Altered Phage P1 Attachment to Strains of *Escherichia coli* Carrying the Plasmid ColV, I-K94

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SUMMARY

Phages P1vir and P1cmclrl00 failed to form plaques on or multiply in *Escherichia coli* strains carrying the ColV, I-K94 plasmid; with P1cmclrl00, the effect occurred both with phage from the lytic cycle and with that induced from a lysogen. The effect was on attachment, these P1 phages attaching poorly to ColV, I-K94+ strains. This receptor defect appeared to result mainly from the presence of ColV-encoded transfer and colicin components in the cells carrying ColV, I-K94 and it was specific to this plasmid. Phage Mu (which uses an attachment mechanism similar to that of phage P1) in the G(+) form attached to both Col- and ColV, I-K94+ strains but the G(−) form attached to neither type.

INTRODUCTION

The ColV, I-K94 plasmid is a large element which confers derepressed transfer properties, the synthesis of colicin V and colicin Ia components and the formation of the VmpA outer membrane protein (Hardy, 1975; Rowbury et al., 1985).

Recent studies of ColV+ strains (Reakes et al., 1987) have indicated that plasmid ColV, I-K94 causes abortive infections by the T4-like phage Mel. During studies of sensitivity to further phages, it was found that phage P1vir was unable to form plaques on ColV, I-K94+ strains. The present paper reports an investigation of this phenomenon.

Plasmids and prophages frequently alter the bacteriophage sensitivity of *Escherichia coli* and related organisms. Plasmid-conferred resistance can result from a number of mechanisms. First, the presence of the plasmid may alter the structure of the receptor, preventing phage attachment (Derylo et al., 1975). Second, the resident plasmid (or phage) may lead to superinfection exclusion whereby the incoming phage can attach but the injection process is aberrant (Susskind et al., 1971, 1974). Third, the plasmid may encode a restriction system which ensures the destruction of incoming phage or other foreign DNA (Modrich, 1979; Yuan, 1981; Kessler & Holtke, 1986). Fourth, the plasmid may induce an abortive infection process (Duckworth et al., 1981). The plasmid-induced effect described here, involving the plasmid ColV, I-K94 and the phage P1vir, appears to involve an altered receptor, the phage attaching only weakly to plasmid-carrying cells.

METHODS

*Bacterial strains*. The parental strains used in the present study were strain P678-54 (thr, leu, thi, lacY, gal, xylP, mtl, mal, minA) and its omp42101 derivative, strains 1829 (trp), C600 (thr, leu, thi), CE1062 (thr, leu, thi, pyr, thy, arg, ilv, his, ompC), D2111 [pro, his, trp, glucose-deficient lipopolysaccharide (LPS)], SP749 [his, trp, lys, gal, lac, mal, Δ8, T6*, rpsL, Tn10 (P1cmclrl00)] and W3110. In addition, the plasmid ColV, I-K94 was introduced into most of these and the resulting ColV+ derivatives used. From strains 1829 and P678-54, derivatives carrying other plasmids were also prepared by introduction of the appropriate element from plasmid-containing derivatives of *E. coli* 153 pro met (obtained from the Plasmid Section, NCTC, Colindale, London, U.K.).

*Phages*. Phage P1vir was used for most of this work. It was kindly provided by Dr B. G. Spratt (University of Sussex, Brighton, U.K.). Phage Mu in both the G(+) and G(−) forms was a kind gift of Professor N. Symonds...
For some experiments, phage P1cmclrl00 was used. It was usually obtained by temperature induction of strain SP749 but for some experiments this phage was obtained from the lytic cycle by infection of strain P678-54.

Growth media. For all experiments, cells were grown in TNA broth medium (Difco Bacto-Tryptone broth powder, 10 g/l; NaCl, 8 g/l; glucose, 1 g/l; 5 mM-CaCl₂) on TNA plates (broth medium solidified with 2% w/v Difco Bacto agar); TNA soft agar was TNA broth containing 1% Difco Bacto agar.

Phage assays, attachment studies and growth. Sensitivity to phages (for P1 and Mu derivatives) was tested by growing strains in TNA broth medium overnight at 37 °C and after mixing aliquots containing approx. 10⁸ cells with appropriate phage dilutions in 4 ml TNA soft agar, the mixtures were overlaid on TNA agar plates and incubated at 37 °C.

