Electrophoresis of Composite Molecular Objects. 2. Competition between Sieving and Frictional Effects in Polymer Solutions

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ABSTRACT: We previously showed that labeling one end of single-stranded DNA molecules with a neutral label like streptavidin increases the interband separation of these hybrid DNA molecules when they are electrophoresed in denaturing cross-linked polyacrylamide gels. This separation process is called trapping electrophoresis because the dynamical properties of the analytes are strongly affected by random steric trapping in the gel. End-labeled ssDNA molecules can also be electrophoretically separated in free solution. This process is called end-labeled free solution electrophoresis, and the separation is then due to the extra hydrodynamic friction provided by the streptavidin label. In this article, we present a study of the capillary electrophoresis of DNA molecules end-labeled with streptavidin in the presence of non-cross-linked polymer solutions ranging from semidilute to ultradilute conditions. In the semidilute limit, sieving interactions dominate, and small DNA molecules move faster than larger ones. In the ultradilute limit, however, we recover free-solution-like separations where small molecules are more retarded than larger ones. We also observe a fascinating transition between these two regimes, for intermediate polymer concentrations and DNA sizes, where the competition between trapping, sieving, and frictional effects leads to nonmonotonic mobility−size relationships. Our theoretical analysis suggests a universal relationship between the mobility of labeled and unlabeled DNA molecules in all electrophoretic conditions.

1. Introduction

1.1. Dilute Polymer Solutions. It is normally impossible to electrophoretically separate single- (ssDNA) or double-stranded (dsDNA) DNA fragments of different sizes in free solutions because of the free-draining properties of DNA. On the other hand, DNA molecules must deform when migrating in dense gels because the topological constraints imposed by such sieving matrices are fixed. In matrices like polyacrylamide or agarose gels, a flexible DNA fragment can find its way through the gel like a snake through thick grass; in other words, it is reptating in a reptation tube as originally proposed by De Gennes and Edwards for polymer melts.[1,2] In this situation, the net electrophoretic mobility $\mu(M)$ does decrease with the molecular size M of the DNA fragment, and size-based separation becomes possible.[3−7] Very large DNA molecules (e.g., M > 40 kbp in agarose gels), however, tend to orient in the field direction, and separation then requires the additional use of pulsed fields.[8−10]

The introduction of capillary electrophoresis (CE) allows the use of entangled polymer solutions as sieving matrices. Such matrices cannot be called gels because of the absence of permanent cross-links; instead, we then have a somewhat "dynamic gel" where each "pore" has a finite lifetime.[11] The DNA migration mechanisms are believed to be essentially the same in concentrated polymer solutions and cross-linked gels as long as the DNA residence time in a "pore" is short compared to the lifetime of the latter. The critical advantage of polymer solutions over chemical gels is the possibility of injecting a fresh polymer solution after every run (gels degrade with time). Moreover, this process can be fully automated, and as many as 100 runs can be carried out in a single capillary. Barron et al.[12] discovered that even dilute polymer solutions (whose concentration c is smaller than their overlap concentration c^*) can provide separation of dsDNA fragments. For example, these authors observed successful dsDNA electrophoretic separations even when the hydroxyethylcellulose (HEC) concentration was as low as $c = c^*/100$. (The solution certainly does not look like a gel in that limit.) The separation process, suggested by Barron et al.[12] and first modeled by Hubert et al.,[13] appears to be a dynamic capture/release mechanism. While the mobility of the small fragments is not really affected by the presence of the free HEC polymer chains (the probability of colliding with the polymer coils and the subsequent contact time are both very small), the mobility loss experienced by the large DNA fragments is sufficient to observe the separation of restriction fragments 2−45 kbp in size. Hubert et al.[13] proposed a simple analytical model to explain these results where it is assumed that the DNA molecules collide and temporarily capture HEC molecules during their migration. It is in fact the increase in the total friction coefficient of the DNA−polymer complex that makes separation possible. Assuming that both the DNA and the HEC polymer slide around their common point of contact, they calculated the mean lifetime of this DNA−HEC association. This theoretical model is in fair agreement with the experimental results of Barron et al.[12] for short HEC polymers. Subsequent theoretical[14] and experimental[15,16] investigations of this novel separation process have established that random collisions are indeed responsible for the separation that is achieved, although our understanding of the physics of the polymer−DNA collisions is still incomplete.

