Spectroscopic Study of Orientation Dynamics of DNA during Electrophoresis in Entangled and Dilute Polyacrylamide Solutions

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ABSTRACT: By using linear dichroism (LD) spectroscopy, the electrophoretic migration of DNA in entangled and dilute solutions of linear polyacrylamide of different molecular weights has been characterized in terms of orientation and orientation dynamics. The LD technique enables measurements at such high field strengths as normally used in capillary electrophoresis (typically 100−200 V/cm), and the results obtained here should therefore be relevant for understanding the mechanism of separation in capillary electrophoresis. In a highly entangled polyacrylamide solution of high molecular weight, the LD response to an electric field pulse contains an over- and undershoot in orientation before a steady-state level is reached, showing that DNA reptates with oscillations in a similar way as in agarose gel. The average orientation of the DNA is extremely high, $S \approx 0.7$ at 100−150 V/cm ($S = 1$ corresponds to perfect orientation). At the same polymer concentration (w/w), a polymer solution with a higher molecular weight gives a behavior more similar to that in gel, which indicates that longer polymer chains form a more rigid network than shorter ones. When the polymer concentration is decreased, the reptational behavior becomes less pronounced and the degree of orientation at steady-state decreases. However, even in an ultradilute polymer solution, the orientation of the DNA is considerable high (*e.g.*, $S \approx 0.3$ at 100 V/cm, which is about 100 times higher than in the absence of any polymers).

Introduction

Slab gel electrophoresis has for long time been the preferred method for separation of DNA fragments, but in the last few years there has been an increasing interest in capillary electrophoresis (CE), a separation technique in rapid development. The main advantages of CE over the traditional slab gel electrophoresis are fast separations and high resolution. This is a consequence of the high electric field strengths that can be applied because of the efficient heat dissipation from the thin capillary. Furthermore, the technique is easily amenable to automation, allowing on-line sample detection with great sensitivity. Minute amounts of sample are required, and the reproducibility is high.

Since the mobility of DNA is essentially independent of molecular size in free solution,[1] a kind of sieving medium must be used. Capillaries filled with cross-linked gels can provide excellent size selectivity[2−4] but are difficult to prepare and have a limited lifetime.[5,6] As an alternative to cross-linked gels, solutions of uncross-linked polymers such as linear polyacrylamide,[7−11] hydrophilic cellulose derivatives,[12−18] glucomannan,[19] poly(vinyl alcohol),[8] and liquified agarose[8,20] have been employed as separation media for capillary electrophoresis. These polymer solutions, which allow easy filling, flushing, and refilling of the capillary between the runs, appear to be a promising medium for separation of DNA.

Until quite recently it was assumed that in order to get separation of DNA, the polymer concentration, c, must be above the overlap concentration, c^*, so that an entangled network, similar to that in gel (except that the network is not static but dynamic), is formed. In such entangled networks, both small−intermediate sized and large DNA have been separated in constant[7−11,14−20] and pulsed fields,[21−23] respectively. However, recently Barron *et al.* demonstrated excellent separation of DNA (2.0−23.1 kbp) using CE in hydroxyethyl cellulose solutions with concentrations well below the overlap concentration.[12,13,24,25] Also separation of mega base paired DNA has been obtained in dilute polymer solutions by using pulsed field capillary electrophoresis.[26] An entangled network is thus not an absolute requirement for achieving separation of DNA.

Fluorescence video microscopy has been used to study the migration of individual DNA molecules in polymer solutions both in constant and pulsed fields, but only under conditions where the electrophoretic velocity of DNA is low.[14,27−29] In highly entangled polymer solutions, large DNA molecules were seen to perform an oscillatory migration in constant field electrophoresis, changing between extended and compressed conformations in a way similar to that observed in gel electrophoresis. The migration behavior changes gradually (the degree of extension of the DNA decreases and the conformational changes occur less regularly) as the polymer concentration decreases. In ultradilute polymer solutions ($c \ll c^*$), the motion is significantly different from that in gel. The DNA molecules appear to "dance" around the obstacles in the solution due to the high degree of Brownian motion. Interestingly, even in these ultradilute polymer solutions, the DNA adopts an on-average extended conformation in contrast to the on-average spherical random coil conformation observed in free solution.[29]

Due to the large similarities in the migration behavior observed in the microscopy measurements[14,27−31] and the similar separation characteristics obtained in CE experiments (*e.g.*, compare the separation of *Hae*III digest of ΦX174 in refs 2, 11, 17, 19, and 32) for DNA in entangled polymer solutions and in gels, the separation process in entangled polymer solutions has been suggested to be the same as in gel, *i.e.*, related to the reptation model. In the ultradilute polymer solutions, on the other hand, it is obvious that a completely different theory is needed to describe the DNA migra-

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tion. Barron *et al*. proposed the "transient entanglement mechanism" to explain the separation, which assumes that collisions between DNA and polymer molecules can lead to transient entities with size-dependent mobilities.[24,25] By assuming that a DNA molecule temporarily drags along polymer molecules and by taking into account the mutual disengagement time of the DNA and the polymer, Hubert *et al*. recently presented a theoretical model of DNA migration in ultradilute polymer solutions[33] which agrees well with the experimental results by Barron *et al*.[24,25]

In this study we have used linear dichroism (LD) spectroscopy to characterize the DNA migrational behavior in entangled and nonentangled polyacrylamide solutions in terms of orientation and orientation dynamics. To enable comparison with our previous, corresponding fluorescence microscopy study,[29] the LD study has been performed with the same DNA (YOYO-stained T2 DNA) and the same concentrations and molecular weights of the polyacrylamide as in that study. Comparison with LD measurements on YOYO-stained T2 DNA in 1% agarose gel[34] is also performed. One advantage with the LD technique is that it enables, in contrast to the fluorescence microscopy technique, measurements at the high field strengths normally used in CE (typically 100−200 V/cm). We find that in a highly entangled polyacrylamide solution, the orientation dynamics, as well as the steady-state orientation, are very similar to what is obtained in agarose gel. At polymer concentrations well below the overlap concentration, a relatively high degree of orientation (about 100 times higher than in the absence of any polymers) is still obtained.

Materials and Methods

Chemicals and Sample Preparation. A 50 mM TBE buffer containing 50 mM Tris, 50 mM boric acid, and 1.25 mM EDTA (pH 8.3) was used throughout. The DNA [linear double-stranded T2 DNA (164 kbp)] was obtained from Sigma. Pulsed field gel electrophoresis showed that at least 80% of the DNA molecules are of full length, unaffected by shear degradation. The fluorescent dye YOYO (trade name YOYO-1) was purchased from Molecular Probes. The YOYO−DNA complexes were formed by adding the DNA solution to a newly prepared solution of YOYO in TBE buffer, and heating this mixture at 50 °C for 2 h to ensure homogeneous distribution of dye among the DNA molecules.[35] The mixing ratio YOYO:DNA base has been 1:10, which is approximately the same as the binding ratio due to the high DNA binding constant of YOYO.[36]

Linear polyacrylamide (PA) with a molecular weight of about 18×10^6 and 5×10^6 (degree of polydispersity unknown), here denoted PA18 and PA5, respectively, was kindly provided by AB CDM and Chalmers Teknikpark, Gothenburg, Sweden. A stock solution of the respective polymer was formed by dissolving the polymer in TBE buffer (0.4%−1% w/w) by use of a magnetic stirrer for about 20 h, and the solution was then kept in a refrigerator for 3−5 days to ensure complete dissolution of the polymer. Before the measurements, this polymer stock solution was mixed with the YOYO−DNA solution and, if needed, with TBE buffer to yield a solution with the desired polymer concentration (PA18, 0.003%, 0.1%, and 0.4%; PA5, 0.4% and 1.0%) and DNA concentration (0.1−2 μg DNA/mL).

