A site-specific endodeoxyribonuclease from *Streptomyces albus* CMI 52766 sharing site-specificity with *Providencia stuartii* endonuclease PstI

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Received 25 March 1977

ABSTRACT

A class II site-specific endodeoxyribonuclease (SalPI) was identified in cell-free extracts of *Streptomyces albus* CMI 52766 after high speed centrifugation and fractionation through BioGel A0.5M. SalPI cleaves lambda DNA into at least 18 fragments. Five cleavage sites were located in the linear lambda map by the use of double and triple restriction enzyme digests involving EcoRI, HindIII, SalGI and another new *Streptomyces* enzyme, SacI. The results were indistinguishable from those previously obtained for a *Providencia stuartii* enzyme, PstI, by Smith, Blattner & Davies (Nucleic Acids Res. 1976 **3**, 343). SalPI and PstI were shown by a double digest test to have the same site specificity. None of 34 phages tested was obviously restricted by *S. albus* CMI 52766, and correspondingly DNA from two of them was not cleaved in vitro by SalPI. DNA from a *Streptomyces* phage that does not form plaques on *S. albus* CMI 52766, and plasmid SCP2 DNA from *S. coelicolor* A3(2), were both cleaved.

INTRODUCTION

In the course of a study of the endonuclease SalGI-mediated restriction of phage Pal6 by *Streptomyces albus* G, an independently isolated strain also classified as *S. albus* (CMI 52766) was used as an alternative host which neither restricted nor modified the phage¹. Subsequently, *S. albus* CMI 52766 was also found to contain a site-specific endodeoxyribonuclease active against DNA of coliphage lambda (though not against DNA of Pal6). This paper describes the mapping of some of the cleavage sites on lambda DNA, and the demonstration that the new enzyme shares these sites with the enzyme PstI isolated from *Providencia stuartii*².

MATERIALS AND METHODS

Bacteriophages and their DNA. *Streptomyces* phages used were: ϕ C31³, ϕ 448⁴, VP5⁵, and various phages independently isolated from soil by the method of Dowding⁶ using a range of different host organisms. All these phages were propagated in soft agar overlays¹. Coliphage lambda was propagated by heat induction of *Escherichia coli* strain W3110 (containing

λ C₁857s7 prophage) as previously described¹. Phage DNA was extracted using phenol¹ except that ϕ 448 DNA was isolated by treatment with sodium perchlorate and ethanol⁷.

Restriction enzymes. EcoRI and HindIII were from Miles Laboratories. SalGI (formerly SalI, but here termed SalGI to avoid confusion with SalPI) was isolated from Streptomyces albus G as previously described¹, with further purification on a DEAE cellulose column (Whatman, DE52) as follows. Active fractions from BioGel AO.5M chromatography were pooled and dialysed against extraction buffer¹ to reduce the NaCl concentration to 0.1M, prior to loading on to DEAE cellulose. Elution of bound SalGI was by the application of a 0-1M NaCl gradient. SalGI eluted as a broad peak from 0.15 to 0.3M NaCl. Active fractions were pooled and precipitated by adding ammonium sulphate to 70% saturation; the precipitate was separated by centrifugation, dissolved in 2 ml of extraction buffer containing 10% glycerol, and dialysed against the same buffer. PstI and SacI were used in a crude form obtained after fractionation of high speed supernatants of cell free extracts at 1.0M NaCl through BioGel AO.5M, as previously described for SalGI¹. More highly purified PstI was kindly provided by S. Liddell. SacI preparations contained a low level of a second activity, presumably SacII (J. R. Arrand, R. J. Roberts and P. Myers, unpublished data). (SacI is a newly identified enzyme present in Streptomyces achromogenes ATCC 12767, and is probably identical with SstI, an enzyme found in a Streptomyces sp. isolated as a contaminant of an S. albus G culture: S. Goff, A. Rambach, J. R. Arrand, P. Myers, R. J. Roberts and K. F. Chater, unpublished data.) Streptomyces enzymes and PstI were all stored at 4^o, at which SalGI preparations retained activity for many months, SacI for several weeks, and PstI and SalPI for only a few days. EcoRI and HindIII were stored at -20^oC.

Digestion conditions, agarose slab gel electrophoresis of DNA and microdensitometry were as previously described¹.

Estimation of molecular weights of DNA fragments. Molecular weight standards - usually lambda DNA digested with EcoRI⁸ or HindIII⁹ - were included on all gels. Logarithms of molecular weight were plotted against distance migrated for these standards and molecular weights of unknown fragments were read from this graph.

RESULTS

Detection of SalPI. Cell-free extracts of Streptomyces albus CMI 52766 contained a site-specific endonuclease which was detected after

BioGel A0.5M chromatography, at 1.0M NaCl, of the high speed supernatant of a crude cell-free extract. There was no suggestion from the lambda DNA cleavage patterns of sequential fractions that more than one such enzyme was present. The peak of activity co-eluted with a yellow material, which was clearly visible on the column.

