Properties of the Deoxyribonucleic Acid Contained in the Defective Particle Coliphage 15¹

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Escherichia coli strain 15 TAU, which requires thymine, arginine, and uracil for growth and harbors an apparently defective prophage, was induced by exposure to ultraviolet light (580 ergs/mm²) or to mitomycin C (5 µg/ml). Phage particles (coliphage 15) were recovered from the resulting lysate by treatment with deoxyribonuclease, filtration, and several cycles of differential centrifugation. Analysis of the phage particles obtained by using cesium chloride density gradient centrifugation in a preparative ultracentrifuge resulted in the resolution of three components. The major component had a peak density of 1.52 to 1.53 g/cm³ followed by components with densities of 1.5 and 1.49 g/cm³. The guanine plus cytosine content of coliphage 15 deoxyribonucleic acid (DNA) was determined by both analytical ultracentrifugation in cesium chloride and by thermal denaturation in standard saline citrate buffer. Respective values of 46.4 ± 1% and 46.6 ± 1% guanine plus cytosine content were obtained. Coliphage 15 DNA formed molecular hybrids with messenger ribonucleic acid (RNA) from both uninduced and ultraviolet-induced cultures of *E. coli* 15 TAU, but did not hybridize with *E. coli* ribosomal RNA. The molecular weight of coliphage 15 DNA was determined by constant velocity sucrose density gradient centrifugation to be about 33 × 10⁶ daltons.

It has been established in several laboratories that derivatives of *Escherichia coli* strain 15 produce phagelike particles after exposure of cultures to a variety of inducing agents (4, 5, 12, 18). The particles produced in response to such treatments were identified by electron microscopy, but all attempts to propagate them by infection were unsuccessful. The colicin activity found previously in lysates of induced cultures of *E. coli* 15 (13, 17) was attributed to the lytic activity of these apparently defective phage particles on the host strain. Although examination of lysates indicated the presence of many incomplete phage components, some apparently intact phage particles and phage heads containing nucleic acid were observed.

The purpose of this investigation was to characterize the nucleic acid incorporated into phage-like particles recovered from lysates of *E. coli* 15 TAU. In this paper, we present the results of several methods utilized to characterize the deoxyribonucleic acid (DNA) contained in these particles. Additional information is presented on the

distribution of the DNA-containing particles recovered from these preparations.

MATERIALS AND METHODS

Organism and culture media. A polyauxotroph, *Escherichia coli* strain 15 TAU (9), requiring thymine, arginine, and uracil for growth was used. Cells were grown with aeration at 37 C in medium C (16) supplemented with thymine (20 µg/ml), arginine (38 µg/ml), and uracil (20 µg/ml).

Induction of particles. Ultraviolet (UV) irradiation with stirring was conducted on 50-ml fractions of two- to five-fold, concentrated, washed bacterial suspensions in a sterile Petri dish placed 95 cm from two General Electric germicidal lamps. Irradiated suspensions (580 ergs/mm²) were then restored to the harvesting concentration (usually 5 liters) and incubated for 3 to 4 hr until lysis was complete.

Purification of particles. Lysates were chilled overnight, and the supernatant material was removed by siphoning. The supernatant fraction was treated with deoxyribonuclease (EC 3.1.4.5) (0.4 µg/ml) for 2 hr and then clarified by filtration through a pad of diatomaceous earth (Hyflo Super Cel). The clarified extract was filtered through a Millipore membrane filter (0.45-µm porosity, 142-mm diameter) to remove any remaining bacteria. The phagelike particles were harvested by centrifugation (20,000 × g) for 1 hr, and the pellet was suspended gently in 0.85% (w/v)

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saline. The suspended pellet was subjected to a low-speed centrifugation ($3,020 \times g$) for 10 min, and the pellet was discarded. The supernatant material was centrifuged at high speed ($20,000 \times g$) for 1 hr, and the resulting pellet was again gently suspended in 0.85% saline. The low- and high-speed centrifugations were repeated, and the final pellet was suspended in 0.85% saline. Further purification was performed in some cases by CsCl density gradient centrifugation in an angle head rotor. For these purifications, 1 ml of the final phage suspension was mixed with 8 ml of CsCl ($\rho = 1.574$), yielding a final density of 1.512 g/cm^3 . After capping each tube, the space remaining at the top was filled with mineral oil, and the tubes were centrifuged at an average centrifugal force of $65,745 \times g$ for 20 hr at 15°C in a Spinco model L-2 ultracentrifuge by using a type 50 rotor. Twenty-drop fractions (0.3 ml) were collected by piercing the bottom of each gradient tube. The resulting fractions were diluted with 3 ml of 0.85% saline for determining the absorbancies at 260, 280, and 320 nm. The linearity of the CsCl gradient was monitored by reading the refractive indices of every fifth fraction. Peak fractions were pooled, and the particles were recovered by centrifugation ($20,000 \times g$) for 1 hr. CsCl was removed by dialysis against 0.85% saline or standard saline citrate (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) buffer.

