VIROLOGY

Electron Microscopic Study of DNA Packing in φkz Bacteriophage (*Pseudomonas Aeruginosa*) Irradiated with Laser in the Presence of a Dye

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DNA packing of bacteriophage φkz (*Pseudomonas aeruginosa*) was studied by electron microscopy after gentle destruction of the capsid with powerful laser impulse in aqueous virus suspension in the presence organic dye. Suspension of destroyed phage heads contained complexes of genomic DNA with endogenous capsid proteins characterized by pronounced regular structure. The type of these structures corresponds to one the main model of phage DNA packing, the so-called coil packing.

Key words: *bacteriophage* φkz*; DNA packing; laser irradiation; dye*

Organization of DNA in all living systems from viruses to higher eukaryotic organisms is still a fundamental biological problem. Genomic DNA is tens times longer than the space in cell nucleus, bacterial cytoplasm, or viral envelope. For instance, DNA of bacteriophage P22 is 250 times folded in the virion. Therefore, compactization and decompactization of DNA and its subsequent packing are an important phase of organism development. Compact or condensed DNA is a highly organized structure, which can be quickly decondensed. However, its structure are not completely studied. The key point in the study of DNA condensation is the choice of a convenient biological object and model. We assume that bacteriophage φkz (*Pseudomonas aeruginosa*) can be used as such object. Bacteriophage φkz, a representative of *Myoviridae* family, possesses an central cylindrical protein body co-directed with the main tail axis and possibly involved in DNA packing and condensation [6].

Most electron microscopy data on DNA packing were obtained on phages with partly or completely destroyed capsids. However, these data do not reflect internal organization of phage DNA, because different objects and conditions of destruction show different packing modes. This can be explained by disorganization of packed DNA after destruction of phage envelope.

The aim of the present study was to examine the tertiary DNA structure in bacteriophage φkz head after its partial destruction with ruby or neodymium laser. Previous studies showed that this method allows to achieve mild destruction of the phage head preserving DNA packing inside partly destroyed head.

MATERIALS AND METHODS

Yanus green dye was dissolved in 0.1 M phosphate buffer (pH 7.0) 1 day before the experiment and serial dilutions from 10^{-2} to 10^{-5} M were prepared. Bacteriophage suspension (40 μl) in 0.1 M phosphate buffer was added to the same volume of the dye. A quartz capillary (1 mm in diameter, 4 cm long) was filled

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with the resultant mixture immediately before the experiment.

Structural organization of genomic DNA in bacteriophage φkz was studied by a method of selective destruction of viral envelope with powerful coherent light from neodymium and ruby lasers. In experimental series I, quartz capillaries were irradiated with a ruby laser in free generation regimen with relatively smooth impulses (duration 1 msec, energy up to 10 J, diameter of light spot 6 mm). In experimental series I, capillaries were irradiated with YAG:Nd^{3+} laser operated in a modulated regimen for 1, 5, and 10 sec. Parameters of impulses were 18 nsec duration, 30 mJ energy, 10 Hz repetition frequency. Laser beam was focused on a capillary with a short-focus (70 mm) lens, the diameter of the spot was 0.5 mm.

Preparations for electron microscopy were contrasted with 1-2% uranyl acetate or 2% phosphotungstic acid (pH 7.0). Immediately after irradiation phage suspension was transferred to carbon-strengthened formvar-coated electron microscope grids treated in glow discharge and dried on air. Specimens were examined under a JEM-100C electron microscope at 50,000 magnification and 80 kV accelerating voltage.

The microscope was calibrated using colloid gold particles.

RESULTS

Phage φkz belongs to a group of phages with contracting tail envelope. Hexagonal projection of the phage head is 120 nm in diameter. Negative contrasting with 1% uranyl acetate visualizes cylindrical central body, a bar-shaped spring-like structure attached to two opposite poles of the head (Fig. 1). The central body consists of one or several types of proteins [4]. Its diameter is 26 nm and its length is equal to that of the phage head.

Electron microscopy of phage suspension irradiated in the presence of yanus green and methylene blue revealed partly destroyed phage heads. Partial separation of the capsid (typical destruction) revealed internal periodic structure of the phage (Fig. 2, 3). Almost completely destroyed capsids were also found. The analysis of microphotographs reveals the following regularity: partial separation of phage head envelope was accompanied by a release of fragments with regular structure (Fig. 2, *b*; Fig. 3, *a*, *b*). The filaments (8.6 nm thick) were separated by 2.3-nm intervals. It is interesting that the adjacent regular structures had the same period. This indicates that the fragments separated from partly destroyed phages have common nature and represent condenced genomic phage DNA identical to DNA in the phage head.

