Single Molecule Observation of DNA Electrophoresis in Pluronic F127

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Single molecule fluorescence microscopy is used to follow the motion of long DNA molecules undergoing electrophoresis in Pluronic gels. We find that for low fields most DNA molecules follow tortuous paths through the gels, at an angle up to 90° from the field direction, while some molecules find paths along the field lines. In high fields, virtually all of the DNA molecules follow the field lines. In many cases, the molecules travel as compact coils with optically discernible radii smaller than in free solution. In other cases, the molecules extend and contract or travel in an extended configuration.

Introduction

Electrophoresis is an essential and ubiquitous tool in the biochemistry and molecular biology arsenal, allowing separation of nucleic acids, proteins, and other relevant molecules by size or conformation. In 1997, two groups reported the use of a triblock copolymer known as Pluronic F127 as a medium for the separation of various nucleic acids.[1–6] Rill et al. performed gel electrophoresis (GE) using a vertical glass tubing filled with solutions of Pluronic F127 liquid crystals (18−30%), and successfully separated DNA fragments ranging from 123 bp (base pair) up to about 2.5 kbp. Also, both Rill et al.[2] and Wu et al.[1] inserted the Pluronic solution into microcapillaries in the cold room, and performed capillary electrophoresis (CE) separations of both single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) with fine resolution. Therefore, both GE and CE results surprisingly showed that DNA fragments with a wide range of contour length were capable of passing through a nanoscale packed structure.

Pluronic F127 is a triblock copolymer that at high enough concentration forms a close-packed medium consisting of spherical micelles (18 nm in diameter) in aqueous solution. The nanoscale micelles completely fill space with a locally ordered lattice.[1,7,8] The Pluronic polymer solution is a liquid below 17 °C, but it becomes a solid-like gel at room temperature (see the phase diagram in refs 4 and 9). Pluronic F127 has a chemical form of $PEO_{106}PPO_{70}PEO_{106}$, where PEO is polyethylene oxide and PPO is polypropylene oxide. The difference in the water affinity of PEO and PPO depends on temperature. The difference is less at lower temperatures, and both subunits are generally hydrophilic and soluble in water. At room temperature, however, PPO subunits become preferentially hydrophobic and more insoluble than PEO subunits. As the temperature increases, the PPO block's hydrophobicity increases, so that PPO subunits attract each other but repel water, being screened from water by the PEO subunits. PPO blocks overlap, surrounded by the hydrophilic ends in order to form micelles at room temperature (higher than 17 °C).[10,11] As the concentration increases (>20%), the micelles self-organize into a close-packed structure that does not flow under gravitational potentials, thus acting like a solid.

To better understand DNA electrophoretic migration through the Pluronic gel, we focus on the following question. How does the DNA molecule move through the close-packed micelles? Previous studies suggest two different migration modes. Rill et al.[2] suggested that DNA molecules migrate through interstitial spaces between neighboring micelles or spaces unoccupied by flexible brushes (PEO chains) at spherical edges of micelles, and possibly distort the brushes to make a path through the micelle structure. On the other hand, Åkerman et al.[12,13] discussed the mechanism of migration and showed via linear dichroism (LD) measurements that DNA fragments undergoing separation oriented with the helix axis preferentially perpendicular to the electrophoresis direction for electric fields lower than 11 V/cm. This was interpreted as evidence of DNA migration along grain boundaries in the micellar lattice. While the two studies contributed to an understanding of DNA molecule movement in Pluronic gel, they do not provide direct evidence of the mode of migration. We report the motion of the DNA molecules through the Pluronic medium directly using a fluorescence microscope. Our study shows directly the molecular paths of DNA molecules and also illuminates factors that influence the paths.

Materials and Methods

Materials. Pluronic F127 was purchased from BASF (Mount Olive, NJ). All buffer reagents, Tris, boric acid, and EDTA, were obtained from Fisher Scientific (Pittsburgh, PA). All solutions were prepared in ultrapure water from a Milli-Q Plus purification system (Millipore, Bedford, MA). Pluronic polymers were dissolved into 1× TBE buffer (89 mM Tris, 89 mM boric acid, and 2 mM EDTA in water) in a cold room at 5 °C for a week to make a 30% (w/v) concentration of stock solution. The stock solution was diluted with additional buffer solution to obtain the final desired concentrations (18, 20, 22, 24, 26, and 28%). Pluronic solutions were stored for at least a week in a refrigerator to allow bubbles in the containers to diffuse out. Bacteria phage λ-DNA (48 kbp, Sigma-Aldrich, St. Louis, MO) and T4-DNA (166 kbp, Nippon Gene, Tokyo, Japan) were fluorescently labeled with SYTOX Orange from Molecular Probes (Eugene, OR). The SYTOX Orange dye has excitation absorption and fluorescence emission maxima of 547 and 570 nm, respectively. DNA fragments were prestained with SYTOX Orange at a 5:1 ratio of base pairs to dye molecules before loading. This was recommended by the manufacturer to result in a constant staining ratio of dye molecules per base pair. Upon binding to nucleic acids, DNA−dye complexes exhibit about a

