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# Structure of Native and Chloroform-Methanol-Treated Mycobacteriophage R1

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The morphologies of native and chloroform-methanol-treated mycobacteriophage R1 were compared by electron microscopy, utilizing three negative stains. R1 was determined to be a complex phage. The head appears as an elongated cylinder with a pointed end (93 ± 3 by 42 ± 3 nm) constructed from an orderly arrangement of capsomeres. The phage tail measures 209 ± 11 by 11 ± 1 nm and possesses a striated surface with two base plates at its distal end. Treatment of R1 with chloroform-methanol resulted in disruption of both the head and tail structures and was accompanied by loss of infectivity. However, because no likely lipid-containing structure was observed in native phages, there is the possibility that the mechanism of chloroform-methanol inactivation is something other than lipid extraction.

Mycobacteriophage R1 (5) is a temperate phage carried by *Mycobacterium butyricum* and for which a variety of mycobacterial strains have been used as indicators. In very early work with R1, we observed that the use of chloroform in preparation and purification of phage stocks caused inactivation of the phage. Subsequent studies by Bowman (4), using a standard virological procedure for testing for organic solvent sensitivity, showed that chloroform did inactivate the phage. Fay and Bowman (Bacteriol. Proc., p. 174, 1971) also showed that diethyl ether inactivates R1. Because these latter studies did not compare the morphology of treated and untreated particles, we now report such comparisons. Recently, Soloff et al. (10) studied the morphological effects of a lipid extraction procedure on the phage. Neither Soloff nor ourselves obtained morphological evidence for the presence of a lipid-containing structure(s).

For this study, the buff strain of *Mycobacterium smegmatis* ATCC 607 (hereafter referred to as 607B) was used to assay the phage and to prepare lysates. Cells of 607B growing in Ghys (6) synthetic medium with aeration at 37°C were infected with R1 at a ratio of 5 PFU/colony former. The yield of virus was approximately 4 × $10^{10}$ PFU/ml after 18 h of incubation. Phage was purified by the partitioning method of Albertsson (1). After the removal of debris and dextran sulfate by KCl precipitation, the preparation was centrifuged three or four times at 5,000 × *g* until no visible pellet was obtained.

For electron microscopy, 1 drop of phage suspension was applied to Formvar-carbon-coated grids which had been rendered hydrophilic by high-voltage discharge. After 30 s, a piece of filter paper was used to remove the unevaporated suspension. The preparations were allowed to air dry for 1 min and were stained for 15 s with 1 drop of 2% phosphotungstic acid (PTA), pH 7.0; 2% uranyl acetate (UA), pH 4.5; or 1% ammonium molybdate (AM), pH 7.2. The excess stain was removed with a piece of filter paper, and the grids were air dried and examined in an Hitachi electron microscope (model HU-12).

When stained with UA, the phage head appears as a cylinder 93 ± 3 nm long and 42 ± 3 nm wide with pointed ends (Fig. 1). Tailless heads are swollen or oval. The tail measures 209 ± 11 nm in length and 11 ± 1 nm in width and appears constricted just below the point of attachment to the head. With UA staining, striations are visible on the tail surface, but details of a structure at the end of the tail are indistinct.

When R1 is stained with AM, more of the fine structure of the phage is evident (Fig. 2). Although the phage head appears oval, head capsomeres can be seen. The proximal end of the tail appears to project into the inner space of the head. The structure at the base of the tail appears to consist of two plates. The upper plate measures 17 ± 2 by 7 ± 1 nm, and the lower plate measures 12 ± 1 by 7 ± 1 nm. The tail core, whose width is approximately 2 nm, can be seen to pass completely through the proximal knob and the tail plates. No convincing evidence of tail fibers, spikes, or contractile sheath was observed.

A standard technique was used for determining the susceptibility of viruses to organic solvents (3). A 0.05-ml amount of chloroform-meth-

