Structure of a Marine Bacteriophage as Revealed by the Negative-Staining Technique

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ABSTRACT

VALENTE, ARTRICE F. (Georgetown University, Washington, D.C.), PETER K. CHEN, RITA R. COLWELL, AND GEORGE B. CHAPMAN. Structure of a marine bacteriophage as revealed by the negative-staining technique. J. Bacteriol. 91:819-822. 1966.—The morphology of a marine bacteriophage has been determined by negative-staining techniques and electron microscopy. The virus possesses a head, 600 A in diameter, and a tail which may be from 860 to 1,000 A in length. No tail sheath is seen. The appearance of the terminal tail structure is discussed.

Within the past few years, significant advances in our knowledge of bacteriophage morphology have been made as a result of improvements in techniques of specimen preparation. Notable among these has been the negative-staining technique developed by Brenner and Horne (4). Structural features, such as differences in terminal structures at the tip of phage tails, tail sheaths, tail fibers, collars, or discs, have been clearly defined as a result of application of this technique. These findings are fully cited in the review article of Horne and Wildy (6). Included among these have been the knoblike terminal-end structures found on some of the Pseudomonas and Staphylococcus phages, the pointed tail of the T1 and T5 coliphages and typhoid phage 1, the base plate of the typhoid Vi I phage which is similar to the T-even phages, and the short wedge-shaped tail of the coliphage T3 and Brucella phages (3). Slayter et al. (8) described a Pseudomonas phage, E79, which bears a resemblance to the T-even phages in having six tail fibers attached to an ill-defined plate. Eiserling and Romig (5) described a Bacillus subtilis phage which has a long flexible tail with a fiber bundle attached to its tip. Takeya and Amako (11) described a mycobacteriophage (B-1) with a base plate probably with five spikes and another mycobacteriophage, HP, which possesses a base plate with knobs. Phages without tail structures have also been described (2, 7, 12).

Up to the present time, there has been no published report of the results of a study of marine bacteriophages by use of the negative-staining technique. This report presents the results of such a study.

The bacterial virus was isolated by Spencer (9), from the North Sea, in an area remote from terrestrial contamination. It has been designated NCMB (National Collection of Marine Bacteria) 385, and has been found to be a deoxyribonucleic acid (DNA) bacteriophage (Chen et al., Bacteriol. Proc., p. 19, 1965). It has also been found to be highly specific to its original host NCMB 397, a bacterium originally designated as Flavobacterium sp. by Spencer, but which has been shown by more recent work to be a Cytophaga sp. (Colwell, Citarella, and Chen, Can. J. Microbiol. in press). Host specificity was determined by plating the virus against 26 different strains of microorganisms: 14 marine microorganisms, 6 freshwater microorganisms, and 6 strains of Vibrio. Growth temperature range (0 to 30 C), salt requirements, and thermosensitivity of the phage have been tested, and the results confirm the findings reported by Spencer (10).

MATERIALS AND METHODS

Phage preparation. The bacteriophages were extracted from artificial seawater-agar plates, after growth, by the double-layer technique (1) in phosphate buffer (pH 7.0). Bacterial cells and agar debris were removed by two low-speed centrifugations, and the virus particles were then formed into a pellet at 4 C with a Sorvall refrigerated centrifuge (model RC-2) at 18,000 rev/min for 2 hr. Infectivity of the virus was checked before and after high-speed centrifugation. Results showed 75 to 85% recovery of the virus particles. Stability of the virus after resuspension in ammonium acetate was also tested. The virus remained stable in the buffer for several days if maintained at refrigerator temperatures (4 to 5 C).

Electron microscopy. Potassium phosphotungstate (4) was employed as the staining medium. Virus sus-
**FIG. 1.** Field of marine bacteriophages, showing properties of bacteriophages in general, i.e., heads and tails. An empty head displaying the hexagonal shape is seen at arrow. Base plates (A) reveal a triangular configuration. Base plates (B) present a rectangular appearance. × 150,000.

**FIG. 2.** Slightly higher magnification showing both triangular (A) and rectangular (B) configurations. C indicates a homogeneous tail. An extra free tail is seen at D. P designates a prong-type base plate. × 200,000.

