Structure of the Ribonucleic Acid
Bacteriophage R17

CESAR VASQUEZ, NICOLE GRANBOULAN, AND RICHARD M. FRANKLIN

Laboratoire de Microscopie Electronique, Institut de Recherches sur le Cancer, Villejuif, Seine, France, and
Department of Pathology, University of Colorado, School of Medicine, Denver, Colorado

Received for publication 27 July 1966

Abstract

VASQUEZ, CESAR (Institut de Recherches sur le Cancer, Villejuif, Seine, France), NICOLE GRANBOULAN, AND RICHARD M. FRANKLIN. Structure of the ribonucleic acid bacteriophage R17. J. Bacteriol. 92:1779–1786. 1966.—The morphology of bacteriophage R17 was studied by electron microscopy of negatively stained virions. The hexagonal shape, the presence of a maximum of 10 units at the periphery, and especially the observation of central fivefold points of symmetry with neighboring five and six coordinated units indicated icosahedral symmetry with 32 morphological units. Although the exact shape of the polyhedron could not be specified, the number of morphological units agreed with the chemically estimated number of structural units.

Pseudo-crystalline arrays of intracellular ribonucleic acid (RNA) bacteriophage R17 have been observed in ultrathin sections. The individual particles were composed of a central dense core surrounded by a narrow zone which was less dense. The diameter of the virion was about 200 A, and the center to center distance in hexagonal closely packed arrays was 230 A (12). Negatively stained R17 particles attached to fimbriae were spherical, with a diameter of about 200 A. The individual morphological units appeared to be hollow (5). Bradley (1) studied some other RNA phages (ZIK/1, ZJ/1, ZG/1) using the technique of negative staining. The diameter of these phages was about 225 A. The capsid was reportedly composed of 92 units, one of which was believed to have a central hole. These 92 capsomeres displayed icosahedral symmetry.

Thus, the morphology and size of phage R17 seems to be similar to that of other small RNA viruses. Detailed structural analysis by use of both X-ray diffraction and electron microscopy has been carried out on two such viruses, namely, poliomyelitis virus (9, 15, 21) and turnip yellow mosaic virus (TYMV) (10, 16, 17, 19, 20, 23). The present investigation deals with the structure of the R17 capsid, by use of the negative staining technique (2). Two fractions were studied: one corresponding to the complete virus particle, and the other to the “top component” composed of empty shells. The structure of R17 was compared with the structures of poliomyelitis virus and TYMV.

Materials and Methods

Growth and purification of the virus. The Escherichia coli (λ-) strain used as host cell has been described, as well as the media and details of the growth of R17 (12).

Growth of large batches of virus and their purification is based on published procedures (8, 25). Batches (14-liter) of cells grown in MS broth to a titer of 5 × 109 cells per milliliter were infected with 10 plaque-forming units (PFU) of R17 per cell. After 2.5 hr of incubation, the cells were chilled and then lysed by shaking with 10% (v/v) chloroform. The lysate was decanted, and 350 g per liter of ammonium sulfate (enzyme grade, Mann Research Laboratories, Inc., New York, N.Y.) was added. After being stirred overnight at 4 C the precipitate was harvested by continuous-flow centrifugation (Szent-Gyorgi-Blum continuous-flow centrifuge; 16,000 rev/min at 4 C). The pellet was resuspended in 300 ml of tris(hydroxymethyl)aminomethane (Tris)-saline (0.1 m NaCl, 0.05 m Tris, pH 7.6 at 25 C) and emulsified by sonic treatment with an equal volume of Genosolv-D (Trifluoro-trichloroethane, Allied Chemical Corp., General Chemical Division, Morrisstown, N.J.). The phases were separated by centrifugation, and the aqueous phase was collected. The Genosolv phase was mixed with 0.5 volume of Tris-saline, sonicated again,
and centrifuged. The second aqueous phase was added to the first. The virus was sedimented by ultracentrifugation, resuspended in 100 ml of Tris-saline containing 10^{-4} M MgCl₂, and treated with a mixture of ribonuclease (5 µg/ml, ribonuclease A, Worthington Biochemical Corp., Freehold, N.J.) and deoxyribonuclease (5 µg/ml, twice crystallized, Worthington Biochemical Corp.) for 30 min at 37 °C. This was followed by digestion with trypsin (0.1 mg/ml, twice crystallized, Worthington Biochemical Corp.) for 60 min at 37 °C. After two cycles of ultracentrifugation, the virus was centrifuged for 48 hr in a CsCl gradient. Some preparations were treated with pronase (25 µg/ml, 30 to 60 min, 25 °C) just before equilibrium centrifugation. The virus band was collected and dialyzed against Tris-saline.

