Mechanism of Electrophoretic Migration of DNA in the Cubic Phase of Pluronic F127 and Water

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Received: May 13, 2003; In Final Form: October 15, 2003

The block copolymer Pluronic F127 and water form a gel-like cubic phase which is useful as sieving media for electrophoretic separation of DNA fragments in capillary electrophoresis because, below a transition temperature of 11 °C, the gel melts to an easily handled liquid solution. Here we study the conformational state and the mode of electrophoretic migration of double stranded DNA in the cubic gel. The DNA remains in B form in the Pluronic F127 gel and functions as a substrate for PCR, and there is no detectable effect on the Pluronic solution−gel transition temperature by the presence of DNA of concentrations up to the overlap concentration. Velocity and coil deformation measurements during electrophoresis show that the mode of DNA migration in this gel is qualitatively different compared to conventional gels. DNA coils are deformed with the helix axis preferentially perpendicular to the field direction, and the electrophoretic mobility varies discontinuously with field strength. At low field strengths (below 11 V/cm in a 30% gel) we find further support for the hypothesis that migration occurs along grain boundaries between crystal domains (Svingen et al. *Langmuir* **2002**, *18*, 8616−8619). This mode of migration is general since it occurs for DNA between 200 and 5400 base pairs (bp) and at gel concentrations between 25 and 30%, and we show that it requires the cubic phase and is not due to the high polymer concentration as such. At about 11 V/cm the electrophoretic mobility of ds DNA increases discontinuously because of reduced friction. We propose that the cause is a reversible perturbation of the cubic structure induced by DNA when migrating at the stronger electric fields.

1. Introduction

The amphiphilic polymer Pluronic F127 forms a cubic phase in aqueous solutions which can be used as a sieving medium in capillary electrophoresis with promising properties regarding easy handling and good size-resolution of DNA fragments.[1,2] Pluronic F127 is a tri-block copolymer with a poly(propylene oxide) core-block and flanking poly(ethylene oxide) blocks[3−5]

$$HO(CH_2CH_2O)_{106}-(CH_2CH(CH_3)O)_{70}-(CH_2CH_2O)_{106}H$$

The polymer changes conformation as the temperature is increased,[6] which leads to a higher degree of hydrophobicity so that micelles form and pack into a gel-like liquid crystal of cubic structure above certain polymer concentrations and temperatures.[3−5] At low enough temperatures, e.g., below 20 °C at a polymer concentration 21% w/w or 11 °C at 30% w/w (Figure 1), the system acts as a free-flowing solution, which is easily filled into a capillary. After raising the temperature, electrophoresis can be performed in the gel state,[1,2] and finally the capillaries are easy to empty since the gelling process is reversible. The latter property also makes Pluronic F127 a promising candidate for preparative applications in electrophoresis since separated components can be recovered by decreasing the temperature or diluting the sample. It is mainly this application we have in mind because the electric field strengths studied here are considerably lower than those used in capillary electrophoresis.

DNA migration in agarose and polyacrylamide gels can now to a large extent be described theoretically[7] for a wide range of DNA sizes. In those hydrogels polymers are cross-linked together, creating a network with aqueous pores having dimensions comparable to typical coil sizes of DNA. If the DNA coil size (radius of gyration R_g) is smaller than the pore radius (R_p), electrophoretic migration occurs mainly in a coiled state with small effect on DNA conformation.[8] By contrast, when the coil radius is large compared to the pore size, the DNA has to "uncoil" in order to be able to migrate, by threading itself with one of the ends seeking out a path between gel fibres. For such long DNA the result is an end-on type of migration (reptation), with the DNA chain being extended along the electric field direction.[8] Migration of long DNA in non-crosslinked polymer solutions in capillary electrophoresis also involves a type of migration where the DNA-helix is oriented along the electric field even well below the polymer overlap concentration.[9]

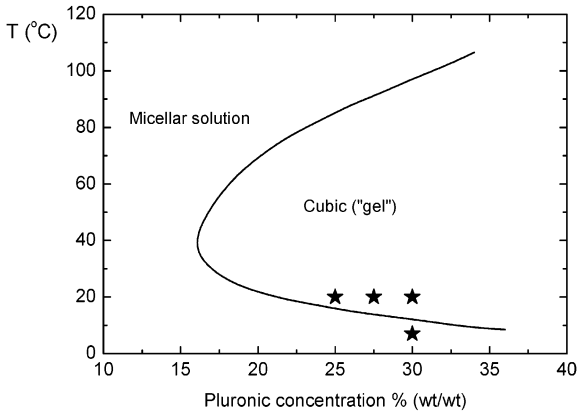

Figure 1. Schematic phase diagram of the Pluronic F127−water system, based on data from ref 1. Stars indicate phase states that have been investigated in this study.

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10.1021/jp035317a CCC: $27.50
Published on Web 02/03/2004

TABLE 1: Coil Parameters for the Used DNA

DNA	L_c[a] (μm)	R_g[b] (nm)
T4 (166 000 bp)	56.4	970
ϕX174 (5386 bp)	1.8	168
1000 bp	0.33	60
236 bp	0.08	20
200 bp	0.07	18

[a] $L_c = 0.34$ nm per base pair. [b] R_g according to wormlike chain model with persistence length 50 nm.

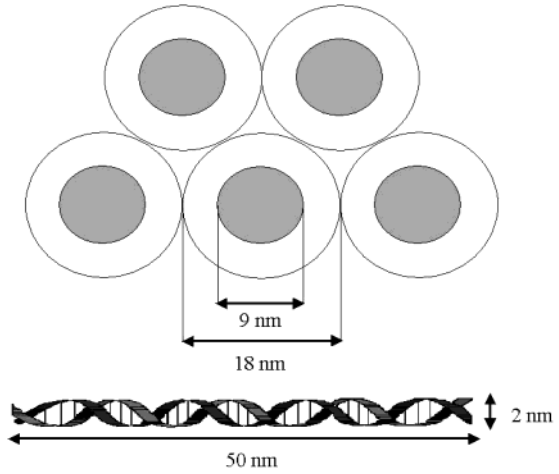


Figure 2. Approximate dimensions of Pluronic F127 micelles in the face-centered cubic phase,[10,11] compared with the helix diameter (2 nm) and persistence length (50 nm) of DNA.

