Characterization of SE1, a New General Transducing Phage of *Salmonella typhimurium*

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A transducing phage, SE1, which is able to infect *Salmonella typhimurium* was isolated from a *Salmonella enteritidis* strain. SE1 is a temperate phage which is heteroimmune with respect to phages P22, L, KB1 and ES18. It is similar in morphology and size to phages P22, L and KB1 and is serologically related to phages P22 and L but not to KB1. Efficiencies of generalized transduction effected by phage SE1 are similar to those for P22HT (*int7*), a mutant which mediates a high frequency of chromosomal gene transduction. The lengths of chromosomal DNA transduced by SE1 and P22HT (*int7*) are similar. Furthermore, the SE1 prophage does not exclude the transducing particles from cells it has lysogenized; consequently it is possible to use both SE1 lysogens and non-lysogenic strains as recipients in SE1-mediated transduction experiments, and obtain similar transduction efficiencies. However, the SE1 prophage gives rise to a lysogenic conversion that decreases the rate of adsorption of SE1 and L phages by about 50%, but does not affect adsorption of P22. Altogether these results suggest that phage SE1 may be a useful tool in the genetic manipulation of *S. typhimurium*.

INTRODUCTION

The most useful transducing vector in *Salmonella typhimurium* is bacteriophage P22, particularly its mutants which display high transduction frequencies. Other generalized-transducing phages such as L (Bezdek & Amati, 1967), KB1 (Boro & Brenchley, 1971) and ES18 (Kuo & Stocker, 1970) have also been reported for *Salmonella*.

Two phenomena modify the efficiency of P22-mediated transduction when the recipients are lysogenic for P22. First, it has been reported that lysogenic conversion by P22 produces a decrease in the adsorption rate of P22 (Zinder, 1958), due to modification of the P22 receptor site by glycosylation of the LPS of *S. typhimurium* (Lindberg, 1973). As a result, there is a slow decrease in the efficiency of P22-mediated transduction. Secondly, P22 has two superinfection exclusion systems. The first, *sieB*, excludes heteroimmune phages (Susskind *et al.*, 1974*b*), while the second, *sieA*, is a nonspecific system that excludes many phages, including MG178, L and even P22 (Susskind *et al.*, 1974*a*). The *sieA* system is also responsible for the exclusion of generalized-transducing particles generated by P22 (Ebel-Tsipis & Botstein, 1971) or other phages. As a consequence, a large decrease in the transduction efficiency is observed (Ebel-Tsipis & Botstein, 1971; Susskind *et al.*, 1971).

Susskind *et al.* (1974*a*) have proposed that the *sieA* exclusion system is a special DNA modification and/or restriction system that acts only on injected DNA, and which also interferes with the classical host restriction system. Thus, both superinfecting, active transducing particles and P22 phages would be able to adsorb normally and inject their DNA, which would then be partially degraded by the *sieA* exclusion system.

In this paper we describe a general transducing phage of *S. typhimurium*, called SE1, which does not express a superinfection exclusion system that adversely affects the efficiency of SE1-

mediated transduction. The physiological and morphological characteristics of SE1 are similar to those of phage P22 and phage L, which is serologically related to phage P22. The transducing properties of SE1 are discussed with reference to those of P22.

METHODS

Bacterial and bacteriophage strains. These are shown in Table 1. Strains lysogenic for SE1 were picked from the centre of the growth in phage plaques, purified by repeated streaking and assayed for the presence of the SE1 prophage.

Media. LB broth, LB agar (Miller, 1972) and soft agar were used for growth of bacterial and bacteriophage strains and for titration of phages. Minimal medium AB (Clark & Maaløe, 1967) supplemented with glucose (0.2%, w/v) and required amino acids (20 µg ml⁻¹) was used for transduction experiments. The components of the rich medium were obtained from Difco, the agar from Oxoid and the salts and the amino acids from Sigma.