1.2. Trapping Electrophoresis. In 1990, Ulanovsky, Drouin, and Gilbert (UDG)[17] published the first paper about trapping electrophoresis (TE). UDG's idea was to

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attach streptavidin, a neutral globular protein, at one end of the DNA to be electrophoresed in order to eliminate the nonseparative regime observed for large oriented DNA molecules (the latter comigrate during gel electrophoresis[3−10]). The labeled end of the hybrid streptavidin-DNA (or S-DNA) molecule exhibits an excess of friction due to the presence of the uncharged streptavidin while the other end is left unmodified. Because of this extra friction, S-DNA molecules are expected to have lower electrophoretic mobilities than naked DNAs. While the bulky label has almost no other effect on small DNA molecules, molecules larger than a critical molecular size M^* experience a surprisingly abrupt mobility drop which improves their separation.[17,18] This dramatic additional effect can be explained by the fact that when a S-DNA molecule moves headfirst through a pore smaller than the size of the trailing uncharged label, the molecule stops its migration since the label cannot traverse this topological constraint. In fact, the molecule needs to backtrack out of this small pore in order to continue its progression into the gel. The pores smaller than the size of the label are thus called "traps". Traps have a larger effect on larger S-DNA molecules since the electric force pulling a S-DNA molecule inside such traps is proportional to the total charge of the molecule.[17−21]

1.3. End-Labeled Free Solution Electrophoresis (ELFSE). The electrophoretic properties of S-DNA molecules have also been studied in free solution. While it is impossible to separate naked DNA in free solution because of the well-known size independence of its charge over friction ratio, the streptavidin label makes separation possible by creating an asymmetry between force and friction. This separation mechanism, first discussed quantitatively by Mayer et al.,[22] is called end-labeled free solution electrophoresis (ELFSE) and can be used for DNA sequencing.[23] This new method presents many advantages, including the fact that free solution separations make the preparation of the capillary system very easy (because there is no need to inject viscous polymer solutions). Another interesting fact is that the resolution is better at high electric field intensity,[24] a property that can also help speed up DNA sequencing. (High fields reduce the resolution when the separation is carried out in a sieving matrix.[4−8])

This new separation process is rather similar to the one observed by Barron et al.[12] in dilute polymer solutions. As mentioned previously, it is the extra friction (or the dragging force) due to the contact between the DNA and the polymer that makes separation possible in dilute polymer solutions. In ELFSE, on the other hand, it is the extra friction due to the presence of streptavidin that makes separation possible. In that sense, we can say that separation in dilute polymer solutions is a stochastic version of the ELFSE process. Even though the fundamental reason for the separation is similar in both cases, the mobility increases with molecular size in ELFSE but decreases in dilute polymer solutions. This is simply due to the fact that the extra friction is the same for every molecule in ELFSE (one streptavidin per DNA fragment) while the average number of HEC polymers in contact with the DNA molecule is a function of the molecular size of the latter: the longer the DNA molecule, the more likely it will drag HEC polymers.[12,13]

1.4. This Study. In the current study, we bridge the gap between the experimental results obtained in the ELFSE regime[23,24] and those characteristic of the dilute polymer solutions.[12] We are interested in understanding how the presence of a small amount of neutral polymers, in conjunction with the frictional properties of the label, will modify the electrophoretic dynamics of ssDNA molecules. It is important to realize that the electrophoretic mobility would decrease with the DNA molecular size M if we added neutral polymer chains to the buffer solution without labeling the DNA. Similarly, we would have the opposite situation if we only labeled the DNA molecules. Obviously, the electrophoretic process will be more complicated when we both label the DNA and use a dilute polymer solution. It is the goal of this paper to study these competing separation processes to acquire new physical insight into the electrophoretic properties of composite molecular objects. First, a model of this new regime, based on the model of Hubert et al.[13] and on our theory of ELFSE, is presented. We then compare our theory to our experimental results.

2. Theory

2.1. Trapping Electrophoresis of S-DNA Molecules. A long streptavidin-DNA molecule that moves in a gel has two possible states: the molecule is either inside a trap (mobility $\mu_{S-DNA,in}$ is zero) or free to move with a mobility $\mu_{S-DNA,out} > 0$ given by

$$\mu_{S-DNA,out}(M) = \mu_{DNA}(M + \alpha)\frac{M}{M + \alpha} \qquad (1)$$

where $\mu_{DNA}(M + \alpha)$ is the mobility of a naked DNA molecule with $M + \alpha$ bases under the same experimental conditions. (The extra size α is due to the increased contour length of the reptation tube of the hybrid molecule in the gel.) The last term in eq 1 can be interpreted as being due to the fact that the friction coefficient of the complex is larger by a factor $(M + \alpha)/M$ compared to that of a DNA molecule of mobility $\mu_{DNA}(M + \alpha)$. The interpretation of the added friction α is thus exactly the same as in free solution (part 1 of the series[26]). The derivation of this simple relation is presented in part 3 of this series.[25]

