Vol. 134, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 14, 1986 Pages 338-343

TRANSCRIPTION BUT NOT TRANSLATION IS REQUIRED FOR EDTA-INDUCED AUTOLYSIS IN ESCHERICHIA COLI

Kolli S N Prasad and Sanjay N Chitnis

Centre for Cellular & Molecular Biology, Uppal Road
Hyderabad 500 007, India

Received December 3, 1985

Rifampicin, but not chloramphenicol or other inhibitors of translation, inhibited EDTA-induced autolysis in Escherichia coli. Inhibition of EDTA-induced autolysis in E. coli was also observed with nalidixic acid and novobiocin, inhibitors of topoisomerase II. Rifampicin or nalidixic acid-resistant mutants of E. coli were resistant to the inhibitory effect of the respective antibiotic on EDTA-induced autolysis. The implications of these studies in regard to our understanding of the regulation of autolysis in E. coli are discussed.

Autolysis can be induced in E. coli by a variety of means (1). Leduc et al (1) showed that protein synthesis is required for autolysis induced (e.g. by penicillin) in growing E. coli cells, but neither RNA synthesis nor protein synthesis is required for autolysis induced (e.g. by 1 mM EDTA) in harvested E. coli cells; rifampicin had no effect on EDTA-induced autolysis in E. coli.

We have carried out a detailed study on the effect of rifampicin and other antibiotics on EDTA-induced autolysis in E. coli. We find that EDTA-induced autolysis is inhibited by rifampicin, nalidixic acid and novobiocin but not by chloramphenicol. Our observations are consistent with the hypothesis that transcription but not translation is required for EDTA-induced autolysis in E. coli.

MATERIALS AND METHODS

Materials: Rifampicin, novobiocin, nalidixic acid, chloramphenicol and streptomycin sulphate were from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals used were of analytical grade. The strain E. coli C90 (2) was used in this study.

EDTA-induced autolysis. A modification of the method described by Leduc et al (1) was used in this study. E. coli cells grown in LB medium (3) in a shaking water bath at 37°C were harvested by filtration. The cells (1.0-2.0 x 10^9) collected on the filter were suspended in 2.5 ml of 1 mM EDTA (pH 6.5) by vortexing

0006-291X/86 $1.50

for 30 sec, transferred to a cuvette maintained at 37°C, and autolysis followed by continuously recording the decrease in optical density at 600 nm (OD_{600}). The rate constant of the initial rate of autolysis was calculated as described earlier (4).

Effect of antibiotics on EDTA-induced autolysis. E. coli cells were grown in LB medium in a shaking water bath at 37°C to reach an OD_{600} of 0.4. The antibiotic was added to obtain the desired final concentration and the culture incubated again at 37°C. Appropriate aliquots of the culture were processed for autolysis as described above at the required time points.

Effect of antibiotics on the viability of E. coli cells. E. coli cells were grown and treated with the antibiotic as described above. At various time points, 1-ml aliquots of the culture were spun in an Eppendorf centrifuge, the cell pellet suspended in minimal A medium (3) without glucose by vortexing, and 0.1 ml of an appropriate dilution of the cell suspension plated on LB agar (3) to determine the viable count.

Isolation of antibiotic-resistant mutants. Rifampicin-resistant mutants were isolated as follows: 10^9 E. coli cells were inoculated in 10 ml of minimal A medium containing rifampicin (100 μg/ml), and incubated for 16 to 24 h at 37°C. The culture that grew in the presence of rifampicin was then streaked on a minimal A agar (3) plate containing rifampicin (100 μg/ml). Single colonies picked from this plate were tested for rifampicin-resistance in LB medium.

Nalidixic acid-resistant mutants were isolated by plating 10^9 E. coli cells on a minimal A agar plate containing nalidixic acid (100 μg/ml). Individual colonies picked from the plate were tested for nalidixic acid-resistance in LB medium.

RESULTS

Figure 1A shows the effect of rifampicin, chloramphenicol and streptomycin on EDTA-induced autolysis in E. coli. At all concentrations - except 10 μg/ml at which very little inhibition of growth was seen (data not shown) - rifampicin showed strong inhibition of EDTA-induced autolysis. Significant inhibition was observed at 5 min, the earliest time point studied, and the maximal inhibition at 20-30 min.

In contrast to rifampicin, chloramphenicol (200 μg/ml) showed a slight stimulation of EDTA-induced autolysis; streptomycin (200 μg/ml) showed slight inhibition over a period of 1 h. At the concentrations used, chloramphenicol was bacteriostatic showing 100% inhibition of growth while streptomycin was bactericidal killing 90% of the cells in less than 30 min (data not shown).

Rifampicin, even at 50 μg/ml, had no inhibitory effect on EDTA-induced autolysis in a rifampicin-resistant mutant of E. coli (Fig. 1B). Similar results were obtained with three other independently isolated rifampicin-resistant mutants.

