Conformation of Double-Stranded DNA during Agarose Gel Electrophoresis: Fractionation of Linear and Circular Molecules with Molecular Weights between 3×10^6 and 26×10^6†

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ABSTRACT: To answer several questions concerning the mechanisms of DNA fractionation during agarose gel electrophoresis, the electrophoretic mobility (μ) of double-stranded DNA has been measured as a function of (1) DNA topological conformation (linear, open circular, closed circular) and molecular weight (M_r) (molecular weights were between 2.9×10^6 and 26.4×10^6), (2) gel concentration (A) and temperature, and (3) voltage gradient. It was found that μ extrapolated to an A of 0 (μ_0') was independent of DNA conformation. The effect of temperature was to raise values of μ_0' in inverse proportion to buffer viscosity. Semilogarithmic μ vs. A plots for linear DNAs had curvature that was opposite to the curvature for spherical particles (plots for linear DNA were concave). As A approached 0, the plots became increasingly linear. For the larger DNAs, the negative slope (K_R) in the region of linearity was decreased as voltage gradient increased. These and other data indicate deformation of linear DNA random coils during agarose gel electrophoresis. The data suggest both an asymmetric and a symmetric collapse of linear DNA random coils during agarose gel electrophoresis. However, end-first migration of linear DNA, previously suggested by others, does not explain the data. The semilogarithmic μ vs. A plots were more linear for closed and open circular DNAs than they were for linear DNAs. Closed circular DNAs had K_R's lower than K_R's of either open circular or linear DNAs of the same molecular weight. At the lower voltage gradients, open circular DNA had the same K_R as linear DNA of the same molecular weight. However, as voltage gradient and molecular weight increased, the K_R of open circular DNA became smaller than the K_R of linear DNA (of the same molecular weight). This and the concave curvature of semilogarithmic μ vs. A plots for linear DNA resulted in a previously unreported reversal of the relative migration of linear and open circular DNAs as A increased.

Gel electrophoresis is used to fractionate double-stranded DNA by molecular weight (M_r) and topological conformation (Fisher & Dingman, 1971; Dingman et al., 1972; Flint & Harrington, 1972; McDonell et al., 1977; Mickel et al., 1977; Johnson & Grossman, 1977; Fangman, 1978; Southern, 1979; Serwer, 1980; Schwartz et al., 1982). To improve separations achievable by gel electrophoresis and to increase the capacity of this technique for characterizing DNA, it is desirable to learn as much as possible about the dependence of electrophoretic mobility (μ) on the molecular weight and conformation of double-stranded DNA. Studies presented in the past have revealed the following contradictory or unexplained phenomena.

(1) Because the μ of linear, double-stranded DNA is independent of molecular weight when determined in the absence of a gel (Olivera et al., 1964), it is usually assumed that gel sieving is the mechanism of DNA separation by molecular weight or conformation. However, previously presented data suggest that μ extrapolated to an agarose percentage (A) of 0 (μ_0') is lower in magnitude for linear DNA than for closed or open circular DNAs of the same molecular weight [Figure 3 of Dingman et al. (1972); Figure 2 of Johnson & Grossman (1977)]. This suggests that either factors other than sieving influence separation of linear and circular DNAs or there is a flaw in the data analysis of these previous studies. [When μ_0' is corrected for electroosmosis, it is referred to as μ_0 (Serwer & Hayes, 1982).]

(2) It has been found that the μ of duplex DNA in agarose–polyacrylamide gels increases with increasing temperature (Fisher & Dingman, 1971). However, the data presented did not reveal whether changes in μ_0' or changes in sieving caused the change in μ with temperature. Apparently, studies of the dependence of μ on temperature in agarose gels (without polyacrylamide) have not been made. Agarose gel electrophoresis of duplex DNA is usually performed at temperatures controlled to no better than ±3 °C (often room temperature).

(3) Some studies of the relative mobility of linear and circular DNA have revealed that open circular DNA always migrates less rapidly than linear and closed circular DNAs of the same molecular weight (Mickel et al., 1977; Johnson & Grossman, 1977). However, in our preliminary studies conditions were found such that some open circular DNAs migrated more rapidly than linear DNAs of the same molecular weight. Migration of single-stranded circular DNA more rapid than migration of single-stranded linear DNA (of the same molecular weight) has previously been observed during gel electrophoresis (Dingman et al., 1972).

