Formation of hairpins and band broadening in gel electrophoresis of DNA

A. N. Semenov[1,2] and J.-F. Joanny[2]
[1]*Department of Applied Mathematics, University of Leeds, Leeds LS2 9JT, United Kingdom*
[2]*Institut Charles Sadron, 6 rue Boussingault, Strasbourg 67083 Cedex, France*
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The dynamics of a long DNA molecule undergoing constant-field gel electrophoresis is considered theoretically on the basis of the reptation theory. A generalized theoretical approach taking into account the possibility of a branched tube structure is presented. It is shown that in a wide range of electric fields a branched conformation is more stable than a linear conformation assumed by the standard biased reptation model. The process of nucleation of a branched structure from a linear structure is considered analytically in detail. The field dependencies of both longitudinal and transverse diffusion constants are discussed within the framework of the proposed two-states model. It is predicted that the longitudinal diffusion constant shows a sharp maximum in the weak-field regime. [S1063-651X(96)09612-2]

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I. INTRODUCTION

The interpretation of the dynamics of long DNA molecules undergoing constant-field gel electrophoresis is a fascinating problem that attracts considerable attention [1–8]. The reptation model [9], which assumes that a chain is moving along a curvilinear tube, has been successfully used [1,4–6] to predict the molecular weight and the field dependence of the DNA mobility. However, a much more complex dynamics has been recently observed both in computer [8] and real [10] experiments: if the field is strong enough, the chain often forms hernias (hairpins), and also often appears as hooked around obstacles (the network strands). A fractal randomly branched treelike conformation has been proposed to describe a chain with hernias [7]. The fractal structure [7] imposes a mobility independent of molecular weight. However a detailed theoretical description of the polymer dynamics in the branched conformation is lacking. More importantly, there is an obvious discontinuity between the reptation theories for weak fields and the speculations concerning hooked and branched conformations [7,8] for stronger fields.

The aim of this paper is to partially fill this gap. The main idea of the paper is that branched conformations might be important also for weak fields. We show that hairpins are irreversibly nucleating from a linear structure with a field-dependent rate. The branched conformation that develops from this nucleation is stable if the reduced field ϵ is higher than a critical value $\epsilon^{**} \sim 1/N^{5/6}$. In the range $\epsilon^{**} < \epsilon < 1$ we propose a two-state model where one state is a linear conformation and the other one a branched conformation. One of the important implications of the model is that the random diffusive motion responsible for band broadening is dramatically enhanced in the region where both states are in competition. As a result the diffusion constant shows a peak as a function of the field ϵ.

In Sec. II we consider the field dependence of the longitudinal and transverse diffusion constants for the linear state (with no hairpins) using the biased reptation model with fluctuations (BRF) [4–6]. Section III is devoted to the dynamics of circular DNA molecules, which is very similar to that of linear DNA molecules in a branched conformation considered in Sec. IV. The problem of hairpin nucleation is addressed in Sec. V. The results are then used to predict the field dependence of the diffusion constants.

II. BIASED REPTATION WITH FLUCTUATIONS AND DIFFUSION

We discuss here the random diffusive motion of a linear chain undergoing constant-field electrophoresis. The longitudinal diffusion constant D_x has been already calculated within the BRF model [6]. With a generalized treatment presented below we obtain both D_x and the transverse diffusion constant D_y.

A. The model and the scaling arguments

Let us consider a long DNA molecule immersed in large porous gel: the pore size a is larger than the Kuhn segment b. The molecule can then be considered as a Gaussian chain of $N \gg 1$ subunits of size a (these blobs are called segments below). The reptation concept reduces the effect of the gel strands on the chain motion to a virtual curvilinear tube confining the chain. The tube diameter is a, and its length is $L = Na$. An electric field (applied in the x direction) induces a drift along the tube axis with curvilinear velocity [1]

$$v = \epsilon \frac{h_x}{N\tau_0}, \tag{1}$$

where τ_0 is the segment relaxation time, h_x is the projection of the tube end-to-end distance onto the field direction, and $\epsilon = qEa/(k_BT)$ is the reduced field (here q is the charge per segment). The center-of-mass velocity is then

$$\dot{x} = v\frac{h_x}{L} = \frac{\epsilon a}{\tau_0}\frac{h_x^2}{(Na)^2}. \tag{2}$$

Assuming that τ_0 is the unit time, and a the unit length, we get the mobility [1] $\mu \equiv \langle \dot{x} \rangle / \epsilon = \langle h_x^2 \rangle / N^2$. In a weak field the chain statistics is nearly Gaussian, so that $\langle h_x^2 \rangle = Na^2/3$ and the mobility is $\mu = 1/(3N)$.

The effect of the electric field induces an orientation of the new parts of the tube that are being created at the chain head; the orientation order parameter $\eta \equiv \langle \cos\theta \rangle = \langle h_x \rangle / N$ is predicted by the BRF theory [4,5] in a self-consistent way as $\eta \sim \epsilon^{1/2}$ if $1 > \epsilon > 1/N$. Therefore in this electric-field range $h_x \simeq \eta N a \gg N^{1/2} a$, and the tube is stretched along the x axis on many Gaussian sizes. Thus the mobility is proportional to the square of the order parameter:

$$\mu \simeq \eta^2 \sim \epsilon. \tag{3}$$

However the center-of-mass velocity is not exactly constant in this regime since the end-to-end chain projection is still fluctuating: $h_x = \eta N + \delta h_x$, where $\langle \delta h_x^2 \rangle \sim N a^2$, as for a Gaussian chain. The corresponding fluctuation of the center-of-mass velocity is [see Eq. (2)]

$$\delta \dot{x} = 2 \epsilon \eta \frac{\delta h_x}{N}. \tag{4}$$

The characteristic relaxation time of the velocity fluctuation is clearly the drift time taken by the chain to reptate over the whole tube length; $\tau \sim N/v$. The longitudinal diffusion constant, defined as

$$D_x \equiv \int_0^\infty \langle \delta \dot{x}(0)\, \delta \dot{x}(t) \rangle dt \tag{5}$$

can thus be estimated using Eq. (2) as [6]

$$D_x \sim \tau (\delta \dot{x})^2 \sim \epsilon \eta \sim \epsilon^{3/2}. \tag{6}$$

A similar estimate is also possible for the transverse diffusion constant

$$D_y = \int_0^\infty \langle \dot{y}(0) \dot{y}(t) \rangle dt. \tag{7}$$

The transverse velocity is

$$\dot{y} = v \frac{h_y}{N} \tag{8}$$

and $\langle h_y^2 \rangle = N a^2/3$. We thus obtain $D_y \sim D_x \sim \epsilon^{3/2}$.

B. Quantitative treatment

These scaling arguments suggest that the longitudinal and the transverse diffusion constants are of the same order. Therefore we expect their ratio to be a universal constant, which we calculate below.

We rely on the results obtained in Ref. [5]. The distribution density function $\rho(h_x, t)$ for the end-to-end projection obeys the following master equation:

$$\frac{\partial \rho}{\partial t} = \frac{\partial}{\partial h_x} \left\{ \frac{\partial}{\partial h_x} (D^* \rho) - v^*(h_x) \rho \right\}, \tag{9}$$

where $D^* = v/3$, and

$$v^*(h_x) \simeq v(-h_x/N + (\text{const}/h_x) \epsilon^{2/3} h_x^{2/3} N^{1/3}) \simeq -\beta_x (\delta h_x),$$

where $\beta_x = \frac{4}{3} v/N$, $\delta h_x = h_x - h_x^{(0)}$, and $h_x^{(0)} = \langle h_x \rangle$ is approximately defined by the condition $v^*(h_x^{(0)}) = 0$. The first term in the curly brackets accounts for the random conformation of newly created parts of the tube, whereas the second term describes the (weak) orientation due to the field. A similar equation can also be written for the distribution of the y projection of the end-to-end vector h_y; the only difference is that $v^*(h_y) = -v(h_y/N) = -\beta_y h_y$ as there is no field orientation in the transverse direction. By rescaling of Eq. (9) we get

$$\int \langle \delta h_x(0)\, \delta h_x(t) \rangle dt = C \frac{D^*}{\beta_x^2}, \tag{10}$$

where C is a universal numerical factor. Using also Eqs. (4) and (5) we thus get back the scaling estimate, Eq. (6).