In the liquid crystal structure of Pluronic F127, the characteristic dimensions of the "pores" are smaller than the DNA coils used here (cf. Table 1). The hydrophobic poly(propylene oxide) core of the micelles is approximately 9 nm in diameter, and the poly(ethylene oxide) chains extend in a corona to give a total micelle diameter of 18 nm.[10,11] When micelles pack in a cubic lattice, their dimension results in interstitial spaces of aqueous pores of about 4−9 nm in diameter (Figure 2) depending on whether the poly(ethylene oxide) corona is included or not.[10,11] Migration in these channels has been suggested as a mode of migration for double-stranded DNA,[1,10] and since the DNA-helix is only 2 nm in diameter, end-on motion is indeed possible in principle (Figure 2). However, during electrophoretic migration in a 30% w/w Pluronic F127 gel a 5386 bp DNA exhibited an orientation of the helix axis perpendicular to the field direction,[12] in disagreement with an end-on reptation-type of DNA migration. Although made for a comparatively large DNA size, this observation may explain the findings by Rill et al.[2] and Wu et al.[1] that the experimental velocities of DNA fragments between 872 bp and 1353 bp disagreed with predictions of the reptation model. On the basis of the observations on the 5386 bp DNA in 30% Pluronic F127, we proposed[12] that this DNA molecule does not enter the cubic structure but instead migrates in grain boundaries between crystal domains in the polycrystalline samples. To further test this hypothesis and to be able to compare with earlier velocity measurements on smaller DNA,[1,2] we here extend our studies of the electrophoretic velocity and DNA coil deformation by investigating the effect of DNA size as well as gel concentration on the behavior of DNA during electrophoresis in Pluronic F127.

2. Methods

2.1. Materials. All experiments were performed on double-stranded DNA. The HaeIII digest of ϕX174 DNA from Amersham Pharmacia Biotech Inc. contained fragment sizes 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, and 72 base pairs (bp). A 236 bp DNA fragment produced by PCR was a gift from Jennie Sandström at LightUp Technologies AB. Circular ϕX174 RFII DNA, 5386 bp, was used as received (more than 90% nicked circular form) or linearized with restriction enzyme Eco1471 (both from Fermentas). T4 DNA, 166000 bp, and calf thymus DNA (about 6000 bp) were from Sigma and used without further purification. Electrophoresis buffer was TBE (50 mM in boric acid and 50 mM in Tris, 1.25 mM in EDTA in deionized water, pH 8.2). Pluronic F127 was obtained from BASF as a dry powder and mixed with TBE buffer by stirring at 4 °C, and the solutions were stored at 5 °C for at least 4 days before use. Agarose D-1 Low EEO was from Pronadisa. Metaphor agarose was from BioWittaker Molecular Applications.

2.2. Electrophoresis. The electrophoresis experiments were performed essentially according to procedures described in detail for agarose gels by Jonsson et al.,[13] but modified as detailed here for measurements in Pluronic F127, either in the cubic phase (referred to as the gel) or the liquid micellar phase. A birefringence-free silica cuvette with internal dimensions of 3 × 30 × 100 mm^3 was mounted vertically between two electrophoresis buffer chambers, in which an electric field was generated using platinum electrodes. A 5 °C solution of Pluronic F127 in TBE-buffer was poured into the pre-cooled cell, which was subsequently kept on ice for 2 h in order to ensure a homogeneous solution before the cubic phase was induced. To prevent leaking of the Pluronic F127 solution before gelation, a 30 mm high 1% agarose plug was formed at the bottom of the cuvette. Gel formation was accomplished through an abrupt temperature increase by putting the cuvette in a 45 °C water bath for 15 min. A sample well was then created manually by forming a cavity in the top of the gel with a spatula. The sample volume was 20 μL containing 3 μg ϕX174 DNA-HaeIII digest or 1 μg of either the 236 bp fragment or the ϕX174 DNA, and 3% of the polysaccharide Ficoll in TBE buffer to enhance density for sample loading. Bromphenol blue, conventionally used to visualize the sample for easier loading, was avoided because it formed a white precipitate in the Pluronic gel.

The Pluronic F 127 cubic phase cannot be in equilibrium with a water-rich phase such as the electrophoresis buffer. Therefore the gel slowly dissolved from the ends of the cuvette, but not faster than that the same gel could be used at least for several days with the same DNA response. We have not performed detailed structural studies of the formed gels. They were always highly transparent and optically isotropic (as determined by crossed polarizers), as expected for the cubic phase.

After loading, the DNA samples were introduced into the gel by electrophoresis at 7.5 V/cm for 4 h in order to have the DNA zones located well into the bulk of the gel at a position suitable for spectroscopic experiments. The introductory electrophoresis could only resolve the 11 restriction fragments of the ϕX174 DNA-HaeIII digest into two DNA zones in 30% Pluronic F127 (not shown). The distribution of the different fragment sizes between the two zones as determined by subsequent analysis on agarose gels is as follows. The DNA was stained in situ in the Pluronic gel by running electrophoresis at 7.5 V/cm for 3 h with 1 μg/mL ethidium bromide in the buffer. The zones were then observed with a UV lamp, and the two separated zones were retrieved and subjected to submarine electrophoresis in separate wells on a 3% Metaphor agarose gel at 3 V/cm for 2.75 h. Comparison with a separately loaded ϕX174 DNA HaeIII digest ladder showed that the faster of the

two DNA zones on the Pluronic gel contained fragments 310-72 and the slower zone the fragments 1353-603. The mixture of DNA fragments in the slowly migrating zone will here be referred to by its average fragment size of 1000 bp and that in the faster migrating zone accordingly as 200 bp.

In Figure 1 it has been identified which points in the phase diagram have been studied by spectroscopic measurements of DNA coil alignment and velocity during electrophoresis. The vertical electrophoresis cell was used for both types of experiments so that they could be performed on the same DNA-gel sample, and in both cases an elevator device was used to move the gel so that after the introductory electrophoresis the DNA zone of interest was positioned in the horizontal light beam. The extent of Joule heating was monitored through an increase in current with time, a 3% current increase corresponding to a 1 °C increase in temperature in TBE buffer.[13] The highest field strength investigated (30 V/cm) during the longest pulse (300 s) gave rise to an 8 °C increase.

Since the Pluronic is nonionic, there is no electroosmosis in the gel. Potential electroosmosis from the cuvette-walls will be neglible at the position of the DNA zone (which is about 1 mm from the wall) because of hydrodynamic quenching of fluorescence by the tight cubic phase. In the micellar solution electroosmosis may contribute to reduce DNA alignment.

