Atypical Sieving of Open Circular DNA during Pulsed Field Agarose Gel Electrophoresis†

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ABSTRACT: Pulsed field agarose gel (PFG) electrophoresis, originally used to improve the resolution by length of linear DNA [Cantor et al. (1988) *Annu. Rev. Biophys. Biophys. Chem. 17*, 287–304], is found here to cause atypical sieving of 48.5–97.0-kb open circular DNA. Two procedures of PFG electrophoresis are used: rotating gel electrophoresis with rotation of 2π radians [2πRGE; Serwer, P., & Hayes, S. J. (1989) *Appl. Theor. Electrophor.* (in press)] and field inversion gel electrophoresis [FIGE; Carle, G. F., Frank, M., & Olson, M. V. (1986) *Science 232*, 65–68]. During 2πRGE at 6 V/cm, the electrophoretic mobility (μ) of 48.5-kb open circular DNA increases in magnitude as agarose percentage (A) increases from 0.4 to 1.5. The sieving revealed by this μ vs A relationship is highly atypical (possibly unique) for any particle. The extent of this atypical sieving increases as electrical potential gradient, DNA length, and pulse time increase. In some cases a maximum is observed in a plot of μ's magnitude vs A. The μ of open circular λ DNA is smaller in magnitude than the μ of equally long linear λ DNA. Atypical sieving has also been observed by use of FIGE. As pulse times used during FIGE decrease below those achievable by 2πRGE, the progressive loss of circular DNA's atypical sieving is accompanied by both a dramatic increase in μ's magnitude at the lower A values and a decrease in μ's magnitude at the higher A values. At the lower A values, open circular DNA sometimes migrates more rapidly than linear DNA of the same length. The sieving of a solid sphere (intact bacteriophage T3) is not modified by use of FIGE; intact T3 has been used as a normalization standard for μ. The atypical sieving observed here is explained by the following hypothesis: (1) Projections from the gel thread, and therefore arrest, open circular DNA during agarose gel electrophoresis. (2) This arrest is relieved by changing the direction of the electrical potential gradient. (3) The gel's threading capability increases as A decreases. Application of this hypothesis to the sieving of linear DNA explains previously found inconsistencies of theory and experiment.

To quantitatively explain the sieving of linear DNA during agarose gel electrophoresis, theories have been based on the assumption that the DNA is in a smooth-walled, nonbinding crooked tube (Lumpkin & Zimm, 1982; Lumpkin et al., 1985; Slater & Noolandi, 1986; Slater et al., 1987). By quantifying elevated electrical potential gradient induced elongation of the DNA's random coil (reptation), these theories have explained why electrophoretic mobility (μ)[1,2] increases in magnitude for DNA as the electrical potential gradient (E) increases [see also Hervet and Bean (1987)]. The above theories also have been used, together with the observed reptation of the DNA, to predict that an agarose gel's pore radius (P_E) varies with agarose percentage (A) by $A^{-0.15}$ (Hurley, 1986). This result is inconsistent with the conclusion, deduced from the sieving[3] of solid spheres, that P_E varies by $A^{-0.7}$–$A^{-0.9}$ [reviewed in Stellwagen (1987); see also Griess et al. (1989)]. Thus, at least one aspect of the above theories for DNA appears inaccurate.

One possible source of inaccuracy in the above theories is the assumption of smooth, noninteracting walls in the DNA-containing tube. An experimental observation that suggests inaccuracy of this assumption is an elevated E induced total arrest of open circular DNA (Mickel et al., 1977; Levene & Zimm, 1987; Serwer & Hayes, 1987). This arrest is relieved by periodically either turning off (Serwer & Hayes, 1987) or inverting (Levene & Zimm, 1987) the electrical field. Procedures for periodically varying either the direction or magnitude of E during agarose gel electrophoresis (collectively referenced as pulsed field gel, or PFG, electrophoresis) have also been used to improve the fractionation by length of linear DNA [reviewed in Anand (1986), Cantor et al. (1988), and Lai et al. (1989)].

