Entropic Trapping of DNA During Gel Electrophoresis: Effect of Field Intensity and Gel Concentration

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We present an experimental study of single-stranded DNA electrophoresis in polyacrylamide gels. We demonstrate the existence of an entropic trapping regime, situated between the Ogston and reptation regimes, in which the mobility scales as $1/M^{1+\gamma}$, where M is the DNA molecular size. The exponent $\gamma > 0$ increases for denser gels but decreases for higher fields. Entropic trapping disappears for electric fields E exceeding a critical size-dependent value $E^*(M)$. We also present various estimates of the gel's mean pore size. Finally, we propose a phase diagram describing the observed DNA migration regimes. [S0031-9007(97)04044-1]

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The dynamics of polymers in gel-like media is often described by the Rouse and reptation models [1,2]. The Rouse polymer does not feel strong topological constraints and its diffusion coefficient is predicted to decrease as $D \sim 1/M$, where M is the polymer's molecular size. The topological constraints force a long molecule to move mostly along its contour, and the reptation model then predicts $D \sim 1/M^2$. A third migration mechanism was also predicted from computer simulations [3]. When the polymer's radius of gyration $R_g(M)$ is comparable to the gel's mean pore size $\overline{a}$, the molecule selectively hops between the largest pores which thus act like "entropic traps" where the chain maximizes its conformational entropy. We then get $D \sim 1/M^{2+\gamma}$ (with $\gamma > 0$) [3,4], although this "entropic-trapping" (ET) regime does not necessarily lead to a power law. The effects of ET are still ill understood and largely underestimated.

DNA gel electrophoresis (GE) is one of the most important tools of molecular biology. The physics of GE has been studied using various theoretical, numerical, and experimental approaches [5]. When $R_g < \overline{a}$, the DNA probably retains a random coil conformation and the gel then acts as a molecular sieve. In this so-called Ogston regime, the electrophoretic mobility (μ) data are often analyzed using the relation $\mu \sim \exp(-[R_g/\overline{a}]^2)$, which derives from an old calculation of Ogston [6]. In the opposite $R_g \gg \overline{a}$ limit, the Einstein relation between μ and D (valid for low fields) predicts that if the DNA reptates, one must have $\mu \sim MD \sim 1/M$, which agrees with experimental results over a fair range of sizes M [5,7]. Recently, however, three groups have reported data that cannot be explained by these models [7–9]. In each case, the low-field mobility was observed to vary like $\mu \sim 1/M^{1+\gamma}$, with $\gamma > 0$; the Einstein relation $D \sim \mu/M$ then strongly suggests the existence of ET. For large molecules, we can thus use the exponent γ as a semiquantitative measure of the "strength" of the entropic effects, with $\gamma = 0$ giving the reptation limit.

In this article, we report new ET data for single-stranded DNA fragments electrophoresed under typical sequencing conditions. We study γ as a function of field intensity (E) and gel concentration ($\%T$). We show that ET is eliminated by strong fields, and that denser gels favor ET probably because they are more heterogeneous [10]. We also show that one can actually use the transitions between the Ogston, ET, and reptation regimes to obtain estimates of the gel's mean pore size. Finally, we propose a first schematic phase diagram for this system.

The DNA samples were radioactively labeled 50, 100, or 250 base-pair (BP) ladders (Pharmacia) prepared as described in [9], as well as the products of DNA sequencing reactions of single-stranded pBluescript SK+ DNA (Stratagene) obtained using the T7 primer and T7 Sequencing Kits (Pharmacia). Polyacrylamide (PAA) gels were obtained by polymerization of acrylamide (AA) and N, N'-methylene-bisacrylamide (BA) in (denaturing) 8M urea and 0.5X trisborate EDTA buffer (TBE) [9]. The gel concentration $T \equiv (w\mathrm{AA} + w\mathrm{BB})/100$ mL was varied between 3.25% and 12%, and the crosslinker concentration was $C \equiv w\mathrm{BA}/(w\mathrm{AA} + w\mathrm{BA}) = 5\%$. The 52.0 cm long, 0.36 mm thick gels were prerun at the operating temperature (40 °C or 55 °C) and field intensity E (in V/cm) for a duration $t_{\mathrm{PRERUN}} = (154/E)$ h (e.g., for 16.0 h when the field is $E = 9.62$ V/cm) [9]. Because of the presence of a field gradient in the first few cm of the gel, the electrophoretic mobilities were measured using our differential method [9]. The voltages were measured with a dc high voltage probe (model HV231; Wavetek).