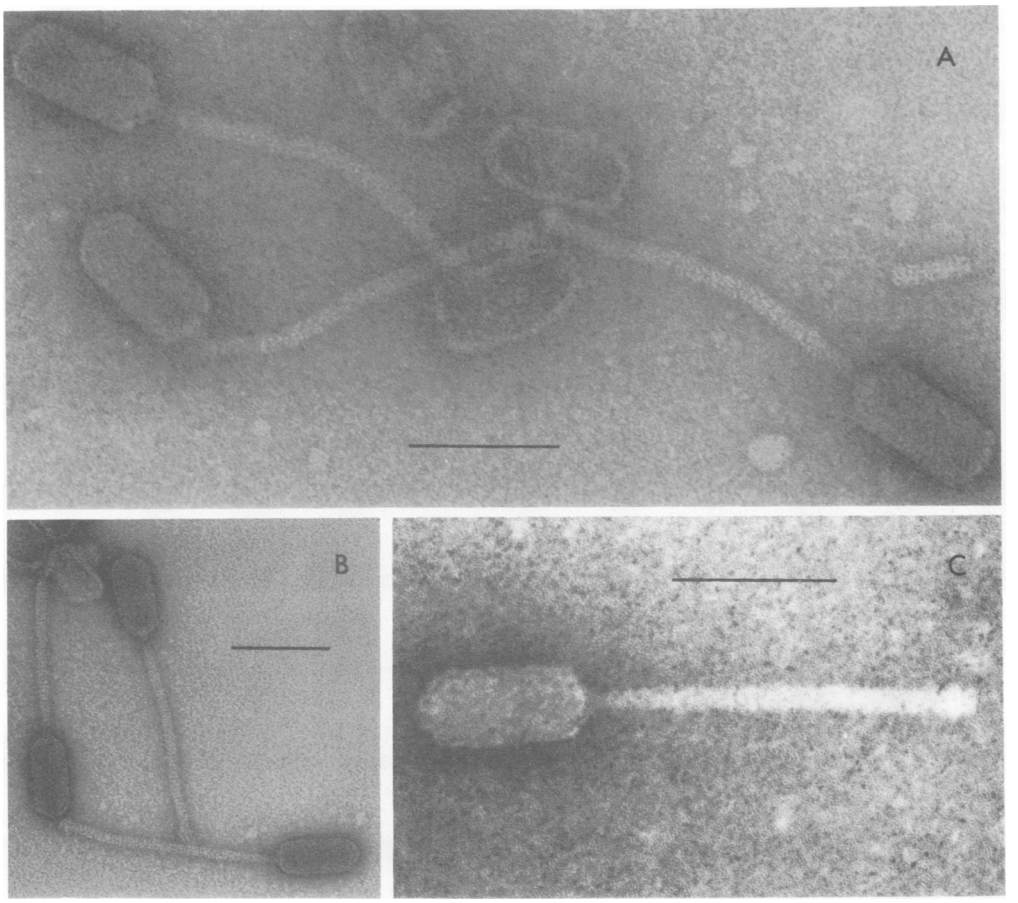


FIG. 1. *Mycobacteriophage R1 stained with 2% UA showing native phages (A), striated tail surface (B), and enlarged view of tail constriction (C). Bar = 100 nm.*

anol (CM) was added to 2 ml of phage suspended in buffer, and the mixture was shaken on a reciprocal shaker at 160 strokes per min for 10 min. The phage suspensions were assayed for infectivity and immediately applied to Formvar grids, stained, and examined as described above. Infectivity decreased by about 99% during CM treatment, and a corresponding amount of morphological damage occurred (Fig. 3), leaving few complete particles. The treated preparations consist mostly of free swollen heads and free tails or tail fragments. Ghosted heads are seen, and many free heads assume irregular shapes. Amorphous, unidentifiable structures, the size of phage heads, are also present. In control experiments, shaking R1 in buffer alone decreased infectivity approximately 30%, and the only observed morphological changes were tail separations and swelling of the heads. The head-tail separations which occurred during shaking, either in the presence or absence of CM, were due to breaks in the constricted area of the tail.

Unaltered heads are most consistently seen with the UA stain, whereas AM causes swelling. However, the use of AM allows the visualization of the capsomeric structure of the head and fine structure of the base plates. PTA causes such distortion of the phage morphology that little information can be derived. The success of Soloff et al. (10) with PTA staining may have been due to their use of PTA at a 1% concentration.

