rapid kinetics of deacetylation observed in vitro with this material (Hay & Candido, 1983). Perry & Chalkley (1982) have proposed that the regular acetylation and deacetylation of histones could allow chromatin to be decondensed for a variety of cellular functions.

An attractive working hypothesis at this stage is that histone deacetylase may be attached to a large matrix (possibly the chromosome scaffold) located at the base of chromatin loops. This would account for the high molecular weight of the deacetylase complex we observe, and also for the finding that the enzyme does not reversibly dissociate during the course of its reaction (Hay & Candido, 1983). The enzyme might then encounter its substrate nucleosomes in a processive manner. The high molecular weight histone deacetylase complex outlined in this report may thus provide a useful system to help elucidate the functions of histone acetylation and possibly of higher order nuclear structures.

Registry No. Histone deacetylase, 9076-57-7; β-mercaptoethanol, 60-24-2; neocuproine, 484-11-7.

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Accurate Molecular Weight Determinations of Deoxyribonucleic Acid Restriction Fragments on Agarose Gels†

Nancy C. Stellwagen

ABSTRACT: The electrophoresis of various DNA restriction fragments ranging in size from 47 to 6000 base pairs has been examined as a function of agarose concentration, electric field strength, and time of electrophoresis. A typical sigmoidal curve was obtained when the logarithm of the molecular weight was plotted as a function of mobility. The logarithms of the mobilities of all fragments were a linear function of gel concentration, if the mobilities of fragments ≥1000 base pairs were first extrapolated to zero electric field strength. The slopes of the lines, called the retardation coefficients, were found to be linearly proportional to the effective hydrodynamic surface areas of the fragments, as predicted by the Ogston theory of pore size distribution. The logarithm of the mobility of native DNA fragments was inversely proportional to $M_r^{0.8}$ over the entire molecular weight range, if the mobilities of fragments larger than 1000 base pairs were first extrapolated to zero electric field strength. The logarithm of the mobility of denatured, single-stranded DNA molecules was inversely proportional to the square root of molecular weight. The agreement of these results with the Ogston theory argues against a reptation mechanism for the movement of DNA molecules ≤6000 base pairs through agarose gels.

Despite the importance of gel electrophoresis in the preparation and purification of DNA restriction fragments, very little is known about the actual mechanism of the migration of DNA fragments through the gel. Obviously, molecular sieving 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). Lerman & Frisch (1982) have suggested that DNA molecules migrate through polyacrylamide gels by means of a reptation mechanism (deGennes, 1971). An equation describing such a wormlike or snakelike migration has been derived by Lumpkin & Zimm (1982).

The classical theory of gel electrophoresis, based on the Ogston model for a random meshwork of linear fibers (Ogston, 1958), predicts that the electrophoretic mobility of a macro-

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molecule is directly proportional to the volume fraction of the gel the macromolecule can enter (Laurent & Killander, 1964; Morris, 1966; Rodbard & Chrambach, 1970). The logarithm of the mobility is predicted to be a linear function of gel concentration, with a slope, called the retardation coefficient, characteristic of the effective surface area of the macromolecule (Rodbard & Chrambach, 1970). The theory of electrophoresis is discussed in detail in several reviews (Rodbard & Chrambach, 1971; Rodbard, 1976; Morris & Morris, 1976).

Very few studies of the electrophoresis of DNA fragments in agarose gels have been concerned with the quantitative relationship between mobility and molecular weight. Southern (1979) proposed empirically that a plot of molecular weight vs. (mobility)$^{-1}$ was linear over a much larger molecular weight range than the conventional graph of log (molecular weight) vs. mobility. Serwer & Hayes (1981), studying the electrophoresis of spherical virus particles in agarose gels, found that the retardation coefficients could be described by the Ogston model of pore size distribution. Bearden (1977) studied DNA fragments 2.8–170 kilobase pairs (kbp)[1] in size and proposed that the mobilities were proportional to $M^{-2/3}$ over a restricted range of experimental conditions.

In the present work, a quantitative study has been made of the agarose gel electrophoresis of DNA restriction fragments ranging in size from 47 to 6000 base pairs. The agarose concentration and applied electric field were varied and Ferguson plots constructed for the different fragments. Denatured single-stranded DNA fragments were also studied. The mobilities were found to be proportional to the effective surface areas of the fragments and can be interpreted in terms of the Ogston model of pore size distribution.