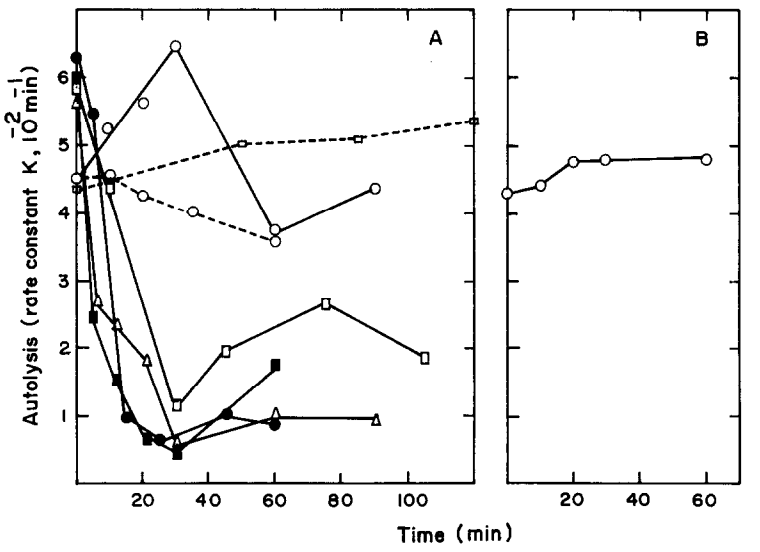

Figure 1A: Effect of rifampicin, chloramphenicol and streptomycin sulphate on EDTA-induced autolysis in wild-type E. coli, O—O, □—□, Δ—Δ, ●—●, ■—■, rifampicin (10, 20, 30, 40 and 50 μg/ml, respectively); □-□, streptomycin sulphate (200 μg/ml); O--O, chloramphenicol (200 μg/ml).

Figure 1B: Effect of rifampicin on EDTA-induced autolysis in a rifampicin-resistant mutant of E. coli. O, rifampicin (50 μg/ml).

Nalidixic acid, an inhibitor of topoisomerase II (5), behaved like rifampicin in showing strong inhibition of EDTA-induced autolysis in E. coli (Fig. 2A). Significant inhibition could be seen at the lowest concentration (10 μg/ml) tested and at the earliest time point studied (10 min). At nalidixic acid concentrations of 25μg/ml and above, maximal inhibition was observed in 20 min.

The viability of nalidixic acid-treated E. coli cells remained unchanged for about 30 min and then showed a decline (Fig. 2A, inset). However, as reported by others (6), the cell mass, measured by determining the OD_{600}, continued to increase (data not shown).

A nalidixic acid-resistant mutant of E. coli was found to be much less sensitive to the inhibitory effect of low concentrations of nalidixic acid on EDTA-induced autolysis (Fig. 2B). Significant inhibition was observed for this strain at a nalidixic acid concentration of 100 μg/ml. Similar results were obtained with two other independently isolated nalidixic acid-resistant mutants of E. coli.

