Tube Model of Field-Inversion Electrophoresis

T. A. J. Duke
Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE, United Kingdom
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The dynamics of long chain macromolecules in field-inversion gel electrophoresis is studied by Monte Carlo simulation, using a reptation model which includes tube length fluctuations. A sharp minimum in chain mobility is observed as the pulse duration is varied, occurring at longer pulse periods for larger chains. Detailed examination of the dynamics shows that the reduced mobility results from the chain being trapped in long-lived compact configurations which have no net translational motion.

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Electrophoresis is a commonly used technique for separating long chain macromolecules according to size. Its effectiveness has recently been improved by using various types of pulsed fields.[1,2] In field-inversion gel electrophoresis (FIGE), pulses of unequal duration are applied periodically in opposite directions. A remarkable nonmonotonic molecular-weight dependence of the chain mobility has been observed,[2] some chains having a substantially reduced mobility compared to the constant-field case. A mechanism which may account for this effect has recently been outlined by Viovy.[3] It is based on the reptation concept,[4] which envisages the gel fibers confining the macromolecule to a tube so that the dominant chain motion is one-dimensional diffusion along its contour. In addition, the "breathing" mode of the chain, which causes the tube length to fluctuate,[5] is considered to play an important part. Here I present a numerical study which confirms that the reptation model can indeed account for dramatic changes in chain mobility as the pulse duration is varied.

The dynamics of macromolecules undergoing FIGE is modeled using a modified form of Rubinstein's "repton" model,[6] which was developed to study the role of tube length fluctuations in the standard reptation process. Here, the gel is pictured as dividing space into cells of size a, the average pore diameter, and the molecule is considered to be a flexible chain passing from cell to cell. The model is discretized in the following way. A chain of contour length L is divided into N segments each of length $l=L/N$ chosen such that their blob size is approximately the pore diameter [Fig. 1(a)]. We then look to see in which cell the midpoint of each segment lies. Most pores contain only one segment, but where the chain makes unentangled loops a cell may house two or more. Being interested in diffusion in the field direction alone, I project this onto a one-dimensional lattice parallel to the field producing a connected walk of N "reptons," each corresponding to a segment of the chain [Fig. 1(b)]. Chain connectivity requires that neighboring reptons occupy either the same or adjacent lattice points. The situation of neighbors occupying the same site corresponds to extra "stored length" in the gel pore.

The dynamics is given by reptons hopping to an adjacent lattice point without violating the connectivity constraint. This represents stored length l diffusing along the tube, as in the original reptation model of de Gennes.[4] Suppose that the molecule is uniformly charged so that each repton carries a charge q. To model their pref-

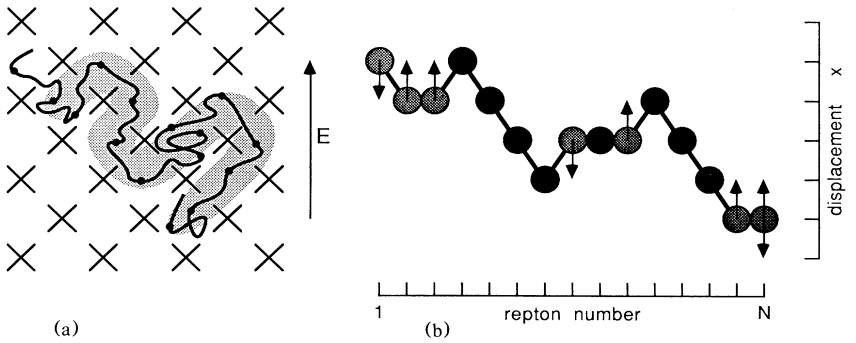

FIG. 1. (a) Two-dimensional representation of a macromolecule in a gel. Crosses represent gel fibers around which the chain is entangled. Dots mark the midpoints of individual segments. The current tube, which I define as the sequence of cells occupied by the chain *excluding* its end segments, is shown shaded. (b) Repton representation. Each repton corresponds to a segment in (a). Reptons that can move at the next time step are shown hatched and their possible jumps are indicated by an arrow. Repton 1 is an example of an end repton outside the tube, while repton N is inside the tube.

erential motion in the direction of the applied electric field E, I make the assumption of local detailed balance:[7]

$$w_{x\to x+1}/w_{x+1\to x}=\exp[-(U_{x+1}-U_x)/kT],$$

where $w_{x\to x+1}$ is the transition rate from site x to site $x+1$ and U is the electrostatic energy. This is implemented by the Metropolis choice of hopping probability:

$$p=\begin{cases}1, & \text{hopping in direction of field,}\\ \exp(-\Delta), & \text{hopping against field,}\end{cases} \quad (1)$$

where $\Delta=qEa/kT$ is the scaled energy difference between reptons on adjacent lattice points.

