mDNA by PLK chromatography is obtained by the selective elution of nDNA from the PLK complex. As this elution step is very critical, it is recommended that the elution molarity of nDNA be determined by the use of analytical PLK columns. These methods allow the purification of milligram quantities of both n- and mtDNA, free of contaminants, in a single working day. The yield of DNA by PLK chromatography is as high as the best isolation procedures reported to date. DNA (and RNA) prepared by PLK is suitable for hybridization experiments without further purification. The capacity, simplicity, and reproducibility of PLK should recommend it as a method for nucleic acid purification, especially from organisms such as yeast where the DNA concentration is low and where the DNA is readily degraded by host nucleases.

References

Bernardi, G., Faures, M., Piperno, G., and Slonimski, P. P. (1970), *J. Mol. Biol. 48*, 23.

Bernardi, G., Piperno, G., and Fonty, G. (1972), *J. Mol. Biol. 65*, 173.

Blamire, J., Cryer, D. R., Finkelstein, D. B., and Marmur, J. (1972a), *J. Mol. Biol. 67*, 11.

Blamire, J., Finkelstein, D. B., and Marmur, J. (1972b), *Biochemistry 11*, 4848.

Bleeg, H. S., Leth Bak, A., Christiansen, C., Smith, K. E., and Stenderup, A. (1972), *Biochem. Biophys. Res. Commun. 47*, 524.

Cohen, M., Casey, J., Rabinowitz, M., and Getz, G. S. (1972), *J. Mol. Biol. 63*, 441.

Corneo, G., Moore, C., Sanadi, D. R., Grossman, L. I., and Marmur, J. (1966), *Science 151*, 687.

Grossman, L. I., Goldring, E. S., and Marmur, J. (1969), *J. Mol. Biol. 46*, 367.

Marmur, J., and Doty, P. (1962), *J. Mol. Biol. 5*, 109.

Michaelis, G., Douglass, S., Tsai, M. J., Burchiel, K., and Criddle, R. S. (1972), *Biochemistry 11*, 2026.

Schatz, G., Halsbrunner, E., and Tuppy, H. (1964), *Biochem. Biophys. Res. Commun. 15*, 127.

Schweizer, E., MacKechnie, C., and Halvorson, H. O. (1969), *J. Mol. Biol. 40*, 261.

Studier, F. W. (1965), *J. Mol. Biol. 11*, 373.

Gel Electrophoresis of Deoxyribonucleic Acid†

Dennis H. Flint*,‡ and Rodney E. Harrington

ABSTRACT: The electrophoretic mobility of duplex DNA from T-2, T-5, and T-7 bacteriophages of molecular weights ranging from 8.5×10^4 to 1.2×10^8 was investigated in 2.5–6% w/v polyacrylamide and 8.7–10% w/v starch gels. DNAs of all molecular weights investigated exhibit significant electrophoretic mobility in the above gel systems. These mobilities are strongly dependent on the field strength of the experiment; this is in contrast with proteins, whose mobilities are independent of field strength in gel electrophoresis. The electrophoretic mobility of DNAs we investigated was a function of molecular weight up to 10^6 in polyacrylamide gels and 5×10^6 in starch gels. Molecular weight resolution was achieved on a preparative scale with DNA of molecular weights between 8.5×10^4 and 3.5×10^5. If ethidium bromide is present during the polymerization of acrylamide gels, it is covalently incorporated into the gel. DNAs migrate through such gels with a greatly reduced mobility. This reduction in mobility of DNA in such gels almost certainly is due to interactions between the incorporated ethidium moiety and DNA since a change in absorbance of the incorporated ethidium is seen in the region of migrating DNA bands. The mobilities of all investigated DNAs in such gels at field strengths above 20 V cm^{-1} are dependent on the molecular weight of the DNAs, thus enabling molecular weight resolution to be achieved with DNAs of molecular weights up to at least 1.2×10^8.

Polyacrylamide gel electrophoresis and starch gel electrophoresis have been widely used for the fractionation of proteins (Davis, 1964; Ornstein, 1964; Smithies, 1955), RNA (Peakcock and Dingman, 1968; Loening, 1969), ribosomes (Dessev *et al.*, 1969), oligonucleotide length DNA (Elson and Jovin, 1969), and recently for circular and linear DNA of higher molecular weights (Fisher and Dingman, 1971; Dingman *et al.*, 1972). We also have investigated the usefulness of this technique for fractionating high molecular weight DNA.

Methods

Polyacrylamide Gels. Acrylamide, $CH_2CHCONH_2$, was recrystallized from hot acetone and dried *in vacuo*. *N,N'*-Methylenebisacrylamide, $(CH_2CHCONH)_2CH_2$, was used as the cross-linking reagent. The polymerization initiator system was ammonium peroxydisulfate, $(NH_4)_2S_2O_8$, and *N,N,N',N'*-tetramethylenediamine, $(CH_3)_2NCH_2CH_2N(CH_3)_2$, at concentrations of 0.07 and 0.4% w/v, respectively, in the final gel forming solution.

The buffer system used was discontinuous. The reservoir buffer was 0.01 M sodium aspartate (pH 8.8), and the gel buffer was 0.0157 M NaCl–0.006 M Tris, pH adjusted to 8.8

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with HCl. The top reservoir buffer also contained 0.00005% w/v Bromophenol Blue. No sample or stacking gel was used.

