Morphology of Actinophage \( \Phi 17 \)

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SUMMARY

The negative staining technique was used to study the morphology of actinophage \( \Phi 17 \) which infects Streptomyces chrysomallus strain s17. It was found that the particle does not possess the characteristic tail of the bacteriophages, and that its capsid is built of distinct subunits.

INTRODUCTION

Some streptomycete phages have already been described (see references in Bacq & Dierickx, 1962); they comprise a head of about 800 Å diameter and a tail up to 2800 Å long. Mach (1962) observed a substructure in the tail of a Streptomyces olivaceus phage; this consisted of discs 45 to 50 Å thick, but was not contractile. The present paper deals with observations made on the phage \( \Phi 17 \) which infects strains of streptomycyes from the \( S. griseus \) group (Welsch, Corbaz & Ettlinger, 1957). The normal host strain used for its isolation and propagation is an actinomycin C-producing \( S. chrysomallus \) s17 (Welsch, Minon & Schönfeld, 1955).

METHODS

A concentrated and highly purified preparation of phage \( \Phi 17 \) was obtained using methods described elsewhere (Bacq & Dierickx, 1962). The material examined contained \( 10^{13} \) plaque-forming units (pfu)/ml. and was prepared for electron microscopic observation by the negative staining technique according to Brenner & Horne (1959).

RESULTS

Electron micrographs revealed particles of \( 625 \pm 30 \) Å average diameter (60 measurements) and of hexagonal outline (Pl. 1, fig. 1). No appendage in the form of a tail structure was visible. Actinophage \( \Phi 17 \) is normally stable in phosphate buffer (pH 6.5, ionic strength 0.1) but was found to be very sensitive to dialysis against ammonium acetate buffer or distilled water and only empty capsids (Lwoff, Anderson & Jacob, 1959) were seen after dialysis. This preparation had been kept frozen (\( -80^\circ \)); this did not modify its infectivity but increased its sensitivity towards further treatments. The complete particles (Pl. 1, fig. 1) were observed when the preparation was fixed with 1% (w/v) formaldehyde in phosphate buffer for 20 min. and dialysed overnight against distilled water before negative staining. The
percentage of empty capsids was in good agreement with the degree of inactivation calculated for the initial suspension from the measurement of the absorption at 260 μm per infective particle (Bacq & Dierickx, 1962). The empty capsids kept the polygonal shape of the intact particles; measurements on electron micrographs indicate an average diameter of 595 ± 25 Å. The crude lysate when examined by the same methods revealed particles of identical morphology; hence the tailless character is probably not an artifact due to the preparation procedures. Empty capsids prepared by dialysis against distilled water appeared to be composed of subunits or capsomeres (Pl. 1, fig. 2). It was not possible to determine the precise number of capsomeres forming the shell. When the phage was lyophilized and resuspended in distilled water before examination, the empty capsids seemed to aggregate by means of short projections borne by the capsomeres (Pl. 2, fig. 3). These projections may be like those observed in electron micrographs of ΦX174 bacteriophage by Tromans & Horne (1961). The capsomeres have a centre-to-centre spacing of 70 Å. As estimated from the pictures of the empty particles, the protein shell is 20–35 Å thick. These small dimensions of the surface components and a close packing would explain that the subunits are difficult to resolve on the entire particle because the phosphotungstate could not penetrate between them.

Sometimes, when specimens were mixed with the stain at pH 4.5 without previous dialysis, an isolated structure of hexagonal outline was observed (Pl. 2, fig. 4). The external diameter is estimated at 105 –110 Å, with a central hole of a diameter of 35 Å. Thin fibres of 15 Å diameter and up to 140 Å long are attached to the hexagonal structure. A tentative interpretation of the isolated hexagonal plate is that it forms a type of fixation plate as observed in other phages (Brenner et al. 1959; Bradley & Kay, 1960). The arrow (Pl. 2, fig. 3) points to a ring-like design on the capsid, the diameter of which is slightly larger than the isolated hexagonal component, and which could be its site of localization on the complete particle. It remains to be seen whether these two structural features are related to an adsorption mechanism. From Pl. 2, figs. 3, 4, the plate structure appears to be located most probably on a vertex of the polygonal head outline.

**DISCUSSION**

The position of the plate structure raises the question of the three-dimensional shape of the virus particle, for in the case of T2 phage the idea has been put forward that the various symmetries of the head and tail components are related (Horne & Wildy, 1961). The plate structure being hexagonal, a vertex of the particle is characterized by a sixfold axis of symmetry; consequently the shape of the total phage should be that of an hexagonal bipyramidal prism, but less elongated than in the T2 phage head, to give a more hexagonal profile. Pictures of empty capsids as those shown in Pl. 2, fig. 6(a, b), have been interpreted in other instances (Bradley & Kay, 1960) as derived from icosahedral capsids, but this type of symmetry seems unlikely in the case of Φ17.

In conclusion, Φ17 is different from actinophages hitherto described. It can be associated with other tailless phage particles, such as bacteriophage P22 studied by Anderson (1961), to form a new morphological group. The interesting feature in Φ17 is the substructure of its capsid, which suggests that it is built of small
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subunits according to symmetry patterns described in other viruses (Horne & Wildy, 1961). The capsomeres or subunits are probably of very small dimensions and different from those observed on coliphage T5 by Bradley & Kay (1960).

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REFERENCES


EXPLANATION OF PLATES

Electron micrographs of negatively stained preparations of actinophage Φ 17.

PLATE 1.

Fig. 1. Particles fixed with 1 % (w/v) formaldehyde. × 115,000.

Fig. 2. Empty shells obtained by dialysis against distilled water. × 202,500.

PLATE 2

Fig. 3. Shells of lyophilized phages. × 300,000.

Fig. 4. Isolated hollow structure. × 360,000.

Fig. 5. Particles fixed with 1 % (w/v) formaldehyde, showing a fixation structure. × 187,500.

Fig. 6. (a, b, c). Different aspects of empty shells. × 300,000.