The analogous correlator for the y component is

$$\int \langle h_y(0) h_y(t) \rangle dt = C \frac{D^*}{\beta_y^2}. \tag{11}$$

The ratio of the diffusion constants is then

$$D_x / D_y = 4 \beta_y^2 / \beta_x^2 = 9/4.$$

This ratio seems to be in good quantitative agreement with the measurements [11] on λ-DNA in 0.7% agarose gel in the field region $E = 1-2$ V/cm.

III. DYNAMICS OF A LONG RING POLYMER UNDER AN ELECTRIC FIELD

In this section we give a simple description of the gel electrophoresis of a ring polymer using reptation ideas. We discuss only usual open ring polymers and do not consider more complex topological states such as supercoiled rings.

A. Equilibrium and reptation dynamics in the absence of electric field

A ring polymer immersed in a network, but not permanently entangled with the network, adopts a branched treelike conformation at equilibrium [12]. That is why it is a natural first step to consider the dynamics of a ring polymer before analyzing the branched structures formed by linear polymers. The excluded-volume interactions do not play any significant role for the DNA conformation (unless the molecular weight is extremely large) since this molecule is very rigid: the ratio of the Kuhn segment b to the chain thickness d is ~ 50. We therefore use the results obtained for ideal rings [12]. These are summarized below.

The typical conformation of a ring in a network (gel) is a branched structure similar to that arising in the classical gelation theory [13]. (We assume that the gel is homogeneous throughout the paper. The effect of gel inhomogeneities might be important for very weak fields; however in the regime of moderate and strong fields, $\epsilon > 1/N$, the inhomogeneities just lead to a slight renormalization of the pore size.) The only difference is that the ring polymer passes twice (in the forward and backward directions) by each bond of the corresponding classical ''tree'' (Fig. 1).

A treelike conformation is characterized by its spatial size $r(N) \sim N^{1/4}$ and its backbone length $s(N)$ that can be defined as the longest primitive path connecting distant polymer seg-

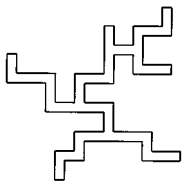

FIG. 1. Classical treelike structure of a ring polymer in a network.

ments. [Thus $N \propto r(N)^4$: a classical tree is a fractal with dimension $D=4$.] For a classical tree $s(N) \sim N^{1/2}$ in contrast to $s(N) \sim N$ for a linear chain. There is another important difference between linear and treelike conformations: for a linear chain the primitive path-length fluctuations are weak, $\delta s/s \sim 1/N^{1/2}$, whereas for a ring these fluctuations are large, $\delta s/s \sim 1$. The spatial statistics of a primitive path (of a given length) itself is Gaussian and $r(N) \sim s(N)^{1/2}$.

The ring structure is dominated by small branches of length 1 (i.e., a): the number of these branches is of order N. The number of branches of length $s(N) \sim N^{1/2}$ is order of 1.

The reptation dynamics of a ring in a network (with no external field) is a theoretical issue discussed in a number of publications [14–16]. The basic dynamic quantity is the longest relaxation time of the chain conformation, $\tau(N)$. All theories predict a scaling behavior, $\tau(N) \sim N^z$, however, various exponents have been obtained $z=3$ [14,15] and $z=2.5$ [16]. It is probably possible to show rigorously that $z \geqslant 2.5$, and also one could hardly expect that $z>3$. Hence most probably $2.5 \leqslant z \leqslant 3$. To be definite, in all the estimates below we use the exponent $z=2.5$, which seems to be in closer agreement with computer simulations [16]. The self-diffusion constant of a ring can be obtained using the assumption that during one relaxation time the ring moves over its own size [16]: $D_s \sim r^2(N)/\tau(N) \sim N^{0.5-z} = N^{-2}$.

B. Gel electrophoresis of ring polymers

In the presence of an electric field the total electric force is $f=\epsilon N$ (here we assume that $k_B T$ is the energy unit). In the limit of an extremely weak field $\epsilon \to 0$, the center-of-mass velocity of the polymer driven by the force is $\dot{x}=D_s f$, and the mobility scales as

$$\mu \sim N^{-1}. \tag{12}$$

Equation (12) is valid if the ring conformation is nearly unperturbed by the field. In order to estimate the effect of the field on the conformation we compare two situations: (1) free motion of a ring polymer; (2) conformation of a ring with a *fixed* segment—both in the presence of the field. It is natural to assume that the conformation of a free chain is less affected by the field. Therefore the second situation provides an upper boundary for the chain deformation. The extension of the ring with a fixed segment in the field direction is negligible if the typical potential energy $U \sim r(N) f \sim \epsilon N^{1.25}$ is smaller than one. Hence Eq. (12) is valid in the region $\epsilon < \epsilon^* = N^{-1.25}$.

Using Eq. (12) it is easy to see that in the regime $\epsilon < \epsilon^* = N^{-1.25}$ the time during which the chain drifts in the field direction on a distance of order of its size, $r(N)$ is much

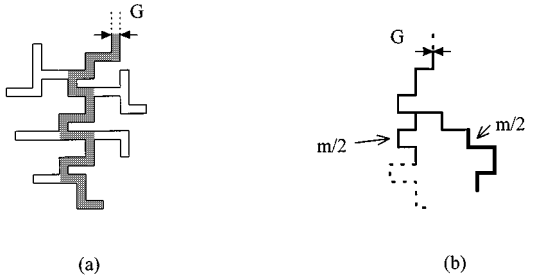

FIG. 2. (a) The ring is adiabatically squeezing through the gates G: the number of segments n in the shown part of the ring is slowly increasing. The primitive path is shown by gray. (b) Rearrangement of the primitive path on the scale $m>g$: the activation state (only the primitive path is shown). The vacated part of the primitive path is shown by dots; the new part is bold.

longer than the relaxation time $\tau(N)$. In this regime we thus anticipate no qualitative difference between the chain conformation in cases (1) and (2). On the other hand, during the time $\tau(N)$ the ring should already come to a virtual equilibrium with the field. The chain extension in the field direction Δx can be calculated using equilibrium arguments, assuming a balance between the elastic energy of the ring elongation $F_{\mathrm{el}} \sim [\Delta x/r(N)]^2$ and the potential energy $-f\Delta x$. This leads to $\Delta x \sim r^2(N) f$, so that

$$\Delta x(N) \sim \epsilon N^{1.5}. \tag{13}$$

We now consider the regime of stronger fields, $1 > \epsilon > \epsilon^* = N^{-1.25}$. Let us represent the chain as a set of N/g blobs, where g is defined by the condition $\epsilon g^{1.25}=1$. If the head blob (i.e., the most advanced in the field direction blob) were free it would move with the velocity

$$\dot{x}_{\mathrm{free}} = \mu(g)\epsilon \sim \epsilon/g \sim \epsilon^{9/5} \tag{14}$$

and would create an oriented (along the field) primitive path. The orientation parameter of its primitive path is [see Eq. (13)]

$$\eta = \eta_b \sim \frac{\Delta x(g)}{s(g)} \sim \epsilon g \sim \epsilon^{1/5}, \tag{15}$$

where the index b refers to the branched conformation. The blobs behind the head blob cannot force it to move faster since the tension along the primitive path of the chain is always positive so that the tension force must be oriented backward (if other blobs are moving faster than the head blob, they will leak to the sides creating new branches).

