Anomalous Electrophoresis of Deoxyribonucleic Acid Restriction Fragments on Polyacrylamide Gels†

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ABSTRACT: A detailed study has been made of the polyacrylamide gel electrophoresis of DNA restriction fragments obtained from two plasmids, pBR322 and p82-6B. Variables studied were molecular weight, gel concentration, temperature, and electric field strength. The retardation coefficients of the larger fragments (>800 base pairs) were independent of molecular weight. The retardation coefficients of the smallest fragments (≤300 base pairs) were proportional to $M_r^{1/3}$, and therefore to the mean geometric radii of the fragments. The logarithm of the relative mobility of all fragments was also proportional to $M_r^{1/3}$. The anomalous migration of certain fragments on polyacrylamide gels was found to be "transportable" into fragments generated by different restriction enzymes. Anomalous migration was enhanced at lower temperatures and disappeared upon increasing the temperature. A fragment which migrated anomalously slowly migrated even more anomalously when dimerized; dimerizing a normally migrating fragment resulted in the normal migration of the dimerized fragment. Anomalously migrating fragments were found to be localized in distinct regions of the pBR322 circle.

Electrophoresis of double-stranded DNA fragments on polyacrylamide gels has long been known to result in the anomalous migration of certain fragments (Mertz & Berg, 1974; Maniatis et al., 1975; Thomas & Davis, 1975; Reddy et al., 1978). These anomalous fragments appear to migrate faster or slower than expected on the basis of their known molecular weights. A similar phenomenon is observed in reverse-phase chromatography; some double-stranded DNA fragments are eluted from the column sooner or later than expected on the basis of their known molecular weights (Patient et al., 1979a,b). Those fragments which are eluted later than expected from reverse-phase chromatographic columns, or which migrate more slowly than expected on polyacrylamide gels, have higher A-T[1] contents than fragments of the same molecular weight which migrate and/or elute as expected (Zeiger et al., 1972; Patient et al., 1979b; Stellwagen, 1982; unpublished observations).

Recently, an electric birefringence study was made of two 147 bp restriction fragments obtained from the *Msp*I digestion of pBR322 (Stellwagen, 1982). These two fragments migrated as separate bands on 6% polyacrylamide gels and were collected and studied separately. The more slowly migrating fragment, which had the higher A-T content (Stellwagen, 1982), appeared to orient in the electric field by a permanent dipole mechanism. The faster migrating fragment oriented in the electric field by the expected induced dipole mechanism. In a second electric birefringence study (Stellwagen, 1981), a DNA restriction fragment which migrated faster than expected on polyacrylamide gels was found to have a significantly lower "electrical orientation factor" than other DNA fragments. In both of these cases the overall lengths of the electrophoretically anomalous fragments were about the same as expected on the basis of their molecular weights. [For a discussion of the electric birefringence experiment, see Fredericq & Houssier (1973).]

Since both electric birefringence and gel electrophoresis involve the interaction of DNA molecules with an electric field, it was thought that these two phenomena might be related. Therefore, as a prelude to a later electric birefringence investigation, an extensive study was made of the polyacrylamide gel electrophoresis of restriction enzyme fragments of pBR322. In particular, it was desired to learn whether the anomalously slow migration of fragment 12A was "transportable" into fragments produced by other restriction enzymes.

A companion study of the agarose gel electrophoresis of DNA restriction fragments (Stellwagen, 1983) showed that no anomalous migration is observed on agarose gels. The electrophoretic behavior of restriction fragments ranging in size from 47 to 6000 base pairs (bp) can be described by the classical theory of pore size distribution (Rodbard & Chrambach, 1970; Rodbard, 1976), which is based on the Ogston model for a random network of linear fibers (Ogston, 1958).

The actual mechanism of migration of DNA molecules through polyacrylamide gels has not been established. Molecular sieving obviously plays an important role, since the diffusion coefficients of double-stranded DNA molecules in free solution are independent of molecular weight (Olivera et al., 1964). "End-on" migration of the DNA molecules through polyacrylamide gels has been suggested (Fisher & Dingman, 1971; Dingman et al., 1972; Flint & Harrington, 1972) on the basis that the average pore size (Ornstein, 1964; Fawcett & Morris, 1966) is much less than the persistence length of a DNA molecule (Frontali et al., 1979; Rizzo & Schellman, 1981; Borochov et al., 1981; Kam et al., 1981; Cairney & Harrington, 1982). Recently, Lerman & Frisch (1982) have suggested that DNA molecules migrate through polyacrylamide gels by means of a reptation mechanism (deGennes, 1971; Doi & Edwards, 1978). An equation describing such a wormlike or snakelike motion has been described by Lumpkin & Zimm (1982).

In the present work, a quantitative study has been made of the polyacrylamide gel electrophoresis of pBR322 restriction fragments ranging in size from 20 to 6000 bp. A variety of different restriction enzymes were used to generate the restriction fragments. Electrophoresis was studied as a function of polyacrylamide concentration, temperature, and electric field

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[1] Abbreviations: bp, base pairs; T, total polyacrylamide concentration; R_f, relative mobility; E, electric field strength; M_r, molecular weight; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; Bis, *N,N'*-methylenebis(acrylamide); TEMED, *N,N,N',N'*-tetramethylethylenediamine; A, adenine; T, thymine; G, guanine; C, cytosine; Tris, tris(hydroxymethyl)aminomethane.

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strength. The apparent mobilities of the high molecular weight fragments were found to be inversely proportional to molecular weight, as predicted by reptation theory. The logarithm of the apparent mobility was found to be proportional to $M_r^{1/3}$ for all fragments. Anomalously migrating sequences were found to be transportable into fragments produced by different restriction enzymes. Anomalously migrating fragments were also found to be localized in distinct regions of the pBR322 circle.

Materials and Methods

Plasmids and Enzymes. Plasmid p82-6B (Hartley & Donelson, 1980) was grown and the DNA isolated as previously described (Stellwagen, 1981). Plasmid pBR322 was a gift from D. Quick of the DNA Recombinant Laboratory here. The restriction fragments 12A and 12B (160 bp) and their multimers were prepared as previously described (Stellwagen, 1982).

*Hae*III, *Hha*I, and *Tha*I were purchased from Bethesda Research Laboratories. *Msp*I was prepared by T. Gregori of the DNA Core Laboratory. All other enzymes were purchased from New England Biochemicals.

