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Sensitization of *Escherichia coli* to Actinomycin D by the Arrest of Protein Synthesis

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Escherichia coli is generally impermeable to charged molecules for which it does not possess specific transport mechanisms (2). This permeability barrier is thought to be the cause of the resistance of *E. coli* to actinomycin, since this antibiotic inhibits deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) synthesis in in vitro extracts prepared from *E. coli*, but has no effect on RNA synthesis in growing cells even at a concentration of 100 μg/ml (1). This view is supported by the demonstration (3) that mild treatment with ethylenediaminetetraacetic acid (EDTA), which causes a release of components of cell wall lipopolysaccharide, renders cells of *E. coil* sensitive to actinomycin. Mutant strains of *E. coli* which are sensitive to actinomycin without pretreatment with EDTA have been isolated (5); it was concluded that these strains have a more permeable cell wall.

In the present report, we describe another condition under which *E. coli* becomes sensitive to actinomycin, namely, when RNA synthesis proceeds in the absence of protein synthesis. One way of achieving this condition is by amino acid starvation of an *E. coli* strain possessing relaxed control of the RNA synthesis (RC^{rel}) phenotype (6). Residual RNA synthesis during arginine starvation of an RC^{rel} strain was actinomycin-sensitive (Fig. 1). Actinomycin had no effect on RNA synthesis in the exponentially growing, arginine-supplemented culture, but it inhibited residual RNA synthesis in the arginine-starved cultures; a concentration of 50 μg/ml caused a greater inhibition of residual RNA synthesis than a concentration of 10 μg/ml. It appears that it takes at least 30 min after the onset of amino acid starvation for the inhibition of RNA synthesis to manifest itself. Until that time, the kinetics of incorporation of ^{14}C-uracil into trichloroacetic acid-precipitable material in the presence or in the absence of actinomycin were similar. Figure 1 also presents the results of an experiment similar to that just described, except that ^{14}C-uracil was added 45 min after resuspension of the cultures in an arginine-free medium. The effect of actinomycin on residual RNA synthesis was more pronounced at this later time.

To test whether the difference in ^{14}C-uracil incorporation between starved and exponentially growing cultures of strain CP79 results from actinomycin sensitivity, ^{3}H-actinomycin was added to both arginine-starved and exponentially growing cultures (Table 1). The uptake of ^{3}H-actinomycin was followed with the procedure of Sekiguchi and Iida (5). In 2 hr, the arginine-starved bacteria took up 40 times more actinomycin than did the exponentially growing bacteria, demonstrating that the reduced incorporation of radioactive uracil in the arginine-starved culture probably derives from greater uptake of the antibiotic (Table 1).

E. coli strains possessing the RC^{str} phenotype do not synthesize appreciable amounts of RNA during amino acid starvation (4). To test whether these strains become permeable to actinomycin during amino acid starvation, another experiment was performed in which a culture was starved in the presence of actinomycin for 45 min. After that time, the required amino acid was restored and uracil incorporation was measured (Fig. 2). First, the presence of actinomycin caused an initial reduction in the rate of ^{14}C-uracil incorporation when arginine was added to the starved RC^{rel} strain CP79. At later times, however, ^{14}C-uracil incorporation appeared to proceed at a rate which gradually approached normal. The initial delay in RNA synthesis must reflect the presence of actinomycin inside the starved RC^{rel} bacteria at the moment of arginine addition. A possible interpretation for the eventual resumption of RNA synthesis appears to be the gradual restoration of cell wall impermeability and the exclusion of extracellular actinomycin once arginine has been resupplied, and the dilution of intracellular actinomycin. Second, actinomycin caused no delay in the resumption of RNA synthesis in the RC^{str} strain, suggesting that it is not present in the starved RC^{str} bacteria at the moment of arginine addition. Hence, it can be inferred that RC^{str}

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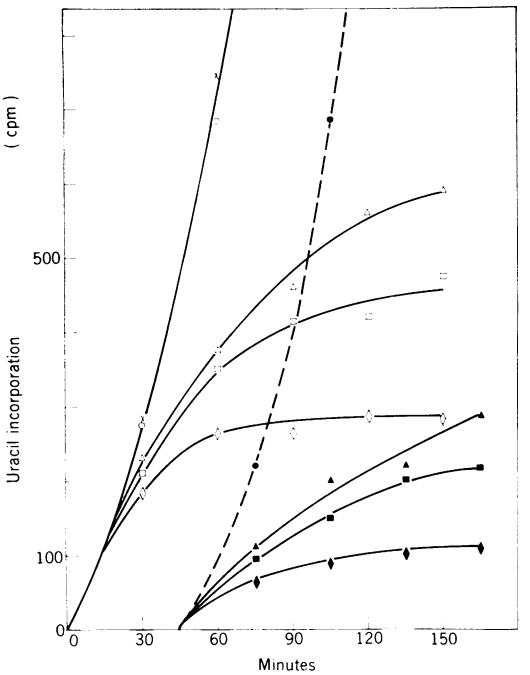