2.3. LD-Spectroscopy. Linear dichroism (LD) is a technique to study DNA helix orientation, and is defined as the difference in absorption of light polarized parallel ($A_{||}$) and perpendicular ($A_{\perp}$) to the direction of the electric field

$$\mathrm{LD} = A_{||} - A_{\perp} \tag{1}$$

As an average over all bases in its common B-form, DNA has an effective transition moment at 260 nm, which is in the plane of the bases and at an effective angle of 86° to the helix axis.[13,14] This results in a negative sign of the LD signal when a DNA molecule is aligned with its helix axis along the electric field (defined as the parallel direction). Division by the absorbance A_{iso} of the sample when in an isotropic state converts the LD into reduced linear dichroism LD^r, which is a quantification of the degree of alignment of a macroscopically oriented sample. For B-form DNA perfect field alignment corresponds to $LD^r = -1.5$, an isotropic sample has $LD^r = 0$, whereas a completely perpendicular helix has $LD^r = +0.75$.

The LD experiments were performed with the light beam positioned at the center of the DNA zone, using an elevator positioning device. Three types of experiments were performed, where the LD was measured vs time, wavelength, or gel-distance. The kinetics of DNA alignment was followed at a fixed wavelength of 260 nm, while potential gel orientation was measured at 300 nm where the Pluronic but not DNA absorbs. The gel was subjected to a rectangular field pulse, and the buildup, the steady-state level, and the field-free decay of the DNA alignment was measured by an oscilloscope. Routinely, each experiment was performed with two pulses of the same duration but with the field reversed, to avoid net migration of the DNA zone out of the light beam. The field-free decay of the LD signal was analyzed by fitting data to a sum of up to three exponentials, and an offset y_0. The DNA orientation behavior in the micellar liquid phase was measured by putting the electrophoresis cell on ice for 10 min to bring a 30% cubic phase gel into the micellar state (Figure 1), and then LD responses to the same field pulse were recorded.

Second, LD spectra of DNA migrating in the gel state were recorded after a steady-state alignment had been obtained, as evidenced by an LD signal (at 260 nm) that was constant in time. Typically an LD spectrum between 240 and 320 nm was recorded in 1 min at 7.5 V/cm.

Third, the spatial LD profile along the gel was scanned in the absence of field in order to determine to what extent the DNA remained in an aligned state over a time-scale of hours after the electrophoresis was ended. This is difficult to determine from field-free relaxation time-traces because of the possibility of a slight drift in the baseline of the instrument over such long time periods. By contrast, in a gel-scan the essentially flat baseline-LD of the surrounding DNA-free gel can be subtracted from the total LD measured in the zone, to obtain the LD contribution from the DNA itself. The LD^r trace is constructed by taking the ratio of the DNA-LD profile and the corresponding A_{iso} scan.

2.4. Velocity Measurements. Pluronic gels are difficult to run in conventional submarine mode because the cubic phase is not stable in contact with a buffer reservoir. DNA velocity v at a given field strength was therefore measured in the electrophoresis cuvette by a spectroscopic technique using a UV−Vis spectrophotometer in which the elevator positioning device could be fitted. In this case the light beam was instead positioned at one edge of the DNA zone and the absorption gradient (dA/dx) was obtained at 260 nm by scanning the electrophoresis cell through the light beam at a known velocity with the field turned off. The cell was then locked in position with the light beam at the zone edge, and the DNA zone was moved through the light beam by electrophoresis, and the slope (dA/dt) of the so obtained absorption time-profile could then be calculated. The DNA migration velocity was then obtained as $v = (dA\ dt^{-1})/(dA\ dx^{-1}) = dx/dt$, using a portion of the zone where both slopes were constant. Measurements on ϕX174 DNA in 1% agarose gels gave electrophoretic velocities in very good agreement with velocities measured by Hervet and Bean[15] using conventional submarine gels (see Supporting Information). The typical temperature increase by less than 8 °C had no detectable effect on the velocity, since dA/dt remained constant and showed no upward curvature during the pulse as would be expected if there was an effect of temperature.

2.5. Fluorescence Microscopy. Fluorescence microscopy of individual T4 DNA molecules in the Pluronic gel was performed by use of a YOYO-staining protocol and an electrophoresis cell as described elsewhere.[16] The main perturbing effect of the dye is to lengthen the DNA.[17]

2.6. CD. Circular dichroism (CD) measurements in the absence of an electric field were made with 300 μM of calf thymus DNA in 30% Pluronic F127 at room temperature on a Jasco J720 spectropolarimeter. The DNA was mixed with the liquid Pluronic at 5 °C, and the cubic phase was formed in the 1 cm spectroscopic cuvette by our standard temperature-raise protocol (see above).

2.7. PCR. PCR experiments were performed on a Light-Cycler. The reaction mixture contained 5 mM Mg, 400 μM dNTP, 300 mM primers, 0.2 mg/mL BSA, 0.05 units/μL Hotstart Taq polymerase. A quantity of 200 000 copies of a 409 bp target DNA was added to each reaction vial where Pluronic F127 concentrations were 0, 5, or 10%, respectively. After the PCR reaction the yield was quantified by electrophoresis in 1% agarose gel.

2.8. Effect of DNA on Pluronic Phase Behavior. The effect of the presence of DNA on the transition temperature between cubic and micellar phases was studied for 30% Pluronic F127, using DNA concentrations of 0, 30, 100, 200, 300, 500, or 1000 μg/mL. Cylindrical glass vials with an inner diameter of 1 cm were prepared to contain 0.6 g of Pluronic F127 powder, 1.4 g

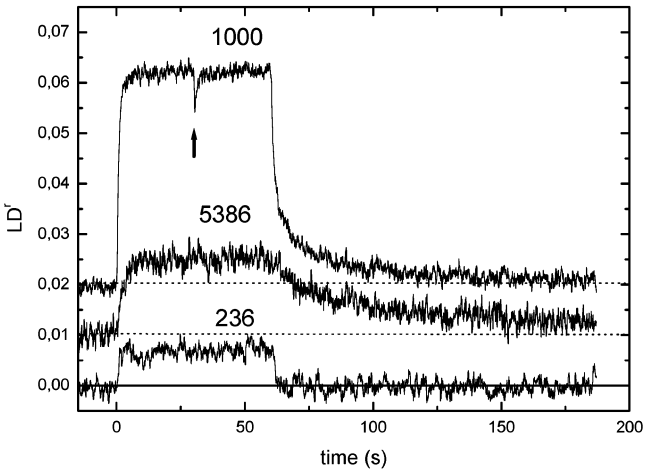


Figure 3. LD^r response to a 7.5 V/cm field pulse in a gel of 30% Pluronic F127 of DNA of indicated sizes (in base pairs). Field is reversed at arrow for all three sizes. The curves for 5386 and 1000 bp have been shifted vertically by 0.01 and 0.02 units, respectively, for clarity. (The 1000 bp sample contains several restriction fragment sizes; see Materials.) DNA concentrations are approximately 50 μM phosphate. Optical path is 3 mm.

