Host-dependent Properties of Coliphage $\phi$W and its Female-specific Host-range Mutants $\phi$3 and $\phi$4

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(Accepted 22 February 1974)

SUMMARY

Coliphage $\phi$W and its female-specific host range mutants $\phi$3 and $\phi$4 were grown on Escherichia coli B and strain D31, an LPS mutant of E. coli K12. Comparison of phage samples from centre and halo of single plaques indicates that host-range mutants of $\phi$3 type are enriched in halos on strain D31. For $\phi$W there was a decreased adsorption to full-grown cells, for $\phi$3 there was only a minor change. Caesium chloride gradients have shown that $\phi$3 is denser than $\phi$W and $\phi$4. After growth on strain D31 both $\phi$W and $\phi$3 produced non-infectious tail-less particles, and burst sizes were reduced correspondingly. It is suggested that the assembly of the tail could be blocked by an assumed LPS precursor.

INTRODUCTION

Dettori, Maccacaro & Piccinin (1961) isolated the first female-specific phage, and later some previously known phages, notably T3, T7 and $\phi$II were found to be female-specific (Schell et al. 1963, Mäkelä, Mäkelä & Soikkeli, 1964; Cuzin, 1965). These phages have found a wide use as tools, but few studies only have been concerned with the mechanism behind their host-specificity. It now appears that growth of female-specific phages can be halted on at least the following molecular levels. (1) At the stage of the adsorption to lipopolysaccharide (LPS) which is the receptor (Monner, Jonsson & Boman, 1971). (2) At the DNA level where a prophage nuclease can interfere with phage formation (S. G. Skogman & G. R. Björk, unpublished observations). (3) At the stage of translation where two F-factor-mediated proteins are believed to interfere with the synthesis of most phage-specific proteins (Morrison & Malamy, 1971).

We have in a previous communication (Monner & Boman, 1970) shown that the phage commonly referred to as $\phi$II in fact is a host-range mutant of the original phage $\phi$II described by Wollman (1947). To avoid unnecessary confusion due to terminology we have renamed the original phage $\phi$W and will also refer to it as the Wollman phage. Phage $\phi$W must be propagated on Escherichia coli, strain B, which has an LPS core structure containing heptose and glucose but lacking galactose and rhamnose (Monner et al. 1971). Propagation on K12 strains, which have an LPS core with a more complex structure containing also galactose and rhamnose, selects host-range mutants. We also observed large differences between female K12 strains of different origin (Monner & Boman, 1970) and this aspect was further analysed by Williams & Meynell (1971).

We now report some results obtained from a comparative study of $\phi$W and two host-range

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mutants, $\phi_3$ and $\phi_4$. Growth on two different host strains showed that non-infectious, tail-less particles could be produced either by a mutation in the phage or by a mutation affecting the LPS of the host.

**METHODS**

*Bacterial strains and media.* For most of the experiments only two strains of *Escherichia coli* were used, strain B maintained in the stock collection of this department, and strain D31 an ampicillin-resistant LPS mutant of K12. Chemical analyses of LPS from strain D31 has shown that the contents of glucose, galactose and rhamnose were decreased compared to the composition of wild-type LPS (Monner et al. 1971). As indicator we have used also strain D11 which has an LPS with wild-type composition. Details for the relationship between D11 and D31 have been published (Boman et al. 1971).

Bacteria were always grown in the basal medium E (Vogel & Bonner, 1956) supplemented with LB broth (Bertani, 1951) and 0.2% glucose. All growth experiments were performed in a rotary shaker at 37°C.

*Bacteriophages.* The isolation of phages $\phi_3$ and $\phi_4$ was described by Monner & Boman (1970). The parental phage $\phi W$ was isolated by Wollman (1947). All three phages were maintained by propagation on *Escherichia coli*, strain B. Phage stocks were prepared according to Adams (1959). Plates were normally read after 3 to 5 h incubation at 37°C, that is, before the halos start to develop.