Determination of guanine plus cytosine (GC) content of DNA. CsCl density gradient centrifugation and the subsequent calculations were conducted by the method described by Mandel, Schildkraut, and Marmur (10). The buoyant density was calculated from a microdensitometer tracing of the phenol-purified defective particle DNA (1 to 2 μg) and an internal reference standard of bacteriophage SP8 DNA (1.742 g/cm^3).

The UV absorbance-temperature profile was determined by the method described by Mandel and Marmur (11). A Zeiss PMQ II spectrophotometer was used, and temperature was measured by a YSI thermistor-thermometer calibrated against a National Bureau of Standards mercury thermometer. The temperature was raised by a Neslab temperature programmer connected to a Lauda water bath. The GC content was calculated by the difference in melting temperature between the phenol-purified DNA obtained from the particle preparation and a purified reference DNA.

Molecular weight determination. Small cultures (30 ml) of *E. coli* 15 TAU were labeled for one generation with thymine- $2\text{-}^{14}\text{C}$ (2 μC per 5 μg per ml) before induction with mitomycin C (5 $\mu\text{g/ml}$). After addition of the mitomycin C, cultures were held for 15 min without aeration before subsequent harvesting and washing procedures. Postinduction growth was accomplished in media containing the same level of radioactive thymine until lysis was complete. The particles were partially purified by a low-speed centrifugation ($3,020 \times g$) for 15 min followed by centrifugation ($20,000 \times g$) of the supernatant material for 2 hr in a Spinco number 30 angle head rotor. The pellet was gently suspended in buffer

[0.9 M NaCl, 0.01 M tris(hydroxymethyl)amino-methane (Tris), 0.01 M MgCl_2 , pH 7.4]. Further purification was accomplished using stepwise CsCl gradients (22). After removal of CsCl by dialysis against 0.85% saline, a portion of the phage suspension was layered over an exponential sucrose gradient of the constant velocity type (14) with a sucrose concentration of 5% by weight (w/w) at the meniscus and 1 ml of 2.5 M sodium perchlorate on top of the gradient as described by P. M. Corry (Ph.D. dissertation, The University of Texas, 1968). Sodium dodecyl sarcosinate (0.15%) was added to the perchlorate to aid in lysing the particles. T2 phage, labeled and purified by methods similar to those used for coliphage 15, was utilized as a molecular weight standard in these determinations. Centrifugation in a Spinco SW 25.1 rotor was done at 24,000 rev/min for 16 hr at 20°C. Fractions (1 ml) were collected by piercing the bottom of the tubes, and radioactivity was determined by collecting cold trichloroacetic acid (5% w/v)-insoluble material on Millipore membrane filters (0.65- μm porosity, 25-mm diameter). The dried filters were counted in a Packard model 3375 Tri-Carb scintillation spectrometer. A computer program described by P. M. Corry (Ph.D. dissertation, The University of Texas, 1968) was used to calculate actual $S_{20,w}$ and molecular weight values based, respectively, on the equations of Noll (14) and Studier (21).

