Transport of Probe Ions in Solutions of Biological Polyelectrolytes

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Transport of monovalent cations was studied in solutions of biological polyelectrolytes, the sodium (or potassium) salts of anionic polysaccharides ι-, κ-, λ-carrageenan, dextran sulfate, and chondroitin sulfate, by steady-state voltammetric reduction of the probe ions Tl+ and H+ at mercury film and platinum disc microelectrodes, respectively. Diffusion coefficients of the electroactive probe ion are determined rapidly and precisely from steady-state, transport-limited current at microelectrodes in solutions with large excess and without supporting electrolyte over a wide range of polyelectrolyte and probe ion concentrations. Electrostatic interactions between polysaccharide anion and probe ion decrease the value of the diffusion coefficient of the probe ion with respect to the value without the polyelectrolyte, with the most pronounced effect in solutions without electrolyte. These interactions are quantified by the transport ratio, D/D_0, of the diffusion coefficient of the probe ion with polyelectrolyte, D, to that in solution without polyelectrolyte, D_0. The values of D/D_0 without supporting electrolyte for thallium ion are 0.59, 0.67, 0.50, 0.35, and 0.70, for ι-, κ-, λ-carrageenan, dextran sulfate, and chondroitin sulfate, respectively. For the hydrogen probe ion these transport ratios are 0.52, 0.79, 0.57, and 0.26, for ι-, κ-, λ-carrageenan and dextran sulfate, respectively. Experimental results are compared with theoretical predictions based on Manning's linear charge model and the Poisson−Bolzmann cylindrical cell model. According to both theories the dimensionless transport of the counterion in deionized solution is related to the charge separation in the polyelectrolyte: the higher the linear charge density, the greater the interaction and the smaller the transport ratio. Interactions in solutions of these biological polyelectrolytes are in accord with both theories, to a reasonable degree of accuracy, considering the uncertainty in the distance between charges.

Introduction

Transport of ions in solutions of polyelectrolytes, M_nP, is of interest for the description of many biological and synthetic systems, such as cellular membranes, biological matrices, ion-exchangers, and colloidal foods. The transport of counterions is suppressed in such systems due to long-range electrostatic interactions between polyions, P^{n-}, and counterions, M^{+}.[1−3] These interactions are the strongest in solutions of low ionic strength. At higher ionic strength the overall charge of the polyion is shielded by the electrical double layer formed by the ions of the supporting electrolyte, and the interactions between polyion and counterion become negligible.

According to Manning's line charge model,[4−7] the self-diffusion coefficient of the counterion depends on the dimensionless charge density, λ, given by

$$\lambda = e_0^2/4\pi\epsilon^\circ\epsilon bkT \qquad (1)$$

where e_0 is the elementary charge, $4\pi\epsilon^\circ\epsilon$ is the dielectric constant of the solvent, b is the length of the polyion per ionized group, k is the Boltzmann constant, and T is the absolute temperature.

Manning's theory treats polyions as infinitely long line charges. Counterions are assumed to condense onto the polyion chain as required to avoid exceeding the critical charge density, λ_c (for monovalent counterions $\lambda_c = 1$). These condensed counterions are inside the hydration layer; therefore their

mobility is that of the polyion, usually negligible in comparison with the mobility of free counterions. Uncondensed counterions and coions are subject to Debye−Hückel interactions with the polyions. The diffusion coefficient ratio for monovalent counterions is[5]

$$D_{PE}/D_0 = 1 - (1/3)A(\lambda;X) \quad \text{for} \quad \lambda < 1 \qquad (2)$$

$$D_{PE}/D_0 = [1 - (1/3)A(1; \lambda^{-1} X)] \quad \text{for} \quad \lambda > 1 \qquad (3)$$

where D_{PE} and D_0 are diffusion coefficients of monovalent counterions in solutions with and without polyelectrolyte, respectively, X is the ratio of the equivalent concentration of polyelectrolyte (i.e., the molar concentration of polyion times the charge per polyion) to the concentration of added monovalent salt, $X = C_{PE}/C_{SE}$, and $A(\lambda;X)$ is given by

$$A(\lambda;X) = \sum_{\substack{m_1=-\infty \\ (m_1,m_2)\neq(0,0)}}^{\infty} \sum_{m_2=-\infty}^{\infty} [\pi\lambda^{-1}(m_1^2 + m_2^2) + 1 + 2X^{-1}]^{-2} \qquad (4)$$

where m_1 and m_2 are integers.

In salt-free solution, $X \rightarrow \infty$,

$$D_{PE}/D_0 = 1 - (1/3)A(\lambda;\infty) \quad \text{for} \quad \lambda < 1 \qquad (5)$$

$$D_{PE}/D_0 = \lambda^{-1} [1 - (1/3)A(1;\infty)] \quad \text{for} \quad \lambda > 1 \qquad (6)$$

where $A(\lambda;\infty)$ can be obtained from eq 4 by setting $X^{-1} = 0$.

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TABLE 1: Reported Values of the Charge Separation Distance, *b*, for Polyelectrolytes

PE	b/nm			ref	average b-value/nm		
	coil	helix	double helix		coil	helix	double helix
ι-car	0.50^a		0.22^a	31,39,40			
	0.50^b	0.43^b	0.22^b	30	0.47	0.43	0.22
	0.42^b			41			
κ-car	1.0^a		0.41^a	39,40			
	$1.03^{\ b}$			42			
	1.03		0.43^b	31	1.01		0.41
			0.41^b	43			
	1.0^a		0.41^a	44,45			
λ-car	$0.48^{b,c}$			45	0.48		
DS	$0.25^{b,c}$			46			
	$0.25^{b,c}$			47	0.25		
ChS	0.47^b			48			
	0.65^b		0.29	49	0.58		0.28
	0.61^b		0.27	50			

a From fully extended molecular models for the coil conformation and from X-ray diffraction data on oriented fibers for the helix conformation. *b* From X-ray diffraction data. *c* Does not form helix or double helix.

