Evidence for Entropic Barrier Transport of Linear, Star, and Ring Macromolecules in Electrophoresis Gels

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The mobility of a flexible probe chain depends strongly on the configurational freedom afforded by its environment. The dilute solution defines one limiting state, with chain motion successfully described by the Zimm model after accounting for excluded volume. The opposite extreme, when confinement is very severe, as within a melt or highly cross-linked gel, defines another limiting state. Here, the dynamics of a long linear probe molecule apparently follow the reptation model. Chains confined at levels between these limits obey complicated molecular dynamics that are not yet well understood. Nondilute solutions, porous solids, and lightly cross-linked gels present local environments in which unknown or unverified crossover dynamics may dominate. In the present contribution we will discuss how electrophoresis experiments with linear, star, and ring macromolecules provide strong support for a recently proposed thermodynamic theory for chain mobility over this crossover regime. The thermodynamic depiction, termed "entropic barriers", was first proposed in a series of papers[1-4] by Muthukumar and co-workers.

Electrophoresis is a convenient tool to monitor the motions of probe molecules in complex media.[5] The principal experimental parameter is the electrophoretic mobility μ related at low fields to the diffusion coefficient D through the standard Einstein relationship. If the charge Q on the probe chain is proportional to its length N, we find

$$\mu \sim DQ \sim DN \tag{1}$$

where D is the diffusion coefficient in the absence of long-ranged intramolecular hydrodynamic interactions (in an electrophoresis experiment these interactions are screened by counterions except at very small N[6]). It is emphasized that μ and D follow eq 1 only when μ is measured at a low enough applied field that the flexible probe chain remains undistorted. Such distortion occurs more readily in denser systems and the range of low-field behavior is consequently reduced. In actual practice, many electrophoretic separations are conducted at field strengths too large to apply eq 1 or with time-dependent applied fields; we are not concerned here with these more complex situations. (A prefactor involving the ionic strength has been neglected in writing eq 1. We have also assumed that the ionic strength is sufficiently large to ensure flexible coil behavior for the probe molecule, present at dilute concentrations.)

In a dilute solution of unconfined probe chains $D \sim N^{-1}$ from Rouse theory, so eq 1 predicts that μ is independent of N; this independence has been verified for a number of polyelectrolytes.[7-11] In a very dense system, reptation predicts $D \sim N^{-2}$ and $\mu \sim N^{-1}$ for linear molecules, in accord with many diffusion and electrophoresis measurements at large N.[12-15] These results confirm that the N dependence of μ reflects solely the configurational interactions of the probe chain with its environment. For simplicity, the electrophoresis experiments discussed here were performed in highly porous agarose gels; the concepts developed will apply equally well to any medium providing comparable degrees of chain confinement. Low-field conditions were always verified by duplicating measurements at widely separated field strengths.

The tranditional depiction of molecular motion in gel electrophoresis, known as the Ogston model,[16-18] considers the motion of a mobile sphere through the interstitial voids of a fixed array of randomly oriented and randomly placed rods. The mobility, normalized by its free solution value μ_0, is equated to the overall volume fraction of the rod/void array that is accessible to a spherical object of radius R:

$$\mu/\mu_0 = \exp[-A\phi(R + r)^2] \tag{2}$$

where ϕ is the rod volume fraction, r is the rod radius, and A is a coefficient of formal value $2/r^2$. The parameter R plays the role of an effective sphere size for the mobile chain and presumably should scale with the molecular weight in the normal fashion, $R \sim N^{\nu}$, where ν is the excluded volume exponent. In some cases, but not all, eq 2 has been satisfactorily fit to μ vs N data using μ_0, r, and A as free parameters.[19] When independently measured values for these parameters are employed, however, fits are not so satisfactory.[20] Experimentally, at large N and/or in dense gels, μ scales as N^{-1}, a form not derivable from eq 2. Agarose gels appear in electron micrographs as heterogeneous fibrillar networks with fiber diameters of 5–10 nm and average mesh sizes in the range 30–200 nm, depending on gel concentration.[21-23] From the micrographs, a random rod model for the matrix structure appears a reasonable first approximation.

