REVERSAL OF RESTRICTION FOR HOST MODIFIED T2 AND T4 DNA UPON CONVERSION OF NON-PERMISSIVE ESCHERICHIA COLI TO SPHEROPLASTS

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In 1954 Sinsheimer found that glucosylation of the hydroxymethylcytosine residues of T-even phage DNA's confers to oligonucleotides resistance to hydrolysis by pancreatic DNase and snake venom phosphodiesterase. Two years earlier Luria and Human (1952) had noted an apparently unrelated phenomenon, that growth of T2 and T6 on certain strains of E. coli B/4 modified phage progeny so that they no longer could be detected on E. coli B, but appeared as normal phage upon infection of a Shigella species. Infection of Shigella reversed the modification induced by B/4 and allowed phage progeny to infect E. coli B. Thus the modification imposed by B/4 on T2 and T6 is phenotypic and dependent upon the last host infected, and for that reason has been called host-induced modification.

Hattman and Fukasawa (1963), Shedlovsky and Brenner (1963), Symonds, Stacey, Glover, Schell, and Silver (1963) found that the molecular mechanism of host-induced modification in T-even phages is deficient glucosylation of phage DNA due to a mutation in the modifying host which results in loss of the glucose donor uridine diphosphoglucose (UDPG). Since

T-even phages code for transfer of glucose from UDPG to the matured DNA's of their progeny, but not for the synthesis of UDPG, they are dependent upon their bacterial host for a glucosyl donor. Hence species which lack UDPG modify T2, T4, and T6.

It has been suggested (Fukasawa, 1964a) that an enzyme hydrolyzing nonglucosylated DNA is responsible for restriction of modified phage in non-permissive hosts. This communication verifies Fukasawa's suggestion in that conversion of restricting bacterial cells to spheroplasts, which results in quantitative loss of periplasmic nucleases (Neu and Heppel, 1964), also reverses restriction for nonglucosylated T2 and T4 DNA.

MATERIALS AND METHODS

Bacterial stocks were obtained as follows: E. coli strain B from Dr. S. Benzer, E. coli K12 strain W4597 from Dr. T. Fukasawa, and Shigella sonnei from ATCC (#31). Phages T2r+ and T4r+ were obtained from departmental stocks.

Modified phage were produced by infecting log phase UDPG-less bacteria with T2 or T4 at a multiplicity of about one and lysing the infected bacteria with chloroform after 90 minutes at 37C. Nonglucosylated T2 (T*2) was produced on E. coli B/4 or S. sonnei Sh/4; T*4 was produced on W4597.

Spheroplasts were produced by gently rocking 5 ml of washed log phase bacteria at ten-fold concentration with 0.5 mg lysozyme for 1 minute, then with 1 mg EDTA for 30 seconds. They were used after 10 minutes standing at room temperature. In order to infect spheroplasts, T-even phages were treated with 8M urea to form "Protoplast-infecting" (π) particles (Fraser, Mahler, Shug, and Thomas, 1957).

RESULTS

Efficiencies of plating of glucosylated and non-glucosylated phages on E. coli B (B) and S. sonnei (Sh) were compared with the recovery of urea-treated preparations of these phages on spheroplasts of B and Sh.

TABLE I. Recovery of phage on whole cells compared with ϒ on spheroplasts.

	pfu/ml intact phage on cells		pfu/ml ϒ on whole cells		pfu/ml ϒ on spheroplasts after 90 mins.	
	B	Sh	B	Sh	B	Sh
T4	1.8×10^{10}	1.8×10^{10}	1.5×10^{5}	1.2×10^{5}	3.6×10^{7}	5.9×10^{6}
T*4	2.3×10^{4}	1.8×10^{7}	<10	10	3.3×10^{5}	1.9×10^{4}
T2	3.2×10^{11}	3.2×10^{11}	1.8×10^{4}	4.0×10^{4}	1.2×10^{8}	1.2×10^{7}
T*2[a]	4.6×10^{4}	2.7×10^{7}	<10	<10	4.2×10^{3}	8.6×10^{2}
T*2[b]	2.1×10^{4}	8.0×10^{8}	30	1.4×10^{4}	1.3×10^{5}	2.7×10^{6}

pfu/ml = plaque-forming unit per ml
[a]T2·B/4, i.e. T2 progeny of E. coli B/4 infection
[b]T2·Sh/4

Modified T*2 and T*4 infected E. coli B with about 10^{-3} efficiency of S. sonnei, whereas recoveries on B spheroplasts often surpassed those on Sh spheroplasts. This lower recovery on spheroplasts of Sh is due to its poor "spheroplast-forming" ability. If, however, ratios of glucosylated and nonglucosylated ϒ infection of B to Sh spheroplasts are compared (Table II) it may be seen that recovery on spheroplasts of the restricting species is roughly the same regardless of glucosylation.

TABLE II. Recoverability of ᾔ on spheroplasts compared with efficiencies of plating intact phage on whole cells.

	pfu/ml intact phage on whole cells B/Sh	pfu/ml after 90 mins. incubation of ᾔ with spheroplasts B/Sh
T4	1.0	6.0
T*4	1.3×10^{-3}	17.4
T2	1.0	10.0
T*2(T2·B/4)	1.9×10^{-3}	4.9
T*2(T2·Sh/4)	2.6×10^{-5}	0.047

Data derived from Table I.

T*2 produced from infection of Sh/4 were modified to a greater degree than from B/4 because reinfection of Shigella is not restricted permitting several cycles of multiplication. If T*2 or T*4 are grown for at least one cycle of infection on Sh, phage progeny plate with equal efficiencies on Sh and B. When modified phage, normally restricted on E. coli B, are converted to ᾔ, they infect E. coli B spheroplasts without restriction (or at least 10^3-fold less restriction in the case of T2·Sh/4).

DISCUSSION

We interpret the above results to mean that non-glucosylated T-even phages are no longer restricted from infecting spheroplasts of normally restrictive hosts. Fukasawa (1964b) demonstrated that restriction of non-glucosylated DNA occurs only at the time of infection and has shown (1964a) a quantitative difference in the amount of

nucleases present in restricting and non-restricting cells. Neu and Heppel (1964) have shown that conversion of cells to spheroplasts results in release of those nucleases found between the cell wall and membrane in E. coli. Thus it seems likely that these nucleases, located in the periplasm, are responsible for the degradation of nonglucosylated T2 and T4 DNA, but are inhibited, perhaps sterically, from hydrolyzing glucosylated DNA.

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