Because acrylamide solutions decrease in volume upon polymerization (D. H. Flint, unpublished observations), the gel interface forming technique of Davis (1964) often produces a concaved gel–water interface. This in turn results in the formation of hyperbolic-shaped bands during electrophoresis. The positions of such bands are difficult to locate exactly, which results in some uncertainty in the measurement of the migration distance. To circumvent this problem the following gel forming technique was devised.

After insertion of the gel tube (0.7 cm i.d. × 15 cm) into a serum cap and addition of enough gel forming solution to fill the tube to within 3 cm of the top, a Plexiglass interface forming plug (see Figure 1) was inserted and pushed to the bottom of the tube. Tygon tubing (3 cm long) was then placed over the open end of the tube and additional gel forming solution was added to bring the liquid level to within 1 cm of the top of the tygon tubing. After completion of polymerization, the tube was removed from the serum cap, the tygon tubing was removed, and the protruding gel was cut flush with the end of the glass tube. The Plexiglass plug was then removed with tweezers, and the thin film of gel that had formed between the plug and the side of the tube was removed by rimming and washing with a bent needle attached to a hypodermic syringe. This procedure always gave a flat interface on the end of the gel that had formed against the plug. The sample was subsequently applied to this end of the gel and would migrate in a disk-shaped band.

The general procedures given by Chrambach and Rodbard (1971) for reproducible formation of gel pore size were always followed.

The polyacrylamide electrophoresis apparatus used was similar to the one described by Gordon (1969) in which the gel tubes are completely immersed in lower reservoir buffer. By careful design and construction, the final apparatus was such that reproducibility of results for successive runs was satisfactory among the various gel tube positions, and in fact comparable to the reproducibility obtained for successive runs involving the same tube position.

After the gel tubes were loaded in the apparatus and the reservoir buffer was added, 2–5 μg of DNA in 20- to 100-μl volume (previously dialyzed against 0.01 M Tris–0.01 M NaCl–0.0125 M EDTA (pH 7.0) and made 1% w/v in sucrose) were layered underneath the top buffer onto the top of each gel. The loading device was a 100-μl disposable pipet (1.5 mm in diameter) attached to a 0.25-ml syringe with a piece of tubing. Loading flow rate was 10 μl sec^{-1}.

After loading, the voltage was adjusted to a value that gave 0.04 W of power/gel tube until the Bromophenol Blue dye had stacked (usually about 10 min); then, it was readjusted to the particular value desired for the run.

After the dye had migrated approximately 11 cm, the run was terminated, and the gels were removed after loosening them for the glass tubes by inserting a 6-in. 20-gauge needle through which a constant flow of water was maintained between the gel and the sides of the tubes. The position of the Bromophenol Blue dye was marked, and the DNA was visualized by staining using the procedure of Elson and Jovin (1969). The relative migration rate, R_m, of DNA to Bromophenol Blue was measured.

Starch Gels. The apparatus and procedures used in starch gel electrophoresis was essentially that described by Gordon (1969). The buffers used were the same as used with polyacrylamide gels except the reservoir buffer was twice as concentrated. The DNA samples were added to the gel by dipping 0.5 × 0.5 cm pieces of Whatman No. 4 filter paper into the DNA solutions, and then loading these papers into a slit made in the gel. The method used for visualization of the DNA bands in starch gels was the same as that used for polyacrylamide gels.

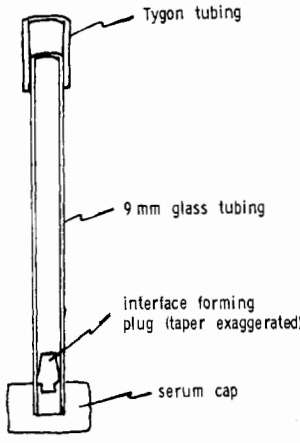

FIGURE 1: Gel-forming apparatus. Gel tube height is 6 in. Taper angle is 2°.

Preparation of DNA. DNA was obtained from T-2, T-5, and T-7 phage by phenol extraction. A homologous molecular weight series was generated from these native bacteriophage DNAs by shearing and sonicating the DNAs using the methods previously described (Harrington, 1966; 1970).

Molecular weights were obtained from intrinsic viscosity measurements and the Crothers–Zimm intrinsic viscosity molecular weight equation (Crothers and Zimm, 1965), or by sedimentation velocity measurements and the Crothers–Zimm sedimentation velocity molecular weight equation (Crothers and Zimm, 1965).

Results

All sizes of duplex DNA molecules investigated in the present study exhibited significant mobility in polyacrylamide gel electrophoresis. The R_m of the largest DNA molecule we investigated, T-2 native DNA with a molecular weight of 1.2 × 10^8, is shown as a function of gel concentration in Figure 2. T-2 DNA has a R_m of almost 0.1 in 6% gels. This result is striking when it is compared to the R_m in the same

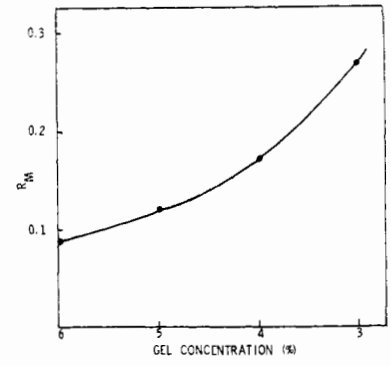

FIGURE 2: Relative mobility of native T-2 bacteriophage DNA as a function of polyacrylamide gel concentration. Field strength used was 14 V cm^{-1}.