(4) It is known that the μ of linear, double-stranded DNA increases as a function of voltage gradient (Fisher & Dingman, 1971; Flint & Harrington, 1972; Lishanskaya & Mosevitsky, 1973) and that an accompanying loss in resolution by molecular weight occurs (Aaij & Borst, 1972; Lishanskaya & Mosevitsky, 1973; Hayward, 1974; Kaplan & Wilcox, 1977; McDonnell et al., 1977; Fangman, 1978; Serwer, 1980). This phenomenon has been explained by end-first migration of linear DNAs oriented by the electrical field (Fisher & Dingman, 1971; Lerman & Frisch, 1982), a phenomenon sometimes referred to as reptation (Lerman & Frisch, 1982; deGennes, 1971). However, as pointed out by Lumpkin & Zimm (1982), the pore sizes of agarose gels used for the electrophoresis of DNA are too large to promote this type of reptation (to be referred to as primary reptation). The pore sizes used by Lumpkin & Zimm (1982) were obtained from Righetti et al. (1982), who used an agarose with pores even smaller than the pores of the agarose normally used for

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0006-2960/84/0423-0922$01.50/0

electrophoresis of DNA (Serwer & Allen, 1983). An alternative possible mechanism for the voltage gradient dependence of the μ of linear DNA is elongation of the envelope of a DNA random coil and reptation of the elongated coil (see Figure 3B) (to be referred to as secondary reptation). However, in none of the previous studies was the possibility of voltage gradient-independent DNA condensation in gels discussed. In addition, several possible tests of reptation hypotheses have not been made. In past studies, a limitation in interpretating gel sieving data for DNA is absence of knowledge concerning the behavior of solid spheres in the gels used for electrophoresis of DNA.

In summary, the following questions are not yet answered. What are the μ_0' values of the different DNA conformers? What is the effect of temperature on μ_0' and gel sieving? How do the shapes of plots of μ as a function of A differ for the different DNA conformers using conditions which include conditions for which open circular DNA migrates more rapidly than linear DNA of the sample molecular weight? During electrophoresis, what is the effective radius of the various duplex DNA conformers as a function of molecular weight? Does reptation occur? If so, what type of reptation occurs? Preliminary studies indicated that answering of these questions required the electrophoresis of double-stranded DNAs in gels with pores larger than pores in gels previously used to study DNA conformers. Agarose gels have larger pores and are stronger than usable polyacrylamide gels (Righetti et al., 1982). Therefore, to answer the above questions, agarose gels have been used, and the A has been extended below the lowest A used in previous studies of DNA conformers, 0.6 (Lishanskaya & Mosevitsky, 1973; Johnson & Grossman, 1977; Mickel et al., 1977). In addition, improved procedures have been used for (1) controlling voltage gradient and temperature (Serwer, 1983), (2) measuring μ as a function of A in gels of low A (Serwer, 1981), and (3) determining the effective DNA radius from such measurements by comparing results with those obtained by using solid spheres (Serwer et al., 1983; Serwer & Allen, 1983). The results of these studies are presented here.

Materials and Methods

Buffers and Reagents. Tris/EDTA[1] buffer contained 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.4, and 0.001 M EDTA. The electrophoresis buffer was 0.05 M sodium phosphate, pH 7.4, and 0.001 M EDTA; sample buffer was 0.005 M sodium phosphate, pH 7.4, 0.001 M EDTA, and 400 μg/mL bromophenol blue, with sucrose as described below. The viscosity (η) of electrophoresis buffer at any temperature was assumed to be the η of water, obtained from Weast (1978).

ME and HGT[P] grades of agarose were obtained from Marine Colloids, Rockland, ME. Some characteristics of the preparations used (HGT[P], lot 60689; ME, lot 90739) are given in Serwer et al. (1983). Some other lots of HGT[P] agarose have been found not to yield sharp DNA banding patterns during agarose gel electrophoresis. HGT[P] agarose was used because its comparatively high gel strength (1260 g/cm^2 for a 1% HGT[P] gel, compared to 960 g/cm^2 for a 1% ME gel; determined by the manufacturer) suggested easier handling of the comparatively dilute gels necessary for the present study. The sieving of HGT[P] agarose and ME agarose (ME agarose is the more usual agarose for DNA electrophoresis) differs only slightly (Serwer et al., 1983). ME agarose gels as dilute as 0.075% have been successfully used in the multigel procedure below.

DNAs. Closed circular pBR322 DNA was purchased from P-L Biochemicals, Inc. Open circular pBR322 DNA was found as contaminant of the pBR322 closed circular DNA. The identification of these two DNAs was made by agarose gel electrophoresis (Mickel et al., 1977). Linear pBR322 DNA was obtained by digestion of circular DNA with restriction endonuclease *Pst*I (Sutcliffe, 1978).

