Tube leakage during electrophoresis retards reptating DNA in unmodified and hydroxyethylated agarose gels

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The resistance towards DNA-loop formation is considerably higher in 2% hydroxyethylated agarose gels (average pore radius $P_E = 380$ Å) than in 1% ordinary agarose gel ($P_E = 1000$ Å) [B. Åkerman, preceding paper, Phys. Rev. E **54**, 6685 (1996)]. This is exploited here to study how DNA-loop formation affects the electrophoretic migration of native $T2$-DNA in agarose gels between 0.5 and 25 V/cm. Linear dichroism (LD) spectroscopy shows that the migration is cyclic in nature in both gels, but the period time is 30% longer in the hydroxyethylated gel at high fields, indicating a 30% higher tube friction coefficient than in unmodified agarose. Together with an observed reduced tube orientation, as expected from the smaller pores according to reptation theory, this explains why the electrophoretic mobility is lower than in unmodified agarose at all fields. Analysis of the field-free decay of the LD shows that in both gels the degree of electrophoretic stretching of the molecules along the path in the gel increases and saturates with increasing field in parallel with the mobility. However, the field enhancement of the mobility occurs for lower fields in the hydroxyethylated agarose because there are fewer loops to be consumed by the stretching in this gel. This observation supports that the loops contribute to the resistance towards migration at intermediate fields. [S1063-651X(96)01412-2]

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I. INTRODUCTION

Experimental studies of the global motion of DNA in both concentrated polymer solutions [1] and gels (for review see [2]) support the central reptation concept of end-on motion. Simulations of the Brownian motion of polymers indicate, however, that under certain conditions the polymer can leak out through the tube wall [3] with fundamental implications for the understanding of polymer behavior in restricted media. In polymer solutions the neighboring chains that define the tube of the probe chain move too [4], and since such constraints release requires a self-consistent treatment of all chains, tube leakage is difficult to model in these systems. In addition it may be experimentally difficult to distinguish tube leakage from effects due to constraints release in such systems. Simulations with fixed obstacles show more accentuated reptation of the probe chain, but leakage out of the tube is still observed [5]. Experimental systems such as DNA in agarose gels, where the obstacles of the matrix can be considered immobile on the time scale of probe diffusion, should thus constitute a better model systems to test reptation theories for tube-leakage effects.

The reptation tube barrier can be considered to have entropic [6,7] and enthalpic (DNA bending) contributions arising as a polymer is to form a loop (or a ''hernia'') of doubled-over chain between two obstacles. In agarose gels the height of this barrier is comparatively low for DNA, in the sense that an electric field of only a few V/cm is enough to allow loops to form more or less without delay [8], in good agreement with theoretical expectations [9]. Here we use this system to test reptation theory for tube leakage effects in the presence of an electric field, with applications to electrophoretic migration and separation of DNA.

As expected from the barrier height, biased reptation models without tube leakage have been successful in describing the electrophoretic migration in fields below about 1 V/cm [10,11]. In stronger fields hernia formation has to be included [11], but the enhanced field has the additional effect of giving rise to field-induced tube-length fluctuations. The reason for this is a transient anchoring of the molecules around a gel fiber, as the two ends temporarily force the coiled molecules to move into a U-shaped conformation. If the field is strong enough, the ends move so quickly that the molecule can unravel the entanglement only by being dragged out of it by the longest arm of the U. In this process the molecule becomes strongly extended before it contracts into a compact coil after the escape. The result is a cyclic extension-contraction mode of migration [12–14], which has important consequences for the mobility of the DNA molecules [2].

In order to understand this high-field regime, where the electrophoretic separations usually are performed, it is important to study the relative importance of field-driven tube length fluctuations and tube leakage. It is possible to decrease the degree of tube leakage with retained field-induced tube length fluctuations by using hydroxyethylated agarose gels, since in this system hernia formation is suppressed [8] but (as shown here) the migration is still cyclic. Hernia formation is slower because the pores are considerably smaller than in unmodified agarose, with sizes comparable to pore sizes in polyacrylamide gels [15]. For our purposes the nusieve gel has the advantage over polyacrylamide that the chemical composition is comparatively close to that of unmodified agarose. In fact, trapping of long DNA [8] and DNA-induced electro-osmotic flows (this work) indicates that the nature of the interaction between the DNA helix and the hydroxyethylated gel fibers is the same as between DNA

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and unmodified agarose. The observed differences are therefore most likely caused by the changes in effective pore size. In particular we study how suppressed hernia formation affects the field dependence of the electrophoretic mobility, in order to test the hypothesis [2] that hernias increase the effective drag against migration through the gel.

II. MATERIALS AND METHODS

Chemicals

*T*2-DNA was prepared for spectroscopy as described previously [16], and was used as received from Sigma for mobility experiments. *Hae*III restriction fragments of ΦX174-DNA were used as received from Promega. All experiments have been performed in 0.5× TBE buffer at 22 °C in 1% agarose (Biorad DNA-grade) or 2% nusieve agarose (FMC).

Pore sizes

Slater *et al.* [17] used Ogston theory to evaluate the average pore radius (P_E) of an agarose gel of a certain concentration as the radius of gyration of the DNA molecular size, which has a mobility equal to half the free solution mobility at that gel concentration. The result at different gel concentrations A can be summarized as

$$P_E = (89\ \text{nm}) A^{-0.7}, \tag{1}$$

which gives a pore radius of 890 Å in 1% agarose. The same approach has been employed here to estimate the pore size of the 2% nusieve gel, using Ferguson plots based on the *Hae*III restriction fragments of ΦX174-DNA [see Eq. (9) in the Results section].

Linear dichroism experiments

Linear dichroism (LD) experiments have been performed as described [18] with the exception that the vertical electrophoresis cell contained a multigel, the top half being 1% agarose, the lower 2% nusieve. In this way the same DNA sample could be studied in both gels under identical field and buffer conditions, and furthermore field inversion gel electrophoresis (FIGE) ($T^+ = 3$ s, $T^- = 1.5$ s, 7.5 V/cm) could be used to separate intact *T*2-DNA from fragments as the sample was introduced into the measurement position of the top agarose gel.

Mobility measurements

Electrophoretic mobility was measured by slab gel electrophoresis in 7-cm strips of sample gel (hydroxyethylated or unmodified agarose) attached to 3-cm 1% ordinary agarose strips. Aliquots of the DNA sample were loaded in wells in the 1% agarose part of ten identical such combistrips, and degraded DNA was separated by FIGE for 3 h, which delivered a zone of intact *T*2-DNA at the top of each sample strip (and a zone of degraded DNA further down the sample strip). The strips were run at different constant field strengths and subsequently stained with ethidium-bromide, using one strip to determine the starting position. Mobility measurements for Ferguson plots were performed as described earlier [19].