Because of steric trapping, there are two critical times in this system: τ_0, the time it takes to fall into a trap, and τ_d, the time spent (or "lost") inside a trap. The equation for the net mobility of the reptating streptavidin-DNA complex is then given by

$$\mu_{S-DNA}(M) = \mu_{DNA}(M + \alpha)\frac{M}{M + \alpha}\frac{\tau_0}{\tau_0 + \tau_d} \qquad (2)$$

where $\tau_0/(\tau_0 + \tau_d)$ simply represents the fraction of the time the molecule is actually moving in the gel. Since detrapping is a thermally activated process, the equation for the detrapping time τ_d follows an Arrhenius form; i.e., τ_d increases exponentially with the electric field (E) and the DNA molecular size (M), explaining the dramatic decrease of the mobility for molecular sizes $M > M^*(E)$.[17−21]

In the weak trapping regime, the time spent inside a trap is negligible compared to the time spent outside the trap and $\tau_0/(\tau_0 + \tau_d) \cong 1$. The mobility of the streptavidin-DNA complex is then related to the reptation mobility of naked DNA by the relation $\mu_{S-DNA}(M) \approx \mu_{S-DNA,out}(M)$ and eq 1.

2.2. End-Labeled Free Solution Electrophoresis. The streptavidin label plays two roles in a gel: it

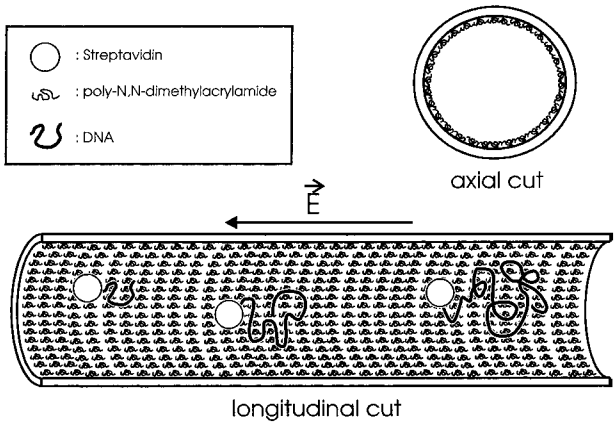

Figure 1. Schematic representation of the migration of the streptavidin-DNA molecules in free solution capillary electrophoresis. The poly(*N*,*N*-dimethylacrylamide) is self-coating the walls in order to reduce the electroosmotic flow.

increases the friction retarding the DNA, and it generates a steric trapping phenomenon. In free solution, it is solely the increase of the total friction which makes ELFSE separation possible. Figure 1 shows a schematic representation of the main elements of our capillary electrophoresis experiments. The buffer solution contains TAPS ions (*N*-tris[hydroxymethyl]methyl-3-aminopropanesulfonic acid) and enough urea (3 M) to keep the DNA denatured, while a dilute solution of poly(*N*,*N*-dimethylacrylamide) (Performance Optimized Polymer, Applied Biosystems) is added to the buffer. This polymer is slightly hydrophobic and sticks to the wall of the capillary, thus reducing the electroosmotic flow[23,24,27] (this process is commonly called dynamic coating). As discussed in the preceding article,[26] the relation between the mobility of naked DNA and the mobility of the corresponding streptavidin-DNA complex is then given by

$$\mu_{S-DNA}(M) = \mu_{DNA}(M)\frac{M}{M+\alpha} \tag{3}$$

Since $\mu_{DNA}(M) \equiv \mu_0$ is independent of the DNA molecular size M, this relationship can also be rewritten as $\mu_{S-DNA}(M) = \mu_{DNA}(M + \alpha)[M/(M + \alpha)]$. Surprisingly enough, this relation between the free-flow DNA and S-DNA mobilities is the one found in the weak trapping regime (eq 1[25])! The value of α is now the size (in number of DNA bases) of a DNA coil having the same hydrodynamic radius as the streptavidin label (i.e., $R_H(\alpha) = R_s$). In other words, α is a *relative* friction coefficient.

2.3. Dilute Polymer Solutions. We have seen that the relation between the mobilities of naked DNA fragments and streptavidin-DNA complexes is the same in a gel (in the weak trapping regime) and in free solution. We will now examine the electrophoretic behavior of DNA and S-DNA molecules in a dilute (nonentangled) polymer solution. Collisions with the free polymer chains slow the DNA molecules and make separation possible in this case.[12,13] The addition of streptavidin will make collisions more likely to occur since the extra label increases the collision cross section. Let us assume that the steady-state average number (n) of polymers dragged by the analyte is the same for a M-base long S-DNA fragment and a naked DNA fragment comprising $M + \beta$ bases. The mobility of the corresponding naked DNA fragment would be given by[13]

$$\mu_{DNA}(M+\beta)E = \frac{QE - nF_{drag}}{(M+\beta)\,\xi} \tag{4}$$

where $Q \sim M + \beta$ is the charge of the DNA molecule, ξ is the friction coefficient of one DNA base, E is the electric field intensity, $F_{drag} = \mu_{DNA}(M + \beta)E\xi_p$ is the average frictional force acting on the DNA when the latter drags one polymer chain, and ξ_p is the polymer friction coefficient. On the other hand, the mobility of the S-DNA fragment would be given by

