Isolation and Characterization of Bacteriophage T4 Base Plates

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A method for isolating bacteriophage T4 base plates from lysates of Escherichia coli B cells infected with the ts mutant in gene 19, ts B31 has been developed. By electrophoresis in polyacrylamide gel with sodium dodecyl sulfate the base plates have been shown to contain five to seven protein components with molecular weights of 36,000, 53,000, 66,000, 81,000, 87,000, and probably about 100,000. Electron microscope studies have demonstrated that base plates may occur in two structural states: in the form of hexagons or stars. Star rays and short fibrils are not radial or elongated and are turned sideways at an angle to the radius. Base plates do not complement in vitro with free tail cores isolated after disintegration of particles of the wild-type bacteriophage.

Base plates are the central tail element of a particle of T-even bacteriophages to which long fibrils, tail cores, sheaths, and short fibrils attach. They occupy the central position and, therefore, play a leading part in bacteriophage morphogenesis (6). Due to this, base plates have long been attracting the attention of investigators. However, because of technical difficulties involved in their isolation, extensive studies of base plates have commenced only recently (2, 8). Some electron microscope investigations have provided evidence that base plates may exist in two states (H. Fernandez-Morgan, 1962, Symp. Int. Soc. Cell Biol., vol. 1, p. 411; references 1 and 9). As a constituent of an intact phage particle with an extended tail sheath the base plates form a thin hexagonal plate of 310 to 400 nm in maximal diameter. In the center of the plate there is a plugged hole surrounded by a hexagonal disc with a diameter of 180 nm situated on the inner side of the plate (2, 6, 9). In response to sheath shortening, base plates transform into their second state and change their form from hexagons to hexagonal stars with a maximal diameter of 500 to 600 nm.

In their recent work Cummings et al. (2) have shown, by using separation on the agarose column, that base plates are composed of three proteins with molecular weights of 17,000, 31,000, and 53,000. These authors have also advanced a model of a base plate built of tetrad complexes of protein subunits. Also, recently Kozloff and co-workers (8) have isolated T4 base plates and have shown that the phage-induced dihydrofolate reductase of molecular weight 30,000 is a tail plate component.

The present study deals with isolation of base plates from the ts mutant of a T4 phage damaged in gene 19 and the investigation of the base plates by electron microscopy and physicochemical methods.

MATERIALS AND METHODS

Base plates were produced with the aid of a temperature-sensitive mutant T4 B31 damaged in gene 19, kindly provided by King (6). The mutant was grown on Escherichia coli B at 42°C, using a synthetic medium and intensive aeration in a 100-liter fermentor. E. coli B was grown to a concentration of 2.10⁸ cells/ml and then infected with phage with a multiplicity of 5, superinfected 15 min later with an equivalent amount of bacteriophage, and incubated for another 40 min. The bacterial mass was centrifuged and resuspended in a small amount of the culture liquid to the final volume of 1.5 liters. Cells were lysed with chloroform and kept overnight at 4°C. The resultant lysate was supplemented with deoxyribonuclease (40 µg/ml) in the presence of Mg²⁺ and ribonuclease (100 µg/ml) and kept for 2 hr at 37°C with continuous stirring. The bacterial cell debris were separated by centrifugation at 6,000 × g for 30 min. Elements of phage particles that remained in the supernatant fluid were precipitated by further centrifugation at 78,000 × g for 7 hr. The residue was suspended in 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer, pH 7.4, in the presence of 0.001 M ethylenediaminetetraacetic acid (EDTA). The resulting suspension contained, in addition to minor cell structures, a mixture of structural elements of a phage particle (heads, polysheaths,
base plates, base plates plus cores, fibrils) and was used to isolate and purify base plates by means of stepwise centrifugation in the sucrose gradient. In the first stage, heads and polyshells were roughly separated from base plates. For this purpose 5 ml of 60% sucrose was poured into a centrifuge tube, and then 10 ml of 20% sucrose was added and 20 to 30 ml of a suspension of bacteriophage structural elements was layered on top of the sucrose solution. As a result of centrifugation at 40,000 × g for 1 hr, two layers were formed. The upper layer contained mainly base plates, empty discs with a diameter that equaled the core diameter, and a small amount of heads and polyshells. The lower layer included a large accumulation of heads, polyshells, and intact phage particles. Both layers were collected with a syringe and centrifuged at 165,000 × g for 5 hr. The residues were suspended with 0.01 M Tris-hydrochloride buffer, pH 7.4, containing 0.001 M EDTA. The residue of the upper layer was then centrifuged at 40,000 × g for 4 hr in the linear sucrose gradient at a concentration of 5 to 20% with a bottom cushion of 60% sucrose. This resulted chiefly in the formation of three opalescent layers. The lower layer that was on the cushion contained a mixture of polyshells and heads; the middle layer included base plates and base plates plus cores (forks); the upper layer showed empty discs that were probably core fragments or connectors. Base plates from the middle layer were precipitated by centrifugation at 165,000 × g for 7 hr and resuspended in distilled water at pH 6.0. The resultant preparation of base plates was occasionally passed through the linear sucrose gradient.