Let us now consider the possibility that the head blob is moving slower than in a free state because the other blobs pull it backward. An extreme situation of that kind is schematically shown in Fig. 2.

The head part of the ring of size $g \sim \epsilon^{-4/5}$ is only moderately affected by the field, and thus can easily come to equilibrium with the field. Therefore it is stretched in the field direction by a factor of order 2. A rearrangement of the primitive path on larger scales is much more difficult. The only possibility to change the primitive path on a scale $m \gg g$ is to create a hairpin near segment m (counting from the head); the hairpin then grows and forms a new primitive path

(''eating'' the old one). The activation state corresponds to the situation shown in Fig. 2(b) where the lengths of the old and new primitive paths are comparable. The corresponding barrier is $U_{\rm act}(m)\sim\epsilon m[x(m)-x(m/2)]$, where $x(m)$ is the averaged projection of the end part with m segments. Obviously $U_{\rm act}(m)\gg U_{\rm act}(g)\sim 1$ since $m\gg g$. Therefore the relaxation on scales $m\gg g$ is exponentially slow, and thus is negligible unless the ''velocity'' dn/dt is exponentially small, which is not the case. Hence a linear primitive path with a nearly homogeneous orientation $\eta_b\sim r(g)/s(g)\sim\epsilon^{1/5}$ is formed. The one-dimensional tension σ along the primitive path is not homogeneous however: it is increasing with the distance from the head as $\sigma(m)\sim\epsilon x(m)$. This tension strongly stretches the middle blobs along the primitive path. Obviously the stretched middle blobs would move faster than the unstretched head blob. In order to be more quantitative, let us consider a completely stretched blob that is thus equivalent to a part of a linear chain. Its velocity in the x direction is $\dot{x}\sim\mu_{\rm lin}\epsilon\sim\eta_b^2\epsilon\sim\epsilon^{7/5}$, where $\mu_{\rm lin}$ is the linear chain mobility defined by Eq. (3) with the order parameter η_b determined by the leading blob. The velocity is much larger than that of the free head blob, Eq. (14). Thus the blobs behind the head blob cannot slow it down as they are moving faster themselves, in contradiction with the original assumption.

We thus come to the conclusion that the head blob is moving neither faster nor much slower than in a free state. The average velocity of the ring center of mass $\dot{x}$ must coincide with the velocity of the head blob. Therefore the mobility of a ring chain is

$$\mu=\mu_b\sim\dot{x}/\epsilon\sim\epsilon^{4/5} \tag{16}$$

if $\epsilon>N^{-5/4}$.

Another important quantity is the time $\tau(\epsilon,N)$ during which the primitive path (connecting the head and the tail of the ring) is renewed. This time is estimated here from general arguments that use only the fact that the primitive path is oriented with an order parameter $\eta\sim\epsilon^{1/5}$, Eq. (15). We thus avoid a detailed description of the complex chain conformation. The primitive path can be represented as a sequence of s gates confining the ring. Let $n(t)$ be the number of segments that passes through a given gate. On the average $n(t)$ is the same for all gates. The dissipation rate is $\mathcal{D}=\zeta(dn/dt)^2$, where ζ is the effective friction constant that is proportional to the total length of the primitive path $\zeta\sim s$.

The last statement can be verified by calculating the relaxation time of a free ring (in the absence of field, $\epsilon=0$) assuming that $\zeta\sim s$. A complete relaxation is possible if $n(t)\sim N$. The effective diffusion constant (for the coordinate n) is $D_n\sim 1/\zeta\sim 1/N^{1/2}$. This leads to the correct relaxation time: $\tau(N)\sim N^2/\zeta\sim N^{2.5}$.

The motion of the ring under electric field is driven by the electric force; the dissipated energy is therefore equal to the work of this force, $W\sim\epsilon n(t)\eta s$ (here ηs is the projection of the primitive path onto the field direction). This condition can be written as $\int\mathcal{D}(t)dt\sim\zeta n^2/t\sim W$. The primitive path renewal time is obtained with $n\sim N$:

$$\tau(\epsilon,N)\sim\frac{N}{\epsilon\eta}\sim\frac{N}{\epsilon^{6/5}}. \tag{17}$$

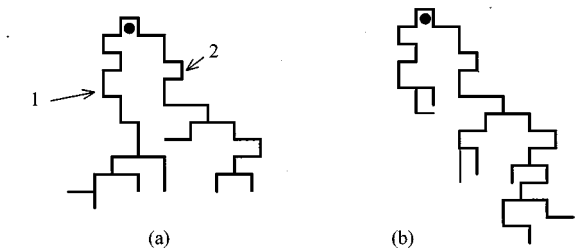

FIG. 3. Typical conformations of a ring hooked over an obstacle on the largest scale (the masses of parts 1 and 2 are comparable).

The ring moves over its size during the primitive path renewal time. This gives an estimate of the ring size in the x direction:

$$h_x\sim\dot{x}\tau(N,\epsilon)\sim N\epsilon^{3/5}. \tag{18}$$

On the other hand, $h_x\sim\eta s$; thus the primitive path length is $s\sim N\epsilon^{2/5}$. Assuming Gaussian statistics of the primitive path in the transverse direction we get the transverse size

$$h_y\sim s^{1/2}\sim N^{1/2}\epsilon^{1/5}. \tag{19}$$

Note that $h_x\sim h_y\sim N^{1/4}$ for $\epsilon\sim\epsilon^*=N^{-5/4}$, and $h_x\gg h_y$ for stronger fields.

Let us proceed to the calculation of diffusion constants for a ring polymer in the regime $\epsilon\gg\epsilon^*$. The crucial notion is that both h_x and h_y always strongly fluctuate. The large fluctuations of h_y are due to the Gaussian statistics of the primitive path in the y direction. The longitudinal fluctuations δh_x are large because the ring often hooks over an obstacle thus creating two competing subtrees of comparable sizes. An example is given in Fig. 3: both h_x and the center-of-mass velocity are smaller in conformation (a) than in conformation (b). The typical time in a hooked state is again $\tau(\epsilon,N)$. The size fluctuation is thus $\delta h_x\sim h_x$.

The fluctuations of the velocity $\delta\dot{x}$ on the time scale $\tau(\epsilon,N)$ are thus of order of the mean velocity: $\delta\dot{x}\sim\delta h_x/\tau(\epsilon,N)\sim\epsilon^{9/5}$. A similar estimate for the transverse velocity gives: $\delta\dot{y}\equiv\dot{y}\sim h_y/\tau(\epsilon,N)\sim N^{-1/2}\epsilon^{7/5}$. Using Eqs. (5) and (7) we obtain scaling estimates for the diffusion constants:

$$D_x\sim(\delta\dot{x})^2\tau(\epsilon,N)\sim N\epsilon^{12/5},\quad D_y\sim(\delta\dot{y})^2\tau(\epsilon,N)\sim\epsilon^{8/5}. \tag{20}$$

IV. DYNAMICS OF A LINEAR CHAIN IN THE BRANCHED STATE

When a hairpin forms on a linear chain undergoing gel electrophoresis, the head of the hairpin behaves similarly to the head of a ring polymer as the chain in the hairpin adopts a branched conformation. We now study the influence of this branched structure on the linear chain dynamics.

