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ELECTRON MICROSCOPIC STUDIES ON ϕ80 AND ϕ80 pt_1 PHAGE VIRIONS AND THEIR DNA

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SUMMARY The fine structures of ϕ80 and ϕ80 pt_1 phage virions were studied in electron microscopy. ϕ80 virion consists of a head with a regular hexagonal outline and a long flexible tail. The hexagonal head has a long axis of 71 mμ. The tail has about 20 subunits arranged periodically along its tail core; the core is approximately 180 mμ in length and 6 mμ in width. From the distal end of the tail, tail fibers about 50 mμ long protrude and at its proximal end, a disc is connected. ϕ80 pt_1 virion is similar to ϕ80 in morphology. Besides this particle, a small number of spherical particles of 56 mμ diameter without a tail were observed in a partially purified preparation of ϕ80 pt_1 phage. The significance of these small particles is unknown.

The DNA extracted with phenol from the two phages were studied in electron microscopy according to Kleinschmidt's technique. Both DNA are usually linear and form circular monomers and polymers or linear polymers when they are heated at 75°C for 10 minutes and allowed to cool slowly. The average lengths of the circular monomers of ϕ80 and ϕ80 pt_1 DNA are 13.8 ± 1.1 μ and 15.8 ± 0.5 μ, respectively. The tryptophan region carried in ϕ80 pt_1 DNA seems to be inserted linearly into the phage genome and responsible for the difference in the two DNA.

Assuming the DNA to be in the B crystalline form, the length measurements give molecular weights of 27×10^6 for ϕ80 DNA and 30×10^6 for ϕ80 pt_1 DNA.

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INTRODUCTION

ϕ80 phage is a specialized transducing phage which transduces the tryptophan region of *Escherichia coli* (MATSUSHIRO, 1963). ϕ80 pt_1 phage is a non-defective derivative of ϕ80 and carries parts of the tryptophan operon (MATSUSHIRO *et al.*, 1964; SATO and MATSUSHIRO, 1965). We compared the structures of virions and DNA of the two phages, to investigate the alteration of phage associated with the incorporation of the tryptophan operon.

MATERIALS AND METHODS

1. *Preparation of phages*

Phage stocks of ϕ80 and ϕ80 pt_1 (supplied by the courtesy of Dr. A. MATSUSHIRO) were grown on W 3102, prototroph, a strain of *Escherichia coli* K 12, in 100 ml of L-broth (LENNOX, 1955). Phage λ_c stock (kindly supplied by Dr. H. OZEKI) was grown on *E. coli*, strain C 600 S (kindly supplied by Dr. H. OZEKI), in 100 ml of lambda broth (polypeptone 10 g, NaCl 2.5 g, total volume 1000 ml, pH 7.2). These lysates were stored overnight in the cold. They were purified by a differential centrifugation of 6,000 rpm for 10 min and 22,000 rpm for 45 min. and the resulting pellets were suspended in 3 ml of T_1 medium (6×10^{-4} M $MgSO_4\cdot7H_2O$, 5×10^{-4} M $CaCl_2$, 1×10^{-3}% gelatin, 6×10^{-3} M Tris-HCl buffer, pH 7.3). 2.38 g of CsCl were dissolved in these phage suspensions, which were centrifuged at 23,500 rpm for at least 18 hours at 10°C using a Hitachi RPS 40 swinging bucket rotor. The band of phage particles formed by this density gradient centrifugation was separated by drop collection and dialysed against 0.1 M NaCl-phosphate buffer (pH 7.1) to remove CsCl. The purified phage suspension was used for the preparation of phage DNA.

2. *Extraction of phage DNA*

To a phage suspension containing 0.2% Duponol, was added an equal volume of 0.1 M NaCl-0.1 M phosphate buffer (pH 7.1)—saturated phenol and the phage DNA was extracted by rotation at 60 rpm for 30–60 minutes at 6°C, as described by FRANKEL (1963). After low speed centrifugation, the phenol layer was pipetted off. This treatment was repeated 3 times. The auqeous layer was transferred into a dialysis-tube by decantation and phenol was removed by dialysing against 0.15 M NaCl-0.015 M sodium citrate (SSC). This DNA solution was used for observation in electron microscopy.

3. *Thermal treatment of phage DNA*

The DNA solution (10 μg/ml) in 0.6 M NaCl-0.05 M phosphate buffer (pH 6.7) was incubated at 75°C for 10 minutes and allowed to cool slowly to room temperature to form circular molecules of DNA.

4. *Electron microscopy*

Phage virions purified by 2 cycles of differential centrifugation were diluted dropwise with distilled water to approximately 10^{10} pfu/ml and mixed with an equal volume of 2% phosphotungstic acid (pH 7.2) and observed in a Hitachi HU 11 B type electron microscope.

