Mutants of Bacteriophages T2 and T6 Defective in α-Glucosyl Transferase

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Received January 6, 1965

The DNA of T-even bacteriophages contains glucose associated with the hydroxymethylcytosine residue. In T2 DNA 70% of the hydroxymethylcytosine residues are associated with glucose as an α-glucosyl residue, 5% are linked to a β-glucosyl-α-glucosyl residue, and 25% are non-glucosylated. In T6 DNA 72% of the hydroxymethylcytosine residues are associated with glucose as a β-glucosyl-α-glucosyl residue, 3% are linked to an α-glucosyl residue, and 25% are non-glucosylated (Lehman and Pratt, 1960). Single cycle growth of the T-even bacteriophages in bacterial strains defective in their ability to synthesize uridine-diphosphoglucose (UDPG) yields host-modified progeny phages, designated T*, which have little or no glucose on their DNA (Hattman and Fukasawa, 1963; Shedlovsky and Brenner, 1963; Symonds et al., 1963; Fukasawa and Saito, 1964; Davison and Freifelder, 1964; Erikson and Szybalski, 1964). The T* phages can grow on S. dysenteriae strain Sh, the permissive host, but not on various E. coli strains, including E. coli B (restrictive hosts; Luria and Human, 1952). These observations provided the basis for the selective isolation of phage mutants deficient in their ability to initiate production of α-glucosyl transferase (Kornberg et al., 1961). The DNA of such mutants should resemble that of T* bacteriophages in containing little or no glucose and, consequently, the mutants should grow in the permissive host S. dysenteriae strain Sh but not in E. coli B.

Isolation of mutants

T2 and T6 bacteriophages were treated with a freshly prepared hydroxyl-

amine solution containing 0.1 M sodium phosphate buffer pH 7.5, 0.5 M hydroxylamine-HCl neutralized with 0.45 M NaOH, and 0.45 M NaCl (Freese et al., 1961). After incubation at 37° for 10 hours the reaction was quenched by a 50-fold dilution into tryptone broth containing 2% acetone. Phage survival was 2×10^{-2}. The mutagenized stocks were freed of mutational heterozygotes by a cycle of growth on S. dysenteriae Sh (Tessman, 1959; Vielmetter and Wieder, 1959). Plating with mixed indicators, the key step in the isolation procedure, was then carried out in the following manner. Aliquots of the lysates, containing approximately 500 phage particles, were mixed with 2×10^8 cells of S. dysenteriae Sh str-r in 0.5 ml of broth and kept for 10 min at 37°; then 10^7 cells of E. coli B str-s were added with 2 ml of soft agar and the mixtures were poured onto tryptone-yeast-extract agar plates. After overnight incubation at 37° small turbid plaques were picked into saline and spotted on Sh cells on plates with streptomycin and on E. coli B cells on plates without streptomycin. Phages which grew on S. dysenteriae Sh and not on E. coli B were thereby recognized. The mutant frequency was about 1 mutant phage per 10^4 phages examined. Mutants of independent origin were obtained from a single mutagenized stock by isolating each mutant from the progeny phage derived by independent single-growth cycles in Sh bacteria.

Properties of the mutants

Six mutants of T2 and 5 mutants of T6 have been isolated. They are designated T2gt and T6gt respectively. Reverse mutation frequencies vary from 10^{-4} to 10^{-8}. Two of the 11 mutants revert readily to a genotype that produces tiny plaques on E. coli B. When any two T2gt or T6gt mutants are crossed in Sh cells, gt^+ recombinants are produced.

The ability of the mutant phages to form α-glucosyl transferase and other 'early' enzymes (Flaks and Cohen, 1957; review by Cohen, 1961) was examined; the results are summarized in figure 1 and table 1. Upon

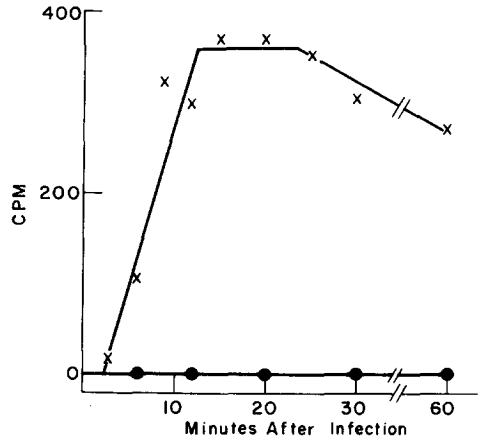

Figure 1. α-Glucosyl transferase activity in S. dysenteriae after infection with T2, x - x; T2gt-1 or T2gt-2, ● - ●.

