Chain Length Determination of Small Double- and Single-Stranded DNA Molecules by Polyacrylamide Gel Electrophoresis†

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ABSTRACT: We describe the use of polyacrylamide gel electrophoresis to estimate chain lengths of double- and single-stranded DNA molecules in the size range 20–1000 base pairs (or nucleotides). Double-stranded DNA molecules of known length produced either by organic synthesis or by restriction endonuclease digestion of viral DNAs were used as standards. The relative electrophoretic mobilities of these standards were examined on both nondenaturing (aqueous) polyacrylamide gels and on denaturing gels containing 7 *M* urea or 98% formamide. Electrophoretic mobility of DNA is a linear function of the log of molecular weight if appropriate conditions are used, although exceptions are noted. Chain lengths can be conveniently estimated by using as standards bacteriophage λ DNA restriction fragments or commerically available tracking dyes.

Several methods have been used to estimate molecular weights of small DNA molecules (Elson and Jovin, 1969; Clark and Felsenfeld, 1971; Danna and Nathans, 1971; Dingman et al., 1972b; Prunell and Bernardi, 1973; Luk et al., 1973; Jeppesen, 1973; Maniatis and Ptashne, 1973a,b). For molecules of 10–1000 base pairs or nucleotides the method of polyacrylamide gel electrophoresis provides the highest degree of resolution and is, in principle, the simplest. In practice this method requires well-characterized molecular weight standards which are sometimes difficult to prepare. We have studied the behavior of DNA molecules with known chain lengths in nondenaturing and denaturing polyacrylamide gels in order to establish a more generally applicable method of chain length determination.

We use as standards synthetic duplex DNAs (22–77 base pairs) with chain lengths determined by chemical analysis (Khorana et al., 1972), and restriction endonuclease fragments generated from viral DNAs (Danna and Nathans, 1971). Restriction fragments of 80–400 base pairs were obtained by digesting ^{32}P-labeled SV40 and ϕX174 RFI DNAs with Hin[1] restriction endonuclease. The chain lengths of these molecules were deduced from their relative amounts of radioactivity (Danna and Nathans, 1971; Edgell et al., 1972; Johnson and Sinsheimer, 1974; Table I). DNA fragments of 400–1000 base pairs were produced by cleavage of ^{32}P-labeled SV40 DNA with Hin II and III as described by Danna and Nathans (1971). These workers measured the chain lengths of SV40 Hin fragments relative to full length SV40 DNA in the electron microscope and demonstrated that their electrophoretic mobilities on polyacrylamide gels are a linear function of the log of their molecular weight. We confirm their observations and show that the linear mobility–log molecular weight relationship is also valid for molecules smaller than 400 base pairs and for single-stranded DNA on denaturing polyacrylamide gels. We also determined the electrophoretic mobilities of two dyes relative to DNA standards for use as chain length markers. A preliminary account of this work has appeared elsewhere (Maniatis and Ptashne, 1973a; Maniatis et al., 1973).

Materials and Methods

Enzymes. Hin II and III, Hpa II, and Hae III restriction endonucleases from *Haemophilus influenzae, Haemophilus parainfluenzae,* and *Haemophilus aegyptius,* respectively, were prepared as previously described (Maniatis et al., 1973; Sharp et al., 1973; Middleton et al., 1972). T_4 polynucleotide kinase was prepared according to published procedures (Richardson, 1965). Bacterial alkaline phosphatase (BAP) was purchased from Worthington Biochemicals.

^{32}P-Labeled DNA. ^{32}P-labeled ϕX174 RFI DNA was prepared as described by Godson and Vapnek (1973), except that the DNA was not subjected to alkaline sucrose sedimentation equilibrium (Hudson et al., 1969). ^{32}P-labeled bacteriophage λ DNA was purified as described by Pirrotta et al. (1971). Uniformly ^{32}P-labeled SV40 DNA was a gift from J. Sambrook. Unlabeled SV40 DNA (a gift from J. Mertz) was digested with Hin and the ends were labeled with ^{32}P using T_4 polynucleotide kinase as described elsewhere (Maniatis et al., 1975).

Polyacrylamide gel electrophoresis was carried out on a slab gel apparatus similar to the one described by Dewachter and Fiers (1971). Native duplex DNA molecules were examined on slab gels (20 cm × 20 cm × 0.3 cm) containing Tris-borate-magnesium (TBM) buffer (0.09 *M* Tris-borate (pH 8.3)–5 m*M* $MgCl_2$) (Maniatis and Ptashne, 1973a) or in Tris-borate-EDTA (TBE) buffer (0.09 *M* Tris-borate (pH 8.3)–2.5 m*M* EDTA) (Peacock and Dingman, 1969). The same buffers were used in electrophoresis reservoirs. To prepare the gels 3 ml of 1.6% ammonium per-

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[1] Abbreviations used are: TBE, Tris-borate-EDTA buffer; Hin, Hpa II, Hae III, restriction endonucleases isolated from *Haemophilus influenzae, Haemophilus parainfluenzae,* and *Haemophilus aegyptius,* respectively; BAP, bacterial alkaline phosphatase; TBM, Tris-borate-magnesium buffer; Temed, *N,N,N′,N′*-tetramethylethylenediamine.