TABLE I: Voltage Dependence of Mobility of DNA in Starch Gels.

DNA Samples	Mol Wt	Distance Migrated at 125 V for 3 hr (mm)	R_m T-5 Son. 6 min	Distance Migrated at 62.5 V for 6 hr (mm)	R_m T-5 Son. 6 min
T-2 N	1.2×10^8	7	0.26	4.5	0.16
T-5 N	7.5×10^7	7	0.26	4.5	0.16
T-7 N	2.5×10^7	7	0.26	4.5	0.16
T-2 Sh,[a] 850 rpm	3.8×10^7	7	0.26	4.5	0.16
T-2 Sh, 1220 rpm	2.8×10^7	7	0.26	4.5	0.16
T-2 Sh, 1600 rpm	2.0×10^7	7	0.26	4.5	0.16
T-5 Sh, 2000 rpm	6.2×10^6	7	0.26	4.5	0.16
T-5 Sh, 8000 rpm	1.2×10^6	15	0.56	14	0.48
T-5 Son.,[b] 30 sec	5.8×10^5	22	0.82	22	0.76
T-5 Son., 6 min	2.0×10^5	27	1.00	29	1.00

[a] Sh means sheared at the listed blade rpm. [b] Son. means sonicated for the indicated time.

gel system of ribulose 1,5-diphosphate carboxylase, 0.13, and ferritin, 0.19, two proteins whose molecular weights are 5.0×10^5 and 4.5×10^5, respectively (Pon, 1967; Richter and Walker, 1967).

Field Strength Dependence of R_m. Figure 3 shows the R_m of some DNAs in polyacrylamide gels as a function of field strength. Proteins are believed to have a R_m in polyacrylamide gel electrophoresis that is independent of field strength (Ornstein, 1964). Therefore, the behavior of DNA is quite different from that of proteins in this respect. Under our experimental conditions, there is an apparent linear relation between the R_m of the DNAs studied and the square root of the field strength, as is shown in Figure 4. We do not understand the exact significance of this if, indeed, there is any. Nevertheless, it is interesting to note that the zero field strength intercept of high molecular weight DNA in Figure 4 is at a negative value of R_m. The possible implications of this finding are discussed below.

Our experimental technique with starch gel electrophoresis did not permit measurement of R_m, so a R_m *vs.* field strength analysis was not done in starch gels. Nevertheless, the data in Table I show that the mobility of DNA in starch gels is dependent on field strength because at different field strengths the DNAs have a different mobility with respect to each other. This is also the case in polyacrylamide gels as can be seen from Figure 3.

One possible cause of the field strength dependence of mobility could be that the DNA is sheared to lower molecular weights by migration through gels, the limiting molecular weight to which it is sheared being a function of the field strength of the run. However, we have found that DNA migrates the same distance through the gels if high then low field strengths are applied for certain periods as when the same field strengths are applied in the reverse order for the same periods. This result seems to rule out shearing as an explanation for our field strength dependence of mobility results.

Physical State of the DNA During Electrophoresis. Of particular significance to the interpretation and future application of these studies is an assurance of the duplex state of DNA under our conditions of gel electrophoresis. Two results we

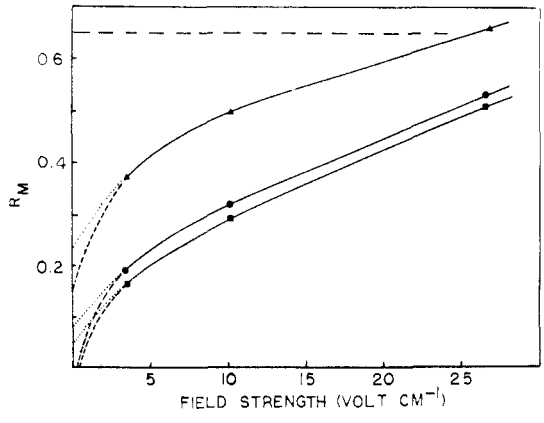

FIGURE 3: Relative mobility of DNA in polyacrylamide gel electrophoresis as a function of field strength. Molecular weights of DNAs used: (■) 1.2×10^8, (●) 1.3×10^6, (▲) 7×10^5. Small dashed lines are zero field strength extrapolations taken from Figure 4. Large dashed line is a hypothetical curve for protein. Dotted lines are merely free-hand zero field strength extrapolations.

