Characterization of Bacteriophage S13 *su*N15 Single-Strand and Replicative-Form Deoxyribonucleic Acid

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The deoxyribonucleic acid (DNA) of bacteriophage S13 was shown to be single-stranded by the criteria of reactivity with formaldehyde, dependence of optical density on ionic strength, broad temperature-absorbance profile, and lack of molar equivalence of the purine and pyrimidine bases. The DNA has a molecular weight of 1.8×10^6 daltons, an S_{20}^0 of 24.6 in SSC (0.15 M NaCl plus 0.015 M sodium citrate), and a buoyant density of 1.726 g/cc in CsCl. Electron microscopy showed the molecule to be circular. S13 replicative-form DNA was shown to be a double-stranded, circular molecule with a molecular weight of 3.5×10^6 daltons, an S_n of 20.7 in SSC, and a buoyant density in CsCl of 1.710 g/cc. The finding that S13 DNA is slightly more pyrimidine-rich than ϕ X174 DNA but is indistinguishable by all other parameters supports the close genetic relationship between the two bacteriophages.

Bacteriophage S13 has been shown to be closely related to bacteriophage ϕ X174 by serological tests (35), genetic recombination experiments (32), comparison of the coat proteins (22), and complementation tests (13). S13 deoxyribonucleic acid (DNA) has been shown to be single-stranded by ³²P decay studies (33) and hydroxylamine inactivation (30, 31), but no other chemical or physical characterization of the DNA has been reported. Most authors have assumed the properties of S13 DNA (molecular weight, base ratios, single-stranded character, and circularity of the molecule) to be identical to ϕ X174 DNA (1, 31). The extensive genetic studies of bacteriophage S13 have influenced the selection of this organism for structural studies (6). To obtain basic information needed in these and other studies on the primary structure of S13 DNA, the single-stranded nature of the molecule, the size and shape of both the single-stranded and replicative-form DNA, the base compositions, and some physical properties of both forms of S13 DNA have been investigated. Comparison of the data obtained with that for ϕ X174 (7, 10, 15, 26, 28) has added chemical support to the comparative genetic studies on the two phages (13, 32, 35).

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MATERIALS AND METHODS

Bacterial and bacteriophage strains. *Escherichia coli* C, the nonpermissive host for phage S13 suppressible (*su*) mutants, *Shigella dysenteriae* Y6R, the permissive host for suppressible (*su*) mutants, and the lysis-defective amber mutant (gene E) (2) bacteriophage S13 *su*N15 were kindly supplied by I. and E. Tessman.

Growth of phage and preparation of DNA. Phage S13 *su*N15 was grown by infection of *E. coli* C in tryptone broth (29), released by treatment with lysozyme (27), and purified as described previously (6).

S13 DNA was released from the purified phage by phenol extraction (27) and dialyzed against SSC (standard saline citrate, 0.15 M NaCl-0.015 M sodium citrate, pH 7.0). The suspension was then mixed with sarkosyl NL97 (Geigy Industrial Chemicals) to a final concentration of 0.1%, CsCl was added to a density of 1.72 g/cc, and the mixture was centrifuged for 48 hr at 38,000 rev/min (Spinco rotor type 50) in polyallomer tubes. The DNA, dialyzed against SSC and stored at 4°C, contained no detectable protein (16) and was infective to *S. dysenteriae* Y6R spheroplasts (11).

In some preparations, the phenol extract was dialyzed against 0.01 M tris(hydroxymethyl)amino-methane (Tris)-hydrochloride buffer, pH 8.1, CsCl was added to a density of 1.71 g/cc, and the mixture was centrifuged for 60 hr at 35,000 rev/min (Spinco rotor type 50) in polyallomer tubes. The DNA was

then dialyzed against SSC or 0.2 M NaCl in 0.001 M sodium phosphate buffer, pH 7.5.

Replicative-form S13 DNA (RF DNA) and ^{32}P -labeled RF DNA were prepared as described previously (6) with the use of the TG-P medium of Shleser et al. (25). RF DNA eluted from methylated-albumin-kieselguhr was dialyzed against SSC, further purified by CsCl density gradient centrifugation as described above, dialyzed against SSC, and stored at 4°C. The purified RF DNA was infective to *S. dysenteriae* Y6R spheroplasts (11).

Electron microscopy. Electron micrographs of purified phage S13 were prepared by J. W. Costerton, Department of Microbiology, Macdonald College, McGill University. Samples were negatively stained with 1.0% sodium zirconium glycolate, pH 7.1, in 0.001 M sucrose (J. W. Costerton, unpublished data).

