Unified Description of Electrophoresis and Diffusion for DNA and Other Polyions†

Earle Stellwagen, Yongjun Lu, and Nancy C. Stellwagen*

Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242

Received July 9, 2003; Revised Manuscript Received August 15, 2003

ABSTRACT: The electrophoretic mobilities and diffusion coefficients of single- and double-stranded DNA molecules up to 50 000 bases or base pairs in size have been analyzed, using mobilities and diffusion coefficients either measured by capillary electrophoresis or taken from the literature. The Einstein equation suggests that the electrophoretic mobilities (μ) and diffusion coefficients (D) should be related by the expression $\mu/D = Q/k_BT$, where Q is the charge of the polyion ($Q = ze_o$, where z is the number of charged residues and e_o is the fundamental electronic charge), k_B is Boltzmann's constant, and T is the absolute temperature. If this equation were true, the ratio μ/zD should be a constant equal to e_o/k_BT (39.6 V^{-1}) at 20 °C. However, the ratio μ/zD decreases with an increase in molecular weight for both single- and double-stranded DNAs. The mobilities and diffusion coefficients are better described by the modified Einstein equation $\mu/N^mD = e_o/k_BT$, where N is the number of repeat units (bases or base pairs) in the DNA and m is a constant equal to the power law dependence of the diffusion coefficients on molecular weight. The average value of the ratio μ/N^mD is 40 ± 4 V^{-1} for 36 single- and double-stranded DNA molecules of different sizes, close to the theoretically expected value. The generality of the modified Einstein equation is demonstrated by analyzing literature values for sodium polystyrenesulfonate (PSS). The average value of the ratio μ/N^mD is 35 ± 6 V^{-1} for 14 PSS samples containing up to 855 monomers.

The electrophoretic mobility of a polyelectrolyte in an electric field is determined by

$$\mu = Q/f \tag{1}$$

where Q is the total charge and f is the friction factor. According to the Einstein equation

$$f = k_BT/D \tag{2}$$

the friction factor can be related to the diffusion coefficient (D), where k_B is Boltzmann's constant and T is the absolute temperature (*1*). Combining eqs 1 and 2 leads to

$$\mu/D = Q/k_BT \tag{3}$$

which is sometimes called the Nernst−Einstein equation (*2*), although that designation is more properly applied to the analogous equation relating conductivity and diffusion (*1*). It has previously been shown that eq 3 does not hold for high-molecular weight, free draining DNA molecules (*2*, *3*), because the mobilities are independent of molecular weight (*4*, *5*) while the diffusion coefficients, measured in the presence (*2*) or absence (*2*, *6*, *7*) of an electric field, decrease with an increase in molecular weight.

To illuminate more clearly the relationship between electrophoretic mobility and diffusion, it is useful to replace Q in eq 3 with ze_o, leading to

$$\mu/zD = e_o/k_BT \tag{4}$$

where z is the number of charged residues in the polyion and e_o is the fundamental electronic charge. If every monomeric unit in the polyion carries a single positive or negative charge, $z = N$, the number of monomers. Equation 4 predicts that the ratio μ/zD should be equal to 39.6 V^{-1} at 20 °C, after substituting known values of the constants into the right-hand side of the equation.

In the study presented here, the ratio μ/zD has been calculated for a variety of small and large single- and double-stranded DNA molecules, using mobilities measured by capillary electrophoresis and diffusion coefficients either measured by capillary electrophoresis or taken from the literature. The results indicate that the ratio μ/zD is not constant, but decreases monotonically with an increase in molecular weight. However, the mobilities and diffusion coefficients can be described by a modified version of the Einstein equation, $\mu/N^mD = e_o/k_BT$, where N is the number of repeat units (bases or base pairs) and m is a constant equal to the power law dependence of the diffusion coefficients on molecular weight. The generality of the modified Einstein equation is demonstrated by analyzing the mobilities and diffusion coefficients of sodium polystyrenesulfonate (PSS)[1] molecules of various sizes, using data taken from the literature.

EXPERIMENTAL PROCEDURES

DNA Samples. Single-stranded DNA oligomers containing 2−15 residues and approximately equal AT/GC ratios were synthesized by standard methods (Integrated DNA Technologies, Coralville, IA) and purified by polyacrylamide gel

† This work was supported in part by Grants GM29690 and GM61009 from the National Institute of General Medical Sciences.

* To whom correspondence should be addressed. Fax: (319) 335-9570. E-mail: nancy-stellwagen@uiowa.edu.

[1] Abbreviations: ss, single-stranded; ds, double-stranded; PSS, sodium polystyrenesulfonate.

