Outer Membrane Protein-specific Bacteriophages of *Salmonella typhimurium*

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INTRODUCTION

Much recent research has been directed to outer membrane proteins (OMP) of both *Escherichia coli* and *Salmonella typhimurium* (Stocker & Makela, 1978). Some of these proteins are called 'major proteins' because of their large amounts (10⁵ to 10⁶ molecules) in each bacterial cell. At least some of the major proteins are responsible for the hydrophilic permeability of the outer membrane, and these are termed porins. They include protein I (divisible into Ia and Ib) in *E. coli* (Hindennach & Henning, 1975) and the '34K,35K,36K complex' in *S. typhimurium* (Nakae, 1976; Nikaido et al., 1977). This complex consists of three proteins with apparent molecular weights of 34000, 35000 and 36000, as determined by sodium dodecyl sulphate (SDS) gel electrophoresis.

In order to be able to isolate mutants of the major outer membrane proteins of *S. typhimurium* we have looked for bacteriophages that would use these proteins as receptors. PH51, which uses the 34K protein receptor, was isolated in this way. It is probably a recombinant between phage ES18 and a prophage of *S. typhimurium* LT-2 (Siitonen et al., 1977). A number of other OMP-specific phages have subsequently been adopted for use in our laboratory. Because of their usefulness in both the isolation and preliminary characterization of mutants, we report here the basic properties of these phages.

METHODS

Two lines of *S. typhimurium* were used: LT-2, subline s14 (Spicer & Datta, 1959), containing prophage Fels1 (Yamamoto, 1967) and obtained from B. A. D. Stocker, Stanford University, California; and line Q received from J. Cameron (Wellcome Reagents Ltd, London) and containing no known prophages (Boyd & Bidwell, 1959). Rough mutants of both lines were isolated by selection for resistance to lipopolysaccharide (LPS)-specific phages (Wilkinson, Gemski & Stocker, 1972) obtained from B. A. D. Stocker. A rfaJ mutant, sn5014, of s14 (Nurminen et al., 1976 and unpublished) was used as the parent from which we isolated OMP mutants by selection for resistance to OMP-specific phages.

The parental phages P221 (Yamamoto, 1967) and ES18 (Kuo & Stocker, 1970) were obtained from B. A. D. Stocker and maintained by growth on derivatives of the s14 subline. Phage methods used have been described previously (Adams, 1959; Nurminen et al., 1976; Siitonen et al., 1977). OMP-specific phages were searched for by screening for phages that would grow on a 'heptoseless' *S. typhimurium* mutant s1102 (Wilkinson et al., 1972). This strain has a very defective LPS and consequently is not sensitive to the common phages attaching to LPS. Particular OMP-specific phages were looked for by using 34K−, 36K− or 34K−36K− mutants as indicators; these mutants lack one or two of the proteins of the 34K,35K,36K complex (Nurminen et al., 1976).
RESULTS

PH105 and PH31

An extensive search for OMP-specific phages in local sewage was not successful. We then looked for such phages in faeces of patients infected with Salmonella. Several phages were obtained. In initial tests they behaved alike in respect of their receptor specificity and only PH105 has been studied further. Like P221 (Siitonen et al., 1977) it uses the 36K protein as receptor: PH105-resistant mutants of sh5014 are resistant to both PH105 and P221 (Table 1, lines 6 to 9) and unable to adsorb them. Extracts of the envelopes of these mutants did not inactivate PH105 and P221 and lacked protein 36K. Furthermore, purified protein 36K, when cut out from an SDS gel after electrophoresis, renatured in water enough to inactivate PH105 (unpublished observations). Thus, 36K− mutants, which lack the 36K protein, can be isolated by selecting for resistance to PH105.

When 10⁹ phages of PH105, grown on the sdi14 line, were plated on a rough PH105-resistant mutant, sh6221, a few plaques developed. These were picked, purified by single plaque isolation, and tested on indicator strains. Since several such isolates appeared to be similar, only one, PH31, was retained. Its activity on OMP mutant strains was opposite to that of its parent, PH105: it gave plaques on 36K− mutants but was without action on 34K− mutants (Table 1, lines 5, 8 and 9). Also, 34K− mutants of sh5014 did not inactivate the phage, whereas sh5014 and its 36K− mutants did. The 34K protein therefore seemed to be the receptor of this phage, as well as of PH51 previously described (Siitonen et al., 1977).