A. Weak electric fields, $\epsilon<1$

A possible way to form a branched structure is shown in Fig. 4: the middle part of chain (n) that leaked out of the original tube adopts a treelike conformation identical to that of a ring polymer comprising $n+1$ segments. This illustrates

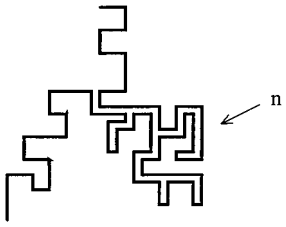

FIG. 4. The middle chain part leaks out of the tube.

the general nonlinear conformation of a linear chain that includes a treelike core with two tails. Since the mobility of a treelike part is higher than that of linear parts [compare Eqs. (16) and (3)], the branched core must be ahead of the tails that follow the primitive path created by the core. The orientation parameter of this primitive path is thus $\eta=\eta_b\sim\epsilon^{1/5}$. The length of the tails is determined by the fact that they must move with the same velocity as the branched part $\dot{x}=\mu_b\epsilon$, where $\mu_b\sim\epsilon^{4/5}$. Let us consider the balance of forces acting on a tail consisting of M segments by projecting all the forces onto the primitive path. The relevant forces are the electric force $f_{\rm el}$, the friction force $f_{\rm fr}$, and the tension forces at the head (near the branched part) $\sigma(M)$, and at the very end $\sigma(0)$:

$$f_{\rm el}=f_{\rm fr}+\sigma(0)-\sigma(M). \tag{21}$$

The end tension force, $\sigma(0)=\sigma_0\sim1$ (that is kT/a), was first considered by Doi and Edwards (see Chap. 6, p. 208 of Ref. [9]). The nature of this force is purely entropic: it is favorable for a chain tail to go out of any given tube. It is the force of Doi and Edwards that prevents a chain from a collapse inside the tube during reptation. Some interesting dynamic implications of this force have been considered in Ref. [17]. In our case the force of Doi and Edwards suppresses formation of hairpins in the tail parts: the tails are almost completely extended along the primitive path, $s(M)\sim M$. Obviously the tension must vanish near the branched part: $\sigma(M)=0$.

The friction force is $f_{\rm fr}\sim v M$, where $v\sim\dot{x}/\eta_b=\mu_b\epsilon/\eta_b\sim\epsilon^{8/5}$ is the curvilinear velocity. The electric force is $f_{\rm el}\sim\epsilon\eta_b M\sim\epsilon^{6/5}M$. The friction force is thus negligible in comparison with the electric force since $\epsilon<1$. Then Eq. (21) can be rewritten as $f_{\rm el}\simeq\sigma(0)$, so that the equilibrium tail length is $M\sim\epsilon^{-6/5}$. The condition $M=N/2$ defines a critical field $\epsilon^{**}\sim N^{-5/6}$. The branched conformation is unstable for $\epsilon<\epsilon^{**}$ but is stable for higher fields. In the regime $\epsilon<\epsilon^{**}$ the tails grow until the head branched core disappears, and finally the chain goes back to a linear conformation. The whole process is driven by the force of Doi and Edwards. On the other hand, if $\epsilon\gg\epsilon^{**}$ then the tails are short, $M\ll N$, so that nearly the whole of the chain is branched (see Fig. 5).

The mobility and the diffusion constants in these regimes are the same as for a ring polymer (it is easy to show that the effect of short tails on these parameters is negligible): they are given by Eqs. (16) and (20). The x projection of the tail part is $M\eta_b\sim1/\epsilon$, i.e., it is larger than the corresponding size of the branched part $N\epsilon^{3/5}$ in the region $\epsilon^{**}<\epsilon<N^{-5/8}$. The tails are completely negligible if $\epsilon>N^{-5/8}$.

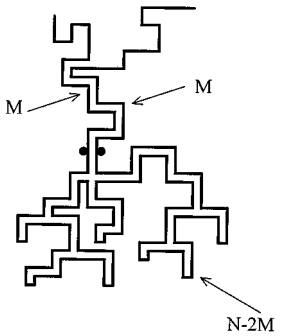

FIG. 5. A typical conformation of a linear chain in the branched state: branched middle part $(N-2M)$ with two parallel tails (M) of nearly equal length. The very end parts of each tail are random Gaussian coils; the length of these parts is determined by tube-length fluctuations.

B. Large electric field, $\epsilon>1$

For $\epsilon\sim1$ the results obtained for weak fields can still be used within scaling accuracy; they lead to the following estimates for the mobility, the relaxation time, the diffusion constants, and the chain sizes

$$\mu\sim1,\quad \tau(1,N)\sim N,\quad h_x\sim N,\quad h_y\sim N^{1/2},$$

$$D_x\sim N,\quad D_y\sim1.$$

The chain is almost completely extended in the x direction for $\epsilon\sim1$, although its conformation is highly branched. (The fractal branched chain structure was originally proposed in the regime of strong fields in Ref. [7].) A chain cannot be extended more than its contour length, and its mobility cannot become larger than the mobility of one free segment $\mu_1\sim1$. Therefore for $\epsilon>1$ we expect that $\mu\sim1$, $h_x\sim N$ in agreement with the results of Ref. [7]. The relaxation time is $\tau(\epsilon,N)\sim N/\dot{x}=N/\mu\epsilon\sim N/\epsilon$. The fluctuations of the center-of-mass velocity $\delta\dot{x}$ are of the order of the average velocity $\dot{x}\sim\epsilon$ for the very same reasons as discussed for weaker fields in Sec. III B. Therefore, the longitudinal diffusion constant can be estimated as $D_x\sim(\delta\dot{x})^2\tau(\epsilon,N)\sim\epsilon N$. The transverse diffusion constant is $D_y\sim h_y^2/\tau(\epsilon,N)$.

In order to estimate the transverse size h_y we consider the head part (of length n) that is falling down the field with velocity $v\sim\epsilon$. The electric force acting on this part is $f_{\rm el}=\epsilon n$, the friction force is $f_{\rm fr}=-v n$ (since the friction constant is n in the reduced units). We assume that $\langle v\rangle=C\epsilon$, where $C<1$ is a numerical factor accounting for the slowing down effect of the hooked conformations (see Fig. 3). Therefore the total force $f=f_{\rm el}+f_{\rm fr}\sim\epsilon n$ is directed along the field (this force is balanced by the chain tension). A deviation of the very head point of the falling hairpin on distance y in a transverse direction would result in a restoring force $f_y=-(y/n)f\simeq-\epsilon y$. Although the falling part is almost completely straight, the gel does create a transverse force acting on this part as the gel pore structure is random. A force created on the scale of one pore is of the order of 1 and is randomly oriented. The total typical force acting on the n part is thus $f_{\rm gel}\sim\sqrt{n}$. Assuming virtual equilibrium between $f_{\rm gel}$ and f_y we get $y\sim\sqrt{n}/\epsilon$. Therefore the chain size in the

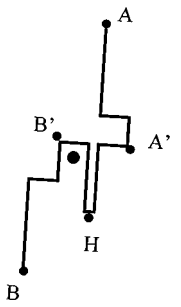

FIG. 6. A hairpin nucleus in the middle of a reptating chain. The hairpin is oriented in the field direction. The chain tension vanishes at point H. The parts AH and HB are virtually independent.

transverse direction is $h_y \sim (N/n)^{1/2}\sqrt{n}/\epsilon \sim \sqrt{N}/\epsilon$. The results for $\epsilon \gg 1$ can be thus summarized as

$$\mu \sim 1, \quad \tau(\epsilon,N) \sim N/\epsilon, \quad D_x \sim \epsilon N, \quad D_y \sim 1/\epsilon,$$

$$h_x \sim N, \quad h_y \sim N^{1/2}/\epsilon. \tag{22}$$

V. NUCLEATION OF HAIRPINS

As the hairpins play a dominant role in the chain structure at high electric fields, it is important to discuss how they appear. This is due to a nucleation process that is described in this section.