Spectroscopic Measurements and Electrophoresis Cell. Linear dichroism (LD) is defined as differential absorbance for light polarized linearly parallel and perpendicular, respecively, to the electric field direction in the electrophoresis cell. (For reviews of LD, see refs 37 and 38.) The orientation factor $S = (3\langle\cos^2\theta\rangle^{-1})/2$, where θ represents the angle between the local helix axis and the field direction, is obtained from the reduced dichroism value, $LD^r = LD/A_{iso}$, where A_{iso} is the absorbance of the isotropic sample. $S = 1$ in a sample where the molecules are perfectly oriented parallel to the reference direction and $S = 0$ in a sample where they are randomly oriented. In the measurements on the YOYO-stained DNA we have used the light absorption of the long-axis polarized transition moment in YOYO at 490 nm.[39] Measurement at 490 nm was found to give the same result as measurement at 260 nm (linear dichroism of the DNA bases) and has therefore been preferred because of a higher signal-to-noise ratio due to the lower degree of light scattering in the visible wavelength region. The LD has been measured on a Jasco J-500 spectropolarimeter, and the isotropic absorbance on a Cary 2300 spectrophotometer.

As an electrophoresis cell we have used a modified version of a cell specially designed and previously used in our laboratory to measure by LD electrophoretic orientation of DNA in free solution. This cell, which is described in detail in ref 40, has been modified by increasing the distance between the electrodes to 4 cm to allow for longer field pulses without risk for depletion of DNA. The cell is constructed of Perspex, except for the middle part, which forms the zone on which the LD measurements are performed. This part consists of a 2 cm long, open rectangular (0.4 cm × 1 cm) quartz cell (optical pathlength 1 cm). A temperature probe is mounted in the electrophoresis compartment close to this cell. The temperature rise during an electric field pulse was relatively high at the highest field strengths (typically 4 °C for a 2 s pulse at 150 V/cm), and the temperature has varied between 22 and 30 °C in the experiments. This variation in temperature may influence, for example, the dynamics in the orientation, but since no obvious correlation between the measured parameters and the temperature was found, this effect seems to be smaller than the experimental uncertainty in the LD measurements. The sample solution was replaced after about 5−15 pulses (depending on field strength and pulse duration), since a decrease in the absorbance at 490 nm, indicating loss or destruction of YOYO, was observed on samples exposed to a large amount of electric field pulses. Furthermore, subsequent analysis of those samples by pulsed-field gel electrophoresis showed that in some cases degradation of the DNA also had occurred.

The LD was measured during square-formed voltage pulses at field strengths between 3 and 150 V/cm. The rise and decay times of the electric field were less than 1 ms as measured over the cell electrodes. The durations of the pulses were chosen so that they permitted determination of the dynamics of the build-up of the orientation as well as the steady-state orientation. The LD signals were recorded on the oscilloscope card PC-Scope (Capax Instrument AB) using a response time of 2 ms. The waiting time between the electric field pulses was 10 min. The data points presented are averages of measurements in two to four polymer solutions.

Characterization of the Polyacrylamides and the DNA. The polyacrylamides were characterized in terms of overlap concentration, radius of gyration, and the "dynamic pore size" of the entangled network they form at concentrations above the overlap concentration, by using theories for polymers in good solvents. The overlap concentration, c^*, is defined as the concentration at which the polymer coils begin to overlap and is thus expected to be the same as the local concentration inside a single coil:[41]

$$c^* = \frac{M}{\frac{4}{3}\pi {R_g}^3 N_A} \tag{1}$$

where M is the molecular weight of the polymer, R_g is the radius of gyration of isolated chains in a dilute solution ($c \ll c^*$), and N_A is Avogadro's number. The overlap concentration can also be estimated from

$$c^* = \frac{1.5}{K} M^{-a} \tag{2}$$

where K and a are characteristic constants in the empirical Mark−Houwink equation ($[\eta] = KM^a$, $[\eta]$ = intrinsic viscosity) for a given polymer−solvent system.[41,42] For PA in water, K

Table 1. Approximate Values of Overlap Concentration and Radius of Gyration for the Polyacrylamides and the DNA Used in This Study[a]

	c^*	R_g/nm
PA18	0.037% (w/w)	268
PA5	0.104% (w/w)	124
T2 DNA	48 μg/mL	963
YOYO-T2 DNA	33 μg/mL	1094

[a] PA18 and PA5 denote polyacrylamide with molecular weight 18×10^6 and 5×10^6, respectively.

Table 2. Screening Length (ξ) for Entangled Polyacrylamide Solutions of Different Concentrations

c/% (w/w)	ξ/nm
0.1	64
0.4	22
1.0	11

and a have been determined to be $K = 6.3 \times 10^{-3}$ mL/g and $a = 0.8$.[42] Thus, c^* can be calculated from eq 2, and then R_g can be obtained from eq 1.

For comparison, R_g and c^* for the YOYO-stained T2 DNA were also calculated. For a wormlike chain, like DNA, R_g is given by[43]

$$\langle R_g \rangle^2 = 2P_\infty L_c \left[\frac{1}{6} - \frac{P_\infty}{2L_c} + \left(\frac{P_\infty}{L_c}\right)^2 - \left(\frac{P_\infty}{L_c}\right)^3 (1 - e^{-L_c/P_\infty}) \right] \quad (3)$$

where P_∞ is the persistence length and L_c the contour length of the DNA. At the buffer concentration used here (50 mM), P_∞ for native DNA is about 50 nm.[44] For T2 DNA (164 kbp), L_c is 56 μm. Since staining with YOYO (YOYO:DNA base ratio 1:10) increases the contour length by 30% but has no major influence on the flexibility,[34] $L_c = 72$ μm and $P_\infty = 50$ nm were used in the calculations of R_g for the YOYO-stained T2 DNA. c^* was then calculated from eq 1.

For entangled polymer solutions the screening length, ξ, which can be viewed as the average distance between entanglements, has been proposed as a measure of the dynamic pore size of the network formed by the entangled polymer chains.[45,46] This length, which does not depend on the molecular weight of the polymer for $c > c^*$, varies with the concentration according to[41,47]

$$\xi = 0.5 R_g \left(\frac{c}{c^*}\right)^{-3/4} \qquad c > c^* \quad (4)$$

All calculated values are shown in Tables 1 and 2.

The concentration at which a polymer becomes entangled can be determined experimentally by measuring the specific viscosity, η_{sp}, at different concentrations, and plotting log of η_{sp} vs log of concentration. For dilute solutions with noninteracting polymer molecules, theories predict that the slope of such a plot should be approximately 1.0.[46] As the polymer coils begin to interact, the slope is expected to increase. To check if this method gives an overlap concentration that agrees with the theoretically calculated one, η_{sp} of PA solutions of different PA concentrations was determined by comparing the efflux time (t) required for a specified volume of the polymer solution to flow through a capillary tube (Ostwald viscosimeter, thermostated to 25 °C) with the corresponding efflux time of pure buffer solution (t_0). The specific viscosity $\eta_{sp} = (\eta - \eta_0)/\eta_0$ was calculated from $\eta_{sp} = (t - t_0)/t_0$.

Results

Characterization of the Polyacrylamides and the DNA. The results of the theoretical calculations of the radii of gyration and the overlap concentrations of the two polyacrylamides and the DNA are presented in Table 1. At the PA concentrations where the present study has been performed only the 0.003% PA18 solution has a concentration that is well below the overlap concentration. In all the other solutions the polymers thus form more or less entangled networks. The calculations of the screening length of these solutions are shown in Table 2.

