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The hydrodynamic frictional coefficient of polysaccharides: the role of the glycosidic linkage

Oliver Zamparo and Wayne D. Comper*
Biochemistry Department, Monash University, Clayton, Victoria 3168 (Australia)

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ABSTRACT

In semi-dilute solutions, the chains of polysaccharides exert differential resistance or obstruction to the movement of water. The nature of the glycosidic linkages was correlated strongly with the magnitude of the frictional coefficient that describes the dynamic polysaccharide–water interaction. This correlation has been confirmed by analysis of the velocity of sedimentation for polysaccharides having widely varied linkages, and comparison with previous data. The results are discussed in terms of the possible role of the secondary structure of the polysaccharide and its degree of flexibility. Minor influences on the magnitude of the frictional coefficient were manifested by chemical and negative-charge substitution on the chain.

INTRODUCTION

The viscous dissipation of water over polysaccharide chains or the relative diffusional displacement of water to the polysaccharide solute can be described by a hydrodynamic frictional coefficient[1]. This frictional coefficient is a central parameter that governs such biological hydrodynamic processes as osmosis[2], diffusion[1,3], and hydraulic conductivity[3–5]. These hydrodynamic processes occur often in semi-dilute polysaccharide(glycosaminoglycan)-containing regions in tissues. In cartilage, the chondroitin sulfates endow the tissue with resistance to compression at high mechanical loads through their high frictional coefficient[3,4], whereas, in basement membranes, the heparin-like polysaccharides have relatively lower frictional coefficients which allow for a high flow of water, as in the kidney glomerular basement membrane[6].

At present, a quantitative interpretation of the frictional coefficient in terms of the structure of the solute is still in its early stages. The dynamic interaction of water with polymer solute should depend on the surface-to-volume ratio of the polymer chain[7] and the nature of the interaction of water with the surface of the polymer. Studies[3–5] of the hydrodynamic frictional coefficient, which involved a wide range of polymers with different volumes and surfaces, have borne out these expectations. For polysaccharides with molecular weights $>10\,000$ and at concentrations of >15 mg/mL, the frictional coefficient in mass units is independent of the molecular weight, which indicates that there are critical lengths of segments associated with dynamic interaction with water[1,3,4,8]. For polymers with relatively low osmotic pressures, good agreement is obtained

* Author for correspondence.

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between the relative dependence on concentration of the frictional movement of the polymer in water as compared to the diffusion of tritiated water around the polymer chain[5]. Anomalous results were obtained, however, with polymers that have relatively high osmotic pressures such as chondroitin sulfate and poly(ethylene glycol).

A striking feature of the comparative studies performed hitherto is the suggestion that not all polysaccharide chains exert the same resistance or obstruction to the movement of water. The differences have been correlated with the nature of the glycosidic linkage[4,9] and to be more or less independent of the degree of sulfation. Glycosaminoglycans with alternating β-(1→3) and β-(1→4) linkages showed the most resistance per unit mass or anhydrous volume.

We now report an extension of this analysis to dermatan sulfate [β-(1→3) and β-(1→4) (α for L sugars) linkages], carboxymethylcellulose [β-(1→4) linkages], and dextrin [α-(1→4) and α-(1→6) linkages] (Table I) in semi-dilute solutions.

EXPERIMENTAL

Dextrin (Type iv, Lot No. 76F-0633) and dermatan sulfate (bovine mucosa, Lot No. 119F0680) were obtained from Sigma. Carboxymethylcellulose was prepared from cotton linters by modification of the reported method[10,11]. Cotton linters (20 g) were powdered by freezing in liquid nitrogen and passage through the 20-mesh attachment in a Wiley Intermediate Mill. The powder was stirred with a mixture of 2-propanol (350 mL) and 3.85M NaOH (40 mL) for 50 min at 5° in an Omni-Mixer blender. Sodium chloroacetate (18 g) was then added, the mixture was stirred for 60 min at 5° under nitrogen, and the resulting slurry was stored at 25° under nitrogen for 72 h, neutralised, washed with aqueous 80% ethanol, and dried. In order to determine the degree of substitution (d.s.), solutions of the sodium salt were dialysed exhaustively against many changes of distilled water and analysed at 589 nm for sodium content using a Varian Techtron Model 1000 atomic absorption spectrophotometer. The average d.s., based on the dry weight of the sample, was 0.68 ionisable group per saccharide unit. The weight-average molecular weight for similar preparations was in the range of 200 000[12]. All other reagents were of analytical quality grade.