Isolation of the phage and preparation of lysates. Phage SE1 was obtained from a strain of *Salmonella enteritidis* isolated from a hospital patient. The strain was grown in LB broth at 37 °C. The culture was then centrifuged and the supernatant assayed for phage with *S. typhimurium* LT2 as indicator.

Phage lysates were routinely obtained by incubating a mixture of *S. typhimurium* LT2 and the desired phage at 37 °C with aeration. Phages were added at an m.o.i. of 1 p.f.u. per c.f.u. Lysates were maintained over chloroform. Titres of up to 10¹⁰ p.f.u. ml⁻¹ were usually obtained by this method.

When necessary, lysates were concentrated and partially purified by repeated centrifugation at 40000 g, followed by resuspension of the pellets in phage buffer (Clowes & Hayes, 1968).

One-step growth curve and Doermann experiment. These experiments were done as described by Clowes & Hayes (1968), except that cells were lysed with chloroform.

Phage antisera. To obtain specific antisera against phages SE1, L, P22 and KB1, 100 ml crude phage lysate was concentrated by high-speed centrifugation and the pellets resuspended in 10 ml 0.4 M-NaCl. Samples (1 ml) of these suspensions were emulsified with 1 ml Freund's incomplete adjuvant (Sigma), and the mixtures were used to

Table 1. *Bacterial and bacteriophage strains*

Strain	Phenotype	Source*
<i>S. enteritidis</i> NA2673	Amp ^R Carb ^R Clm ^R Tet ^R , lysogenic for SE1	G. Prats
<i>S. typhimurium</i> LT2	Wild-type	J. L. Ingraham
UA1260	LT2 but lysogenic for SE1	This work
UA1020	Rough	This laboratory
UA1120	Semirough	
UA1160	LT2 Lys ⁻	
UA1180	LT2 Leu ⁻	
UA1201	LT2 Thy ⁻	
UA1219	LT2 Trp ⁻	
UA1190	LT2 Thr ⁻	
UA1197	As UA1190 but lysogenic for P22	This work
UA1192	As UA1190 but lysogenic for P22 <i>sieA</i>	
UA1193	As UA1190 but lysogenic for SE1	
UA1010	LT2 His ⁻ Met ⁻	This laboratory
UA1121	As UA1010 but lysogenic for P22	This work
UA1122	As UA1010 but lysogenic for P22 <i>sieA</i>	
UA1123	As UA1010 but lysogenic for SE1	
UA1124	As UA1123 but His ⁺	
UA1125	As UA1124 but Met ⁺	
Phage ES18	Wild-type	J. R. Roth
KB1	Wild-type	J. L. Ingraham
L	Wild-type	J. Casadesús
P22	Wild-type	J. R. Roth
P22HT <i>int7</i>	Int ⁻ , high transduction efficiency	J. L. Ingraham
P22 <i>sieA</i>	SieA ⁻	J. Casadesús
SE1	Wild-type	This work

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immunize rabbits by two intramuscular injections of 0.5 ml (Hudson & Hay, 1978). The rate of inactivation of phages P22, L, KB1 and SE1 by antisera raised against these phages was determined by the procedure of Adams (1959).

Density gradient centrifugation. CsCl gradients were prepared by resuspending the phage, after purification by high-speed centrifugation, in 4.0 M-CsCl, 10 mM-MgSO₄, 10 mM-Tris (pH 8) and 0.1 mM-Na₂EDTA in a 13 × 51 mm Beckman cellulose nitrate centrifuge tube (Davis *et al.*, 1980). After centrifugation at 34500 r.p.m. at 20 °C for 16 h in a Beckman 80 Ti rotor, fractions were collected from the bottom of the gradient. Each fraction was assayed for *A*₂₆₀, CsCl density and phage titre. The different phages in a mixture were identified by titration against bacterial strains lysogenized with each one of them and against a P22 *sieA* mutant.