The reptons at the ends of the chain must be treated differently. I make a distinction between an end repton that occupies the same site as its neighbor, which represents a chain segment inside the tube, and one which does not, which corresponds to a "free" chain end outside the tube (see Fig. 1). Since a free end may occupy any of the pores that lie next to the end of the tube, it has a greater configurational weight, according to the lattice coordination.[6] Assuming that each pore in the gel is surrounded by z others, half of which are upfield and half downfield, the equilibrium weightings are in the ratio

$$\text{inside:outside}=1:(z/2)\exp(-\Delta)+(z/2)\exp(\Delta).$$

Thus, the Metropolis hopping probability for the end reptons is

$$p_{\text{end}}=\begin{cases}1, & \text{hopping out of tube,}\\ (1/z)\cosh(\Delta), & \text{hopping into tube.}\end{cases} \quad (2)$$

When an end repton leaves the tube, forming a new free end, it may hop in either the forward (+) or the reverse (−) direction. The choice is made with Boltzmann probability:

$$p_{\text{out}\pm}=1/[1+\exp(\mp 2\Delta)]. \quad (3)$$

Equations (2) and (3) together ensure local detailed balance amongst the three possible configurations of an end repton: inside, outside upfield, and outside downfield.

The precise simulation procedure is, at each time step, to choose N reptons at random; if a selected repton *can* hop to an adjacent site, it is moved with the appropriate probability given by (1)–(3). I choose $z=6$ to correspond with random packing in three dimensions. In all cases, the chain is initially equilibrated in zero field.

We might expect this model to be valid for $\Delta<1$. First, the molecule must remain a random coil on the scale of a repton; otherwise motion occurs by transmission of tension along the backbone rather than diffusion of stored length by thermal fluctuation. Second, at higher electric field strengths, the gain in energy for an excursion out of the tube outweighs the loss in entropy and a recent simulation based on the Langevin equation has shown that the dynamics is then most unlike reptation.[8] Typical FIGE experiments on DNA fragments in agarose gels (with $a\sim 100$ nm) at fields 1–10 V cm^{-1} have $\Delta\sim 0.1$–1.

The experimentally observed mobility μ of macromolecules undergoing electrophoresis in a uniform field displays two main features: At low molecular weights M and low values of the electric field E, there is a linear regime with $\mu\sim 1/M$; as M increases, the mobility tends to a constant value. This has been interpreted by reptation theory, assuming that the molecule becomes aligned with the field.[9] Figure 2 shows the results of the numerical simulation for the constant-field case. At low values of Δ, the inverse variation with chain length is reproduced for small N, and as N becomes large the mobility assumes a constant value. At $\Delta=0.2$ band inversion is apparent, with a minimum in the mobility at $N=20$. This has previously been predicted by the biased reptation model (BRM) of Noolandi *et al.*,[10] and observed experimentally. It is interesting that it also occurs in the present model, which has different dynamics.

As well as not including tube length fluctuations, the BRM assumes that the curvilinear diffusion rate of the chain along its tube depends only on the end-to-end vector. It does not distinguish between uniformly extended molecules and chains pinned around an obstacle. The chain motion is seen as a series of jumps in the tube, of step length a, which occur with time duration t and go either forwards (+) or backwards (−) with probabilities $P_\pm$ given by[10]

$$P_\pm=1/[1+\exp(\mp 2\delta)],\quad t\sim N\tanh(\delta)/2\delta, \quad (4)$$

where $\delta=\Delta|x_N-x_1|/2$ and $|x_N-x_1|$ is the end-to-end distance.

The corresponding result for the repton model, obtained by calculating the probability of a repton moving from one end of the chain to the other (neglecting tube

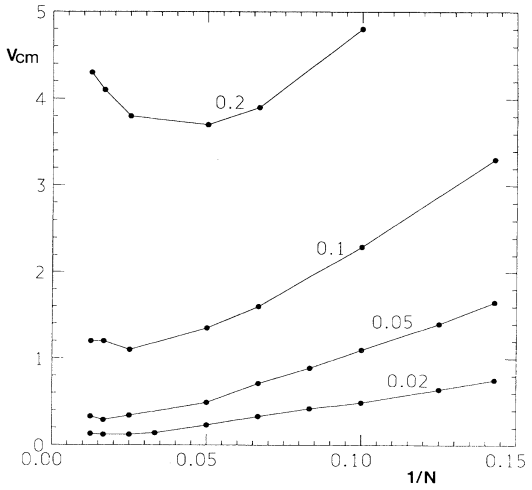

FIG. 2. Variation of the average drift velocity V_{cm} with chain length N for different values of a steady electric field Δ.

