TRANSIENT REPRESSION BY AMP IN ESCHERICHIA COLI

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SUMMARY

Transient repression of the synthesis of tryptophanase and β-galactosidase occurs when 0.05 mM AMP is added to induced cultures of Escherichia coli. The repression by AMP is overcome by cyclic AMP and it is not due to a generalized inhibition of protein synthesis. It appears that AMP inhibits and cyclic AMP stimulates tryptophanase synthesis at the level of initiation of transcription.

The repression of catabolic enzymes synthesis by glucose can be separated in E. coli into a permanent type of repression (catabolite repression) which occurs when cells grow on glucose in the presence of the proper inducer (1), and a severe, transient repression which takes place when glucose is added to induced cultures growing on other carbon sources (2). Recent studies (3) have shown that adenosine-3', 5'-monophosphate (cAMP) overcomes both permanent and transient repression. Therefore, these two types of repression can be regarded as related processes. This paper reports that adenosine-5'-monophosphate (AMP) produces a transient repression of the synthesis by E. coli of two inducible enzymes, tryptophanase and β-galactosidase, such a repression being also antagonized by cAMP.

MATERIAL AND METHODS

Experiments were routinely conducted with E. coli RS1, a strain selected from E. coli K 12 (NCIB 9483) for its increased sensitivity to rifampicin. Where indicated, the parent strain and E. coli 3.000 have

been also used. Cells were aerobically grown at 37° in a mineral base (4) supplemented with 30 mM glycerol and 0.6 μM vitamin B_1.

The synthesis of enzyme was elicited by the addition of the proper inducer to exponentially growing cultures. At the desired times samples were withdrawn and placed in the cold with 50 mM N_3Na and 100 μg/ml chloramphenicol. Cells were harvested by centrifugation, washed with 0.1 M potassium phosphate buffer, pH 7.8, and resuspended in the same buffer. This suspension was assayed for enzyme activity. Tryptophanase was measured in toluenized cells by a modification of the method of Gunsalus et al. (5). For estimation of β-galactosidase the cell suspension was disrupted by ultrasonic treatment. The resulting extract was clarified by centrifugation, and the enzyme assayed in the supernatant according to the method of Wallenfels (6).

To determine the incorporation of leucine, exponentially growing cells were incubated with 0.1 mM L-leucine-^{14}C (0.2 mCi/mmole). At the desired times 0.5 ml samples were taken and added to 0.15 ml of a solution containing 20% trichloroacetic acid (TCA) and 0.1 M unlabelled leucine. After heating at 85-90° for 15 min, the precipitate was collected on Whatman filters (GF/C) previously soaked in a solution containing 5% TCA and 40 mM unlabelled leucine, and washed with this solution. The filters were glued on planchets, dried and their radioactivity measured in a gas flow counter.

RESULTS AND DISCUSSION

The effect of AMP on enzyme induction has been tested in E. coli cells exponentially growing on glycerol, which had been previously induced for tryptophanase or β-galactosidase synthesis. The nucleotide was added twenty minutes after the inducer, when the enzyme was being synthesized exponentially, to prevent any possible interference with the internal accumulation of the inducer. Fig. 1 shows that under these conditions 0.05 mM AMP causes an almost complete inhibition of tryptophanase synthesis for about 15 minutes. Following this lag enzyme production resumes at a rate similar to that of the control culture. When AMP is added together with cAMP no transient repression occurs