synthetic oligonucleotides	no. of nucleotides in each strand	ref
CCCGCACACCGCGCATCAGCCATCGCGCGAGGGAATCGTACCCTCTCAGAGGCCAAGCTAAGGCCTGAGCAGGTGGT-^{32}P ^{32}P-GGGCGTGTGGCGCGTAGTCGGTAGCGCGCTCCCTTAGCATGGGAGAGTCTCCGGTTCGATTCCGGACTCGTCCACCA	77/77	1
GTTTCCCTCGTCTGAGATTTAGACGGCAGTAGCTGAAGCTTCCAAGCTTAGGAAGGGGGTGGTGGT-^{32}P ^{32}P-GAGCAGACTCTAAATCTGCCGTCATCGACTTCGAAGGTTCGAATCCTTCCCCCACCACCA	66/60	2
GAATCGTACCCTCTCAGAGGCCAAGCTAAGGCCTGAGCAGGTGGT-^{32}P ^{32}P-GCTCCCTTAGCATGGGAGAGTCTCCGGTTCGATTCCGGACTCGTCCACCA	45/50	1
GTTTCCCTCGTCTGAGATTTAGACGGCAGTAGCTGAAGCT-^{32}P ^{32}P-GAGCAGACTCTAAATCTGCCGTCATCGACTTCGAAGGT	38/40	2
TCCAAGCTTAGGAAGGGGGGGGTGGT-^{32}P ^{32}P-TCGAATCCTTCCCCCCCCACCA	26/22	2
CCCGCCACCGCGCATTCAGCCA-^{32}P ^{32}P-GGGCGGTGGCGCGTAAG	22/17	3
TCCAAGCTTCAGCTACTGCCGTCTAAATC-^{32}P	29	6
ACCACCACCCCCTTCCTAAGCT-^{32}P	22	6
ACCACCACCCCCTTCCTAA-^{32}P	19 (RNA)	7
GGGTACGATTCCCTCG-^{32}P	16	4, 8
GCATCAGCCA-^{32}P	10	5
TTTTTTTTTT-^{32}P	10	10
AAAAAAAAAA-^{32}P	10 (RNA)	11

FIGURE 1: Synthetic polynucleotides used to calibrate polyacrylamide gels. The syntheses of fragments 1–5 are described in: (1) Caruthers et al., 1972a; (2) Caruthers et al., 1973; (3) Van deSande et al., 1972b; (4) Buchi and Khorana, 1972; (5) Caruthers et al., 1972b; (6) was a gift from P. Loewen; (7) was obtained from an RNAse T digest of *E. coli* tyrosine tRNA by Dr. U. L. RajBhandary; (8) was a gift from Dr. H. Buchi; (9) was a gift from Dr. M. Caruthers; (10) was obtained from chemical polymerization of dT; and (11) was obtained from an enzyme digest of poly(A).

Table 1: Chain Length Measurements for ϕX 174 Hind Fragments.[a]

Fragment No.	% Total Radioactivity	Chain Length A	Chain Length B
R1	19.1	999	930
R2	15.0	784	730
R3	12.5	653	640
R4	9.0	470	440
R5	7.1	371	370
R6	18.5	323	320
R7.1	11.0	287	290
R7.2			270
R8	3.6	188	195
R9	2.7	141	150
R10	1.5	78	80
Total no. of base pairs		5227	5050

[a] The fragment numbers are the same as those originally presented by Edgell et al. (1972). ^{32}P-labeled fragments are fractionated on a 3.5% polyacrylamide gel. The portions of the gel containing the fragments were localized by autoradiography, excised, and counted as described by Danna and Nathans (1971). The chain lengths in column A were calculated from the percent total radioactivity assuming a molecular weight of 3.4×10^6 for full length ϕX174 RFI DNA and a molecular weight of 650 for each base pair. The chain lengths in column B were determined by polyacrylamide gel electrophoresis using SV40 Hind restriction fragments and the synthetic duplex molecules of Figure 1 as markers (see Figures 3 and 5). The values of column A are in good agreement with those presented by Johnson and Sinsheimer (1974).

sulfate, 7.5 ml of ten times concentrated gel buffer, and an appropriate volume of 30% acrylamide (1 g of *N,N'*-methylenebisacrylamide + 29 g of acrylamide/100 ml) were mixed and the final volume was brought to 75 ml with water. After degassing, the solution was polymerized by the addition of 25 μl of *N,N,N'N'*-tetramethylethylenediamine (Temed) (Eastman Kodak Co.). Samples were ethanol precipated prior to electrophoresis to remove salt and buffer; 0.1 volume of 20% sodium acetate, 20 μg of yeast tRNA carrier, and 2 volumes of 95% ethanol were added to the sample; the mixture was placed in an ethanol–Dry Ice bath for 15 min and centrifuged at top speed in an eppendorf centrifuge for 5 min. The precipitate was rinsed two times with 70% ethanol and resuspended in 25 μl of ten times diluted electrophoresis buffer containing 5% glycerol, 0.025% xylene cyanol FF (Edward Gurr Ltd., London), and 0.025% Bromophenol Blue (Matheson Coleman Bell). The presence of salt reduces the mobility of DNA and leads to inaccurate molecular weight estimates. Electrophoresis was carried out at room temperature at constant voltage (200 V) (10 V/cm).