Experimental conditions as in Table 1. Aliquots for enzymatic assay were taken at 3, 6, 9, 12, 15, 20, 25, 30, and 60 minutes after infection.

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infection of S. dysenteriae Sh the mutants fail to initiate the synthesis of α-glucosyl transferase, whereas deoxycytidylate hydroxymethylase and thymidylate kinase are produced in essentially normal amounts.

In its reactions with the restrictive host E. coli B, T2gt-1 behaves similarly to T*2. Adsorption to E. coli B is normal, but the killing efficiency per particle is only one-half to one-third. Small amounts of deoxycytidylate hydroxymethylase and thymidylate kinase are formed. In mixed infection on E. coli B the mutant T2gt-1 can complement amber mutants of cistron 42 (hydroxymethylase-defective), but not amber mutants of cistron 44 (function unknown) (Wiberg et al., 1962; Epstein et al., 1963). As with T*2 multiplicity activation occurs at high multiplicities of infection (Hattman, 1964).

Experiments with the α-glucosyl transferase of T2 have indicated that DNA isolated from T2gt-1 is as good a substrate for this enzyme as

Table 1

Relative Activity of Various Early Enzymes Induced by T2 and T6 and Their gt Mutants in S. dysenteriae

Phage	α-Glucosyl transferase	Thymidylate kinase	Deoxycytidylate hydroxymethylase
T2	100	100	100
gt-1	< 0.5	210	90
gt-2	< 0.5	220	100
gt-5	< 0.5	260	105
gt-6	< 0.5	300	100
gt-7	< 0.5	210	115
gt-3	< 0.5	240	110
T6	100	100	100
gt-1	< 0.5	160	100
gt-2	< 0.5	140	100
gt-3	< 0.5	120	110
gt-4	< 0.5	100	90
gt-5	< 0.5	120	100

S. dysenteriae cells at 2.5 x 10^8/ml in tryptone-yeast-extract broth were infected with about 5 phages per bacterium and incubated at 37°. In all mixtures there were less than 4% survivors measured at 5 minutes. At 20 minutes 2 ml aliquots were pipetted into cold tubes containing 80 μmoles mercaptoethanol, 10 μmoles EDTA, 20 μmoles NaN_3 and 50 μmoles Tris buffer pH 7.5. The cells were frozen overnight, thawed, and sonicated for 1 minute in an MSE ultrasonic disintegrator at 10 kcycles. The sonic extracts were spun at 10,000 xg for 20 minutes at 4° and the clear supernatant solutions were assayed for enzymatic activity: α-glucosyl transferase was assayed as described by Josse and Kornberg (1962); thymidylate kinase by the procedure of Wiberg et al. (1962), and deoxycytidylate hydroxymethylase according to Wiberg and Buchanan (1964).

The activities of the enzymes induced by T2 or T6 have been set at 100 in each series.

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is T*2 DNA. Glucosylated T2 DNA does not serve as a substrate for this enzyme (Kornberg et al., 1959).

Although the glucose content of the gt DNA has not yet been measured directly, the foregoing circumstantial evidence suggests that the mutant DNA, like that of T* phages, contains little or no glucose.

Experiments on mapping the α-glucosyl transferase gene in T2 and T6 and experiments on the production of 'phenotypically mixed' DNA from these phages are in progress.

ACKNOWLEDGMENTS

This work was supported by grants from the National Science Foundation (GB-535) and the National Institutes of Health (AI-03038). One of the authors, S. Hattman, is a trainee under Microbiology Training Grant 5 T1 GM-602 from the National Institutes of Health. The authors wish to acknowledge the excellent technical assistance of Mrs. Joan Campbell.

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