CYCLIC 3',5'-ADENOSINE MONOPHOSPHATE AND CATABOLITE REPRESSION IN ESCHERICHIA COLI

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Received April 4,1975

SUMMARY: Several strains of E. coli were grown on different sources of carbon and β-galactosidase activity as well as intracellular and extracellular concentrations of c-AMP were determined. There was a good (inverse) correlation between extracellular concentrations of c-AMP and the intensity of catabolite repression, whereas the relationship between intracellular c-AMP levels and catabolite repression was not clear-cut.

Cyclic 3',5'- adenosine monophosphate (c-AMP) is known to be involved in the control of a variety of functions in prokaryotes as well as in eukaryotes (1,2). In the past few years it was shown to affect induced enzyme synthesis in bacterial systems, reversing in vivo the catabolite repression of such synthesis in E. coli (3,4) and counteracting the glucose effect at the transcription level (5). The nucleotide stimulates the synthesis of the lac specific mRNA by increasing the frequency of initiation of lac mRNA chains. In combination with a specific protein called CAP, the catabolite gene activator protein (6), or CRP, the c-AMP receptor protein (7), c-AMP facilitates the formation of the proper initiation complex necessary for an efficient transcription of genes sensitive to catabolite repression.

Although hardly any doubt persists about the importance of c-AMP in the regulation of the synthesis of enzymes sensitive to catabolite repression, sufficiently accurate data concerning the intracellular levels of c-AMP in E. coli growing on different carbon sources are rare. The present study was performed to obtain more detailed information about the intracellular and extracellular levels of c-AMP and to establish to what extent the metabolism of c-AMP influences the degree of catabolite repression in E. coli.

MATERIALS AND METHODS

The strains of E. coli employed here are listed in Table 1. The bacteria were cultivated aerobically at 37°C in a mineral salt medium (8) containing 10 mM glucose, 15 mM succinate or 20 mM glycerol. When using a mixture of glucose and sodium gluconate, the final concentration of each was 5 mM. L-Methionine and vitamin B_1 were added to a final concentration of 0.4 mM and 1 µg/ml, respectively, when required.

Assay of c-AMP. The protein binding assay of Gilman (9) was employed with the modification described previously (10).

Cultivation and sampling for the assay of c-AMP and β-galactosidase. Bacteria were pregrown on the carbon source to be tested for at least 10 generations. When the cells reached the middle of the log phase of growth, they were diluted with fresh medium of the same composition and allowed to grow to a density corresponding to 200 µg of protein/ml. For c-AMP determination, 5 ml samples of such cultures were filtered through membrane filters (Synpor 6, pore size 0.4 µm, diameter 50 mm) and, without any washing, immediately immersed in 4 ml of 0.05 M HCl, extracted by heating in a boiling-water bath; the pH was adjusted to 4 with 200 mM acetate buffer and the c-AMP was determined. The filtrate, obtained from the same culture, was used without any further processing for the determination of extracellular c-AMP concentrations. At the same time, 1 ml aliquots were used for the β-galactosidase assay. Just before harvesting the cells, carrier-free [^{3}H]-c-AMP was added to the culture to a final concentration of about 150,000 dpm/ml. This was done to determine the contamination of the filters with the culture medium. It was found to be important to harvest the cells after a sufficiently long period of growth in the log phase (at least 10 generations) to obtain reproducible results.

Assay of β-galactosidase. β-Galactosidase was assayed according to Muller-Hill et al. (11), methyl-thio-β-D-galactoside (TMG) at a concentration of 1 mM serving as the inducer of the enzyme.

Chemicals. Cyclic AMP was purchased from Sigma Chemical Co. or Serva and [^{3}H]-c-AMP (28 or 30 Ci/mmol), from the Radiochemical Centre, Amersham.

RESULTS AND DISCUSSION

Levels of c-AMP during catabolite repression. The degree of catabolite repression was modified by using different carbon sources. Highest repression was obtained with the mixture of glucose and gluconate, followed by glucose, and finally glycerol and succinate (Table 1).

Simultaneously with β-galactosidase activity, the amount of intracellular and extracellular c-AMP was determined. Cyclic AMP was found to be secreted into the medium, as already described with certain strains of E. coli (12,13). The amount of extracellular c-AMP varies inversely with the degree of catabolite repression (Table 2). It is lowest in the medium supplemented with the mixture of glucose and gluconate, where the most pronounced repression was

Table 1. Specific activity of β-galactosidase in E. coli strains grown on different carbon sources

Strain	Glucose + gluconate	Glucose	Glycerol	Succinate
AB 257 CR^+, met^-, PDE^+	112	690	12675	19100
AB 257 PC-1 CR^-, met^-, PDE^-	1450	9000	15600	20000
B CR^+, PDE^+	1845	4537	9320	11427
Crookes strain CR^-, PDE^-	2255	8070	6800	8170
K_{12}-3000 CR^+, PDE^+	746	4280	10450	7825
K_{12}-3300 CR^+, PDE^+	2250	5350	13500	11500
K_{12}-LA 12G CR^-, PDE^-	2125	7125	6930	not tested