A. Mechanical picture

We study the reptation of a linear chain along a linear tube in the weak-field regime, $1/N < \epsilon < 1$. The tube is oriented along the field with an order parameter $\eta \sim \epsilon^{1/2}$ (see Sec. II A). We want to discuss the stability of the reptation motion with respect to formation of hairpins. This is done by considering a small hairpin of size n located in the middle of the chain (Fig. 6).

The chain tension σ vanishes at the very head point (H) of the hairpin. Therefore the parts AH and HB are moving nearly independently. The total electric force (projected onto the tube axis) acting on the part AH is $f_1 = \epsilon\eta(N-n)/2 + \epsilon n/2$, where the first term corresponds to the part AA' [consisting of $(N-n)/2$ links], and the second term is due to the half of the hairpin ($A'H$), which is assumed to be completely stretched along the field. (This simplifying assumption is actually irrelevant for the final result. It can be easily checked that it is the stretched configuration that leads to the lowest-energy barrier.) The effective electric force acting on the second part (HB) is $f_2 = \epsilon\eta(N-n)/2 - \epsilon n/2$. The curvilinear velocity of the part AH is $v_1 = [f_1 - \sigma(A) + \sigma(H)]/\zeta$, where $\zeta = N/2$ is the curvilinear friction constant; the velocity of the second part is $v_2 = [f_2 + \sigma(B) - \sigma(H)]/\zeta$. The hairpin growth rate dn/dt is equal to $v_1 - v_2$. Taking into account that $\sigma(A) = \sigma(B) = \sigma_0 \sim 1$ (the force of Doi and Edwards) and $\sigma(H) = 0$ we get

$$\frac{dn}{dt} = \frac{\epsilon n - 2\sigma_0}{\zeta}. \tag{23}$$

Thus the hairpin tends to disappear if $n < n^*$, but it grows if $n > n^*$, where $n^* \sim 1/\epsilon$ is the critical nucleus size.

Therefore the reptation motion is only metastable for any fixed ϵ as the critical hairpins should appear with a finite rate Γ that depends on ϵ. Once created the critical hairpins grow and develop into a treelike branched structure considered in the Sec. IV; this treelike structure is stable if $\epsilon > \epsilon^{**}$. Finally after a long enough time $t \sim 1/\Gamma$ nearly all the chains take a treelike conformation. (See Sec. VII for more discussion of this point.)

The formation of a critical hairpin is an activation process. The corresponding activation barrier U is equal to the work that an external force $f_{\rm ext}$ applied to the tip-point H should do against the force of Doi and Edwards. In the presence of the external force the right-hand side (rhs) of Eq. (23) takes the form $(\epsilon n + f_{\rm ext} - 2\sigma_0)/\zeta$, so that the adiabatic condition ($dn/dt = 0$) leads to $f_{\rm ext} = 2\sigma_0 - \epsilon n$, and

$$U = U_0 = \int f_{\rm ext}(n)\,dn \sim \epsilon (n^*)^2 \sim \sigma_0^2/\epsilon. \tag{24}$$

Using the same kind of arguments it is possible to show that U does not nearly depend on the position of the nucleus along the chain (although hairpin formation is slightly promoted near the chain head). Therefore the rate of critical hairpin formation is

$$\Gamma_0 \propto \exp(-U) \propto \exp(-C/\epsilon), \tag{25}$$

where C is a numerical constant.

In Sec. V B we consider another mechanism of hairpin formation that leads to a much higher rate Γ.

B. The effect of tube orientation fluctuations

The reptation motion of a linear chain is driven by the *effective* electric force $f = \epsilon\eta$ per unit length of the tube (the primitive path). So far we have assumed that the tube orientation is *homogeneous*, so that $\eta = \eta(s)$ is constant. In reality, however, the tube conformation is random and is oriented only on the average. Therefore the order parameter fluctuates along the tube: $\eta(s) = \eta_0 + \delta\eta(s)$, where $\eta_0 \sim \epsilon^{1/2}$ is the mean value. The effective force $f(s) = \epsilon\eta(s)$ is thus also fluctuating giving rise to fluctuations of the tension σ along the tube: in some parts the tension is higher than the average value $\sigma_0 \sim 1$, in some parts it is lower. The barrier for hairpin formation is proportional to the square of the *local* tension: in the general case σ_0 in Eq. (24) should be replaced by the local tension σ and the hairpin formation is dramatically accelerated in the regions of lower tension. Actually, the optimal way to create a hairpin is to wait for a large enough tube orientation fluctuation leading to zero tension in some part of the tube—the hairpins would then immediately appear in this part: below we show that the rate of creation of such fluctuations is much higher than the rate Γ_0 given by Eq. (25).

Let us estimate the typical tension fluctuations $\delta\sigma$ induced by the fluctuations of tube orientation on a scale n along the tube. The total effective force acting on the chain fragment inside the tube section of length n (say between the points $s = 0$ and $s = n$, where s is the coordinate along the tube) is $F = \epsilon h(n)$, where $h(n) = \int_0^n \eta(s)\,ds = h_0 + \delta h$ is the x projec-

tion of the tube section of length n and $h_0=\eta_0 n$. The Gaussian statistics of the tube fragments implies that

$$\langle(\delta h)^2\rangle=\tfrac{1}{3}n. \tag{26}$$

The typical fluctuation of the force is $\delta F=\epsilon\delta h\sim\epsilon\sqrt{n}$. The curvilinear velocity of the chain section is $v=F/\zeta=F/n$, where $\zeta=n$ is the friction constant. The average velocity is $v_0=\epsilon\eta_0\sim\epsilon^{3/2}$. The typical velocity fluctuation is $\delta v\sim\delta F/n\sim\epsilon/\sqrt{n}$. Note that the force δF is acting on the chain section during the time t it passes the given tube part $0<s<n$: $t\sim n/v_0=n/\epsilon\eta_0$. The fluctuation of the position along the tube of the chain section of n monomers, which is induced by δF, can be thus estimated as $\delta s\sim t\delta v\sim\sqrt{n}/\eta_0$. Since conformations of different tube parts are statistically independent, the effective electric forces δF acting on neighboring chain sections (of length n) are not correlated. Therefore δs can also be interpreted as a typical change of the curvilinear distance between the neighboring sections of size n. The corresponding fluctuation of tension is $\delta\sigma\sim\delta s/n\sim n^{-1/2}/\eta_0$. Note that $\delta\sigma$ is increasing as the scale n is decreased.

The above estimate is valid if $\delta\sigma$ is smaller than the original force fluctuation δF, that is if $n>n_0\sim 1/\epsilon\eta_0$. On shorter scales, $n<n_0$, the electric force fluctuation is directly balanced by the fluctuation of the tension force: $\delta\sigma\sim\delta F\sim\epsilon\sqrt{n}$. Therefore $\delta\sigma$ is dominated by fluctuations on the scale n_0: the typical tension fluctuation is $\delta\sigma\sim\delta\sigma(n_0)\sim\epsilon^{1/4}$, or $D_\delta\equiv\langle\delta\sigma^2\rangle\sim\epsilon^{1/2}$.

If the tension vanishes at some point (which implies a large fluctuation $\delta\sigma=\sigma_0$), then a hairpin starts to grow at this point. It grows up to a size of order n_0 during the time $t_0\sim n_0/v_0$, which is simultaneously the Rouse relaxation time of an n_0 fragment ($t_0\sim n_0^2$). The hairpin then continues to grow (the growth being driven by the electric field) since $n_0\sim 1/\epsilon^{3/2}$ is much larger than the critical nucleus size $n^*\sim 1/\epsilon$. [Note that the effect of thermal noise is negligible: the typical thermal fluctuation of the curvilinear length of an n fragment in $\delta s\sim n^{1/2}$, so that $\delta\sigma_{\rm therm}\sim\delta s/n\sim n^{-1/2}$; for the scale $n\sim n^*$ we thus get $\delta\sigma_{\rm therm}\sim\epsilon^{1/2}$, which is much smaller than the typical fluctuation ($\epsilon^{1/4}$) induced by tube orientation fluctuations. Thermal fluctuations on smaller scales are irrelevant as they cannot possibly lead to the formation of a critical hairpin of size n^*.]

