THE EFFECT OF CYCLIC 3',5'-AMP ON CATABOLITE REPRESSION OF β-GALACTOSIDASE SYNTHESIS IN ESCHERICHIA COLI

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Perlman and Pastan (1968) reported that cyclic 3',5'-adenosine monophosphate (cyclic 3',5'-AMP) reversed glucose repression of β-galactosidase synthesis when added to ethylenediamine tetraacetic acid (EDTA)-treated cultures of Crooke's strain of Escherichia coli. This finding was corroborated in our laboratory using a K12 strain of E. coli. Catabolite repression by glucose of β-galactosidase synthesis in this strain was reversed to a great extent by the addition of cyclic 3',5'-AMP. If, however, glucose-6-phosphate (G-6-P) or a combination of glucose plus gluconate was the repressor source instead of glucose, cyclic 3',5'-AMP had only limited ability to reverse catabolite repression. This suggests that cyclic 3',5'-AMP functions at a catabolic level effecting the conversion of glucose to G-6-P, and not at a level involving the transcriptional or translational events that govern β-galactosidase synthesis.

Materials and Methods -- Isopropyl-β-D-thiogalactoside (IPTG), O-nitrophenyl-β-D-galactoside (ONPG), G-6-P (disodium), and cyclic 3',5'-AMP (free acid) were purchased from Mann Research Laboratories. L-leucine-^{14}C (u.l.) was purchased from New England Nuclear Corp. 2,5-Diphenyloxazole (PPO) and 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) were products of Packard Inst. Co.

E. coli K12-701 (White, 1968) was used in this study, and grown at all times with aeration at 37°. Cultures were grown overnight in a mineral salts medium (Okinaka and Dobrogosz, 1967) containing a 0.01 M concentration of

the particular substrate used in each experiment. These cultures were harvested by centrifugation, suspended in 0.05 M sodium phosphate buffer (pH 7.5) and then inoculated into 20 ml of the AG medium described by Perlman and Pastan (1968). The glycerol included in this medium by these workers was omitted in the present study. The cultures were allowed to grow for 120 minutes, and each flask was then harvested by centrifugation at room temperature, washed with a buffer containing 0.12 M tris (pH 8.0), 0.5% glycerol and 10^{-3}M sodium phosphate. The cells were then resuspended in 10 ml of this buffer, and incubated at 37°C for 2 minutes with 10^{-3}M EDTA (Leive, 1965). One ml of this suspension was then diluted into 20 ml of fresh, pre-warmed (37°) media containing the various additions described in Figure 1 and 2. The starting cell concentration was 30-70 μg dry weight/ml at this time and β-galactosidase synthesis was started. Growth was measured by the rate of

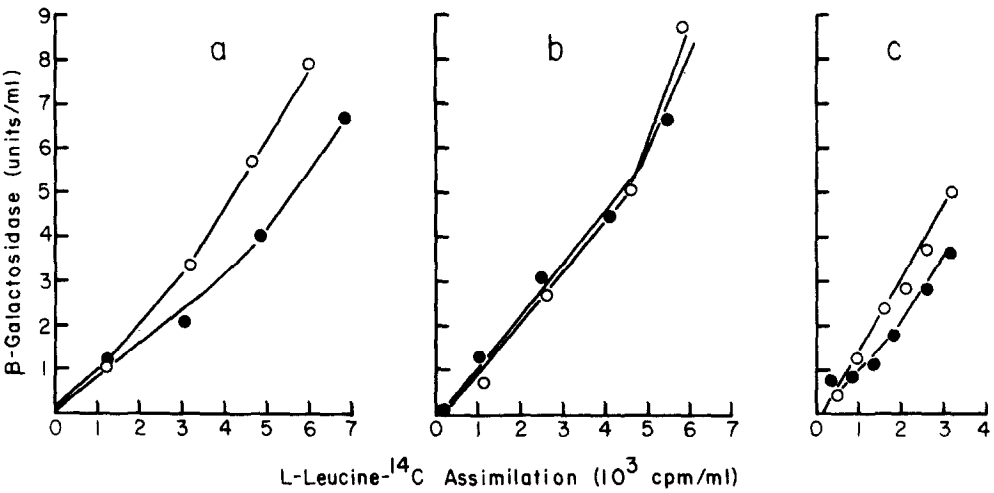

Figure 1. The effect of cyclic 3',5'-AMP on the rate of β-galactosidase synthesis during growth on weak repressor sources.