To study phage attachment (adsorption), approx. 10⁸ cells/ml and 10⁷ phage/ml were statically incubated for 15 min in TNA broth at 37 °C. After dilution and chloroform treatment, samples were assayed for residual free phage using strain W3110 as an indicator.

To study phage growth in sensitive strains, bacteria and phage (10⁷/ml) were incubated in TNA broth medium as for attachment and, following dilution (1 in 100) in TNA, incubated at 37 °C with aeration, samples being removed at intervals for phage assays after chloroform treatment.

For studies on the effect of phage on the growth of bacterial strains, cultures (at approx. 2 × 10⁷ cells/ml) growing exponentially in TNA broth were incubated at 37 °C for 4 to 5 h with phage (approx. 4 × 10⁷/ml), samples being taken at intervals for optical density readings.

For thermal induction of phage P1cmclrl00, overnight cultures of strain SP749 or P678-54 ColV,I-K94 (P1cmclrl00) grown at 30 or 32 °C overnight in TNA medium, were diluted 1 in 50 in the same medium and grown to approx. 1 × 10⁸ cells/ml at 30 or 32 °C with aeration. Cultures were then shifted to 40 °C for 45 min and back to 30 or 32 °C for 90 min prior to assay of phage on strains W3110 and P678-54.

RESULTS

Phage P1vir fails to form plaques on ColV,I-K94+E. coli strains

During an attempt to transduce genetic material from Col⁻ and ColV⁺ strains using phage P1vir, it was noticed that this phage failed to form plaques on strain 1829 ColV,I-K94 whereas plaque formation was efficient on strain 1829 (Table 1). The effect was due to the plasmid (not to the preferential conjugal introduction of the plasmid into a phage P1vir-resistant mutant) because a cured (Col⁻) derivative regained sensitivity. A ColV,I-K94⁺ strain produced by transformation with plasmid DNA also became phage P1vir-resistant.

<table>
<thead>
<tr>
<th>Plasmid(s) in E. coli strains</th>
<th>Incompatibility group(s)</th>
<th>Transfer properties</th>
<th>Response to phage P1vir*</th>
</tr>
</thead>
<tbody>
<tr>
<td>In strain 1829</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>FI</td>
<td>Derepressed</td>
<td>Resistant</td>
</tr>
<tr>
<td>ColV,I-K94†</td>
<td>FI</td>
<td>Derepressed</td>
<td>Sensitive</td>
</tr>
<tr>
<td>ColV-M50(1)</td>
<td>FI</td>
<td>Transfer-deficient</td>
<td>Sensitive</td>
</tr>
<tr>
<td>ColV-K30†</td>
<td>FI</td>
<td>Derepressed</td>
<td>Sensitive</td>
</tr>
<tr>
<td>ColB-K98</td>
<td>FIII</td>
<td>Repressed</td>
<td>Sensitive</td>
</tr>
<tr>
<td>R124</td>
<td>FIV</td>
<td>Repressed</td>
<td>Sensitive</td>
</tr>
<tr>
<td>ColV,I-K94, R124</td>
<td>FI and FIV</td>
<td>Repressed</td>
<td>Sensitive</td>
</tr>
<tr>
<td>ColV,I-K94, ColB-K98</td>
<td>FI and FIII</td>
<td>Repressed</td>
<td>Sensitive</td>
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<tr>
<td>In strain P678-54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>FI</td>
<td>Derepressed</td>
<td>Resistant</td>
</tr>
<tr>
<td>ColV,I-K94</td>
<td>FI</td>
<td>Derepressed</td>
<td>Sensitive</td>
</tr>
<tr>
<td>ColV-M40(5)</td>
<td>FI</td>
<td>Derepressed</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>

* Sensitive, plaques were formed at 37 °C on lawns of the strain and the strain failed to grow in broth at 37 °C in the presence of excess phage; resistant, no plaque formation and good growth in broth with an excess of phage. † ColV,I-K94 and ColV-K30 are both from clinical E. coli isolates (Hardy, 1975). Both confer derepressed transfer properties and encode the VmpA protein and colicin V; ColV,I-K94 also encodes colicin Ia (Hardy, 1975; Rowbury et al., 1985). The aerobactin iron chelation system is encoded by ColV-K30 but not by ColV,I-K94 (Williams, 1979).
Phage P1 attachment to E. coli ColV,I-K94+

The ColV,I-K94 plasmid had the same effect on several other E. coli strains. The ColV,I-K94-bearing derivatives of strains P678-54 (Table 1), P678-54 ompA2101, CE1062 (ompC), C600 and D21fl all showed no plaque formation with phage P1vir whereas the Col- parents were sensitive.