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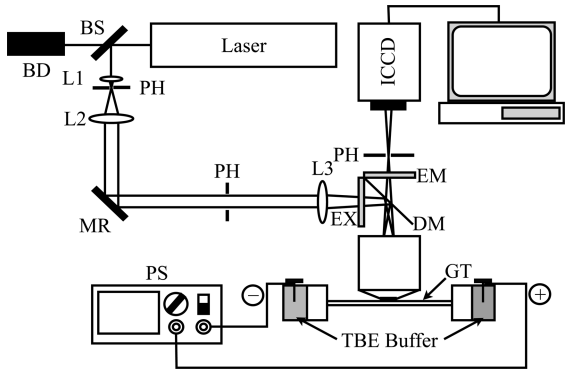


Figure 1. Schematic diagram of the laser-induced fluorescence electrophoresis setup: BS, beamsplitter cube,; MR, mirror; BD, beam dump; HP, half-wave plate; PH, pinhole; L1, lens (f = 16.7 mm); L2, lens (f = 75 mm); L3, lens (f = 175 mm); EX, exciter filter centered at 531 nm with 40 nm bandwidth (green); EM, emitter filter centered at 593 nm with 40 nm of bandwidth (orange); DM, dichroic mirror with 499−555 nm of reflection band (green); ICCD, intensified CCD camera; 100× objective lens (infinity corrected, NA = 1.32); PS, power supply; GT, square glass tubing (ID = 0.9 mm × 0.9 mm × 75 mm).

450-fold fluorescence enhancement over the background of free dye molecules of the same concentration.

Preparation of Tube Gel Electrophoresis. DNA molecules were electrophoresed in a square glass tube purchased from Fiber Optic Center Inc. (New Bedford, MA). The tube was cut to a length of 75 mm using a diamond tip pencil and precooled in a refrigerator before use. The inner dimensions of the tube were 0.9 mm × 0.9 mm, and the thickness of the tube was 0.1 mm. Since capillary forces were insufficient to fill the tube with the high viscosity Pluronic solution, a glass syringe was used to inject the solution. With a steady force, the Pluronic solution was injected to completely and uniformly fill two-thirds of the tube from one end. The tube was then brought up to room temperature, and the Pluronic solution gelled within a few minutes. Prior to loading DNA, the prestained DNA solution was mixed with bromophenol blue that is conventionally used as a gel loading solution (Sigma-Aldrich, St. Louis, MO), at a mixing ratio (v/v) of 5:1. About 1 μL of the DNA solution was added to the two-thirds-full tube from the empty end. Cold Pluronic solution was injected carefully to fill the rest of the tube. Thus, a single blue band of DNA solution could be seen in the middle of the tube, and the blue band was about 1 mm in thickness. The tube was slightly overfilled with Pluronic solution to avoid air bubbles trapped at both ends.

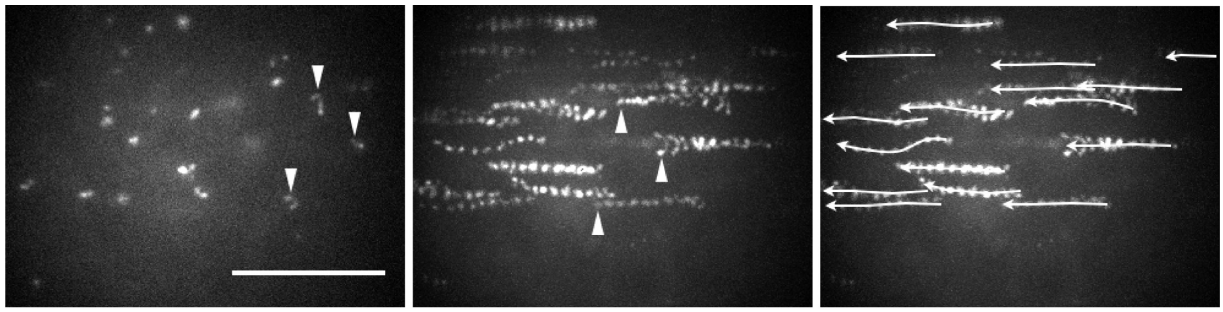
Figure 1 shows a schematic of the experimental setup. The tube was placed horizontally, bridging two buffer trays. A Pluronic gel (30%) block was formed in the inner half of each buffer tray to prevent gel in the tube from dissolving into the buffer solution. 1× TBE buffer solutions filled the outer parts, and Platinum wires were glued at the edges. The distance between the wires was 10 cm.

