sediment sizes, but no effects were seen on the depositional pattern. For the plastic particles, the ratio was 0.1. These particles moved as bedload on the sloping canyon, but were deposited immediately after the drop in shear stress.

The fan deposits formed in the experiments were two-dimensional because the currents were not able to spread laterally. Many turbidite environments do involve significant lateral flow, but there are a number of cases where turbidity flows are fairly two-dimensional over significant distances. For instance, the major channel of the Rhone river fan (*18*) shows significant spillover from the channel to the sides, but the channel has remained in the same place during thousands of years of deposition, being simply displaced vertically.

On the basis of our observations, a continuous, channelized turbidity current driven by fine material and experiencing a hydraulic jump in the proximity of a canyon-fan transition drops most of its bedload immediately downstream of the jump. The suspended load can be expected to respond more gradually to the change in flow regime, with the resulting deposit spread out over several hundred meters to several kilometers. This result seems to agree with field observations that show a zone of hemipelagic shale separating channel mouth deposits from lobe deposits in submarine fans (*2*) and supports the idea of sediment bypassing put forward by Mutti (*19*). The significance of the shale interval in hydrocarbon exploration is that it could act as a permeability barrier between lower fan lobes and other potential reservoir facies in the upper and middle fan (*2*).

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20. This research was supported by the National Science Foundation grant NSF/EAR-8517747. D. Hansen and P. Thompson provided the Coulter counter.

24 March 1989; accepted 5 June 1989

Understanding the Anomalous Electrophoresis of Bent DNA Molecules: A Reptation Model

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In polyacrylamide gel electrophoresis, the retardation of DNA molecules containing regions of intrinsic curvature can be explained by a novel reptation model that includes the elastic free energy of the DNA chain. Computer simulations based on this model give results that reproduce the dependence of anomalous mobility on gel concentration, which is quantified by new experimental data on the mobilities of circularly permuted isomers of kinetoplast DNA fragments. Fitting of the data required allowing for the elasticity of the gel.

INTRINSIC CURVATURE OF THE DNA double helix, due to structural polymorphism in DNA, as in the case of natural and synthetic DNA sequences containing tracts of oligo(dA)-oligo(dT) (*1–5*), or induced by the binding of specific proteins (*6*), has been demonstrated in a number of systems. These "bent" DNA molecules have dramatically reduced mobilities for a given size in polyacrylamide gel electrophoresis but essentially normal mobilities in agarose gels (*1*). The reduced mobility is correlated with a decrease in the overall dimensions of a DNA molecule, as has been established by independent hydrodynamic measurements made with the use of rotational diffusion techniques (*1, 4, 7*). However, a valid quantitative treatment relating mobility and overall molecular dimensions has remained elusive. We report the results of calculations of the electrophoresis of semiflexible polyelectrolyte chains containing intrinsic bends that provide a model for understanding the anomalous electrophoretic behavior of bent DNA molecules.

Current theories of the electrophoresis of linear DNA are based on the reptation model of deGennes (*8*) and Doi and Edwards (*9*), in which DNA chains migrate in a snakelike fashion among the fibers of an electrophoresis gel. The gel fibers constrain the motion of the chain, confining it largely to translation along the local axis of a "tube" (*9*). The tube is composed of a sequence of segments, which lie between consecutive points of contact between the DNA chain and gel fibers. The conformation of the chain changes as it moves along the tube, entering new tube segments at the leading end of the chain and abandoning tube segments at the trailing end. In an applied electric field this process is biased, with more moves being made in the downfield direction than upfield; hence there is a net displacement of the chain's center of mass. Lerman and Frisch (*10*) and Lumpkin and Zimm (*11*) applied reptation models to the electrophoresis of DNA by equating the component of the electrophoretic force on the DNA acting along the tube axis to the frictional resistance for translation along the tube. The mobility of the chain, μ, is then given by the component of the center-of-mass velocity of the chain in the field direction, $\dot{x}_{cm}$,

$$\mu = \langle \dot{x}_{cm} \rangle / E = \frac{Q}{\zeta} \langle h_x^2 / L^2 \rangle \qquad (1)$$

where the field of strength E is along the x axis, Q is the total electrophoretic charge on the DNA, ζ is the friction constant for motion along the tube, h_x is the component of the tube's end-to-end vector, **h**, in the field direction, L is the contour length of the tube, and the angle brackets denote an average over an ensemble of conformations.