5. *The DNA specimen for electron microscopy*

The DNA specimen for electron microscopy was prepared according to Kleinschmidt's technique (KLEINSCHMIDT and ZAHN, 1959). The DNA (1 μg/ml) in 0.01% cytochrome C and 2 M ammonium acetate was spread on a water surface. The cytochrome C monolayer was picked up onto formvar-covered grids and shadowed with Pt-Pd in different directions. Photographs were taken under a direct magnification of 7000. The magnification was checked with a grating replica. The lengths of the DNA were measured on enlarged prints using a map meter.

RESULTS

1. *The structure of ϕ80 and ϕ80 pt_1 phage virions*

Fig. 1 shows the general appearance of ϕ80 virions negatively stained. The virion consists of a head and a flexible tail. The head has a nearly regular hexagonal outline, the maximum diameter of which is 71 mμ. Such an outline suggests that the head may be an icosahedron. The tail length is approximately 180 mμ. A fine tail fiber of about 50 mμ length protrudes from the distal end of the tail and a disc is connected at the proximal end. The number of the tail fibers appears one although the exact number is undefinite at present. The

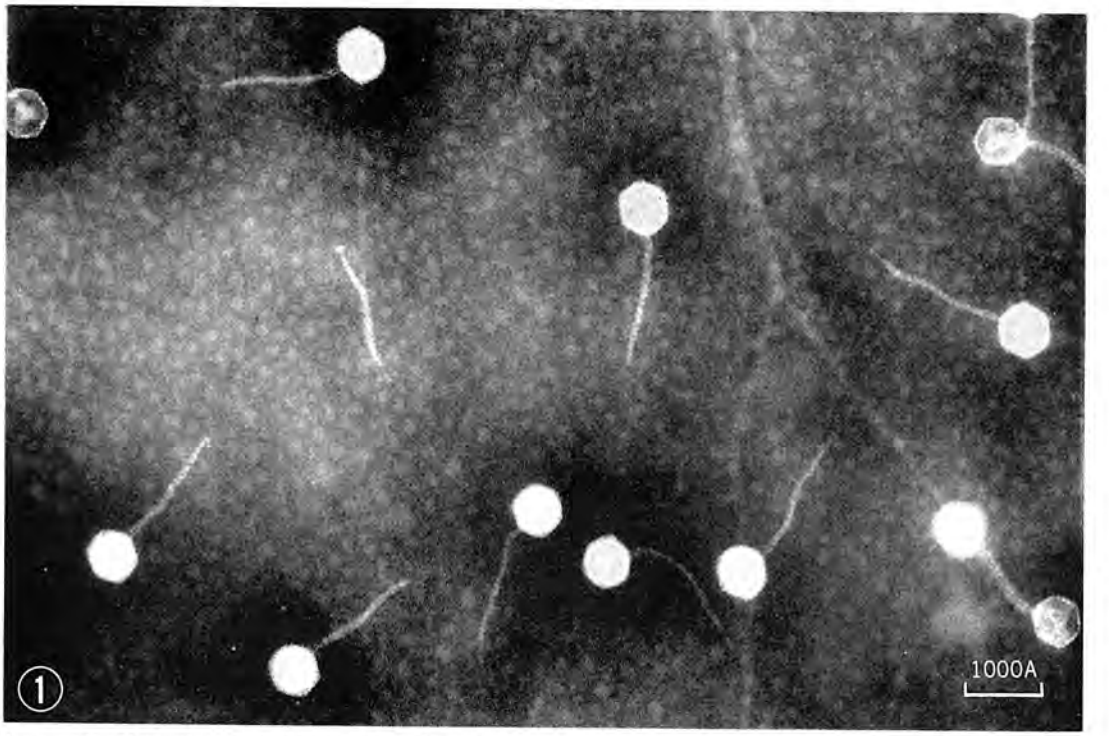

FIGURE 1 General appearance of ϕ80 phage virions, negatively stained with phosphotungstic acid. The virion consists of a head with a regular hexagonal outline and a long tail. Sometimes free tails were observed. The tail has a disc at the proximal end and fibers at the distal end.

disc is localized on a vertex of the head within the head and clearly seen in empty heads. Often free tails with a disc and a fiber are seen. The diameter and thickness of the disc are about 14 mμ and 4 mμ, respectively.

In Fig. 2, ϕ80 phage virions are shown at a higher magnification. The heads appear to be composed of morphological subunits. In virions with empty heads, hollow tail cores are clearly seen. The outer and inner diameters of the tail core are approximately 6 mμ and 2–3 mμ, respectively. About 20 subunits of disc-like structure with about 10 mμ diameter and about 5 mμ thickness are arranged periodically along the tail core. The subunit appears to have a double layer structure.

In partially purified preparations of ϕ80 pt_1, two kinds of particles are observed, as shown in Fig. 3. Large particles are similar to ϕ80 in their morphology and predominate. Small particles have no tail and are 56 mμ in diameter.

2. *Electron microscopy of ϕ80 phage DNA*

Fig. 4 is an electronmicrograph of a DNA preparation of phage ϕ80. The DNA appears to be a continuous unbranched linear structure or a multilooped strand. The distribution of the lengths of the DNA molecules is shown in Fig. 5. A peak at 12 μ is seen. A small number of molecules about 1.5 to 2 times longer than 12 μ are observed; these are probably polymers formed during storage. Shorter fragments of DNA molecules which are probably produced by shearing during preparation, are also observed.