Numerical evaluation of the series results gives $A(1;\infty) = 0.40$, and therefore

$$D_{PE}/D_0 = 0.867\lambda^{-1} \quad \text{for } \lambda > 1 \tag{7}$$

The Poisson−Boltzmann equation has been used extensively to describe properties of polyelectrolyte systems with different geometries.[1−3,8−10]

$$\nabla^2\psi = - (1/\epsilon°\epsilon) \sum_i z_i e n_i \exp(-z_i e\psi/kT) \tag{8}$$

where ψ denotes the average potential, n_i and z_i are the number concentration at the cell boundary and the valence of ion species i, and the other symbols have their usual meaning.

Calculations according to the Poisson−Boltzmann cylindrical cell model were performed in cylindrical coordinates using a program written originally for spherical polyelectrolyte particles.[11] The following parameters characterizing polyelectrolyte units were used: macroion radius = 0.5 nm (the same for all polyelectrolytes), number of monomers per polyelectrolyte unit = 50, equivalent concentration of the polyelectrolyte 20 mM, and the average value of the charge separation distance for coil forms of polyelectrolytes from Table 1. A temperature of 25 °C and the dielectric constant of water, $\epsilon = 78.3$, were used in calculations. Equation 8 was used to calculate the quantity $1 − f$, the number fraction of counterions in the deionized solution that have an energy of attractive electrostatic interaction with the macroion greater than kT. These counterions are considered to neutralize the anionic sites of the macroion, and their transport is limited by the diffusion of the macroion, which has a diffusion coefficient 2 orders of magnitude less than that of the free counterion. The fraction of ions f is free to diffuse. The value of f is identified with the D/D_0-value in Manning's theory and with the experimentally determined transport ratio in deionized solution.

The inference of ionic interactions from transport data requires measurements over a wide range of concentrations, extending to solutions nominally without supporting electrolyte. We have demonstrated the effectiveness of steady-state voltammetry at microelectrodes for transport studies in polyelectrolyte systems.[12−18] Small electrode size (diameter in the range of several micrometers) yields steady-state voltammograms on time scales of seconds and makes measurements possible in resistive media, such as solutions without supporting electrolyte. In addition, the steady-state current at microelectrodes is proportional to the flux of reactant. Thus the voltammetric signal is very sensitive to changes in the value of the diffusion coefficient. This method has advantages of simplicity, low cost, and very high throughput in comparison with NMR, which is also used to study these systems.

It has been shown that the transport rate of the singly charged cations of hydrogen and thallium, in solution of the synthetic polyelectrolyte poly(styrenesulfonic acid), PSSA, without supporting electrolyte, is only one-third that in solution without polyelectrolyte.[12−14] This result agrees well with the predictions of the two major theories for polyelectrolytes, Manning's linear charge model and the Poisson−Boltzmann cylindrical cell model described above. Comparison of voltammetric results and pulsed-field-gradient spin−echo NMR results for transport of [205]Tl(I) in PSSA has shown that the gradient diffusion coefficient measured by voltammetry is identical with the self-diffusion coefficient from NMR experiments.[15] Furthermore, the identity of these effects for counterions and for traces of other ions added as probes has been shown.[13,14] To test the predictive power of these theories and to analyze experimental results in light of existing models, we have studied a group of polyelectrolytes that have a range of charge separation distances, and hence of charge densities. This work also addresses the need for a fast and precise method for quantitative studies of interactions in solutions of natural, unmodified biological samples of polyelectrolytes.

An important group of biological polyions are anionic polysaccharides; the ones chosen for these studies are shown in Figure 1. Carrageenans are sulfated polysaccharides produced by many genera from the Rhodophyta (marine red algae).[19,20] They are used in industry to form gels and highly viscous solutions. They are composed of alternating 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose units modified by substitution with sulfate ester groups or cyclization into 3,6-anhydro-α-D-galactopyranose residues. Three major groups of carrageenans have been discerned: iota- (ι-car), kappa- (κ-car), and lambda-carrageenan (λ-car). They differ in chemical composition, which affects their charge density and their solution properties, such as gelling behavior. Thus, ideally, κ-car in solution has one charge per disaccharide, ι-car has two, but λ-car can bear two or three (on the average 2.7) charges per disaccharide unit.[21,22] Depending on the ionic strength and temperature, ι-car and κ-car can undergo a coil−helix transition,[23,24] whereas λ-car does not.[20] Dextran sulfate, DS, is a moderately branched anionic polyelectrolyte with up to three sulfate groups per monomer unit. It is a D-glucose polymer with α(1→6)-glycosidic linkages. This compound has antiviral activity, and it inhibits human inmmunodeficiency virus in vitro.[25] Its chains are in a randomly coiled state independent of ionic strength and temperature, because the polymer backbone is flexible and the sulfate groups are randomly distributed.[26] Chondroitin sulfate, ChS, is a polyanionic glucosaminoglycan found in the extracellular matrix of mammalian connective tissues. It is composed of repeating disaccharide units, glucuronic acid and glucosamine (N-acetygalactosamine). Its primary function is to resist and absorb compressive stresses, whereas the associated collagen is believed to resist and transmit tensile stresses.[27] It has been suggested that the cation-exchange properties of proteoglycans and glucosaminoglycans may be significant in the distribution and storage of extracellular cations and in the deposition of calcium during calcification.[28,29]
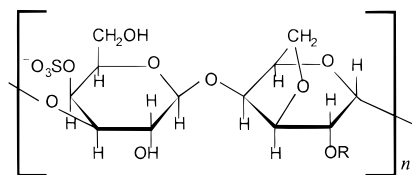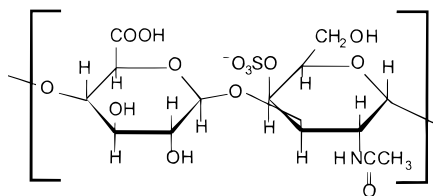
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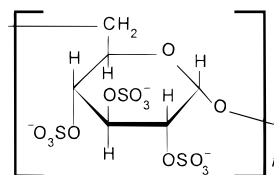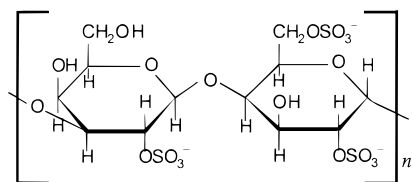
Figure 1. Repeating monomer units of polyelectrolytes used in this study.