LD theory

Linear dichroism is used to measure the orientation of the DNA molecules that result from the migration through the gel [20], in terms of an orientation factor S:

$$S = \tfrac{1}{2}(3\langle \cos^2\theta \rangle - 1), \tag{2}$$

where θ is the angle between the helix axis and the field direction. The time-dependent LD directly reflects the orientational dynamics of the DNA helix axis [20] since DNA remains in the *B* form during agarose gel electrophoresis [21].

Within the reptation model for the migration of DNA in gels, the orientation factor in turn factorizes into two contributions [22,14]

$$S = S_{\text{tube}} S_{\text{local}} \tag{3}$$

with

$$\begin{aligned} S_{\text{tube}} &= \tfrac{1}{2}(3\langle \cos^2\delta \rangle - 1), \\ S_{\text{local}} &= \tfrac{1}{2}(3\langle \cos^2\beta \rangle - 1), \end{aligned} \tag{4}$$

where δ is the angle between the local tube axis and the field direction, and β the angle between the DNA helix axis and the local tube axis. When the field is turned off the LD will decrease as an effect of both a diminished degree of stretching of the DNA along the tube (S_{local}) and because the DNA diffuses out of the originally field-aligned tube by reptation, which decreases S_{tube}.

For long DNA reptation is much slower than destretching, so the overall LD decay is markedly biphasic [22]. For the slow phase we will follow Weill and co-workers [22] and assume that reptation gives rise to a single exponential decay for S_{tube}, with time constant τ_3. For the relaxation of the stretching component they employed a stretched exponential, but we will adhere to the two-exponential analysis (time constants τ_1 and τ_2) that proved fruitful in a study of shorter DNA [23], and that has found support in an analysis of the effect of agarose concentration on the LD relaxation [16]. The orientation factor S was thus assumed to have the following time dependence:

$$\begin{aligned} S(t) &= S_{\text{tube}}(t) S_{\text{local}}(t) \\ &= S^0_{\text{tube}} e^{-t/\tau_3} [S^r_{\text{local}} + (S^0_{\text{local}} - S^r_{\text{local}})(a_1 e^{-t/\tau_1} \\ &\quad + a_2 e^{-t/\tau_2})]. \end{aligned} \tag{5}$$

Here a_1 and a_2 are the relative amplitudes of the two stretching components ($a_1 + a_2 = 1$). S^0_{tube} and S^0_{local} are the degrees of tube orientation and DNA stretching, respectively, when the field is turned off. S_{tube} will relax to zero when the DNA chain has completely left the field-aligned tube. S_{local}, on the other hand, generally does not relax to zero, but to a finite value S^r_{local}, which is the orientation factor for the helix with respect to the tube axis when the DNA chain is locally relaxed in the tube. The chain is defined to be locally relaxed when the contour length of DNA per tube segment (length segment $a = 2P_E$, the average pore diameter) is such that its equilibrium end-to-end distance equals a. Since the end-to-end vector of the subchain in a given tube segment will be

oriented parallel to the tube axis, $S_{\rm local}$ for the locally relaxed state is not zero (irrespective of the orientation of that tube segment), because, on the average, the segments of a polymer chain in equilibrium have a preferential orientation with respect to the end-to-end direction of the polymer coil [24]. (For a wormlike chain of persistence length 500 Å $S^r_{\rm local}$ can be estimated to 0.11 if the pore diameter is 2000 Å [20].) It is convenient to define a parameter $\Delta S_{\rm local}=S^0_{\rm local}-S^r_{\rm local}$, which quantifies how overstretched the chain is compared to the locally relaxed state.

Fitting of the field-free decay

The total orientation factor when the field is turned off is $S_{\rm SS}=S(t=0)=S^0_{\rm tube}S^0_{\rm local}$. Following Eq. (5) the decay of the normalized orientation factor $S^n(t)=S(t)/S_{\rm SS}$ was fitted to

$$S^n(t)=e^{-t/\tau_3}[A_3+A_1e^{-t/\tau_1}+A_2e^{-t/\tau_2}]. \tag{6}$$

Identification with Eq. (5) shows that

$$A_i=a_i\,\frac{\Delta S_{\rm local}}{S^0_{\rm local}},\quad i=1,2,$$

$$\frac{A_i}{A_1+A_2}=\frac{a_i}{a_1+a_2}, \tag{7}$$

$$A_3=S^r_{\rm local}/S^0_{\rm local}.$$

Multiplication by the experimentally known steady-state level $S_{\rm SS}$ gives the absolute amplitudes

$$A^a_i=A_iS_{\rm SS}=a_i\Delta S_{\rm local}S^0_{\rm tube},\quad i=1,2,$$

$$A^a_3=A_3S_{\rm SS}=S^0_{\rm tube}S^r_{\rm local}, \tag{8}$$

$$(A^a_1+A^a_2)/A^a_3=\Delta S_{\rm local}/S^r_{\rm local}.$$

In the fitting procedure A_3 and τ_3 were first determined from the semilogarithmic plot of $S^n(t)$, which was distinctly linear for long times because the tube renewal process is much slower than the decay of the stretching ($\tau_3\gg\tau_1,\tau_2$). This fact was exploited in the subsequent fitting of the stretching components by approximating $e^{-t/\tau_3}=1$ in Eq. (6), and fitting the early decay to two exponentials and an offset that was fixed at the value of A_3 obtained from the zero-time intercept of the long-time linear portion. (Fits of the early decay to a single exponential and an offset were very poor, and no visible improvement resulted from using three exponentials with or without an offset.) This method of analysis was a consequence of the fact that the complete relaxation had to be measured in two portions. The fast decay components could not be measured faithfully with the high instrument time constant (1 s) that was necessary to measure the low-amplitude decay at long times, and the early decay was therefore measured in a separate experiment with a 64-ms time constant. The consistency between the two data sets for each decay was checked by comparing the decay profile in an overlapping intermediate time window.

Operational definition of electrophoretic stretching

We will adopt the operational definition of stretching as the fast-relaxing component (A_1+A_2) of the biphasic field-free orientation relaxation [22]. Referring to Eq. (8) the amplitudes $(A^a_1+A^a_2)/A^a_3$ and A^a_3 will be used to monitor the overstretching $\Delta S_{\rm local}$ and the tube orientation $S^0_{\rm tube}$, respectively, but the proportionality factors are not known since $S^r_{\rm local}$ is not experimentally accessible without independent information on the tube orientation [14]. However, on theoretical grounds [20] it can be assumed that $S^r_{\rm local}$ is lower in 1% ordinary agarose (than in 2% nusieve) because the wider pores allow the DNA to undulate more inside the tube when it is locally relaxed.