One-step growth curves were performed essentially as in Adams (1959) with the omission of the serum-neutralization step. The host strain was grown to approx. $2 \times 10^8$ cells/ml, concentrated 5 times and infected with the appropriate phage at a multiplicity of infection of 0.05. After 5 min of adsorption samples were taken for assay of unadsorbed phages and dilutions of $10^4$ and $10^5$ made into warm medium from which phage growth was assayed at 1 or 2 min intervals. Burst size was calculated after correction for background of unadsorbed phage.

*Caesium chloride gradient analyses* were performed as described by Meselson & Stahl (1958). Phage lysates were first freed from bacterial debris by three 15 min sedimentations at 7000 g. Phages were then concentrated by centrifuging for 90 min at 30000 g into a 'pillow' of caesium chloride in 0.01 M-phosphate buffer, pH 6.8, and 0.15 M-NaCl (density 1.5 g/ml). The 'pillows' were collected and dialysed overnight against the same buffer. The dialysates were centrifuged for 15 min at 7000 g. To 3.25 g of caesium chloride in a centrifuge tube was added 4.25 ml of the phage purified as above, and the remainder of the tube filled with mineral oil. Samples were centrifuged in a Spinco ultracentrifuge with the SW 40 Ti rotor using the conditions specified in the legends to the respective figure. Density determinations of fractions were made at 25°C using a Zeiss refractometer. Assuming the density of phage P2 to be 1.440 g/ml (G. Bertani, personal communication), it was used as standard in the calculation of densities at 25°C.

**RESULTS**

*Properties of phage grown on Escherichia coli, strain B*

Table 1 gives data from a series of one-step growth curves performed on *Escherichia coli*, strain B and on strain D31, an ampicillin-resistant LPS mutant of *E. coli* K12. When strain B was used as a host, the average burst size for the Wollman phage was about 180, while for the mutant $\phi_3$ it was less than half that amount. An intermediate value was obtained for phage $\phi_4$. If $\phi W$ was propagated on strain D31 the burst size was reduced to
Table 1. One-step growth-experiments with phages $\phi$W, $\phi$3 and $\phi$4

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Phage</th>
<th>Host strain</th>
<th>Latent period (min)</th>
<th>Burst size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Observed</td>
<td>Average</td>
</tr>
<tr>
<td>1</td>
<td>$\phi$W</td>
<td>B</td>
<td>11</td>
<td>150</td>
</tr>
<tr>
<td>2</td>
<td>$\phi$W</td>
<td>B</td>
<td>12</td>
<td>210</td>
</tr>
<tr>
<td>3</td>
<td>$\phi$W</td>
<td>D31</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>$\phi$W</td>
<td>D31</td>
<td>13</td>
<td>71</td>
</tr>
<tr>
<td>5</td>
<td>$\phi$3</td>
<td>B</td>
<td>11</td>
<td>95</td>
</tr>
<tr>
<td>6</td>
<td>$\phi$3</td>
<td>B</td>
<td>11</td>
<td>67</td>
</tr>
<tr>
<td>7</td>
<td>$\phi$3</td>
<td>D31</td>
<td>11</td>
<td>110</td>
</tr>
<tr>
<td>8</td>
<td>$\phi$3</td>
<td>D31</td>
<td>11</td>
<td>73</td>
</tr>
<tr>
<td>9</td>
<td>$\phi$4</td>
<td>B</td>
<td>13</td>
<td>125</td>
</tr>
<tr>
<td>10</td>
<td>$\phi$4</td>
<td>B</td>
<td>12</td>
<td>130</td>
</tr>
</tbody>
</table>

Experiments performed according to Adams (1959) except for details given in Methods. Burst size is given as p.f.u./infected cell for each experiment and as the average.

less than half of that found with strain B. There was no change in burst size of $\phi$3 grown on D31. Phage multiplication on this strain will be further treated in the next section.