We report here on the transport of the monovalent probe cations, Tl^+ and H^+, in solutions of these five polyions of different charge spacing. The effect of polyions on the transport of probe ions is measured by the ratio D_{PE}/D_0 for solutions without added electrolyte. The main objective of this work is to find out how the strength of electrostatic interactions depends on the charge density (or charge separation distance) of the polyion. The secondary objective is to compare experimental findings with existing theoretical models.

Experimental Section

Chemicals and Solutions. Iota-carrageenan (ι-car, MW 20 000 ± 5000), kappa-carrageenan (κ-car, MW 20 000 ± 5000), dextran sulfate (DS, MW 15 000 ± 5000), and chondroitin sulfate (ChS, MW 20 000 ± 5000) sodium salts were purchased from Fluka. Lambda-carrageenan (λ-car, 15 000 ± 5000) mixed sodium−potassium salt was purchased from Sigma. The samples were dialyzed (Spectra/Por 3, MWCO 3,500) against ultrapure water (Milli-Q, Millipore Corporation) for 4 days. The water was changed approximately five times per day. Ultrapure water was also used in all rinses and preparations of solutions. The water obtained from the Milli-Q purification system has a conductivity of about 0.4 μmho cm^{-1}. Final solutions were passed through 5.0 μm filters (Whatman) and lypholized under vacuum at the temperature of liquid nitrogen. Purified compounds were stored at 5 °C to inhibit bacterial growth. This previously used purification procedure[24,30−32] is reported to result in pure, good quality samples of polysaccharides. However, for ι-, κ-, and λ-carrageenan the amperometric titration of 20 mM solutions of these polyelectrolytes with perchloric acid using a Pt microelectrode shows no current for hydrogen ion reduction for the addition of acid below 0.2 mM. This may indicate the presence of some basic species in the solution, or it is the result of partial protonation of the polyelectrolyte. This effect does not exist for dextran sulfate and chondroitin sulfate, which suggests that it is not due to hydrolysis of sulfate groups. Concentrations of polyelectrolyte solutions reported in this work are equivalent polyelectrolyte concentrations, that is, the concentration of the monovalent counterion. All other reagents were of reagent grade purity and were used as received.

Voltammetry. Electrochemical measurements were carried out with a three-electrode system in a glass cell enclosed in an aluminum Faraday cage. Staircase voltammetry was applied with a Model 273 potentiostat (EG&G PARC) connected with a current amplifier (Keithley, Model 427) and controlled via a computer. Staircase voltammetry parameters were as follows: step height (ΔE) 5 mV, frequency (f) 1 Hz. Under these conditions the limiting current for thallium cation reduction at disc electrodes of 15 μm radius does not exceed the steady-state value by more than 3%.[33]

The working microelectrodes were 4.6 μm radius platinum disc (Project Ltd., Warsaw, Poland) and mercury film (silver-amalgam-based) disc microelectrodes of 15 μm radius. The method of preparation of these microelectrodes has been described in detail.[12,13,34] Optical inspection of the state of the electrode surface was accomplished with an inverted metallurgical microscope (Leitz Wetzlar, Germany).

A platinum quasi-reference electrode of area 2.5 cm^2 was chosen over a standard reference electrode in order to avoid introduction of electrolyte from the reference electrode junction. These electrodes in aqueous solutions for the potential range of Tl^+ and H^+ reduction drift about 5 mV/h in use.[16] As we are interested only in transport-limited currents, modest changes in potential or in reversibility of the wave do not affect the quality of the data. The counter electrode was a platinum wire. All solutions were deoxygenated before voltammetric scans and blanketed with water-saturated argon. Experiments were performed at 25 °C.

