NOTES

Disruptive Action of Potassium Phosphotungstate on ϕ T, the Temperate Bacteriophage of *Bacillus megaterium* 899a

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Although untreated ϕ T may be dried on grids without damage, they were disrupted when negatively stained with potassium phosphotungstate. The potassium phosphotungstate reacted with phage suspended in phosphate buffer, and the altered but still-viable phage was disrupted only on drying. Stability of altered phage was not recovered by dialysis against 10^{-3} M Mg^{2+} .

Many bacteriophages of *Bacillus* spp. are known to be unstable under a variety of conditions (6). One type of instability is that to potassium phosphotungstate (PTA) used in negative staining for electron microscopy. For example, *B. stearothermophilus* ϕ TP84 (2) and T ϕ 3 of *B. stearothermophilus* (3) were not successfully stained by PTA and were disrupted into heads and tails. Likewise, Phycovirus LPP-1 was unstable in PTA but could be partially protected by 1 M Mg^{2+} ; hence it was postulated that chelation of Mg^{2+} resulted in the disruption (5). These three bacteriophages were successfully stained with uranyl acetate.

The above studies did not determine how the bacteriophage were disrupted during the preparation of the PTA smears. A disruption could have occurred by chelation of Mg^{2+} , by drying in PTA in the preparation of an electron microscopic (EM) grid, or by rapidly mixing the bacteriophage with the PTA reagent.

Temperate ϕ T of *B. megaterium* 899a is sensitive to chloroform, osmotic shock, and is also disrupted when negatively stained by PTA, ammonium molybdate, or uranyl acetate. Prefixation in osmium vapor or glutaraldehyde solution did not stabilize ϕ T in PTA. Phage T was prepared by induction of vegetative cells of *B. megaterium* 899a with 0.35 μ g of mitomycin C per ml, purified by differential centrifugation, concentrated on a CsCl gradient, and dialyzed against a buffer A containing 0.1 M NaCl, 0.01 M K_2HPO_4 at pH 7.2, and 10^{-3} M $MgCl_2$. The optical cross section (4) of the phage preparation was found to be 0.59×10^{-11} cm². A few

pieces of cell wall were noted as impurities. Equal volumes of 1% PTA (pH 6.9) and ϕ T in buffer A were mixed immediately before application on an EM grid.

Figure 1a shows a PTA smear of ϕ T with separated heads and tails. Attached to the tails are filaments, presumably the DNA. Shadowing was used to visualize the intact phage. Equal quantities (50 μ liters) of a suspension of ϕ T and either PTA, 1% EDTA at pH 6.9, or buffer A (control) were mixed for 20 min and then diluted to 1 ml with buffer A. Each mixture was then placed on a 3.9-ml preformed stepped gradient of CsCl, density from 1.7 to 1.3, and centrifuged at $100,000 \times g$ for 60 min.

The gradient was scanned at 254 nm in a gradient analyzer (ISCO model D with UV analyzer), the bacteriophage fraction was collected, dialyzed against buffer A, and titered on *B. megaterium* KM, Sp⁻ as an indicator. The profiles of the gradients are grouped in Fig. 2: (a) shows the control, ϕ T treated with buffer only, (b) the PTA-treated ϕ T, (c) the PTA control with the PTA remaining at the top of the gradient, and (d) ϕ T treated with 1% EDTA. Each of the main peaks is at the same position in the gradients. Titters of (a) and (b) fractions were the same (0.35 ml, containing 1.35×10^{10} ϕ T/ml); however, the peak fraction from (d) contained no viable phage after dialysis. The addition of EDTA to ϕ T disrupted it rapidly (note light peak) and rendered it nonviable, whereas with PTA treatment, it remained viable with no apparent alteration.

The recovered ϕ T samples were dried on a

FIG. 1. a, PTA-negative strain of ϕT showing disrupted phage. What appears to be DNA extruded through the phage tail lies between the two arrows; $\times 56,000$. b, Carbon replica of tungstate-shadowed ϕT showing intact phage; $\times 56,000$. c, Two views from one grid of a carbon replica of tungstate shadowed, PTA-treated ϕT showing disrupted phage. The extruded DNA is again indicated by an arrow; $\times 56,000$.

FRACTION OF GRADIENT LENGTH

FIG. 2. Optical density scans of *CsCl* gradients containing (a) untreated ϕT , (b) PTA-treated ϕT , (c) PTA alone, and (d) EDTA-treated ϕT . In (b) and (c) the PTA remained at the top of the gradient, and in (b) one could recover PTA-treated ϕT , free of PTA.

grid, shadowed at 14° with tungsten oxide, and a carbon replica was made. Figure 1b shows the control ϕT which is almost entirely composed of intact phage. Figure 1c shows the reisolated viable PTA-treated phage which consists mainly of disrupted heads and tails, and tails with extruded DNA fibers attached. Note the similarity of Fig. 1a and c.

Tungstate alone, at the concentration used, did not disrupt ϕT but rendered it unable to

withstand the forces of drying. Thus, the action of PTA is different from that of EDTA. Tungstate-altered ϕT dialyzed against 10^{-3} M Mg^{2+} is not stable on drying, nor is ϕT protected by inclusion of 10^{-2} M Mg^{2+} in the PTA, prior to the preparation of an EM grid.

If the K_2HPO_4 in buffer A is changed to Tris-hydrochloride at pH 7.2, the disruptive effect of PTA is greatly reduced so that 70% of the ϕT may remain intact on the EM grid. A further protective effect against PTA disruption is noticed when the $MgCl_2$ concentration is raised to 5×10^{-3} M in Tris-hydrochloride.

The lethal effect on many bacteriophages of triphosphate and other chelating agents, and the protective effect of Mg^{2+} has been noted (1). However, under our experimental conditions the action of PTA is more clearly demonstrated. Because tungstates and molybdates are known to react with protein, we suggest that PTA combines with some positively charged phage protein groups, causing the still-viable phage to be unstable to drying on the EM grid. Phosphate buffers enhance this effect, and low concentrations of Mg^{2+} (10^{-3} M) cannot reverse it.

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