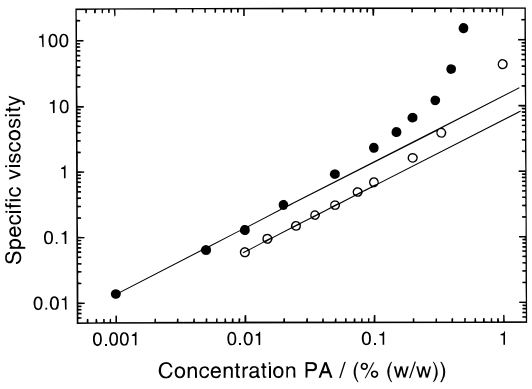

Figure 1. Dependence of the specific viscosity, η_{sp}, of PA18 (●) and PA5 (○) solutions on PA concentration. Deviation from the approximately straight line at the low PA concentrations indicates the overlap concentration, c^* [~0.02−0.06%(w/w) for PA18 and ~0.07−0.2% (w/w) for PA5]; temperature, 25 °C.

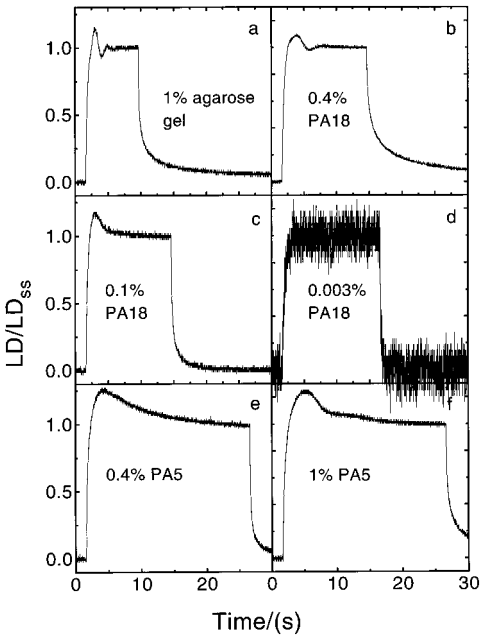

Figure 2. LD signals of YOYO-stained T2 DNA in (a) 1% agarose gel, (b) 0.4% PA18, (c) 0.1% PA18, (d) 0.003% PA18, (e) 0.4% PA5, and (f) 1.0% PA5 upon application of an electric field pulse (20 V/cm). The field-free time before the pulses were applied was 10 min. The LD signals are normalized with respect to their values at steady-state. DNA concentration was 2 μg/mL in b, c, e, and f, 0.2 μg/mL in d, and 13 μg/mL in a.

The dependence of the experimentally determined specific viscosity, η_{sp}, of the PA−buffer solutions on PA concentration is shown in Figure 1. The slopes of the lines passing through the first points are 0.97 and 0.98 for PA18 and PA5, respectively, in agreement with the value of 1 expected from dilute solution theories.[46] Deviation from these lines occurs at concentrations between 0.02 and 0.05% (w/w) for PA18 and between 0.07 and 0.2% (w/w) for PA5, indicating the onset of entanglement in these concentration ranges. This agrees well with the theoretically calculated values in Table 1 ($c^*_{PA18} = 0.04\%$ (w/w), $c^*_{PA5} = 0.1\%$ (w/w)).

Orientation Measurements. Figure 2 shows representative LD responses upon single rectangular field pulses of 20 V/cm applied to buffer solutions containing YOYO-stained T2 DNA and varying amounts of PA18

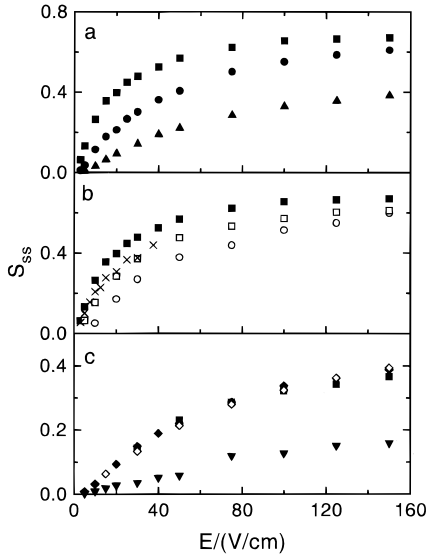

Figure 3. The orientation factor at steady-state orientation, S_{ss}, versus field strength for YOYO-stained T2 DNA in polyacrylamide solutions of different concentrations and molecular weights and, for comparison, in 1% agarose gel (data from ref 34): (a) 0.4% PA18 (■), 0.1% PA18 (●), 0.003% PA18 (▲); (b) 0.4% PA18 (■), 0.4% PA5 (○), 1.0% PA5 (□), 1% agarose gel (×). (c) The DNA concentration dependence of S_{ss} versus field strength is shown for YOYO-stained T2 DNA in 0.003% PA18: 0.1 μg/mL (■), 0.2 μg/mL (◆), 0.5 μg/mL (◇), and 2 μg/mL (▼).

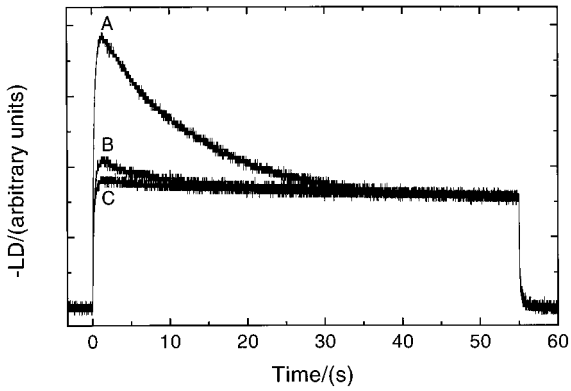

Figure 4. Dependence of LD response of YOYO-stained T2 DNA (2 μg/mL) in 0.003% PA18 on the number of applied field pulses (field strength 30 V/cm). (A) First field pulse, (B) second field pulse (6 min of field-free time), and (C) ninth field pulse (12 min of field-free time).

or PA5, and, for comparison, a corresponding response from the DNA in 1% agarose gel. The contributions from orientation effects of the polymers or the gel were found to be negligible in comparison with the DNA orientation, so the LD responses can be considered to originate entirely from DNA. The LD signals are in all cases negative, showing that the orientation factor S is positive; *i.e.*, the helix axis of DNA is oriented in the direction of the electric field. To better illustrate the difference in orientation dynamics, the signals are normalized with respect to their values at steady-state. For large DNA in agarose gel it is well-known that the orientation exhibits an overshoot followed by a minor undershoot before a steady-state level is reached[34,48−50] (Figure 2a). This behavior has been explained by the oscillatory elongation−contraction motion of reptating DNA, and although the over- and undershoot are seen only in the initial part of the LD response, their presence evidences ongoing cyclic migration, as found by analysis of LD data[51] and observed in fluorescence microscopy studies.[52] The LD response from the DNA in the entangled 0.4% PA18 solution (Figure 2b) is similar to that in agarose gel, displaying an over- and undershoot, indicating that the DNA reptates in this solution in a way very similar to that in the agarose gel. At a concentration of PA18, which is lower (0.1%) but still above the overlap concentration, the undershoot disappears (Figure 2c), and at a concentration (0.003%) which is well below the overlap concentration, neither an overshoot nor an undershoot can be seen (Figure 2d) (at this field strength and DNA concentration). In 0.4% and 1.0% solutions of PA5 (concentrations above the overlap concentration), an overshoot but no undershoot is observed (Figure 2e,f).