Results and Discussion

Electroactive probe ions in polyelectrolyte solutions, previously employed for metal cations such as Tl^+, Cd^{2+}, and Pb^{2+} in PSSA solution,[14,15] can be used in solutions of sodium salts of polyanions. The concentration of the probe ion should be much lower than the equivalent concentration of polyelectrolyte to expect the same interactions between polyion and both counterion (i.e., Na^+ from the dissociation of sodium salt of a polyanion) and probe ion. To verify this assumption, we calculated, based on Manning's theory, the changes in the D/D_0-value for the monovalent counterion expected due to the addition of ions of the same charge to a low ionic strength solution of
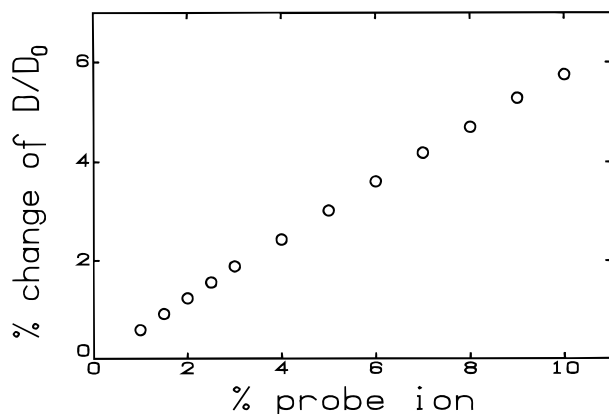
Figure 2. Calculated influence of added monovalent probe ions on changes of the transport of counterions in polyelectrolyte solution without supporting electrolyte, according to Manning's theory.

Figure 3. Steady-state voltammograms for Tl^+ probe reduction in 20 mM solution of sodium salt of λ-carrageenan without added supporting electrolyte. Tl^+ concentration/mM: (a) 0.1, (b) 0.3, and (c) 0.5. Mercury film disc microelectrode, $r = 15$ μm.

polyelectrolyte, with results as shown in Figure 2. As one can see, the addition of 2% of probe ion changes the interactions between counterion and polyanion by less than 1%. We assume of course that there are no specific interactions between the polyanion and either the counterion or probe ion. Therefore, the addition of a very low level of probe ion does not change significantly the strength of electrostatic interactions in polyelectrolyte solution. Additionally, if the concentration of the probe ion is no more than 2% of the concentration of counterion, the counterion acts as supporting electrolyte for the probe ion. Thus, even in solutions without added electrolyte, the contribution of migration to the transport-limited steady-state current for reduction of the probe cation is not larger than 0.5% of the diffusional value.[35−37]

The group of anionic polysaccharides selected for our studies, ι-car, κ-car, λ-car, DS, and ChS, all have sulfate acidic groups but differ in the number of these groups per monomer unit. The key parameter in theoretical calculations of interactions between simple ions and polyions is the charge separation distance of the polyelectrolyte, b (eq 1). Table 1 presents b-values for these polysaccharides. Charge separation distances in λ-car and DS are reported for their coil form, since they do not undergo phase transitions. For ι-car, κ-car, and ChS, polysaccharides that are known to undergo coil−helix transition as a result of changes in ionic strength of solution or temperature, b-values are given for their coil, helix, and double- helix forms. The charge separation distance for the coil form of these polyelectrolytes is greater than that for the helix or double-helix form. Under the conditions of temperature and concentration employed in this work, only the coil form is present.[23,24]

Our studies are based on the comparison of the diffusion coefficient of the probe ion in polyelectrolyte solution with that in solution without polyelectrolyte. The diffusion coefficient of electroactive probe ion, D, can be determined from the diffusion-limited steady-state current at disc microelectrodes[38]

$$I_d = 4nFCDr \qquad (9)$$

where C is the concentration of electroactive ion, r is the radius of the microelectrode, n is number of electrons transferred, and F is the Faraday constant. Diffusion coefficients are determined from slopes of concentration calibration plots; that is, $D = (1/4nFr)(\delta I_d/\delta C)$.

Thallium Cation as a Probe. Figure 3 presents steady-state voltammograms for the reduction of thallium ion at a mercury film disc microelectrode in solutions of 20 mM sodium salt of λ-car without added electrolyte. As one can see, steady-state
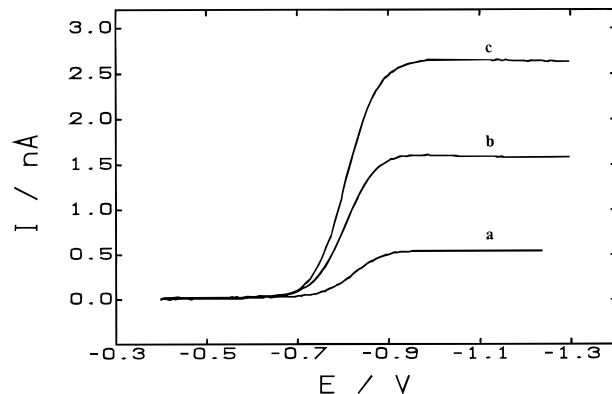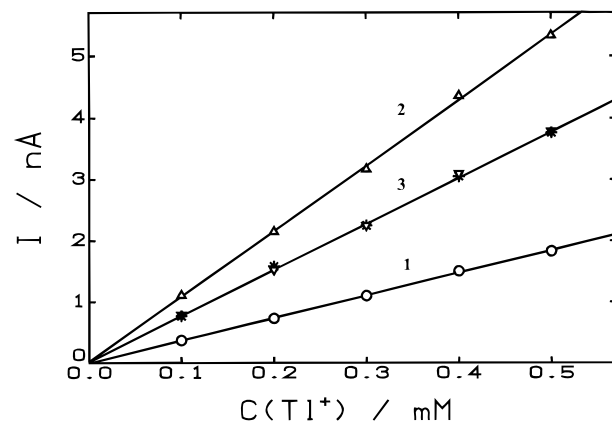
Figure 4. Concentration calibration plots for Tl^+ probe ion in ($\bigcirc$) 20 mM dextran sulfate without supporting electrolyte, ($\triangle$) 20 mM NaCl, (*) 20 mM dextran sulfate, 2 M LiClO$_4$, and ($\triangledown$) 2 M LiClO$_4$.

waves of Tl^+ reduction are very well-defined. They look similar for all polyelectrolytes and equivalent solutions of NaCl with and without added electrolyte. The reproducibility of limiting currents is very good for all systems, with relative standard deviation, rds, not higher than 2%.