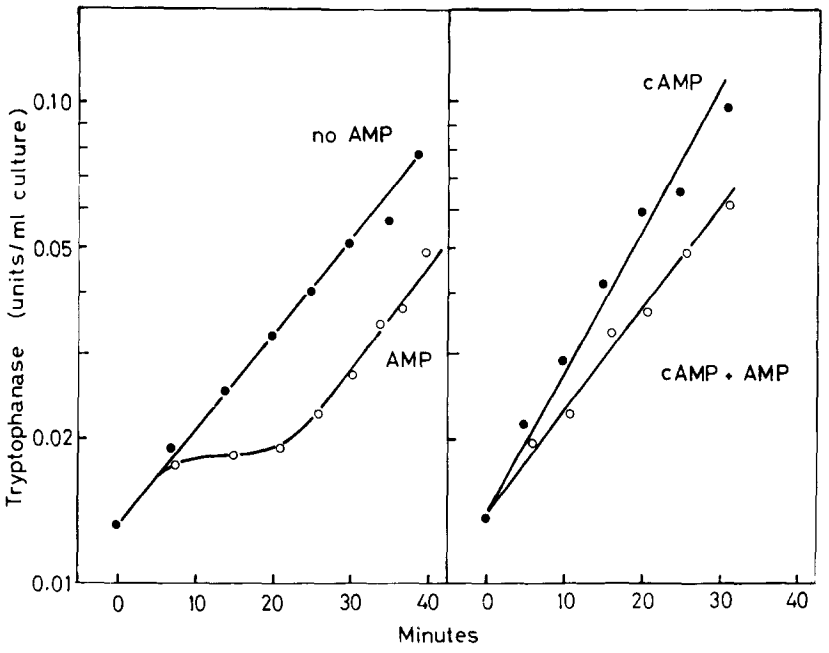

Fig. 1. Effect of AMP on the synthesis of tryptophanase in the presence and in the absence of cAMP. AMP (0.05 mM) and cAMP (5 mM) were added at zero time; tryptophanase synthesis was induced 20 minutes before the addition of the nucleotides with 1.25 mM L--tryptophan.

and the level of tryptophanase continues its exponential increase although at a rate somewhat lower than in a culture with cAMP alone (Fig. 1). The glucose effect on tryptophanase synthesis has been assayed under our experimental conditions, and the transient repression caused by this compound closely resembles the AMP effect. AMP also represses the synthesis of β-galactosidase, the inhibition being abolished by the cyclic nucleotide (Fig. 2).

The strain used for these experiments was selected by its increased sensitivity to rifampicin and it is probably a permeability mutant. However, repression by AMP is not restricted to this particular strain as evidenced by experiments which show that the inhibition of tryptophanase synthesis by AMP and its reversion by cAMP also occur in E. coli K12 (NCIB 9483) and E. coli 3.000.

AMP is not the only nucleotide able to produce repression of

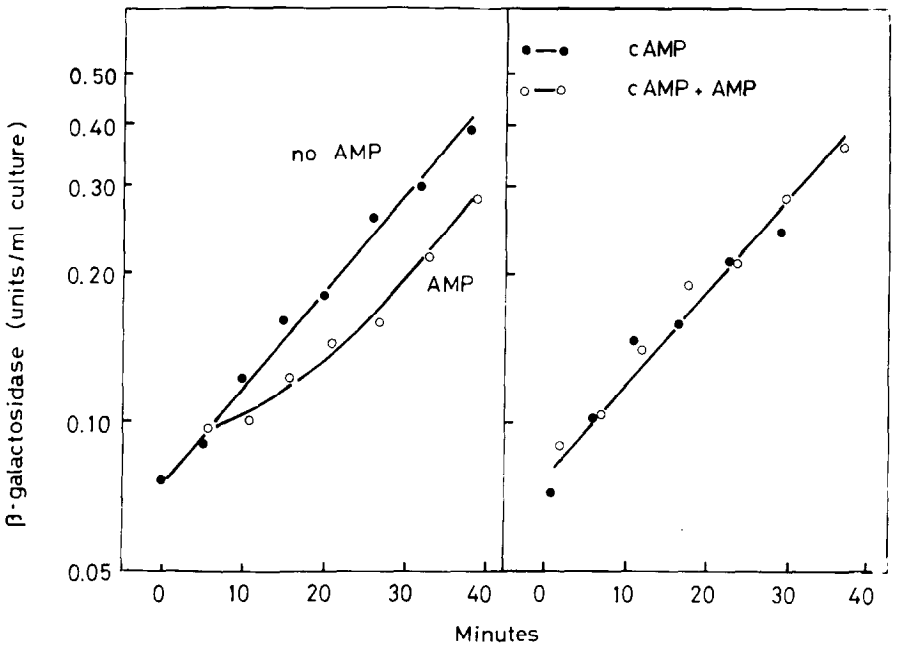

Fig. 2. Effect of AMP on the synthesis of -galactosidase in the presence and in the absence of cAMP. AMP (0.1 mM) and cAMP (5 mM) were added at zero time; -galactosidase synthesis was induced 20 minutes before the addition of the nucleotides with 0.5 mM isopropyl- -D-thiogalactopyranoside.

tryptophanase synthesis: other nucleotides, 2'-AMP, 3'-AMP, 5'-ADP, 5'-ATP, 2'deoxi-5'-AMP, 5'-GMP, 5'-GDP, 5'-UMP, 5'-UDP, NADH and NADPH, have been also tested and all of them inhibit to a degree the synthesis of this enzyme. Whether this inhibition is also relieved by cAMP has been tested for 2'-AMP, 3'-AMP, 5'-ATP, 2'-deoxi-5'-AMP and 5'-UMP. In all these cases the cyclic nucleotide reverses the inhibition.

