Capillary Electrophoresis of DNA in Uncrosslinked Polymer Solutions: Evidence for a New Mechanism of DNA Separation

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The electrophoretic separation of DNA molecules is usually performed in thin slabs of agarose or polyacrylamide gel. However, DNA separations can be achieved more rapidly and efficiently within a microbore fused silica capillary filled with an uncrosslinked polymer solution. An early assumption was that the mechanism of DNA separation in polymer solution—capillary electrophoresis (PS-CE) is the same as that postulated to occur in slab gel electrophoresis, i.e., that entangled polymer chains form a network of "pores" through which the DNA migrates. However, we have demonstrated that large DNA restriction fragments (2.0–23.1 kbp) can be separated by CE in extremely dilute polymer solutions, which contain as little as 6 parts per million (0.0006% (w/w)) of uncrosslinked hydroxyethyl cellulose (HEC) polymers. In such extremely dilute HEC solutions, far below the measured polymer entanglement threshold concentration, pore-based models of DNA electrophoresis do not apply. We propose a transient entanglement coupling mechanism for the electrophoretic separation of DNA in uncrosslinked polymer solutions, which is based on physical polymer/DNA interactions. © 1996 John Wiley & Sons, Inc.

Key words: capillary electrophoresis of DNA

INTRODUCTION

Increasing the rate of DNA restriction mapping is a major obstacle in the current international effort to map and sequence the 3 billion base pairs (bp) of the human genome. One of the slowest steps in the restriction mapping of chromosomal DNA is the electrophoretic separation of DNA restriction fragments ranging in size from a few thousand to a few million base pairs. Currently, DNA fragments ranging from 2 to 40 kilobase pairs (kbp) in length are separated in agarose slab gels using small voltage gradients, requiring 1–2 h of electrophoresis. DNA larger than ~40 kbp cannot be separated by steady-field electrophoresis; size separation is achieved by the application of low-voltage, pulsed electric fields, which are applied alternately in two directions (Schwartz and Cantor, 1984). Pulsed-field electrophoresis typically requires 12–24 h of electrophoresis. The high-resolution separation of small DNA fragments (under 2 kbp) is necessary for fine restriction mapping and is typically achieved in crosslinked polyacrylamide gels in 1–2 h. In this article, we demonstrate the potential of the technique of polymer–solution capillary electrophoresis to vastly increase the rate of DNA restriction mapping for DNA in the size range of 0.7–23.1 kbp.

Slab Gel Electrophoresis

DNA electrophoresis is typically performed on slab gels, in which several samples are run in parallel lanes alongside DNA molecular weight standards. Because DNA chains of different lengths have different electrophoretic mobilities within the gel matrix, they form separate bands on the gel. To allow detection of these bands, the DNA molecules are labeled with fluorescent or radioactive compounds before, during, or after the electrophoretic separation. Relatively large samples of DNA (up to 10 μg) are required, because while the fluorophores or radiolabels emit in all directions, the signal is only collected by imaging the gel in two dimensions (e.g., by exposing one side of the gel to X-ray film). Because gels are prepared and loaded manually, slab gel electrophoresis is labor intensive; furthermore, the separation step is slow, requiring from 1 to 24 h of electrophoresis, depending on the conditions, desired resolution, and size range of DNA to be analyzed. Advantages of slab gel electrophoresis include the ability to make side-by-side comparisons of related DNA samples and molecular weight standards (necessary for applications such as restriction mapping, DNA sequencing, mobility shift assays of protein binding to DNA, etc.) and the ability to separate and subsequently collect from the gel relatively large amounts of DNA for further manipulation.

Capillary Electrophoresis

There are several practical advantages to performing DNA
difficult to cast within a capillary, are optically opaque, and yield relatively poor and irreproducible separations (Chan et al., 1993; Cohen et al., 1988; Guttman et al. 1990). Small capillaries have been developed (Albin et al., 1992; Altria and analytical technique.

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capillary electrophoresis may be accomplished on-line (by 
 running the capillary directly through a detector), either by 
 UV absorbance (Hjertén, 1967) or by laser-induced fluores- 
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 various methods to collect separated DNA bands from cap- 
 illaries have been developed (Albin et al., 1992; Altria and 
 Dave, 1993; Cohen et al., 1988; Guttman et al. 1990). Small 
 sample volumes (2–100 nL) are required for high- performance capillary electrophoresis, so only small amounts of DNA are required (less than 50 ng). Hence, capillary electrophoresis serves extremely well as an analytical technique.