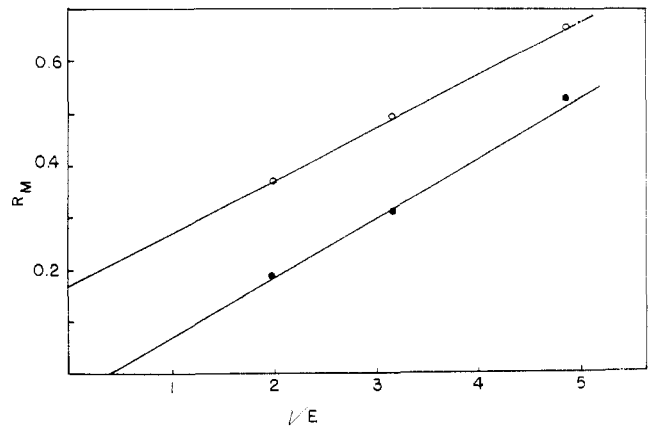

FIGURE 4: Relative mobility of DNA in polyacrylamide gel electrophoresis as a function of the square root of the field strength. Molecular weights of DNAs: (●) 1.2×10^8 (○) 7×10^5. Note negative R_m intercept for 1.2×10^8 molecular weight DNA.

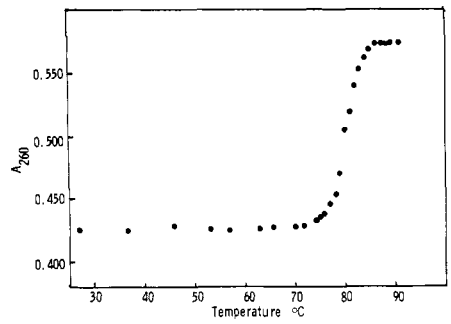

FIGURE 5: Melting curve of T-5 DNA, mol wt 2.5×10^5. This DNA was eluted from the polyacrylamide gel of a preparative electrophoresis run the results of which are shown in Figure 7. This particular sample of DNA was taken from tube 9. Na^+ concentration was 0.1 M in buffer used in this melting run.

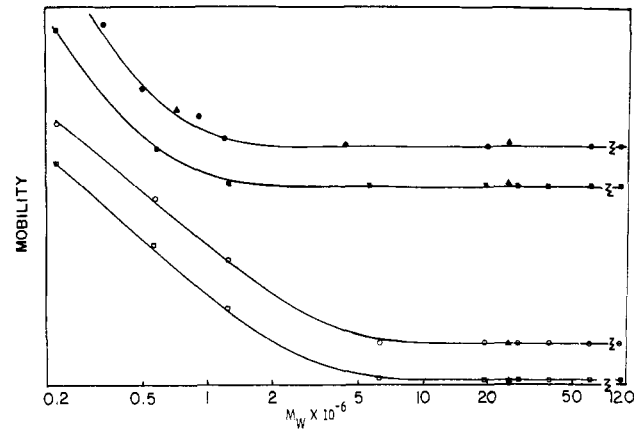

FIGURE 6: Mobility of DNA as a function of molecular weight. (●) T-2 or T-5 DNA in 2.5% polyacrylamide gels; (■) T-2 or T-5 DNA in 4% polyacrylamide gels; (○) T-2 or T-5 DNA in 8.7% starch gels; (□) T-2 or T-5 DNA in 10% starch gels; (▲) T-7 DNA. The data were plotted so that the same mobility difference exists in each gel regime between the 3×10^5 and 1×10^8 mol wt DNAs. Zero mobility is at a different position for each gel.

have obtained we believe are evidence that the duplex structure of DNA is maintained during electrophoresis.

The first piece of evidence comes from electrophoresis studies on heat-denatured, unbroken T-2 DNA and T-5 DNA. When heat denatured, these DNAs migrate at about 10% the rate of native DNA, which indicates that denatued DNA migrates very differently from the native DNAs placed on the gels. Thus, the native DNAs do not behave as if they were denatured during electrophoresis.

The second piece of evidence comes from melting studies that were made on DNA eluted from polyacrylamide gels subsequent to gel electrophoresis. A result typical of this kind of study is shown in Figure 5. The melting profile is completely normal, indicating that the duplex state has been maintained during electrophoresis in polyacrylamide gels.

Molecular Weight Dependence of Gel Electrophoretic Mobility. Figure 6 summarizes our results concerning the molecular weight dependence of the gel electrophoretic mobility in polyacrylamide and starch gels. The electrophoretic mobility of DNA in polyacrylamide gels is dependent on molecular weight up to molecular weights of approximately 10^6, above which the mobility becomes essentially independent of molecular weight. The electrophoretic mobility of DNA in starch gels is dependent on molecular weights up to approximately 5×10^6, above which the mobility also becomes independent of molecular weight. The region of loss of molecular weight resolution does not seem to be affected by changes in either field strength or gel concentration as is shown in Figure 6.

One exception to the above statement is found with unbroken T-7 DNA in polyacrylamide gels. As shown in Figure 6, T-7 DNA migrates faster under the conditions described in the legend than unbroken T-2 or T-5 DNA molecules, or T-2 and T-5 DNA fragments of molecular weights similar to that of unbroken T-7 DNA. Under conditions where the acrylamide to *N,N'*-methylenebisacrylamide ratio was higher (50:1 and 40:1), the T-7 DNA would migrate *more slowly* than unbroken and broken T-2 and T-5 DNA. T-7 DNA did not exhibit this anomalous behavior in starch gels. We are presently unable to explain these results, but perhaps it is due to differences in base composition. However, it does seem that under some circumstances polyacrylamide gel electrophoresis can be used to resolve certain high molecular weight DNA mixtures, but in general, the technique is limited in its usefulness to DNA of molecular weights below approximately 10^6 (or 5×10^6 in the case of starch gels).