Figure 3a shows how the steady-state orientation, S_{ss}, of DNA in entangled (0.4% and 0.1%) and nonentangled (0.003%) PA18 solutions depends on the field strength. At a certain field strength, the steady-state orientation increases with increasing polymer concentration, and for all polymer concentrations, the orientation increases with increasing field strength, although the field strength dependence is different. For the highest polymer concentration, the increase with field strength is strongest at low fields but weakens rapidly at higher fields, and the orientation reaches a plateau level already around 100 V/cm with an orientation factor $S_{ss} \approx 0.7$. In 0.1% PA18, the increase in S_{ss} with field strength is somewhat less, but S_{ss} continues to grow also at higher fields and begins to saturate first at 150 V/cm, where it reaches $S_{ss} \approx 0.6$. At the lowest polymer concentration (0.003%, well below the overlap concentration), the orientation of DNA is smaller than in the entangled polymer solutions, but nevertheless considerable. It increases with increasing field strength and reaches $S_{ss} \approx 0.4$ at 150 V/cm.

In Figure 3b, a comparison is made between the field strength dependence of S_{ss} in the entangled PA18 and PA5 solutions and 1% agarose gel. At the same polymer concentration (0.4%) S_{ss} is higher in PA18 than in PA5, demonstrating that not only the polymer concentration but also the molecular weight of the polymer influences the migration behavior. S_{ss} increases when the concentration of PA5 is increased to 1.0%, but it is still lower than in 0.4% PA18 at corresponding field strengths.

In the nonentangled 0.003% PA18 solution, the steady-state orientation of DNA depends on the DNA concentration if the DNA concentration is larger than 0.5 μg/mL (Figure 3c). Furthermore, the orientational response is dependent on the number of earlier applied field pulses (Figure 4). Even at low DNA concentrations (0.1−0.5 μg/mL) where no dependence of S_{ss} on DNA concentration is noticed, an overshoot is obtained at high electric field strengths (not shown). The overshoot seems to be more pronounced and appear at lower field strengths the higher the DNA concentration is. No dependence on the DNA concentration, neither in steady-state orientation nor degree of overshoot, was observed in the entangled PA solutions for DNA concentrations ≤ 2 μg/mL (the highest DNA concentration used in our studies).

The influence of the field strength on the kinetics of the build-up of the overshoot in the DNA orientation, characterized by the time to reach the overshoot (t_p), is shown in Figure 5a for DNA in the entangled PA solutions and, for comparison, in 1% agarose gel. In the PA solutions as well as in the agarose gel, t_p decreases with increasing field strength (a stronger field pulls the molecule faster through the conformational changes).

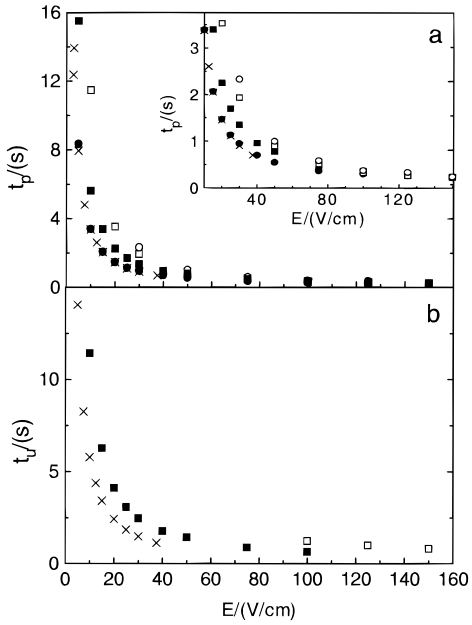

Figure 5. Field strength dependence in (a) the time to the overshoot, t_p, and (b) the time to the undershoot, t_u, for YOYO-stained T2 DNA (2 μg/mL) in 0.4% PA18 (■), 0.1% PA18 (●), 0.4% PA5 (○), 1.0% PA5 (□), and, for comparison, in 1% agarose gel (data from ref 34) (×). Inset: enlargement of the same data.

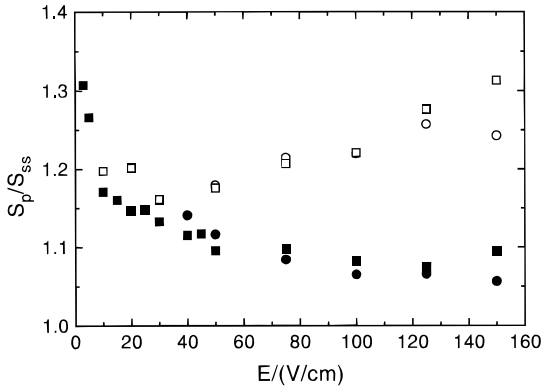

Figure 6. The orientation factor at the overshoot, S_p, relative to that at steady-state, S_{ss}, versus field strength for YOYO-stained T2 DNA (2 μg/mL) in 0.4% PA18 (■), 0.1% PA18 (●), 0.4% PA5 (○), and 1.0% PA5 (□).

At corresponding field strengths, t_p is longer at the higher polymer concentration in the PA18 solutions, but approximately the same in the two PA5 solutions. Comparison between PA5 and PA18 at the same polymer concentration (0.4%) shows that t_p is longer in the solution of the polymer with the smaller molecular weight (PA5).

In 0.4% PA18 solution and in the gel, where the overshoot in the orientation is followed by an undershoot (seen at fields up to 100 V/cm in PA18), the time to reach the undershoot, t_u, is longer in PA18 than in gel (Figure 5b). In both cases t_u decreases with the field in a manner very similar to that for t_p (Figure 5).

The degree of orientation at the overshoot, S_p, depends on the field strength. This dependence in the entangled PA solutions is shown in Figure 6. It is seen that in 0.4% PA18, S_p normalized to S_{ss} is large at low fields and then decreases rapidly with increasing field and reaches a constant value at about 60 V/cm. In 1% PA5, S_p/S_{ss} also decreases at low fields, but from about 30 V/cm, the decrease is turned to a steady increase. In 0.1% PA18 and 0.4% PA5, a reliable quantification of S_p/S_{ss} was possible only at fields above 40 V/cm, but it can be seen in the figure that at these fields the ratio in the respective solution is almost the same as in the corresponding solution with the higher polymer concentration.

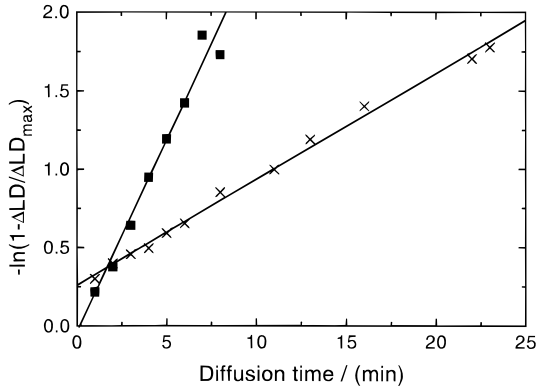

Figure 7. Semilogarithmic plot of the ΔLD recovery for YOYO-stained T2 DNA in 0.4% PA18 (■) and, for comparison, in 1% agarose gel (data from ref 34) (×). The slopes correspond to $\tau_{rec} = 4.1$ min and $\tau_{rec} = 14.2$ min for the polymer solution and the gel, respectively.