Hybridization experiments. Ribonucleic acid (RNA)-DNA hybridizations were performed on nitrocellulose membrane filters by the method of Gillespie and Spiegelman (6). The messenger RNA (mRNA) fractions used in the hybridization analyses were obtained from cultures of *E. coli* 15 TAU and *E. coli* B/r. When mid-logarithmic growth was attained in an enriched medium (C medium supplemented with 1% Casamino Acids), the bacteria were harvested, washed, and transferred to regular C medium, thus effecting a shift-down in growth conditions. After 5 min of incubation at 37°C, tritiated uridine (2.5 μC per 0.5 μg per ml) was added, and incubation continued for 10 min. Incorporation was stopped by the addition of sodium azide (10^{-2} M) and rapid chilling of the cultures with crushed ice. Induced cultures were exposed to UV light (580 ergs/ mm^2) before growth and labeling under the shift-down conditions. Harvested cells were disrupted in a French pressure cell in the presence of bentonite (2 mg/ml) and ribonuclease-free deoxyribonuclease (2 $\mu\text{g/ml}$). RNA was purified by phenol deproteinization and precipitation with ethyl alcohol. The final product was dialyzed against standard saline citrate buffer. Ribosomal RNA (rRNA) was prepared from bacteria grown in a glucose-salts medium in the presence of tritiated uridine (18.5 μC per 1.5 μg per ml) for 90 min followed by growth for 30 min in a medium containing an excess of unlabeled uridine (100 μg per ml). Ribosomal RNA was purified from a ribosomal pellet ($105,000 \times g$) by methods similar to those used for the preparation of mRNA.

Chemicals. All chemicals used in media and buffers, etc., were reagent grade compounds from commercial sources. Mitomycin C was obtained from Calbio-

chem, Los Angeles, Calif. Radioactive compounds were obtained from New England Nuclear Corp., Boston, Mass. Optical grade CsCl was obtained from the Harshaw Chemical Co., Cleveland, Ohio. Sucrose (ribonuclease-free) was a product of Schwarz BioResearch Inc., Orangeburg, N.Y. Deoxyribonuclease (EC 3.1.4.5) type I from bovine pancreas was obtained from Worthington Biochemical Corp., Freehold, N.J. Sodium dodecyl sarcosinate (Sarkosyl NL 97) was a product of Geigy Industrial Chemicals, Ardsley, N.Y.

RESULTS

Characterizations of particles in the purified preparation. The types of particles obtained in the purified phage pellets were characterized as to their densities in CsCl. The distribution of particle densities obtained under the purification conditions described here is shown in Fig. 1. Three peaks were resolved, the largest peak, designated peak I, banded at a density in CsCl of 1.52 to 1.53 g/cm³, whereas peaks II and III had peak densities in CsCl of 1.5 and 1.49 g/cm³, respectively. The material in each peak was pooled, concentrated by centrifugation, stained negatively with uranyl acetate, and observed by electron microscopy. The particles observed in peak I consisted entirely of phage heads with the neck region attached. Peak II consisted of apparently normal phage particles, whereas the particles in peak III were indistinguishable from those in peak II. The morphology of these particles was the same as had been described in previous reports (4, 5, 12, 18). The head appeared hexagonal in outline in all photomicrographs with a distinct neck region and

a rigid contractile tail. Measurements made on these purified particles indicated that the head has a diameter of 42 nm and the tail is 100 nm long and about 10 nm wide.

Determination of GC content of coliphage 15 DNA. DNA isolated from purified phage pellets was subjected to analytical CsCl density gradient analysis (10). A microdensitometer tracing of the banded coliphage 15 DNA with phage SP8 reference DNA is shown in Fig. 2. The average density of coliphage 15 DNA calculated from two separate preparations of DNA was 1.7055 g/cm³. This density value yielded a calculated GC content of $46.4 \pm 1\%$ for coliphage 15 DNA.

Although CsCl density gradient centrifugation is the preferred technique for estimating the GC content of DNA, the value obtained by this method was corroborated by measuring the UV absorbance-temperature profile (T_m) of the DNA. In Fig. 3, the melting temperature is presented for both coliphage 15 DNA and *E. coli* DNA in standard saline citrate buffer. The absorbance measurements at 260 nm were corrected for the concentration dilution caused by the thermal expansion of water, and the corrected absorbancies at each temperature are shown plotted versus temperature. The 1.4-degree lower melting temperature, for the particle DNA, calculated to a GC content of $46.6 \pm 1\%$ for coliphage 15 DNA. This value corresponds to the value obtained from its buoyant density in CsCl, indicating the probable absence of unusual bases in this DNA.

Hybridization experiments. Further analysis of the DNA contained in the phage particles was made by using the RNA-DNA hybridization technique. The ability of coliphage 15 DNA to form molecular hybrids with mRNA synthesized between 5 and 15 min after induction and rRNA from *E. coli* is shown in Fig. 4. Tritium-labeled mRNA purified from either uninduced or UV-induced cultures of *E. coli* TAU readily formed molecular hybrids with DNA from the particles. However, mRNA from *E. coli* B/r which does not carry the prophage also hybridized with the particle DNA but to a lesser extent. Ribosomal RNA binding to the phage DNA was completely absent at levels well above saturation.