Room temperature (25 °C) electrophoresis was carried out with applied voltages ranging from 20 V up to 400 V across the 10 cm. Prior to measurements, prerunning at 5 V/cm for between 30 min and an hour was performed to drive the DNA molecules from the blue band into the gel medium. During the experiments, some of the 30% Pluronic gel blocks dissolved, but never completely to the square tube. Hydrogen and oxygen gas bubbled from the appropriate electrodes in the buffer solutions due to electrolysis. At the low currents used in the experiments (ranging from 10 to 450 μA), the rate of hydrolysis at the electrodes was very low and the temperature change of the buffer was less than 2 °C in all cases. The entire electrophoresis apparatus was mounted on an xyz-axis stage so that the glass tube and the DNA within could be brought into focus with an external microscope. Using a 100× oil immersion microscope objective (PL Fluotar, 100×/1.32, Leica, Germany), the inner surface of the tube was brought into focus. By moving the tube closer to the objective by at least 100 μm or deeper from the inner surface, fluorescing DNA molecules were imaged.

Fluorescence Microscopy. The laser-induced fluorescence microscope was home-built as shown in Figure 1. An argon ion laser (Innova 70, Coherent, Inc. Santa Clara, CA) provides a high-intensity source of monochromatic and linearly polarized light (λ = 514.5 nm). The polarization direction can be changed by rotating a quartz half-wave plate (HP in Figure 1) placed between the argon laser and a polarizing beam splitter cube (BS). The BS splits the polarized beam into transmitted and reflected beams with S polarization (perpendicular component) and P polarization (parallel component), respectively. The intensity ratio depends on the angle between HP and the input polarization. The transmitted beam with the majority of the laser power is dumped into the beam dump (BD). The reflected beam is redirected with the BS into a 5× microscope objective L1 that focuses through a 25 μm diameter pinhole. L1 and the pinhole constitute a spatial filter passing only the TEM 00 mode. Lens L2 collimates the beam with a diameter of 6 mm. Mirror M directs the beam through an aperture and then to a lens L3 (f = 175 mm) which illuminates the 100× oil immersion microscope objective through a cube filter with an expanding beam. The cube filter (CY3-4040B, Semrock, Inc. Rochester, NY) consists of a dichroic mirror in the middle and two band-pass filters corresponding to the excitation and emission spectra of SYTOX Orange stained on DNA molecules.

For fluorescence imaging, the ICCD camera (Cascade 650, Roper Scientific, Inc., Tucson, AZ) was used, since it is highly sensitive to the low intensity of incident light and transfers data from the image sensor frame by frame to on-chip storage at high speed. Because there is no mechanical shutter in the camera, the sensor detects incident light at each pixel for an exposure time controlled by a LabVIEW program. The exposure time is manually adjusted depending on experimental conditions such as the mobility of DNA, turbidity of medium, photobleaching of dye molecules, and intensity of illumination. Frame rate is determined by the sum of the exposure time and the readout time for the frame transfer from the sensor to the storage. For example, if the exposure time is 10 ms and the readout time is 34.8 ms, the frame rate is 22.3 fps (1 frame/44.8 ms). In these experiments, frame rates were adjusted from 10 to 0.25 fps. The camera has a spatial resolution of 653 × 492 pixels at a 7.4 μm pixel pitch. A 2 × 2 binning (combining neighboring pixels) was performed by combining the light from all four pixels into one to enhance the fluorescent signal from the sample, but the spatial resolution of the sample is reduced to 326 × 246 pixels. Using a stage micrometer (10 μm/division) for calibration, the image represents 26.1 × 19.7 μm with 0.08 μm per pixel.

Image Processing. Stored frames were exported as sequential jpeg-format images, and a LabVIEW program was designed to track the motion of single molecules. This program stores time-sequential positions of a specific spot representing a DNA molecule brightly fluorescing against a dark background frame by frame. Once the positions of a molecule are obtained in sequence, an average velocity of the molecule is calculated using a least-squares fitting algorithm. Position x is defined as the



(a) (b) (c)

Figure 2. Fluorescently labeled T4-DNA molecules undergoing electrophoresis at $E = 2$ V/cm in a 20% gel of Pluronic F-127: (a) A snapshot showing several DNA molecules in relatively compact bundles. (b) The z-projection image (see Materials and Methods) for a sequence of 10 images selected at equal intervals of 2.19 s. The first image in this sequence is subfigure a. (c) Lines connect presumed centers of separate DNA molecules seen in the z-projection image. The scale bar in Figure 2a represents 10 μm.

center-of-mass of the molecule along the x -axis. A set of m data points consists of experimentally measured values (t_1, x_1), (t_2, x_2), ... , (t_m, x_m) at a time $t_m = m\tau$, where m is the number of frames and τ is the time difference between adjacent frames. The velocity of the DNA molecule in the field direction was determined by best-fitting a straight line to the plot of x vs t and by calculating the slope from the method of least squares, i.e.,

$$v_x = \sum_{i=1}^{m} (t_i - \bar{t})(x_i - \bar{x}) / \sum_{i=1}^{m} (t_i - \bar{t})^2$$

where $\bar{x} = \sum_{i=1}^{m} x_i/m$ and $\bar{t} = \sum_{i=1}^{m} t_i/m$. An averaged velocity ($v_x$) was obtained from sampling v_x of 5−10 different molecules that were representative of each experimental condition.