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 Core Laboratory here. The restriction fragments 12A and 12B (160 bp) and their multimers were prepared as previously described (Stellwagen, 1982). *Hae*III 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 pBR322 DNA in 50 μL of buffer. For digestion with *Taq*I, *Hph*I, *Hae*III, *Msp*I, *Tha*I, or *Bst*NI, the 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. For digestion with *Hin*fI, *Alu*I, or *Eco*RI, the buffer was the same except that the concentration of NaCl was 0.05 M. All solutions were incubated at 37 °C for $2^1/_4$ 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 known, the molecular weights of all restriction fragments are also known.

Preparation of Agarose Gels. All agarose gels were prepared by using BRL electrophoresis grade agarose (lot no. 20105A) (a typical lot analysis taken from the BRL catalog: gelling temperature at 1% concentration 36–40 °C; sulfate <0.35%; ash <0.75%; electroendosmosis 0.10–0.15). Gels were cast in a 15 × 28 cm horizontal gel form, with 2.5-cm vertical wicks extending from the gel bed into the buffer chambers. The agarose (0.3–1.5% w/v) was dissolved in TAE buffer (TAE, 0.04 M Tris base and 1 mM EDTA, brought to pH 7.9 with glacial acetic acid) by boiling in a microwave oven. All gels contained 2.5 μg/mL ethidium bromide, added to the warm solution just before pouring. Gels prepared without ethidium bromide gave similar results to gels containing ethidium bromide. However, staining the gels in an ethidium bromide solution after electrophoresis caused diffusion of the smaller DNA fragments, smearing the bands and making accurate measurements impossible. Therefore, all results reported here were obtained with gels containing ethidium bromide.

Electrophoresis. Electrophoresis was carried out at constant voltage with a HeathKit Model SP-2717A regulated power supply. Experiments were performed at room temperature, 25 ± 1 °C. The running buffer in all cases was TAE.

Samples containing 5–400 ng of DNA were diluted to 5 μL with T0.1E buffer, and 0.5 μL of a solution containing 2 μg/μL bromphenol blue marker dye in 50% glycerol was added. The samples were layered under the TAE buffer in 1 × 3 × 5 mm sample wells. Samples cut with different restriction enzymes were run in parallel lanes. Denatured DNA samples were prepared by adding 0.1 volume of 1 N NaOH to the sample just before applying it to the gel.

After electrophoresis the gels were 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 typical agarose gel is shown in Figure 1.

Distances were measured from the sample wells to the centers of the migrating bands, because considerable band spreading sometimes occurred, especially with small fragments in relatively dilute gels. Since the dye band was frequently too diffuse to measure accurately, the mobility of each fragment was calculated relative to the mobility of the 12A or 12B fragment (160 bp). The R_f values were generally reproducible within ±0.01 unit (±3% or better).

Dependence on Time. The rate of migration of the various DNA fragments was independent of time at all agarose concentrations and all electric field strengths used here. In addition, samples applied to the gel at different times migrated at the same rates as samples previously applied. Therefore, the mobilities were independent of the duration of the experiment. The mobilities were also independent of the age of the gel.

Dependence on DNA Concentration. The various restriction digests were usually applied to the gel at concentrations of about 80 ng/μL (total load about 400 ng of DNA). Relatively high loads were necessary in order to be able to visualize the smaller restriction fragments. For the largest fragments, *Eco*RI digest of pBR322 and p82-6B, sample concentrations of 20–40 and 5–10 ng, respectively, were used. Changing the DNA concentration in the applied samples, changing the total DNA load, and/or changing the thickness of the gel did not cause changes in mobility which were greater than sample-to-sample variations in the same gel. Therefore, all results were averaged.

[1] Abbreviations: bp, base pairs; kbp, kilobase pairs; T, gel concentration; R_f, relative mobility; M_r, molecular weight; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; E, electric field; Tris, tris(hydroxymethyl)aminomethane.