**FIG. 3.** Higher magnification of a virus particle. Individual subunits are not detected, but a definite structure is apparent. × 400,000.
pensions were either mixed directly with the phosphotungstate and applied to the support grids by touching the grid to the surface of the mixture or were applied as droplets directly to the grid and then subjected to the phosphotungstate (PTA). Carbon-coated colloidion grids of 150 or 200 mesh were used as the specimen supports. The preparations remained on the grids for 20 to 30 sec before the excess liquid was removed with filter paper. After drying, the grids were ready for examination in the electron microscope. The grids were examined in a Siemens Elmiskop I electron microscope equipped with a 50-μ objective aperture. Micrographs were taken at electronic magnifications of 15,000, 20,000, and 40,000.

RESULTS AND DISCUSSION

The heads of the bacterial viruses were hexagonal in shape and approximately 600 Å in diameter. The hexagonal shape of the head becomes particularly prominent in ghost, i.e., empty heads (arrow, Fig. 1). The tails varied in length from 860 to 1,000 Å, and were generally straight. Occasionally, however, a tail with a slight bend was seen. There was no evidence of a contractile sheath, but an unusual terminal tail structure or base plate was prominent. The portion of the tail proximal to this base plate appeared homogeneous in some viruses (Fig. 3). In others, it gave the appearance of being composed of spherical subunits arranged in a linear array (Fig. 1 and 4). The apparent periodicity sometimes noted in the tail may be a longitudinal density variation arising from such a linear array; it may reflect a true periodic structure, or it may simply result from the formation of puddles of PTA. The first of these possibilities is favored by the authors, after examination of a great many micrographs and in consideration of the nature of these preparations. Tails which appear homogeneous may be in a state of decomposition (C, Fig. 2; upper arrow, Fig. 3) or they may represent a failure of the staining procedure.

Several interpretations may be placed upon the configurations presented by the terminal structure. This structure may be composed of several subunits disposed so as to give either a triangular or rectangular configuration when seen in profile (Fig. 1, 2, and 4). Whether it appears as a rectangular or triangular structure, the base plate usually appears to be composed of two rows of three individual subunits (Fig. 1, 2, and 4). The difference in appearance seems to be determined by the relationship of these two rows to the last subunit comprising the tail. Therefore, a triangular configuration results when the last subunit is more proximal to the base plate, but not in superposition with the base plate, and a rectangular appearance results when the last subunit is more distal to the base plate or in superposition with it. When subunits are visible, three subunits are always seen in each of the two rows comprising the base plate, when the particle is viewed from the side. Therefore, it seems possible that we are dealing, in the case of the base plate, with a structure composed of at least six subunits. Such a structure would account for the arrangement that is consistently seen. Also, in support of the interpretation of individual subunits would be the measurements of the terminal structure, which indicate that each row is 200 Å long. This figure is approximately the sum of the postulated three individual subunits seen on each of the rows. The configurations noted could also arise from a bending or flattening of the tail so that the base plate is viewed directly rather than in profile. This interpretation seems less likely, for the general straightness of the tail suggests a rigidity rather than a flexibility. An additional element of confusion in the manner of interpretation of the base plate is provided by the several phage particles in which a base plate with three prongs appears (P, Fig. 2; upper arrow, Fig. 4). This is, of course, a frequently seen

FIG. 4. Arrows indicate the proximal portion of tails where the arrangement of individual subunits is seen. The longitudinal density variation is apparent. X 172,000.
configuration in the T series of phages. It may be interpreted as a chance disposition of the six subunits discussed above. Further work is in progress which is designed to determine which of these interpretations is correct.

The terminal structures appear to be relatively fragile, and loss of morphological integrity was often seen (Fig. 2), as well as structures which were intact but in which it was impossible to detect individual subunits (Fig. 3). The term subunits has been used in this preliminary study to represent the smallest morphological unit distinguishable. There has been no attempt to equilibrate this structure with the more detailed terminology in this field. However, it seems quite likely that our "subunit" corresponds to the term "capsomere" or "morphological unit," as these terms generally apply to the smallest structure visible by electron microscopy.

In summary, this first study of a marine bacteriophage by means of electron microscopy of negatively stained specimens has revealed a particle with a hexagonal head and a tail, possessing a base plate but no tail sheath. The base plate presents a somewhat enigmatic appearance, reflecting, perhaps, a polymorphic nature or a lability rather greater than is ordinarily encountered.

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Literature Cited