The Structure of Bacteriophage ϕ R

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SUMMARY

Bacteriophage ϕ R was examined in the electron microscope by the metal shadowing and negative-contrast techniques. The particle was a very small icosahedron and appeared to possess an extremely small tail. Some information was also obtained about the structure of the protein coat.

INTRODUCTION

The history of bacteriophage ϕ R which attacks *Escherichia coli* and *Salmonella typhi* was described by Kay (1961). It resembles the minute bacteriophages S13 (Zahler, 1958) and ϕ X-174 (Sinsheimer, 1959) which are themselves closely related and it possesses in common with them a single-stranded form of deoxyribonucleic acid in contrast to the double helical type found in the larger phages. Hall, Maclean & Tessman (1959) examined ϕ X-174 in the electron microscope by shadowcasting and by the negative-contrast technique of Brenner *et al.* (1959). They found that virus particle was an extremely small polyhedron 248 ± 10 Å across with a pentagonal symmetry and no apparent tail. Little information was obtained about the three-dimensional structure of the particle. The present paper describes information on bacteriophage ϕ R obtained by using a variety of different electron microscope specimen preparation techniques.

METHODS

Preparation of the phage suspension. The methods used for the growth and purification of the phage used in this work were described by Kay (1962). In the final stage of purification, the phage was eluted from a diethylaminoethyl cellulose column in 0.2M-ammonium acetate at pH 7.4. The eluate contained 2.9×10^{12} plaque-forming particles/ml. This preparation had the advantage of being in a completely volatile buffer; as can be seen in Fig. 4 which is typical of many fields examined, it contained very little material other than phage particles.

Electron microscope preparation techniques

The phage particles were examined by the following methods: platinum metal shadowing, platinum+carbon shadowing (Bradley, 1959), a negative contrast method with lanthanum acetate; a negative contrast method with uranyl

acetate (Bradley & Kay, 1960). Details are as follows. Platinum shadowing was carried out by drying a suspension of phage in ammonium acetate on freshly cleaved mica (Hall & Litt, 1958) followed by shadowing with platinum. The shadowing layer was backed with a film of evaporated carbon and the composite film floated on a water surface from which it was picked up on support grids. Most of the phage particles remained attached to the mica so that a replica was in fact obtained.

Platinum + carbon shadowing was carried out according to the methods of Bradley (1959) using platinum + carbon rods of average composition 60 % carbon and 40 % platinum at a working distance of 4 cm. with a 2 mm. intermediate aperture and an evaporating angle of 2:1. The particles were first mounted on freshly cleaved mica as with the platinum shadowing. The platinum + carbon film was floated on water and then mounted on grids.

The preparation with lanthanum acetate was carried out by mixing an equal volume of phage suspension (in 0.2M-ammonium acetate) with a 1 % (w/v) solution of lanthanum acetate (pH 4.5). The resulting suspension (pH 5.2) was dried on a carbon-coated grid.

In the case of uranyl acetate, a similar procedure was used, a 1 % (w/v) solution of uranyl acetate (pH 3.5) replacing the lanthanum acetate. The mixture was at about pH 4.2.

RESULTS

Platinum-shadowed particles

Micrographs similar to those of Hall *et al.* (1959) were obtained. Plate 1, fig. 1, shows that, in spite of a particularly granular shadowing layer, the six knobs observed by these authors were present in the ϕ R specimen. Most of the particles were orientated so that one knob is surrounded by five others in regular pentagonal array. A number of particles exhibited a completely different orientation, however; one of these is illustrated in Pl. 2, fig. 2, where eight knobs can be seen. This was not observed by Hall *et al.* (1959) who found that all their particles were in the first orientation. The appearance of the particle is exactly consistent with an icosahedron having twelve subunits. (An icosahedron is a regular solid body which has twenty equiangular triangular faces, twelve apices and a 5:3:2 symmetry.) In the first case (Pl. 1, fig. 1) it is viewed down its five-fold symmetry axis, and in the second (Pl. 1, fig. 2) down its two-fold axis. This implies that the phages are standing either on an apex or an edge. It will also be noticed that there is an appreciable gap between subunits, particularly in Pl. 1, fig. 2, indicating that, in all probability, the knobs do not represent the single subunits of a protein coat.

