BIKEN JOURNAL Vol. 14, 197-201, 1971

SHORT COMMUNICATION

SOME PROPERTIES OF THE PHAGE OF THE *CORYNEBACTERIUM DIPHTHERIAE* PARK WILLIAMS NO. 8 STRAIN[1]

MORIHIRO MATSUDA, CHIE KANEI and MASAHIKO YONEDA

Department of Tuberculosis Research I, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka

(Received February 8, 1971)

A recent discovery by Maximescu (1968) of indicator strains (*C. ulcerans* strains 9304 and 298G) for the phage of the well-known Park Williams No. 8 (=PW8) strain of *C. diphtheriae* has made it possible to study the nature of the PW8-carried phage, whose knowledge would facilitate the approach to the problem of the phage-host relationship in diphtheria toxinogenesis in this unusually highly toxinogenic organism. In her study (1968), Maximescu revealed the spontaneous liberation, by all the substrains of the PW8 strain tested, of phage which was active against the particular *C. ulcerans* strains described above. She also showed the host-range activity, plaque morphology of the phage and the converting ability to toxinogenesis. This communication briefly describes some other profiles of the PW8 phage: the inducible nature of the prophage, the morphology of the phage released and its serological property, which is distinct from those of classical tox^+ corynebacteriophages of the β group.

The bacterial strains employed were a substrain (Biken) originally derived by single colony isolation from another substrain (Toronto-Harvard) of *Corynebacterium diphtheriae* Park Williams No. 8 strain (Park and Williams, 1896), a nonlysogenic nontoxinogenic strain C7(−) of *C. diphtheriae* (Barksdale and Pappenheimer, 1954) and the two *C. ulcerans* strains, 9304 and 298G, kindly provided by Drs. Saragea and Maximescu. Modified Pope's medium (Yoneda and Matsuda, 1961) was used for the PW8 and two *C. ulcerans* strains, PGT medium (Barksdale and Pappenheimer, 1954) for the C7(−) strain. The conditions for growing these organisms and other experimental conditions are given in the legends to the accompanying figures. The phage techniques employed were as described by Adams (1959). Plating bacteria for phage assay were prepared as follows; the C7(−) and *C. ulcerans* 9304 (or 298G) strains were grown in each medium containing 0.3 μg Fe^{++} per ml, the cells were harvested from actively growing cultures (3×10^8 cells/ml) by centrifugation. A well homogenized suspension containing about 10^9 cells per ml was made by shaking the packed cells vigorously with glass beads in an appropriate small volume of fresh medium. Aliquots of 0.1 ml of the homogenized suspension were applied to tryptose agar plates and phage was assayed as plaque forming units (PFU).

Fig. 1 shows plots of the numbers of infective centers of a PW8 culture on a lawn of *C. ulcerans* strain 9304 as a function of the dose of ultraviolet light. As can be seen, appropriate

1 This work was reported at the 22nd Annual Meeting (Kansai District) of the Japan Bacteriological Society at Tsu, on September 28, 1969.

UV-irradiation caused a marked increase in the size of the phage-producing fraction of the PW8 culture. This clearly indicates the inducible nature of the PW8-carried phage. Under the conditions employed, the optimal dose of irradiation for induction was about 100 sec and at this dose some three hundred-fold increase in the number of infective centers (2×10^6/ml) was observed. On the other hand, even after irradiated with this optimal dose only partial lysis was observed on incubation at 33 C with gentle shaking for 5 hr. The lysate obtained was assayed for free phage on *C. ulcerans* lawn and was found to contain 1.5×10^8 plaque forming units per ml. Calculation revealed that the induction rate was very low (about 1.4%) but the average burst size (56) was comparable to both that on UV-induction of a normal lysogenic C7 (β) strain and on infection of the C7(−) strain with nonlysogenizing phage β^{hv64} (Matsuda and Barksdale, 1967), suggesting strongly the non-defective nature of the PW8-carried phage. The plaques formed by the phage (tentatively designated here as ω) on *C. ulcerans* lawns were all small and turbid as described by Maximescu (1968).

Plating of a UV-lysate of the PW8 strain on a C7(−) lawn gave a few occasional plaques (Barksdale, 1959; Miller et al., 1966; Maximescu, 1968) and these were also small. The phage in the UV-lysate responsible for the formation of these few plaques was tentatively designated as ω_7. Phage ω_7 was purified by extensive single plaque isolations on C7(−) lawns on which phage ω cannot produce plaques. The purified preparation formed plaques on *C. ulcerans* lawns only at very low efficiency (about 10^{-2} of that on C7(−) lawns).