After EDTA-treatment cultures were inoculated into 20 ml of fresh media containing 0.04 M glycerol (a), 0.02 M fructose (b) and 0.04 M succinate (c). The media in each case also contained 2.5×10^{-3}M IPTG, 4×10^{-4}M L-leucine, and 2 μc L-leucine-^{14}C. Cultures on each substrate were grown with (o——o) and without (•——•) 10^{-3}M cyclic 3',5'-AMP. Samples were taken at 10 min. intervals for determination of units of β-galactosidase/ml of culture and cpm/ml of L-leucine-^{14}C (u.l.) incorporated into the 5% TCA insoluble cell fraction. The rate of β-galactosidase synthesis was plotted against the rate of L-leucine-^{14}C assimilation into the same culture.

incorporation of ^{14}C-leucine (u.l.) into cells that were collected and dried on membrane filters (Bac-T-Flex, type B-6 Schleicher and Schuell Co.) after extraction and washing with 5% trichloroacetic acid (TCA). Counting was done with a Packard Scintillation counter (Packard Inst. Co.) using a scintillation fluid composed of 0.40% PPO and 0.10% POPOP in toluene.

β-Galactosidase was induced by the addition of 2.5 x 10^{-3}M IPTG, and was assayed as previously described (Dobrogosz, 1965). One unit of enzyme was defined as the amount of enzyme that hydrolyzed 1 μmole of ONPG/hr at 30°C in the presence of 2 x 10^{-3}M ONPG, 1.24 x 10^{-4}M reduced glutathione, and 0.05 M sodium phosphate buffer, pH 7.5. Neither cyclic 3',5'-AMP nor G-6-P had any effect on β-galactosidase activity under the conditions used. The rate of

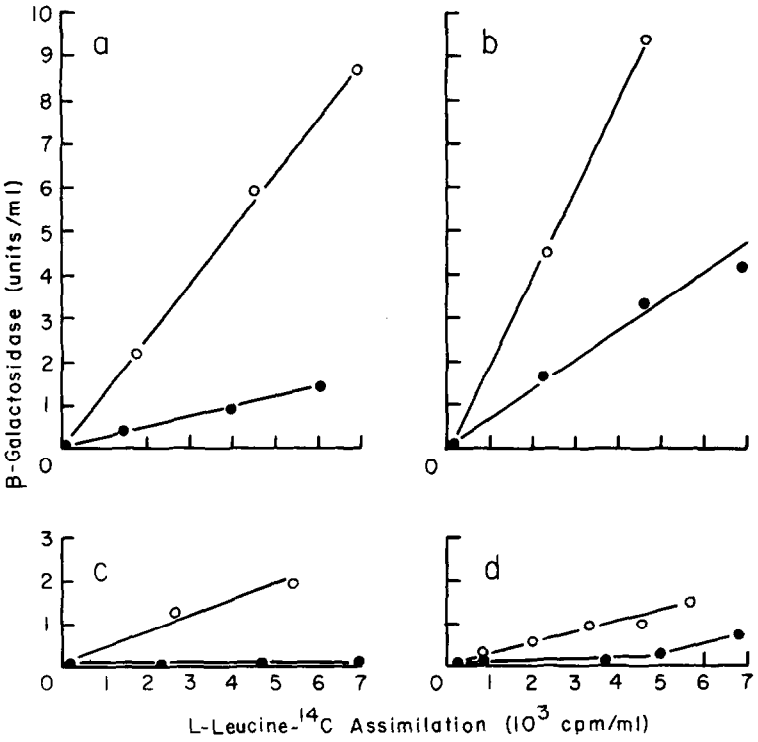

Figure 2. The effect of cyclic 3',5'-AMP on the rate of β-galactosidase synthesis during growth on strong repressor sources.

EDTA-treated cultures were inoculated into four flasks containing 0.02 M glucose (a), 0.02 M mannitol (b), 0.02 M glucose plus 0.02 M gluconate (c) and 0.86 x 10^{-2}M G-6-P (d). The cultures were grown as described in Figure 1 with (o—o) and without (•—•) 10^{-3}M cyclic 3',5'-AMP.