Preparation of Restriction Fragments. One unit of enzyme was added to 5 μg of DNA in 50 μL of buffer. For digestion with *Taq*I, *Hph*I, *Hae*III, *Msp*I, *Tha*I, and *Bst*NI, the digestion buffer contained 0.01 M Tris buffer, pH 7.4, 0.01 M NaCl, 6 mM mercaptoethanol, 6 mM $MgCl_2$, and 1 mg/mL autoclaved gelatin. With *Hin*fI, *Alu*I or *Eco*RI, the same digestion buffer was used except that the NaCl concentration was 0.05 M. All solutions were incubated at 37 °C for $2^1/_2$ h (10× overcut). The completeness of the cut was checked by electrophoresis on agarose gels. After the cut was complete, the solutions were heated 15 min at 65 °C, cooled, ethanol precipitated at −20 °C, redissolved in T0.1E buffer (10 mM Tris, pH 8.1, and 0.1 mM EDTA), and stored frozen at −20 °C. Since the nucleotide sequences of pBR322 (Sutcliffe, 1978) and p82-6B (J. L. Hartley, personal communication), are both known, the molecular weights of the restriction fragments are also known.

Preparation of Polyacrylamide Gels. For all experiments reported here, 15.5 × 26.5 cm slab gels were used, usually with 1.6-mm spacers. All gels were prepared in TBE buffer (0.032 M boric acid, 0.05 M Tris base, and 1 mM EDTA) by using 3% *N,N'*-methylenebis(acrylamide) (Bis) as cross-linking agent. The running buffer was also TBE. The ratio of Bis to acrylamide was kept constant, because electrophoretic mobility is dependent on this ratio (Fawcett & Morris, 1966; Hedrick & Smith, 1968; Weber & Osborn, 1969; Rodbard & Chrambach, 1971; Richards & Lecanidou, 1971).

The desired quantitaties of acrylamide and Bis were weighed out, separately dissolved in water, and diluted to the proper concentration with TBE buffer. Then 0.1% (w/v) freshly dissolved ammonium persulfate and 0.1% (v/v) TEMED (*N,N,N',N'*-tetramethylethylenediamine) were separately added, and the gel mixture was poured slowly into the previously prepared gel form. Gelation occurred within 5–15 min, depending on the acrylamide concentration. All gels were allowed to age overnight in a gel cabinet containing TBE buffer, because it is known that unpolymerized ultraviolet absorbing material can be eluted from polyacrylamide gels for at least 24 h after polymerization (Bishop et al., 1967). Total polyacrylamide concentration (% *T*) was calculated as the sum of the acrylamide and Bis concentrations.

The acrylamide, Bis, and ammonium persulfate used in the preparation of the gels were Fisher Scientific reagent grade. The TEMED was Eastman or Fisher Scientific electrophoresis grade. Tris was Sigma Trizma base, reagent grade. All other chemicals were reagent grade.

Electrophoresis Procedure. Electrophoresis was carried out at constant voltage with a HeathKit Model SP-2717A regulated power supply. All gels were preelectrophoresed at least 2 h, to remove polar impurities (Chrambach & Rodbard, 1971) which bind to DNA (Elias & Eden, 1981). During the preelectrophoresis the current decreased from 20–30 mA (depending on polyacrylamide concentration) to 5–20 mA and remained relatively constant thereafter. Unless otherwise specified, all electrophoresis experiments were carried out at room temperature, 25 ± 1 °C.

Restriction digests containing 2–400 ng of DNA were diluted to 3 μL with T0.1E buffer, and 0.3 μL of a solution containing 10 mg/mL bromphenol blue marker dye in 60% glycerol was added. The samples were layered under the TBE buffer in 3 × 5 mm sample wells by using a 5-μL Dade accupipet. For some experiments, wider sample wells were used, requiring correspondingly more DNA sample. Restriction fragments cut with different enzymes were run in parallel lanes. After electrophoresis the gels were soaked 15 min in $1^1/_2$ L of a 3 μg/mL ethidium bromide solution, illuminated with a Mineral-light Model R-52 short wavelength ultraviolet light (Ultraviolet Products, Inc.) and photographed with a Polaroid MP-4 Land camera with Polaroid Type 57 high speed film and an orange filter. A photograph of a typical polyacrylamide gel is shown in Figure 1.

Distances were measured from the sample wells to the leading edges of the centers of the migrating bands. Apparent relative mobilities (R_f) were calculated from the ratio of the distance migrated by the fragment compared with the distance migrated by the marker dye. The apparent relative mobilities were generally reproducible within ±0.02 R_f unit.

Downward curvature was sometimes observed for some of the bands on the gel, especially when low electric fields (≤1.9 V/cm) were used. This effect seemed to be due to the DNA preferentially migrating at the corners of the wells and could be minimized by decreasing the load or by using wider wells. In some experiments, the same marker fragment was placed in several wells, with no apparent effect on the mobility of the marker fragment or the other fragments.

Dependence on Time. The distance migrated by the DNA fragments was linear with time. The same R_f values were obtained within experimental error when the marker dye was allowed to run one-third of the way down the gel as when it ran nearly all the way down the gel. The apparent mobility of the marker dye was also independent of time.

Dependence on DNA Concentration. A small dependence of the apparent mobility of the various DNA fragments on DNA concentration was noted. When the total DNA concentration in a restriction digest was increased from 1 to 4 μg, the apparent mobilities increased by about 0.01 R_f unit. Since this increase was less than the sample-to-sample variation in the same gel, concentration effects were ignored. Most restriction digest samples contained about 500 ng of DNA, except *Eco*RI digests of pBR322 and p82-6B, which contained 20–40 and 5–10 ng, respectively.

Dependence on Electric Field Strength. The apparent mobilities of some of the larger restriction fragments increased with increasing field strength, as previously noted (Fisher & Dingman, 1971; Lishanskaya & Mosevitsky, 1973). This dependence on electric field strength was observed for fragments larger than 1000 bp in 4.6% gels and for fragments between 300 and 2100 bp in 6.9% gels. In 9.3% gels the apparent mobilities were approximately constant at electric