10.1021/bi035203p CCC: $25.00
Published on Web 09/19/2003

electrophoresis. Double-stranded DNA restriction fragments were prepared by standard methods (*8*) using a variety of restriction enzymes, as described previously (*9*). Some of the restriction fragments were denatured by heating in a boiling water bath for 5−15 min, followed by rapid cooling in a water/ice mixture. All DNA samples were dissolved in T0.1E buffer [10 mM Tris-HCl buffer and 0.1 mM EDTA (pH 8.1)] and stored at −20 °C until they were needed. AMP (sodium adenosine 5′-monophosphate) and ATP (sodium adenosine 5′-triphosphate) were obtained from Sigma and used without further purification.

Capillary Electrophoresis. Capillary zone electrophoresis was carried out with a Beckman Coulter P/ACE MDQ Capillary Electrophoresis System run in the reverse polarity mode with UV detection at 254 nm, using procedures described previously (*5*). Migration times and peak areas were analyzed with 32 Karat software. The neutral eCAP-coated capillaries used for these experiments were 40.0 cm in length (29.8 cm to the detector) and had internal diameters of 100 μm. The running buffer was 40 mM Tris-acetate, prepared by titrating Tris base to a pH of 8.3 using glacial acetic acid. The ionic strength of the buffer was calculated from the measured pH and the pK_a of Tris, using the Henderson−Hasselbach equation (*8*). The electroosmotic flow of the buffer in the coated capillaries was negligible. DNA electrophoretic mobilities were calculated from

$$\mu = d/Et \tag{5}$$

where μ is the mobility, d is the distance from the injection site to the detector in centimeters, E is the applied electric field strength in volts per centimeter, and t is the migration time in seconds. Diffusion coefficients of selected single-stranded oligomers were measured by the stopped migration method, which is described in detail elsewhere (*7*, *10*). All mobility and diffusion measurements were taken at 20.0 ± 0.1 °C.

RESULTS AND DISCUSSION

DNA Electrophoretic Mobility. The electrophoretic mobilities observed for single-stranded DNA (ssDNA) molecules first increase with an increase in molecular weight, pass through a maximum at ∼10 nucleotide residues, and then decrease slowly until reaching a constant value at high molecular weights, as shown by the empty circles in Figure 1. The plateau mobility observed at high molecular weights (2.84×10^{-4} cm^2 V^{-1} s^{-1}) is close to the value of 3.11×10^{-4} cm^2 V^{-1} s^{-1} observed by Costantino et al. (*11*) for heat-denatured calf thymus DNA in 25 mM NaCl, after correcting their mobility for the difference in the viscosity of water at 4 and 20 °C. The agreement between the two values is satisfactory, considering the large temperature correction.

The mobility maximum observed for ssDNAs at ∼10 nucleotide residues has not been observed previously for random-sequence DNAs, although single-stranded polythymidine oligomers exhibit a similar mobility maximum at ∼15 nucleotide residues (*3*). A ssDNA molecule containing 10 nucleotide residues would be ∼4.3 nm in length, assuming the rise per nucleotide to be 0.43 nm (*12*). Since the persistence length of ssDNA ranges from 3 to 7 nm in solutions of moderate ionic strength (*13*−*16*), depending on the sequence and method of measurement, the mobility maximum occurs for oligomers approximately one persistence length in size. The slow decrease in mobility that is observed for larger ssDNAs may be due to the gradual onset of coiling with an increase in molecular weight. Unlike a rod, the interior of a coil is permeable to the solvent. Counterions in the interior of a coil would tend to migrate toward the cathode when an electric field is applied, while the negatively charged DNA molecules would migrate toward the anode. The gradual increase in the drag force due to this "electrophoretic effect" and/or the concomitant deformation of the counterion cloud (or the polyion itself) in the electric field (*3*, *17*) may be responsible for the subsequent decrease in the mobility with an increase in molecular weight.

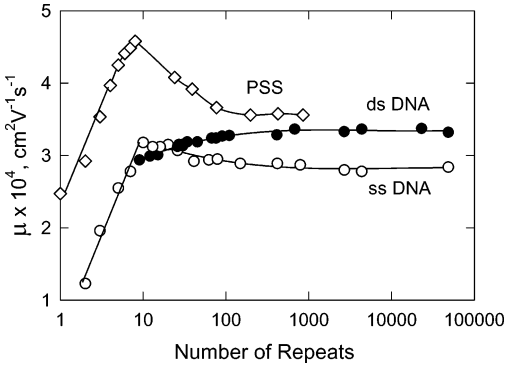

FIGURE 1: Log−log plot of mobility as a function of the number of repeat units (bases, base pairs, or styrenesulfonate residues) in each polyion: (○) single-stranded DNA molecules in 40 mM Tris-acetate buffer (I = 20 mM) (measured in this study), (●) double-stranded DNA molecules measured in 40 mM Tris-acetate buffer (*5*), and (◇) sodium polystyrenesulfonates (PSS) measured in sodium borate buffer (I = 20 mM) (*45*). The literature values for dsDNA and PSS were corrected to a temperature of 20 °C using the ratio of the viscosity of water at the two temperatures.