For a long DNA in agarose gel, the relaxation of the orientation after the field is turned off contains two phases, one fast and one very slow.[49,51] The fast process is believed to relieve the stretching of the DNA along the path in the reptation tube, leading to a relaxed chain in the still oriented tube.[51,53,54] The slow step has been shown to be well-described by reptation of the chain into an unoriented tube.[49,51,53,54] It can be seen from Figure 2 that the LD relaxation of DNA in the entangled PA solutions (Figure 2b,c,e,f) is very similar to that in the agarose gel (Figure 2a), indicating similar relaxation behaviors. We have not analyzed the fast relaxation but have studied the slow component for the DNA in 0.4% PA18 solution by a technique developed earlier for DNA in agarose gel.[49] If a second pulse of the same polarity and field strength is applied shortly after the first pulse is turned off, the over- and undershoot (peak-to-trough value = ΔLD) in the LD response is much less pronounced than in the response to the first field pulse if this is applied to the DNA in its equilibrium state in the gel. With increasing waiting time between the pulses, ΔLD increases until finally the value (ΔLD_{max}) corresponding to the equilibrium state of the DNA is recovered. In our study on the DNA in the entangled PA18 solution a similar effect was found. The recovery of the ΔLD was analyzed according to ref 49 (gel) by plotting $-\ln(1 - \Delta LD/\Delta LD_{max})$ versus the waiting time, and it can be seen from Figure 7, which shows this plot and a corresponding plot for the DNA in 1% agarose gel, that the ΔLD recovery for the DNA in both the polymer solution and the gel can be described by a single exponential process. The relaxation time, τ_{rec}, calculated from the slope of the lines, is however much shorter in the polymer solution, only 4.1 min compared to 14.2 min in the gel.

Figure 8 shows LD responses of DNA in 0.4% and 0.1% PA18 when the field is rapidly reversed in the middle of the field pulse. In agreement with the behavior in agarose gel, the field reversal first leads to a dip in the LD signal, followed by an overshoot which is smaller and takes a longer time to reach than the overshoot in the initial part of the pulse.

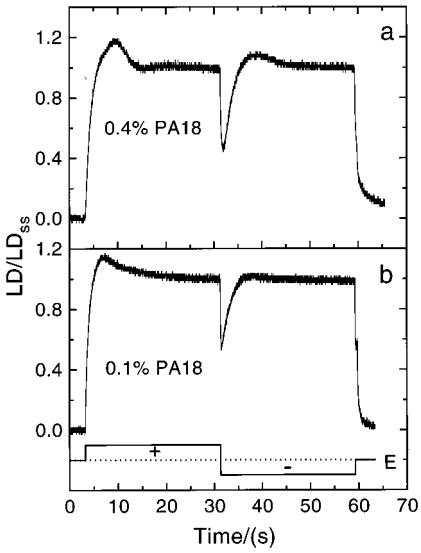

Figure 8. LD responses of YOYO-stained T2 DNA (2 μg/mL) in (a) 0.4% and (b) 0.1% PA18 to an electric field pulse where the direction is reversed after half the time. The step-formed curve in the bottom part of the figure shows turn-on (at 3.3 s), field-reversal (at 31.3 s), and turn-off (at 59.3 s) of the electric field (9 V/cm).

Discussion

In this study, linear dichroism spectroscopy has been used to characterize the migration of DNA during electrophoresis in polymer solutions. Previously, fluorescence videomicroscopy has been used to investigate this migration.[14,27−29] However, even if the microscopy technique gives excellent qualitative information about the global behavior of individual DNA molecules, it is laborious to get accurate quantitative information about the average behavior of all molecules in a sample, and in this respect the LD technique is superior, since it directly and sensitively quantifies this behavior. The time resolution in the video images is low (generally 25 images/s) and at the high field strengths normally used in capillary electrophoresis of DNA (100−200 V/cm), the DNA molecule moves microscopically so fast that it is not possible to obtain any details of the migration. In the studies where the aim has been to observe the conformational behavior of the molecules, the microscopy experiments have therefore been performed either at low fields (<25 V/cm)[14,29] or by decreasing the velocity of DNA by increasing the microviscosity of the polymer solutions by adding a large amount of sucrose.[27,28] LD has a high temporal resolution (~1 ms in the equipment we have used); therefore, it is able to follow the fast orientational dynamics of the DNA also at the high field strengths and in polymer−buffer solutions typically used in CE, which makes comparison with CE more relevant.

The electrophoresis cell used in this study was originally constructed to measure electrophoretic orientation of DNA in pure free solution.[40] The cell has been found to work without measurable effects of electroosmosis or heat convection at experimental conditions similar to those used here.[40] The cross section in the part of the cell where the measurements are performed (0.4 cm × 1.0 cm) is however much larger than the cross section of the thin capillaries utilized in CE (inner diameter typically 50 μm). Molecules close to walls may experience a different microenvironment and thus behave differently than those in the bulk of the solution. The possibility that the average behavior of DNA observed here differs somewhat from that during CE cannot thus be ruled out, since wall effects should have a negligible influence in our wide cell but may have a certain effect in CE due to the high surface-to-volume ratio of the capillaries.

In the entangled PA solutions studied here, the polymer chains form networks with pore sizes (expressed as screening lengths) about 20−100 times smaller than the radius of gyration of the YOYO-stained T2 DNA (Tables 1 and 2). In an agarose gel such small pore sizes relative to the DNA coil size would force the DNA molecules to uncoil and reptate through the gel.[51] At fields where it has been possible to study the migration with fluorescence microscopy (<25 V/cm), the conformational behavior of the DNA in the entangled PA solutions was also found to strongly resemble that observed for reptating DNA in agarose gel.[29] However, the entangled polymers form a much less rigid network than the cross-linked agarose, and one of the aims with this study was to find out if the entanglements are strong enough to withstand the force from the DNA molecules also at high electric fields or if they then rupture so quickly that the molecules never need to uncoil and reptate to be able to migrate through the network.

LD and Oscillatory Reptation. In agarose gel, the presence of over- and undershoot in the build-up of the DNA orientation (Figure 2a) is evidence for oscillatory reptation.[51] The following scenario explains their presence. When the electric field is applied to the DNA in its coiled state (LD = 0) and the molecule begins to migrate, hooking on gel fibers leads to the formation of a U-shaped conformation where both arms of the U move in the field direction (increasing LD). After the arms have reached their maximum extension (the overshoot in LD) the longer arm takes the lead and pulls the shorter arm backward around the hooking point, and the U-conformation changes via a J-conformation to an I-conformation. The tail of the I-conformation then soon catches up the head so that the chain collapses into a coil again (the undershoot in LD). Initially there is a coherence in the stretching and coiling between the molecules, but this is soon lost (due to a wide distribution in the period times[52,55]), and as a consequence of the ensemble-averaging in LD, only the cycle-averaged orientation is observed (steady-state level in LD), even if each molecule continues to oscillate. According to this scenario the time to reach the undershoot, t_u, in LD is equal to the average period time of the oscillation cycle, which has found support in the good agreement between LD values and average period times based on statistics from fluorescence microscopy observations on reptating DNA.[34,52] The time to reach the overshoot, t_p, in the LD is the duration of the phase of the oscillation cycle in which the DNA develops from its coiled state into an overstretched U-conformation; the phase we henceforth will call the extension phase. The difference ($t_u - t_p$) is a measure of the duration of the phase of the oscillation cycle during which the overstretched U-conformations slide off the hooking points and the molecule coils up again, the phase we will call the contraction phase.