Although Eq. 1 is often cited to explain the anomalous gel electrophoresis behavior of intrinsically bent DNA molecules, logical inconsistencies can arise in applying the underlying model to explain the anomalous mobilities of bent DNAs. If the path taken by the chain is rigidly fixed in space and

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determined by random fluctuations of the orientation of the leading segment, for example, then the conformation of the tube will be independent of the conformation of any of the internal tube segments. An intrinsic bend will therefore affect the mobility of the chain in this model only if it is located at one end of the chain. This prediction is contradictory to what is observed in circular permutations of DNA molecules containing a single bending locus, which display the greatest anomaly when the bend is located near the center of the molecule and has the most effect on molecular dimensions (*2, 3*).

The main distinction between agarose and polyacrylamide electrophoresis gels appears to be that of mean interfiber spacing, or "gel pore size." Recent electron microscopy studies of agarose gels (*12*) present a persuasive picture for the gel as a network of fibers with a broad distribution of open spaces, including some very large voids with plane-projected open diameters of the order of 3000 Å. In accord with this number, measurements of the mobilities of spherical particles indicate that the "mean pore diameter" of gels consisting of 0.2 to 4.0% agarose ranges from 3900 to 420 Å (*13*). In contrast, similar measurements in polyacrylamide gels suggest that this quantity ranges from 10 to 80 Å (*14*) and depends on the concentrations of cross-linking reagent and acrylamide monomer. The characteristic length that measures the stiffness of the DNA chain is the persistence length, P, normally taken to be 500 Å under conditions of moderate ionic strength (*15*). P is thus smaller than, or similar to, the interfiber spacing of agarose gels but is many times larger than the interfiber spacing in polyacrylamide gels.

We describe the results of computer simulations of the electrophoresis of a charged DNA chain containing intrinsic bends in a "tight" gel, a gel in which the mean spacing between the fibers is smaller than the persistence length of the intrinsically straight polyion, P. The DNA chain is assumed to move by pure reptation along a tube in an idealized gel of rigid fibers with constant interfiber spacing, a; the structure of the gel is ignored except as it determines the effective size of the segments in the tube. The number of segments, that is, interfiber spaces, in the tube, N, is taken to be a constant; we believe that this is an appropriate assumption for stiff chains confined to a tight gel, in contrast to those in a loose gel such as agarose (*16*). The chain makes discrete steps along the tube, simultaneously entering a new tube segment at the head and vacating a segment at the tail. We simulate the tube's conformation and position as they evolve in time.

For the particular case of a tight gel, the dynamics of the tube are expected to involve the elastic free energy of the DNA chain because the chain must accommodate deformations as it moves around the obstacles in the gel. As the chain moves through the gel, the new tube conformation that is generated may depart from the minimum free-energy chain conformation to a greater or lesser extent than the previous conformation; thus changes in the elastic free energy of the chain accompany motion of the chain through the gel network. We take the conformation of the tube to be given by the orientation of N tube segments of length a, numbered from 0 to $N - 1$; $N - 1$ pairs of angles, indexed from 1 to $N - 1$, specify the relative orientations of the tube segments. These angles, θ_i and ϕ_i, are the usual polar and azimuthal angles between segments i and $i - 1$, respectively. The elastic free energy of the chain in the tube, g, is given by the Hooke's law expression (*17*)

$$g(\theta_1, \ldots, \theta_{N-1}) = B\sum_{1}^{N-1} (\theta_i - \theta_i^0)^2 \quad (2)$$

where θ_i^0 is the minimum free-energy angle for the chain elements in tube segments i and $i - 1$ and B is a bending force constant. We relate this force constant to the flexibility of the chain through the ratio of tube segment length to chain persistence length, a/P (*17, 18*).