Concentrated, suspensions of phages $\phi$W, $\phi$3 and $\phi$4 were compared by equilibrium sedimentations in caesium chloride. The results in Fig. 1 show first that the active phage particles of $\phi$3 have a higher density than $\phi$W and $\phi$4, and second that the mutant $\phi$3 produced an inactive band with a density higher than that found for the active phage. Electron microscopy (not shown) has revealed that the inactive band contains phage particles lacking the tail.

To verify the apparent density differences seen in Fig. 1 between active $\phi$3 and phages $\phi$W and $\phi$4, the active phage bands of $\phi$3 and $\phi$4 were each re-run at lower concentrations in combination with phage $\phi$W. The differences between $\phi$W and $\phi$3 were large enough to permit separation of the two phages even in a mixture. On the other hand, $\phi$W and $\phi$4 were found in one peak. The results from three density determinations are summarized in Table 2. Each phage was centrifuged to equilibrium in CsCl together with phage P2 as a density marker. Whereas phages $\phi$W and $\phi$4 probably had the same density, $\phi$3 clearly had a higher density than its two relatives.

Phage propagation on the strain D31, an LPS mutant of K12

It is a characteristic of the Wollman phage that with continued incubation it forms plaques with large halos on sensitive hosts. The halos which develop on strain B are quite regular (Fig. 2a, b), while those which develop on strain D31 are somewhat irregular, often with small satellite plaques (Fig. 2c, d). To investigate this phenomenon the Wollman phage, pre-grown on Escherichia coli B, was plated on strains B and D31. From the centre and the halo of a plaque on each strain a sample of phage was taken by picking with a sterile toothpick and rinsing into 1 ml broth. After shaking with chloroform these phage samples were assayed for their e.o.p. on strains D11 and D31 using E. coli B as reference. Table 3 shows that phage samples from the centre and the halo of a plaque on E. coli B grew well on strain D31 and not at all on strain D11 (e.o.p. difference $> 10^8$). However, the phage sample from the halo formed on strain D31 grew on strains D11 and D31 with only a tenfold difference in e.o.p., while the sample from the centre of the same plaque grew poorly on D11 compared to D31 (difference in e.o.p. nearly $10^5$). Thus phage $\phi$W
grown on *E. coli* B retained its narrow host range, while growth on strain D31 gave rise to progeny with an extended host range intermediate between φW and φ3. Since phage φ3 was originally isolated on strain D11 (Monner & Boman, 1970), control experiments were made with phage samples from a plaque formed on this strain. The results showed that both the centre and the halo contained phage with properties similar to those of phage φ3.

The results in Table 3 could be explained by assuming that the indicator strain changed its properties when the plates grew older and that this alteration was the basis for the
Properties of phages \( \phi W, \phi 3 \) and \( \phi 4 \)

Table 2. Buoyant densities of phages \( \phi W, \phi 3 \) and \( \phi 4 \)

<table>
<thead>
<tr>
<th>Phage</th>
<th>Density in gradient at 4 °C (g/ml)</th>
<th>Calculated density at 25 °C (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \phi W )</td>
<td>1.480</td>
<td>1.498</td>
</tr>
<tr>
<td>( \phi 3 )</td>
<td>1.487</td>
<td>1.505</td>
</tr>
<tr>
<td>( \phi 4 )</td>
<td>1.478</td>
<td>1.496</td>
</tr>
<tr>
<td>P2</td>
<td>1.423</td>
<td>1.440</td>
</tr>
</tbody>
</table>

Approx. 5 \( \mu l \) with \( 10^8 \) p.f.u. of \( \phi W, \phi 3 \) and \( \phi 4 \), grown on \textit{Escherichia coli}, strain B, were added to 5 ml of CsCl in 0.01 M-phosphate buffer, pH 6.8 and 0.15 M-\( \text{NaCl} \) with a density of 1.49 g/ml. The samples were then centrifuged 20 h at 26000 rev/min at 4 °C in the Spinco ultracentrifuge using the SW 40 Ti rotor. Ten-drop fractions were collected from the bottoms of the tubes. In all these experiments phage P2 (Bertani & Bertani, 1970) was included as an internal density reference.

