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GUANOSINE-5'-DIPHOSPHATE-3'-DIPHOSPHATE INHIBITS THE IN VITRO SYNTHESIS OF β-LACTAMASE FROM pBR322 DNA

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SUMMARY

The in vitro synthesis of β-lactamase directed by pBR322 DNA is inhibited by guanosine-5'-diphosphate-3'-diphosphate.

INTRODUCTION

Amino acid starvation in E. coli results in the rapid inhibition of the synthesis of ribosomal RNA, tRNA, ribosomal proteins and elongation factors (1,2). This response to amino acid starvation is referred to as the "stringent effect" and is believed to be mediated by guanosine 5'-diphosphate 3'-diphosphate (ppGpp). This unique nucleotide functions at the level of transcription (1,2) although the exact mechanism by which ppGpp alters the specificity of RNA polymerase remains unknown. There is evidence that ppGpp affects initiation of transcription (3-8) as well as chain elongation (9).

A previous study has shown that ppGpp stimulates the in vitro synthesis of chloramphenicol acetyltransferase directed by P1CM DNA (10). However, little else is known about the effect of ppGpp on the expression of other non-chromosomal prokaryotic genes. The present study shows that the DNA dependent in vitro synthesis of β-lactamase is inhibited by ppGpp.

MATERIALS AND METHODS

The isolation of plasmid DNA was carried out as described elsewhere (11). The preparation from E. coli Z19i^{q} (provided by G. Zubay, Columbia University) of the ribosomal wash, washed ribosomes and DEAE salt eluate has been reported previously (12,13). ppGpp was purchased from P.L. laboratories, Milwaukee, Wisconsin.

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DNA-directed in vitro protein synthesis. The incubation components and conditions are essentially as described previously (13). The complete system (35 µl) contained 10 mM Tris-acetate (pH 8.0), 10 mM Na-dimethylglutarate (pH 6.0), 30 mM NH_4-acetate, 2 mM dithiothreitol, 9 mM Mg acetate, 2.9 mM ATP, 0.7 mM UTP, CTP and GTP, 29 mM phosphoenolpyruvate, 0.5 µg of pyruvate kinase, 29 mM K-acetate, 0.8 mM spermidine, 1.25 mg of polyethylene glycol 6000, 160 pmoles of $N^{5,10}$-methenyl-H_4-folate, 25 µg of E. coli B tRNA, 0.12 mM each of 19 amino acids (minus methionine), 1 nmol of methionine and 20 pmol of L-[^{35}S]methionine (Amersham 1210 Ci/mmol), 15 pmoles of NH_4Cl-washed ribosomes and saturating amounts of pBR322 DNA as template (2.8 µg). The protein factors for the in vitro protein synthesizing system were supplied by the addition of 13.4 µg of ribosomal salt wash, 157 µg of 0.25 M DEAE salt eluate (12) and 0.8 µg of E. coli RNA polymerase. The reaction mixtures were incubated in the presence or absence of ppGpp at 37°C for 50 min.

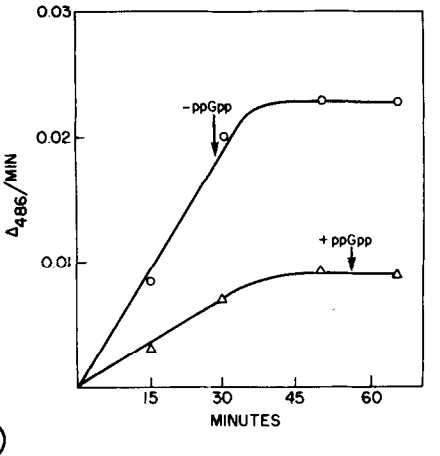
Assay for β-lactamase activity. Aliquots of the reaction mixtures (15 µl) were assayed for β-lactamase activity using a chromogenic cephalosporin substrate by a previously described procedure (14) with the following modifications. The final volume of the incubations was 0.5 ml and the substrate concentration was 0.04 mM. The results are expressed as the change in absorbancy at 486 nm per minute.

Slab gel electrophoresis. 5 µl aliquots of the incubations were removed for analysis of the radioactive products by radiofluorography after slab gel electrophoresis on 15% polyacrylamide gels in the presence of 0.1% sodium dodecyl sulfate.

RESULTS AND DISCUSSION

During studies on the effect of ppGpp on the in vitro expression of ribosomal protein genes directed by the plasmid pNF1337 (15), it was noted in control studies, in which pBR322 DNA was used as template, that the synthesis of a gene product of about 32,000 daltons was inhibited by ppGpp. From the molecular weight, it appeared that this gene product was the precursor of β-lactamase (16). Based on this preliminary finding, the effect of ppGpp on the in vitro synthesis of β-lactamase directed by pBR322 was investigated further. For these studies, the β-lactamase activity was assayed colometrically (14). Figure 1 shows the time course for the in vitro synthesis of β-lactamase in the presence or absence of ppGpp. Synthesis is linear for 30 min and in the presence of 200 µM ppGpp there is about a 60% inhibition of β-lactamase formation.