In order to indentify the regimes, we used the "reptation plot" of $3\mu M/\mu_0$ vs M. The DNA free-solution ($T = C = 0$) mobility μ_0 was measured using a set of small single-stranded DNA oligomers and the standard Ferguson plot approach [9,11,12]; we obtained $\mu_0 = 2.9 \times 10^{-4}$ cm^2/V s at 40 °C, and $\mu_0 = 3.8 \times 10^{-4}$ cm^2/V s at 55 °C (results not shown), consistent with the 2%/°C decrease of the viscosity of water. If

M_a is the molecular size of an unperturbed DNA molecule for which $R_g(M_a) = \overline{a}$, the Ogston regime predicts $\mu/\mu_0 = \exp(-[M/M_a]^2)$ for $M < M_a$, while the reptation model, which should be valid for $M > \beta M_a$ (with $\beta \approx 1.4$ according to [12]), predicts $\mu/\mu_0 = M_a/3M + f(E)$, where $f(E)$ is a field-dependent term due to DNA orientation [5,9,12]. In the latter case, the reptation plot should give a straight line with slope $3f(E) \geq 0$ and intercept M_a; this thus provides a simple way to estimate M_a. Since M_a is a function of $\overline{a}$, we will use M_a as a measure of the mean pore size $\overline{a}$. This mean pore size, which is more precisely the mean size of the reptation blobs (or primitive segments [2]) during the migration, corresponds solely to the pores visited by reptating DNA molecules, which is a subfraction of all gel pores. In the Ogston regime, the reptation plot gives a monotonically increasing curve with a negative curvature. In the ET regime, which may exist only between the other two regimes, this plot shows a *decreasing* function of M. Therefore, all three regimes should be clearly distinct on the reptation plot, and the Ogston/ET transition should show up as a maximum at $M \approx M_a$. In this last case, M_a is the size of the largest molecule that can migrate through a percolating (connected) pathway made of pores of sizes $a \geq R_g(M_a)$. The values of M_a obtained from the reptation intercept and from the Ogston/ET transition should differ since they characterize different mechanisms. To avoid confusion, we will denote them $M_{a\mathrm{RP}}$ and $M_{a\mathrm{ET}}$, respectively.

Figure 1 shows a typical low-field reptation plot for three different gel concentrations. A log[mobility] vs log[M] plot (inset) does not show clear regimes. The reptation plot, however, demonstrates that we have three distinct migration regimes identified by three different symbols. The maximum between the Ogston and ET regimes defines $M_{a\mathrm{ET}}$ for these conditions. As expected, $M_{a\mathrm{ET}}$ decreases for larger concentrations. We studied the (negative) slope of the ET regime on log-log plots in order to find the exponent γ defined previously. Figure 2 shows that γ increases with gel concentration and vanishes at $T \approx 2\%$. This suggests that a low concentration gel contains percolating pathways made of large pores, while a high concentration one contains a distribution of pore sizes with some isolated pores large enough to favor ET where some larger DNAs could hop around without deforming too much at low electric fields. Note that below $T \approx 2\%$, PAA gels simply do not form [13].

The effect of the field intensity E was also studied. Figure 3 shows some results for $T = 7.0\%$ gels at 40 °C. While ET is observed for $E \leq 25.0$ V/cm, no region with a negative slope follows the Ogston regime at higher fields. For $E = 27.8$ V/cm, the intercept of the linear fit gives $M_{a\mathrm{RP}} \approx 56$ bases. In some cases, a reptation regime coexists with ET (e.g., see Fig. 1 for $T = 4.0\%$ and $T = 7.0\%$). Therefore, the critical field $E^*(M)$ necessary to force the molecules to reptate appears to decrease as the molecular size M increases, in qualitative agreement with recent computer simulations [14]. The inset of Fig. 2 shows the evolution of $1 + \gamma$ as E is increased. ET is predicted to disappear entirely when E exceeds about 30 V/cm for these conditions.

Figure 4 shows two reptation plots with a detailed analysis of the characteristic sizes M_a. At low field intensity, a value of M_a can be defined using the Ogston/ET transition; here, we get $M_{a\mathrm{ET}} \approx 265$ bases, as indicated. At high field intensity, however, one observes reptation (a positive slope). The intercept of the linear fit gives $M_{a\mathrm{RP}} \approx 119$ bases, while the Ogston/reptation transition is found at $M \approx 171$ bases, thus indicating that $\beta \approx 1.44$. All our data show the same three features: (i) For a given temperature and gel concentration, the value of $M_{a\mathrm{ET}}$ determined at the Ogston/ET transition (low fields) is larger