Sieving of gels during electrophoresis is usually quantified by measuring μ as a function of A [reviewed in Chrambach (1985), Tietz (1987), and Stellwagen (1987)]. As indicated in these reviews, the magnitude of μ always decreases as a function of A. During preliminary attempts to detect gel–DNA interactions that could (1) explain the above inconsistency of theory and experiment and (2) improve the separation of DNA by its conformation (linear, circular, branched, for example), the opposite relationship of μ to A (atypical sieving) was observed during the PFG electrophoresis of open circular DNA. In the present study, the dependence of this atypical

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[1] Abbreviations: μ, electrophoretic mobility; E, electrical potential gradient; P_E, agarose gel's pore radius; A, agarose percentage; PFG, pulsed field gel; FIGE, field inversion gel electrophoresis; t_f, time of each forward electrophoresis during FIGE; RGE, rotating gel electrophoresis; 2πRGE, RGE by use of rotation through an angle of 2π radians; t_r, time of rotation during 2πRGE; μ_N, normalized μ; oc, open circular DNA; lin, linear DNA; t_a, average time required to arrest open circular DNA during agarose gel electrophoresis.

[2] μ is the migration velocity divided by the electrical potential gradient, E.

[3] As used here, "sieving" refers to any gel-induced retardation that depends on either the size or shape of the particle sieved.

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sieving on A, E, and pulse times is investigated. Implications of the results for explaining the above inconsistency are discussed.

Materials and Methods

Buffers and Reagents. Bacteriophages were stored in Tris/Mg buffer: 0.2 NaCl, 0.01 M Tris-HCl, pH 7.4, 0.001 M $MgCl_2$. DNA was stored in NET buffer: 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.4, 0.001 M EDTA. Electrophoresis buffer was 0.01 M sodium phosphate, pH 7.4, and 0.001 M EDTA. Seakem LE agarose was used to fractionate DNA in the running gels described below. All agarose was received from the Marine Colloids Division of FMC Corp. (Rockland, ME).

DNAs and a Spherical Standard. Linear DNA from bacteriophages T4 (approximately 170 kb; Kutter et al., 1983), T5 (approximately 120 kb; Son et al., 1988), and T7 (39.9 kb; Dunn & Studier, 1983) was obtained by diluting bacteriophage, purified by procedures previously described (Griess et al., 1989), into NET buffer with 0.1% Sarkosyl NL97 and subsequently incubating at 75 °C for 10 min. This treatment released most DNA from its associated capsid. To obtain purified DNA from bacteriophage λ, this bacteriophage was purchased from Stratagene (La Jolla, CA). For release of λ's DNA (48.5 kb; Sanger et al., 1982) and cyclization of this DNA by annealing of its complementary single-stranded ends, procedures used were those previously described (Serwer & Hayes, 1987). Bacteriophage T3, a spherical (icosahedral) standard with a radius of 30.1 nm, was grown and purified by procedures previously described (Griess et al., 1989).

Electrophoresis. All agarose gel electrophoresis was performed in horizontal agarose gels submerged beneath electrophoresis buffer. The gel rested on a raised platform between two buffer tanks in an apparatus with the inner dimensions of the Aquebogue Machine and Repair Shop Model 850 (Aquebogue, NY). The version of this apparatus used for field inversion gel electrophoresis (FIGE; Carle et al., 1986) has been previously described, as have the modifications needed to pour the multigels used for FIGE below (Serwer, 1986). To perform FIGE, electrical power was applied through one of the four ports of a field-inverting device from DNAStar (Madison, WI). The time of each forward electrophoresis (t_f) was always 3 times the time of reverse electrophoresis; only t_f is indicated below.

For rotating gel electrophoresis (RGE), the electrophoresis apparatus (above) has been modified to rotate the gel on a circular disk. Electrophoresis in one direction was periodically interrupted by rotation through an angle that, for all experiments reported here, was 2π (2πRGE; Serwer & Hayes, 1989). The direction of a rotation was opposite to that of the previous rotation. The electrophoresis apparatus and the means for rotating the disc have been previously described (Serwer & Hayes, 1989). The time of rotation (t_r) was, for all experiments described here, equal to the time of stationary electrophoresis.

To initiate electrophoresis, DNA in NET buffer was brought to 3–4% sucrose and 100 μg/mL bromphenol blue and was layered in sample wells. At first, an electrical potential gradient was applied at 0.5 V/cm for 30 min, thereby driving all of the DNAs used into the gel. Subsequently, either RGE or FIGE was performed by use of the E and times described below. During electrophoresis, buffer was circulated between the buffer tanks at the two ends of the electrophoresis apparatus. The buffer was circulated through a constant-temperature circulating bath that maintained the gel's temperature at 25 ± 2 °C for RGE and at 15 ± 2 °C for FIGE.