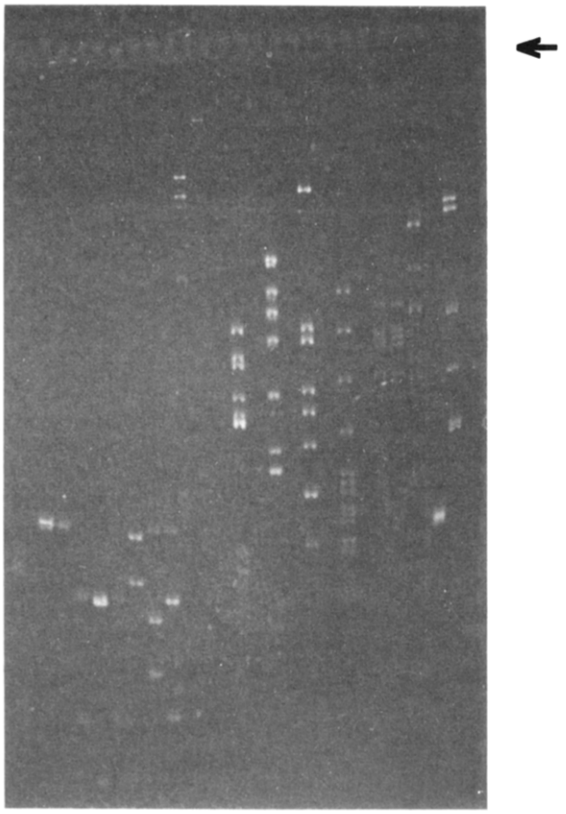

FIGURE 1: Photograph of typical polyacrylamide electrophoresis gel. 6.9% T; E = 11.8 V/cm; duration of electrophoresis = 4 h. The restriction digests (with the molecular weights which can be separately identified given in parentheses) are from left to right: lane 1, 12B (161); lane 2, 12A (161); lane 3, *Alu*I cut 12B (104, 57); lane 4, *Hha*I cut 12B (114, 47); lane 5, *Alu*I cut 12B (104, 57); lane 6, *Hgl*AI cut 12B (161, 114, 47); lane 7, *Hha*I cut 12A (161, 89, 72); lane 8, *Ava*II cut 12A (161, 102, 59); lane 9, *Rsa*I cut pBR322 (2117, 1564, 681); lane 10, *Eco*RI cut pBR322 (4362); lane 11, *Tha*I cut pBR322 (581, 473, 452, 372, 355, 341–329, 145, 129, 122, 115, 104, 97); lane 12, *Alu*I cut pBR322 (910, 659, 655, 520, 403, 281, 257, 224, 136, 100); lane 13, *Hin*fI cut pBR322 (1630, 516, 506, 396, 344, 298, 221, 154); lane 14, *Msp*I cut pBR322 (622, 527, 403, 309, 242, 238, 217, 201, 190, 180, 160, 12A, 12B, 122, 110); lanes 15 and 16, *Hae*III cut pBR322 (587, 540, 504, 457, 434, 267, 234, 213, 197, 184); lane 17, *Hph*I cut pBR322 (1124, 853, 557, 407, 396, 282, 226-7, 221); lane 18, 12B (161); lane 19, *Taq*I cut pBR322 (1443, 1307, 616, 368, 315, 312). The arrow indicates the origin of electrophoresis; the direction of migration is downward.

field strengths of 1.9 and 3.8 V/cm and then increased with increasing E. In all cases where a systematic dependence on electric field strength was noted, the apparent mobilities were extrapolated linearly to $E = 0$. In most cases the extrapolated values differed by less than 10–15% from the values measured at E = 1.9 V/cm. An abrupt decrease in mobility such as noted by Lishanskaya & Mosevitsky (1973) for very large DNA molecules was not observed for the samples studied here.

Retardation coefficients (see below) were calculated for the different DNA fragments with and without extrapolating the apparent mobilities to $E = 0$. The retardation coefficients agreed within ±5%. This observation is consistent with that of Dingman et al. (1972), who noted that the retardation coefficients of very high molecular weight DNA molecules were independent of E. In the calculations described below, the retardation coefficients calculated from R_f values extrapolated to $E = 0$ were used when a systematic dependence on electric field strength was observed. Averaged R_f values were used in the other cases. None of the results to be described depended on whether or not extrapolated values were used.

The apparent mobility of the marker dye, bromthymol blue, was independent of electric field strength but depended on the gel concentration. The apparent mobility of the dye decreased linearly with increasing gel concentration, as shown in the insert of Figure 6a. This effect can probably be attributed to the affinity of polyacrylamide gels for aromatic compounds (Fawcett & Morris, 1966); it was not observed in agarose gels, where the apparent mobility of bromthymol blue was independent of gel concentration (data not shown).

Theory of Electrophoresis. The Ogston (1958) model of pore size distribution in a random meshwork of linear fibers has been applied to gel electrophoresis by Rodbard & Chrambach (1970). This theory predicts that the electrophoretic mobility of a macromolecule should be proportional to the ratio of the cross-sectional area of the macromolecule to the average pore size of the gel. Since the average pore size of a gel is proportional to gel concentration (Ornstein, 1964; Laurent & Killander, 1964; Rodbard & Chrambach, 1970; Rodbard, 1976), electrophoretic mobility is predicted to vary with gel concentration according to

$$\log R_f = \log Y_0 - K_R(T) \quad (1)$$

where R_f is the relative mobility, Y_0 is the mobility in free solution, T is the total gel concentration, and K_R is called the retardation coefficient (Chrambach & Rodbard, 1971; Rodbard, 1976). A semilogarithmic plot of this equation, called a Ferguson plot, is linear for proteins (Ferguson, 1964) and for nucleic acids under some conditions (Rodbard, 1967; Johnson & Grossman, 1977; Serwer, 1980; Stellwagen, 1983).

The retardation coefficients, K_R, can be related to molecular parameters by

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

where a and b are empirically determined constants, R is the molecular radius, and r is the radius of the fibers in the gel (Rodbard & Chrambach, 1970; Rodbard, 1976). Equation 2 has been verified for compact, globular proteins and for proteins in SDS denaturing gels (Rodbard & Chrambach, 1971; Rodbard, 1976). The molecular radius was taken to be the geometric mean radius (proportional to $M_r^{1/3}$) and the radius of gyration (proportional to the mean square radius) in these two cases, respectively (Rodbard, 1976). For highly asymmetric molecules such as DNA restriction fragments, the correct value to use for R has been a matter of debate (Rodbard, 1976). However, Stellwagen (1983) showed that R was proportional to effective surface area for DNA restriction fragments in agarose gels.

Results

Logarithm of Molecular Weight Is a Sigmoidal Function of Mobility. A semilogarithmic plot of molecular weight vs. distance migrated is shown in Figure 2 for the gel illustrated in Figure 1. Since the electrophoresis of most fragments can be described by a single curve, the apparent mobility is independent of the nature of the ends of the fragments ("blunt" or "sticky" with one to four unpaired bases). Changes in polyacrylamide concentration did not change the shape of the curve in Figure 2 but shifted its position with respect to the axes, as shown by the double logarithmic plots in Figure 3.

At the low molecular weight end of Figure 3, the curves appear to approach a horizontal slope at molecular weights below about 25–30 bp. The limiting slopes of the lines at the high molecular weight end of Figure 3 are −1, indicating that the apparent mobilities are inversely proportional to molecular weight. The reciprocal of this relationship (molecular weight proportional to the inverse of mobility) was noted empirically

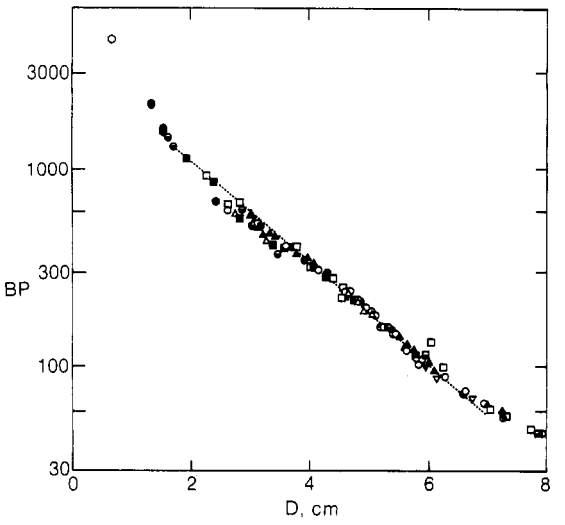

FIGURE 2: Plot of the electrophoresis experiment in Figure 1. The logarithm of molecular weight, expressed in base pairs (BP; M_r = 660 × BP) is plotted as a function of the distance migrated on the gel, D. The different symbols represent fragments cut by different restriction enzymes. The dotted line represents a straight line drawn through many of the points. 6.9% T; E = 11.3 V/cm.