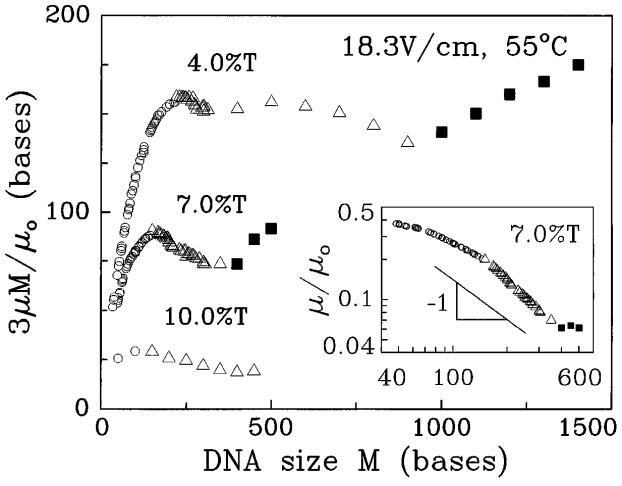

FIG. 1. A reptation plot for gel concentrations $T = 4.0\%$, 7.0%, and 10.0%. The temperature was 55 °C and the field $E = 18.3$ V/cm. The inset shows a log-log plot of μ/μ_0 vs M for $T = 7.0\%$. The empty circles (o) correspond to Ogston sieving, the empty triangles (△) to entropic trapping, and the filled squares (■) to reptation.

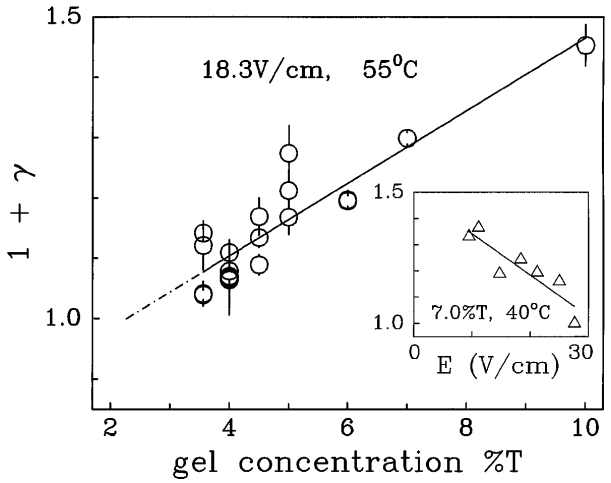

FIG. 2. Exponent $1 + \gamma$ vs gel concentration $\%T$ for experiments done at 55 °C with a field $E = 18.3$ V/cm. Inset: Exponent $1 + \gamma$ vs field E for a $T = 7.0\%$ gel at 40 °C.

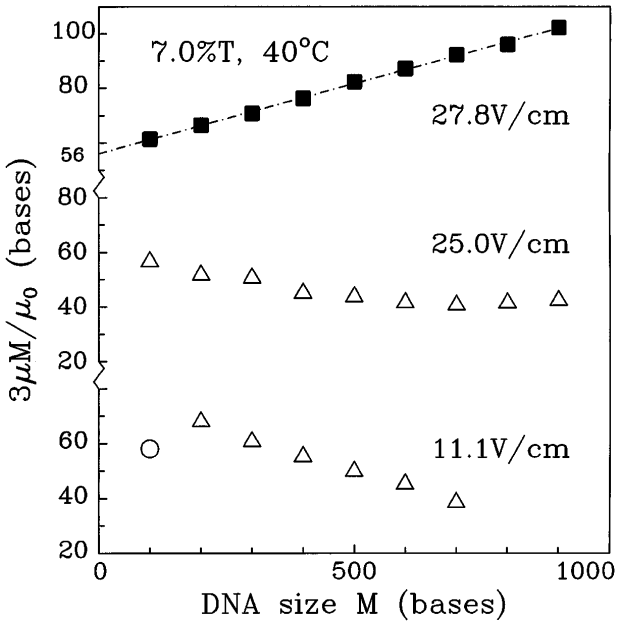

FIG. 3. A reptation plot for three different field intensities. The 7.0%T gels were at a temperature of 40 °C. The linear fit at $E = 27.8$ V/cm is $y(x) = 0.051x + 56$. See legend of Fig. 1 for the description of the symbols.