CR^+, catabolite repression sensitive; CR^-, catabolite repression resistant on glucose. PDE, c-AMP phosphodiesterase. All strains used are inducible for β-galactosidase except for K_{12}-3300, which is constitutive. Specific activity of β-galactosidase is expressed in nmoles of ONPG hydrolyzed per min per mg of protein at 37°C.

found in all tested strains. The highest values were obtained in media with glycerol or succinate as carbon sources where the catabolite repression was low.

No similar substantial differences in the intracellular content of c-AMP could be detected in cultures grown on different carbon sources (Table 2). The lowest content of c-AMP was always found in bacteria grown on the glucose-gluconate mixture. In addition, the so-called catabolite repression resistant cultures always contained higher amounts of intracellular c-AMP, approaching those detected in the same cultures grown on glycerol or succinate.

Table 2. Extracellular and intracellular concentrations of c-AMP

Strain	Glucose + gluconate		Glucose		Glycerol		Succinate	
	A	B	A	B	A	B	A	B
AB 257	26	0.7	52	0.7	128	1.3	205	2.5
AB 257 PC-1	54	2.1	146	4.3	243	4.0	136	3.5
B	30	1.2	52	1.4	115	1.7	154	1.4
Crookes	46	2.0	154	1.9	192	2.5	148	2.3
K_{12}-3000	34	2.1	49	1.7	58	3.3	87	3.3
K_{12}-3300	25	0.6	46	0.6	184	2.4	282	2.0
K_{12}-LA 12G	42	0.8	76	0.7	56	2.3	not tested	

Column A - extracellular concentrations of c-AMP expressed in pmoles per 1 ml of the media

Column B - intracellular concentrations of c-AMP expressed in 10^{-5} M. The calculation is based on the fact that the E. coli cell represents a volume of 0.75×10^{-12} ml. The assay was done when the cultures reached 5×10^{8} cells/ml.

The discrepancy of the values shown in Table 2 with other published results is probably due to inadequate techniques used for the extraction of c-AMP, together with the harvesting of cells (14). Collecting the cells by techniques other than filtration without any further washing can cause an increase in the intracellular level of c-AMP and, hence, incorrect values might be obtained. It is also very important to know the portion of the extracellular c-AMP remaining on the filters after collection of cells. This should be subtracted from the determined values (13).

Overall synthesis of c-AMP during growth. It was of interest to investigate the course of c-AMP synthesis during growth, the results obtained in a

typical experiment being shown in Fig. 1. As long as the culture was in the logarithmic phase of growth, the synthesis of c-AMP was a linear function of newly formed proteins. Once the culture reached the stationary phase, due to exhaustion of the carbon source, c-AMP was synthesized at a much higher rate (not shown here). These results would suggest that the metabolism of the carbon sources is involved in the regulation of the activity of bacterial adenylate cyclase. So far, no data are available to explain these findings.

The findings reported here seem paradoxical: differences in the concentration of extracellular c-AMP found when various sources of carbon were employed, were in good agreement with the hypothetical role of c-AMP in catabolite repression. The intracellular concentrations of c-AMP, however, varied only little and inconsistently with different sources of carbon. A role of the c-AMP of the medium in mediating catabolite repression is unlikely in view of the relative impermeability of E. coli to exogenous c-AMP [starving bacteria take up exogenous c-AMP no more effectively than do non-starving bacteria (Rickenberg, personal communication)]. In view of these findings the possibility must be considered that the transcriptional machinery responds to slight, threshold changes in intracellular c-AMP concentrations and that these changes are too small to be measured accurately by the techniques currently employed for the assay of c-AMP. Alternatively, the possibility should be con-

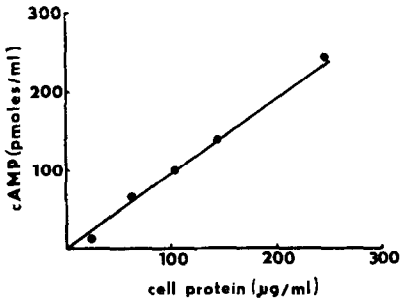

Figure 1. Total synthesis of c-AMP during growth of Crookes strain of E. coli in the mineral salt medium supplemented with glucose.

sidered that regulation is based on the sum total of the intracellular and extracellular c-AMP in the sense that the extracellular cyclic nucleotide repre sents the spill-over of c-AMP previously active inside the cell. The present results support the hypothesis that c-AMP regulates the rate of initiation of transcription of operons sensitive to catabolite repression.

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