Dependence on Polymer Concentration (PA18 Solutions). 0.4% PA18. In this solution the build-up of orientation of DNA exhibits an overshoot followed by a minor undershoot before a steady-state level is reached (Figure 2b). This behavior is seen at all fields up to 100 V/cm, showing that the mode of migration of the DNA up to this field is oscillatory reptation. At

higher fields no clear undershoot is visible, but this does not necessarily mean that the DNA then moves without oscillations in the conformation. The amplitudes of the over-and undershoot are small relative to the total LD signal and decrease gradually with increasing field, and at 100 V/cm the amplitude of the minor undershoot is so small that it is barely seen above the noise in the LD signal, indicating that at higher fields the undershoot may be hidden under the noise. Furthermore, in the field strength interval covered by our measurements (up to 150 V/cm), the trends of the field dependencies in the orientation at the overshoot relative to that at steady-state (Figure 6), and in the time to reach the overshoot (Figure 5a), show no changes around 100 V/cm, or at any other field, that would indicate a change in mode of migration of DNA. This suggests that the polymer network is so robust also at the highest fields that it does not let through the DNA molecules as coils, but instead forces them to uncoil and reptate as in a cross-linked gel.

From fluorescence microscopy observations (low electric fields), the electrophoretic mobility of the DNA in the 0.4% PA18 solution has been found to be approximately the same as in 1% agarose gel.[29] Comparison of the orientational behavior in these two media (at fields up to 40 V/cm; the highest field where measurements have been possible in the gel) shows that this behavior also is very similar (Figures 3b and 5) although the dynamics in the oscillations is somewhat slower (longer t_p and t_u; Figure 5) in the PA solution. t_p and t_u have, however, approximately the same field strength dependence in the polymer solution ($t_p \propto E^{-1.25}$, $t_u \propto E^{-1.23}$) as in 1% agarose gel[34] ($t_p \propto E^{-1.21}$, $t_u \propto E^{-1.26}$). These similarities are surprising in view of the fact that the polymer screening length, which has been proposed as a measure of the pore size in entangled polymer solutions, in the PA18 solution is nearly 10 times smaller (22 nm, Table 2) than the average pore diameter in 1% agarose gel (~200 nm[56]). Such a small pore size would in an agarose gel, with the trends observed at increasing gel concentration,[49] give a much lower mobility and orientation and a slower orientational dynamics than those obtained in the PA solution. This shows that the screening length of the entangled polymers cannot be used as an equivalent to the pore size in a cross-linked gel in the description of the electrophoretic behavior of DNA. The similar behavior in the PA solution and the 1% agarose gel instead indicates that the effective "pore size" in the PA solution is close to that in the 1% agarose gel, *i.e.*, about 10 times the screening length. It is probably so that the DNA molecules in the PA solution, although they reptate as in a cross-linked gel, at the same time are able to deform and drag along the polymer obstacles. Such effects have been used to explain why the mobility observed in tightly entangled hydroxypropyl cellulose solutions is much larger than predicted by reptation models.[14] A detailed analysis of our orientation data (see below) also supports this picture.

It is surprising that the time period t_u of the oscillation cycle is of the same size order and even somewhat longer in the polymer solution than in the gel (Figure 5b). The polymer chains are much closer to each other than the gel fibers, and since the mobility is almost the same in the two media, the DNA molecules should encounter obstacles much more often in the polymer solution than in the gel. They should therefore more frequently get stuck and form coils, and thus also more frequently be forced to repeat the reptation cycle in the polymer solution. However, the elasticity in the polymer network may allow the DNA molecules to migrate a longer time before they get stuck by permitting them to drag along or push away polymer chains. Furthermore, the entanglements between the polymer chains are not permanent like the cross-links in the gel, since the thermal motion of the chains ensures that the entanglements dissociate and renew themselves continuously. This may make it easier, than in a permanent network, for the heads of the migrating DNA molecules to find openings so that the molecules remain in their stretched states a longer time before they collapse into coils.

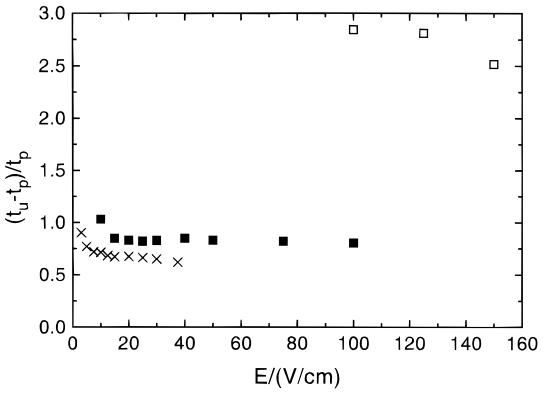

Figure 9. Field strength dependence in the ratio $(t_u - t_p)/t_p$ for YOYO-stained T2 DNA in 0.4% PA18 (■), 1.0% PA5 (□), and in 1% agarose gel (×) (data from ref 34).

If the ratio $(t_u - t_p)/t_p$ is plotted versus field strength (Figure 9), it can be seen that this ratio is about 0.85 in the 0.4% PA solution except at low fields, where it is somewhat higher. A similar trend is observed in the gel (which further confirms the similar behavior of the DNA in the gel and the polymer solution) but the value is consistently lower, about 0.65, at the higher fields. This difference is interesting since the ratio reflects how asymmetrical the oscillation cycle is with respect to the durations of the contraction ($t_u - t_p$) and extension phases (t_p). Ratios <1 means that the duration of the extension phase is longer than the duration of the contraction phase. That a large fraction of the cycle time in agarose gel is used to build up U-conformations has been observed by fluorescence microscopy.[52] The larger $(t_u - t_p)/t_p$ ratio in the polymer solution compared to agarose gel shows that the duration of the contraction phase relative to the duration of the extension phase is longer in the polymer solution. This demonstrates that the contraction phase of the oscillation cycle is more drawn out in the polymer solution, which suggests that it is easier for the heads of the stretched migrating DNA molecules to find openings in a deformable and transient network than in a permanent network, and thus delay the coiling of the molecules. The observation in the fluorescence microscope that the head of the DNA molecule is less bunched in the PA solution than in the agarose gel[29] confirms this interpretation, since a plausible reason for bunching is that the head has difficulties in finding openings in the network. An alternative or complementary explanation to the long contraction phase can be that the stretched U-conformations drag along the polymer chains.

0.1% PA18. At low fields DNA shows oscillatory reptation also in this solution according to the fluorescence microscopy studies, but the extension of the molecule and the periodicity in the oscillations are less

pronounced,[29] which is understandable since the pore size (screening length) of the entangled network is 3 times larger than in the 0.4% solution (Table 2). Furthermore, in contrast to the behavior in gel (and essentially also in 0.4% PA18) the U-conformations were seen to often move downfield before they change to I-conformations, indicating that they drag along polymer chain/chains.[29] This can be explained by the less rigid polymer network due to the longer distances between entanglement points. In our LD responses no undershoot is observed (neither at low nor high fields), but the similar field dependencies in S_p/S_{ss} (Figure 6) and in t_p (Figure 5a), compared with those in the 0.4% solution, indicate that oscillatory reptation is the mode of migration also at higher fields. The absence of undershoot is understandable, since the less periodicity in the oscillations in this solution mean that the molecules come out of phase more rapidly.