than the one determined by the reptation extrapolation (high fields), i.e., $M_{a\mathrm{ET}} > M_{a\mathrm{RP}}$; (ii) the Ogston/reptation transition is found at $M = \beta M_{a\mathrm{RP}}$, where $\beta = 1.5 \pm 0.2$; (iii) the values of $M_{a\mathrm{RP}}$ are essentially field independent (see also Fig. 6). The field dependence of $M_{a\mathrm{ET}}$ cannot easily be studied because, as the field increases, the maximum in the curve becomes flatter (see Fig. 3) and the error made in estimating $M_{a\mathrm{ET}}$ increases; indeed, one can say that the intrinsic uncertainty on $M_{a\mathrm{ET}}$ diverges when $E \to E^*$ (see Fig. 6). Figure 5 shows an aggregate of all our results for the characteristic sizes M_a vs gel concentration $\%T$. Four data sets are shown. The empty symbols are above the filled ones because $M_{a\mathrm{ET}} > M_{a\mathrm{RP}}$. The ET data appear to scale like $M_{a\mathrm{ET}} \sim T^{-z}$, with $z = 1.5 \pm 0.2$. The reptation data (filled symbols) are characterized by a larger exponent $z = 2.0 \pm 0.3$. The inset shows the distribution of $M_{a\mathrm{RP}}$ values for 39 different 4%T gels. No clear correlation between the field intensity E and the value of $M_{a\mathrm{RP}}$ was observed (not shown); therefore, the width seen here may be related to the fundamental difficulty in preparing gels with the same microscopic heterogeneity (i.e., pore size distribution).

For a DNA molecule migrating in a gel, we can write $M_a \sim \overline{a}^x$, where $x = 1$ for a rigid DNA chain, and $x = 2$ for a random coil. Let us assume that $\overline{a} \sim T^{-y}$, with $y = \frac{1}{2}$ for a random network of linear fibers [6], and $y = \frac{3}{4}$ for a semidilute solution of flexible polymers [1]; experimental results actually give $y \approx 0.60 \pm 0.05$ for 5%C PAA gels [11,15]. Combining these two scaling laws, we obtain that our exponent $z = xy$. Our result, $z_{\mathrm{ET}} = 1.5 \pm 0.2$, is consistent with $x = 2$ and $y = \frac{3}{4}$, indicating that the DNA is flexible on the length scale of one pore size (required for ET to exist). In fact, our ET results rule out the $x = 1$ case; in other words, the persistence length p of single-stranded DNA must be smaller than the average pore size of polyacrylamide gels up to approximately $T = (7\text{–}10)\%$ (our last two concentrations), in agreement with the results of Pluen [16]. The reptation value of $z_{\mathrm{RP}} \approx 2$ is also consistent with $x = 2$, but implies that $y \approx 1$. The scatter in the data, however, does not rule out the possibility that $z_{\mathrm{ET}} = z_{\mathrm{RP}}$. Note, also, that the decoupling $z = xy$ may not be valid for this problem.

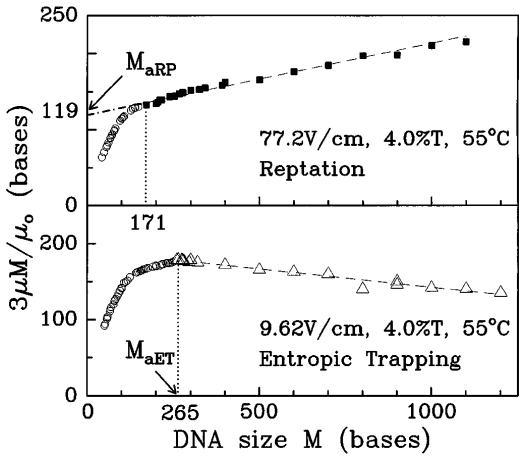

FIG. 4. A reptation plot for two different field intensities showing how the critical molecular size M_a can be determined in the presence of entropic trapping (lower curve) or reptation (upper curve). The linear fit is $y = 0.094x + 119$. The gel concentration was $T = 4.0\%$ and the temperature was 55 °C. See the legend of Fig. 1 for the description of the symbols.

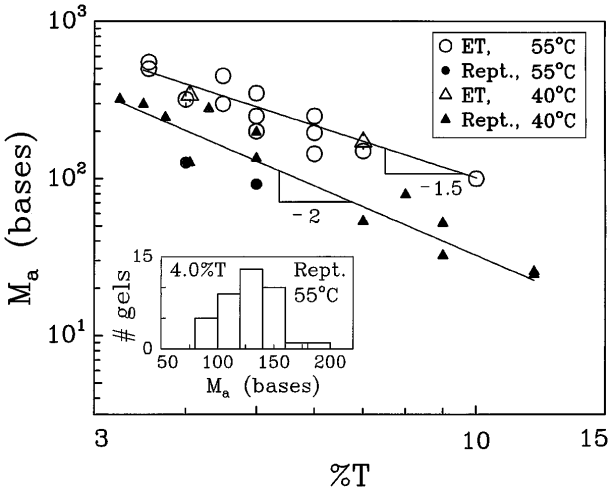

FIG. 5. Log-log plot of the characteristic molecular size M_a vs gel concentrations $\%T$. Four sets of data are shown, corresponding to two different temperatures and two values of M_a measured using the Ogston/ET transition or the reptation intercept, as indicated. Inset: Distribution of the values of $M_{a\mathrm{RP}}$ for 4.0%T gels. The most probable value is $M_{a\mathrm{RP}} = 125$, while the average is $M_{a\mathrm{RP}} = 127 \pm 4$.