Electron microscopy. Crude phage lysates were concentrated by high speed centrifugation and purified by CsCl gradient centrifugation. A drop of phage suspension was negatively stained with uranyl acetate on carbon-coated copper grids for electron microscopy (Dawes, 1971). Excess liquid was removed with filter paper and the grids were allowed to dry in air. The preparations were examined and photographed in a Hitachi electron microscope at 20000–60000 magnification.

Transduction procedure. Recipient cells for transduction were grown at 37 °C in LB broth (20 ml) inoculated with a 24 h culture of bacteria to give a cell density of about 2×10^8 c.f.u. ml⁻¹. Phage were usually grown in *S. typhimurium* LT2 as propagation strain. Mixtures (1:1) containing one phage per recipient bacterium were incubated for 30 min at 37 °C in aerobic and static tubes of LB broth. Samples of the mixtures (0.1 ml) were plated on minimal salts medium with glucose (0.2%, w/v) and supplements when appropriate, and incubated at 37 °C for 48 h (Ebel-Tsipis & Botstein, 1971). Controls for spontaneous reversion were made simultaneously and samples were plated to determine the number of p.f.u. and c.f.u. in the mixtures. Transduction frequencies are given as T/P ratios, i.e. the ratio of the number of recombinant transductants to the number of p.f.u.

Determination of phage adsorption rates. Adsorption rates were determined by measuring the unadsorbed phages (Adams, 1959). Exponential bacterial cultures were infected at an m.o.i. of 0.1 p.f.u. per c.f.u. and kept at 37 °C without shaking. Samples were periodically withdrawn and treated with chloroform. When the bacterial strains were lysogenic, a control for spontaneous phage induction was made simultaneously and the result was taken into consideration in the calculations.

RESULTS AND DISCUSSION

General characterization of phage SE1

Morphological and physical characteristics and growth kinetics of phage SE1 were studied and compared with those of several phages previously described in *S. typhimurium*. The mature SE1 virion was a hexagonal particle of 60 nm diameter, with a short base plate of 12 nm which showed six-fold symmetry (Fig. 1). This morphology is similar to that of phages P22 (Susskind & Botstein, 1978), L (Bezdek & Amati, 1967) and KB1 (Brenchley, 1971). Nevertheless, SE1 was heteroimmune with respect to phages P22, L, KB1 and ES18 because it was able to grow on cells lysogenic for these phages (data not shown).

Phage SE1 had the same buoyant density as phage P22, which was higher than that of phage L (Fig. 2). The density values obtained for phages L (1.505 g cm⁻³) and P22 (1.515 g cm⁻³) were in accordance with the values reported by Bezdek & Amati (1967).

The one-step growth and Doermann experiments with phage SE1 showed similar growth kinetics to those reported for phages P22 (Grabnar & Hartman, 1968) and L (data not shown). The minimal latent period for phage SE1 was 42 min, while that for phage P22 under the same conditions was 36 min, in agreement with the data reported by Grabnar & Hartman (1968). However, the burst size of SE1 was about 300 p.f.u. per infected bacterium, three times the value obtained for phages P22 and L.

Antigenic properties

Table 2 presents the *K* values obtained in cross-serological tests between phages P22, L, SE1 and KB1 and their respective antisera. For comparison, the *K* values are expressed as percentages, where 100 indicates the inactivation by an anti-phage serum against the same phage.

Anti-P22 serum inhibited the plaque-forming ability of phages L and SE1 (Table 2), but it had a more inhibitory effect on phage L than on phage SE1. Similarly, the anti-L serum and the anti-

Fig. 1. Electron micrograph of phage SE1. Bar, 75 nm.

Fig. 2. Phage titre after CsCl density gradient centrifugation of phages P22 (▼), L (◆) and SE1 (□). The CsCl density gradient is also shown (○).

SE1 serum reacted with phage P22 with different efficiencies. Phages SE1 and L were shown to be serologically related, because the anti-L and anti-SE1 serum inhibited the plaque-forming ability of phages SE1 and L respectively (Table 2).

