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High-Resolution Separation and Accurate Size Determination in Pulsed-Field Gel Electrophoresis of DNA. 2. Effect of Pulse Time and Electric Field Strength and Implications for Models of the Separation Process†

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ABSTRACT: Bacteriophage DNAs annealed into linear oligomeric concatemers were used to examine the quantitative pulsed-field gel electrophoretic behavior of different-sized DNAs as a function of electrical field strength and pulse time. Three zones of resolution are observed for increasingly larger DNAs. In the first two zones, the electrophoretic mobility decreases linearly with increasing DNA size. The separation in zone 2 is roughly twice that in zone 1. The largest DNA molecules do not resolve at all and migrate in a compression zone. Mobility in zone 1 increases linearly with the electric field strength and decreases with the inverse of the pulse time. The behavior of DNA in zone 2 is qualitatively similar. However, the effect of field strength and pulse time on the separations in each zone is quite different. The results for zone 1 are generally consistent with the predictions of several existing physical models of pulsed-field gel electrophoresis, but no model accounts for all of the observed behavior in the three zones.

Conventional agarose gel electrophoresis of DNA, like gel filtration, is a sieving technique. The driving forces, gravitational in gel filtration and electrical in electrophoresis, are proportional to the size of the macromolecule. Frictional forces are also proportional to size, and so the velocity of particles in both techniques is size independent (Olivera et al., 1984). Separation of molecules smaller than the maximum pore size of the matrix occurs because the smaller the molecule, the larger the fraction of pores it can enter. The size limit for sieving of DNA is a few hundred base pairs (bp), where DNA lengths approach the size of the pores. Larger molecules move through the matrix in a stretched out form by a reptation process with a velocity that is inversely proportional to length (Lumpkin & Zimm, 1982).

DNA molecules with sizes above about 20 000 base pairs (20 kb)[1] do not resolve well in conventional agarose gel electrophoresis at finite field strengths because of electrical orientation effects (Lumpkin et al., 1985). However, the introduction of pulsed fields dramatically extends the range of agarose gel electrophoretic separations (Schwartz et al., 1983). In this technique, DNA is subjected alternately to two electrical fields at different angles for a time called the pulse time, τ. The molecules must presumably change direction prior to net translational motion each time the field is switched. Larger molecules take longer to change direction and have less time to move during each pulse, so they migrate slower than smaller molecules. We can operationally define a reorientation time as that portion of the pulse that does not result in net translational motion. The exact meaning of this time will depend on the particular physical model used to describe the reorientation process.

Pulsed-field gel electrophoresis (PFG) should resolve molecules that can complete reorientation within the pulse time. Larger molecules, which are unable to reorient rapidly enough, will presumably achieve an average orientation midway between the two applied fields (Schwartz & Cantor, 1984). Molecules so small that their reorientation time is short

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[1] Abbreviations: kb, kilobase pairs; Mb, megabase pairs; τ, pulse time; E, electric field strength; TBE, 0.1 M Tris, 0.1 M borate, and 0.2 mM EDTA, pH 8.4; μ, electrophoretic mobility; N, molecular size; N_i, size of DNA species at the inflection point between zones 1 and 2; N_c, size of the smallest DNA species in the compression zone; μ_i, mobility of the DNA species at the inflection point between zones 1 and 2; μ_c, mobility of the compression zone; m, number of pulses; h, length of the DNA molecule; θ, angle between the midline of the gel and the applied field; r, distance moved in PFG; r_0, distance moved in continuous electrophoresis; μ_0, mobility of DNA in continuous electrophoresis.

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compared to the pulse time will spend most of the pulse duration in conventional electrophoretic motion where size resolution is quite limited. As a result of this, resolution in PFG is likely to be optimal for molecules with reorientation times comparable to the pulse time. At applied field strengths of about 10 V cm^{-1}, 0.1-s pulse times resolve DNA optimally in the 5-kb size range, while pulse times of 1000 s at 3 V cm^{-1} are used to resolve 3–7-Mb molecules (Smith et al., 1987). The angle between the alternating electrical fields can be constant or variable [see Anand (1986) and Cantor et al. (1987) for a review of available systems].

A variety of parameters influence PFG performance. We have systematically examined a number of these to understand the separation process and to obtain data necessary for the critical testing of different theories of PFG. This investigation should also aid in the determination of optimal running conditions for different types of separations. Here we report on the effects of field strength, E, and pulse time, τ. The influence of agarose, concentration, temperature, field geometry, and DNA topology are presented in accompanying papers (Mathew et al., 1988a,b; Cantor et al., 1988).

Materials and Methods

DNA Samples. Bacteriophage DNA concatemers were prepared by tandemly annealing DNA from P2, λcI_{857}, and λvir bacteriophages (Mathew et al., 1988a).