Measurement of μ vs A. To avoid fluctuations in E and temperature among different gels used to determine μ vs A, all of these gels (running gels) were embedded in a single frame of agarose. The composite of running gels and frame is called a multigel. For FIGE, the embedding procedure was the procedure previously described (Serwer, 1986), except that 18 running gels (0.3 cm wide) were used instead of 9 running gels. For 2πRGE, a template for embedding seven running gels was constructed (design modifications required are available from the authors on request). The comb for 2πRGE formed two sample wells and the comb for FIGE formed one sample well at the origin of each running gel. During 2πRGE, running gels with A values below 0.4 were difficult to use without rotation-induced breakage. To prevent breakage of even gels with A values of 0.4–0.6, these gels were poured closer to the multigel's center than the more concentrated gels. To help avoid misinterpretation caused by the ordering of running gels for 2πRGE, A values are indicated at the top of each running gel.

Although open circular λ DNA can be discriminated from linear λ DNA by comparing μ to the μ of linear T4, T5, and T7 DNAs, in all cases of RGE and FIGE the following procedure was used to confirm identification of open circular DNA during the determination of μ vs A. After either 2πRGE or FIGE, the gel was rotated by 0.5π radians from the average direction of electrophoresis and was then subjected to orthogonally oriented electrophoresis with an invariant E (6 V/cm). Circular DNA was arrested during this second, orthogonally oriented electrophoresis; linear DNA was not (Mickel et al., 1977; Levene & Zimm, 1987; Serwer & Hayes, 1987). In the case of FIGE, rotation of the gel for the second electrophoresis was accomplished by lifting the gel from its support. In the case of 2πRGE, the gel was rotated by its supporting disc (Serwer & Hayes, 1989). All preparations of cyclized λDNA contained some linear DNA. Unless otherwise indicated, the monomeric linear λ DNA always formed a band further from the origin than the band of monomeric open circular λ DNA. In some, but not all, preparations of cyclized λ DNA, a band of open circular DNA was observed closer to the origin of electrophoresis than the band of monomeric open circular λ DNA. The DNA that formed the origin-proximal band was a dimeric λ open circle [see Serwer and Hayes (1989)]. To normalize μ (μ_N), division by the μ (normalizing μ) of another particle was performed. Both μ's were obtained in the same multigel. Details of two different procedures of normalization used are in the Results.

Results

Atypical Sieving for Open Circular λ DNA: Effect of Electrical Potential Gradient and DNA Length. When 2πRGE, previously used to fractionate open circular λ DNA (Serwer & Hayes, 1989), was used at 6 V/cm (t_r = 50 s) to determine μ vs A for open circular λ DNA, an atypical (unique in the authors' experience) observation was made. The magnitude of μ increased monotonically as A increased in a multigel (atypical sieving). The range of A values was 0.4–1.5 (Figure 1a, right lane of each running gel; linear T4, T5, and T7 DNAs are in left lanes; for all running gels, the open circular monomer is indicated by a horizontal line at the right). After using as normalizing μ the μ of the linear λ DNA in the most dilute running gel, μ_N was determined for the monomeric open circular λ DNA in Figure 1a and was plotted as a function of A in Figure 2 (oc-1, 6 V/cm). When E was lowered to 3 V/cm, an atypical μ vs A plot was observed for A between 0.4 and 0.6. However, the typical decrease in normalized μ occurred as A increased for A values between