In the CsCl gradient two viral components were often isolated. The major component (fraction I) was the complete virus, which banded at ρ = 1.40 (25 °C), and the minor component (fraction II), which banded at a buoyant density slightly lower than fraction I, was empty shells. The buoyant density of 1.40 is in agreement with the value of 1.38 reported by Strauss and Sinsheimer (25) for the equilibrium centrifugation of concentrated suspensions of the closely related bacteriophage MS2.

Characteristics of a typical preparation are shown in Table 1. The absence of RNA in fraction II is indicated by the ratio of optical density at 260 µm to that at 280 µm and the low ratio of PFU to optical density at 260 µm. Furthermore, in several studies on bacteriophage preparations labeled with radioisotopes, the following was found. If H³-uridine was used as label, only fraction I was labeled, whereas, if H³-amino acids were used, both fractions I and II were labeled.

Three separate preparations of fraction I had the following particle-PFU ratio: 5.56, 5.67, and 2.78.

Samples of fraction I dialyzed against distilled water for 12 hr were used for the negative staining, and fraction II was used without dialysis.

Electron microscopy. Formvar membranes with holes in them were prepared, and these membranes were strengthened with a layer of carbon. Droplets of virus samples (fractions I and II) were placed on grids covered with the Formvar membranes. The grids were stained with 3% potassium phosphotungstate (Prolabo, Paris, France) adjusted to pH 7. These negatively stained preparations were immediately examined and photographed, especially over holes in the Formvar-carbon substrate, by use of a Siemens Elmiskop I microscope with double-condenser illumina
tion at direct magnifications of 40,000 and 60,000 X. Some grids were negatively stained with potassium phosphotungstate at different concentrations (1 to 4%) or at different pH values from 3 to 12.5. The contrast of individual morphological units was not improved by these variations in phosphotungstate (PTA) concentration or in pH.

RESULTS AND DISCUSSION

Because of stretching of the PTA film over the holes, many of the virus particles were compressed and distorted. Despite this distortion, resolution of the surface detail of virions was much better over holes than over the Formvar substrate, where the noise of the granular background was of the same order of magnitude as the size of the morphological units forming the capsid.

At low magnification, the virus particles and empty shells appeared as spherical or hexagonal in shape (Fig. 1a and b). Two-dimensional crystalline arrays of virus, in a diamond-type lattice, were seen over the holes when the virus solution was applied to grids at an abnormally high concentration (Fig. 1b).

The diameter of virions was about 230 A when measured on isolated particles and 250 A from measurements of the interparticle distance in crystals. As is well known, the diameter of viruses appears larger in negatively stained preparations than in ultrathin sections. The diameter reported for R17 phage is smaller than that of poliomyelitis virus [300 A (15)] and TYMV [248 A (23), 280 to 300 A (16), and 280 A (10, 17)].

Since virus particles are completely enveloped in PTA (10), there is a superposition of detail from the top and bottom of the virion, leading to difficulties in interpreting the arrangement of morphological units. In the majority of the particles, only a few of the morphological units over the capsid were clearly seen, and only a small fraction of the total number of capsids was suitable for analysis. Nevertheless, it was often easy to count the number of morphological units at the periphery of the virus, and their number was never greater than 10 (Fig. 2a, b, c, and e). In most cases, it was only possible to distinguish clearly six to seven morphological units, the

<table>
<thead>
<tr>
<th>Fraction</th>
<th>OD³ at 260 µm</th>
<th>OD³ at 280 µm</th>
<th>Ratio of OD³ at 250 to OD³ at 280 µm</th>
<th>FFU/ml</th>
<th>FFU/OD³ at 260 µm</th>
<th>Particles per ml³</th>
<th>Ratio of particles to FFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6.56</td>
<td>3.66</td>
<td>1.79</td>
<td>2.1 × 10¹⁴</td>
<td>3.2 × 10¹²</td>
<td>1.2 × 10¹⁴</td>
<td>5.6</td>
</tr>
<tr>
<td>II</td>
<td>0.62</td>
<td>0.48</td>
<td>1.29</td>
<td>9.6 × 10¹⁰</td>
<td>1.5 × 10¹²</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

³ Optical density.