Pulsed-Field Gel Electrophoresis. All of the results presented were obtained on 33- and 55-cm apparatus, the LKB Pulsaphor and a Pulsaphor prototype, respectively, using electrode positions described elsewhere (Mathew et al., 1988a). PFG experiments were typically 40–50 h in duration and utilized relatively intense electric fields (~10 V cm^{-1}). Consequently, the running solution was strongly buffered and the concentration of current carriers kept low to minimize heating effects. We routinely use a modified TBE buffer (100 mM Tris and 100 mM borate, pH 8.4, containing 0.2 mM Na_2EDTA). On the 33-cm apparatus run at 10 V cm^{-1}, currents ranged from ~200 mA at the start to ~300 mA after 40 h. Near optimal running conditions for the separation of up to 1×10^6 base pairs (1 Mb) of DNA have been empirically found to be E = 9–10 V cm^{-1} and τ = 100 s at 15 °C. In the experiments reported here, the field strength was varied from 2.5 to 12.5 V cm^{-1} with the pulse time fixed at either 100 or 25 s. Pulse times were also varied from 25 to 400 s with the electric field strength fixed at either 5 or 10 V cm^{-1}. Mobilities were calculated as described in Mathew et al. (1988a). One percent agarose was used because this offers a reasonable compromise between resolution and running time.

Results

To study the quantitative dependence of PFG mobility on DNA size, we examined the behavior of a set of concatemers of linear bacteriophage DNAs. Plots of mobility (μ) vs molecular size (N) exhibit three zones of separation (Mathew et al., 1988a; Vollrath & Davis, 1987; Southern et al., 1987). The first two zones show a linear decrease in mobility with increasing molecular size. Very large DNA molecules do not resolve at all and bunch together in the compression region or zone 3. The molecular size at the inflection point between zones 1 and 2 and the behavior of different DNAs in zone 2 can differ for different bacteriophage concatemers (Mathew et al., 1988a). Here λcI_{857} concatemers were used for analyses of the inflection point and zone 2.

Variation of Electrical Field Strength. Figure 1 presents a montage of PFG experiments using a pulse time of 100 s and nominal electrical field strengths ranging from 2.5 to 10.0

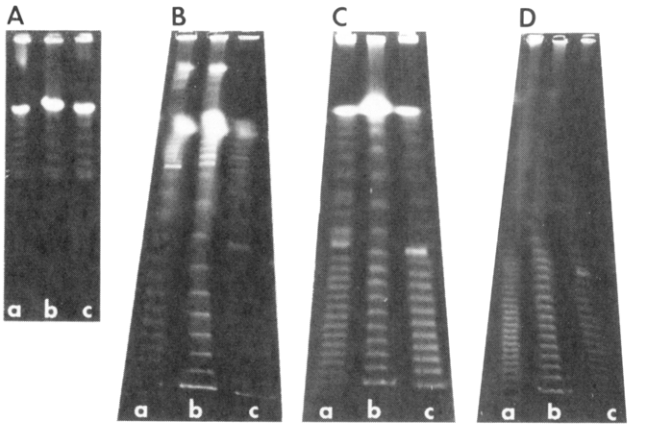

FIGURE 1: Electric field strength dependence of PFG mobility. Bacteriophage DNA ladders were analyzed in 1% agarose at a pulse time of 100 s and a temperature of 15 °C: (A) 2.5 V cm^{-1} for 250 h; (B) 5 V cm^{-1} for 80 h; (C) 7.5 V cm^{-1} for 60 h; (D) 10 V cm^{-1} for 40 h. Lanes contain (a) P2 DNA (33 kb); (b) λcI_{857} DNA (48.5 kb), and (c) λvir DNA (40.5 kb).

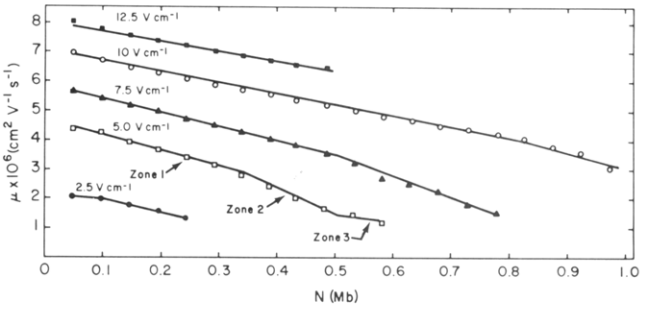

FIGURE 2: Mobility of λcI_{857} DNA as a function of electric field strength. The results are plotted from the data of Figure 1. The various zones of resolution are indicated. (●) 2.5 V cm^{-1}; (□) 5 V cm^{-1}; (▲) 7.5 V cm^{-1}; (○) 10 V cm^{-1}; (■) 12.5 V cm^{-1}.

