Stalked Bacteria: Properties of Deoxyribonucleic Acid Bacteriophage ϕ CbK

NINA AGABIAN-KESHISHIAN AND LUCILLE SHAPIRO

Department of Molecular Biology, Division of Biological Sciences, Albert Einstein College of Medicine, Bronx, New York 10461

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The *Caulobacter crescentus* bacteriophage ϕ CbK was studied with respect to the physical and chemical properties of both the phage and its deoxyribonucleic acid (DNA). Electron micrographs reveal the phage to be among the largest DNA bacteriophages reported, with head dimensions of 64 by 195 nm and a flexible tail 275 nm in length. The phage is composed of 57% DNA. This DNA is double-stranded as indicated by (i) the sharp increase in extinction coefficient over a narrow range of temperature increase, (ii) an increase in density in CsCl upon thermal denaturation, and (iii) the equivalence of guanine and cytosine as well as adenine and thymine, determined by chemical analysis. ϕ CbK DNA cosediments with *Escherichia coli* phage T2 DNA and has therefore been assigned an $S_{20,w}$ value of 63.5S. The size of the phage and its DNA and the percentage of DNA indicate that the ϕ CbK phage head is relatively loosely packed. The properties of the DNA from bacteriophage ϕ CbK are similar to those of host *C. crescentus* DNA with respect to buoyant density, thermal transition point, and guanine plus cytosine content.

The *Caulobacter* are dimorphic eubacteria characterized by the possession of a stalk. The bacteria undergo asymmetric cell division which results in the production of a stalked cell and a fully formed swarmer cell (7). The swarmer cell possesses a single polar flagellum and several pili (10); it must differentiate into a stalked cell before it is capable of cell division. In this obligatory differentiation step, a stalk, which is continuous with the body of the cell, is formed from the base of the flagellum (7) and both pili and flagellum are subsequently lost. These gram-negative bacteria are easily grown under laboratory conditions in defined media and represent a workable system with which to study the molecular basis of differentiation. Genetically determined functions expressed by the stalked cell, but not the swarmer, include proliferation of a polar mesosome (8), flagellum formation at the pole opposite the stalk location, and formation of a transverse septum followed by cell division. An apparent genetic function expressed solely by the swarmer cell is stalk proliferation involving stalk membrane and wall formation.

To utilize genetic techniques for the elucidation of differentiation in *C. crescentus*, it would be helpful to develop a transduction system. For this reason, the isolation and characterization of *Caulobacter* DNA phages are in progress.

We report here the chemical and physical properties of one of these phages, ϕ CbK, which specifically infects the swarmer cells of the *C. crescentus* strains CB13B1a, CB15, and KA2. A unique feature of the phage is its very large size. The physical parameters of the phage deoxyribonucleic acid (DNA) were studied and compared with those of the host DNA.

MATERIALS AND METHODS

Growth conditions. *C. crescentus* strain CB13B1a was cultured in a nutrient broth (PYE) containing 0.2% peptone, 0.1% yeast extract, and 0.02% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in tap water (7). Liquid cultures were incubated at 30°C on a rotary shaker. The strain was routinely used as a host for the DNA bacteriophage ϕ CbK, which gave tiny plaques (about 1 mm in diameter) on PYE soft agar.

Escherichia coli B/T⁻ was used as a host for the bacteriophage T2. The bacteria were grown in Casamino Acids broth supplemented with 0.025 M KPO_4 (pH 7.0) and 25 μg of thymine per ml.