Figure 4 presents concentration calibration plots obtained for Tl^+ reduction in solutions of 20 mM dextran sulfate and 20 mM NaCl, with excess (line 3) and without added supporting electrolyte (lines 1 and 2). The concentration range of thallium ion was 0.1−0.5 mM. Slopes of concentration calibration plots were reproducible for each polyelectrolyte solution, with an rds of 4−7% ($n \geq 3$). The lowest correlation coefficients from linear regression analyses was 0.9993. The x-intercept for all concentration calibration plots was insignificant, lower than 1.5 $\times 10^{-3}$ mM. As one can see from Figure 4, the slopes of the concentration calibration plots are the same in solutions with 2 M excess electrolyte both with and without polyelectrolyte. This indicates the same diffusion coefficient value of Tl^+ in both systems, which means that there are no significant electrostatic interactions between polyanion and thallium probe ion. A completely different situation exists in solutions without added electrolyte. The slope of the concentration calibration plot in polyelectrolyte solution is much smaller than that in solution of the same concentration of Na^+ ions but with chloride as anion. This difference is attributed to the decrease in Tl^+ diffusion coefficient due to electrostatic interaction of Tl^+ with the polyanion.

Table 2 summarizes the results obtained from concentration calibration plots for all five polyelectrolytes in solutions without

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TABLE 2: Concentration Calibration Plots, $\partial I/\partial C_{(Tl^+ \text{ or } H^+)}$, in PE (or NaCl) Solution with Tl^+ or H^+ as Probe[a]

| PE	$\partial I/\partial C_{Tl^+}$ (nA mM^{-1})	$\partial I/\partial C_{H^+}$ (nA mM^{-1})	$D^b/10^5$ (cm^2 s^{-1}) Tl^+ probe	$D^b/10^5$ (cm^2 s^{-1}) H^+ probe
ι-car	6.28	8.35	1.08	4.70
κ-car	7.14	12.69	1.23	7.15
λ-car	5.33	9.15	0.92	5.16
DS	3.69	4.18	0.64	2.35
ChS	7.45		1.29	
NaCl	10.65	16.06	1.84	9.05

[a] Steady-state voltammetry; H^+ reduction: Pt disc $r = 4.6$ μm; Tl^+ reduction: Hg film disc $r = 15$ μm, 25 °C; no added electrolyte. [b] Calculated from $D = (\delta I/\delta C)/4nFr$.

added electrolyte. In solutions containing excess supporting electrolyte, 2 M LiClO$_4$ ($C_{SE}/C_{PE} \geq 100$), slopes of concentration calibration curves ($\delta I/\delta C_{Tl^+}$) are almost the same for all 20 mM solutions of polyelectrolytes and NaCl, with the mean value of 7.77 nA mM^{-1} and rds of 5% ($n = 12$). Table 2 also lists D-values for the Tl^+ probe in all solutions without supporting electrolyte. As one can see, slopes of concentration calibration plots, and consequently D-values for Tl^+ in solutions without electrolyte, differ depending on the identity of the polyelectrolyte.

Diffusion coefficient values depend on viscosity, which might in turn depend on concentration of polyelectrolyte. We used oxidation of the neutral electroactive radical TEMPO, 2,2,6,6-tetramethyl-1-pipperidinyloxy, as a measure of changes in viscosity. As an uncharged species, TEMPO does not interact electrostatically with the polyanion. Therefore, the only reason to expect changes in the D-value of TEMPO is differences in the viscosity of solutions. The oxidation of TEMPO takes place around $+0.52$ V versus SCE, and the diffusion-limited current can be monitored using a Pt disc microelectrode. We measured limiting currents for TEMPO for all of the cases shown in Table 2 as well as for these solutions with 2 M LiClO$_4$ (large excess electrolyte). In 20 mM solutions of all biological polyelectrolytes without added electrolyte and 20 mM NaCl we observed the same height of the oxidation wave of TEMPO. The average diffusion coefficient calculated according to eq 9 was 6.4×10^{-6} cm^2 s^{-1} with an rds of 4.5%. In solutions with 2 M LiClO$_4$, the diffusion coefficient of TEMPO was the same for all solutions with an average value of 4.8×10^{-6} cm^2 s^{-1}, as expected. This experiment shows that the changes in the macroscopic viscosity of solution due to the presence of polyelectrolyte do not influence the diffusion coefficient of small electroactive probes. Thus the changes in the diffusion coefficient of the Tl^+ probe are due entirely to the strong electrostatic interactions with polyanion.