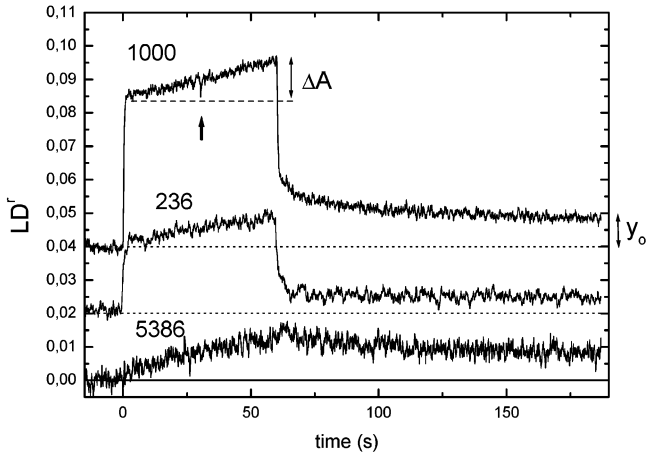


Figure 4. LD^r response to a 30 V/cm field pulse in a gel of 30% Pluronic F127 of DNA of indicated sizes (in base pairs). (The 1000 bp sample contains several restriction fragment sizes; see Materials.) Field is reversed at arrow for all three sizes. The curves for 1000 and 236 bp have been shifted vertically by 0.04 and 0.02 units, respectively, for clarity.

of TBE solutions, and with calf thymus DNA added and mixed until homogeneous. The vials were sealed and left refrigerated for 48 h yielding homogeneous 30% Pluronic F127 solutions. The samples were mounted in a water bath where the temperature was changed in steps of 0.3 °C, both in decreasing and increasing temperature direction. The sample holder was tilted 45°, and if the interface retained the tilt after 5 and 10 min, the sample was considered to be in the gel state.

3. Results

3.1. No Effect of DNA on Gelling Temperature of Pluronic. The gelling temperature for a series of 7 Pluronic F127 samples (30%) containing DNA concentrations between 30 and 1000 μg/mL was 11.0 ± 0.3 °C and the same as for the DNA-free control. We also note that ocular inspection of the electrophoresis gel revealed no perturbation of the gel at the position of the DNA zone, and the gel was isotropic when viewed through crossed polarizers.

3.2. Linear Dichroism of DNA in F127 Gel. Figure 3 compares the LD^r time-responses at 260 nm of the 236, 1000, and 5386 bp DNA samples in a 30% F127 gel to an electric field pulse of 7.5 V/cm. The 236 bp fragment exhibits a fast (seconds) build-up of a positive LD as the field is applied and a similarly fast relaxation of the LD as it is turned off. The 1000 bp (Figure 4) sample also displays a fast positive LD response upon field application, but the magnitude is about 5 times higher than for the smaller fragment. As the field is turned off, the 1000 bp sample initially has a fast relaxation, but in contrast to the shorter fragments, it is followed by a slower component. The largest DNA size (5386 bp) also exhibits a positive LD, as observed earlier,[12] but with a somewhat slower build-up and relaxation than the 1000 bp fragment. In contrast to the case with the smaller sizes, the LD of the 5386 bp DNA does not relax completely during 2 min of field-free conditions, as evidenced by a remaining offset. This indicates that there is a slow relaxation component for the largest DNA size that is not present for the smaller DNA samples.

At the higher field of 30 V/cm (Figure 4), all sizes of DNA studied here (including 200 bp, not shown) again exhibit a positive LD, but now with the fast buildup being followed by a slowly increasing positive LD which lasts as long as the field is applied (at least 3 min, data not shown). The relaxation behavior is similar to that at the low field, except that at the high field all DNA sizes exhibit an offset after 2 min.

3.3. Origin of Positive LD Signal. The positive LD indicates that DNA migration in the Pluronic gel occurs with a perpendicular helix orientation relative to the field direction. This was unexpected[13] and we therefore performed control experiments to exclude other possible sources for a positive LD. During a field pulse of 7.5 V/cm there was no detectable LD response at 300 nm where DNA does not absorb light (see Supporting Information). This observation strongly indicates that the LD response observed at 260 nm during the same field pulse (Figure 3) is not due to a gel deformation, which otherwise can result in LD of either sign.[13] A positive LD could also reflect an altered secondary structure with a significant tilt of the bases with respect to the helix axis,[18] so several experiments were performed to investigate the secondary structure of DNA when in the Pluronic cubic phase. There is no measurable difference in the CD spectrum of DNA in 30% F127 Pluronic and in TBE buffer (see Supporting Information), which shows that the DNA retains its B-form in the Pluronic, at least in absence of field. Second, the LD spectrum of 5386 bp DNA migrating at an applied field of 7.5 V/cm in a 30% Pluronic F127 gel has a shape which is very similar to the absorption spectrum of DNA when present in the TBE buffer (see Supporting Information). This supports the finding that the B-form is attained also during migration.[13,19] Third, PCR experiments showed that the DNA acted as template for the enzyme *Taq* polymerase also in the presence of Pluronic. With 5% Pluronic the yield of amplification was the same (99 ± 2%) as without Pluronic present, whereas at 10% the yield was somewhat lower, 84%.

Fluorescence microscopy of individual T4-DNA molecules in a 30% Pluronic gel showed coil-like DNA molecules similar to those observed in TBE buffer solution and in agarose gels.[16]

3.4. Linear Dichroism in the Micellar Solution Phase. Figure 5 compares the positive LD response of 5386 bp DNA to a 7.5 V/cm pulse in a 30% Pluronic F127 cubic phase (top curve) with the one obtained if the gel is reversed to the micellar solution phase by decreasing the temperature to 5 °C (lower curve). The response to the same pulse now has a negative sign, indicating a DNA migration with the helix axis preferentially parallel to the electric field, and no positive contribution is

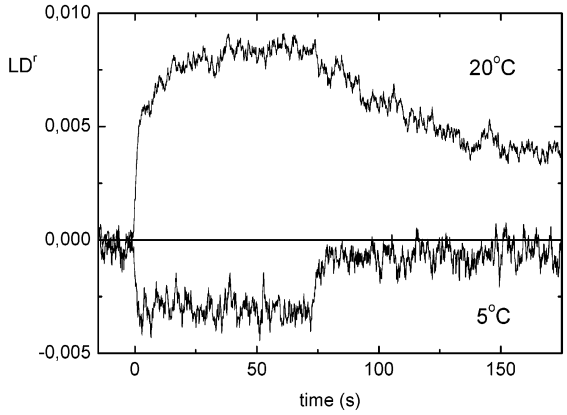


Figure 5. LD^r response to a 7.5 V/cm pulse for 5386 bp DNA in 30% Pluronic F127 sample at 20 °C (cubic gel phase) and approximately 5 °C (micellar solution phase). Field is turned on at time 0 and off at 75 s.