Two kinds of denaturing gels were used. DNA fragments in the range of 10–100 nucleotides were analyzed on 12% polyacrylamide TBE gels containing 7 *M* urea. The gels were prepared by mixing 31.5 g of urea (Schwarz/Mann ultrapure), 7.5 ml of 10 × TBE buffer, 30 ml of 30% acrylamide, 3 ml of 1.6% ammonium persulfate, and enough water to bring the final volume to 75 ml. After degassing the gel was polymerized with 25 μl of Temed. Larger DNA fragments were analyzed on 5% polyacrylamide gels containing 98% formamide. The gels were prepared using a modified version of the procedure of Staynov et al., (1972); 99% formamide (Matheson Coleman Bell) was deionized by stirring with a mixed bed ion exchange resin (Bio-Rad AG 501-X8) (5 g of resin/100 ml of formamide) for 0.5 hr and the resin removed by filtration; 1 ml of a solution containing 100 mg of ammonium persulfate, 170 mg of dibasic sodium phosphate (anhydrous), and 40 mg of monobasic sodium phosphate (monohydrate) (final $NaPO_4$ concentration was 0.02 *M*, pH 7.5) was mixed with 74 ml of deionized formamide containing 3.19 g of acrylamide and 0.56 g of bisacrylamide. The solution was polymerized by the addition of 150 μl of Temed. The running buffer, which was circulated between reservoirs during the run, was 0.02 *M* sodium phosphate (pH 7.5). Pinder et al. (1974b) have shown that aqueous reservoir solutions do not penetrate the gel appreciably during the run. Gels were stored with deionized formamide in the sample wells which was replaced with electrophoresis buffer immediately prior to sample applications. For both 7 *M* urea and formamide containing gels, ethanol-precipitated samples were suspended in 25 μl of 98% formamide, placed in a boiling water bath for 2 min,

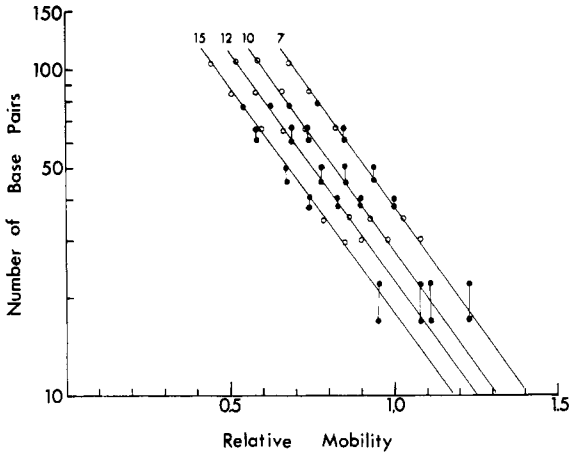

FIGURE 2: Chain length calibration on polyacrylamide TBM gels. The solid circles represent the synthetic polynucleotide duplexes shown in Figure 1. Duplex molecules containing unequal number of nucleotides in the two strands are represented by two solid circles joined by a line. The open circles represent double-stranded operator DNA fragments isolated from bacteriophage (Maniatis and Ptashne, 1973a). The chain lengths of these operator fragments were determined by comparing their electrophoretic mobility on native and denaturing polyacrylamide gels relative to the synthetic DNA markers and were confirmed by quantitative analysis of pyrimidine tract data. The chain length of the 30 base pair operator fragment was confirmed by DNA sequence analysis (Maniatis et al., 1974). The mobility plotted on the abscissa is relative to that of xylene cyanol FF. The numbers above each line indicate the concentration of acrylamide in the gel.

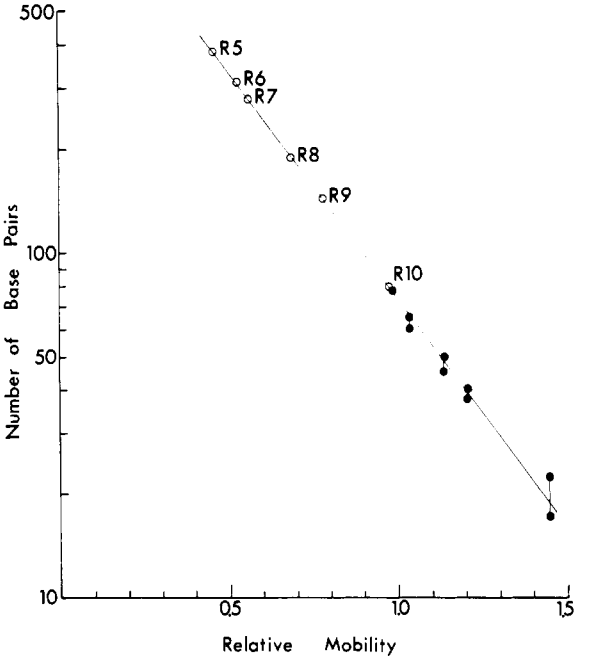

FIGURE 3: Relative mobilities of synthetic duplex DNA molecules and ϕX174 Hin fragments on a 5% polyacrylamide TBM gel. The mobilities are plotted relative to xylene cyanol FF which comigrates with a DNA duplex of 75 base pairs. In 5% polyacrylamide TBE gels the xylene cyanol FF comigrates with a duplex of 260 base pairs while the Bromophenol Blue migrates with a 65 base pairs duplex.

and quickly cooled in ice. After the addition of marker dyes the gel was run at room temperature at a constant voltage (200 V) (10 V/cm).

Autoradiography. Wet gels were covered with Saran Wrap (Dow Chemical Co.) and exposed to Kodak no screen X-ray film.

Synthetic Oligonucleotides. The oligonucleotides used for molecular weight markers are shown in Figure 1 along with the references describing their syntheses. The polynucleotides were labeled with ^{32}P by first dephosphorylating by treating a reaction mixture (50 μl) containing 5–10 pmol of the compound in 0.01 *M* Tris-HCl (pH 8.0) with 0.03 unit of BAP at 70° for 15 min. A second aliquot of BAP (0.033 unit) was added and incubation continued for another 10 min. The BAP was denatured by the addition of NaOH to pH 13.0 and the reaction mixture left at room temperature for 20 min after neutralization. Phosphorylation was then carried out under previously described conditions (Van deSande et al., 1972b). Unincorporated ATP was removed by gel filtration through Sephadex G-50 (1 × 12 cm) in 0.05 *M* triethylammonium bicarbonate.