Preparative Gel Electrophoresis Studies. We have attempted to fractionate large quantities of DNA using a Buchler Poly Prep (Buchler Instruments, Fort Lee, N. J.). The results of a successful run are shown in Figure 7. It is clear that polyacrylamide gels can be used to fraction DNA on a preparative basis. However, attempts to fractionate DNA samples of molecular weight greater than approximately 5×10^5 on a preparative basis were unsuccessful because of a strange yet perhaps revealing phenomenon. When DNA of these higher molecular weights entered the gel, the top of the gel would contract. The region of contraction would then move down through the gel. If the field strength was shut off during the run and the gel removed, the region of contraction did not relax; rather, it was stable for days. If the gel was stained, the presence of a DNA band in the region of the gel contraction was confirmed. The severity of the contraction seemed to be directly related both to DNA concentration and to the molecular weight of the sample.

This phenomenon of gel contraction prevented the success-

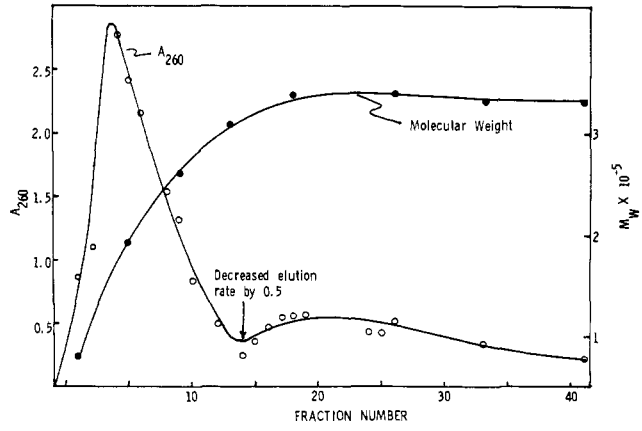

FIGURE 7: Results of a preparative gel electrophoresis run. Gel concentration used was 5%. Buffers and technique were as described in manual for Buchler Poly Prep with the exception that no stacking gel was employed. Gel concentration was 5%; 88 ml of gel forming solution was used. T-5 DNA (21 mg) that had previously been sonicated for 2 min (8, 9) was added in a volume of 30 ml. Molecular weights were determined from sedimentation velocity measurements.

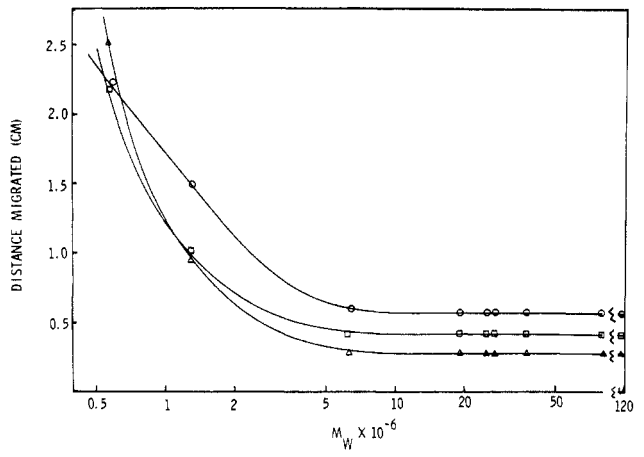

FIGURE 8: Mobility of DNA in starch gel electrophoresis as a function of molecular weight and ethidium bromide concentration. Gel concentration was 8.7%. (○) No ethidium bromide, (□) ethidium bromide concentration 10^{-5} M, and (Δ) ethidium bromide concentration 10^{-4} M.

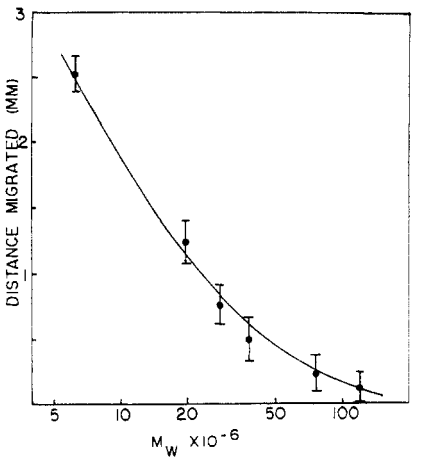

FIGURE 10: Mobility of DNA in ethidium polyacrylamide gels as a function of molecular weight. Acrylamide concentration was 4% and ethidium concentration was 10^{-5} M. Temperature was 0° and run time was 1.3 hr. Field strength used was 23 V cm^{-1}.

ful preparative fractionation of DNA above about 5×10^5 in molecular weight because the contraction would disrupt the adhesion between the gel and the glass column. This adhesion was required to keep the gel in place so that the proper volume could be maintained in the elution chamber below the gel.

Gel Electrophoresis in the Presence of Ethidium Bromide. During our investigations on the possible mode of migration of DNA through polyacrylamide and starch gels, we did some investigations on the mobility of DNA in these gels in the presence of ethidium bromide. Ethidium is known to bind to DNA and alter its length and flexibility (Freifelder, 1971). The results of these studies in starch gels are shown in Figure 8. No change in the molecular weight region where loss of molecular weight resolution occurred was found.