Electron micrographs of the two corynebacteriophages, ω and β^{hv64}, negatively stained with uranyl acetate, are shown in Figures 2 (a) and 2 (b). Fig. 2 (a) is a preparation of phage ω from an irradiated culture of the PW8 strain. The material for electron microscopic examination was a lysate containing 1.5×10^9 PFU/ml prepared by the method described in Fig. 1 without procedures such as ultracentrifugaiton and resuspension which could affect phage morphology. As seen in the photograph, the preparation of phage ω consisted almost entirely

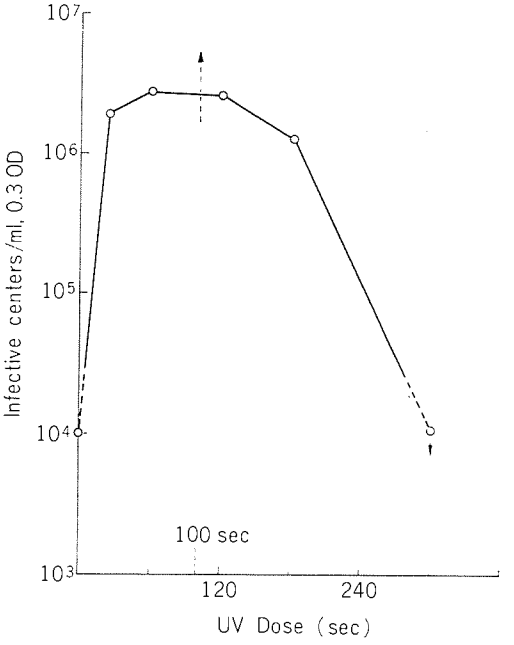

FIGURE 1. *Induction of the PW8 strain of Corynebacterium diphtheriae with ultraviolet light. Cells of the PW8 strain were grown overnight in Pope's medium containing 0.1 μg iron/ml to a final OD of 1.0 (1.3×10^9 cells/ml). The cells were diluted in the same medium containing 0.3 μg iron/ml to OD 0.1 and incubated with shaking (160 rotations/min) at 35 C and allowed to grow to OD 0.3. Then 5 ml aliquots of culture were irradiated in Petri dishes under a Toshiba 15 w germicidal lamp (GL-15) at a distance of 45 cm. Samples of 0.1 ml were taken at intervals and after appropriate dilution were plated in the dark on tryptose agar using C. ulcerans, strain 9304, as indicator cells for assay of infective centers. For preparing a phage lysate containing a high titer of free phage, after irradiation for 100 sec under the conditions described above, the cells were chilled and centrifuged in the cold and the packed cells were resuspended in one-tenth of the original volume of fresh medium. Then they were centrifuged and 5 ml aliquots were incubated in shielded 100 ml Erlenmeyer flasks with gentle shaking (130 rotations/min) at 33 C for 5 hr. The yield of free phage from the original irradiated culture was calculated by dividing the yield of free phage thus obtained after concentration by 10.*

of complete phage particles with a polyhedral head and long slender tail, and their shape appears almost indistinguishable from that of β^{hv64} phage shown in Fig. 2 (b). The appearance of phage ω is also similar to those described previously of β, B, Bh and the particles found in " UV-lysate " of a substrain of the PW8 strain (Mathews et al., 1966) and of other corynebacteriophages (Holmes and Barksdale, 1970). Measurements of the dimensions of phages ω and β^{hv64}, however, showed that phage ω was somewhat smaller than β^{hv64}; the average dimensions of the head (width/length) and tail (length) of phage ω were 42 mμ/46 mμ and 222 mμ while those of β^{hv64} were 48 mμ/51 mμ and 274 mμ, respectively. Thus phage ω appears to be smaller than the other corynebacteriophages described by Holmes and Barksdale (1970).

Fig. 3 shows data on the kinetics of neutralization of phages by anti β^{hv64} rabbit serum (K=17.5). As can be seen, the anti β^{hv64} serum neutralizes the infectivity of all the phages tested but the rate of neutralization is much lower with phage ω than with the other phages. The K value for phage ω was 5.7 while those for phages β and ω_7 and for the homologous phage β^{hv64} were all 17.5. This clearly indicates that phage ω is serologically related, but not identical, with phage ω_7 and phages of the β group such as α, β, β^{hv64} and P, which are neutralized at the same rate by