Results

Electrophoretic Mobility of 20–100 Base Pairs Duplex DNA. A chain length calibration of polyacrylamide TBM gels containing 7, 10, 12, and 15% acrylamide is shown in Figure 2. The log of the number of base pairs of the synthetic DNA molecules of Figure 1 is plotted vs. electrophoretic mobility relative to that of the xylene cyanol FF tracking dye. The relative mobilities of DNA and dye are reproducible to within 5% if exactly the same conditions including pH, $MgCl_2$ concentration, acrylamide concentration, and volts/cm are used. The best resolution in this size range is obtained using 12% polyacrylamide gels. When using these gels, the xylene cyanol FF dye is run between 13 and 14 cm from the origin and the chain length of the duplex can be read off the graph of Figure 2 by its mobility relative to the dye. When the smaller duplexes (Figure 1) were examined on gels containing EDTA (TBE), some of the radioactivity migrated with the single strands derived from these duplexes. We did not observe this phenomenon with duplex molecules larger than 30 base pairs or when magnesium (5 m*M*) was included in the buffer.

Electrophoretic Mobility of 80–400 Base Pairs Duplex DNA. DNA molecules in this size range were resolved on polyacrylamide gels containing 3.5–5% acrylamide. In Figure 3 the mobilities of ϕX174 DNA fragments produced by digesting closed circular RFI DNA with Hin restriction endonuclease are compared to the mobilities of various synthetic DNAs. Using the synthetic DNAs as standards, values of 80, 150, and 195 base pairs for the smallest ϕX174 Hin fragments are deduced by extrapolation. These chain length estimates are in good agreement with those derived from the relative amounts of radioactivity in ϕX174 Hin fragments (see Table I) demonstrating the linear relationship between mobility and log of molecular weight for 20–200 base pairs molecules on 5% TBM gels. A similar relationship was observed using 5% TBE gels, except that the absolute mobility of DNA is greater (see legend to Figure 3). For DNA of 150–400 base pairs, we compared the mobilities of ϕX174 and SV40 Hin fragments on 3.5% polyacrylamide TBE gels (Figure 4). The chain lengths of these fragments span the size range between the largest synthetic DNA molecule (77 base pairs) and the smallest SV40 Hin fragment (400 base pairs) whose chain length was determined by electron microscopy (Danna and Nathans, 1971). As previously shown by Danna and Nathans (1971), the electrophoretic mobilities of the SV40 Hin fragments are linearly related to the log of their chain length (Figure 5). The chain lengths of the ϕX174 Hin fragments deduced from Figure 5 are in good agreement with those determined

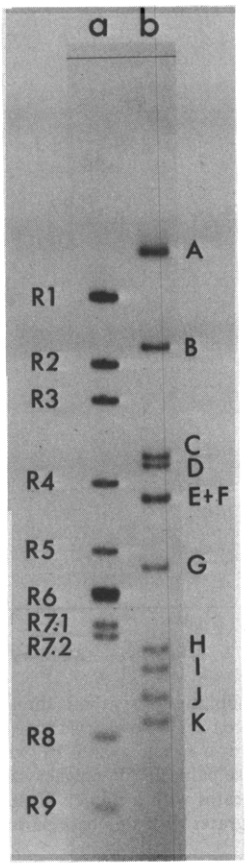

FIGURE 4: Autoradiogram showing the relative mobilities of $\phi X174$ and SV40 Hin fragments on a 3.5% polyacrylamide TBE gel. (a) $\phi X174$ Hin fragments. The numbering is the same as that used by Edgell et al. (1972). Fragment R10 is not shown. (b) SV40 Hin fragments. The letter assignments are the same as those used by Danna and Nathans (1971). The dimensions of the gel were 40 cm × 20 cm × 0.3 cm.

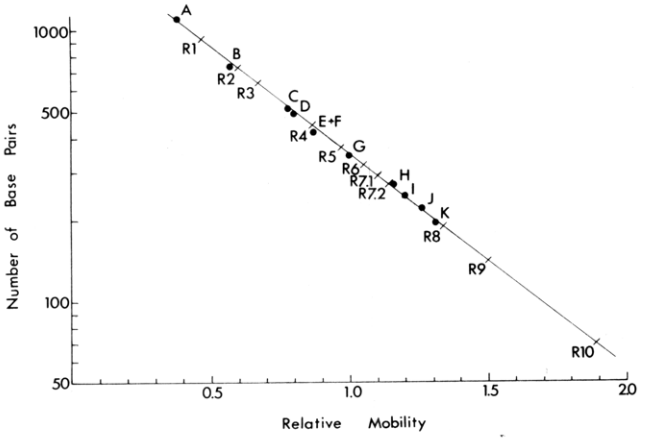

FIGURE 5: Plot of electrophoretic mobility vs. log of chain length for the DNA fragments of Figure 4. The positions of SV40 and $\phi X174$ Hin fragments are represented by solid circles and lines, respectively. The mobility plotted on the abscissa is relative to that of the SV40 Hin G fragment.

by comparison to the mobilities of synthetic DNAs (Figure 3), and with those estimated from relative amounts of radioactivity (Table I). Thus, the linear relationship between electrophoretic mobility and log of molecular weight is valid for duplex DNA molecules of 20–1000 base pairs.