Yeast plasmid closed circular pYE(CEN3)41 DNA (Clarke & Carbon, 1980) was received purified from Dr. Donna L. Montgomery. Open circular pYE(CEN3)41 DNA was found as a contaminant of these preparations and was identified as described above. For $A \geq 1.2$, pYE(CEN3)41 open circular DNA formed a doublet band, if the voltage gradient was $\geq$0.7 V/cm; a singlet band was formed for all A's at 0.34 V/cm. The reason for the doublet is not known, and its occurrence does not have an effect on calculations made here. Linear pYe(CEN3)41 DNA was obtained by digestion with restriction endonuclease *Bgl*II (Clarke & Carbon, 1980).

Bacteriophage T7 DNA was obtained by phenol extraction from bacteriophages purified as described in Serwer (1980). Phenol-extracted DNA from bacteriophage λ deletion mutant 590 (Murray et al., 1977) was received purified from Dr. Montgomery. The molecular weights of the above DNAs are given in Table I.

Bacteriophage λ590 DNA was made to form open circles (Wang & Davidson, 1968) as follows. A DNA sample containing 0.5 μg/mL λ590 DNA in Tris/EDTA buffer was incubated at 70 °C for 10 min (to disassociate end-to-end aggregates), followed by 45 °C for 7.0 h (to circularize the DNA). The circularized DNA was kept at 4 °C.

Electrophoresis. Electrophoresis in agarose slab gels was performed as previously described (Serwer, 1980). To measure μ as a function of A, electrophoresis of a sample through several gels of HGT[P] agarose (running gels), each of a different A and all embedded in a single ME agarose frame, was performed. (The composite gel, consisting of frame and running gels, is referred to as a multigel.) A sample in Tris/EDTA buffer was diluted with either a 2.5× amount of sample buffer containing 3% sucrose [pBR322 or pYE(CEN3)41 DNAs] or a 0.2× amount of sample buffer containing 15% sucrose (T7 and λ590 DNAs). After 50 μL of this mixture was layered at the origins of the running gels, in a multigel prepared in electrophoresis buffer as described in Serwer & Hayes (1982), electrophoresis was performed. During electrophoresis, temperature was controlled to ±1 °C, as previously described (Serwer, 1983). Voltage gradient was controlled to 1–2% at 0.7–1.0 V/cm, as previously described (Serwer, 1983). At 0.34 and 0.17 V/cm, control of voltage gradient was accurate ±6% and ±12%, respectively. The variable accuracy of voltage gradient is caused by variable accuracy of the power supply (a Hewlett-Packard 6207B). Gels were stained with ethidium bromide and photographed as previously described (Serwer, 1980). The amount of each DNA used per running gel was 8–25 ng. By use of these DNA amounts, the shape and position of DNA bands are independent of the amount of DNA used (Serwer, 1980).

Analysis of Data. A value of μ for DNAs in each running gel of a multigel was determined from the distance of a band center from the origin of electrophoresis. A semilogarithmic plot of $-\mu$ as a function of A was made. The intercept on the μ axis (μ_0') and the negative slope of the plot (K_R), when linear, were determined by linear regression analysis. K_R's were reproducible ±3%. In all cases r values were at least 0.999.

[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

Values of μ_0 were determined from μ_0' as described in Serwer & Hayes (1982).

Theoretical Considerations. To assist understanding of the conformation of double-stranded DNAs during their fractionation by electrophoresis in agarose gels, semilogarithmic plots of μ vs. A for double-stranded DNAs will be compared to the plots expected of particles with previously defined properties. These particles include (1) solid spheres and (2) random coils with the configuration of double-stranded DNA outside of a gel.

(1) Intact, spherical bacteriophages and related particles that approximate solid spheres (radius = 13.3–41.9 nm) have been used to study sieving during agarose gel electrophoresis with the following results. Semilogarithmic plots of $\mu(A)/\mu_0'$ are linear for $A \leq 0.9$, but become increasingly nonlinear at higher A's. In the region of nonlinearity, ln $\mu(A)/\mu_0'$ decreases progressively more rapidly as a function of A than it does for $A \leq 0.9$ (i.e., the plots have convex curvature) (Serwer & Hayes, 1981; Serwer et al., 1983). From the region of linearity, the K_R (calculated by using natural logarithms) is related to particle radius (R) [Serwer et al., 1983; see also Rodbard & Chrambach (1970)]:

$$K_R^{1/2} = c(R + r) \quad (1)$$

where r is the effective radius of a gel fiber and c is a second constant whose origin is discussed in Rodbard & Chrambach (1970) and Serwer et al. (1983). For the agarose used here (HGT[P] agarose), c is 12.4×10^{-3} nm^{-1}, and r is 25.3 nm when measured in a buffer the same as the electrophoresis buffer used here, except that 0.001 M $MgCl_2$ replaces the 0.001 M EDTA (Serwer et al., 1983). Semilogarithmic μ vs. A plots for double-stranded DNA are not detectably altered by this difference in buffers (data not shown). For double-stranded DNA during agarose gel electrophoresis, R_{EG} is defined here as the radius of a sphere with a K_R equal to the K_R of the DNA (i.e., the radius of an equivalent sphere in the gel).