An active fraction was dialysed against extraction buffer containing 10% glycerol to remove NaCl, and its relative activity on lambda DNA determined at 0, 3, 6 and 10mM MgCl_2 and at 0, 20, 50 and 100mM NaCl (Fig.1). Most complete digestion was at 10mM MgCl_2 and 50mM NaCl. Some activity was observed even in the ostensible absence of MgCl_2 .

After storage at 4° for 1-2 weeks, most of the activity was lost. Addition of 10mM MgCl_2 did not prevent activity loss.

Following the recommendations of Smith and Nathans¹⁰, the new enzyme

Fig. 1. 1% agarose gel showing effects of NaCl and MgCl_2 concentrations on SalPI activity on lambda DNA. Digestion was with 5 μl extract at 37° for 1 h, using 1.3 μg lambda DNA per digest.

has been termed SalPI, as strain CMI 52766 was first accurately described and named by Puntoni¹¹. In order to avoid confusion with SalI from S. albus G, it is proposed that the latter enzyme should be designated SalGI in future.

Analysis of fragments produced by SalPI digestion of lambda DNA. SalPI cleaves lambda DNA into at least 18 fragments (Table 1, Figs.1,2). When completed digests were not heated to 60°C and then rapidly cooled in ice, fragments A and F almost completely disappeared and were replaced by a single fragment of molecular weight approximately equal to the sum of the molecular weights of the missing fragments. This suggested that fragments A and F possessed the single stranded cohesive ends of the lambda DNA molecule.

Fragments A,B,C,D and F were located within the linear lambda DNA molecule by agarose gel analysis of the fragments arising from further digestion of lambda/SalPI digests with other restriction enzymes that cleaved lambda DNA in known positions (Fig.3). Fig.4 gives cleavage maps of lambda DNA by all the enzymes concerned, to clarify the arguments that follow.

Fragment A was located at the conventional right end of the linear map, because it would not otherwise have been cleaved by either EcoRI or HindIII. In further support for this hypothesis, fragments in each of these double digests co-migrated with the two EcoRI and HindIII fragments closest to the right end, none of which should be cleaved by SalPI with the suggested arrangement.

SalGI cleaves lambda DNA at 67.3% and 68.3%¹². SalPI fragment C (9.8%)

Fig.2. Microdensitometer scan of track (50mM NaCl, 10mM MgCl₂) from Fig.1. Letters correspond to fragment designations in Table 1.

Table 1. Fragments generated by SalPI digestion of lambda DNA.

Fragment	MW(Md)	% of lambda DNA
A	7.5	24.5
B	3.3	10.9
C	3.0	9.8
D	2.8	9.2
E	1.9	6.3
F	1.8	5.8
G	1.7	5.5
H	1.7	5.5
I	1.5	4.8
J	1.4	4.5
K	1.1	3.7
L	0.74	2.4
M	0.70	2.3
N	0.47	1.5
O	0.31	1.0
P	0.29	0.95
Q	0.22	0.72
R	0.18	0.59
	<u>30.61</u>

Fig.3. Diagrammatic representation of the results of digestion of lambda DNA by SalPI together with other restriction enzymes.

Fig.4. Cleavage sites (arrows) on lambda DNA of restriction enzymes SacI (unpublished results), SalGI¹², EcoRI⁸, HindIII⁹, PstI² and SalPI (see text). Relevant fragments are identified by letters.

was the only one cleaved by SalGI, giving a new fragment of 7.8% and presumably two smaller fragments of about 1% each, which were undetected. The SalPI sites generating fragment C were thus at either 66.3% and 76.1%, or 59.5% and 69.3%. Only in the latter case would the SalGI/SalPI 7.8% fragment be cleaved by EcoRI. It was not, so that the former alternative is favoured.

SacI cleaves lambda DNA at 51% and 53.4% (K. F. Chater, unpublished results). SalPI fragment D (9.2%) was the only one cleaved by SacI, giving a new fragment of 5.2%, and presumably two smaller fragments of 2.4% and about 1.6%, which were not detected. The cleavage sites generating fragment D were thus at either 45.8% and 54.9%, or at 49.5% and 58.6%. The former possibility was favoured because HindIII fragment E, but not F, was cleaved by SalPI; on the second possibility HindIII fragment F but not E would have been cleaved.

The remaining large fragment, B (10.9%), could now be accommodated either in the leftmost 46% of the map or between fragments C and D, i.e. between 54.9% and 66.3%. Since it was cleaved by HindIII, the latter location was indicated.

The summed observed molecular weights (16.6×10^6) of fragments A, B, C and D are in close agreement with the value 16.6×10^6 for the whole of the region to the right of the cleavage site proposed at 46%. There must

therefore be few, if any, undetermined SalPI cleavage sites in this region, so that virtually all the unmapped fragments are located in the region 5.8% to 46%.

