SELECTIVE REPLICATION OF DIPLOPHAGE Dp-4 DEOXYRIBONUCLEIC ACID IN 6-(*p*-HYDROXYPHENYLAZO)-URACIL TREATED *STREPTOCOCCUS PNEUMONIAE*

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1. Introduction

Pneumococcal phages have been isolated in [1–3]. Transfection in this system has been described in [4,5]. We have reported the availability of Dp-4 DNA labeled at a high specific activity [6] and also some of the physicochemical properties of this DNA [7] which allows studies on the fate of *Streptococcus pneumoniae* bacteriophage DNA both by infection and transfection. For these purposes, host DNA replication may be selectively inhibited without affecting phage DNA synthesis. This selective inhibition has not been achieved yet in streptococci.

Semiconservative replication of *Bacillus subtilis* DNA is inhibited by 6-(*p*-hydroxyphenylazo)-uracil (HPUra) [8] which specifically affects DNA polymerase III [9] whereas the replication of some phages in HPUra-treated *B. subtilis* is not affected [10–12]. In contrast, HPUra inhibits the replication of *Streptococcus sanguis* as well as of it temperate bacteriophage ϕ227 [13].

Here, we show that HPUra selectively inhibits *S. pneumoniae* DNA synthesis without affecting bacteriophage Dp-4 DNA replication. We also use HPUra to determine the time of initiation of Dp-4 DNA replication.

2. Materials and methods

The pneumococcal strain used to study the replication of Dp-4 was the nuclease-deficient mutant (Mo end 1–exo 2) [5] designed as *nuc*$^-$ hereafter; 470 strain (kindly supplied by Professor S. Lacks) was used to label Dp-4 [6]. Conditions for growth of the host bacteria and obtaining purified pneumococcal phages are in [3,5]. The lysis of the cultures was carried out as in [14]. To 3 ml lysate, enough solid CsCl (suprapure grade, Merck) was added to give a refractive index at 25°C (η_D^{25}) of 1.3980. *S. pneumoniae* DNA, 10 μg (ρ = 1.6995 g/cm^3) and 10 μg Dp-4 DNA (ρ = 1.666 g/cm^3) [7] were included as density references. Centrifugation was carried out in a Sorvall TV-865 ultravertical rotor at 40 000 rev./min for 20 h in a Sorvall OTD-65 ultracentrifuge equipped with an automatic rate controller apparatus (ARC-1). Fractions of 3 drops were collected by puncturing the bottom of the tube and treated as in [7]. Fluorodeoxyuridine (FudR), nalidixic acid, and hydroxyurea were purchased from Sigma. HPUra from Imperial Chem. Indust., was a gift of Dr M. Piechowska.

3. Results and discussion

Several antibiotic inhibitors of DNA synthesis were assayed to differentially suppress host metabolism without affecting phage replication. Table 1 shows that nalidixic acid and hydroxyurea were unable to affect either host DNA synthesis or phage development. On the other hand, both FudR and HPUra dramatically blocked the incorporation of [^{3}H]-thymidine into *S. pneumoniae* DNA but only HPUra did not affect phage production. These findings suggest that phage DNA synthesis possibly does not require the DNA polymerase III of the host cell.

We next studied in detail the specific inhibition of bacterial DNA synthesis by HPUra as well as the onset of Dp-4 DNA replication in infected *S. pneumoniae* cells. Figure 1 demonstrates that HPUra (100 μM) blocks the incorporation of [^{3}H]thymidine

Table 1
Effect of antibiotic inhibitors on *S. pneumoniae* DNA synthesis and on the production of phage Dp-4

Treatment		Host DNA synthesis (%)[a]	Phage titer (p.f.u./ml)[b] (%)
None (control)		100	100
FudR	(1 μg/ml)	23	27
	(5 μg/ml)	10	16
	(10 μg/ml)	5	10
	(20 μg/ml)	4	8
HPUra	(10 μM)	15	124
	(50 μM)	12	130
	(100 μM)	4	90
	(150 μM)	3	80
Nalidixic acid	(10 μg/ml)	100	100
	(100 μg/ml)	90	96
Hydroxyurea	(10 mM)	100	100

[a] *nuc*⁻ strain (5 × 10^7 c.f.u./ml) received 5 μCi [^{3}H]thymidine/ml (20.3 Ci/mmol) in the presence of the indicated concentrations of metabolic inhibitors. Incubation was continued for 90 min at 37°C. Samples of 0.5 ml culture received 2 mg unlabeled thymidine/ml and were centrifuged (10 000 × *g*, 5 min), resuspended, precipitated with 10% trichloroacetic acid, filtered and counted (100% = 53 695 cpm)

