Characteristics of a Group IV Cholera Phage

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Cholera bacteriophages have been classified into four distinct groups by Mukerjee (1963a). Of these, the group IV phages can differentiate *Vibrio cholerae* and *V. eltor* organisms (Mukerjee, 1963b) and are of practical importance. The hitherto unreported physiological and physico-chemical properties of the group IV cholera phage, φ149, have been investigated by us and the results are presented below.

Cholera bacteriophage φ149 was propagated on the host *V. cholerae* strain OGAWA 154 following in general the method of Hershey, Kalmanson & Bronfenbrenner (1943). The phage stock thus obtained usually contained about 10¹¹ p.f.u./ml. as assayed by double agar layer technique (Adams, 1959). This phage yielded clear plaques of diameter between 0.5 and 1.5 mm. after 16 hr incubation at 37°C. Longer incubation produced a surrounding halo. In all cases, bacteria were grown in nutrient broth (NB) containing, in 1 l. of distilled water: bactopeptone (Difco), 10 g.; NaCl, 5 g.; beef extract (ACAS, Italy), 10 g.; pH 7.4.

Kinetics of adsorption was studied at an m.o.i. of 0.001 or 0.01 following in general the method described by Adams (1959). Adsorption was biphasic with rate constants of 1.026 × 10⁻⁹ ml./min, up to 81% adsorption and 9.950 × 10⁻¹⁰ ml./min. thereafter. Similar biphasic adsorption kinetics was also reported for the phage 7V of *Pseudomonas aeruginosa* (Feary, Fisher & Fisher, 1964), phage χ of *Escherichia coli* (Schade & Adler, 1967) and the phage φW14 of *P. acidovorans* (Kropinski & Warren, 1970). Adsorption of this phage φ149 depended significantly on the pH of the medium. In NB media of pH 6, 7.4, 8 and 9, the percentages of phage adsorbed in 15 min. at 37°C were 72, 85, 64 and 60%, respectively, with a m.o.i. of 0.001. No significant adsorption to the *V. eltor* strain MAK 757 could be obtained even after 20 min. incubation in NB of pH adjusted to any of the above values. *V. eltor* was known to be resistant to group IV cholera phage but the basis of this resistance was unknown. It has now been shown that the cell wall of *V. eltor* does not have the specific phage receptors.

Cell wall and lipopolysaccharide (LPS) were isolated from the host cell, following in general the methods of Keeler et al. (1966) and Westphal, Lüderitz & Bister (1952), respectively. The purity of cell-wall preparation was checked by electron microscopy, u.v. and visible extinction (Sur & Chatterjee, 1970) and that of LPS by u.v. extinction, electron microscopy and chemical analysis (Chatterjee, Adhikari & Raychaudhuri, 1971; Adhikari, 1971). Assays of phage adsorption to cell walls (10/µg./ml.) and to LPS (10/µg./ml.) were done by the methods of Chatterjee (1969) and Lindberg (1967), respectively. Adsorption of cholera phage φ149 to isolated cell wall and LPS followed first-order reaction kinetics resulting in 70% inactivation by cell wall within 30 min. and 50% by LPS within 60 min. These results agree satisfactorily with those reported for mycobacteriophage GS-7 (Imaeda & San Blas, 1969). The presence of phage receptors in these structures is in conformity with the observations of Levine & Frisch (1934) for *Enterobacteria* and of Lindberg (1967) for *Salmonella minnesota*. After treatment with 0.5% (w/v) sodium deoxycholate for 1 hr at 37°C, cell wall as well as LPS largely lost their phage-inactivating capacity. The 50% phage-inactivating concentration (IC50) of LPS increased from the normal value of 7 µg./ml. to about 3.6 mg./ml. after sodium deoxycholate treatment. The loss of phage-inactivating capacity might be due to the fact that sodium deoxycholate dissociates LPS of Gram-negative bacteria into very small units with subsequent loss of biological activity (Ribi et al. 1966; Lindberg, 1967).
Kinetics of thermal inactivation of phage $\phi 149$ was studied in NB medium at 50°, 60° and 65° following in general the method of Pollard & Reaume (1951), the initial phage titre in all cases being about $4 \times 10^8$ p.f.u./ml. The rate constant ($K$), free energy ($\Delta F$), enthalpy ($\Delta H$) and entropy ($\Delta S$) were determined (Friedman & Cowles, 1953) and are shown in Table 1. At all temperatures inactivation obeyed the first order reaction kinetics. The half-life at different temperatures was similar to that reported (Guice & Newman, 1969) for a number of unclassified cholera phages. The thermodynamic constants of phage $\phi 149$ are similar to those of coliphage $T_2$ (Pollard & Reaume, 1951). Phage $\phi 149$ was rapidly inactivated below pH 4 and above pH 11. In NB media adjusted to pH 4, 5, 6, 7, 8, 9, 10 and 11, phage survivals after 1 hr at 37° were 35, 82, 92, 100, 100, 97, 94 and 98 %, respectively. Inactivation of phage $\phi 149$ by different chemicals was studied following in general the method of Kuo, Huang & Chow (1969) with an initial phage titre between $10^6$ and $10^8$ p.f.u./ml. No significant loss of viability could be observed after 1 hr at 37° with any of the following chemicals: tris (100 $\mu$M, pH 8); tris (100 $\mu$M, pH 8) + EDTA (50 $\mu$g./ml.); tris (100 $\mu$M, pH 8) + lysozyme (50 $\mu$g./ml.); sodium deoxycholate 1 % (w/v), trypsin (500 $\mu$g/ml.) in 0.1 M-phosphate buffer (pH 7-6); chloroform (0.5 ml./20 ml. of phage suspension); 0.1 M-phosphate buffer (pH 7); furazolidone (5 $\mu$g./ml.) and mitomycin C (5 $\mu$g./ml.) in NB. The phage could be stored over chloroform at 4° for over one year without any significant loss of titre. However, sodium lauryl sulphate (SLS) rapidly inactivated it and its half-life in 0.005, 0.05 and 1 % w/v SLS in 0.1 M-phosphate buffer (pH 7) was 3.3, 2 and 1 min., respectively.

The average burst size as determined by the single-step growth experiment (Ellis & Delbrück, 1939) was 58 p.f.u./cell and by the single burst experiment (Adams, 1959) 51 p.f.u./cell.
The latent period of infection and rise period were 36 and 24 min., respectively. The eclipse period as determined by inducing premature lysis of the infected cells (Sechaud & Kellenberger, 1956) was 20 min.

The effect of drugs on phage yield was studied following in general the method of Watanabe & August (1967). As shown in Fig. 1 the yield was reduced to 50% of the control value by 0.15 µg./ml. of mitomycin C or 0.05 µg./ml. of furazolidone.

Fig. 2. (a) and (b). High magnification reproductions of the negatively stained cholera phage φ149 showing knob (K) and prongs (arrow) at the end of the tail.

Phage φ149 was shown by electron microscopy to have a hexagonal head measuring 75 × 85 nm. and a flexible tail of length 155 nm. and width 11 nm. terminating in a knob 13 nm. in diameter with prongs (Fig. 2). It thus belongs to group B of Bradley’s (1967) classification and its morphology agrees satisfactorily with that reported previously for a different strain of group IV cholera phage (Chatterjee, Das & Barua, 1965).

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Department of Biophysics
School of Tropical Medicine
Calcutta-12, India

M. MAITI
S. N. CHATTERJEE

REFERENCES
Short communications


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