Attempts to perform capillary electrophoresis of DNA in gel-filled capillaries have focused almost exclusively on the use of polyacrylamide gels and have met with limited success. The preparation of gel-filled capillaries of uniform quality and stability has proven difficult and remains a difficult problem (Drossman et al., 1990; Swerdlow and Geste- 
 land, 1990; Yin et al., 1990). Furthermore, the structure of crosslinked polyacrylamide is such that it can only separate DNA smaller than ~2 kbp, making it inadequate for most chromosome mapping separations, though it can provide excellent separation of small DNA fragments (15–500 bases) for DNA sequencing. Agarose gels, though useful in a slab geometry for restriction mapping of large DNA, are poor media for capillary electrophoresis, because they are difficult to cast within a capillary, are optically opaque, and yield relatively poor and irreproducible separations (Chan et al., 1993). Thus, no crosslinked gel matrix is effective for the capillary electrophoresis separations of DNA restriction

ration. The magnitude of the current which may be applied to a slab gel is limited, because it is important that the gel remain at a relatively low and uniform temperature during electrophoresis, to prevent sample or gel degradation and/or thermal convection of DNA molecules which can be caused by temperature gradients. The maximum voltage which can be applied to conventional slab gels is in the range of 5–40 
 cm, depending on the thickness of the gel (thin gels dis- 
sipate heat more efficiently). A microbore fused silica cap- illary (inner diameter 50–100 μm) has an internal volume of 
 only 1–2 μL and an intrinsically high electrical resistance, 
 so that the current and resultant Joule heat generated during 
 the application of even very high voltage gradients are rela- 
tively low in magnitude. The small amount of Joule heat 
 produced during capillary electrophoresis is dissipated with 
great efficiency because of the high surface-area-to-volume 
 ratio of the capillary; hence voltage gradients of up to 700 
 V/cm may be applied without appreciable temperature rise 
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Figure 1. Schematic representation of a capillary electrophoresis apparatus.

CA) was used to drive the electrophoresis. Current was measured over a 1-kΩ resistor in the return circuit of the power supply using a digital multimeter (model 3465B, Hewlett-Packard, Palo Alto, CA). On-column detection was by UV absorbance at 260 nm using a modified variable-wavelength detector (model 783, Applied Biosystems, Foster City, CA). Data were collected using an integrator (model 3390, Hewlett-Packard, Palo Alto, CA).

Materials

A nonstoichiometric mixture of λ-HindIII and ΦX174-HaeIII restriction fragments (λ-HindIII fragments are present at a lower concentration, so that peaks of the larger DNA fragments in this digest will not dwarf those of the ΦX174-HaeIII digest) was obtained from Pharmacia LKB Biotechnology (Alameda, CA) at a concentration of 500 µg/mL. In experiments with uncoated capillaries, the time it took a neutral species, mesityl oxide (Aldrich Chemical Co., Milwaukee, WI), to move from the entrance of the capillary to the detection window was used to calculate the neutral marker’s average velocity, assumed to be the electroosmotic velocity. The buffer used in all experiments with DNA was 89 mM Tris, 89 mM borate, and 5 mM ethylenediaminetetraacetic acid (EDTA), with a pH of 8.15 (all buffer reagents purchased from Sigma Molecular Biology, St. Louis, MO). Measured amounts of hydroxyethyl cellulose (HEC) were used to make extremely dilute solutions. The HEC samples used had manufacturer-reported number-average molecular masses of $M_n = 90,000–105,000$ g/mol and $M_n = 24,000–27,000$ g/mol and were obtained from Polysciences, Inc. (Warrington, PA).

Capillary Electrophoresis

DNA samples containing λ-HindIII and ΦX174-HaeIII restriction fragments were preheated for 5 min at 65°C and then stored on ice (λ-HindIII restriction fragments of 4361 and 23,130 bp have cohesive termini). All DNA samples were premixed with a minute amount of mesityl oxide and injected without dilution. In the electropherograms, peaks were identified by integration of peak areas. A representative plot of peak area as a function of the number of DNA base pairs can be found in Barron et al. (1993).