The observed free structures can be divided into two types.

Type A consists of concentric rings converging to the center. Four rings most distant from the center are clearly seen. This structure can represent a left-handed helix (Fig. 2, *b*).

Type B can be subdivided in subtypes I and II. Type I structures projected to a plane are a isosceles trapezoid with ellipse-shaped top and bottom sides, which probably attests to truncated cone-shaped spatial structure. The top and bottom diameters are 44 and 150 nm, 7 turns are easily distinguished.

Subtype II structure projected to a plane looks like a vertically crossed circle (Fig. 2, *a*). The most probable spatial analogue of this structure is a spherically shaped stack of disks. Seven disks are clearly seen. The diameters of the smallest and the largest (fifth disk from the top) disks are 43 and 115 nm, respectively. As mentioned above, striation periods in type A and B structures are similar.

Bacteriophage φkz used in this study was chosen as a perspective model for investigation of genomic DNA packing and condensation. This phage is the only described bacteriophage with easily visualized central body participating in DNA packing [4]. Core elements of DNA-containing phages, which do not undergo proteolysis during phage maturation, were previously described for bacteriophage T7 (*P. aeruginosa*) [15] and phage 21 (*P. aeruginosa*) [6]. However, in these phages the central body can be visualized by election microscopy only after special preparation procedure (equilibrium centrifugation of phage preparations in cesium chloride and sucrose density

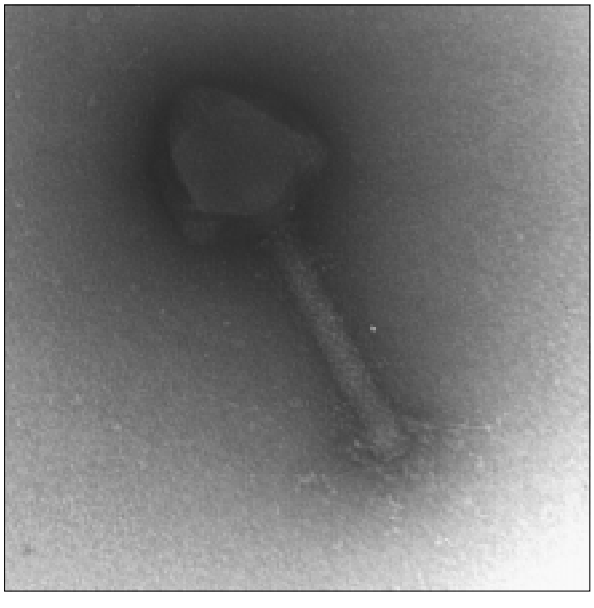

Fig. 1. Electron microscopy of mature bacteriophage φkz (*P. aeruginosa*). Negative contrasting with 1% uranyl acetate, ×200,000.

gradient). In contact, the central body of bacteriophage φkz can be visualized by negative contrasting in 1% aqueous solution of uranyl acetate without preliminary preparation.

Powerful sources of coherent laser light were often used in biological studies for destruction of macromolecules [1]. The use of a dye absorbing laser-emitted light allowed to destroy mitochondria without damaging other organelles. This method was used for the study of DNA packing in DNA-containing bacteriophage S_d [2]. The following physical mechanism can underlie laser destruction of biological objects in the presence of a dye: dye molecules interact with proteins of the viral capsid, absorbed energy is transformed into heat, the heat is released and caused destruction of some capsid structures [5]. The type of destruction of viral particles in the present study confirms this assumption. Fragments of destroyed virion have regular character and include genomic DNA. The packing density of phage DNA is known to be the highest among all other biological systems. For instance, packing density of genomic DNA of bacteriophage T7 is about 450 mg/ml, which 5-fold surpasses packing density of chromatin [8]. This fact provides an evidence that after partial destruction of virion DNA tends to release itself from the envelope. It should be also noted that phage head contains internal proteins and the content of these proteins greatly varies in different phages. The role of these proteins is not completely understood. Thus, the head of mature bacteriophage T4 contains 1000 protein molecules. Some molecules can participate in DNA compactization inside the capsid [7]. We assume that the size of DNA turns in all observed structures significantly exceeds that of pure DNA molecule because it is bound with internal bacteriophage proteins. It cannot be excluded that the number of internal proteins including histon-like proteins in bacteriophage φkz is higher than in well studied T-even phages. This assumption is supported by the presence of central protein body in the capsid of the virus φkz [4].