The trajectory of the model chain is calculated from the time evolution of its tube, which is obtained with a dynamic Monte Carlo algorithm (*19*). Importance sampling techniques (*20*) were used to improve the efficiency of the calculation.

In the range of field strengths and chain lengths studied, the chain moves by weakly biased diffusion, and the field acts as a small perturbation. We therefore take the unit time in the simulation to be the time, $\tau_D = a^2/2D$, required for a chain of length L to diffuse, in the absence of electrical and bending forces, an average distance a, which corresponds to the distance between gel fibers, where D is the curvilinear diffusion constant (the diffusion constant of the chain along the tube axis) for a chain of contour length L. A step in either the downfield (with the field) or the upfield (against the field) direction is chosen with probability 1/2. The rate of curvilinear motion of the chain is a random variable characterized by a transition probability, which we take to be the probability that the chain has advanced a distance a during the time τ_D. The transition probability is computed from a solution of the diffusion equation in which the drift term contains contributions from the force due to the electric field and also from the change in the elastic free energy of the chain accompanying the trial move. We assume a uniform distribution of chain ends over a space of length a at time $t = 0$; the fraction of this population leaving the space a in time τ_D in the downfield or upfield direction is denoted d or u, respectively, and is given by

$$\left.\begin{matrix} d \\ u \end{matrix}\right\} = \frac{1}{2}\left\{ (1 \mp VX + \Delta g)\right.$$

$$\operatorname{erfc}\left[\frac{(1 \mp VX + \Delta g)}{\sqrt{2}}\right]$$

$$\pm (VX \mp \Delta g)\operatorname{erfc}\left[\frac{(VX \mp \Delta g)}{\sqrt{2}}\right]$$

$$+ (2/\pi)^{1/2}\exp\left[-\frac{(VX \mp \Delta g)^2}{2}\right]$$

$$\left.- (2/\pi)^{1/2}\exp\left[-\frac{(1 \mp VX + \Delta g)^2}{2}\right]\right\} \quad (3)$$

where $V = QEa/k_BT$, $X = |h_x|/L$, Δg is the change in elastic free energy for the trial move in units of k_BT (where k_B is Boltzmann's constant and T is the absolute temperature), and erfc is the complement of the error function. The upper sign corresponds to d and the lower to u. The trial move is accepted if the transition probability is greater than a uniformly distributed random number between 0 and 1 and otherwise rejected.

The use of an explicit time dependence in the model was stimulated by the work of Noolandi *et al.* (*21*), who showed that the appropriate averages for dynamical variables in gel electrophoresis are time averages rather than ensemble averages; these averages diverge as the field increases (*22*). The model presented here also neglects tube orientation effects of the type considered by Lumpkin *et al.* (*23*) and Slater and Noolandi (*24*); field-induced orientation effects on the leading segment are negligible in the cases considered here, where a/P is much less than unity. The velocity of the chain is computed from the elapsed time associated with the trajectory and the sum of the displacements of the tube center of mass.

The dependence of mobility on the intrinsic bending angle at the center of a chain is shown along with the dependence of $\langle h^2 \rangle$ for a corresponding equilibrium ensemble of free chains (Fig. 1). The calculated relative mobilities are more strongly dependent on the extent of intrinsic bending than are the overall dimensions of these chains, $\langle h^2 \rangle$. With increasing values of the reduced interfiber spacing a/P, the mobilities of the bent chains begin to approach the mobilities of unbent chains, implying that the mobility reduction is larger for an intrinsically bent

chain in a concentrated gel than for the same chain in a more dilute gel. This behavior reproduces an important experimentally observed property of bent DNA molecules: these molecules migrate anomalously in concentrated gel systems, such as polyacrylamide, but have essentially normal mobilities in dilute gels, such as agarose (*1*). The gel-concentration effect is due to coupling of the dynamics to the elastic free energy of the chain in this model; the transition probability density depends approximately on the exponential of the change in the elastic free energy of the chain and thus is quite sensitive to the value of the force constant, B, in the free-energy expression (Eq. 2). This force constant increases linearly with the ratio, P/a, in the limit of large P/a.

