Affinity Gel Electrophoresis of DNA

Björn Åkerman*

Contribution from the Department of Physical Chemistry, Chalmers University of Technology, S-412 96 Göteborg, Sweden

Received December 1, 1998

Abstract: The principles of DNA affinity gel electrophoresis are investigated experimentally using velocity and linear dichroism spectroscopy measurements. As a model system we use 1% agarose gels covalently modified with either ethidium bromide between 0 and 30 μM or biotin using the same immobilization chemistry. The method allows the immobilized ethidium bromide to interact with the double-stranded DNA by an intercalation type of binding leading to a well-defined DNA−matrix interaction which is reversible, whereas biotin captures avidinated DNA irreversibly. At 1 μM immobilized ethidium bromide T2 DNA undergoes a weakly perturbed version of the cyclic reptation which is typical of unmodified gels and the velocity is retarded by 35%. At 10 μM the velocity is retarded by 80% and the mode of migration is strongly perturbed. In both cases the DNA becomes strongly field-aligned due to the transient affinity anchoring to the gel which also causes the velocity retardation. Some fundamental aspects of affinity electrophoresis are studied. The affinity effect on the migration disappears when the field force is strong enough to overcome the summed DNA−gel interactions, which indicates that migration is slower than in unmodified gels because a fraction of the applied electric force is used to overcome the attraction between DNA and affinity label. Second, DNA migration processes are retarded if they occur on time scales similar to the dissociation time of the DNA−gel affinity complex, whereas processes which are much slower are unaffected. Finally, irreversible capture of end-avidinated DNA shows that the long DNA used here encounters the affinity label with high efficiency, perhaps through a directed search by sliding around the labeled gel fibers.

Introduction

Motion through gels is commonly used for the analysis of the size and structure of DNA and proteins. Additional separation motifs can be introduced by modifying the gel matrix with affinity labels for the macromolecule of interest. This approach is common in chromatography, but the potential of affinity electrophoresis[1] has been exploited to a much lesser extent, especially in studies of DNA. Polyadenine-modified agarose gels were used to enhance separation according to base composition[2], and ethidium bromide immobilized in polyacrylamide gels was used to modulate separation according to size[3] or tertiary structure.[4] More recently biotin-modified gels have been used to capture avidinated DNA electrophoretically,[5] and gels containing immobilized DNA[6] or PNA[7] have been used to sequence-distinguish oligonucleotides or to capture double-stranded DNA by triple helix capture.[1] In view of these promising applications of affinity electrophoresis, a mechanistic understanding of the electrophoretic migration of DNA in affinity-modified gels is desirable in order to design more optimal separation procedures. Several of the previously used systems are less suitable for such studies because the structure of the immobilized probe is poorly characterized[3,4,7] or the interaction of the probe with DNA is not fully understood.[2,6] By contrast, the triazine bridging protocol used to immobilize biotin[5] gives a well-defined gel adduct[8] and the interaction with the avidin on the DNA is well studied.[9] The biotin−avidin binding is essentially irreversible, however, whereas a reversible DNA−gel affinity has the obvious advantage that the retained component can be eluted after separation. A model system based on a label with a well-characterized reversible DNA interaction was therefore a desirable complement to the irreversible biotin−avidin capture system.

Ethidium bromide (EB) is a monovalent cationic dye which intercalates between the base pairs of the DNA helix, a well-understood reversible process.[10] We have recently reported that ethidium bromide immobilized to agarose by the triazine chemistry can interact in a manner very similar to the intercalation between free EB and DNA.[8] Since both the thermodynamic and the kinetic properties of the EB−DNA complex have been studied in detail in solution,[11] immobilized EB provides a good model system for mechanistic studies of affinity electrophoresis. Here we use such gels to study fundamental aspects of the motion of DNA in affinity-modified gels. We investigate the role of both stability and dynamics of the DNA−matrix complex and determine to what extent the migration is different from that in unmodified (native) gels. The focus is on DNA molecules which are long enough to undergo end-on motion, so-called

* Author to whom correspondence should be addressed. Phone: 46317723052. Fax: 46317723858. E-mail: baa@phc.chalmers.se.

(1) Ito, T.; Smith, C. L.; Cantor, C. R. *Genet. Anal.: Tech. Appl.* **1992**, *9*, 96−99.
(2) Yashima, E.; Suehiro, N.; Miayuchi, N.; Akashi, M. *J. Chromatogr.* **1993**, *654*, 151−158.
(3) Flint, D. H.; Harrington, R. E. *Biochemistry* **1972**, *11*, 4858−4864.
(4) Stellwagen, A.; Stellwagen, N. C. *Biopolymers* **1990**, *30*, 309−324.
(5) Åkerman, B. *J. Chem. Soc., Chem. Commun.* **1996**, 661−662.
(6) Baba, Y.; Sawa, T.; Kishida, A.; Akashi, M. *Electrophoresis* **1998**, *19*, 433−436.
(7) Igloi, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 8562−8567.
(8) Åkerman, B. *Bioconjugate Chem.* Submitted for publication.
(9) Bayer, E. A.; Wilcheck, M. *Methods Enzymol.* **1990**, *184*, 49−67.
(10) Bloomfield, V. A.; Crothers, D. M.; Tinoco, I. In *Physical Chemistry of Nucleic Acids*; Harper & Row: New York, 1974.
(11) Wilson, W. D.; Krishnamoorthy, C. R.; Wang, Y.; Smith, J. C. *Biopolymers* **1985**, *24*, 1941−1961.

10.1021/ja984154e CCC: $18.00
Published on Web 07/29/1999

reptation, for which a good understanding of the migration in native gels has been gained in the past decade.[12−15] The present approach can easily be extended to studies of smaller DNA, because the triazine chemistry can also be applied to hydroxyethylated agarose, which has pore sizes[16] comparable to the polyacrylamide gels commonly used for small DNA. We also report some work based on the irreversible biotin−avidin system,[17] which was used in order to design more well-defined experiments on how the DNA encounters the affinity label and how the DNA conformation is affected by the affinity interaction.

Materials and Methods

Chemicals. Agarose gels (Biorad, DNA-grade, and low melt temperature-grade) were 1%. T2 DNA (166 000 base pairs), cyanuric chloride (2,4,6,-trichloro-1,3,5-triazine), ethidium bromide, and DAPI from Sigma, λ-DNA from Pharmacia, biotinylated DNA oligo 5′-GGGCGGCGACCT-Biotin-3′ from Medprobes, biotin-ethylenediamine, streptavidin, and streptavidin-conjugated fluorescein from Molecular Probes, and T4 ligase from Boehringer were all used as received. DNA concentrations in nucleotides are determined by absorption at 260 nm using the extinction coefficient 6600 M^{-1} cm^{-1}. Oxaxole yellow (YO) was a kind gift of Per Lincoln, Chalmers University of Technology. If not otherwise stated the experiments have been performed in TBE buffer (50 mM Tris, 50 mM borate, 1.25 mM EDTA, pH = 8.2) at 20 °C and with native (nonstained) DNA.

Affinity Modification of Gels. Agarose gels (1%) were covalently modified with ethidium bromide (EB) and biotin as described in detail elsewhere.[8] Briefly[5] amino groups on the affinity label are rendered reactive toward hydroxyl groups on the agarose gel fibers by activation with cyanuric chloride. In the case of EB modification, activation was performed at a 1:2 stoichiometric ratio of cyanuric chloride to EB, which promotes monofunctional attachment of the EB to the gel,[8] whereas biotin was activated at a 1:1 ratio. After reaction with gel samples cut in approximately 5 mm sized pieces, nonreacted affinity label was removed by thorough washing in electrophoresis buffer. Such stock gels were diluted to the desired degree of affinity modification by remelting the pieces and mixing with the appropriate volumes of liquid (50 °C) 1% unmodified agarose.