We assume that the data on free type I and II fragments are the most valuable information on DNA conformation in bacteriophage φkz. In our experiments not only partial, but also complete destruction of phage capsid was observed. However, heating of phage suspension with the dye has local nature and only proteins bound with dye molecules are destroyed, therefore, the internal structures of the head, which had no contact with the dye before irradiation, remain intact even after complete envelope destruction. Possibly, type A and B structures present genomic DNA

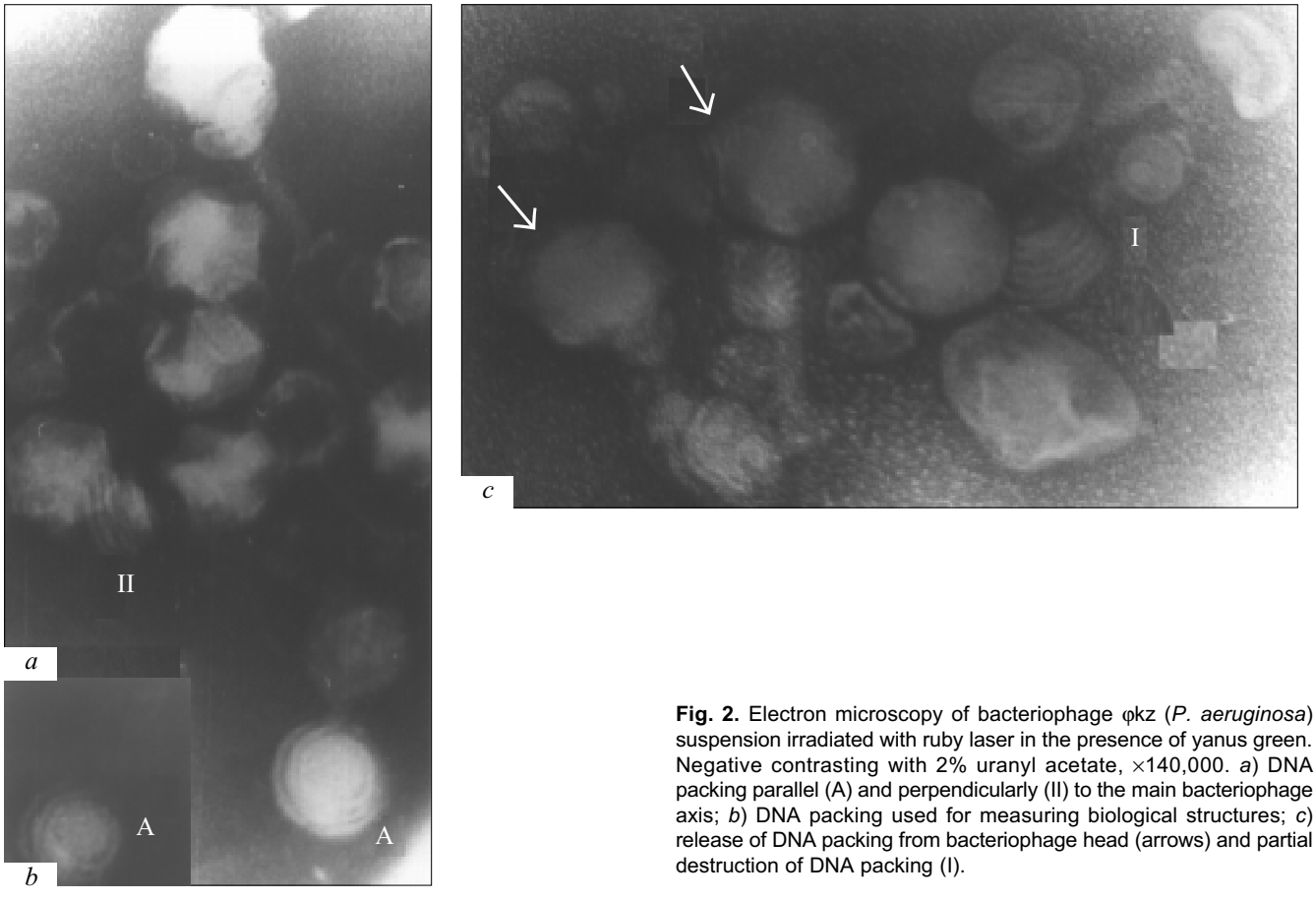

Fig. 2. Electron microscopy of bacteriophage φkz (*P. aeruginosa*) suspension irradiated with ruby laser in the presence of yanus green. Negative contrasting with 2% uranyl acetate, ×140,000. *a*) DNA packing parallel (A) and perpendicularly (II) to the main bacteriophage axis; *b*) DNA packing used for measuring biological structures; *c*) release of DNA packing from bacteriophage head (arrows) and partial destruction of DNA packing (I).