The effect of an intrinsic bend on the detailed dynamics of the chain is shown in typical time series for the position of the tube center of mass along the field axis, x_{cm}, and the square of the component of the tube end-to-end vector along the field direction, h_x^2 (Fig. 2). In the case of the unbent chain, the velocity fluctuates rapidly in time, and large jumps in the center-of-mass position generally coincide with large values of h_x^2. This response is very different with the bent chain, where the velocity is weakly correlated with h_x^2; here, there are long periods where there is little downfield migration despite sizable simultaneous values of h_x^2. There are long plateaus in the time series of h_x^2 of the bent chain; these correspond to a long sequence of moves rejected in the simulation as the chain remains confined to a local free-energy minimum.

The tendency for a chain to fluctuate about favorable tube conformations in this model accounts for the major difference in the behavior of intrinsically bent and intrinsically straight chains. The presence of a single intrinsic bend imposes a large barrier to the motion of a chain if the tube conformation is close to the minimum free-energy conformation of the chain.

Calculated mobilities were compared with experimentally measured values for a set of circularly permuted DNA fragments derived from *Leishmania tarentolae* kinetoplast DNA (*3*) (Fig. 3); these fragments were chosen because the extent of bending is accurately known from hydrodynamic measurements (*7*). We found that reasonable fits to the data were obtained only by using an independently adjustable elastic force constant for the tube, B_{eff}, which we take to be an effective elastic modulus for the chain-gel combination. The computed mobilities of the set of permuted model chains agree reasonably well with the experimental data, provided that a value of B_{eff} is chosen that is much smaller than the value estimated from a/P based on values of the mean interfiber spacing of polyacrylamide gels and apparent free-solution values of P for the DNA fragments of interest.

The low value of B_{eff} can be rationalized in terms of a departure from our highly idealized model of the gel matrix as an array

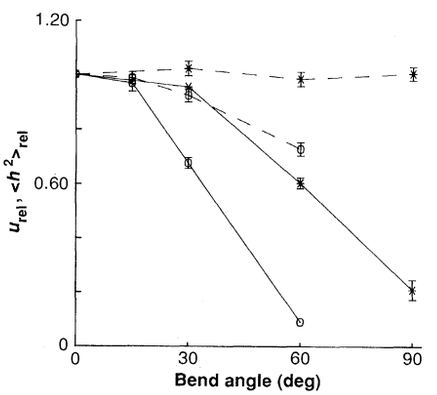

Fig. 1. Mobilities, u_{rel} (———), and ratios of equilibrium mean-square end-to-end lengths, $<h^2>_{rel}$ (– – – –), relative to an unbent chain for intrinsically bent chains as a function of bend angle at the center of the chain, $\theta^0_{N/2}$. Values of the reduced parameters are as follows: (*) $L/P = 2.0$, $P/a = 2.0$, $E' = 0.0625$; (o) $L/P = 2.0$, $P/a = 4.0$, $E' = 0.0156$; the two sets of data thus simulate the effect of a twofold reduction in the interfiber spacing a. The effective field in these calculations is given by $E' = q_0Ea^2/2Pk_BT$, equivalent to the reduced, dimensionless electric field as described in (*23*), where q_0 is the charge contained in a tube segment of length P. B was determined by calculating the Boltzmann average by numerical integration of the cosine of θ corresponding to a pair of tube segments with θ^0 equal to 0, substituting this value into the expression, $P/a = (1 - <\cos\theta>)^{-1}$, and continuing this process iteratively until the desired value of P/a was obtained. Each mobility value is the average of 1,000 independent trajectories of 16,000 trial moves computed on a CRAY X-MP/48. The $<h^2>$ values were obtained from Monte Carlo calculations on an equilibrium ensemble of 1,000 free chains, based on the use of the same parameters as above. Tests of the simulation with no applied field gave values of $<h^2>$ similar to the equilibrium Monte Carlo results within statistical uncertainty. Error bars (shown wherever larger than the symbols denoting the points) indicate 90% confidence limits.