$$\mu_{S-DNA}(M)E = \frac{Q'E - nF'_{drag}}{(M+\alpha)\xi} \tag{5}$$

where $Q' \sim M$ takes into account the fact that the charge is smaller here (streptavidin is neutral). Note that $F'_{drag} = \mu_{S-DNA}(M)E\xi_p \neq F_{drag}$. From eqs 4 and 5, it is easy to show that

$$\frac{M+\beta}{\mu_{DNA}(M+\beta)} = \frac{M}{\mu_{S-DNA}(M)} + \frac{\beta-\alpha}{\mu_0} \tag{6}$$

where $\mu_0 = Q'/M\xi$ is the free-flow mobility of a free-draining DNA molecule. Since $M \gg \beta - \alpha$ and $\mu_0 > \mu_{DNA} > \mu_{S-DNA}$ in all realistic cases, this equation can be simplified to $\mu_{S-DNA}(M) \cong \mu_{DNA}(M + \beta)[M/(M + \beta)]$, which is exactly the same as eq 1. As we will see later, our experimental results actually suggest that $\alpha \cong \beta$. According to Hubert et al.'s theory,[13] the parameter n is only proportional to the collision cross section between the analyte and the free polymers. Since the streptavidin label is (hydrodynamically) equivalent to adding a blob of α extra nucleotides, one would indeed expect that $\alpha \cong \beta$. Therefore, we can again write that

$$\mu_{S-DNA}(M) \approx \mu_{DNA}(M+\alpha)\frac{M}{M+\alpha} \tag{7}$$

To a good approximation, this universal relation should thus apply to all three electrophoresis regimes (free solution, dilute polymer solutions, and gels).

As discussed in section 2.5 of the first paper of this series,[26] we actually have good reasons to believe that our S-DNA complexes are not in a stretched conformation when $E = 333$ V/cm (the highest field obtained with our apparatus). This field is much smaller that the critical electric field required to stretch S-DNA molecules. Therefore, we do not have to worry about such effects here.

2.4. From ELFSE to TE. Figure 2 provides a schematic diagram showing the possible electrophoresis situations with both naked DNA and S-DNA. It is assumed that the relation $\mu_{S-DNA}(M) = \mu_{DNA}(M + \alpha)[M/(M + \alpha)]$ is always valid. We have shown the following regimes:

(A) *DNA in a Cross-Linked Gel*: The DNA molecule reptates in the gel. The mobility decreases like $\mu_{DNA} \propto 1/M$ and then reaches a plateau where the mobility is molecular size independent.[3−7]

(B) *S-DNA in a Cross-Linked Gel*: We now have trapping electrophoresis. The mobility of the streptavidin DNA complex decreases like $\mu_{S-DNA} \propto M/(M + \alpha)^2$ for small molecules in the weak trapping regime. This is followed by an abrupt mobility drop for large trapped molecules ($M > M^*$).[17−21,26]

(C) *DNA in a Tight (Entangled) Polymer Solution*: The dynamics in a tight polymer solution is similar to

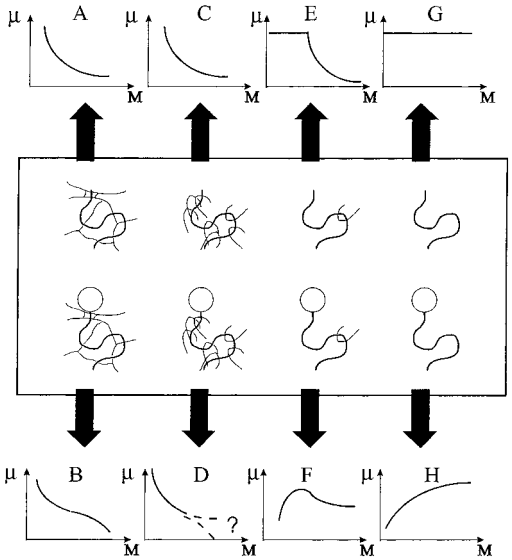

Figure 2. Schematic representation of the different electrophoretic separation mechanisms for DNA and S-DNA molecules.

the one observed in a cross-linked gel and $\mu_{DNA} \propto 1/M$ (see regime A).[11]

(D) *S-DNA in a Tight (Entangled) Polymer Solution*: The mobility decreases like $\mu_{S-DNA} \propto M/(M + \alpha)^2$ as in (B) for weak trapping. However, the existence of strong trapping can be questioned since small pores have a finite lifetime, and hence there are no truly permanent topological constraints. We have been unable to observe steric trapping.