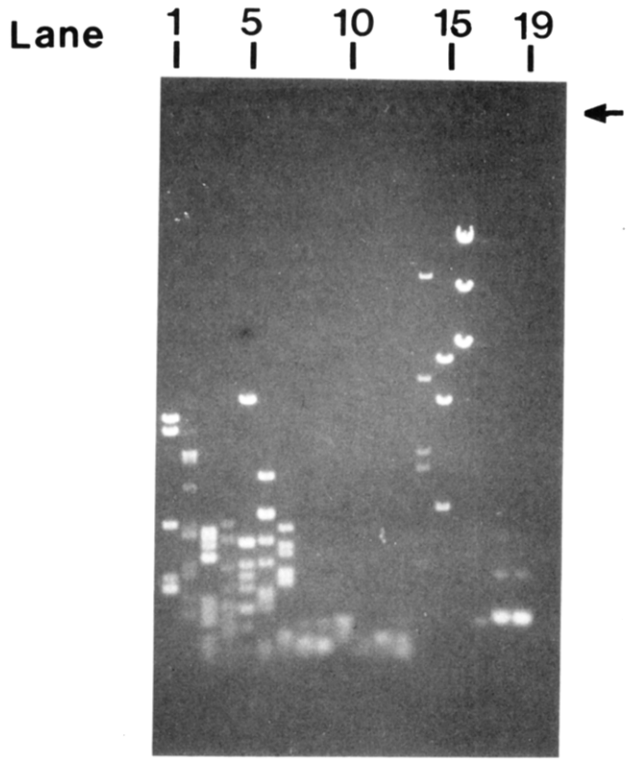

FIGURE 1: Photograph of typical agarose electrophoresis gel. 1.0% agarose, TAE running buffer, E = 3.3 V/cm, duration of electrophoresis = 5 h. The restriction digests (with the molecular weights which can be separately identified given in parentheses) are from left to right: lane 1, *Taq*I cut pBR322 (1443, 1307, 616, 368, 312–315); lane 2, *Hph*I cut pBR322 (1124, 853, 557, 407, 396, 282, 221–227); lane 3, *Hae*III cut pBR322 (589, 540, 504, 457–434, 184–227, 124, and smaller); lane 4, *Msp*I cut pBR322 (622, 527, 403, 309, 242, and smaller); lane 5, *Hin*fI cut pBR322 (1631, 506 and 516, 396, 344, 298, 220 and 221, 154); lane 6, *Alu*I cut pBR322 (910, 655 and 659, 520, 403, 226–281); lane 7, *Tha*I cut pBR322 (581, 473, 452, 329–372); lane 8, *Ava*II cut 12A (161, 102, 59); lane 9, *Hha*I cut 12A (161, 89); lane 10, *Hgl*AI cut 12B (161, 114); lane 11, *Alu*I cut 12B (104, 57); lane 12, *Hha*I cut 12B (114, 49); lane 13, *Alu*I cut 12B (104, 57); lane 14, *Eco*RI cut pBR322 and *Bst*NI cut pBR322 (4362, 1857, 1060, 928, 383); lane 15, *Rsa*I cut pBR322 (2117, 1564, 681); lane 16, *Eco*RI cut p82-6B (6000, 3911, 2407); lane 17, 12A (161); lanes 18 and 19, 12B plus multimers (480, 320, 161). The arrow indicates the origin of electrophoresis; the direction of migration is downward.

Dependence on Electric Field Strength. The relative mobilities of fragments smaller than 1000 bp were independent of electric field strength (E) at agarose concentrations up to 1.5%, as previously observed by McDonell et al. (1977). Therefore, the R_f values of these fragments were averaged. The relative mobilities of restriction fragments larger than 1000 bp increased with increasing electric field strength; for these samples, the observed R_f values were extrapolated linearly to $E = 0$.

Theory of Electrophoresis. The Ogston (1958) model of pore size distribution states that the volume fraction, f, of pores in a three-dimensional random network of linear fibers, large enough to admit a sphere of radius R, is

$$f = 4\pi\nu LR \exp(-2\pi\nu LR^2) \quad (1)$$

where ν is the average number of fibers per unit volume of gel, and L is the average fiber length. The product, νL, is assumed to be proportional to T, the gel concentration (Rodbard & Chrambach, 1970). Rodbard & Chrambach (1970) pointed out that the volume fraction derived by Ogston is equivalent to the area fraction of a random cross section of the gel. Since the exponential term in eq 1 is proportional to the surface area

