Biochemical and Biophysical Properties of *Hyphomicrobium* Bacteriophage Hy ϕ 30

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Hyphomicrobium bacteriophage Hy ϕ 30 and its nucleic acid were studied to determine some of their biochemical and biophysical properties. The molecular weight of the phage is 55.4×10^6 , and its buoyant density is 1.508 g/ml. The nucleic acid of Hy ϕ 30 is linear, double-stranded DNA with a molecular weight of 29.7×10^6 . The guanine-plus-cytosine content of the DNA was 62% as determined from its melting temperature and buoyant density.

Hyphomicrobium vulgare is an aquatic prosthete bacterium that has been isolated from many sources (9). It is an unusual organism in that its reproductive cycle differs from that of true bacteria (8, 10, 11, 16, 17, 27), which divide by binary fission; *Hyphomicrobium* reproduces by budding. During this process the mother cell produces a hypha, which in turn produces a bud, usually at the tip of the hypha. The bud enlarges, becomes flagellated, and detaches from the hypha. The flagellated daughter cell swims away from the mother cell and eventually loses its flagellum. At this point the daughter cell begins to grow a hypha and the cycle repeats. Since there are many different morphological forms in the reproductive cycle of the organism, *Hyphomicrobium* is an excellent model for the study of cellular morphogenesis.

At present, Hy ϕ 30 is the only bacteriophage reported to be active on *Hyphomicrobium* (2, 8). Because this phage seems only to infect cells in certain stages of the life cycle, it may be useful in studying the mechanisms involved in the morphogenesis of *Hyphomicrobium* (24). In this paper we report some of the biochemical and biophysical properties of Hy ϕ 30 and its nucleic acid.

(This paper is taken in part from a dissertation submitted by R.L.K. in partial fulfillment of the requirements of the Ph.D. degree at West Virginia University.)

MATERIALS AND METHODS

Bacterial strain and bacteriophage. The isolation of the host bacterium *Hyphomicrobium* Hy30 (24) and the bacteriophage Hy ϕ 30 (6) has been reported.

Medium and buffers. Medium MH-69 (6) was used throughout for growth of Hy30 and for dilution of

Hy ϕ 30. When a solid medium was required, 15.0 g of Special Agar Noble (Difco) was added to 1 liter of MH-69 before autoclaving.

Phosphate buffer with added magnesium (PBM) consisted of 1.74 g of Na_2HPO_4 , 1.06 g of KH_2PO_4 , and 0.29 g of MgSO_4 per liter. The composition of the saline-sodium citrate (SSC) buffer was 8.76 g of NaCl and 4.41 g of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ per liter. Before use both buffers were adjusted to pH 7.0.

Phage propagation. To propagate large batches of Hy ϕ 30, a mid-log culture of Hy30 was inoculated with phage at a multiplicity of infection of 0.1 and incubated on a rotary shaker at 30°C. After 40 h the cells were removed by centrifugation at $6,800 \times g$ for 30 min. The supernatant fluid was decanted, and the crude phage lysate was stored at 4°C.

Phage concentration and purification. Hy ϕ 30 was concentrated by using polyethylene glycol at a 10% (wt/vol) concentration according to the method of Yamamoto et al. (26). After incubation overnight at 4°C, the phage was pelleted by centrifugation at $6,800 \times g$ for 30 min, resuspended in PBM, and dialyzed against PBM. After dialysis, the phage was pelleted by centrifugation in a Beckman fixed-angle 30 rotor at 25,000 rpm for 3 h, resuspended in PBM, and sedimented through a 10 to 40% sucrose gradient, using a Beckman SW25.1 rotor at 20,000 rpm for 1 h. The phage band was withdrawn and further purified by extensive dialysis against PBM. The phage suspension was then filter sterilized, titered, and stored at 4°C.

Phage assay. Viable numbers of phage were determined by a modification of the agar layer method (3). Assay plates were incubated at 30°C for 72 h before scoring.

Extraction of DNA. Phage DNA was extracted from purified Hy ϕ 30 with phenol according to the method of Mandel and Hershey (13). The aqueous extract was dialyzed against SSC at 4°C to remove the phenol. The DNA was stored at 4°C.

Determination of DNA and protein. Qualitative and quantitative DNA determinations were performed by the diphenylamine reaction as described by Burton (4). Calf thymus DNA was used as a standard. All quantitative protein determinations

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were done by the method of Lowry et al. (12), with bovine serum albumin as a standard.