As a control, cross-reactions with phages P22 and KB1 were tested. In agreement with previous data (Brenchley, 1971), the results indicated that phages P22 and KB1 were not related. Furthermore, analogous results were obtained with both anti-SE1 and anti-L sera with respect to phage KB1, showing that phages SE1 and L were not serologically related to KB1.

Table 2. Cross-serological tests between phages P22, L, SE1 and KB1

Phage antiserum	Percentage K value*			
	P22	L	SE1	KB1
P22	100	55	38	0
L	79	100	50	0
SE1	34	55	100	0
KB1	0	0	0	100

* The K values for the reaction between each phage and its own antiserum were 55 min⁻¹ for P22, 67 min⁻¹ for SE1, 84 min⁻¹ for L and 26.5 min⁻¹ for KB1.

Table 3. Adsorption constants of phages SE1, L and P22 on SE1 lysogenic and non-lysogenic *S. typhimurium* strains

Bacteriophage	K (ml c.f.u. ⁻¹ min ⁻¹)	
	LT2	UA1260
SE1	1.6×10^{-9}	8.0×10^{-10}
L	7.5×10^{-10}	1.8×10^{-11}
P22	4.9×10^{-9}	4.3×10^{-9}

Adsorption rate

Phage SE1 probably adsorbs to LPS, since it was unable to infect either semirough or rough mutants of *S. typhimurium* strains (data not shown).

Table 3 shows the adsorption constants of phage SE1 on both an SE1 lysogen (UA1260, a derivative of LT2) and non-lysogenic strain LT2. The SE1 prophage affected the adsorption rate of phage SE1, giving a lower adsorption constant on the lysogenic strain UA1260 than on the non-lysogenic strain LT2. A similar effect was observed in the adsorption constants of phage L, which were lower on the SE1 lysogen than on the non-lysogenic strain. However, the SE1 prophage did not affect the adsorption rate of P22 (Table 3), suggesting that phages P22 and SE1 have different *S. typhimurium* receptor sites.

These results suggest that the SE1 prophage causes a lysogenic conversion, similar to that reported for phage P22 (Gough & Scott, 1972), which is due to the presence of a new antigen (factor 1), encoded by the prophage, on the surface of *S. typhimurium*. The lysogenic conversion produced a twofold decrease in the rate of SE1 adsorption. A similar effect was seen for the rate of L adsorption. Further studies are necessary to establish the connection between phages SE1 and L and also to determine the basis of the modification to the surface of *S. typhimurium*, and probably *S. enteritidis*.

Transduction test

Preliminary results from transduction tests with phage SE1 showed that it can accomplish generalized transduction in *S. typhimurium*. Results from cotransduction tests with SE1 and P22HT (*int7*) showed that these phages can transport the same length of bacterial DNA in their virions, since they gave the same cotransduction frequency for *thy* and *lys* markers (40%). Schmieger & Backhaus (1973) have shown that P22 can carry 26×10^6 to 27×10^6 Da, which represent about 1/100 of the total *Salmonella* chromosome (Sanderson & Hartman, 1978).

Table 4 shows the results obtained for transduction of various markers by phages SE1 and P22HT (*int7*) at an m.o.i. of 1. The results indicated that both phages had similar transduction frequencies with non-lysogenic recipients and that the transduction efficiency of phage SE1 depended on which marker was transduced. A similar phenomenon has also been reported for phage P22 and has been attributed to the fact that bacterial DNA contains *pac* or *pac*-like sequences which can be recognized by the phage-packaging complex as a signal to start a series

Table 4. *Transduction frequency of various markers by phages SE1 and P22HT (int7)*

Marker	Position*	Transduction frequency†	
		P22HT (<i>int7</i>)	SE1
<i>leu</i>	2	2.8×10^{-6}	2.6×10^{-6}
<i>met</i>	87	3.5×10^{-5}	7.9×10^{-5}
<i>his</i>	42	1.5×10^{-4}	5.5×10^{-6}
<i>thr</i>	0	1.8×10^{-5}	8.1×10^{-6}
<i>thy</i>	61	9.3×10^{-7}	2.6×10^{-7}
<i>trp</i>	34	6.4×10^{-6}	3.6×10^{-6}

* Map position according to Sanderson & Roth (1983).