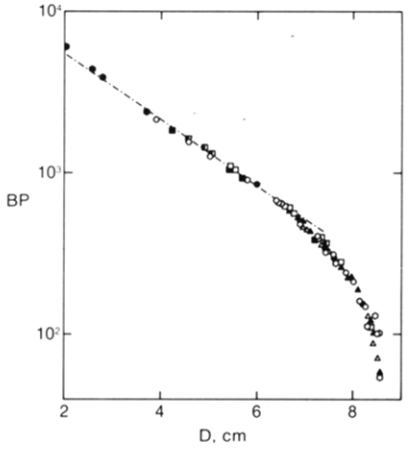

FIGURE 2: Typical electrophoresis curve, with the logarithm of molecular weight plotted vs. distance migrated on the gel, D. The distances are calculated from the photograph in Figure 1. Molecular weights are expressed by the number of base pairs (BP) in the fragment; $M_r = 660 \times$ bp. The different symbols represent fragments cut by different restriction enzymes. The dashed line represents a straight line drawn through many of the points. 1.0% agarose; E = 3.3 V/cm.

of a sphere, the electrophoretic mobility should be proportional to the ratio of the surface area of the sphere to the cross-sectional area of the pores, and hence to f:

$$f = R_f = M/M_0 \quad (2)$$

where M is the observed mobility, M_0 is the mobility in free solution, and R_f, the relative mobility, is the mobility of the macromolecule divided by the mobility of a reference dye or other marker.

Equations 1 and 2 provide a theoretical basis for a linear relationship between the logarithm of mobility and gel concentration (Rodbard, 1976)

$$\log R_f = \log Y_0 - K_R(T) \quad (3)$$

where K_R is called the retardation coefficient (Chrambach & Rodbard, 1971; Rodbard, 1976) and Y_0 is the free mobility. A semilogarithmic plot of this equation is called a Ferguson plot and is linear for proteins (Ferguson, 1964) and for nucleic acids under some conditions (Rodbard, 1976; Johnson & Grossman, 1977; Serwer, 1980). The slope and intercept, respectively, determine K_R and Y_0.

The retardation coefficients, K_R, can be related to molecular parameters by combining eq 1 and 3 (Rodbard, 1976):

$$K_R = a + bR^2 \quad (4)$$

This analysis has worked well for compact, globular proteins and for proteins in SDS denaturing gels (Rodbard & Chrambach, 1971; Rodbard, 1976), where the molecular radius has been taken to be proportional to $M_r^{-1/3}$ or $M_r^{-1/2}$, respectively. However, for asymmetric particles, the correct value to use for R is not clear (Rodbard, 1976). Various values have been suggested such as the radius of gyration, the radius of a sphere of equal volume, or the effective surface area. From the results presented below, it will be seen that the effective hydrodynamic surface area is the parameter which is related to K_R.

Results

Logarithm of Molecular Weight Is a Sigmoidal Function of Mobility. A semilogarithmic plot of molecular weight vs. distance migrated, calculated from the electrophoresis ex-

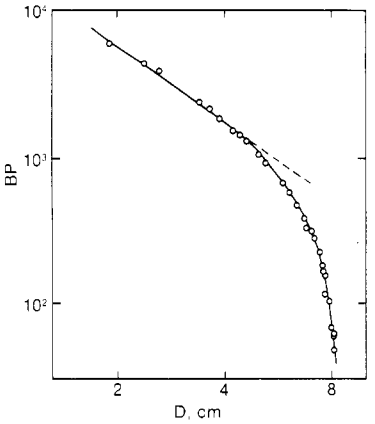

FIGURE 3: Double logarithmic plot of the data in Figure 2: the logarithm of the molecular weight (in BP) is plotted vs. the logarithm of the distance migrated, D. The dashed line represents the relationship suggested by Southern (1979). 1.0% agarose; E = 3.3 V/cm.