Tethering of DNA. (A) Biotinylation of DNA. Biotinylated 12 base oligomers were hybridized to the 12-base single-strand overhangs at the cos-L site in one end of the λ-DNA. Ten micrograms (0.31 pmol molecules) of λ-DNA in 100 mM Tris HCl/10 mM $MgCl_2$ (pH 7.5) was heated at 65 °C for 5 min to break up λ-oligomers formed due to the complementary overhangs. Then 2 μL (100 pmol) of biotinylated oligo was hybridized for 1 h at 37 °C to overcome the activation barrier for the 12-base hybridization.[17] The hybrids were ligated by addition of 4 units of T4 ligase in a total of volume of 60 μL and incubation for 40 min at 37 °C. The ligation mixture was diluted with 1.5 mL of 1% low-melt agarose (at 40 °C) in electrophoresis buffer, to give a final DNA concentration of 6.7 μg/mL. Cylindrical agarose plugs 5 mm in diameter (0.1 μg of DNA/mm) were formed by solidification of the DNA−gel mixture in plastic tubing. The gel plugs serve to protect the DNA from shear degradation and as convenient DNA containers since the λ-DNA in practice is immobilized in the plugs due to its very slow diffusion in gels.

(B) Avidination and Immobilization of DNA. For DNA avidination, the gel plugs were first incubated in large excess of 1 M NaCl for 2 h. This step removes nonhybridized biotin-oligo, and the high salt concentration reduces the nonspecific binding of streptavidin.[9] The biotinylated DNA was avidinated by incubating the gel plugs at 20 °C for 24 h, in 3 mL of 1 M NaCl (pH = 7.5) to which 23 μg (1μg/μL in phosphate buffered saline) of streptavidin had been added (100-fold excess of streptavidin molecules over DNA ends). The streptavidin is small enough to penetrate into the gel plugs and bind to the DNA-bound biotin. Finally the gel plugs are washed in electrophoresis buffer (0.5× TBE) for 24 h in order to remove excess streptavidin. The net yield of avidination was found to be 0.41 ± 0.05, by electrophoretic capture in biotinylated gels.[8] The avidinated DNA was tethered by electrophoretic migration through the biotinylated agarose (*5*).

Spectroscopy. Absorption and turbidity spectra of modified gels formed in standard cuvettes were recorded on a Cary spectrophotometer, as described elsewhere.[8] Linear dichroism (LD) spectroscopy was performed in a previously described[18] vertical electrophoresis cell, containing a frame gel of unmodified agarose in which a 4 cm long insert of modified gel was formed (both 1% agarose). One well was formed in the modified gel and one in the frame gel, which was used for reference measurements on DNA in unmodified gel. In the case the insert contained EB, identical liquid samples of native (unstained) T2 DNA were loaded in the two wells. With biotinylated inserts the gel plugs with streptavidinated λ-DNA were put in the sample well and nonavidinated DNA in the nonbiotinylated gel. The DNA was introduced in the gels by electrophoresis, until the DNA had reached a suitable measuring position for LD. For EB gels this was determined by scanning the gel at 260 nm to find the DNA peaks in the two lanes. For biotin gels the position of the tethered DNA is determined by the position of the biotinylated insert, and in this case electrophoresis was continued until the zone of unsuccessfully avidinated DNA was observed well below the insert (again by scanning at 260 nm). Since the DNA was tethered at the first encountered (i.e., top) part of the insert, the biotinylated gel was covered with a layer of agarose in order to ensure that the zone of tethered DNA is not too close to the interface between the gel and the liquid in the well.

LD can be used to measure the average degree of DNA−helix orientation in terms of an orientation factor S which is related to the angle θ between the helix axis and the field direction[14]

$$S = \langle 3\cos^2\theta - 1\rangle/2 \qquad (1)$$

S is equal to 1 if all molecules are perfectly aligned with the field ($\theta = 0°$), and for a random orientation, $S = 0$. The orientation factor reflects both the degree of alignment of the path of the DNA between the gel fibers (the so-called reptation tube) as well as how much the DNA is stretched along this path,[19] and should be viewed as a time-dependent function $S(t)$. The field-free decay of the DNA alignment was analyzed as described earlier.[16]

Results

DNA Mobility in EB Gels. Figure 1 (squares) shows how the electrophoretic velocities of T2 DNA molecules are affected by gel-bound EB. The DNA is retarded to an extent which is stronger the higher the concentration of gel-bound dye. The turbidity of the gel at 600 nm (which is outside the EB absorption band) was used to monitor potential effects of the EB modification on the gel structure.[8] The turbidity data in Figure 1 (circles) show that EB modification does perturb the gel structure, but to a detectable extent only above about 10 μM. It is thus clear that the bound EB retards DNA also when the gel structure is not perturbed.

The Cases of Strong and Weak DNA−Gel Interactions. To gain a better understanding of the mechanism behind the DNA retardation caused by the immobilized EB, we studied the DNA conformation dynamics during electrophoresis by linear dichroism. In Figure 2 the LD response of T2 DNA to a

(12) Lumpkin, O. J.; Déjardin, P.; Zimm, B. H. *Biopolymers* **1985**, *24*, 1573−1593.
(13) Slater, G. W.; Noolandi, J. *Biopolymers* **1986**, *25*, 431−454.
(14) Nordén, B.; Elvingson, C.; Jonsson, M.; Åkerman, B. *Q. Rev. Biophys.* **1991**, *24*, 103−164.
(15) Viovy, J.-L. *Mol. Biotechnol.* **1996**, *6*, 31−46.
(16) Åkerman, B. *Phys. Rev. E.: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.* **1996**, *54*, 6697−6707.
(17) Wang, J. C.; Davidsson, N. *J. Mol. Biol.* **1966**, *15*, 111−123; *19*, 469−482.
(18) Jonsson, M.; Åkerman, B.; Norden, B. *Biopolymers* **1988**, *27*, 381−414.
(19) Åkerman, B. *Electrophoresis* **1996**, *17*, 1027−1036.

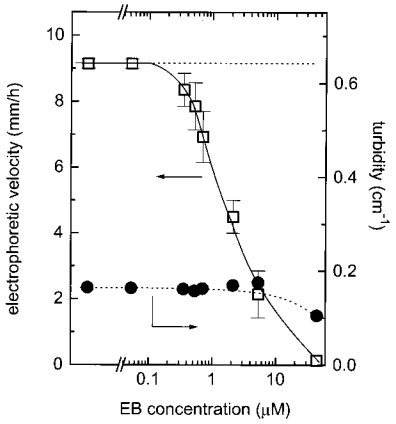

Figure 1. Electrophoretic migration of T2 DNA in ethidium bromide-modified agarose gel: squares, DNA velocity at 5 V/cm vs concentration of bound ethidium bromide in 1% agarose; circles, gel turbidity at 600 nm. Notice that the single data point to the left of the axis break corresponds to unmodified gel. Bars indicate the approximate widths of zones. DNA concentration: 20 μM phosphate.

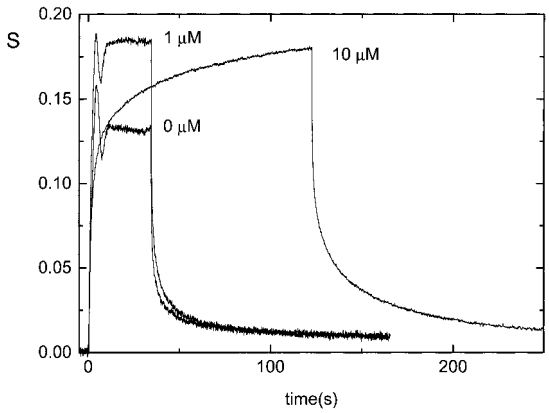

Figure 2. Orientation S of T2 DNA vs time at indicated concentrations of immobilized ethidium bromide in 1% agarose. The electric field of 7.5 V/cm was applied at time zero and turned off after 35 s (0, 1 μM) or 130 s (10 μM). DNA concentration: 120 μM phosphate.

pulse of constant electric field in the presence of 1 or 10 μM of immobilized EB is compared to the response observed in unmodified gel. The orientation factor *S* is seen to be positive in all three cases, which shows that the DNA molecules in the modified gels are preferentially aligned along the electric field as is the case in nonmodified agarose.[20] At 1 μM EB the response grows in an oscillatory manner similar to the behavior in the unmodified gel, whereas at 10 μM EB the LD grows monotonically and much more slowly than in the reference gel. The degree of steady-state orientation is seen to be quite similar at the two degrees of modification and significantly higher than in the reference gel. Finally, the field-free LD relaxation is again considerably slower with 10 μM than with 1 μM EB, where the decay is similar but not identical to that in the reference gel. Taken together these orientation characteristics indicate that the migration behavior at 1 μM EB is similar albeit not identical to that in nonmodified gel, whereas the migration is strongly affected at the higher degree of modification. The two cases were therefore studied in more detail, as representative cases of weak and strong interactions between DNA and gel. Figure 1 shows that the two conditions correspond to the DNA being retarded by 35% and 80%, respectively, compared to the reference gel.