On the molecular level, stretching refers to any perturbation of the chain from its local equilibrium conformation within the tube. One component of the stretching of the DNA is the field-induced lengthening of the tube, which results from the transient anchoring during U formation, as can be quantified from fluorescence microscopy images of migrating DNA [14]. Combined LD and microscopy data indicate, however, that at 8 V/cm a substantial fraction of the stretching resides at the pore level, below the resolution of the microscope, where the equilibrium conformation is locally perturbed by the field in terms of field-aligned hernias. Available data support that both stretching components (field-induced tube-lengthening and field-aligned hernias) fulfill the operational definition by relaxing rapidly. Electrophoretic mobility [25] and orientation experiments [21], and detrapping of long DNA [26], in high-frequency modulated fields indicate that the response time of the hernias is as short as about 0.1 s at 5 V/cm in 1% agarose, as expected from the short length scale involved. Direct observation in the microscope shows that the field-induced tube lengthening relaxes in typically 10 s for $T2$-DNA in 1% agarose [27]. It is thus clear that both types of stretching contribute to the fast LD relaxation component (A_1+A_2), but it is not possible to evaluate their relative contributions on the basis of field-free relaxation analysis. The tube lengthening most likely relaxes by a wide spectrum of relaxation times, partly overlapping with the time domain of the hernia relaxation, and in particular there is no foundation for assigning the two stretching amplitudes A_1 and A_2 to any particular type of stretching.

III. RESULTS

Linear dichroism responses

Figure 1(a) shows a gallery of LD responses of $T2$-DNA in agarose and nusieve gels, at four different field strengths. The LD oscillations observed in agarose reflect the fact that the migration of long DNA is cyclic in nature [14], and since an overshoot is observed also in nusieve it is clear that $T2$-DNA oscillates during migration in nusieve gels. A prominent difference is that the degree of orientation is considerably lower in nusieve at any given field strength. By normalizing the LD to the steady-state orientation [Fig. 1(b)], it is also clear that the oscillation amplitude is smaller and the buildup slower in nusieve at any given field strength. The smaller pores in this gel thus have a marked effect on the orientation dynamics during migration.

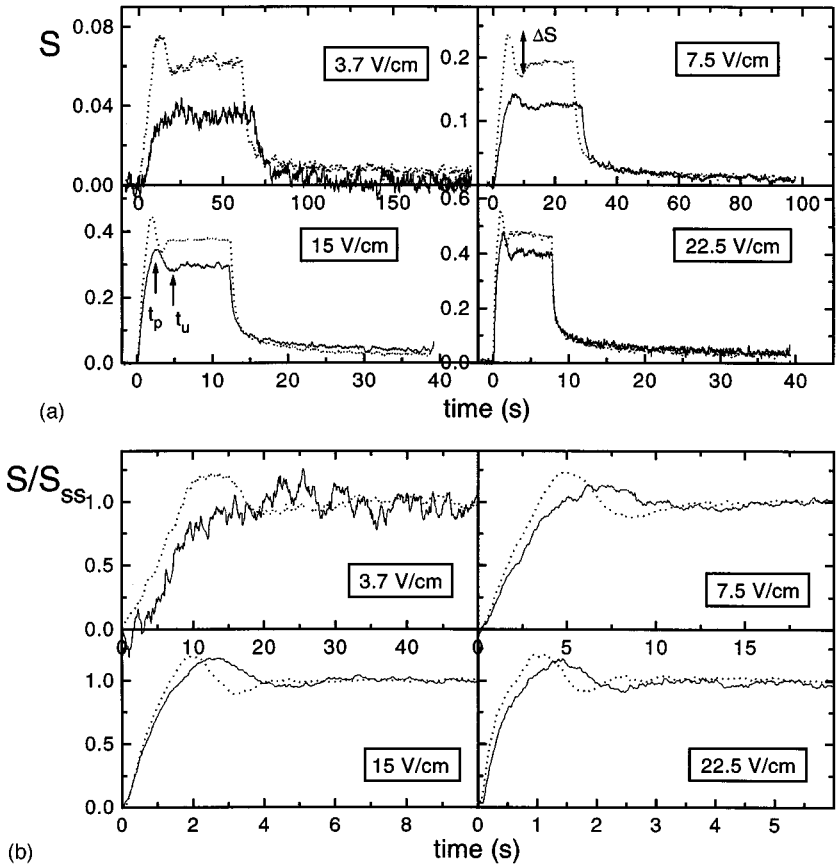

FIG. 1. (a) LD response of $T2$-DNA (in terms of orientation factor S, see text) in 1% agarose (dotted) or 2% nusieve gel (full) at four different field strengths (indicated). The DNA samples had been under field-free conditions for 30 min before the field was applied. The LD response parameters t_p (time to overshoot), t_u (time to undershoot), and ΔS (overshoot amplitude) are defined. (b) Responses in (a) normalized to the steady-state value S_{SS}.

If the beam position was moved away from the maximum of the DNA zone (the position at which the responses in Fig. 1 were obtained), a true steady-state LD was not reached. There was a slow (minutes) LD signal, which at a given position changed sign when the field was reversed, and that had opposite sign on opposite sides of the zone maximum for a given field direction (results not shown). A slow LD with these characteristics in unmodified agarose has been ascribed to a slow gel deformation caused by DNA-induced electroosmotic flows [18]. The slow LD component in the nusieve gel has the same cause, as was confirmed by the observations that the same polarity-dependent and slowly growing LD component (but not the oscillatory DNA response) was observed when LD was measured at 300 nm (where the DNA does not absorb), but not if the LD was measured (at 260 or 300 nm) in the absence of DNA. These results indicate that the nature of the interaction between the DNA helix and the gel fibers is the same in the two gel types, as expected from hydroxyethylation being only a minor chemical modification.

Buildup kinetics and steady state LD

The kinetics of the buildup of the DNA response can be characterized by several parameters (see below). However, in order to have a comparable data set for the two gel types it is important that the molecules are allowed to equilibrate in the gel before the field is applied, since the buildup profile is sensitive to the starting conformation [16]. Here the molecules were allowed to relax without field for 30 min because longer equilibration did not change the characteristics of the LD response, in agreement with earlier observations [16].

The time to the overshoot decreases with increasing field strength (Fig. 2) according to a power law $t_p \sim E^{-a}$. The exponent a is equal to 1.3 ± 0.1 in normal agarose, in good agreement with earlier results [16,22,28], and significantly higher (1.6 ± 0.1) in the nusieve gel. At 3.7 V/cm t_p is almost twice as long in nusieve (Fig. 2, inset) but the ratio decreases to about 1.25 at the highest field strength. Also the time to the undershoot decreases with increasing field in both gels (Fig. 3), although the ratio is less field dependent than for t_p (Fig. 3, inset). In agarose the overshoot amplitude (ΔS) increases with increasing field strength (Fig. 4, bottom) in good agreement with earlier observations [16]. In nusieve ΔS is strongly suppressed at low fields by as much as 60% Fig. 4, top), but approaches that observed in agarose at 22.5 V/cm.