Electrophoretic Mobility of 400–1000 Base Pairs Duplex DNA. We determined the chain lengths of bacterio-

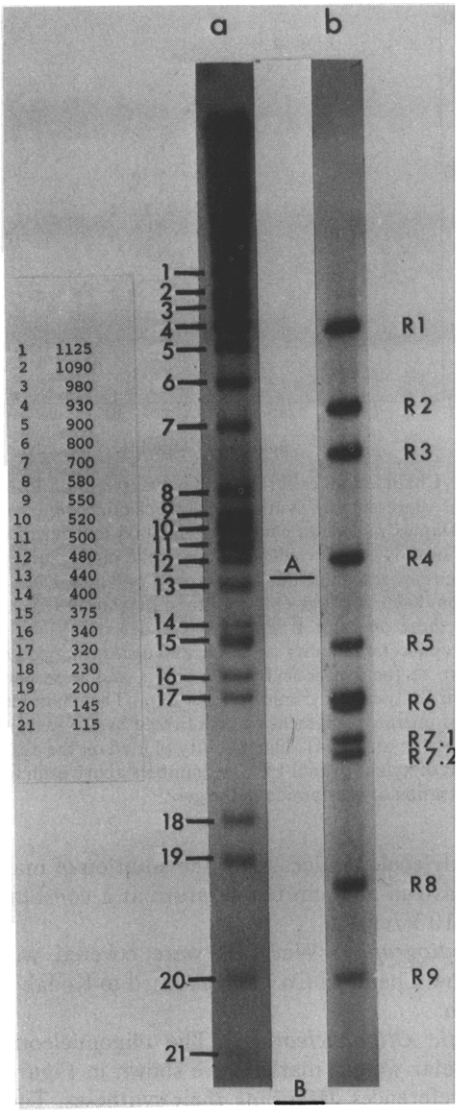

FIGURE 6: Autoradiogram showing the relative mobilities of $\phi X174$ and λ Hin fragments on a 3.5% polyacrylamide TBE gel. (a) λ DNA; (b) $\phi X174$ DNA. The chain lengths of the λ DNA fragments determined from the relative mobilities are indicated on the figure. Horizontal lines indicate the positions of the two dye markers xylene–cyanol FF (A) and Bromophenol Blue (B).

phage λ DNA Hin restriction fragments of 100–1000 base pairs by comparing their mobilities to $\phi X174$ Hin fragments on a 3.5% polyacrylamide gel. As seen in Figure 6, digestion of λ DNA with Hin produces a number of well-resolved DNA fragments in this size range. The chain length of the fragment labeled 1 in Figure 6 as determined by electron microscopy is 1100 ± 50 base pairs (R. Schleif, personal communication), in excellent agreement with that deduced from Figure 6. The linear relationship between electrophoretic mobility and log of molecular weight observed in Figures 4 and 5 was also demonstrated using the λ Hin fragments. ^{32}P-labeled λ DNA fragments with chain lengths shown in Figure 6 were recovered from gels and digested with either Hpa II or Hae III, and the products were examined on a 3.5% polyacrylamide gel. The data presented in Table II show that in every case the sum of the chain lengths of the Hpa II and Hae III digestion products is very close to that of the undigested Hin fragments. Similar observations have been reported by others (Lebowitz et al., 1974).

Table II: Additivity of Chain Lengths.[a]

Fragment No.	Chain Length	Restriction Enzyme	Chain Length of Digestion Products	% of Total Chain Length
1	1125	Hpa II	600	107
			430	
			180	
1	1125	Hae III	330	
			205	
			200	104
			195	
			135	
			120	
5	900	Hae III	740	
			170	101
6	800	Hae III	460	
			180	99
			155	
9	550	Hae III	400	
			125	95
13	440	Hae III	295	91
			105	
17	320	Hpa II	175	100
			145	

[a] ^{32}P-labeled λ DNA fragments produced by digestion with Hind restriction endonuclease were fractionated on a 3.5% polyacrylamide TBE gel and recovered from the gel as previously described (Maniatis et al., 1973). The fragments were then digested with either Hpa II or Hae III and examined on a 3.5% polyacrylamide TBE gel along with the undigested Hind fragments. The chain lengths of the digestion products were determined from their mobilities relative to the undigested fragments. The fragment numbers are those shown in Figure 6.

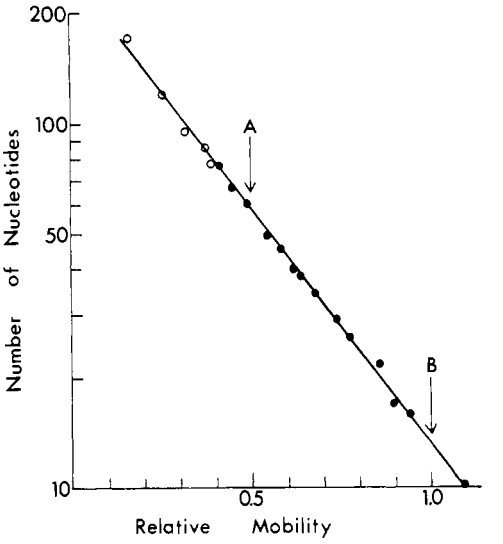

FIGURE 7: Chain length calibration of 12% polyacrylamide TBE gel containing 7 *M* urea. The closed circles represent the synthetic DNA markers shown in Figure 1. The open circles represent RNA molecules of known chain length. RNA markers (from smallest to largest) are glycine tRNA, mixed tRNA, and serine tRNA from *Staphylococcus epidermidis* provided by R. Roberts; 5S and 7S RNA purified from 60S rRNA subunits from *A. polyphenus* by R. Gelinas. The chain lengths of these molecules are 75, 85, 89, 120, and 168 nucleotides, respectively. The plotted mobility is relative to that of Bromophenol Blue. The position of the Bromophenol Blue (A) and xylene cyanol FF (B) dyes which coelectrophorese with oligonucleotides 13 and 58 long, respectively, are indicated.

