Restriction Mapping and Close Relationship of the DNA of *Streptomyces erythraeus* Phages 121 and SE-5

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The biological properties and genome structure of two actinophages, 121 and SE-5, infecting *Streptomyces erythraeus* were characterized. They had the same host range (limited to *S. erythraeus*) and similar DNA G + C contents (around 60 mol %). Restriction maps of their genomes also showed many similarities. The close relationship between the two phages was confirmed by DNA hybridization experiments: large parts of their genomes were homologous, except for a segment in the middle of the map, where no hybridization was detected.

INTRODUCTION

The actinophages 121 and SE-5 studied in this work are virulent phages able to lyse an industrially important strain, the erythromycin producer *Streptomyces erythraeus*. Phage 121 was described first by Retinskaya & Rautenstein (1960) and further characterized, by Rautenstein & Retinskaya (1963), together with three other phages infecting this *Streptomyces* species. All these phages were very similar in morphology, kinetics of inactivation by UV light, citrate sensitivity and serological properties. Five other actinophages (including SE-5), described by Ostrowska-Krysiak *et al.* (1971) and Ostrowska-Krysiak (1974), were serologically related to those studied by Rautenstein & Retinskaya (1963): for example, antiserum against phage SE-5 also inactivated 121 particles. These phages differed mainly in their degree of virulence (reflected, for example, by plaque morphology and burst size) and in *S. erythraeus* strain specificity (some of the phages were able to lyse strains that were selected for resistance to another of the phages; Gurkau & Ostrowska-Krysiak, 1972). Finally, in all the cases studied, the host range was limited to erythromycin-producing strains of *S. erythraeus*.

Here we present further evidence for a close relationship at the DNA level between two of these actinophages.

METHODS

Phages and micro-organisms. *Streptomyces erythraeus* 64-575 (Gurkau & Ostrowska-Krysiak, 1972) was used as a host for phage amplification. Phage 121 was originally isolated from a lysogenic strain of *S. erythraeus* (Retinskaya & Rautenstein, 1960). Phage SE-5 was isolated from a lysed, large-scale culture (Ostrowska-Krysiak, 1974). Both phages and *Streptomyces* strains were kindly provided by Drs B. Ostrowska-Krysiak and A. Błońska (Institute of the Pharmaceutical Industry, Warsaw, Poland).

Media and culture conditions. For actinophage amplification and host strain propagation, a medium containing (per litre) 10 g yeast extract (Difco), 0.5 g K₂HPO₄ and 10 g glucose and adjusted to pH 6.5 was used either as a liquid medium, or solidified with 0.8% or 1.5% (w/v) Bacto agar.

For DNA extraction, phages were amplified in liquid medium as follows. Spores of *S. erythraeus* 64-575 were suspended in liquid medium, briefly (10 min) heated at 55 °C and inoculated into 100 ml liquid medium. The culture was incubated at 30 °C with vigorous shaking for 36 h, after which it had reached late exponential phase.

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Then, the culture was inoculated with actinophage suspension in phage buffer (50 mM-Tris buffer, pH 8.0, 10 mM MgSO_4) containing 2.5×10^9 p.f.u. After 30 min preadsorption, the culture was diluted with fresh medium up to 1 litre and incubated overnight at 30 °C with vigorous shaking. Usually, complete lysis was observed. The titre was around 10^{10} p.f.u. ml^{-1} . Mycelial debris were removed by low-speed centrifugation.

Phage particles were precipitated with 10% (w/v) polyethylene glycol (Carbowax 8000, Fisher) in the presence of 0.5 M-NaCl (Yamamoto *et al.*, 1970). Purification was achieved by centrifugation in a CsCl step gradient followed by flotation of phage particles on a CsCl layer (Davies *et al.*, 1980).

Biological properties. The host range was determined by plating up to 10^8 p.f.u. on a lawn of the strain to be tested. Adsorption efficiencies were determined similarly, as described by Adams (1959): cells from the early exponential phase of growth (approx. 10^7 c.f.u. ml^{-1}) were infected with phage at low m.o.i. (< 0.1) and incubated in medium supplemented with 1 mM-NaCl and 1 mM- $\text{Ca}(\text{NO}_3)_2$ at 30 °C with gentle shaking. After 30 min incubation, samples were centrifuged and tested for free phage titre.

