ON THE NATURE AND SIGNIFICANCE OF BACTERIOPHAGY

REPORT XIII*. ULTRA-THIN SECTIONS OF PHAGES

(METHOD AND PRELIMINARY DATA)

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Regardless of the considerable progress in utilization of the ultrathin section technique, in particular in sectioning of bacteria [3,4], this technique did not offer the means to obtain clear pictures of internal structure of phage particles. This condition is dependent on the results of fixation, dehydration, and imbedding of phage particles which are restricted by a series of highly significant difficulties. To the latter belong, first of all, the difficulties connected with obtaining of highly concentrated phage filtrates and the preparation from them of a residue of phage particles adequate for fixation.

Investigating the conditions for preparation, we developed a procedure which allows one to obtain relatively simply samples of ultra-thin section of phage particles. The completed electron microscopic investigations of samples led to obtaining of electron photograms which disclosed the internal structure of phage particles.

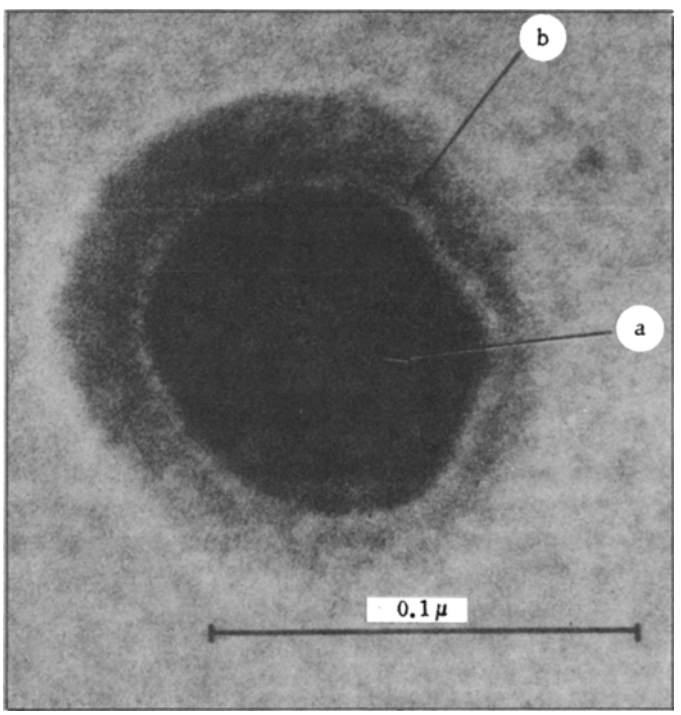

Fig. 1. Ultra-thin section of phage head; magnified 572,000. a) internal contents; b) outer layer.

* For Report XIII, see Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii, 2, 29 (1959).

Description of the developed procedure and of the obtained data comprises the contents of this article.

Phage 1321, lysing cells of Aerobacter aerogenes 1321, was used as the source for ultra-thin sections. The initial concentration of phage, as determined by the method of agar overlay, was 4×10^8 particles per ml.

The important feature of the proposed method consists of preliminary addition of antiphage serum which assures agglutination of phage particles. Another feature is connected with dehydration of phage particles which is attained, not by transferring of samples through different dilutions of alcohol, but by lyophilization. For imbedding one uses material obtained after lyophilization.

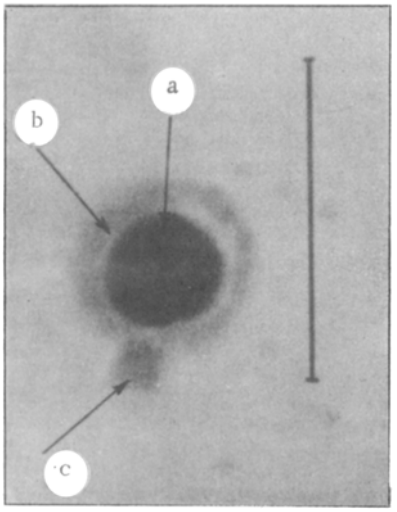

Fig. 2. Ultra-thin section of phage particle; magnified 432,000: a) internal contents; b) outer layer; c) tail.

In practice, the method consists of the following. One and a half ml of antiphage serum and 3.5 ml of 1% solution of OsO_4 are added to 3.5 ml of phage filtrate. The mixture is held for 4 hours at room temperature, one hour at 37°, and is then placed overnight in a refrigerator. After this, 1 ml portions of the material are distributed in ampules and lyophilized in frozen state. Material dried in ampules has the appearance of large-grain dark mass. Subsequently, the dry material is transferred for 4 hours, at room temperature, into a mixture of p-butyl methacrylate and methyl methacrylate (1:9) with 1% benzoyl peroxide. After holding in methacrylate, small grains of the material are transferred into capsules and are covered with polymerized methacrylate mixture with benzoyl peroxide. The blocks were dried for the usual length of time at 48°. They were then sliced, using glass knives, on Shestrand's ultramicrotome.

The study of prepared samples in electron microscope UEMB-100 made it possible to conclude that the described method allowed one to obtain systematically ultra-thin sections of free phage particles whose pictorial analysis offers a means of estimating their internal morphology.

One can see from a photomicrograph (Fig. 1), the head of a phage particle of the internal homogeneous material, characterized by sharply expressed osmiophilic nature, and external circular layer of poor contrast. On the photomicrograph of ultra-thin section of phage head and tail (Fig. 2), one can observe that the latter is a continuation of the external circular layer.

The obtained photomicrographs allow one to measure accurately the components of a phage particle. According to the measurements, the diameter of the head is about 900 A°, of which the diameter of the internal mass equals about 600 A° and the thickness of the external layer is about 300 A°. Thickness of the tail on the section equals about 350 A°. It should be noted that in similar electromicroscopic studies of the intact phage particles shadowed with chromium, it was found that diameter of the head equals 520-680 A°.

Thus the results of our experiments confirm that there is a positive solution to the problem of preparation of ultra-thin sections of phage particles. The availability of a method by means of which it is possible to "disclose" a phage particle offers a means for direct verification of biochemical representation of structure of phages and particularly of a detailed study of their internal structure.

The detailed structural-functional characterization of disclosed structures and their comparison with individual cell components of bacteria (nucleus, cytoplasm, cell wall) will form the subject of a separate report. It must be pointed out that even at this time, the obtained electron photomicrographs of phage sections allow one to negate definitely a consideration of the phage particle as being a spiral aggregate of spherical albumin macro-molecules [1, 2].

SUMMARY

A method is suggested for preparing ultra-fine sections of free phage particles. After adding antiphage serum and OsO_4 to the phagofiltrate the material is lyophilized in a frozen state. Later the material is placed into a metacrylate mixture with benzoyl peroxide, and is afterwards embedded by the usual method. Preliminary data are presented on the electron microscopic picture of phage particles (the ultra-fine sections).

LITERATURE CITED

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