We thus need to estimate the probability, $P(0)$, that σ vanishes at some point. The tension fluctuation is proportional to the size fluctuation: $\delta\sigma\propto\delta F\propto\delta h(n_0)$. On the other hand, the distribution of $\delta h(n_0)$ is Gaussian in the region $\delta h^2/D_h\ll n_0$, where $D_h=\langle\delta h^2\rangle$. Therefore $\delta\sigma\equiv\sigma-\sigma_0$ is also characterized by a Gaussian distribution in the region $\delta\sigma^2/D_\sigma\ll n_0$, i.e., $\delta\sigma\ll 1/\epsilon^{1/2}$: The probability density function is $P(\sigma)=\text{const}\times\exp[-(\sigma-\sigma_0)^2/(2D_\sigma)]$. Hence the probability that the tension vanishes at some point is $P(0)\propto\exp(-\tilde{U})$, where

$$\tilde{U}=\frac{\sigma_0^2}{2D_\sigma}\sim\epsilon^{-1/2}. \tag{27}$$

Note that the apparent barrier $\tilde{U}$ is much lower than U_0, Eq. (24). Therefore the hairpin formation process is dominated by the fluctuations of the tube orientation.

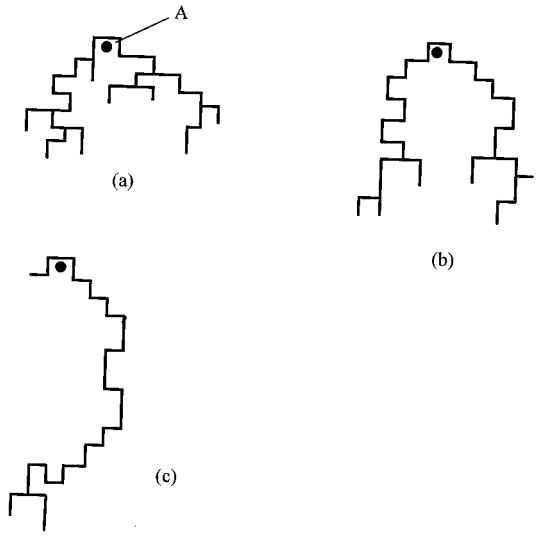

FIG. 7. (a) The chain is hooked over an obstacle A. (b) After some time both parts will extend and become essentially linear. (c) Then one (larger) part wins and a linear conformation is finally created.

This scaling analysis is supported by the quantitative treatment of the linear tension fluctuations presented in the Appendix, where it is shown that $D_\sigma=\frac{1}{6}\sigma_0(\epsilon/\eta_0)\epsilon^{1/2}$ and $\tilde{U}\simeq 2.1\sigma_0^{1/2}\epsilon^{-1/2}$.

We thus conclude that the rate of nucleation of a hairpin from a linear state is

$$\Gamma\propto\exp(-2.1\sigma_0^{1/2}/\epsilon^{1/2}). \tag{28}$$

The parameter σ_0 depends on the gel structure. However, ideally it does not depend on the gel-pore size a if the gel has large pores, $a\gg b$. A naive tube model representing the tube as a sequence of Gaussian blobs of size a implies that $\sigma_0=3$ (since the elastic energy of the nth blob is $3k_BT[s(n)-s(n-1)]^2/(2a^2)$.

VI. DISCUSSION

In the previous sections we considered the mechanisms of formation of a branched chain structure from a linear configuration, and the effect of this transition on the electrophoretic mobility. The theoretical treatment was based on an implicit assumption that the branched structure, once appeared, remains stable. Let us discuss this assumption in more detail focusing on the weak field regime $\epsilon^{**}<\epsilon\lesssim 1$.

To clarify the point let us try to imagine a process of transformation of a treelike structure into a linear one. One simple possibility is shown in Fig. 7: the chain in the branched conformation is hooked over an obstacle, so that two chain parts are competing. If the parts are almost equal, they both will move forward thus increasing the tension near point A, and finally the chain conformation becomes linear.

Two points are important here. First, the time during which the whole mass will flow to the winning (larger) part is $\tau(\epsilon,N)$, whereas the time needed for a chain to extend itself to a linear conformation is much longer since this process implies that the treelike chain will move on many its own sizes. The second point: even if the chain happens to

transform to a linear state, there is no reason for the tube orientation parameter η right after the transformation to be the same as for equilibrium linear state η_l. In fact, one should anticipate that the tube orientation at first will be of the order of $\eta_b \gg \eta_l$. When the chain starts to reptate in this more oriented (and also inhomogeneously oriented) tube, it moves faster than an equilibrium linear chain and thus creates new tube parts with even weaker orientation that η_l (the tube order parameter at the tip is inversely proportional to the curvilinear velocity [5]). Therefore the head part of the tube is much less oriented than the tail part, so that the effective electric force per segment of the head part is smaller than for the tail part. But both parts have to move with the same curvilinear velocity. Therefore, the tail part will push the head part, the pushing force being proportional to the total friction, that is proportional to molecular weight. Obviously the pushing force could easily decrease the linear tension in the middle of the chain to formally negative values, which means that hairpins leak out of the tube, and that the treelike structure develops again. We thus conclude that an irreversible transformation of the treelike structure to a linear one must be extremely unlikely. The transformation implies that not only the chain conformation should become linear, but also that the tube must be homogeneously oriented with the order parameter η_l, which is much lower than that of the branched structure. Therefore, each blob of size $g \sim \epsilon^{-4/5}$ in the branched structure must be appreciably less oriented than on the average. For each blob the probability p_1 to have a low orientation remains finite (it is not exponentially small). The number of primitive path segments per blob is $l_1 = g^{1/2}$ (see Sec. III A). If we ignore the correlations between the orientations of the different tube parts, the probability that the whole tube has a lower orientation is $p_{bl} \propto (p_1)^{N/l_1} \sim \exp(-\text{const} \times N\epsilon^{2/5})$. (In other words, we assume here that a lower orientation of a blob does not increase the probability that a new blob appearing at the tip is also less oriented than on the average. Actually we expect an opposite tendency: at least we know that it is opposite for linear chains [5].) This probability is always much smaller than the probability of the reverse transformation (linear to branched) which is $p_{lb} \propto \exp(-\text{const} \times \epsilon^{-1/2})$, see Eq. (28), since $N\epsilon^{2/5} \gg \epsilon^{-1/2}$ in the region $\epsilon > \epsilon^{**} \sim N^{-5/6}$, where the branched structure itself is stable. Thus transitions from the branched to the linear state can be neglected in this region.

The main results of this paper are partially based on the analysis of the effect of the electric field on the orientation parameter of a circular polymer chain given in Sec. III B. We showed that the effect becomes noticeable if the typical potential energy of a ring in the field $\epsilon N r(N)$ where $r(N)$ is the ring size, becomes larger than 1 ($k_B T$). This leads to the critical field $\epsilon^* \sim N^{-5/4}$. Note that this criterion is not valid for linear chains: it would imply that $\epsilon^* \sim N^{-3/2}$ as for linear chains $r(N) \sim N^{1/2}$, instead of the correct result predicted by the BRF model, $\epsilon^* \sim 1/N$ [4,5]. The difference is connected with the fact that in the linear case the electric field orients effectively not the whole chain but only its end part that fluctuates forwards and backwards along the tube trying its different conformations. The length of this part is of the order $N^{1/2}$, i.e., it is much shorter than the whole tube. In the case of a ring polymer the situation is different: here both the average primitive path length and its typical fluctuation are of the same order: $s(N) \sim \delta s(n) \sim N^{1/2}$, so that the field effectively orients the whole chain.