If λ Hin fragments cannot be prepared, chain length estimates can be made by examining the mobility of DNA relative to the two tracking dyes, Bromophenol Blue and xylene cyanol FF. These two dyes consistently coelectrophorese with duplex DNA of 100 and 450 base pairs, respectively, in 3.5% polyacrylamide TBE gels.

Electrophoretic Mobility of Denatured DNA on Urea and Formamide Gels. The mobilities of denatured DNA and RNA molecules of 10–150 nucleotides are a linear function of the log of molecular weight on 12% polyacrylamide TBE gels containing 7 *M* urea (Figure 7). The deoxy- and ribodecanucleotides which also differ in base composition (Figure 1) have indistinguishable mobilities. The mobilities of single-stranded DNAs in denaturing gels are consistent with the mobilities of double-stranded DNAs in nondenaturing gels. This consistency has been observed for a number of DNA molecules with base composition varying from 35 to 53% A-T. The chain lengths of a number of RNA and DNA molecules have been determined by their mobility relative to the two dyes and subsequently verified to within 1–5 nucleotides by sequence analysis (Maniatis et al., 1974, 1975; Gilbert and Maxam, 1973; Pribnow, 1975).

We have also analyzed molecules in this size range using 12% polyacrylamide gels containing 98% formamide and either barbital (Maniatis and Ptashne, 1973a; Pinder et al., 1974b) or phosphate (Maniatis et al., 1973) buffer. Zone disturbances which lead to inaccurate and irreproducible chain length estimates have been observed in formamide gels containing barbital buffer (T. Maniatis, unpublished; Pinder et al., 1974b). These disturbances are characterized by a "funneling" effect in which the DNA appears as a band near the top of the gel and as a spot near the dye front. In addition, DNA tends to pile up at the dye front so that fragments which are ordinarily well separated appear as a single band or spot. We have not observed this phenomenon with formamide gels containing phosphate buffer. A linear relationship between the mobility of single-stranded DNAs of 10–100 nucleotides was observed in 12% polyacrylamide–98% formamide gels (not shown), but 7 *M* urea gels are preferred because they afford slightly better resolution and are easier to prepare.

We found that complete denaturation of longer DNA molecules (>200 nucleotides) could not be consistently maintained in polyacrylamide gels containing 7 *M* urea. However, complete denaturation and high resolution can be achieved using gels containing 98% formamide. An autoradiogram of $\phi\chi$174 Hin fragments fractionated on a 5% polyacrylamide–98% formamide gel is shown in Figure 8b. The relative mobilities of most of the denatured fragments are similar to those observed for the corresponding duplex molecules on nondenaturing gels. However, as shown in Figure 8a, the mobilities of two fragments (R3 and R4) deviate significantly from the curve derived from chain lengths determined on nondenaturing gels. R3, which appears to be 640 base pairs long on nondenaturing gels, appears to be 600 nucleotides long on denaturing gels. Our estimate from relative amounts of radioactivity is 653 base pairs. The corresponding values for R4 are 440 base pairs, 470 nucleotides, and 470 base pairs. We have not observed significant differences in mobilities on nondenaturing and denaturing gels with SV40 or phage λ Hin fragments. One λ DNA fragment, generated by the combined activities of Hin and Hpa II, appears to be 150 base pairs long on nondenaturing gels and 165 nucleotides on formamide gels. The actual chain length of this molecule from sequence analysis is 164 base pairs.

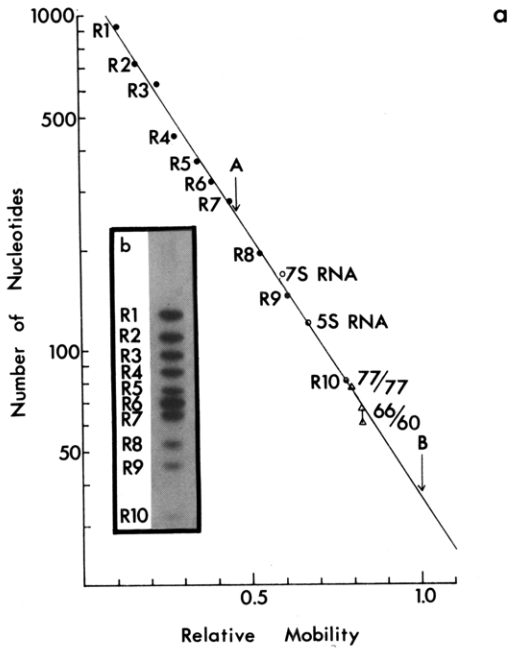

FIGURE 8: (a) Relative mobilities of denatured DNA fragments on a 5% polyacrylamide gel containing 98% formamide. The triangles represent synthetic duplex markers, the open circles RNA markers (see legend to Figure 7) and the closed circles $\phi\chi174$ Hin fragments. The mobility is plotted relative to Bromophenol Blue. A, xylene cyanol FF, B, Bromophenol Blue. (DNA 255–260 nucleotides long migrates with the xylene cyanol FF dye.) (b) Autoradiogram showing the resolution of ^{32}P-labeled $\phi\chi174$ Hin fragments on the gel of Figure 8a.