Hydrogen Ion as a Probe. Steady-state voltammograms obtained for the reduction of hydrogen ion at a platinum disc microelectrode in solutions of 20 mM sodium salts of all polyelectrolytes with and without supporting electrolyte are very well-defined and reproducible with an rds not higher than 6%. Figure 5 presents voltammograms for reduction of H^+ probe ion in solution of ι-car. The potential window for the solution of the ι-car sodium salt is very similar to that for NaCl solution. After addition of 0.2 mM hydrogen ion (from HClO$_4$ solution), a well-defined wave for hydrogen ion reduction is observed. For higher concentrations of hydrogen ion the reduction current is proportional to the concentration of H^+. We used the method of concentration calibration plots to determine the diffusion coefficient of H^+ probe as described above for Tl^+. The concentration range of hydrogen ion was 0.2–0.6 mM. Slopes of concentration calibration plots were reproducible for each
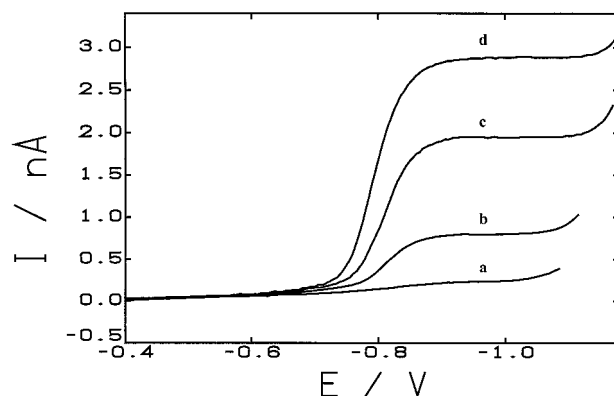

Figure 5. Steady-state voltammograms for H^+ probe reduction in 20 mM solution of sodium salt of ι-carrageenan without added electrolyte. C_{H^+}/mM: (a) 0.2, (b) 0.3, (c) 0.45, (d) 0.55. Platinum disc electrode, $r = 4.6$ μm.

TABLE 3: Transport Ratios, D/D_0, for Various Polyelectrolytes without Supporting Electrolyte

			D/D_0 calculated		D/D_0 experiment this work		D/D_0 experiment	
PE	b^a/nm	λ^b	Manning^c	PB^d	Tl^+	H^+	other [ref]
ι-car	0.47	1.52	0.54	0.43	0.59	0.52	0.73 [30]
							0.65 [43]
							0.64 [51]
							0.50 [46]
							0.65 [47]
κ-car	1.01	0.71	0.93	0.76	0.67	0.79	0.75 [52]
							0.73 [43]
							0.80 [51]
λ-car	0.48	1.49	0.58	0.46	0.50	0.57	0.49 [51]
DS	0.25	2.86	0.31	0.26	0.35	0.26	0.27 [47]
							0.38 [52]
							0.28 [46]
ChS	0.58	1.23	0.63	0.52	0.70		0.80 [53]
							0.54 [16]
							0.50 [50]
							0.74 [48]
PSSA	0.28	2.56	0.33	0.31	0.35	0.34	see [13, 16]

[a] Reported values from Table 1. [b] Calculated from b-value using eq 1. [c] Calculated from eq 7 or eq 5. [d] Calculated according to ref 11 using parameters given in the text.

polyelectrolyte solution, with an rds of 6–8% ($n \geq 3$). The lowest correlation coefficients from linear regression analyses for those plots was 0.9990. In solutions with 2 M LiClO$_4$, slopes of concentration calibration plots, $\delta I/\delta C_{H^+}$, are almost the same with and without polyelectrolytes, with the mean value of 12.1 nA mM^{-1} and rds of 7% ($n = 8$). Thus H^+ has the same value of diffusion coefficient in all solutions with excess electrolyte. In solutions without added electrolyte slopes of concentration calibration plots differ significantly; they are much smaller in 20 mM polyelectrolyte solutions than in 20 mM NaCl solution, and they differ between polyelectrolytes. Slopes of concentration calibration plots for hydrogen probe ion in 20 mM solutions of ι-car, λ-car, κ-car, and DS and D-values for H^+ are presented in Table 2. As expected, the diffusion coefficient of hydrogen ion depends on the identity of the biological polyelectrolyte.

Transport Ratio, D/D_0. Interactions between counterion and polyelectrolyte are evaluated from changes in the transport ratio, D/D_0, of the probe ion, which is affected in the same way as the counterion (eqs 2 and 3). Table 3 presents transport ratios, D/D_0, for Tl^+ and H^+ probes in solutions of the biological
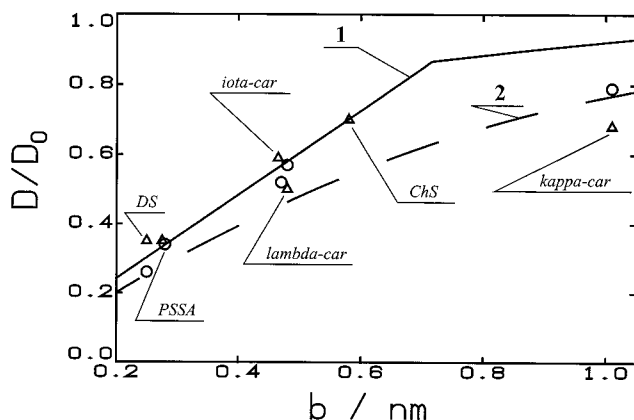
Figure 6. Transport ratio, D/D_0, as a function of the charge separation distance, b, according to Manning's (1) and the Poisson−Boltzmann (2) model, together with experimental D/D_0-values for Tl^+ ($\triangle$) and H^+ ($\bigcirc$) in polyelectrolyte solutions without electrolyte.

polyelectrolytes, and in addition for PSSA, which we studied previously.[12−14] These transport ratios are calculated from Table 2, with D_0 the value in 20 mM NaCl. Table 3 also shows values of the charge separation distance, b, as reported in Table 1, and the linear charge density, λ, calculated from the b-value according to eq 1. As one can see, the transport ratio depends on the charge separation in the polyelectrolyte; the smaller the b-value, the smaller the D/D_0 ratio. Also included are data from the literature on the transport of monovalent counterions in biological polyelectrolytes. These values do not differ statistically, although the range of values reported for each polyelectrolyte is uncomfortably large.