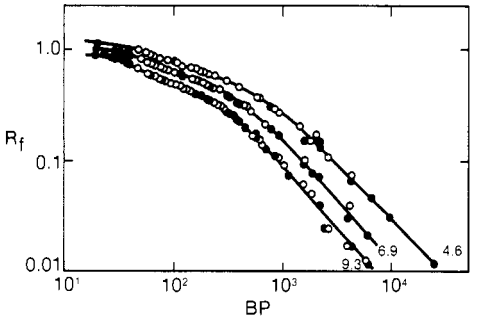

FIGURE 3: Double logarithmic plots of molecular weight (in BP) vs. relative mobility, R_f. The numbers refer to the polyacrylamide concentration. (○) E = 3.8 V/cm; (●) E = 1.9 V/cm.

by Southern (1979). On the basis of a reptation mechanism, Fischer & Lerman (1982) and Lumpkin & Zimm (1982) have predicted theoretically that the mobility of high molecular weight double-stranded DNA molecules should be proportional to M^{-1}, as observed.

Anomalous Electrophoretic Migration Is a Transportable Feature. Many of the points in Figure 2, especially in the 350–700 bp size range, fall below the straight line drawn through the points describing the electrophoretic behavior of the smaller fragments (90–350 bp). Molecular weights of these anomalously migrating fragments, calculated from their electrophoretic behavior, would be 10–20% larger than their sequenced molecular weights. Similar results are observed in 9.3% gels. Almost all anomalous migration is eliminated in 4.6% gels with E = 11.3 V/cm (data not shown). No anomalous mobilities are observed in agarose gels (Stellwagen, 1983).

Anomalous electrophoretic mobilities are enhanced at lower temperatures, as shown in Figure 4, where the anomalous points fall 30–35% below the straight line describing the electrophoretic behavior of the smaller fragments. Anomalous mobilities are completely eliminated at 37 °C (data not shown), with all points falling on a single smooth curve. The anomalous delay of some A-T rich fragments on reverse-phase chromatography columns is also enhanced at lower temperatures (Patient et al., 1979a).

If anomalously migrating fragments are identified from plots such as shown in Figure 2 or Figure 4, and the location of the

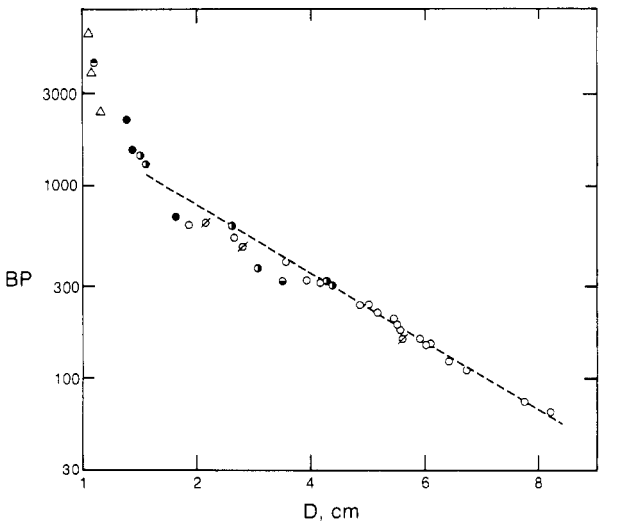

FIGURE 4: Changes in electrophoretic behavior of DNA fragments under different conditions. The logarithm of molecular weight (in BP) is plotted as a function of the distance migrated on the gel, D. The different symbols refer to fragments cut with different restriction enzymes. 6.9% polyacrylamide; E = 3.8 V/cm; temperature 4 °C.

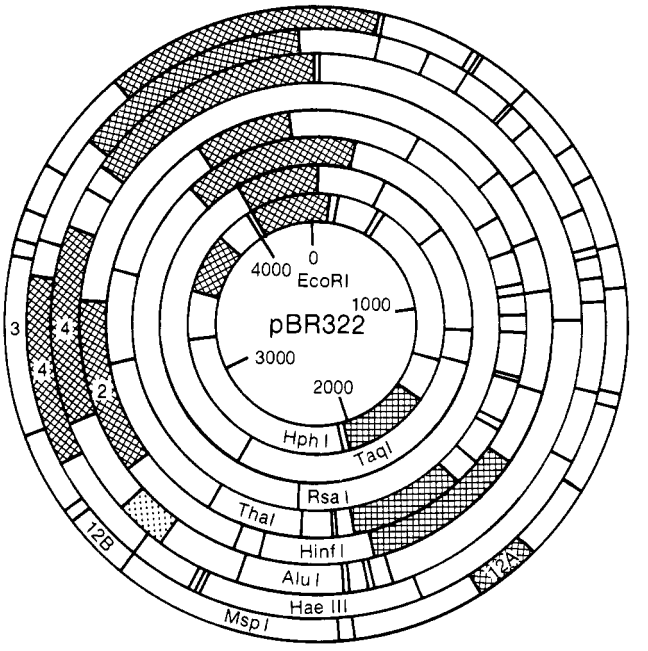

FIGURE 5: Schematic diagram of pBR322 sequence (Sutcliffe, 1978). Segments migrating anomalously slowly (by at least 10%) in 6.9% T gels are crosshatched; the fragment migrating anomalously fast is dotted.

fragment is identified on the schematic diagram of the pBR322 sequence (Sutcliffe, 1978), the result is shown in Figure 5. Anomalously migrating fragments are found clustered around three sites: one site centered near the *Eco*RI site at the top of the circle, a second site near the large inverted repeat at 3220 bp, and a third encompassing the 12A fragment generated by *Msp*I; this fragment is the one exhibiting an apparent permanent dipole moment in the electric birefringence experiment (Stellwagen, 1982). As the gel concentration is increased, more and more fragments in these same general regions migrate anomalously. Anomalously migrating fragments do not seem to be found in the first third of the pBR322 sequence.