If ethidium bromide is present in acrylamide gel forming solutions during polymerization with the initiator system we used, the ethidium apparently becomes covalently incorporated into the gel structure since it cannot be removed by diffusion or electrophoresis after polymerization has taken place.

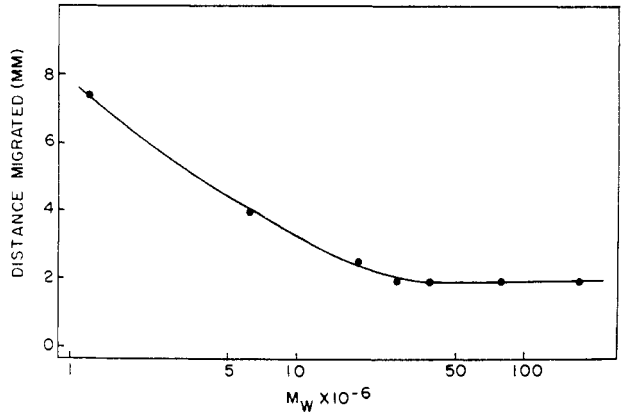

FIGURE 9: Mobility of DNA as a function of molecular weight in ethidium polyacrylamide gels. Field strength is 10 V cm^{-1}, temperature 25°, run time 2.5 hr. Ethidium concentration was 10^{-5} M and acrylamide concentration was 4%.

The electrophoretic behavior of DNA in gels in which ethidium was covalently incorporated was quite different from that observed in normal gels. In ethidium-containing gels, DNA seemed still able to interact with ethidium molecules even though they were covalently bound to the gel as evidenced by the greatly reduced mobility of the DNA and the noticeable shift in the absorbance spectrum of the ethidium in the region of the DNA band. (This shift was visible only if the ethidium concentration was above 5×10^{-5} M.) Some preliminary results of the electrophoresis of DNA in these ethidium polyacrylamide gels are shown in Figures 9 and 10. Two results of this study are striking. The mobility of the DNA is strongly dependent upon field strength, and molecular weight resolution of DNA in these gels occurs with DNA up to 10^8 in molecular weight at high field strengths. Using this technique, molecular weight resolution and perhaps molecular weight determinations can be made over almost the entire spectrum of DNA molecular weights normally studied. Further studies are presently being conducted to find the optimum conditions for DNA resolution in ethidium polyacrylamide gels and to find if the base composition of DNA in these gels influences its mobility.

Discussion

The mechanism whereby DNA migrates in polyacrylamide and starch gels is evidently very complex. For example, the largest DNA we worked with, T-2 bacteriophage DNA, has a free solution root-mean-square radius of gyration of approximately 10^4 Å (based on a calculation in which the statistical segment length is assumed to be 10^3 Å); yet, Fawcett and Morris (1966) found by gel permeation studies that the radius of molecules for which 50% of the gel volume was available in 6.5% gels (acrylamide to *N,N'*-methylenebisacrylamide ratio 20:1), conditions similar to some of those in the present studies, was 20 Å. Nevertheless, T-2 DNA has significant mobility in these gels. In fact, as was pointed out previously, it has approximately the same mobility in 6% gels as two proteins whose radius is about 10^2 Å. It appears, then, that DNA must undergo rather drastic perturbations from its average free solution configuration during migration in the gels used in this study. The ability to undergo such perturbations must be a reflection of the lack of a rigid tertiary struc-

ture in duplex DNA in contrast to the comparatively rigid tertiary structure that exists in the proteins. Duplex DNA can, without crossing large activation energy barriers, assume a profile that in two dimensions is essentially independent of molecular weight and roughly 20 Å in diameter; thus, one would predict that very large molecules are able to migrate through holes much smaller than the free solution root-mean-square radius of gyration of the molecules.

Some energy, however, must be put into DNA upon coil expansion to overcome entropic forces that arise. Approximate calculations of the forces involved in such perturbations indicate that they are on the same order of magnitude as the electrical forces imposed by the field strengths employed in these studies (Flint, 1972). The negative intercept on the R_m axis of the extrapolation of the high molecular weight data shown in Figure 4 could then be interpreted as deriving from the necessity of a certain critical force being applied before sufficient distortion arises for permeation to take place.

In certain respects, it is enlightening to compare the results of gel electrophoresis of RNA and DNA. RNA, unlike duplex DNA, contains regions of hairpin bends that render it unable to assume a profile as small as 20 Å. Thus, RNAs of much lower molecular weights than certain DNAs might be expected to have a lower gel mobility than the DNAs because of their inability to assume a small two-dimensional profile. This is, indeed, exactly the case. Peakcock and Dingman (1968), for example, find that RNAs with moecular weights of greater than 7×10^5 will not permeate 5% polyacrylamide gels; yet, DNA with a molecular weight more than two orders of magnitude greater has considerable mobility in 6% polyacrylamide gels.