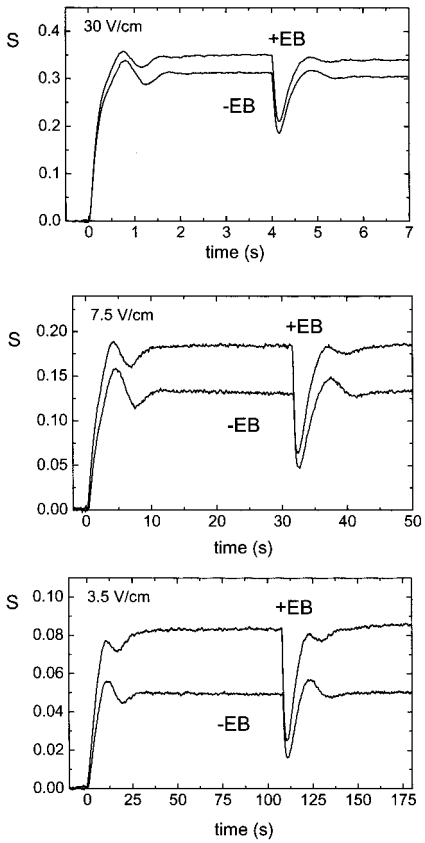

Figure 3. Orientation responses $S(t)$ of T2 DNA to onset of field (time zero) and reversal of field (after 4, 32, and 110 s from top to bottom) at indicated field strengths. Nonmodified (−EB) or 1 μM ethdium bromide immobilized (+EB) in 1% agarose gel. DNA concentration: 120 μM phosphate.

DNA Dynamics with Weak Affinity. The migration at 1 μM EB was investigated by measuring the LD response of T2 DNA at field strengths between 3 and 30 V/cm. In addition to the response to the onset of a field (as in Figure 2), the response if the field direction is reversed in the steady state was also investigated. Both types of experiments have been helpful in understanding DNA migration in native agarose gels.[19−21] Figure 3 presents responses (+EB) at three representative field strengths (3.5, 7.5, and 30 V/cm) together with the corresponding responses in the reference gel (−EB). The build-up of orientation from equilibrium is oscillatory at all three field strengths, but the overshoot is less pronounced in the presence of EB than in the unmodified gel. At the lowest field strength, the maximum is even below the steady-state level, but this deviation from the reference response becomes progressively less pronounced as the field is increased. The response to a field reversal is again similar in nature in the modified and unmodified gels. There is a deep orientation minimum followed by an overshoot, and again there is a pattern with a less pronounced overshoot especially at low fields. Interestingly, even though the immobilized EB alters the magnitude of the overshoot, it has very little effect on how fast the overshoot occurs. Figure 4 shows that the time t_p to reach the overshoot after the field is applied is the same with or without EB within experimental uncertainty (size of symbols). The inset shows that in both gels t_p follows the power law dependence $E^{-1.2 \pm 0.1}$ in the field as has been reported earlier for T2 DNA in native gels.[20−22]

(20) Åkerman, B.; Jonsson, M.; Norden, B.; Lalande, M. *Biopolymers* **1989**, *28*, 1541−1571.

(21) Platt, K. J.; Holzwarth, G. *Phys. Rev. A.: At., Mol,. Opt. Phys.* **1989**, *40*, 7292−7300.

(22) Mayer, P.; Sturm, J.; Weill, G. *Biopolymers* **1993**, *33*, 1347−1357.

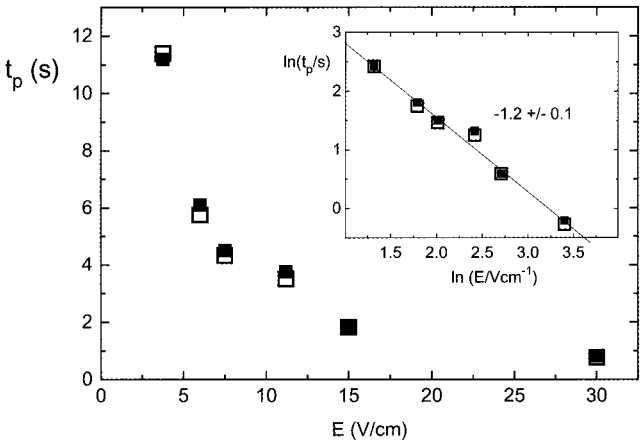

Figure 4. The time to reach the orientation overshoot for T2 DNA (from Figure 3) vs field strength with 1 μM (open) and 0 μM (closed) immobilized ethidium bromide. The inset shows a double logarithmic plot of the data. The least-squares linear fit has a slope of -1.2 ± 0.1 in both cases.

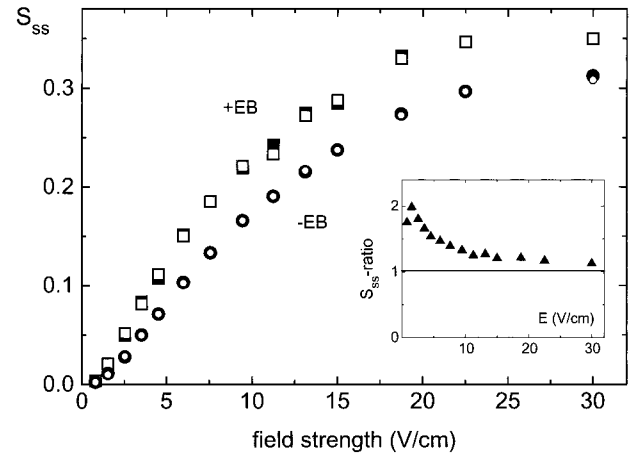

Figure 5. The steady-state orientation S_{ss} (from Figure 3) of T2 DNA vs electric field strength with 1 μM (squares) and 0 μM (circles) of immobilized ethidium bromide, with two independent data sets at each concentration (open and closed symbols). The inset shows the ratio $S_{ss}(+EB)/S_{ss}(-EB)$ with and without ethdium bromide.

Figure 3 also shows that the steady-state degree of orientation (S_{ss}) is higher in the presence of EB at a given field strength, but also in this respect, the difference tends to decrease with increasing field strength. This is more clearly seen from the collected data from all studied field strengths (Figure 5). The main part of the figure shows that S_{ss} increases in a sigmoidal fashion with increasing field strength in both gel types. The ratio $S_{ss}(+EB)/S_{ss}(-EB)$, however, decreases from a value of about 2 at low fields to an asymptotic value close to 1 at high fields (Figure 5, inset, solid symbols). This shows in a quantitative way how the migration in the two gels becomes more and more similar (at least in terms of S_{ss}) as the field becomes stronger, in an interesting parallel with the overshoot behavior becoming more similar too.