Uncoated Capillaries

Each new, uncoated capillary was treated with 1 M NaOH for 3 h and then rinsed with water before initial use to etch the fused silica surface clean of adsorbed impurities. Thereafter, before each filling of the capillary with a given polymer solution, the uncoated inner capillary wall was conditioned first with 1 M NaOH for 10 min, then with 0.1 M NaOH for 10 min, and finally with the electrophoresis buffer for 10 min before being filled with the polymer solution.
specific time which depended on the buffer viscosity, to introduce approximately 3 nL (3 × 10⁻⁶ cm³) of sample for each run. After the sample was drawn into the capillary, the anodic end of the capillary was replaced in the electrophoresis buffer, together with the anodic electrode, and the electrophoretic voltage of 265 V/cm was applied. The uncoated capillary was enclosed in a plexiglass box and surrounded by convected air at a temperature of 30.0 ± 0.1°C during all experiments.

Absolute electrophoretic mobilities are calculated by subtracting the electroosmotic mobility (determined from the elution time of the neutral marker) from the apparent electrophoretic mobility of the DNA band, since the electroosmotic flow and the DNA move in opposite directions; a mathematical description of this calculation can be found elsewhere (Grossman et al., 1989). Electroosmosis of the buffer results in uniform flow with a flat velocity profile (Grossman and Colburn, 1992). This flow exerts an equal force on all DNA molecules in the sample, regardless of chain length. This is demonstrated by the fact that when electrophoresis is performed in free solution, in the absence of polymers in the buffer, all DNA molecules migrate at the same velocity and elute as a single peak.

Coated Capillaries
Polyacrylamide-coated capillaries were produced according to the method of Hjertén (1985). When coated capillaries were used, DNA was injected electrokinetically at the cathodic end of the capillary by placing both the capillary and the platinum wire electrode into the DNA sample and applying a voltage of 265 V/cm for 3–4 s. The capillary and cathodic electrode were then placed in a vial containing the electrophoresis buffer, and the electrophoretic voltage of 265 V/cm was applied. Because the coated capillaries require the polarity of the electrodes in relation to the detection window to be reversed, limitations of the apparatus required experiments to be run at room temperature. Thus, a temperature correction was used to compare electrophoretic mobilities with uncoated capillary experiments (Barron et al., 1995).

MODELING OF DNA GEL ELECTROPHORESIS
Theories describing the electrophoretic separation of DNA are based on slab gel electrophoresis. Without any separation medium, the electrophoretic mobilities of DNA molecules are independent of molecular size (Olivera et al., 1964) and no separation of DNA occurs. The “DNA sieving” capability of gels is thought to arise from the presence of a network formed by the interstices between gel structures, termed “pores,” through which the negatively charged DNA molecules are forced to migrate by the application of an electric field. The two models commonly employed for steady-field DNA gel electrophoresis, the Ogston and reptation models, are both based on this assumption.

Ogston Model
The Ogston model (Chrambach and Rodbard, 1971; Rodbard and Chrambach, 1970) describes the gel as a random network of fibers having some average pore radius. A DNA molecule is assumed to migrate through this network as a spherical random coil, diffusing laterally until it encounters a pore large enough to allow its passage. DNA electrophoretic mobility is assumed to be proportional to the volume fraction of gel pores that will permit its passage. The Ogston model is successful in fitting experimental data for small DNA fragments in the limit of low electric fields (Viovy et al., 1992). However, DNA with radii of gyration much larger than the average gel pore radius still migrate rapidly through the gel during electrophoresis, implying that the assumptions inherent in the Ogston model are invalid for larger DNA. Tietz and Chrambach developed an “extended Ogston model” (Tietz, 1988) to account for the behavior of larger DNA fragments. “Local retardation coefficients” for large DNA are determined, which depart from the original Ogston model by allowing the gel concentration to affect the equivalent radius of the DNA and the gel fiber radius.

