Properties of the Deoxyribonucleic Acid of the Thermophilic Bacteriophage TP-84*

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ABSTRACT: The physical and chemical properties of the nucleic acid of the thermophilic bacteriophage TP-84, specific for certain strains of *Bacillus stearothermophilus*, have been investigated. Phenol-extracted TP-84 DNA has an $s^{50}_{20,w}$ of 30.0 and a base composition of 42% guanine + cytosine, ρ = 1.705 g/cc. The molecular weight of the DNA determined from sedimentation and intrinsic viscosity measurements is 22,600,000 and 22,400,000, respectively. The length of the TP-84 DNA molecules is 13.9 μ, corresponding to a molecular weight of 27,000,000 in the B crystallographic form. Denaturation of TP-84 DNA by heat or alkali results in the formation of two new bands in a CsCl density gradient: ρ_L = 1.717 and ρ_H = 1.729 g/cc. Isolation of the L and H strands is accomplished by stepwise elution chromatography on a methylated albumin kieselguhr column. The isolated strands give melting profiles characteristic of single-stranded polynucleotides, are not self-renaturable, and form only one band at ρ = 1.705 g/cc after mixing in equimolar quantities and renaturing at 45° for 2 hr. The H and L strands differ in their ultraviolet absorption properties, sedimentation coefficients, and nucleotide compositions. The data suggest that native TP-84 DNA exists as a duplex with a limited number of noncomplementary strand interruptions in the individual polynucleotide chains.

In a previous paper (Saunders and Campbell, 1965b) we have presented data on the biological and physicochemical properties of the thermophilic bacteriophage TP-84. Briefly, this phage (1) is specific for only certain strains of *Bacillus stearothermophilus*, (2) is stable in broth at 65° for 12 hr, (3) has an hexagonal head 53 mμ in diameter and a tail 130 mμ long, (4) has a particle molecular weight of 50,000,000 daltons, and (5) has a DNA base composition of 42% guanine + cytosine.

Marmur and co-workers (Marmur and Cordes, 1963; Marmur and Greenspan, 1963; Marmur *et al.*, 1963) have studied the DNA of several phages which lyse certain *Bacillus* species. They found that the DNA of some of the phages exhibited a single band when centrifuged to equilibrium in a CsCl density gradient but after denaturation a bimodal distribution was obtained. The DNA strands of two of the phages (phage α for *B. megaterium* and phage SP-8 for *B. subtilis*) were separated and the nucleotide composition of the complementary strands determined. This paper describes the properties of phage TP-84 DNA and the separation and physicochemical properties of the DNA strands.

Materials and Methods

Phage Preparation. TP-84 phage was prepared as described by Saunders and Campbell (1965b). In order to increase the quantity of phage that can be purified during one centrifugation in a CsCl density gradient fixed angle rotors were used (Fisher *et al.*, 1964). The CsCl-phage preparation was centrifuged for 24 hr at 26,000 rpm in a Model L ultracentrifuge with a No. 30 rotor. The phage forms a sharp white band and is collected by puncturing the tubes and dripping out the contents.

Electron Microscopy. TP-84 DNA was prepared for electron microscopy by extracting a phage suspension of 2×10^{12} plaque-forming units/ml (pfu/ml)[1] in 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0 (SSC, standard saline citrate), with an equal volume of 0.01 M KH_2PO_4-(pH 6.7) buffered phenol (HMP-phenol). The mixture was extracted in a Kontes chromatography column (9 mm in diameter × 150 mm high without a fritted glass disk) by gently allowing the liquid to run the length of the column nine times. After shaking, the phenol phase was removed. The DNA solution was drained into a dialysis bag and dialyzed against 4000 volumes of double strength (2×) SSC. To examine the

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[1] Abbreviations used in this work: pfu, plaque-forming units; HMP, phosphate-buffered phenol; SSC, standard saline citrate; MAK, methylated albumin kieselguhr.

phage DNA grids were prepared by the method of Lang *et al.* (1964) as modified by Chandler *et al.* (1964). The preparations were shadowed with uranium oxide and examined at a magnification of 10,000×.

The electron micrographs were enlarged and the lengths of the DNA molecules were determined with the aid of a cartographer's map-measuring device. To determine the molecular weight of the DNA it was assumed that the molecules were maximally hydrated and therefore equivalent to the B crystallographic form (Langridge *et al.*, 1960). In this form, according to MacHattie and Thomas (1964), the sodium salt of the molecule should have a linear density of 192 daltons/angstrom.

DNA Isolation. Phage DNA was prepared by extracting a purified phage preparation with HMP-phenol (Grossman *et al.*, 1961). An equal volume of HMP-phenol was added to the phage suspension in saline–EDTA (0.15 M NaCl–0.1 M ethylenediaminetetraacetate, pH 8) and shaken for 30 min at 4°. The aqueous phase was extracted three times with diethyl ether to remove traces of phenol and the DNA was precipitated with two volumes of 95% ethanol. The DNA fibers were dissolved in 0.1 SSC, precipitated with redistilled 2-propanol, and dissolved again in 0.1 SSC. The purified DNA was dialyzed overnight at 4° against SSC. The DNA preparations were adjusted to a concentration of 200–800 μg/ml.