Molecular weight determinations. Three separate constant velocity (isokinetic) sucrose density gradient analyses were performed on coliphage 15 DNA with bacteriophage T2 DNA included in each analysis as a reference. The average sedimentation coefficient ($S_{20,w}$) calculated for coliphage 15 DNA was 35.2 and the average molecular weight value for this DNA was $33 \pm 2 \times 10^6$ daltons. This molecular weight value was not confirmed by an alternative method, but it is presumed to be reasonably correct since the aver-

FIG. 1. Distribution of DNA-containing particles in CsCl. Absorbance at 260 nm versus fraction number (○). Density of CsCl versus fraction number (broken line), and peak density values for peaks I, II, and III are indicated.

age $S_{20,w}$ value for the T2 reference DNA was 58.2. This value corresponds closely to the predicted value of 57.2 for T2 DNA made by Studier, which was based on a molecular weight of 134×10^6 calculated from an analytical zone sedimentation analysis of single-stranded T2 DNA (21).

DISCUSSION

The results of a recent investigation on DNA homologies in lysogenic systems complement the findings of the present study. Using thermal elution chromatography of DNA-DNA hybrids, Cowie and Szafranski (2) measured the homologies existing between the DNA of lysogenic bacteriophages, including coliphage 15, and their hosts as well as the homologies between various phage types. Similar to other lysogenic bacteriophages, coliphage 15 DNA exhibited some homology with the host cell DNA (2). The degree of homology displayed, however, was not as great as that exhibited by bacteriophage lambda DNA, and no evidence was found of interphage homology between lambda and coliphage 15 (2). Also, a slightly lower elution temperature was noted for the reaction between coliphage 15 DNA and *E. coli* B DNA than for either homologous reaction (2). This event was attributed to either a lower average GC content for homologous portions of the phage DNA or to imperfect base sequence homologies between the two DNA molecules (2).

An increase in 6-methylaminopurine (6-methyladenine) content of bacterial DNA has been noted when *E. coli* 15T⁻ (thymineless) is grown in a medium containing low levels of thymine or thymine analogues (3). Exposure to inducing agents such as mitomycin C or nalidixic acid (Medoff and Schwarz, *Bacteriol. Proc.*, p. 115, 1967; Yudelevich and Gold, *Fed. Proc.* 26:450, 1967) produced results similar to the thymine analogues. The degree to which coliphage 15 DNA also contains 6-methylaminopurine in its DNA has not been established. However, the close agreement in base composition data obtained from the density gradient analysis and temperature denaturation curves argues against the occurrence of substantial levels of unusual bases in coliphage 15 DNA. The apparent asymmetry of the coliphage 15 DNA band shown in the microdensitometer tracing (Fig. 2), is an artifact introduced by a technical problem in the recording microdensitometer. The thermal denaturation curve of coliphage 15 DNA (Fig. 3) shows a sharp transition profile differing from the broad transition observed in bacteriophage lambda DNA (8). This result suggests the absence of intramolecular base composition heterogeneity in

FIG. 2. Microdensitometer tracing of UV-absorption photograph of 1.2 μ g of coliphage 15 DNA obtained from total phage pellet and 0.5 μ g bacteriophage SP8 DNA in CsCl. Centrifugation for 21 hr at 25 C.

FIG. 3. Thermal denaturation curves of coliphage 15 DNA (●) and *E. coli* DNA (○) in standard saline citrate buffer. The absorbance at 260 nm, corrected for the volume expansion of water above 25 C, plotted versus temperature. The arrows indicate the midpoint of each transition (T_m).

coliphage 15 DNA similar to that found in lambda DNA (8).