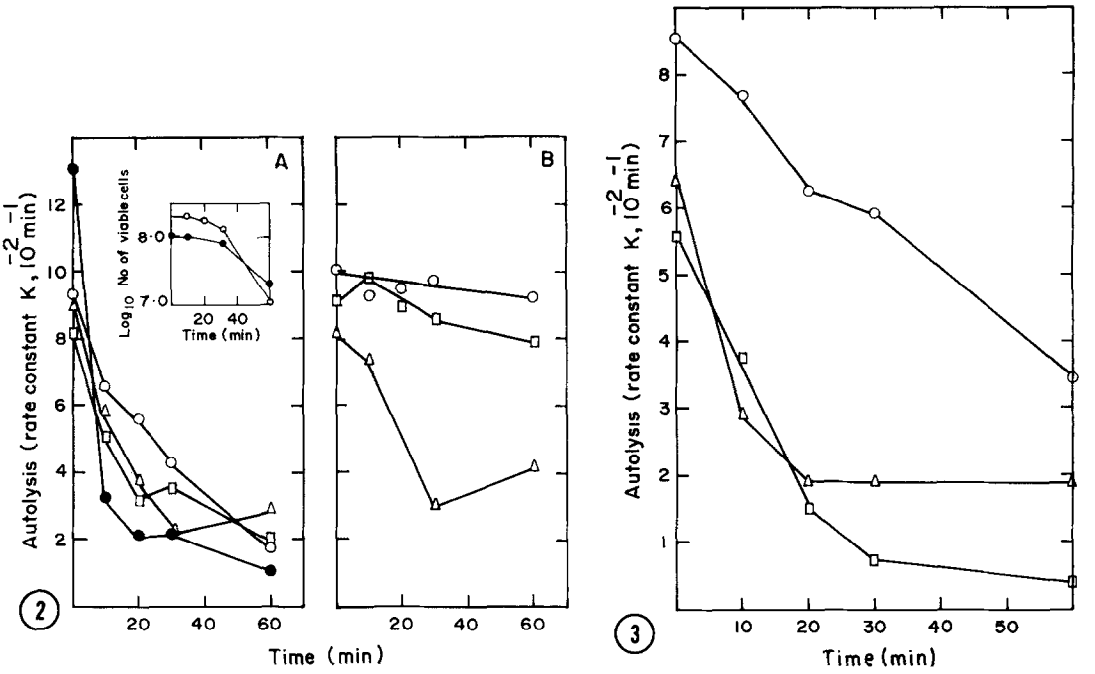

Figure 2A: Effect of nalidixic acid on EDTA-induced autolysis in wild-type E. coli. ○, □, △, ●, nalidixic acid (10, 25, 50 and 100 μg/ml, respectively). Inset: Effect of nalidixic acid on viability of E. coli cells. ○, ●, nalidixic acid (10 and 50 μg/ml, respectively).

Figure 2B: Effect of nalidixic acid on EDTA-induced autolysis in a nalidixic acid-resistant mutant of E. coli. ○, □, △, nalidixic acid (10, 50 and 100 μg/ml, respectively).

Figure 3: Effect of novobiocin on EDTA-induced autolysis in E. coli ○, □, △, novobiocin (10, 50 and 100 μg/ml, respectively).

Novobiocin, another inhibitor of the enzyme topoisomerase II (5), behaved like nalidixic acid with regard to its effect on EDTA-induced autolysis in E. coli (Fig. 3).

DISCUSSION

Our results on the effect of chloramphenicol and rifampicin on EDTA-induced autolysis in E. coli are very similar to those obtained by Leduc et al (1). However, the conclusion of Leduc et al (1) that rifampicin has no effect on EDTA-induced autolysis is at variance with ours. This may be because of the fact that Leduc et al (1) studied the effect of rifampicin only at 10 μg/ml, and although their data do actually indicate slight inhibition by rifampicin, the authors concluded that this antibiotic had no effect on EDTA-induced autolysis. Had they tried higher

concentrations of rifampicin they would perhaps have found a more prominent inhibition of EDTA-induced autolysis.

Our results show that rifampicin, nalidixic acid and novobiocin strongly inhibit EDTA-induced autolysis in E. coli; streptomycin shows only a slight inhibition. By contrast, chloramphenicol shows slight stimulation of the autolysis. Lack of inhibition by chloramphenicol and only a slight inhibition by streptomycin clearly shows that protein synthesis is not required for EDTA-induced autolysis.

How do rifampicin, nalidixic acid and novobiocin inhibit EDTA-induced autolysis? The fact that rifampicin-resistant and nalidixic acid-resistant mutants of E. coli are resistant to the inhibitory effect of the respective antibiotics on EDTA-induced autolysis, suggests that RNA polymerase and topoisomerase II activities are somehow required for EDTA-induced autolysis. Although we have not mapped the mutations, we have shown the absence of inhibition in several independently isolated rifampicin-resistant and nalidixic acid-resistant mutants. According to Miller (3), all rifampicin-resistant and nalidixic acid-resistant mutants of E. coli, resistant to high concentrations of the antibiotics, are RNA polymerase and topoisomerase mutants respectively. In addition, the fact that nalidixic acid and novobiocin, which inhibit topoisomerase II by interacting with different subunits of the enzyme (5) behaved similarly with regard to their inhibitory effect on autolysis, supports the contention that topoisomerase II activity is required for EDTA-induced autolysis.

It is known that inhibition of topoisomerase II activity by nalidixic acid or novobiocin results in inhibition of replication (5). Inhibition of RNA polymerase activity by rifampicin also results in inhibition of DNA synthesis (7). However, it seems unlikely that the inhibition of replication of DNA by these antibiotics is responsible for their inhibitory effect on EDTA-induced autolysis, in view of our observation that chloramphenicol, which also inhibits chromosomal replication in E. coli (7), had no effect on EDTA-induced autolysis.

The primary effect of rifampicin is inhibition of RNA synthesis. Although nalidixic acid does not inhibit RNA synthesis primarily, both nalidixic acid and novobiocin have been shown to inhibit transcription of certain genes in E. coli

(5,8). Considering these facts, it seems reasonable to hypothesise that nalidixic acid, novobiocin and rifampicin exert their inhibitory effect on EDTA-induced autolysis by inhibiting the synthesis of certain RNA(s). Since the inhibition of translation, obtained by treatment with chloramphenicol, had no effect on EDTA-induced autolysis (at least for several hours), the above hypothesis would imply that EDTA-induced autolysis either involves one or more RNAs or is coupled to the transcription of certain, as yet unidentified, genes.

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