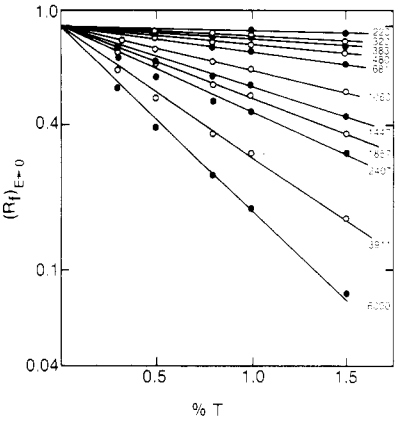

FIGURE 4: Ferguson plots of DNA restriction fragments on agarose gels. The logarithm of the relative mobility, R_f, extrapolated to zero electric field strength, is plotted vs. agarose concentration, T. The slopes of the lines are the retardation coefficients. Each point is the average of three to six individual determinations. Above 1000 bp, each point represents the value of R_f extrapolated to E = 0. The numbers beside each line represent the number of base pairs in the fragment.

periment illustrated in Figure 1, is shown in Figure 2. A small upward curvature is noted for the high molecular weight fragments (≥2500 bp), while a sharp downward curvature is noted for the smaller fragments. The smallest fragments tended toward a constant mobility; they would have been impossible to distinguish individually except for the fact that different restriction digests were run in parallel lanes. Figure 2 illustrates why small restriction fragments are very difficult to separate on agarose gels. Changes in agarose concentration did not change the shape of the curve in Figure 2 but shifted the molecular weight region of apparent linearity [data not shown; also see, e.g., Hayward & Smith (1972), Helling et al. (1974), Lehrach et al. (1977), and Serwer (1980)].

If the data in Figure 2 are plotted as molecular weight vs. reciprocal mobility, as suggested by Southern (1979), a nearly straight line is obtained. However, this is a fortuitous result, brought about by the compression of data near the origin. If the same data are plotted on a double logarithmic scale, as shown in Figure 3, the inverse relation between distance migrated and molecular weight is seen to be valid only for fragments larger than about 1400 bp, as shown by the dashed line.

Logarithm of Electrophoretic Mobility Is a Linear Function of Gel Concentration. The relative mobilities of the different fragments were found to be a semilogarithmic function of the agarose gel concentration, as shown in Figure 4. Similar observations have been made by others (Rodbard, 1976; Johnson & Grossman, 1977; Serwer, 1980). The different lines extrapolate to values very close to R_f = 1.0. Thus, the DNA restriction fragments obey the Ferguson (1964) relationship, as predicted by the Ogston (1958) theory of pore size distribution. The intercept at zero gel concentration should be equal to the mobility of free DNA molecules in solution (Rodbard, 1976).

Apparent Mobility of the DNA Fragments at Zero Gel Concentration Is Close to the Free Solution Value. The apparent electrophoretic mobility, μ, was defined as $\mu = d/(Et)$, where d is the distance migrated in cm, E is the electric field strength in volts per centimeter, and t is the time in seconds. Apparent mobilities were calculated for the various DNA fragments, extrapolated to zero gel concentration and zero voltage, if necessary, and averaged. The resulting value, averaged for 14 fragments ranging in size from 75 to 6000 bp, was $(1.36 \pm 0.01) \times 10^{-4}$ cm^2/(V·s). This value agrees with the value of 1.4×10^{-4} cm^2/(V·s) obtained by Johnson & Grossman (1977) for linear PM2 DNA at zero agarose gel concentration. However, it is somewhat lower than the free solution values of 1.51×10^{-4} (Olivera et al., 1964) and 1.85×10^{-4} cm^2/(V·s) (Ross & Scruggs, 1964) observed in 0.1 M NaCl. From the ionic strength dependence of the free solution mobility given by Olivera et al. (1964), it can be estimated that the mobility of DNA in a solution of ionic strength = 0.025 (the ionic strength of the TAE buffer used in the electrophoresis experiments) would be 1.9×10^{-4} cm^2/(V·s). The 30% lower value obtained by extrapolating the apparent mobility to zero gel concentration can probably be attributed to electroendosmosis (Ghosh & Moss, 1974).

Effective Hydrodynamic Surface Areas of DNA Fragments Can Be Calculated from Electric Birefringence Data. Electric birefringence and electric dichroism techniques have been used to measure the relaxation or reorientation times of DNA restriction fragments in solution (Hogan et al., 1978; Stellwagen, 1981; Hagerman, 1981; Elias & Eden, 1981a,b; Diekmann et al., 1982). The relaxation times, τ, are related to "effective hydrodynamic lengths", L_h, according to eq 5:

$$\tau = \frac{\pi\eta(L_h)^3}{18kT[\ln(2P_h - \gamma)]} \quad (5)$$

where L_h is the effective length, P_h is the corresponding effective axial ratio (see below), η is the solvent viscosity, k is Boltzmann's constant, T is the absolute temperature, and γ is a correction factor for end effects (Broersma, 1960; Elias & Eden, 1981b). Since the τ values are experimental measurements, the values of L_h are known without making further assumptions about model systems.

