Theory of Capillary Electrophoretic Separation of DNA Using Ultradilute Polymer Solutions

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ABSTRACT: We present a model of DNA electrophoresis in unentangled polymer solutions based on a new separation mechanism in which the DNA drags along polymer molecules it encounters during migration. Taking into account the deformation and the hydrodynamic resistance of the polymers in the flow, the mutual disengagement time of the DNA and the polymer, and the average number of polymers dragged by one DNA, we build a self-consistent theory leading to predictions for the DNA velocity as a function of the experimental conditions. Our results agree with the data of Barron et al. (1994), and important separation regimes are also identified.

1. Introduction

Modern genetics relies heavily on electrophoresis to separate DNA molecules of different molecular sizes. In free liquid, DNA migrates at a velocity independent of its molecular weight, so that size separations are generally obtained in gels.[1] The separation process is usually described (at least qualitatively) by either the so-called Ogston sieving model[2] (when the DNA molecules are small compared to the typical pore size of the gel) or the reptation model[3−5] (which applies to larger molecules which must migrate end first through the gel structure). Gel electrophoresis is labor-intensive and poorly reproducible, so considerable efforts are currently focused on using capillary electrophoresis (CE) where DNA separation can be achieved in nongelled, entangled polymer solutions ($c > c^*$).[6] The mechanism of this process was shown to be related to reptation.[7] Recently, the experimental results of Barron et al.[8] have demonstrated that separation can also be achieved in unentangled, ultradilute polymer solutions ($c \ll c^*$) of hydroxyethyl cellulose (HEC). For example, large DNAs (2−23 kbp) can be separated by CE under a high field (270 V/cm) using HEC polymer concentrations as low as 0.0006%, although c^* was estimated to be near 0.40% for this sample (90 000−105 000 MW HEC). Since a network of entanglements cannot exist for $c \ll c^*$, standard models cannot explain these surprising results.

Figure 1 presents a schematic illustration of the separation process, as suggested by Figure 7 of ref 8. In this model, we assume that a DNA molecule temporarily "captures" the polymers with which it collides during its electrophoretic drift. Clearly, these polymers resist the flow and slow down the migration of the DNA. However, the captured polymers also deform under the flow. This, in turn, affects their resistance to the flow. The net electrophoretic velocity of the DNA molecule and the hydrodynamic properties of the polymers are thus intimately related. In this article, we describe the main features of this process, and we focus our attention on the 24 000−27 000 average MW HEC case studied in ref 8. For simplicity, we work in the frame of reference where the HEC molecules are at rest, which means that the contribution of the electroosmotic flow does not appear explicitly; our results can therefore be compared directly with those of ref 8.

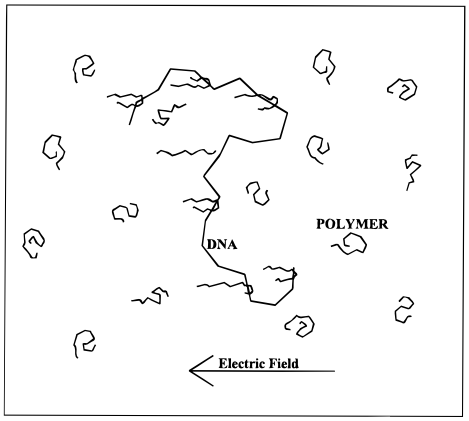

Figure 1. Schematic illustration of the DNA molecule during electrophoresis in a solution of polymers (e.g., 24 000−27 000 average MW HEC) as suggested by Figure 7 of ref 8. We note that the radius of gyration of the polymer is smaller than the persistence length of the DNA. The electroosmotic flow is not taken into account.

2. Theory

The capture and release of polymers (e.g., HEC) is at the heart of the phenomenon. We denote the mean number of polymers in contact with a DNA molecule by n. Thus, the (instantaneous) electrophoretic velocity of a DNA molecule with exactly n polymers attached to it is defined as

$$V_n = \frac{QE - nF_{\mathrm{drag}}}{M_{\mathrm{DNA}}\xi} \tag{1}$$

where Q is the effective charge of the DNA, E is the electric field intensity, F_{drag} is the average drag force acting on the DNA due to one polymer, M_{DNA} is the molecular size of the DNA (in base pairs, or bp), and ξ is the DNA friction coefficient per bp. Equation 1 simply says that the net force applied on the DNA molecule, $QE - nF_{\mathrm{drag}}$, equals the dissipative (frictonal) force

