Structure and Composition of *Xanthomonas pruni* Bacteriophage

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This communication deals with the detailed morphology, buoyant density, and chemical composition of *Xanthomonas pruni* phage XP5 (Mandell & Eisenstark, 1953).

In agreement with earlier descriptions (Thornberry, Eisenstark & Anderson, 1948; Hickman, 1955), electron micrographs revealed that the phage has a polyhedral head, which, via a neck region, connects with a contractile tail. The phage head has a hexagonal outline, 600 Å across opposite apices.

The ‘neck’ connecting the phage head and tail is 80 Å in length, but the exact point at which it connects to head and tail is not clearly defined. The contractile tail is composed of an outer sheath and a central core, terminating with a base plate. Attached to the base plate are six tail spikes, each 100 Å long. In its extended form the sheath is 800 × 200 Å, with 20 cross bands (Pl. 1, fig. 1, 2). After contraction, the sheath dimensions are changed to 400 × 260 Å with 10 cross bands. On the exposed portion of the central core in the contracted particles 11 cross bands can be counted. This portion of the central core is 440 × 100 Å. The central canal through the core is 30 Å in diameter (Pl. 1, fig. 4, 6).

Plate 1, fig. 3, is a schematic diagram summarizing the gross construction of phage XP5 with dimensions of the various structures. Plate 1, fig. 7, shows two contracted particles with emptied and collapsed heads. Plate 1, fig. 8, shows two contracted particles where the tail sheaths are half way off the central cores.

No tail fibres similar to those of the T-even phages of *Escherichia coli* were found on phage XP5.

The size and shape of phage XP5 are in close conformity with earlier observations of Thornberry et al. (1948) and Hickman (1955), except for the tail length. This difference is probably due to the fact that they did not recognize the tail spikes which they may have included in their measurements of the tail.

For buoyant density and chemical studies, as well as for electron microscopy, an important step in the purification of phage samples was to treat them with antiserum against host cells. *Xanthomonas pruni* produced a pigmented capsular material which was difficult to separate from phage particles, even in density gradient centrifugation. After antiserum treatment, however, this contaminating material was easily separated upon suspension in caesium chloride and centrifuging. Phage bands were removed from centrifuge tubes with a tuberculin syringe and dialysed against several changes of distilled water or 5 × 10⁻⁴ M magnesium acetate.

For determination of densities, phage suspensions were layered on preformed CsCl gradients and centrifuged in Spinco Model L SW39 rotor at 28,000 rev./min. After 24 to 36 hr of centrifugation, drops were collected for subsequent plaque titration, determination of density, and chemical analysis. Buoyant density of phage XP5 was determined by centrifuging concentrated phage preparations (10¹³ p.f.u./ml) in a
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cæsium chloride density gradient. This resulted in the formation of four to six distinct bands. Samples from each of the bands were collected in separate tubes, plaque titrated, and their density measured. The band with maximum absorption and density of 1.45 g. cm.−3 showed the highest phage titre and obviously consisted of intact phage particles. The other bands contained particles with contaminating materials. Phage purified twice in CsCl density gradient formed a single sharp, white, opaque band, free of the yellow pigment present in the original phage preparation.

Phage T5 of Escherichia coli was used as a density marker in all the experiments since it was initially thought to resemble Xanthomonas pruni phage in size and shape. The buoyant density of T5 in CsCl solution was 1.508 g. cm.−3, calculated from the refractive index. This value is close to the average of buoyant density of DNA (1.7 g. cm.−3) and of protein (1.3 g. cm.−3), suggesting that phage T5 contains approximately equal amounts of DNA and protein. Phage XP5, though nearly equal in size to T5,

Table 1. Comparison of some chemical and physical properties of XP5 phage with that of its host and other phages