Enzyme treatment of phage nucleic acid. Hy ϕ 30 nucleic acid (10 μ g/ml) in PBM was treated with DNase I (1.0 μ g/ml), obtained from Sigma Chemical Co. The optical density at 260 nm was followed with time, using a Gilford spectrophotometer 240 with an attached recorder. The procedure was repeated with bovine pancreatic RNase (Sigma) that had been heated for 30 min at 80°C just before use to inactivate any trace amounts of DNase.

Formaldehyde treatment of phage DNA. To determine whether Hy ϕ 30 DNA was single or double stranded, it was reacted with formaldehyde according to the method of Sinsheimer (22). Known single-stranded DNA (heat-denatured calf thymus) and double-stranded DNA (native calf thymus) were run as controls.

Melting temperature of phage DNA. The mol% guanine plus cytosine (G+C) of Hy ϕ 30 DNA was determined from its thermal denaturation temperature (T_m) by a modification of the method of Marmur and Doty (15). Calf thymus DNA was used as a control.

Buoyant density of phage DNA. Buoyant density was determined by isopycnic density gradient centrifugation in CsCl (21). *Bacillus licheniformis* DNA was used as a control.

Buoyant density of phage particles. The buoyant density of Hy ϕ 30 was determined by isopycnic density gradient centrifugation in CsCl. After centrifugation, the bottom of the tube was punctured, fractions were collected, the density of every fifth fraction was determined, and each fraction was assayed for PFU.

Sedimentation velocities. Sedimentation coefficients (S values) were determined by the boundary sedimentation method, using a Spinco model E analytical ultracentrifuge equipped with a schlieren optical system. S values were corrected to zero concentration and water at 20°C ($s_{20,w}$).

Molecular weights. The molecular weight of Hy ϕ 30 was determined from its $s_{20,w}$ value, using the equation of Pitout et al. (18). The molecular weight of Hy ϕ 30 DNA was determined from its $s_{20,w}$ value by the equation of Freifelder (5).

RESULTS

Biochemical and biophysical properties of Hy ϕ 30 phage particles. The buoyant density, molecular weight, and chemical composition of Hy ϕ 30 were determined. The average buoyant density of the phage, calculated from six separate determinations, was 1.508 g/ml. Dilution of phage from CsCl resulted in inactivation of the phage. More than 6 logs of phage infectivity was lost. If phage dilutions were made in MH-69 containing 40% sucrose, only 4 logs of infectivity was lost.

The sedimentation coefficient of Hy ϕ 30 was 492S. This value yielded a molecular weight of 55.4×10^6 when substituted into the equation published by Pitout et al. (18). The bacteri-

ophage sedimented as a single symmetrical peak during a 20-min run, indicating that the phage preparation was homogeneous.

When the amounts of DNA and protein in a suspension of purified phage were determined, Hy ϕ 30 was found to contain 53% DNA and 47% protein. No chemical analyses were done to determine whether lipid was present.

Phage nucleic acid. Hy ϕ 30 has been reported to contain double-stranded DNA on the basis of fluorescent staining with acridine orange (6). To confirm this, the nucleic acid extracted from Hy ϕ 30 was treated with nucleases (Fig. 1). Digestion of the phage nucleic acid by DNase but not by RNase indicated that it was DNA.

Formaldehyde has been shown to react with single-stranded but not with double-stranded DNA (7, 22). Treatment of Hy ϕ 30 DNA with formaldehyde did not appreciably effect its UV adsorption profile even after 24 h of incubation (Fig. 2A). However, heat-denatured phage DNA reacted with the formaldehyde (Fig. 2B), showing the increase in adsorbance at 260 nm and the shift of the adsorbance peak towards 265 nm, which are characteristic of single-stranded DNA reacted with formaldehyde. These two experiments demonstrated that Hy ϕ 30 contains double-stranded DNA.

Thermal denaturation of phage DNA. Hy ϕ 30 DNA melted over a narrow temperature range. Calf thymus DNA was melted simultaneously as a control and showed, as expected, a

FIG. 1. Treatment of Hy ϕ 30 nucleic acid with nucleases. Phage nucleic acid was treated with either DNase I, bovine pancreatic RNase, or bovine serum albumin. The optical density (OD) of the mixture was followed with time.

greater heterogeneity in its melting profile. Duplicate samples from three different phage preparations were melted. By inserting the average T_m value of Hy ϕ 30 DNA, 93.9°C, into the equation of Marmur and Doty (15), a mol% G+C value of 60.0 was calculated. The average T_m for calf thymus DNA was 87.05°C; the literature reports 87.0°C for this DNA (15).