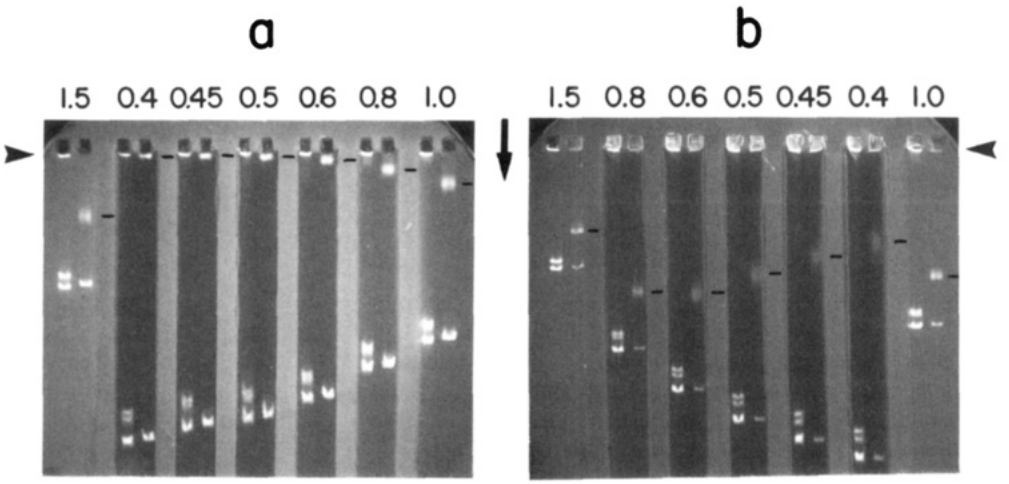

FIGURE 1: Determination of μ vs A for linear and open circular DNA: 2πRGE. The following samples were subjected to 2πRGE (t_r = 50 s), by use of either (electrical potential gradient, followed by the time) (a) 6 V/cm, 5 h, or (b) 3 V/cm, 13 h. The left sample well of each running gel was loaded with a mixture of T4, T5, and T7 DNAs (15–25 ng each); the right sample well was loaded with 40 ng of cyclized λ DNA. The position of monomeric open circular λ DNA is indicated by a horizontal line at the right. The A of running gels is indicated at the top. The origins of electrophoresis are indicated by arrowheads; the direction of electrophoresis is indicated by the arrow.

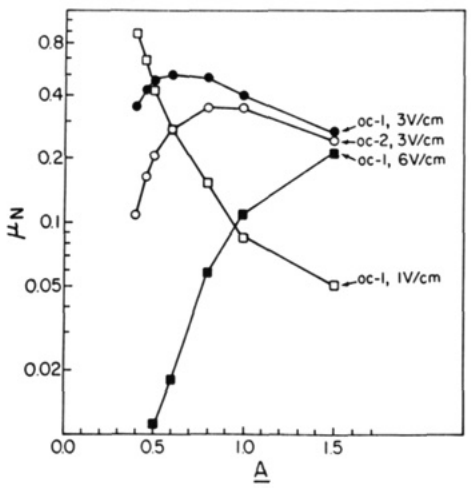

FIGURE 2: Normalized μ vs A. After 2πRGE with t_r = 50 s, μ_N vs A was determined for several E values and two DNAs: monomeric open circular λ DNA (oc-1) and dimeric open circular λ DNA (oc-2). Values of μ_N (log scale) were plotted as a function of A. Values of E and types of DNA are indicated at the right. The normalizing μ for μ_N was that of linear λ DNA in the running gel with A = 0.4.

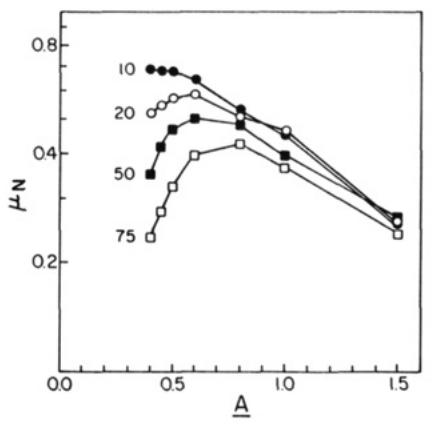

FIGURE 3: Effect of t_r. After 2πRGE at 3 V/cm, μ_N vs A was determined by use of several t_r values. Values of μ_N (log scale) were plotted as a function of A for the t_r values (seconds) indicated at the left.

0.6 and 1.5 (Figure 1b, right lanes; Figure 2; oc-1, 3 V/cm). Thus, at 3 V/cm a peak in μ_N was observed for A equal to 0.6. The atypical sieving of monomeric open circular λ DNA was eliminated by further lowering E to 1 V/cm (Figure 2; oc-1, 1 V/cm). Thus, atypical sieving of open circular DNA decreased as E decreased between 6 and 1 V/cm.

Although not visible in Figure 1, dimeric λ open circles were sometimes observed. At 3 V/cm, the normalized μ vs A plot for this dimer was qualitatively similar to that of the monomeric λ open circle. However, the range of A values in the atypical region of the μ_N vs A plot was greater for the dimeric open circular DNA (Figure 2; oc-2, 3 V/cm). Thus, atypical sieving also increased as the length of open circular DNA increased.

Dependence of Atypical Sieving on t_r. During 2πRGE, as t_r increased from 10 to 75 s for monomeric open circular λ DNA, the extent of atypical sieving increased (Figure 3; t_r is indicated at the left of each plot). However, even when t_r was 10 s, an atypical plateau was observed for A between 0.4 and 0.5 (Figure 3). Thus, atypical sieving increased as t_r increased.

