO concentration (this is an underlying assumption in the generation of eq. 3) are shown in Table 1. By varying the concentration of HTO by a thousand-fold resulted in essentially the same percentage transfer of HTO from the bottom compartment in the diffusion cell. The percentage transfer is used here as a transport parameter in order to quantitate the HTO transport at low concentrations.

3.2.2. Preparation of solutions

All polymer solutions were made up by weight from solids of known moisture content in phosphate buffered saline which consisted of 0.14 mol dm^{-3} NaCl, 2.68×10^{-3} mol dm^{-3} KCl, 1.5×10^{-3} mol dm^{-3} KH_2PO_4 and 8.1×10^{-3} mol dm^{-3} Na_2HPO_4 (pH 7.5).

Table 1

The percent transfer of HTO in 100 mg/ml chondroitin sulfate solutions as a function of HTO concentration

Initial concentration of HTO in lower chamber of diffusion cell (dpm/ml)	Percent transfer	
	4 h	8.2 h
4.6×10^4	25.65 ± 2.49	33.54 ± 2.08
3.5×10^5	28.54 ± 2.73	38.02 ± 2.78
3.7×10^6	28.61 ± 0.67	36.93 ± 1.62
3.6×10^7	25.64 ± 0.71	36.22 ± 2.83

4. Results

The diffusion of tritiated water has been measured in chondroitin sulfate solutions up to 150 mg/ml in concentration or to a volume fraction of ~0.07 (Fig. 1). The degree of obstruction offered to HTO diffusion was compared to other polymers of different chemical composition namely dextran and poly(ethylene glycol) (PEG). When compared on a volume fraction basis, there was no significant difference offered by these polymers to tritiated water diffusion. At relatively high volume fractions, in the range of 0.07–0.09, we recorded a 20–30% reduction in diffusion. Similar results have been previously obtained [4,7].

The similar obstruction offered by these polymers to the diffusion of tritiated water is quite different to that observed with hydraulic conductivity as shown in Fig. 2. In this type of experiment for a given volume fraction, the degree of flow resistance is in the order of chondroitin sulfate > poly(ethylene glycol) > dextran. (Measurements of $k \leq 10^{-15}$ cm^2 by sedimentation analysis in the Analytical Model E are difficult due to the very low sedimentation rate and the prolonged use of high centrifugation speeds.)

5. Discussion

With the diffusion data from Fig. 1 and the hydraulic conductivity data from Fig. 2, we are in a position to examine the reciprocal relationship which relates the hydrodynamic frictional coefficient from exchange diffusion in eq. (5) that can be used to calculate k in eq. (6). Using a value of $D_2^{*\circ}$ of 192×10^{-7} cm^2 s^{-1} and a reduced diffusion coefficient ($D_2^*/D_2^{*\circ} = 0.8$) then the hydraulic conductivity is calculated to be 5.65×10^{-16} cm^2. This value is an order of magnitude lower than the values of k we have measured. In fact, from the theoretical values shown in Fig. 3 of the predicted k for a given $D_2^*/D_2^{*\circ}$ it is clear that the values k are low over a wide range of concentrations. This does establish that the measurements of HTO diffusion and hydraulic conductivity do not give rise to the same '$c_1 f_{12}$' term.

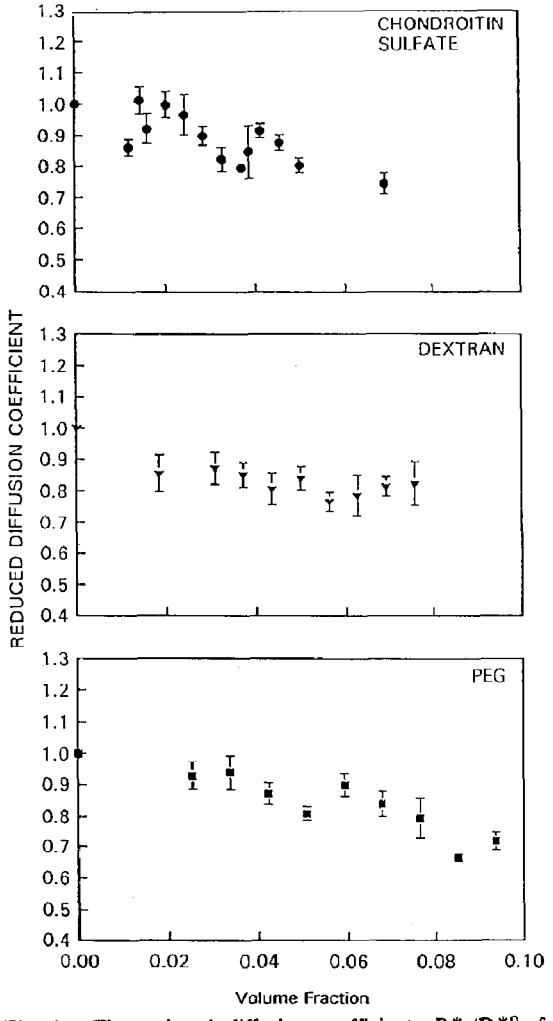

Fig. 1. The reduced diffusion coefficient, $D_2^*/D_2^{*\circ}$, for tritiated water diffusion in solutions of chondroitin sulfate, dextran and poly(ethylene glycol).