Both PH105 and PH31 attacked smooth (Table 1, lines 1, 13) and rough (Table 1, lines 2 to 4, and 14) strains of both the tT-2 and Q lines, whereas the previously described phages P221 and PH51 did not give plaques on the smooth strains. Lysogeny for phages Fels2, Fels1, P22, ES18, P221 or PH51 did not affect the sensitivity (for example, Table 1, lines 4, and 10 to 12). Both PH105 and PH31 are virulent phages and we have been unable to obtain strains lysogenic for them. Their plaques are small and they are easily killed by heat (99% killed in 5 min at 70 °C) befitting a B phage category (Boyd & Bidwell, 1959).

PH41 and PH42

Since P221 plated on 36K− mutants of sh5014 with an efficiency of approximately 10⁻⁵ compared with the parent sh5014, its receptor was believed to be the 36K protein (Nurminen et al., 1976; Siitonen et al., 1977). The plaques obtained on the 36K− mutants, which varied in number in separate P221 stocks, were thought to be its host range mutants or recombinants (probably with an unidentified prophage) occurring at a fairly high frequency. To test this hypothesis, such plaques were isolated, purified by single plaque isolation on sh5014, and tested on indicator bacteria (Table 1). They indeed turned out to have an altered host range in that they now attacked 36K− mutants as well as the parent sh5014, but not its 34K− mutants (Table 1, lines 5, 8 and 9). They thus resembled phages PH51 and PH31 found earlier. Only one, PH41, was studied further.

To test for further versatility of the P221 phage group, we then plated PH41 on sh5551, a 34K− mutant of sh5014. A few plaques were obtained from the undiluted phage stock; one was purified by single plaque isolation on sh5014, and designated PH42. To our astonishment, PH42 plated with full efficiency on sh5014, as well as on its 34K− or 36K− derivatives but not on mutants lacking both the 34K and 36K proteins (Table 1, lines 8 and 9). Although other explanations are possible, our working hypothesis is that PH42 can use as its receptor either protein 34K or 36K.

The phages PH41 and PH42 resemble their parent P221 in attacking only rough forms (Table 1), probably because of steric hindrance by the O polysaccharide (Lindberg, 1973). Their plaque morphology is similar (small B type plaques). They are even more sensitive
Table 1. Sensitivity of various *S. typhimurium* strains to the OMP-specific phages

<table>
<thead>
<tr>
<th>Category</th>
<th>Strain</th>
<th>Parent</th>
<th>Isolated as:</th>
<th>OM protein present†</th>
<th>Sensitivity to OMP-specific phages‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Resistant to</td>
<td></td>
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<td></td>
<td></td>
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<td>Lysogenic for</td>
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<tr>
<td>LPS indicators of the <strong>sd14</strong> line*</td>
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</tr>
<tr>
<td>1. st696</td>
<td>sh5014</td>
<td>PH51</td>
<td>S</td>
<td>++-</td>
<td>++-</td>
</tr>
<tr>
<td>2. st733</td>
<td>sh5014</td>
<td>PH105</td>
<td>Rb2</td>
<td>-</td>
<td>++-</td>
</tr>
<tr>
<td>3. st1102</td>
<td>sh5014</td>
<td>PH105</td>
<td>Rb2</td>
<td>-</td>
<td>++-</td>
</tr>
<tr>
<td>4. sh5014</td>
<td>sh5014</td>
<td>PH51</td>
<td>Rb2</td>
<td>-</td>
<td>++-</td>
</tr>
<tr>
<td>5. sh5551</td>
<td>sh5014</td>
<td>PH51</td>
<td>Rb2</td>
<td>-</td>
<td>++-</td>
</tr>
<tr>
<td>6. sh6017</td>
<td>sh5014</td>
<td>PH105</td>
<td>Rb2</td>
<td>-</td>
<td>++-</td>
</tr>
<tr>
<td>7. sh6015</td>
<td>sh5014</td>
<td>PH105</td>
<td>Rb2</td>
<td>-</td>
<td>++-</td>
</tr>
<tr>
<td>8. sh6263</td>
<td>sh5551</td>
<td>PH105</td>
<td>Rb2</td>
<td>-</td>
<td>++-</td>
</tr>
<tr>
<td>9. sh6260</td>
<td>sh6017</td>
<td>PH51</td>
<td>Rb2</td>
<td>-</td>
<td>++-</td>
</tr>
<tr>
<td>Isolated as resistant to the OMP-specific phages§</td>
<td></td>
<td></td>
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<tr>
<td>10. sh5420</td>
<td>sh5014</td>
<td>ES18</td>
<td>Rb2</td>
<td>+</td>
<td>++-</td>
</tr>
<tr>
<td>11. sh5554</td>
<td>sh5014</td>
<td>PH105</td>
<td>Rb2</td>
<td>+</td>
<td>++-</td>
</tr>
<tr>
<td>12. sh6191</td>
<td>sh5014</td>
<td>PH105</td>
<td>Rb2</td>
<td>+</td>
<td>++-</td>
</tr>
<tr>
<td>Q line†</td>
<td>cn5235-q1</td>
<td>P221</td>
<td>Rb2</td>
<td>+</td>
<td>++-</td>
</tr>
</tbody>
</table>