CsCl Equilibrium Centrifugation. CsCl (7.7 M) solutions (0.6–0.65 ml) containing the DNA sample and a density marker (^{15}N-*Pseudomonas aeruginosa* DNA, buoyant density, ρ = 1.742 g/cc) were centrifuged 18–24 hr at 44,770 rpm at 24° in a Spinco Model E analytical ultracentrifuge. The centrifuge cell was a standard cell fitted with a 1° negative wedge window. At various time intervals photographs were taken using the standard Spinco ultraviolet camera. The resulting photographs were traced with a Joyce-Loebl double-beam recording microdensitometer. Buoyant densities and base compositions were calculated using the equations of Sueoka (1961) and were based on ρ = 1.713 g/cc for the DNA of *Escherichia coli*.

Melting Temperature. Thermal denaturation of DNA was followed in an Optica 3 spectrophotometer equipped with a heated cell compartment and a temperature readout assembly. Base compositions were calculated from the T_m using the equation of Marmur and Doty (1962): % guanine + cytosine = $(T_m - 69.3) \times 2.439$.

Enzymatic Hydrolysis and DNA Base Composition. Purified DNA was dialyzed into TM buffer (1×10^{-3} M MgCl and 1×10^{-2} M Tris-HCl, pH 7.3) for hydrolysis. Degradation was accomplished by adding to 1.6 ml of ^{32}P-labeled phage DNA 0.25 ml (400 μg) of highly polymerized salmon sperm DNA (Mann Research Laboratories, Inc.) in TM buffer and 0.25 ml of DNAse I (3.2 mg/ml in TM buffer), and incubating at 37° for 1 hr. The carrier salmon sperm DNA of known base composition was added to obtain sufficient absorbancy units for the identification of the nucleotide regions and to provide controls for losses of individual nucleotides. The amount of each nucleotide was calculated by using known extinction coefficients. Complete hydrolysis was obtained by adding 0.06 ml of 1 M magnesium acetate, 0.1 ml of Tris–acetate, pH 9.1, and 0.1 ml of venom phosphodiesterase (5 mg of crude Worthington enzyme/ml in demineralized H_2O). The mixture was then incubated at 37° for 4 hr.

The mononucleotides were separated on a Dowex 1-X8 (200–400 mesh) formate column according to the method of Canellakis and Mantsavinos (1958). The sample was charged onto a column (5 ml pipet) with a bed volume of approximately 4.5 ml. The eluate was collected in 5-ml fractions and the absorbancy at 260 mμ was determined for each fraction. The radioactivity of each fraction was determined by liquid scintillation counting.

Sedimentation Velocity. Sedimentation velocity studies were performed in a Spinco Model E analytical ultracentrifuge at 20°. The sample was always equilibrated against the solvent (SSC) by dialysis prior to ultracentrifugation. All runs were made in a 12-mm standard cell fitted with a Kel F centerpiece. The phage DNA sedimentation was followed by ultraviolet absorption optics. The resulting photographs were traced as described above.

The molecular weight of TP-84 DNA was calculated using the equation of Burgi (1963) and Burgi and Hershey (1963).

$$s_{20,w} = 0.080M^{0.35}$$

Viscosity. Viscometric measurements were made in a Ubbelohde multigradient capillary viscometer immersed in a water bath regulated at 20°. Flow times for each sample were measured at three gradients for each of three dilutions of DNA in SSC between 10 and 35 μg/ml. To determine the specific viscosity an extrapolation to zero shear was made for each concentration. The intrinsic viscosity $[\eta]$ of the DNA sample was found by extrapolating the reduced specific viscosity to infinite dilution.

The empirical equation of Doty *et al.* (1958) relating intrinsic viscosity to molecular weight for a series of sonic-treated samples of calf thymus DNA in 0.2 M NaCl is

$$[\eta] = 1.45 \times 10^{-6}M^{1.12}$$

DNA Fractionation on Methylated Albumin Kieselguhr (MAK) Columns. One layer MAK columns were prepared as described by Sueoka and Cheng (1961). The column used for separation of TP-84 DNA strands was a 25-mm inside diameter column where 25 ml of the MAK suspension was poured directly onto the fritted glass disk (the filter paper powder layer was omitted). The column was washed exhaustively with 200 ml of 0.1 M NaCl–0.05 M phosphate at pH 6.7, 100 ml of 0.85 M NaCl–0.05 M phosphate, 50 to 80 ml of 1.5 M NaCl–0.05 M phosphate, and then again with 150 ml of 0.85 M NaCl–0.05 M phosphate.