The fluorescent spots of λ- or T4-DNA move from right to left in a series of frames. Overlapping a series of frames into a single image is convenient for visualizing and tracing the moving molecules. The overlapped images were automatically generated with ImageJ software (W.S.Ransband, ImageJ, U.S. National Institutes of Health, Bethesda, MD, http://rsweb.nih.gov/ij). The ImageJ's z-projection function with an option of "maximum intensity" creates a single output image consisting of many stacked images each of whose pixels contains the maximum value over all images in the stack at the particular pixel location. For example, if three images are stacked, the z-projection function selects the brightest spot by comparing among three spots at the specific pixel and the brightest spot is placed on the projected image. The spot that is white in color has an intensity value of 255, but the spot that is black is at 0. As we will later demonstrate, the stacked images can show DNA movements in a Pluronic gel as a series of spots in a row.

Results

Long DNA (λ (48 kbp) and T4 (166 kbp)) were electrophoresed in a supporting medium of Pluronic gel at room temperature (∼25 °C). Results were reported for several concentrations of Pluronic solution (ranging from 18 to 30%) and for several electric field strengths (ranging from 2.0 to 40.0 V/cm).

Pluronic gel acted like a viscous liquid for the lowest concentration studied (18%) but like a solid for the higher concentrations. The 18% gel flowed upon being tilted in a gravitational field. The 20% gel flowed slowly, over several hours, in response to a tilt. The higher concentrations did not flow for the experimental time scales. These results are entirely consistent with rheological measurements on a similar range of concentrations and phase diagrams.[9–11]

Before turning on the voltage source, fluorescing DNA molecules were observed in the bromophenyl blue marked band in the middle of the tube with the microscope. The fluorescing DNA molecules came into focus and left the field of view on a subsecond time scale. They moved rapidly up and down and side to side, apparently in random motion.

Upon turning on the voltage, the DNA molecules were driven into the Pluronic gel from the loading solution. Prerunning was carried out at 5.0 V/cm for 30 min (Pluronic concentration ≤24%) or 1 h (Pluronic concentration ≥26%). During this time, the bromophenyl blue electrophoresed away from the DNA molecules. As soon as the field was turned on, the molecules moved freely toward the positive anode, antiparallel to the field direction. Upon reaching the edge of the Pluronic gel, the molecules stacked and slowly entered the gel. Before prerunning, the DNA spanned about 1 mm in the glass tube, and after prerunning, it spanned about 2 mm. On a microscopic scale, the DNA molecules became separated enough to be individually observable.

Following the prerunning, each sample was run for a significant length of time (30 min typical) at discrete field strengths starting at 2.0 V/cm and increasing up to 40.0 V/cm. The field strengths were increased in steps of 1 V/cm from 2.0 V/cm up to 10.0 V/cm and in steps of 2.0 V/cm from 12.0 V/cm to 40.0 V/cm. Recording began at least 15−20 min after changing the applied field between steps. A complete data set for each concentration of gel typically took several hours, often stretching to 10 or more.

Figures 2 and 3 show migration patterns of fluorescently labeled DNA undergoing electrophoresis in Pluronic copolymer solution in various electric field strengths and Pluronic concentrations. In the images, the DNA molecules move toward the positive electrode to the left (defined as a positive x-direction). Up is defined as the positive y-direction. As shown in the images, the motion of the molecules represents the electrophoretic behavior under the given experimental conditions.

Molecular Path in Pluronic Gel. Figure 2 shows T4-DNA molecules electrophoresing in 20% Pluronic under the influence of an applied electric field (2.0 V/cm). The DNA molecules migrate through the gel and toward the positive electrode. The shape of the DNA molecules appears primarily compact and bright with slight variations in shape (see the down-pointing cursor indicating a semicircle in Figure 2a). As seen in this figure, the average size of the DNA molecules occupies 10−15