FIGURE 2 Details of ϕ80 phage virions at a higher magnification, negatively stained with phosphotungstic acid. The tail has about 20 subunits arranged periodically along its tail core of approximately 180 mμ length and 6 mμ width. The tail core is clearly observed in the virions with empty heads.

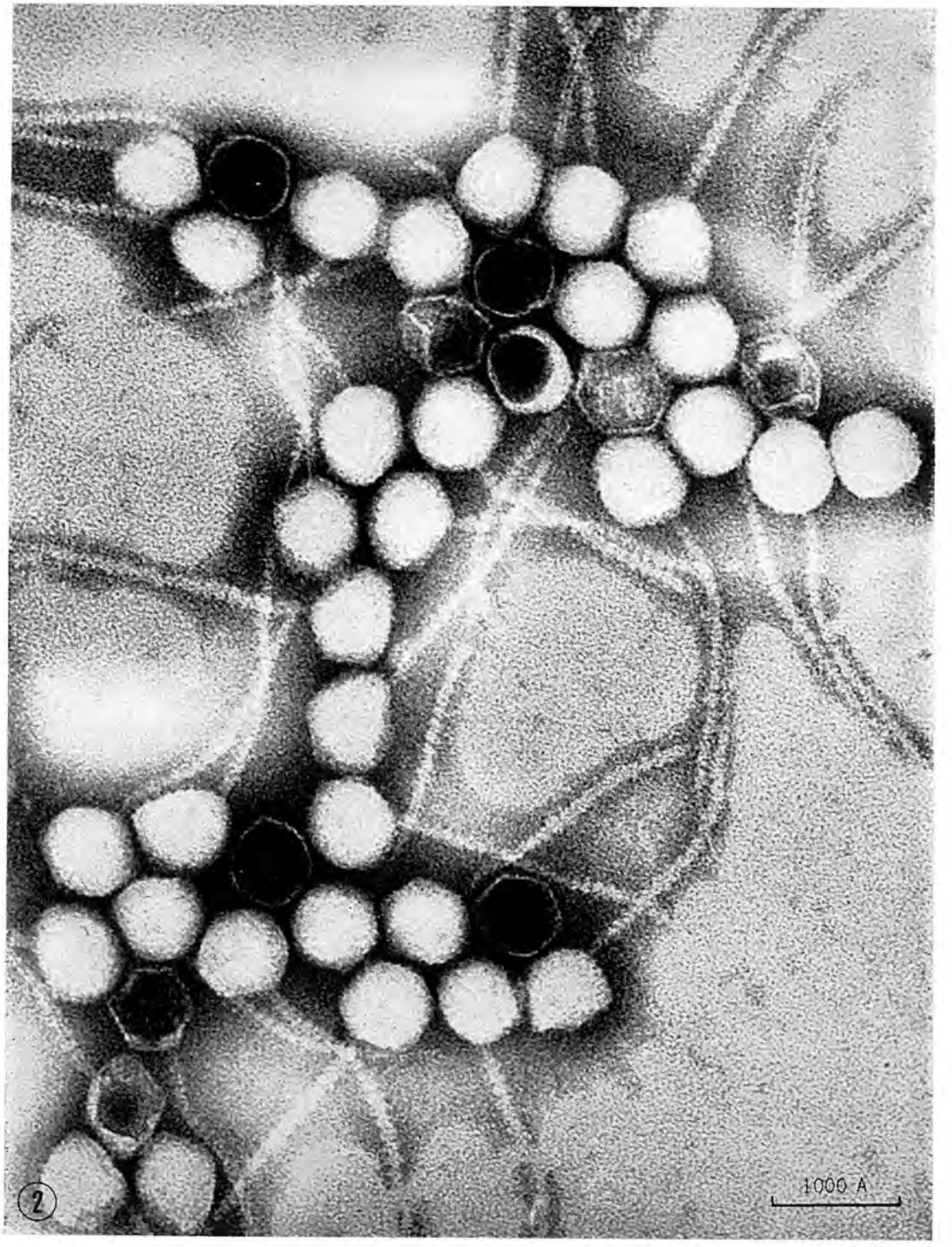
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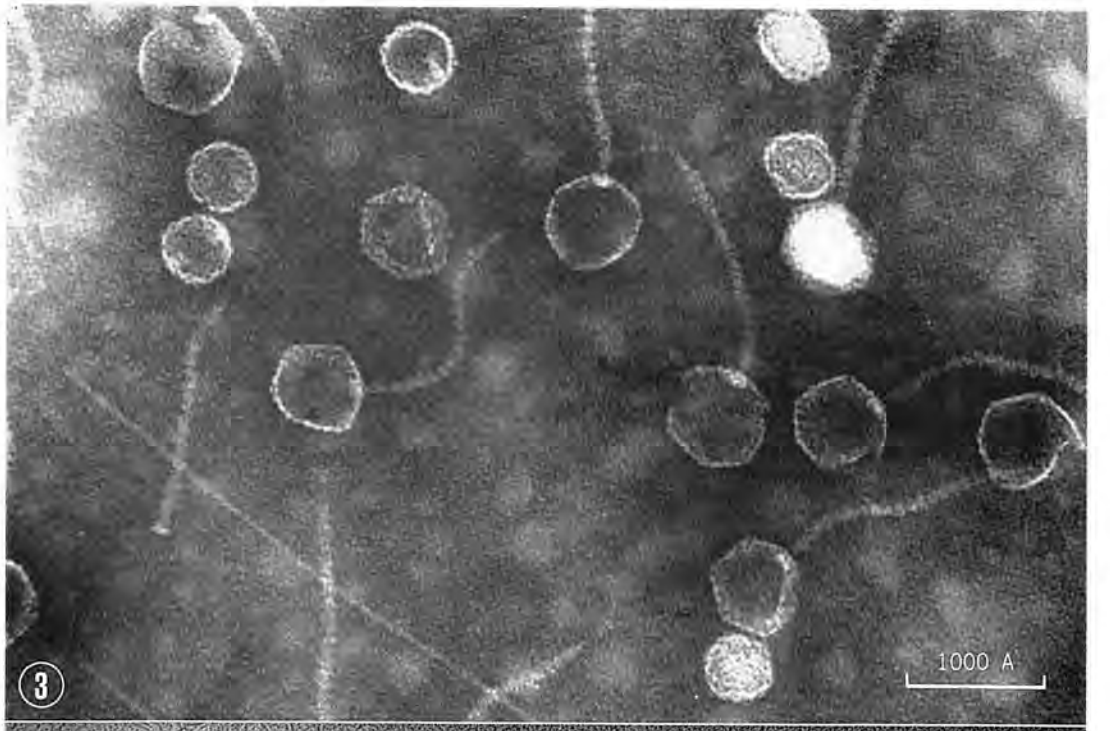
FIGURE 3 A partially purified preparation of ϕ80 pt_1 phage. Two kinds of particles with different sizes are observed. The morphology of the large particles, which are predominant, is similar to that of ϕ80 virions. The small particles have no tail and a diameter of 56 mμ.