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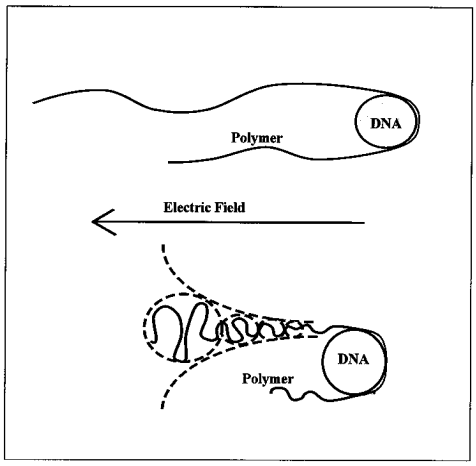

Figure 2. Schematic illustration of the conformations of a polymer which has been captured by a DNA molecule. The upper part represents the high field intensity/large velocity limit where the polymer is almost completely stretched. The bottom part represents the low field intensity/large MW polymer limit, where the polymer is in a "trumpet-like" conformation. In both cases, the longest arm of the polymer "pulley" grows at the expense of the small one until the polymer disengages from the DNA molecule. At the same time, the DNA also forms a pulley and slides around the polymer.

$M_{DNA}\xi V_n$ that slows down the motion of the DNA molecule in the buffer.

When $n \gg 1$ (the most important regime), we can assume that we have a steady-state situation with constant n. In such cases, the mean velocity V_{DNA} of a DNA molecule is approximately equal to V_n:

$$V_{DNA} \approx V_n \tag{2}$$

When $n < 1$, the polymer/DNA collisions are separated by periods during which the DNA drifts freely, and we have, instead,

$$V_{DNA} = \frac{V_{n=1}\tau + (\tau_{coll} - \tau)V_{n=0}}{\tau_{coll}} \tag{3}$$

where $V_{n=1}$ and $V_{n=0}$ are given by eq 1, τ is the mean lifetime of one polymer−DNA contact, and τ_{coll} (with $\tau_{coll} \geq \tau$) is the mean time between collisions. Assuming that both the DNA and the polymer slide around their point of contact (see Figure 2), the mean lifetime τ is given by

$$\tau = \left(\frac{1}{\tau_p} + \frac{1}{\tau_{DNA}}\right)^{-1} \tag{4}$$

where τ_p is the time required for the polymer to disengage from the DNA, and τ_{DNA} is the time required for the DNA to disengage from the polymer. Therefore, in order to calculate V_{DNA}, we must estimate the release times τ_{DNA} and τ_p, the collision time τ_{coll}, the mean drag force F_{drag}, and, finally, the mean aggregation number n.

The aggregation number n is found by equating the collision rate with the mean escape rate. The result is

$$n \approx V_{DNA} c \tau S \tag{5}$$

where c is the (number) polymer concentration and S is the polymer−DNA collision cross-section. The latter depends on the length and flexibility of the two macromolecules. For polymers with a radius of gyration, R_p (≈27 nm for the 24 000−27 000 MW HEC used in ref 8), smaller than one DNA persistence length p (≈60 nm, or about 176 bp), the collision cross-section is given by

$$S \approx R_p \times \max[M_{DNA}b, R_p] \tag{6}$$

where b (≈0.34 nm) is the contour length of one DNA bp. Other limits also exist. For instance, we note that $R_p \approx p$ for the largest HECs used in ref 8; in this case, the collision process itself might be different. In this article, we will restrict our study to cases where $p > R_p$ and $R_p < M_{DNA}b$.

The escape time of a (pulley-like) DNA molecule around an obstacle has been studied by many authors;[9−11] simple arguments indicate that it simply scales as

$$\tau_{DNA} \approx \frac{M_{DNA}b}{2V_n} \tag{7}$$

Both the release time τ_p and the mean drag force F_{drag} are related to the hydrodynamic properties of the polymer during the escape process. The work of Brochard-Wyart et al.[12] suggests that for low field intensity/large polymer size, one should expect the polymer to assume a "trumpet-like" conformation (see Figure 2) because the drag forces are not sufficient to fully stretch the polymer. This case will be treated in a forthcoming article. For high field intensities, the polymer stretches almost completely (see Figure 2). This is also a pulley-like problem where the difference in the frictional forces on the two arms of the captured polymer causes the longest arm to grow at the expense of the short one until the polymer escapes. This problem is similar to a DNA molecule moving around an obstacle where the electric forces are replaced by drag forces. Thus, the escape time of the polymer simply scales as

$$\tau_p \approx \frac{L}{2V_n} \tag{8}$$

whereas the average drag force due to one polymer scales as

$$F_{drag} \approx \eta L V_n \tag{9}$$

where η is the viscosity of the buffer solution and L is the contour length of the polymer.