DNA extraction and analysis. After dialysis of phage CsCl stocks against 10 mM-Tris buffer, pH 8.0, 10 mM-EDTA, DNA was purified by phenol-chloroform extraction. DNA was precipitated with ethanol, and was dried and dissolved in H_2O .

Restriction enzymes were used as recommended by the suppliers. DNA fragments were separated on 0.7–1.5% agarose gels or on 3.5–10% polyacrylamide gels.

The G + C content was estimated by determination of the thermal denaturation temperature (Mandel & Marmur, 1968), and by UV spectroscopy (Ulitzur, 1972).

For hybridization analysis, DNA fragments, separated on agarose gels, were blotted onto Schleicher and Schuell nitrocellulose BA 85 sheets, according to Southern (1975), as modified by Smith & Summers (1980). Prehybridization and hybridization were done at 42 °C in 50% (v/v) formamide, 0.1% (w/v) SDS, 0.9 M-NaCl, 0.005 M-EDTA, 0.05 M-sodium phosphate buffer pH 7.7. Heparin (500 $\mu\text{g ml}^{-1}$) was included for background control (Singh & Jones, 1984). Final washing was done at 50 °C in 0.1% (w/v) SDS, 0.018 M-NaCl, 10^{-4} M-EDTA, 10^{-3} M-sodium phosphate buffer pH 7.7. For hybridization probes, we used nick translated DNA (Rigby *et al.*, 1977) of total phage or restriction fragments separated on a low melting-point agarose (BRL) gel and purified on a NACS Pre-Pac column (BRL).

RESULTS

Biological properties

Phage 121 was isolated by Retinskaya & Rautenstein (1960) as a virulent variant of a prophage present in a lysogenic erythromycin-producing strain, *S. erythraeus* 8594. However, in our experiments, we did not observe the abundant secondary growth of mycelium in the phage plaque, as described by these authors. Instead, plaque morphology was identical with that described by Ostrowska-Krysiak *et al.* (1971). This difference is probably due to the choice of host strain, as the latter authors described different plaque morphologies for phage 121 on various indicator strains. The plaque morphology observed for phage SE-5 was the same as for phage 121.

Both phages have a narrow host range. We failed to obtain plaques on the following strains: *S. albus* G., *S. lividans* 66, *S. coelicolor* A3(2), *S. fradiae*, *S. vinaceus*, *S. azureus*, *S. parvulus*, *S. griseus*, *S. cattleya*. In the case of *S. fradiae* and *S. albus* G., failure to act as hosts was correlated with the virtual absence of adsorption of phage 121 in conditions giving 92% adsorption to *S. erythraeus*.

Estimation of the G + C content of phage DNA

By measurement of the melting temperature of 121 and SE-5 DNA we concluded that the G + C content was 59 and 57.5 mol % respectively. Slightly higher values were obtained by the spectrophotometric method: 62.5 and 59.5 mol % respectively. These fall into the range of values found for *Streptomyces* phages (Lomovskaya *et al.*, 1980) and are quite different from the G + C content of host DNA (around 70 mol % G + C).

Genomic organization of phages 121 and SE-5

Genome length, calculated as the sum of the restriction fragment lengths, is 41.9 kb for 121 DNA and 42.2 kb for SE-5 DNA. Sizes of DNA fragments produced by *Bgl*II, *Pvu*II, *Xba*I, *Sal*I, *Sst*II and *Stu*I are shown in Fig. 1. Both phage DNAs contain cohesive ends. This was shown by the tendency of terminal fragments to associate to give a fragment of a length equal to the sum of the two fragments (Fig. 2).

