CYCLIC AMP AS AN ANTAGONIST OF CATABOLITE REPRESSION IN *ESCHERICHIA COLI*

Agnès ULLMANN and Jacques MONOD
Collège de France, Institut Pasteur, Paris, France

Received 25 October 1968

1. Introduction

Cyclic 3′5′ AMP (CyAMP) * is known to be a mediator in a variety of hormonal systems [1]. The observation of Makman and Sutherland [2] that glucose-starved *Escherichia coli* cells accumulate large amounts of CyAMP, focussed our attention on a possible role of this nucleotide as a mediator of the so-called glucose effect (catabolite repression [3]) in bacteria.

In the present paper we wish to report some experiments which show that CyAMP does, under proper conditions, antagonize the repression of enzyme synthesis by glucose.

2. Materials and methods

CyAMP was purchased from Calbiochem and Sigma Chemical Companies.

The bacterial strains were grown at 37°C in minimal salt medium, supplemented with vitamin B_1. If not otherwise stated glucose was used as carbon source.

β-galactosidase was assayed as described elsewhere [4]; bacterial dry weight was calculated from optical density measurements at 600 mμ.

3. Results

As is well known [3,5] the severity of the catabolic repression effect depends in particular upon the relative availability of the carbon and nitrogen sources in the medium. If CyAMP antagonizes catabolic repression more or less specifically, one would expect a marked effect of the nucleotide under conditions of severe repression, and little or no effect under conditions where it is minimized. These expectations are fulfilled, as may be seen from table 1. For instance, in the presence of succinate as carbon source and NH_4^+ as nitrogen source, the differential rate of β-galactosidase synthesis is maximal and is not increased by addition of CyAMP. With NH_4^+ and glucose, the rate drops to 33% of that on succinate, and it is more than doubled in the presence of CyAMP. With a dipeptide as nitrogen source and glucose as carbon source, repression is extremely severe: the rate amounts to less than 1% of maximal, and it is increased some fifty-fold in the presence of CyAMP.

It is seen also from table 1 that marked effects are observed only in the presence of fairly high concentration of CyAMP. This may reflect poor permeability of the cells towards the nucleotide, or also the fact, reported by Makman and Sutherland [2] that CyAMP appears to be actively excreted from the cells upon addition of glucose to the medium.

Since the wild type strain used in the experiments reported above is inducible with respect to β-galactosidase, the possibility should be considered that CyAMP may act by favoring the permeation and accumulation of the inducer. Several constitutive strains, grown in the absence of inducer were therefore tested, in comparison with inducible strains. It will be seen (table 2) that:

a) The effect of CyAMP is present in all strains and appears to be greater in those strains where the differential rate of β-galactosidase synthesis is lowest in the absence of the nucleotide.

b) Its effect in a cryptic strain is about the same as in the wild type.

* Abbreviations used: 3′5′ cyclic AMP = CyAMP; isopropyl-β-D-thiogalactoside = IPTG.

Table 1

Strain 3000 (Hfr $i^+z^+y^+$) was induced with IPTG 5×10^{-4} M and the differential rate of β-galactosidase synthesis was followed for a period of one to two generation times. The CyAMP (at concentrations mentioned in the table) was added to the cultures 5 minutes prior to the induction. The results are expressed in units of β-galactosidase/mg dry weight bacteria. (Abbreviations: Gly-glu = glycyl-glutamate; His-glu = histidyl-glutamate).

Carbon source	Nitrogen source	Addition of 3′5′ cyclic AMP	Concentration (M)	U/mg β-galactosidase
Glucose	$(NH_4)_2SO_4$	–	–	4,400
Glucose	$(NH_4)_2SO_4$	+	2×10^{-4}	4,400
Glucose	$(NH_4)_2SO_4$	+	10^{-3}	7,000
Glucose	$(NH_4)_2SO_4$	+	5×10^{-3}	10,500
Glucose	His-glu	–	–	60
Glucose	His-glu	+	5×10^{-3}	2,340
Glucose	Gly-glu	–	–	52
Glucose	Gly-glu	+	5×10^{-3}	2,700
Glycerol	$(NH_4)_2SO_4$	–	–	7,600
Glycerol	$(NH_4)_2SO_4$	+	5×10^{-3}	9,500
Succinate	$(NH_4)_2SO_4$	–	–	13,500
Succinate	$(NH_4)_2SO_4$	+	5×10^{-3}	13,400

Table 2

The cultures were grown in minimal medium in the presence of glucose. The i^+ strains were induced with IPTG (5×10^{-4} M), the i^- strains were assayed without induction. The cultures were grown for two generations in the absence or presence of CyAMP (5×10^{-3} M). The results are expressed in differential rate of enzyme synthesis.