Based on A at 260 µm = 8.20 cm²/mg and a virion particle weight of 4.19 × 10⁶ daltons (7).
FIG. 1. Low magnification of negatively stained R17 bacteriophage. (a) Empty shells of bacteriophage R17 with spherical profiles (fraction II); peripheral units are seen. × 150,000. (b) Two-dimensional crystalline array of virus particles (fraction I) in a diamond-type lattice. × 200,000.
others being blurred. Some of these peripheral units were thicker than others. It was very difficult to count the peripheral units in virus particles in crystalline arrays. The hexagonal shape of the R17 phage and the evidence of a preponderance of 10 peripheral units suggested that the morphological units were arranged as a deltahedron with 5:3:2 rotational symmetry, and that there...
were 32 subunits (6). A model was made and compared with the images of the negatively stained particles. It was possible to check three main orientations of the model with the images of the virus. In these positions, 0, 10, or 6 to 7 peripheral units were clearly seen. These cases corresponded to the threefold (Fig. 4c), fivefold (Fig. 2d), and twofold (Fig. 2f) positions, respectively (see legend to Fig. 2). In the latter view, a superposition of morphological units made some of them look thicker than others (Fig. 2f).

The finding of 10 peripheral units is a fortiori against the 92-unit structure suggested by Bradley (1) for three other RNA phages. In the pictures published by Bradley, the contribution from the supporting membrane to the overall pattern of morphological units appears so great that it seems impossible to make any conclusion from these photographs.

The examination of numerous complete R17 particles from fraction I shows that there is more than one central unit, eliminating the possibility of a 12-unit structure, as has been suggested for the small deoxyribonucleic (DNA) bacteriophage \( \phi X \) 174 (14, 26).

Could not a 42-unit structure show the same arrangement of 10 peripheral units? Generally, in this case, about 12 peripheral units are counted (18), and, in our investigation, a maximum of 10 was found. Being aware of the necessity of finding a correlation between these peripheral units and the pattern towards the center of the particle, we tried to identify unequivocally the central morphological units. Because of superposition of details, it was difficult to distinguish them clearly, and many particles showed diffused patterns. In some, however, apparently hollow morphological units were seen, close to the direction of the fivefold (Fig. 2h, i) and the twofold axis (Fig. 2j, k). The diameter of these morphological units was about 60 Å, which was not consistent with the 30-Å diameter measured on the peripheral units. However, it is well known that superposition of a morphological unit on one side of the capsid with another on the opposite side can result in the appearance of one or two morphological units which are larger and more prominent than the others. When the superposition of both sides is alike, especially along the twofold axis, the morphological units can appear to be hollow. If this is the case, we must admit that in bacteriophage R17 they are similar to those of TYMV (10).

Nevertheless, patterns of five- and six-coordinated units having the same diameter as the peripheral units were observed, and no evidence of holes was seen. In Fig. 3, images with regular detail of arrangement of the morphological units are shown. Positive and reverse prints and a model of 32 morphological units (the same as that of Fig. 2, now embedded in black cotton) are shown.

In Fig. 3a, b, and c, one central unit is surrounded by five more units, corresponding to a particle viewed along the fivefold symmetry axis. In this case, peripheral units are distinctly visible, and two neighboring five-coordinated morphological units can be identified by comparing the photographs of virus (Fig. 3a, b) with the model having the same orientation (Fig. 3c). The arrangement of the six-coordinated morphological units in their neighborhood is also discernible.

In Fig. 3d, e, and f, the same arrangement is found, but the fivefold point of symmetry has another orientation, slightly rotated with respect to the central axis of the particle. The distance between the five-coordinate units seems to be in the order of 95 ± 5 Å and between the six-coordinated units, 65 ± 5 Å.