Sedimentation velocity. — Sedimentation coefficients were measured[4] in an analytical ultracentrifuge at 20°. Normally, the solutions were so concentrated that the concentration gradient that developed and moved away from the air/solution interface refracted light completely out of the cell. The result was that a band developed that moved according to the sedimentation of the material. The sedimentation was recorded by monitoring the movement of the median of the band by a series of the photographs taken during 48 h. Measurements of the movement of the front, middle, or rear of the band yielded similar sedimentation coefficients; the accuracy was within ±3%. Due to the low sedimentation coefficients, the distances moved by the boundary during the measurements were small, so that radial dilution effects were insignificant. Essentially similar results were obtained for the sedimentation coefficient whether it was estimated by sedimentation away from the meniscus (as routinely performed), or by recording the

movement of the maximum peak associated with the introduction of a small concentration gradient within the cell as studied for chondroitin sulfate proteoglycan[3] and dextran[1]. The sedimentation experiments were performed at high rotor speeds where the sedimentation force was considerably greater than the macroscopic osmotic pressure of the solution. Quantitative agreement on the magnitude of the hydrodynamic frictional coefficient has been obtained in semi-dilute solutions through sedimentation and diffusion analysis[1], and sedimentation and ultrafiltration analysis[4].

The expression[1] for the sedimentation coefficient (s_1) in a volume fixed frame of reference is

$$(s_1)_v = (1-\rho v_1)(1-\phi_1)M_1/f_{12}, \qquad (1)$$

where ρ is the solution density, v_1 is the partial specific volume of the solute 1, ϕ_1 is the volume fraction and M_1 is the molecular weight of 1, and f_{12} is the hydrodynamic frictional coefficient between 1 mol of solute and solvent (component 2). The biologically relevant parameter in equation *1* is the term M_1/f_{12}, which can be incorporated in the specific hydraulic conductivity (k) parameter that represents resistance to flow of membranes and biological compartments[13]:

$$k = (1-\phi_1)^2\eta_2 M_1/f_{12}C_1, \qquad (2)$$

where η_2 is the solvent viscosity and C_1 is the concentration of component 1 (mass/volume units).

Densities of solutions. —These were measured on a DMA 55 density meter (Anton Paar).

Preparation of solutions. —Carboxymethylcellulose was dialysed extensively against phosphate-buffered saline (PBS) which comprised 0.14M NaCl, 2.68mM KCl, 1.5mM KH_2PO_4, and 8.1mM Na_2HPO_4 (pH 7.5). Solutions of dermatan sulfate were made by adding the solid material directly to PBS. Solutions of dextrin were made up in distilled water.

Analytical procedures. — Carboxymethylcellulose and dextrin were analysed for hexose, using the anthrone assay[14].

RESULTS

The specific hydraulic conductivity data for dermatan sulfate, dextrin, and carboxymethylcellulose, as a function of concentration and volume fraction, are shown in Figs. 1a and 1b, respectively. The data of Sundelöf and Nyström[15] for hydroxyethylcellulose (see also ref. 16) have been included and appear to be similar to those for carboxymethylcellulose. Data[4,5,9] for various polysaccharides have also been included in Fig. 1. Both Figs. 1a and 1b show the same trend of the dependence of k on the concentration; however, it is evident from Fig. 1b that there appear to be three groups of data which are distinguished by the nature of the linkages in the polysaccharide. The

