Stimulation of Galactokinase Synthesis in *Escherichia coli* by Adenosine 3', 5'-Cyclic Monophosphate

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Adenosine 3', 5'-cyclic monophosphate (cyAMP) stimulates the rate of synthesis of galactokinase in glycerol-grown *Escherichia coli* both when production of the enzyme is induced by D-fucose and when it is repressed by glucose in the presence of inducer. cyAMP also stimulates the synthesis of galactokinase in constitutive strains B78A (R-) and R10 (O+), and overcomes the transient repression of galactokinase synthesis caused by glucose.

Makman and Sutherland (4) demonstrated the production of adenosine 3', 5'-cyclic monophosphate (cyAMP) in *Escherichia coli*. The enzyme, adenyl cyclase, which catalyzes the formation of this cyclic nucleotide, was subsequently isolated from *E. coli* by Ide (2) and by Tao and Lipmann (10). Recently, Pastan and his co-workers (6-9) and Ullman and Monod (11) have shown that the nucleotide can reverse catabolite repression (3) of the inducible enzymes, β-galactosidase and tryptophanase. It was of interest to determine whether this action of cyAMP is a general phenomenon in catabolite repression. We wish to report experiments on the stimulatory effect of cyAMP on another inducible enzyme, galactokinase of the galactose operon. While this work was in progress, a similar observation was reported by Perlman et al. (8).

MATERIALS AND METHODS

D-Fucose and D-galactose were purchased from Sigma Chemical Co., St. Louis, Mo., and D-galactose-14C was purchased from New England Nuclear Corp., Boston, Mass. Crooke's strain (ATCC 8739) of *E. coli* was supplied by the American Type Culture Collection. Strain B78A (R-) was given to us by Herman Kalckar, and strain R10 (O+) by Peter Starlinger.

The medium for cell cultivation contained 14 g of KH_2PO_4, 6 g of KH_2PO_4, 2 g of (NH_4)_2SO_4, 0.2 g of MgSO_4·7H_2O, and 5 ml of glycerol per liter of distilled water. The microorganisms were grown overnight with shaking at 37 C. Before each experiment, the overnight culture was diluted with, or centrifuged and resuspended in, fresh medium. Galactokinase was induced by the addition of 5 mM d-fucose to 10 ml of the cell suspension (about 10^8 cells/ml) growing at 37 C. Samples were withdrawn at different time intervals; they were diluted with a buffer containing 0.01 M tris(hydroxymethyl)aminomethane-HCl, 1 mM dithiothreitol, and 30 µg of chloramphenicol per ml, and were kept at 0 C. Galactokinase activity was determined by the method of Williams and Paigen (12). Enzyme activity is expressed as nanomoles of galactose-1-P formed per hour at 37 C.

RESULTS AND DISCUSSION

The growth of Crooke's strain of *E. coli* in the presence and in the absence of cyAMP is shown in Fig. 1. In the experiments reported, cyAMP did not affect the rate of growth of any of the organisms used. Pastan and Perlman have reported a similar observation for other strains of *E. coli* (6). Figure 2 shows the effect of cyAMP on galactokinase induction by D-fucose in Crooke's strain of *E. coli* grown in glycerol medium. The stimulation of induced galactokinase synthesis caused by the addition of 1 mM cyAMP ranged from 30 to 60% of the control rate.

Glucose, added simultaneously with inducer, repressed the production of enzymes required for galactose utilization. The inhibition of induced galactokinase synthesis by glucose could be partially reversed by cyAMP. Figure 3 shows that, initially, little enzyme synthesis occurred. However, the rate of synthesis increased after about 1 hr of induction. cyAMP more than doubled the rate of synthesis of galactokinase in the presence of glucose. The prolonged lag phase is probably due to the time required for inducer accumulation. Adhya and Echols (1) have shown that glucose interferes with the uptake of inducer into the cells.

To exclude the possibility that cyAMP might also affect the uptake of inducer, we have further studied the action of this cyclic nucleotide on constitutive mutants by the use of repressor (R-) and operator (O+) strains where an inducer
X8047 to be as high as 10^{-1} M. The discrepancy may be due to the difference in permeability to the cyclic nucleotide of various strains of E. coli. It is also possible that the distribution of cyAMP phosphodiesterase in different strains

FIG. 1. Growth of Crooke's strain of E. coli in the presence and in the absence of cyAMP. The cells were grown in minimal salt medium containing 0.5% glucose alone (○) or in the presence of 5 mM cyAMP (●). Similar results were obtained for E. coli strains B78A and R-10.

FIG. 2. Effect of cyAMP on galactokinase induction in Crooke's strain. The organism was grown in glycerol medium. At zero time, 5 mM inducer D-fucose was added together with (or without) 1 mM cyAMP.

is not required for galactokinase synthesis. cyAMP also stimulated the synthesis of galactokinase in strain B78A (R^-), the stimulation ranging from 30 to 60% over the control rate (Fig. 4). A similar observation was obtained when an Oe mutant was used. That this should occur in constitutive strains of E. coli also suggests that the nucleotide does not act on the repressor-operator interaction.

Figure 5 shows how the rate of galactokinase synthesis in strain B78A (R^-) varies when different cyAMP concentrations are used. The optimum was found to be at 10^{-3} M. Recently, Perlman et al. (8) reported the concentration of cyAMP required for maximal stimulation of galactokinase synthesis in strains X7700 and

FIG. 3. Effects of cyAMP and glucose on galactokinase induction in Crooke's strain. The organism was grown in glycerol medium. At zero time, 10 mM glucose and 5 mM D-fucose were added together with (or without) 1 mM cyAMP.

FIG. 4. Effect of cyAMP on galactokinase synthesis in strain B78A (R^-). The organism was grown in glycerol medium. At zero time, 5 mM cyAMP was added to one of the samples and distilled water was added to the other.
of bacteria may influence the concentration required for maximal stimulation.

The addition of glucose to E. coli cells grown in glycerol leads to a transient repression of galactokinase synthesis (5). The effect of cyAMP on transient repression was studied with constitutive strains of E. coli. As shown in Fig. 6, when glucose was added to strain B78A (R-) growing in glycerol, a severe transient repression of galactokinase synthesis occurred which lasted about 30 min, at which time enzyme synthesis resumed at a slower rate. However, cyAMP not only reversed glucose repression but, at 5 mM, caused an enzyme synthesis greater than that of the control and similar to that observed in glycerol medium. A similar result was obtained with the O+ mutant of E. coli (Fig. 7). As Makman and Sutherland (4) have shown, glucose causes the rapid excretion of the cyclic nucleotide into the medium when it is added to cells with high initial content of cyAMP. It is possible that the phenomenon of transient repression is in part due to the sudden lowering of the concentration of cyAMP in the cells.

Since cyAMP acts on repressor-negative as well as operator-constitutive mutants, one can conclude that repressor-operator interactions are not involved, nor are there changes in the cellular uptake of inducer. These results confirm the findings of others and suggest that cyAMP may play a general role in catabolite and transient repression in E. coli. However, the molecular mechanism of action of cyAMP still remains to be determined.

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LITERATURE CITED