The steady-state orientation (S_{SS}, Fig. 5) grows in a sigmoidal fashion with increasing field in both gel types, but from the ratio (Fig. 5, top) it is clear that in nusieve the orientation falls off much more strongly with decreasing field (below about 15 V/cm) in nusieve than in agarose. On the other hand, at the highest fields used here the molecules reach almost the same degree of average orientation in nusieve as in agarose. In order to understand these differences in the total helix orientation between the two gel types the tube and stretching contributions have to be resolved, and to this end the field-free relaxation was studied.

Field-free decay of LD

Figure 6 shows the LD relaxations in terms of the orientation factor S normalized to its value S_{SS} at the time of field removal ($t=0$). In both gel types there is a fast decay (sec-

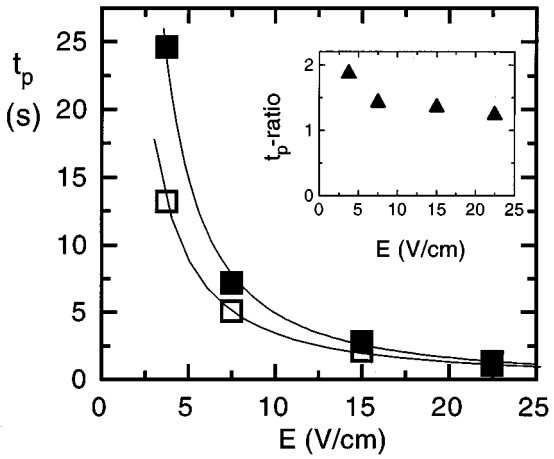

FIG. 2. Time to LD overshoot t_p vs field strength in 1% agarose (open symbols) and 2% nusieve gel (filled symbols). Curves are best fit to power-law dependencies $t_p \sim E^{-a}$, with $a=1.3$ in 1% agarose and $a=1.6$ in nusieve gel. Inset: The ratio between t_p in 2% nusieve and in 1% agarose.

onds to tens of seconds) of large amplitude followed by a comparatively slow component of low amplitude, which has not relaxed after 40 s. This overall biphasic nature of the relaxation is well established in agarose [22], and has been ascribed to a fast decay of the DNA stretching and slow reptation out of the field-aligned tube (see LD theory). The orientation relaxation of $T2$-DNA retains the biphasic pattern in the nusieve gel, but it is seen that the intermediate phase of the relaxation (in the range of tens of seconds) is clearly slower in nusieve, whereas the very initial decay is less affected. From Fig. 6 it is clear that the relaxation is not complete after 40 s, and the long-term relaxation was followed in a separate experiment lasting over 1000 s. Figure 7 shows that the corresponding semilogarithmic plots are linear after about 200 s, and that this long-time monoexponential component is quite similar in the two gel types. This is borne out by the similarity of the fitted relative amplitudes

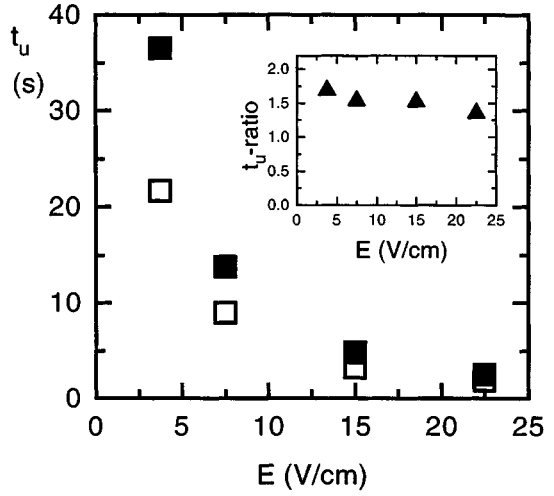

FIG. 3. Time to LD undershoot t_u vs field strength in 1% agarose (open symbols) and 2% nusieve gel (filled symbols). Inset: The ratio between t_u in 2% nusieve and in 1% agarose.

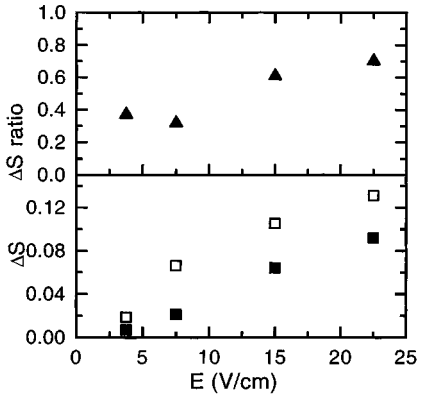

FIG. 4. (Bottom) Overshoot amplitude ΔS in 1% agarose (open symbols) and 2% nusieve gel (filled symbols). (Top) The ratio between ΔS in 2% nusieve and in 1% agarose.

A_3 [Fig. 8(a), bottom] and time constants τ_3 [Fig. 8(b), bottom] in the two gels. The observed relative amplitude of about 5% and the time constant of about 500 s are in good agreement with earlier observations [22] on $T2$-DNA in 1% agarose. There is considerable scatter in the time constants in particular, which reflects the substantial noise level in the original data obtained by LD, which generally is a less sensitive method [20] than the birefringence technique used by Mayer, Sturm, and Weill [22].

The short-time relaxation was fitted to two exponentials as described in Sec. II. Figure 8(b) shows that the rate of the fastest component (τ_1, top) indeed is very similar in the two gels, whereas the second component (τ_2, middle) is considerably slower in nusieve, as was indicated by inspection of the raw data (Fig. 6). There is a marked decrease in τ_1 with increasing field (in both gels) whereas τ_2 is less affected by the field strength in agarose, and a weak increasing trend is observed in nusieve. There is an overall shift in the relative amplitudes towards the faster stretching component with increasing field [as seen from the increase in the relative amplitude A_1 at the expense of A_2 in Fig. 8(a)] and the decreasing value of τ_1 [Fig. 8(b), top] most likely reflects a similar

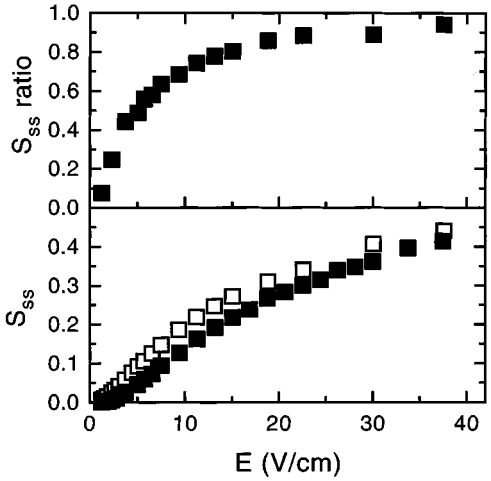

FIG. 5. (Bottom) Steady-state orientation S_{SS} in 1% agarose (open symbols) and 2% nusieve gel (filled symbols). (Top) The ratio between S_{SS} in 2% nusieve and in 1% agarose.