Platinum + carbon shadowing

A heavily shadowed replica was prepared so that the combination of high contrast and sharp shadows obtained under such conditions would provide information about the geometrical form of the phage particle. Smith & Williams (1958) first used the analysis of shadow shape to determine the form of a virus particle. The same specimen was shadowed from two directions so that each particle cast two shadows. By comparing the shadow shapes with those obtained from models illuminated from different directions, it was possible to show that the virus in question, *Tipula* iridescent virus, was an icosahedron. It is not in fact necessary to shadow from two

directions, since a particle orientated suitably gave unmistakable characteristic shadow shapes. This fact was also recognized by Harrison & Nixon (1960) in a study of small plant viruses.

The heavily shadowed preparation of phage ϕR did not show up the knobs seen with the platinum-shadowed particles, because they were obscured by the thick shadowing film. A few particles did, however, exhibit the shadow shape shown in Pl. 1, fig. 3, the flat-topped one being typical of an icosahedron lying on a face, and the pointed one also lying on a face, but differing by 60° in orientation with respect to the shadowing direction. By far the greater majority of particles exhibited pointed shadows, since the preferred orientation was the same as with the platinum shadowed preparation, there being no difference in the method of deposition onto the mica substrate.

Particles shadowed more lightly with platinum + carbon showed up the knob structure, and also, in this particular replica, large areas of regularly packed phages were found (Pl. 2, fig. 4). It will be noticed that the packing is not truly hexagonal (the angle between the lines made by the particles is 108° instead of 120°) as would be expected with icosahedra. A likely explanation is that some distortion had taken place during drying; the significance of this will be discussed later.

Particles embedded in lanthanum acetate

Particles embedded in lanthanum acetate are illustrated in Pl. 2, fig. 5. There appears to be no preferential staining of the deoxyribonucleic acid or protein coat, in fact true 'negative staining' was achieved. The first most obvious point about this preparation is that there is no sign of the knobs seen in the shadowed specimens. The particles exhibit a well-defined hexagonal outline, their edges being straight and the angles sharp. The hexagonal outline is consistent with an icosahedron viewed down either its two- or three-fold symmetry axis. The rather coarse background of lanthanum acetate precludes the direct visualization of any small protein subunits.

Particles embedded in uranyl acetate

It can be seen from Pl. 3, fig. 6, that the hexagonal outline is apparent when uranyl acetate is used but that is not so obvious as in the lanthanum acetate preparation. It is clear that the staining mechanism with uranyl acetate is different and that some positive-staining has taken place in certain parts of the phage. The majority of the particles in Pl. 3, fig. 6, are outlined by a dark line; within this is a pale band, and the centre is noticeably darker than the general background. The large knobs found with the shadowed particles are absent, but a number of phages possess black specks arranged in the same way (Pl. 3, fig. 6, arrowed; Pl. 3, fig. 7).

None of the micrographs studied so far has shown any regularly arranged subunits as demonstrated by Horne, Brenner, Waterson & Wildy (1959) with adenovirus, though in a very few cases, as in Pl. 3, fig. 8, some indication of organization appeared.

It has been thought up to now that this type of phage possesses no tail. The appearance of a small protrusion on a number of particles was therefore unexpected. One is shown in Pl. 2, fig. 5, and a few are discernible in Pl. 3, fig. 6, but those in

Pl. 3, fig. 9, are very clear and a figure can be given for their length, namely about 50 Å. More evidence in favour of the phage possessing a tail, or at least a unique point capable of combining with the host bacterium, is given in the preceding paper (Kay, 1961).