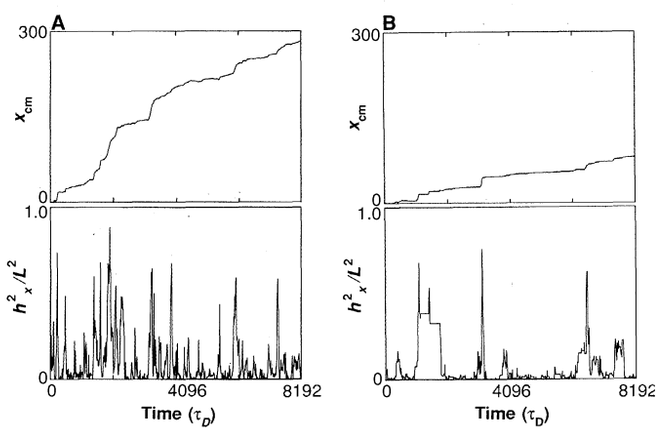

Fig. 2. Typical time series of $x_{cm}(t)$ and $h_x^2(t)$; the time is given in units of τ_D, as described in the text. (**A**) An unbent chain, $L/P = 4.0$, with $N = 8$ and $E' = 0.0313$. (**B**) A chain with the same parameters but with $\theta_4^0 = 60°$.

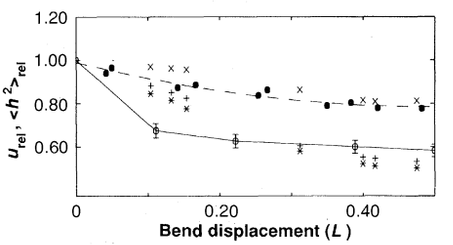

Fig. 3. Comparison of the computer-simulation results and mobilities predicted from the Lumpkin-Zimm formula (Eq. 1) with experimental mobilities of intrinsically bent DNA fragments. Shown here are experimentally measured mobilities relative to an electrophoretically normal 240-bp DNA fragment, u_{rel}, for circular permutations of a 240-bp fragment from *L. tarentolae* in 4.0% (×), 8.0% (+), and 12.0% (*) polyacrylamide gels. Also shown are corresponding relative values of $<h^2>_{rel}$ for Monte Carlo ensembles of free chains (●) and computed relative electrophoretic mobilities for once-bent tube models with an intrinsic bend of 66.0° (○). Error bars for the data from the electrophoresis simulation give 90% confidence levels, as in Fig. 1. The value of the intrinsic bend angle used in the simulation was determined from previous data on the temperature dependence of the rotational relaxation times of two circularly permuted isomers of the 240-bp fragment (*7*) and used the junction-bending model with a junction angle θ_J of 7.0°. Other parameters in the simulation were $N = 18$ and $E' = 0.0128$, and $B_{eff}/k_BT = 0.33$ rad^{-2}. This value compares with the value $B = 4.66$ determined from the ratio $a/P = 0.1$, as described in the caption to Fig. 1. DNA fragments were obtained from digestions of plasmid pHW132 with restriction endonucleases cleaving at unique sites, as described in (*3*). The resulting digestion products were subjected to electrophoresis in polyacrylamide gels (3.5% bisacrylamide:acrylamide monomer) at 13.0° ± 0.5°C with 50 mM tris-borate, 1 mM sodium EDTA, pH 8.3, as the electrolyte. Free-chain values of $<h^2>$ were computed by Monte Carlo methods from the DNA sequence of the fragment for ensembles of 1000 free chains with the use of the same junction-bending parameters and a value for the chain persistence length of 500 Å. The bend displacement for the kinetoplast fragment is measured relative to the center of bending identified in (*3*).

of rigidly fixed, regularly spaced obstacles. Polyacrylamide gels are actually elastic media. We have assumed that the network forces the DNA chain to bend as it migrates through the gel; however, if the network forces the chain to bend, then the chain must also exert a force that can deform the network. The actual local elastic free-energy barriers encountered by a DNA chain in tight gels are probably much smaller than predicted from the mean spacing of rigid fibers, a; hence the low value found for B_{eff}.

