Structure of *Erwinia carotovora* Temperate Bacteriophage 59 and Its DNA

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The temperate phage 59 from *Erwinia carotovora* and its DNA were studied. The phage particles have an icosahedral head and a long noncontractile tail with a base plate. The virus DNA makes up 50% of the total virus and exists as a linear molecule (molecular weight, $2.6 \times 10^6$). A model of virus structural organization is presented.

The study of the biological, structural, and physicochemical properties of *Erwinia carotovora* temperate phages is of taxonomic importance, for these are not presented in the modern classification of bacterial viruses (1, 13). The need for such an investigation is dictated also by the interests of microbiological industries: *E. carotovora* 268 is an active producer of L-asparaginase (an enzyme with antileukemic effect) (14) and appears to be polylysogenic (15). We have previously described certain morphological peculiarities of the temperate phage 59 from *E. carotovora* (9). The aim of this work was to study the macromolecular organization of phage 59 virions, the physicochemical characteristics of the virus, and its DNA.

Indicatory cultures of *Erwinia horticola* 450 were grown in broth containing 1 volume ofaminopeptide (Leningrad Pharmaceutical Plant) and 2 volumes of 0.015 M NaCl. Phage 59 was obtained by the bilayer agar technique, concentrated with polyethyleneglycol (PEG: molecular weight, 6,000) (19), purified in a step gradient of CsCl (1.14 to 1.60 g/cm³), and dialyzed against 0.05 M Tris buffer, pH 7.2. Lambda phage was obtained from the temperature-inducible culture *Escherichia coli* 34 (λC1857) by the traditional method.

Virion DNA was isolated by detergent-phenol extraction (17). For the electron microscopic studies, DNA was obtained according to Freifelder (4), and DNA from *Micrococcus lysodeikticus* was obtained according to Marmur (12).

Purified phage preparations were stained with a 2% alcohol solution of uranyl acetate. DNA preparations for electron microscopy were made as described elsewhere (10) and shadowed with platinum/palladium (3:1). To estimate the true magnification of the microscope, tobacco mosaic virus preparations and a diffraction grating (1,200 lines per mm) were used. Lengths of DNA molecules and their molecular weights were measured according to Wilkins (18).

Virus and DNA sedimentations were done in an analytical ultracentrifuge, MOM 3170-B (Hungary). The rotor speed used for virus sedimentation was 12,000 rpm, and 40,000 rpm for DNA. DNA and virus buoyant densities were estimated according to Mandel et al. (11), using DNA from *M. lysodeikticus* and lambda phage as marker patterns. Phage buoyant density was determined by centrifugation in the preformed CsCl gradient (1.4 to 1.6 g/cm³) at 37,000 rpm for 24 h with subsequent gradient fractionation.

In the preparations of phage 59, particles with a hexagonal head and a long noncontractile tail ending in a base plate were found. Virion heads either had the shape of a regular hexagon (I type) or were more round (II type) or oblong (III type); their diameters were 57 by 63.8 ± 1.0, 57 by 60.2 ± 1.0, and 57 by 66.3 ± 0.7 nm, respectively (Fig. 1a, b, and c).

In some preparations, heads lacking the inner contents were discovered; in these, separate morphological subunits composing the capsid could be distinguished (Fig. 1d). The diameter of a subunit was close to 5.5 nm, and the distance between the centers of two neighboring subunits was almost the same.

The phage tails on the micrographs are straight or arc shaped, 168 ± 5 nm long, and 13.1 ± 0.5 nm in diameter. After the prolonged storage of the phage suspension, empty capsids as well as capsids lacking tails and separate tails can be found in the preparations; here, thickened proximal parts of the tails are visible (Fig. 1f). The phage tail consists of small subunits near 4.0 nm in diameter arranged in 45 ± 4 horizontal disks that are seen as light bands. From the diameter of the tail and the size of a single subunit, one can conclude that one disk contains 6 subunits, the total number of the tail.
FIG. 1. Preparation of phage from *E. carotovora* 268. (b) Heads oriented along different axes of symmetry (5:3:2) are marked with figures. The arrows indicate base plates in different positions on the plane (a, b, and e), tail channel (a and f), and tail proximal parts and elements connecting tail to phage head (f).
subunits being 270 ± 24. In some preparations, the inner channel of the tail can be seen (Fig. 1a).

The base plate of phage 59 has the shape of a trident in lateral projection and of a six-petaled flower in frontal view. This structure, with a size of 24 ± 0.4 nm, consists of six subunits, each connected to an ellipsoid 9.5 nm long. The central hole of the plate, 2.9 nm in diameter, corresponds to the diameter of the tail channel.

The sedimentation coefficient of phage 59 preparations is 406 ± 10S. The relative particle weight of the virion was calculated to be $6 \times 10^7$ daltons. Both preparative and analytical centrifugation in CsCl showed the buoyant density of the virus to be 1.500 g/cm$^3$.

Electron micrographs of DNA from phage 59 revealed linear molecules (Fig. 2). Analysis of 110 DNA molecules isolated by the detergent-phenol method and 115 molecules isolated by the sodium perchlorate method showed the modal contour length of the molecules to be 14.3 ± 0.23 μm in the first case, and 14.61 ± 0.53 μm in the second case, the molecular weights for these molecules being $28.3 \times 10^6 \pm 0.45 \times 10^6$ and $28.66 \times 10^6 \pm 1.04 \times 10^6$, respectively. Thus, both methods of DNA isolation yielded similar results.

The sedimentation coefficient of phage 59 DNA is 30S. According to an empirical formula (3), the average molecular weight of DNA is $26 \times 10^8$. Buoyant density of virus DNA was determined to be 1.702 ± 0.001 g/cm$^3$. From the data for the buoyant density of the virion (1.500 g/cm$^3$) and DNA (1.702 g/cm$^3$) and assuming protein buoyant density to be 1.300 g/cm$^3$, we conclude that the virion contains about 50% nucleic acid (5).

As stated above, electron microscopic analysis of virus particles revealed hexagonal heads of three different types. As the shapes of these heads correspond to the projections of the icosahedron oriented along different axes of symmetry (5:3:2), we conclude that the phage 59 capsid is a regular icosahedron. From the diameter of morphological subunits composing the capsid (5.5 nm) and the icosahedron rib length calculated according to Hosaka (8), one can conclude that six subunits are located on the rib of the virion head. By the method of Horne and Wildy (6, 7), we determined the total number of the capsomeres to be 252. If we assume capsomeres on the vertices (12 in all) to be pentamers and all others hexamers, the total number of the structural subunits would be 1,500. The number of the structural subunits is 60T (T, the triangulation number), hence $T = 25$.

Based upon the morphological and structural peculiarities of phage 59 E. carotovora, this virus can be referred to the fourth group according to the classification of Tikhonenko (16) or to group B according to Bradley (2), and, if the
structure of the tail end is considered, to the third type of such formations (16).

According to the data given above, we propose the model of the macromolecular structure of phage 59 from *E. carotovora* shown in Fig. 3.

**LITERATURE CITED**