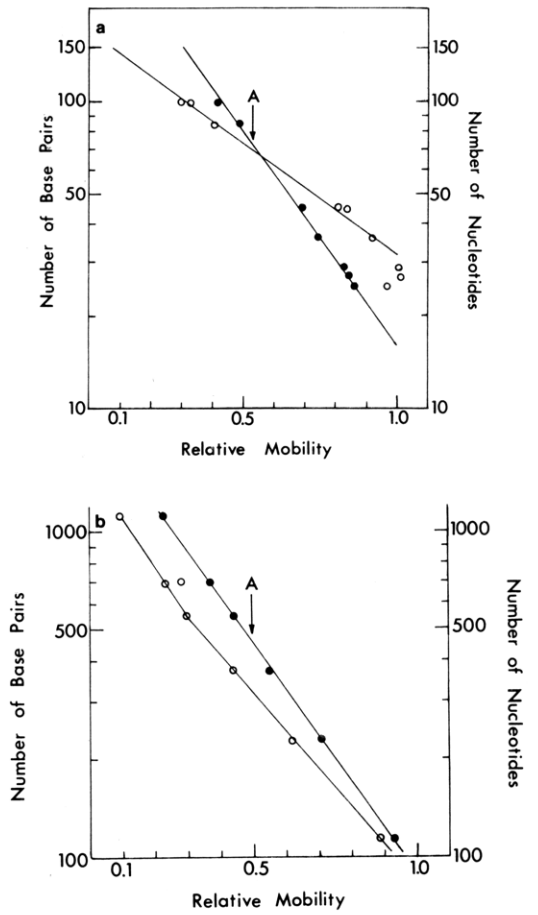

FIGURE 9: Relative mobilities of native and denatured duplex DNA in polyacrylamide TBE gels. The mobility is plotted relative to that of Bromophenol Blue. (a) 30–100 base pairs molecules electrophoresed on a 12% polyacrylamide gel. Native and denatured DNA are represented by closed and open circles, respectively. The chain lengths of the 29 and 45 base pairs molecules are known from sequence analysis (Maniatis et al., 1975). The other DNA molecules were obtained by digesting λ DNA with three different restriction enzymes and their chain lengths estimated as described in Figure 2 and in the text. (b) 100–1000 base pairs molecules electrophoresed on a 3.5% polyacrylamide gel. Native and denatured DNA are indicated as in Figure 9a. The DNA molecules are Hin fragments (see Figure 6). In both (a) and (b) DNA was denatured by boiling in formamide and quickly cooled. The letter A indicates the mobility of the xylene cyanol FF tracking dye.

DNA and RNA molecules of the same molecular weight exhibit different relative mobilities on formamide gels. For example, one RNA molecule of 168 nucleotides consistently appears to be 155 nucleotides relative to DNA markers on formamide gels. A similar observation has been made by Noll (1974) and Efstradiadis et al. (1975). Even larger differences between the mobilities of RNA and DNA molecules were observed when barbital buffer was used in the formamide gels instead of sodium phosphate (not shown). We therefore conclude that RNA and DNA markers cannot be used interchangeably on formamide gels.

Comparison of Mobilities of Native and Denatured DNA in Aqueous Polyacrylamide Gels. We compared the electrophoretic mobilities of native and denatured DNA of known molecular weights on aqueous gels. Figure 9a shows the relationship between mobility and chain length for native and single-stranded DNA in the size range of 20–100 base pairs (nucleotides). The curves relating mobility to log of chain lengths are linear, they have different slopes, and they intersect at 68–70 base pairs (nucleotides). Thus, for molecules smaller than 68 base pairs, single strands move faster than the duplex molecules from which they were derived, whereas the opposite is true for molecules larger than 68 base pairs. A similar analysis of fragments in the 100–1000 base pairs size range is shown in Figure 9b. The curves for native and denatured DNAs have different slopes but in every case the denatured fragments have a mobility less than that of duplex DNA.

Discussion

The experiments presented here show that the electrophoretic mobilities of double- and single-stranded DNA molecules of 20–1000 base pairs (nucleotides) are a linear function of the log of their chain lengths in polyacrylamide gels if the appropriate conditions are used. Chain lengths of double- and single-stranded DNA molecules 20–100 base pairs long can be determined to within 5–10 nucleotides by comparing their electrophoretic mobilities with those of tracking dyes on 12% polyacrylamide gels. Single-stranded DNA and RNA molecules of the same chain length migrate identically on 7 *M* urea–polyacrylamide gels.

Double-stranded DNAs with chain lengths of 100–1000 base pairs are well resolved on 3.5% polyacrylamide gels. An examination of the relative electrophoretic mobilities of DNA molecules with chain lengths known from nucleotide sequence analysis and experiments such as those presented in Table II indicate that chain length estimates for 100–400 base pairs DNA molecules are accurate to within 5%. The accuracy of chain length estimates for molecules of 400–1000 base pairs is limited by the accuracy of Danna and Nathans (1971) contour length measurements of the SV40 Hin fragments we used as mobility standards (±50 base pairs).