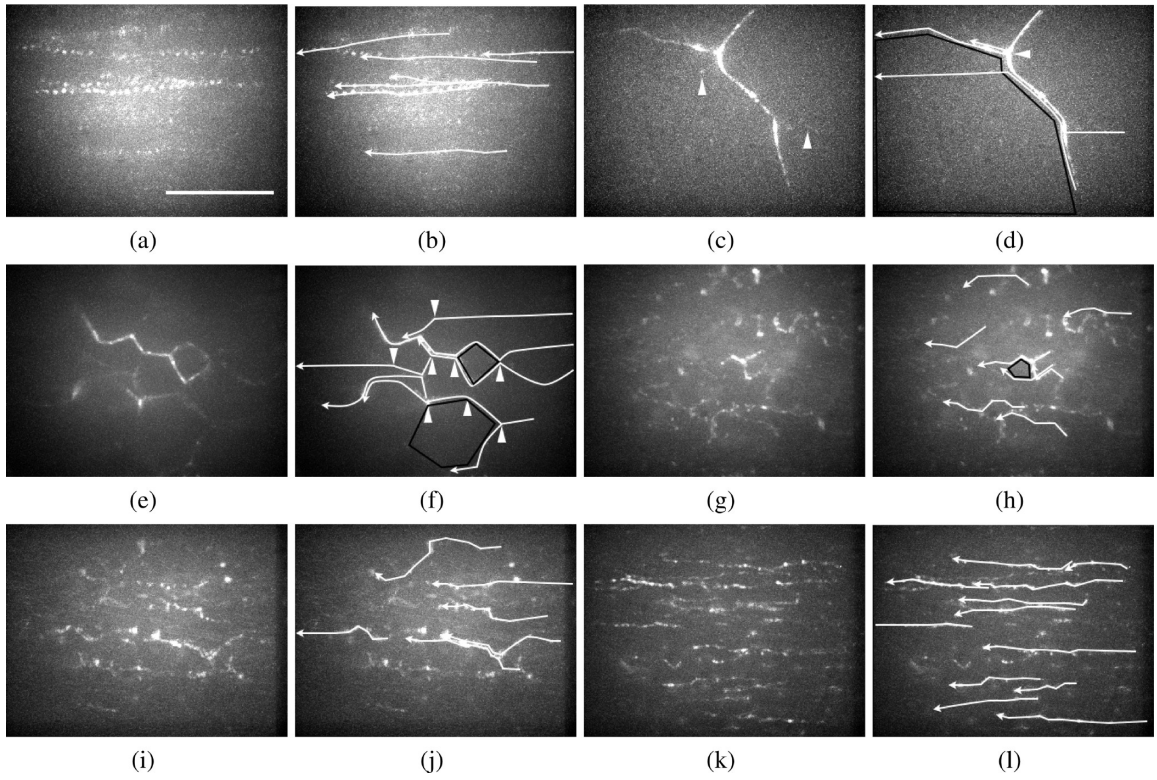


Figure 3. The scale bar in part a represents 10 μm. All images are shown at the same magnification. Each picture represents a 20-image stack highlighting the brightest pixels within the stack (see text). This technique highlights the motion of the fluorescent λ-DNA undergoing electrophoresis. Pictures are repeated in pairs (a−b), (c−d), (e−f), (g−h), (i−j), and (k−l) with the second picture in each pair annotated with white lines indicating the path taken by a DNA molecule during the time represented by the stack. Black lines indicate representative domain boundaries (see text). The electrophoresis parameters shown are (a−b) in 18% Pluronic F-127 at 2 V/cm and 0.37 frames per second (fps); (c−d) in 22% at 2 V/cm and 0.1 fps; (e−f) in 26% at 2 V/cm and 0.1 fps; (g−h) in 30% at 2 V/cm and 0.05 fps; (i−j) in 30% at 4 V/cm and 0.12 fps; (k−l) in 30% at 10 V/cm and 0.5 fps.

pixels square, corresponding to 0.8 × 0.8 to 1.4 × 1.4 μm^2 (0.08 μm per pixel).

While DNA molecules moved through the gel, some remained compact but others changed their conformation to become extended or compressed. A DNA molecule that was initially compact and marked by the right-most down-pointing cursor in Figure 2a became vertically extended to a hook-shaped molecule that is marked by the right-most up-pointing cursor in Figure 2b. On the other hand, two other DNA molecules that were initially hook-shaped (marked by the left down-pointing cursors in Figure 2a) became compact or slightly oriented with the field direction (marked by the two left-most up-pointing cursors of Figure 2b).

Figure 2c shows the same stack as Figure 2b, with arrows superimposed, indicating the path taken by several individual DNA molecules. The length of the arrow thus represents the distance traveled during the 10 images (each separated by 2.19 s).

Field Dependence of Molecular Path in Pluronic Gels. Figure 3 shows the paths of λ-DNA molecules in four concentrations of Pluronic gel and three different electric fields. All images in this figure are the projected images created by stacking 20 sequential frames so that a series of 20 successive spots traces the path of a λ-DNA molecule. White solid arrows in the second and fourth columns were added to aid in following DNA paths shown in the first and third columns. The arrows were, in general, drawn on the traces, but some lines were shifted down or up near the traces to differentiate when more than two lines were stacked or merged at the same locations.

For the same applied electric field and varying concentrations, the paths of DNA molecules in higher concentrations became more tortuous than those in lower concentrations of gel. This is shown by comparing the patterns in Figure 3a for 18%, Figure 3c for 22%, Figure 3e for 26%, and Figure 3g for 30% concentration of Pluronic gel at 2.0 V/cm. Figure 3a and b shows λ-DNA molecules moving through a medium of 18% gel at 2.0 V/cm. Most of the DNA molecules are not stretched or elongated but compact. Their paths are primarily aligned with the field direction and seen unrelated to paths traveled by other DNA molecules. For higher concentrations, individual DNA molecules often stretched out and followed paths created by the earlier passage of a DNA molecule.