Electron micrographs of S13 DNA and RF DNA were prepared by M. Fiantt in the laboratory of W. Szybalski, McArdle Laboratory, University of Wisconsin, using the method of Westmoreland, Szybalski, and Ris. (34). Contour lengths were measured by laying silk threads over the photographs.

Spectral analyses. Temperature-absorbance profiles of DNA samples in SSC or 7.2 M sodium perchlorate-0.05 M ethylenediaminetetraacetate (EDTA) were measured in a Gilford model 220 spectrophotometer fitted with a Beckman DU monochromator and thermospacers. Temperature was controlled by a Haacke circulating bath, and the temperature in the cuvette was measured by means of a digital read-out thermosensor (5). All measurements were corrected for thermal expansion. Ultraviolet spectra were measured in a Gilford model 2000 spectrophotometer.

Sedimentation analyses. Velocity sedimentation analyses were performed at 20°C in a Beckman model E analytical ultracentrifuge, equipped with ultraviolet optics. S13 DNA was sedimented at concentrations from 10 to 40 $\mu\text{g}/\text{ml}$ in SSC and 0.2 M NaCl in 0.001 M sodium phosphate buffer, pH 7.5 (26); RF DNA, at concentrations from 12 to 50 $\mu\text{g}/\text{ml}$ in SSC. Sedimentation coefficients were corrected to S_{20}^0 .

Buoyant densities of the DNA preparations were determined by isopycnic centrifugation in CsCl at 44,000 rev/min for 20 hr at 25°C in a Beckman model E analytical ultracentrifuge with the use of Kel F centerpieces and an An-D rotor (20). Densitometer tracings of the photographs were obtained with a Beckman Acta III spectrophotometer fitted with a scanning device using the 0.05-mm slit assembly.

Base ratio analyses. DNA samples (0.4 mg/ml) were incubated with 20 μg of pancreatic deoxyribonuclease I (EC 3.1.4.5., grade D, Worthington Biochemical Corp.) per ml in 0.005 M Tris-hydrochloride buffer, pH 7.6, containing 0.005 M MgCl_2 , for 19 hr at 37°C. The solution was then adjusted to 0.01 M Tris-hydrochloride-0.03 M MgCl_2 , pH 8.8; 25 μg of snake venom phosphodiesterase (EC 3.1.4.1., grade VPH, Worthington Biochemical Corp.) per ml was added, and the mixture was incubated for 7 hr at 37°C. The digestion mixture was lyophilized, dissolved in 0.5 ml of 0.1 M ammonium formate, pH 3.2, and

chromatographed on Dowex AG 50 WX4 (3). The pooled 5'-nucleotides were measured spectrophotometrically in a Gilford model 2000 spectrophotometer. 5'-Nucleotides from ^{32}P -labeled RF DNA samples, digested and fractionated as described above, were analyzed by mixing 1-ml portions of the pooled fractions with 12 ml of scintillation solution (4) and measuring the radioactivity in a Beckman LS 250 liquid scintillation spectrometer for 50 min or to 1% error. Counts/minute were converted to disintegrations/minute, and all results were adjusted for ^{32}P decay.

RESULTS

Morphology. An electron micrograph of bacteriophage S13 is shown in Fig. 1. The virus appears as a knobby polyhedral object approximately 26 nm in diameter and confirms the close morphological similarity to bacteriophage ϕX174 previously observed by McLean and Hall (18).

Properties of S13 DNA. Electron micrographs of S13 DNA molecules are shown in Fig. 2, with RF molecules shown in Fig. 3. The DNA is circular, and the mean contour length of 13 measured circles was $1.90 \pm 0.05 \mu\text{m}$. Assuming a mass per unit length of 0.95×10^6 daltons/ μm (10), the length corresponds to a molecular weight for S13 DNA of 1.8×10^6 daltons.

Densitometer tracings of velocity sedimentation analyses of S13 DNA are shown in Fig. 4a. The DNA sedimented as a single sharp boundary with $S_{20}^0 = 24.6$ in SSC and $S_{20}^0 = 23.3$ in 0.2 M NaCl in 0.001 M sodium phosphate buffer, pH 7.5. A densitometer tracing of an isopycnic centrifugation of S13 DNA in CsCl, with λ DNA as marker, is shown in Fig. 5a. The buoyant density of the λ DNA marker, 1.7093 g/cc, was based on *E. coli* DNA, 1.7100 g/cc (19), and the calculated buoyant density of S13 DNA was 1.726 g/cc.

The base composition of S13 DNA is shown in Table 1, and the results are compared with the base compositions of single-stranded DNA from other phages. The DNA is pyrimidine-rich and shows no molar equivalence between adenine and thymine or guanine and cytosine. The buoyant density of S13 DNA calculated from the base composition by the formula derived by Riva et al. (23) is 1.724 g/cc, in excellent agreement with the measured value.