The t_p values are shorter than in 0.4% PA18 in spite of the fact that, according to microscopy observations,[29] the hooked U-shaped DNA molecules migrate some distance before they begin to slide off the polymer chains (Figure 5a). The effect of this migration on t_p is probably counteracted by a faster unentanglement of the coiled state since the coil in this loose network can be expected to be less entangled with the polymer chains. A short residence time in the coiled, immobile state and the larger pore sizes (fewer collisions with the network) may also explain the higher electrophoretic mobility in this solution compared to that in the 0.4% solution.[29]

At low fields the steady-state orientation, S_{ss}, is lower than in the 0.4% solution (Figure 3a), but the difference decreases with increasing field strength, since S_{ss} continues to increase at least up to 150 V/cm, whereas S_{ss} in 0.4% PA18 begins to saturate around 100 V/cm. The DNA molecule can only reach a certain degree of maximum orientation with increasing field, which has to occur in the U-conformation of the oscillation cycle, and the overshoot orientation, S_p, will therefore sooner or later saturate. If there is no change in the fraction of the cyclic life of the molecules spent in this high-orientation state, S_{ss} (the average orientation during the cycle) should saturate at the same field as S_p. Figure 6 shows that in the 0.4% PA18 solution the ratio S_p/S_{ss} is almost constant at fields above 100 V/cm, the field where S_{ss} saturates. This shows that S_p also saturates at about 100 V/cm in that solution, thus suggesting that the U-conformations have reached their maximum orientation at that field. In the 0.1% PA18 solution, S_p/S_{ss} is also almost constant at high fields (Figure 6), but since there is no saturation in S_{ss} (Figure 3a), there is no saturation in S_p. This difference in the behavior of DNA in the two solutions is probably due to the different strength of the two polymer networks. In the less concentrated and thus less entangled polymer solution, the network is more easily deformed and the resistance in it will not be built up to the value that is needed for the hooked U-conformations to reach their maximal possible orientation before they begin to slip off the hooking points.

0.003% PA18. Even in this ultradilute solution with a concentration well below the overlap concentration (Table 1), DNA migrates with oscillations between U-, J-, and I-conformations, as observed by fluorescence microscopy at low fields.[29] However, in contrast to the behavior in the entangled solutions, the degree of extension of these conformations was found to be considerably lower and the changes in conformation very irregular. Furthermore, the migration cannot be characterized as reptation since considerable transversal and rotatory motions were observed. Our LD measurements also reveal that the orientation is lower than in the entangled polymer solutions but still considerably high (Figure 3a). The high orientation must be caused by the presence of the polymer chains, because S_{ss} for the DNA in pure buffer is about 100 times lower, as found here (not shown) and in earlier LD measurements.[40] This large enhancement of the orientation caused by the polymer is quite surprising in view of the fact that the average distance between two polymer coils in 0.003% PA18 is about 10 μm and the radius of gyration of the polymer and the DNA only is about 0.3 and 1 μm, respectively. Only a small number of the DNA molecules should therefore simultaneously be involved in collisions with the polymer coils. However, fluorescence microscopy observations have shown that about 30% of the DNA molecules are in U- and J-conformations at a given moment,[29] explaining the high degree of orientation. This indicates that when a DNA molecule has collided with a polymer chain, they will stay together for a while, which indeed is confirmed by the microscopy observations that once a U-conformation is formed it can migrate in that shape for tens of microns.[29] The absence of an over- and undershoot in our LD responses shows a rapid loss of coherence in the oscillatory migration, which is understandable since Brownian motion has a considerable effect on the motion as revealed by the transversal and rotatory motion seen in the microscope.

Dependence on Molecular Weight of the Polymer. The fluorescence microscopy studies showed that the mode of migration of DNA is oscillatory reptation also in the entangled solutions of the shorter PA5 polymer, but the same high degree of reptation-like motion as in 0.4% PA18 was not observed, not even in 1% PA5.[29] Our LD responses show overshoots in both 1% and 0.4% PA5, but undershoots are observed only in 1% PA5 and only at fields $\geq$100 V/cm. However, the field dependence in the orientational parameter t_p is at both concentrations very similar and also similar to that in 0.4% PA18 solution, indicating that the oscillatory reptation seen at low fields in the microscope also is the mode of migration at higher fields.

The time to the overshoot, t_p, is consistently longer in both 0.4% and 1% PA5 compared to in 0.4% PA18 (Figure 5a). The difference at 0.4% is interesting, because in entangled polymer solutions, the dynamic pore size, characterized by the screening length, is independent of the polymer length and only depends on the polymer concentration. Thus, one expects the same orientational behavior, and thus the same t_p, in 0.4% PA5 and PA18. However, the PA5 chain is almost 4 times shorter and thus 4 times lighter than the PA18 chain. Furthermore, at the same concentration of the two chains (same screening length) the number of entanglement points per chain are 4 times less for PA5. Even in 1% PA5, where the screening length is about half of that in the 0.4% solution (Table 2), the number of entanglement points per chain is about 2 times smaller than in 0.4% PA18. The polymer networks of the PA5 solutions are thus less rigid and more easily deformed than the networks of the PA18 solutions. The reason for the longer t_p may therefore be that the DNA molecules in their U-conformations can more easily stretch and drag along the PA5 chains than the PA18 chains. Such an effect finds support in the microscopy

studies, where the U-conformations more frequently are seen to move in the field direction in the PA5 solutions than in the PA18 solutions.[29]

The overshoots are broader in PA5 than in PA18 (Figure 2). A broader overshoot indicates a broader distribution of the cycle times in the DNA sample, which is known from computer simulations to give an earlier loss of the coherency in the oscillations.[55] The fact that undershoots still turn up at high fields in 1% PA5 suggests that the coherency is more pronounced at high fields, which is in accordance with observations made in agarose gel where at high fields the initial over- and undershoot often can be seen to be followed by a second, although smaller, overshoot before the steady-state level is reached.[49]

We have used t_p and t_u in 1% PA5 to form, in the same way as in 0.4% PA18 and agarose gel, the ratio $(t_u - t_p)/t_p$. Figure 9 shows that the ratio is much larger in PA5 (2.7) than in PA18 (0.85) and gel (0.65). The trend in the values shows that the less rigid the surrounding network is, the longer will the contraction phase of the DNA cycle be relative to the length of the extension phase (for interpretation, see "Dependence of Polymer Concentration" above). This indicates that the $(t_u - t_p)/t_p$ ratio in 0.4% PA5 should be even larger than in 1% PA5 (2.7), which most likely is the main reason for the total absence, also at high fields, of undershoots in this solution; the contraction phase lasts so long that the coherency in the cycles has been lost before the molecules begin to coil.

Figure 6 shows that, in the entangled polymer solutions, the ratio S_p/S_{ss} at fields from 50 to 150 V/cm is almost constant in PA18 but grows steadily with the field in PA5. S_{ss} is the average orientation of all conformations that appear during the cycle. Since S_p is the orientation of the stretched U-conformations (the conformations with the highest orientation in the cycle), S_p/S_{ss}, reflects the relative weight of the orientation of these conformations in the average orientation. If they are long-lived and/or have high orientations so their contribution to S_{ss} dominates over those from the other conformations, then S_p/S_{ss} will have a lower value than if the opposite case applies. When the field is increased, the DNA molecules will be pulled faster (shorter t_p and t_u; Figure 5) and with higher average orientation (larger S_{ss}; Figure 3) through the cycle. The fact that S_p/S_{ss} remains almost constant in PA18 indicates that these changes in this case only have a limited effect on the relative life times and orientations of the cycle conformations and thus also on the distribution of the cycle time on the contraction $(t_u - t_p)$ and the extension (t_p) phases of the cycle. This conclusion finds support in the fact that the $(t_u - t_p)/t_p$ ratio (Figure 9) in 0.4% PA18 is constant at high fields within the range of fields where it could be determined (up to 100 V/cm). At low fields $(t_u - t_p)/t_p$ decreases with increasing field until it reaches its constant value, *i.e.*, the contraction phase becomes gradually shorter relative to the extension phase, and Figure 6 shows that this is accompanied by a decrease in S_p/S_{ss}. Is the opposite effect, *i.e.*, an increase in $(t_u - t_p)/t_p$, the explanation for the increase in S_p/S_{ss} in PA5 with increasing field? A comparison between the two ratios is here possible only at fields higher than 100 V/cm and in 1% PA5 ($(t_u - t_p)/t_p$, Figure 9) and this shows that $(t_u - t_p)/t_p$ is constant at these fields in spite of the fact that S_p/S_{ss} increases. This indicates that DNA in this case instead spends, with increasing field, a smaller and smaller fraction of the total cycle time in its overstretched U-conformation.