Purification of phage. Lysates of ϕ CbK were prepared by inoculating early-log-phase cultures of CB13B1a with a multiplicity of infection of 0.1. Infection was allowed to proceed 7 to 12 hr, and then the lysate was cleared by centrifugation for 10 min at 9,000 rev/min in the Sorvall RC2-B centrifuge. Maximum titers obtained in this manner were 10^{11} to 2×10^{11} phage per ml. Phage ϕ CbK was concentrated from the crude lysate by precipitation with 70%

saturated ammonium sulfate. The precipitate was collected by centrifugation at 17,500 rev/min for 20 min, and the resulting pellet was suspended in 0.01 M ammonium acetate. The phage suspension was then centrifuged in a Spinco model L ultracentrifuge in a fixed-angle Beckman 30 rotor at 30,000 rev/min for 5 hr. The resulting pellet was suspended in 2 to 4 volumes of SSC (0.15 M NaCl and 0.015 M Na citrate) and then centrifuged in a density gradient of CsCl (1.5 g/cm³). Centrifugation was first carried out for 30 min at 25,000 rev/min in the Beckman 30 rotor; at this point, cell debris was observed at the bottom and the top of the gradient. The middle layer was transferred to cellulose nitrate tubes, overlaid with paraffin, and centrifuged for 24 hr at 50,000 rev/min in the Spinco model L ultracentrifuge. Fractions were obtained from a hole in the bottom of the gradient. An opalescent band of phage was observed at $\rho = 1.53$ g/cm³, and the optical density profile paralleled that of plaque-forming units (PFU). The recovery of PFU after centrifugation in CsCl was routinely 50 to 70%.

Lysates of T2 were prepared by inoculating cultures of *E. coli* B/T⁻ supplemented with 25 μ g of thymine per ml at a multiplicity of infection of 8.0. Just before phage infection, ³H-thymine (100 μ Ci) was added to the bacterial culture (200 ml), and infection was allowed to proceed for 2 hr. Chloroform was added and incubation on the rotary shaker was continued for 1 hr. Phage were then purified by differential centrifugation.

Electron microscopy. Purified phage were stained with 1% phosphotungstate at pH 7.0 in a manner similar to that described by R. W. Horne (2). A Siemens Elmiskop IA electron microscope at a voltage of 80 kv was used for this study.

Preparation of *C. crescentus* DNA. *C. crescentus* was grown in PYE broth until late-stationary phase. Under these conditions more than 95% of the cell population consisted of stalked cells (7). The cells were collected by centrifugation for 15 min at 9,000 rev/min in the Sorvall centrifuge, and the pellets were frozen. The frozen cells were ground in a mortar and pestle with an equal volume of alumina; the resulting paste was suspended in 2 volumes of SSC. The cell-free extract was treated with an equal volume of redistilled phenol, and the DNA was then further purified by the procedure of Marmur (4).

Preparation of phage DNA. Phage ϕ CbK purified by CsCl centrifugation was dialyzed against SSC for 18 hr. The DNA was either purified by phenol extraction followed by the procedure of Marmur (4) or released from the phage by incubation at 45 C for 10 min in the presence of 2% sodium dodecyl sulfate.

Zone centrifugation. Sedimentation of bacteriophage DNA was carried out in an SW41 swinging-bucket rotor in a Spinco model L2-65B ultracentrifuge using a 5 to 30% linear glycerol gradient. The gradient contained 0.1 M NaCl, 0.02 M tris (hydroxymethyl) aminomethane (pH 7.8), and 0.001 M ethylenediaminetetraacetic acid.

CsCl density-gradient centrifugation. Analytical sedimentation was carried out at 25 C in the Beckman

model E ultracentrifuge as described by Meselson, Stahl, and Vinograd (6). The density marker was DNA from either *Clostridium perfringens* (density = 1.691 g/cm³) or *E. coli* (density = 1.710 g/cm³). The ultraviolet photographs were scanned with a Joyce-Loebl double-beam recording microdensitometer.

Analysis of base composition. ³²P-labeled DNA was degraded to 5'-nucleoside monophosphates by treatment with venom phosphodiesterase and pancreatic deoxyribonuclease as described by Wu and Kaiser (18). The mononucleotides were separated by electrophoresis at pH 3.5 in pyridine-acetate-water (1:10:89) for 2 hr at 5,000 v.