V cm^{-1}. The field strengths are computed as the applied voltage divided by the size of the buffer chamber. Because of the complex electrical field shape used, the actual local field at any point will differ from the applied field by a constant, voltage-independent ratio. The variation in local field strength throughout the trajectory followed by a particular molecule is small (less than 10% over the whole gel or under 5% through any one zone) (Cantor et al., 1988). Thus, we can ignore these effects and use the nominal electric field strengths.

The mobility of selected DNA species from Figure 1 is plotted in Figure 2 as a function of molecular size. There is a marked effect of field strength on absolute mobilities and on the position of the inflection point between the two zones of linear resolution. The DNA size at the inflection point decreases with decreasing electric field strength. At 2.5 V cm^{-1}, the inflection point lies between the first two points of the λ ladders. The slopes of all the plots in Figure 2 are similar, illustrating that, in PFG, resolution in zone 1 is essentially independent of the field strength.

$$\partial\mu/\partial N_\tau \propto E^0 \qquad (1A)$$

In addition, the apparent mobility of the particles is proportional to the applied field for relatively short DNAs at field strengths of 5 V cm^{-1} or greater (Figure 3). These two results imply that the mobility must have the functional form

$$\mu = AE - BN \qquad (1B)$$

where A and B are independent of N and E.

Variation of Pulse Time. Figure 4 presents a montage of PFG experiments run at various pulse times ranging from 50

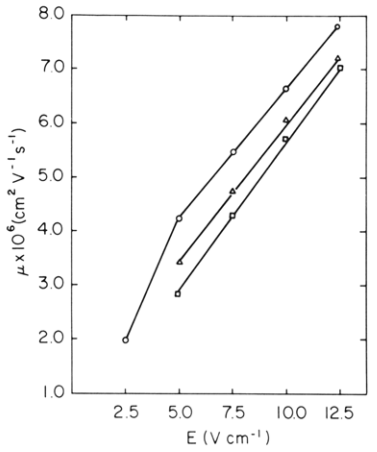

FIGURE 3: Variation of the estimated PFG mobility of λcI_{857} DNA with electric field strength. Data from the initial linear portion of the μ vs N plots in Figure 2 were replotted to show the field dependence explicitly. (○) 97 kb; (△) 242 kb; (□) 340 kb.

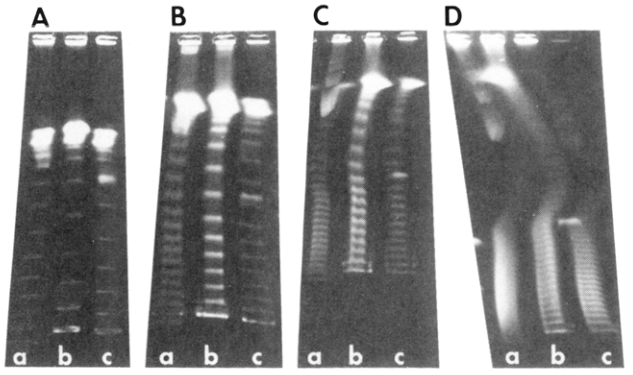

FIGURE 4: PFG analyses of bacteriophage ladders run in 1% agarose at 5 V cm^{-1} and 15 °C for 85 h. Pulse times: (A) 50 s; (B) 100 s; (C) 200 s; (D) 400 s. Lanes contain (a) P2 DNA, (b) λcI_{857} DNA, and (c) λvir DNA.

to 400 s at 5 V cm^{-1}. Plots of mobility vs molecular size from these experiments are shown in Figure 5. The slopes of these plots are strongly dependent on pulse time. Figure 6A shows that, for a given size of DNA in zone 1, the PFG mobility decreases inversely proportionally to the pulse time. Furthermore, the slope of this decrease increases linearly with the inverse pulse time and with molecular size as shown in Figure 6B,C. This suggests that in PFG the mobility of DNA should have the functional form

$$\mu = C - DN/\tau \quad (1C)$$

where the constants C and D are independent of N and τ. Combining eq 1B with 1C allows us to summarize the bulk of the experimental results for separation in zone 1 by the simple functional relationship

$$\mu = AE - DN/\tau \quad (1D)$$

Behavior of DNA at the Inflection Point between Zones 1 and 2. For most of the PFG experiments reported here, the mobility decreases linearly with DNA size in two regimes designated zone 1 and zone 2 (see Figure 2). We define N_i as the size of the DNA molecule at the inflection point between the two zones of linear resolution. N_i is quite sensitive to both pulse time and field strength (Figure 7A,B). However, the mobility of the inflection point, μ_i, is only weakly dependent on pulse time and field strength (Figure 7C,D).