Reptation and Biased Reptation Model
Experimentally, DNA smaller than ~40 kbp undergo steady-field size separation in agarose; larger DNA migrates as a single band and can only be separated by pulsed electric fields (Fangman, 1978; Schwartz and Cantor, 1984). The reptation model was developed to explain these results (Lumpkin et al., 1985). It is assumed that spherically coiled DNA molecules too large to fit through a pore undeformed will migrate head first, snake-like, through “tubes” formed by the pore network of the gel. This model has the advan-

Figure 2. Separation by capillary electrophoresis of a mixture of λ-HindIII and ΦX174-HaeIII restriction fragments (in nonstoichiometric mixture) (a) in 0.15% (w/w) HEC (90,000–105,000 g/mol). The far left peak corresponds to a neutral marker (mesityl oxide), used to determine the velocity of electroosmotic flow in the capillary. Peak identification: 1 = 23,130 bp, 2 = 9416 bp, 3 = 6557 bp, 4 = 4361 bp, 5 = 2322 bp, 6 = 2027 bp, 7 = 1353 bp, 8 = 1078 bp, 9 = 872 bp, 10 = 603 bp, 11 = 310 bp, 12 = 281 bp, 13 = 271 bp, 14 = 234 bp, 15 = 194 bp, 16 = 118 + 72 bp. (b) In 0.00125% (w/w) HEC (90,000–105,000 g/mol). The far left peak corresponds to a neutral marker (mesityl oxide), while the second peak at left is an impurity present in the DNA sample. Peak identification: 1 = 23,130 bp, 2 = 9416 bp, 3 = 6557 bp, 4 = 4361 bp, 5 = 2322 bp, 6 = 2027 bp, 7 = 1353 bp, 8 = 1078 bp, 9 = 872 bp, 10 = 603 bp, 11 = 310 bp, 12 = 281 bp, 13 = 271 bp, 14 = 234 bp, 15 = 194 bp, 16 = 118 + 72 bp. The far left peak corresponds to a neutral marker (mesityl oxide), while the second peak at left is an impurity present in the DNA sample. Peak identification: 1 = 23,130 bp, 2 = 9416 bp, 3 = 6557 bp, 4 = 4361 bp, 5 = 2322 bp, 6 = 2027 bp, 7 = 1353 + 1078 + 872 + 603 bp, 8 = 310 + 281 + 271 + 234 + 194 + 118 + 72 bp.
tage of providing a theoretical explanation for the experimentally observed regime in which electrophoretic mobility is inversely related to DNA size. The biased reptation model (Slater and Noolandi, 1985, 1989) modifies the basic reptation model to explain the behavior of DNA at high fields and/or for DNA larger than 40 kbp. It is believed that under these conditions field-induced DNA orientation extends the stretching periods of the DNA fragments, strongly biasing the random walk in the forward direction so that DNA is stretched to a rod-like conformation. Since all DNA molecules in this rod-like conformation no longer take a tortuous path through the gel, the size-based separation ability of the gel is lost for DNA larger than 40 kbp.

While the Ogston model envisions DNA as a spherical random coil, and the reptation model treats it as an extended, snake-like molecule, both assume that it moves through topologically static “pores” having a certain average radius. H.-J. Bode (1979) has questioned this static concept of a gel, particularly in the case of polyacrylamide, a vinyl polymer which would be expected to form a highly flexible and easily deformable network. Bode showed that experimental results from polyacrylamide gel electrophoresis of macro-ions, interpreted earlier as substantiation of the “rigid-pore” model of a gel, could be equally well explained by a viscosity model based on the assumption that gel structures function as obstacles which are moved aside by the electrokinetic pressure of the migrating macroions.

Application of Gel Electrophoresis Models to Capillary Electrophoresis in Polymer Solutions

Although it was originally thought that capillary-based separations of DNA would be impossible without the sieving effect of a high-density gel, it was later shown that solutions of uncrosslinked polymers are capable of separating DNA (Heiger et al., 1990). The use of uncrosslinked polymers for the separation of macroions was pioneered by Bode (Bode, 1976) and later applied to capillary electrophoresis of DNA by Zhu et al. (Zhu et al., 1989). The mechanism of DNA separation in uncrosslinked polymer solutions has been a matter of debate. Some researchers assert that the mechanism is essentially the same as that in traditional slab gel electrophoresis, i.e., that transient “pores” are formed in the polymer solutions (Grossman and Soane, 1991; Pulyaeva et al., 1992), while others attribute separation to the attraction and interaction of DNA fragments with the polymers in the buffer, without specifying these interactions (Chin and Colburn, 1989; Strege and Lagu, 1991). A version of the biased reptation model, modified to include constraint release, has been proposed by Viovy and Duke (Viovy and Duke, 1993; Viovy et al., 1991) to account for the dynamic nature of the uncrosslinked polymer network. This model includes tube dimension fluctuations due to the movement of the polymer fibers. Grossman and Soane (1991) proposed that, in order for uncrosslinked polymers to be effective for DNA separation, they must be entangled and that the physical similarity of this entangled network to the pore network of a gel allows size-dependent separation of DNA. A polymer solution is entangled if it is well above the overlap threshold concentration (Φ*) (de Gennes, 1979), i.e., the concentration at which the polymer chains begin to interact strongly in solution. This concentration is determined from a log-log plot of solution viscosity vs. polymer concentration, as evidenced by a large increase in slope upon the formation of an entangled network in solution (Barnes et al., 1989).