(E) *DNA in a Dilute Polymer Solution*: Very small DNA fragments do not see the polymers at all (they are too small to drag free polymers), and their mobility is size independent, much like in free solution (see regime G), while larger fragments are retarded by the collisions with free polymers.[12−16]

(F) *S-DNA in a Dilute Polymer Solution*: We should observe a situation like that in (H) below for DNA fragments too small to entangle with free polymers and a situation like (E) above for larger DNAs. We thus predict what is usually referred to as band inversion (or peak inversion in a capillary), although this situation is unlike any other reported previously.

(G) *DNA in Free Solution*: We do not observe any separation (i.e., all molecules move at mobility μ_0) since the charge over friction ratio is constant.[6−8]

(H) *S-DNA in Free Solution (or ELFSE)*: The mobility of the S-DNA molecules increases monotonically like $\mu_{S-DNA}(M) = \mu_{DNA}(M + \alpha)[M/(M + \alpha)] = \mu_0[M/(M + \alpha)]$.[23−27]

3. Materials and Methods

3.1. Electrophoresis Solutions. Solutions containing 3 M urea (BDH), 0.1 mol/L of TAPS (*N*-tris[hydroxymethyl]methyl-3-aminopropanesulfonic acid) (Sigma), and various concentration (% w/v) of poly(*N*,*N*-dimethylacrylamide) polymer (POP polymer; Applied Biosystems) were used as separation buffers. The POP polymer, which is also used to dynamically coat the capillary walls,[20,21] has a molecular weight of MW = 10^6 g/mol, a polydispersity index PI ≃ 3, and an overlap concentration $c^* \simeq 1.5\%$ (w/v).[26] This solution was buffered to pH = 8.4 (the pK_a of TAPS) with NaOH.

3.2. DNA and S-DNA Samples. We used Pharmacia's 100 bp ladder for our experiments. The two ends of these double-stranded DNA fragments are different. This allowed us to label only one of the strands. One end has a 5′ TCGG 3′ overhang whereas the other end has a 5′ CCGA 3′ overhang. The first step of the labeling procedure consisted in blocking the GG site of the first strand by adding deoxycytosine. We mixed 5 μL (5 μg) of 100 bp ladder, 5 μL of 5 × Amplitaq buffer (Applied Biosystems), 5 μL of dCTP (5 pmol/μL), 0.5 μL of Amplitaq FS, and 9.5 μL of dH_2O, and we allowed the filling reaction to proceed at 60 °C for 20 min. Unincorporated nucleotides were then removed using Centrisep columns (Princeton Separations). In the second step, we mixed the modified 100 bp ladder (17 μL) obtained in step one with 8 μL of 5 × Amplitaq buffer, 2 μL of dideoxy C-term Big Dye terminator (Applied Biosystems; 21.5 pmol), 4.5 μL of biotin-dUTP (148.5 pm), 0.5 μL of Amplitaq FS, and 8 μL of dH_2O (for a total volume of 40 μL), and we allowed the reaction to proceed at 60 °C for 20 min. Centrisep columns were then used to remove the unincorporated nucleotides.

The solution thus obtained was diluted 10 times with distilled water. The final DNA samples were produced by mixing 3 μL of this dilute solution with 10 μL of formamide and 6 μL of dH_2O. The streptavidin-labeled DNA (S-DNA) samples were obtained by adding an appropriate quantity of purified streptavidin to this solution[23] and heating at 65 °C for 5 min to separate the two strands. These denatured samples were subsequently kept on ice until they were used.

3.3. Electrophoresis Conditions. The running temperature (30 °C) was controlled through the thermostatic plate of the ABI PRISM 310 genetic analyzer. Experiments were performed on 45 cm long capillaries, and the applied voltage was 15 kV (E = 333 V/cm). Sample injection was performed at 15 kV for 5 s. The distance between the injection end of the capillary and the detector was 34 cm.

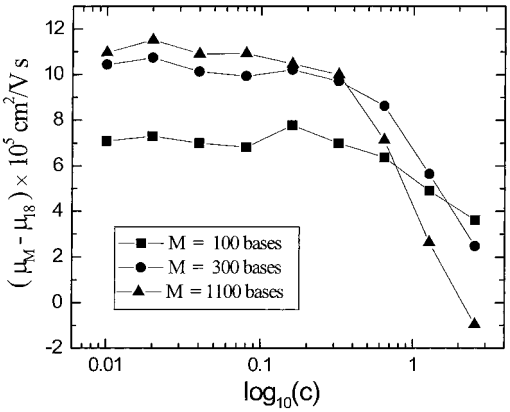

Figure 3. Difference between the mobility of three S-DNA molecules and a 18-base S-DNA fragment vs the polymer concentration c (in %). These represent results of single experiments; subtracting the mobility of the 18-base fragment corrects for the slight variations in temperature and EOF from run to run.

