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Resistance of Bacteriophage PBS2 Infection to 6-(*p*-Hydroxyphenylazo)-Uracil, an Inhibitor of *Bacillus subtilis* Deoxyribonucleic Acid Synthesis

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6-(*p*-Hydroxyphenylazo)-uracil (HPUra), an inhibitor of semiconservative deoxyribonucleic acid (DNA) synthesis in *Bacillus subtilis*, does not prevent (but slightly reduces the rate of) replication of the uracil-containing DNA phage PBS2. Our observations are consistent with the hypothesis that all *B. subtilis* phages which are resistant to HPUra are able to induce a new DNA polymerase activity.

6-(*p*-Hydroxyphenylazo)-uracil (HPUra) has been shown by Brown (1-3) to be a specific inhibitor of semiconservative deoxyribonucleic acid (DNA) replication in *Bacillus subtilis*. However, DNA synthesis and virus production by some *B. subtilis* lytic phages is essentially unaffected by HPUra. These include the hydroxymethyluracil-containing DNA phages SP8 (1), ϕe (4), and SPO1 (*unpublished data* cited in reference 7), and the thymine-containing DNA phages SP3 (1), SPO2 c_1 (1, 7), and ϕ29 (C. Schachtele, *personal communication*). In contrast, the thymine-containing DNA phage ϕ105 is blocked in its DNA replication by HPUra (7). Since SPO1 (8) and SPO2 c_1 (7) have been shown to induce a new DNA polym-

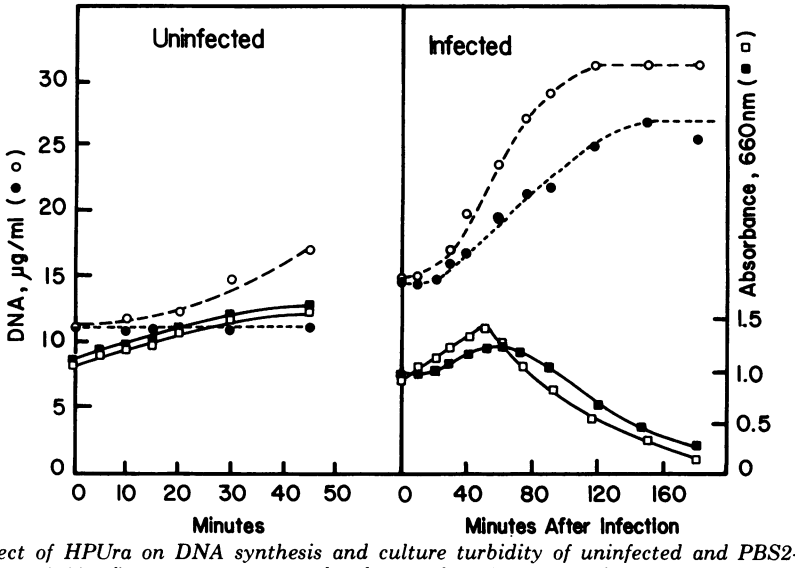

FIG. 1. *Effect of HPUra on DNA synthesis and culture turbidity of uninfected and PBS2-infected B. subtilis. Cultures (500 ml) were grown to an absorbance of 1.0 (at 660 nm in a 1-cm cuvette), and 5 ml of dimethylsulfoxide was added with or without HPUra (to give a final concentration of 400 µM). After 2 min, some cultures were infected with PBS2 at a multiplicity of 4.5 phage per cell (90 ml of PBS2 lysate). At the indicated times, samples were removed for measurement of the culture absorbance (□, ■) or for colorimetric DNA determination (6) (○, ●). Controls, ○ and □; HPUra-treated, ● and ■.*

erase activity, whereas ϕ105 does not (7), it has been proposed that all *B. subtilis* phages resistant to HPUra may induce a new DNA polymerase (7). Since we had previously demonstrated that the uracil-containing DNA phage PBS2 induced a new DNA polymerase activity after infection of *B. subtilis* (5), we tested the sensitivity of PBS2 infection to inhibition by HPUra.