FIGE: Sieving of a Solid Sphere. To use pulse times and A values lower than those used during 2πRGE, atypical sieving was also explored by use of FIGE. The use of FIGE for open circular DNA is also of practical significance because FIGE is more efficient in time and cost per sample than 2πRGE. If t_f was 24 s during FIGE at 3 V/cm, then atypical sieving of monomeric open circular λ DNA was observed at A values between 0.15 and 0.60 (Figure 4a). When t_f was lowered to 3 s (1) atypical sieving was eliminated, in qualitative agreement with results obtained by use of 2πRGE, and (2) near comigration of linear and open circular monomeric λ DNA was observed (Figure 4b). Reducing t_f even further to 0.3 s caused crossing of the μ vs A plots for linear and open circular, monomeric λ DNA (Figure 4c, lanes 1–12). At the lower A values in Figure 4c (A = 0.15–0.40), the open circular λ DNA migrated more rapidly than the linear DNA.

To determine whether solid spheres also experienced atypical sieving during FIGE, bacteriophage T3 was analyzed in the same multigels used for analysis of DNA (an example is in Figure 4c, lanes 13–18). After measuring μ vs A for T3, values of P_E vs A were calculated by procedures previously used [eq 1 in Griess et al. (1989)]. The result was no atypical sieving and no t_f dependence of sieving (Figure 5).

Obtaining and Analysis of μ_N from FIGE. Because t_f had no detectable influence on the sieving of bacteriophage T3, the μ of bacteriophage T3 was used as the normalizing μ when results obtained at different t_f values (and, therefore, in dif-