DNA Dynamics with Strong Affinity. Figure 6 shows the LD response of T2 DNA to field pulses of four different strengths at the higher degree of EB modification (10 μM). The lack of overshoot is observed at all studied fields, and the build-up kinetics was well-described by two exponentials with the time constants τ_1 and τ_2 given in Figure 7, panels a and b, respectively. The fast component is about 10 times faster than the slow and occurs on the same time scale as the overshoot time at 1 μM EB (Figure 4). The double-logarithmic plot in

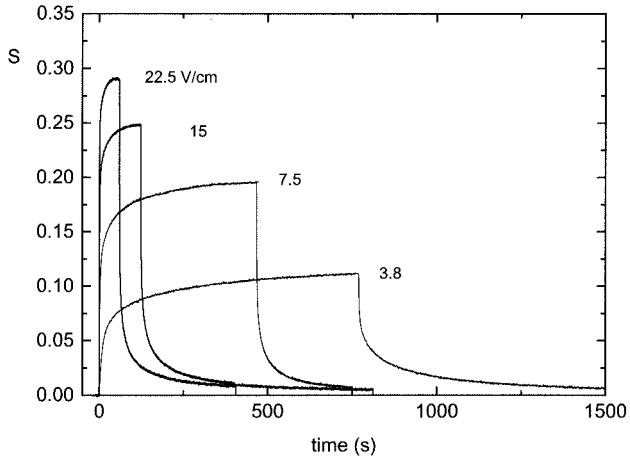

Figure 6. Orientation responses $S(t)$ of T2 DNA to onset of field (time zero) of indicated strengths; 10 μM of ethdium bromide immobilized in 1% agarose gel. DNA concentration: 120 μM phosphate. The field was turned off after (top to bottom) 60, 120, 470, and 770 s, respectively.

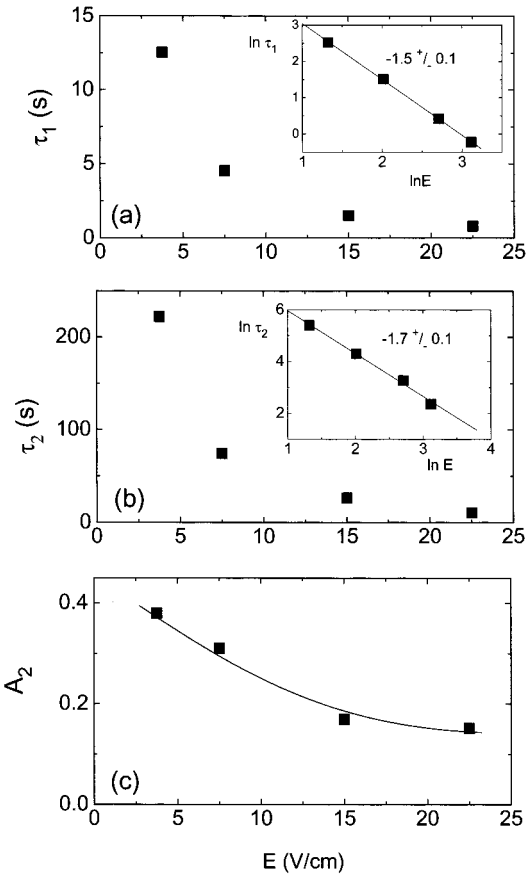

Figure 7. Analysis of build-up kinetics in Figure 6. Fast rise time (a), slow rise time (b), and relative amplitude A_2 (relative S_{ss}) of the slow component vs field strength (c). The insets in a and b show semilog-arithmic plots of data. The least-squares linear fits have slopes of -1.5 ± 0.1 (a) and -1.7 ± 0.1 (b).

the insets of Figure 7a,b shows that τ_1 and τ_2 follow approximate powerlaw dependencies $E^{-1.5 \pm 0.1}$ and $E^{-1.7 \pm 0.1}$, respectively. The relative amplitude A_2/S_{ss} of the slow component decreases with increasing field, as shown in Figure 7c.

Figure 8 shows the field-free decays of orientation at 7.5 V/cm from Figure 2, normalized with respect to the steady-state orientation (S_{ss}) at the time of field removal for the sake of comparison. It is seen that the rate of relaxation in the presence

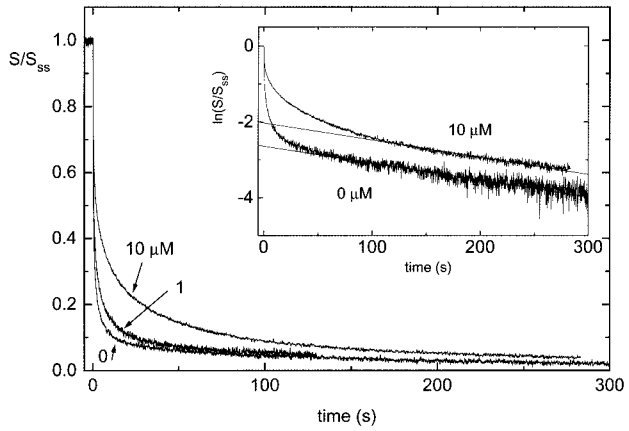

Figure 8. Field-free decay of T2 DNA starting from steady-state orientation at 7.5 V/cm, with 0, 1, and 10 μM immobilized ethidium bromide in 1% agarose gel; a plot of S normalized to the steady-state orientation S_{ss}. Inset: Semilogarithmic plot of data at 0 and 10 μM ethidium bromide. The least-squares best fits at long times have slopes 220 ± 2 s (10 μM) and 224 ± 2 s (0 μM). Intercepts at time zero of linear fits give relative amplitudes A_3 for the monoexponential component of 0.13 (10 μM) and 0.07 (0 μM).

of 10 μM EB is considerably slower than in the reference gel and that at 1 μM EB the relaxation is intermediate between those at 10 and 0 μM. For a more quantitative analysis, a semilogarithmic plot of the decays at 10 and 0 μM (inset) shows that the decay is not monoexponetial in either case, confirming earlier results in the case of nonmodified gel.[22] For long times the decay is well described by a single exponential, however, and the time constants evaluated from the slopes are very similar, 220 ± 2 s in the presence of EB and 224 ± 2 s in the unmodified gel. The effect of the immobilized EB is thus to slow down the faster parts of the relaxation, whereas it has negligible effect on the long-term relaxation which corresponds to end-on motion (reptation) of the DNA (see discussion). The amplitude A_3 of the slow process relative to S_{ss} (using the notation of ref 16) is 0.13 and 0.07, as determined from the zero-time intercepts of the straight-line fits. The remaining relative relaxation amplitude $(1 - A_3)$ corresponds to a much faster destretching relaxation process,[22] and its absolute value $S_{stretch} = (1 - A_3)S_{ss}$ is a measure of the degree of stretching of the DNA along its path between gel fibers in the steady state. Using S_{ss} values from Figure 5 gives $S_{stretch}(+EB) = 0.152$ and $S_{stretch}(-EB) = 0.121$; that is, DNA is more stretched in the presence of EB than without.

Affinity Capture of Avidinated DNA in Biotinylated Gels. DNA that is end-modified by streptavidin can be captured irreversibly by electrophoresis through a biotin-modified agarose gel.[5] Some experiments with this extreme case of affinity electrophoresis were included here for two reasons. The irreversible nature of the affinity means that the resulting DNA distribution in the gel reflects the first successful encounter between DNA and label and not the average effect of many such encounters. Second, the orientation behavior of permanently tethered DNA is helpful in interpreting the orientation dynamics of DNA in the EB gels. End-tethering gives a more well-defined anchoring situation than a DNA molecule which is attached to the gel at several points at unknown positions along the DNA.

Figure 9a shows a scan of the DNA distribution in a gel after electrophoretic capture of avidinated λ-DNA in a biotin insert.[5] The tethered DNA (T) is found as a zone in the upfield part of the biotinylated insert (arrow), whereas the free-moving DNA molecules (F), which were not successfully avidinated, are found

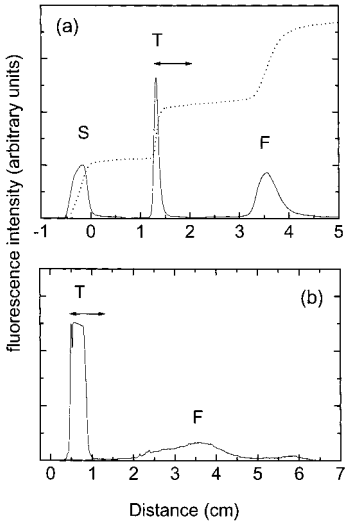

Figure 9. (a) Electrophoretic capture of streptavidinated λ-DNA in biotinylated gel: scan of fluorescence vs distance after YO staining of gel after electrophoresis for 7 h at 2.6 V/cm. The positions of sample plugs (S), biotinylated gel-insert (double-arrow), and zones of tethered (T) and free-moving (F) DNA are indicated. The insert contains 200 nM gel-bound biotin. The 5 mm long sample plugs originally contained 0.7 μg of avidinated λ-DNA at a concentration of 20 μM phosphate. A 45% yield of end-avidination was calculated from the integrated intensity (dotted) of T and F. (b) Electrophoretic capture of streptavidin−fluorescein conjugate in biotinylated gel insert: scan of fluorescence vs distance of fluorescein fluorescence after electrophoresis for 4 h at 3 V/cm. The positions of biotinylated gel-insert (double-arrow) and zones of captured (T) and free-moving (F) streptavidin are indicated. The insert contains 430 nM gel-bound biotin, and the loaded amount of streptavidin was 5 μg. In both a and b, electrophoresis started at distance = 0.