The temperature-absorbance profile of S13 DNA (Fig. 6) shows a broad temperature transition from 30 to 70°C, with a 20% hyperchromic shift typical of thermally denatured or single-stranded DNA. Additional evidence for the single-stranded nature of S13 DNA was shown by the dependence of the optical density on ionic strength (Fig. 7), the extinction at 260 nm decreasing with

FIG. 1. *Electron micrograph of bacteriophage S13. $\times 560,000$.*

FIG. 2. *Electron micrographs of S13 DNA molecules. $\times 42,000$.*

FIG. 3. *Electron micrographs of S13 replicative-form DNA molecules. $\times 42,000$.*

FIG. 4. Densitometer tracings of analytical velocity sedimentation analyses of S13 DNA and S13 replicative-form DNA. Sedimentation was performed at 48,300 rev/min in SSC at 20 C. (a) S13 DNA, 40 µg/ml; 4-min intervals. (b) S13 replicative-form DNA, 50 µg/ml, 8-min intervals.

increasing salt concentration. Incubation of S13 DNA in SSC containing 1.8% formaldehyde at 37 C (Fig. 8) resulted in a hyperchromic shift which was rapid in the first 30 min of incubation and complete within 16 hr of treatment. The reactivity with formaldehyde indicates that the amino groups of the purine and pyrimidine rings are accessible and not involved in a tightly hydrogen-bonded structure (26). The extinction at 260 nm per mole of phosphorus in SSC at 30 C was 8,500, a value typical for a disordered strand of DNA.

Properties of RF DNA. S13 RF DNA molecules (Fig. 3) are circular, as expected for the replicative-form of a single-stranded circular DNA phage. The mean contour length based on the measurement of 23 molecules was 1.83 ± 0.02 µm. Assuming a mass per unit length of 1.92×10^6 daltons per µm (17), this corresponds to a molecular weight for RF DNA of 3.5×10^6 daltons. This value is in good agreement with the calculated value of twice the weight of S13 DNA, 1.8×10^6 daltons.

Densitometer tracings of velocity sedimentation analyses of RF DNA are shown in Fig. 4b. The main component sedimented as a single boundary, but about 10% of the sample sedi-

FIG. 5. Buoyant density in CsCl of S13 DNA and S13 replicative-form DNA. Densitometer tracings of ultraviolet absorption photographs of DNA samples at equilibrium in a CsCl gradient. (a) S13 DNA, 5 µg/ml, and λ DNA marker, 3 µg/ml. (b) S13 replicative-form DNA, 0.5 µg/ml, and S13 DNA marker, 3 µg/ml. Densities are relative to *E. coli* DNA, 1.7100 g/cm³ (19).

mented more slowly and is probably due to nicked RF II molecules in the preparation. The concentration of this component was too low for accurate measurement of an *S* value. The S_{20}^0 value for the RF DNA was 20.7 in SSC. A densitometer tracing of an isopycnic centrifugation of RF DNA in CsCl is shown in Fig. 5b, with S13 DNA as marker. The calculated buoyant density of RF DNA was 1.710 g/cc.

Temperature-absorbance and renaturation profiles of RF DNA, in both SSC and 7.2 M sodium perchlorate-0.05 M EDTA, are shown in Fig. 9. The T_m in SSC is 87.3 C, and in 7.2 M sodium perchlorate, 46 C. The profiles in both solvents display the sharp transition typical of a double-

TABLE 1. Base composition of single-stranded bacteriophage DNA

Bacteriophage	Molar ratios				Reference
	Adenine	Guanine	Thymine	Cytosine	
S13.....	1.00	0.98	1.42	0.86	—
ϕ X174.....	1.00	0.98	1.33	0.75	26
ϕ R.....	1.00	1.03	1.41	0.83	14
fd.....	1.00	0.81	1.40	0.89	12
M13.....	1.00	0.90	1.53	0.85	24
S13, moles per cent ^a .	23.5 $\pm$ 0.5	23.1 $\pm$ 0.5	33.3 $\pm$ 0.3	20.1 $\pm$ 0.1	—

^a Values are $\pm$ the standard error of the mean based on three separate analyses.

FIG. 6. Temperature-absorbance profile of S13 DNA. $E_{260}^{1\text{ cm}}$ at 20 C, 0.536. All measurements were corrected for thermal expansion.

stranded DNA molecule, and the DNA renatures almost completely, which is typical of small circular double-stranded DNA molecules. The RF DNA showed no optical density changes with change in ionic strength at salt concentrations with which S13 DNA showed large effects, nor was there any hyperchromic change after incubation with formaldehyde.