Failure of phage P1vir to multiply in liquid medium in ColV,I-K94+ strains was also demonstrated. When inoculated at approx. 10^3 p.f.u./ml, the phage multiplied extensively at 37 °C in broth cultures of strains 1829 or P678-54 but not in their ColV,I-K94+ derivatives. ColV,I-K94+ strains were able to grow in broth at 37 °C apparently unhindered by the presence of excess phage P1vir.

Attachment of phage P1vir to Col- and ColV,I-K94+ strains

Studies of phage adsorption indicated that phage P1vir attached poorly to several ColV,I-K94+ strains; for strains 1829 ColV,I-K94 and P678-54 ColV,I-K94, attachment was reduced sixfold compared to the Col- strains. This low level of attachment to ColV+ strains may be due partly to non-specific adsorption. There was essentially no multiplication of phage in the ColV,I-K94+ strains although a small proportion of cells may allow attachment and phage multiplication.

The ColV,I-K94+ strains were also resistant to phage P1cmclr100, as indicated by the failure of this phage to form plaques on ColV,I-K94+ strains and by the ability of these strains to grow well with excess phage.

The above experiments with phage P1vir were exclusively with phage from the lytic cycle. Tests were made with phage P1cmclr100 produced both by induction of strain SP749 and by lytic infection of strains P678-54. Both preparations formed plaques on Col- strains but ColV,I-K94+ strain were resistant to both.

If it is only an attachment defect which makes ColV,I-K94+ strains Pl-resistant, then prophage ought to be maintained and remain normally inducible in ColV,I-K94+ strains. This was tested by making strain P678-54 ColV,I-K94 (P1cmclr100). The phage and ColV,I-K94 were stably maintained; i.e. after growth in broth at 30 °C, all the cells retained the chloramphenicol resistance characteristic of the phage and the properties characteristic of ColV,I-K94. On temperature induction of this ColV,I-K94+ lysogen, infective phage was formed, indicating that P1cmclr100 can multiply in ColV+ cells.

Plasmid components and phage P1vir resistance

Resistance to phage P1vir, involving reduced attachment, was specific to ColV,I-K94; none of the other plasmids tested showed the effect. Derivatives of strain 1829 carrying ColV,K30 or ColV-8, were phage-sensitive; only a few plaques were formed on strain 1829 ColV-41 but attachment was efficient. None of a wide range of other plasmids (Flac, R386, R1, ColB-K98, R124, R124-F2, R483, pIP40a) affected sensitivity to phage P1vir.

Three findings suggest that transfer components are involved in resistance. First, repression of transfer properties by the fi+ R124 or ColB-K98 abolish the ColV,I-K94 effect (see strains 1829 ColV,I-K94, R124 and 1829 ColV,I-K94, ColB-K98; Table 1). Second, a mutant derivative of ColV,I-K94 [ColV-M50(1)] which fails to encode transfer properties (Tewari et al., 1986) had no effect on the sensitivity of strain 1829 to phage P1vir (Table 1) or on adsorption of this phage.

Third, cultures of strain 1829 ColV,I-K94 grown at 25 °C and then infected at 37 °C were sensitive to phage P1vir; transfer components are not formed at 25 °C (Tewari et al., 1985). The VmpA protein, which is encoded by ColV,I-K94, fails to form in the presence of MgSO4 (Tewari et al., 1986). To test the involvement of this protein in Pl-resistance, cells of 1829 and 1829 ColV,I-K94 were grown statically at 37 °C with 0-05 M-MgSO4. Strain 1829 grown in this way proved sensitive to phage P1vir whereas strain 1829 ColV,I-K94 was resistant, thus showing that the VmpA outer membrane protein had no influence on the phenomenon investigated.