FIGURE 4 The native ϕ80 phage DNA, phenol-extracted. The DNA molecules and either unbranched linear structures or multi-looped strands.



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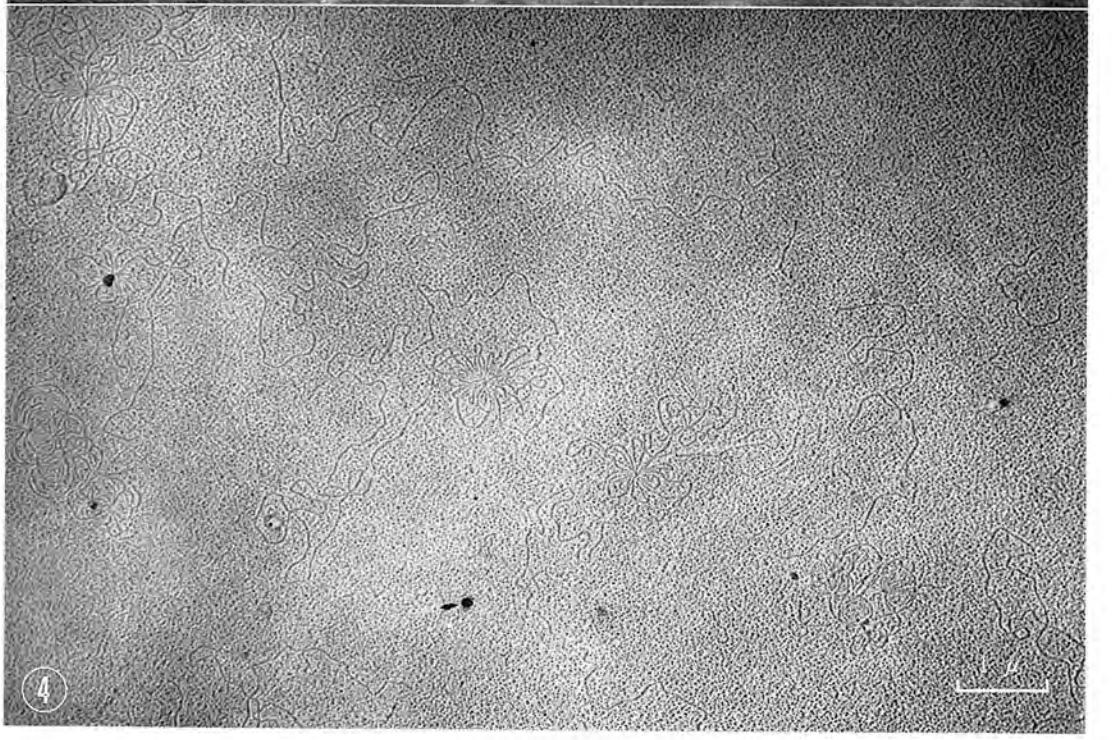