The mobilities of double-stranded DNA (dsDNA) molecules of different molecular weights (filled circles in Figure 1) increase monotonically with an increase in molecular weight, before leveling off and becoming constant at molecular weights of more than ∼400 bp (*5*). No mobility maximum is observed, possibly because of the inherently greater stiffness of dsDNA; the persistence length is ∼50 nm in the buffers typically used for electrophoresis (*9*, *18*). The mobility curves observed for ssDNA and dsDNA molecules intersect; the crossover occurs for DNA molecules containing ∼18 repeat units (bases or base pairs). Hence, one cannot use electrophoretic mobility to distinguish between single- and double-stranded DNA molecules in this molecular weight region.

DNA Diffusion Coefficients. Diffusion coefficients were measured for AMP, ATP, and several small DNA oligomers by capillary electrophoresis, using the stopped migration method (*7*, *10*). The diffusion coefficients are compared in Table 1 with values in the literature obtained by other methods (*19*−*25*). In general, the correspondence between the results is very good. The diffusion coefficients measured for AMP and ATP are equal within experimental error, as observed previously (*20*), and equal to the diffusion coefficient of the CpA dinucleotide, which contains a single phosphate residue. Hence, the effective hydrodynamic radii of these three analytes must be similar.

The dependence of the diffusion coefficients of single- and double-stranded DNA molecules of various sizes on the

Table 1: Diffusion Coefficients of Small DNA Oligomers

DNA	D ($\times 10^6$, cm^2 s^{-1}), CE[a]	D ($\times 10^6$, cm^2 s^{-1}), other methods[b]
AMP, ATP	3.36	3.35 (19), 3.3 (20), 4.2 (21)
CpA	3.36	–
ss 10-mer	2.05	–
ss 16-mer	1.95	1.6 (22)[c]
ss 20-mer	1.52 (10)	1.5 (22)[c]
ds 20-mer	1.05 (7), 1.07 (10)	1.09 (23), 1.10 (24), 1.05 (25)

[a] Measured by the stopped migration method (*7*, *10*). The estimated accuracy of the results is ±10%. [b] Measured diffusion coefficients at a temperature T corrected, if necessary, to 20 °C using the equation $D_{20} = D_T(293\eta_T/T\eta_{20})$ (*1*). [c] Phosphothioate linkers between the bases, instead of phosphate residues.

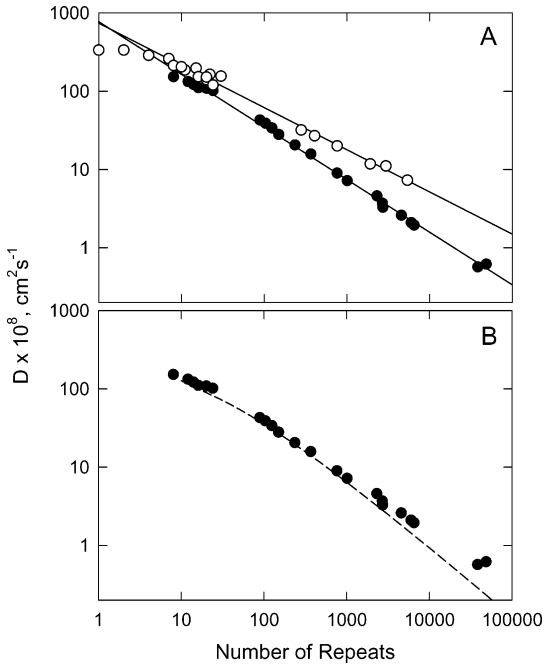

FIGURE 2: (A) Log−log plot of the diffusion coefficients (D) of (○) ssDNA and (●) dsDNA as a function of the number of repeat units (bases or base pairs) in each polyion. The data for the small oligomers were taken from Table 1; other values were taken from refs *6*, *14*, and *19*−*37*. Various buffers were used in the literature studies; most solutions ranged from 50 to 200 mM in ionic strength. The straight lines represent linear least-squares fits of the data. The equations of the lines are as follows: $D_{ssDNA} = 7.38 \times 10^{-6} \times N^{-0.539}$ ($r^2 = 0.992$) and $D_{dsDNA} = 7.73 \times 10^{-6} \times N^{-0.672}$ ($r^2 = 0.997$), where N is the number of repeat units (bases or base pairs) in each polyion. (B) Comparison of the measured diffusion coefficients of dsDNA (●) with diffusion coefficients calculated from the equation of Tirado and García de la Torre (eq 6) (− − −). The diameter of dsDNA was assumed to be 2.5 nm and the rise per base pair to be 0.34 nm. The calculated diffusion coefficients are not sensitive to the assumed DNA diameter (not shown).