The methods for the growth and titering of *B. subtilis* SB19 and PBS2 phage have been described (5). The HPUra was the gift of Neal Brown and of Bernard Langley (Imperial Chemical Industries); it was dissolved in dimethylsulfoxide at a concentration of 40 mM (9.3 mg/ml).

Figure 1 shows the effect of 400 μM HPUra on uninfected and PBS2-infected cells. In agreement with previous results (1), HPUra caused an immediate cessation of DNA synthesis (without preventing cell growth) in uninfected *B. subtilis*. Even 40 μM HPUra is sufficient to stop cellular DNA synthesis (1). In confirmation of previous data (6), we found that PBS2 infection also results in the cessation of host DNA synthesis, and new phage DNA synthesis begins within 20 min. During infection in the presence of 400 μM HPUra, the rate and the amount of synthesis of phage DNA was slightly decreased (Fig. 1). Lysis of the culture was also delayed.

Figure 2 indicates that HPUra also reduced somewhat the rate and level of induction of the viral thymidylate phosphohydrolase (6). The rate of phage production was also slower, but the final burst of progeny phage was essentially the same in the presence and absence of HPUra (Fig. 2). Thus, infection of *B. subtilis* by PBS2 phage is essentially resistant to inhibition by HPUra under conditions in which *B. subtilis* DNA synthesis is completely prevented. The relatively small effects of 400 μM HPUra on viral enzyme, DNA, and progeny phage production may be caused by impurities in the HPUra preparation employed, or may be nonspecific effects of high HPUra concentrations.

The mechanism of *B. subtilis* DNA synthesis inhibition by HPUra appears to involve competition by a reduced metabolite of HPUra with deoxyguanosine triphosphate in the semiconservative DNA replication catalyzed by a DNA polymerase activity present in *pol*$^-$ cells lacking the major *B. subtilis* DNA polymerase I (3; Neal Brown, *personal communication*). *B. subtilis* phages like PBS2 which are resistant to HPUra probably induce a new DNA polymerase which is unaffected by the HPUra metabolite (7). In support of this hypothesis, we

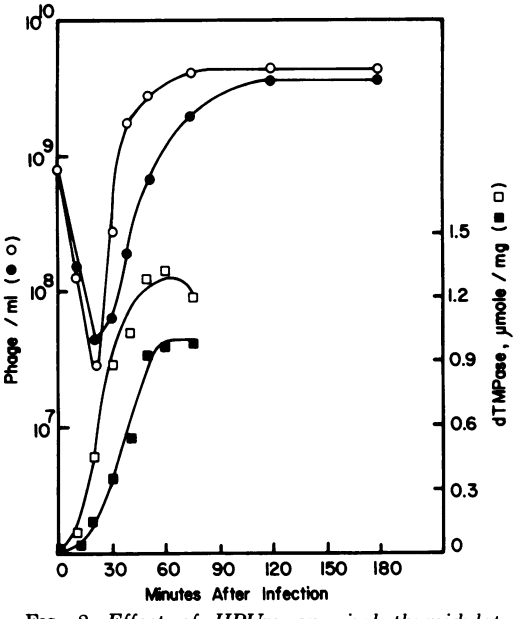

FIG. 2. *Effect of HPUra on viral thymidylate phosphohydrolase induction and phage production in PBS2-infected B. subtilis. Cultures infected as in Fig. 1 were sampled at the indicated times; 0.10 ml was removed for lysis in medium containing chloramphenicol (100 μg/ml) and lysozyme (100 μg/ml) to measure total phage (6) (○, ●); or 35 ml was removed for preparation of cell-free extracts and assay (6) of thymidylate phosphohydrolase activity (micromoles of phosphate released in 15 min per milligram of protein) (□, ■). Controls, ○ and □; HPUra-treated, ● and ■.*

found that the time course of PBS2 DNA polymerase induction (5) in the presence and absence of HPUra was similar to that of thymidylate phosphohydrolase shown in Fig. 2. Furthermore, the PBS2 DNA polymerase activity in crude extracts of *B. subtilis* 1306 *pol*$^-$ (5) was not inhibited by 400 μM HPUra nor by 400 μM reduced HPUra (prepared with dithiothreitol in toluene-treated cells as described in reference 3).

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