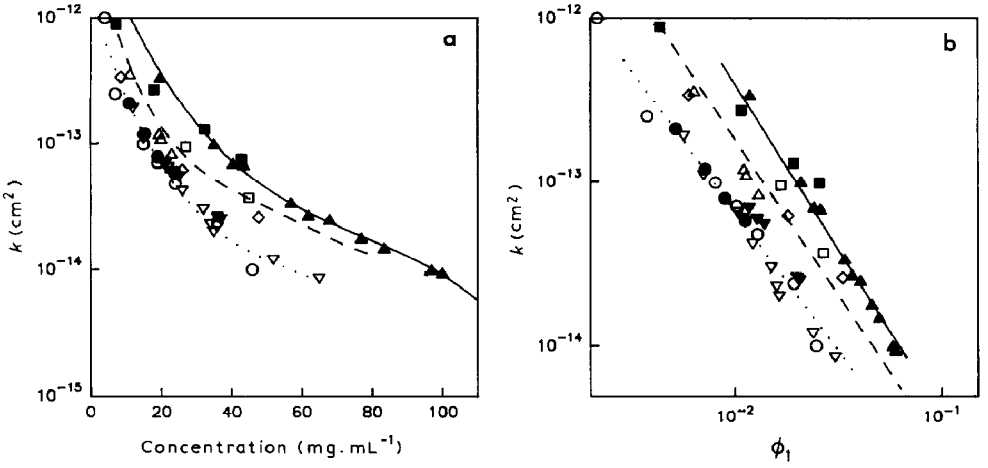

Fig. 1. The variation of specific hydraulic conductivity (k) for solutions of dextrin in water (■), carboxymethylcellulose in PBS (△), and hydroxyethylcellulose[15] (◇). Other data[4,5,9] are for solutions of dextran in water (▲), dermatan sulfate in PBS (●), desulfated heparin in PBS (□), chondroitin sulfate in PBS (▽), chondroitin (○), and carboxyl-reduced chondroitin (▼), as a function of (*a*) mass concentration and (*b*) volume fraction. Lines drawn are those of best fit.

group of data showing the lowest values of k corresponds to polysaccharides with alternating β-(1→3) and β-(1→4) linkages. The middle group corresponds to polysaccharides with β-(1→4) linkages or α-(1→4) and β(α for L sugars)-(1→4) linkages as in the heparin-like polysaccharides. The third group of data that exhibit the highest value of k correspond to polysaccharides with either α-(1→6) linkages, like dextran [which also contains a minor proportion of (1→3) linkages], and α-(1→4) and α-(1→6) linkages for dextrin.

The differentiation of these three major groups, based on the nature of the glycosidic linkage, appears to be independent of differences in the nature of the substituents on the polysaccharide chain (Table I). For example, there is little difference between hydroxyethylcellulose and carboxymethylcellulose. Moreover, these cellulose derivatives are similar to the heparin-like polysaccharides (Table I). For polysaccharides with alternating β-(1→3) and β-(1→4) linkages, a marked variation in the structure and molecular weight of the repeating disaccharide unit (Table I) does not change significantly the overall trend in the variation of k with concentration in terms of the major groupings. In addition, for both β-(1→3)- and β-(1→4)-linked polysaccharides and β-(1→4)-linked cellulose derivatives, the transformation of a polyanionic to a neutral molecule, whilst introducing a moderate change in k (see, for example, carboxyl-reduced chondroitin), did not alter the overall discrimination between the differently linked polysaccharide units.

DISCUSSION

It is not clear why the glycosidic linkages of a polysaccharide should have such a relatively profound influence on the magnitude of the frictional coefficient. In previous

TABLE I

Chemical and physical characteristics of polysaccharides[a]

Polysaccharide	*Linkage*	*Molecular weight of disaccharide unit (sodium form)*	*Partial specific volume (mL/g)*	*Anhydrous volume of disaccharide unit (mL/mole)*
Dextran	α-(1→6) (major)	324	0.60	194
Dextrin	α-(1→4) (major)	324	0.60	194
Carboxymethyl cellulose	β-(1→4)	409	0.57	233
Desulfated heparin	α-(1→4),β(and α-L)-(1→4)	430	0.51	219
Chondroitin sulfate	β-(1→3),β-(1→4)	504	0.47	237
Chondroitin	β-(1→3),β-(1→4)	402	0.54	217
Carboxyl-reduced chondroitin	β-(1→3),β-(1→4)	364	0.56	203
Dermatan sulfate	β-(1→3),β(and α-L)-(1→4)	504	0.47	237

[a] From refs. 3, 4, and 9. Dermatan sulfate is assumed to be the same as chondroitin sulfate.

studies of the conformation of the polysaccharide chain and its possible role in differentiating the hydrodynamic frictional coefficient, it is apparent that (*a*) the role of immediate water interaction on the conformation of the chain has largely been ignored and (*b*) the conformation of the chain, predicted from models and X-ray diffraction on condensed films, does not provide an obvious differentiation between the polysaccharide groups. For example, β-(1→4)-linked polysaccharides can be regarded as extended ribbons, α-(1→4)-linked polysaccharides as coiled springs, and α-(1→6)-linked polysaccharides as flexible coils with well recognised conformational flexibility, and β-(1→3)-linked polysaccharides may give rise to hollow helices[17]. The β-(1→3) linkage has been regarded as extended and flexible[18]. In glycosaminoglycans, where there is an alternating arrangement of β-(1→3) and β-(1→4) diequatorially linked residues, it is predicted that the conformation of the chain may range from undulating ribbons to extended hollow helices[17].