The DNA bands above the inflection point are broader than those below it. The broadening is not an inherent property of the DNA sample. The same band, say the 388-kb band

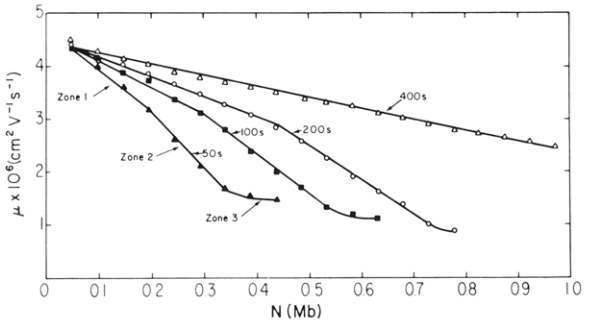

FIGURE 5: Size dependence of λcI_{857} DNA mobility during PFG with different pulse times. The curves were obtained by plotting the data in Figure 4. (▲) 50 s; (■) 100 s; (○) 200 s; (△) 400 s.

of the λcI_{857} ladder, can be either sharp or smeared at 5 V cm^{-1} depending on its position relative to N_i (Figure 1). For example, at τ = 200 s with N_i at 440 kb, this band is sharp, while at τ = 100 s with N_i at 350 kb, this same band is smeared. At a fixed pulse time, increasing the electric field sharpens the band because it shifts N_i (Figure 4). The 388-kb band of the λcI_{857} ladder is sharp at τ = 100 s and a field of 10 V cm^{-1} because N_i in this case is at 800 kb.

All DNAs larger than those found in zone 2 migrate together in a broad band called the compression region. Assuming that separation occurs only for DNA fragments with reorientation times comparable to or smaller than the pulse time, τ, the compression region should contain all molecules with longer reorientation times.

One should be able to use information from the compression region to estimate DNA reorientation times. However, the size of the DNA at the inflection point between zones 1 and 2 is better defined than that at the compression region. Thus, we have used N_i to estimate the dependence of the DNA reorientation time, τ_R, on DNA size and electrical field strength. It seems reasonable to expect that PFG resolution of DNA might be optimal for molecules with reorientation times comparable to the pulse time, τ. PFG separation of DNA above the inflection point is observed to be much better than below it, typically by a factor of 2. Thus, the inflection point may be related to the DNA reorientation time, and we speculate that the inflection point may occur when the reorientation time, τ_R, is some fraction k of the pulse time.

As shown by the results in Figure 8, N_i is very well fit by the relationship

$$N_i \propto \tau E^2 \quad (2A)$$

Substituting τ_R/k for τ in eq 2A and rearranging the result yield

$$\tau_R \propto N_i/kE^2 \quad (2B)$$

However, N_i can be varied at will by a suitable choice of pulse time and field strength. If k is size independent, eq 2B should be valid for any length of DNA and the subscript i can be dropped. This yields an estimate of the expected functional dependence of the DNA relaxation time

$$\tau_R \propto N/kE^2 \quad (2C)$$

where k is a constant and N is the size of the DNA.

Behavior of DNA in Zone 2. Zone 2 is the second linear zone of separation with a slope almost twice that in zone 1. It is bounded by N_i at the low molecular size limit and the compression zone at the other. We define N_c as the size of the smallest DNA species in the compression zone. We have seen that N_i and hence the size range of zone 1 increase with both pulse time and electric field strength. The size range of

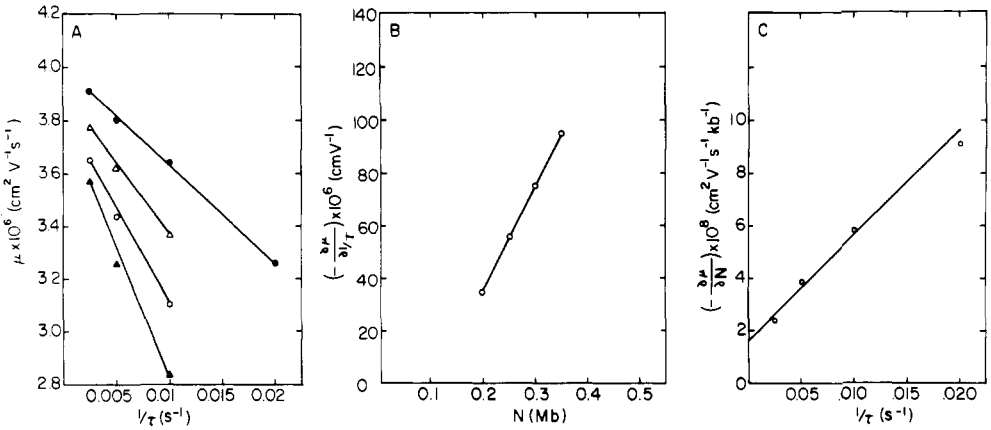

FIGURE 6: Dependence of PFG mobility on pulse time. (A) Mobility of λcI_{857} DNA as a function of inverse pulse time. (▲) 388 kb; (O) 340 kb; (△) 291 kb; (●) 194 kb. (B, C) Slopes of the curves in (A) plotted as a function of DNA size and inverse pulse time, respectively.