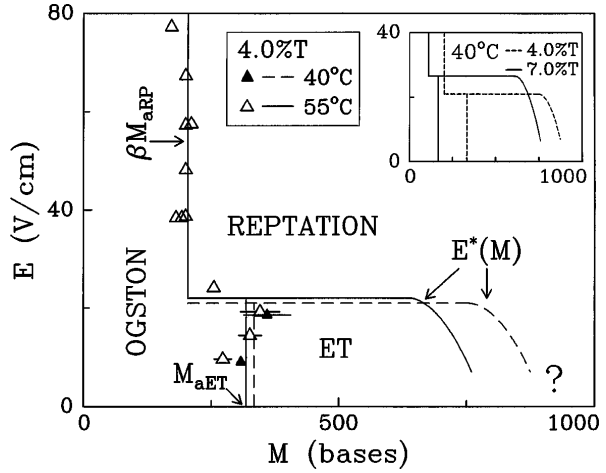

FIG. 6. Schematic phase diagram for single-stranded DNA electrophoresis in our PAA gels. The Ogston, entropic trapping (ET) and reptation regimes are shown. The three critical lines $\beta M_{a\mathrm{RP}}$, $M_{a\mathrm{ET}}$, and $E^*(M)$ are discussed in the text. Inset: A schematic phase diagram showing the effect of gel concentration.

According to Pluen [16], the persistence length of single-stranded DNA is $p \approx 5$ nm. If we use $R_g \approx (pL/3)^{1/2}$, where $L = 0.43M$ nm is the DNA contour length, and $M_{a\mathrm{ET}} \approx 3200/T^{1.5}$ (the fit shown on Fig. 5), we obtain $\overline{a}_{\mathrm{ET}} = R_g(M_{a\mathrm{ET}}) \approx 48\ \mathrm{nm}/T^{0.75}$. As an example, we obtain $\overline{a}_{\mathrm{ET}} = 17$ nm for $4\%T$ gels. The mean pore size thus becomes smaller than the DNA Kuhn length $2p = 10$ nm for $T > 8\%$, in fair agreement with [16]. When this happens, a major change in behavior is expected; for instance, there is no orientation overshoot observed in such high concentration gels [16,17].

Using our results, we propose in Fig. 6 a schematic phase diagram for this problem (a few data points are also shown). At high fields, the Ogston and reptation regimes are separated by the vertical $M = \beta M_{a\mathrm{RP}}$ line. The low-field Ogston/ET transition is possibly vertical (as discussed before, the data do not allow us to confirm this). The $E^*(M)$ line that separates ET from reptation probably has a weak negative slope. The situation for low fields and large sizes is not clear (hence the question mark). Indeed, it has proven extremely difficult to demonstrate the existence of reptation in computer simulations of long polymers in disordered systems [3,18]. For lower gel concentrations, the diagram shifts towards the right (inset). The dynamics of a molecule situated close to the triple point is expected to be quite sensitive to the gel heterogeneity.

In conclusion, ET is an important phenomenon for the interpretation of low-field mobility data. However, it must be mentioned that it is quite difficult to observe ET without using the differential method [9]. The reptation plot allows us to determine the conditions under which ET exists, the transitions between the regimes, and two estimates of the mean pore size of the gel, $M_{a\mathrm{ET}}$ and $M_{a\mathrm{RP}}$. Our finding that $M_{a\mathrm{ET}} > M_{a\mathrm{RP}}$ suggests that entropic effects are more important in the low-field ($E < E^*$) regime. In other words, the DNA molecules select larger pores in the ET regime than in the reptation regime. Therefore, gel heterogeneity appears to be of less consequence in the reptation regime. We also found that the reptation regime essentially starts when the molecule is composed of about 1.5 reptation blobs. Our study thus establishes the experimental conditions under which the various regimes, including ET, dominate the dynamics. The phase diagram proposed here is still tentative, but we hope that it will catalyze further research on this puzzling polymer system.

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