<table>
<thead>
<tr>
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<th>Phage</th>
<th>Host</th>
<th>Phage</th>
<th>Phage</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>XP5</td>
<td>X. pruni</td>
<td>T5</td>
<td>T2</td>
</tr>
<tr>
<td>Density (phage particle)</td>
<td>1.450</td>
<td>—</td>
<td>1.508</td>
<td>—</td>
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<tr>
<td>Density (DNA) in CsCl</td>
<td>1.722</td>
<td>1.725</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Calculated GC%</td>
<td>61.5</td>
<td>61.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Density (DNA) in Cs2SO4</td>
<td>1.430</td>
<td>1.432</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Calculated GC%</td>
<td>64.5</td>
<td>65.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tm(°C)</td>
<td>95.5</td>
<td>96.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Calculated GC%</td>
<td>61.9</td>
<td>66.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DNA content (µg./p.f.u.)</td>
<td>0.856 x 10−10</td>
<td>—</td>
<td>2 x 10−10</td>
<td></td>
</tr>
<tr>
<td>Protein content (µg./p.f.u.)</td>
<td>1.83 x 10−10</td>
<td>—</td>
<td>3 x 10−10</td>
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</tbody>
</table>

was found to be much lighter with a buoyant density of 1.45 g. cm.−3. This indicated the possibility of some difference in overall chemical composition when compared to T5. Low density of XP5 could be attributed to either: (a) the fraction of DNA in the intact phage is less in XP5 than in T5, or (b) low fraction of guanine + cytosine to adenine + thymine (GC/AT) in the phage DNA. These two possibilities were examined.

To calculate the GC/AT content of the DNA in XP5, buoyant density and melting temperature (Tm) measurements were made.

When centrifuged in CsCl (final density 1.70 g. cm.−3) at 25,000 rev./min. for 72 hr in the Spinco ultracentrifuge model L, SW39 rotor, DNA from XP5 banded at a distance of 23 mm. from the bottom of the tube and had a buoyant density of 1.72 g. cm.−3 (Table 1).

In determinations of melting-points (Marmur & Doty, 1962), XP5 DNA had a sharp melting profile with the midpoint Tm at 95.5° (Table 1). The helix-coil transition upon heating, was over a comparatively narrower range (only 6°) than that observed in bacterial DNA from Xanthomonas pruni, which was used as a control.

The value of mole percentage of guanine + cytosine (G+C) in the DNA calculated from the linear relation between Tm and G+C agreed with the value calculated from that between buoyant density and G+C, within the expected probable error (Table 1).

For chemical analyses, samples of XP5 phage of known titre were hydrolysed with formic acid for 1 hr at 175°. Protein and DNA were then determined colorimetrically (Lowry et al. 1951). Phage T2 of Escherichia coli was used as a control.
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Hydrolysed samples of purified phage XP5 DNA gave four distinct spots on two-dimensional chromatography. Spectrophotometric analysis of samples eluted from these spots and comparison of the data thus obtained with that of standard samples revealed that the phage contained the usual four bases of DNA. No unusual base was detected in a series of experiments.

The ratio of protein and DNA in T2 phage used as control was 1.5:1, whereas in XP5 it was observed to be approximately 2:1 (Table 1). This higher ratio could be accounted for by the amount of sheath and head proteins needed to encase a smaller phage with resulting lower density. There was no evidence of any abnormal DNA content that might contribute to the lower density.

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REFERENCES


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EXPLANATION OF PLATE

Preparations for fig. 1 to 5 and 9 to 11 were embedded in 1% uranyl acetate, while those in fig. 7 and 8 were embedded in 2% potassium phoshotungstate.

Fig. 1. An extended particle of phage XP 5 with discernible capsomeres, sheath cross-bands, sheath polymeric subunits and five to six tail spikes.

Fig. 2 is similar to fig. 1, except that some variation is seen in the number and placement of the tail spikes.

Fig. 3. Diagram in two dimensions showing the gross structure of phage XP 5. All of the numbers are in Å units.

Fig. 4. A contracted particle with the base plate separated, exposed central core and a few tail spikes attached to the base plate.

Fig. 5. Two contracted particles. In one, the base plate appears to be detached and lost. On the particle on the right, 11 cross-bands can be counted on the exposed portion of the central core, and 10 cross-bands on the sheath. Also, a detached central core may be seen.

Fig. 6 is similar to fig. 5. It shows the correspondence between the sheath subunits and the core subunits, as discussed in this paper.

Fig. 7. Two contracted particles with emptied and collapsed heads, and a ‘monster’ which is probably an artifact.

Fig. 8. Two contracted particles in which the sheath has moved away from the central core.
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