† No. of recombinant transductants/no. of p.f.u.

Table 5. *Transduction frequency of phages SE1 and P22HT (int7) on lysogenic and non-lysogenic strains*

Marker	Prophage in recipient cell	Transduction frequency*	
		P22HT (<i>int7</i>)	SE1
<i>his</i>	—	1.5×10^{-4}	5.5×10^{-6}
<i>his</i>	SE1	2.3×10^{-4}	1.2×10^{-6}
<i>his</i>	P22	3.6×10^{-6}	
<i>his</i>	P22 <i>sieA</i>	4.9×10^{-5}	
<i>met</i>	—	3.5×10^{-5}	7.9×10^{-5}
<i>met</i>	SE1	4.0×10^{-5}	2.2×10^{-5}
<i>met</i>	P22	5.9×10^{-7}	
<i>met</i>	P22 <i>sieA</i>	1.4×10^{-5}	
<i>thr</i>	—	1.9×10^{-5}	8.1×10^{-6}
<i>thr</i>	SE1	1.3×10^{-5}	1.5×10^{-6}
<i>thr</i>	P22	1.0×10^{-7}	
<i>thr</i>	P22 <i>sieA</i>	5.2×10^{-6}	

* No. of recombinant transductants/no. of p.f.u.

of sequential packaging events (Schmieger, 1982). Thus, for a given chromosomal marker, the probability of being encapsulated depends on its distance from a *pac* signal.

A *sieA* superinfection exclusion system that excludes transducing particles has been described in phage P22 (Susskind *et al.*, 1974a). In recipients lysogenic for P22 this system effects a marked decrease, which is marker-dependent, in the transduction efficiency of P22 (Ebel-Tsipis & Botstein, 1971). To study this phenomenon in phage SE1, transduction experiments with SE1 and P22HT (*int7*), using SE1 lysogens and non-lysogenic strains as recipients, were done. Transductions with P22HT (*int7*), using recipients lysogenic for P22 and P22 *sieA*, were done as controls.

It can be seen that the SE1 transduction frequencies decreased by only about four- to fivefold when the recipient was an SE1 lysogen (Table 5). On the other hand, the transduction frequencies of P22HT (*int7*) decreased significantly when the cells were P22 lysogens. However, when the recipient was a P22 *sieA* lysogen the decrease was only about fourfold. These results confirmed the operation of the *sieA* system in the transduction experiments with P22 and P22 lysogens. In addition, they suggested that phage SE1 does not encode a superinfection exclusion system similar to *sieA* of P22.

Although the SE1 prophage produced a decrease in the transduction efficiency of phage SE1, it did not lower the efficiency of transduction mediated by P22. The P22 *sieA* prophage caused a decrease in the P22HT (*int7*) transduction efficiencies. Both phenomena are similar and can be attributed to the decrease in the rate of SE1 adsorption (Table 3) on SE1 lysogens and the lysogenic conversion of the P22 prophage respectively.

These results indicated that it is possible to use SE1 lysogens as recipients in phage SE1-mediated, successive transductions. To test this possibility we performed transduction experiments, at an m.o.i. of 1, with UA1123 as recipient, selecting first for the phenotype His⁺. One such His⁺ transductant was isolated and used as a recipient in a second SE1 transduction experiment, selecting for Met⁺. The efficiencies of transduction obtained were similar to those obtained using phage SE1 as a transducing vector and an SE1 lysogen as recipient (Table 5).

We have shown that phage SE1 is able efficiently to mediate successful transduction to an SE1 lysogen and so we propose that it may be a useful tool in genetic studies of *S. typhimurium*, mainly when the transduction frequency of phage P22 is greatly decreased by the *sieA* exclusion system of the P22 prophage.

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