One must also keep in mind that our understanding of the reptation motion of ring polymers remains rather rough. All the scaling laws derived here are based on the assumptions that are compatible with the scaling model proposed by Obukhov, Rubinstein, and Duke [16]. The model may turn out not to be exact. This would affect the numerical values of the exponents predicted in the present paper. However, we believe that the main qualitative results would remain valid, in particular that the orientation parameter is higher for a branched conformation.

We also assumed that the statistics of DNA molecules is ideal, i.e., we did not take into account any swelling of DNA coils by excluded volume interactions. For a coiled conformation (zero electric field) the degree of swelling is determined by the Fixman parameter $z \sim N_b^{1/2} B_2 / b^3$, where $B_2 \sim b^2 d$ is the typical excluded volume for two Kuhn segments, d is the thickness of the DNA chain, and N_b is the number of Kuhn segments per chain. Therefore $z \sim 0.02 N_b^{1/2}$. The swelling might be important if $z \gtrsim 1$, that is for $N_b \gtrsim 2500$; this length corresponds to $\sim 10^6$ bp (base pairs). The excluded volume interactions are less important in the regime of high fields, where the conformation is extended. On the other hand, binary contacts are more probable in the more compact branched state. Since the chain is extended on the average on scales larger than the blob size $g \sim \epsilon^{-4/5}$ we only need to estimate the effective Fixman parameter for a ''branched blob'' g (assuming that $a \sim b$): $\tilde{z} \sim g^2 B_2 / r(g)^3$, where $r(g) \sim b g^{1/4}$. Thus $\tilde{z} \sim (d/b) 1/\epsilon$, so that the effect of the excluded volume interactions is negligible ($\tilde{z} < 1$) if $\epsilon > d/b \sim 0.02$.

Note also that we assumed that the network structure is ideal. In particular we neglected any effect of dangling ends (tails) in the gel, which might slow down the dynamics of a circular polymer in a weak field regime [22].

Finally we discuss the ϵ dependencies of the diffusion constants D_x and D_y, assuming that the transition to the treelike conformation is controlled by experimental time t, i.e., by the product Γt. The fraction of chains in the linear state is roughly $e^{-\Gamma t}$, and in the branched state, $1 - e^{-\Gamma t}$. The transverse diffusion constant is simply the weighted average between the linear ($\epsilon^{3/2}$) and the branched ($\epsilon^{8/5}$) values:

$$D_y \sim e^{-\Gamma t} \epsilon^{3/2} + (1 - e^{-\Gamma t}) \epsilon^{8/5}.$$

Thus D_y is increasing with ϵ in the region $1/N < \epsilon < 1$ except in the vicinity of the transition point ($\Gamma t \sim 1$) where it decreases by a factor $\epsilon^{3/2}/\epsilon^{8/5} = \epsilon^{-1/10}$. The cross-over field ϵ_c, determined by condition $\Gamma t \sim 1$, is small if the experimental time is large compared with the segmental time τ_0:

$$\epsilon_c \sim 1/(\ln t)^2 \tag{29}$$

[see Eq. (28)]. In the region of strong fields ($\epsilon > 1$) the transverse diffusion constant decreases as the field is further increased: $D_y \sim 1/\epsilon$.

The behavior of the longitudinal diffusion constant is more complicated. Here we should take into account the fact that in the region $\Gamma t \sim 1$, where the two states (linear and treelike) compete, the electrophoretic band is additionally broadened because each chain part of the time is moving

with the velocity $v_l=\mu_l\epsilon$, and part of the time—with the velocity $v_b=\mu_b\epsilon$. As a result the chain displacements are distributed (more or less homogeneously) between $\Delta x_l=\mu_l\epsilon t$ and $\Delta x_b=\mu_b\epsilon t$. The apparent diffusion constant in this region is thus

$$D_x\sim(\Delta x_b-\Delta x_l)^2/t\sim\epsilon^2(\mu_b-\mu_l)^2t\sim\epsilon^{18/5}t. \qquad (30)$$

On the other hand, in the regions $\Gamma t\ll 1$ and $\Gamma t\gg 1$ the diffusion constant is always increasing with the field: In these regions D_x is determined by the dynamics in the linear and branched states, respectively, hence $D_x\sim\epsilon^{3/2}$ for $\Gamma t\ll 1$, $D_x\sim N\epsilon^{12/5}$ for $\Gamma t\gg 1$ [see Eqs. (6) and (20)]. This qualitative behavior persists in the regime of strong fields, $\epsilon>1$, where $D_x\sim\epsilon N$ [see Eq. (22)].

We assume that the experimental time t is longer than the drift time $\tau=N/v\sim N/\epsilon^{3/2}$ of the linear state. Therefore D_x shows a maximum in the region $\Gamma t\sim 1$ since Eq. (30) implies that $D_x>N\epsilon^{2.1}$, i.e., it is larger than the value $D_x\sim N\epsilon^{2.4}$ inherent for the branched conformation at $\epsilon\gg\epsilon_c$. This prediction is in agreement with some experiments [18] and computer simulations [19].

Note that in the regime $\epsilon>\epsilon_c$ the longitudinal diffusion constant is always larger than the transverse diffusion constant by a factor proportional to N. The ratio D_x/D_y is increasing with ϵ in the region $\epsilon>\epsilon_c$. This result is in a qualitative agreement with observations for λ-DNA [11].

VII. CONCLUSIONS

The main result of this paper is that the normal reptation motion of a charged polymer chain driven by an electric field is not absolutely stable even if the field is weak, $\epsilon<1$: hairpins nucleate and develop into a treelike branched structure with the rate Γ defined in Eq. (28).

The branched structure is a fractal as suggested in Ref. [7]. In the region of weak fields ($\epsilon<1$) the structure is characterized by an additional scale—the blob size $g=g(\epsilon)\sim\epsilon^{-4/5}$. Inside these blobs (on a scale $\Delta n<g$) the conformation is nearly isotropic and is characterized by a unique fractal dimension $D=4$ in both longitudinal and transverse directions: $\Delta r_x(\Delta n)\sim\Delta r_y(\Delta n)\propto(\Delta n)^{1/4}$. For larger scales $\Delta n\gg g$ the structure is strongly anisotropic: $\Delta r_x\propto\Delta n$, $\Delta r_y\propto(\Delta n)^{1/2}$, so that the fractal dimensions are different: $D_x=1$, $D_y=\frac{1}{2}$. The internal fractal characteristics of the structure are also different on short ($\Delta n<g$) and long ($\Delta n>g$) scales. In particular, the longest primitive path length of a branch Δs, scales as $\Delta s\propto(\Delta n)^{D'}$, where $D'=\frac{1}{2}$ inside the g blobs, and $D'=1$ for larger scales.

The treelike structure is stable if $\epsilon>\epsilon^{**}\sim N^{-5/6}$. Thus after a relaxation time $1/\Gamma$ nearly all chains would transform to a treelike state. The mobility in the branched state $\mu_b\sim\epsilon^{4/5}$ is higher than the mobility in the linear state, $\mu_l\sim\epsilon$. Therefore in *equilibrium* conditions we expect that the mobility as a function of ϵ should increase in a vicinity of ϵ^{**} by a factor $\epsilon^{-1/5}\sim N^{1/6}$, which is at least formally large (actually however it is hardly larger than three as normally $N\lesssim 10^3$). This increase should also show in the dependence of mobility vs N for $N^{**}\sim\epsilon^{-6/5}$. This result thus provides an alternative explanation of the band inversion phenomenon observed in many experiments: molecules that are longer than N^{**} are moving faster than slightly shorter chains.