Figure 3c and d shows the locally confined paths followed by λ-DNA molecules electrophoresed in a medium of 22% gel. One of the DNA molecules marked by an upright cursor on the right side of Figure 3c moved from right to left along the electric field line (2.0 V/cm). When the DNA molecule arrived at the locally confined tilted path, it became more compact and deformed to fit in the path. The DNA molecule followed along the tilted path almost entirely in the positive y-direction for a short distance. In addition, two paths merge at a point marked by a left-pointing cursor where one path approaches from below and the other path comes down from the upper right-hand area. The merged path continues to the left. The DNA became compressed at the merging point and then elongated along the merged path to move forward.

On these locally confined paths, DNA stretches and shrinks to move forward, which is analogous to the motion of an inchworm; its head moves forward, the body stretches out, and the tail approaches the head, subsequently. Several DNA molecules followed along the locally confined and tilted paths successively. Some DNA molecules were able to escape from the locally

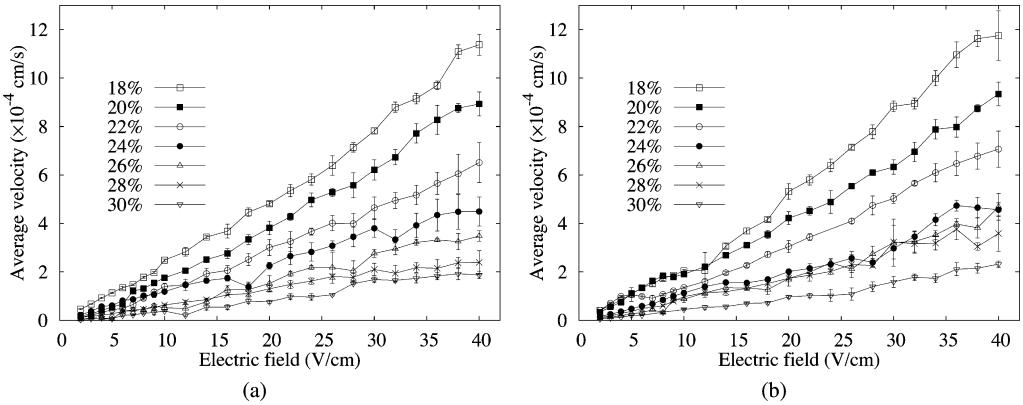


Figure 4. Average velocities of (a) λ-DNA and (b) T4-DNA vs electric fields increasing from 2 to 40 V/cm in various concentrations of Pluronic gel at 18, 20, 22, 24, 26, 28, and 30%.

confined paths. For example, a DNA molecule, marked by an upright cursor on the left side of Figure 3c, emerged from the curved channel to the right. The DNA molecule was compact before emerging and extended after leaving the channel.

With an increased field, the likelihood of emerging from the locally confined paths also increased. The frequency of escaping increased as the field strength increased. For fields ≥8.0 V/cm, it was observed that DNA molecules emerged from several places along locally confined paths at the same time; i.e., DNA molecules did not follow along the channels but moved alone along the field direction. Thus, the channels restricting the paths of DNA molecules disappeared at large field strengths (data not shown). When the field strength was reduced below 8.0 V/cm, on the other hand, the channels came into view at the same place as they were before disappearing. DNA molecules followed along the resurgent paths, again (data not shown).

For further discussion, we refer to the areas where the DNA molecules do not follow channels as domains and the channel locations as domain boundaries. Within the domains, DNA molecules primarily move parallel to the field direction and subsequent DNA molecules seem to move independently of the paths of earlier molecules. On the other hand, when traveling along domain boundaries, subsequent DNA molecules seem to follow identical paths as earlier molecules, even to the extent of being delayed at certain locations. In the domain boundaries, the DNA molecules primarily extend and contract like an inchworm.

Figure 3e shows the results of DNA electrophoresis carried out in 26% gel at 2.0 V/cm. DNA molecules followed along domain boundaries that had well-defined large angle changes. As indicated by upright and down-pointing cursors shown in Figure 3f, DNA molecules move through domains along the field direction and change their direction at boundaries. Some DNA molecules emerged from domain boundaries and traveled through domains. Up-pointing cursors mark places where subsequent DNA molecules followed domain boundaries involving a significant angular change (apparent discontinuity in slope). Figure 3g and h shows DNA molecules in 30% gel at 2.0 V/cm. The domains are smaller and the paths are more tortuous than those in lower concentration gels.