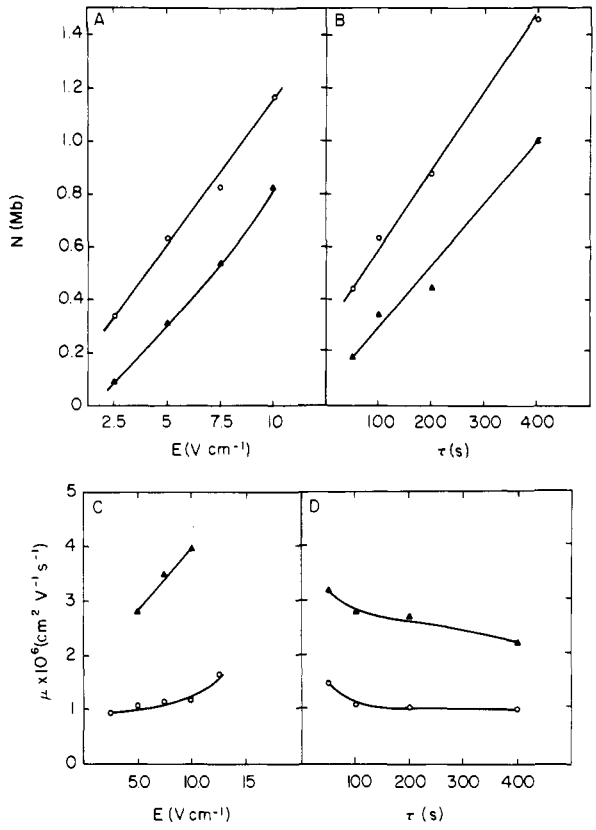

FIGURE 7: Behavior of DNA in the compression region and at the inflection point. (A) Variation of DNA size at the inflection point, N_i (▲), and of the smallest DNA species in the compression region, N_c (O) with electric field strength. The pulse time was kept constant at 100 s. (B) Variation of N_c (O) and N_i (▲) with pulse time at a field strength of 5 V cm^{-1}. Variation of the mobility of the compression zone, μ_c (O), and the inflection point, μ_i (△), with field strength at a pulse time of 100 s. (D) Variation of μ_c (O) and μ_i (▲) with pulse time at a field strength of 5 V cm^{-1}.

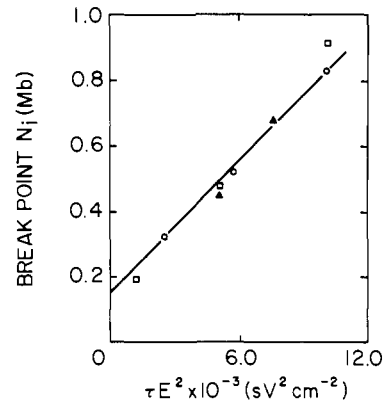

FIGURE 8: Variation of the DNA size, N_i, at the inflection point between the two linear separation zones in PFG as a function of pulse time and electric field strength. The symbols correspond to different sets of experiments from which the data are derived.

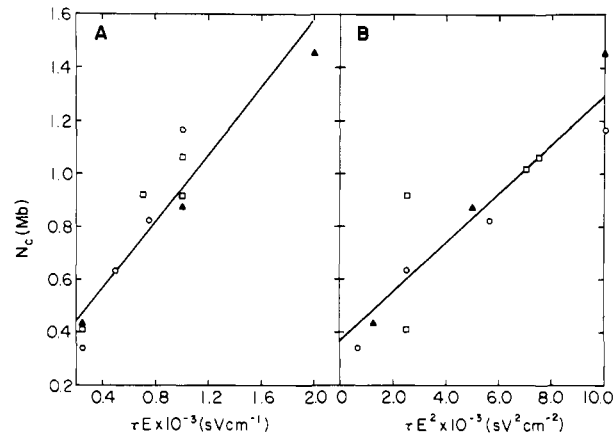

FIGURE 9: Variation in the minimum size of DNA in the compression zone, N_c, with pulse time and electric field strength. (A) N_c as a function of τE. (B) N_c as a function of τE^2. The lines drawn correspond to a linear least-squares fit of the data. The symbols correspond to different sets of experiments from which the data are derived.

zone 2, $N_c - N_i$, also increases with both pulse time and field strength, but much less so (Figure 7A,B). The size of zone 2 varies from 250 kb at 2.5 V cm^{-1} and 100 s to 450 kb at 5 V cm^{-1} and 400 s. N_i, on the other hand, varies from 100 to 1000 kb over the same range of experimental conditions.