(2) Linear DNA random coils (not in a gel) are assumed to have a spherical envelope (see Figure 3A), and the radius of gyration (R_G) of random coil associated mass (not constrained in a gel) has been calculated with the following expression [eq 5–36 of Bloomfield et al. (1974), with higher order terms neglected]:

$$R_G = \left[\frac{a}{3}(L - 3a)\left(\frac{L}{2a}\right)^{\epsilon}\right]^{1/2} \quad (2)$$

with a, an empirical parameter referred to as the persistence length, $\ll L$, the total length of the random coil. The factor $(L/2a)^{\epsilon}$ corrects an idealized random coil R_G for excluded volume effects. The exponent, ϵ, is also an empirical parameter. For the electrophoresis buffer used here, $[Na^+] = 0.085$ M; therefore, a is 50 ± 5 nm and ϵ is 0.11 ± 0.01 (Rizzo & Schellman, 1981; Borochov et al., 1981; Hagerman, 1981).

The effective hydrodynamic radius of a random coil, R_{EH}, is $0.665R_G$ (Bloomfield et al., 1974, p 218). Thus, for linear DNA

$$R_{EH} = 0.384\left[a(L - 3a)\left(\frac{L}{2a}\right)^{\epsilon}\right]^{1/2} \quad (3)$$

That is, R_{EH} is the radius of a sphere with the same frictional coefficient as the DNA random coil when the DNA is free of a gel (or any other solid support). To assist in understanding the conformation of double-stranded DNA during agarose gel electrophoresis, values of R_{EG} and R_{EH} will be compared.

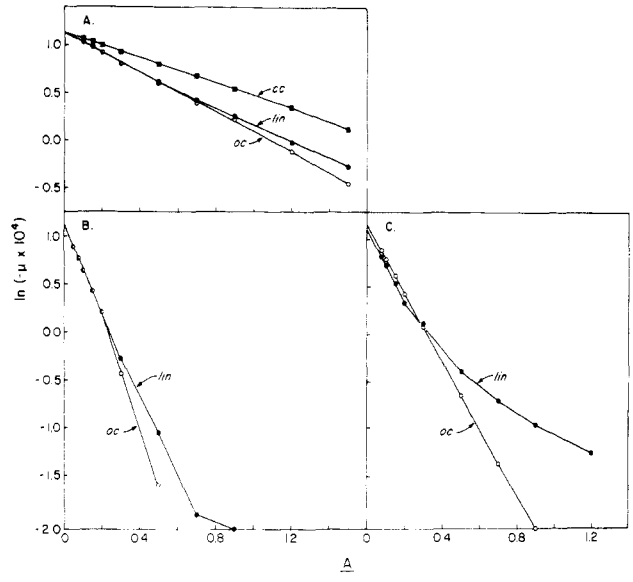

FIGURE 1: μ as a function of A. Values of μ were determined as a function of A by electrophoresis in multigels, at 25 °C, as described under Materials and Methods. (A) pBR322 linear, open circular, and closed circular DNAs, 1.0 V/cm; (B) T7 linear and λ590 open circular DNAs, 0.34 V/cm; (C) T7 linear and λ590 open circular DNAs, 1.0 V/cm. Abbreviations: cc, closed circular; oc, open circular; lin, linear.

Results

Electrophoresis of pBR322 DNA Topological Conformers. To determine whether or not μ_0' depends on the conformation (linear, open circular, closed circular) of duplex DNA, the ln $-\mu(A)$ for different pBR322 conformers was determined by using A values as low as 0.075, lower than the minimum A (0.6) previously used for similar plots in Dingman et al. (1972) and Johnson & Grossman (1977). It was found that the μ_0' values for linear, open circular, and closed circular pBR322 DNAs were indistinguishable from each other (Figure 1A). The value of μ_0 for duplex DNA at 25 °C, calculated from μ_0' as described under Materials and Methods, was $-3.1 \pm 0.15 \times 10^{-4}$ cm^2/(V·s). This value of μ_0 is indistinguishable from the μ_0 (viscosity corrected) obtained by electrophoresis in a metrizamide density gradient using the electrophoresis buffer used here (Serwer & Watson, 1981).