Figure 3g, i, and k shows electrophoresis for increasing fields at a fixed 30% gel concentration. At 2.0 V/cm, DNA molecules mostly followed along the domain boundaries. At 4.0 V/cm shown in Figure 3i, some DNA molecules were more aligned with the field direction. The higher the electric field, the more DNA molecules escaped from the boundaries and crossed the domains. At 10.0 V/cm shown in Figure 3k, DNA molecules mostly moved along the field direction. In detail, the movements of DNA molecules were occasionally interrupted by domain boundaries while they traveled. This interruption appeared when a molecule at a domain boundary hooked with both ends moving away from the boundary independently. The molecule would slow down for a while and suddenly move away from the boundary. As the field strength was increased, these interruptions became weaker. For fields larger than 14.0 V/cm, there was no sign that the DNA molecules followed along domain boundaries: DNA molecules were extended and aligned with the field direction, and DNA molecules moved entirely parallel to the field direction (data not shown).

Figure 4 shows the average velocities of (a) λ-DNA and (b) T4-DNA as the strength of the electric field was increased from 2.0 to 40.0 V/cm. Each velocity shown was obtained by averaging many x positions (pixels converted to distance) versus time (frame number converted to elapsed time) of at least five individual molecules. Shown as error bars are the standard deviations of the averages. The average velocities increased monotonically as the strength of the electric field increased. The velocities for T4-DNA in 30% gel are consistent with those from Svingen and Åkerman,[12,13] though their discrete change in slope was not seen. Although it does not dominate the graph, there is a transition voltage at which the domain boundaries did not influence the path of the DNA and they disappeared. The transition voltage was in the range 8.0−14.0 V/cm, depending on the concentration of the gel.

Discussion

Fluorescently labeled DNA molecules in Pluronic gel are apparently more compact than they are in free buffer solution. The measured diameters of DNA spots are, on average, $d \simeq 0.52 \pm 0.08$ μm (6−7 pixels, measured full width at baseline of the intensity profile of the DNA spots) for λ-DNA molecules in 18% concentration and $d \simeq 1.04 \pm 0.2$ μm (10−15 pixels, measured full width at baseline of the intensity profile of the DNA spots) for T4-DNA molecules in 20% concentration. For the Gaussian chain model of the DNA molecules in free solution, the mean end-to-end distance for λ-DNA is $\bar{R} \simeq 1.28$ μm and for T4-DNA $\bar{R} \simeq 2.38$ μm, and the persistence length is approximately 50 nm.[14] The size of the DNA molecules is more often described by the radius of gyration R_g rather than by $\bar{R}$, where $R_g = \bar{R}/\sqrt{6}$. Thus, the radii of gyration for the λ- and T4-DNA molecules are, theoretically, 0.52 and 0.97 μm, respectively. As a result, the measured sizes of the DNA molecules in Pluronic gel are about half of the theoretical values of ideal chains in free solution. This is even more surprising

considering that the fluorescent labels will, if anything, stiffen the backbone, increasing the persistence length.

DNA coils in free solution. The coiled DNA can collapse in a polymer solution, depending on the interactions between the segments, and between segments and solvent. From experimental observations, a DNA chain shrank in PEO polymer solution at high enough concentrations to less than half of its size in free solution.[15,16] Because of the hydrophilic character of PEO chains, the interaction between DNA segments and water molecules becomes less favorable. This less favorable effect puts DNA into a poor-solvent environment, while the interaction between segments within the DNA chain becomes relatively attractive. The gel used in this project consists of spherical micelles with a hydrophobic core (PPO) surrounded by hydrophilic brushes (PEO), and DNA molecules are external to micelles so that they are in indirect contact with the PEO chains. Thus, DNA molecules apparently collapse in their local PEO environment.

The local structure formed by a Pluronic solution depends on temperature and concentration. At room temperature, the critical micelle concentration (CMC) is quite low. With increasing concentration, the intermicelle distances become small and neighboring micelles interact strongly. At about 20%, the spherical micelles in a gel pack closely enough together that they do not flow. The micelles aggregate and arrange themselves into ordered locally cubic lattices. By analyzing the scattering patterns from small-angle X-ray scattering and small angle neutron scattering measurements, the size of the PEO core ranges from 4 to 5 nm and the center to center distance of neighboring micelles ranges from 17.5 to 20.5 nm, depending on the concentration of the solution.[1,8,17,18]

The micellar order has been observed by X-ray diffraction to exhibit negligible temperature dependence after the transition from fluid to gel.[1] This is also supported by viscoelastic measurements[19] indicating uniform saturated viscosity for temperatures higher than the transition. Chu et al. tested the mobility of DNA versus temperature in capillary electrophoresis in 21.2% Pluronic F127 that showed an increase in mobility with increasing temperature for the range 25−60 °C.[4] A thermocouple measurement of the temperature of our square tube apparatus did not indicate a temperature increase for any experiments. Considering the total volume of electrolyte of about 7.1 cm^3 and the maximum power 150 mW dissipated of 400 V at 450 μA, the calculated rate of temperature increase without equilibration to the surroundings is about 6.1×10^{-3} °C/s. However, the buffer temperature stayed constant for these conditions, indicating the heat loss to surroundings was sufficient to dissipate this power.

