CONTROL OF URACIL TRANSPORT BY CYCLIC AMP IN *E. COLI*

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1. Introduction

Previous work from this laboratory indicated that the addition of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) to the culture medium decreases the growth rate of *E. coli*. The cyclic AMP concentration necessary for growth inhibition is the same as that required for the reversal of permanent catabolite repression [1]. On the other hand, growth inhibition was not observed in mutants defective in the cyclic AMP receptor protein or when the cyclic nucleotide was replaced by other nucleoside-phosphates [2].

The cyclic AMP-promoted inhibition of growth rate could be due to a decrease in the activity of some enzymatic steps required for the metabolization of certain carbon sources or, in turn, to a more general phenomenon involving for example membrane transport, macromolecular synthesis (DNA, RNA or protein), etc. In fact this inhibition varies with the type of carbon source used in the culture medium. However, all the evidence obtained indicates that the absence of cyclic-AMP effect on the growth rate of bacteria cultured in succinate, malate or glycerol as carbon sources is the consequence of the relatively high levels of the nucleotide within the cells [2].

This paper provides evidence indicating that the culture of *E. coli* in the presence of cyclic AMP leads to an impairment in the transport of uracil.

2. Materials and methods

E. coli 3000 (HfrH, thy$^-$), a prototrophic *E. coli* K12 strain was used throughout this work. Bacteria were grown in minimal medium containing 14 g of K_2HPO_4, 6 g of KH_2PO_4, 2 g of $(NH_4)_2SO_4$, 0,2 g of $MgSO_4$ and 5 mg of thiamine per liter, supplemented with glucose (25 mM) as carbon source.

Cultures were started by dilution of an overnight culture in fresh medium to a density of 1.5×10^7 cells per milliliter. Growth was carried out at 37°C with shaking (100 cycles/min). The increase in cell density was measured at 560 nm.

Determinations of total uracil uptake were performed on 0.1 ml aliquots of exponentially growing cultures ($O.D._{560}$ 0.15–0.30) incubated with 7 μM [^{3}H]uracil (specific activity 250 μCi/μmole) at 37°C for the indicated periods of time. The total volume was 0.115 ml. Incubations were stopped by dilution with 4 ml of cold minimal medium. The mixtures were rapidly filtered through nitrocellulose filters (Selectron, 0.45 μpore) and washed twice with 4 ml of fresh medium. Dilution, filtration and washings took no more than 15 sec.

Incubations for [^{3}H]uracil or [^{14}C]leucine incorporation into trichloroacetic acid (TCA) insoluble material were similar to that described for total uracil uptake. The concentration of [^{14}C]leucine was 40 μM (specific activity 0.25 mCi/μmole). Reactions were stopped by the addition of cold 5% TCA. The mixture was then filtered through nitrocellulose filters and washed with the acid solution.

Filters were dried and counted using a toluene-Omnifluor (New England Nuclear) mixture. Radioactivity incorporated was expressed as nmoles incorporated/ml per $O.D._{560}$ unit. The uptake of [^{3}H]uracil into the acid soluble fraction was calculated as the difference between total uptake and incorporation into the TCA-insoluble fraction.

Table 1
Effect of cyclic AMP on the rate of [^{3}H]uracil and [^{14}C]leucine incorporation into the acid insoluble cell fraction

Time of culture (min)	[^{3}H]uracil incorporation		[^{14}C]leucine incorporation	
	No additions	Plus 5 mM cyclic AMP	No additions	Plus 5 mM cyclic AMP
75	0.59	0.44	8.0	8.72
105	0.45	0.13	6.70	4.96
165	0.46	0.14	6.45	4.80
195	0.58	0.16	7.18	6.49

Aliquots of bacterial cultures carried out in the presence or absence of 5 mM cyclic AMP were taken at the indicated times and assayed for incorporation of precursors. The assays were performed at 37° C for 5 min as indicated in Materials and methods. Results were expressed as nmoles of precursor incorporated in 5 min per ml of culture per O.D.$_{560\ nm}$.