Buoyant density of phage DNA. The second method used to define the base composition of Hy ϕ 30 DNA was to correlate buoyant density with mol% G+C according to the method of Schildkraut et al. (21). The average buoyant density of Hy ϕ 30 DNA was 1.723 g/ml; this yielded a mol% G+C value of 64.2 when substituted into the equation given by Schildkraut et al. (21). *B. licheniformis* DNA, run as a control, had a density comparable to that reported in the literature.

Sedimentation velocity of phage DNA. The $s_{20,w}$ value for Hy ϕ 30 DNA was determined to be 34.5. When this $s_{20,w}$ value was substituted into Freifelder's equation (5), a molecular

weight of 29.7×10^6 was calculated. The bacteriophage DNA sedimented as a single sharp peak during an 80-min run, indicating that the preparation was homogeneous and that the DNA molecules had not been sheared during extraction. On the basis of the molecular weights of the complete phage particle and its DNA, Hy ϕ 30 is 54% DNA and 46% protein.

DISCUSSION

Some of the biochemical and biophysical properties of *Hyphomicrobium* bacteriophage Hy ϕ 30 and its nucleic acid have been determined. Table 1 summarizes their properties. Calculation of the molecular weight of the phage particle from its $s_{20,w}$ value gave $55.4 \times 10^6 \pm 2.7 \times 10^6$. This value is in good agreement with the molecular weights of other bacteriophage such as T7 and PL-25, which have a similar morphology and size (18).

Physical and chemical determinations are in agreement as to the composition of Hy ϕ 30. The phage is approximately 54% DNA and apparently contains only double-stranded DNA and protein.

The G+C content of the phage DNA was determined to be 60.0 and 64.2% when calculated from melting temperature and density determinations, respectively. Since the average values for the two methods varied by about 4% G+C and in some cases individual values were within 1% G+C, it was felt that these determinations gave a true indication of the base composition of Hy ϕ 30 DNA. The similarity of the results, obtained by the two different methods, suggests that few, if any, unusual bases are present in Hy ϕ 30 DNA. Based on the inherent inconsistency in hand-collecting the fractions used in the buoyant density determinations, the G+C content is probably more accurately represented by the thermal melting determinations. It may be of interest here to note that 70 strains of *hyphomicrobia* have been examined for G+C content and all were found to contain between 59.2 and 66.8% G+C (14).

Calculation of the molecular weight of Hy ϕ 30 DNA yielded $29.7 \times 10^6 \pm 3 \times 10^6$. This agrees with the molecular weights of DNA molecules obtained from a variety of other bacteriophage

FIG. 2. Formaldehyde treatment of Hy ϕ 30 DNA. The solution of phage DNA was scanned in the UV range, and the optical density (OD) was plotted against the wavelength. Samples were scanned before and after treatment with formaldehyde. (A) Native Hy ϕ 30 DNA; (B) denatured Hy ϕ 30 DNA.

TABLE 1. Biochemical and biophysical properties of Hy ϕ 30 and its DNA

Bacteriophage	Density (g/ml)	$s_{20,w}$	Mol wt ($\times 10^{-6}$)	Composition	T_m (°C) in SSC
Hy ϕ 30 particle	1.508	492.0	55.4	54% DNA 46% protein	
Hy ϕ 30 DNA	1.723	34.5	29.7	62% G+C	93.9

(PL-25, gh-1, P22, T7) that have approximately the same head size as Hy ϕ 30 (1, 18-20, 23).

West et al. have recently reported on the characterization of bacteriophage ϕ Cdl (25). This phage infects *Caulobacter*, a bacterium that also has various morphological forms. The characteristics of ϕ Cdl and its DNA reported by West et al. are similar to the characteristics reported for Hy ϕ 30 and its DNA in this paper and by others (6).

ACKNOWLEDGMENTS

We thank Harold Resnick for his help with the model E work.

This investigation was supported in part by Public Health Service General Research Support Grant 5 S01 RR 05347-13.

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