Restriction of a Bacteriophage in *Streptomyces albus* P (CMI 52766) by Endonuclease *Sal*PI

By K. F. CHATER AND A. T. CARTER*

John Innes Institute, Colney Lane, Norwich NR4 7UH

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INTRODUCTION

Restriction has been hard to show in streptomycetes. For example, in *Streptomyces coelicolor* A3(2) (a strain sensitive to many actinophages), restriction could be demonstrated only in a hybrid strain (*S. coelicolor* A3(2) × *S. griseus* Kr.15) which possessed phage receptors from *S. griseus* Kr.15 and a restriction system from *S. coelicolor* A3(2) (Lomovskaya *et al.*, 1977).

Unlike *S. coelicolor* A3(2), *S. albus* G is resistant to many wide host-range phages. However, a few of these phages – through mutation – can acquire the ability to form plaques efficiently on it. After pre-growth on *S. albus* P (see below), such a mutant of one of these phages (Pa16) was restricted by *S. albus* G, by the action of the class II restriction enzyme *Sal*GI (Chater & Wilde, 1976). Mutants lacking *Sal*GI are sensitive to most of the phages to which their restriction-proficient progenitor is resistant (K. F. Chater, unpublished), showing that restriction is very important in determining phage sensitivity in *S. albus* G.

The *S. albus* P strain used in the *S. albus* G work is a suggested new designation for *S. albus* strain CMI 52766 (first described by Puntoni in 1931). All the wide host-range phages tested by Chater (1977) gave high efficiencies of plating on *S. albus* P, despite the presence in its cells of a class II site-specific endodeoxyribonuclease. This enzyme (*Sal*PI) had the same site-specificity as *Pst*I, an endonuclease discovered in *Providencia stuartii* by Smith *et al.* (1976). The nucleotide sequence recognized by *Pst*I is 5'-CTGCAG-3' (Sanger *et al.*, 1977).

This paper reports the restriction and modification by *S. albus* P of a wide host-range temperate *Streptomyces* phage, R4, and it is shown that the agent of restriction is the endonuclease *Sal*PI.

METHODS

The temperate phage R4 was isolated from soil and (unless stated otherwise) propagated on strain J1074, a mutant of *S. albus* G deficient in restriction and modification and described by Chater & Wilde (1976) (K. F. Chater & A. T. Carter, unpublished). General methods for R4 were the same as for Pa16 (Chater & Wilde, 1976) except that Difco nutrient agar was supplemented with 25 mM-Ca(NO₃)₂, 10 mM-MgSO₄ and 1 % (w/v) glucose, and R4 DNA was extracted by the perchlorate-ethanol procedure of Wilcockson & Hull (1974). All other phages, strains and methods were as in Chater (1977), except that *S. albus* CMI 52766 is here called *S. albus* P (see Introduction).

Efficiency of plating (e.o.p.) was determined throughout in relation to the number of plaques formed on strain J1074.

RESULTS

Restriction of an R4 mutant by S. albus P

Although phage R4 formed plaques efficiently on most streptomycetes tested, distinct plaques occurred at a very low e.o.p. (about 10⁻⁵) on *S. albus* P. (Sometimes we detected

* Present address: Brunel University, Uxbridge, Middlesex.

Table 1. *Efficiencies of plating of R4 and R4P phages on S. albus P*

Phage	Last host	E.o.p. on <i>S. albus P</i>	Kind of phage obtained from plaques on <i>S. albus P</i>
R4	J1074	10^{-5}	R4P mutants (10/10 tested)
R4P	J1074	1	R4P
R4P	<i>S. albus P</i>	1	R4P

Ten independent R4P mutants have been tested and all behaved as in lines 2 and 3.

minute plaques at an e.o.p. of up to 10^{-2} .) Since the low e.o.p. might have resulted from restriction, phages from 10 independent single plaques of R4 on *S. albus P* were tested further. All proved to be R4 mutants (termed R4P) that had a high e.o.p. (about 1) on *S. albus P* even after propagation on a restriction- and modification-deficient *S. albus G* strain (J1074), suggesting that the initial low e.o.p. of R4 on *S. albus P* was not due to restriction (Table 1).

This conclusion was complicated by observations made with a second class of R4 mutants (termed R4G) isolated by their ability to form plaques at a high e.o.p. on the wild-type (restriction- and modification-proficient) *S. albus G* (K. F. Chater & A. T. Carter, unpublished). The e.o.p. of R4G mutants was relatively high (5×10^{-4} to 3×10^{-2}) when they were first tested on *S. albus P*. Further tests (Table 2) showed that the e.o.p. of R4G mutants on *S. albus P* was dependent on their last host. It was about 1 after propagation on *S. albus P* but 10^{-2} to 10^{-1} after propagation on *S. albus G* strain J1074. Thus R4G, unlike R4P, mutants were subject to restriction and modification by *S. albus P*. For certain mutants (R4G1 and R4G4) the initial e.o.p. on *S. albus P* was lower than that obtained after passaging the phages through *S. albus P* and *S. albus G* strain J1074, indicating that further mutations had been selected which enhanced the ability of the unmodified phages to grow on *S. albus P*. For other mutants (R4G2, R4G3, R4G5) the e.o.p. obtained initially and after passage through *S. albus P* did not change significantly.

Effects of a SalPI-containing extract of S. albus P on DNA from various R4 phage preparations

Fractions of a cell-free extract of *S. albus P* were obtained after chromatography on BioGel A-0.5 M and incubated with λ DNA as previously described (Chater, 1977). After agarose gel electrophoresis of the reaction mixture, fractions were identified that contained the class II site-specific endodeoxyribonuclease *SalPI* (Chater, 1977), which cleaves λ DNA in about 20 places. In agreement with previous results, no additional site-specific cleavage of λ DNA was detected which would have indicated the presence of other enzymes of this kind.