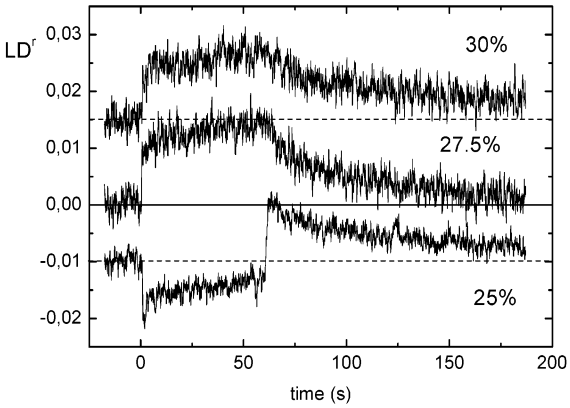


Figure 6. LD^r response to a 15 V/cm pulse for 5386 bp DNA in Pluronic F127 gels at indicated concentrations. The curves for 30% and 25% have been shifted vertically by 0.015 and −0.01 units, respectively, for clarity.

observed. The amplitude of the negative LD is smaller and the relaxation is faster than for the positive LD observed in the cubic phase.

3.5. Effect of Gel Concentration. Figure 6 shows the LD^r response of 5386 bp DNA at 15 V/cm in cubic gels at Pluronic F127 concentrations of 30, 27.5, and 25%. The LD^r curve at 27.5% is similar to that observed in a 30% gel. In the 25% gel, by contrast, there is a fast negative initial response preceding a comparatively slow positive LD contribution similar to the only contribution observed at the higher Pluronic concentrations. The negative component is present throughout the pulse as can be seen when the field is turned off: there is first a fast relaxation corresponding to the negative component, which is followed by a slow positive relaxation, again very similar to that observed for the higher concentrations.

3.6. Velocity Measurements. Figure 7 shows that the velocity of the 200, 236, and 1000 bp DNA samples in the 30% Pluronic F127 gel increases in a linear fashion with increasing field strength. At a given field, the velocity increases with decreasing size, in agreement with the size-separation which has been reported earlier.[1,2] Our velocity of 9.2 μm/s for the 236 bp DNA at 30 V/cm is comparable to a value of about 6 μm/s, which can be calculated from the mobility of 2×10^{-5} $cm^2/V/s$ obtained by Chu and co-workers[1] for a 271 bp DNA in 30% F127. The difference may be due to the fact that our DNA is somewhat smaller. The velocity behavior of larger DNA molecules (Figure 8a) is qualitatively different. Both 5386 bp and 166000 bp DNA exhibit two regimes of linear dependence

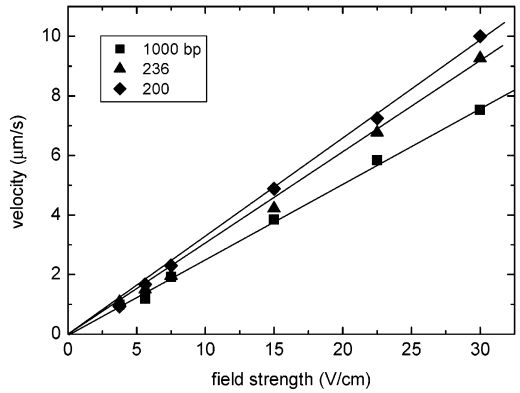
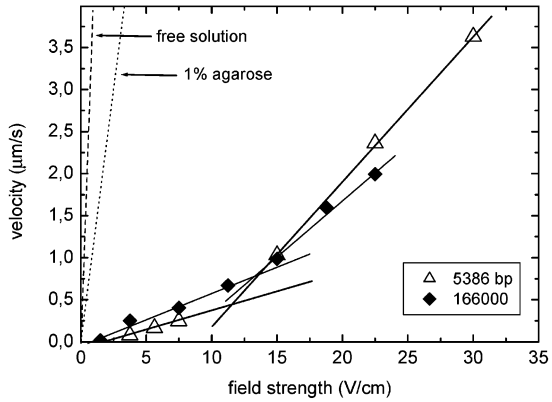


Figure 7. Electrophoretic velocity vs field strength in 30% Pluronic F127 for 200, 236, and 1000 bp DNA (indicated). (The 200 and 1000 bp sample contains several restriction fragment sizes; see Materials.) Straight lines are least-mean-square fits, and correspond to mobilities 3.4×10^{-5} $cm^2/V/s$ (200 bp), 3.1×10^{-5} $cm^2/V/s$ (236 bp), and 2.6×10^{-5} $cm^2/V/s$ (1000 bp).



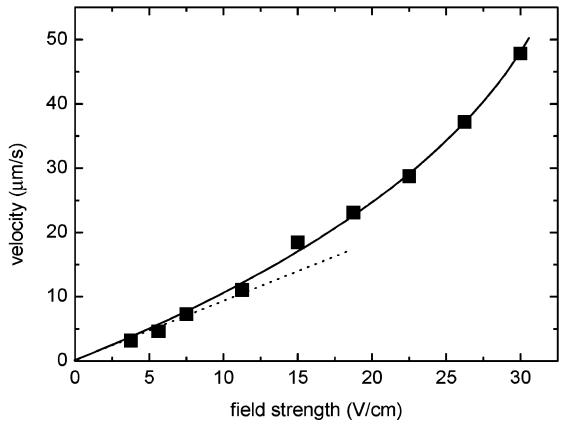


Figure 8. Electrophoretic velocity vs field strength in (a) 30% Pluronic F127 for 5386 bp DNA (open symbols) and 166 000 bp DNA (solid), and (b) in 1% agarose for 5386 bp DNA. Straight lines in (a) are least-sqaure fits to low- and high-field regions. Dashed line shows velocity of DNA (of any size) in TBE buffer (ref 17). Dotted line shows velocity of linear 5386 bp in 1% agarose gel, taken from part (b) where the low-field mobility (slope of dotted line) is 1.2×10^{-4} $cm^2/V/s$.

on field strength E, with a breakpoint (at approximately 12 and 13 V/cm, respectively) that is not observed for the smaller DNA fragments (Figure 7). For the sake of comparison Figure 8b shows the velocity of the 5386 bp DNA in 1% agarose gel. Also here the velocity increases in a nonlinear manner with increasing field, but in contrast to the Pluronic case in a smooth fashion. Importantly, in all cases the velocity was the same whether the studied field range was investigated by increasing or decreasing the field strength.