4. Results

4.1. The Transition Region. If we plot the mobility of the S-DNA fragments vs the POP polymer concentration (Figure 3), we observe a remarkable transition. (Note that we actually plotted the difference between the mobility of three different molecular sizes (100, 300, and 1100 bases) and the mobility of a 18-base S-DNA fragment in order to eliminate the small fluctuations in absolute elution times between runs.) The relative mobility of the 1100 base S-DNA fragment (triangles) is almost constant for concentrations ranging from 0.01% to about 0.32% but decreases quickly for larger concentrations. This is due to the fact that the probability of encountering a POP polymer is too small to affect these DNA fragments for $c \lesssim 0.32\%$. In fact, we observe a transition from ELFSE ($c < c^*$) to (possibly) reptation in semidilute polymer solutions ($c > c^*$). A

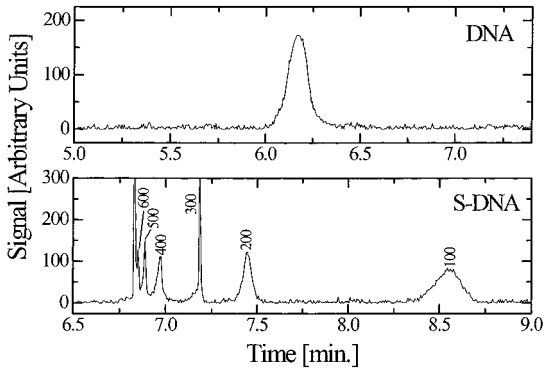

Figure 4. Electropherograms for DNA and S-DNA fragments in a low POP concentration buffer (c = 0.04%). The peaks (bottom pannel) are identified with the corresponding DNA sizes (in bases).

similar transition is observed for the other molecular sizes. For instance, we see that the large molecules move faster than the small ones ($\mu_{1100} > \mu_{300} > \mu_{100}$) for low polymer concentrations (e.g., 0.01%), typical of the ELFSE regime, while we have the opposite situation ($\mu_{100} > \mu_{300} > \mu_{1100}$) at high concentrations $c \gtrsim 2.56\% > c^*$, indicating the presence of electrophoretic sieving. Therefore, there is a remarkable intermediate region where clear band inversion (peak inversion) occurs ($\mu_{300} > \mu_{100} > \mu_{1100}$). This was one qualitative prediction of eq 7 (see Figure 2F). Clearly, such a regime, while fascinating from a fundamental point of view, is to be avoided in practice.

4.2. ELFSE Separations. Figure 4 (top) shows the electropherogram of our naked DNA sample for a polymer concentration c = 0.04%, which is much lower than the overlap concentration $c^* \cong 1.5\%$. We observe no separation; i.e., DNA fragments of various sizes all comigrate and reach the detector at essentially the same time. Figure 4 (bottom) also shows the electropherogram for the S-DNA sample in the same conditions. The smaller S-DNA molecules now move slower than the larger ones. Figure 5 presents a plot of mobility vs molecular size for both naked and labeled DNAs. We observe an horizontal line for naked DNA while the mobility increases with molecular size for S-DNA.

We will now examine whether eq 3 holds in the ELFSE regime for the large ssDNA sizes chosen here (eq 3 was verified previously for smaller ssDNA sizes[23,26]). First, we note that this relation can be rewritten as

$$\frac{\mu_{\mathrm{DNA}}(M)}{\mu_{\mathrm{S-DNA}}(M)} = 1 + \frac{\alpha}{M} \qquad (8)$$

Therefore, if we plot $\mu_{\mathrm{DNA}}/\mu_{\mathrm{S-DNA}}$ vs $1/M$, we should get a straight line with a slope α that crosses the origin at 1. Figure 6 shows that our data satisfy this relation with $\alpha \cong 34.2$ (in agreement with previous estimates for a streptavidin label[23,26]) and a y-intercept of 1.05 (which is very close to the expected value of 1).

At an intermediate concentration $c = 0.64\% \simeq c^*/2$, separation also occurs for naked DNA. As we can see in Figure 7 (top panel), smaller DNA fragments elute first. In other words (Figure 8), the mobility decreases smoothly for all DNA sizes. The evenly distributed peaks (for >200 bases) in the top pannel of Figure 7 is in fact fully consistent with the prediction $\mu \sim 1/M$ of the biased reptation model.[3-8] The situation is somewhat complex for the S-DNA molecules, and the resulting electropherogram (Figure 7, bottom panel) is rather strange. The 100-base fragment is one of the slowest fragments. The mobility increases for small molecules (M < 300 bases) and reaches a maximum for $M \cong 300$ bases (see also Figure 8). The mobility then starts to decrease much like for naked DNA. To test whether eq 7 is valid in this regime, we plotted in Figure 9 the ratios of the mobilities at c = 0.64% and c = 0.04% for both S-DNA and DNA as a function of the effective molecular size (M for DNA and $M + \alpha$ for S-DNA). Our results