Strain	Characteristics	Addition of 3′5′ cyclic AMP	U/mg β-galactosidase
3000	Hfr $i^+z^+y^+$	–	7,200
		+	15,500
300P	Hfr $i^+z^+y^-$	–	5,700
		+	15,300
3300	Hfr $i^-z^+y^+$	–	1,900
		+	14,600
2E01	F^- $i^-z^+y^-$	–	4,600
		+	19,000

c) The effect of CyAMP is, if anything, more marked in the two constitutive than in the wild-type strains and therefore is independent from inducer permeation or, from inducer-repressor interaction.

Other enzyme systems sensitive to catabolite repression are also sensitive to the antagonistic effect of CyAMP. This could be shown, qualitatively, in a simple and striking way. As one of us found many years ago, the growth of bacteria in certain mixtures of two carbohydrates is "diauxic", i.e. exhibits two complete cycles, separated by a more or less prolonged lag [8]. This is known to be due to the repressive effect of one of the carbohydrates (generally glucose) upon the synthesis of the enzyme system required for the metabolism of the other. We therefore tested the effect of CyAMP upon growth in mixtures of glucose + maltose, glucose + xylose, and glucose + lactose. In all cases, the characteristic stationary phase separating the two growth cycles observed in the control, was virtually suppressed in the presence of CyAMP. An example is shown in fig. 1.

Other adenine nucleotides (see table 3) were also tested under these various conditions, and found to be completely inactive.

In connection with these observations, we wish to report briefly on the properties of an interesting mutant strain which appears to be affected in some mechanism related to the glucose effect. This strain (3 ARY), derived (apparently in one step) from Hfr 3000, is unable to grow, or only exceedingly slowly, on any carbohydrate except glucose. Since the carbohydrates in question include disaccharides such as maltose and lactose, whose metabolism involves the liberation of glucose, the lesion, in this strain, must be attributed to one of the earliest steps in the mechanism of carbohydrate dissimilation, namely either to a pleiotropic defect in permeation, or in the

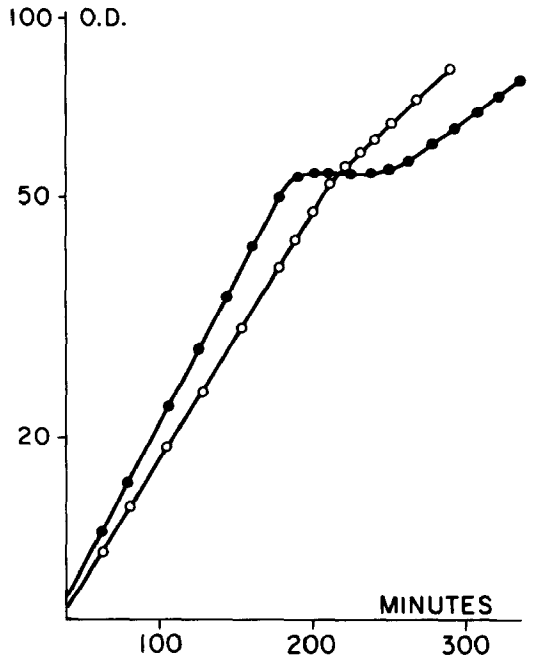

Fig. 1. An overnight culture of strain 3000 was diluted in 63 minimal medium supplemented with vitamin B_1 and a mixture of glucose (0.02%) and maltose (0.1%). Optical density at 600 mμ was measured:

●——● Control

○——○ 8×10^{-3} M CyAMP added at $T = 0$.