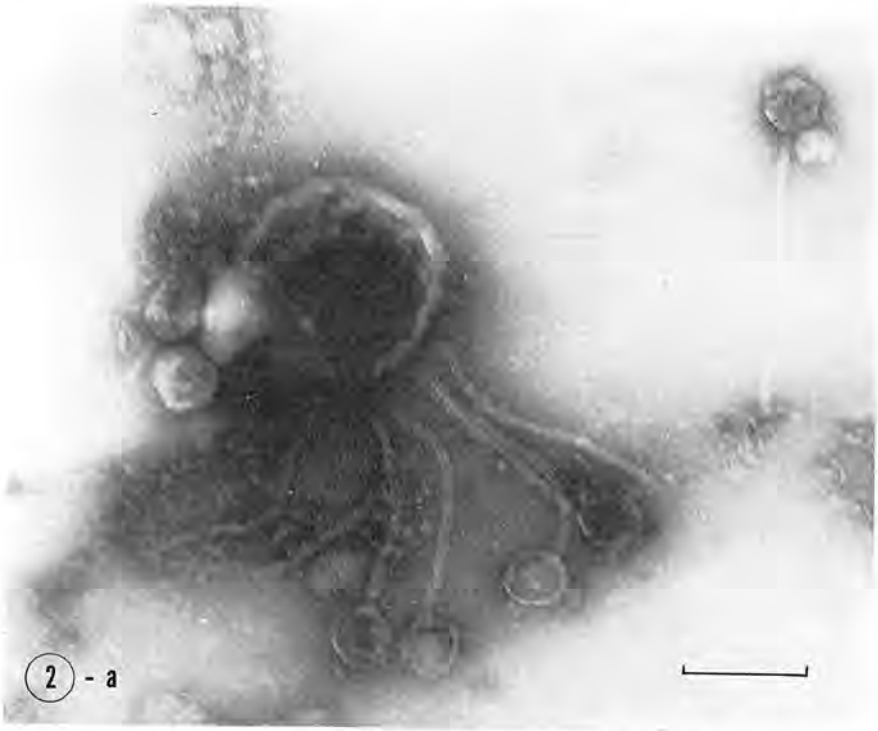

FIGURE 2 (a). *Electron micrograph of bacteriophage (ω) released by induction of the PW8 strain of Corynebacterium diphtheriae with ultraviolet light. Initial magnification* $\times 41{,}000$. *Marker=ca 1,000 A. A UV-lysate was mounted on carbon-supported collodion films, dried in air and washed with distilled water. Then it was negatively stained with 1% uranyl acetate and examined under a Hitachi electron microscope, type HU-11D-S. The method of preparation of the UV-lysate is described in the legend for Fig. 1.*

anti β^{hv64} serum (Matsuda and Barksdale, 1967).

Incidentally, the bacteria lysogenized with phage ω or ω_7 were found to be immune to the infection by phage β and those with phage β, to the infection by phage ω or ω_7. In any event, from what we observed on the profiles (host range activity, UV-inducibility and serological property), it is clear that ω and ω_7 are different kinds of phages though they were derived from a single source, the PW8 strain.

The toxin produced by various lysogenic strains of different phages (ω, ω_7 and β) has so far been found to be a single molecular species in terms of immunochemical specificity and electrophoretic mobility in acrylamide gel. The question of whether this indicates the bacterial origin of the "toxin gene" must await further study.

ACKNOWLEDGEMENTS

We are indebted to Drs. Saragea and Maximescu for their kindness in providing strains of *C. ulcerans*. We are also grateful to Dr. Y. Hosaka, Mr. T. Fukuoka and Mr. I. Shirahase for their help in electron microscopic examinations.