It is also interesting to compare the gel electrophoretic behavior of circular DNA with that of linear DNA. Aaij and Borst (1972) and Dingman *et al.* (1972) have found that the electrophoretic mobility of circular DNA is higher than linear DNA at low gel concentration when the pore size of the gel is large enough for passage of a hairpin bend in a duplex DNA chain. However, at higher gel concentrations where the pore size of the gel admits passage of a linear double-helical chain but not a hairpin bend in a double-helical chain, the mobility of the linear DNA is much greater than the circular.

These considerations seem to suggest that noncircular DNA migrates in a linear "threading" fashion through pores in the gels. If DNA does migrate in this way, the rate of migration of the ends of the molecule would determine the rate of migration of the complete molecule because either end would have to "lead." Such a mechanism of migration might give rise to the loss of molecular weight resolution observed at high molecular weights if segments at increasingly greater distances away from the ends of the molecules exert decreasingly smaller effects on the velocity of the ends.

The origin of the field strength dependence of the mobility of DNA in gel electrophoresis is not fully understood by us. However it seems likely for the following reasons that the dependence of the gel on mobility on field strength could arise from an interaction between the DNA and the gel strands. In ethidium polyacrylamide gels, an interaction between the ethidium, which is part of the gel strand, and the DNA almost certainly occurs, and the mobility of DNA in such gels is field strength dependent. This is possibly due to the field strength influencing the binding constant of the DNA to the ethidium, an effect similar to the second Wien effect (Onsager, 1934).

Realizing then that mobility can be a function of field strength if DNA–gel interactions take place as apparently is the case in ethidium polyacrylamide gels, the question arises whether some interaction occurs between DNA and polyacrylamide or starch gels in the absence of ethidium. At least for polyacrylamide gels, this seems to be the case since severe contractions of the gel takes place in the presence of large amounts of DNA with molecular weights greater than about 5×10^5. Since this contraction is somewhat molecular weight dependent, it probably results from some kine of cooperative interaction. If this interaction is field strength dependent, it could, then, give rise to a field strength dependence of the gel electrophoretic mobility.

The ability to make polyacrylamide gels with ethidium covalently bound to them raises the possibility of using gel beads of this material in column chromatography to fractionate DNA by binding it to the gel beads then eluting with ethidium. As of now, we have not been able to find gelling conditions such that high molecular weight DNA will bind to the surface of gel beads that contain ethidium, presumably because the DNA does not penetrate the beads enough to come into intimate contact with the ethidium present. However, it seems worthwhile to search for gelling conditions such that DNA is bound to such gel beads. In any case, it seems likely that column chromatography of low molecular weight DNA might be effective using ethidium polyacrylamide gel beads since low molecular weight DNA should easily penetrate the beads.

Acknowledgment

Thanks are due to the Department of Biochemistry and Biophysics, University of California, Davis, Calif., whose cooperation in the degree program of D. H. F., and in providing certain important facility and equipment items, was invaluable to this work.

References

Aaij, C., and Borst, P. (1972), *Biochim. Biophys. Acta 269*, 192.
Chramback, A., and Rodboard, D. (1971), *Science 172*, 440.
Crothers, D. M., and Zimm, B. H. (1965), *J. Mol. Biol. 12*, 525.
Davis, B. (1964), *Ann. N. Y. Acad. Sci. 121*, 404.
Dessev, G. N., Venkov, C. D., and Tsanev, R. G. (1969), *Eur. J. Biochem. 7*, 280.
Dingman, C. W., Fisher, M. P., and Kakefuda, T. (1972), *Biochemistry 11*, 1242.
Elson, E., and Jovin, T. M. (1969), *Anal. Biochem. 27*, 193.
Fawcett, J. S., and Morris, C. J. O. R. (1966), *Separ. Sci. 1*, 9.
Fisher, M. P., and Dingman, C. W. (1971), *Biochemistry 10*, 1895.
Flint, D. H. (1972), Ph.D. Thesis, University of California, Davis, Calif.
Freifelder, D. (1971), *J. Mol. Biol. 60*, 401.
Gordon, A. H. (1969), Electrophoresis of Proteins in Polyacrylamide and Starch Gels, New York, N. Y., American Elsevier Publishing Co.
Harrington, R. E. (1966), *J. Polym. Sci., Part A-1 4*, 489.
Harrington, R. E. (1970), *J. Amer. Chem. Soc. 92*, 6957.
Loening, U. E. (1969), *Biochem. J. 113*, 131.
Onsager, L. J. (1934), *J. Chem. Phys. 2*, 599.
Ornstein, L. (1964), *Ann. N. Y. Acad. Sci. 121*, 321.
Peakcock, A. C., and Dingman, C. W. (1968), *Biochemistry 7*, 6591.

Pon, N. G. (1967), *Arch. Biochem. Biophys. 119*, 179.
Richter, G. W., and Walker, G. F. (1967), *Biochemistry 6*, 2871.
Smithies, O. (1955), *Biochem. J. 61*, 629.