N_c is sensitive to variations in either the pulse time or the field strength, varying almost linearly with both as shown in Figure 7A,B. There is, however, some uncertainty in the determination of N_c, especially at high field strengths or long pulse times. Thus, it is difficult to determine whether N_c is fit better with a function of τE or τE^2 (Figure 9). In contrast to the behavior of N_c, the mobility of the compression zone, μ_c, is only weakly dependent on pulse time and field strength (Figure 7C,D).

The separation in zone 2 can be estimated as $(\mu_i - \mu_c)/(N_c - N_i)$. Figure 10A shows that the separation in zone 2 increases with increasing field strength up to a plateau at about 10 V cm^{-1}. Figure 10B demonstrates that separation decreases linearly with increasing pulse time before a plateau is reached at about 400 s. Hence, in order to achieve large separations of DNA molecules in zone 2, relatively short pulse times and

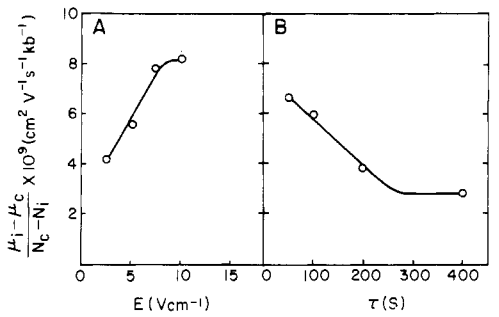

FIGURE 10: Separation in zone 2 (A) as a function of electric field strength and (B) as a function of pulse time. Here separation is measured as the total change in mobility divided by the size range between the inflection point between zones 1 and 2 and the compression zone.

high field strengths are needed. Thus, zone 2 is really most useful for DNAs of less than 1 Mb. The ratio of the separation in zone 2 to that in zone 1 varies with both pulse time and electric field strength. At τ = 100 s the ratio decreases from 1.75 at E = 5 V cm^{-1} to 1.55 at E = 10 V cm^{-1}, while at E = 5 V cm^{-1} it increases from a value of 1.45 at τ = 50 s to 1.90 at τ = 200 s.

DISCUSSION

Optimization of Experimental Performance. The size of the largest resolved DNA species increases with increasing pulse time (Figure 7A). At a given pulse time, an increase in the electric field should decrease the reorientation time (eq 2C) and hence resolve larger fragments. This is indeed the case where runs of adequate quality have been obtained (Figures 1 and 2). However, other factors militate against attempts to use very high fields, say 30 V cm^{-1}, to resolve megabase-sized DNAs. In general lower fields are required to achieve quantitative recovery of megabase DNA in sharp, well-resolved bands (Smith et al., 1987). At fields of more than 10 V cm^{-1}, poor recovery and smeared bands are usually observed. It is not clear if this is due to failure of the large molecules to enter the gel pores at high field or to shear damage. Such damage would be expected to occur preferentially at high fields and with the largest molecules in a population. Deterioration in PFG performance at high field is a particularly serious problem with bacteriophage ladders that are annealed and not ligated and so have a number of potential weak points.

PFG separations at high field strength are also subject to much more extensive lateral spreading than those at lower fields. However, at a given field strength the DNA separation range can always be adjusted by a suitable choice of pulse time. Thus, in selecting the field strength for a particular experiment, a compromise between run time and resolution has to be made.

DNA size separation in zone 2 is better than that in zone 1 by a factor between 1.5 and 2. Hence, it can be useful to adjust parameters so as to bring the region of interest into zone 2. Resolution in this zone can be optimized by using pulse times of less than 200 s and field strengths of 7.5–10 V cm^{-1}.

Estimation of DNA Reorientation Times. Two features of DNA behavior in PFG must be clarified before an adequate theory can be developed. These are the configurations of DNA molecules in the gel and the mechanism of DNA reorientation in response to an altered electric field direction. The two features may be tightly related. Furthermore, they may be different in the three zones of PFG behavior seen under typical conditions and even different in various types of PFG systems.

DNA tends to align parallel to an applied electric field (Åkerman et al., 1985; Stellwagen, 1985). Switching the direction of the applied field requires the molecule to reorient between two roughly linear, highly extended configurations. Such a reorientation should have a time constant dependent on DNA length. In the biased reptation model for PFG, this reorientation time depends on a parameter called the stretching time. The stretching time for an oriented chain in a matrix with average pore size less than the average end-to-end length of the chain should be proportional to N^1E^{-2} (Viovy, 1987), in exact agreement with eq 2C.