Whereas analysis of the conformation of the chain in relation to the frictional coefficient appears to be inconclusive, interesting data have been generated with regard to inter-residue hydrogen bonding, especially for the mammalian glycosaminoglycans studied in solution in dimethyl sulfoxide[19–22]. For glycosaminoglycans with alternate β-(1→3) and β-(1→4) linkages, hydrogen-bonding groups as well as the glycosidic oxygens are equatorial. This allows the formation of a co-operative hydrogen-bonding pattern between residues. For hyaluronate, four different hydrogen bonds within a repeat trisaccharide unit have been identified[19–22], namely, from HO-4 of GlcNAc to O-5 of GlcA, acetamido C=O to HO-2 of GlcA, HO-3 of GlcA to O-5 of GlcNAc, and NH to COO^- of GlcA. For chondroitin sulfates, only three hydrogen bonds can form due to the fact that the bond from the axial HO-4 of GalNAc to O-5 of GlcA cannot form[20]. These inter-residue hydrogen bonds would be expected to decrease the flexibility of the critical hydrodynamic segments. The derivatives of chondroitin sulfate, as described in Fig. 1, should retain this inter-residue hydrogen-bonding pattern. These results may indicate a major discriminating factor that characterises the effect of the glycosidic linkage on the hydrodynamic frictional coefficient in terms of the flexibility of the polysaccharide segment. Consistent with this interpretation is the fact that hyaluronate has a k value similar to that of chondroitin sulfate[4]. On the other hand, polysaccharides that have the highest value of specific conductivity, like α-(1→6)-linked dextrans, have a flexible structure. For β-(1→4)-linked polysaccharides such as heparan sulfate, two possible inter-residue hydrogen bonds may form[22], yet the structure will be flexible because of the presence of the iduronate residue[23]. A further point is that a flexible segment of a chain may represent a larger radius for a given volume fraction, which would be predicted[7] to increase k. These conclusions, associated with the relation of the flexibility of segments to the value of k, have been suggested in a theoretical study of polymer self-diffusional motion, although the physical basis for such a treatment remains to be established[24].

On the basis of comparative data, it has been suggested[5] that the mobility of segments will be important in governing k and that local chain–solvent interactions may play an important role in the viscous dissipation of water over the chain. It was

demonstrated that, for a given type of glycosidic linkage, the characteristic chain segment that governs the hydrodynamic interaction was insensitive to the substituents attached thereto. This conclusion has been confirmed in the present study. It appears that, apart from the glycosidic linkage that governs k, the size of the critical segment of the chain will be important also. However, there is still a paucity of information on this hydrodynamic interaction, but additional factors may be relevant (see below).

The solubility of polysaccharides has been related to the types of glycosidic linkage, with the least soluble being β-(1→4)-linked (*e.g.*, cellulose) and the most soluble being α-(1→6)-linked or with alternating β-(1→3) and β-(1→4) linkages[25]. Appropriate substituents on the cellulose chain create a more soluble polysaccharide that appears to give a solubility-independent-related frictional factor through the comparison of data in Fig. 1.

The differential hydrophobicity of glycosaminoglycans has been used for the separation of these materials[26], although at present its possible relationship to hydrodynamical interaction is unclear.

Bettelheim and co-workers[27,28] have established, from water sorption vapour isotherms, the high-affinity binding of 1–2 water molecules per disaccharide unit for a limited number of glycosaminoglycans. The order of binding (mass units) was demonstrated to be chondroitin 6-sulfate > heparin > dermatan sulfate > chondroitin 4-sulfate. The relationship between high-affinity binding of water and the effective hydrodynamic dimension of the polysaccharide remains to be established. Further, it is difficult to predict the effect that the binding of water to the polysaccharide chain may have on k.

Another aspect of the close proximity of water with the polysaccharide chain is the nature of the dynamic properties of the fluid flow over the chain (microviscosity). The fact that a mobility gradient of the solvent, which exemplifies this microviscosity, could exist over a finite distance from the segment has been discussed[29]. Whereas the specific nature of the mobility gradient was not defined, more recent studies[5] indicated that an osmotic gradient may exist for materials of relatively high osmotic activity. This conclusion was based on the lack of agreement between the relative reduction of the diffusion of tritiated water and the specific conductivity for such osmotically active materials as poly(ethylene glycol) and chondroitin sulfate. It is evident, however, that the backbone of the polymer makes a significant contribution in determining k, as indicated by the data in Fig. 1, and that factors that affect the charge-derived osmotic gradients were not enough to differentiate between the values determined by the type of glycosidic linkage.

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