Figure 2 shows the effect of increasing ppGpp and GDP concentrations on the synthesis of β-lactamase. GDP which is structurally related to ppGpp is without effect on the in vitro synthesis of β-lactamase whereas as



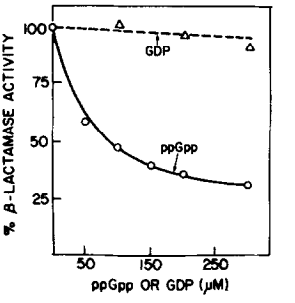


Fig. 1: Time course of the DNA-dependent in vitro synthesis of β-lactamase in the presence (Δ) or absence (0) of 200 μM ppGpp. A 15 μl aliquot of the incubation was assayed for β-lactamase activity.

Fig. 2: The effect of ppGpp and GDP on the in vitro synthesis of β-lactamase. Various concentrations of ppGpp (0) or GDP (Δ) were added to the reaction mixtures and after 50 min of incubation the amount of β-lactamase synthesized was determined in a 15 μl aliquot (see Methods).

little as 50 μM ppGpp gives significant inhibition of β-lactamase synthesis. The concentration of ppGpp used to obtain inhibition in these in vitro studies is similar to that previously shown to inhibit ribosomal protein synthesis under similar conditions (15). However, the in vitro synthesis of ribulose-bisphosphate carboxylase, directed by the plasmid pSoe3101 (17) that carries the spinach chloroplast gene for this protein, was not inhibited by ppGpp (data not shown).

As indicated above, the effect of ppGpp on the in vitro synthesis of β-lactamase could be demonstrated by slab gel analysis of the products formed. As seen in Figure 3 (Lane 2, control), the major [^{35}S] protein product synthesized in vitro is a protein of approximately 32,000 daltons that is consistent with the product being the precursor of β-lactamase (16). In the presence of ppGpp (100 μM and 200 μM, Lanes 3 and 4), there is a marked drop in the amount of this product formed whereas in the presence of 200 μM GDP there is no inhibition of β-lactamase synthesis (Lane 5).

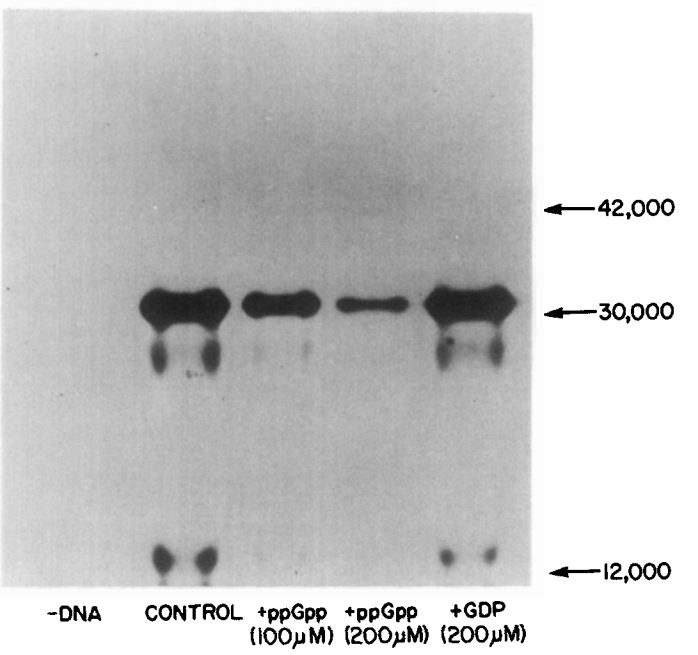


Fig. 3: Slab gel electrophoresis of the in vitro synthesized products. The reaction mixtures were incubated in the absence (Lane 2) or presence of 100 μM and 200 μM ppGpp and 200 μM GDP. Aliquots of the reaction mixtures were removed and slab gel analysis performed (see Methods).

The E. coli chromosomal β-lactamase operon contains an attenuator region that is involved in the cell's ability to coordinate the synthesis of β-lactamase with growth rate (18). The coordination between growth rate and synthesis has also been observed with other E. coli factors, e.g. ribosomal proteins, elongation factors, and rRNA. The synthesis of these components is affected by ppGpp (stringent control) and the present study indicates that the synthesis of β-lactamase from a plasmid gene is also regulated by ppGpp.

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