A previous report from this laboratory noted that when a uracil-requiring derivative of *E. coli* 15T⁻ was exposed to inducing agents, a stimulated incorporation of radioactive uridine into RNA ensued under shift-down growth conditions (5). Since the synthesis of rRNA and transfer RNA are temporarily inhibited by these growth

conditions, this increased incorporation of uridine into mRNA was thought to reflect the synthesis of phage-specific RNA (5). Incorporation of increased levels of radioactive uridine by induced cultures was evident 10 min after the commencement of incubation under the shift-down growth conditions (5). The labeling differential between induced and uninduced cultures increased during 50 min of subsequent incubation (5). In the present study, an effort was made to characterize qualitatively the mRNA synthesized early after the induction of *E. coli* 15 TAU. By utilizing labeling and growth techniques similar to those just described, mRNA synthesized between 5 and 15 min after induction was tested for its ability to form ribonuclease-resistant molecular hybrids with coliphage 15 DNA. A limiting problem in the present investigation was the amount of coliphage 15 DNA available for this purpose. The yields of DNA-containing particles from 5-liter lysates of *E. coli* TAU were too low to supply saturating levels of DNA similar to those used in hybridization studies conducted after the depression of lambda prophage (7, 20). The RNA loading curves shown in Fig. 4 indicate, therefore, that the RNA synthesized by *E. coli* 15 TAU early after induction seems to have only a slightly greater capacity for binding to coliphage 15 DNA than the RNA prepared from uninduced cells. This result is in agreement with the study made by Sly, Echols, and Adler (20) on a mitomycin C-induced culture of *E. coli* W 3104 lysogenic for lambda. These workers demonstrated that phage-specific RNA did not increase greatly during the first 30 min after induction, but was evidenced by approximately a 60- to 70-fold increase 50 min after induction (20). Similarly, as in the case of lambda prophage repression in which a basal level of phage-specific RNA has been detected in uninduced cells (7, 20), it seems quite probable that the greater binding capacity of mRNA from uninduced cultures of *E. coli* 15 TAU when compared to the level displayed by the mRNA from *E. coli* B/r, reflects the operation of a repression mechanism similar to the one present in the lambda system. Since homology exists between coliphage 15 DNA and *E. coli* DNA (2), mRNA from *E. coli* B/r would be expected to demonstrate some binding capacity with the phage DNA even though the prophage is absent. More extensive quantitative data, particularly at later periods after induction, will be required before the kinetics of coliphage 15-specific RNA synthesis can be accurately compared to the lambda system.

The absence of detectable rRNA cistrons in the viral DNA (Fig. 4), along with previously discussed data on the composition of this DNA,

strengthens the conclusion that coliphage 15 DNA is different from the host cell DNA. Therefore, the inability of coliphage 15 particles to replicate on any of the bacterial strains tested (4, 5, 12, 18) cannot be ascribed to an exclusive incorporation of host cell DNA into phage heads similar to the situation in bacteriophage PBSX which is inducible in cultures of *Bacillus subtilis* (15, 19). It is evident that coliphage 15 belongs to a class of nonreplicating phagelike particles different from bacteriophage PBSX. The reason that coliphage 15 is unable to replicate is unknown. Presumably, mutations in the prophage leading to structural defects in the mature particles could be re-

FIG. 4. RNA-DNA hybridization curves showing the binding of messenger RNA to coliphage 15 DNA (5 µg per membrane). The messenger RNA fractions from UV-induced cultures of *E. coli* 15 TAU (●) and from uninduced cultures of *E. coli* 15 TAU (○) had respective specific activities of 8,215 counts/min per µg and 6,334 counts/min per µg, whereas the messenger RNA from *E. coli* B/r (■) had a specific activity of 6,150 counts/min per µg. The actual counts/min values were normalized on the basis of 6,000 counts/min per µg for each fraction before plotting, and the resulting values are depicted in this graph. The ribosomal RNA (□) had a specific activity of 43,400 counts/min per µg. Hybrids were formed by incubation for 16 hr at 66°C in double-strength standard saline citrate buffer (2 × SSC).

sponsible for this inability. Mennigmann (12) observed that the attachment site of the phage tail to the neck region appears to be particularly vulnerable to separation. The distribution of particles shown in Fig. 1 confirms this observation since the largest peak of DNA-containing particles (peak I) is composed of phage heads having only the neck region attached. The small peak designated as peak III was observed in numerous gradient separations of these particles. The particles isolated in this peak, although indistinguishable from those in peak II by the staining technique used, may contain either slightly more protein or less DNA to account for their lower buoyant density. Studies are being continued on the lytic capacities of the three groups of particles isolated by CsCl density gradient centrifugation.

Bradley (1) has stated that bacteriocins consist of either nonparticulate entities or of larger particles which are phagelike or consist of phage subunits. Depending upon one's choice of terminology, therefore, coliphage 15 can be classified as either a defective bacteriophage or as a colicin, since the detached tail structures present in culture lysates were demonstrated to be responsible for "colicin" activity (18).

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