number of hydrodynamic repeat units (bases or base pairs) in each molecule is illustrated in Figure 2A. The diffusion coefficients were taken from Table 1 and various studies in the literature (*6*, *14*, *19*−*37*). For ssDNA (empty circles in Figure 2A), the diffusion coefficients exhibit a power law dependence on the number of nucleotides, over a more than 1000-fold range of molecular weights. The terminal slope of the line is −0.54, close to the power index of −0.52 measured by Hadden et al. for ssDNA using pulsed field gradient NMR (*38*), but somewhat larger than the power index of −0.49 obtained by Tinland et al. for dye-labeled DNAs in 8 M urea, using fluorescence recovery after photobleaching. A power index of −0.50 is expected for random coils without excluded volume; a value of −0.60 would be expected for random coils if excluded volume effects were important (*2*, *14*). The observed power index of −0.54 is intermediate between these two values, suggesting that excluded volume effects may be somewhat important for ssDNA. Alternatively, ssDNA could be stiffer than expected for a true random coil-like molecule, leading to an intermediate power index.

The diffusion coefficients of ssDNAs containing three or fewer nucleotides fall significantly below the straight line describing the diffusion coefficients of the larger molecules, indicating that the equation of the line cannot be used to estimate the diffusion coefficients of very small DNA oligomers. The deviation from a power law dependence in this molecular weight range may be due to hydrodynamic coupling between translational and rotational diffusion (*39*).

The slope of the straight line describing the dependence of the diffusion coefficients of dsDNA on molecular weight (filled circles in Figure 2A) is −0.67, very close to the value of −0.70 that can be calculated (*30*) from the theory of Yamakawa and Fujii (*40*) for worm-like chains without excluded volume. The observed power index is larger than the value of −0.57 obtained by Nkodo et al. (*2*) for dye-labeled DNAs, using the technique of fluorescence recovery after photobleaching. The diffusion coefficients of dsDNAs of moderate size can also be described by an equation derived by Tirado and Garcia de la Torre (*39*, *41*):

$$D = \frac{k_B T[\ln(L_c/d) + \nu]}{3\pi\eta L_c} \quad (6)$$

where η is the viscosity of the solvent, L_c is the contour length of the DNA, d is the diameter, and ν is a correction for end effects, as shown in Figure 2B. The theoretical and experimental curves agree well for DNA molecules containing 4−1000 bp, even though eq 6 was derived for short, rod-like DNA molecules. The divergence between theory and experiment at high molecular weights is most likely due to the coiling of very large DNA molecules.

Comparison of DNA Electrophoretic Mobility and Diffusion using the Einstein Equation. The ratio μ/zD was calculated for the single- and double-stranded DNA molecules whose mobilities are given in Figure 1. Diffusion coefficients were calculated for DNAs of exactly the same size from the equations of the lines in Figure 2A, except for the CpA dinucleotide, for which the measured value of D (Table 1) was used. The calculated values of μ/zD are plotted as a function of the number of repeat units (bases or base pairs) in each DNA in Figure 3. It can be seen that the ratio μ/zD is not constant for either large or small, single- or double-stranded DNA molecules. Therefore, the Einstein equation (eq 3) is not obeyed, and the physical meaning of an "effective charge" calculated from DNA mobilities and diffusion coefficients using the Einstein equation is not clear.

Unified Description of Electrophoretic Mobility and Diffusion. The mobilities and diffusion coefficients observed for single- and double-stranded DNA molecules can be collapsed onto a common curve by using a modified version of the Einstein equation:

$$\mu/N^m D = e_o/k_B T \quad (7)$$

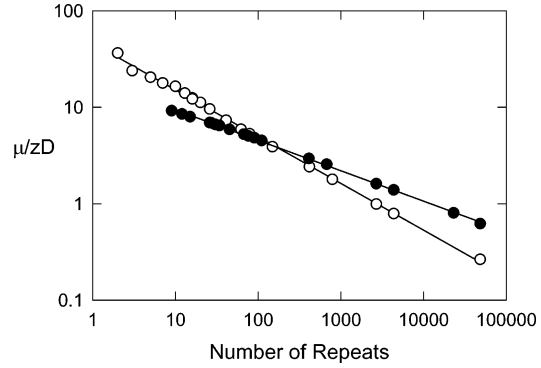

FIGURE 3: Log−log plot of the Einstein ratio (μ/zD) as a function of the number of repeat units (bases or base pairs) in the molecule: (○) ssDNA and (●) dsDNA.

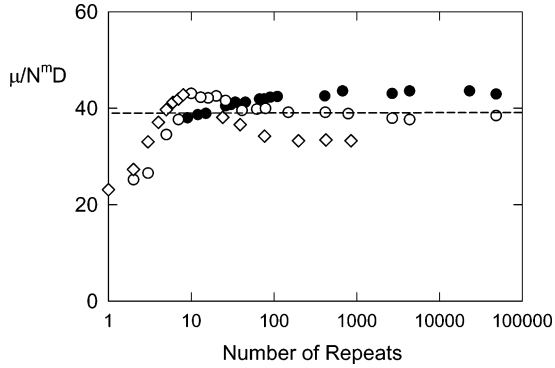

FIGURE 4: Modified Einstein ratio (μ/N^mD) plotted as a function of the logarithm of the number of repeat units (bases, base pairs, or styrenesulfonate residues) in each molecule: (○) ssDNA, (●) dsDNA, and (◇) PSS. The dashed line corresponds to the theoretical e_o/k_BT value of 39.6 V^{-1} at 20 °C.

where N corresponds to the number of charged repeat units in the polyion (bases or base pairs), m is a constant corresponding to the power law dependence of the diffusion coefficients on molecular weight, taken from the slopes of the lines in Figure 2A, and the other terms have been defined above.