The phage DNA was denatured by raising the pH to

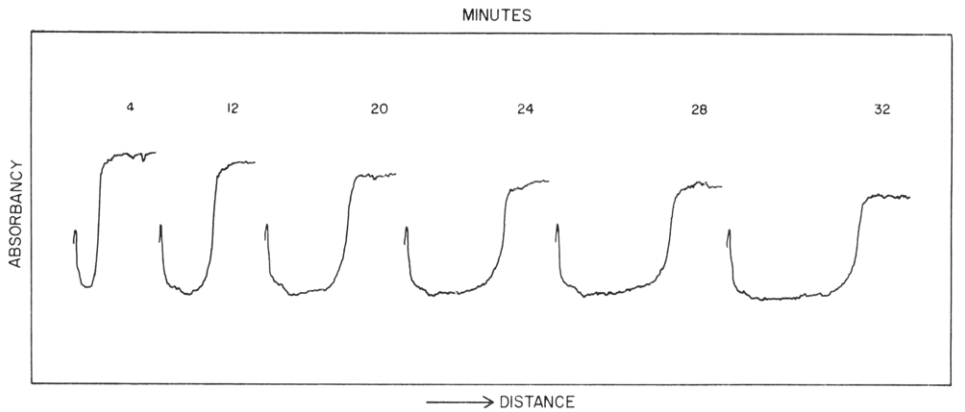

FIGURE 1: Sedimentation pattern of TP-84 DNA. Conditions of centrifugation: DNA concentration = 20 μg/ml; rotor speed = 36,588 rpm; solvent = SSC buffer; exposure time = 17 sec.

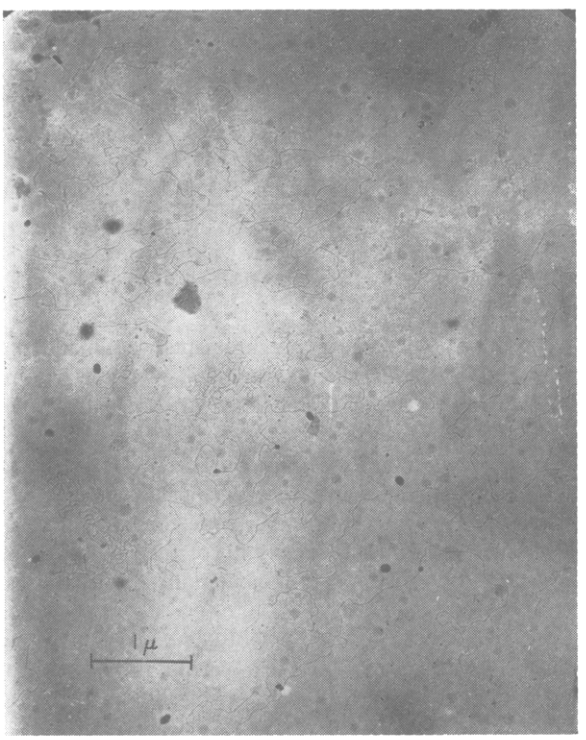

FIGURE 2: Electron micrographs of TP-84 DNA molecules. The method of Kleinschmidt, as modified by Chandler *et al.* (1964), was used for electron-microscope preparations.

12.0 with 0.1 M KOH and then diluting in 0.85 M NaCl–0.05 M phosphate to a DNA concentration of 20 μg/ml. After adjusting the DNA solution to pH 6.7 the sample was charged onto the MAK column. One fraction of DNA came through the column with the filtrate, and the column was washed with the charging buffer solution until the absorbancy at 260 mμ dropped to 0.010. Elution of a second ultraviolet absorbing fraction was effected by raising the salt concentration to 1.5 M NaCl–0.05 M phosphate. The capacity of the column is about 800 μg of denatured TP-84 DNA. With higher amounts of DNA an effective separation was not obtained. The isolated DNA fractions were concentrated by precipitation with three volumes of ethanol, suspended, and dialyzed against the desired buffer.

Results

Native TP-84 DNA. Phenol-extracted TP-84 DNA has a sedimentation coefficient $s^{50}_{20,w} = 30.0$ in SSC. Using the relationship of Burgi and Hershey (1963), $s = 0.080M^{0.35}$, this corresponds to a molecular weight of 22,600,000. The sedimentation profile (Figure 1) of dilute solutions of TP-84 DNA suggests a remarkable homogeneity in size of the DNA molecules.

The DNA molecular weight calculated from the intrinsic viscosity is the same as that obtained from sedimentation velocity measurements. The relative viscosity of mean shear gradients from 80 sec^{-1} to 1300 sec^{-1} was measured with efflux times for water between 400 and 500 sec. The intrinsic viscosity found for TP-84 DNA was 248 dl/g. From the equation of Doty *et al.* (1958) this corresponds to a molecular weight of 22,400,000.

Electron micrographs of TP-84 DNA molecules prepared by gentle phenol extraction detailed in the methods section are shown in Figure 2. No strand interruptions were observed in the molecules photographed. The absence of small puddles or pools indicates that the molecule is a rigid polymer composed of presumably two strands and contains no extensive single strand regions. Four complete chains were found. The lengths of these molecules were measured on the enlarged photographs and corrected for the total magnification. The calculated molecular lengths were 14.2, 14.0, 13.8, and 10.4 μ. Multiplying the measured lengths by the linear density of the molecule (192 daltons/angstrom) the molecular weights were calculated to be 27,300,000, 26,900,000, 26,500,000, and 20,000,000. It is probable that a piece of the DNA