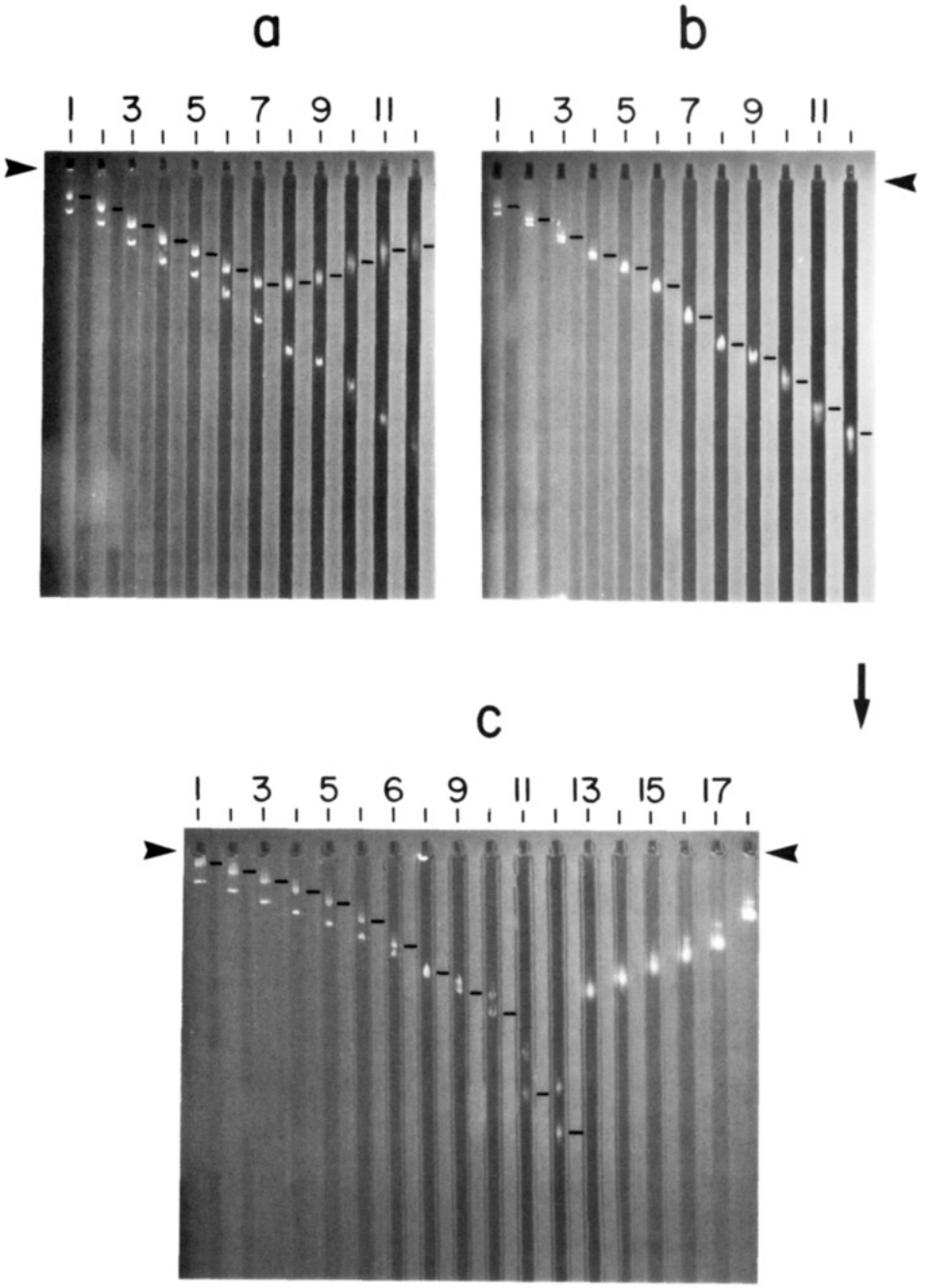

FIGURE 4: Determination of μ vs A for linear DNA, open circular DNA, and a spherical standard by FIGE. Two samples were subjected to FIGE at 3 V/cm for 10 h: cyclized bacteriophage λ DNA (lanes 1–12; see also Figure 1) and 2 μg/lane of bacteriophage T3 (lanes 13–18). The values of t_f were (a) 24, (b) 3, and (c) 0.3 s. The A values of running gels were (lane number, followed by A) as follows: (1) 2.5; (2) 2.0; (3) 1.5; (4) 1.2; (5) 1.0; (6) 0.80; (7) 0.60; (8) 0.45; (9) 0.40; (10) 0.30; (11) 0.20; (12) 0.15; (13) 0.20; (14) 0.40; (15) 0.60; (16) 0.80; (17) 1.0; (18) 1.5. The position of monomeric open circular λ DNA is indicated by a horizontal line at the right. The origins of electrophoresis are indicated by arrowheads; the direction of electrophoresis is indicated by the arrow. The origin-proximal minor T3 band (lanes 16–18) is formed by a dimer of T3.

ferent multigels) for linear and open circular λ DNA were compared. For $A = 0.15$, the μ_N of open circular λ DNA decreased dramatically as t_f increased during FIGE. In contrast, the μ_N of linear DNA increased slightly as t_f increased at this A value (Figure 6a; A is indicated in the figure). The behavior of linear DNA was approximately the same for A values of 0.6 and 2.5 as it was for an A value of 0.15. In contrast, the behavior of the open circular DNA progressively inverted as A increased (Figure 6a). That is, as A increased, μ_N progressively became an increasing function of t_f. For monomeric open circular λ DNA, the A dependence of μ_N vs t_f is shown in more detail in Figure 6b. The differential in response to t_f indicates that the changing of t_f between first and second dimensions of a two-dimensional agarose gel electrophoresis will assist separation of DNA by its conformation.

The procedure of FIGE used here was initially developed to improve the resolution by length of linear DNA (Carle et al., 1986). The optimal resolution for $A = 1.5$ when separating T7 and T4 DNAs with the conditions of Figure 6 occurred for $t_f = 12$ s (D. Louie, G. A. Griess, and P. Serwer, unpublished data). A slight dip in the plot for linear λ DNA at $A = 1.5$ was observed at this t_f (the vertical arrow in Figure 6a indicates the position of the same dip for $A = 2.5$). A larger dip occurred at this t_f for linear DNAs longer than λ DNA (T4 DNA, for example), and this phenomenon produces the

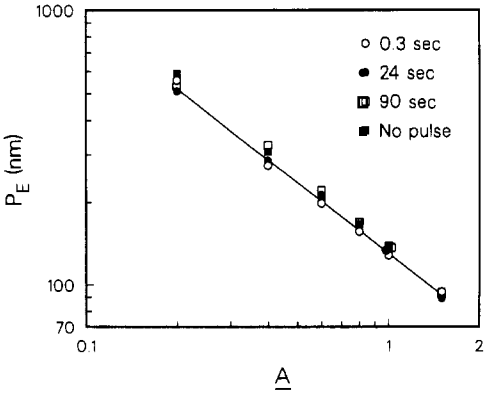

FIGURE 5: P_E vs A during FIGE. The μ vs A relationship was determined for intact bacteriophage T3 during either electrophoresis with invariant E (indicated by no pulse) or FIGE at 3 V/cm. From μ vs A, the P_E vs A relationship was calculated by procedures described in Griess et al. (1989). The t_f (seconds) and absence of pulse are indicated on the figure.