[b] *nuc*⁻ strain (5 × 10^7 c.f.u./ml) were infected with Dp-4 at m.o.i. 3, in the presence of the different drugs. Incubation was continued for 20 min at 37°C when the cultures were centrifuged (10 000 × *g*, 10 min) to eliminate unadsorbed phages. The cultures were resuspended in fresh CpH 8 medium [15] plus the different drugs and incubation proceeded for 120 min. Samples (1 ml) were centrifuged (10 000 × *g*, 10 min) and phages were assayed in the supernatants (100% = 2.9 × 10^9 p.f.u./ml)

in uninfected cells whereas in infected cultures, the synthesis of DNA starts after a lag of 15 min. In addition, Dp-4 affects only partially host DNA synthesis in the absence of HPUra.

To know whether the DNA synthesized in phage-infected HPUra-treated *S. pneumoniae* corresponds to Dp-4 DNA we isolated and analysed in a CsCl gradient the DNA obtained from infected cultures. Figure 2 demonstrates that only Dp-4 DNA was

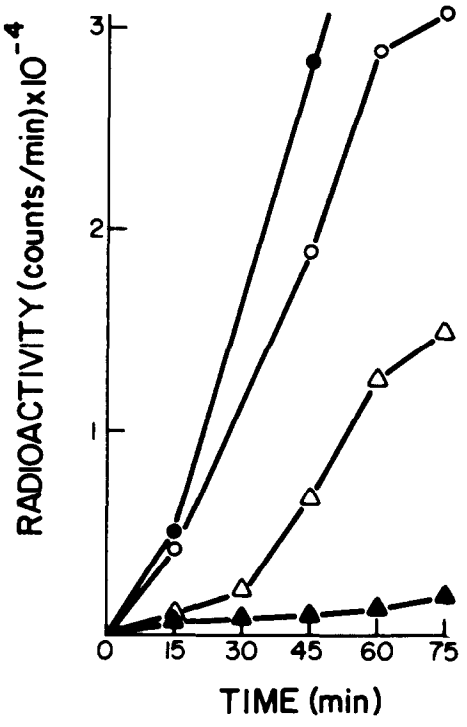

Fig.1. Effect of HPUra on DNA synthesis in uninfected and phage Dp-4-infected *S. pneumoniae nuc*⁻ strain. [^{3}H]Thymidine (5 μCi/ml) was added at 0 time. At different times, samples (0.5 ml) were withdrawn and the trichloroacetic acid-precipitable radioactivity was determined as indicated in table 1[a]. (•) Uninfected culture; (○) Dp-4-infected cells; (▲) uninfected culture plus HPUra (100 μM); (△) Dp-4-infected cells plus HPUra (100 μM).

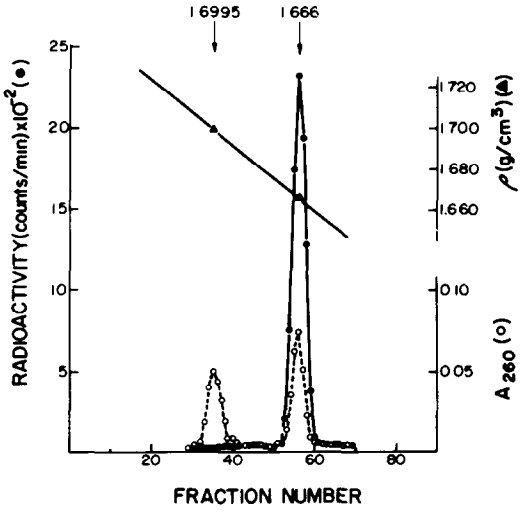

Fig.2. Equilibrium CsCl density gradient centrifugation of the DNA isolated from Dp-4 infected *nuc*⁻ strain in the presence of HPUra. Bacteria (5 × 10^7 c.f.u./ml) were infected with Dp-4 (m.o.i. 3) in the presence of HPUra (100 μM). [^{3}H]Thymidine (20 μCi/ml) was added and incubation continued for 45 min at 37°C when 2 mg unlabeled thymidine/ml was added. The culture was centrifuged, lysed and analysed in CsCl density gradients as in section 2.

labeled with [^{3}H]thymidine in the presence of HPUra.

From the above results we can conclude that HPUra inhibits *S. pneumoniae* DNA synthesis without affecting either Dp-4 DNA replication (fig.1,2) or the production of complete phage particles (table 1). In these conditions, the onset of phage replication was found 15 min after the addition of the phage.

Most important, these results appear to be of immediate importance to us and other groups involved in the study of DNA synthesis of pneumococcal phages since nothing has been reported on the replication of streptococcal phages.

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