3. Results and discussion

As a first approach to the study of the factors responsible for the effect of cyclic AMP on bacterial growth, the action of this compound on RNA and protein synthesis was studied. The rate of [^{3}H]uracil and [^{14}C]leucine incorporation into acid insoluble material was taken as a gross measure of these biosynthetic processes. Table 1 shows that whereas incorporation of [^{14}C]leucine was slightly affected by the presence of cyclic AMP in the culture medium, that of [^{3}H]uracil seemed to be strongly influenced by the nucleotide. Indeed, bacterial cultures carried out in media containing 5 mM cyclic AMP incorporation uracil at rates of about three times lower than those measured in cultures carried out in the absence of the cyclic nucleotide. The inhibition of uracil incorporation did not appear to be an immediate consequence of the presence of cyclic AMP in the culture medium. As it can be observed in table 1, this inhibition was only evident 105 min after the addition of cyclic-AMP. This period was roughly equivalent to one generation time.

A decrease in the rate of uracil incorporation into the acid insoluble cell fraction could be due to two facts: to an inhibition of the rate of RNA synthesis or, in turn, to an impairment in the transport of the precursor. The experiment shown in fig. 1 indicates that the addition of 5 mM cAMP inhibited precursor transport, measured either as total [^{3}H]uracil uptake

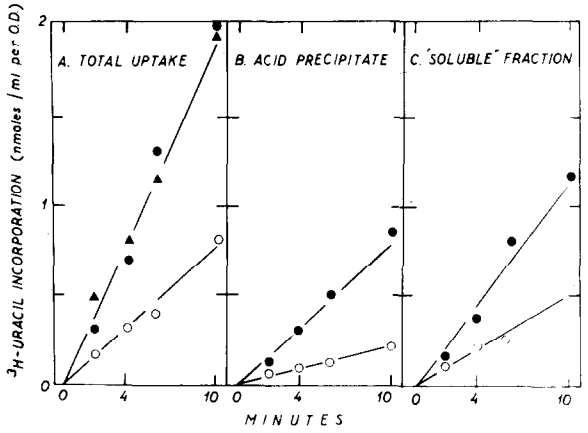

Fig. 1. Effect of cyclic AMP on uracil uptake in bacterial cultures. The cultures were grown in media with (open circles) or without (closed circles and triangles) 5 mM cyclic AMP. Aliquots of these cultures were assayed for uracil uptake (A) or incorporation into the TCA insoluble fraction (B). The difference between the values in A and B were plotted in C ('soluble fraction'). Aliquots from the culture carried out in the absence of cyclic AMP were also assayed for uracil uptake in reaction mixtures containing 5 mM of the cyclic nucleotide (A, triangles). Other conditions were as those indicated under Materials and methods.

or as incorporation into the acid-soluble fraction.

The latter result was confirmed studying the precursor uptake in the absence of RNA synthesis. Such condition was attained after preincubation of the bacterial cells with the antibiotic rifampicin. As it

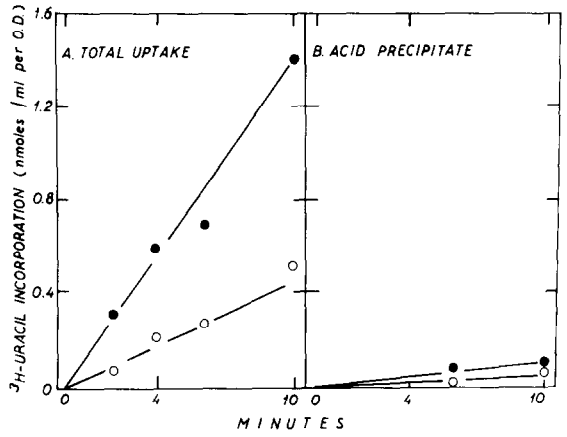

Fig. 2. Effect of cyclic AMP on uracil uptake in bacterial cultures treated with rifampicin. Bacteria were grown in the presence (open circles) or absence (solid circles) of 5 mM cyclic AMP. Prior to the assay for total uracil uptake or for incorporation into the TCA insoluble fraction the cultures received 100 μg/ml of rifampicin and were further incubated for ten minutes at 37°C. Other conditions were as those indicated in fig. 1.

is shown in fig. 2 under conditions leading to a 90% inhibition of RNA synthesis the impairment of the precursor transport in cultures containing cyclic AMP was still evident.