Tail cores of bacteriophages T2 and T4 were obtained according to the previously developed procedure (Poglavov and Nikoljskaia, 1969). In the case of bacteriophage T4, cores emerged attached to the shortened sheaths and connectors (so called grenades).

The complementation experiments were carried out in accordance with the method of Edgar and Wood (4). The preparation of bacteriophage T4 ts B31 base plates was mixed with that of bacteriophage T2L cores or T4D in equal volumes. The mixture was added to the lysate of phage T4D-infected bacterium, incubated for 3 hr at 30 C, and then examined in the electron microscope. The lysate was prepared in the following way. Bacteriophage T4D was grown in 200 ml of Hottinger broth, the lysis being delayed for 40 min. Without lysing the bacteria, they were precipitated at 6,000 × g for 1 hr. The residue was resuspended in 25 ml of phosphate buffer (0.039 M Na2HPO4 + 0.022 M KH2PO4 + 0.07 M NaCl + 0.02 M MgSO4) and lysed by freezing and thawing. Cell debris and intact phage particles were removed by centrifugation at 30,000 × g for 1 hr. The supernatant fluid was used as lysate in complementation experiments.

Electron microscope examinations were performed in a Hitachi 12 microscope with a magnification of ×50,000. The specimens were stained with 2% sodium phosphotungstate and placed on the Formvar film support.

The sedimentation analysis was conducted in the Spinco E centrifuge equipped with a scanning system adjusted for 230 nm. For sedimentation experiments, base plates were placed in 0.01 M Tris-hydrochloride buffer, pH 7.4, to bring the solution Σm density to 1. The centrifugation velocity was 26,000 rev/min. Electrophoresis in polyacrylamide gel with sodium dodecyl sulfate (SDS) was carried out according to Maizel (Maizel, 1969; Laemmli, 1970). The protein solution with a concentration of 100 to 200 μg/ml was placed in 0.031 M Tris-hydrochloride buffer, pH 6.8, containing 1% SDS, 5% glycerol, 2.5% mercaptoethanol, and 0.0025% bromophenol blue. The mixture was kept for 1.5 min in a boiling bath, cooled, and placed on the gel. The separating polyacrylamide gel was used in the 10% concentration. The separation was continued for 5 to 7 hr using a current of 3 amps.

RESULTS

Base plates were isolated using the above procedure with constant electron microscopy and sedimentation analysis to control their purification. The only admixture that occurred occasionally with base plates was core material; in this case we saw not only base plates but also base plates connected with cores. Figure 1 shows a typical electron micrograph of a base plate fraction.

The sedimentation analysis confirmed homogeneity of the base plate preparation yielding the only peak that corresponded to a sedimentation coefficient of 69S.