TABLE 2: Steady-State Alignment Parameters for DNA in 30% Pluronice F127 Gel[a]

	linear ϕX174		1000 bp		236 bp		200 bp	
field strength (V/cm)	τ_{ss} (s)	L_τ (μm)	τ_{ss} (s)	L_τ (μm)	τ_{ss} (s)	L_τ (μm)	τ_{ss} (s)	L_τ (μm)
3.75	38	3.0	12.8	12.7	4.25	4.6	3.2	2.9
5.6			12.6	14.9	3.95	5.9	3.6	6.0
7.5	29.7	7.4	7.1	13.7	1.4	2.7	1.8	4.1
15	0.5	0.5	2.5	9.6	1.3	5.5	2.8	13.6
22.5			1.75	10.2	1.1	7.5	1.7	12.3
30	0.3	1.1	1.65	12.4	1.2	11.1	3.9	39

[a] τ_{ss} is the time to reach a steady LD (Figures 3, 4; see text); $L_\tau = \tau_{ss} \times v$ is the corresponding distance where the velocity v is obtained from Figures 7 and 8.

TABLE 3: Field-Free Decay of DNA Alignment in 30% Pluronic F127 Gel[a]

	236 bp		1000 bp		5386 bp	
	7.5 V/cm	30 V/cm	7.5 V/cm	30 V/cm	7.5 V/cm	30 V/cm
y_0	0	0.19	0.03	0.13	0.23	0.39
t_1 (s)	1.0	1.76	1.54	0.32	34.3	32.8
A_1	1.0	0.81	0.67	0.13	0.77	0.61
t_2 (s)			20.3	5.8		
A_2			0.29	0.14		
t_3 (s)				59		
A_3				0.6		
scan LD^r [a]	0		0		0.25−0.3	

[a] Fits of normalized LD^r/LD$^r_{(t=0)}$ decays to sum of exponentials (see Materials and Methods). [b] Maximum LD^{r}evaluated from scan of gel in absence of field as in Figure 9. y_0 is residual LD^r in Figure 4.

3.7. Alignment Kinetics. The rate of LD buildup was used to measure the length of time time and the distance the DNA molecules have to travel in order for their coils to become deformed. At low fields (Figure 3) the rate of coil deformation was characterized by the time τ_{ss}, needed to reach a steady-state orientation LD_{ss}, whereas at the higher field τ_{ss} was instead defined as the time needed to reach the slow linearly increasing component (Figure 4). By combining τ_{ss} with the velocity data (Figures 7,8), the distance L_τ the DNA coils have to traverse to become deformed can be derived. The results (Table 2) show that at low fields L_τ has values of approximately 5−15 μm for all DNA sizes. At the higher fields L_τ remains in this range for the smaller DNA sizes, but for the 5386 bp DNA L_τ falls below 1 μm.

The field-free relaxation of the LD after the field is turned off was used to monitor the rate of Brownian motion of the DNA molecules inside the gel. Table 3 shows field-free decay times for the DNA samples 236, 1000, and 5386 bp after field pulses of 7.5 and 30 V/cm. At the lower field strength the 1000 bp fragment exhibits a double exponential decay, whereas the relaxation of the 236 bp and 5386 bp DNA could be fitted to a single exponential decay. However, the 5386 bp DNA in addition exhibits a substantial anisotropy remaining after 120 s ($y_0 = 0.23$), which indicates the presence of a second slow relaxation process. This interpretation was supported by scans of LD and absorbance along the gel performed 360 min after the field (7.5 V/cm) was tuned off (Figure 9). The peak in the absorbance profile (260 nm) shows the position of the 5386 bp DNA zone on the gel. The LD peak associated with it shows that the DNA sample remains in a significantly anisotropic state (LD^r of about 0.25−0.30) for at least 6 h after the electrophoresis has ceased. Importantly, when performed on the smaller 236 and 1000 fragments, the same type of gel-scan showed that these DNA sizes were completely randomized (results not shown).

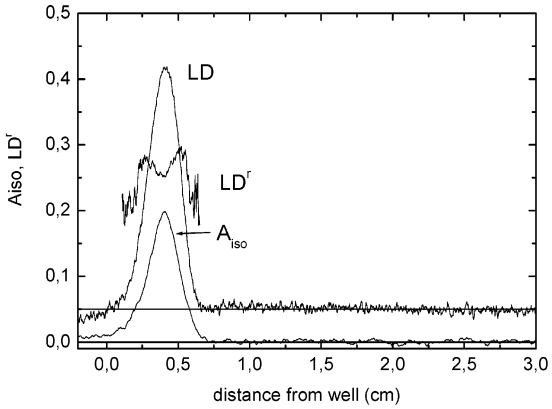


Figure 9. Absorption A_{iso}, LD, and LD^r profiles at 260 nm along a gel of 30% Pluronic F127 containing a zone of 5386 bp DNA, recorded 360 min after the field (7.5 V/cm, 4 h) was turned off. Position of the well is at the origin. A_{iso} has been corrected for a small gel absorbance so that it corresponds to the DNA absorbance and shows the position of the zone. The LD trace contains both DNA and gel contributions. The latter (LD_{gel}) is essentially constant along the gel as evidenced by the constant LD outside the DNA zone, with $LD_{gel} \approx 0.05$. The LD^r of the DNA is calculated as $LD^r = (LD - LD_{gel})/A_{iso}$ and is only shown for the parts of the gel where $A_{iso} \neq 0$.

These observations have been included in Table 3. After pulses at the higher field strength of 30 V/cm, all three DNA samples exhibit significant long-term offsets (y_0 in Table 3), in contrast to the situation at the lower field where only the largest DNA did. This indicates that migration in the high field has brought all three DNA sizes to a strongly confining environment in the gel.

4. Discussion

4.1. A New Mode of DNA Migration. The nonzero DNA-LD we observe during electrophoresis in gels of Pluronic F127 (Figures 3 and 4) evidences a migrative DNA coil perturbation. Studies in agarose and polyacrylamide gels have shown that DNA coils become deformed during migration if the radius of gyration, R_g, is comparable to or larger than the pore size.[8] A coil deformation is therefore expected also in the Pluronic case given the nanometer dimension of the aqueous channels of the cubic phase and that the DNA used here have R_g values in the range between 18 and 168 nm (Table 1). Below the break-point in Figure 8a, the electrophoretic mobility $\mu = v/E$ for 5386 bp DNA is 4.5×10^{-6} cm^2/V/s, which is 100 times lower than its mobility for the free solution (4.5×10^{-4} cm^2/V/s)[20] and about 30 times lower than the low-field mobility of 1.2×10^{-4} cm^2/V/s in 1% agarose (Figure 8b). These values show that the Pluronic cubic phase offers a strong resistance to migration, in agreement with substantial steric interactions between the Pluronic cubic matrix and a strongly confined DNA molecules.