We suggest that the inadequacies of the Ogston model primarily result from the flexibility of the probe chain. Backbone flexibility imparts configurational entropy to a polymer, and in a heterogeneous matrix environment, this entropy depends on the location of the polymer's center of mass. Local entropy gradients are most pronounced when the coil size nearly matches the size of inhomogeneities in the matrix; here, the chain center-of-mass preferentially partitions itself into the more open void spaces, as these regions permit a greater number of configurations. A pictorial explanation of this thermodynamics effect is shown in Figure 1. A flexible-coil molecule, residing in a void of the inhomogeneous gel, is weakly driven by the electric field from left to right. Its movement is impeded by a confinement which must be passed before the chain can reside in the next open region. The confinement creates an entropic barrier possessing a height that controls the rate of transport between the two open regions. Barrier passage becomes an activated process, with the passage rate expressed as the product of an attempt frequency and an exponential of the chain's dimensionless entropy decrease during confinement in the barrier.

A real gel creates an inhomogeneous three-dimensional network, so accurately specifying the structure and location of individual entropic barriers is a very difficult task; in such a complex system, an entropic barrier probably should not be regarded as a discrete entity. In fact, a value for the confinement entropy can be specified at every point in the system available to the polymer's center-of-mass; the full set of these entropies then defines a three-dimensional entropy surface. From this vantage, polymer transport by the entropic barrier mechanism maps into an equivalent problem, that of the field-biased Brownian motion of a point particle over the entropy surface. The

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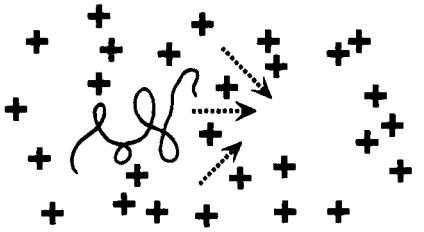

Figure 1. Motion of a flexible coil chain through a random gel under a weak external field driving the chain from left to right (matrix constraints are represented by the cross hairs). The dotted arrows indicate potential transport through entropic barriers as the chain moves from one cavity to the next.

field "tilts" the surface so that motion is preferentially toward the direction of lower potential. In the low-field limit, this preference constitutes a small, nonlocal perturbation on the locally rough surface. It is stressed that the entropy surface depiction is merely a conceptual convenience: modeling actual transport requires detailed knowledge of, or specific models for, the local gel structure and the flexible probe molecule conformation. Simulations of the diffusion coefficient of a flexible linear chain, for example, were conducted on a lattice generated by the site percolation method;[24] results for the diffusion coefficient were subsequently rationalized using a scaling picture in conjunction with a simpler model for the matrix.[1-4] Although only simplified models for the gel structure and probe chain conformation will be considered in the subsequent discussion, the basic principles of entropic barriers transport are more general.

Two features distinguish entropic barrier transport from more conventional theories for gel electrophoresis or diffusion. First, if the molecular weight dependence of the mobility is written $\mu \sim N^{-\beta}$, β becomes a nonconstant parameter that may exceed 1 as N increases; in fact, the μ vs N relationship is predicted to more closely approach an exponential than a power law.[1-4] Values greater than 1 have not been observed for poly(styrenesulfonate) or DNA in agarose gels[25] but have been noted for poly(styrenesulfonate) in polyacrylamide gels.[26] Large N exponents have also been determined for the self-diffusion coefficient in moderately concentrated polymer solutions and gels.[27-30] Second, and most importantly for the present discussion, the entropic barrier approach differs from alternative models in the influence of a probe chain's topology on its mobility. With the Ogston model one expects two chains of equal N, but of different topology, to possess distinct mobilities characteristic of their different molecular radii: linear chains will move most sluggishly because of their large size. With reptation the trends are opposite: the motion of topologically complex polymers such as rings and stars will be substantially hindered in comparison to linear chains of equal total N.[31,32]