RESULTS

Dilute and Ultradilute Solution Separation of DNA

We find, however, that excellent DNA separations in HEC solutions are possible at extremely dilute concentrations, far below the HEC overlap threshold (Barron et al., 1993). Figure 2a depicts the separation by capillary electrophoresis of a mixture of ΨX174-HaeIII and λ-HindIII DNA restriction fragments, ranging between 72 bp and 23.1 kbp, in a 0.15% (w/w) HEC solution. This represents less than half of the measured HEC overlap threshold concentration (Φ* ~ 0.37% (w/w)) for HEC (Mn = 90,000–105,000 g/mol). Figure 2b shows the separation of the same restriction digest in a 0.025% (w/w) HEC solution. At this concentration, resolution is lost for DNA smaller than 600 bp but retained for larger restriction fragments. Even at concentrations as low as 0.00125% (w/w), (12 parts per million), resolution of larger DNA fragments is achieved (see Fig. 2c). Resolution is only completely lost when HEC concentration is reduced below 1.5 parts per million.

Figure 3a gives a plot of DNA electrophoretic mobility (μ) as a function of HEC concentration for this mixture of ΨX174-HaeIII and λ-HindIII restriction fragments. For fragments larger than 2 kbp, there is a distinct concave curvature at low HEC concentrations. Although the measured overlap threshold concentration of the HEC is ~0.37% (w/w), no distinguishable changes in the behavior of the electrophoretic mobilities occur at this concentration. Figure 3b shows the ultradilute solution data on an expanded scale; the transition from the equal electrophoretic mobilities of all DNA fragments in free solution to size-dependent electrophoretic mobilities is seen to occur at ultradilute HEC concentrations. For smaller HEC (Mn = 24,000–27,000 g/mol), we find that dilute separations of large DNA (>2 kbp) is not possible, though DNA smaller than 2 kbp is separated at concentrations well below the entanglement threshold (Φ* ~ 1.80% (w/w)) (Barron et al., 1994). At concentrations two orders of magnitude below the overlap threshold, HEC chains remain relatively isolated in solution. Although chains may collide and interact transiently, HEC does not form an entangled network. Nothing resembling the “tubes” or “pores” which are assumed to exist in the reptation and Ogston models would be expected to exist. Yet, DNA larger than 2 kbp can be readily separ-
Figure 3. Plot of DNA electrophoretic mobility vs. HEC concentration (HEC 90,000–105,000 g/mol) for DNA restriction fragments ranging from 72 bp to 23,130 bp in length. (a) Normal scale. (b) Plotted on an expanded scale to show detail at ultradilute HEC concentrations. Data points at each HEC concentration are the average of 3–5 individual determinations. Average run-to-run variation in calculated electrophoretic mobilities: ±0.46%. The entanglement threshold for this HEC is shown (\(\Phi^* = 0.37\%\) (w/w)).

rated at these concentrations. This suggests that the mechanism of DNA separation in dilute, uncrosslinked polymer solutions must be quite different from that postulated for gels. In an extremely dilute uncrosslinked polymer solution, no obstacle is permanent on the time scale of DNA motion (Bae and Soane, 1993; Barron et al., 1993). Thus, the controlling factor in DNA electrophoretic mobility may be the local resistance of polymer chains to dislocation and deformation, which would depend on the relative sizes of the DNA and the polymer chains, as well as other polymer
properties such as stiffness, hydrophilicity, and polydispersity.