Fig. 2. Upper part (a and b): photographs of plaques of phage \( \phi W \)-B on \textit{Escherichia coli} B after incubation for 5 h and 20 h, respectively. Lower part (c and d): \( \phi W \)-D3L plated on strain D31. Incubation times the same as in a and b, respectively.
Table 3. E.o.p. values for samples of φW-B isolated from centres and halos of plaques, respectively

<table>
<thead>
<tr>
<th>First indicator</th>
<th>Plaque area</th>
<th>Second indicator</th>
<th>E.o.p. on second indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> B</td>
<td>Centre</td>
<td>JDII</td>
<td>&lt; 4 \times 10^{-7}</td>
</tr>
<tr>
<td><em>Escherichia coli</em> B</td>
<td>Halo</td>
<td>JDII</td>
<td>&lt; 8 \times 10^{-7}</td>
</tr>
<tr>
<td>Strain D31</td>
<td>Centre</td>
<td>JDII</td>
<td>6 \times 10^{-4}</td>
</tr>
<tr>
<td>Strain D31</td>
<td>Halo</td>
<td>JDII</td>
<td>6 \times 10^{-2}</td>
</tr>
</tbody>
</table>

The initial phage, φW-B was mixed in a soft agar overlay with the first indicator bacteria listed. Phage samples were picked from centres and halos of the resulting plaques and assayed for plating efficiencies on the second indicator. Each sample contained per ml 10^4 to 10^6 p.f.u. E.o.p. values were calculated using *Escherichia coli* B as reference.

Fig. 3. Adsorption of phage φW to strains D31 (○, ●) and B (△, ▲) at different stages of growth. At the times and Klett values indicated (●, ▲) samples of bacteria were withdrawn and diluted to 100 Klett units in pre-warmed medium (no dilution was made of the first samples, which were taken at 100 Klett). 0.5 ml of bacteria were mixed with 0.5 ml of a pre-warmed solution of phage in media containing 4000 p.f.u./ml. The adsorption mixture was incubated for 5 min on the shaker, chilled in an ice bath, and centrifuged for 5 min at 3000 rev/min. Triplicate assays for unadsorbed phage were made from the supernatant fluid. Percentage of added φW adsorbed to D31 (○---○) and to strain B (△---△).
Properties of phages \( \phi W \), \( \phi 3 \) and \( \phi 4 \)

Fig. 4. Caesium chloride gradient analyses of phages \( \phi W \) and \( \phi 3 \) propagated on the LPS mutant, strain \( D_31 \), a derivative of \emph{Escherichia coli} K12. The samples were centrifuged 20 h at 26000 rev/min at 4 °C. Fractions of 5 drops were collected from the bottoms of the tubes. To each fraction was added 0.5 ml of phosphate buffer, after which assays for phage activity (\( \circ \cdots \circ \)) and u.v. extinction (\( \bullet \cdots \bullet \)) were made. Density (g/ml), \( \circ \cdots \circ \).

selection of the mutant. We have therefore investigated the adsorption of phage \( \phi W \) to strains B and \( D_31 \) at different stages of growth. The results in Fig. 3 show that when the bacteria were leaving the logarithmic phase of growth the cells were altered in such a way that \( \phi W \) was no longer adsorbed. When adsorption was tested with \( \phi 3 \) there was only a moderate decrease from 70 to 50% adsorption. Thus, the difference in adsorption to full-grown cells can explain the enrichment of \( \phi 3 \)-like mutants. However, chemical analysis of known carbohydrates and fatty acids in the LPS did not show any differences between samples from exponentially dividing and full-grown cells.