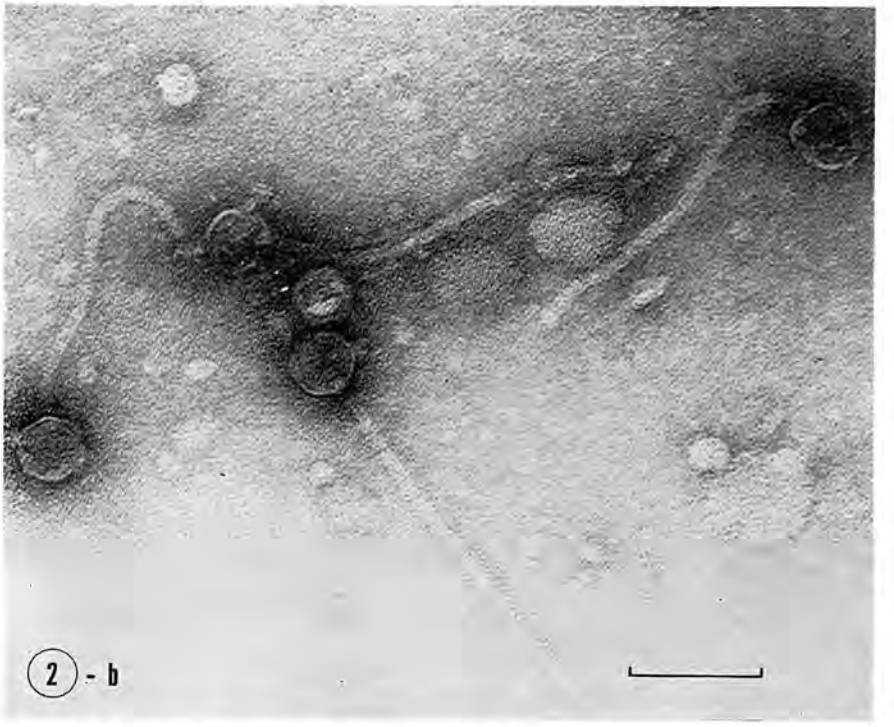

FIGURE 2 (b). *Electron micrograph of corynebacteriophage* β^{hv64}. *Initial magnification* × *41,000. Marker=ca 1,000 A. Conditions for electron microscopic examination were as described in the legend for Fig. 2 (a). The* β^{hv64} *lysate employed was prepared as described by Matsuda and Barksdale (1967).*

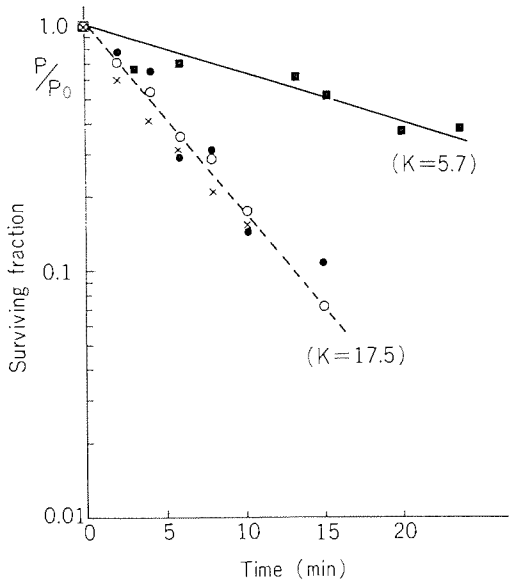

FIGURE 3. *Kinetics of neutralization of corynebacteriophages by anti-β^{hv64} phage rabbit serum. Neutralization experiments were carried out at 37 C at an anti-phage serum dilution of 1 : 100. PW8-phage ω was propagated in C. ulcerans cells (■——■), ω_7 was purified by several successive single plaque isolations from a very few particles, which formed plaques on C7 (−) cells, and was propagated in C7 (−) cells (○——○). Phage β was propagated in C. ulcerans cells, $\beta_{(ulc)}$ (●——●) and in C7 (−) cells, $\beta_{(7)}$ (×– – –×). The anti-β^{hv64} phage serum employed in these experiments was prepared as described by Matsuda and Barksdale (1967). The indicator cells for phage and $\beta_{(ulc)}$ were C. ulcerans strain 9304 and for phage ω_7 and $\beta_{(7)}$ were C7 (−) strain.*

REFERENCES

Adams, M. H. 1959. Methods of study of bacterial viruses, p. 443–522. *In* M. H. Adams, Bacteriophages. Interscience Publishers, Inc., New York.

Barksdale, L. 1959. Lysogenic conversions in bacteria. Bacteriol. Rev. 23 : 202–212.

Barksdale, W. L. and A. M. Pappenheimer, Jr. 1954. Phage-host relationships in nontoxigenic and toxigenic diphtheria bacilli. J. Bacteriol. 67 : 220–232.

Holmes, R. K. and L. Barksdale. 1970. Comparative studies with tox^+ and tox^- corynebacteriophages. J. Virol. 5 : 783–794.

Mathews, M. M., P. A. Miller and A. M. Pappenheimer, Jr. 1966. Morphological observations on some diphtherial phages. Virology 29 : 402–409.

Matsuda, M. and L. Barksdale. 1967. System for the investigation of the bacteriophage-directed synthesis of diphtherial toxin. J. Bacteriol. 93 : 722–730.

Maximescu, P. 1968. New host strains for the lysogenic *Corynebacterium diphtheriae* Park Williams No. 8 strain. J. Gen. Microbiol. 53 : 125–133.

Miller, P. A., A. M. Jr. Pappenheimer and W. F. Doolittle. 1966. Phage-host relationships in certain strains of *Corynebacterium diphtheriae*. Virology 29 : 410–425.

Park, W. H. and A. W. Williams. 1896. The production of diphtheria toxin. J. Exp. Med. 1 : 164 :185.

Yoneda, M. and M. Matsuda. 1961. Immunological and biological properties of a subcellular component of *Corynebacterium diphtheriae* having the capacity to bind antitoxin. Biken's J. 4 : 171–186.