Materials. *C. perfringens* DNA, used as a marker in analytical sedimentation in CsCl, was a generous gift of J. Marmur. Optical-grade CsCl was purchased from Gallard-Schlesinger Chemical Manufacturing Corp., N.Y., and spectro-quality reagent-grade glycerol was purchased from Matheson, Coleman and Bell, East Rutherford, N.J. Inorganic ³²P was obtained from Tracer Labs, Waltham, Mass., and ¹⁴C-thymine was purchased from Schwarz Bio-Research, Mt. Vernon, N.Y. Bovine pancreatic ribonuclease and deoxyribonuclease and venom phosphodiesterase were obtained from the Worthington Biochemical Corp., Freehold, N.J. T₁ ribonuclease was purchased from Calbiochem, Los Angeles, Calif.

RESULTS

Properties of bacteriophage ϕ CbK. PFU of ϕ CbK banded at a density of 1.53 g/cm³ in a preparative CsCl density gradient. The density of ³²P-labeled phage in CsCl was also determined by using ¹⁴C-labeled bacteriophage lambda [$\rho = 1.508$ g/cm³ (16)] as a reference marker. In these experiments, the buoyant density of ϕ CbK was 1.536 g/cm³.

Phage ϕ CbK prepared for electron microscopy by negative staining appear to have an elongated polygonal head with a long, flexible tail (Fig. 1). A dense region was routinely observed at the junction of the head and the phage tail. The average of many measurements revealed that the phage has an overall length of 470 nm. The head is 195 nm in length and 64 nm in diameter; the tail is 275 nm in length.

Sedimentation velocity experiments of ϕ CbK were carried out in a Beckman model E analytical ultracentrifuge by using schlieren optics. A sample of purified ϕ CbK in SSC buffer, containing 174 μ g of viral protein per ml, was used to fill a 4° single sector cell, 12 mm in length. The phage could be seen to sediment in a 20-min run at 13,410 rev/min. The observed *S* value, corrected to 20 C and the viscosity of the buffer, was 1,165S. The purified phage appeared homogeneous since it formed a single, symmetrical peak in the analytical ultracentrifuge. The content of DNA in the same phage preparation was

FIG. 1. Electron micrograph of bacteriophage ϕCbK dried down in neutral phosphotungstic acid. (a) Magnification, $\times 140,000$; (b) magnification, $\times 187,000$.

57%. Thymus DNA of known phosphorus content and bovine serum albumin were used as standards in the diphenylamine (1) and the Lowry (3) reactions, respectively.

Properties of bacteriophage ϕCbK DNA. The buoyant density in CsCl of purified native ϕCbK

DNA banded at 1.721 g/cm^3 . Heat-denatured DNA formed a single band at 1.734 g/cm^3 . The increase in buoyant density obtained upon heating the DNA at 100°C for 10 min in 0.1 SSC would be expected if the phage DNA were double-stranded (9). The mole per cent of guanine plus

cytosine (G+C) was 62.3%, calculated from the native buoyant density by using the relationship $\rho = 1.660 + 0.098 (G+C)$ derived by Schildkraut, Marmur, and Doty (9).

The G+C content was also calculated from the thermal transition profiles. The DNA melted sharply with a thermal denaturation temperature (T_m) of 95 C in SSC. The T_m was dependent on ionic strength, and the relative absorbance of the denatured DNA was 1.33. The mole per cent G+C calculated by the method of Marmur and Doty (5) by the equation $T_m = 69.3 + 0.41 (G+C)$ was 62.6% for the DNA in SSC. The correspondence of the G+C content derived from the density of the DNA in CsCl and its T_m in SSC suggested that ϕ CbK DNA does not contain significant quantities of unusual bases.