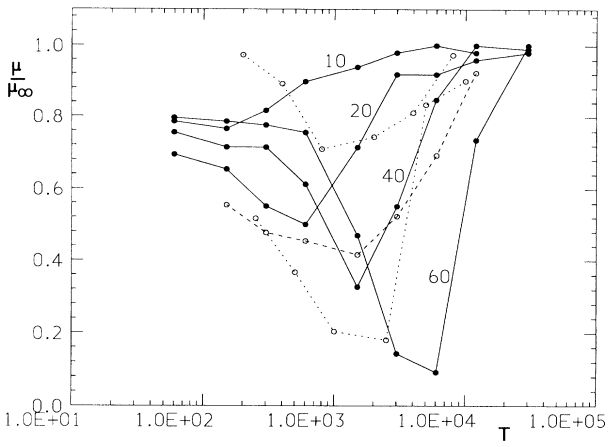

FIG. 3. Chain mobility as a function of pulse period for different sizes of macromolecule and different pulse asymmetries. Solid lines, labeled by N, are for $\Delta=0.5$, $t_2/t_1=1/2$. Dotted lines are for $N=40$, $\Delta=0.5$; the upper has $t_2/t_1=1/3$; the lower $t_2/t_1=2/3$. The dashed line is for $N=40$, $\Delta=0.2$, $t_2/t_1=1/2$. Mobility is expressed as a fraction of its value μ_∞ in the low-frequency limit ($T\rightarrow\infty$). T is measured in units of the basic repton jump time.

fluctuations), is[11]

$$P_{\pm}=1/[1+\exp(\mp 2\delta)], \quad (5)$$

$$t\sim\sum_i \exp(-\Delta x_i)/[\exp(-\Delta x_1)+\exp(-\Delta x_N)], \quad (6)$$

where x_i is the lattice coordinate of the ith repton (x increasing in the direction of the field). So we see that the curvilinear diffusion rate depends on the entire chain configuration. If a chain is uniformly extended (which is typical in high-field electrophoresis), (6) reduces to

$$t\sim N\tanh(\delta)/[|x_N-x_1|(\exp\Delta-1)]$$

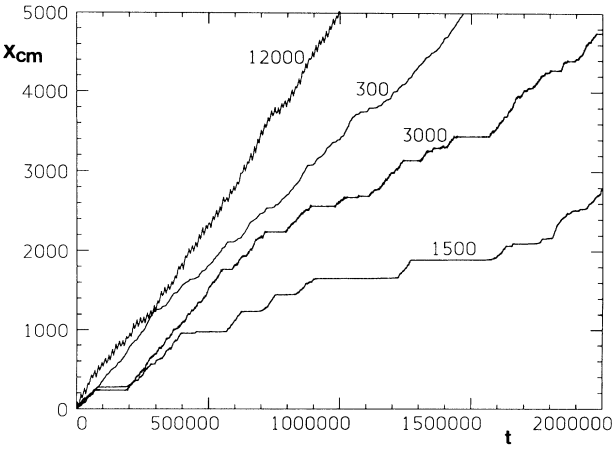

FIG. 4. Displacement of the center of mass as a function of time for different pulse periods. $N=40$, $\Delta=0.5$ $t_2/t_1=1/2$; curves labeled by T.

and so the BRM result (4) is recovered for $\Delta\ll 1$. If, however, a long chain is in a hairpin configuration, with its central portion at coordinate $x_{\min}$ and ends pointing in the field direction, (6) gives

$$t\sim\exp[\Delta\min(x_1-x_{\min},x_N-x_{\min})] \quad (7)$$

for $\Delta(x_1-x_{\min}),\Delta(x_N-x_{\min})\gg 1$, and the chain is pinned for long times, in agreement with a previous theory.[12] As we shall see, this occurs in FIGE.

To simulate an inversion experiment, an electric field is applied in the forward direction for time t_1, then reversed for time t_2, and this cycle is repeated. Figure 3 shows the mobility of chains of various lengths as a func-

