SHORT PAPERS

SITE OF ACTION OF 3',5'-CYCLIC ADENOSINE MONOPHOSPHATE IN PRODUCTION OF TRYPTOPHANASE IN *ESCHERICHIA COLI*

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ABSTRACT

3',5' cyclic-AMP (cAMP) will stimulate the rate of tryptophanase synthesis in *Escherichia coli* cultures induced with tryptophan. Adding cAMP after the initiation of messenger RNA synthesis was blocked by rifampicin, did not stimulate tryptophanase synthesis. This indicates that cAMP acts at initiation of either transcription or translation and not at the level of chain elongation of either the messenger or the polypeptide chain.

EXPERIMENTS using actinomycin D to block RNA synthesis suggested that cAMP affects the translation of the tryptophanase messenger (PASTAN and PERLMAN 1969). On the other hand, when RNA synthesis was blocked by rifampicin, it was suggested that cAMP affects transcription of the tryptophanase messenger (DEL CAMPO, RAMIREZ and CANOVAS 1970). Since experiments with a temperature-sensitive mutant of *Escherichia coli* that fails to synthesize functional α and β -galactosidase or tryptophanase in the absence of cAMP at 43°, pointed out the possibility that the synthesis of these three enzymes is affected similarly by cAMP (SIMON and APIRION, manuscript in preparation), we decided to further investigate the site of action of cAMP in stimulation of tryptophanase synthesis.

RESULTS

When the effect of cAMP on tryptophanase production was measured in cultures grown at 30° and 43°, we found that cAMP enhanced tryptophanase production up to ten fold at 43°C (Figure 1) and therefore we conducted our experiments at 43°C.

In previous studies on the site of action of cAMP on tryptophanase synthesis, different levels of L-tryptophan and cAMP were used. Therefore, we determined the effect of various concentrations of L-tryptophan and cAMP on tryptophanase production and found that using L-tryptophan 0.25 to 5.0 mM did not much change the level of tryptophanase, which was about 0.25 units (PARDEE and PRESTIDGE 1961) per ml of culture of A_{560} of 0.4. Using 5.0 mM L-tryptophan we tested the effect of cAMP (0.2 to 10.0 mM), and found that the level of enzyme

FIGURE 1.—Effect of cAMP on tryptophanase synthesis at 30° and 43°C. Strain 112-130 was grown at 30°C in glycerol medium (minimal medium + 0.2% glycerol, + 1% vitamin-free casamino acids, L-leucine, L-cysteine, and L-methionine, 50 μ g/ml each). The culture was divided, and grown at 30°C and 43°C respectively to an A_{560} between 0.3 and 0.4. Both cultures were divided again into three portions. To one portion L-tryptophan (5 mM) and cAMP (5 mM) were added at various times as indicated, aliquots were withdrawn, rifampicin (300 μ g/ml) was added, and the samples were further incubated for 20 min at the temperature of incubation and then assayed for tryptophanase at 43°C according to the method outlined by PARDEE and PRESTIDGE (1961). To the other portion, L-tryptophan (5 mM) only was added followed by rifampicin (300 μ g/ml) and treated as above. On the third portion (no additions) the basal level of tryptophanase was measured. One unit of enzyme is the amount that will produce 1 nanomole of indole/min at 43°C. The strain used in these experiments is an *E. coli* K12 strain, which is auxotrophic for L-cysteine and L-leucine and is missing RNase I.

TABLE 1

Synthesis of tryptophanase after short periods of induction with L-tryptophan at 43°C

	1 minutes	Tryptophanase (units/ml) after induction for			
		2 minutes	3 minutes	4 minutes	20 minutes
cAMP	.010	.015	.012	.015	.008
tryp	.022	.026	.123	.126	.252
tryp + cAMP	.097	.148	.217	.517	2.383

An overnight culture of strain 112-130 in glycerol medium was diluted and grown at 43°C to A_{560} of 0.35. 0.2 ml samples of culture were added to tubes containing (a) cAMP (5 mM) (b) L-tryptophan (5 mM) and (c) both together, followed by the addition of rifampicin (300 μ g/ml) at various times thereafter as indicated. Cultures were incubated at 43°C for 20 min and then assayed for tryptophanase.

was about the same in this range of cAMP concentration (it was around 2 units per ml of culture of A_{560} of 0.4). In these experiments L-tryptophan or L-tryptophan and cAMP were added to the cultures 20 min before samples were assayed for tryptophanase (see Figure 1). In all further experiments the concentrations of both L-tryptophan and cAMP were 5 mM.

