Suppression of Adsorption Properties of a z Mutant of Bacteriophage T4 by r Mutations

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The products of bacteriophage T4 r genes influence the organization of the phage-adsorption apparatus in a noncatalytic way.

The z mutants of T4D (13) are mutated in gene 37, which codes for a major component of the tail fibers (7). The z mutants of T4B are mutated in gene 19, the product of which is required for conversion of the baseplate to the core baseplate (11). Crosses between T4B and T4D generate z hybrids which derive gene 19 from T4D and gene 37 from T4B. The z phage have altered adsorption and lysis properties (13). Freshly liberated lysates of z mutants adsorb at a strongly reduced rate compared to fresh wild-type phage.

Figure 1 and Table 1 show the difference in adsorption of freshly liberated T4D w and zHA10. The z mutant used throughout the experiments reported here is zHA10, a revertible mutant of T4D induced with hydroxylamine (13). Unexpectedly, fresh phage carrying the rII mutation r47 (rIIA) (6) in addition to the z mutation adsorb seven to eight times faster than the z mutant.

The increased adsorption rate of zr double mutants could depend on an absence or alteration of cellular breakdown products which normally attach to z phage and prevent adsorption, or to the fact that the adsorption apparatus of zr phage is structurally different from that of z phage.

By measuring the adsorption in a lysate from cells mixedly infected with z and zr, one should be able to distinguish between the above alternatives. If the first one is correct, both z and zr should adsorb slowly. If the second alternative is

TABLE 1. Adsorption constants of freshly liberated T4D w, z, and zr47 (rIIA) calculated from Fig. 1

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<table>
<thead>
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<tbody>
<tr>
<td>w</td>
<td>z</td>
<td>zr47</td>
</tr>
<tr>
<td>19</td>
<td>1.4</td>
<td>11</td>
</tr>
</tbody>
</table>

Values are expressed as K_{ads} per 10^{10} per milliliter per minute.

![Figure 1](http://jvi.asm.org/) Adsorption of freshly liberated T4D w, z, and zr47 (rIIA) (6). An overnight culture of Escherichia coli B was diluted in tryptone broth (13) to 10^{8} cells per ml, and 5-ml portions were infected at a multiplicity of 5 to 8. After 2 hr on a shaker at 37 C, bacteria and debris were spun down. Adsorption of the phage lysate was measured on the same day. Lysate at a multiplicity of 0.2 to 1 was added to overnight E. coli B at a concentration of about 2.5 × 10^{8} cells per ml. At 2, 4, and 6 min, 0.1-ml samples were taken into 5 ml of dilution medium (13) with 0.5 ml of chloroform added, and the mixture was shaken. After dilution, platings were made on E. coli B.
Table 2. Adsorption of freshly liberated T4D w, z, and zr47 (rIIA), z and zr47 from a mixed infection, zr73 (rIIB) (6), z and zr73 from a mixed infection, zr48 (rI) (5), and z and zr48 from a mixed infectiona

<table>
<thead>
<tr>
<th>w</th>
<th>z</th>
<th>zr47b</th>
<th>z from mixed infection with zr47a</th>
<th>zr47 from mixed infection with z</th>
<th>zr73b</th>
<th>z from mixed infection with zr73</th>
<th>zr73 from mixed infection with z</th>
<th>zr48b</th>
<th>z from mixed infection with zr48</th>
<th>zr48 from mixed infection with z</th>
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</thead>
<tbody>
<tr>
<td>28</td>
<td>2.3</td>
<td>12</td>
<td>6.6</td>
<td>7.9</td>
<td>17</td>
<td>4.4</td>
<td>5.7</td>
<td>13</td>
<td>4.3</td>
<td>9.0</td>
</tr>
</tbody>
</table>

a Adsorption was measured as described in the legend of Fig. 1. Values are expressed as $K_{ads}$ per $10^{10}$ per milliliter per minute.

b The zr double mutants were obtained by crossing zHA10 at a multiplicity of 8 and the r mutant at a multiplicity of 2 in E. coli C600. The progeny was plated on E. coli B; r plaques were picked and spotted on E. coli A, where z phage do not plaque (15).

c Single-burst experiments showed that at least 75% of the bacteria infected with z and zr gave rise to mixed bursts. The adsorption of reference z and zr lysates was not changed when they were mixed and adsorbed together.

Table 3. Adsorption of freshly liberated T4D w, z, and zr62 (rIIA) (6), z and zr62 from a mixed infection, zrIII (14), and z and zrIII from a mixed infectiona

<table>
<thead>
<tr>
<th>w</th>
<th>z</th>
<th>zr62b</th>
<th>z from mixed infection with zr62</th>
<th>zr62 from mixed infection with z</th>
<th>zrIII</th>
<th>z from mixed infection with zrIII</th>
<th>zrIII from mixed infection with z</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>3.0</td>
<td>11</td>
<td>7.5</td>
<td>9.9</td>
<td>8.7</td>
<td>7.9</td>
<td>8.6</td>
</tr>
</tbody>
</table>

a Adsorption was measured as described in the legend of Fig. 1. Values are expressed as $K_{ads}$ per $10^{10}$ per milliliter per minute.

b The zr double mutants were prepared as described in Table 2.

correct, one expects phenotypic mixing (1, 12, 18); i.e., z and zr should adsorb at a rate in between that of z lysates and zr lysates.

Table 2 shows the result of one such experiment. It is seen that z from a mixed infection with zr47 adsorbs considerably faster than z from a nonmixed lysate. In the mixed lysate, zr47 adsorbs more slowly than zr47 in the reference lysate, although not at a rate identical with that of z in the same lysate. The same results were obtained when the zr mutant had a mutation in the rIIB cistron, the rI gene (Table 2), the rIII gene, or another mutation in the rIIA cistron (Table 3).

Fresh z phage was treated in a number of ways to remove possible cell debris. The following treatments were tried and had no effect on the adsorption rate: heat (15); distilled water (15); incubation in 0.5, 1, 2, and 4 mM CsCl; incubation at pH 4.6 and 9.0; dialysis against 0.02 mM buffer; incubation with deoxyribonuclease, ribonuclease, Pronase, lysozyme (3), chymotrypsin (0.5 mg/ml), pancreatic lipase (1 mg/ml), or shaking with chloroform.

What is known about the physiology of the rII gene points to, or is at least compatible with, an involvement of the rII product in cell membrane repair and biosynthesis (2, 4, 8, 9, 10, 16). I propose a dual function for the r genes. Early in infection the r products associate with the cell membrane participating in its repair; later they provide parts of a structure on which phage assembly occurs. There is an indication that assembly of T4 may occur at the cell membrane, starting with the baseplate (17).

LITERATURE CITED