downfield from the insert. Some DNAs remain at the position of the sample gel plugs (S), most likely because they were trapped during the formation of the gel plugs. For our present purposes the most important observation in Figure 9a is that the avidinated DNA is captured in the first encountered part of the insert with essentially no smear in the downfield part of the insert and that the zone is narrow compared to that of the free-moving DNA. Figure 9b shows that a similarly sharp zone is formed by streptavidin itself (modified with fluorescein for visualization) after electrophoresis through a biotinylated insert (arrow) formed in the same way. The streptavidin was loaded without gel plugs, and therefore, no sample was left in the well (distance = 0).

Alignment of Permanently Tethered DNA. The conformation dynamics of tethered DNA was studied by performing LD measurements on the zone of immobilized DNA in the biotinylated insert (T in Figure 9a). Figure 10a shows the orientation response of tethered and free-moving DNA in an agarose gel when a field of 15 V/cm is turned on with the molecules in the equilibrium state. The responses have been normalized with respect to their respective steady-state values S_{ss}, which are given in Figure 10b. It is seen that the free λ-DNA (F) responds with an orientation overshoot, albeit less pronounced than for T2 DNA because λ-DNA is shorter,[21] whereas the tethered DNA (T) exhibits no oscillations. As expected both free-moving and tethered DNA are aligned with the field ($S > 0$), but the tethered DNA is considerably more aligned, as seen by the higher value for S_{ss} at any given field strength (Figure 10b). Figure 10c shows that the ratio is as high as 9 at low fields and then decreases toward a value of 2 at high fields.

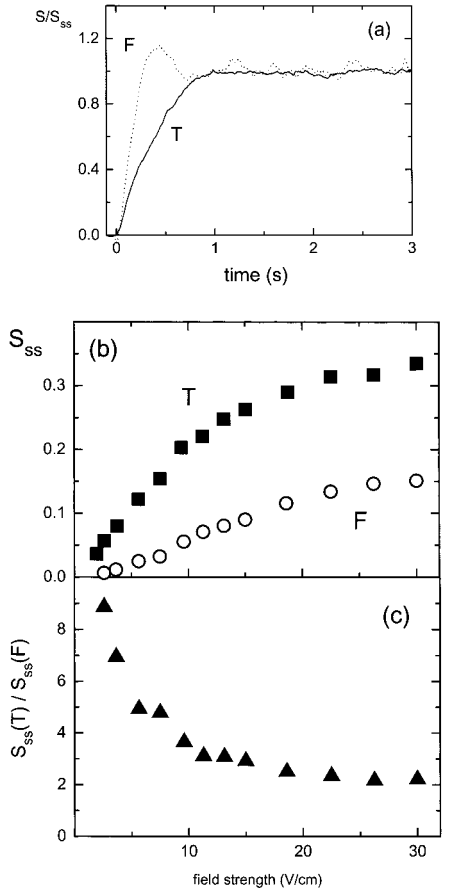

Figure 10. (a) Orientation responses $S(t)$ of T2 DNA of tethered (T, solid) and free-moving (F, dotted) λ-DNA to a constant field of 15 V/cm applied at time $t = 0$. (b) Steady-state orientation S_{ss} of tethered and free-moving λ-DNA vs electric field-strength. (c) Ratio $S_{ss}(T)/S_{ss}(F)$ of steady-state orientation for tethered and free-moving DNA vs field strength.

Discussion

Gel-Immobilized Ethidium Bromide Affect DNA Migration by Direct Interaction. The purpose of this study was mechanistic studies of DNA affinity electrophoresis. It is therefore important to know by which mechanism the affinity label affects the DNA migration. When the cationic ethidium bromide is immobilized, its counterions will cause an electroosmostic flow of water in the gel, which in principle could influence DNA migration. However, the micromolar gel charge added by the immobilized EB is low compared to the typical concentration of 200−400 μM of negative charges present in native agarose as bound sulfate groups,[23] so the change in electroosmosis due to the EB is small. Furthermore, the EB-induced flow will be directed toward the positive electrode (negative counterions), so it cannot explain the retardation of DNA (Figure 1) which moves in the same direction. Together with the observation that DNA is retarded even if the degree of modification is so low that the gel structure is unaltered (Figure 1), this shows that the immobilized ethidium bromide affects DNA migration not by altering the gel properties but by direct interaction with the DNA.

It has recently been shown that the modified form of EB used here is able to intercalate into DNA when not gel-bound and that, when immobilized, it exhibits the increase in fluorescence in the presence of double-stranded DNA,[8] which is characteristic of intercalation of unmodified EB.[10] Furthermore it has been shown that the agarose gel environment itself has negligible effect on the binding constant for nonimmobilized EB or on the association or dissociation kinetics.[24] We will therefore base the discussion of our results in EB-modified gels on the properties of the DNA intercalation complex of nonmodified EB. The affinity constant is 3.4×10^6 M^{-1} under present conditions,[25] a level which can be expected to result in a strong DNA−gel interaction if the EB is immobilized. This would explain the retardation and the increased zone widths in Figure 1. Furthermore the intercalation of EB in DNA is reversible, which is consistent with the DNA forming zones, as opposed to a smear extending from the well.

The average number N_L of gel-bound EBs which are available to a T2 DNA molecule at different EB concentrations (C_L) can be estimated as the number of EBs inside the volume of the (spherical) DNA coils, using the equilibrium radius of gyration[10] (R_G) as an effective radius.

$$N_L = \frac{4\pi}{3}\left(\sqrt{\frac{P(L-P)}{3}}\right)^3 C_L \qquad (3)$$

where L and P are the contour and persistence lengths of DNA. For T2 DNA, $L = 58$ μm, and at the present ionic strength $P = 500$ Å[10] which gives $R_G = 1.0$ μm. Since all available EBs most likely are not accessible for intercalation at the same time (see below), the N_L values should be considered as an upper limit to the actual number of interactions. The lowest EB concentration which gives a detectable retardation effect on the DNA (0.35 μM, Figure 1) corresponds to 830 EB per T2 coil, or 0.5 EB per 100 base pairs on the average. By comparison, at 30 μM gel-bound EB, which in effect arrests the DNA, there is an average of 71 000 EB/coil. This corresponds to 0.42 gel-bound EB per base pair, which is close to the saturation value of nearest-neighbor excluded intercalation (0.5 EB/base pair). Interestingly, similar near-saturation values of nonimmobilized EB slow T2-sized DNA in 1% agarose by only 15%, an effect which is ascribed to a reduction of the electrokinetic charge. [25]

The Mode of DNA Migration in Affinity Gels. The mode of DNA migration in affinity-modified gels will be discussed on the basis of the results for weak (1 μM) and strong (10 μM) affinity in Figure 2, which correspond to N_L values of 1.4 and 14 EB per 100 base pair, respectively. The migration of T2 DNA in unmodified agarose gels is well understood.[19−22,26−28] It basically occurs by reptation, an end-on type of motion along an imagined cylinder (the reptation tube), which represents how the gel fibers which are closest to the DNA prevent its sideways motion. However, often both ends of the DNA try to lead the way, which leads to a migration of highly dynamic nature. Due to steric interactions with the gel fibers, the initially coiled-shaped molecule splits between at least two pores so that the two ends move the molecule into an extended U-shaped conformation hooked on a gel fiber. Driven by one of the ends, the molecule slides around the hooking point and contracts back to a coiled state from where the process starts over. The result is migration by cyclic conversions between coiled and extended states.[29,30] The cyclic migration is reflected as an oscillatory LD response as the field is turned on (as in Figure 2), which

