NOTE

Inhibition of Ribonucleic Acid Bacteriophage Release from Its Host by Rifampin

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Rifampin, in addition to interfering with intracellular growth of the ribonucleic acid-containing phage MS2, also inhibits the release of mature phage particles from *Escherichia coli* cells.

The antibiotic rifampin blocks ribonucleic acid (RNA) synthesis in *Escherichia coli* (5-7) by acting on the core of the enzyme deoxyribonucleic acid (DNA)-dependent RNA polymerase (4, 9, 10). It was reported that this drug also inhibits RNA phage development in *E. coli* when applied prior to or within a few minutes after infection (5, 8), whereas its addition 5 min after infection permitted near normal growth of RNA phage (5). No effect on RNA phage development was detected when a mutant of *E. coli* possessing a rifampin-resistant DNA-dependent RNA polymerase was used as an host (8), and it was suggested that host DNA-dependent RNA polymerase is involved in phage RNA replication either directly (5, 8) or indirectly (2).

Here we report that rifampin interferes not only with intracellular growth of the RNA-containing phage MS2 but also inhibits the release of mature phage particles from *E. coli* cells.

A culture of *E. coli* AB301 (Hfr met λ^+), rifampin-sensitive, (kindly supplied by R. Gesteland) was grown with aeration at 37 C in tryptone broth medium (3). Cells at a density of 2.10^8 ml were infected with MS2 phage at a multiplicity of infection of 10. Rifampin, at a final concentration of 100 μ g/ml, was added at various times postinfection. Forty and 150 min after infection, the cultures were centrifuged at 10,000 rev/min for 20 min at 4 C. The supernatants were titrated (1) for extracellular phage content. The intracellular phage content was determined as follows. The pellets obtained by centrifugation were resuspended to starting volume in fresh medium and lysed by mixing 10- μ l portions with 1 ml of lysozyme ethylenediaminetetraacetic acid

(EDTA) solution (containing 1 mg of lysozyme per ml in 0.05 M EDTA at pH 8.3), to which a few drops of chloroform were added. The mixtures were then shaken at 4 C for 10 min, and samples were titrated (1). To determine total

FIG. 1. Effect of rifampin on the yield of MS2 phage. Intra- and extracellular phage yield was determined 40 and 150 min, respectively, postinfection. Total phage yield was estimated 40 and 150 min postinfection. Symbols: ●, intracellular; ○, extracellular; ×, total.

phage yield, samples of the cultures were withdrawn and lysed in the lysozyme-EDTA-chloroform solution as described above.

The effect of rifampin on extracellular, intracellular, and total phage yields is illustrated in

TABLE 1. *Effect of rifampin on the intracellular and extracellular distribution of MS2 phage*

Time (min) of addition of rifampin postinfection	Total (PFU/ml)	Extracellular ^a		Intracellular ^a	
		PFU/ml	Per cent of total	PFU/ml	Per cent of total
0	6.9×10^{10}	1.7×10^9	2.5	6.7×10^{10}	97.0
10	3.4×10^{11}	2.0×10^{10}	6.0	3.2×10^{11}	95.0
20	7.1×10^{11}	8.5×10^{10}	12.0	6.4×10^{11}	90.0
No rifampin	7.0×10^{11}	6.5×10^{11}	93.0	6.5×10^{10}	9.0

^a Intracellular and extracellular phage yield was determined 150 min postinfection. PFU, plaque-forming units.

Fig. 1. The extracellular phage content in the control (without rifampin) was maximal at 150 min after infection. At this time, the titer of the extracellular phage was only 0.25% of the control titer when rifampin was applied together with the infecting phage. The reduction of extracellular phage yield was dependent on the time of addition of the drug, being 0.25, 12, 25, and 50% of the control yield when rifampin was applied at 0, 20, 30, and 40 min, respectively, postinfection. Since it was previously reported that rifampin inhibits intracellular phage synthesis (5, 8), the reduction in extracellular phage yield could be a result of low phage production. This seems most unlikely since the inhibitory effect of rifampin on extracellular phage yield could be completely separated from its effect on phage synthesis. In our experiments, phage synthesis was unaffected when the drug was applied 20 min or later postinfection. Even when the antibiotic was added at the time of infection, which reduced phage synthesis by a factor of 10, only 2.5% of the total phage yield was found in the extracellular medium (Table 1).

As demonstrated (Table 1), the reduction of extracellular phage content in the presence of rifampin was accompanied by an equivalent intracellular accumulation of progeny phage particles. For example, when the drug was added at 20 min postinfection, only 12% of the extracellular phage content was obtained, whereas 90% of the total phage particles were intracellularly located. Therefore, the diminished extracellular phage represents an inhibition of phage release from the host.

These data indicate that rifampin, in addition to its known effect of interfering with RNA phage synthesis (5, 8), also inhibits the release of progeny phage particles from the host cells. The release process itself may involve a step for which

the host DNA-dependent RNA polymerase is required. This possibility is supported by the observation that MS2 plates normally on mutants possessing rifampin-resistant DNA-dependent RNA polymerase (8). As a consequence, one may speculate that host-controlled protein(s) participate in the release of RNA phage particles from bacteria. This could be relevant to the mechanism of phage release.

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