We have assumed that the reorientation time of a molecule at the inflection point is some fraction, k, of the pulse time. Using eq 2 and data from Figure 5 for 500-kb DNA at 5 V cm^{-1}, one can set an upper limit at 200 s for τ_R by setting k = 1. This is much larger than viscoelastic relaxation times that range from 0.5 s for 150-kb DNA to 10 s for 1500-kb DNA (Kavenoff et al., 1974). Thus, earlier suggestions that PFG reorientation may be related to viscoelastic relaxation (Schwartz & Cantor, 1984) seem quite unlikely.

Estimation of PFG Mobility in Zone 1. To a first approximation, net translation of the DNA in PFG will occur only after reorientation. Hence, the PFC mobility should be proportional to the fraction of the pulse time remaining after reorientation. We can write

$$\mu = \mu_0(1 - \tau_R/\tau) \quad (3A)$$

where μ_0 is the mobility once the molecule has completed reorientation. Using eq 2C, we can rewrite eq 3A as

$$\mu = \mu_0(1 - N/kE^2\tau) \quad (3B)$$

Equation 3B is derived from a very simple physical picture and estimates of DNA reorientation times from inflection point behavior. For eq 3B to be consistent with the bulk of the experimental results summarized by the second term of eq 1D, μ_0 must be independent of τ and N and be proportional to E^2. This is just what is expected in the high-field limit of the biased reptation theories (Lumpkin et al., 1985). However, with this choice of μ_0 the first term of eq 3B is predicted to scale as E^2 for small DNA, while what is actually observed in an E^1 dependence (eq 1D). Thus, the picture we have developed still needs further refinement.

Implication for Models of DNA Reorientation in PFG. Three classes of models have been proposed to provide a quantitative explanation of PFG. These are a biased reptation model (Slater et al., 1987) recently modified by Viovy (1987), a switchback model (Southern et al., 1987), and a hairpin extension model (Deutsch, 1987).

The first class considers DNA to be confined in tubes of about the pore size of the agarose, larger than the persistence length of the DNA but much smaller than the equilibrium end-to-end distance. Disengagement from the tube, for a molecule previously aligned by a field, scales with a stretching time (Slater & Noolandi, 1985; Viovy, 1987) proportional to N^1E^{-2}. This scaling is experimentally supported by our data (Figure 8 and eq 2C), assuming a direct relation between the stretching and reorientation times. Separation is predicted to be optimal for a small range of sizes, selectable by a suitable choice of pulse time. This might represent the zone of enhanced separation we see for sizes above the inflection point. However, outside this region, separation is predicted to depend logarithmically on DNA size, which is contrary to what we observe.

A critical parameter in the biased reptation theory is the size of the tube through which the DNA moves. This is related to the pore size of the agarose matrix. Indeed, we find that the mobility of DNA in PFG electrophoresis is sensitive to the pore size (Mathew et al., 1988a). It is not clear, however, that

the theory would predict the stepwise change in resolution seen with pore size (Mathew et al., 1988a). Moreover, biased reptation models predict that the mobility is proportional to E^2, whereas, as discussed earlier, an E^1 relation is observed.

The other classes of model assume linear configurations of DNA in the gel once reorientation is complete (Southern et al., 1987; Deutsch, 1987) but differ in the configuration adopted during reorientation. Deutsch (1987) proposed that when the field is rotated, a nucleation step occurs with hairpin kinks budding out at variation points on the DNA chain. These kinks then compete with each other, some growing longer at the expense of the others, ending up with a single loop. This, in turn, may, in time, unfold to yield a linear molecule. This model predicts an activation energy for reorientation. The formation of kinks may require the local melting of DNA, consistent with the large temperature dependence of DNA mobility in PFG (Mathew et al., 1988a).

The pattern of kink formation should depend on the agarose matrix, and it is conceivable that the kink theory could explain the observed dependence of resolution on pore size (Mathew et al., 1988a). Finally, a spread of reorientation times is postulated even for a homogeneous population of DNA. It would appear that molecules with kinks still present when fields are switched will form complex structures with extremely low mobility. This should lead to enhanced resolution and smearing and perhaps explain the zone of enhanced separation we observe above N_i.

The switchback model postulates that moving a kink through the gel would require more energy than moving an end (Sothern et al., 1987). It suggests that, for each alternate pulse, the leading and trailing ends of the chain must reverse if the electric fields are at obtuse angles. Reorientation occurs as the new leading end pulls the rest of the molecule out of its previous configuration and into a new orientation. The reorientation time would thus be proportional to the length of the DNA and inversely proportional to its velocity, consistent with our findings. Furthermore, net separation for DNAs with sizes below N_i would be proportional to differences in length and to the number of times the field is switched.