The ranges of Pluronic concentrations studied are all well below the concentrations needed for crystalline ordering in a hard sphere solution. In general, growth of single crystals is difficult and multidomain crystals are much more common. Normally, at the phase transition, crystallites nucleate in many planes and grow until they interact with another crystallite. Ordering of Pluronic micelles occurs following the temperature driven reduction in the CMC that occurs at about 18 °C. It is very likely that this self-organizing leads to domains of ordered micelles separated by domain boundaries. The domains will probably have randomly oriented axes. If the tilted paths followed by DNA molecules are domain boundaries in this sense, then the stacked photos in Figure 3 give information about the size and structure of the domains.

In Figure 3d, f, and h, black solid lines are shown that seem representative of domain boundaries. The enclosed area for 22% gel is about 260 μm^2. The enclosed areas for 26% gel are 10 and 30 μm^2, and for 30% gel, the enclosed area is 3 μm^2. Therefore, domain boundaries apparently exist in Pluronic gel and the size of the domain decreases as the gel concentration increases.

If the domain boundaries in Pluronic gel are analogous to domain boundaries in crystalline systems (either atomic or colloidal crystals), the micellar order will be disrupted at the boundaries for a few lattice spacings transverse to the boundary. That corresponds to 100−200 nm at most. This disrupted region may be expected to exert less friction on a DNA molecule passing along it, particularly if the DNA molecule is extended as a reptating polymer. The DNA molecules were observed to travel as 500−1000 nm diameter spots when passing through domains. While traveling along domain boundaries, the motion consisted of an end rapidly extending from a spot, traveling along the domain boundary for a while (10−20 μm typical) and then hesitating while the rest of the molecule caught up. Where extended, the mobility is higher, but they travel in fits and starts with an average velocity quite similar to DNA "spots" moving through domains.

Gel electrophoresis has been conventionally carried out in porous media, i.e., physical gels and entangled polymer solutions made of agarose and polyacrylamide. These porous media form a mesh network that provides a torturous path consisting of a series of pores through which DNA molecules migrate. The pores of agarose or polyacrylamide gels have a broad distribution of sizes on the order of 20−500 nm.[20−22] The mesh size of entangled polymer solutions ranges from 3 to 19 nm.[23] If DNA molecules have a radius of gyration larger than the pore size, they need to deform and stretch out in order to pass through the smaller hole. This migrating behavior has been theoretically well-described in analogy to the motion of a snake (reptation); its body and tail follow along the track of the head.[24−26] Reptation has been experimentally observed using fluorescence microscopy.[27−29]

Unlike in conventional media, we did not observe the reptative behavior of DNA in Pluronic gels; i.e., one end of a DNA molecule moves through the gel medium and the rest of the molecule follows. Instead, we observed that DNA molecules kept their compact shape and randomly deformed or stretched while they passed through. Micelles can be thought of as spherical obstacles opposing the motion of DNA. Although the micelles pack closely and form a crystalline structure, this does leave space; the interstitial space between micelles is estimated at about 2−3 nm for face centered cubic packing of spherical beads of 18 nm in diameter. Compared with a DNA molecule's cross section that is about 2.0 nm, the DNA molecule has a chance to pass through space between micelles. Although the persistence length of DNA is about 50 nm in free solution, it is apparently much shorter in the Pluronic gel due to the poor solvent condition. Finally, it is reasonable to consider that a DNA chain is external to the micelles and it squeezes into interstitial spaces among micelles while it moves.

This research was intended to expand understanding of how the DNA molecules migrate through the Pluronic system. Not only did DNA molecules follow along domain boundaries where micelles are randomly packed and arranged, but they also entered into domains where micelles are close-packed and arranged regularly, depending on the gel concentrations and the field strengths. As the concentration of Pluronic gel increases, the DNA molecules follow along domain boundaries, rather than directly through domains. As the strength of the electric field is increased, the DNA molecules move through domain regions

along the field direction, rather than follow along domain boundaries. At low concentrations (≤20%), DNA molecules mostly formed compact shapes and traveled across the medium along the applied electric field lines. At higher concentrations (≥22%), domain boundaries between crystalline domains appeared in the medium as paths along which DNA molecules traveled. At low electric fields, most DNA molecules followed along the domain boundaries. While the DNA molecules moved along the boundaries, they extended and shrank repeatedly in an inch-worm fashion to move forward. Domain sizes decreased as gel concentrations increased. At high electric fields, DNA molecules were elongated and DNA paths became more oriented with the field direction. In such conditions, the domain boundaries were not significant because most DNA molecules traveled directly through without following along the boundaries. When the electric field increased beyond 10−15 V/cm, most of the DNA molecules moved through the domain, rather than along the boundaries. This research shows directly the molecular paths of DNA molecules and also the factors that influence the paths, i.e., field strength and gel concentration.

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