The base composition of RF DNA is shown in Table 2. The experimental results are in good agreement with values calculated from the base composition of S13 DNA (Table 1). The molar ratios are those expected of a double-stranded DNA molecule. Confirmation of the base composition of the RF DNA was obtained by calculation from the T_m value in SSC, 87.3 C, which corresponds to 43.9% guanine plus cytosine (21).

FIG. 7. Variation of extinction at 260 nm per mole of phosphorus of S13 DNA with ionic strength.

DISCUSSION

The molecular weight of 1.8×10^6 daltons and S_{20}^0 of 23.3 of S13 DNA compared with the values for ϕ X174 DNA, molecular weight 1.7×10^6 daltons and $S_{20} = 23.8$ in 0.2 M NaCl in 0.001 M sodium phosphate buffer, pH 7.5 (26), indicate no significant differences between the DNA of the two phages. Freifelder (9) has discussed the use of electron microscopy for molecular-weight measurements and pointed out that until the dependence of mass per unit length on ionic strength and the composition of the supporting medium on base composition is better defined an error of 10% must be considered. The buoyant density of S13

FIG. 8. Effect of 1.8% formaldehyde on the ultraviolet absorption of S13 DNA, 27 $\mu\text{g/ml}$, in SSC at 37 C. Symbols: $\circ$, 0 min; $\bullet$, 30 min; $\blacktriangle$, 16 hr.

DNA in CsCl, 1.726 g/cc, is almost identical to the value of 1.725 g/cc for ϕX174 DNA (26, 28).

The base composition of S13 DNA is closer to the values reported for the DNA of phages ϕR (14) and fd (12) than those for ϕX174 DNA (26). The presence of more pyrimidines in S13 DNA, 2,935 compared to 2,815 for ϕX174 DNA (8), is reflected in the pyrimidine oligonucleotide catalogues of the DNA of the two phages (6, 8).

The contour lengths of the S13 RF DNA molecules, from electron microscopy, are well within the range of values reported for ϕX174 RF DNA, from $1.64 \pm 0.11 \mu\text{m}$ (15) to $1.89 \pm 0.04 \mu\text{m}$ (7). The calculated molecular weight of S13 RF DNA, 3.5×10^6 , is also in the range of 3.2×10^6 daltons (15) and 3.6×10^6 daltons (7) reported for ϕX174 RF DNA. The S_{20}^0 value of S13 RF DNA, 20.7 in SSC, corresponds closely with the 21S value reported for ϕX174 RF DNA (7), and the buoyant density in CsCl, 1.710 g/cc, compares with the value of 1.709 g/cc for ϕX174 RF DNA (28), values relative to that of *E. coli* of 1.7100 g/cc.

That S13 DNA is a single-stranded molecule was demonstrated by its temperature-absorbance profile, dependence of the optical density on ionic strength, reactivity with formaldehyde, and lack of molar equivalence of the purines and pyrimidines in the base composition analysis. The same parameters were used to identify S13 RF DNA as double-stranded: temperature-absorbance profiles and renaturation characteristics, lack of depend-

FIG. 9. Temperature-absorbance profiles of S13 replicative-form DNA in SSC ($E_{260}^{1\text{cm}}$ at 20 C, 0.515) and 7.2 M sodium perchlorate-0.05 M EDTA ($E_{260}^{1\text{cm}}$ at 20 C, 0.510). All measurements were corrected for thermal expansion. Symbols: $\bullet$, denaturation in SSC; $\circ$, renaturation in SSC; $\blacktriangle$, denaturation in sodium perchlorate; $\triangle$, renaturation in sodium perchlorate.

TABLE 2. *Base composition of S13 suN15 replicative-form DNA*

Source	Moles per cent				Molar ratios		
	Adenine (A)	Guanine (G)	Thymine (T)	Cytosine (C)	Pu/Py	A/T	G/C
Predicted from Table 1.....	28.4	21.6	28.4	21.6	1.00	1.00	1.00
Experimental values.....	27.9	22.2	28.5	21.4	1.00	0.98	1.04

ence of optical density on ionic strength, lack of reactivity with formaldehyde, and the base composition showing molar equivalence of the purines and pyrimidines. The results confirm the previous assumption that S13 DNA is in all chemical and physical parameters almost identical to ϕ X174 DNA and adds support to the evidence for a close genetic relationship between the two phages (13, 32, 35). The results also provide support for the use of a molecular weight of 1.8×10^6 daltons, corresponding to 5,500 nucleotides (of which 2,935 are pyrimidines), per molecule of single-stranded S13 DNA in the computation of tract numbers of pyrimidine oligonucleotides (6).

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