The anomalous electrophoretic behavior of fragment 12A is enhanced by dimerizing the fragment: a head-to-tail dimer of fragment 12A migrates 10–15% more slowly than a head-

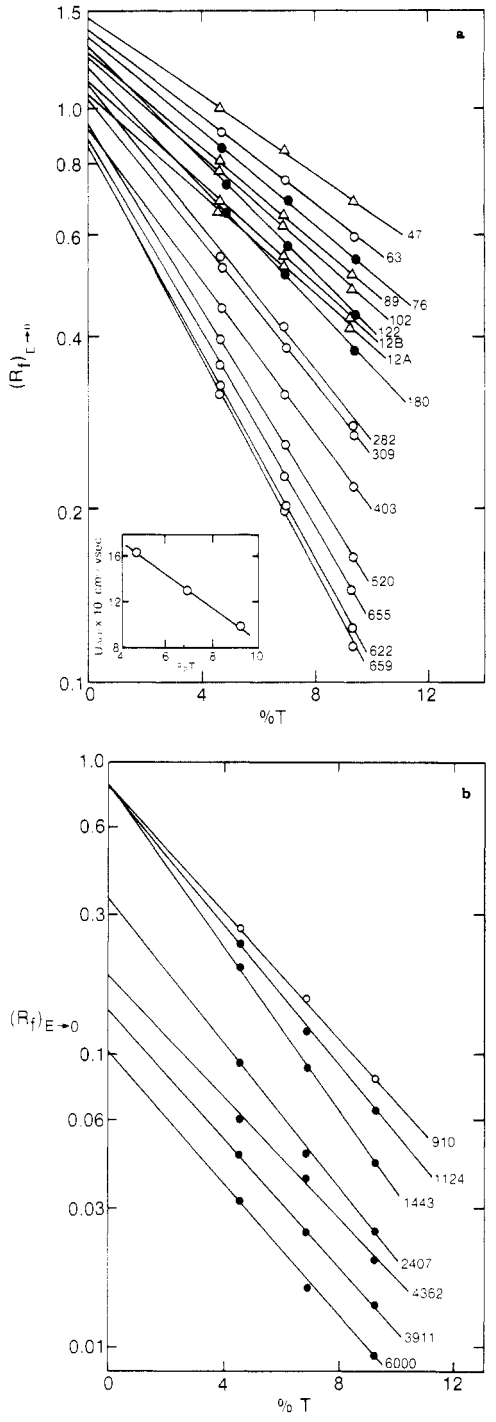

FIGURE 6: Typical Ferguson plots of DNA restriction fragments. The logarithm of the relative mobility extrapolated to zero electric field strength, $(R_f)_{E\to 0}$, is plotted as a function of total polyacrylamide concentration, T. The numbers refer to the molecular weight (in BP) of each fragment. The slope of each line is the retardation coefficient (K_R) of that fragment; the intercept of the line with the axis of zero gel concentration is the Y_0 value (see text). (a) Low molecular weight fragments; (b) high molecular weight fragments. (●) Fragments with sticky ends; (○) fragments with blunt ends; (△) fragment with two different sticky ends. The insert shows the apparent mobility of the marker dye, U_{APP}, as a function of gel concentration, T.

to-tail dimer of fragment 12B, depending on experimental conditions, even though the monomers differ by only 3–5% in apparent mobility. If fragment 12A is cut by a restriction enzyme such as *Hha*I, the pieces of it incorporated in the adjacent restriction fragments do not migrate anomalously.

Although fragment 12A, which migrates anomalously on polyacrylamide gels, has a higher A-T content than fragment 12B (47% vs. 39%), A-T content alone is not a criterion for

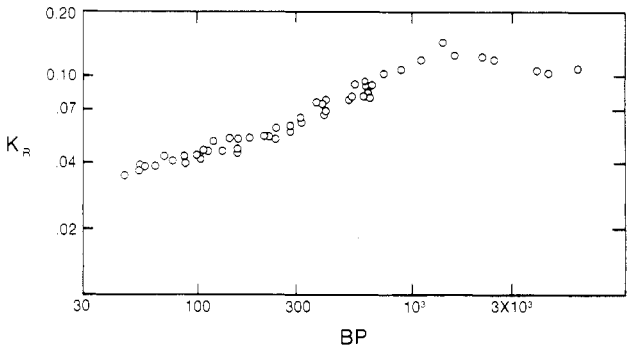

FIGURE 7: Double logarithmic plot of the dependence of the retardation coefficients, K_R, of the DNA fragments on molecular weight (in BP).

anomalous migration. For example, in the *Msp*I digest of pBR322, fragment 3, 403 bp, has an A-T content of 67% but migrates normally on 6.9% gels, while fragments with slightly different ends generated by *Hae*III (fragment 4), *Alu*I (fragment 4), or *Hin*fI (fragment 2) migrate anomalously slowly (see Figure 5). The *Msp*I fragments which migrate most anomalously, 622 and 527 bp, have A-T contents of 55% and 44%, respectively.

Logarithm of Electrophorectic Mobility Is a Linear Function of Gel Concentration. The apparent relative mobilities of the different fragments were a semilogarithmic function of the polyacrylamide gel concentration, as shown in Figure 6. Similar results have been observed by Fisher & Dingman (1971) and by Lishanskaya & Mosevitsky (1973) for much higher molecular weight DNAs in polyacrylamide–agarose composite gels. Linear Ferguson plots, such as those in Figure 6, can also be calculated from the data of Maniatis et al. (1975) for DNA fragments of the size studied here.

The slopes of the lines, called the retardation coefficients, K_R, were approximately equal for fragments 850 bp and larger and did not extrapolate to an R_f of 1.0, as shown in Figure 6b. Similar results have been observed by Fisher & Dingman (1971). The retardation coefficients of fragments smaller than 850 bp were an increasing function of molecular weight, as shown in Figure 6a. The lines appeared to extrapolate to a common intercept at a hypothetical polyacrylamide concentration of about −3%. The intercept of the apparent mobility with the axis of zero gel concentration (Y_0) was not 1.0 but gradually approached a value of 1.45 with decreasing molecular weight. Similar results have been observed with proteins (Rodbard, 1976) and suggest a specific interaction between the aromatic DNA molecules and the polyacrylamide gel (Fawcett & Morris, 1966).