* Wilkinson *et al.* (1972); S, smooth; R, rough, Ra with the complete core, Rb2 with less complete core, Re with very incomplete, "heptoseless" core (Lüderitz, Jann & Wheat, 1968).

† Only 34K and 36K proteins recorded; +, present in approximately wild-type amounts; -, absent or present in much reduced amounts.

‡ ±, Full efficiency of plating of the phage; −, efficiency of plating reduced to 0 to $< 10^{-6}$.

§ Nurminen *et al.* (1976); Lounatmaa & Nurminen (1977); unpublished results.

to heat than P221; 99% of the plaque-forming units were inactivated in 30 min at 60 °C while 30% of those of P221, and 10% of PH105 and PH31 withstood this treatment. Like P221 they are co-immune with P22, P221, ES18, PH51 and each other (for example, Table 1, lines 10 to 12).

DISCUSSION

The bacteriophages described here, together with the previously found P221 (Yamamoto, 1967) and PH51 (Siitonen et al., 1977), form a set of OMP-specific phages that have proved useful in our laboratory for isolating and characterizing porin mutants of S. typhimurium (unpublished results). Several phages described for a similar purpose in E. coli (Henning & Haller, 1975; Schnaitman, Smith & Forn de Salas, 1975; Skurray, Hancock & Reeves, 1974) could not be used since they do not attack S. typhimurium strains (unpublished observations). Two of the phages are specific for the 36K protein, and three for the 34K protein. PH105 (specific for the 36K protein) and its derivative PH31 (specific for the 34K protein) are also active on smooth bacteria, and are the best phages to use both for mutant isolation and characterization. Another advantage is that they are not sensitive to immunity caused by a number of lysogenizing S. typhimurium phages. P221 (36K-specific) as well as PH51 and PH41 (34K-specific) can be used to characterize rough strains.

PH42 may be a very interesting phage, whose receptor requirements appear to be less strict: both the 34K and 36K proteins seem capable of serving as its receptor. Such a broad specificity is perhaps not too surprising because the 34K–36K proteins correspond functionally to protein I of E. coli, in which the amino acid sequences of Ia and Ib have been shown to be very similar (Schmitges & Henning, 1976). In fact the strict specificity of the other phages may be more surprising. To understand the implications of this finding, a more detailed knowledge of the respective Salmonella OMP structures will be necessary. It should also be pointed out that we have not formally excluded the trivial possibility that the PH42 phage has two kinds of tail fibres on one phage particle. A mixture of two types of phage, however, seems to be excluded by the complete inactivation of phage PH42 by 34K−36K+ bacteria (unpublished observations).

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REFERENCES


NURMINEN, M., LOUNATMAA, K., SARVAS, M., MÄKELÄ, P. H. & NARAS, T. (1976). Bacterio-