A thorough study of electron micrographs warranted the following major conclusions concerning the preparation and the structure of base plates. In addition to hexagonal base plates, the preparation also contained a small number of stars, which varied from experiment to experiment, apparently depending on the effect of certain damaging factors attending the isolation of base plates and their preparation for electron microscopy. It was noted that a small increase of alkalinity favored an irreversible transformation of hexagonal base plates into stars. This process occurred most intensively in a narrow pH zone of 10.5 to 11.0. At early stages of alkalization, beginning from 10.0, a small number of tetradces was seen to appear in the medium, a phenomenon already referred to by Cummings et al. (2). An increase in pH above 11.0 brought about a dissociation of base plates and disappearance of tetradces. To study the reversibility of dissociation of base plates, an alkalinized solution was neutralized by adding HCl or by dialysis against neutral buffer. In either case we failed to find reconstructed base plates, and amorphous aggregates of subunit material were seen to form in the solution instead.

A statistical study shows that the largest diameter of hexagonal base plates is approximately 360 nm and of star base plates about
Fig. 1. Electron micrograph of a base plate fraction. ×565,000.

480 nm. In the center of hexagons we discrimi- 
nated a plug and a hexagonal disc of about 
180 nm in diameter. The disc was very clear when 
the base plates that formed dimers or combined 
with cores were turned sideways (Fig. 2). Besides, 
hexagonal base plates (Fig. 3a) displayed a sub-
structure situated along the edge of the plate 
which looked like tilted gear teeth. A schematic
representation of a hexagonal base plate is given in Fig. 4a. So far we have accumulated no direct evidence but we can suppose that these teeth are none other than a projection of short fibrils. If this is true, then short fibrils cannot run radially in relation to the center of a base plate but should be disposed at a certain angle. It should be noted that stars (Fig. 3b and c) also exhibit not straight radial rays but slightly inclined rays running at an angle to the radius. Accordingly, the long fibrils that are attached to them are also turned in one direction. A schematic diagram of the star outline is presented in Fig. 4b.

Simple electron microscope examination of base plates demonstrate that they are composed of at least four different elements (short fibrils, a plug, the plate itself, and a disc located in the inner part of the base plate). The protein composition was verified through studying dissociated base plates with the aid of electrophoresis in polyacrylamide gel with SDS. In most experiments we clearly distinguished five bands that corresponded to proteins with molecular weights of 36,000, 53,000, 66,000, 81,000, and 87,000. We also had an impression that the band of 53,000 is a double one. This idea needs, however, further investigation. Furthermore, we often observed protein material with a molecular weight of slightly more than 100,000. Thus, according to our results, the base plates are composed of 5 to 7 protein components. Nevertheless, we cannot rule out the possibility that some protein components occurring in minor amounts such as the phage dihydrofolate reductase are beyond the sensitivity of our method.

Owing to the fact that the first product with which the base plate interacts during the process of bacteriophage particle assembling is a core protein and also because the mechanism of its polymerization on a base plate are unknown, we explored the possibility of a base plate combining in vitro with free tail core in the presence of extracts from phage T4D-infected cells. These experiments were designed to verify the hypothesis that a core may begin to get assembled only on a base plate rather than getting attached to it in a free form (6). In these experiments, purified base plates were mixed with isolated cores of bacteriophages...
A base plate is the most complicated structural element of the tail of T-even bacteriophages. Fifteen genes are involved in its formation (3, 5). Some of them produce structural proteins and others play a regulatory, catalytic part. Our electrophoretic experiments have clearly revealed 5 to 7 protein fractions, although we can suppose the existence of additional minor proteins which we have failed to discriminate.

It is obvious that the electrophoretic separation we have employed is more sensitive than the separation on the agarose column used by Cummings et al. (2). Apparently, this has enabled us to discriminate additional protein components of a base plate—proteins with molecular weights of 36,000 and 53,000. More detailed studies on the proteins of the base plate will be reported by King and Lacmml (personal communication).