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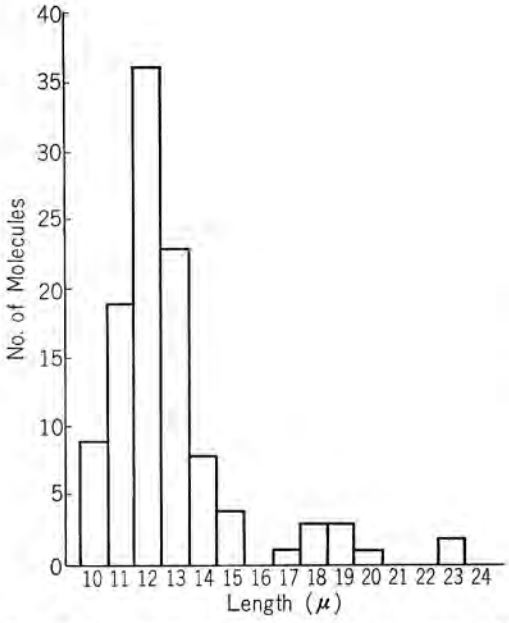


FIGURE 5 Distribution of lengths of native ϕ80 DNA molecules.

3. *Electron microscopy of ϕ80 phage DNA after thermal treatment*

It was found in electron microscopy that many circular molecules were formed after heating and slow cooling of ϕ80 DNA. Fig. 6 shows a circular molecule thus formed. The distributions of the lengths of the circular and linear molecules in the treated preparation are shown in Fig. 7 a and 7 b. The circular molecule forms a sharp peak at 14 μ and a small peak at 27 μ but has no DNA lengths outside of the peaks, while the linear molecule has a broad peak around 13 μ, tailing off towards the shorter lengths. The mean length of the circular molecules forming the 14 μ peak is 13.8$\pm$ 1.1 μ (calculated from 31 molecules).

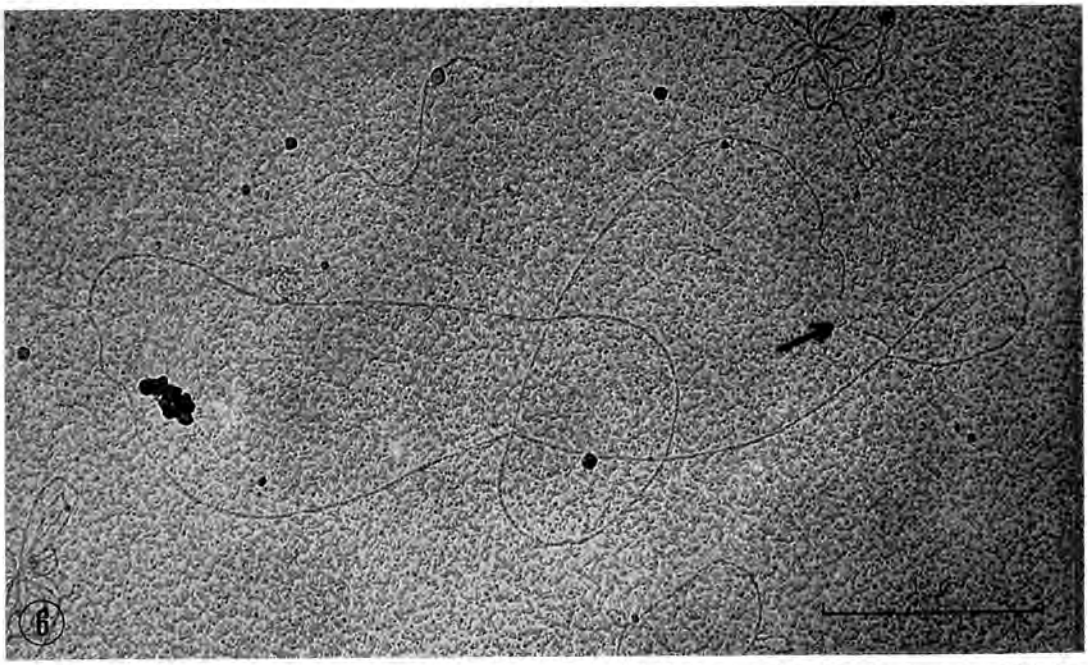


FIGURE 6 A circular monomer of ϕ80 phage DNA. When ϕ80 DNA, phenolextracted, is heated at 75°C for 10 minutes and slowly cooled, it forms a circular molecule. The discontinuity is marked by an arrow and indicates the probable cohesive site of the DNA.

As shown in Fig. 6, some circular molecules have a discontinuous region. The lengths of these discontinuous regions vary from molecule to molecule and range from 0.1 to 0.4 μ.

The formation of circular molecules of DNA about 14 μ in length but not shorter, suggests that the DNA of this length has cohesive sites at both ends and that it is an intact molecule of ϕ80 DNA (monomer).