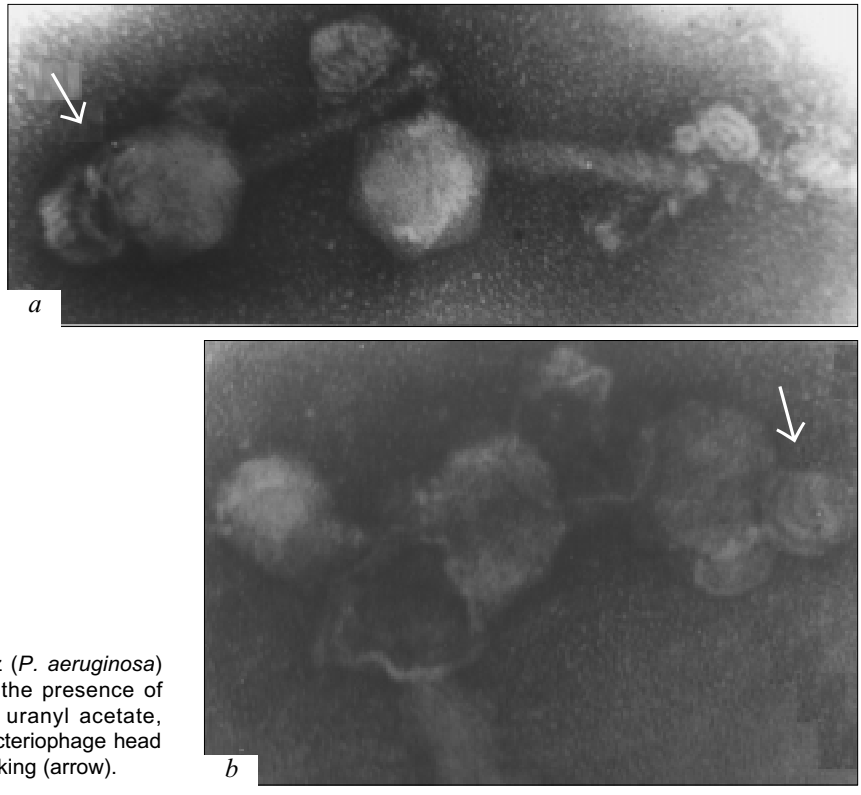

Fig. 3. Electron microscopy of bacteriophage φkz (*P. aeruginosa*) suspension irradiated with neodymium laser in the presence of methylene blue. Negative contrasting with 2% uranyl acetate, ×105,000. *a*) initial phase of DNA release from bacteriophage head (arrow); *b*) released and partly destroyed DNA packing (arrow).

combined with internal proteins and released after complete capsid destruction. Types A and B represent projections perpendicular and parallel to the tail axis, respectively. In this case spatial model of packed DNA can be presented as a coil [13]. Four main models of phage DNA packing were proposed. According to the first model, packed genomic DNA has a spool-like structure [9-11]. The second model in known as "spiral-fold". In this model double DNA helix is folded into sheets and packed in parallel layers in a capsid [7]. According to the third model capsid DNA is packed like in DNA crystals [14]. The last model is known as "folded toroid" [12]. Our data most agree with the first coil model of DNA organization. The axis of this structure coincides with the 5th order axis of the phage capsid, which connects two opposite peaks, including one phage tail. We assume that in bacteriophage φkz DNA complexated with internal capsid proteins infolded in layers organized perpendicularly to the main tail axis. The number of DNA turns around the central body is limited by capsid walls. Thus, the first layer (most distant from the tail) is formed by one turn of DNA-protein complex around the central body. Its diameter (43 nm) is equal to the diameter of the central body plus double thickness of DNA-protein complex (26 nm+2×8.6 nm). The diameter measured on microphotographs was 44 nm. Thus, the selected model agrees with experimental data. The second layer is formed by two turns of DNA-protein thread, layers 3 and 4 by 3 and 4 turns, respectively, layers 5 and 6 are formed by 5 turns, because they are located in the central part of the capsid. This can explain that structure A contains not more than 5 concentric turns, because it presents a projection of DNA layers on a plane perpendicular to the main tail axis (or the tail—central body axis). Thereafter, the number of turns then decreased to one. Calculations show that the expected number of layers is 10. However, on the photos of type I and II structures reflecting the number of DNA-protein layers we could observe maximum 7 layers, which may point to extreme instability of the DNA structure and its destruction in the absence of stabilizing effect of the capsid. The character of DNA destruction (disappearance of lower layers in subtypes I and II, and increase in diameter of the lower layers in subtype II to 150 nm) suggests that this process starts from the end of the molecule. This confirms the conclusion that spatial model of DNA packing in bacteriophage φkz can be imaged as a coil [13].

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