66. Lytic Enzyme Activity Associated with Double-stranded RNA Bacteriophage $\phi 6$

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Bacteriophage $\phi 6$ of *Pseudomonas phaseolicola* has a double-stranded ribonucleic acid genome and is surrounded by a lipid-containing envelope.^{1),2)} The infective cycle of this bacteriophage was recently observed under electron microscope.^{3),4)} During infection of *P. phaseolicola* the $\phi 6$ envelope and the bacterial outer membrane fuse to form a bulge, subsequently the nucleocapsid penetrates the cytoplasmic membrane. In the step of penetration the peptidoglycan layer of the host cell is expected to be digested by the nucleocapsid. In this paper we describe a lytic enzyme activity associated with the $\phi 6$ virion.

Materials and methods. Bacteriophage $\phi 6$ and its host *Pseudomonas phaseolicola* HB10Y were kindly supplied by Dr. A. K. Vidaver. The MSC (minimal salts/casein) medium⁵⁾ was used for bacterial growth and phage production. The phage was purified from 300 ml lysate. After removal of cell debris by low speed centrifugation, the lysate was centrifuged at $75,000\times g$ for 60 min. The pellet was suspended in 5 ml of 10 mM potassium phosphate (pH 7.1) containing 1 mM $MgSO_4$ (buffer A). This solution was layered onto a 10%–35% sucrose gradient in buffer A and centrifuged at $52,000\times g$ for 100 min. The peak fractions of infectivity were collected and centrifuged at $75,000\times g$ for 120 min. The purified phage was suspended in 1 ml of buffer A (1.0×10^{13} plaque forming units (PFU)/ml).

Results and discussion. Lytic enzyme activity was demonstrated by spot test on a chloroform treated bacterial lawn. The cell wall of gram-negative bacteria is sensitive to hydrolysis by a lytic enzyme after the cells are killed by chloroform vapor.⁶⁾ When 5 μ l aliquots of the purified $\phi 6$ solution at different concentrations were spotted on bacterial lawns of several species, clear lytic spots were observed. Where *P. phaseolicola* HB10Y R-4 (Fig. 1B) is a $\phi 6$ resistant mutant of *P. phaseolicola* HB10Y (Fig. 1A), and *Caulobacter crescentus* CB13 (Fig. 1C) is a non-host bacterium. Similar lytic spots were also observed with *Escherichia coli* K38 (photograph

Fig. 1. Lytic activity and infectivity of bacteriophage $\phi 6$ examined on agar plates of several bacteria.

For lytic activity test, each bacterial lawn was prepared by the usual top agar (0.4%) overlayer technique. *P. phaseolicola* HB10Y wild type and R-4 were incubated overnight at 25°C on MSC-agar plate, and *C. crescentus* was incubated at 30°C on PYE-agar plate (9). After the lawn was exposed to chloroform vapor by adding 1 ml of chloroform to the cover of inverted plate for 20 min, each 5 μ l of the solution containing phage $\phi 6$ in different concentrations ((1) the original stock, 1.0×10^{13} PFU/ml, (2) 4-fold dilution, (3) 4^2 -fold dilution, (4) 4^3 -fold dilution, (5) 4^4 -fold dilution of the original stock) was spotted on the lawns. The plates were incubated for additional 3 hr at 25°C. (A) *P. phaseolicola* HB10Y wild type, (B) *P. phaseolicola* HB10Y R-4, (C) *Caulobacter crescentus* CB13. For infectivity test each of 5 μ l of phage $\phi 6$ preparation at the same concentrations as described above was spotted on the top agar (0.4%) layer soon after the top agar containing indicator cell has solidified. Each indicator cell was grown overnight in the same agar-medium at the same temperature as described above. (D) *P. phaseolicola* HB10Y wild type, (E) *P. phaseolicola* HB10Y R-4, (F) *C. crescentus* CB13.

not shown). Infectivity spot tests using $\phi 6$ and these species of bacteria, gave positive results with only the susceptible host *P. phaseolicola* HB10Y, and not others (Fig. 1, D–F). This indicated that the lytic activity on chloroform treated lawns was due to a lytic enzyme in $\phi 6$, and not due to infection. This detection method is so sensitive that crude lysate showed a lytic activity, whereas after the removal of phage particles by centrifugation no activity was detected.

Lytic enzyme activity was also measured using lyophilized *E. coli* cells as substrates.⁷⁾ Lytic activity increased markedly when the intact

Fig. 2. Increase of lytic activity of $\phi 6$ virion by treatment with Triton X-100 or with chloroform.

A fresh suspension of lyophilized *E. coli* cells (0.2 mg/ml) in 10 mM Tris (pH 7.4)-50 mM NaCl was stored overnight in the refrigerator. The cell suspension (0.98 ml) was mixed with 0.02 ml of phage $\phi 6$ preparation (1.0×10^{13} PFU/ml) at 25°C and the decrease of the absorbance at 350 nm was monitored for the first 10 min. Buffer A as a control ○, $\phi 6$ ●, $\phi 6$ treated with 0.5% Triton X-100 for 5 min at 25°C ●, $\phi 6$ treated with chloroform-saturated buffer A for 5 min at 25°C ▲.

phage was treated with non-ionic detergent Triton X-100 or with chloroform (Fig. 2). These treatments resulted in a loss of infectivity and in the removal of phage envelope.^{4),8)} These results indicate that the lytic enzyme is localized within the virion of phage $\phi 6$ and is not a contamination from the lysates.

The association of lytic enzyme activity with the $\phi 6$ particle suggests a possible role of the enzyme at the early infection step. When the envelope of $\phi 6$ is removed during the course of the fusion to the host cell membrane, this enzyme may hydrolyse the peptidoglycan of the host cell for the easy penetration of the nucleocapsid into the cell wall. The characterization of the lytic enzyme associated with $\phi 6$ is now in progress.

Summary. Lytic enzyme activity was found in the purified virion of double-stranded RNA bacteriophage $\phi 6$ of *Pseudomonas phaseolicola*. This activity increases after treatment of phage particles

with non-ionic detergent Triton X-100 or with chloroform.

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