Various unmodified R4 DNA preparations were incubated with a fraction containing *SalPI* activity and examined by agarose gel electrophoresis (Fig. 1). Unmodified DNA from R4 and R4G2 was cleaved, but R4P1 DNA was not (even though λ DNA present in the same digestion mixture was completely digested). Cleavage generated a fragment of about 8.5×10^6 molecular weight and a larger one of undetermined size (Fig. 1). There were no lower molecular weight bands, even on heavily loaded gels where fragments down to 1.5×10^5 molecular weight were detected in adjacent tracks with similar loadings of λ DNA digested with *SalPI*. DNA from R4G2 modified by growth on *S. albus P* was relatively resistant to cleavage, though the preparation contained a fraction (about 10 to 20 %) that could be cleaved. This is shown by the detection of the 8.5×10^6 molecular weight fragment at only 10 to 20 % of the relative amount found in *SalPI* digests of the unmodified DNA (Fig. 1). The resistance to cleavage was not due to inhibition of *SalPI* because λ DNA present in the same digestion mixture was completely digested.

Fig. 1. Agarose gel electrophoresis of *Sal*PI-digested DNA from various R4 phage preparations. All DNA was unmodified for *Sal*PI sites unless marked 'mod'. All tracks were from the same slab gel except the right-hand two. Electrophoresis was from top to bottom (photographs) or left to right (microdensitometer tracings). A, B, C and D indicate the positions of the four largest fragments of λ DNA digested with *Sal*PI (Chater, 1977). Microdensitometer tracings are of the tracks marked with asterisks. Note that the faster running R4 band (arrows) is present in only very small amounts with R4G2mod DNA and is absent with R4P1 DNA.

Table 2. Restriction and modification of R4G mutants by *S. albus* P

Mutant	Initial e.o.p. on <i>S. albus</i> P	(a)	(b)	Restriction coefficient (Ratio <i>b/a</i>)
		E.o.p after passage through <i>S. albus</i> P	E.o.p. after passage through <i>S. albus</i> P then through J1074	
R4G1	1×10^{-8}	9×10^{-1}	1.5×10^{-1}	0.17
R4G2	3×10^{-2}	8×10^{-1}	4×10^{-2}	0.05
R4G3	3×10^{-2}	1.3×10^0	4×10^{-2}	0.03
R4G4	5×10^{-4}	9×10^{-1}	3×10^{-2}	0.03
R4G5	2×10^{-2}	1×10^0	6×10^{-2}	0.06

DISCUSSION

R4G mutants underwent restriction and modification in *S. albus* P. Two observations showed that restriction was mediated by the class II site-specific endodeoxyribonuclease *Sal*PI: fractions of a cell-free extract containing *Sal*PI activity cleaved R4G DNA efficiently only when it was unmodified, and the DNA of an R4 mutant (R4P) insusceptible to restriction and modification in *S. albus* P was not cleaved by *Sal*PI.

The availability of a phage sensitive to restriction by *S. albus* P opens up the possibility of isolating restriction-deficient mutants and then of further biological and genetical studies of class II restriction enzymes in streptomycetes.

Certain peripheral observations require discussion. Our first impression, gained from the restriction-resistance of R4P lysates, had been that the low e.o.p. of wild-type R4 on *S. albus* P was a consequence of a host-range effect entirely unrelated to restriction. However, the finding that R4 DNA was cleaved *in vitro* by *Sal*PI necessitates reconsideration of this conclusion. A plausible unifying explanation of all our observations can be given by making four assumptions. (1) Cleavage by *Sal*PI of unmodified DNA at its single *Sal*PI site is the major cause of any reduction in e.o.p. on *S. albus* P of R4 or its derivatives pre-grown on other streptomycetes. (2) Modification of the *Sal*PI target site in R4 DNA is extremely inefficient during the normal phage life cycle (perhaps due to rapid packaging of nascent phage DNA). (3) R4G mutants have acquired a recessive mutation which results in much more efficient (80 to 90 %) modification of the *Sal*PI target site (newly synthesized phage DNA might be exposed to modification enzyme for somewhat longer prior to DNA packaging). (4) R4P mutants have a mutation altering or deleting the *Sal*PI target site present in R4 and R4G DNA.

The following course of events follows from these assumptions. Unmodified R4 phage DNA entering *S. albus* P has a low probability of a successful primary infection because of cleavage of its DNA by *Sal*PI. Owing to the inefficiency of modification, nearly all the progeny of the rare successful primary infections are unable to initiate further infections because they are restricted. This severely constrains plaque formation and, in the absence of mutations affecting the interaction of the phage and the host's restriction-modification system, at best only very small plaques are formed. (These correspond to the minute plaques we observed in such circumstances; see Results.) However, two groups of mutants of this kind have been described, i.e. R4P and R4G. An R4P mutant occurring during minute plaque formation can overgrow the plaque, and a normal large plaque then results. [Such specific mutations should be rare; but if we take, for example, a frequency of occurrence of 10^{-6} and an average number of 1000 phage particles per minute plaque, then 1 in every 1000 tiny plaques will be overgrown by an R4P mutant. Thus the observed frequency of R4P plaques in these circumstances (10^{-5} ; Table 1) is not incompatible with a low primary mutational frequency.] R4G mutants are rarely found among the large plaques when R4 is plated on *S. albus* P, because, on the hypothesis adopted, R4G mutations are recessive and therefore

an R4G particle released from the cell in which the primary mutational event occurred is usually unmodified and is thus usually eliminated by restriction at its next attempted infection.

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