Since their effective hydrodynamic lengths are less than their contour lengths, the DNA fragments must have radii larger than the 13 Å usually associated with the Watson–Crick helix. Assuming the DNA molecules to be extended ellipsoids or cylinders of revolution, "effective hydrodynamic radii" can be calculated for the fragments if an assumption can be made about molecular volume. For simplicity, it will be assumed here that the molecular volume of each fragment is equal to the volume of a rigid rod of a given number of base pairs with a radius of 13 Å and a rise/base pair of 3.4 Å. Under the assumption that the shorter, fatter structures actually present in solution can be approximated as right circular cylinders, an effective hydrodynamic radius can be calculated from

$$R_h = [V/(\pi L_h)]^{1/2} \quad (6)$$

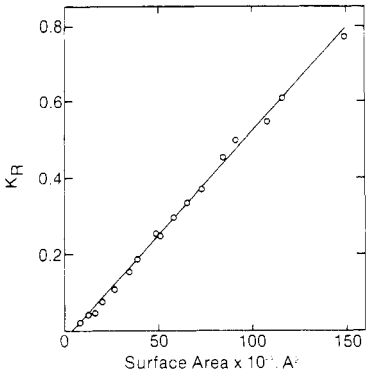

FIGURE 5: Dependence of the retardation coefficient, K_R, on the effective hydrodynamic surface area of each fragment.

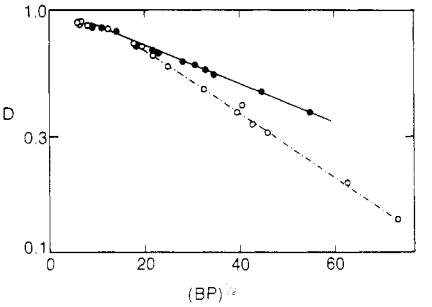

FIGURE 6: Logarithm of the distance migrated, D, of native and denatured DNA fragments as a function of $M_r^{1/2}$ (expressed as $BP^{1/2}$). (○) Native, double-stranded fragments; (●) denatured, single-stranded fragments. The denatured fragments were produced by making the solution 0.1 N in NaOH (see text). 1.5% agarose; E = 3.3 V/cm.

where V is the volume of the particle calculated from the molecular weight. The "effective hydrodynamic axial ratio", P_h, is then

$$P_h = L_h/(2R_h) \quad (7)$$

and the "effective hydrodynamic surface area", SA, is

$$SA = 2\pi R_h L_h \quad (8)$$

This surface area is proportional to $M_r^{0.8}$, except at low M_r (graph not shown). It is not related to the surface area of an equivalent sphere, which would be proportional to $M_r^{2/3}$.

Retardation Coefficients Are a Linear Function of Effective Hydrodynamic Surface Area. Retardation coefficients, K_R, were calculated for the various fragments from the slopes of the lines in Figure 4 (Rodbard & Chrambach, 1971). Attempts were made to correlate the retardation coefficients with different molecular parameters such as molecular weight, radius of an equivalent sphere, hydrodynamic length, and hydrodynamic surface area by using log–log plots (see Figure 3 as an example). The retardation coefficients did not correlate with the radii of equivalent spheres, because of a systematic deviation at low molecular weights (data not shown). The only correlation found to be valid over the entire range of molecular weights studied here was the correlation of K_R with effective hydrodynamic surface area, calculated from eq 8. As shown in Figure 5, the retardation coefficients are linearly proportional to the effective hydrodynamic surface areas of the DNA molecules at all molecular weights. Figure 5 provides a verification of the Ogston theory of pore size distribution for relatively stiff asymmetric molecules.