Some experiments were performed in order to characterize further this inhibition. As it is shown in fig. 1 the phenomenon is not merely a competition between [^{3}H]uracil and cyclic AMP for the entry into the cell, since the presence of 5 mM cyclic AMP in the assay mixture did not alter the precursor uptake. This result confirms a similar observation made by Perlman and Pastan [3] showing that under the conditions employed for the reversion of transient catabolite repression the cyclic nucleotide did not affect uracil incorporation into acid insoluble material. Furthermore, the result is also consistent with data of table 1 indicating a time requirement of about one generation for the establishment of the inhibition.

Inhibition of precursor uptake is a specific phenomenon in terms of the dependence for cyclic AMP. No effect was observed in cultures supplemented with ATP, ADP or AMP (table 2). Fig. 3 shows the influence of different amounts of cyclic AMP added to the culture medium on the [^{3}H]uracil uptake. Maximal inhibition was observed at 5 mM, and half

Table 2
Specificity of cyclic AMP effect

Initial rates of [^{3}H]uracil uptake nmoles/min per ml per O.D.$_{560\ nm}$				
No additions	Cyclic AMP	AMP	ADP	ATP
0.22	0.13	0.23	0.18	0.19

Cultures were carried out in the presence of the indicated additions as described in Materials and methods. The concentrations of nucleotides was 5 mM.

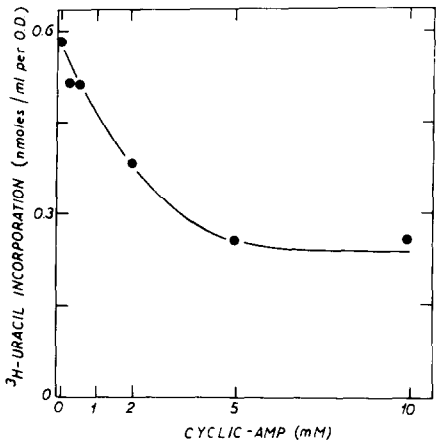

Fig. 3. Uracil uptake of cultures performed in media containing different concentrations of cyclic AMP. Incubations for the assays of precursor uptake were carried out at 37°C for 3 min.

maximal effect was attained at about 1.5 mM cyclic AMP. This range of cyclic nucleotide concentration is similar to that required for the relief of permanent catabolite repression of β-galactosidase synthesis in *E. coli* [4].

If the rate of precursor uptake is a function of the intracellular level of cyclic AMP, it could be expected that under conditions such as those imposed by growth in succinate or malate, leading to the intracellular accumulation of the nucleotide [5,6], the presence of cyclic AMP in the culture medium should not modify uracil uptake. As it is shown in table 3 this seemed to be the case. The impairment of precursor uptake, as well as the stimulation of β-galactos-

Table 3
Effect of cyclic AMP on growth rate, uracil uptake and β-galactosidase synthesis in *E.coli* Hfr 3000 cultured in different carbon sources

Carbon source	Cell growth (% of doubling time increase)	Uracil uptake (% of inhibition)	β-Galactosidase induction (% of stimulation)
Glucose	41.5	48	121
Malate	0.0	1	14
Succinate	2.4	0	2

Concentration of glucose, malate or succinate 25 mM. Rate of β-galactosidase induction was measured as previously described [1]. Per cent differences were referred to the cultures carried out in the absence of cyclic AMP.

idase synthesis and the inhibition of bacterial growth, was observed in glucose-containing media, but not in cultures carried out in succinate or malate.

The results reported in this paper indicate that uracil uptake in bacteria is under the control of cyclic AMP. The implicance of this regulatory mechanism in the control of cell multiplication is not obvious. In fact, the inhibition of bacterial growth rate by cyclic AMP was observed in prototrophic strains growing in minimal medium [1–2]. However, as it was suggested for eucariotic systems [7,8] under certain conditions transport of metabolite precursors could be limitant for cell growth.

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