In the regime of very weak fields the relaxation time $1/\Gamma$ becomes exponentially large, and can easily exceed the time t of the experiment or computer simulation. Then a nonstationary mobility would be observed: $\mu\simeq\mu_l+\Gamma t\mu_b$, where $\Gamma t\ll 1$ is the fraction of chains that transform to the branched state during t. Then the region where the mobility increases with the field is determined by the time t, rather than by the molecular weight.

An experimental test of the possibility of the linear chain to the branched chain transition would be very desirable. While a direct observation of the DNA conformation might not be easy, we suggest the following tentative experiment intended to verify the main prediction in the weak field regime $\epsilon^{**}<\epsilon<1$: Let us apply an oscillating field ϵ to a system of DNA molecules in a gel. The period of oscillations should be longer than the tube renewal time of a linear chain, $\tau\sim N/\epsilon^{3/2}$. Then the amplitude of the induced DNA oscillations should saturate during a few periods. However if a field-induced transformation to the branched state does happen, then the amplitude should appreciably change (increase) on a much longer time scale $1/\Gamma$, which is exponentially dependent on ϵ. Thus measurements of the time and the field dependence of the amplitude would reveal a conformational change.

We hope that future experiments and computer simulations can provide a more quantitative basis to the main prediction of this paper that the nonlinear treelike conformations are important even for weak fields.

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APPENDIX A: THE CHAIN TENSION FLUCTUATIONS

We calculate here the mean-square fluctuation D_σ of the chain tension in a linear chain conformation. The biased reptation dynamics (including Rouse fluctuation modes inside the tube) is governed by the following master equation that was analyzed in Refs. [20,21,5]:

$$\zeta_1\frac{\partial s(n,t)}{\partial t}=-\frac{\partial\sigma}{\partial n}+f(s), \qquad (A1)$$

where $s(n,t)$ is the curvilinear coordinate of the nth segment (the segments are counted from the chain head to the tail) $\sigma=-\sigma_0\partial s/\partial n$ is the linear chain tension, $\zeta_1=1$ is the friction constant per segment (in units $k_BT\tau_0/a^2$), and $f(s)=\epsilon\eta(s)$ is the effective electric force per segment. (We ne-

glect a dependence of ζ_1 on one-dimensional density $\partial n/\partial s$ since the typical fluctuations of the density are small.) The boundary conditions are

$$\sigma(0)=\sigma(N)=\sigma_0. \tag{A2}$$

The random *thermal* force acting on the segments is neglected as explained in Sec. V B. Equation (A1) is still stochastic since the force $f(s)$ is random: $f(s)=f_0+\delta f(s)$, where $f_0=\epsilon\eta_0$ is the mean force, and $\delta f(s)=\epsilon\delta\eta(s)$. Since orientations of the tube segments are statistically independent, the random source $\delta\eta(s)$ is characterized by the following correlation function:

$$\langle\delta\eta(s)\delta\eta(s')\rangle=\tfrac{1}{3}\delta(s-s'). \tag{A3}$$

Note that Eq. (A3) is entirely consistent with Eq. (26).

The system of equations (A1)–(A3) can be rescaled to a standard form using the following linear substitutions: $s\to S=s/n_0$, $n\to\nu=n/n_0$, $t\to\tau=t/t_0$, and $\delta f\to\xi=\delta f/f_0$:

$$\frac{\partial S(\nu,\tau)}{\partial\tau}=\frac{\partial^2 S}{\partial\nu^2}+1+\xi(S), \tag{A4}$$

$$\frac{\partial S}{\partial\nu}=-1 \quad \text{for } \nu=0, \quad \nu=N/n_0, \tag{A5}$$

$$\langle\xi(S)\xi(S')\rangle=D_\xi\delta(S-S'), \tag{A6}$$

where $n_0=\sigma_0/f_0$, $t_0=\sigma_0/f_0^2$, and

$$D_\xi=\frac{\epsilon^2}{3f_0\sigma_0}. \tag{A7}$$

If we formally put $\xi\equiv 0$, then the solution of Eqs. (A4) and (A5) is trivial: $S_0(\nu,\tau)=\tau-\nu$. Let us consider the random force ξ as a perturbation that slightly affects the chain motion (this is true since according to the analysis given in Sec. V B the typical tension fluctuation $\delta\sigma$ induced by the random force, is small): $S(\nu,\tau)=\tau-\nu+w(\nu,\tau)$. Up to first order in the perturbation approach, we can substitute $\xi(S)$ by $\xi(S_0)=\xi(\tau-\nu)$. The quantity of interest is $D_\sigma(\nu)=\langle\delta\sigma^2\rangle=\sigma_0^2\langle(\partial w/\partial\nu)^2\rangle$. It is proportional to D_ξ:

$$D_\sigma=\sigma_0^2 D_\xi I(\nu)/2,$$

where ν is the reduced distance from the head, and

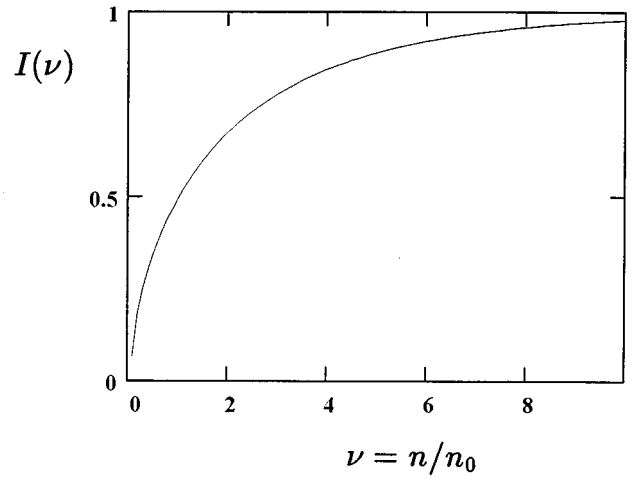

FIG. 8. The dependence of the reduced mean square of the linear tension I as a function of the reduced distance from the chain head $\nu=n/n_0$.

$$I(\nu)=\frac{2}{D_\xi}\left\langle\left(\frac{\partial w}{\partial\nu}\right)^2\right\rangle$$

is a universal function if $N/n_0\gg 1$. The system of Eqs. (A4)–(A6) with $\xi(\tau-\nu)$ instead of $\xi(S)$ can be solved using the standard Rouse-mode analysis. The result is

$$I(\nu)=1+\frac{4}{\pi}\int_0^\infty\frac{x\,\sin(\nu x)}{1-x^2}\left[e^{-\nu x}-\frac{2}{1+x^2}\,e^{-\nu x^2}\right]dx. \tag{A8}$$

In particular $I\to 1$ for $\nu\to\infty$. The plot of $I(\nu)$ is shown in Fig. 8.

Therefore the lowest barrier $\tilde{U}$ for the hairpin formation, defined by Eq. (27), corresponds to large ν:

$$\tilde{U}=\frac{\sigma_0^2}{2D_\sigma}=\frac{1}{D_\xi I(\infty)}=\frac{3f_0\sigma_0}{\epsilon^2}.$$

Finally we take into account that $f_0=\epsilon\eta_0$, where the mean order parameter is [5] $\eta_0\simeq 0.71\sigma_0^{-1/2}\epsilon^{1/2}$. Thus we get

$$\tilde{U}\simeq 2.1\,\frac{\sigma_0^{1/2}}{\epsilon^{1/2}}. \tag{A9}$$

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