To test involvement of colicin components, strain P678-54 ColV-M40(5) was tested for sensitivity to phage P1vir. This mutant plasmid is deleted for the region which encodes colicin V and Ia components (Tewari et al., 1986). Strain P678-54 ColV-M40(5) was sensitive to phage
Plvir when plaque formation and growth in broth were tested (Table 1) and studies of attachment indicated that the process was only slightly reduced (compared to strain 1829) for this strain.

**Effect of ColV,I-K94 on sensitivity to phage Mu**

Phage Mu closely resembles phage P1 in its attachment properties (Toussaint *et al.*, 1978; Iida, 1984). Accordingly, ColV,I-K94+ strains were tested with Mu. The G(+) form of Mu formed plaques on strains 1829, P678-54 and their ColV,I-K94+ derivatives. In contrast, none of these strains was sensitive to the G(−) form.

**DISCUSSION**

Phage P1 vir formed in the lytic cycle failed to form plaques on lawns of ColV,I-K94+ strains. Appreciable phage multiplication failed to occur in the ColV,I-K94+ strains and these strains grew essentially unhindered in the presence of excess phage. Measurements of phage attachment indicated that this was markedly reduced in ColV,I-K94+ strains; the 10% attachment observed presumably represents, at least in part, non-specific surface adsorption. Support for an attachment lesion comes from transduction measurements. Transduction by Plvir of strains P678-54 and P678-54 ColV,I-K94 to the Thr+ Leu+ phenotype showed a 30-fold reduction in frequency for the ColV+ strain. This may indicate that the attachment lesion is not absolute but that phage can specifically attach to approx. 3% of ColV+ cells.

Although phage P1 derivatives cannot attach to ColV,I-K94+ cells, the two elements can be stably maintained together and Plcmclrl00 can be induced from a ColV,I-K94+ lysogen. Accordingly, there is probably no inhibition of multiplication of P1 and its derivatives in ColV,I-K94+ strains.

The receptor for phage P1 is LPS (Franklin, 1969; Ornellas & Stocker, 1974) and it is most probable that there is a change in primary LPS structure or in the association of LPS with other envelope components which makes an essential receptor group on the LPS unavailable for the attachment of phage Plvir. Such a LPS change is also suggested by the marked changes in inhibitor sensitivity observed in ColV,I-K94+ strains (Rowbury *et al.*, 1985; Davies *et al.*, 1986).

Phage resistance appears to depend on the presence of both transfer and colicin components in the ColV,I-K94+ outer membrane (Table 1) but the absence of the VmpA protein did not abolish resistance. The presence of transfer and colicin components may indeed affect LPS structure or insertion; the sensitivity to rifampicin observed in ColV,I-K94+ strains is dependent on the presence of both transfer and colicin components (Davies *et al.*, 1986) and probably results from a LPS alteration. The results with other plasmids with derepressed transfer properties are in accord with those obtained with ColV-M40(5).

The phage Plvir used for most experiments was obtained by lytic infection and, therefore, consisted solely of the C(+) form (Van de Putte *et al.*, 1980). The finding that ColV,I-K94+ strains were also resistant to phage Plcmclrl00 produced by induction of a lysogen indicates that ColV,I-K94+ strains are not sensitive to the C(−) form either [induction of lysogens produces a mixture of C(+) and C(−) forms; Daniell *et al.*, 1973; Van de Putte *et al.*, 1980; Iida *et al.*, 1982].

Although phages Mu and P1 show marked differences in their mechanisms of multiplication and lysogeny, the genetic regions governing host range both have an invertible segment (Iida, 1984) and these regions are sufficiently similar to allow complementation of one phage by the other (Toussaint *et al.*, 1978). Accordingly, ColV,I-K94+ strains were tested for sensitivity to phage Mu. The G(+) form of the virus formed plaques on strains 1829, P678-54 and on their ColV,I-K94+ derivatives. The G(−) form failed to form plaques on any of the strains. This was expected since this form cannot attach to K12 derivatives of *E. coli* (Van de Putte *et al.*, 1980).

**REFERENCES**


Phage P1 attachment to E. coli ColV,I-K94+


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