β-galactosidase synthesis was plotted against the rate of assimilation of leucine-^{14}C into cell protein.

Results -- Perlman and Pastan (1968) induced for β-galactosidase synthesis in a medium containing glycerol or glycerol plus glucose in order to determine the rates of enzyme synthesis under non-repressed and catabolite repressed conditions respectively. They observed that 10^{-3}M cyclic 3',5'-AMP stimulated the differential rate of β-galactosidase synthesis in cultures grown on a relatively weak repressor source such as glycerol. Similar results were obtained in this laboratory when E. coli K12 was grown in the presence of weak repressor sources as shown in Figure 1. Cyclic 3',5'-AMP stimulated the differential rate of enzyme synthesis during growth on glycerol and succinate, but did not stimulate enzyme synthesis during growth on fructose. All enzyme assays were made at 10 minute intervals up to 60 minutes after the EDTA treatment. During this period the cells were still recovering from their prior treatments, resulting in some variability (also noted by Perlman and Pastan, 1968) and non-linearity in the differential plots that were obtained under these conditions.

The results presented in Figure 2 showed the effect of cyclic 3',5'-AMP on the rate of enzyme synthesis during growth in the presence of substrates that produce catabolite repression. The strong repression produced by glucose and the repression exhibited by mannitol were both largely eliminated by addition of cyclic 3',5'-AMP. However, when the cells were grown on a combination of glucose plus gluconate, the addition of cyclic 3',5'-AMP resulted in only a partial reversal of repression, and when G-6-P was the repressor substrate, cyclic 3',5'-AMP produced very little reversal of catabolite repression. The rate of enzyme synthesis in the presence of G-6-P and cyclic 3',5'-AMP was no greater than that observed during normal glucose repression. On this basis it was concluded that although cyclic 3',5'-AMP can enhance the rate of β-galactosidase synthesis under certain conditions, it is unable to reverse catabolite repression.

Discussion -- Hsie and Rickenberg (1967) described a mutant of E. coli whose β-galactosidase system had become resistant to catabolite repression by glucose or gluconate. These authors found, however, that catabolite repression still occurred in this mutant if it was grown in the presence of G-6-P or a mixture of glucose and gluconate. Rickenberg, Hsie, and Janecek (1968) later showed that two catabolite repression resistant mutants of E. coli isolated by Loomis and Magasanik (1967) were indeed resistant to catabolite repression by glucose, but were fully sensitive to repression when G-6-P or a mixture of glucose and gluconate was included in the growth medium. Okinaka and Dobrogosz (1967) had pointed out earlier that whereas an anaerobic shock could temporarily reverse catabolite repression produced by glucose or gluconate when these substrates were included alone in the culture medium, this reversal did not occur if the cultures were grown in medium containing a combination of glucose plus gluconate.

These studies point out very clearly that environmental or mutational changes that produce an insensitivity to glucose repression cannot necessarily be interpreted as having produced an insensitivity to catabolite repression per se. That is, the insensitivity need not be due to some profound change in the transcriptive or translational process that regulates activity of the lac operon. It can be due in some cases to a more trivial or catabolic event that alters the production or maintenance of a low molecular weight "effector" molecule that is derived from the repressor substrate. In fact, all known examples in which a system is made insensitive to glucose repression appear to fall in this latter category. In a system that has been rendered insensitive to glucose repression, as has been shown to be the case with the cyclic 3',5'-AMP reversal of glucose repression, it is desirable to determine if this is in fact a trivial or a profound effect. The studies described in this report showed that although cyclic 3',5'-AMP could reverse repression by glucose, it had only limited ability to reverse repression by G-6-P or by a combination of glucose and gluconate. It would thus appear that the ability of cyclic 3',5'-AMP to reverse glucose repression is due to some metabolic inter-

ference in the conversion of glucose to the level of G-6-P, and is not due to a more profound effect on the activity of the lac operon or on the protein synthesizing machinery involved in β-galactosidase synthesis.

Perlman and Pastan (1968) mentioned some unpublished evidence that in E. coli cyclic 3',5'-AMP acts at the level of translation. While our experiments do not rule out this possibility, they do indicate that whatever mechanism is involved in the stimulatory effect of cyclic 3',5'-AMP on β-galactosidase synthesis, it is unable to cause reversal of catabolite repression.

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