In both Figs. 7 a and 7 b, a small peak at about 27 μ is seen. It probably represents the dimer of ϕ80 DNA. It has two discontinuous regions which divide the total length into two equal parts.

The circularized molecules become linear again when they are heated at 75°C for 10 minutes and rapidly cooled.

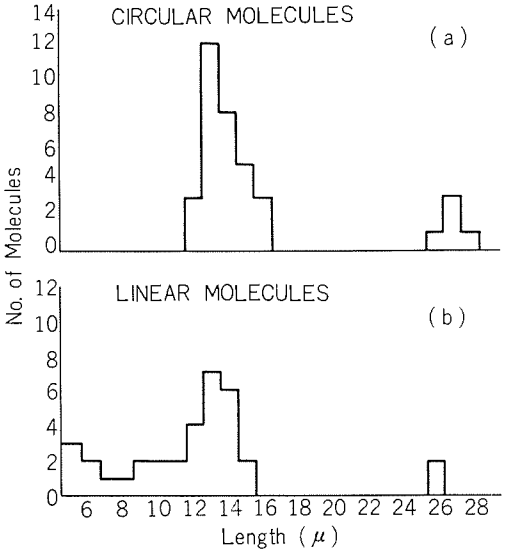


FIGURE 7 Distribution of lengths of ϕ80 DNA molecules after thermal treatment: (a) circular molecules; (b) linear molecules.

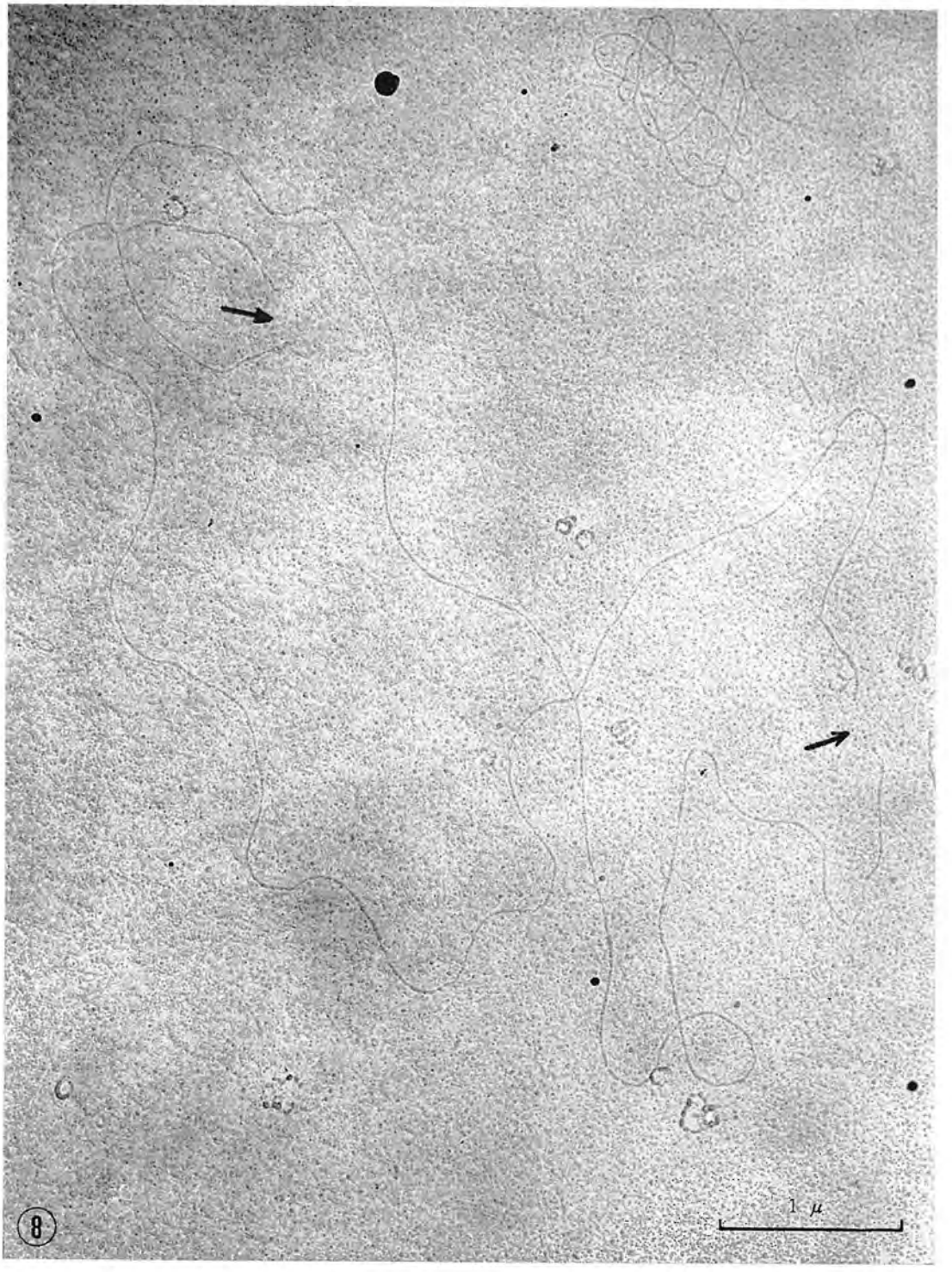
4. *Electron microscopy of ϕ80 pt_1 phage DNA*