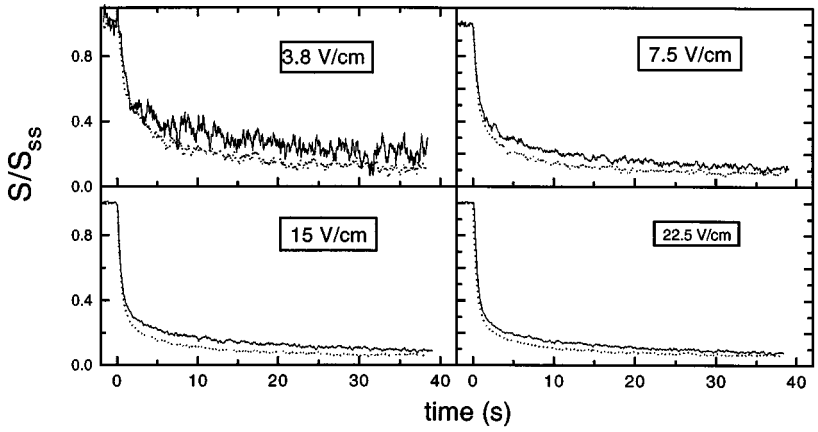

FIG. 6. Short-time field-free relaxation of S normalized to steady-state level (S_{SS}) in 1% agarose (dotted) and 2% nusieve gel (full) at four different field strengths (indicated).

shift towards the faster components in a distribution of fast relaxation processes here represented by a single average relaxation time, τ_1. Such a trend is generally observed for the relaxation of the overstretching of long DNA in agarose gels, whether analyzed as a stretched exponential [22] or in terms of discrete exponentials [19]. However, for all field strengths the fast component (A_1) dominates the relaxation of the stretching, amounting to 70% or more of the stretching amplitude (A_1+A_2).

In Fig. 9 absolute relaxation amplitudes have been obtained according to Eq. (8) by multiplying the relative amplitudes in Fig. 8(a) with the steady-state orientation factor S in Fig. 5. In 1% agarose the overstretching $(A_1^a+A_2^a)/A_3^a$ increases with increasing field up to about 15 V/cm, and then levels off. A strong increase in the overstretching has been reported for intermediate fields by Weill and co-workers [22], but their data were limited to fields below 10 V/cm. The same trend of increase and saturation is observed in the nusieve gel, although the leveling off at high fields is less accentuated. Also A_3^a increases with increasing field in both gels, but the amplitude is overall lower in nusieve. According to Eq. (8) this indicates a lower degree of tube orientation in the modified gel since S_{local}^r is higher there, if anything.

Electrophoretic mobility

As expected from the small pore size, the hydroxyethylated agarose gel is a poor separation medium for long DNA. The mobility becomes size insensitive already at about 4 kilo-base-pairs (kbp), whereas (as is well known) the separation is retained up to about 50 kbp in the unmodified agarose under otherwise identical conditions (Fig. 10). Of main interest in this study is how the mobility of long DNA (in our case 164 kbp) varies with field strength. Figure 11 shows that in both gel types the mobility increases strongly with field but tends to level out at 15–20 V/cm, as has been reported earlier for unmodified agarose [2]. The mobility in hydroxyethylated agarose is consistently lower, however, and in relative terms especially at low fields. In fact, the ratio of the mobility in nusieve to that in ordinary agarose is as low as 0.3 below 1 V/cm (Fig. 11, top) from which it increases strongly between 1 and 5 V/cm and then levels out at approximately 0.7 above 10 V/cm.

Ferguson plots

Figure 12 shows that the Ferguson plots of the *Hae*III–ΦX174-DNA fragments are linear for all DNA sizes used. The mobility at zero gel concentration for the different DNA sizes extrapolated to zero molecular weight (inset) gave a free solution mobility of 33×10^{-5} $\text{cm}^2\,\text{V}^{-1}\,\text{s}^{-1}$ in good agreement with earlier results [29]. The average pore radius, calculated from the DNA sizes exhibiting half the free solution mobility, varies as

$$P_E \sim A^{-1.4} \tag{9}$$

between 1.1% and 2.5% gel concentration, with a pore diameter of 380 Å in 2% nusieve agarose. With this strong concentration dependence [compared to unmodified agarose, Eq. (1)] the pore sizes could have been made considerably smaller (presumably giving a stronger suppression of the hernias) by increasing the nusieve concentration. However, spectroscopic studies of native DNA (requiring UV) is considerably hampered by the accompanying strong increase in gel turbidity, and studies of electrophoretic mobility (this work) and loop barriers [8] were therefore restricted to 2% nusieve.

IV. DISCUSSION

The mode of migration is the same in hydroxyethylated and unmodified agarose

The presence of DNA-induced electro-osmotic flows (evidenced by the polarity-dependent slow LD component), and

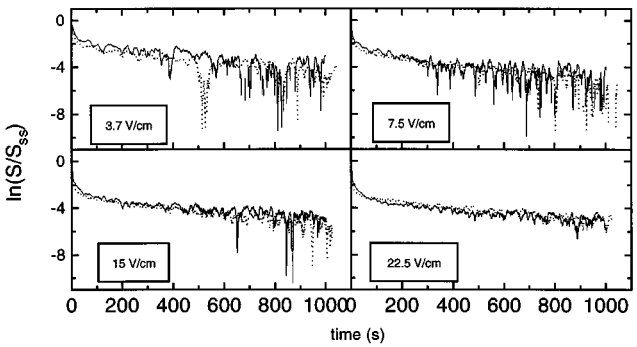

FIG. 7. Semilogarithmic plot of the long-time field-free relaxation of S normalized to steady-state level (S_{SS}) in 1% agarose (dotted) and 2% nusieve gel (full) at four different field strengths (indicated).