(23) Cock, R. B. In *Electrophoresis '81*; Allen, R. C., Arnauld, P., Eds.; De Gruyter: New York, 1981; pp 219−228.
(24) MacGregor, R. B.; Clegg, R. M. *Bioplymers* **1987**, *26*, 2103−2106.
(25) Reese, H. R. *Biopolymers* **1994**, *34*, 1349−1358.
(26) Holzwarth, G.; McKee, C. B.; Steiger, S.; Crater, G. *Nucleic Acids Res.* **1987**, *23*, 10031−10045.
(27) Holzwarth, G.; Platt, K. F.; McKee, C. B.; Whitcomb, R. W.; Crater, G. D. *Biopolymers* **1989**, *28*, 1043−1058.
(28) Larsson, A.; Åkerman, B. *Macromolecules* **1995**, *28*, 8, 4441−4454.
(29) Deutsch, J. M. *Science* **1988**, *240*, 922−924.

can be used to characterize the average dynamics of the conformational cycle.[28] The time t_p to the orientation overshoot reflects the average time to go from the coiled state to the U, where the DNA is maximally extended (highest S). The undershoot corresponds to the situation where the coil (lowest *S*) has formed again, so the time to the minimum thus represents the average cycle duration.

The oscillatory nature of the LD response in the presence of 1 μM EB (Figure 3) indicates strongly that the migration is of a similar cyclic nature as in the unmodified gel. This is further supported by the several shared features in the modified and native gels, such as a deep minimum followed by an oscillatory response after a field reversal (Figure 3), as well as the similar overshoot time (Figure 4), field-free relaxation behavior (Figure 6), and sigmoidal field dependence in S_{ss}. (Figure 5). The essentially retained mode of migration means that existing theories for cyclic DNA migration developed for nonaffinity agarose[15] can be used to analyze the differences that do exist in this case of weak affinity gel electrophoresis.

By contrast, with 10 μM label (Figure 6) the orientation response is monotonic in the same range of field strengths. The small decrease in gel turbidity compared to the native and weakly modified gel (Figure 1) suggests a somewhat altered gel structure, but control experiments indicate that this cannot explain the drastically different conformation behavior in the strongly modified gel. When the same triazine chemistry was used to modify the gel with 10 μM fluorescein (which is negatively charged at the present pH and therefore has no interaction with the DNA), there was no effect on DNA mobility even though the gel structure was perturbed to a similar extent.[8] We thus ascribe the monotonic and slow nature of the response to the enhanced affinity interaction with the gel due to the higher EB concentration.

The lack of overshoot bears a resemblance to the monotonic response of tethered DNA (T in Figure 10a), but the cause cannot be the same. Tethered DNAs lack an overshoot since immobilized DNA cannot slide and perform the cyclic migration, whereas DNAs in EB gels do undergo migration also at 10 μM EB (although they are retarded; see Figure 1). In fact the DNA may still be undergoing cyclic migration. In native gels the migration is cyclic because[31] the T2 coil size is larger than the average pore size, so the molecule will split between several pores and has to form a U. This basic premise for cyclic migration is likely to be upheld in the modified gel since intercalation of immobilized EB will increase the DNA coil size if anything (by increasing the contour length *L* through helix extension)[10] and the gel structure is only weakly affected. However, the conformation cycles of individual molecules can be detected in the spectroscopic ensemble averages only as long as the molecules respond to an applied electric field with some degree of coherence, as is possible by having the molecules starting from similar equilibrium coil conformations.[20,28] All responses presented here (Figure 2, 3, and 6) were therefore measured after a field-free period, which is long enough to equilibrate the sample as determined by established procedures.[20] The magnitude and number of LD oscillations will then depend on how well and for how long the migration cycles of the different molecules are in phase.[20] In the unmodified gel, approximately one LD oscillation is observed, which shows that most of the cycle coherence between different molecules is lost after approximately one cycle. This is because the distribution of period times is as wide as the average period time,[28] and single-molecule analysis indicates that this distribution width reflects the range of lifetimes of the transient U-formed DNA states. An added distribution of EB-caused DNA−gel interactions is likely to widen the distribution of the U-state lifetimes and should cause the molecules to respond with less coherence. The suppressed amplitude of the oscillations at 1 μM EB (Figure 3) and their absence at 10 μM EB (Figure 6) may thus reflect a progressively lower degree of coherence as the EB concentration is increased.

Forces on DNA during Migration in Affinity Gels. The N_L values of 1.4 and 14 EB per 100 base pair, respectively, for weak and strong DNA−gel affinity in Figure 2 should be compared with the electrophoretic charge of 50 unit charges per 100 base pairs of DNA.[32] This comparison illustrates the competition between the affinity and the electric forces acting on the DNA, a fundamental aspect of affinity electrophoresis. Here we analyze this competition by modeling the EB interactions as an extra source of resistance against DNA motion, which adds to the resistance in the unmodified gel provided by hydrodynamic friction against the solvent in the gel pores and by steric interactions with the gel fibers. The latter two contributions will collectively be expressed as an effective friction coefficient ζ (per unit length of DNA), here assumed to be same as in the absence of affinity label. In the steady state (constant velocity), the electric force acting on the DNA will be balanced by the sum of the gel/solvent friction force and the resistance force provided by the attractive DNA−gel interactions, which gives the following equation:

$$\rho EL = \zeta L v + \alpha C_L L \qquad (4)$$

where ρ and L are the linear charge density and the contour length of the DNA, E is the field strength, and v is the steady-state velocity. The accessibility of the EB label is measured by the parameter α, such that αC_L is the effective number of complexes between DNA and affinity label per unit length of DNA. As discussed below, α can be expected to depend on C_L and possibly also on the velocity v. Equation 4 is based on a picture of local force balance, a model for polymer electrophoresis which recently has come under scrutiny.[33,34] An important part of the hydrodynamic resistance is the friction resulting from the solvent flow induced by the counterions of the polymer, a flow which is directed in the opposite direction of DNA motion. In free solution this so-called retardation effect can have strong influence on DNA behavior in electric fields, in particular for immobilized DNA where the ion-induced flows act on the global scale of the molecule[33] so that a local force balance is inadequate. In our case the DNA is indeed in effect anchored, even if only transiently in most cases. We will still adopt a local force balance, because in the gel global ion-induced solvent flows can be expected to be screened to a large extent by the gel network[33,35] and to occur mainly on the length scale of the pore size.

If, for the sake of the argument, α is assumed to be independent of v, eq 4 shows that the ratio between the velocities in the modified and unmodified ($C_L = 0$) gels is given by the following:

(30) Smith, S. B.; Aldridge, P. K.; Callis, J. B. *Science* **1989**, *243*, 203−206.

(31) Magnusdottir, S.; Åkerman, B.; Jonsson, M. *J. Phys. Chem.* **1994**, *98*, 2624−2633.

(32) Schellman, J. A.; Stigter, D. *Biopolymers* **1977**, *16*, 1415−1434.

(33) Stigter, D. *Biopolymers* **1991**, *31*, 169−176.

(34) Long, D.; Viovy, J.-L.; Adjari, A. *Phys. Rev. Lett.* **1996**, *76*, 3858−3860.

(35) Doi, M.; Edwards, S. F. In *The Theory of Polymer Dynamics*; Clarendon Press: New York, 1986; p 173.