Fig. 1. Cleavage maps of 121 and SE-5 DNA. Sizes (kb) are mean values of at least four determinations. Larger fragments were determined indirectly (as a sum of smaller fragments created by double or triple digestions). In SE-5 DNA, the 5.4 kb *PvuII* fragment localized on the left end of the map was arbitrarily assigned as D; the right-end *PvuII* fragment was assigned as E. Small fragments (less than 0.5 kb) were identified on 5% acrylamide gels. All the other fragments were identified on 0.8–1.4% agarose gel. Dotted lines indicate areas of less intense hybridization.

Fig. 2. Phage DNA (prepared as described in Methods) was digested with restriction endonucleases and either loaded directly on the gel (lanes *b* and *d*) or heated for 5 min to 80 °C and quickly cooled on ice before loading (lanes *a*, *c* and *e*). The samples were electrophoresed on a 1% agarose gel for 4 h at 90 V. Lanes *a* and *b*, 121 DNA digested with *BglII*; lanes *c* and *d*, SE-5 DNA digested with *BglII*; lane *e*, SE-5 DNA digested with *PvuII*.

For the cleavage map of phage 121 DNA, the single *Xba*I site was taken as a reference point. The *Bgl*/II map was deduced by combining the results of partial *Bgl*/II digestion and *Bgl*/II-*Xba*I double digestion. The *Pvu*II map was determined by partial digestion as well as by *Bgl*/II-*Pvu*II and *Xba*I-*Pvu*II double digestions. Finally, the position of the single *Stu*I site was deduced from double digestions. Sites for *Sal*I and *Sst*II are absent in 121 DNA.

In phage SE-5 DNA, the position of *Sst*II, *Sal*I and *Xba*I sites was determined by single and double digestions. Double digestions with *Bgl*/II and one of the above enzymes located the *Bgl*/II fragments A and F. The *Bgl*/II fragment B was identified as a terminal fragment (reduced intensity without heating and rapid cooling; Fig. 2, lanes *c* and *d*). The *Pvu*II map was deduced from double or triple digestions. However, from all these experiments, the position of *Bgl*/II fragments C, D, E, G and H could not be established. Therefore, *Bgl*/II fragments F and G were extracted from low melting-point agarose, nick-translated and used as hybridization probes for Southern blot analysis of DNA fragments from partial and total *Bgl*/II and *Pvu*II digests of SE-5 DNA (one of the results is shown in part in Fig. 3, lanes 9, 10). The following order of *Bgl*/II fragments could be derived: E-D-G-H-C-F (Fig. 1). SE-5 DNA contains no site for *Stu*I. Among other enzymes examined but not used for mapping, *Eco*RI gave 14 fragments with 121 DNA and 17 with SE-5 DNA, *Bcl*I gave 16 and 13, *Clal* gave 9 and 7 and *Hind*III gave 8 and 13, respectively, while *Hind*II gave more than 25 fragments with DNA of both phages. Neither 121 nor SE-5 DNA contained sites for *Bam*HI, *Xho*I, *Sph*I, *Aha*III *Nco*I and *Sac*I.

Cross hybridization between 121 DNA and SE-5 DNA

Since the restriction maps of the two phage DNAs show some similarities and since we detected many fragments of identical size when digesting 121 and SE-5 DNA with restriction enzymes that cut frequently, for example *Alu*I and *Sau*3A (data not presented), Southern blotting experiments were done to test for cross-hybridization between the two DNA molecules. Restriction fragments of phage 121 DNA were separated on an agarose gel, transferred to nitrocellulose and probed with nick-translated SE-5 DNA (Fig. 3, lanes 1, 2, 3). Restriction fragments of phage SE-5 DNA, treated in the same way, were hybridized with labelled 121 DNA (Fig. 3, lanes 4, 5, 6, 7).