For $A \leq 0.5$, the plots for open circular and linear pBR322 DNAs in Figure 1A were linear and coincident. At higher A's, the plot for linear DNA acquired slight concave curvature. In contrast, the plots for open circular and closed circular DNAs were linear for $A \leq 1.2$; slight convex curvature was observed at $A = 1.5$. The K_R for closed circular pBR322 DNA was 0.62 × the K_R for open circular DNA. The results of Figure 1A were obtained at 25 °C by using a voltage gradient of 1.0 V/cm. The plots of Figure 1A were not significantly altered at 25 °C by the use of a voltage gradient of 0.34 V/cm (data not shown).

Electrophoresis of λ Open Circular and T7 Linear DNAs. For DNAs with $M_r > 10 \times 10^6$, voltage gradient dependent μ's have been reported (introduction). Therefore, it was expected that, in contrast with the results obtained with pBR322 DNA, the plots of Figure 1A would be voltage gradient dependent if obtained with the longer λ590 open circular and T7 linear DNAs (these latter two DNAs were chosen because of the near quality of their molecular weights; Table I). At 0.34 V/cm, T7 linear DNA and λ590 open circular DNA comigrated with $A \leq 0.2$; these DNAs separated with $A \geq 0.3$ (Figure 2A). That the slower migrating DNA with $A \geq 0.3$ is the λ590 circular DNA was shown by electrophoresis

Table I: Values of R_{EG} and R_{EH}[a]

DNA	M_r (×10⁶)	K_R[f]	R_{EG} (nm)[g]	R_{EH}[h] (nm)	R_{EG}/R_{EH}
pBR322 closed circular	2.89[b]	0.73_8	43.9		
pBR322 open circular		1.1_9	62.7		
pBR322 linear		1.1_9	62.7	120	0.52
pYE(CEN3)41 closed circular	6.1[c]	1.1_5	61.2		
pYE(CEN3)41 open circular		1.8_9	85.6		
pYE(CEN3)41 linear		1.8_9	85.6	179	0.48
λ590 open circular	26.3[d]	5.3_1	161		
T7 linear	26.44[e]	5.3_1	161	412	0.39

[a] For definition of symbols, see the text. [b] From Sutcliffe (1978). [c] From Clarke & Carbon (1980). [d] Calculated by assuming λ590 to have a genome 18% shorter than the wild-type λ genome (Murray et al., 1977) and that wild-type λ DNA has M_r 32.11×10^6 (Sanger et al., 1982). [e] From Dunn & Studier (1983). [f] Determined as described under Materials and Methods by using a temperature of 25 °C and a voltage gradient low enough so that further lowering of the voltage gradient does not significantly alter K_R (see Results). K_R is accurate ±4%. [g] Calculated from K_R as described under Materials and Methods. [h] Calculated for linear DNAs from eq 3 by using an L (in nm) determined by multiplying molecular weight by 5.14×10^{-4}.

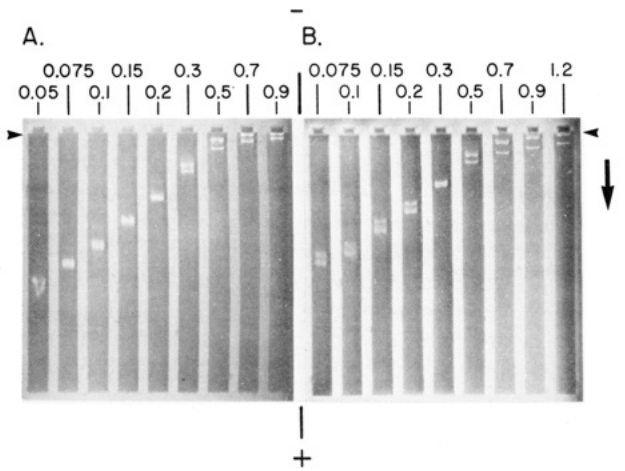

FIGURE 2: Multigel electrophoresis of λ590 open circular and T7 linear DNA. A mixture of λ590 DNA and T7 DNA (20 ng each) was prepared and subjected to electrophoresis at 25 °C in a multigel, as described under Materials and Methods: (A) 0.34 V/cm, 20.0 h; (B) 1.0 V/cm, 6.5 h. The values of A used for running gels are indicated at the top. The arrowheads indicate the origins of electrophoresis; the arrow indicates the direction of electrophoresis. The 0.05% agarose gel in (A) was partially damaged during handling after electrophoresis, causing partial blurring of the DNA band.