The modified Einstein equation can be rationalized as follows. The fitting equations describing the straight lines in Figure 2A have the general form $D = D_1N^{-m}$, where D_1 is the diffusion coefficient corresponding to a hypothetical monomer of the target polyion. Upon substitution into eq 7, $\mu/N^mD = \mu/N^mD_1N^{-m} = \mu/D_1$, which will be a constant whenever the mobility is independent of N. In fact, the ratio μ/N^mD is approximately constant for all the DNA molecules that have been examined, as shown by the empty and filled circles in Figure 4. The average value of μ/N^mD is 38 ± 5 V^{-1} for 18 ssDNA samples ranging in size from 4 to 49 000 nucleotides, and 42 ± 2 V^{-1} for 18 dsDNA samples ranging in size from 9 to 49 000 base pairs. These values are close to the theoretical value of 39.6 V^{-1} calculated for the ratio e_o/k_BT at 20 °C. Hence, the modified Einstein equation (eq 7) provides a good description of the relationship between the electrophoretic mobility and diffusion of single- and double-stranded DNA molecules.

The constancy of the ratio μ/N^mD for both single- and double-stranded DNA molecules is somewhat surprising, since $N = z$ for ssDNA and $N = 2z$ for dsDNA, where z is the number of charged nucleotide residues. However, the constant ratio can be understood as follows. For ssDNA, the modified Einstein equation is $\mu/N^mD = \mu/z^mD = \mu/z^mD_1N^{-m} = \mu/z^mD_1z^{-m} = \mu/D_1$, as described above. For dsDNA, the modified Einstein equation becomes $\mu/N^mD = \mu/(2z)^mD$, when expressed in terms of z, the number of charged residues. A plot of the diffusion coefficients of double-stranded DNA as a function of z (not shown) gives the same slope, m, as determined for dsDNA in Figure 2A but a smaller intercept at $z = 1$, termed D_1'. Comparison of the intercepts shows that $D_1' = 2^{-m}D_1$. Hence, the fitting equation for the diffusion coefficients becomes $D = z^{-m}D_1'$, and therefore, $\mu/N^mD = \mu/(2z)^mD = \mu/2^mz^mD_1'z^{-m} = \mu/2^m2^{-m}D_1 = \mu/D_1$. The results indicate that the modified Einstein equation (eq 7) gives the same value of the ratio μ/N^mD for both single- and double-stranded DNA molecules, despite the fact that N is defined differently for the two DNAs.

Physical Meaning of the Modified Einstein Equation. The modified Einstein equation (eq 7) suggests that the effective charge of a polyion in solution is smaller than its formal charge, probably because the polyion exists in three-dimensional space, not one-dimensional space, which strengthens the interaction between charged residues. In the original Einstein equation (eq 3), the total charge of a polyion is assumed to be the sum of the charges of the monomers ($Q = \sum q_i = N\alpha$, where N is the number of monomer units and α is the charge per monomer unit). The modified Einstein equation is an empirical equation that takes into account the fact that the total charge of the polyion is reduced according to the relation $Q = \sum z_i = N^m\alpha$.

Classical studies of electrophoretic mobility (*42*) have suggested that the effective charge of an analyte depends on the thickness of the ion atmosphere according to the relation $Q_{measured} = Q_{true}[\kappa^{-1}/(\kappa^{-1} + r)]$, where κ^{-1} is the Debye−Hückel length and r is an effective radius. For most of the DNA molecules considered here, the effective radius is much larger than κ^{-1} ($\kappa^{-1} \approx 15-20$ Å in solutions with ionic strengths of 20−50 mM), leading to the equation $Q_{measured} = Q_{true}\kappa^{-1}/r$. Substituting $N\alpha$ for Q_{true} and assuming that the effective radius is proportional to N lead to the relation $Q \sim N\alpha\kappa^{-1}/N$, which is constant. Hence, ionic strength effects do not contribute to the decrease in the effective charge with an increase in molecular size that is observed for DNA.