Retardation Coefficients of Fragments Smaller Than 300 Base Pairs Are Proportional to the Geometric Mean Radius. The dependence of the retardation coefficients on molecular weight is shown in a double logarithmic plot in Figure 7. A line drawn through the points in this figure would have a slope of about 0.3 at the low molecular weight end and be nearly horizontal at the high molecular weight end. This suggests that the retardation coefficients of fragments smaller than about 300 bp are proportional to $M_r^{1/3}$, or equivalently, to the geometric mean radius, $\bar{R}$, of a sphere of equivalent volume. When the volume (V) of a DNA molecule is taken to be that of a right circular cylinder of radius 13 Å and length 3.4 Å × BP, the geometric mean radius, $\bar{R}$, is calculated to be

$$\bar{R} = 3(V)/(4\pi) = 7.55(\mathrm{BP}) \qquad (3)$$

Figure 8 shows that the retardation coefficients of small DNA fragments are linearly proportional to $\bar{R}$. This result is rather unexpected, since the solution conformation of DNA

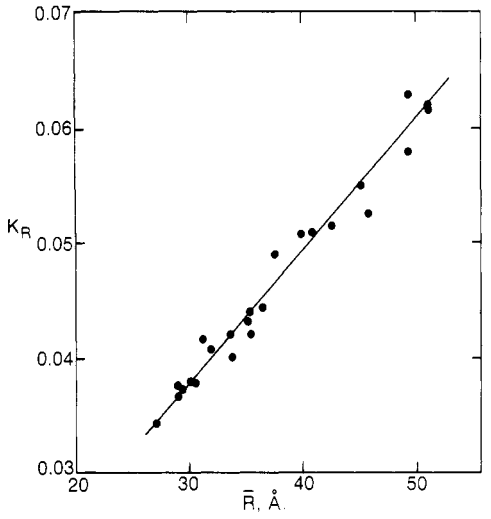

FIGURE 8: Dependence of the retardation coefficients, K_R, of small DNA fragments (≤350 bp) on mean geometric radius, $\bar{R}$.

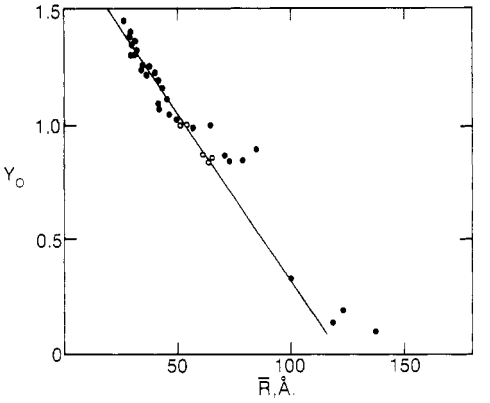

FIGURE 9: Dependence of the Y_0 values (intercepts of the lines in Figure 6 with the axis of zero gel concentration) on mean geometric radius, $\bar{R}$. (○) Anomalously migrating fragments; (●) "normally" migrating fragments.

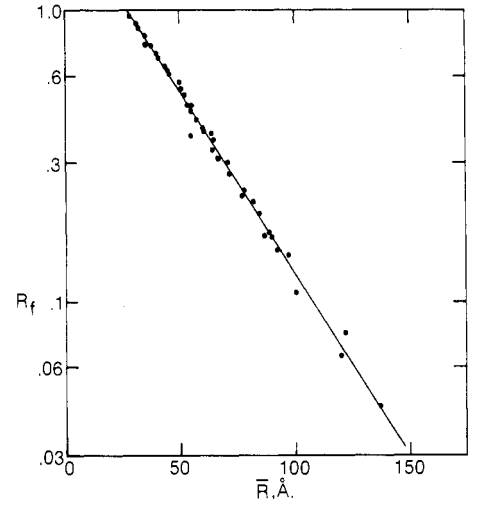

FIGURE 10: Logarithm of the relative mobility, R_f, of native double-stranded DNA fragments as a function of $\bar{R}$. 4.6% T; E = 3.8 V/cm.

restriction fragments of this size can in no way be described as spherical (Crothers, 1980; Stellwagen, 1981; Hagerman, 1981; Elias & Eden, 1981a,b; Diekmann et al., 1982). Alternatively, the points at the low molecular weight end of Figure 7 can be compressed by plotting $K_R^{1/2}$ as a function of the mean geometric radius (data not shown). $K_R^{1/2}$ appears to be a linear function of $\bar{R}$ until it becomes approximately constant for fragments larger than about 800 bp. Both results are in marked contrast to the electrophoresis of DNA fragments in agarose gels, where the retardation coefficients of fragments ranging from 160 to 6000 bp are linearly proportional to effective surface area (i.e., $M_r^{0.8}$) over the whole molecular weight range (Stellwagen, 1983). There is no correlation between the K_R values and the anomalously fast or slow migration of some of the fragments.

If the line describing the dependence of $K_R^{1/2}$ on $\bar{R}$ is extrapolated to $K_R^{1/2} = 0$, the radius of the acrylamide fibers in the gel can be estimated (Rodbard & Chrambach, 1970, 1971; Rodbard, 1976). This radius is estimated to be 38 Å, between the values of 8 Å, estimated by Fawcett & Morris (1966), and 100 Å, estimated by Richards & Lecanidou (1971) for gels of similar composition.

Retardation Coefficients of Fragments Larger Than 800 Base Pairs Are Independent of Molecular Weight. The slopes of the Ferguson plots of fragments larger than about 800 base pairs were roughly independent of molecular weight, as shown in Figure 6b. Therefore, the electrophoretic mobilities of these particles were not determined by the distribution of pore sizes in the gel (Ogston, 1958; Rodbard, 1976). Similarly, Fisher & Dingman (1971) and Dingman et al. (1972) found that the retardation coefficients of very large DNA fragments were independent of molecular weight and suggested end-on migration through the gel. A lack of dependence of the retardation coefficients on molecular weight may be a reasonable experimental criterion for reptation.

Apparent Relative Mobility of the DNA Fragments at Zero Gel Concentration Is Inversely Proportional to the Mean Geometric Radius. The dependence of the intercepts of the Ferguson plots at the axis of zero gel concentration (Y_0 values) on DNA molecular weight is shown in Figure 9. The Y_0 values are inversely proportional to the mean geometric radius, $\bar{R}$, although a certain amount of scatter exists, especially among the larger fragments. To eliminate this scatter would require a much more detailed study using gel concentrations designed to accurately measure the mobility of the larger fragments. The observed dependence of Y_0 on $\bar{R}$ can be anticipated from the apparent dependence of K_R on $\bar{R}^2$ and the equations for the motion of a particle through a random network of fibers (Ogston, 1958; Rodbard & Chrambach, 1970). There is no correlation of the Y_0 values with anomalously fast or slow migration of different restriction fragments on polyacrylamide gels, as also shown in Figure 9.

In free solution, the mobility of DNA molecules is independent of molecular weight (Olivera et al., 1964). The dependence of the apparent mobility at zero gel concentration on molecular weight may be due to the interaction of the DNA bases with the polyacrylamide gel matrix. The marker dye bromthymol blue (see Materials and Methods) and another small aromatic molecule, (dinitrophenyl)ethanolamine (Fawcett & Morris, 1966), both interact with polyacrylamide gels.