When using 24 000−27 000 MW HEC and $n \gg 1$, eqs 1, 2, and 4−9 give

$$\frac{V_{DNA}}{V_{n=0}} \approx \frac{1}{1 + \gamma C\left(1 + \beta\frac{L}{M_{DNA}b}\right)^{-1}} \quad \text{for } R_p < M_{DNA}b \text{ and } p > R_p \tag{10}$$

where C is the HEC concentration in the units of ref 8. Here, γ scales as $(\rho_{buffer}/\rho_{HEC}) \times (N_{HEC})^{1+\nu}$, where ρ_{buffer} and ρ_{HEC} are the densities of the buffer and the polymer, respectively, $N_{HEC} \propto L$ is the degree of polymerization of the polymer, and $\nu \approx 3/5$ is Flory's exponent, while β is the ratio of the (unknown) scaling numerical prefactors found in eqs 8 and 7, respectively (therefore, it is expected to be of order unity).

3. Results

We will now compare our results to those of Barron et al.[8] for the 24 000−27 000 MW HEC polymer solu-

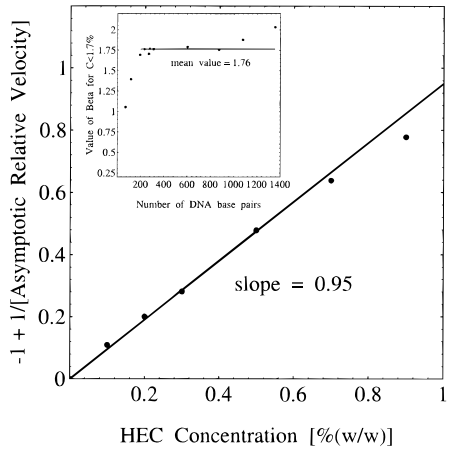

Figure 3. Plot of $[-1 + V_{n=0}/V_{DNA}(M_{DNA}\rightarrow\infty)]$ vs the HEC concentration C; the slope gives the parameter γ. The asymptotic velocities $V_{DNA}(M_{DNA}\rightarrow\infty)$ were estimated from the raw data of ref 8. The insert shows the value of the parameter β vs DNA molecular size; the average value for $M_{DNA} \geq 200$ bp is 1.76.

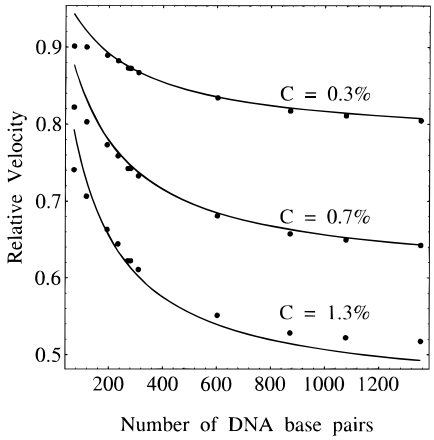

Figure 4. Plot of the relative velocity, $V_{DNA}(M)/V_{n=0}$, vs DNA molecular size M_{DNA} (in bp). The experimental points come from ref 8. The solid lines are obtained from eq 10. The concentrations are, from top to bottom, $C = 0.3\%$, 0.7%, and 1.3%.

tions where eq 10 applies. The parameter γ can be estimated as follows. Figure 3 shows $[V_{n=0}/V_{DNA}] - 1$ vs C for $M_{DNA}\rightarrow\infty$. According to eq 10, this plot should yield a straight line of slope γ. The figure is in good agreement with this prediction where we obtain $\gamma \approx 0.95$. In order to obtain β, we use eq 10 to fit ref 8's velocity vs concentration (C) data for various DNA molecular sizes M_{DNA} with $\gamma \approx 0.95$ and $L_{HEC=24000} \approx 51.5$ nm. As can be seen in the insert of Figure 3, β is essentially constant for DNA molecules longer than one persistence length ($M_{DNA} > 200$ bp). The figure also indicates the existence of a different regime for smaller, stiffer DNA molecules; this will not be studied here. Using the mean value $\beta = 1.76$, eq 10 yields the solid lines found on Figures 4 and 5. We can see that our expression is in good agreement with the experimental results when $C \ll C^*$ ($\approx$1.8% for this case) and $M_{DNA} >$ 200 bp. Deviations are found for very low concentrations C; this is possibly due, in this regime, to the fact that eq 2 is no longer valid and should be replaced by eq 3. Since we have been using scaling arguments, the numerical prefactors in eqs 5−9 are unknown; therefore, it is not possible to use the values of γ and β found above to obtain a reliable estimate of the average number n of polymers dragged by a single DNA molecule. Our