**Coated and Uncoated Capillaries**

Since our demonstration of the separation of DNA fragments at concentrations significantly below the entanglement threshold was shown in uncoated fused silica capillaries, we considered the possibility that adsorption of DNA to the wall or some other wall effect might play some role in the separation. To investigate the importance of the wall, we compared DNA separations carried out in polyacrylamide-coated and uncoated capillaries using HEC polymer solutions. The polyacrylamide coating prevents electroosmotic flow in the capillary by essentially having an infinite viscosity in the polyacrylamide coating layer. The surface charge of the capillary is not completely masked (Hjertén, 1985). We found that the mobilities of the DNA fragments were practically equal at all concentrations, and the shapes of the mobility vs. DNA size curves were the same in coated and uncoated capillaries, as shown in Figure 4 (Barron et al., 1995). We therefore conclude that the separation of DNA in dilute polymer solutions is only a function of polymer–DNA interactions. An interesting observation is that while the electrophoretic mobilities of DNA fragments are equivalent in coated and uncoated capillaries, the presence of electroosmotic flow in uncoated capillaries effectively increases the residence time of the DNA in the capillary and allows for better temporal resolution for the same length of capillary.

The electrophoretic separation of DNA in dilute and ultra-dilute polymer solutions suggest that a mechanism of separation different from those postulated by the Ogston and reptation models exists in capillary electrophoresis. To account for the observations that separation only occurs in solutions containing polymers and that DNA separations are equivalent in coated and uncoated capillaries, any proposed model must be based on interactions between the polymer and DNA molecules and be based on knowledge of polymer and DNA properties. Any model must also explain the nonlinear size dependence of these interactions that allow for separation to occur. HEC is a linear, uncharged cellulose derivative, having bulky, ethylene oxide side chains terminating in hydroxyl groups. These hydrophilic side groups force HEC chains into an extended, stiff conformation, as evidenced by its Porod–Kratky persistence length of 83 Å (roughly 10 times that of a flexible, random-coil polymer) (Brown, 1961). Double-stranded DNA is even more stiff and extended in solution, with a Porod–Kratky persistence length of 450 Å in 0.2 M buffer (Cantor and Schimmel, 1980). At comparable concentrations, stiff, extended polymers exhibit the effects of entanglement coupling much

![Figure 4](image-url)

*Figure 4.* Log-log plot comparing the electrophoretic mobilities of λ-HindIII and ΦX174-HaeIII restriction fragments as a function of DNA size in polyacrylamide-coated and uncoated capillaries. The mobilities in coated and uncoated capillaries are essentially equal at all three HEC concentrations of 0.00125%, 0.025%, and 0.15% (2/2) HEC (90,000–105,000 g/mol); the shapes of the mobility vs. DNA size curves are also the same. We have corrected for the difference in run temperature for uncoated and coated capillaries.
more strongly than flexible, random-coil polymers (Bueche, 1962).

DISCUSSION

Transient Entanglement Coupling

We propose a mechanism of DNA separation based upon transient entanglement coupling (Barron et al., 1994). When DNA molecules encounter isolated HEC molecules in solution, it is likely that they undergo transient entanglement coupling with the polymer molecules. Hence, DNA molecules are forced to drag the uncharged HEC molecules along with them during electrophoresis, resulting in a decrease of DNA electrophoretic mobility. Larger DNA molecules have a higher probability of encountering and entangling with HEC molecules. Figure 5 is a schematic illustration of DNA motion in a dilute HEC solution, showing the relative sizes of large DNA (9461 bp) and small DNA (118 bp) compared to HEC. It has been demonstrated theoretically by Bueche (1962) that the apparent molecular friction factor of a polymer in solution is much increased by entanglement coupling with other polymers. Therefore, this type of DNA/HEC entanglement coupling interaction could cause a size-dependent increase in the apparent molecular frictional coefficients of DNA molecules during electrophoresis. One of the advantages of this model for the mechanism of DNA separation is that it requires no theoretical constructs such as "pores" or "tubes." Recently, Morris and co-workers used epifluorescence microscopy to show that yeast chromosomal DNA (225 kbp–1.9 Mbp) electrophoresing through polymer solutions both above and below the entanglement threshold underwent conformational changes from compact to U-shaped conformations, qualitatively similar to the motions in gels (Shi et al., 1995). The existence of the U-shaped conformations, especially at dilute concentrations, provide evidence that the DNA is entangling with polymer molecules.