In our opinion, the component with a molecular
weight of 17,000 (2) should be referred to the core protein because we also have detected it (molecular weight = 19,000) mainly in the fraction of base plates plus cores (forks).

As mentioned above, the electron microscopy data suggest the presence of at least four structural elements in a base plate (short fibrils, a central disc, the plate itself, and a plug). The plug protein occurs undoubtedly in the least amount. The base plate itself contains certain special subunits which are in contact with the disc and with short and long fibrils. The central disc can certainly be considered an integral part of base plates, as follows from the recent experiments with double mutants (48−, 18−) carried out by King (6).

We have failed to reconstruct base plates in vitro after their dissociation. The results of these experiments serve to emphasize a complex organization of a base plate and point to the need to implicate some additional regulatory factors. We do not know as yet how to explain the fact that base plates cannot complement with free cores. Considering that we have experimented with highly purified base plates and added all necessary cofactors into the reaction mixture with the lysate, it remains to be assumed that during isolation the artificially produced cores may lose a component which should lie at the end of a core and contact with the base plate.

Of great interest is the structural transformation of a hexagonal base plate into a star base plate, although the mechanism and function of the process remain unknown. It may be supposed that the conversion is accompanied by a conformational transformation of base plate proteins and their transfer to an active state necessary for an interaction with the cell envelope and a liberation of the core tail. The spinning of star rays and nonradial arrangement of short fibrils we have observed correlate to a certain extent with the shape of an extended tail sheath in the cross section found by Klug and DeRosier (7).

LITERATURE CITED
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Volume 10, no. 4, p. 810, abstract: Change the second sentence, “By electrophoresis in polyacrylamide gel with sodium dodecyl sulfate the base plates have been shown to contain five to seven protein components with molecular weights of 36,000, 53,000, 66,000, 81,000, 87,000, and probably about 100,000.” to read “By electrophoresis in polyacrylamide gel with sodium dodecyl sulfate the base plates have been shown to contain seven protein components with molecular weights of 30,000, 36,000, 46,000, 60,000, 86,000, and about 100,000 and 115,000.”

P. 813, left column, beginning with line 21: Change “In most experiments we clearly distinguished five bands that corresponded to proteins with molecular weights of 36,000, 53,000, 66,000, 81,000, and 87,000. We also had an impression that the band of 53,000 is a double one. This idea needs, however, further investigation. Furthermore, we often observed protein material with a molecular weight of slightly more than 100,000. Thus, according to our results, the base plates are composed of 5 to 7 protein components. Nevertheless, we cannot rule out the possibility that some protein components occurring in minor amounts such as the phage dihydrofolate reductase are beyond the sensitivity of our method.” to read “In most experiments we clearly distinguished five bands that corresponded to proteins with molecular weights of 30,000, 36,000, 46,000, 60,000, and 86,000. We also had an impression that the band of 46,000 is a double one. This idea needs, however, further investigation. Furthermore, we often observed protein material with a molecular weight of about 100,000 and 115,000. Thus, according to our results, the base plates are composed of 7 protein components. Nevertheless, we cannot rule out the possibility that some protein components occurring in minor amounts are beyond the sensitivity of our method.”

P. 814, right column, line 16: Change “of 36,000 and 53,000.” to read “of 31,000 and 53,000.”

Latency of Human Measles Virus in Hamster Cells

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Volume 10, no. 5, p. 995, column 2, line 7: Change “The Schwarz measles virus, vaccine lot no. 94217, was obtained from Merck, Sharp & Dohme. This virus was passaged eight times in BSC-1 cells.” to read “The virus employed was obtained commercially from The Dow Chemical Co. It was derived from the Schwarz strain but had been passed eight times in BSC-1 cells at 37 C prior to its use in this study.”

Ribonuclease H: a Ubiquitous Activity in Virions of Ribonucleic Acid Tumor Viruses

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Volume 10, no. 6, p. 1137, second column, line 11: Omit completely “0.03 M Tris-hydrochloride (pH 7.8), 0.1 M (NH4)2SO4.”