Manning (*43*) and Record (*44*) have proposed that the localization of counterions near the surface of a highly charged polyion such as DNA effectively reduces the charge per phosphate residue. The model of counterion condensation has been very successful in explaining many of the polyelectrolyte properties of DNA (*45*). According to this model, the effective charge of a polyion is not Q, the total charge, but is reduced to $(1 - \theta_1)Q$ after counterion condensation, where θ_1 is 0.76 for dsDNA and 0.40 for ssDNA in solutions containing monovalent cations (*43*). If the factor $(1 - \theta_1)N$ is used in eq 7 to express the reduced charge of DNA, instead of the factor N^m, the modified Einstein equation would become $\mu/(1 - \theta_1)ND = e_o/k_BT$. Log−log plots of the ratio $\mu/(1 - \theta_1)ND$ as a function of the number of DNA repeats are essentially identical to Figure 3, except for displacement of the data on the vertical axis (not shown). Hence, calculation of the renormalized charge according to the postulates of counterion condensation theory cannot reconcile the mobilities and diffusion coefficients of single- and

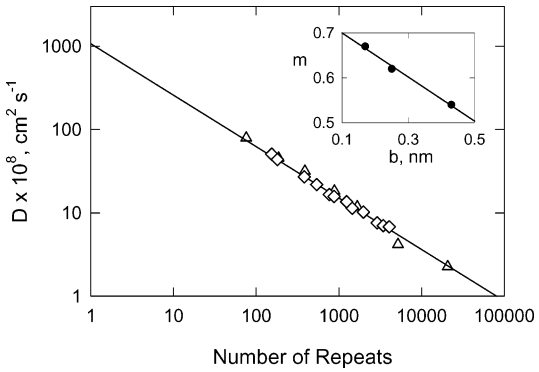

FIGURE 5: Log−log plot of the diffusion coefficients of sodium polystyrenesulfonate in (◇) 0.05 M NaCl (*48*) and (△) 0.1 M $NaNO_3$ (*49*) corrected to a temperature of 20 °C. The straight line is a linear least-squares fit of the data in 0.05 M NaCl. The equation of the line is $D_{PSS} = 10.72 \times 10^{-6} \times N^{-0.617}$ ($r^2 = 0.996$). The inset shows the slopes of the lines (m) observed for the power law dependence of the diffusion coefficients of ssDNA, dsDNA, and PSS on molecular weight, plotted as a function of the average axial distance between the charged residues in each polyion (b). The equation of the line is $m = -0.491b + 0.749$ ($r = 0.993$), with b in nanometers.

double-stranded DNAs. This result suggests that the decrease in the effective charge of the DNA expressed by the term N^m is due to another factor, such as the coiling of the polyion in three-dimensional space.

Application of the Modified Einstein Equation to PSS. To demonstrate the generality of the modified Einstein equation, the mobilities and diffusion coefficients of another well-studied highly charged polyion, sodium polystyrenesulfonate (PSS), were analyzed, using data taken from the literature. The mobilities of PSS molecules of different molecular weights (*46*) are plotted as the empty diamonds in Figure. 1. The mobilities go through a maximum with an increase in molecular weight, similar to that observed for ssDNA but larger in amplitude. The increased amplitude may be due in part to the fact that the PSS samples were analyzed in borate buffer; electrophoretic mobilities in borate buffers are known to be larger than those measured in Tris buffers with comparable ionic strengths (*5*). The mobility maximum for PSS occurs for oligomers containing ∼10 styrenesulfonate residues. If it is assumed that the rise per residue is ∼0.25 nm (*47*), the mobility maximum is observed for oligomers with a length of 2.0−2.5 nm, somewhat smaller than the persistence length of ∼3−7 nm observed for PSS under low-ionic strength conditions (*48*, *49*).

The dependence of the diffusion coefficients of PSS on molecular weight (*49*, *50*) is illustrated in Figure 5. A power law dependence of −0.62 is observed, closer to that of dsDNA (−0.67) than ssDNA (−0.54), for reasons that are not clear. It is possible that excluded volume effects are more important for PSS than ssDNA, leading to a larger power index for PSS even though the persistence lengths of the two polyions are similar. Alternatively, it is possible that the power index is related to the axial charge densities of highly charged polyions, rather than their persistence lengths. On average, negatively charged residues occur every 0.43 nm for ssDNA (*12*), every 0.25 nm for PSS (*47*), and every 0.17 nm for dsDNA. These linear charge densities are correlated with the slopes of the lines describing the power law dependence of the diffusion coefficients on molecular weight, as shown in the inset of Figure 5.

The modified Einstein equation (eq 7) was used to calculate the ratio μ/N^mD for the various PSS samples in Figure 1, with the results shown as the empty diamonds in Figure 4. The average value of the ratio μ/N^mD is 35 ± 6 V^{-1} for 14 PSS samples containing up to 855 styrenesulfonate residues, somewhat lower than the expected value of 39.6 V^{-1} at 20 °C. The relatively low value of the ratio μ/N^mD may be due in part to the fact that the PSS polymers were not fully charged; the manufacturer characterized the PSS samples as "more than 88% sulfonated" (*46*). If the PSS samples are assumed to contain 88% charged residues, and the observed electrophoretic mobilities are increased to reflect the mobilities that would have been observed if the PSS polyions had been fully sulfonated, the average value of the ratio μ/N^mD would become 40 ± 6 V^{-1}.