The importance of polymer properties and the relative sizes of the HEC and DNA molecules is shown by comparing electrophoretic separation in small and large HEC (\(M_n\) values of 27,000 and 105,000 g/mol). Unlike the large HEC, the solutions of the smaller HEC cannot separate DNA larger than 600 bp very well even at low concentrations (Barron et al., 1994). DNA larger than 1353 bp does not separate in any concentration of the smaller HEC, which implies that large HEC molecules are required to separate large DNA. It is likely that if the HEC molecules are much smaller than the DNA molecules, they may be easily displaced by the larger, stiffer DNA restriction fragments, and there would not be an increase in the polymer–DNA interactions with increasing DNA size. In this case, there would be an upper size limit of DNA for which transient entanglement coupling with small HEC molecules would be able to introduce a size-dependence to the molecular friction factor of larger DNA, as is observed experimentally. It is not known what effects the polydispersity of the HEC solutions are on the separation in dilute solutions, but good separations were obtained with very polydisperse HEC (\(M_n/M_M = 12.5\) for \(M_n = 105,000\) g/mol).

Effect of Polymer Concentration on DNA Separation

Using the data displayed in Figure 3a, we may determine the optimum HEC concentration for the separation of each set of two adjacent DNA peaks. This provides a "window of separation," i.e., the HEC concentration range which is appropriate for a given range of DNA sizes. This is accomplished by calculating the difference in DNA electrophoretic mobilities (\(\Delta \mu\)) for each set of adjacent peaks as a function HEC concentration. Figure 6a is a plot of \(\Delta \mu\) vs. HEC concentration for all of those DNA restriction fragments larger than 1353 bp. Clearly, the optimum \(\Delta \mu\) for these larger fragments is obtained at quite low HEC concentrations, in the range 0.03–0.08% (w/w) HEC. Figure 6b is the same plot for those DNA restriction fragments in the range 603–1353 bp. It can be seen that smaller DNA requires much higher HEC concentrations to effect good size separation. For those DNA fragments smaller than 310 bp, the optimum HEC concentration was not yet reached at 0.55% (w/w), as shown in Figure 6c.
Figure 6. Plot of the difference in electrophoretic mobilities ($\Delta \mu$) between adjacent DNA peaks as a function of HEC concentration (HEC 90,000–105,000 g/mol): (a) for DNA ranging in size from 1353 to 23,130 bp; (b) for DNA ranging in size from 310 to 1353 bp; and (c) for DNA ranging in size from 234 to 310 bp. The $\Delta \mu$ was calculated from the data plotted in Fig. 2a. These plots allow us to determine the optimum HEC concentration to separate DNA as a function of DNA size. For the large DNA (1353–23,130 bp), the optimum HEC concentration lies between 0.03 and 0.08% (w/w). For DNA 310–1353 bp, the optimum lies between 0.10 and 0.45% (w/w), while for smaller DNA the optimum HEC concentration is greater than 0.55% (w/w). The entanglement threshold for this HEC is shown [$\Phi^* = 0.37%$ (w/w)].

However, at concentrations of HEC (MW 90,000–105,000) greater than 0.45% (w/w), peaks for DNA larger than 872 bp begin to merge, and separation is lost as concentration is increased. This may be because at high concentrations, large DNA can no longer move easily through the solution as a random coil, as free space between HEC chains vanishes. The DNA is then likely to undergo a conformation change and become elongated in the field direc-
tion, such as is postulated to occur in the reptation model. However, the easy dislocation of the uncrosslinked HEC chains, in comparison to the semirigid structures of a gel, may effectively place the reptating DNA in a rapidly dilating "tube" which constantly expands and changes, allowing DNA to pass. When DNA migrates in this manner, the dependence of its frictional properties on molecular length would be decreased (Barron et al., 1993).

CONCLUSIONS
Our observations of DNA separations in ultradilute solutions of hydroxyethyl cellulose suggests that a new approach must be taken to understand DNA electrophoresis in such uncrosslinked polymer solutions and that the models used for traditional gel electrophoresis are not appropriate. Based upon a transient entanglement coupling model for DNA electrophoresis in dilute solutions, it is not clear what will be the upper size limit for DNA separations by capillary electrophoresis in a constant field; this remains to be explored. At field strengths as high as those used in our capillary electrophoresis experiments (265 V/cm) DNA as large as 23.1 kbp cannot be separated in an agarose slab gel under a steady electric field. Pulsed fields can also be applied to capillaries for the separation of DNA by capillary electrophoresis in dilute polymer solutions. Whether steady or pulsed fields are applied to capillaries filled with dilute polymer solutions, we anticipate a dramatic speed increase in DNA restriction mapping separations of large DNA using this technique.

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