Interactions of \(N^6-(\Delta^2\text{-Isopentenyl})\)adenine with Cyclic AMP on the Regulation of Growth and \(\beta\)-Galactosidase Synthesis in \(Escherichia coli\)

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INTRODUCTION

Cyclic adenosine 3':5'-monophosphate (cyclic AMP) has a marked inhibitory action on the growth of \(Escherichia coli\) (Judewicz, De Robertis & Torres, 1973), and it also affects \(lac\)-operon expression and \(\beta\)-galactosidase synthesis in the organism (Ullmann & Monod, 1968; Perlman & Pastan, 1968; Perlman, De Combrugghe & Pastan, 1969). These two phenomena may be considered as typical and reproducible responses of some \(E. coli\) strains to cyclic AMP.

During studies of the role of cytokinins (\(N^6\)-derivatives of adenine) in microbial metabolism, we obtained experimental evidence which suggested that there may be an interaction between cytokinins and cyclic AMP (Coppola, Zoina & Marino, 1975b): cyclic AMP concentrations in \(E. coli\) were halved if cytokinin was added to the culture medium. Gallo et al. (1972) have suggested that adenine \(N^6\)-derivatives can act through the cyclic AMP system, while Hecht, Faulkner & Hawrelak (1974) and Kull & Kuhn (1975) have shown that cytokinins can influence cyclic AMP metabolism in higher organisms. Therefore, it seemed interesting to test the capacity of exogenous cytokinin to modify the effects of cyclic AMP on growth and \(\beta\)-galactosidase biosynthesis in \(E. coli\). Our results show that \(N^6-(\Delta^2\text{-isopentenyl})\)adenine (2iP), a cytokinin found in \(E. coli\), can, at \(10^{-8}\) M, overcome the effects of 5 mM-cyclic AMP.

METHODS

Organism. \(Escherichia coli\), strain b/b, kindly provided by Dr M. L. Barnett, University of Cambridge, was used.

Substrate and growth conditions. Bacteria were grown aerobically at 37 °C in AG minimal medium (Judewicz, De Robertis & Torres, 1973) containing (per litre): \(K_2HPO_4\), 14 g; \(KH_2PO_4\), 6 g; \((NH_4)_2SO_4\), 2 g; \(MgSO_4\), 0·2 g; and glucose, 50 mM, or glycerol, 68 mM, as carbon source. A stationary-phase population was used to inoculate (to a final cell density of 1·0 \(\times\) 10⁷ cells ml⁻¹) four types of media: (i) minimal medium; (ii) minimal medium + 5 mM-cyclic AMP; (iii) minimal medium + \(10^{-6}\) M-2iP; and (iv) minimal medium + 5 mM-cyclic AMP + 2iP (\(10^{-6}\) to \(10^{-9}\) M).

Bacterial growth was measured by recording the increase in extinction at 680 nm, and by direct counts in a Petroff–Hauser chamber at 30 min intervals.

\(\beta\)-Galactosidase assay. The rate of enzyme synthesis was determined in samples removed from exponentially-growing cultures, after induction with 0·5 mM-isopropyl-\(\beta\)-D-thiogalactoside. \(\beta\)-Galactosidase was assayed in toluenized cells according to Wallenfels'...

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Fig. 1. The effects of cyclic AMP and 2iP on the growth of E. coli B/b. Cultures were grown, as described in Methods, in the following media: AG medium, ○; AG minimal medium + 5 mM cyclic AMP, ●; AG minimal medium + 5 mM cyclic AMP + 10⁻⁸ M-2iP, □; AG minimal medium + 5 mM cyclic AMP + 10⁻⁷ M-2iP, ■; AG minimal medium + 5 mM cyclic AMP + 10⁻⁶ M-2iP, △; AG minimal medium + 10⁻⁶ M-2iP, ▲.

method (1962). (One unit of enzyme activity is defined as the amount of enzyme that hydrolyses 1 μmol substrate in 1 min under standard conditions.) Protein was determined by the method of Schacterle & Pollack (1973).

RESULTS AND DISCUSSION

The growth of E. coli B/b, Hfr 3000 and other strains, reported by Judewicz et al. (1973), was strongly inhibited by 5 mM-cyclic AMP in the medium. The presence of cytokinin at concentrations in the range 10⁻⁸ to 10⁻⁶ M did not affect the growth of E. coli (Supniewski et al., 1957; Kernell, 1960; Coppola et al., 1972), but when 2iP was added to cultures inhibited by cyclic AMP, the inhibition was overcome (Fig. 1). The relief of inhibition was proportional to the concentration of the cytokinin. No inhibition by cyclic AMP was observed when E. coli was grown in the presence of 10⁻⁶ M-2iP.

The following observations were made concerning the ability of the cytokinin to modify the effects of cyclic AMP on β-galactosidase biosynthesis: (1) E. coli strain B/b, a catabolite repression sensitive strain, produced 69.9 units (mg protein)⁻¹ when grown on 50 mM-glucose as carbon source, and 240.5 units (mg protein)⁻¹ on 68 mM-glycerol. (2) Addition of 5 mM-cyclic AMP to the minimal medium supplemented with glucose derepressed β-galactosidase synthesis and 225.9 units (mg protein)⁻¹ were produced. (3) N⁶-(Δ²-isopentenyl)adenine strongly reduced the enzyme induction promoted by cyclic AMP: 125.1 units (mg protein)⁻¹ were produced in the presence of 5 mM-cyclic AMP + 10⁻⁶ M-2iP. Enzyme synthesis and bacterial growth were not modified by the cytokinin alone: control (minimal medium), 66.9 units (mg protein)⁻¹; minimal medium + 10⁻⁶ M-2iP, 69.7 units (mg protein)⁻¹.

Concentrations of about 1 mM-cyclic AMP are reported to be necessary both to overcome glucose repression of β-galactosidase synthesis and to inhibit growth of E. coli (Perlman & Pastan, 1968; Judewicz et al., 1973; De Robertis, Judewicz & Torres, 1973). In addition to
the above mentioned data, we found that 2iP, at concentrations of $10^{-6}$ to $10^{-7}$ M, overcame the effects of cyclic AMP at every concentration at which cyclic AMP was capable of modifying growth and catabolite repression in *E. coli* b/b.

In different strains of *E. coli*, De Robertis et al. (1973) have shown that certain intracellular concentrations of cyclic AMP are needed for optimal growth, while higher concentrations of the nucleotide inhibit growth. The inhibitory nucleotide concentrations vary with the different intracellular concentrations of cyclic AMP. Thus there is a balance between cellular functions and growth rate, correlated with cyclic AMP concentrations. N6-(2-isopentenyl)adenine can interfere with this balance, and the interference seems to occur through an effect exerted on the regulation of intracellular levels of cyclic AMP. As suggested by our other findings (Coppola, Marino & Zoina, 1975a), this action probably occurs through a stimulation of cyclic AMP degradation, i.e. promoting the activity rather than the biosynthesis of cyclic AMP-phosphodiesterase. An experimental verification of this hypothesis is in progress.

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REFERENCES