The DNA of ϕ80 pt_1 phage usually had an unbranched linear configuration but formed a circular structure by heating at 75°C for 10 minutes with subsequent slow cooling; this happened as well as with ϕ80 phage DNA. The distribution of lengths of circular DNA is shown in Fig. 9. The mean length of ϕ80 pt_1 DNA is 15.8±0.5 μ (calculated from 33 molecules). The circularized DNA became linear again by heating at 75°C for 10 minutes with subsequent rapid cooling, as in the case of ϕ80 DNA. Fig. 10 shows a circular molecule of ϕ80 pt_1 DNA.

DISCUSSION

The fine structures of ϕ80 and ϕ80 pt_1 phages have been elucidated in the present studies. We proposed from present data a morphological model for phage ϕ80 as presented in Fig. 11. It consists of the following components differentiated morphologically: a head, a tail core, 20 subunits arranged along the tail core, a disc at the proximal end of the tail and a tail fibers at the distal end of the tail. We could not detect a contractile system in the phage tail such as has been demonstrated in the T-even phages. The general appearance of ϕ80 phage is similar to λ phage (KELLENBERGER, 1961) and T5 phage (KAY and BRADLEY, 1960), in that it consists of a head with a regular hexagonal outline and a long flexible tail. We wish to point out two morphological features of the ϕ80 virions; first the presence of a disc at the proximal end of the tail within the head, an entity as yet unknown in other phages, but which may have a role in fixing the tail to the head; the second, the size of the head, relative to its DNA content. The head of the ϕ80 virion is larger than that of the λ virion (KELLENBERGER, 1961; CUMMINGS et al., 1965) by more than 10 μ, but contains a smaller amount of DNA than the λ virion (BURGI and HERSHEY, 1963; RIS and CHANDLER, 1963; MACHATTIE and THOMAS, 1964; CARO, 1965). This find-

FIGURE 8 A circular dimer of ϕ80 phage DNA (the same preparation as in Fig. 6). The discontinuities are marked by arrows. They divide the total length of the molecule into two equal parts of 13.5 μ (monomer length).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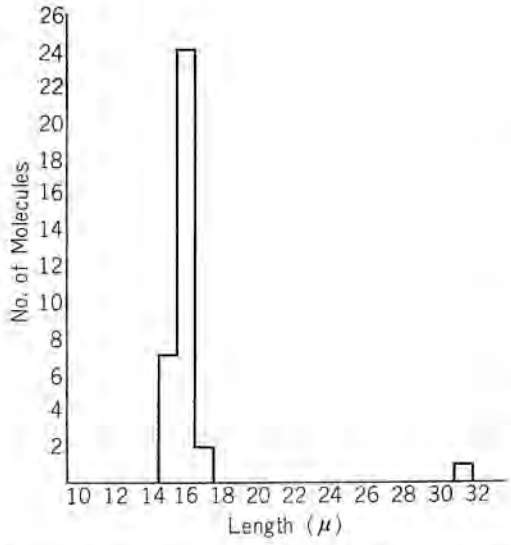

FIGURE 9 Distribution of lengths of circular $\phi 80$ pt_1 DNA molecules after thermal treatment.

ing is consistent with that of OZEKI (personal communication) who postulated it from the densities of the phenotipic mixings between λ and $\phi 80$ phages.

The small particles without tails were frequently observed in the partially purified preparations of $\phi 80$ pt_1 (more than 10% of the total phage particles) but rarely observed in that of $\phi 80$. The presence of these small particles may be a characteristic of $\phi 80$ pt_1 phage.

YAMAGISHI *et al.* (1966) showed that $\phi 80$ DNA forms a band which sediments more rapidly in sucrose zone centrifugation after heating and slow cooling than it does before such treatment. They suggested that this band corresponds to circular molecules of $\phi 80$ DNA. We confirmed their suggestion in electron microscopy and showed that $\phi 80$ and $\phi 80$ pt_1 DNA have thermal cohesive sites at both ends. We interprete that the cohesive sites of the DNA