Size measurements

The average diameter of the phage ϕR was measured from micrographs calibrated with monodisperse suspensions of Dow polystyrene latex. The size of these lattices is known to within 10 %, the diameters being 1880 Å and 880 Å. The size of the phage was measured in four different ways, first from shadowed specimens (with sharper shadows than those shown here) using shadow length and the width of the shadow, secondly from the lanthanum acetate preparation, and thirdly from the uranyl acetate preparation. The results are as follows:

(1) From shadow widths:	335 Å
(2) From shadow lengths:	295 Å
(3) From the lanthanum acetate preparation:	300 Å
(4) From the uranyl acetate preparation:	275 Å

It is considered that the error due to the shadowing layer in (1) and (2) is small, but in all cases it is not reasonable to expect to measure to an accuracy beyond the limitations of resolution imposed by both specimen and microscope. These factors, together with the limitation of the latex itself, mean that an accuracy of better than 10 % is not possible. Thus the final size indicated from the above figures is 300 ± 30 Å. This is rather larger than the figure given by Hall *et al.* (1959) for phage $\phi X-174$.

DISCUSSION

There seems no doubt that phage ϕR has the form of an icosahedron, but the number of subunits which compose the protein coat is at present uncertain. The origin of the knobs found in the shadowed specimens is difficult to ascertain, though they do not represent single subunits. There are, in fact, 12 of these knobs, arranged in the 5:3:2 symmetry of an icosahedron and it is likely that they are in some way related to the packing either of the deoxyribonucleic acid core or of the protein coat. The fact that a monolayer of particles does not show the hexagonal packing expected of icosahedra strongly suggests that distortion has taken place during drying and this might well cause the partial collapse of the particle, thus producing the knobs at the apices. It is interesting to compare the shadowed particles with the plant viruses studied in the same way by Harrison & Nixon (1960). These viruses are the same size and shape as phage ϕR , but they do not show any apical knobs, confirming that it is not preferential granulation of the shadowing material which causes the knobs.

The interpretation of the micrographs of uranyl acetate-stained phage (Pl. 3) is made difficult because of the dual properties of the strain. There is evidence that true negative staining has taken place, because the particles are surrounded by a dark layer, but, since the centres of the particles are also darker than the background, it is believed that the deoxyribonucleic acid within the particles has been positively stained. The dark specks seen in some of the particles indicate the presence of uranium and cannot be confused with the background granularity because of their greater contrast. They could be due to the negative stain penetrating be-

tween the subunits, or positive staining of the subunits, or the stained nucleic acid showing between the subunits. More information on the behaviour of uranyl acetate is required to settle this matter.

There seems little doubt from the evidence of univalency that the tail shown in Pl. 2, fig. 5, and Pl. 3, figs. 6 and 9, is genuine. Though it is extremely small, there is no reason why this tail should not carry out the functions of absorption and injection of the phage deoxyribonucleic acid. There is adequate space in the tail for a hollow core down which the nucleic acid could pass, especially as it is a single strand and not a double one.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Bacteriophage ϕ R shadowed with platinum; $\times 170,000$.

Fig. 2. Bacteriophage ϕ R observed down its two-fold symmetry axis; platinum shadowed; $\times 330,000$.

Fig. 3. Bacteriophage ϕ R heavily shadowed with platinum+carbon showing the characteristic shadows of an icosahedron; $\times 330,000$.

PLATE 2

Fig. 4. Particles of bacteriophage ϕ R arranged in a nearly hexagonal array, platinum+carbon shadowed; $\times 157,000$.

Fig. 5. Bacteriophage ϕ R embedded in lanthanum acetate; $\times 330,000$.

PLATE 3

All the micrographs in this plate are of particles prepared in uranyl acetate.

Fig. 6. Particles clearly showing hexagonal outline; $\times 270,000$.

Fig. 7. A single particle showing possible subunits; $\times 330,000$.

Fig. 8. Some organization is visible on the arrowed particles; $\times 415,000$.

Fig. 9. Three particles clearly showing the presence of a tail; $\times 330,000$.