of unmixed, λ590, and T7 DNAs in slab gels (data not shown). This is a pattern similar to the pattern observed above for pBR322 DNA, except that the separation of linear from open circular DNA occurred at a lower A (0.5 for pBR322). At 1.0 V/cm, λ590 open circular DNA also migrated less rapidly than T7 linear DNA for $A \geq 0.3$. However, in contrast to the results at 0.34 V/cm, the open circular λ DNA migrated more rapidly than the linear T7 DNA for $A \leq 0.2$. (Identity of the DNAs has been confirmed by electrophoresis on slab gels; data not shown.) This voltage gradient and molecular weight dependent relative mobility reversal has apparently not previously been observed for open circular and linear double-stranded DNAs. However, a relative mobility reversal of closed circular and linear double-stranded DNAs has been observed (Mickel et al., 1977).

Table II: K_R as a Function of Voltage Gradient[a]

DNA	voltage gradient (V/cm)	K_R
pYE(CEN3)41 linear	0.34	1.8_8
	0.70	1.9_1
	1.00	1.9_4
pYE(CEN3)41 open circular	0.34	1.8_8
	0.70	1.8_2
	1.00	1.7_7
pYE(CEN3)41 closed circular	0.34	1.1_5
	0.70	1.1_2
	1.00	1.1_1
T7 linear	0.17	5.2_3
	0.34	5.3_1
	1.00	4.4_4
λ590 open circular	0.17	5.2_3
	0.34	5.3_1
	1.00	4.1_8

[a] Determined at 25 °C as described under Materials and Methods.

Semilogarithmic plots of μ as a function of A for λ circular and T7 linear DNAs in parts B (0.34 V/cm) and C (1.0 V/cm) of Figure 1 were linear for $A \leq 0.2$. However, as A increased above 0.2, the plots for the linear DNA progressively acquired concave curvature, and this curvature was considerably more pronounced at 1.0 V/cm than it was at 0.34 V/cm. The plots for the circular DNA were more linear than the plots for the linear DNA but did have a comparatively small amount of convex curvature at the higher A values.

The K_R's in the region of linearity of Figure 1B,C were higher at 0.34 V/cm than they were at 1.0 V/cm (Table II). However, further decreasing the voltage gradient to 0.17 V/cm did not further significantly increase K_R (Table II). Because the K_R's of all pBR322 DNAs were independent of voltage gradient between 0.34 and 1.0 V/cm, the voltage gradient induced decrease in K_R observed for λ590 and T7 DNAs apparently is an increasing function of molecular weight.

Electrophoresis of pYE(CEN3)41 DNA Topological Conformers. Experiments similar to those performed in the previous sections were also performed with the conformers of pYE(CEN3)41 DNA, a DNA with a molecular weight intermediate to the molecular weights of the DNAs used above (Table I). By use of 1.0 V/cm, the regions of linearity were as follows: closed circular, $A \leq 1.2$; open circular, $A \leq 0.7$; linear, $A \leq 0.5$. For $A \geq 0.5$, the plot for the linear DNA had concave curvature; the curvature was less than it was for T7 DNA and more than it was for linear pBR322 DNA. For $A \geq 0.7$, the plot for open circular DNA had convex curvature slightly greater than the curvature for pBR322 open circular DNA (data not shown). The mobility inversion observed above for linear and open circular DNAs was also observed with linear and open circular pYE(CEN3)41 DNA at 1.0 V/cm, but the maximum separation obtained at the lower A's was only 0.38 × the separation obtained with T7 linear and λ590 open circular DNAs (data not shown). Thus, qualitatively, the plots at 1.0 V/cm for pYE(CEN3)41 DNA were intermediate to the plots for the smaller pBR322 and the larger T7 and λ590 linear and open circular DNAs. At 0.70 and 0.34 V/cm the mobility inversion for open circular and linear pYE(CEN3)41 DNAs found at 1.0 V/cm was no longer visible. That is, the linear and open circular DNAs comigrated for $A \leq 0.5$ (data not shown).

Quantitatively, values of K_R for all conformers changed with decreasing voltage gradient to an extent less than the exper-

Table III: μ_0' and K_R as a Function of Temperature

T (°C)[a]	$-\mu_0'$ [b] [cm²/(V·s) × 10^{-4}]	$-\mu_0'\eta$ [cP·cm²/(V·s) × 10^{-4}]	K_R closed circular	K_R linear
20	2.89 ± 0.15	2.90	1.14	2.06
25	3.22	2.87	1.11	1.94
32	3.80	2.91	1.10	1.92
40	4.85	3.16	1.17	1.98

[a] Temperature of electrophoresis ±0.3 °C. [b] Determined at 1.0 V/cm for closed circular pYE(CEN3)41 DNA, as described under Materials and Methods, using data for $A \leq 0.7$. Values of μ_0' for linear and open circular DNA did not differ significantly from the values of closed circular DNA.

imental error in K_R determinations (Table II). However, the occurrence of the relative migration reversal of linear and open circular pYE(CEN3)41 DNAs (above) indicates that there is a significant effect of voltage gradient on the K_R's of pYE(CEN3)41 DNAs in this range of voltage gradients. (The K_R of the open circular DNA was probably a decreasing function of voltage gradient.)