SalPI and PstI are isoschizomers. There is close similarity between the positions of SalPI cleavage sites in lambda DNA and those reported for the Providencia stuartii enzyme PstI². Lambda DNA digested with crude PstI taken from a BioGel A0.5M column gave a pattern on agarose gel electrophoresis that was indistinguishable from that obtained with lambda DNA digested with SalPI. Fig.5 shows the identical patterns obtained after digesting lambda DNA with SalPI and purified PstI separately and together,

Fig.5. Agarose gel electrophoresis of lambda DNA digested with PstI (left), SalPI (right) and both enzymes (centre).

confirming that SalPI recognises and probably cleaves the same base sequence as does PstI: the enzymes are thus isoschizomers (the term of R. J. Roberts). The identity of all three patterns shows that the minor discrepancy between the size of the leftmost lambda fragment reported here and that given by Smith *et al.*² is probably a function of the particular lambda DNA used.

Search for restriction of phages by *S. albus* CMI 52766 in vivo and in vitro. Of 34 Streptomyces phages tested on *S. albus* CMI 52766 only two (VP5⁵ and Ø448⁴, propagated on *S. lividans* 66, both of which have very narrow host ranges) failed to form plaques. The remaining 32 all gave at least as many plaques on *S. albus* CMI 52766 as on the strain on which they had previously been propagated, except for the temperate phage ØC31³ which gave 10- to 20- fold fewer plaques than on the alternative host (*S. lividans* 66) irrespective of which of the two strains was the previous host. The other 31 phages tested were all virulent and comprised VP11, VP12 and VP13 (isolated and propagated on *S. coelicolor* A3(2)⁶); f6 (isolated on a neutrophilic Streptomycete by S. T. Williams and K. I. Sykes and propagated on *S. coelicolor* A3(2)); Pal6¹ and Pal11 (isolated and propagated on *S. albus* G); six phages isolated and propagated on a restriction and modification deficient mutant of *S. albus* G; six isolated and propagated on *S. griseus* ATCC 23345; six isolated and propagated on *S. achromogenes* ATCC 12767; one isolated and propagated on *S. rimosus* NRRL 2234; and six isolated on *S. albus* CMI 52766 itself, and propagated on *S. coelicolor* A3(2).

DNA from two of these phages - VP12 last propagated on an unidentified wild-type Streptomycete (John Innes strain No.1158), and Pal6 last propagated on *S. albus* G - was not cleaved by SalPI in vitro, indicating that the resistance to restriction of at least these two phages was a direct result of DNA structure and was not due, for example, to the specification by the phage of a protein protecting its DNA against endonucleolytic attack as with coliphages T3 and T7¹³. Since both VP12 DNA and Pal6 DNA are cleaved by a variety of other restriction endonucleases (ref.1. and unpublished results), resistance to SalPI cleavage cannot be due to generalised DNA modification such as glucosylation or methylation.

A single cleavage site was detected in DNA of Ø448 propagated on *S. lividans* 66: this was one of the two phages that failed to form plaques on *S. albus* CMI 52766. Four sites were also detected in *S. coelicolor* A3(2) SCP2 plasmid DNA^{14,15}. Thus resistance to restriction by *S. albus* CMI 52766 of at least those eleven of the phages tested that had been propagated on *S. lividans* 66 or *S. coelicolor* A3(2) was not due to the presence in the

previous hosts of enzymes modifying the SalPI recognition site.

DISCUSSION

It has been demonstrated that PstI and SalPI share site specificity. The single PstI recognition site in ϕ X174 DNA has recently been determined¹⁶ as: 5'-C T G C A↓G-3'. The occurrence of shared specificity should not necessarily be taken to indicate any particular relatedness between the two enzymes, particularly in the absence of any knowledge of their physical properties and precise modes of cleavage.

The presence of at least 18 SalPI sites in lambda DNA (molecular weight 30.8×10^6), and of four sites in S. coelicolor A3(2) SCP2 plasmid DNA (molecular weight $18-20 \times 10^6$),^{14,15} suggested that DNA from most phages should possess sites. However, none of 32 phages that formed plaques on S. albus CMI 52766 was restricted by it. (Of five of these phages examined electron microscopically all were of lambdoid or larger dimensions.) This result emphasises the limitations of using phage tests to look for the presence of restriction enzymes.

Providencia stuartii is potentially pathogenic, usually being found associated with human urinary tract and enteric infections. On the other hand Streptomyces albus CMI 52766 is (as far as is known) non-pathogenic and is very easy to handle in the laboratory. One litre of S. albus CMI 52766 culture yields at least several thousand units of SalPI (1 unit completely digests 1 μ g lambda DNA in 1 h at 37°C). SalPI may therefore provide a useful alternative to PstI in some circumstances.

ACKNOWLEDGEMENTS

I am grateful to Lynn Wilde for technical assistance; to Mervyn Bibb for providing S. coelicolor plasmid DNA; to John Arrand for helping with the PstI experiment and to Georgina Moore for typing the manuscript. Some of the phage isolation and testing for in vivo restriction was done by Jonathan Ward and Tony Parkin.

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