FIG. 1. *Inhibition of uracil incorporation in arginine-starved cells of strain CP79 by different concentrations of actinomycin D. Bacteria growing exponentially in minimal medium supplemented with required amino acids (100 μg/ml) and thiamine (2 μg/ml) were filtered, washed, and resuspended with ^{14}C-uracil (10 μg/ml; 0.01 μc/ml) in minimal medium either containing all required amino acids or lacking arginine. As indicated below, some cultures contained actinomycin D. At intervals, samples were taken in order to follow the incorporation of ^{14}C-uracil into trichloroacetic acid-precipitable material. Symbols: ○, with arginine, without actinomycin; ×, with arginine, with actinomycin (50 μg/ml); △, without arginine, without actinomycin; □, without arginine, with actinomycin (10 μg/ml); ◇, without arginine, with actinomycin (50 μg/ml). In the case of the curves which start at 45 min, ^{14}C-uracil was added after 45 min of exponential growth or after 45 min of starvation for arginine, either in the presence or in the absence of actinomycin. The counts which represent the uracil incorporation of the starved cultures are normalized to the optical density of the exponentially growing culture at the time label was added. Symbols: ●, with arginine, without actinomycin; ▲, without arginine, without actinomycin; ■, without arginine, with actinomycin (10 μg/ml); ◆, without arginine, with actinomycin (50 μg/ml).*

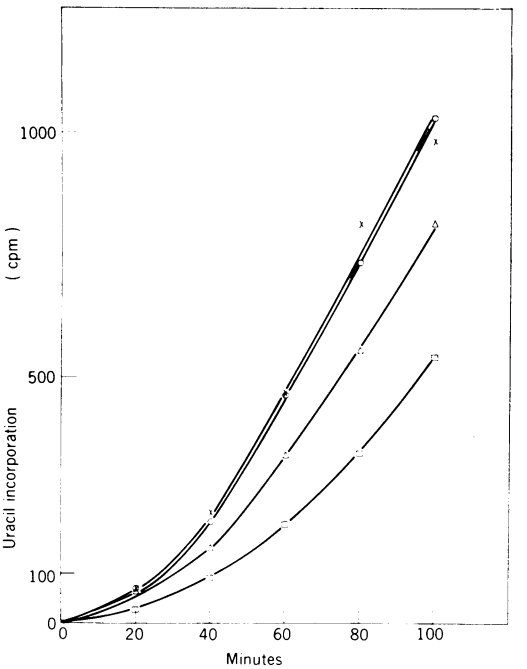

FIG. 2. *Effect of actinomycin treatment during arginine starvation on RNA synthesis after arginine restoration. Exponentially growing cultures of strain CP78 and CP79 were filtered, washed, and resuspended in growth medium lacking arginine. After 45 min of incubation at 37 C, actinomycin was added to one half of each culture, to a final concentration of 50 μg/ml. After an additional 45 min of incubation, arginine and ^{14}C-uracil (10 μg/ml, 0.01 μc/ml) were added to each culture, and the incorporation of radioactivity into trichloroacetic acid-precipitable material was followed. Symbols: ×, CP78 without actinomycin; ○, CP78 with actinomycin; △, CP79 without actinomycin; □, CP79 with actinomycin.*

bacteria do not become permeable to actinomycin during arginine starvation.

Another way of achieving RNA synthesis in the absence of protein synthesis is by treatment with chloramphenicol (4). Chloramphenicol was added to an exponentially growing culture of strain CP78, to a final concentration of 100 μg/ml. The culture was divided into two parts, and ac-

TABLE 1. *Uptake of ^{3}H-actinomycin D by exponentially growing and arginine-starved bacteria of strain CP79*[a]

Culture	Radioactivity (counts per min per 10^9 bacteria) after incubation in the presence of actinomycin for	
	30 min	120 min
With arginine.....	5	25
Without arginine.....	250	1,000

[a] ^{3}H-actinomycin D was added at a concentration of 1 μg/ml (specific activity, 3 c/mmole). After the indicated periods of incubation at 37 C, samples were diluted into ice-cold M9 medium containing nonradioactive actinomycin to a final concentration of 5 μg/ml, centrifuged, and washed with more ice-cold M9 (*see* reference 5). The bacteria were then collected on filters, washed again with M9, and counted for ^{3}H activity.

tinomycin (final concentration, 50 μg/ml) was added to one. The incorporation of ^{14}C-uracil into trichloroacetic acid-precipitable material was then followed. The results of this experiment were analogous to those presented in Fig. 1, in that actinomycin was found to cause a strong inhibition of RNA synthesis within 30 min after the arrest of protein synthesis by chloramphenicol.

We examined other RC^{rel} strains for sensitivity to actinomycin when starved for a variety of amino acids other than arginine. In each case, we observed that actinomycin sensitivity arises during the period of amino acid starvation or after chloramphenicol treatment of either RC^{str} or RC^{rel} strains.

The findings presented here can be explained by envisaging that the intracellular accumulations of RNA in bacteria of RC^{rel} strains under conditions which do not permit formation of cell wall polypeptides result in a distortion of the bacterial cell wall which renders it permeable to actinomycin. The 30-min delay observed in the establishment of actinomycin sensitivity after the onset of amino acid starvation or after chloramphenicol addition would then reflect the time required to produce the necessary permeability change.

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