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INFLUENCE OF NOVOBIOCIN ON THE INDUCTION KINETICS OF β-GALACTOSIDASE IN ESCHERICHIA COLI

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SUMMARY: Novobiocin (0.05 μg/ml) reduced the growth rate of cultures of Escherichia coli strain DK6 by about a factor of 2. The lag in appearance of β-galactosidase-forming capacity was extended from 50 sec to 85 sec by the drug. This appeared to be the result of a reduced rate of nascent mRNA elongation.

In Escherichia coli, chromosomal DNA is normally maintained in an underwound, or negatively supercoiled, condition. This is presumably the end result of the action of the DNA topoisomerases (reviewed in reference 3). In this organism, the two best characterized topoisomerases are DNA gyrase, which introduces negative supercoils into circular duplex DNA, and ω protein, which unwinds negative supercoils. The existence of specific inhibitors of DNA gyrase have made it possible to investigate the influence of chromosome topology on various cellular processes. Novobiocin and coumermycin are structurally related antibiotics that seem to bind to the same site on one of the two subunits of DNA gyrase. Mutants isolated for their resistance to coumermycin have a gyrase that is insensitive to both drugs. Treatment of intact E. coli cells with coumermycin leads to decreased supercoiling of chromosomal DNA (5) and also to inhibition of the synthesis of both DNA and RNA (reviewed in reference 4). Recent studies, both in vivo and in vitro, have shown that inhibition of DNA gyrase influences initiation of mRNA synthesis in a promoter-specific fashion (15,17,20). In the study of β-galactosidase induction reported here, we have asked whether the

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topologically underwound condition is necessary for optimal rates of RNA polymerase progression, as well as for normal initiation.

EXPERIMENTAL PROCEDURES:

E. coli strain DK6 (10) is a derivative of strain AS19 (16). AS19 was originally isolated for its permeability, and hence high sensitivity, to actinomycin D, but was subsequently found to be quite permeable to a spectrum of antibiotics including fusidic acid, rifampicin and streptolydigen (13), and, as shown here, low concentrations of novobiocin.

Cultures were grown in medium (11) supplemented with 0.2% glucose and a synthetic amino acid mixture (12) with shaking at 37°C. For the induction of β-galactosidase, glycerol replaced glucose as the carbon source.

RESULTS:

Control cultures of strain DK6 grew with a generation time of 29-33 min. When treated with novobiocin at a partially inhibitory concentration (0.05 μg/ml), growth progressed at the control rate for 30-40 min before slowing (Fig. 1A). After the new rate was established (generation times of 56-80 min in various experiments at this drug concentration), exponential growth continued in the presence of the drug for at least 6 hrs. After exposure of the cells to novobiocin, DNA synthesis was affected first, being inhibited by more than 50% in 10 min (Fig. 1B). This was followed by inhibition of RNA synthesis (Fig. 1C) and finally protein synthesis (Fig. 1D).

The inducer of β-galactosidase, isopropylthiogalactoside (IPTG), was added to cultures growing in the presence and absence of novobiocin. At timed intervals, samples were taken into solutions containing the antibiotic streptolydigen to block continued RNA synthesis (2,9,13). After further incubation to allow translation of the completed mRNA molecules, β-galactosidase was assayed (Fig. 2). The final rate of accumulation of enzyme-forming capacity was reduced by a factor of approximately 3, consistent with the previously reported influence of novobiocin on the frequency of initiation of lac operon transcription (15,20). In addition, the lag prior to the appearance of active mRNA was about 35 sec longer in the drug-treated culture. This increased lag, generated by drug treatment, could be due either to a reduced rate of mRNA elongation or to a slower rate of action

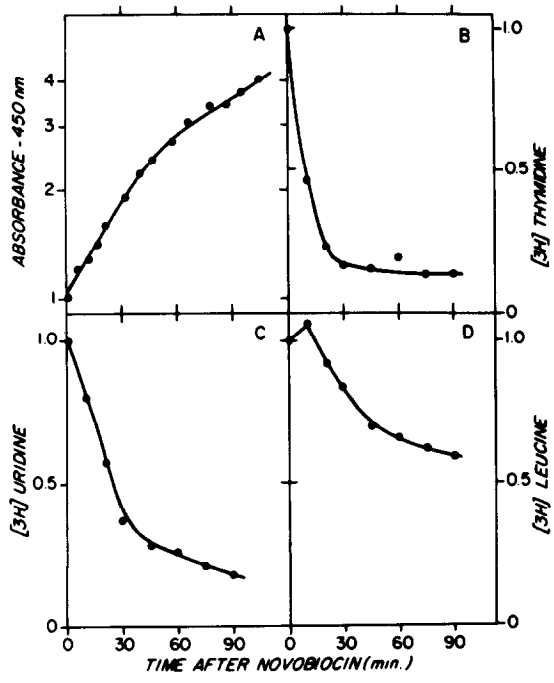

Fig. 1. At 0 time, novobiocin (0.05 µg/ml) was added to bacterial cultures. A. Growth was followed by measuring the absorbance at 450 nm; the A_{450} at 0 time was arbitrarily defined as 1.0 and the values were normalized accordingly. For the following experiments (B, C and D), radioactive precursor incorporation was measured into trichloroacetic acid insoluble material in two-min labeling periods. The radioactivity incorporated was corrected for turbidity of the culture and normalized to the incorporation of control cultures prior to drug addition. B. ^{3}H-Thymidine (5 Ci/mM) was added at a level of 50 µCi/ml of culture. Incorporation in control, untreated cultures was 4.8 x 10^5 cpm/ml/A_{450}. C. ^{3}H-Uridine (0.75 Ci/mM) was added at a level of 2.8 µCi/ml culture. Incorporation in control cultures was 6.8 x 10^5 cpm/ml/A_{450}. D. ^{3}H-Leucine (52 Ci/mM) was added at a level of 50 µCi/ml of culture. Incorporation in control cultures was 3.7 x 10^5 cpm/ml/A_{450}.