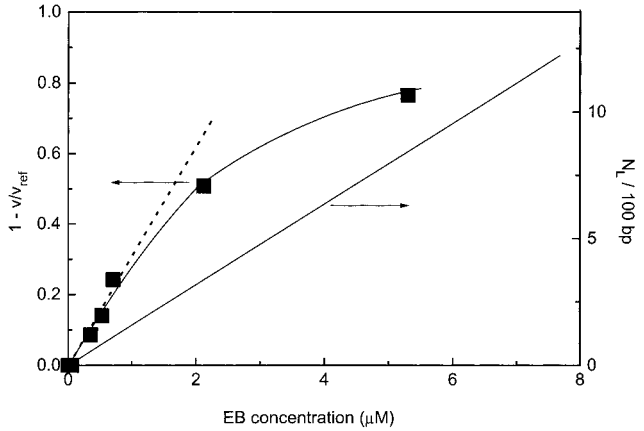

Figure 11. Velocity data of Figure 1 plotted as relative retardation $1 - v/v_{ref}$ (cf. eq 5) vs concentration C_L of immobilized ethidium bromide (squares) and best linear fit to the first four points (dotted). The solid line shows the average number of EB labels N_L available to a T2 DNA molecule vs C_L (eq 1).

$$\frac{v}{v_{ref}} = 1 - \frac{\alpha C_L}{\rho E} \tag{5}$$

where v_{ref} is the electrophoretic velocity in the unmodified gel and the second term reflects the ratio between affinity and electric forces. Within this approximation, the velocity ratio is predicted to decrease linearly with the concentration of EB. Figure 11 shows the velocity data in Figure 1 replotted as the relative retardation $1 - v/v_{ref}$ on a linear EB concentration scale. It is seen that initially the degree of retardation is proportional to C_L, in agreement with eq 5 with a constant α. For higher EB concentrations, the retardation increases more slowly, which indicates that the retarding efficiency per EB is lower at high EB concentrations than at low concentrations. This is not surprising since the probability that the DNA chain has a conformation that allows interaction with all available affinity labels will decrease when saturation numbers of EB are approached.

Gel Affinity Enhances the Electrophoretic Alignment of DNA. The migrative alignment of DNA in gels is an important effect, which is exploited for example in pulsed field electrophoresis to enhance separation.[14,15] This potential for DNA manipulation may be even larger in affinity electrophoresis, since the migrative orientation is even stronger than in native gels (higher S values in Figures 2 and 5). The overall higher degree of DNA orientation in the presence of EB can be understood as an anchoring effect, in analogy with the enhanced field alignment resulting from the biotin−avidin tethering (Figure 10b). Those parts of a DNA molecule which do not interact with EB molecule will move downfield relative to the parts bound to EB labels, which act as anchors. This proposal is supported by the increased DNA stretching, which was deduced from the analysis of the relaxation components (Figure 8, inset). Anchoring is expected to lead to enhanced DNA stretching but to have a comparatively small effect on the reptation tube orientation[28] because it is governed by the orientation behavior of the other, free, end. The EB anchoring is not permanent, however, which explains why the field alignment is enhanced by not more than a factor of 2 with reversible affinity anchoring (Figure 5) but by as much as a 9 with permanent tethering (Figure 10c). Increasing the number of anchor points should thus increase the strength of anchoring and enhance alignment even more but only until the affinity forces are comparable to the electric force, and then the anchoring effect on orientation is expected to saturate. This may explain why orientation is not higher at 10 μM EB than at 1 μM (Figure 2).

The Effect of Field Strength in Affinity Electrophoresis. The concept of a force competition can also be used to analyze the effect of increasing the electric force at a constant C_L. Equation 5 predicts that affinity effects on the migration should disappear if the field force is strong compared to the affinity forces, since v/v_{ref} approaches 1 in the limit of strong fields. Since the EB-enhanced alignment is due to the same transient anchoring which causes retardation, also the alignment behavior is expected to approach that in the nonmodified gel at high fields. Indeed, with increasing field both the magnitudes of the LD oscillations and the steady-state orientation (Figure 3) progressively approach those values observed in the native gel. More quantitatively the S_{ss} ratio in the inset of Figure 5 shows that the steady-state orientation approaches that measured in the native gel at about 10 V/cm. In the force balance picture, this behavior can be understood as the retarding forces from the gel-bound EB being more easily overcome as the electric force acting on the DNA becomes stronger, and the migration therefore becomes more and more similar to that in the absence of the affinity label. In agreement with this proposal, with tethered DNA the S_{ss} ratio does not reach unity in the same range of field strengths (Figure 10c), because in this case the anchor holds also at high fields. An interesting conclusion from these observations is that the field strength can be used to modulate the affinity effect.

Dynamic Aspects of Affinity Electrophoresis. The force picture is helpful in understanding such aspects as the enhanced alignment and the effect of field strength, but it is incomplete because the migration is treated as a stationary process. Even in a constant field the migration is highly dynamic, with its ongoing cyclic changes in the DNA conformation. A molecular model of affinity electrophoresis of DNA therefore will have to take into account (1) how the conformation of the DNA affects the affinity interaction and (2) how the rate of change between different conformations interplays with the association and dissociation kinetics of the DNA−label complex. Understanding these aspects will require advanced kinetic models, and eq 4 can only be a first simple approach.

However, the complex process of cyclic DNA migration in gels can also be viewed as a good experimental system to investigate dynamic aspects of affinity electrophoresis in general. Because the steady-state orientation (Figure 5) and velocity (Figure 1) are affected, immobilized EB molecules are interacting with the DNA also during migration, and since the molecules do move over macroscopic distances (Figure 1), the DNA−gel interactions obviously are being replaced continuously with new ones, a process which is an integral part of migration in affinity electrophoresis in general. The affinity dynamics can be suitably probed by the conformational changes of migrating DNA because they occur on time scales (milliseconds to hours) which are both much longer and considerably shorter than the dissociation time for (nonimmobilized) EB from DNA, which is 0.1 s at the present ionic strength of 50 mM.[11] This value is not affected by the presence of the gel itself,[24] most likely because the dissociation is governed by highly localized base pair dynamics, and we will assume it is valid as the intrinsic dissociation time constant also in the case of immobilized EB. On the other hand, the characteristic association rates for nonimmobilized EB are fast compared to most DNA processes in gels, because they occur on the microsecond time scale in view of the affinity constant being about 10^6 M^{-1}. However,

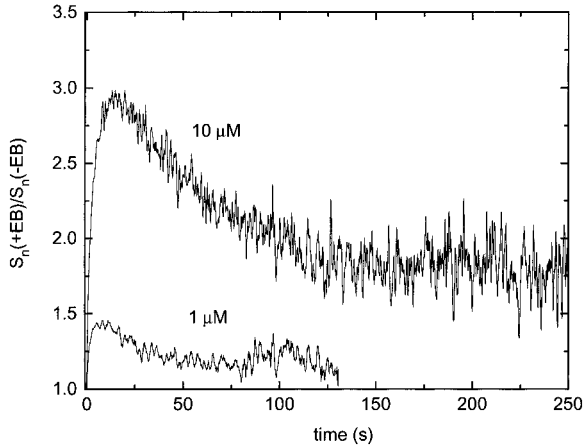

Figure 12. Plot of ratio of normalized relaxation curves ($S_n(t) = S(t)/S_{ss}$, where S_{ss} is the steady-state orientation) with and without ethidium bromide at indicated concentrations. Data from Figure 8.

since the EB is not a diffusing species when immobilized to the gel, the association process in affinity electrophoresis is principally different from that with free EB. An interesting aspect is that the encounter between DNA and affinity label is most likely coupled to the mode of DNA migration as discussed below.

The dynamic aspects were studied either by starting from an equilibrium state and monitoring the approach of the steady migrative state or by following the field-free relaxation from the migrative state toward equilibrium. The degree of DNA−gel interaction may be quite different in the equilibrium and migrative states. For instance, it is possible that the affinity effects disappear in the limit of high fields not only because the force then is strong enough to break the interactions but also because the velocity is too high for them to have time to reform. For the moment we have no direct way to monitor the number of contacts, and both types of kinetic studies were therefore performed at fields (7.5 V/cm in most cases) where there exists a substantial number of interactions also in the migrative state.

Dynamics of DNA−Gel Interactions in Absence of Field. For T2 DNA, the field-free decay in the unmodified gel involves both relaxation of the stretching of the molecules, which occurs by motion along the field-aligned reptation tube, as well as end-on reptation of the whole molecule out of the field-aligned tube in order to create an isotropic one.[22] The latter process occurs by a set of processes with a wide range of characteristic time constants.[35] The slowest one, characterized by the reptation time τ_d, is much slower than the destretching processes,[22] however, and its monoexponential characteristic[35] therefore dominates the final part of the relaxation. This is seen in Figure 8 (inset), where the final time constant in the unmodified gel agrees well with values for the reptation time in other studies.[22] The slope at 10 μM EB shows that the effect of EB on the reptation time is negligible, whereas the destretching is markedly retarded. The relaxation at 1 μM EB (Figure 8) exhibits a similar pattern of EB retardation of destretching at intermediate times but little effect on the long-term relaxation.