Since the CD, PCR, and LD control experiments reported here (see Supporting Information) allow us to rule out alternative explanations, the positive sign of the LD shows that DNA migrates in the Pluronic gel with a preferential perpendicular orientation of the DNA helix axis relative to the field. Such a mode of migration is in contrast to the parallel helix alignment which would be expected for DNA migrating end-on between micelles (Figure 10a), and which indeed is observed for DNA migrating end-on in the conventional gels.[8] We here show that the nonreptative migration-mode is general because it is observed for several Pluronic concentrations (Figure 6) and for a range of DNA sizes (Figures 3 and 4). Our size range includes

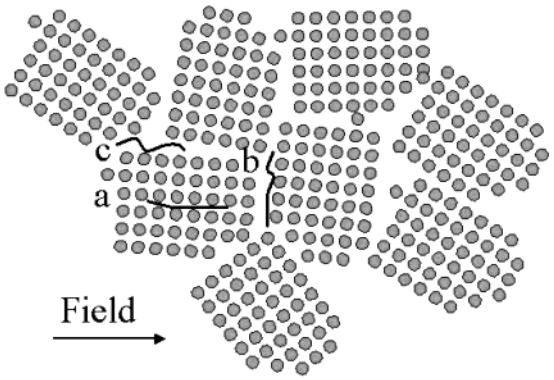


Figure 10. Schematic presentation of modes of DNA migration (and the predicted sign of the LD) in an electric field *E* through a polycrystalline sample of the cubic phase of Pluronic F127. Dimensions of micelles and crystal domains are not drawn to scale. (a) DNA molecule migrating end-on inside the cubic crystallite (LD < 0). (b) DNA flattened out against a blocking domain of cubic crystal (LD > 0). (c) DNA molecule migrating along grain boundary between domains (LD < 0).

those studied by Wu et al.[1]and Chu et al.[2], and our observations, therefore, explain why reptation models failed to describe their data on the electrophoretic velocities for DNA between 872 and 1353 bp.

4.2. The Role of the Cubic Structure. Migration in the liquid micellar phase (Figure 5, lower trace) occurs with a preferential parallel helix alignment, and a field-free relaxation which is fast compared to in the gel state (Figure 5, upper trace). This behavior is characteristic for DNA molecules migrating entangled in a concentrated polymer solution,[9] and the distinctly different behavior above and below the Pluronic transition temperature (cf. Figure 1) therefore shows that the nonconventional mode of DNA migration in Pluronic F127 is not the result of the high concentration of polymer as such. It only occurs under conditions where the cubic phase forms, and one question is then to what extent the cubic structure can be expected to remain in the presence of DNA undergoing electrophoresis.

Chu and co-workers[11] have shown that addition of the TBE buffer has negligible effect on the phase diagram of F127 Pluronic and water. The gel phase we use is transparent and isotropic, as expected for the cubic phase, also in the parts that contain the DNA zone. Furthermore, when present throughout a gel-sample at concentrations as high as its overlap concentration (150 μg/mL) fX174 DNA does not change the melting temperature for 30% Pluronic F127. Thus, even at concentrations typical of that inside individual coils, DNA does not seem to affect the gel state (as monitored by the transition temperature), suggesting that the DNA does not perturb the gel structure. Although only one aspect of the phase diagram has been studied, it is reasonable to assume that the equilibrium gel-structure is the same as for DNA-free cubic phase. The situation may be different during migration, however.

4.3. Matrix−DNA Interactions. Balance between electric and friction forces during steady-state migration gives $\mu = Q/f$, where Q is the effective electrophoretic charge and f the friction coefficient. The increase in mobility (slope) above the breakpoint in the biphasic velocity (Figure 8a) most likely reflects a decrease in friction between DNA and the Pluronic F127 gel, rather than an increase in effective charge, because the electrophoretic velocity in free solution is proportional to field strength throughout the range studied here.[21] The presence of a breakpoint then indicates an abrupt change (decrease) in friction between DNA and gel. This altered DNA−gel interaction is likely to be caused by a perturbation of the gel structure rather than of the DNA coils. Deformation of DNA coils due to interactions with the (rigid) agarose gel network is known to give rise to reduced friction,[22] but in this case the velocity (Figure 8b) is a smoothly nonlinear function of field[15] because the degree of coil deformation increases (and friction decreases) in a gradual fashion.[13] By contrast, the cubic phase is not cross-linked and could yield to the force from a migrating DNA coil if it is large enough. Rheological studies in the related Pluronic F108[23] show that shearing at low stresses leads to a flow of intact cubic-phase domains (crystallites) that slide relative to each other along grain-boundaries. At higher flow-rates also the cubic crystal domains are affected by the shear forces, so that the cubic structure become liquidlike ("melts"). We propose by analogy that the (polycrystalline) cubic structure is retained at low field strengths, but that above a certain field strength the electric force from DNA on the gel is strong enough to lead to a perturbation of the lattice-structure of the individual crystals, perhaps akin to a local melting. A cubic lattice that yields can be expected to cause less steric hindrance to the DNA motion than an intact one. Still, the viscosity from the 30% Pluronic polymers will be substantial, in agreement with the finding that the mobility (slope) of the 5386 bp DNA above the breakpoint (Figure 8a) is 1.7×10^{-5} cm^2/Vs and thus still low compared to the value 4.5×10^{-4} cm^2/Vs in aqueous solution.

The breakpoints for T4 DNA (166 000 bp) and ΦX174 DNA (5386 bp) occur at essentially the same field strength (12 and 13 V/cm, respectively, Figure 8a), which is surprising since the former carries about 30 times more charge and thus exerts correspondingly higher electric force on the gel at given field strength. However, both DNA coils have radii of gyration (Table 1) which are large compared to the spacing of the cubic lattice. Whatever the nature of the gel-perturbation, many micellar contacts would thus have to be broken more or less in concert, which could explain the rather abrupt change in friction that is evidenced by the discontinuous increase in mobility. The fact that the electric force from the DNA will be distributed over many micelles may also explain why the critical field-strength for breakdown is independent of DNA size: the force from the DNA will increase, but so also will the number of involved micelles.