To gain information about which step of tryptophanase production is affected by AMP we made use of rifampicin, an antibiotic which blocks the initiation of mRNA synthesis without affecting its elongation or polypeptide synthesis (7, 8). Tryptophanase was induced for a 3 minute pulse and incubation was prolonged for 30 more minutes. The increase in tryptophanase activity after the induction pulse reflects indirectly the amount of tryptophanase mRNA initiated -but not trans-

TABLE 1

Effect of AMP and cAMP on the synthesis of tryptophanase after an induction pulse

Nucleotide added		Tryptophanase activity (milliunits/ml culture)	
At the beginning of the induction pulse	At the end of the induction pulse	At the end of the induction pulse	Increase in the post-induction period
None	None	2.9	2.6
None	AMP	2.9	2.4
None	cAMP	2.9	3.0
AMP	None	2.8	0.7
AMP	cAMP	2.8	0.8
cAMP	None	3.3	6.1
cAMP, AMP	None	3.2	4.0

The induction pulse was started with 1.25 mM L-tryptophan and stopped 3 minutes later by rifampicin (250 ug/ml). Incubation was prolonged 30 minutes more after rifampicin addition. AMP (0.05 mM) and/or cAMP (5.0 mM) were added together with tryptophan (beginning of the induction pulse) or with rifampicin (end of the induction pulse), as indicated.

lated- during the 3 minutes of induction. The results, summarized in Table 1, show that there is little effect of AMP and cAMP when they are only present after rifampicin addition. Therefore, these compounds do not seem to alter the steps of enzyme production which follow the initiation of mRNA synthesis. By contrast, AMP strongly represses tryptophanase synthesis when present during the 3 minute induction pulse. Considering that the AMP effect is not due to an inhibition of the entry of tryptophan into the cells as can be inferred from the experiments reported in Fig. 1, this result suggests that the step where the nucleotide interferes is the initiation of transcription. cAMP does not relieve the inhibition by AMP if added after the induction pulse, which agrees with the hypothesis that the effect of AMP has already taken place. If both AMP and cAMP are present during the induction pulse the inhibition by AMP is partly overcome. On the other hand stimulation of tryptophanase production by cAMP clearly occurs when this nucleotide is added before rifampicin, but not if provided at the end of the induction pulse. These results suggest that both nucleotides exert their effects at the level of initiation of tryptophanase mRNA synthesis. Therefore, it appears that AMP inhibits tryptophanase production by interfering with the same step of protein synthesis where transient repression by glucose takes place. The available data from the literature indicate that this sugar inhibits β-galactosidase mRNA synthesis (9), precisely at its initial step (10). On the other hand the apparent stimulation of transcription by cAMP described in this paper although similar to that found in the β-galactosidase system (10), conflicts with the conclusion of Pastan and Perlman (11) that cAMP acts at the level of the polysome to increase the rate of tryptophanase production.

The possible physiological significance of the repression by AMP reported in this publication is at the present moment difficult to evaluate. It is not even clear yet whether AMP enters the cell or remains bound to the cell outer layers. It has been reported (12) that mutant strains of E. coli deffective in sugar transport are even more sensitive to transient repression by glucose than their parent

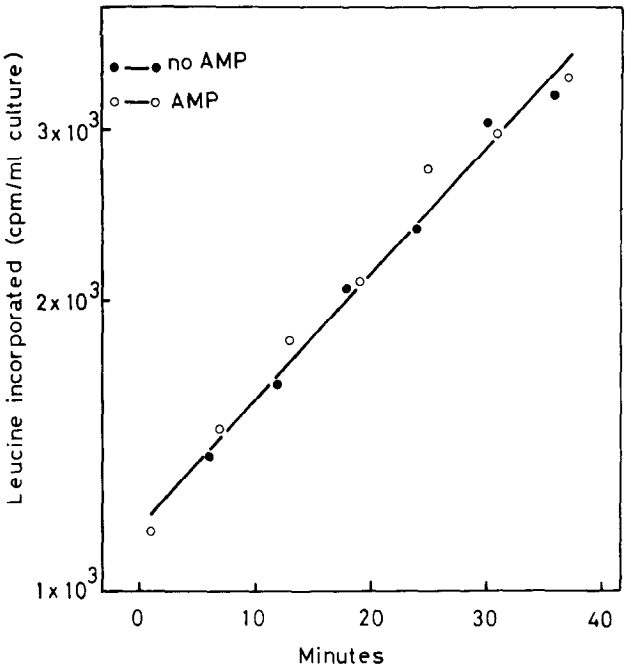

Fig. 3. The absence of effect of AMP on the incorporation of leucine. AMP (0.05 mM) was added at zero time and L-leucine -^{14}C 20 minutes before the nucleotide.

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strains. This may indicate that transient repression is produced by the interaction of glucose with some membrane component, a hypothesis that could be extended to the AMP effect.

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