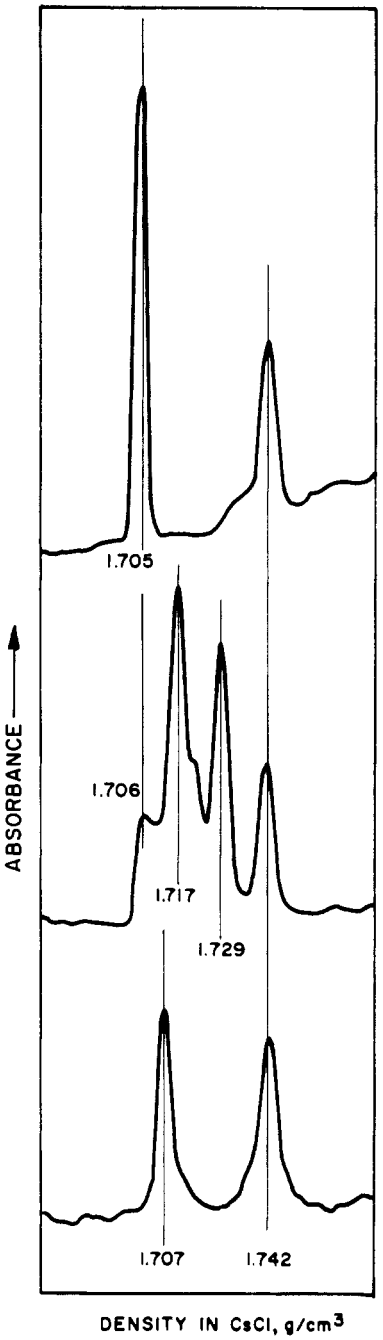

FIGURE 3: Banding of native, heat-denatured, and renatured TP-84 DNA at equilibrium in a CsCl density gradient. Top curve: native TP-84 DNA and reference ^{15}N-*P. aeruginosa* DNA (ρ = 1.742 g/cc). Middle curve: heat-denatured TP-84 DNA and reference DNA. Lower curve: renatured band; DNA obtained by incubating heat-denatured TP-84 DNA at 20 μg/ml in SSC at 45° for 2 hr. Centrifugation conditions: rotor speed, 44,770 rpm in AnD rotor; temperature, 25°; time, 22 hr.

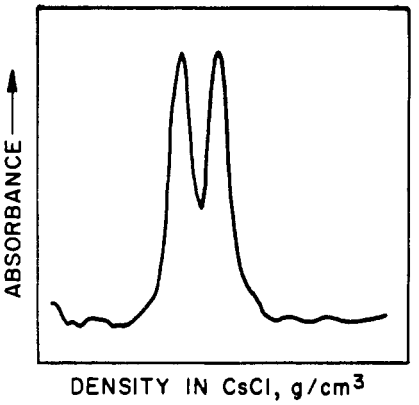

FIGURE 4: Bands of denatured TP-84 DNA in an alkaline CsCl density gradient, pH 12.0. Rotor speed, 44,770 rpm for 24 hr; input CsCl–DNA solution, ρ = 1.765 g/cc; temperature, 25°.

molecule was sheared off giving rise to the 20,000,000 piece. The other three molecules are approximately the same size, so it may be concluded from the limited sample that by electron microscopy the molecular weight of TP-84 DNA is about 27,000,000 daltons. Although this value is in good agreement with those obtained by sedimentation and intrinsic viscosity measurements it should only be regarded as tentative. A detailed examination of TP-84 DNA by electron microscopy is now in progress in collaboration with A. K. Kleinschmidt.

Figure 3 (top curve) shows the characteristic sharp band of TP-84 DNA when centrifuged to equilibrium in a CsCl density gradient. The sharpness of the DNA band suggests that the preparation is markedly homogeneous with respect to molecular weight and buoyant density. The narrow DNA band also suggests that the DNA is of high molecular weight since the band width at equilibrium is inversely proportional to the molecular weight (Meselson *et al.*, 1957). The difference in buoyant density of TP-84 DNA (ρ = 1.705 g/cc) and the host *Bacillus stearothermophilus* DNA (ρ = 1.713 g/cc) facilitates the detection of host DNA in TP-84 DNA preparations and the converse (Saunders and Campbell, 1965b).

Denatured TP-84 DNA. The thermal denaturation of TP-84 DNA results in the presence of two new bands in a CsCl density gradient (Figure 3, middle curve) at mean buoyant densities of ρ_L = 1.717 and ρ_H = 1.729 g/cc; these bands will be referred to as the L strand and H strand, respectively. Heat denaturation of the DNA was accomplished by holding the DNA solution (20 μg/ml in 0.1 SSC) in a boiling water bath for 12 min and then quickly immersing the flask in ice sprinkled with NaCl crystals. The appearance of two bands suggests that the DNA has a compositional bias in which one strand is relatively purine rich and the other relatively pyrimidine rich. The small band at ρ = 1.706 of the middle curve probably represents a fraction of the DNA