Hoagland and co-workers have reported that over a broad crossover regime, coinciding with comparable probe chain radius of gyration and gel mean mesh spacing ξ, the experimentally determined mobility of a flexible polyelectrolyte depends strongly on total molecular weight N and not on topology.[20] Parts A and B of Figure 2 present data sets illustrating this independence, linear vs star poly(styrenesulfonate) and linear vs ring DNA, respectively; the mobilities were measured in dilute agarose gels at low applied field ($E \leq 0.3$ V/cm). The functionality f of the star polymers are generally in the range 1–12, and the arm lengths N_{arm} varied over approximately 1 order of magnitude. Details on sample preparation and experimental conditions are found in refs 15 and 20. Behavior consistent with reptation is observed only for the largest linear chains in the most dense gels. The unexpected

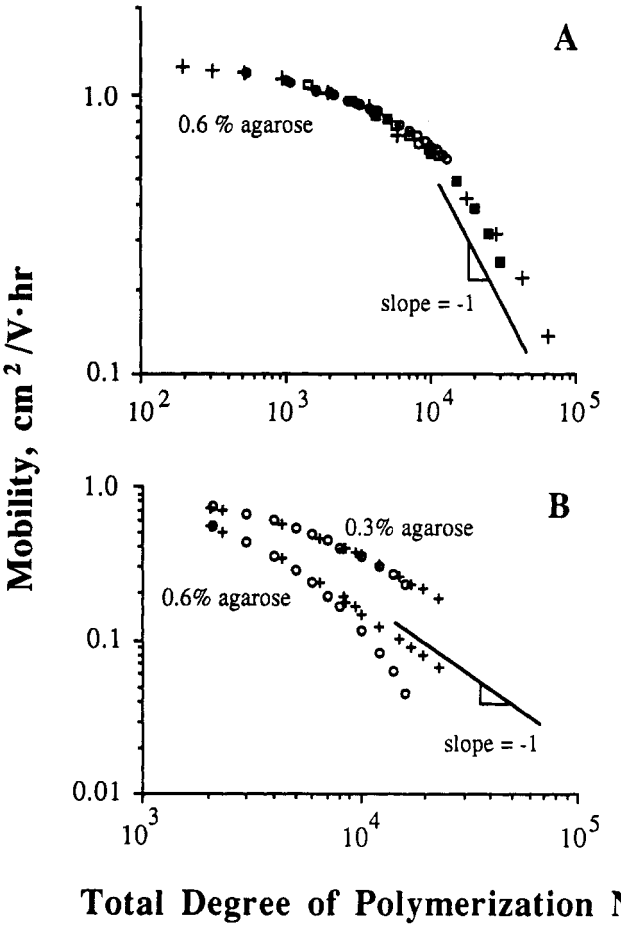

Figure 2. Comparison of the electrophoretic mobilities for linear, star, and ring polymers. Linear chains are indicated by "+" symbols. (A) Star topology = poly(styrene sulfonate): (●) N_{arm} = 540 ($1 < f < 8$); (○) N_{arm} = 1000 ($1 < f < 12$); (□) N_{arm} = 1400 ($1 < f < 8$); (■) N_{arm} = 5000 ($1 < f < 6$). (B) Ring topology = relaxed plasmid DNA: (○) ring DNA, $N = 2.07 \times 10^3$, 2.97×10^3, 3.99×10^3, 5.01×10^3, 6.03×10^3, 7.05×10^3, 8.07×10^3, 10.10×10^3, 12.14×10^3, 14.17×10^3, 16.21×10^3.

topological independence of μ at smaller N for flexible probe species is to be explained through the entropic barriers model. The weak influence of chain topology on confinement entropy, at intermediate to high degrees of spatial confinement, forms the core of this argument.

Consider first a linear, star, or ring polymer of size R enmeshed in a gel of uniform confinement spacing L, which is much less than R. The polymer's conformational dynamics at length scales less than L effectively will be those of an unconfined linear chain; a portion of the chain with spatial dimension L and chain length N_b thus constitutes a "blob". To good approximation the chain can be analyzed as a necklace of these independent blobs.[31] With L constant, the total configurational entropy is obtained by multiplying the blob entropy by the total number of blobs N/N_b required to mimic the size and structure of the confined molecule.[33] This procedure implies that the conformational entropy, to good approximation, is an extensive function of N; a small N-independent correction to the confinement entropy will arise from the constraint that the blobs be connected properly to represent the probe chain structure (i.e. ring, branched, linear, etc.).