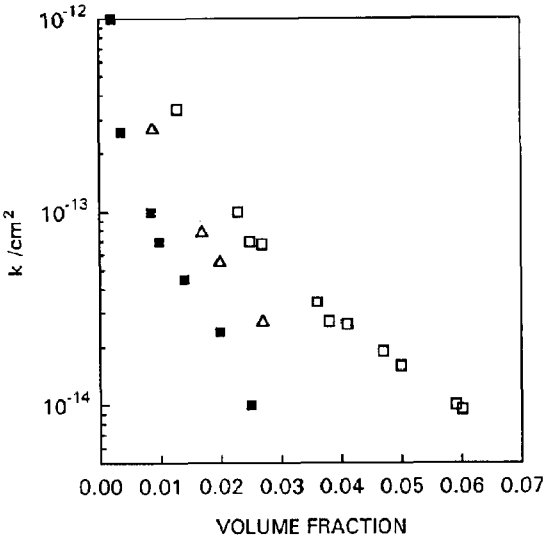

Fig. 2. Variation of the hydraulic conductivity, k, as a function of volume fraction for the polymers, dextran (□), chondroitin sulfate (■), and poly(ethylene glycol) (△). Data from refs. [2–4].

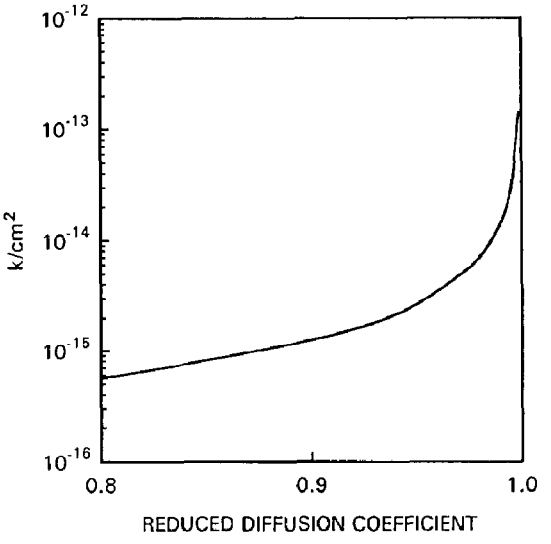

Fig. 3. The predicted values of k as a function of the reduced diffusion coefficient as determined by eqs. (5) and (6).

The difference is likely to be reflected in the effective segmental length of the polymer (or corresponding effective molecular weight of the segment) specifically associated with each type of measurement. We assume that the frictional term, f_{12}, does not vary for a given concentration from one type of measurement to another.

If we take a value of the reduced diffusion coefficient of HTO as 0.92 ± 0.04 (a value that corresponds to $\phi = 0.02$ for chondroitin sulfate with a measured $k = 2.4 \times 10^{-14}$ cm^2) then this corresponds to a calculated k of 1.63×10^{-15} cm^2 (range from 3.44×10^{-15} to 1.04×10^{-16}) which yields the ratio of $M_1^k/M_1^d = 14.7$ (range 5.8 to 24.0). This is interpreted qualitatively in terms that water exchange diffusion is seeing a small segment of the polymer chain as compared to water interaction associated with hydraulic flow. This correlates, too, with the relaxation times of each transport process where HTO exchange diffusion may be two orders faster than that associated with sedimentation. It is also apparent from Fig. 1 the relatively faster exchange diffusion process is indiscriminate with respect to the obstruction it sees in relation to size of the segments seen with the linear polymers studied. It is apparently too small to reflect long range structural differences at the secondary and tertiary level. On the other hand, with the relatively slow process of the viscous dissipation of water over the polymer chain associated with the unidirectional flow of sedimentation larger chain segments are seen that incorporate the specific characteristics of the higher order structure of the polymer. These may reflect structural and thermodynamic differences [4]. Certainly the differences seen in k for chondroitin sulfate and dextran have been correlated to the nature of the glycosidic linkage and the presence of interresidue hydrogen bonds [3]. For chondroitin sulfate, it has been suggested that an average of three inter sugar residue hydrogen bonds span a trisaccharide repeat structure which would provide extra rigidity within the chain [8]. For dextran, however, no interresidue hydrogen bonding has been identified and as such, it is thought to be a highly flexible polymer. Recent experiments have demonstrated that increasing the flexibility of the chondroitin sulfate chain by limited periodate digestion (which cleaves the carbon (2)–carbon (3) bond of the uronic acid residue) markedly increased the value of k [9]. We have also suggested that non electrostatic osmotic properties of the chain correlate with k and that this too may be associated with chain–water interaction and chain rigidity [4].

Previous attempts to evaluate the actual size of the segments [7] have related the reduced diffusion coefficient to the f_{12} term from mutual diffusion D_1 ie.,

$$D_1 \approx (M_1/f_{12})(\partial \Pi/\partial C_1)$$

where Π is the osmotic pressure and C_1 the mass concentration. This type of calculation, however, has assumed a value of M_1 corresponding to the individual molecule in the osmotic expression. It appears that the only direct way of obtaining segment lengths is a titration of the experimental parameter associated with water transport with varying molecular weight fractions of the polymer.

An obstruction and excluded volume model of the reduced diffusion coefficient of small molecules in polymer fiber matrices has used only parameters associated with molecular radii of the interacting species [10]. The major finding in this study is that segment length is also an important operational parameter in the dynamics of fiber–small molecule interaction and it will vary depending on the type of dynamic measurement performed.

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