INHIBITION OF E. COLI β-HYDROXYDECANOYL THIOESTER DEHYDRASE BY ppGpp

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SUMMARY: Guanosine-5'-diphosphate-3'-diphosphate was found to inhibit β-hydroxydecanoyl thioester dehydrase of E. coli at concentrations corresponding to those generated in vivo during amino acid starvation of rel$^+$ cells.

INTRODUCTION

In bacterial cells a regulatory mechanism termed stringent control reduces the rate of RNA accumulation whenever protein synthesis is limited by the absence of any required amino acid (1,2) or by the failure to activate an amino acid (3). Two nucleotides, ppGpp and pppGpp*, have been implicated as the mediators of this stringent control (4,5). These compounds coordinate a general inhibition of macromolecular synthesis, and of several other enzymatic processes (6-10).

β-hydroxydecanoyl thioester dehydrase is the enzyme that furnishes the initial olefinic products for elongation to the long-chain unsaturated fatty acids in E. coli (11). We were involved in an investigation of inhibitors of the E. coli dehydrase when Lane and coworkers (12) reported that stringent control is exercised at the level of fatty acid synthesis, and that ppGpp specifically inhibited the carboxytransferase component of acetyl-CoA carboxylase from E. coli. Since dehydrase is essential for the synthesis of long-chain unsaturated fatty acids, we have tested ppGpp as a negative effector of this enzyme.

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* The abbreviations are: ppGpp, guanosine 5'-diphosphate-3' diphosphate , pppGpp, guanosine 5'-triphosphate-3' diphosphate , and NAC, N-acetylcysteamine.

MATERIAL AND METHODS

Nucleotides were purchased from PL Biochemicals. ppGpp was a gift from Ricardo Bloch of the Biological Laboratories. Dehydrase was purified from E. coli B cells as described previously (13).

The enzyme was assayed spectrophotometrically at 25° on a Cary 118 spectrophotometer by measuring the increase in absorbance at 263 nm with time after addition of cis-3-decenoyl-NAC*. The assays were performed in a total volume of 0.25 ml in 1 mm path-length cuvettes. Each assay contained 0.03 nmoles (1.1 ug) dehydrase in 0.01 M sodium phosphate, pH 7.2, nucleotides, and 2.44 x 10^{-4} M cis-3-decenoyl-NAC. These additions were made in the order: buffer, nucleotide, enzyme and substrate, which were mixed quickly and then transferred to the cuvette with a 0.5 ml syringe, fitted with a 2 inch #20 needle.

RESULTS AND DISCUSSION

In addition to ppGpp, several other nucleotides were examined for their effect on dehydrase activity. The spectrophotometric assays had to be carried out in unusually small volumes due to the high absorbance of most nucleotides at the wavelength where dehydrase activity is monitored (263 nm). At these high absorbance levels, artifactual inhibitory effects can be observed due to the presence of stray light (14,15). The use of cuvettes of 1 mm path length negates this effect by decreasing the total absorbance of the solutions in which the assays are performed.

Figure 1 demonstrates a significant inhibitory effect of ppGpp on dehydrase. The other nucleotides tested, AMP, GMP, GDP, UDP and CTP had little effect; at a concentration of 3 mM less than 20% inhibition occurred. The inhibitory effect of ppGpp, on the other hand, is already pronounced at 0.5 mM, and at the highest concentration tested, 2.5 mM, there was a 60% inhibition of enzyme activity.

The fact that relatively high concentrations of ppGpp were necessary for inhibition is consistent with Lane's observation (12) that ppGpp gave maximal

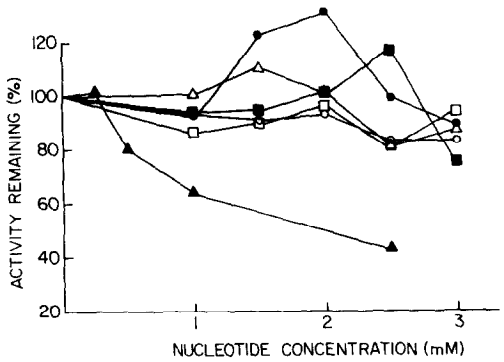

Figure 1. Dehydrase activity in the presence of increasing concentrations of various nucleotides. With the exception of ppGpp, all values represent the average of two or more assays. All activities are expressed relative to control samples containing no nucleotides. Symbols: (□), AMP; (○), GMP; (●), GDP, (△), UDP; (■), CTP; (▲), ppGpp.

inhibition of acetyl-CoA carboxylase at concentrations of 1-2 mM. Significantly, maximal inhibition of these reactions is achieved at concentrations of ppGpp which correspond to those generated *in vivo* (up to 4 mM) during amino acid starvation of *rel*$^+$ cells (15). The finding that ppGpp inhibits dehydrase at physiological concentrations follows reports of its inhibition of the synthesis of lipids (7), glycolytic intermediates (6), nucleotides (10), the uptake of exogenous metabolites (8), and four other enzymes, acetyl-CoA carboxylase (12), RNA polymerase (9), and both adenine and guanine phosphoribosyltransferases (16). Many biosynthetic processes may therefore be under coordinate control in the microbial cell.

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