The susceptibility of a virus to CM (or other lipid solvents) is frequently interpreted as indicating the presence of structural lipids. Soloff et al. (10) have reported the extraction of lipids from R1 preparations and concluded that the inactivation of R1 by CM is associated with lipid removal. However, there are alternative explanations for the effect of organic solvents on phages. Amako and Yasunaka (2) reported the inactivation of filamentous phage Pf-1 by CM, but attributed the effect to disruption of hydrophobic bonds between coat proteins. Although coliphage T5 was inactivated by chloroform,

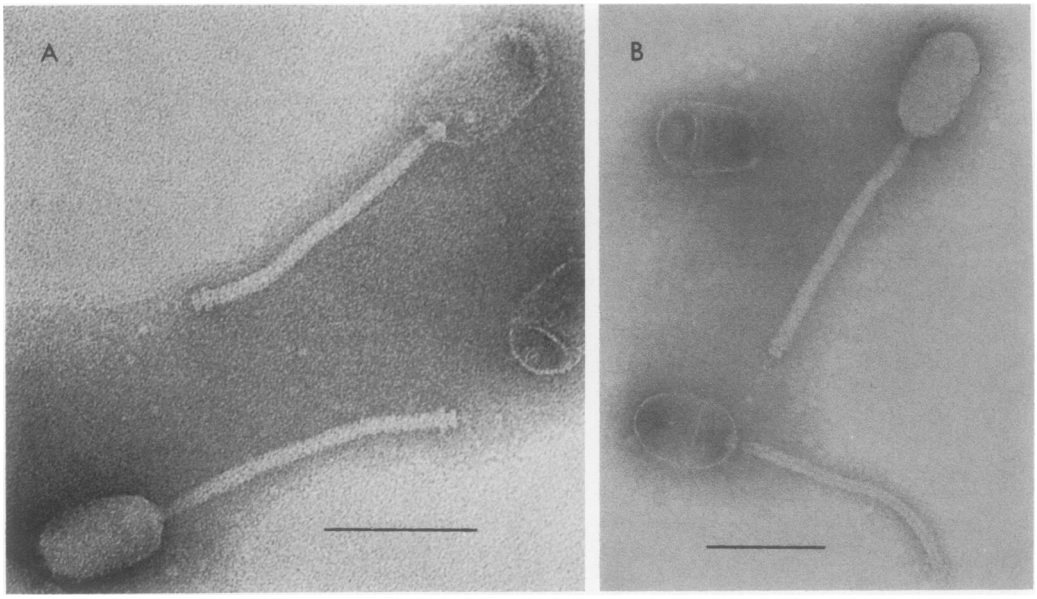


FIG. 2. *Mycobacteriophage R1 stained with 2% AM. (A) Double base plate; (B) capsomeric substructure of head and staining of the tail core. Bar = 100 nm.*

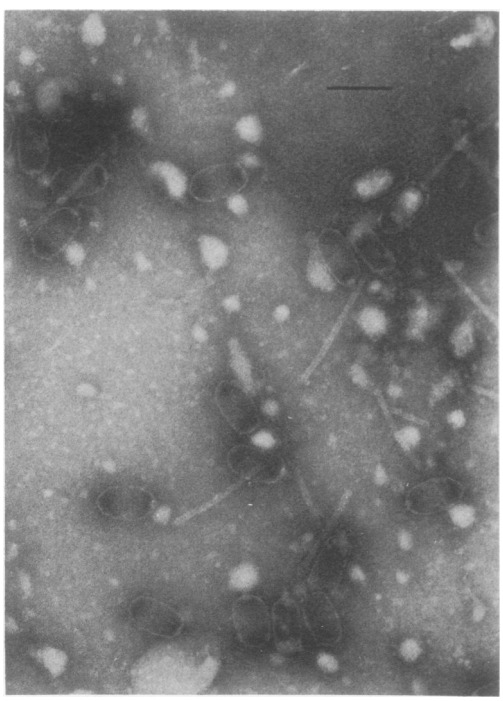

FIG. 3. *CM-inactivated mycobacteriophage R1 stained with 2% UA. Phage particles in buffer plus 2.5% CM were shaken for 10 min on a reciprocal shaker at 160 strokes per min. Bar = 100 nm.*

Schäfer et al. (9) assumed that a direct denaturation of phage proteins occurred at the solvent-water interface. Jones et al. (8) reported the presence of two lipids in the CM-sensitive mycobacteriophage D29; Schäfer et al. (9) were unable to confirm the presence of a lipid(s) and suggested that the inactivation of D29 may be due to a mechanism similar to that of phage T5.

Lipid-containing structures of viruses are frequently recognizable by electron microscopy. These include the lipid bilayer of *Pseudomonas* phage PM-2 (7) and the envelopes of animal viruses. Despite the susceptibility of R1 to CM and other organic solvents and the use of three negative stains, no morphological evidence was obtained to suggest that R1 possesses a structure likely to contain extractable lipids. Thus, the mechanism for its inactivation by organic solvents remains unanswered.

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