SPECIFIC INACTIVATION OF INFECTIOUS λ DNA BY SONICATES OF RESTRICTIVE BACTERIA WITH R FACTORS

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In the studies on the host-controlled modification (Arber, 1965a) of bacteriophage λ, certain aspects of two fundamental functions of host bacteria have been elucidated: Modification is a function by which viral DNA is subjected to some chemical alterations (Arber, 1965b). Restriction is another function which recognizes the modification pattern or the host specificity and serves to restrict the phage multiplication depending on the host specificity (Arber and Dussoix, 1962). These functions are directed not only by bacterial genomes (Boyer, 1964; Wood, 1966) but also by episomal elements; such as phage P1 (Lederberg, 1957; Glover et al., 1963; Wood, 1966) or a class of R factors (Watanabe et al., 1964), namely $\underline{fi}^-$ R ($\underline{fi}$ represents "fertility inhibition"). The biochemical nature of the restriction is still obscure. A remarkable finding in the study on the restriction is that the DNA injected into the restrictive cells undergoes an extensive degradation to small fragments becoming soluble in acid (Dussoix and Arber, 1962; Watanabe et al., 1966). It is reasonably assumed that some enzymes, whose synthesis are directed by the genomes of

restrictive bacteria, fi⁻ R or P1, are involved in the restriction: The hypothetical enzymes, referred to as "restriction enzymes" in this paper, recognize the host specificity of entering DNA and restrict its replication depending on the host specificity, either by destructing it or by some other means which eventually result in degradation of the restricted DNA. We have tried to search for such enzyme(s) in cell-free preparations from restrictive bacteria using purified λ DNA as a substrate on the basis of the following facts: 1) Biological activity of phenol-extracted λ DNA can be assayed on helper-infected cells of Escherichia coli (Kaiser, 1962). 2) The host specificity survives the phenol treatment in some cases that have been studied (Dussoix and Arber, 1965). However, normal cells of E. coli B and K-12 contain various DNA-specific nucleases (Shortman and Lehman, 1964) which would presumably disturb the detection of the specific restriction enzyme(s) by non-specifically destroying the infectivity of the isolated λ DNA. Among them, endonuclease I (endo I) (Lehman, Roussos and Pratt, 1962) is known to be the major component (Shortman and Lehman, 1964). Fortunately, we have been able to employ an endo I-deficient mutant of E. coli K-12 which has recently been isolated by Hoffmann-Berling. By the use of this mutant, we have obtained the following findings, which will be reported in this paper: 1) Phenol-extracted DNA from λ grown on K-12 with fi⁻ R retains the host specificity, in agreement with Dussoix and Arber (1965). 2) Restriction and degradation of λ DNA occurs in this endo I-less mutant carrying fi⁻ R to an extent equal to that in the wild type with the same R factor. 3) Infectious DNA from unmodified λ is specifically inactivated by cell-free extracts of the endo I-less cells carrying fi⁻ R. We suggest that the observed specific inactivation is due to the proposed restriction enzyme(s) whose synthesis may

be controlled by the genome of the restrictive R factors. Analogous experiments are now under way in the system of P1-controlled restriction in our laboratories.

EXPERIMENTS AND DISCUSSION

1) Host specificity of phenol-extracted DNA from phage λ grown on various hosts with or without R factors

The infectious DNA from intact phage λ was prepared by the ultraviolet induction of E. coli CSH-2, a substrain of K-12, with or without R factors, according to the method described by Kaiser (1962). λ DNA as 0.87 mμmoles of phosphorus in 0.1 ml of Tris buffer (pH 7.4, $MgCl_2$ 10 mM) per tube was incubated with 0.2 ml of helper λ_{i434}-infected competent bacteria, E. coli C600 (λ_{i434}), at 37° C. At 30 minutes after the infection with DNA, the reaction

Table 1. Infectivity of λ DNA from the phage grown on E. coli CSH-2, a substrain of K-12, with or without a fi⁻ R, N-3

λ	Helper *	Plaque-forming units per tube on	
		C600(λ_{i434})	C600(λ_{i434})(N-3)
DNA from λ	None	2.1×10^3	1.0×10^1
	λ_{i434} modified by N-3	3.4×10^5	1.7×10^3
DNA from λ modified by N-3	None	2.4×10^3	5.5×10^2
	λ_{i434} modified by N-3	2.7×10^5	2.8×10^5
Intact phage λ	None	8.9×10^9	3.0×10^7
Intact phage λ modified by N-3	None	7.8×10^9	8.8×10^9

* Multiplicity of input: 10.0

was interrupted by the addition of pancreatic DNase (3 μg/tube) with additional incubation for 5 minutes at 37° C. As the results are shown in Table 1, phenol-extracted DNA from λ grown on CSH-2 with or without a fi⁻ R, N-3, shows the restrictive host specificity.

2) Restriction of phage λ in an endo I-less mutant of K-12 carrying a fi⁻ R factor

An endo I-less mutant of E. coli K-12, strain 1100, and its parental strain 1000 were supplied to us by Dr. H. Hoffmann-Berling. This mutant strain contains about 1 % of the endo I activity of the wild type (Hoffmann-Berling, personal communication). At first it was thought necessary to see if endo I of the host bacteria is invovled under any circumstances in the restriction controlled by the restrictive R factors, since an RNA-inhibitable endonuclease of E. coli B was once suggested to play a role in the restriction of T*4 (Fukasawa, 1964). The efficiency of plating (e. o. p.) of phage λ grown on C600 was assayed on the endo I-less strain carrying a fi⁻ R, N-3, under the conditions described by Arber and Dussoix (1962). As seen in Table 2, there was no difference in the e. o. p. of λ on the endo I-less strain carrying N-3 and the wild type strain carrying the same R factor. We conclude that R-controlled restriction system is independent of endo I of the host bacteria.