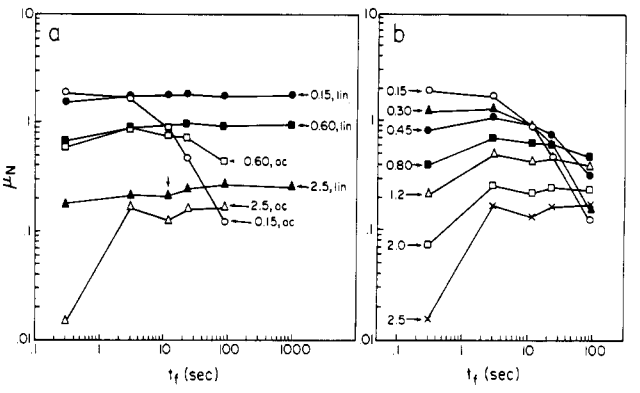

FIGURE 6: μ_N vs t_f relationship for linear and open circular λ DNA: dependence on A. By electrophoresis in multigels (see Figure 4), μ vs A was determined for both intact bacteriophage T3 and cyclized λ DNA. Values of μ_N were calculated by use of the μ of T3 (A = 0.2) as the normalizing μ. The μ_N vs t_f relationship is plotted at several A values (indicated on the figure) for (a) both linear (lin) and open circular (oc) DNA (results for three A values are shown) and (b) open circular DNA alone (results for seven A values are shown). For linear DNA, the point furthest to the right was obtained by electrophoresis without pulsing.

improved resolution by length of linear DNA reported in Carle et al. (1986).

Discussion

From observation of a solid sphere's sieving during agarose gel electrophoresis, P_E always decreases as A increases. This relationship has been found during all previous studies [reviewed in Stellwagen (1987); see also Griess et al. (1989)] and also during studies made here by use of FIGE. The theories for the sieving of random coils discussed above all predict that μ decreases in magnitude as P_E decreases. This prediction is also a consequence of theories for the sieving of solid spheres [reviewed in Tietz (1987)]. Thus, at present no quantitative theory accounts for the atypical sieving observed here during either 2πRGE or FIGE.

To explain this atypical sieving, at least one of the basic assumptions of the above theories must be incorrect. Without attempting to investigate all of the possibilities, we propose that the incorrect assumption is nonbinding of DNA to the gel. Because linear DNA does not experience the atypical sieving of open circular DNA and because atypical sieving can be prevented by decreasing pulse time, the binding that occurs is assumed to be nonchemical. Therefore, the working hypothesis made here is as follows: (1) During either 2πRGE or FIGE, projections from the gel thread loops of DNA. (2) When threaded, open circular DNA is arrested until the direction of E changes; in contrast, linear DNA is not thus arrested because linear DNA can pass an end around a projection. (3) In the region of atypical sieving, on the average DNA is arrested by threading in a time (t_a) less than that of the pulse time. (4) The value of t_a decreases as A decreases. To obtain an estimate of t_a, the approximations are made that (1) linear DNA is not slowed by threading for the higher pulse times (>12 s) and (2) the μ of open circular DNA would be the same as the μ of equally long linear DNA in the absence of threading. For the following reason, assumption 2 is believed to be no more than 30% inaccurate. As A increases during 2πRGE (i.e., as threading decreases), the μ of open circular DNA approaches that of linear DNA, within 30% in Figure 1b. In addition, conditions of FIGE exist for the comigration of linear and open circular λ DNA (Figure 4b); possibly threading is eliminated in Figure 4b. For E = 6 V/cm, A = 1.0, and t_r = 50 s (Figure 1a, furthest right lane), the ratio of the μ of open circular DNA to the μ of linear DNA was 0.18 and t_a would be 9.0 s. From this t_a and the μ of the linear DNA (5×10^{-5} cm^2/V·s; obtained from the data in Figure 2), the open circular DNA would on the average migrate 2.7×10^4 nm before arrest. This distance is about 100 times the $2 \cdot P_E$ from Figure 5. Thus, the conclusion is drawn that the open circular DNA migrated through more than one pore (1100 pores if t_a is accurate) before arrest. The same conclusion would be drawn for A = 1.5 in Figure 1a and also for any gel subjected to 2πRGE at 3 V/cm. A more detailed analysis of t_a is not attempted here because of uncertainty in the accuracy of approximations 1 and 2. This analysis would require either addition of threading to the theories discussed in the introduction or development of new theories that include threading.