Effects of Temperature. Because of effects of temperature on buffer viscosity, an increase in $-\mu_0'$ with increasing temperature is expected. Experimentally, an increase was observed when pYE(CEN3)41 DNA was used as the sample (Table III). Values of $\mu_0'\cdot\eta$ did not vary significantly with temperature for temperature between 20 and 40 °C. Thus, all detectable effects of temperature on μ_0' are explained by a decrease in buffer η. The K_R's did not vary significantly with temperature (Table III). In addition, curvature of the plots followed the pattern outlined above at all of the above temperatures (data not shown).

Semilogarithmic plots of μ as a function of A were also made at 20, 25, and 32 °C for T7 linear and λ circular DNAs. Values of μ_0' for these DNAs also varied in inverse proportion to η (not shown). The K_R's were independent of temperature.

Comparison with Data Obtained in the Absence of a Gel. To compare the conformation of linear double-stranded DNA during agarose gel electrophoresis with the conformation of linear double-stranded DNA in the absence of a gel, values of R_{EG} were obtained from K_R and were compared to values of R_{EH}, calculated as described above. Voltage gradients used to determine R_{EG} were low enough so that further lowering would not affect R_{EG} (see above). Values of R_{EG} were 0.52, 0.48, and 0.39 times values of R_{EH} for pBR322, pYE(CEN3)41, and T7 linear DNA, respectively (Table I). Thus, values of R_{EG} were significantly lower than values of R_{EH} for all of the above linear DNAs.

Discussion

The data presented here indicate that μ_0' is the same for linear, open circular, and closed circular DNAs of a given molecular weight and also is independent of molecular weight [see also Serwer (1980)]. Nonlinearity of semilogarithmic μ vs. A plots for $A \geq 0.5$ was observed, and this nonlinearity would have obscured the equality of μ_0' values if data had only been taken for $A \geq 0.5$. Data were taken only for $A \geq 0.5$ in previous studies, and this is the probable reason for the apparent conformer dependence of μ_0' [Figure 3 of Dingman et al. (1972); Figure 2 of Johnson & Grossman (1977)]. The use in the present study of multigels has assisted the use of comparatively dilute gels and the obtaining of accurate data. The improved control of voltage gradient and temperature used here was necessary for the accuracy achieved in measuring μ_0'.

Values of $-\mu_0'$ were an increasing function of temperature in inverse proportion to buffer η. The K_R's were independent of temperature, as was the curvature of semilogarithmic μ vs. A plots. These data suggest that the usual ±3–5 °C variation in ambient temperature will not have a significant effect on relative values of μ. If so, control of temperature is not necessary when absolute values of μ are not needed. Relative values of μ will, however, be altered by electroosmosis (Johnson et al., 1980; Serwer & Hayes, 1982). The only known effect of electroosmosis is to alter μ_0' (Serwer & Hayes, 1982). If so, changes in electroosmosis should not change the sequence in which different DNAs band as a function of distance from the origin of electrophoresis. This sequence has been shown to be invariant by using the closed circular, open circular, and linear forms of bacteriophage φX174 DNA ($M_r = 3.56 \times 10^6$) in agarose preparations of varying electroosmosis (Johnson et al., 1980). However, different preparations of agarose may vary in properties other than electroosmosis, and 10–20% variable K_R's have been observed for spherical particles in different agarose preparations [compare ME agarose and HGT[P] agarose in Serwer et al. (1983)]. This could result in some agarose preparation dependent variability in the K_R's or shapes of semilogarithmic μ vs. A plots for double-stranded DNAs.