The size of ϕ CbK DNA was determined by zone sedimentation in either 5 to 30% glycerol gradients or 5 to 20% sucrose gradients. The DNA was released from a mixture of ^{32}P - ϕ CbK and ^{14}C -T2 phage particles by treatment with sodium dodecyl sulfate at 45 C and then gently applied to a glycerol gradient. ϕ CbK DNA cosedimented with T2 DNA (Fig. 2). In other experiments, DNA was released from both phages by treatment with alkali directly on top of a sucrose gradient (19). In all cases the DNA molecules from ϕ CbK and T2 cosedimented. Since ϕ CbK DNA is double-stranded as indicated by analysis of its density, thermal transition, and base composition, it can be assigned an $S_{20,w}$ value of 63.5S.

The base composition of ϕ CbK DNA determined by chemical analysis is shown in Table 1. The content of guanine was equivalent to the content of cytosine, and the content of adenine equalled that of thymine. The total radioactivity in a preparation of ^{32}P -labeled DNA hydrolysate migrated as the four common deoxyribonucleoside 5'-monophosphates during electrophoresis at pH 3.5. The mole per cent G+C content was 65% determined by this method. The difference in G+C content as determined by CsCl banding or thermal denaturation parameters varies from

that found by chemical analysis and is unexplained. An even more striking discrepancy between G+C content determined by chemical methods and that determined from density and T_m parameters is exhibited by the host cell DNA (see below).

Properties of *C. crescentus* DNA. The buoyant density of *C. crescentus* swarmer and stalked cell DNA was determined by analytical sedimentation in CsCl (Fig. 3). The two cell types were separated by differential centrifugation as described by Schmidt (10). DNA from both types of cells banded in CsCl at 1.718 g/cm³ corresponding to a mole per cent G+C content of 59%. A satellite band routinely appeared in stalked cell DNA preparations at $\rho = 1.703$ but was never detected with DNA isolated from swarmer cells.

FIG. 2. Sedimentation profiles of ^{32}P -bacteriophage ϕ CbK DNA and ^{14}C -bacteriophage T2 DNA. A mixture of ^{32}P - ϕ CbK phage and ^{14}C -T2 phage in 1 ml of SSC was incubated with 0.01 ml of 10% sodium dodecyl sulfate at 45 C for 10 min. The solution was then layered on a 5 to 30% glycerol gradient as described in Materials and Methods. After centrifugation for 150 min at 41,000 rev/min at 4 C, fractions were collected and analyzed for radioactivity in a Mark I scintillation counter (Nuclear-Chicago Corp.). Less than 1 μg of DNA was used in each case.

TABLE 1. Base composition of phage and host DNA

DNA	Mole per cent ^a				
	Cytosine	Adenine	Guanine	Thymine	Guanine plus cytosine
ϕ CbK.....	32.5 $\pm$ 0.1	17.4 $\pm$ 0.1	32.7 $\pm$ 0.1	17.4 $\pm$ 0.3	65
<i>C. crescentus</i>	33.5 $\pm$ 0.3	16.5 $\pm$ 0.1	33.7 $\pm$ 0.4	16.4 $\pm$ 0.2	67

^a Errors are expressed as standard deviations from the mean of three estimations.

The thermal transition profiles of *C. crescentus* stalked cell DNA revealed a T_m of 94 C in SSC corresponding to a G+C content of 60%. The denatured DNA had a relative absorbance of 1.36, and the denaturation temperature was dependent on ionic strength. The shape of the

curves was monophasic and indicative of double-stranded DNA; the concentration of the satellite DNA was too low to be detected. The base composition determined by chemical analysis yielded a value for G+C content of 67% (Table 1). Guanine and cytosine were equivalent as were adenine and thymine, and during electrophoresis at pH 3.5 all the radioactivity migrated as either adenine, cytosine, guanine, or thymine mononucleotides.

A summary of the physical properties of bacteriophage ϕ CbK DNA and its host, *C. crescentus* CB13B1a, is shown in Table 2. It can be concluded that although minor differences exist, the phage and host DNA are similar with respect to buoyant density, thermal transition point, and base composition.