The distance r migrated by DNA in the switchback model is (Southern et al., 1987)

$$r = r_0 \cos\theta - mh \cos\theta \tag{4}$$

where r_0 is the distance moved in conventional electrophoresis, θ is the angle between the midline of the gel and the applied field, m is the number of pulses, and h is the contour length of the molecule, which is proportional to the molecular size N. Dividing eq 4 by the nominal electrical field strength and time of the run ($m\tau$) yields

$$\mu = \mu_0 \cos\theta - h \cos\theta / E\tau \tag{5}$$

where μ_0 is the mobility in conventional electrophoresis.

Since large DNA molecules are not separated by continuous field electrophoresis, the first term of eq 5 must be independent of N. It follows that

$$\left(\frac{\partial\mu}{\partial h}\right)_E = \left(\frac{\partial\mu}{\partial N}\right)_E \propto -\frac{1}{E\tau} \tag{6}$$

The pulse-time dependence of PFG separation predicted by eq 6 is totally consistent with our data as summarized by eq 1D. However, the predicted field dependence is inconsistent because our data reveal that separation is essentially independent of field strength.

The switchback theory is based on the assumption that all molecules with sizes below N_i can complete reorientation within the pulse time τ. For molecules with sizes somewhat above N_i random variation in end-to-end distances would allow them to complete reorientation on some proportion of pulses (Southern et al., 1987). This leads to a predicted separation larger for sizes above N_i than below, in agreement with experiment. However, a serious disagreement arises for the behavior of molecules in the compression zone. The switchback theory predicts that these molecules should not move at all. In practice, the compression zone has a mobility about half that of the inflection point. This mobility increases with both the electric field strength and the pulse time (Figure 7).

None of the above models explains the separations achieved by field inversion gel electrophoresis, where the angle between fields is 180° (Carle et al., 1986). It is possible that the mechanisms of reorientation when fields are inclined at mildly obtuse angles (110–140° in these experiments) are different from those that operate at much larger angles. Experiments run at a wide variety of angles will be needed to investigate this.

In conclusion, no class of existing models fits all of the available PFG data. Most of the data are consistent with molecules adopting highly extended configurations, but the mechanism of reorientation is unclear. However, the data presented here should serve to stimulate the refinement of these theories and, if necessary, the generation of new ones.

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High-Resolution Separation and Accurate Size Determination in Pulsed-Field Gel Electrophoresis of DNA. 3. Effect of Electrical Field Shape†

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ABSTRACT: The resolution of pulsed-field gel electrophoresis is dramatically affected by the number and configuration of the electrodes used, because these alter the shape of the applied electrical fields. Here we present calculations and experiments on the effect of electrode position in one of the most commonly used pulsed-field gel electrophoresis configurations. The goal was to explore which aspects of the electrical field shape correlate with improved electrophoretic resolution. The most critical variable appears to be the angle between the alternate electrical fields. The most effective electrode configurations yield angles of more than 110°. A continually increasing angle between the fields produces band sharpening that greatly enhances the resolution.

In pulsed-field gel electrophoresis (PFG) DNA molecules moving in an agarose gel are forced to change their direction of migration, periodically, by alterations in the applied electrical field (Schwartz et al., 1983; Schwartz & Cantor, 1984; Carle & Olson, 1984). In the accompanying papers, we show that the pulse time and the electrical field strength can be adjusted to tune the size range of effective PFG resolution (Mathew et al., 1988a,b). It has been apparent from the very first PFG experiments that the shape of the electrical field also strongly influenced the separation pattern achieved by PFG (Schwartz & Cantor, 1984). In these early studies best results were obtained by using electrical fields that contained field gradients such as those that are generated when the positive and negative electrodes are of very different lengths (i.e., by using inhomogeneous fields). The effect of these field gradients in improving resolution was only partially understood. More recent studies have revealed that large DNAs can be resolved by PFG without field gradients when large angles between alternating applied fields are used (Carle et al., 1986; Cantor et al., 1986; Chu et al., 1986; Anand, 1986; Gemmill et al., 1987; Serwer, 1987; Southern et al., 1987).

In an attempt to optimize the quality of PFG separations and to provide a more rational basis for understanding the PFG phenomenon, we have calculated the electrical field shapes in some experimental electrode configurations that appear to provide generally excellent PFG results on a wide range of DNA sizes. Our goal was to identify the critical aspects of field shape responsible for high-resolution separations.

Materials and Methods

DNA Samples. Yeast chromosomal DNAs were prepared as described previously (Schwartz & Cantor, 1984), except that the DNA concentrations were typically 10 μg/mL. Details of the preparation of bacteriophage λ DNA concatemers are given elsewhere (Smith et al., 1986a,b; Mathew et al., 1988a). The sizes of all these DNAs are known (Mathew et al., 1988a).

PFG Electrophoresis. All of the results presented were obtained on horizontal submarine PFG apparatus using arrays of platinum point electrodes normal to the gel surface and connected through diodes to the power supply as described

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