That there is change in the migration at about 12 V/cm is further supported by the difference in the nature of the coil alignment behavior below (Figure 3) and above (Figure 4) this field strength. Assuming that there is not yet another mobility-discontinuity at even lower fields, we will discuss the DNA migration behavior below the breakpoint in terms of an intact cubic phase, while above we will allow for a possible distortion of it. It should be noted that the fields used here are weak compared to those used in capillary electrophoresis.

4.4. Migration at Low Fields. *Grain-Boundary Migration.* The results presented here lend some further support for our suggestion[12] that at low field strengths DNA molecules do not enter the cubic structure but instead migrate in interstitial spaces (grain boundaries) between crystallites of cubic structure (Figure 10b). This hypothesis arose from the observation[12] that the average distance L_τ the 5386 bp DNA coils had to migrate to become deformed was on the order of micrometers, i.e., well above any typical length-scale of the cubic lattice, but a possible size of the domains themselves.[3] The results obtained in this study (Table 2) show that the L_τ values are on the order of micrometers independently of DNA size. This is expected if the characteristic length for migrative coil deformation is determined by a length-scale in the gel such as domain size, and not by the DNA size as is the case for coil deformation

during reptation.[22] In this picture the positive LD signal can then be interpreted as DNA coils that are migrating in the grain boundaries are being flattened out perpendicular to the field as they reach a blocking crystal domain (Figure 10b), after migrating a distance corresponding to the average domain size (Figure 10c).

Experiments on circular DNA shines further light on the possible modes of DNA migration. Since they lack ends, circular DNA molecules have to form loops between the micelles in order to penetrate the cubic crystals. The free-energy cost for double-helical DNA to form such loops is about 10 kT,[24] because the effective pore size is about 10 times smaller than persistence length of the DNA helix (Figure 2). The fields used here are too weak[25] to force the DNA to bend into such a tight loop, so the fact that circular 5386 bp DNA has a nonzero velocity in 30% Pluronic gels[12] indicates that it is possible for DNA molecules to migrate through the Pluronic cubic phase by another route than between the micelles in the cubic crystal. The fact that the LD response was similar to that observed for the corresponding linear form[12] suggests that the two DNA forms share this alternate mode migration.

Grain-Boundary Width and DNA Velocity. Field-free relaxation in gels can be used to monitor the nature of the environment where the DNA resides at the time when the field is turned off.[26] Even for the shortest fragment the relaxation is on a time scale (seconds, Table 3) which is much longer than the μs relaxation times observed in free solution for DNA molecules of this size.[26] This shows that the relaxation occurs in a strongly confining environment with cavity sizes comparable to the radius of gyration (R_g), which is 20 nm for the smallest DNA studied here (Table 1). The even slower relaxation of 5386 bp DNA (Figure 9) is not surprising in view of the coil size ($R_g = 168$ nm) being considerably larger than the widths of the grain boundaries suggested by the relaxation-rates of the shorter fragments. The grain-boundaries can thus act as a molecular sieve[7] for DNA coils, which could explain the size separation that is observed for the DNA sizes studied here (Figure 7,8a). Interestingly, the estimated channel widths are of the same order as the persistence length of DNA (50 nm). This may explain why the linear 5386 bp DNA migrates considerably faster than its circular form,[12] which has to form loops inside the channels.

4.5. Migration at High Fields. For fields above the breakpoint in the velocity data (Figure 8a), there is an additional slow positive LD component (Figure 4) that continues to increase at least for several minutes, which corresponds to a migrated distance of hundreds of micrometers. This length scale is long compared to the characteristic micrometer distance that governs the migration at lower fields, and cannot be related to any obvious typical length in the gel or DNA. A second observation which supports the finding that the mode of migration is different compared to low fields is that all DNA sizes exhibit a slow component in the field-free relaxation represented by a relaxation offset (Figure 4 and Table 2), even the smaller DNA sizes which do relax fully in a few seconds after migration at low field. This indicates that the migration drives the DNA molecules into a more confining environment than at low fields, so that also the short DNA fragments become strongly hindered in their Brownian motion.

It is clear that the gel perturbation we propose to underlie this altered migration behavior at the higher fields is not a full melting of the cubic liquid crystal structure. Under conditions where the Pluronic state is liquid (liquid micellar solution; Figure 5) or likely to be closer to that state (25% Pluronic at room temperature, Figure 6) the LD response exhibits a negative component which is qualitatively different from the additional positive one observed at high fields in 30% Pluronic. Second, when the field is reversed (indicated by an arrow in Figure 4) the slowly growing coil deformation that has accumulated during the forward pulse is retained as the DNA molecules turn around. If the cubic phase had melted, the DNA molecules would have time to partially relax as they were turning around and encounter a melted phase behind them, because in the micellar solution (Figure 5) the relaxation occurs on the seconds time-scale. In fact, the absence of any relaxation of the alignment during field-reversal is in contrast to a dip in the LD that can be observed upon field reversal in more open agarose gels,[27] and suggests (in agreement with the field-free relaxation data) an even stronger DNA confinement in the Pluronic gel after migration in stronger fields. Notably, the velocity experiments indicate that the perturbed gel indeed returns to the cubic phase sooner or later after the DNA molecules have passed, because the velocity is the same whether the field range is investigated by increasing or decreasing the field. In summary, there are indications that DNA perturbs the Pluronic structure reversibly at high enough fields, leading to a stronger confinement of the DNA coils than migration at low (nonperturbing) fields.

Concluding Remarks. Spectroscopic investigations of DNA migration in the cubic phase of Pluronic F127 have revealed a new mode of DNA migration at low electric fields. A full understanding of the nature and importance of perturbations of the Pluronic cubic phase by migrating DNA at higher fields will require complementing methods to study the structure of the Pluronic phase during electrophoresis.

Acknowledgment. Finacial support from the Swedish Research Council is thankfully acknowledged. Sara Nordgren and Jenni Sandström at LightUp Technologies AB are thanked for performing the PCR experiments and providing the 236 bp DNA samples. Malin Zackrisson at Göteborg University is gratefully acknowledged for help with the gel−sol experiments, and Ann-Sofie Winge for performing the experiments of Figure 10. Paschalis Alexandridis and Ulf Olsson are thanked for helpful discussions, and inspiring comments by Amanda Åkerman and Aleksandra Hulisz are greatly appreciated. A preliminary account of these results was presented on Surfactants in solution in Barcelona, June 2002.

Supporting Information Available: Presents results of control experiments that validate the spectroscopic method to measure DNA velocity and that confirm the B-form secondary structure of DNA undergoing electrophoresis in the Pluronic F127 cubic phase. This material is available free of charge via the Internet at http://pubs.acs.org.

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