The last series (Fig. 3g, h, and i) shows one morphological unit surrounded by six more, corresponding to a particle viewed along the threefold symmetry axis. In this direction there is a superposition effect at the periphery, and the individual peripheral units cannot be clearly distinguished. In all three cases, the model was rotated to have approximately the same orientation as the virus.

From the preceding findings, we suggest that the capsid of R17 phage is composed of 32 morphological units, 12 located on the fivefold and 20 on the threefold axis of symmetry.

A central diamond, corresponding to the twofold axis of symmetry was extremely rare; the rarity of this orientation may eliminate the rhombic triacontahedron as a possible structure, since this orientation should be most frequently found for this polyhedron according to studies made on TYMV (16) and poliomyelitis virus (21). The pentagonal dodecahedron, with pentagonal pyramids placed on each face, has been proposed for the K rat virus (27); in this case, knobs and concavities deformed the hexagonal shape of the capsid. Although protuberances and concavities were sometimes seen on the R17 virions, they were not at all regular. Therefore, they were probably either the result of a distortion of the particles or due to irregularities of staining.

The polyhedra just discussed, as well as others having the same arrangement of symmetry axes, cannot be easily distinguished in the electron microscope. For this reason, with the information available, it is not possible to definitively choose between them. We can only propose that there are 32 morphological units, probably situated on the lattice points of a \( T = 3 \) icosahedral surface lattice, according to the formula \( 10T + 2 \) (3, 4).
Fig. 3. Three viral particles (arrows), corresponding reverse prints, and a model embedded in black cotton in approximately the same orientation as the particles. The model is based on the icosahedron having 32 columnar units (see Fig. 2d, f). Five-coordinated morphological units are marked with an X. (a, b, c) A particle viewed along the fivefold symmetry axis, showing 10 peripheral units. With reference to the model, it is possible to recognize on the reverse print two neighboring five-coordinated units and the six-coordinated units. × 640,000. (d, e, f) Other particles with a fivefold axis of symmetry slightly rotated with respect to the central axis of the virus. × 640,000 (g, h, i) A threefold axis of symmetry is shown in these pictures. Note that, viewed along this axis, the peripheral units form an almost continuous contour. × 900,000.
With the resolution available, it was not possible to demonstrate that these 32 morphological units are composed of clustered structural units. According to Caspar and Klug (3, 4), there should be 12 pentamers and 20 hexamers and, therefore, a total of 180 structural units. Our model of 12 pentamer and 20 hexamer units (Fig. 4) is suggested by the following considerations. The best estimate for the molecular weight of the protein subunit is 14,200 (8). The total molecular weight of protein per virion is $2.5 \times 10^6$ (7, 11). Therefore, there should be 176 subunits ($2.5 \times 10^6 / 1.42 \times 10^9$), which fits very well the 180 structural units expected ($12 \times 5 + 20 \times 6$) for an icosahedron with $T = 3$ (3, 4).

Thus, the RNA bacteriophage R17 is likely to be in the class of viruses with 32 regularly disposed morphological units, approximately equal in size and arranged in icosahedral symmetry. It is the first bacteriophage to be assigned to this class which already includes one RNA animal virus, poliomyelitis virus (21); one RNA plant virus, TYMV (10, 16, 17, 20, 23); and one DNA animal virus, K rat virus (27).

ACKNOWLEDGMENTS

We are very much indebted to W. Bernhard for his critical remarks, and to A. Nivel due for his excellent technical assistance.

This investigation was supported by grant B-14646 from the National Science Foundation and by Public Health Service grant AI-05320-VI from the National Institute of Allergy and Infectious Diseases.

One of us (C. Vasquez), member of Carrera del Investigador (Consejo Nacional de Investigaciones Cientificas y Tecnicas, Argentina), undertook these investigations during the tenure of an Eleanor Roosevelt Cancer Fellowship of the American Cancer Society awarded by the International Union Against Cancer.

LITERATURE CITED


Fig. 4. A model of the R17 bacteriophage composed of 32 hollow morphological units, 12 pentamers on the 12 fivefold symmetry vertices, and 20 hexamers with threefold symmetry on the surface of the triangles of a regular icosahedron. (a) Viewed along a twofold symmetry axis; (b) viewed along a fivefold symmetry axis; (c) viewed along a threefold symmetry axis.