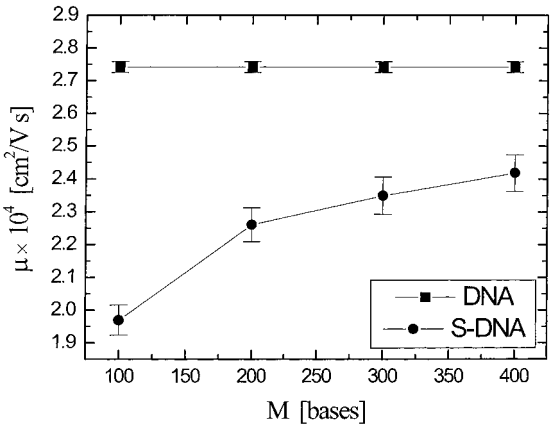

Figure 5. Mobility μ vs ssDNA molecular size M for naked DNA (squares) and S-DNA (circles) molecules.

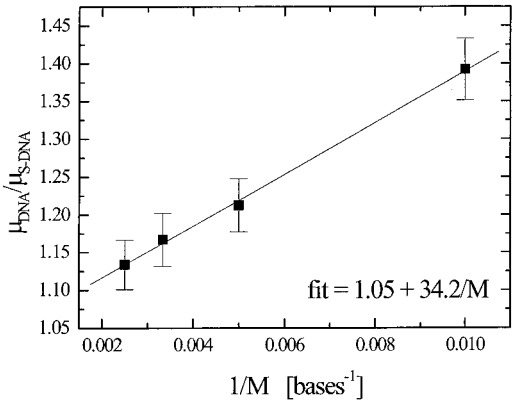

Figure 6. $\mu_{\mathrm{DNA}}/\mu_{\mathrm{S-DNA}}$ vs $1/M$ for a low concentration buffer (c = 0.04%). The linear behavior demonstrates that eq 3 is verified. The error bars for M > 400 bases are too large to provide meaningful data points on this type of diagram.

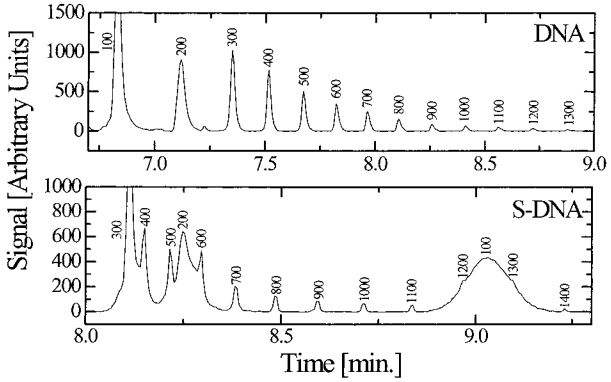

Figure 7. Electropherogram for DNA and S-DNA fragments in an intermediate POP concentration buffer (c = 0.64%). The peaks are identified with the corresponding DNA sizes (in bases). For the lower panel, peaks were assigned based on their relative height/surface area and by the fact that reptating DNA molecules lead to (roughly) evenly spaced peaks.[6-8]

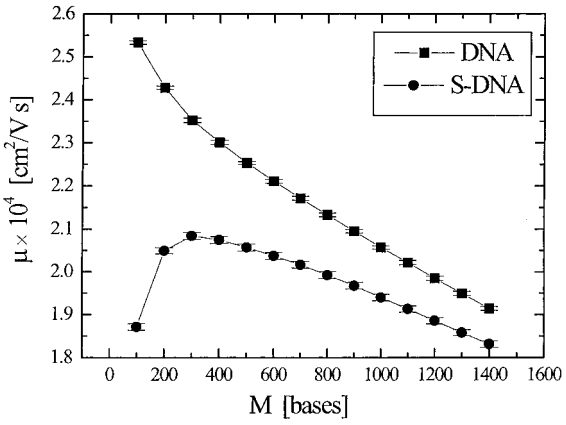

Figure 8. Mobility μ vs ssDNA molecular size M for naked DNA (squares) and S-DNA (circles) molecules. The predicted band inversion is clearly observed for S-DNA.