Concluding Remarks. Figure 4 indicates that the modified Einstein equation (eq 7) provides a good description of the relationship between the mobilities and diffusion coefficients of ssDNA, dsDNA, PSS, and presumably other highly charged polyions. Strictly speaking, we expect the modified Einstein equation to be valid for only high-molecular weight polyions, where the mobilities are independent of molecular weight. If the ratio μ/N^mD is calculated only in this free draining region, the limiting values become 39 ± 1 V^{-1} for ssDNA, 43 ± 1 V^{-1} for dsDNA, and 34 ± 0.5 V^{-1} for PSS (39 ± 0.5 V^{-1} if the observed PSS mobilities are corrected for 88% sulfonation). The limiting values observed for ssDNA and 100% sulfonated PSS are very close to the expected value of 39.6 V^{-1} at 20 °C. For dsDNA, the average value of the ratio μ/N^mD is ∼8% high, for reasons that are not clear.

The ratio μ/N^mD goes through a maximum for small ssDNA and PSS molecules, reflecting the mobility maxima observed for these two polyions in Figure 1. It is possible that the deviation of the ratio μ/N^mD from a constant in this region of molecular weights is due to the fact that the diffusion coefficients are not adequately described by the power laws given in the legends of Figures 2 and 5. Alternatively, the deviation may result from the fact that these relatively small polyions are not significantly larger than the thickness of the ion atmosphere, leading to a reduction of the effective charge (*42*). A detailed comparison of the mobilities and diffusion coefficients of small, single- and double-stranded DNA molecules as a function of ionic strength will be needed to determine the accuracy of the modified Einstein equation in this range of molecular weights. Such studies are currently underway in this laboratory.

The deviation between the measured and expected values of the ratio μ/N^mD may also be due, in part, to the fact that the electrophoretic mobilities were measured in buffers with ionic strengths of 20 mM, while most of the diffusion coefficients were measured in solutions with ionic strengths ranging from 50 to 200 mM. It is known that the electrophoretic mobilities of DNA, PSS, and other highly charged polyions decrease with an increase in ionic strength (*3*, *46*, *51*, *52*). However, the dependence of the diffusion coefficients on ionic strength is not clear. The diffusion coefficients of ssDNA (*14*) and PSS (*29*, *53*) were found to increase with an increase in ionic strength, while the diffusion coefficients of ATP (*20*) and some proteins (*54*) decrease with an increase in ionic strength. The variation of the

diffusion coefficients with ionic strength may also be buffer-dependent, since diffusion is coupled to ion transport through the electrostatic forces needed to maintain electroneutrality in the solution (*55*). The rate of ion transport is, in turn, determined by the transference numbers, or equivalently the limiting ionic mobilities, of all the ions in the solution. Further studies are needed to determine whether the diffusion coefficients of single- and double-stranded DNA molecules depend on ionic strength and/or buffer composition.