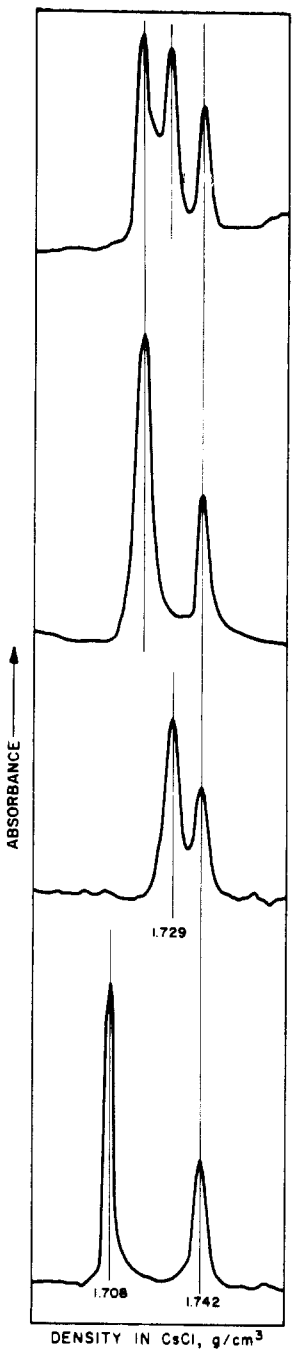

FIGURE 5: Banding patterns of alkali-denatured TP-84 DNA, isolated TP-84 DNA strands, and renatured TP-84 DNA. Top curve: alkali-denatured TP-84 DNA, dialyzed against SSC buffer to pH 7.0, and reference ^{15}N-*P. aeruginosa* DNA (ρ = 1.742 g/cc); second curve: purified L strand of TP-84 DNA, ρ = 1.717 g/cc; third curve: purified H strand of TP-84 DNA, ρ = 1.729 g/cc; bottom curve: renatured TP-84 DNA prepared by alkaline denaturation, dialysis to pH 7.0 against SSC, incubation at 45° for 2 hr. Conditions for ultracentrifugation were the same as in Figure 3.

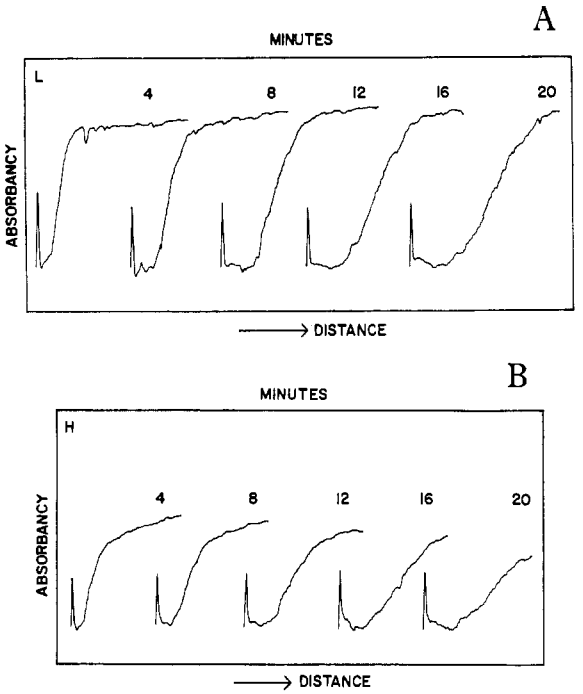

FIGURE 6: Sedimentation velocity profile of the isolated strands of TP-84 DNA. Conditions of centrifugation: DNA concentration, A, light strand, 29 μg/ml; B, heavy strand, 37 μg/ml; rotor speed, 42,040 rpm; solvent, 0.02 M Tris–HCl, pH 7.4. Photographed at 4-min intervals with an exposure time of 12 sec.

population whose strands either did not separate at all or were denatured such that renaturation was the favored state upon fast-cooling. The shoulder on the heavy side of the peak at ρ = 1.717 also indicates incomplete denaturation. The appearance of this shoulder can be interpreted as showing that the population of L-strand molecules is inhomogeneous with respect to size and affinity for some complementary regions on the H strand. A double-stranded region would result in a decrease in buoyant density and conceivably pull the band from the more dense position almost into the L-strand band. The fact that the two denatured DNA bands are unequal in area under the peaks lends further support to this view.

Incubation of the heat-denatured DNA at 45° in 2× SSC for 2 hr results in the appearance of only one band at approximately the same buoyant density as the native TP-84 DNA (Figure 3, lower curve). The renatured band is not as sharp as that of native material (Figure 3, upper curve). This suggests that more heterogeneity now exists in the preparation and the mean molecular weight has probably been reduced. High temperatures have been shown to cause some breakage of the DNA backbone and also some degree of depurination (Greer and Zamenhof, 1962).

In order to achieve complete denaturation without resorting to a more rigorous heating treatment a dif-