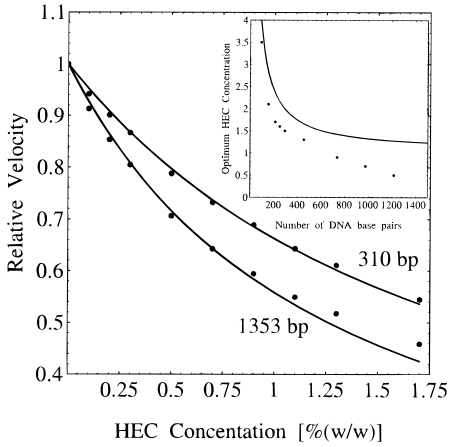

Figure 5. Plot of the relative electrophoretic velocity of the DNA molecule, $V_{DNA}/V_{n=0}$, vs the HEC concentration C. The experimental points come from ref 8; upper points for $M_{DNA} =$ 310 bp, and lower points for $M_{DNA} = 1353$ bp. The solid lines are obtained from eq 10. The insert shows a plot of the optimum HEC concentration vs the size of the DNA molecule to be separated (in bp). The experimental points are from ref 8. The line is from eq 11.

best (rough) estimates indicate that $0.1 < n < 10$ for the conditions corresponding to Figures 3 and 4.

From eq 10, we can compute the optimum HEC polymer concentration $C_{optimum}$ for separating DNA molecules of size M_{DNA}. The separation between molecules of sizes M_{DNA} and $M_{DNA} + 1$ (in bp) is proportional to their relative velocity $\partial[V_{DNA}]/\partial M_{DNA}$; the optimal concentration to separate these molecules can thus be found by solving the equation $\partial^2[V_{DNA}]/\partial C\partial M_{DNA} = 0$. We obtain

$$C_{optimum} = \frac{1}{\gamma} + \frac{\beta L}{\gamma M_{DNA} b} \qquad (11)$$

The insert of Figure 5 shows that eq 11 is in fair agreement with experimental results considering the number of approximations made, the fact that we are comparing second-order derivatives, the uncertainty on the data points, and the fact that this comparison involves no curve fitting. Note in particular that our results agree with the fact that the optimal concentration appears to plateau for very large DNA sizes (one of the most important results in ref 8; in the latter, the data for the 95 000−105 000 MW HEC polymer solutions shows a more obvious plateau). Since $\gamma \propto L^{8/5}$, we also predict that the optimal concentration for the separation of large DNA molecules $C_{optimum}(M_{DNA}\rightarrow\infty)$ will decrease as the 8/5th power of the molecular size of the HEC molecules. For the 95 000−105 000 average MW HEC used in ref 8, we thus predict that the plateau value $C_{optimum}(M_{DNA}\rightarrow\infty)$ should be a factor of about $4^{8/5} \approx 9$ smaller than for the 24 000−27 000 MW HEC polymer solutions. Barron et al.[8] found roughly $C_{optimum} \approx 0.5\%$ in the latter case and 0.05% in the former case, giving a ratio of about 10, which compares well with our predictions.

4. Conclusions

Our model can be generalized to treat numerous other regimes. For instance, large polymers have different collision cross-sections with DNA, while small DNAs are too rigid to form pulley conformations and "slide" around the point of contact with the polymer. Lower field intensities and/or smaller DNA velocities may lead to

cases where a long polymer assumes a trumpet-like conformation which will greatly modify the escape time τ_p and the mean drag force F_{drag}. If the polymer concentration C is very low, one has, on average, less than one polymer attached per DNA molecule and eq 3 must be used instead of eq 2. In the latter case, the velocity of the DNA changes during the escape process, which means that the escape times, the mean drag force, and the mean DNA velocity must be calculated self-consistently. In this limit, one must also take into account the finite relaxation time of the DNA between two collisions since this may lead to a collision cross-section S that changes with time. Finally, the polymer−polymer and DNA−DNA hydrodynamic interactions have been neglected.

The simple model presented here with the scaling relations for the escape times, mean aggregation number, and mean drag force per polymer successfully explains the surprising results reported by Barron et al.[8] for 24 000−27 000 average MW HEC polymer solutions. In particular, it predicts that the optimum polymer concentration $C_{optimum}$ decreases with the 8/5th power of the molecular size of the neutral polymer. These results could not be expected from a simple extension of either the Ogston or the biased reptation model. Therefore, we have mathematically described a yet unknown separation mechanism, consistent with the qualitative picture proposed by Barron et al.,[8] which is based on hydrodynamics, molecular collisions, and drag forces. At the present time, the limits of this new separation mechanism are unknown. We are exploring the various scenarios one might use to increase the correlation between the total drag force and the molecular size of the DNA molecules. However, it is already clear that the net velocities permitted by this mechanism are much larger than with standard gel-based separations; if a similar resolution could be achieved, this would represent a major advance in DNA separation techniques. Because of the type of molecular conformations the DNA and the polymers assume during their association (Figure 2), it is possible that pulsed fields may reduce the mean lifetime of the polymer−DNA contacts, thus offering an alternative method to modulate the effect of the drag forces on selected DNA molecules. This, as well as various other constant field regimes, will be studied in a forthcoming full-length article using the ideas described above.

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