ISOLATION AND PRELIMINARY CHARACTERIZATION OF T4 MUTANTS WITH NONGLUCOSYLATED DNA[1]

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Received June 12, 1967

Infection with T-even phages causes the production of enzymes that transfer glucosyl residues from UDPG to the hydroxymethyl cytosine (HMC) groups of the phage DNA (Kornberg et al., 1961). Phages T2 or T6, whose DNA glucosylation depends on an α-glucosyl transferase, yield gt mutants defective in induction of this enzymatic activity (Revel et al., 1965). These mutants, as well as the nonglucosylated T* forms of T-even phages (Hattman and Fukasawa, 1963), grow on S. dysenteriae Sh (permissive host) but not on E. coli B or K-12 (restricting hosts). Attempts to isolate T4gt mutants by the procedure of Revel et al. (1965) were unsuccessful, presumably because T4 elicits two glucosyl transferases, α and β, which can glucosylate 70 and 100% of the HMC groups in T4 DNA respectively (Kornberg et al., 1961; Lehman and Pratt, 1960). Each of these two enzymes alone may provide adequate glucosylation to permit phage growth in hosts that restrict T2gt and T6gt.

Hosoda (1967) accidentally discovered in a stock of a T4 amber mutant a second mutation which abolishes α-glucosyl transferase activity; by appropriate crosses Hosoda prepared a phage stock, T4 αgt 1, that carries this mutation and not the amber mutation. As predicted, T4 αgt 1 grows quite well on E. coli B and K-12.

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1 Supported by grants to Dr. S. E. Luria from the National Institutes of Health (AI-03038) and the National Science Foundation (GB-5304X).

2 Trainee under Microbiology Training Grant 5 T1 GM 602 to the Department of Biology, Massachusetts Institute of Technology.

Starting from T4 *agt* 1, using mutagenesis with NH_2OH according to Hall and Tessman (1966) and the selection method of Revel *et al.* (1965) I have isolated many mutants that grow on Sh bacteria but not on B or K-12. Some examples are shown in Table 1. These mutants are defective in both glucosyl transferase functions and are called T4 *agt bgt*. The defect in production of β-glucosyl transferase activity (at 37°) is not always complete; intermediate levels of activity are encountered.

Table 1

Properties of Phage Mutants

Phage strain	E.o.p. on *E. coli* B	α-glucosyl* transferase	β-glucosyl* transferase
1. T4 wild type	1.0	(100)	(100)
2. T4 *agt* 1	0.5	< 2	> 90
3. T4 *agt* 1 *bgt* (12 isolates)	$< 10^{-4}$	< 2	2
4. T4 *agt* 1 *bgt* (4 isolates)	$< 10^{-4}$	< 2	5-20
5. T4 *agt*$^+$ *bgt* (revertants from class 3)	1.0	50-100	< 2
6. T4 *agt* 1 *bgt*$^+$ (revertants from class 3)	0.15-0.5	< 2	80-100

* Values are in percent of the α or β glucosyl transferase activity induced by T4 wild type phage. *S. dysenteriae* Sh cells at 2 x 10^8/ml in tryptone-yeast extract broth were infected with about 6 phages per bacterium and incubated at 37°. At 20 minutes 2 ml aliquots were added to 2 mg bovine serum albumin in cold tubes and extracts were prepared by sonication for 1 minute in an MSE ultrasonic disintegrator. The assays were done according to the procedures of Josse and Kornberg (1962). The non-glucosylated DNA substrate was T2*gt* DNA. C^{14}-labeled UDPG was kindly donated by Dr. H. Revel.

All the stocks tested revert readily to ability to grow on B. Revertants were isolated and classified by their efficiency of plating on B and Sh and their ability to induce α- or β-glucosyl transferases. Some typical findings are included in Tables 1 and 2. Most revertants have regained

Table 2

Properties of Phage Strains and of their DNA

Phage strains	E.o.p. on *E. coli* B	α-glucosyl* transferase activity	β-glucosyl* transferase activity	Glucose on DNA†	Glucose added to DNA by‡	
					α-glucosyl transferase	β-glucosyl transferase
T4 wild type	1.0	(100)	(100)	(100)	< 1	< 1
HA57 = T4 *αgt*57 *βgt*14	$< 10^{-4}$	< 2	< 2	< 2	77	(100)
R4 = revertant from *αgt* 1 *βgt*4	1.0	100	< 2	80	1	16
R4a = revertant from *αgt* 1 *βgt*4	0.4	< 2	< 2	50	24	48
R20 = revertant from *αgt* 1 *βgt*20	1.0	75	< 2	44	22	30
R20a = revertant from *αgt* 1 *βgt*20	0.15	< 2	80	103	1	1
T2 wild type	1.0	(100)	< 2	-	6	31

* Assays as described in Table 1.

