THE EFFECT OF AMINO ACIDS ON THE ABILITY OF CYCLIC AMP TO REVERSE CATABOLITE REPRESSION IN ESCHERICHIA COLI

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Pastan and Perlman (1968) reported that 1 mM adenosine-3',5'-cyclic phosphate (cyclic AMP) completely abolished transient glucose repression of β-galactosidase synthesis in Escherichia coli but had little or no effect on permanent catabolite repression. These authors found later that permanent repression could also be overcome by cyclic AMP if concentrations in the range of 5 mM were used. These findings conflicted with results from experiments conducted in our laboratory which showed that 1 mM cyclic AMP was sufficient to completely abolish permanent catabolite repression produced by glucose or N-acetylglucosamine-6-phosphate (Goldenbaum et al., 1969). Since strains of E. coli K12 were used in all of these studies, and since comparable basal mineral salts media were employed, we felt that the observed difference could possibly be accounted for by some minor alteration in experimental conditions. In our experiments growth was measured by the rate of assimilation of ^{14}C-L-leucine into the cells using 5 x 10^{-4}M L-leucine as a cold carrier while Perlman and Pastan (1968) used culture absorbancy changes as their measure of cell growth. Quite surprisingly, we found that the presence of L-leucine in our culture mediu did in fact account for these apparent discrepancies. The results of a preliminary examination of these findings are presented in this communication.

Materials and Methods -- Isopropyl-β-D-thiogalactoside (IPTG) and O-nitrophenyl-β-D-galactoside (ONPG) were purchased from Mann Research Laboratories. Cyclic 3',5'-AMP (free acid) was purchased from Schwarz BioResearch, Inc.

E. coli K12-701 (White, 1968) was used in this study, and was grown with vigorous aeration at 37°. Cultures were grown overnight in a mineral salts medium (Okinaka and Dobrogosz, 1967) containing a 0.01 M glucose. These cultures were harvested by centrifugation, suspended in 0.05 M sodium phosphate buffer (pH 7.5) and then inoculated into 20 ml of fresh medium (pH 7.2) containing (per liter): K_2HPO_4, 28 g; KH_2PO_4, 8.0 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $(NH_4)_2 SO_4$, 1 g. Glucose (0.02 M) was added just prior to inoculation. After 2 hours of incubation under these conditions the cells were in exponential growth and had a mass of approximately 100 μg cell dry weight/ml of culture. IPTG (2.5×10^{-3}M) and the amino acids to be tested were added at this time and β-galactosidase measurements were initiated. Cyclic AMP was added 40 min after the addition of the inducer. β-Galactosidase was measured as previously described using ONPG as substrate and toluenized cells as enzyme source (Dobrogosz, 1965) and is expressed as the differential rates of enzyme synthesis using cell dry weight as the growth parameter. One unit of enzyme is defined as that amount of enzyme that hydrolyzed 1 μmole of ONPG/hr at 30°C in a reaction mixture containing 2×10^{-3}M ONPG, 1.24×10^{-4}M reduced glutathione, and 0.05 M sodium phosphate buffer, pH 7.5.

Results -- The rapid rate of β-galactosidase synthesis observed when E. coli is grown in a mineral salts medium containing succinate as a carbon and energy source is repressed approximately 50% when glucose serves as the substrate in place of succinate. This catabolite repression by glucose can be overcome by the addition of cyclic AMP as shown by that data summarized in Figure 1. Concentrations of cyclic AMP as high as 5 mM or higher were needed to abolish glucose repression under these conditions. We observed that this

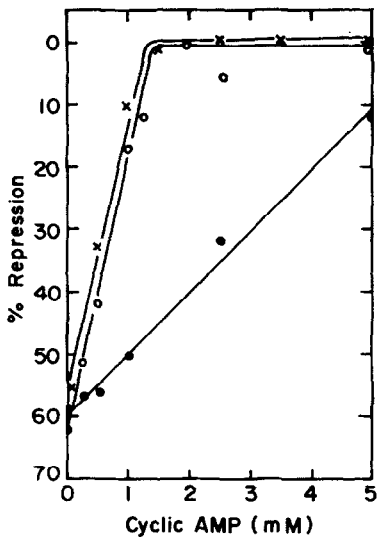

Figure 1. Effect of L-leucine and L-alanine on the ability of cyclic AMP to reverse permanent glucose repression of β-galactosidase synthesis in E. coli.

The differential rate of enzyme formation calculated when cells were grown in medium containing 0.04 M succinate was used to indicate zero percent repression. In the experiment shown here, the cells were grown in medium containing 0.02 M glucose, 2.5×10^{-3} M IPTG and the indicated concentrations of cyclic AMP. The differential rate of β-galactosidase synthesis was measured during an 80 minute period following the addition of cyclic AMP. One culture was grown in unsupplemented minimal medium (•—•); a second culture contained 125 μM L-leucine (o—o); a third culture contained 125 μM L-alanine (x—x).

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catabolite repression could be reversed in the presence of only 1-1.5 mM levels of cyclic AMP if either L-leucine or L-alanine was included in the culture medium. When tested under identical conditions none of the other amino acids listed in Table 1 appeared to have influence on the ability of cyclic AMP to overcome glucose repression. The effect of L-valine could not be tested in this connection because of the severe growth inhibition that resulted when it was added to the medium.

While the additions of L-alanine in concentrations as high as 2.5 mM had no effect on growth of these cultures, some growth inhibition was observed when L-leucine was added. Some inhibition of growth was also detected when the higher concentrations (e.g., 5 mM) of cyclic AMP were used. Preliminary studies have shown that less cyclic AMP is required to produce growth

Table 1. Effect of various amino acids on the ability of cyclic AMP to reverse glucose repression.

Additions*	% De-repression
None	7
L-aspartate	6
L-glutamate	14
L-glycine	11
L-isoleucine	9
L-serine	10
L-threonine	9
L-methionine	10
L-cysteine	8
L-cystine	9
L-lysine	11
L-arginine	11
L-histidine	9
L-phenylalanine	11
L-tryptophan	6
L-tyrosine	5
L-proline	9

*All amino acids were added to the culture medium at a final concentration of 125 μM except L-cystine which was added at a final concentration of 50 μM. Cyclic AMP at a concentration of 1 mM was included in the medium in each case. In all other aspects these experiments were conducted as described in Figure 1.

inhibition when L-leucine or L-alanine are present than when they are absent from the culture medium. This observation indicated to us that these amino acids could possibly enhance the uptake of cyclic AMP into these cells and thereby account for their influence on the ability of cyclic AMP to reverse

catabolite repression. Studies along this line are in progress at the present time.

Conclusions -- The amount of cyclic AMP needed to reverse permanent catabolite repression has been reported to vary with the bacterial strain employed, the nature of the repressor substrate, and the enzyme system whose repression is being measured (De Crombrugghe et al., 1969; Perlman et al., 1969; Goldenbaum and Dobrogosz, 1968). On the basis of the results described in the present communication, we can now conclude that even seemingly subtle alterations in the composition of the basal medium can also have a significant influence on responsiveness of cells to exogenous cyclic AMP. The amount of cyclic AMP required to abolish permanent glucose repression can be significantly decreased by including low levels of L-leucine or L-alanine in the culture medium. We have very little information at the present time concerning either the nature of the "sparing" effect of these amino acids or why the other amino acids tested appeared to play no role in this regard. We can only speculate at the moment that these two amino acids may somehow enhance the permeability of the cells resulting in a more efficient uptake of this cyclic nucleotide.

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