Theoretical Interpretation. We compared experimental results obtained for Tl^+ and H^+ probe ions in biological polyelectrolyte solutions with theoretical predictions of Manning's linear charge model and of the Poisson−Bolzmann cylindrical cell model. Calculations according to both theories were performed with the assumption that the concentration of supporting electrolyte is zero.

The transport ratio, D/D_0, was calculated according to Manning's linear charge model based on eq 7 or eq 5 (for κ-car), using the dimensionless charge density of the polyelectrolyte, λ, calculated according to eq 1. The λ-value is calculated from the charge separation distance, b, presented in Table 1, for all cases the average value for the coil form, $t = 25$ °C, and $\epsilon = 78.3$.

Calculated values of transport ratios, D/D_0, in salt-free solutions from Manning's linear charge model and the Poisson−Boltzmann cylindrical cell model are collected in Table 3 together with experimental results for Tl^+ and H^+ probes. Figure 6 displays these results graphically. The theoretical curves are fairly close at high charge density but differ more strongly at higher b-values, especially close to 0.716 nm, the charge separation distance that results in the charge density value of $\lambda_c = 1$, the critical value from Manning's theory.

The results generally agree better with Manning's theory for $\lambda > 1$, whereas the single result for polyion with $\lambda < 1$ appears to lie closer to the prediction of the Poisson−Boltzmann model. For PSSA and DS the difference between the predictions and the results is about the same as the experimental uncertainty. For the other cases with $\lambda > 1$, the experimental values are within measurement uncertainty of the Manning prediction, but the Poisson−Boltzmann prediction differs by several times the measurement uncertainty. However, because the polyelectrolytes are biological products, less uniform in properties than, for example, PSSA, and because the quantity of data is small,

this should be viewed as a hypothesis for further theoretical examination.

For $\lambda > 1$ apparently Manning's theory can be used to estimate the value of b using diffusion coefficient ratios D/D_0 determined from voltammetric data (eq 7). Using average D/D_0-values for Tl^+ and H^+ probes, we determined b-values as 0.46, 0.45, 0.26, 0.58, and 0.29 nm for ι-car, λ-car, DS, ChS, and PSSA, respectively. These values are within 2, 6, 4, 0, and 4% of the average literature structural b-values for ι-car, λ-car, DS, ChS, and PSSA, respectively. Therefore, under these conditions, the easiest, least expensive, and perhaps most accurate way to determine b might be from diffusion coefficients measured as described here, rather than from structural data. We have shown previously that the limiting relationship of eq 7 predicts quantitatively the dependence of transport ratio on dielectric constant of the solvent, even in the case of mixed solvents.[17] Thus there is some scope for moving systems with large values of b into the range of counterion condensation by adding a second solvent of lower dielectric constant. Some natural samples may contain other electrolytes that are very inconvenient or impossible to remove. In those cases, provided the concentration is not too large in comparison with the counterion concentration, methods based on eqs 3 and 4 can be used to obtain the limiting values of transport ratio.

The experimental techniques and methods employed here require only simple and inexpensive equipment and can be quickly executed. The measurement of steady-state, transport-limited current is robust, as is the theory linking the experimental quantity with the quantity sought, described by eq 9. The probe ion method presents wide choices of electrochemical reactions and avoids complications due to migration, which occurs in salt-free solutions. Thus we not only recommend this approach for analytical purposes but also suggest its use be explored for the physical characterization of polyions and other macroionic solutions and suspensions such as micelles or latexes.

References and Notes

(1) Armstrong, R. W.; Strauss, M. P. *Polyelectrolytes*. In *Encyclopedia of Polymer Science and Technology*; Mark, H. F., Gaylord, N. G., Bikales, N. M., Eds.; Wiley-Interscience: New York, 1969; Vol. 10.

(2) Dautzenberg, H.; Jaeger, W.; Kötz, J.; Philipp, B.; Seidel, C.; Stscherbina, D. *Polyelectrolytes: Formation, Characterization and Application*; Hanser/Gardner Publications, Inc.: Cincinnati, 1994.

(3) *Polyelectrolytes: Science and Technology*; Hara, M., Ed.; Marcel Dekker: New York, 1993.

(4) Manning, G. S. *J. Chem. Phys.* **1969**, *51*, 924.

(5) Manning, G. S. *J. Chem. Phys.* **1969**, *51*, 934.

(6) Manning, G. S. *J. Chem. Phys.* **1967**, *47*, 2010.

(7) Manning, G. S. *Polyelectrolytes*. In *Annual Reviews of Physical Chemistry*; Eyring, H., Christensen, C. J., Johnston, H. S., Eds.; Annual Reviews: Palo Alto, CA, 1972; Vol. 23.

(8) Katchalsky, A. *Pure Appl. Chem.* **1971**, *26*, 327.

(9) Alfrey, T.; Perg, P. W.; Morawetz, H. *J. Polym. Sci.* **1951**, *7*, 543.

(10) Fuoss, R. M.; Katchalsky, A.; Lifson, S. *Proc. Natl. Acad. Sci. U.S.A.* **1951**, *37*, 579.

(11) Roberts, J. M.; Linse, P.; Osteryoung, J. G. *Langmuir*, in press.

(12) Morris, S. E.; Ciszkowska, M.; Osteryoung, J. G. *J. Phys. Chem.* **1993**, *97*, 10453.

(13) Ciszkowska, M.; Osteryoung, J. G. *J. Phys. Chem.* **1994**, *98*, 3194.