The results in Table 1 showed that a low burst size was obtained both when the Wollman phage was grown on strain \( D_31 \) and also when the mutant \( \phi 3 \) was grown on either strain B or strain \( D_31 \). The caesium chloride gradient shown in Fig. 1 revealed inactive particles in \( \phi 3 \) grown on \emph{Escherichia coli} B. We therefore examined \( \phi W \) and \( \phi 3 \) after a one-cycle propagation on strain \( D_31 \). The results in Fig. 4 show that heavier bands with inactive particles were produced in both cases. The inactive bands of \( \phi W \) and \( \phi 3 \) appeared to have the same density. Again there was a difference in density between the infective particles of \( \phi W \) and \( \phi 3 \). E.o.p. tests confirmed that in the gradient with \( \phi W \) there were no detectable particles with the host range of \( \phi 3 \).
DISCUSSION

Phage $\phi W$ was initially isolated on *Escherichia coli*, strain B (Wollman, 1947). When propagated on this strain it consists of homogeneous phage particles with a density of 1.498 g/ml at 25 °C (Fig. 1 and Table 2).

Our caesium chloride gradients (Fig. 1, 4; Table 2) show that phage $\phi 3$ grown on strains B or $D_{31}$ gives two bands of particles, both denser than the parental phage. Electron micrographs (not given) have shown that phages $\phi W$, $\phi 3$ and $\phi 4$ have hexagonal heads with a short tail by which they are attached to the bacteria. The inactive particles produced by phage $\phi 3$ have no detectable tails, an observation which is consistent with their higher density (Table 2) and the lower burst size found for phage $\phi 3$ (Table 1). Linial & Malamy (1970) briefly stated that their phage $\phi II$-T (like our $\phi 3$) produced inactive particles without tails, but no data were given. This was confirmed by Brunovskis, Hyman & Summers (1973) who in agreement with our data (Fig. 1) also found that $\phi W$ grown on *Escherichia coli* B was homogenous.

It has gradually become clear that *Yersinia* (earlier *Pasteurella*) phage H, and coliphages T3, T7, $\phi$W and mutants of the latter are all closely related (Molnar & Lawton, 1969; Linial & Malamy, 1970; Beier & Hausmann, 1973; Brunovskis et al. 1973). It is also known that the tails of T7 contain at least three different proteins (Studier, 1972) and host-range mutants like $\phi 3$ and $\phi 4$ would be expected to have altered tails. Since DNA is denser than proteins the lack of tail explains why inactive particles are denser than $\phi W$ grown on *Escherichia coli* strain B ($\phi W$.B). The fact that $\phi 3$ is denser than $\phi W$.B (but less dense than inactive particles) would be explained by loss of some protein component which would also account for its instability and the production of tail-less particles. Attempts to detect such a missing protein have, however, not been successful.

If bacteria change their cell surface during growth (Fig. 3) it is understandable that mutants can be selected for in a halo, as we have observed for phage $\phi W$ on strain $D_{31}$ (Fig. 2, Table 3). However, the results in Fig. 4 show that inactive tail-less particles were produced also during one cycle of growth of $\phi W$ on log-phage cells of strain $D_{31}$. It should be stressed that under these conditions of propagation there was no enrichment for mutants of the $\phi 3$ type. The production of tail-less particles from phage $\phi W$ grown on $D_{31}$ indicates, therefore, that the host contributes some products which affect the formation or attachment of the tail. Strain $D_{31}$ carries a mutation located in the LPS cluster at 72 min and giving an LPS with reduced amounts of glucose, galactose and rhamnose (Monner et al. 1971; unpublished observations). If this LPS mutation gave rise also to the accumulation of some LPS precursor, defective phage particles may arise because of an affinity between the precursor and the tail proteins such that the assembly of the tail is partly blocked.

We thank Professor G. Bertani for the reference sample of phage P2 and Professor G. Bloom for help with the electron microscopy. The work was supported by grants from the Swedish Cancer Society (Project no. 157) and the Swedish Natural Science Research Council (Dnr 2453).
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(Received 5 January 1974)