Logarithm of the Apparent Mobility of Native, Double-Stranded DNA Fragments Is Proportional to the Mean Geometric Radius. Since the straight lines in the Ferguson plots of the small DNA fragments extrapolated close to a common intercept (Figure 6a), and since the retardation coefficients were proportional to $\bar{R}$ (Figure 8), pore size theory predicts that the logarithm of the apparent mobility also should be proportional to $\bar{R}$. This prediction is illustrated in Figure 10, where the logarithm of the distance migrated on the gel is plotted as a function of $\bar{R}$ (compare with Figure 2). This type of plot was equally linear for the other gel concentrations studied here (data not shown), if the electric field strength was

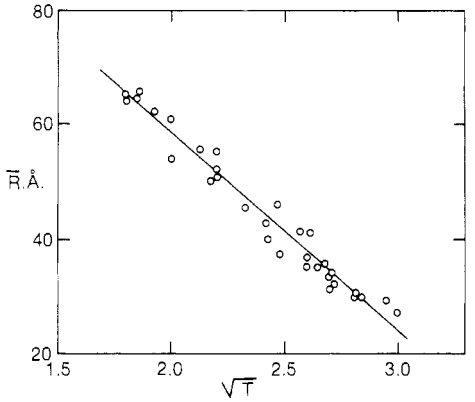

FIGURE 11: Dependence of the mean geometric radius $\bar{R}$, on the square root of the gel concentration, $T^{1/2}$, at which the mobility was half the mobility at zero gel concentration.

3.8 V/cm or below. At higher electric fields, upward curvature was observed at the high molecular weight end of plots like Figure 10, reflecting the anomalously fast migration of high molecular weight DNA samples in strong electric fields (Fisher & Dingman, 1971; Dingman et al., 1972; Flint & Harrington, 1972; Lishanskaya & Mosevitsky, 1973; McDonell et al., 1977).

Mean Pore Size of the Gel Is Proportional to the Square Root of Polyacrylamide Gel Concentration. When the apparent mobility of a macromolecule is equal to half its mobility at zero gel concentration, the mean pore size of the gel should be equal to the average pore size and to the average diameter of the macromolecule (Laurent & Killander, 1964; Fawcett & Morris, 1966; Rodbard & Chrambach, 1970; Chrambach & Rodbard, 1971). In Figure 11, the mean geometric radius of the DNA fragments is plotted as a function of the gel concentration at which the apparent mobility of the fragment was equal to half of its mobility at zero gel concentration. The mean geometric radius, and therefore, the mean pore size, was found to be proportional to $T^{1/2}$, as required by the Ogston model of pore size distribution and other models of gel structure (Rodbard, 1976). For fragments larger than 800 bp, the apparent pore size was constant, reflecting the approximately parallel lines on the Ferguson plots of these fragments. A constant pore size is consistent with end-on migration of the larger fragments through the gel.

For 6.5% and 8.0% polyacrylamide gels, mean pore radii can be estimated from Figure 11 to be 37 and 32 Å, respectively. These values can be compared with other values of 20–22 (Fawcett & Morris, 1966), 38 (Raymond & Nakamichi, 1962), 50 (Ornstein, 1964), and 60–70 Å (Tombs, 1965) estimated for polyacrylamide gels in this concentration range.

Discussion

According to the Ogston model of pore size distribution, the retardation coefficient is a measure of the fractional cross-sectional area available to a macromolecule in a gel (Rodbard & Chrambach, 1970, 1971; Rodbard, 1976). This relationship was found to be valid for DNA restriction fragments in agarose gels, where the retardation coefficients of fragments ranging in size from 160 to 6000 base pairs were found to be proportional to the effective surface areas of the fragments (Stellwagen, 1983). However, for DNA restriction fragments in polyacrylamide gels, the retardation coefficients of fragments larger than 800 bp were independent of molecular weight (Figure 6b), indicating that the electrophoretic migration of these fragments cannot be described by pore size distribution theory. More likely, these fragments migrate through the gels end on, by a reptation mechanism (Fisher & Dingman, 1971; Flint & Harrington, 1972; Lerman & Frisch, 1982; Lumpkin & Zimm, 1982). In accord with this mechanism, the apparent mobilities were inversely proportional to molecular weight (Figure 3). However, the Y_0 values, corresponding to the apparent relative mobilities at zero gel concentration, were inversely proportional to $\bar{R}$, i.e., $M_r^{1/3}$ (Figure 9).

For the smaller DNA fragments (≤800 bp), the analysis presented here suggests that the electrophoresis follows pore size distribution theory, if the size of the DNA fragment is equated with its mean geometric radius. The retardation coefficients are proportional to $\bar{R}$ (Figure 8) or $\bar{R}^2$, while the Y_0 values are proportional to $\bar{R}$ (Figure 9). No theory is presently available to explain why the electrophoretically important parameter for DNA molecules in polyacrylamide gels is $\bar{R}$ (proportional to $M_r^{1/3}$), while in agarose gels the rate of electrophoretic migration is dependent on the effective surface area (proportional to $M_r^{0.8}$). The explanation may lie in the interaction of polyacrylamide with aromatic molecules (Fawcett & Morris, 1966) and/or distortion of the gel matrix by the electric field.

The electric fields used in these electrophoresis experiments are orders of magnitude too low to orient DNA fragments of the molecular weights studied here (Stellwagen, 1981). However, preliminary electric birefringence experiments (N. C. Stellwagen, unpublished results) have shown that acrylamide itself interacts strongly with the electric field, exhibiting a large negative birefringence. Orientation or distortion of the polyacrylamide chains even to a small extent by the electric field (which, although small, is applied continuously) might aid the passage of the DNA molecules through the gel matrix. Interaction of the DNA molecules with the electric field must also be important. Lyamichev et al. (1982) found that the electrophoretic migration of a 1700 bp DNA molecule containing a 70 bp denatured region was strongly retarded on denaturing polyacrylamide gels, while the same 70 bp denatured region in a DNA molecule 4 times larger did not affect the electrophoretic mobility of the parent fragment. Therefore, the larger DNA molecule must interact with the electric field sufficiently strongly that it can overcome the retardation effect of the denatured region.

The anomalous migration of certain DNA fragments has been found to be a transportable feature (Figure 5). The regions near the *Eco*RI site and near the inverted repeat contain the early melting domains of pBR322 (Lyamichev et al., 1982; Pratt et al., 1983). These regions also contain significant numbers of AA and TT base pairs at 10 bp intervals (A. Arnone, personal communication). Trivanov & Sussman (1978) have postulated that such an arrangement of bases could cause the smooth bending of a DNA helix into a 45–50-Å radius superhelix. The *Msp*I 12A fragment (but not the 12B fragment) contains AA and TT base pairs at 7 bp intervals; in such a segment the backbone chain might be twisted ("writhe"). Hence, one might postulate that the anomalous migration of these fragments on polyacrylamide gels is due to a "bent" conformation of the DNA backbone chain (Marini et al., 1982). Such a bent conformation might require a larger "tunnel" through the gel matrix than would be required by an unbent molecule of the same size and therefore account for the anomalously slow migration of these fragments on polyacrylamide gels. However, the data presented here do not provide evidence in favor of this hypothesis: there is no correlation of retardation coefficients or Y_0 values with anoma-

lously fast or slow migration (Figure 9). If the anomalously migrating segments of the pBR322 sequence actually have an atypical backbone conformation, it may be manifested by an atypical interaction of the fragments with the electric field or with the gel matrix. Further studies of "normal" and "abnormal" DNA fragments will be needed to distinguish between these possibilities.