Dependence on DNA Concentration. The influence of DNA concentration on orientation was studied by varying the DNA concentration in the experiments between 0.1 and 2 μg/mL. In the entangled polymer solution no effects were observed on the orientational parameters. However, in the ultradilute polymer solution (0.003% PA18), both orientation and orientational dynamics depend strongly on the DNA concentration if it is larger than 0.5 μg/mL. At 2 μg/mL, the steady-state orientation is less than half of that at 0.5 μg/mL (Figure 3c). Furthermore, the overshoot in the orientational response decreases with the number of applied field pulses, disappears eventually (Figure 4), and cannot be recovered, not even after 24 h of field free conditions, which indicates that the sample changes irreversibly in some way when exposed to an electric field. We have no explanation to this behavior. All DNA molecules should be able to find polymer coils to interact with since even at the highest DNA concentration (2 μg/mL) there are more than 90 polymer chains per DNA chain in the solution. It is unlikely that it is caused by direct interaction between the DNA molecules since the overlap concentration of YOYO-stained T2 DNA is 33 μg/mL (Table 1), *i.e.*, about 16 times higher than we have used in the LD measurements. Furthermore, the irreversibility in the process indicates drastic changes in the solution. Mitnik *et al.* have observed, with a fluorescence microscope, that DNA in hydroxypropyl cellulose solutions segregates into large aggregates upon application of electric fields, even at DNA concentrations lower than the overlap concentration.[57] The effect was favored by high field strengths, large DNA size, and high DNA concentrations. Segregation may be the reason for the DNA concentration effects in our LD measurements, but to elucidate this further studies are required.

Recovery of ΔLD. The diffusional process leading to recovery of ΔLD was found to be faster for DNA in 0.4% PA18 (relaxation time $\tau_{rec} = 4.1$ min) than in 1% agarose gel ($\tau_{rec} = 14.2$ min). In agarose gel τ_{rec} for the overshoot recovery agrees well with the time, τ_R, it takes for the DNA chain to reptate into an unoriented tube after the field has been turned off,[49] and we will assume here that this also holds in the PA solution. τ_R can be estimated from[58]

$$\tau_R = \frac{L_c^3 b\zeta}{a^2\pi^2 kT} \tag{5}$$

where L_c is the contour length of DNA, b is the length of segments of DNA in an equivalent Rouse chain (equal to two persistence lengths), ζ is the translational friction coefficient for a segment, and a is the mesh size of the network.

The mesh size of the PA network is equal to the screening length of the solution (22 nm; Table 2). However, our experiments have shown that the DNA behavior during electrophoresis in the PA solution is very similar to that in 1% agarose gel, which demonstrates that DNA experiences the "pore size" of the PA network as more similar to the pore diameter of the gel (200 nm) than to the screening length. Is this also the case during diffusion? The ratio between the relaxation times of the DNA in the two media is, according to eq 5 ($\tau_{rec} = \tau_R$):

$$(\tau_{rec})_{PA}/(\tau_{rec})_{gel} = (a_{gel})^2/(a_{PA})^2 \tag{6}$$

With $a_{gel} = 200$ nm and $a_{PA} = 22$ nm, the relaxation time would be nearly 100 times longer in the PA solution than in the gel. Our measurement gives a value that instead is almost 4 times shorter than in the gel, showing that also during diffusion in the PA network the DNA molecule experiences a "pore size" that is much larger than the screening length. Insertion of the measured τ_{rec} values and $a_{gel} = 200$ nm gives $a_{PA} = 370$ nm, which shows that this "pore size" is even larger than during electrophoresis.

The results demonstrate that even though the entanglements between the PA chains persist long enough to form tube-like constraints for the DNA (since DNA reptates), they do not dominate the DNA migration to the degree expected from the short distances between them. Because the chains are only entangled, they can diffuse and disentangle and thus allow motions for the DNA that would be forbidden in a cross-linked network. The lifetime of the "pores" in relation to the drift velocity of the DNA is likely to play an important role in the tube renewal, which explains the apparently larger "pore" experienced by the DNA during diffusion compared to that experienced during electrophoresis.

Reorientation. The reorientation dynamics upon changing the field direction for DNA in 0.4% and 0.1% PA18 was also studied (Figure 8). When the field is reversed, the LD in both cases decreases rapidly, whereupon it increases and, in 0.4% PA18, passes through a maximum (smaller than in the first pulse) before a steady-state level of approximately the same magnitude as in the first field pulse is reached. The behavior in 0.4% PA18 is similar to that obtained in agarose gel,[49] except that in the PA solution no undershoot is seen in the second pulse. As in the gel, it takes a longer time to reach the overshoot in the second than in the first field pulse, indicating that when the field direction is switched, the arms of the extended U-conformations first retreat back along their stretched paths, forming a globular coil before they can reextend in the new field direction. Furthermore, fluorescence microscopy observations have shown that, after the collapse to a globular conformation, the DNA usually moves for some time in this conformation before reextending into a new U-conformation.[28]

The reorientation behavior is thus very similar to that in gel, which may explain why field inversion electrophoresis also works in CE.[21−23]

Conclusions

This study has been focused on the migration behavior of DNA during electrophoresis in polymer solutions, knowledge of which is important for understanding the separation mechanism in capillary electrophoresis. By using linear dichroism (LD) spectroscopy, the orientation and orientation dynamics of a long DNA (164 kbp) in solutions of linear polyacrylamide have been quantified for field strengths up to 150 V/cm. In a 0.4% solution of polyacrylamide with molecular weight $= 18 \times 10^6$ (overlap concentration $\approx 0.04\%$), the LD response to an electric field pulse shows an over- and undershoot in orientation before a steady-state level is reached. This shows that DNA in an entangled polymer solution (transient network) migrates in an oscillatory motion in a similar way as it does in agarose gel (permanent network). At 150 V/cm the DNA orientation is extremely high; the orientation factor S is on average during the oscillation cycle almost 0.7 ($S = 1$ corresponds to all molecules being stretched to their full contour lengths and completely aligned with the electric field).

In entangled polymer solutions, the dynamic pore size, taken as the screening length, depends on the polymer concentration only and is thus independent of the molecular weight of the polymer. In spite of this, the LD responses are clearly different in polymer solutions with the same concentration but different molecular weights of the polymer. The polymer with the higher molecular weight gives a behavior more similar to the reptation behavior in gel, which indicates that longer polymer chains form a more rigid network than shorter ones. Also, the duration of the contraction phase of the oscillation cycle (during which the extended U-conformation contracts into a coil) relative to the duration of the extension phase (the coil extends to a U-conformation) was found to depend on the polymer molecular weight. For example, this ratio was 2.7 and 0.85 in polyacrylamide solutions of molecular weight 5×10^6 and 18×10^6, respectively, showing that the contraction phase of the DNA lasts longer relative to its extension phase when the surrounding network is less rigid (in 1% agarose gel the ratio is 0.65). This is important knowledge when choosing pulse times in field inversion capillary electrophoresis.

The degree of reptation-like motion and the degree of orientation decreases as the polymer concentration decreases. However, even very small amounts of polymer have a strong influence on the orientation of the DNA. For example, in ultradilute polyacrylamide solution (0.003%, MW $= 18 \times 10^6$), the orientation factor is about 100 times higher ($S \approx 0.3$) than in pure buffer solution.

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