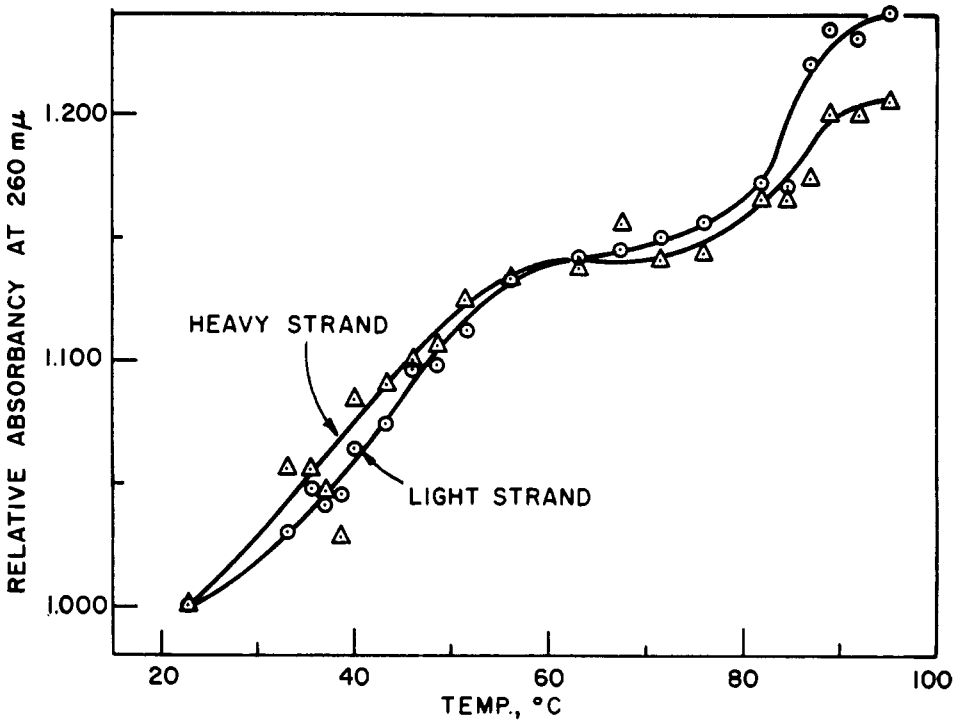

FIGURE 7: Melting curve of the isolated strands of TP-84 DNA in SSC buffer.

ferent method of denaturation was employed. Alkali has been shown to cause the rapid denaturation of DNA of both viral and bacterial origin (Geiduschek, 1962). Elevation of the pH of a TP-84 DNA solution to pH 12.0 and banding the DNA solution in an alkaline cesium chloride density gradient (pH 12.0, ρ midpoint = 1.760) give rise to two peaks of approximately equal areas (Figure 4). The buoyant density difference between the two bands was 0.012 g/cm^3, the same as that found under heat-denaturation conditions. The equivalence in peak size and the absence of a band at a lower buoyant density indicate that upon alkaline denaturation strand separation is virtually complete. It is also clear that a compositional bias will not express itself as a band size bias.

The Isolated Strands of TP-84 DNA. Having established that native TP-84 DNA consists of a duplex structure which upon denaturation dissociates reversibly into two identifiable DNA species, a study was undertaken to effect the physical separation of these molecular species.

TP-84 DNA, denatured by alkali treatment and diluted to 15 μg/ml in 0.85 M NaCl–0.05 M phosphate and the pH adjusted to 6.7, was fractionated on an MAK column. The L strand came through the column with the charging solution and the H strand was eluted with 1.5 M NaCl–0.05 M phosphate, pH 6.7. The fractions were identified by CsCl density gradient centrifugation. In Figure 5, the top curve shows the alkali-denatured TP-84 DNA, dialyzed exhaustively against SSC to bring the pH to 7.0. The second and third curves are traces of the isolated strands. The bottom curve depicts the renaturation of TP-84 DNA after exhaustive dialysis of the alkaline-denatured DNA against SSC followed by incubation at 45° for 2 hr. The curve on the right at a density of 1.742 g/cc is ^{15}N-labeled *Pseudomonas aeruginosa* DNA. The absence of a peak or a shoulder on the DNA bands suggests that the isolated strand preparations are essentially free of contamination from the opposite strand.[2] By this technique strand contamination of less than 2% would probably go undetected.

The disparity in the shape of the bands obtained after renaturation of heat-denatured TP-84 DNA (Figure 3, bottom curve) and alkali-denatured TP-84 DNA (Figure 5, bottom curve) can be traced to the method of denaturation. As mentioned previously, heat denaturation can lead to backbone breakage, lowering of the molecular weight, and hence increasing the band width. Davidson *et al.* (1964) found that no detectable strand scission occurred in coliphage λ DNA and phage B3 DNA specific for *P. aeruginosa* after incubation in 0.1 N NaOH for 4 hr at 25°. The band obtained from renaturation of alkali-denatured TP-84 is hypersharp and appears to be almost identical with the native DNA band (Figure 3, top curve). A slight discrepancy exists between the buoyant densities of the renatured DNA and the native material. A plausible explanation is that some of the shorter DNA segments were free in

[2] Hybridization of the isolated strands with ^{32}P-pulse-labeled RNA from TP-84 phage infected cells has shown that the H strand is responsible for TP-84 phage induced RNA synthesis (G. F. Saunders and L. L. Campbell, unpublished results).