REFERENCES

1. Bockris, J. O., and Reddy, A. K. N. (1998) *Modern Electrochemistry*, 2nd ed., Vol. 1, Ionics, Plenum Press, New York.
2. Nkodo, A. E., Garnier, J. M., Tinland, B., Ren, H. J., Desruisseaux, C., McCormick, L. C., Drouin, G., and Slater, G. W. (2001) *Electrophoresis 22*, 2424−2432.
3. Hoagland, D. A., Arvanitidou, E., and Welch, C. (1999) *Macromolecules 32*, 6180−6190.
4. Olivera, B. M., Baine, P., and Davidson, N. (1964) *Biopolymers 2*, 245−257.
5. Stellwagen, N. C., Gelfi, C., and Righetti, P. G. (1997) *Biopolymers 42*, 687−703.
6. Sorlie, S. S., and Pecora, R. (1988) *Macromolecules 21*, 1437−1449.
7. Stellwagen, N. C., Magnusdóttir, S., Gelfi, C., and Righetti, P. G. (2001) *Biopolymers 58*, 390−397.
8. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1987) *Molecular Cloning, A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Plainview, NY.
9. Lu, Y. J., Weers, B., and Stellwagen, N. C. (2002) *Biopolymers 61*, 261−275.
10. Stellwagen, E., and Stellwagen, N. C. (2002) *Electrophoresis 23*, 2794−2803.
11. Costantino, L., Liquori, A. M., and Vitagliano, V. (1964) *Biopolymers 2*, 1−8.
12. Record, M. T., Jr., Anderson, C. F., and Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103−178.
13. Smith, S. B., Cui, Y., and Bustamante, C. (1996) *Science 271*, 795−799.
14. Tinland, B., Pluen, A., Sturm, J., and Weill, G. (1997) *Macromolecules 30*, 5763−5765.
15. Mills, J. B., Vacano, E., and Hagerman, P. J. (1999) *J. Mol. Biol. 285*, 245−257.
16. Desruisseaux, C., Long, D., Drouin, G., and Slater, G. W. (2001) *Macromolecules 34*, 44−52.
17. Manning, G. S. (1981) *J. Phys. Chem. 85*, 1506−1515.
18. Hagerman, P. J. (1988) *Annu. Rev. Biophys. Biophys. Chem. 17*, 265−286.
19. Hubley, M. J., Locke, B. R., and Moerland, T. S. (1996) *Biochim. Biophys. Acta 1291*, 115−121.
20. Bowen, W. J., and Martin, H. L. (1964) *Arch. Biochem. Biophys. 107*, 30−36.
21. Rymdén, R., and Stilbs, P. (1985) *Biophys. Chem. 21*, 145−156.
22. Li, S. K., Ghanem, A.-H., Teng, C.-L., Hardee, G. E., and Higuchi, W. I. (2001) *J. Pharm. Sci. 90*, 915−931.
23. Eimer, W., and Pecora, R. (1991) *J. Chem. Phys. 94*, 2324−2329.
24. Bonifacio, G. F., Brown, T., Conn, G. L., and Lane, A. N. (1997) *Biophys. J. 73*, 1532−1538.
25. Liu, H., Skibinska, L., Gapinski, J., Patkowski, A., Fischer, E. W., and Pecora, R. (1998) *J. Chem. Phys. 109*, 7556−7566.
26. Lang, D., and Coates, P. (1968) *J. Mol. Biol. 36*, 137−151.
27. Voordouw, F., Kam, A., Borochov, N., and Eisenberg, H. (1978) *Biophys. Chem. 8*, 171−189.
28. Langowski, J., Giesen, U., and Lehmann, C. (1986) *Biophys. Chem. 25*, 191−200.
29. Wang, L. X., and Yu, H. (1988) *Macromolecules 21*, 3498−3501.
30. Sorlie, S. S., and Pecora, R. (1990) *Macromolecules 23*, 487−497.
31. Goinga, H. T., and Pecora, R. (1991) *Macromolecules 24*, 6128−6138.
32. Seils, J., and Dorfmüller, T. (1991) *Biopolymers 31*, 813−825.
33. Gosnell, D. L., and Zimm, B. H. (1993) *Macromolecules 26*, 1304−1308.
34. Rivetti, C., Guthold, M., and Bustamante, C. (1996) *J. Mol. Biol. 264*, 919−932.
35. Lapham, J., Rife, J. P., Moore, P. B., and Crothers, D. M. (1997) *J. Biomol. NMR 10*, 255−262.
36. Kontturi, A.-K., Kontturi, K., and Urtti, A. (2002) *Eur. J. Pharm. Sci. 15*, 379−385.
37. Soda, K., and Wada, A. (1984) *Biophys. Chem. 20*, 185−200.
38. Hadden, D. A., Rill, R. L., McFaddan, L., and Locke, B. R. (2000) *Macromolecules 33*, 4235−4248.
39. Tirado, M. M., and García de la Torre, J. (1979) *J. Chem. Phys. 71*, 2581−2587.
40. Yamakawa, H., and Fujii, M. (1973) *Macromolecules 6*, 407−415.
41. Tirado, M. M., Martínez, C. L., and García de la Torre, J. (1984) *J. Chem. Phys. 81*, 2047−2052.
42. Giddings, J. C. (1991) *Unified Separation Science*, John Wiley & Sons, New York.
43. Manning, G. S. (1978) *Q. Rev. Biophys. 11*, 179−246.
44. Record, M. T., Jr., Anderson, C. F., and Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103−178.
45. Record, M. T., Jr., Zhang, W., and Anderson, C. F. (1998) *Adv. Protein Chem. 51*, 282−353.
46. Cottet, H., Gariel, P., Theodoly, O., and Williams, C. E. (2000) *Electrophoresis 21*, 3529−3540.
47. Sudor, J., and Novotny, M. V. (1994) *Anal. Chem. 66*, 2139−2147.
48. Tricot, M. (1984) *Macromolecules 17*, 1698−1704.
49. Yashiro, J., and Norisuye, T. (2002) *J. Polym. Sci., Part B: Polym. Phys. 40*, 2728−2735.
50. Thielking, H., and Kulicke, W.-M. (1996) *Anal. Chem. 68*, 1169−1173.
51. Stellwagen, E., and Stellwagen, N. C. (2002) *Electrophoresis 23*, 1935−1941.
52. Stellwagen, E., and Stellwagen, N. C. (2003) *Biophys. J. 84*, 1855−1866.
53. Böhme, U., and Scheler, U. (2003) *Colloids Surf., A 222*, 35−40.
54. McBain, J. W., Dawson, C. R., and Barker, H. A. (1934) *J. Am. Chem. Soc. 56*, 1021−1027.
55. McBain, J. W., and Dawson, C. R. (1934) *J. Am. Chem. Soc. 56*, 52−56.

BI035203P