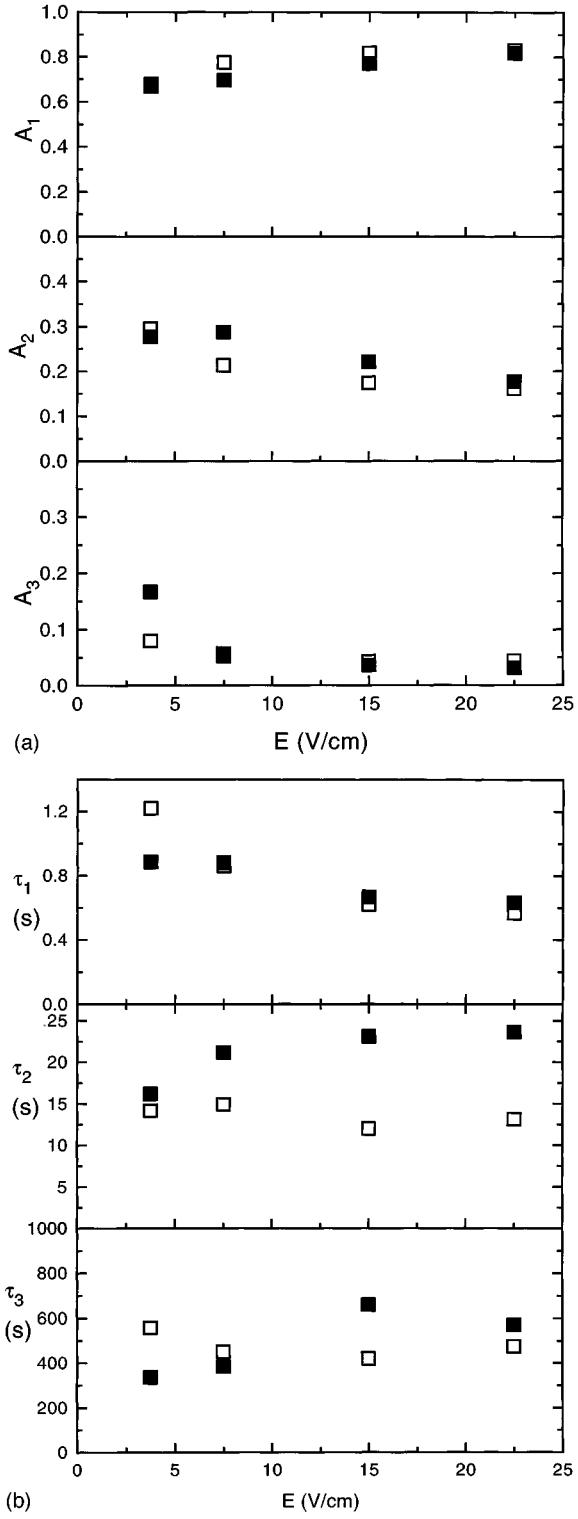

FIG. 8. Amplitudes (a) and corresponding time constants (b) for the best fit of the field-free relaxation of S/S_{SS} (Figs. 6 and 7) to Eq. (5), in 1% agarose (open symbols) and 2% nusieve gel (filled symbols).

the observation that DNA is trapped in hydroxyethylated gels with the similar efficiency as in unmodified agarose [8], indicate that DNA interacts with the gel fibers in a manner that is very similar in the two gels. Furthermore the oscillatory LD shows that $T2$-DNA migrates by the same cyclic mechanism in nusieve as in unmodified agarose. The results

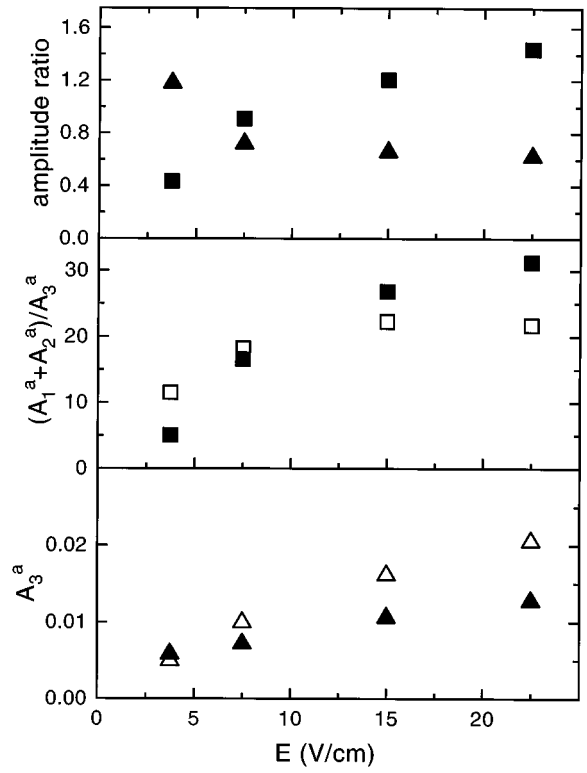

FIG. 9. Absolute amplitude A_3^a (bottom panel) and absolute amplitude ratio $(A_1^a+A_2^a)/A_3^a$ (middle panel) calculated from Figs. 8 and 5 according to Eq. (7), in 1% agarose (open symbols) and 2% nusieve gel (filled symbols). Top panel shows the ratio of the values in 2% nusieve and in 1% agarose for A_3^a (triangles), and for $(A_1^a+A_2^a)/A_3^a$ (squares).

presented here can therefore be discussed within the present theoretical framework for the migration of long DNA in (unmodified) agarose gels.

The sigmoidally shaped increase in relative mobility in Fig. 11 (top) will be interpreted in terms of a transition between two modes of migration, one predominant below 1 V/cm and one dominating above 15 V/cm. Below typically 1 V/cm the migration in normal agarose is dominated by tube

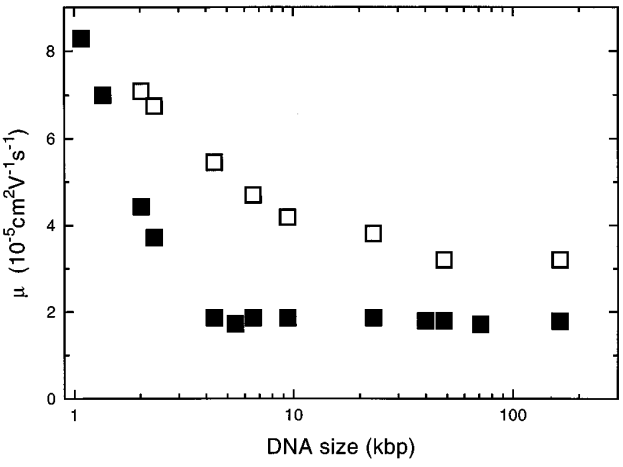

FIG. 10. Constant field electrophoretic mobility of DNA vs molecular weight in 1% agarose (open symbols) and 2% nusieve gel (filled symbols). Field strength 2.6 V/cm.