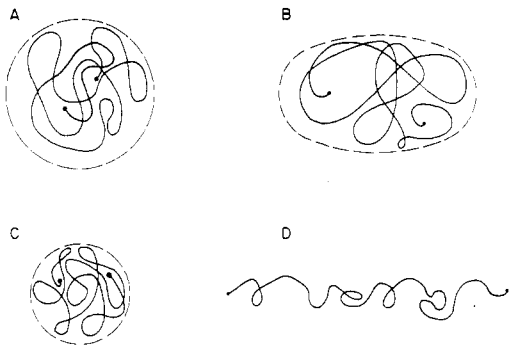

FIGURE 3: Possible conformations of a linear DNA random coil during agarose gel electrophoresis. Schematic views of a DNA random coil: (A) spherical envelope presumed in the absence of a gel, (B) asymmetric collapse during agarose gel electrophoresis at a sufficiently high voltage gradient (secondary reptation), (C) symmetric collapse, and (D) sufficient asymmetric collapse to produce end-first migration (primary reptation).

For linear DNAs, all semilogarithmic μ vs. A plots eventually had concave curvature as A increased. This is opposite to the curvature of these plots for solid, spherical particles (Serwer & Hayes, 1981; Serwer et al., 1983). Thus, the concave curvature for linear DNAs suggests deformation of DNA random coils during agarose gel electrophoresis. Deformation might be either symmetric (i.e., radial collapse; Figure 3C) or asymmetric (i.e., stretching of the DNA in the direction of the electrical field; Figure 3B,D). That at least some of the concave curvature for T7 DNA is caused by asymmetric deformation is indicated by the increase in curvature of semilogarithmic μ vs. A plots with increasing field strength. The most extreme form of asymmetric collapse is primary reptation (introduction; Figure 3D). The minimum pore radius of the most concentrated gel used here ($A = 1.5$) is no smaller than 59 nm, and the minimum pore radius of the least concentrated gel in which concavity first was observed for T7 DNA ($A = 0.3$) is no less than 920 nm [calculated by using eq 2 in Serwer and Allen (1983)]. Because these pore radii are 2–3 orders of magnitude greater than the radius of double-stranded DNA (1.0 nm; Bloomfield et al., 1974), it seems likely that secondary reptation (Figure 3B), not primary reptation (Figure 3D), is causing the concavity in semilogarithmic μ vs. A plots for T7 (linear) DNA.

The following data indicate that λ590 circular DNA also reptates at 1.0 V/cm, and in this case, the reptation must be secondary reptation (because a circular DNA has no ends, it could not possibly undergo primary reptation). (1) The K_R of λ590 circles increased when the voltage gradient decreased from 1.0 to 0.34 V/cm. (2) The curvature of the semilogarithmic μ vs. A plot for λ590 circles became more convex when the voltage gradient was lowered from 1.0 to 0.34 V/cm (Figure 1); this is the change expected if lowering voltage gradient caused conversion of an elongated DNA coil (Figure 3B) to a more spherically symmetric DNA coil (Figure 3A). The mobility inversion of T7 linear and λ590 open circular DNA can be explained by assuming that reptation-induced increase in the magnitude of μ is greater for λ590 open circular DNA than it is for T7 linear DNA when $A < 0.3$ but greater for T7 linear DNA than it is for λ590 open circular DNA when $A > 0.3$.

The occurrence of asymmetric DNA deformation (secondary reptation) does not exclude the occurrence of symmetric deformation. The finding of R_{EH} values for linear DNAs consistently roughly $2R_{EG}$ values (at a voltage gradient low enough so that voltage gradient induced alteration in μ was not occurring) suggests that some symmetric collapse of linear DNAs occurs during agarose gel electrophoresis. Further indication of symmetric collapse is the failure of the semilogarithmic μ vs. A plots for pBR322 linear and open circular DNAs to become convex at $A = 1.2$ (and under conditions such that K_R is independent of voltage gradient), even though the R_{EG} of these DNAs is large enough so that this plot should be convex at $A = 1.2$ and higher if the DNA were a solid sphere (Serwer & Hayes, 1981). A possible explanation of this observation is that the convexity expected was masked by a symmetric DNA collapse that increased in extent as A increased.

For all conditions described here, closed circular DNA migrated more rapidly than open circular and linear DNAs of the same molecular weight, primarily because the R_{EG} of closed circular DNA was smaller than the R_{EG} of either open circular or linear DNA. However, when voltage gradients and A values are made higher than those used here, a relative mobility reversal for linear and closed circular DNA is observed (Mickel et al., 1977). That is, above some critical A linear DNA migrates more rapidly than closed circular DNA. The data presented here indicate that secondary reptation induced concave curvature in the semilogarithmic μ vs. A plot for linear DNA is the reason for this relative mobility reversal.

Acknowledgments

We thank Shirley J. Hayes for performing some of the experiments reported here, Dr. Donna L. Montgomery for generous gifts of pYE(CEN3)41 and λ590 DNA's, and Linda C. Winchester for typing the manuscript.

Registry No. Agarose, 9012-36-6.

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