† Glucose was determined by the glucose oxidase assay of Huggett and Nixon (1957) after hydrolysis of the phage DNA in 2 N HCl at 100°C for 2 hours. The glucose oxidase was from Boehringer and Mannheim; the peroxidase was from Sigma. The values given are the percent of the amount of glucose in equal amounts of T4 wild type DNA.

‡ Phage DNA, isolated as described in the text, was incubated with UDPG-C^{14} and crude extracts of *E. coli* B infected with wild type T4. The values given are in percent of the amount of glucose accepted by equivalent amounts of DNA from phage strain HA57.

either the α- or the β-glucosyl transferase activity, at full or partial levels compared with T4 wild type; no case of reversion in both activities was found. All revertants with α-glucosyl transferase activity (*αgt*$^+$ *βgt*) have an e.o.p. of about 1.0 on B vs. Sh; all those with only β-glucosyl transferase activity (*αgt* *βgt*$^+$) have e.o.p.s ranging from 0.15 to 0.5 on B and form smaller plaques on this bacterial host; this is true also of the original T4 *αgt* 1 *βgt*$^+$ mutant stock. Hence, it appears that β-glucosylation of all the HMC (see Table 2) does not by itself confer full ability to overcome the restriction in *E. coli* B.

From an *αgt*$^+$ revertant I obtained by NH_2OH mutagenesis another mutant, T4 *αgt*57 *βgt*14, which lacks both α- and β-glucosyl transferase activities (see Table 2).

High titer lysates of a selected group of mutants and revertants were prepared on *S. dysenteriae* Sh. (Care must be exerted in preparing *gt* mutant lysates because the revertants tend to overcome the mutants; inoculation from single plaques of T4 *αgt* *βgt* yields lysates with e.o.p. on B less than 10^{-3}). The phage was purified by 2 cycles of high and low speed centrifugation and the DNA, extracted by phenol-ether-dialysis procedure, was analyzed for glucose content and was used as substrate for incorporation of glucose from C^{14}-labeled UDPG. The results are presented in Table 2, which also shows the levels of enzyme activities induced by these phage preparations in *S. dysenteriae* Sh. For several of the mutants the results of the various tests are quite concordant. Thus, HA57, which is restricted by *E. coli* B and lacks both α- and β-glucosyl transferase activities, has DNA with no glucose and is capable of accepting *in vitro* the expected amounts of glucose from wild type transferases. R20a and R4, lacking respectively α or β transferase activity, also behave in the expected manner. The DNA of R20a is fully glucosylated; the DNA of R4 has 80% of the normal complement of glucose and accepts some glucose from the β enzyme. In mutant R20 the α-glucosyl transferase is induced to almost normal levels, but *in vivo* it glu-

cosylates the DNA less effectively than does the wild type enzyme.

Mutant R4a exhibits peculiar properties: its extracts show no glucosyl transferase activities, even when assayed at 24° instead of 37°. Yet, phage R4a has partly glucosylated DNA and plates on E. coli B with an efficiency comparable to that of mutants with active β-glucosyl transferases (see Table 2). Presumably R4a induces a glucose transferase, either α or β, which is fairly active in vivo but inactive in the in vitro assay, due either to instability or to altered requirements.

All T4gt mutants and their revertants which have been tested induce levels of dCMP hydroxymethylase comparable to those induced by T4 wild type.

Preliminary recombination tests in which S. dysenteriae Sh was infected with pairs of mutants (αgt^+ βgt x αgt βgt^+) gave αgt βgt recombinants at a frequency of 7 to 11%. Similar genetic crosses performed in E. coli Kr6 r2,4, a strain permissive for nonglucosylated T-even phages (Revel, 1967), gave αgt βgt recombinants at a frequency of 8 to 11%. Since, however, the mutant stocks used had been mutagenized and carried no known markers by means of which the normality of recombination frequencies could be tested, no firm conclusions are justified.

Further work is in progress on the properties of the mutants, their DNA and glucosyl transferases, as well as on the relation between the amounts and distribution of the glucosyl groups and the ability to withstand restriction by nonpermissive host bacteria.

ACKNOWLEDGMENT

The author would like to express his appreciation to Drs. S. E. Luria and H. R. Revel for helpful criticism, discussions and assistance in preparing the manuscript.

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