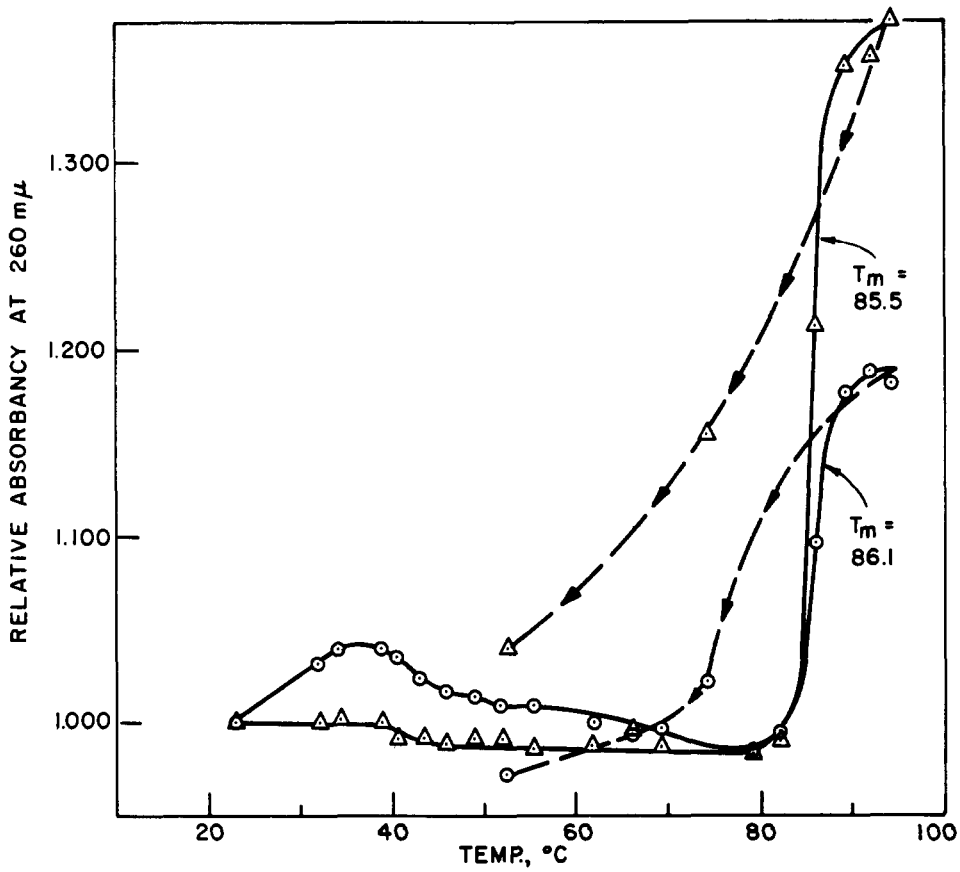

FIGURE 8: Thermal transitions of native and a mixture of the isolated strands of TP-84 DNA. Triangles, native TP-84 DNA in SSC buffer; circles, equimolar mixture of the isolated strands in SSC buffer.

solution and did not find their complementary locations on the larger strand molecules. Since single-stranded DNA is not as buoyant as double-stranded material, the short regions of single strandedness could lead to a slight increase in buoyant density of the DNA band. The reproducibility of the technique of banding in a CsCl density gradient is approximately ±0.001 g/cc.

The evidence for separation of the individual DNA strands of the TP-84 DNA duplex obtained by banding experiments in a CsCl density gradient is at best only presumptive. A study of the physical and chemical parameters of the isolated strands should do much to substantiate the claim of strand separation, particularly in relation to the properties of the duplex. For example, the base composition of the duplex should be equal to the average of the base composition of the individual strands. If native TP-84 DNA consists of two uninterrupted strands the isolated strands should have equivalent sedimentation coefficients as well as approximately the same shapes of their sedimenting boundaries.

The nucleotide base compositions obtained from analyses of phosphodiesterase digests of the isolated TP-84 DNA strands and native TP-84 DNA are given in Table I. The mole per cent of a given base in native TP-84 DNA is approximately midway between the amount of that base in the isolated strands. Pyrimidine excess appears to increase the buoyant density since the H strand is relatively pyrimidine rich and the L strand is relatively purine rich.

TABLE I: Base Composition of TP-84 DNA.

	Mole %				Purines/ Pyrimidines	% G + C
	G	A	C	T		
Native DNA	22.0	27.7	22.1	28.2	0.99	44.1
H strand	18.5	24.0	23.7	33.8	0.74	42.2
L strand	23.1	33.4	20.3	23.2	1.30	43.6

A discrepancy exists in the ultraviolet absorption spectra of the isolated strands (H strand λ_{max} 259 mμ, λ_{min} 234 mμ; L strand λ_{max} 257 mμ, λ_{min} 229.0 mμ). The absorption discordancy between the strands can be explained by the higher absorption maxima of pyrimidines than that of purines.