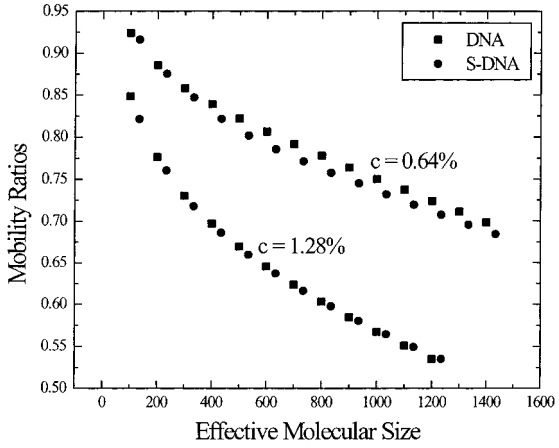

Figure 9. Mobility ratios $\mu_{DNA}(c)/\mu_{DNA}(c=0.04\%)$ (squares) and $\mu_{S-DNA}(c)/\mu_{S-DNA}(c=0.04\%)$ (circles) vs the effective molecular size for polymer concentrations $c = 0.64\%$ and $c = 1.28\%$. The effective DNA molecular size shown on the x-axis is either the number of bases M for naked ssDNA (squares) or the value of $M + \alpha$ for labeled S-DNA complexes (circles), where $\alpha = 34.2$ is the effective size of the label.

indicate that, in agreement with eq 7, we do have

$$\frac{\mu_{S-DNA}(M, c_1)}{\mu_{S-DNA}(M, c_2)} \approx \frac{\mu_{DNA}(M + \alpha, c_1)}{\mu_{DNA}(M + \alpha, c_2)} \qquad (9)$$

Note that there was no fitting involved in obtaining Figure 9 (we simply used the value $\alpha = 34.2$ obtained from Figure 6 at $c = 0.04\%$). Figure 9 also shows the data for $c = 1.28\% \cong c^*$ (using $\alpha = 34.2$ again); we see that even for a concentration close to c^*, eqs 7 and 9 are satisfied.

5. Discussion

In this article, a theoretical model for the migration of end-labeled DNA molecules in dilute polymer solutions was developed. This model generalizes that of Hubert et al.[13] which applied to the migration of naked DNA fragments. Our new theoretical model leads to a surprisingly simple relation between the mobilities of DNA and S-DNA molecules. It turns out that the relation is the same as the one obtained in free solution[26] and in gels.[25] This is a very surprising result since the separation mechanisms are very different in these three cases.

We also obtained experimental confirmation of this universal relationship (eq 7). Our experiments used concentrations of poly(*N*,*N*-dimethylacrylamide) ranging from very dilute ($c = 0.01\%$) to semidilute ($c = 2.56\%$). However, we probably did not study fully entangled solutions since our concentrations did not exceed the overlap concentration c^* very much (although the meaning of the latter is not always clear for very polydisperse solutions). Nevertheless, we were able to see the transition from free solution to what appears to be some sort of gel (sieving) electrophoresis. At low polymer concentrations, the large S-DNA molecules are faster than the small ones while we observed the opposite situation at high polymer concentrations. At intermediate polymer concentrations (c of the order of 0.64%), the small molecules are in a ELFSE-like separation regime while the large molecules are effectively in a gel electrophoresis-like regime where they apparently reptate through the polymer "matrix". This fascinating situation was correctly predicted by our theoretical model.

Furthermore, our results actually demonstrate a crucial point about the conformation of the streptavidin-DNA complexes during ELFSE experiments. In the first paper about ELFSE,[22] it was assumed that S-DNA molecules were stretched when electrophoresed in free solution. This assumption is rather appealing since one does indeed expect the two ends of a streptavidin-DNA complex to have different natural mobilities and hence to lead to DNA stretching. However, this argument neglects the very large internal entropic elasticity of the DNA coil and the hydrodynamic coupling between the DNA and its label. In light of the theories developed by Long et al.,[28−32] the stretching of S-DNA was questioned.[26] According to these theories, the electric field necessary to stretch S-DNA is very large compared to the electric field used (or even achievable) in our experiments. Our results can be used to clarify the situation. If the streptavidin-DNA complex is in its random coil conformation (like naked DNA), the effect of the collisions with the free POP should be the same for both species. On the other hand, if the S-DNA molecules alone were stretched, the probability of colliding with a free polymer chain would be much lower (because of the much reduced collision cross section). Our results indicate that the POP polymers have the same effect on DNA and S-DNA molecules since eq 9 was verified experimentally. This means that those molecules adopt the same conformation, thus establishing that S-DNA is not stretched under these conditions. This is an important conclusion of this work.

It has proven impossible to observe steric electrophoretic trapping[17−21] in concentrated polymer solutions ($c > c^*$). This is not surprising since the current models of trapping electrophoresis are based on the existence of permanent steric traps in the sieving medium. Indeed, deep steric traps are not likely to exist in non-cross-linked polymer solutions. More precisely, the duration of the steric streptavidin−polymer blocking interactions is certainly limited by the dynamics of the polymer solution itself and not by the thermal escape process believed to control the properties of the TE regime.[17−21]

In the next part of this series,[25] we will examine trapping in cross-links gels and establish the validity of eq 7 in this limit. Moreover, we will investigate the role of the gel concentration, and we will use our

experimental results to clarify the nature of the scaled field intensity that one must use to analyze data in the framework of the reptation models.

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