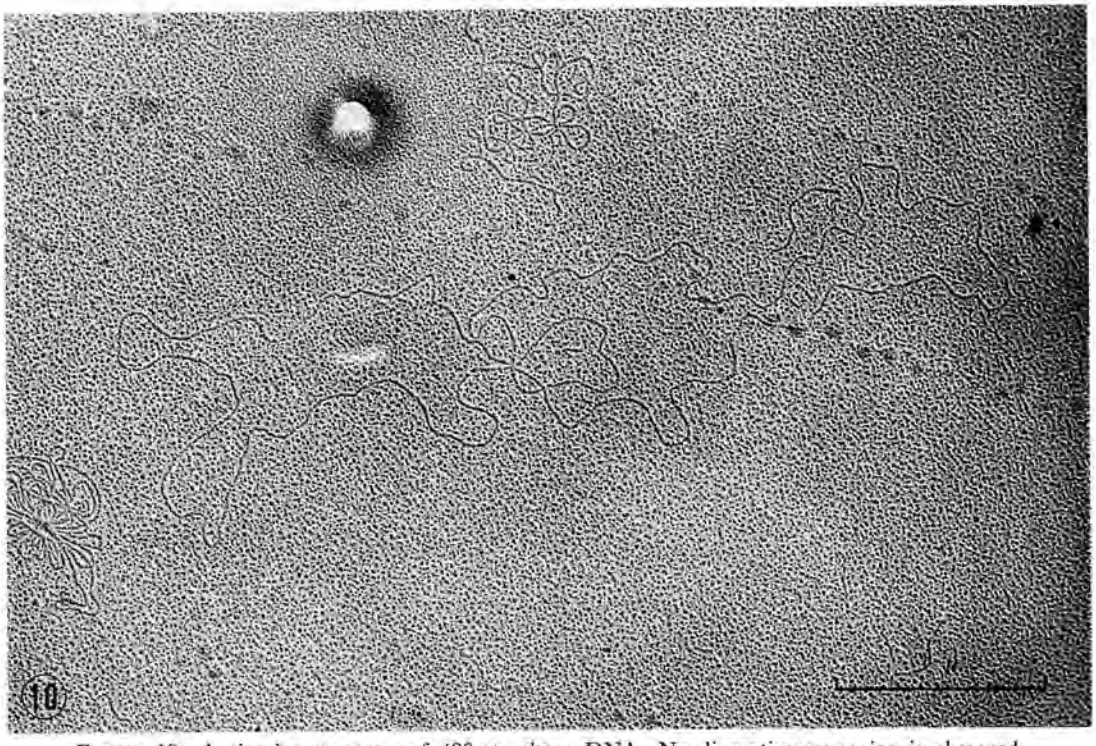

FIGURE 10 A circular monomer of $\phi 80$ pt_1 phage DNA. No discontinuous region is observed in this molecule.

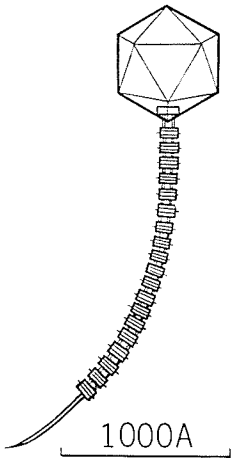

FIGURE 11 A morphological model of ϕ80 virion. It consists of the following components: a head, a tail core, 20 subunits arranged along the tail core, a disc at the proximal and a fiber at the distal end of the tail. The head seems to be an icosahedral arrangement of subunits. The exact number of tail fibers is unknown at present.

are single-stranded and of complementary nature as in the case of λ DNA (STRACK and KAISER, 1965). The discontinuous regions seen in the circular molecules may indicate the sites of cohesion artificially separated during the specimen preparation for electron microscopy, because 1) not all the circular molecules have discontinuous regions and 2) the lengths of the regions vary from molecule to molecule.

The molecular length of ϕ80 pt_1 DNA is longer than ϕ80 DNA by about 2 μ. It was also observed in zone centrifugation by YAMAGISHI *et al.* (1966) that the molecular weight of ϕ80 pt_1 DNA is larger than that of ϕ80 DNA. The difference is probably caused by the incorporation of the host tryptophan region into the phage genome. Since the difference is observed in the circularized molecules and since no branch is observed, this excludes the possibility that the tryptophan operon attaches to the phage genome as a branch. Since the reversible cohesion of ϕ80 pt_1 DNA as well as ϕ80 DNA readily occurs, the structures of both ends of the two DNA molecules are probably the same. These findings lead to the conclusion that the tryptophan region is inserted linearly into a site of the phage genome.

In our measurements of phage DNA, the molecular length of ϕ80 DNA is 13.8 μ and that of ϕ80 pt_1, 15.8 μ. Assuming the DNA to be in the B crystalline form (MACHATTIE and THOMAS, 1964; CARO, 1965), the molecular weights of ϕ80 and ϕ80 pt_1 are $(26.5\pm2.1)\times10^6$ and $(30.3\pm1.0)\times10^6$, respectively. We used the circular molecules of λc phage DNA as a reference for DNA length, because this DNA has been extensively studied in electron microscopy. RIS and CHANDLER (1963) reported that its length is 16.3 μ, MACHATTIE and THOMAS (1964), 16.3 μ and CARO (1965), 15.6 μ. Our measurements of λ DNA gave the length of 15.3 ± 0.9 μ (calculated from 17 molecules). Thus our value of λ DNA is consistent with those of other workers and available as a reference. Furthermore, the fact that the length of ϕ80 DNA is a little shorter than that of λ DNA agrees with the finding that ϕ80 DNA sediments a little more slowly in zone centrifugation than λ DNA (YAMAGISHI *et al.*, 1966).

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