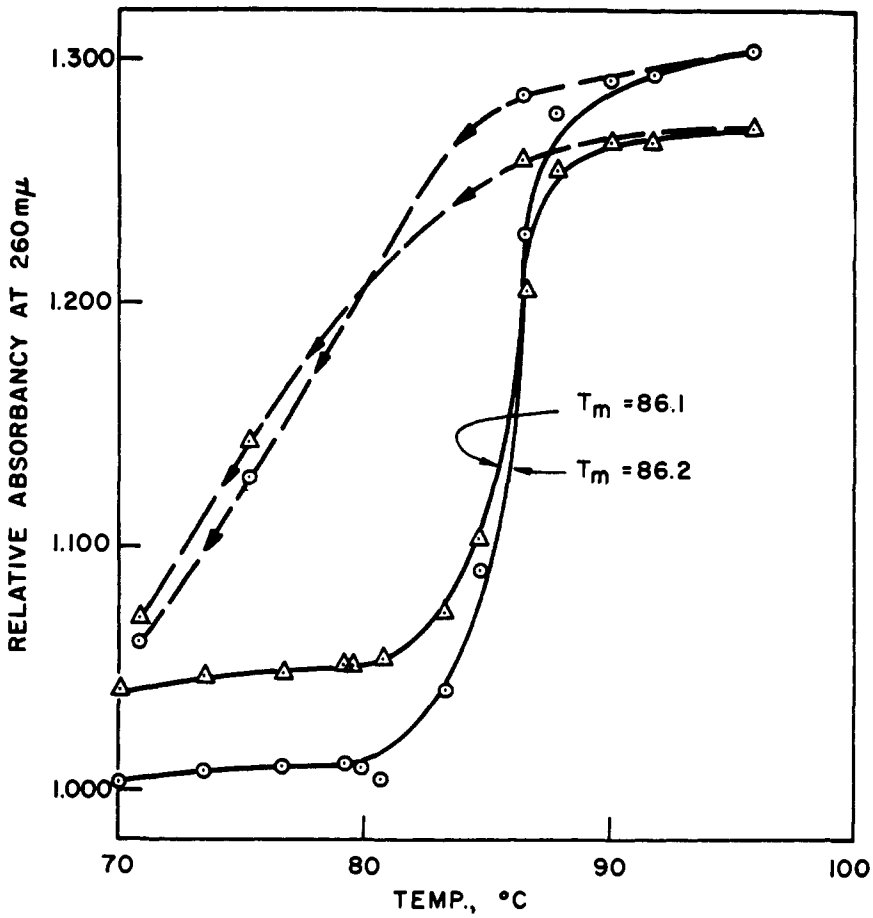

FIGURE 9: Melting curve of renatured TP-84 DNA in SSC buffer. Triangles denote DNA renatured from the isolated strands of TP-84 DNA; circles represent DNA renatured from native TP-84 DNA.

Sedimentation velocity profiles revealed unequal sedimentation coefficients for the two strands (H strand $s^{50}_{20,w}$ = 19.1; L strand $s^{50}_{20,w}$ = 22.1). The broad sedimenting boundaries observed (Figure 6) are indicative of a sample that is heterogeneous with respect to molecular weight. This provides evidence for the existence of intrastrand interruptions in native TP-84 DNA. Since breaks were not observed in the sedimenting boundaries, both preparations probably contain a broad range of molecular weights. Therefore it is probable that native TP-84 DNA contains randomly spaced breaks in both polynucleotide chains. The possibility that the denaturation procedure induced the strand interruptions has not been ruled out.

The melting curve of the isolated strands (Figure 7) shows a profile characteristic of random-coil polynucleotides up to a temperature of 60°. At 60° both strands reach a plateau, but at approximately 80° a 5–8% hyperchromic shift occurs. The melting curve characteristic of native TP-84 DNA (Figure 8) is noticeably absent. Mixing of the isolated strands in equimolar amounts gives the melting curve shown in Figure 8, denoted by open circles. The isolated strands appear to be melting as in Figure 7, but at about 35° the pairing of the complementary regions begins. This lowers the number of individual molecules in solution with a resulting decrease in absorbancy. Both the renatured and native material melt out at the higher temperatures with T_m values of 86.1 and 85.5°, respectively. The reproducibility in the thermal denaturation curve is about ±0.5° (Marmur and Doty, 1962). The cooling curves of both the native and isolated strand preparations drop rapidly, suggesting that the frequency of a given strand colliding with its complementary strand is quite high.

The relatively low hyperchromicity found for the melting of DNA renatured from the isolated strands (Figure 8) could be interpreted as showing that in the strand isolation procedure some fragments of the individual chains were selectively lost. A means of testing this hypothesis would be to carry the DNA renatured on cooling in Figure 8 through a second cycle of heating and cooling. Presumably the sample originating from native TP-84 DNA in Figure 8 contains the full complement of the DNA chains of each strand. Both the material renatured from native DNA and renatured from the isolated strands gave virtually the same melting curves (Figure 9). The difference in the height of the

absorbancy rise does not rule out the possibility that some DNA fragments were lost in the isolation of the individual strands.

Discussion

The DNA of TP-84 was found to consist of two separable polynucleotide components. The components can be purified and reassociated to give a structure possessing many of the physical properties of the original material. By analyses of melting profiles, band widths at equilibrium in CsCl density gradients, and sedimentation coefficients, it may be concluded that the individual polynucleotide strands of TP-84 contain a limited number of noncomplementary strand breaks.

The presence of strand interruptions presents a problem as to the effect breaks might have on replication of the viral DNA. A preferential involvement of the individual DNA strands has been proposed (Jones and Truman, 1964), one in coding for protein synthesis and one for replication. A model for DNA transcription involving single-strand interruptions was proposed by Jones and Truman (1964). They postulated that one of the DNA strands, the transcription strand, is broken into segments. The segments act as templates for the synthesis of one or more RNA messages. The unbroken DNA strand, the reference strand, serves as a reference for the synthesis of a new transcription strand of complementary nucleotide sequence. This proposal is not consistent with the data shown here for TP-84 DNA.

Models proposed by Meselson (1964) and Whitehouse (1963) to explain genetic recombination phenomena, particularly recombination by chromosome breakage and reunion, invoke the presence of noncomplementary intrastrand breaks.

Acknowledgments

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