We have also followed the fate of the phage DNA injected into the endo I-less and the wild type cells carrying a restrictive R factor, N-3, with the procedure described by Dussoix and Arber (1962). Forty to 50 % of the radioactivity of ^{32}P-labeled λ, which was adsorbed by the endo I-less and wild type bacteria carrying N-3, was found in the acid-soluble fraction within 15 minutes after the infection. This observation indicates that endo I is not involved in the degradation of λ DNA

Table 2. Restriction of phage λ grown on E. coli C600 in an endo I-less and wild type strains of E. coli K-12 carrying a fi⁻ R, N-3.

Indicator strain	R factor		e. o. p. of phage λ
	Strain	fi	
1100, endo I-less	R^-		1.00
	222	+	0.94
	N-3	-	1.6×10^{-3}
1000, wild type	R^-		1.00
	222	+	0.95
	N-3	-	2.3×10^{-3}

in the strain K-12 with a restrictive R factor.

3) Inactivation of infectious DNA from phage λ by sonicates of endo I-less and wild type strains of K-12 carrying a fi⁻ R factor

Then, we investigated whether or not the infectious DNA from the unmodified λ is specifically inactivated by the sonicates of the restrictive endo I-less bacteria. The sonicates of the endo I-less mutant cells carrying various R factors were prepared as follows: Cells were grown in a salt-glucose medium enriched with 0.6 % yeast extract (Shortman and Lehman, 1964) to a late logarithmic phase. The collected and washed cells were disrupted in a sonic disintegrator (Ohtake Co.) at 10 kc for 10 minutes. The supernatant samples after centrifugation (at 100,000 x g, for 120 minutes) of the sonicates were filtered twice through Millipore filters (type HA). The reaction consisted of the following two steps: Two tenths ml of the reaction mixture of the first step, containing λ DNA (0.87 mμmoles as phosphorus and 1.6×10^5 plaque-forming activity), sonicates, Tris-HCl buffer (10mM, pH 7.5), and $MgCl_2$ (10 mM) was incubated at 37° C for 10 minutes. The second step reaction was started by adding to the reaction

mixture of the first step 0.2 ml of the helper-infected bacteria to measure the residual activity of the λ DNA. As the results are shown in Figure 1, the infectious DNA of the unmodified λ was inactivated to a greater extent by the sonicates from the restrictive endo I-less bacteria carrying a $\underline{fi}^-$ R, N-3, than by the sonicates from those with a non-restrictive R, 222. The infectious DNA from λ modified by N-3 had a low sensitivity to the inactivation by the restrictive sonicates, but was inactivated to a slightly larger extent by the restrictive sonicates than by the non-restrictive sonicates (Figure 2).

The reason why the infectious DNA modified by N-3 was still partially sensitive to the inactivation by the homologous sonicates

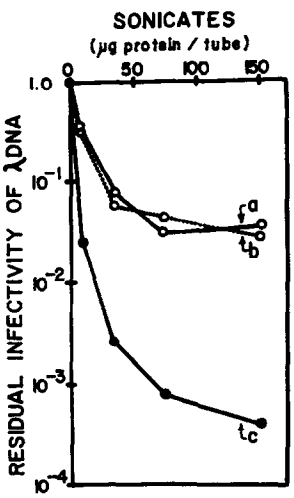

Figure 1. Inactivation of infectious λ DNA by sonicates of an endo I-less mutant of E. coli carrying R.

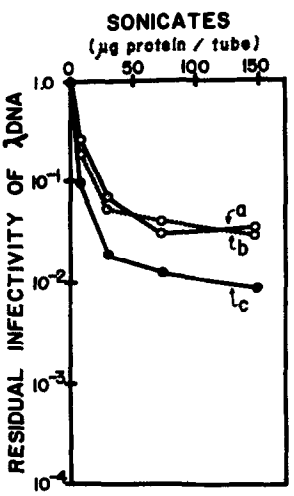

Figure 2. Inactivation of infectious λ DNA modified by N-3 with sonicates of an endo I-less mutant of E. coli carrying R.

Experimental procedures for Figures 1 and 2 are described in the text. The infectious DNA was incubated with the sonicates from (a) 1100 (R^-), (b) 1100 (222), and (c) 1100 (N-3).

is unknown. But the major portion of the observed inactivation with the cell-free preparations is considered specific with regard to the restriction. The non-specific portion of the inactivation might be due to some R-controlled nuclease(s).

Essentially similar results were obtained with another fi⁻ R, R-15, to the above results with N-3. We are now concentrating our efforts on the characterization and purification of the hypothetical enzyme(s).

§ Recent experiments with the endo I-less mutant have indicated that endo I is not involved in the restriction of T* phages: T*2, T*4 and T*6 are restricted in strain 1100 as well as in the wild type bacteria. It has also been shown that T* DNA is broken down extensively to acid-soluble fragments upon infection of the endo I-less cells like in the wild type bacteria.

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