Erythromycin, a Peptidyltransferase Effector†

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ABSTRACT: The effect of erythromycin on peptide-bond formation was studied in a modified fragment reaction. Evidence showed that this reaction was a specific assay for peptidyltransferase. The transfer of monoaminoacyl moieties from tRNA to puromycin was consistently stimulated by erythromycin. On the other hand, the transfer of dipeptidyl moieties such as diphenylalanine, *N*-acetyldiphenylalanine, -prolylglycine, -phenylalanylglycine, and -phenylalanylleucine from tRNA to puromycin was inhibited by the antibiotic. Nevertheless, the transfer of two dipeptidyl moieties, *N*-acetyldiglycine and -glycylproline was stimulated by the antibiotic. The degree of stimulation or inhibition was different depending upon the substrates, and complete inhibition was not observed. The maximal effect of erythromycin was observed at an erythromycin concentration of less than 10^{-6} M. Kinetic studies showed that erythromycin altered the rate of peptide-bond formation. The dose–response curve of peptidylpuromycin formation relating to erythromycin concentration coincided with the erythromycin–ribosome binding curve suggesting that the action of erythromycin on peptidyltransferase is a consequence of its binding to ribosomes; and it has been previously established that the binding of erythromycin to ribosomes inhibits bacterial growth. The above results suggest that erythromycin is an allosteric effector. It binds to the 50S ribosomal subunit in the vicinity of the peptidyl-tRNA binding site, induces a conformational change, and thus affects the reaction rate of peptidyltransferase. Considering the results of various donor substrates, several structural factors such as the number of amino acid residues on the donor tRNA, the hydrophobicity of the aminoacyl moiety of the donor tRNA, and the size of the amino acid side chain, seemed to be related to the expression of erythromycin action on peptidyltransferase.

Erythromycin, a macrolide antibiotic, is known to be an inhibitor of protein biosynthesis in bacterial systems (Taubman *et al.*, 1964; Vazquez, 1966; Mao and Wiegand, 1968), but the exact mechanism of action has not yet been elucidated. Nevertheless, the specific step affected by erythromycin has been narrowed down to either peptide-bond formation (Rychlik *et al.*, 1967; Jayaraman and Goldberg, 1968; Černá *et al.*, 1969) or to translocation (Cundliffe and McQuillen, 1967; Igarashi *et al.*, 1969; Oleinick and Corcoran, 1970). Other macrolides (niddamycin, carbomycin, spiramycin, and tylosin) have been convincingly shown to be inhibitors of peptidyltransferase (Monro and Vazquez, 1967; Černá *et al.*, 1969; Mao and Robishaw, 1971a). Since erythromycin competes with other macrolides for the same binding site on the 50S ribosomal subunits (Wilhelm *et al.*, 1967; Mao, 1971) and since its structure is similar to other macrolides, it has been suggested that the action of erythromycin should also be on peptidyltransferase (Mao and Robishaw, 1971a). The evidence supporting this claim is the inhibition by erythromycin of the transfer of the polylysyl moiety from tRNA to puromycin (Černá *et al.*, 1969, 1971), to CpA-Gly[1] (Rychlik *et al.*, 1967) or to Lys-tRNA (Jayaraman and Goldberg, 1968). Recently Tanaka *et al.* (1971) also showed that erythromycin inhibited the transfer of Phe-Phe from tRNA to puromycin. Puromycin has long been considered an analog of aminoacyl-tRNA and has been widely used to study peptide-bond synthesis. Therefore, inhibition of the transfer of a peptidyl moiety to puromycin is a good indication of inhibition of peptide-bond formation.

There are some inconsistencies in the effects of erythromycin on peptidyltransferase. For instance, the transfer of acPhe (Černá *et al.*, 1971), fMet (Monro and Vazquez, 1967; Mao and Robishaw, 1971a), Gly-Phe, Leu-Phe, and Val-Gly-Phe (Tanaka *et al.*, 1971) from tRNA to puromycin was either stimulated or unaffected by erythromycin. Also, erythromycin characteristically caused the accumulation of short peptides in poly(U)- or poly(A)-directed synthesis of Phe_n or Lys_n (Teraoka *et al.*, 1969; Mao and Robishaw, 1971a). In addition, erythromycin did not inhibit synthesis of $acPhe_2$ but did inhibit $acPhe_3$ synthesis (Oleinick and Corcoran, 1970). At first glance these results suggest an effect on something other than peptidyltransferase. The results can also be explained by assuming that the effect of erythromycin on peptidyltransferase is related to the length and nature of the peptidyl moiety (Mao and Robishaw, 1971a; Černá *et al.*, 1971). However, the number of peptidyl donors tested previously are too few to substantiate this explanation.

The availability of the 50S reaction, a modification of the fragment reaction (Monro, 1967), enabled us to test specifically the effect of erythromycin on peptidyltransferase. Although this reaction containing methanol is carried out under unnatural conditions, there is evidence to support the fact that the reaction mechanism is identical with peptide-bond formation in protein biosynthesis (Monro and Vazquez,

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[1] Abbreviations used are: CpA-Gly, cytidylyl-(3′→5′)-2′(3′)-*O*-glycyladenosine; acGly-tRNA, and acPro-Phe-tRNA, etc., *N*-acetylglycyl-tRNA, and *N*-acetylprolylphenylalanyl-tRNA, etc.; fMet-tRNA, *N*-formylmethionyl-tRNA; erythromycin, erythromycin A; acLeu-ACCAC, cytidylyl-(3′→5′)-adenylyl-(3′→5′)-cytidyl-(3′→5′)-cytidylyl-(3′→5′)-2′(3′)-*O*-L-acetylleucyladenosine.