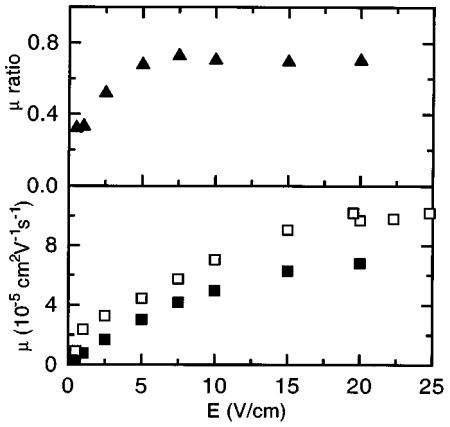

FIG. 11. (Bottom) Constant field electrophoretic mobility μ of $T2$-DNA vs field strength in 1% agarose (open symbols) and 2% nusieve gel (filled symbols). (Top) The ratio between μ in 2% nusieve and in 1% agarose.

orientation effects [11,2], and it is clear from the low mobility ratio at low fields (Fig. 11, top) that the reduced pore size in the hydroxyethylated gel has considerable influence on this type of migration. For stronger fields an analysis [2] of available data on tube orientation and DNA stretching indicates that the electrophoretic mobility in ordinary agarose increases strongly between 1 and 15 V/cm, and tends to saturate between 15 and 20 V/cm (Fig. 11, bottom), because of a similarly strong increase and saturation of the DNA stretching. In Ref. [2] the degree of stretching was monitored indirectly through the amplitude ΔS of the LD oscillations (Fig. 4), which is correlated [2] with the stretching as operationally defined by the orientation relaxation. Here direct evaluation of the stretching amplitude up to fields as high as 22.5 V/cm (Fig. 9, middle) confirms that in 1% agarose the degree of stretching $[(A_1^q+A_2^q)/A_3^q]$ indeed increases up to about 15 V/cm and then saturates. The considerably higher mobility ratio in the stretching-dominated mobility regime (above 15 V/cm in Fig. 11, top) thus indicates that this mode of motion is much less sensitive to pore size. We will return

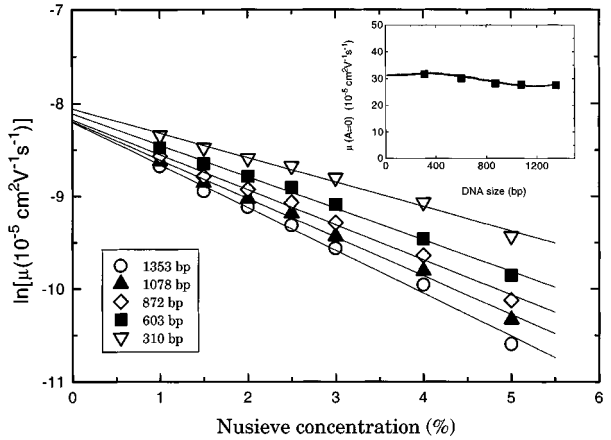

FIG. 12. Ferguson plot for DNA of different sizes (indicated in base pairs) in nusieve gel at 6 V/cm. Straight lines are best fits used to calculate gel concentration at which a certain fragment has half the free solution mobility (see text). Inset: Variation of intercept at zero gel concentration with DNA size.

below to the possible reasons for the differences in effective friction in the low- and high-field limits, and first concentrate on the intermediate field regime, where DNA exhibits strong field dependence in the mobility in both gels.

DNA stretching and mobility in unmodified agarose gels: A hypothesis

The steady-state electrophoretic mobility μ is given by [2]

$$\mu = Q/f, \tag{10}$$

where Q is the electrophoretic charge and f the effective friction coefficient for center-of-mass motion. Since Q can be considered to be independent of field strength in the present range of fields, the increase and saturation of the mobility reflects that the effective friction coefficient f decreases progressively between 1 and 15 V/cm and then saturates [2]. The correlation between mobility and stretching (regarding growth and saturation) therefore suggests that enhanced stretching of the DNA molecules decreases the effective friction coefficient [2]. Here we present and test the hypothesis that DNA loops, leaked or pulled out of the reptation tube into hernias, contribute to the effective friction for center-of-mass motion at intermediate fields. This hypothesis can explain why enhanced stretching of the DNA by an elevated field will reduce the friction, because stretching of the DNA along the tube will consume and reduce the number of hernias, so that the retarding electric force from them will be weaker. The mechanism by which the hernias should retard the migration is very simple. LD and microscopy together [14] show that the hernias are field aligned at the pore level, so for the whole molecule to move forward it has to pull the DNA loops backwards against the field. This hypothesis is first discussed against the present knowledge about hernia formation in unmodified agarose, and then tested by the data presented here on $T2$-DNA migration in nusieve agarose where hernias are known to be suppressed [8].

Field effects on the distribution of DNA segments between loops and the reptation tube in unmodified agarose

The idea that for DNA molecules migrating in (unmodified) agarose gels, loops of DNA are pulled out into hernias has been suggested on the basis of several indirect observations (see [20]). The first direct experimental support for their existence came from combining LD and microscopy data on migrating $T2$-DNA [14], but that study did not address the question of how the strength of the electric field affects the amount and sizes of the hernias. However, hernia formation should be prominent between roughly 1 and 15 V/cm, according to the following observations. (i) Below 1 V/cm hernia formation is suppressed because the energy cost of loop formation is too high [8]. (ii) Above 15 V/cm the stretching saturates most likely because all loops are consumed by the now dominating tube-lengthening contribution. The latter conclusion is strongly supported by an analysis [2] of data on the apparent length of the reptation tube [30], which increases and saturates in good correlation with the stretching (as defined in terms of the LD decay amplitudes), and reaches a length consistent with the contour length of the

DNA [2]. (iii) Finally we note that the combined LD and microscopy study [14] that supported the presence of the loops was conducted at a field strength (8 V/cm) in the middle of the field range where it is suggested here that hernias should be abundant.

The accumulated data thus support that there is a progressive transfer of DNA segments from the hernias into the tube (which becomes longer) when the field is increased between 1 and 15 V/cm. Thus, if the hernias are abundant enough to be of importance for the migration, their impact should be greatest at low fields above about 1 V/cm and diminish progressively as the field is increased, and disappear above about 15 V/cm. This is in good agreement with the mobility behavior as a function of field strength, as required if hernia consumption by stretching shall be able to explain the decrease in friction with increasing field strength.

Mobility and stretching in nusieve agarose

Also in the hydroxyethylated gel the mobility (Fig. 11) and stretching (Fig. 9, middle) tend to grow and level out in parallel between 1 and 20 V/cm, in a way similar to normal agarose. This indicates that the field dependence of the mobility is governed by a similar stretching mechanism as in unmodified agarose, which is expected since the mode of migration is of the same cyclic nature in the modified gel. Nusieve gels can therefore be employed to test if hernia consumption by tube lengthening contributes to making the mobility increase with increasing field strength. Since the tighter pores in the modified gel suppress hernia formation [8], the prediction is that the mobility increase should occur for lower fields in nusieve gels than in unmodified gels, because less tube lengthening (i.e., a lower field) is required to remove the hernias in order to increase the mobility. In accordance with this prediction, the mobility in hydroxyethylated gel increases compared to normal agarose (Fig. 11, top) in the field range of 1–5 V/cm where the hernias should reduce the mobility most severely in unmodified agarose.