The square root of the retardation coefficient, $(K_R)^{1/2}$, was found to be linearly proportional to the effective axial ratio, P_h, defined by eq 7 (data not shown). However, this relationship will not be discussed further, because it does not have a theoretical justification. A similar situation arises in the electrophoresis of spherical proteins on polyacrylamide gels: $K_R^{1/2}$ is proportional to effective radius and can be justified theoretically; K_R is proportional to molecular weight and is seldom discussed [see Rodbard & Chrambach (1971)].

Logarithm of the Electrophoretic Mobility of Denatured Single-Strand DNA Molecules Is Inversely Proportional to $M_r^{1/2}$. In order to demonstrate that the electrophoretic behavior of the DNA restriction fragments described above could be attributed to their stiff, elongated structures in solution, a few experiments were undertaken with denatured, single-stranded molecules (see Materials and Methods). Denatured and undenatured restriction digests containing the same DNA fragments were run in parallel lanes, with the results shown in Figure 6. For the denatured molecules, the logarithm of the mobility was inversely proportional to $M_r^{1/2}$, as expected when the Ogston theory is applied to a Gaussian distribution of randomly coiled molecules (Porath, 1963; Robard, 1976). A similar inverse dependence of mobility on $M_r^{1/2}$ was found by Lehrach et al. (1977) for denatured, single-stranded RNA molecules. For native undenatured DNA fragments, the logarithm of the mobility was inversely proportional to $M_r^{1/2}$ only over a limited molecular weight range, as also shown in Figure 6.

Discussion

According to the Ogston model of pore size distribution, the retardation coefficient is a measure of the fractional volume available to a macromolecule in a gel (Rodbard & Crambach, 1970, 1971; Rodbard, 1976). This relationship, expressed in eq 4, has been found to be valid for proteins having a 1000-fold variation in molecular weight (Rodbard & Chrambach, 1971). In the work reported here, the Ogston model has been applied to stiff, asymmetric DNA restriction fragments and a linear relationship found between the retardation coefficients and the effective hydrodynamic surface areas of fragments ranging from 220 to 6000 bp in size. This result indicates that the electrophoresis of DNA fragments through agarose gels is well described by the Ogston theory of pore size distribution. Accordingly, there is no evidence for "end-on" migration, or reptation, through agarose gels, probably because of the large effective pore size of agarose gels (Ackers & Steere, 1962; Righetti, 1981).

Since the straight lines in the Ferguson plots (Figure 4) extrapolate to a common intercept, and since the retardation coefficients are proportional to effective hydrodynamic surface area, the Ogston model predicts that the logarithm of the mobility should also be proportional to the effective hydrodynamic surface area. This prediction is found to be correct (data not shown). Because the surface areas of fragments larger than about 100 bp are proportional to $M_r^{0.8}$, as discussed above, the logarithm of the mobility should also be proportional to $M_r^{0.8}$. This prediction is verified, as shown in Figure 7, where the data of Figure 2 are replotted as log (distance migrated) vs. $M_r^{0.8}$. This relationship was valid for all agarose concentrations studied.

The straight line obtained in Figure 7 suggests that a plot of log mobility vs. $M_r^{0.8}$ would be very useful in estimating unknown molecular weights of linear DNA fragments, if the mobilities of fragments ≥1500 bp are first extrapolated to zero electric field strength. Likewise, from Figure 6, a plot of log R_f vs. $M_r^{1/2}$ would be useful in estimating unknown molecular weights of denatured single-stranded DNA fragments.

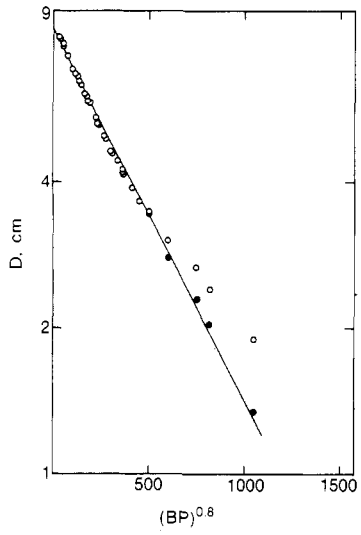

FIGURE 7: Dependence of the logarithm of distance migrated, D, on $M_r^{0.8}$ (plotted as $BP^{0.8}$). (○) The same data as in Figure 2, replotted; (●) values extrapolated to $E = 0$. 1.0% agarose; $E = 3.3$ V/m.

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