A real gel presents an enormously complex and heterogeneous confinement geometry not adequately characterized by a single confinement spacing. A gel can be modeled more satisfactorily as a network of open "cavities" separated by constrictive "bottlenecks". Bottlenecks constitute the entropic barriers. If cavities are large

compared to R, only the confinement entropy within the bottleneck portion of the network will affect μ. Assuming each bottleneck can instantaneously host an entire probe molecule, effects of chain topology on the confinement entropy in certain simple geometries can be obtained via direct calculation. For example, results derived by Casassa and Tagami[34] for linear and star-shaped Gaussian chains in slits and cyclindrical pores reveal the trend

$$\Delta S_{c,linear}(N) - \Delta S_{c,star}(N,f) = g(f) \quad (3)$$

whenever the radius of gyration of the linear chain exceeds about 30–40% of the slit or pore diameter. Under these conditions the confinement entropies for both linear and star chains, $\Delta S_{c,linear}$ and $\Delta S_{c,star}$, are proportional to their total degrees of polymerization N. The function $g(f)$, which increases with star functionality f, will provide a weak topological dependence to chain motion. Comparing linear and three-arm star Gaussian polymers in a slit geometry, for instance, $g(f)$ is about $0.08k$, where k is the Boltzmann constant. The entropic barrier model predicts that the mobility through a hypothetical network of cavities and bottlenecks is proportional to the local partition K [$\equiv \exp(\Delta S_c/k)$] between cavity and bottleneck,[35,36] so for this particular matrix model the mobility of a three-arm star and a linear chain at equal N would differ by about 8%. When confinement effects within the cavities as well as within the bottlenecks are important, μ becomes an even weaker function of topology; if eq 3 holds in each region, the topological dependence of K completely disappears. Including intramolecular excluded volume does not significantly modify these conclusions.[37]

Realistically, a single constriction in a swollen gel can encompass only a small portion of a probe molecule and not the entire chain. An earlier paper[1] discussed this issue in the context of the cavity and bottleneck model, showing that for a linear polymer the height of the entropic barrier is associated with the transfer to an adjacent bottleneck of the segments within a given cavity. The barrier height is not constant but possesses a mean value associated with the average number of chain segments occupying the cavity. The same argument applies, without any modification, to polymers of nonlinear topology—as long as the confinement entropy within the cavities and bottlenecks is linear in the number of segments in the local space, μ is independent of topology.

The topological independence of the electrophoretic mobility constitutes a strong test of the entropic barrier model, with the present data for linear, star, and ring polymers suggesting that the theory applies across a substantial range of confinement conditions. The regime over which $\mu(N)$ superimposes for linear and star, as well as linear and ring, polymers can be roughly bounded $0.1 \lesssim R_g/\xi \lesssim 2$, coinciding with the optimal electrophoretic fractionation conditions in these gels. (Defining a mean mesh spacing ξ for an agarose gel is somewhat problematical,[21-23] as is rigorously relating experimental values for ξ to the sizes of bottlenecks.) It is interesting to note that the value of β determined from the $\mu(N)$ relationship for linear chains never exceeds 1 in these experiments; therefore, entropic barrier transport could not have been deduced from the linear polymer data alone.

The group of Lodge et al. has recently reported tracer diffusion measurements for linear and star poly(styrene)s in toluene-swollen poly(vinyl methyl ether) gels;[30,38] they find that star diffusion is substantially hindered compared to that of linear chains. Their result is not necessarily in disagreement with the present data, as the probe chain confinement in the diffusion experiments was apparently greater than in most of the electrophoresis experiments reported here. As seen in Figure 2B, when confinement conditions become sufficiently severe that linear chains follow a $\mu(N)$ relationship describable as a power law, there develops a substantial gap between ring and linear chain mobilities. We expect star polymers to follow roughly the same trend. The topological independence discussed here will only exist at intermediate confinement levels where the entropic barrier model is appropriate. When the confinement is much stronger, spatial inhomogeneities are squeezed out and a new dynamics dominates.[4]

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