A high level of homology was detected, as almost all the restriction fragments gave a signal on the respective autoradiograms. However, two *Eco*RI fragments from 121 DNA (3.7 kb and 1.45 kb) had a very low intensity on the autoradiogram (Fig. 3, lane 1). Similarly, one SE-5 *Eco*RI fragment (3.5 kb) had a low intensity and two others (1.9 kb and 1.3 kb) gave no signal at all on the blot (Fig. 3, lane 6). This allowed us to estimate the extent of non-homology to be 4–6 kb in length. Most of the non-homologous region should be localized in the middle of the genome, as three central restriction fragments in SE-5 DNA (*Pvu*II fragment G, *Sal*I fragment C and *Bgl*/II fragment F) gave no hybridization signal (Fig. 3, lanes 4, 5, 7). This was confirmed by using one of these fragments (*Bgl*/II fragment F) as a probe. With SE-5 DNA, the fragments predicted from the SE-5 restriction map gave hybridization signals on the autoradiogram (Fig. 3, lanes 9, 10), while no signals were detected with 121 DNA restriction fragments (Fig. 3, lane 8).

Less intense hybridization was observed with some of the 121 DNA *Pvu*II or *Bgl*/II fragments when SE-5 was used as a probe; these fragments are indicated by filled pointers in Fig. 3 and by the dotted line over the 121 restriction map (Fig. 1). It was not clear whether these fragments indicated regions of low homology or of lower G + C composition, or merely indicated non-uniform incorporation of label into the nick-translated probe.

DISCUSSION

Phages 121 and SE-5 are distinct from actinophages previously characterized (Lomovskaya *et al.*, 1980; Walter *et al.*, 1981; Cox & Baltz, 1984; Anné *et al.*, 1984, 1985; Ogata *et al.*, 1985; Greene & Goldberg, 1985), judging from the number of restriction enzyme cleavage sites and from cleavage maps (when available). Phages 121 and SE-5 have similar plaque morphology and behave similarly during growth experiments. Their close relationship has now been confirmed by DNA restriction mapping and Southern blot analysis; there is only one major difference between the genomes of 121 and SE-5 and the overall DNA homology is high.

Fig. 3. Restriction endonuclease fragments (A) and hybridization signals (B) from 121 DNA (lanes 1, 2, 3, 8) or SE-5 DNA (lanes 4, 5, 6, 7, 9, 10) probed with nick translated total SE-5 DNA (1, 2, 3), total 121 DNA (4, 5, 6, 7) or *Bgl*II fragment F from SE-5 DNA (8, 9, 10). The restriction enzymes used were *Eco*RI (1, 6), *Pvu*II (2, 4, 10), *Bgl*II (3, 7, 8), *Sac*I (5) and *Bgl*II + *Pvu*II (9). Filled pointers indicate fragments that are less intense on autoradiograms; open pointers indicate fragments without any corresponding signal on the autoradiograms.

Phage 121 DNA is resistant to digestion by eight restriction enzymes among 19 tested. Of the eight restriction enzymes known to be present in *Streptomyces* species (Roberts, 1984), phage 121 is resistant to five. According to Cox & Baltz (1984), such a pattern seems to be typical for broad host-range actinophages. This may indicate that phage 121 has evolved from a broad host-range phage and that its adaptation to *S. erythraeus* as a sole host is a rather recent acquisition.

Phage SE-5 has a short central segment of DNA with a higher density of restriction target sites (mainly GC-rich sequences). It would be interesting to determine if this segment represents a 'module' (Botstein, 1980) originating from another phage genome or from a *Streptomyces* chromosome.

Our work extends previous findings (Rautenstein & Retinskaya, 1963) that phages isolated on *S. erythraeus* as indicator strain have a narrow host range limited only to this species. This was indirectly confirmed by Cox & Baltz (1984), who isolated and characterized many phages originating from soil samples and used *S. fradiae* and *S. griseofuscus* as hosts; although some of their phages had a very broad host range, none was able to lyse *S. erythraeus*. On the other hand, some broad host range phages that are able to amplify on *S. erythraeus* have been described, for example SH10 (Klaus *et al.*, 1981), P23, CPC, CWK and CT (Anné *et al.*, 1984). However, we have tested three of these phages (P23, CPC and CT) and they did not form plaques on *S. erythraeus* strains 64-575 or ISP 5517 (unpublished data). Therefore, the existence of broad host range *S. erythraeus* actinophages should be carefully reconsidered.

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