Physically, part 4 of the working hypothesis (previous paragraph) implies the following. As A decreases, either the length, shape, stiffness, or number of the gel's (circle-threading) projections changes to increase threading. The nature of this change cannot be deduced from the data obtained here. If threading of loops within linear DNA increases the sieving of linear DNA (though presumably less than it increases sieving for open circular DNA at the higher pulse times), then the inconsistency described in the introduction is qualitatively explained. That is, as A decreases, P_E would increase by $A^{0.7-0.9}$ (Griess et al., 1989), but the A dependence of threading would weaken P_E's apparent dependence on A for DNA.

That threading of loops is a component of linear DNA's improved separation by PFG electrophoresis has been proposed previously (Serwer, 1988). This proposal included flexibility and hysteresis of at least some (but not necessarily all) of the gel's projections. Because the bending of fibers proposed would be accomplished primarily by the leading end of a reptating DNA, a prediction [not made in Serwer (1988)] is that DNA would accumulate at the leading end of a reptating random coil. This prediction is correct; however, another model also predicts the same phenomenon (Smith et al., 1989). The data presented here appear not to discriminate models for linear DNA's improved separation by PFG electrophoresis.

The gel's projections that thread open circular DNA presumably also sieve solid spheres. However, by observation of the sieving of spheres, these projections have not yet been discriminated from other parts of the gel. Open circular DNA has served here as a probe for a region of agarose gels that

would otherwise have remained undetected.

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Characterization of a cDNA for Rat P-450g, a Highly Polymorphic, Male-Specific Cytochrome in the P-450IIC Subfamily†,‡

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ABSTRACT: Cytochrome P-450g (IIC13) is a highly polymorphic, male-specific rat liver isozyme which is a member of the P-450IIC subfamily. A cDNA, c5126 (1737 bp), for P-450g was isolated from a λgt11 library synthesized from (+g) male rat liver mRNA. Sequence analysis of the clone, c5126, revealed an open reading frame of 1473 nucleotides, which encodes for a 490 amino acid polypeptide possessing the 30 NH_2-terminal residues reported for cytochrome P-450 (M-3) (P-450g) [Matsumoto et al. (1986) *J. Biochem. 100*, 1359-1371]. A high degree of sequence similarity (>70%) exists between c5126 and the published sequences of cDNAs for members of the IIC subfamily, while its sequence similarity to other subfamilies (IA, IIB, and IIIA) was much lower (<55%). RNA blot analysis utilizing an oligonucleotide probe specific for P-450g revealed that P-450g mRNA was expressed in livers of male but not female Sprague-Dawley (CD) and ACI rats, indicating that the sex difference was regulated pretranslationally. Furthermore, expression of P-450g mRNA was age dependent in livers of male ACI rats (a homozygous, phenotypically high P-450g strain). However, the mRNA for P-450g was expressed equally in livers of outbred male CD rats representing either the high (+g) or the low (-g) phenotype and of inbred ACI rats (+g) representing the high phenotype, indicating that the defect in (-g) rats does not reflect differences in expression of P-450g mRNA.

Cytochrome P-450 (P-450)[1] is a superfamily of hemoproteins which function in the oxidative metabolism of a variety of endogenous and exogenous substrates (Sato & Omura, 1978). Recently, an ad hoc committee has proposed division of this superfamily on the basis of similarities of amino acid sequence between families (Nebert et al., 1987). Currently, nine families of mammalian P-450s designated P-450I, -II, -III, -IV, -XI, -XVII, -XIX, -XXI, and -XXVI have been

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‡ The nucleic acid sequence in this paper has been submitted to GenBank under Accession Number J02861.

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[1] Abbreviations: P-450, cytochrome P-450; CD, Charles River Sprague-Dawley; IPTG, isopropyl β-D-thiogalactopyranoside; GAR-HRP, goat anti-rabbit IgG, human IgG absorbed, horseradish peroxidase conjugated; RIA, radioimmunoassay; SSC, standard sodium citrate; SDS, sodium dodecyl sulfate.