(14) Ciszkowska, M.; Osteryoung, J. G. *J. Phys. Chem.* **1994**, *98*, 11791.

(15) Ciszkowska, M.; Zeng, L.; Stejskal, E. O.; Osteryoung, J. G. *J. Phys. Chem.* **1995**, *99*, 11764.

(16) Scordilis-Kelley, C.; Osteryoung, J. G. *J. Phys. Chem.* **1996**, *100*, 797.

(17) Ciszkowska, M.; Osteryoung, J. G. *J. Phys. Chem.* **1996**, *100*, 4630.

(18) Ciszkowska, M.; Osteryoung, J. G. *Proceedings of the International Symposium on "New Directions in Electroanalytical Chemistry"*; Leddy, J., Wightman, R. M., Eds.; The Electrochemical Society, Inc.: Pennington, NJ, 1996; pp 263−273.

(19) Stortz, C. A.; Cerezo, A. S. *Int. J. Biol. Macromol.* **1991**, *13*, 101.

(20) *Solution Properties of Polysaccharides*; Brant, D. A., Ed.; ACS Symposium Series 150; American Chemical Society: Washington, DC, 1981.

(21) Slootmaekers, D.; Mandel, M.; Reynaers, H. *Int. J. Biol. Macromol.* **1991**, *13*, 17.

(22) Slootmaekers, D.; van Dijk, J. A. P. P.; Varkevisser, F. A.; Bloys van Treslong, C. J.; Reynaers, H. *Biophys. Chem.* **1991**, *41*, 51.

(23) Manning, G. S. *Macromolecules* **1990**, *23*, 5149.

(24) Nilsson, S.; Piculell, L. *Macromolecules* **1990**, *23*, 2776.

(25) Maderich, A. B.; Sugita, E. T. *J. Chromatogr.* **1993**, *620*, 137.

(26) Rizzo, R. *Makromol. Chem.* **1984**, *185*, 757.

(27) Scott, J. E.; Chen, Y.; Brass, A. *Eur. J. Biochem.* **1992**, *209*, 675.

(28) Comper, W. D.; Zamparo, O. *Biochem. J.* **1990**, *269*, 561.

(29) Maroudas, A. *Biophys. J.* **1970**, *10*, 365.

(30) Piculell, L.; Rymden, R. *Macromolecules* **1989**, *22*, 2376.

(31) Nilsson, S.; Piculell, L.; Jonsson, B. *Macromolecules* **1989**, *22*, 2367.

(32) Austen, K. R.; Goodall, D. M.; Norton, I. T. *Carbohydr. Res.* **1985**, *140*, 251.

(33) Sinru, L.; Osteryoung, J. G.; O'Dea, J. J.; Osteryoung, R. A. *Anal. Chem.* **1988**, *60*, 1135.

(34) Ciszkowska, M.; Donten, M.; Stojek, Z. *Anal. Chem.* **1994**, *66*, 4112.

(35) Amatore, C.; Fosset, B.; Bartlett, J.; Deakin, M. R.; Wightman, R. M *J. Electroanal. Chem.* **1988**, *256*, 255.

(36) Oldham, K. B. *J. Electroanal. Chem.* **1992**, *337*, 91.

(37) Myland, J. C.; Oldham, K. B. *J. Electroanal. Chem.* **1993**, *347*, 49.

(38) Wightman, R. M. *Anal. Chem.* **1981**, *53*, 1125A.

(39) Anderson, N. S.; Cambell, J. W.; Harding, M. M.; Rees, D. A.; Samuel, J. W. B. *J. Mol. Biol.* **1969**, *45*, 85.

(40) Arnott, S.; Scott, W. E.; Rees, D. A.; McNob, C. G. A. *J. Mol. Biol.* **1974**, *90*, 253.

(41) Rees, D. A. *Adv. Carbohydr. Chem. Biochem.* **1969**, *24*, 267.

(42) Snoeren, T. H. M. *Biochim. Biophys. Acta* **1976**, *437*, 264.

(43) Yuryev, V. P.; Plashchina, I. G.; Braudo, E. E.; Tolstoguzov, V. B. *Carbohydr. Polym.* **1981**, *1*, 139.

(44) Nilsson, S.; Piculell, L. *Macromolecules* **1989**, *22*, 3011.

(45) Nilsson, S.; Piculell, L. *Macromolecules* **1990**, *23*, 2776.

(46) Kowblansky, M.; Tomasula, M.; Ander, P. *J. Phys. Chem.* **1978**, *82*, 1491.

(47) Ander, P. In *Water Soluble Polymers*; Shalaby, S. W., McCormic, C. L., Butler, G. B., Eds.; ACS Symposium Series 476; American Chemical Society: Washington, DC, 1991.

(48) Magdalenat, H.; Turq, P.; Trivant, P.; Chemla, M.; Menez, R.; Drifford, M. *Biopolymers* **1979**, *18*, 187.

(49) Yonese, M.; Tsuge, H.; Kishimoto, H. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 20.

(50) Tsuge, H.; Yonese, M.; Kishimoto, H. *J. Phys. Chem.* **1987**, *91*, 1971.

(51) Rinaudo, M.; Karimian, A.; Milas, M. *Biopolymers* **1979**, *18*, 1673.

(52) Huizenga, J. R.; Griegor, P. F.; Wall, F. F. *J. Am. Chem. Soc.* **1950**, *72*, 4228.

(53) Nilsson, L.G.; Nordenskiold, L.; Stilbs, P. *J. Phys. Chem.* **1987**, *91*, 6210.