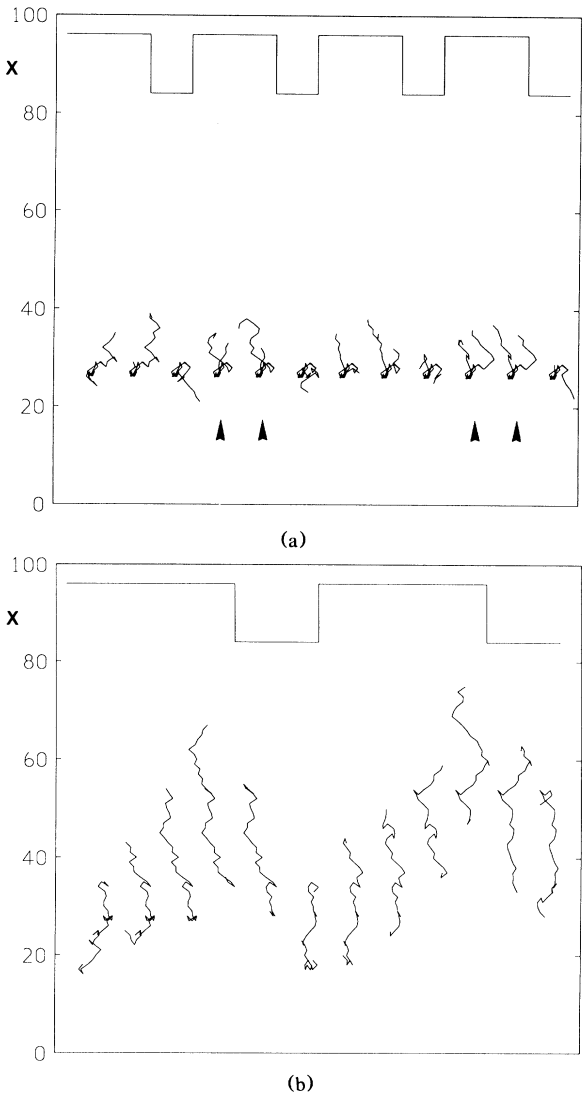

FIG. 5. Snapshots of the tube for $N=40$, $\Delta=0.5$, $t_2/t_1=1/2$, and (a) $T=3000$, during a phase of fast migration, and (b) $T=1500$ (near T_0) during a stationary phase. The time interval between snapshots is 500. Pulses of the electric field are indicated above. Hairpin configurations are arrowed.

tion of the overall pulse period $T=t_1+t_2$. If the field is held constant at $\Delta=0.5$, chains of length $N=10$–60 all migrate at the same speed and so would not be separated by normal gel electrophoresis. They have markedly different responses when the field is pulsed, however. All have a minimum in the mobility curve, but this occurs at different values T_0 of the pulse time T; longer chains have deeper minima located at longer pulse periods. The reduction in mobility can be more than a factor of 10. At lower field values, the minima occur at approximately the same T_0, but are less pronounced and broader. Changing the pulse asymmetry ratio t_1/t_2 also strongly affects the mobility curves; deeper minima are found for $t_1/t_2=2/3$ than for 1/2 or 1/3. These results, for a practically relevant parameter range, seem to be in agreement with experiment.[2]

The center-of-mass motion (Fig. 4) and snapshots of the tube (Fig. 5) indicate that the drop in mobility near T_0 is a result of the chain alternating between stretched configurations, which migrate rapidly, and compact conformations, which are almost stationary. Viovy has described how molecules can collapse.[3] Tube length fluctuations at the "tail" of a stretched chain tend to produce segments ill oriented with respect to the rest of the tube. When the field is reversed, these segments are preserved if the drift induced by the field is large enough (compared to fluctuations), and the pulse period not so long that the chain drifts right out of the original tube. The result is a reduction of the chain orientation.

Having lost its orientation, the molecule's mobility is substantially reduced. A detailed examination of the dynamics reveals why this is so. Notice [Fig. 5(b)] that the typical chain configuration just before switching the field is a compact "ball" with an extended "head" section. On inversion, the head becomes a tail. Reptons jumping into the tube at this end tend to drift towards the ball under the influence of the electric field, causing the tail to retract rapidly. In contrast, the rate of growth of a new head section is controlled by its supply of reptons from the ball. Since there is no net field bias along the unoriented part of the tube, reptons move to the head only slowly, at (of order) the standard reptation rate. Consequently, if the pulse time is long enough for the tail to completely retract, but not so long that the head fully extends, the molecule once again has the ball and head configuration as the field reverts. The process then repeats in the opposite direction. There is no net translational motion of the chain; the ball just "unravels" and "rewinds" at each end. Furthermore, the separation between chain ends becomes small during this motion which allows for reptation at both ends of the tube [Eq. (5)]. Since new tube segments tend to align with the field, hairpin configurations may therefore form by both fluctuations *and* reptation. These states have very slow dynamics [Eq. (7)] and are effectively trapped on the pulsing time scale.

This rich dynamics, involving tube length fluctuations driven by the field and reptation suppressed by pinning, explains why the mobility is depressed more sharply than predicted on the basis of reduced orientation in the BRM.[3] The outcome of the numerical study provides some insight into how to formulate the problem theoretically. How does the lifetime of immobile compact states depend on chain length, pulsing period, and electric field strength? Another interesting question to resolve is which choice of pulse asymmetry parameter best differentiates between different molecular sizes.

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