If the hernias were the only source of resistance the present hypothesis would predict that the mobility should be higher in nusieve than in ordinary agarose at any given field strength. This is clearly not the case (Fig. 11), and this is because the center-of-mass motion of the molecules is retarded also by direct friction with the tube walls (modeled through a tube-friction coefficient) and by detour effects due to lack of perfect field alignment of the reptation tube [10]. These effects can be studied separately from hernia effects, in terms of the approximate constant levels of relative mobility in the limits of low and high fields, where the hernia contribution to the friction should be zero because the hernias are absent (due to loop barriers and DNA stretching, respectively). Results presented below show that the smaller pores in the nusieve gel indeed increase the retarding effect of both these phenomena, and more so at low fields. This explains why the mobility is overall lower in the nusieve gel, and more so at low fields. The particular friction effect of the hernias then will be superimposed on this general and field-dependent decrease of the mobility in the nusieve gel, and their effect can be enhanced by forming the relative mobility. Thus, we suggest here that the relative mobility increases in a sigmoidal fashion between 1 and 5 V/cm because the friction-reducing benefit of the tube lengthening occurs at lower fields in the nusieve gel, since there are fewer hernias to consume.

One important question is whether the smaller pores in the nusieve gel really can reduce the number of hernias substantially in the field range where the relative mobility increases most strongly, which is between 1 and 5 V/cm (Fig. 11, top). This seems reasonable in view of the results on the loop barriers in the two gels, which are presented in the accompanying paper [8]. At the field strength at the ''midpoint'' (2.5 V/cm, Fig. 11, top) the loop nucleation time is about 40 s in the nusieve gel and about 7 s in the 1% unmodified agarose [8]. This should be compared with the period time (which equals t_u [14]) of the cyclic migration, which is about 40 and 20 s in the nusieve and normal agarose, respectively, at the somewhat higher field strength of 3.7 V/cm (Fig. 3). In nusieve the typical hernia thus hardly has time to nucleate during the typical cycle period, whereas in the unmodified gel the nucleation time is short enough to allow loop formation during each cycle. It is also noteworthy that the field strength at the point where the mobility ratio levels out (5 V/cm, Fig. 11, top) is the same field (within experimental uncertainty) where the nucleation time becomes negligible even in the nusieve gel [8]. This is consistent with the relative mobility leveling out because at this stage the hernia contribution to the resistance should be about the same in the two gels.

Orientation measurements support that hernias are suppressed in the modified gel

If the hernias are suppressed in the nusieve gel, one expects a decrease in the measured helix orientation. A DNA loop will be field oriented as a lever, by a similar mechanism that orients the leading DNA segment in biased reptation models [10]. Therefore the DNA helix is field aligned in the hernias [14], while it is likely to be of more random orientation if the corresponding DNA segments are confined to the tube. The total helix orientation S is indeed considerably lower in the hydroxyethylated gel than in the unmodified gel (Fig. 5, bottom) and especially so in the field range below 10 V/cm (Fig. 5, top). One contribution to the lower value of S is that the tube orientation is lower in the modified gel, as is seen from Eq. (8) and the fact that the amplitude of the reptation component of the relaxation and A_3^q is lower in the nusieve gel (Fig. 9, bottom). This result is expected from biased reptation theory because smaller pores give rise to weaker field bias on the first DNA segment as it creates new tube segments [10]. However, the reduced tube orientation is unlikely to be the cause for the strong reduction of the total orientation in the hydroxylated gel below 10 V/cm (Fig. 5, top), since the ratio of the A_3^q amplitudes is essentially independent of field (Fig. 9, top, triangles, where it is seen that it even increases at the lowest field). By contrast, the ratio of the stretching components $(A_1^q+A_2^q)/A_3^q$ in the two gels (Fig. 9, top, squares) exhibits a similar (but less pronounced) decrease when the field is decreased as does the ratio of the total orientation factors (Fig. 5, top). It is also noteworthy that in the modified gel the overshoot-amplitude ratio is much more reduced at low fields than at high (Fig. 4,

top), which also supports that the degree of stretching is reduced compared to the unmodified gel at low fields [2].

The orientation measurements thus support that it is stretching rather than tube orientation that is responsible for the drop in orientation in the nusieve gel below 10 V/cm. This is consistent with the reduction of the number of hernias that was invoked to explain why the mobility grows faster with field in the hydroxyethylated gel than in normal agarose in the same field range. We cannot unequivocally assign the decrease in stretching to suppression of the loops, however, since is not possible to deconvolute the hernia and tube-lengthening contributions to the measured stretching.

Effective friction in the high- and low-field limits

According to the present analysis loops are absent in the limits of high and low fields, where the mobility ratio is constant. That the corresponding levels are less than 1 reflects more severe detour effects (from lower degree of tube orientation) and/or higher resistance from tube friction (because of the smaller pore size) in the modified gel.

Under the assumption that the tube is perfectly aligned in both gel types at the highest field investigated, the increase in tube friction coefficient can be estimated from the mobility ratio, which equals the ratio of the tube friction coefficients [10]. The mobility ratio plateaus at about 0.7 in the limit of strong fields (Fig. 11, top) indicating a 30% higher tube friction coefficient in the modified gel. A similar value can be deduced [31] from the ratio of 1.25 for the overshoot time (Fig. 2, top) in the two gels at the highest field strength. These are upper limits, however, since the A_3^q amplitude indicates a lower tube orientation in the modified gel than in unmodified agarose also at the highest field (Fig. 9, bottom), which will tend to both reduce mobility [10] and increase t_p [31].

The level of the relative mobility in the low-field limit (0.3) is considerably lower than in the high-field limit. In such low fields tube orientation effects govern the mobility [11], and the lower tube orientation in the hydroxyethylated gel is expected to add to the effect of enhanced tube friction and slow down the molecules compared to unmodified gel even more than in strong fields. Quantitative comparison can only be made on the basis of absolute values of the tube orientation, however.

Mechanism of field-free relaxation

The most urgent refinement of the present analysis is to resolve the hernia and tube-lengthening contributions to the stretching. This possibly can be accomplished by a better understanding of the field-free relaxation, and the present investigation offers some interesting aspects of the process. The fact that the slow stretching process (τ_2) is considerably more retarded by smaller pore size than the fast (τ_1) extends a similar trend observed within a more limited range of pore sizes [16]. It also further supports that the two-exponential fitting model corresponds to two experimentally distinguishable relaxation processes for the stretching, as was earlier proposed on the basis of the effect of the molecular weight on the relaxation [23]. The lack of sensitivity of the fast process to average pore size (here) and DNA length [23] indicates a relaxation process localized at the pore level, consistent with the short time scale. Loop retraction is clearly a potential mechanism for the fastest LD relaxation since the enhanced barrier in the nusieve gel should primarily act to slow down loop creation [8] but not loop withdrawal. This possibility is interesting for the question of resolution of hernia contributions to the stretching amplitude, but since the fast destretching component is even more dominating at the highest field [Fig. 8(a)] where hernias are believed to be absent, A_1 itself obviously cannot be used to monitor hernia abundance. The considerably higher sensitivity of the second process to pore size suggests that it involves transport of DNA segments through narrow channels into larger cavities, either situated along the path of the original tube, or as recepients of chain that leaks out of the tube.

ACKNOWLEDGMENT

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