Figure 12 shows an attempt to analyze somewhat more quantitatively the characteristic times of those relaxation processes which are retarded by EB, by forming the ratio between the decay curves with and without EB (from Figure 8). The ratio starts at 1 because of the normalization but soon reaches a maximum level of about 2.9 for 10 μM EB and 1.4 with 1 μM EB. (Note that the abscissa range has been chosen to emphasize the change relative to a ratio of 1.) The ratio then decreases monotonically and at long enough times reaches a constant level which is the ratio of the amplitudes of the reptation contribution to the relaxation (i.e., $A_3(+EB)/A_3(-EB)$ in the notation of ref 16). At this point in the process, the rate of relaxation is the same with and without EB and corresponds to 30 and 125 s at 1 and 10 μM, respectively. Another marked difference is that the maximum value of the ratio (compared to 1) is 7 times higher at 10 μM than at 1 μM, but the interpretation probably has to be based on a model capable of explaining the stretched exponential nature[22] of the destretching. Figure 12 suggests a considerable potential for mechanistic studies if it is possible to exploit the essentially continuos distribution of relaxation times in this manner.

The main observation that fast relaxation processes are more retarded than slow processes is understandable, since DNA alignment relaxes by translational transport either along the tube or out of it. During the fast processes chain displacement occurs on time scales comparable to the dissociation time of the EB−DNA complex (0.1 s) and the chain motion can be expected to be retarded. On the other hand, since the DNA−gel attachments can break and reform many times on the time scale of the reptation relaxation process (minutes), the affinity will probably have a smaller effect on its rate. Furthermore it is known that during migration the DNA polymer is nearly fully stretched in the U-conformation,[28] so initially during field-free relaxation the stretched DNAs slide along the tube essentially as a rope. This is possible only if several retarding gel interactions are broken in a concerted manner, an event which is correspondingly less probable than the random dissociation of one attachment. During reptation the chain is locally relaxed inside the tube,[22] and the individual dissociation−association events will be much less coupled and other energy barriers determine the relaxation kinetics. Notably, it is also known that stretched DNA has a higher affinity for EB than relaxed.[36]

Affinity Dynamics during DNA Migration. Field-free relaxation gives some insight also into the migration dynamics during DNA affinity electrophoresis. For example, the end-driven U-formation after the coil stage is similar to reptation and should be considerably less sensitive to EB retardation than the sliding after U-formation, which is very similar to the in-tube motion which occurs initially during field-free relaxation. Complementary information is provided by studies of field-driven disruption of the equilibrium DNA affinity gel complex, in terms of the rates of orientation build-up from equilibrium at 1 μM (Figure 3) and 10 μM EB (Figure 6).

In unmodified gels the overshoot amplitude S_p is higher than the steady-state value S_{ss}, as expected if S_{ss} is the average of the orientation factors for the different cycle stages, including the most aligned U-formed state with an orientation factor S_p.[28] With 1 μM EB, S_p is instead lower than the steady-state value at low fields (e.g., 3.6 V/cm in Figure 3), which indicates that the first U is different from those formed in the subsequent cycles. Interestingly, a similar type of distorted oscillatory LD response is observed in unmodified gels if DNA molecules are arranged to form several field-aligned loops (i.e., several small U's) before they develop into a globally U-shaped conformation.[37] Each loop contains a head of weakly aligned DNA, so the summed orientation factors of several loops are lower than the orientation factor for the same amount of DNA segments when they form a single U. In the modified gel, transient EB

(36) Smith, S. B.; Finzi, L.; Bustamante, C. *Science* **1992**, *258*, 1122−1126.

(37) Åkerman, B. *Phys. Rev. E.: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.* **1996**, *54*, 6685−6696.

anchoring of the DNA chain at several positions could result in similar loop formation, if the field is low enough so that the attachments are not disrupted much faster than the time to form the single U. In agreement with this proposal, with stronger fields the normal oscillatory response, with S_p larger than S_{ss}, is observed also in the presence of EB.

In the monotonic LD response at 10 μM EB (Figure 6), the fast part of the two-step build has a rise time τ_1 (Figure 7a), which is comparable to the overshoot time at 1 μM EB (Figure 4) at a given field strength. Also at 10 μM EB, certain DNA segments will be tied down by the EB interactions which existed when the field was applied, leaving the intervening free parts of the DNA to move. This intermediate state can be expected to be more long-lived than at 1 μM, since more EB anchors will be involved, potentially to the extent that release and further alignment may be detected as a separate process characterized by the rise time τ_2 of the slow component (Figure 7b). The $E^{-1.7}$ power law for τ_2 (Figure 7b) is significantly stronger than the -1.2 exponent, which is expected for coil deformation by a purely migrative mechanism[19] and which is observed for t_p in the native and weakly modified gels (Figure 4). A deviation from the -1.2 slope is a strong indication of an enhanced interaction with the gel. For example, circular DNAs which are retarded by impalement on gel fibers exhibit a -2 slope, in contrast to the -1.2 exponent exhibited by the linear form which cannot be impaled.[38] In the case of EB affinity gels the deviation probably reflects that the retarding effect of the label is more easily overcome as the field increases, an effect which was explicitly demonstrated at 1 μM EB (see above). In the force picture of eq 4, the higher the field the larger the fraction of the applied force is actually used for transport and migrative deformation of the DNA coil, which therefore occurs by a rate which increases more rapidly than the field itself. Furthermore, such a delay in the release of the molecule from the original conformation may prevent the detection of a cyclic type of motion by ensemble-averaging spectroscopy, as discussed above.

How Does the DNA Encounter the Affinity Label? The present results show how electrophoretic processes on the time scale of seconds and minutes are affected by the dissociation properties of the DNA−label complex, but these experiments reveal less about the association process. One interesting subject is the mechanism by which the DNA encounters the affinity label, and in an attempt to address this question more directly, we exploited the irreversible nature of the biotin capture of avidinated DNA. The distribution of the avidinated DNA in the biotin insert (Figure 9a) reflects where the first successful affinity encounter between DNA and biotin label occurs. The fact that the zone of captured λ-DNA is in the first part of the insert, and that it is sharp compared to that of free-moving DNA, suggests that the process by which the avidinated DNA end finds the biotin is efficient. These experiments were performed with λ-DNA because its single-stranded ends simplify end-avidination, but the results are applicable to T2-DNA as well since both types of DNA undergo the cyclic type of migration. The overshoot in Figure 10a shows that free (i.e., nonavidinated) λ-DNA undergoes cyclic migration under the present conditions (albeit the magnitude is lower than for T2 DNA because λ-DNA is shorter[26]). This is likely to be true also for avidinated DNA before it has been captured because the presence of end-avidin has no detectable effect on the migration of λ-DNA in nonbiotinylated gel as monitored by the velocity.[5] We conclude that T2 DNAs most likely encounter the EB label with a similar degree of efficiency, if the search mechanism is related to the cyclic type of migration. An interesting possibility is that the sliding motion which occurs after U-formation in the cyclic motion provides a particularly efficient search process. This hypothesis may seem to be contradicted by the control experiment where the electropohoretic search by avidin itself (Figure 9b) is of similar efficiency, suggesting that facilitation by DNA sliding is not necessary. The mode by which the avidin finds the biotin label may be completely different in these two cases, however, because streptavidin is small enough to diffuse rapidly in agarose gels compared to the DNA. In fact, diffusive capture of streptavidin[8] in the same type of biotinylated gels used for electrophoretic capture here also reveals a very efficient search mechanism as indicated by the concentration front being very sharp compared to the normally broad profile in nonbiotinylated gels. This approach will be used in further investigations of encounter mechanisms in affinity electrophoresis.

Acknowledgment. This research is funded by the Swedish National Research Council.

JA984154E

(38) Åkerman, B. *Biophys. J.* **1998**, *74*, 3140−3151.