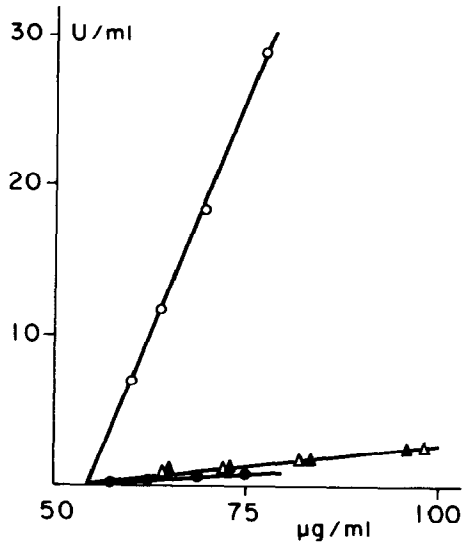

Fig. 2. Exponentially growing cultures were centrifuged, washed and resuspended in minimal medium containing glycyl-glutamate as the only nitrogen source, in the presence of glucose as carbon source. The cultures were induced with 10^{-3} M IPTG at 55 μg/ml dry weight. CyAMP (8×10^{-3} M) was added 5 minutes prior to induction to the cultures labelled ○——○ and △——△

●——● Strain 3000 (control)

○——○ Strain 3000 + CyAMP

▲——▲ Strain ARY (control)

△——△ Strain ARY + CyAMP

Table 3

Strain 3000 was induced with 5×10^{-4} M IPTG in 63 minimal medium in the presence of glucose as carbon source. The nucleotides were added 5 minutes prior to induction at a final concentration of 8×10^{-3} M (the final concentration of adenine was 2×10^{-3} M). The numbers represent differential rate of β-galactosidase synthesis.

Addition	U/mg β-galactosidase
–	6,600
3′5′ cyclic AMP	12,600
Adenine	6,800
5′ AMP	6,700
3′ AMP	5,700
2′3′ AMP	5,900
ATP	5,200

capacity to derepress the synthesis of a whole series of inducible (glucose sensitive) enzyme systems.

Actually the synthesis of β-galactosidase is induced only to very low levels (5% of maximum) in this strain, even in the presence of concentrations of IPTG known to fully induce permeaseless mutants. Moreover, as shown in fig. 2, the differential rate of β-galactosidase synthesis is, in this strain, completely insensitive to CyAMP under conditions where its effect is maximized with wild type strain. This mutant, in other words, might be described as having lost the capacity to respond to "derepressors" of the glucose effect. As is well known, certain mutants (i^s) of the "Lac" regulator gene (i) have lost the capacity to bind galactosides, and are therefore non-inducible [6]. The properties of strain 3 ARY might tentatively and strictly as a working hypothesis be interpreted in a similar way.

In conclusion, the observations reported above suggest that CyAMP may act as a specific mediator,

actually a "derepressor" of the glucose effect. The evidence however, as it now stands, does not exclude a more trivial interpretation: namely that CyAMP might act indirectly as an inhibitor of an enzyme system responsible for the synthesis of certain "catabolic metabolites" which would be more directly involved in the effect.

As our work was in progress, the paper by Perlman and Pastan [7] came to our attention. While the experimental conditions used by these authors were quite different from ours, their observations are, in part, similar to ours, as are their conclusions.

Acknowledgements

Research in the Department of Cellular Biochemistry, at the Pasteur Institute, has been aided by grants from the U.S. National Institutes of Health, the "Délégation Générale à la Recherche Scientifique et Technique", the "Centre National de la Recherche Scientifique" and the "Commissariat à l'Energie Atomique".

References

[1] G.A.Robison, R.W.Butcher and E.W.Sutherland, Ann. Rev. Biochem. 37 (1968) 149.
[2] R.S.Makman and E.W.Sutherland, J. Biol. Chem. 240 (1965) 1309.
[3] B.Magasanik, Cold Spring Harb. Symp. Quant. Biol. 26 (1961) 193.
[4] A.Ullmann, D.Perrin, F.Jacob and J.Monod, J. Mol. Biol. 12 (1965) 918.
[5] J.Mandelstam, Biochem. J. 79 (1961) 489.
[6] G.Willson, D.Perrin, M.Cohn, F.Jacob and J.Monod, J. Mol. Biol. 8 (1964) 582.
[7] R.Perlman and I.Pastan, Biochem. Biophys. Res. Commun. 30 (1968) 656.
[8] J.Monod, Recherches sur la croissance des cultures bactériennes (Hermann Edit., Paris, 1941).