For the purpose of determining the site of action of cAMP we wanted to add cAMP after initiation of transcription was blocked by rifampicin, and after cells had been exposed to the inducer tryptophan for a relatively short time interval. Therefore we measured the level of tryptophanase after cells were exposed to tryptophan for 1 to 4 min. As can be seen from Table 1 measurable levels of tryptophanase can be obtained even after 1 min of exposure of cells to inducer, but 3 and 4 min of exposure result in much higher values of tryptophanase. Also it is evident that cAMP enhances tryptophanase production during each time interval. Therefore we could now test whether the addition of cAMP after rifampicin had been added was effective.

In order to ensure that there would not be more than one round of transcription, rifampicin was added 1 min after tryptophan. As can be seen in Figure 2 adding cAMP even 0.5 min after rifampicin does not enhance the level of tryptophanase production. There is, however, a residual level of tryptophanase synthesis when cAMP is added up to about two minutes after the addition of rifampicin. That this is probably due to incomplete shut off of initiation of RNA synthesis by rifampicin is indicated by the control experiment when rifampicin is added first followed by the addition of tryptophan and cAMP. As can be seen in Figure 2, the residual level of tryptophanase synthesis is quite similar in both cases (when cAMP is added with tryptophan after rifampicin, or when cAMP is added after rifampicin, and tryptophan was added prior to rifampicin). Furthermore, even when the time of exposure to tryptophan was increased to 2 or 4 min, again addition of cAMP 0.5 min after rifampicin did not increase the level of tryptophanase in the cultures.

DISCUSSION

The experiments performed by DEL CAMPO *et al.*, as well as those presented here indicate that the site of action of cAMP for enhancing production of trypto-

FIGURE 2.—Effect of cAMP on tryptophanase synthesis after addition of rifampicin at 43°C. A culture of strain 112-130 was grown in glycerol medium at 43°C to A_{560} of 0.35. A 0.2 ml aliquot was added to a tube containing L-tryptophan (5 mM) followed one minute later by rifampicin (300 $\mu\text{g}/\text{ml}$) and incubated 20 min and assayed for tryptophanase, (a). Another 0.2 ml aliquot was added to a tube containing L-tryptophan (5 mM) and cAMP (5 mM) followed one minute later by rifampicin (300 $\mu\text{g}/\text{ml}$), incubated 20 min and assayed for tryptophanase, (b). 0.2 ml aliquots were added to tubes containing L-tryptophan (5 mM) followed one minute later by rifampicin (300 $\mu\text{g}/\text{ml}$), and at indicated time intervals thereafter cAMP (5 mM) was added, these tubes were further incubated for 20 min and assayed for tryptophanase,

phanase synthesis is at the initiation of transcription of messenger RNA. This was determined by using rifampicin to block initiation of messenger RNA in *E. coli*, and adding cAMP afterwards. In the experiments performed here the amount of tryptophanase produced in a culture where cAMP is added after rifampicin is comparable to that synthesized in a culture where cAMP is not added. If cAMP acts at the initiation of transcription, there should be no cAMP effect when cAMP is added after messenger RNA synthesis has been blocked by rifampicin. If on the other hand the effect is translational, comparable amounts of tryptophanase should be produced whether cAMP is added before or after rifampicin.

Although our data indicate that the cAMP effect occurs at the initiation of transcription, it does not rule out the possibility of an effect of cAMP on early translation (SIMON and APIRION, manuscript in preparation).

One possible way to explain the discrepancy between the experiments of PASTAN and PERLMAN (1969) on one hand, and those conducted by DEL CAMPO, RAMIREZ and CANOVAS (1970) as well as the experiments described here, is that the addition of actinomycin D is very effective in blocking RNA synthesis immediately after cells are treated with EDTA, but if actinomycin is added 10–12 min after EDTA treatment it is not very effective in blocking RNA synthesis (see for instance, KENNEL 1968).

If these results are meaningful and are not due to some interaction between cAMP and rifampicin, at present we know of no enzyme in *E. coli* whose synthesis is known to be affected by cAMP during translation. Therefore, it is quite possible that in bacteria, the effect of cAMP is always dependent on the CRP protein (DE CROMBRUGGHE *et al.* 1971; ZUBAY, SCHWARTZ and BECKWITH 1970) EMMER, DE CROMBRUGGHE, PASTAN and PERLMAN 1970) and is exerted during initiation of mRNA transcription.

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(c). 0.2 ml aliquots were added to tubes containing rifampicin (300 μ g/ml) and at indicated times thereafter, a mixture of L-tryptophan (5 mM) and cAMP (5 mM) was added to each tube, incubated 20 min, and assayed for tryptophanase, (d).

The level obtained in (a) (0.02 units/ml) was subtracted from that obtained in (b) (0.103) to give the total cAMP effect or the 100% value. The value of (a) was subtracted from the (c) and (d) values as well.

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