DISCUSSION

Electron micrographs of the *C. crescentus* DNA bacteriophage ϕ CbK reveal it to be composed of an elongated head with a flexible tail. Schmidt and Stanier have published electron micrographs of a related and structurally similar *C. crescentus* DNA bacteriophage, ϕ Cb13, in which the tail was shown to possess a hollow core (11). The mode of infection for bacteriophage with flexible tails, including the *E. coli* phage T5 (17), is unknown.

The DNA of phage ϕ CbK was prepared by various methods and shown to consistently cosediment with the DNA of bacteriophage T2. It has thus been assigned an $S_{20,w}$ value of 63.5S corresponding to the $S_{20,w}$ value determined for T2 DNA by Studier (14). Since the ϕ CbK phage head has approximately twice the volume of the T2 phage head, and since the *Caulobacter* phage is 57% DNA, it is surprising that both DNA molecules are of about the same size. The *B. subtilis* phages PBS1 and SP15, which are closer in size to the *C. crescentus* phage, possess a DNA molecule of 73.5 and 85.6S, respectively (15). With the *B. subtilis* phages, at least, there does not seem to be a direct correlation between head volume and the size of the DNA. Both of these

FIG. 3. Microdensitometer tracings of DNA in CsCl after 20 hr of centrifugation at 44,770 rev/min. (a) DNA isolated from stalked *Caulobacter* cells which banded at $\rho = 1.718$ g/cm³ and $\rho = 1.703$ g/cm³. (b) DNA isolated from swarmer *Caulobacter* cells which banded at $\rho = 1.718$ g/cm³. *C. perfringens* DNA ($\rho = 1.691$ g/cm³) was used as a reference marker.

TABLE 2. Physical parameters of phage and host DNA

DNA	$S_{20,w}$	Buoyant density in CsCl (g/cm ³)	T_m in SSC ^a	Moles per cent guanine plus cytosine		
ϕ CbK.....	63.5	1.721	95 C	65 ^b	62.3 ^c	62.6 ^d
<i>C. crescentus</i>		1.718	94 C	67 ^b	59.0 ^c	60.0 ^d

^a SSC, 0.15 M NaCl plus 0.015 M sodium citrate.

^b Obtained from chemical analysis shown in Table 2.

^c Calculated from buoyant density in CsCl (9).

^d Calculated from T_m in SSC (5).

phages are of the same size and shape, whereas the size of their DNA varies considerably. The size of the phage and its DNA and the percentage of DNA indicate that the ϕ CbK phage head is relatively loosely packed, as appears to be the case with SP15.

ϕ CbK DNA is double-stranded as indicated by (i) the sharp increase in extinction coefficient over a narrow range of temperature increase, (ii) an increase in density in CsCl upon thermal denaturation, and (iii) the equivalence of guanine and cytosine as well as adenine and thymine, determined by chemical analysis.

In general, the DNA of phage ϕ CbK and its host cell have similar buoyant densities, thermal transition points, and G+C contents. It was observed that DNA isolated from purified *Caulobacter* stalked cells contained a minor DNA component of 44% G+C content. This satellite band was not detected in preparations of DNA from purified swarmer cells. The origin and function of the satellite DNA in stalked cells will be the subject of further investigation. An anomalous observation with the host DNA was that the G+C content determined by chemical analysis was 67%, whereas the G+C content calculated from both density and T_m parameters was approximately 60%. All experiments were performed several times with different DNA preparations, and consistent results were obtained. Perhaps an unusual base is present in *Caulobacter* DNA which comigrated with the four common deoxyribonucleoside monophosphates in electrophoresis at pH 3.5.

The chemical and physical properties of DNA from bacteriophage ϕ CbK are quite similar to those of host *C. crescentus* DNA. It is of primary concern to determine whether ϕ CbK is capable of transducing genetic markers in *C. crescentus*, since no other means of genetic transfer have as yet been described for the *Caulobacter* family. These experiments are currently in progress.

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