Although apparently good agreement is obtained with the mobilities of DNA fragments containing intrinsic bends near the center of the chain, the agreement is significantly worse with molecules bent near the ends. At least some of this disagreement may be due to the assumption of a rigid gel. An elastic gel is expected to accommodate larger deformations for DNA molecules bent near one end than for molecules bent near the center, thus lowering the elastic free-energy barriers for motion of chains bent near one end.

In addition, there are limitations due to modeling intrinsic curvature with a large bend located at a single position. More realistically, the natural curvature at a bending locus, such as that in kinetoplast DNA, would occur over a number of tube segments. We found, however, that distributing an effective 66° intrinsic bend without torsional constraints over two or more segments about the middle of a chain had little effect on the computed mobility. This was determined from simulations on a chain with a series of two or three torsionally unconstrained bends with equal values of θ^0 chosen to give the same free-chain $<h^2>$ values as that of the once-bent chain. Modeling the chain more realistically with several adjacent smaller bends including azimuthal phasing and torsional elasticity (as an additional parameter) encounters a number of difficulties and seems difficult to justify in view of the other approximations in this calculation, such as omission of an explicit treatment of gel elasticity.

Good agreement is also obtained with the predictions of the Lumpkin-Zimm formula (*11*) (Eq. 1) at the lowest gel concentration (Fig. 3). The data shown are values of the ratio of $<h^2>$ for bent chains to that for an unbent chain. Because this ratio pertains to the free chain and not the tube, the model cannot explain the decreasing mobility of intrinsically bent chains with increasing gel concentration.

The picture of the electrophoresis of DNA in concentrated gels presented here suggests that the anomalous mobility of intrinsically bent DNA molecules in concentrated gels such as polyacrylamide can be accounted for by inclusion of an elastic free-energy contribution to the dynamics of the chain. The numerical values of the elastic force constant that successfully fit the data are much smaller than values appropriate for DNA alone; we attribute this to elastic compliance of the gel matrix. An objective of this work is to achieve a rigorous understanding of the gel electrophoresis of bent DNA fragments so that this simple physical technique may be used to accurately characterize DNA bends in systems such as some protein-DNA complexes, which are often not amenable to analysis by electro-optical, or other, methods.

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25. Supported by grant GM 11916 from the National Institutes of Health. Partial support was also obtained from a postdoctoral fellowship from the American Cancer Society and a grant from the National Science Foundation for advanced computing resources (S.D.L.). Calculations were carried out at the San Diego Supercomputer Center. We thank E. P. Geiduschek for helpful comments on the manuscript.

22 February 1989; accepted 25 May 1989

Lignin-Like Compounds and Sporopollenin in *Coleochaete*, an Algal Model for Land Plant Ancestry

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Unusual cell wall structure and resistance to microbial degradation led to an investigation of resistant biopolymers in *Coleochaete* (Chlorophyta, class Charophyceae), a green alga on the evolutionary lineage that led to land plants. In *Coleochaete* that are undergoing sexual reproduction, vegetative cell walls contain material similar to lignin, a substance generally thought absent from green algae, and the zygote wall includes sporopollenin. Knowledge of chemically resistant compounds in *Coleochaete* may facilitate interpretation of the fossil record. Placental transfer cells in *Coleochaete orbicularis* and in the hornwort *Anthoceros* survive acetolysis and contain lignin-like compounds, implying a close relation between these taxa.

ON THE BASIS OF CYTOLOGICAL and chemical similarities, embryophytes (land plants) are thought to have evolved from charophycean green algae (*1*), and the charophycean genus *Coleochaete* is the extant alga with greatest similarity to embryophytes (*2*). *Coleochaete* (Fig. 1) is a tiny (1 mm in diameter) alga occurring in shallow, quiet freshwater habitats, which may be similar to those occupied by the algal ancestors of land plants (*3*). Transition to a land flora probably occurred during the late Ordovician or early Silurian, but interpretation of fossils of the earliest ancestors of plants is difficult because they were incompletely adapted to the terrestrial environment and thus lacked systems characteristic

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