Chain length estimates for single-stranded DNA 100–

1000 nucleotides long are obtained using 5% polyacrylamide gels containing 98% formamide and are accurate to within 5–10%. Using the conditions described here, formamide gels provide a reproducible method for the fractionation and molecular weight analysis of single-stranded DNA. This method has been used to characterize reverse transcripts of various messenger RNAs (Efstradiadis et al., 1975) and to detect single-stranded nicks in duplex DNA (Maniatis and Ptashne, 1973a; Noll, 1974). Three out of the many duplex DNA molecules we have studied have different mobilities relative to molecular weight standards when examined on nondenaturing and denaturing gels (see Figure 8 and text). We do not understand these unusual cases. DNA is completely denatured in 98% formamide at room temperature, and Pinder et al. (1974a,b) have argued that the electrophoretic mobility of nucleic acids in formamide gels is independent of base composition and secondary structure. We presume, therefore, that chain length estimates derived from denaturing gels are more accurate than those derived from nondenaturing gels. In most cases this presumption could not be tested because the differences in chain lengths derived from native and denaturing gels are so small. In the one case where the chain length of a fragment is known from its sequence (a 164 base pairs bacteriophage λ DNA fragment), and different chain lengths are derived by analysis on native and denaturing gels, the value most consistent with sequence data was derived from denaturing gels.

The electrophoretic mobility of double-stranded DNA may be influenced by base composition or DNA sequence. Mertz and Berg (1974) found that the molecular weights of two double-stranded Hin fragments isolated from a mutant SV40 strain, determined from relative amounts of radioactivity, are not in good agreement with molecular weights derived from their mobilities relative to standards on polyacrylamide gels. Although they note the base composition of these fragments are among the highest and lowest in G–C among the 11 SV40 Hin fragments, we note that other fragments which deviate as much or more from the average base composition (see Danna and Nathans, 1971) behave as expected on polyacrylamide gels. Base composition has been shown to influence the electrophoretic mobility of high molecular weight duplex DNA (>12,000 base pairs) (Zeiger et al., 1972), but similar studies on low molecular weight DNA (20–1000 base pairs) have not been reported. It has been shown, however, that double-stranded DNA larger than 1500 base pairs migrates through gels differently than lower molecular weight DNA (Fisher and Dingman, 1971; Dingman et al., 1972a,b; Flint and Harrington, 1972) (DNA >1500 base pairs is thought to migrate through polyacrylamide gels "end on") so base composition may not have the same effect on electrophoretic mobility of the two size classes of DNA. Although it is possible that unusual nucleotide sequences might preferentially interact with the gel (Flint and Harrington, 1972) or might result in altered secondary structures which would influence electrophoretic mobilities of duplex DNA, the aberrant behavior of some of the double-stranded DNA molecules on aqueous polyacrylamide gels described here and those described by Mertz and Berg (1974) cannot be explained without further studies.

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Fluorescence Detected Circular Dichroism Study of the Anticodon Loop of Yeast $tRNA^{Phe}$ †

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ABSTRACT: Fluorescence detected circular dichroism (FDCD) measurements have been used to study the conformations of the anticodon loop of yeast phenylalanine tRNA. To our knowledge this is the first application of fluorescence detected circular dichroism. Much smaller amounts of tRNA are needed for the measurement of FDCD than for the conventionally measured circular dichroism. Furthermore, FDCD is specific for conformational changes near the anticodon loop. The FDCD measurements suggest a transition in the anticodon loop near 20° in 0.01 *M* $MgCl_2$–0.1 *M* NaCl (pH 7). This is followed by a broad transition from 30 to 60° and finally a sharp melting at 75° consistent with the absorbance detected melting of the entire tRNA. Removal of Mg^{2+} from the tRNA at 1° causes nearly a factor of two decrease in the FDCD near 230 nm. This indicates a decrease in conformational rigidity in the anticodon loop on removal of Mg^{2+}.

Many biological processes depend upon very localized structural features or conformational changes, and there is, therefore, a great demand for probes of local conformation. We recently reported a new technique, fluorescence detected circular dichroism (FDCD), which combines the conformational sensitivity of circular dichroism (CD) with the specificity of fluorescence (Turner et al., 1974). This technique is thus capable of providing unique structural information for proteins and nucleic acids. In this paper, the method is used to study the conformations of the anticodon loop of yeast $tRNA^{Phe}$.

The anticodon loop of tRNA is responsible for reading the genetic code during protein synthesis. This important function has generated great interest in its conformation. The structure of $tRNA^{Phe}$ in crystals (Kim et al., 1974; Robertus et al., 1974) provides a starting point for the interpretation of solution studies. It is of course important to determine the conformations in solution and to understand how they depend on the environment.

Several studies have taken advantage of the fact that in yeast $tRNA^{Phe}$ the base immediately adjacent to the 3′ end of the anticocon, called Y base, is moderately fluorescent (RajBhandary and Chang, 1968). Thus the fluorescence intensity has been used to monitor conformational changes of the loop (Beardsley et al., 1970; Eisinger et al., 1970; Robison and Zimmerman, 1971). The fluorescence properties of fragments derived from the loop have also been studied (Maelicke et al., 1973). Phosphorescence of the bases in the loop at low temperature has proved to be another useful probe (Hoover et al., 1974). Another technique which has provided important information is binding of oligonucleotides (Eisinger et al., 1970; Uhlenbeck et al., 1970, Högenauer, 1970; Uhlenbeck, 1972; Pongs et al., 1973; Eisinger and Spahr, 1973; Eisinger, 1971; Eisinger and Gross, 1974.)

Materials and Methods

Materials. Yeast $tRNA^{Phe}$ was purchased from Boehringer-Mannheim. According to their assay, it has an accep-

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