References

Bearden, J. C., Jr. (1979) *Gene 6*, 221–234.

Bishop, D. H. L., Claybrook, J. R., & Spiegelman, S. (1967) *J. Mol. Biol. 26*, 373–387.

Borochov, N., Eisenberg, H., & Kam, Z. (1961) *Biopolymers 2*, 231–235.

Chrambach, A., & Rodbard, D. (1971) *Science (Washington, D.C.) 170*, 440–451.

deGennes, P. G. (1971) *J. Chem. Phys. 55*, 572–578.

Diekmann, S., Hillen, W., Morgeneyer, B., Wells, R. D., & Pörschke, D. (1982) *Biophys. Chem. 15*, 263–270.

Dingman, C. W., Fisher, M. P., & Kakefuda, T. (1972) *Biochemistry 11*, 1242–1250.

Doi, M., & Edwards, S. F. (1978) *J. Chem. Soc., Faraday Trans. 2 74*, 1789–1818.

Elias, J. G., & Eden, D. (1981a) *Macromolecules 14*, 410–419.

Elias, J. G., & Eden, D. (1981b) *Biopolymers 20*, 2369–2380.

Fawcett, J. S., & Morris, C. J. O. R. (1966) *Sep. Sci. 1*, 9–28.

Ferguson, K. A. (1964) *Metabolism 13*, 985–1002.

Fisher, M. D., & Dingman, C. W. (1971) *Biochemistry 10*, 1895–1899.

Flint, D. H., & Harrington, R. E. (1972) *Biochemistry 11*, 4858–4864.

Fredericq, E., & Houssier, C. (1973) *Electric Dichroism and Electric Birefringence*, Clarendon Press, Oxford.

Frontali, C., Dore, E., Ferrauto, A., Gratton, E., Bettini, A., Pozzar, M. R., & Valdeivit, E. (1979) *Biopolymers 18*, 1353–1373.

Hagerman, P. J. (1981) *Biopolymers 20*, 1503–1535.

Hartley, J. L., & Donelson, J. E. (1980) *Nature (London) 286*, 860–864.

Hedrick, J. L., & Smith, A. J. (1968) *Arch. Biochem. Biophys. 126*, 155–164.

Hogan, M., Dattagupta, N., & Crothers, D. M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 195–199.

Kam, Z., Borochov, N., & Eisenberg, H. (1981) *Biopolymers 20*, 2671–2690.

Kovacic, R. T., & van Holde, K. E. (1977) *Biochemistry 18*, 1490–1498.

Johnson, P. H., & Grossman, L. I. (1977) *Biochemistry 16*, 4217–4225.

Laurent, T. C., & Killander, J. (1964) *J. Chromatogr. 14*, 317–330.

Lerman, L. S., & Frisch, H. L. (1982) *Biopolymers 21*, 995–997.

Lindenau, D., & Schnabel, W. (1978) *J. Polym. Sci., Polym. Lett. Ed. 16*, 665–667.

Lishanskaya, A. I., & Mosevitsky, M. I. (1973) *Biochem. Biophys. Res. Commun. 52*, 1213–1220.

Lumpkin, O. J., & Zimm, B. H. (1982) *Biopolymers 21*, 2315–2316.

Lunney, J., Chrambach, A., & Rodbard, D. (1971) *Anal. Biochem. 40*, 158–173.

Lyamichev, V. I., Panyutin, I. G., & Lyubchenko, Y. L. (1982) *Nucleic Acids Res. 10*, 4813–4826.

Maniatis, T., Jeffrey, A., & van deSande, H. (1975) *Biochemistry 14*, 3787–3794.

Marini, J. D., Levene, S. D., Crothers, D. M., & Englund, P. T. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 7664–7668.

McDonell, M. W., Simon, M. N., & Studier, F. W. (1977) *J. Mol. Biol. 110*, 119–146.

Mertz, J. E., & Berg, P. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4879–4883.

Ogston, A. G. (1958) *Trans. Faraday Soc. 54*, 1754–1757.

Olivera, B. M., Baine, P., & Davidson, N. (1964) *Biopolymers 2*, 245–257.

Ornstein, L. (1964) *Ann. N.Y. Acad. Sci. 121*, 321–349.

Patient, R. K., Hardies, S. C., & Wells, R. D. (1979a) *J. Biol. Chem. 254*, 5542–5547.

Patient, R. K., Hardies, S. C., Larson, J. E., Inman, R. B., Maquat, L. E., & Wells, R. D. (1979b) *J. Biol. Chem. 254*, 5548–5554.

Pratt, L. T., Knowles, J. E., & Blake, R. D. (1983) *Biophys. J. 41*, 289a.

Raymond, S., & Nakamichi, M. (1962) *Anal. Biochem. 3*, 23–30.

Reddy, V. B., Thimmappaya, B., Dhar, R., Subramanianm, K. N., Zain, B. S., Pan, J., Ghosh, P. K., Celma, M. L., & Weissman, S. M. (1978) *Science (Washington, D.C.) 200*, 494–502.

Richards, E. G., & Lecanidou, R. (1971) *Anal. Biochem. 40*, 43–71.

Rizzo, V., & Schellman, J. (1981) *Biopolymers 20*, 2143–2163.

Rodbard, D. (1976) in *Methods of Protein Separation* (Catsimpoolas, N., Ed.) Vol. 2, Chapters 3 and 4, Plenum Press, New York.

Rodbard, D., & Chrambach, A. (1970) *Proc. Natl. Acad. Sci. U.S.A. 65*, 970–977.

Rodbard, D., & Chrambach, A. (1971) *Anal. Biochem. 40*, 95–134.

Serwer, P. (1980) *Biochemistry 19*, 3001–3004.

Stellwagen, N. C. (1981) *Biopolymers 20*, 399–434.

Stellwagen, N. C. (1982) *Biophys. Chem. 15*, 311–316.

Stellwagen, N. C. (1983) *Biochemistry* (preceding paper in this issue).

Sutcliffe, J. G. (1978) *Cold Spring Harbor Symp. Quant. Biol. 42*, 77–90.

Thomas, M., & Davis, R. W. (1975) *J. Mol. Biol. 91*, 315–328.

Tombs, M. P. (1965) *Anal. Biochem. 13*, 121–132.

Trivanov, E. N., & Sussman, J. L. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 3816–3820.

Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406–4412.

Zeiger, R. S., Salmon, R., Dingman, C. W., & Peacock, A. C. (1972) *Nature (London), New Biol. 238*, 65–69.