of the inducer. In untreated E. coli, induction of the lactose operon began essentially simultaneously with the addition of IPTG (8,9). This was tested for the drug-treated cells by blocking transcriptional initiation with rifampicin at various times before and after addition of IPTG (9). There was no detectable lag in inducer action either in the presence or absence of novobiocin (Fig. 3). Thus, we interpret the increased delay in appearance of enzyme-forming capacity in cultures treated with novobiocin to be the result of a diminished rate of elongation of nascent mRNA molecules. We estimate the first significant appearance of completed

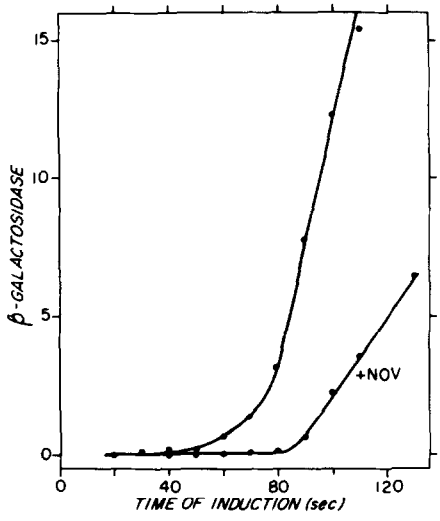

Fig. 2. Cultures were grown in the presence of novobiocin (0.05 μg/ml) until exponential growth was achieved and untreated controls were induced with IPTG at 0 time (9). At the indicated times, samples were taken into streptolydigen, incubated for 20 min at 37°C for expression of enzyme-forming capacity and assayed (9). Enzyme units were defined as nanomoles of o-nitrophenylgalactoside hydrolyzed per minute and were normalized to 1 ml culture at A_{450} = 1.0. To allow better comparison of the curves, the basal uninduced levels (0.92 for control and 0.66 for drug-treated) were subtracted.

β-galactosidase mRNA to be at 50 sec in control cultures and at 85 sec in the drug-treated cultures. A length for the lacZ-gene of 3,063 nucleotides can be estimated from the length of the polypeptide chain (6), and the induction lags convert to elongation rates of 61 and 36 nucleotides per sec for control and drug-treated cultures, respectively.

A mutant of strain DK6 was isolated which was resistant to the growth inhibitory effects of novobiocin at a concentration of 1.0 μg/ml. This strain retained its generalized antibiotic permeability as shown by its sensitivity to low concentrations of actinomycin D and rifampicin. When this novobiocin-resistant strain was treated with the drug at the concentration used in this study (0.05 μg/ml), there was no effect on growth or on the kinetics of β-galactosidase induction.

DISCUSSION:

We conclude that treatment of E. coli with novobiocin slows the rate of mRNA elongation. A similar conclusion has recently been reached for

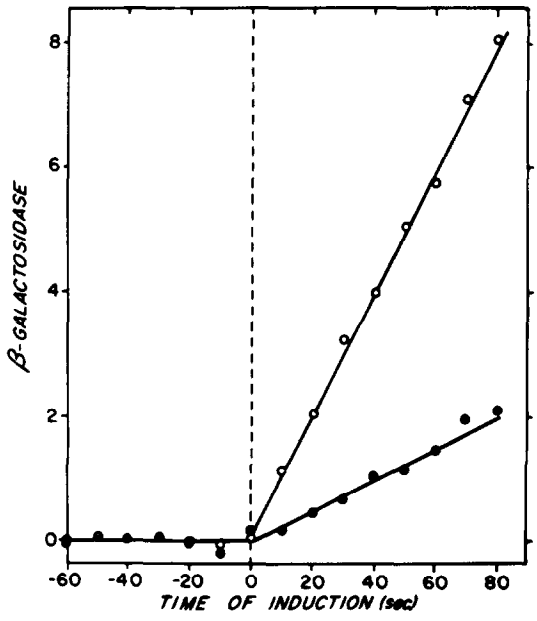

Fig. 3. Cultures of strain DK6 were grown in the presence (●) and absence (○) of novobiocin as described in Fig. 2. Rifampicin (60 μg/ml) was added to samples of the culture at the times indicated on the abscissa of the graph. IPTG was added at 0 time. The samples were then incubated for 15 min at 37°C for expression of enzyme-forming capacity and assayed for β-galactosidase. Enzyme units were defined and normalized as described in Fig. 2.

ribosomal RNA synthesis (18). It is tempting to suggest that RNA polymerase movement on the duplex DNA template requires melting of the helix, and that this is aided by the positive free energy associated with the negatively supercoiled state. However, studies in vitro with isolated E. coli polymerase suggest that the degree of superhelicity of small circular DNA templates influences only initiation and not chain elongation (1,14). It is certainly possible that the intracellular ionic conditions or the presence of DNA binding proteins may modify the situation in vivo. Another possibility is that DNA gyrase is directly involved in RNA polymerization in vivo; we know of no evidence concerning this possibility. Finally, inhibition of DNA gyrase and the resultant changes in the complex structure of the E. coli chromosome (19), may create a direct physical impediment to the movement of RNA polymerase.

Lastly, it should be noted that starvation of an auxotroph for spermidine also slowed the rate of lac mRNA chain elongation (9). Spermidine has been reported to strongly stimulate the activity of DNA gyrase in vitro (7). However the possible relationship between the result obtained in this paper with novobiocin-inhibited cells and that observed with spermidine starvation has not been proven.

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